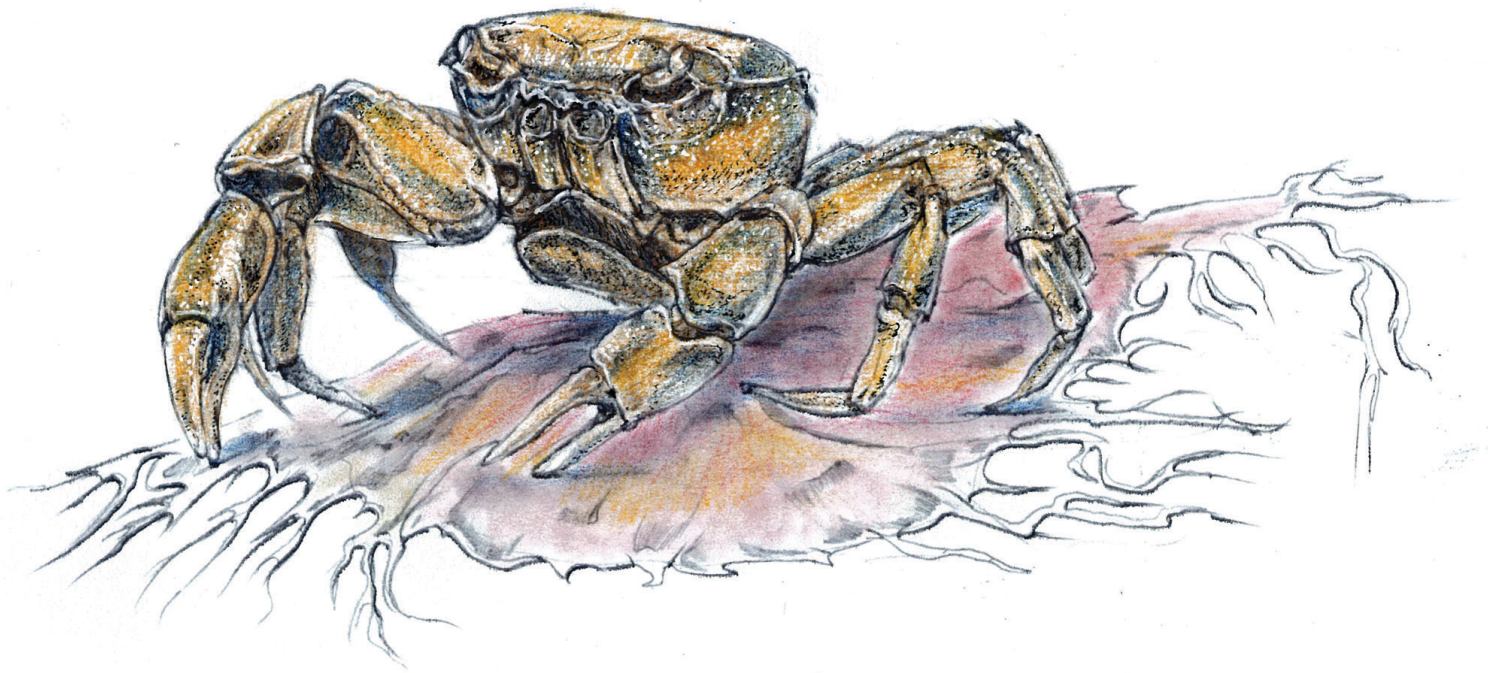
Slovenian Veterinary Research

Slovenski veterinarski zbornik

THE SCIENTIFIC JOURNAL OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA



ISSN 1580-4003

Slov Vet Res, Ljubljana, 2025, Volume 62, Supplement 27, Pages 1–68

Slovenian Veterinary Research

Slovenski veterinarski zbornik

THE SCIENTIFIC JOURNAL OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA

Slov Vet Res, Ljubljana, 2025, Volume 62, Supplement 27, Pages 1–68

The Scientific Journal of the Veterinary Faculty University of Ljubljana

Slovenian Veterinary Research

Slovenski veterinarski zbornik

Previously: RESEARCH REPORTS OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA
Prej: ZBORNIK VETERINARSKE FAKULTETE UNIVERZA V LJUBLJANI

4 issues per year / Izhaja štirikrat letno
Volume 62, Supplement 27 / Letnik 62, 27. tematska izdaja

Published by / Založila	University of Ljubljana Press / Založba Univerze v Ljubljani
For the Publisher / Za založbo	Gregor Majdič, Rector of the University of Ljubljana / Rektor Univerze v Ljubljani
Issued by / Izdala	Veterinary Faculty University of Ljubljana / Veterinarska fakulteta Univerze v Ljubljani
For the Issuer / Za izdajatelja	Breda Jakovac Strajn, Dean of the Veterinary Faculty / Dekanja Veterinarske fakultete
Address	Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia
Naslov	Veterinarska fakulteta, Gerbičeva 60, 1000 Ljubljana, Slovenija
Phone / Telefon	+386 (0)1 4779 100
E-mail	slovetres@vf.uni-lj.si
Sponsored by / Sofinancira	The Slovenian Research Agency / Javna agencija za raziskovalno dejavnost Republike Slovenije
Printed by / Tisk	DZS, d.d., Ljubljana, February/Februar 2025
Number of copies printed / Naklada	220
Indexed in / Indeksirano v	Agris, Biomedicina Slovenica, CAB Abstracts, IVSI Urlich's International Periodicals Directory, Science Citation Index Expanded, Journal Citation Reports – Science Edition
	https://www.slovetres.si/
	ISSN 1580-4003 2385-8761 (on-line)

The cover illustration by Pšenica Kovačič.

Table of Content

Slov Vet Res
DOI 10.26873/SVR-2182-2024
UDC 2-468.6:24-18:591.139:614.217:616-006:636.09
Pages: 5–7

Editorial

Advancing Cancer Therapies Through the One Health Approach: The Role of Veterinary and Comparative Oncology in Human and Animal Patient Care

Napredek pri zdravljenju raka z uporabo pristopa Eno zdravje: vloga veterinarske in primerjalne onkologije pri zdravljenju ljudi in živali

Klementina Fon Tacer[1,2]

[1]Texas Tech University School of Veterinary Medicine, [2]Texas Center for Comparative Cancer Research, Amarillo, Texas, USA

fontacer@ttu.edu

Accepted: 20 December 2024

The landscape of human health problems in the last several decades has markedly changed due to life sciences development. Non-communicable (chronic) diseases have become the leading causes of death, with several types of cancer ranking among the top causes globally (1). As in humans, and also in veterinary medicine, the landscape of the disease burden is changing. In small animal practice in particular. Cancer is among the most common causes of death for dogs (and cats) in the developed world, even though it is uncommon in wildlife and other domestic animals (2). Dogs get cancer at roughly the same rate as humans, while there is less information about the rate of cancer in cats.

Over the past 50 years, there have been significant advances in basic cancer research, leading to improved preventive measures, diagnostic tools, and treatments. However, therapy resistance and progression to metastatic disease continue to limit improvements in patient survival rates, even with the recent advancement of immunotherapy, the latest breakthrough in cancer management (3). Moreover, cancer survivors often experience a lower quality of life due

V zadnjih desetletjih je zaradi napredka v znanosti o življenju prišlo do pomembnih sprememb v razumevanju in obravnavi zdravstvenih težav pri ljudeh. Nenalezljive (kronične) bolezni so postale vodilni vzrok smrti, pri čemer se več vrst raka uvršča med glavne vzroke smrtnosti na globalni ravni (1). Podobno kot pri ljudeh se tudi v veterinarski medicini spreminja narava bremena bolezni, zlasti v praksi malih živali. V razvitem svetu je rak eden najpogostejših vzrokov smrti pri psih (in mačkah), medtem ko se pri divjih živalih in drugih domačih vrstah redko pojavlja. Pojavnost raka pri psih je primerljiva s tisto pri ljudeh, medtem ko je podatkov o pogostosti raka pri mačkah manj.

V zadnjih petdesetih letih je bil dosežen izjemen napredek na področju temeljnih raziskav raka, kar je omogočilo pomembna izboljšanja pri preventivnih ukrepih, diagnostičnih metodah in možnostih zdravljenja. Kljub temu pa odpornost proti zdravljenju in napredovanje bolezni v metastatsko fazo še vedno predstavljata glavno oviro pri izboljšanju preživetja bolnikov, tudi ob nedavnih dosežkih na področju imunoterapije, ki velja za enega najnovejših prebojev v zdravljenju raka (3). Poleg tega se mnogi preživeli bolniki z rakom soočajo

to the systemic effects of tumors and the side effects of treatments (4).

Comparative oncology and collaboration between human and animal cancer researchers and clinicians are critically important to improve the prevention, diagnosis, and treatment of cancer in animals, and translate the research and knowledge between the two fields to benefit people with cancer. Several comparative cancer centers have been at the forefront of comparative cancer research in the United States and Europe. At the University of Ljubljana Veterinary Faculty (my alma mater), there are more than 20 years of comparative oncology research (see Editorial In the spotlight in this issue for more details). Recently, we also launched the Texas Center for Comparative Cancer Research (TC3R) at the Texas Tech University School of Veterinary Medicine (my current position as assistant professor) to help in the efforts to advance the prevention, diagnosis, and treatment of cancers in both humans and animals with evolutionary, transdisciplinary and comparative approaches (5).

At the Slovenian Veterinary Research Journal, we occasionally publish articles related to the field of comparative oncology (6, 7). Given the significance of the topic and to encourage further transdisciplinary collaboration, we have decided to dedicate special issues to comparative oncology, with this being the first. This collection of articles highlights the importance of understanding the similarities and differences in cancer susceptibility, disease aggressiveness, and treatment response between animals and humans, aiming to advance therapeutic opportunities for both.

Nataša Tozon highlights the developments and collaborations in comparative oncology over the past 20 years at the University of Ljubljana. Breznik and coauthors review the literature on the role of the gut microbiome in cancer and its impact on outcomes for both human and animal patients. Švara and collaborators present data on the incidence and types of canine tumors in Slovenia over the last 20 years. Ozmen et al. provide a transcriptomic analysis of canine hemangiosarcoma, while Belma et al. discuss the metastases of ovine pulmonary adenocarcinoma. Lastly, Hatipoglu et al. offer a case report on undifferentiated embryonal rhabdomyosarcoma in a German Shepherd dog.

This is the first set of articles devoted to comparative oncology. If you have exciting research, case reports, or review articles on this topic to contribute, please consider submitting to our next one!

s slabšo kakovostjo življenja, ki je posledica sistemskih učinkov tumorjev in stranskih učinkov zdravljenja (4).

Primerjalna onkologija ter sodelovanje med raziskovalci raka pri ljudeh in živalih ter kliniki igrata ključno vlogo pri izboljšanju preprečevanja, diagnostike in zdravljenja raka pri živalih, hkrati pa omogočata prenos raziskav in znanja med obema področjema v korist bolnikov z rakom. Več centrov za primerjalno onkologijo v Združenih državah Amerike in Evropi je v ospredju primerjalnih raziskav raka. Na Veterinarski fakulteti Univerze v Ljubljani (moji almi mater) se že več kot dvajset let ukvarjajo s primerjalnimi onkološkimi raziskavami (za podrobnosti glej uvodnik V središču pozornosti v tej številki). Nedavno smo na Veterinarski fakulteti Univerze Texas Tech (moje trenutno delovno mesto docenta) ustanovili Teksaški center za primerjalne raziskave raka (TC3R), ki s pomočjo evolucijskih, transdisciplinarnih in primerjalnih pristopov prispeva k napredku na področju preprečevanja, diagnostike in zdravljenja raka pri ljudeh in živalih (5).

V Slovenskem veterinarskem zborniku občasno objavljamo prispevke s področja primerjalne onkologije (5, 6). Zaradi pomembnosti te teme in z namenom spodbujanja nadaljnjega transdisciplinarnega sodelovanja smo se odločili, da primerjalni onkologiji posvetimo posebne številke, pri čemer je ta prva v nizu. Zbirka člankov poudarja pomen razumevanja podobnosti in razlik v dovzetnosti za raka, agresivnosti bolezni in odzivu na zdravljenje med ljudmi in živalmi, da bi se izboljšale terapevtske možnosti za oboje.

Nataša Tozon predstavlja razvoj in dosežke na področju primerjalne onkologije v zadnjih dvajsetih letih na Univerzi v Ljubljani. Breznik s soavtorji prinaša pregled literature o vplivu črevesnega mikrobioma na razvoj raka in izid zdravljenja pri ljudeh in živalih. Švara s sodelavci analizira podatke o pojavnosti in vrstah tumorjev pri psih v Sloveniji v zadnjih dveh desetletjih. Ozmen in sodelavci predstavljajo transkriptomsko analizo pasjega hemangiosarkoma, medtem ko Belma in sodelavci obravnavajo metastaze pljučnega adenokarcinoma pri ovcah. Hatipoglu s soavtorji zaključuje s poročilom o primeru nediferenciranega embrionalnega rabdomiosarkoma pri nemškem ovčarju.

To je prvi sklop člankov, namenjen primerjalni onkologiji. Če imate zanimive raziskave, poročila o primerih ali pregledne članke s tega področja, vas vabimo, da jih predložite za objavo v eni izmed naslednjih številk!

References

1. WHO. Geneva: World Health Organisation, 2024. https://www.who.int/ (20. 12. 2024).
2. Sarver AL, Makielski KM, DePauw TA, Schulte AJ, Modiano JF. Increased risk of cancer in dogs and humans: a consequence of recent extension of lifespan beyond evolutionarily-determined limitations? Aging Cancer 2022; 3(1): 3–19. doi: 10.1002/aac2.12046
3. Vasan N, Baselga J, Hyman DM. A view on drug resistance in cancer. Nature 2019; 575(7782): 299–309. doi: 10.1038/s41586-019-1730-1
4. Miller KD, Nogueira L, Devasia L, et al. Cancer treatment and survivorship statistics, 2022. CA Cancer J Clin 2022; 72(5): 409–36. doi: 10.3322/caac.21731
5. Florke Gee R, Fon Tacer K. Advancing cancer diagnosis and therapy through collaboration and comparative research. Panhandle Health 2024; 34: 19–21.
6. Čater M. Interdisciplinary approaches for oncological treatments: proton therapy at the intersection of physics and medicine. Slov Vet Res 2024; 61(1): 5–8. doi: 10.26873/SVR-1957-2024
7. Rajčević U, Smole A. Preclinical mouse models in adoptive cell therapies of cancer. Slov Vet Res 2022; 59(4): 173–84. doi: 10.26873/SVR-1513-2022

Slov Vet Res
DOI 10.26873/SVR-2180-2024
UDC 303.4:331.472.4:531.14:616-006:616.12:636.09
Pages: 9–12

Translation Oncology Through the One Health Perspective

Translacijska onkologija skozi perspektivo enega zdravja

Key words

one health;
translation oncology;
electrochemotherapy;
gen electrotransfer;
IL-12

Accepted: 17 December 2024

Nataša Tozon

Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenija

natasa.tozon@vf.uni-lj.si

Translational research, including translation oncology, bridges the gap between basic research and clinical practise by bringing together disciplines, resources, expertise and techniques to improve the prevention, diagnosis and treatment of various diseases. Veterinary medicine plays a critical and integral part in helping translate recent advancements in therapeutic opportunities from bench to bedside, helping animal and human patients alike. To connect and involve researchers from all areas of life sciences, it is also important to present the results to promote translational studies and share their importance (1).

Comparative oncology is part of the core concept of One Health which places veterinary medicine alongside human medicine and emphasizes the interconnectedness of animal and human health (2 – 3). This integrated approach is crucial not only in the field of infectious diseases, particularly zoonoses, including the treatment and rational use of antimicrobial and antiparasitic drugs, but also in addressing several non-communicable diseases, such as cardiovascular diseases and cancer, which represent major health challenges in both developed and developing countries. Several animal and human tumours are similar in their epidemiology, characteristics and clinical manifestations. Companion animals share their living environment with humans and are exposed to the same environmental risk factors for cancer. Furthermore, while we gain valuable insights from the similarities across species, we also learn from the differences in cancer susceptibility and pathogenesis (4). For example, translating knowledge of tumour suppressor mechanisms

Translacijske raziskave, vključno s translacijsko onkologijo, zapolnjujejo vrzel med temeljnimi raziskavami in klinično prakso s povezovanjem disciplin, virov, strokovnega znanja in tehnik za izboljšanje preprečevanja, diagnosticiranja in zdravljenja bolezni. Veterinarska medicina ima ključno vlogo pri prenosu novih načinov zdravljenja iz predkliničnih raziskav v klinično prakso, kar prinaša dobrobit tako bolnim živalim, kot ljudem. Pomembno je tudi seznanjanje širše javnosti z rezultati raziskav in njihovega pomena, z namenom spodbujanja translacijskih študij (1).

Primerjalna onkologija je sestavni del koncepta »eno zdravje,« ki veterinarsko medicino postavlja ob bok humani in poudarja medsebojno povezanost zdravja živali in ljudi (2–3). Ta integrirani pristop je ključen ne le pri nalezljivih boleznih, zlasti zoonozah in racionalni uporabi protimikrobnih ter antiparazitskih zdravil, temveč tudi pri obravnavi nenalezljivih bolezni, kot so bolezni srca in ožilja ter rak, ki predstavljajo velik zdravstveni izziv v razvitem in nerazvitem svetu. Veliko živalskih in človeških tumorjev ima podobno epidemiologijo, značilnosti in klinične manifestacije. Družne živali, ki si z ljudmi delijo življenjsko okolje, so posledično izpostavljeni istim okolijskim dejavnikom tveganja za nastanek raka. Poleg podobnosti med vrstami lahko pridobimo dragocena spoznanja tudi iz razlik v dovzetnosti za raka in patogenezi (4). Na primer, prenos znanja o mehanizmih tumorske supresije, pri živalih, na zdravje ljudi je omogočil nove poglede na strategije preprečevanja in zdravljenja

observed throughout the animal kingdom to human health provided novel perspectives on cancer prevention and treatment strategies, underscoring the importance of veterinary medicine and comparative studies in understanding the mechanisms of cancer development (5-9).

The large number of dogs represents a significant group of potential 'candidates' for studying various aspects of tumors, particularly since experts estimate that about half of all dogs are affected by some form of neoplastic disease. Given the absence of 'gold standards' for treatment in veterinary medicine, testing new approaches in dogs offers a faster and more humane way to evaluate novel treatments (10). Dogs have a shorter life expectancy than humans, so all processes are faster. Unlike preclinical studies where tumours are experimentally induced in selected animal models with specific characteristics, such as immunodeficiency, dogs spontaneously develop cancer, which may lead to more comparable disease progression or treatment response as in humans. Additionally, due to their shorter lifespan, dogs allow for the observation of long-term treatment outcomes and are ideal for pre-clinical trials which ultimately can benefit both, humans and dogs. In recent decades, animals, or 'animal patients,' have been recognized as valuable models for translating research to human medicine. The number of clinical trials, particularly involving dogs, is growing exponentially.

In several breed-specific cancers, the same genetic mutations are found as in humans. One such example is colorectal cancer (11). The information on canine cancer genetics and molecular signatures are still lagging behind the human medicine what hinders advancement of comparative cancer research and veterinary oncology. The Integrated Cancer Database (ICDC) (12) was established to further research on human cancers by enabling comparative analysis with canine cancer. It offers insights into the known biological characteristics and similarities between various types of cancer in humans and dogs. The database also includes information on experimental strategies and ongoing basic and clinical studies. In veterinary medicine, the main aim of the standards and guidelines for conducting clinical trials on animals is to ensure the animal's welfare or the best possible quality of life (13). Work with animals is regulated by national legislations and international standards, which are strictly aimed at ensuring good clinical and laboratory practise and, above all, ethical considerations. Clinical trials in dogs are therefore conducted at various stages of drug and treatment development to investigate safety, efficacy, pharmacological properties, biomarker discovery, interactions in combination therapies and minimal residual disease, which should be beneficial for both humans and animals (8, 10). This requires collaboration between human and animal researchers and clinicians.

An example of a successful collaboration and therapeutic strategy implementation is the development of electrochemotherapy and gene electrotransfer with plasmid DNA

raka, kar poudarja pomen veterinarske medicine in primerjalnih raziskav pri razumevanju mehanizmov razvoja raka (5-9).

Velika populacija psov, med katerimi jih vsaj približno polovica zboli za rakom, predstavlja pomembno skupino živali za preučevanje različnih vidikov raka. Zaradi odsotnosti »zlatih standardov« zdravljenja v veterinarski medicini je preizkušanje novih pristopov na psih hitrejše in bolj humano. Psi imajo krajšo življenjsko dobo, kar omogoča hitrejše prepoznavanje uspešnosti zdravljenja. Za razliko od predkliničnih raziskav, pri katerih proučujemo večinoma inducirane tumorje na laboratorijskih živalih z okrnjenim imunskim sistemom, se pri psih rak razvije spontano, kar vodi do bolj primerljivih podatkov o napredovanju bolezni in odzivu na zdravljenje kot pri ljudeh.

Pri nekaterih pasmah psov so bile ugotovljene enake genetske mutacije kot pri ljudeh, na primer raku debelega črevesa in danke (11). Podatki o genskih in molekularnih značilnostih pasjih rakov zaostajajo za humano medicino, kar ovira napredek primerjalne onkologije. Ustanovitev integrirane baze podatkov o raku (ICDC) (12) omogoča primerjalne analize med človeškimi in pasjimi tumorji. Podatkovna baza vključuje tudi informacije o eksperimentalnih strategijah ter bazičnih in kliničnih raziskavah. Glavni cilj smernic za klinične študije na živalih je zagotoviti dobrobit živali in najboljšo možno kakovost življenja (13). Klinične raziskave na psih potekajo v različnih fazah razvoja zdravil, z namenom proučevanja varnosti, učinkovitosti, farmakoloških lastnosti, odkrivanja biomarkerjev, kombiniranega zdravljenja in rezidualne bolezni, kar prinaša korist tako ljudem kot živalim (8, 10).Primer uspešnega sodelovanja med veterinarsko in humano medicino je razvoj elektrokemoterapije (EKT) in genskega elektroprenosa (GET) s plazmidno DNA za IL-12, kar sta ključni področji raziskav na Veterinarski fakulteti, Univerze v Ljubljani. EKT je naša raziskovalna skupina v veterinarsko prakso uvedla pred več kot 25 leti (14, 15). Do danes smo uspešno zdravili več kot 400 živali, vključno s psmi, mačkami (16), konji (17) in eksotičnimi hišnimi ljubljenčki (18, 19). Leta 2016 smo objavili standardni operativni postopek za EKT pri psih in mačkah (20). Ugotovili smo, da je EKT varna in učinkovita alternativa za lokalno zdravljenje različnih vrst tumorjev, hkrati pa ne povzroča funkcionalni motenj in ohranja odličen kozmetični izgled. Številne klinične študije so potrdile varnost in učinkovitost kombiniranega zdravljenja ter pozitiven vpliv na lokalni nadzor tumorja in napredovanje bolezni ter spodbujanja imunskega odziva v boju proti raku (21–27). V zadnji klinični raziskavi smo pokazali, da kombinacija EKT z GET IL-12 pri mastocitomih podaljša čas brez bolezni in brez napredovanja v primerjavi s samo EKT (26–27). Spodbudni rezultati v veterinarski onkologiji so omogočili izvedbo faze I intratumoralnega GET z IL-12 pri bolnikih z bazalnoceličnim karcinomom, objavljene leta 2025 (28). Ti rezultati kažejo, da sodelovanje med raziskovalci in zdravniki na področju humane in veterinarske medicine vodi k razvoju novih terapevtskih možnosti, ki koristijo

for IL-12, key areas of focus for our research group at the University of Ljubljana. Electrochemotherapy (ECT) was introduced in veterinary medicine by our group more than 25 years ago (14, 15).

Since then, we have successfully treated more than 400 animals: dogs, cats (16), horses (17) and exotic pets (18, 19). In 2016, we established and published a Standard Operating Procedure protocol of the ECT treatment in dog and cats (20). We found that ECT is a safe and effective alternative local treatment option for different tumour types in all treated animal species. Beyond providing a long-lasting objective response, including complete or partial response, it also offers excellent cosmetic outcomes, which are often more acceptable to owners than extensive surgery. Furthermore, in dogs, gene electrotransfer treatment (GET) with plasmid-encoded IL-12 was added to increase the local response and stimulate the patient's immune system response against cancer. Numerous clinical studies over the years have confirmed the safety of this novel treatment and positive effects on local tumour control and disease progression (21-27). We found that GET offers patients a good quality of life, as the side effects are minimal. This was the primary reason why owners considered this treatment method suitable and would choose it again if necessary (13). Additionally, in the last clinical study we showed that the combinational treatment of ECT with GET of IL-12 of mast cell tumours, resulted in prolonged disease free and progression free interval in comparison to ECT alone (26-27). Moreover, the encouraging results from veterinary oncology, using gene electrotransfer with IL-12 have facilitated the completion of phase I trial of phIL12 plasmid intratumoral gene electrotransfer in patients with basal cell carcinoma in head and neck region, published in 2025 (28).

Together, these results highlight how successful collaboration between researchers and clinicians in both animal and human medicine leads to the development of novel therapeutic opportunities benefiting both human and animal patients. Additionally, publications emphasizing comparative oncology, such as this special issue of Slovenian Veterinary Medicine, are crucial for sharing findings with the broader community and fostering further collaborations between human and animal medicine.

Founding: the research was supported by Slovenian Research Agency (grant number P4-0053, P3-0003, J4-2546, J3-6796).

tako ljudem kot živalim. Publikacije, kot je tudi ta posebna izdaja Slovenskega veterinarskega zbornika, so ključne za širjenje ugotovitev in spodbujanje nadaljnjega sodelovanja med obema področjema.

Financiranje: Raziskavo je podprla Javna agencija za raziskovalno dejavnost Republike Slovenije (P4-0053, P3-0003, J4-2546, J3-6796).

References

1. Fon Tacer K. Reflecting and looking forward – the role of veterinary scientific journals in veterinary research and medicine. Slov Vet Res 2023; 60(4): 177-9. doi: 10.26873/SVR-1922-2023
2. WHO. One Healt. Geneva: World Health Organisation, 2025. https://www.who.int/news-room/questions-and-answers/item/onehealth (10. 1. 2025).
3. Dujon AM, Brown JS , Destoumieux-Garzón D, et al. On the need for integrating cancer into the One Health perspective. Evol Appl 2021; 14(11): 2571-5. doi: 10.1111/eva.13303
4. Sulçe M, Munga A, Beqiraj D, et al. Applicability of flow cytometry in identifying and staging lymphoma, leukemia and mast cell tumors in dogs: an overview. Slov Vet Res 2022; 59(3): 129-36. doi: 10.26873/SVR-1372-2022
5. Vincze O, Colchero F, Lemaître Jean-F, et al. Cancer risk across mammals. Nature 2022; 601(7892): 263-7. doi: 10.1038/s41586-021-04224-5
6. Seluanov A, Gladyshev VN , Vijg J, Gorburova V. Mechanisms of cancer resistance in long- lived mammals. Nat Rev Cancer 2018; 18(7): 433-41. doi: 10.1038/s41568-018-0004-9
7. Rajčević U, Smole A. Preclinical mouse models in adoptive cell therapies of cancer Slov Vet Res 2022; 59 (4): 173–84 doi: doi.org/10.26873/SVR-1513-2022
8. Lisjak A, Correa Lopes B, Pilla R, Nemec A, Suchodolski JS, Tozon N. A comparison of the oral microbiota in healthy dogs and dogs with oral tumors. Animals(Basel) 2023; 13(23): 3594. doi: 10.3390/ani13233594
9. Milevoj N, Tozon N, Tomsič K. Use of cannabidiol products by pet owners in slovenia: a survey-based study. Slov Vet Res 2022; 59(3): 149-58. doi: doi.org/10.26873/SVR-1294-2022
10. Milevoj N, Nemec A, Tozon N. Metronomic chemotherapy for palliative treatment of malignant oral tumors in dogs. Front Vet Sci 2022; 9: 856399. doi: 10.3389/fvets.2022.856399
11. Wang J, Wang T, Sun Y, et al. Proliferative and invasive colorectal tumors in pet dogs provide unique insights into human colorectal cancer. Cancers (Basel) 2018; 10(9): 330. doi: 10.3390/cancers10090330
12. NIH. Integrated canine data commons. Bethesda: National Cancer Institute, 2025. https://datacommons.cancer.gov/repository/ integrated-canine-data-commons) (10. 1. 2025).
13. Milevoj N, Tozon N, Licen S, Lampreht Tratar U, Sersa G, Cemazar M. Health-related quality of life in dogs treated with electrochemotherapy and/or interleukin-12 gene electrotransfer. Vet Med Sci 2020; 6(3): 290-8. doi: 10.1002/vms3.232
14. Tozon N, Sersa G, Cemazar M. Electrochemotherapy: potentiation of local antitumour effectiveness of cisplatin in dogs and cats. Anticancer Res 2001 ;21(4A):2483-8.
15. Cemazar M, Tamzali Y, Sersa G, et al. Electrochemotherapy in veterinary oncology. J Vet Intern Med 2008; 22(4): 826-31. doi: 10.1111/j.1939-1676.2008.0117.x
16. Tozon N, Pavlin D, Sersa G, Dolinsek T, Cemazar M. Electrochemotherapy with intravenous bleomycin injection: an observational study in superficial squamous cell carcinoma in cats. J Feline Med Surg 2014; 16(4): 291-9. doi: 10.1177/1098612X13507071
17. Tozon N, Kramarič P, Kadunc Kos V, Sersa G, Cemazar M. Electrochemotherapy as a single or adjuvant treatment to surgery of cutaneous sarcoid tumours in horses: a 31-case retrospective study. Vet Rec 2016; 179(24): 627. doi: 10.1136/vr.103867
18. Račnik J, Švara T, Zadravec M, et al. Electrochemotherapy with bleomycin of different types of cutaneous tumours in a ferret (Mustela putorius furo). Radiol Oncol 2017; 52(1): 98-104, doi: 10.1515/raon-2017-0057
19. Račnik J, Švara T, Zadravec M, et al. Electrochemotherapy with cisplatin for the treatment of a non-operable cutaneous fibroma in a cockatiel (Nymphicus hollandicus). N Z Vet J 2019; 67(3): 155-8. doi: 10.1080/00480169.2018.1564393
20. Tozon N, Lampreht Tratar U, Znidar K, Sersa G, Teissie J, Cemazar M. Operating procedures of the electrochemotherapy for treatment of tumor in dogs and cats. J Vis Exp 2016; 24(116): 54760. doi: 10.3791/54760
21. Pavlin D, Cemazar M, Sersa G, Tozon N. IL-12 based gene therapy in veterinary medicine. J Transl Med 2012; 10: 234. doi: 10.1186/1479-5876-10-234
22. Cemazar M, Ambrozic Avgustin J, Pavlin D, et al. Efficacy and safety of electrochemotherapy combined with peritumoral IL-12 gene electrotransfer of canine mast cell tumours. Vet Comp Oncol 2017; 15(2): 641–54. doi: 10.1111/vco.12208
23. Salvadori C, Svara T, Rocchigiani G, et al. Effects of electrochemotherapy with cisplatin and peritumoral IL-12 gene electrotransfer on canine mast cell tumors: a histopathologic and immunohistochemical study. Radiol Oncol 2017; 51(3): 286–94. doi: 10.1515/raon-2017-0035
24. Milevoj N, Tratar UL, Nemec A, et al. A combination of electrochemotherapy, gene electrotransfer of plasmid encoding canine IL-12 and cytoreductive surgery in the treatment of canine oral malignant melanoma. Res Vet Sci 2019; 122: 40–9. doi: 10.1016/j.rvsc.2018.11.001
25. Nemec A, Milevoj N, Lampreht Tratar U, Serša G, Čemežar M, Tozon N. Electroporation-based treatments in small animal veterinary oral and maxillofacial oncology. Front Vet Sci 2020; 7: 575911. doi: 10.3389/fvets.2020.575911
26. Lampreht Tratar U, Milevoj N, Cemazar M, et al. Treatment of spontaneous canine mast cell tumors by electrochemotherapy combined with IL-12 gene electrotransfer: comparison of intratumoral and peritumoral application of IL-12. Int Immunopharmacol 2023; 120: 110274. doi: 10.1016/j.intimp.2023.110274
27. Vilfan M, Lampreht Tratar U, Milevoj N, et al.. Comparison of nucleosome, ferritin and LDH levels in blood with clinical response before and after electrochemotherapy combined with IL-12 gene electrotransfer for the treatment of mast cell tumours in dogs. Animals (Basel) 2024; 14(3): 438. doi: 10.3390/ani14030438
28. Strojan P, Jesenko T, Omerzel M, et al. Phase I trial of phIL12 plasmid intratumoral gene electrotransfer in patients with basal cell carcinoma in head and neck region. Eur J Surg Oncol 2025; 51(5): 109574. doi: https://doi.org/10.1016/j. ejso.2025.109574

Slov Vet Res
DOI 10.26873/SVR-2029-2024
UDC 543.95:576.36:591.434:616-006:616-052:636.09
Pages: 13–26

Review Article

Gut Microbiome in Cancer: the Next big Opportunity for Better Patient Outcomes?

Key words

gut microbiome;
cancer;
treatment outcome;
tumor models;
glioma

Jure Povšin[1,2#], Timotej Sotošek[1,2#], Metka Novak[1], Barbara Breznik[1]*

[1]National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, 121 Večna pot, 1000 Ljubljana, [2]Faculty of Chemistry and Chemical Engineering, University of Ljubljana, 113 Večna pot, 1000 Ljubljana, Slovenia

[#]Shared first authorship
***Corresponding author:** barbara.breznik@nib.si

Abstract: The gut microbiome, a diverse community of microorganisms in the human body, plays an important role in maintaining health and influences various processes such as digestion, immunity, and protection against pathogens. A person's unique gut microbiome, shaped by factors such as birth method, diet, antibiotics, and lifestyle, contributes to bodily functions such as nutrient metabolism, drug processing, and immune regulation. Changes in the gut microbiome are associated with a predisposition to cancer and can influence the effectiveness of cancer treatments. Dysbiosis in the gut microbiome can lead to inflammation, tumor development, and metastasis, highlighting its importance in cancer research and prevention. The gut microbiota significantly influences cancer development and treatment outcomes. Certain bacteria enhance the effects of therapies such as cyclophosphamide and contribute to the body's immune response against tumors. Microbes produce anti-cancer molecules and probiotic compounds, making them potential tools in cancer prevention and treatment. Future research aims to develop targeted antibiotics and explore fecal microbiota transfer to selectively manipulate the microbiota for improved cancer treatment. Due to genetic and physiological similarities, mouse models are invaluable in biomedical research. However, because the gut microbiome of humans and mice and the composition of the tumor microenvironment differ, direct comparison between these two models can be challenging in research. Bridging these gaps is crucial for comparative medicine, especially in cancer research where the microbiome plays an important role in treatment outcomes. One important area where the gut microbiome could offer potential new treatment options is in primary brain tumors such as gliomas. To date, there are no long-lasting effective treatments for this type of cancer, but research in mouse models shows a link between tumor progression and response to treatment with changes in the gut microbiome. Overall, the gut microbiome and its modulation represent an opportunity for more efficient future cancer treatment.

Received: 5 April 2024
Accepted: 21 October 2024

Introduction

The gut microbiome is known to play a crucial role in maintaining normal homeostasis in humans. The microbiome is a collection of all microorganisms, such as bacteria, fungi, viruses, and their genes, that naturally reside on and in our bodies. Often referred to as the "forgotten organ", the microbiome contains a metagenome that is 100 times larger than our own genome and performs key functions that are vital to human health (1). For instance, the human body comprises approximately 40-100 trillion microbial cells, which is ten times more than the number of human somatic cells. A healthy individual's gut contains around 300-500 different species of bacteria (2), although some sources mention up to 3500 bacterial species (3).

Over time, the microbiome and the host have evolved into a complex "superorganism" with their symbiosis benefiting

the host in numerous ways, such as food metabolism, protection against pathogens, and assistance in the development of the immune system. The majority (99%) of microbial mass resides in the gastrointestinal tract (GI) and functions both locally and over long distances. As a result, the gastrointestinal microbiome not only has the most substantial impact on overall health and metabolic status among all microbiomes but is also the most extensively studied microbiome, serving as a model for understanding interactions between the host, microorganisms, and diseases (4, 5).

The role of a normal gut microbiome

A hypothesis suggests that the GI's microbiome plays a significant role in maintaining an individual's gut health and is crucial for overall human health (6, 7). Everyone has a unique gut microbiome profile, which serves specific functions in host nutrient metabolism, xenobiotic metabolism, maintenance of the structural integrity of the intestinal mucosal barrier, immunomodulation, and protection against pathogens.

Under normal circumstances, the host's immune system recognizes markers that are specific to pathogenic microorganisms, making it easier to eliminate them. The fact is that the majority of the host's microbiome is non-pathogenic and lives in symbiosis with the host's immune system. Intestinal bacteria play an important role in this, as they inhibit the growth and spread of pathogens, provide essential nutrients, and assist in nutrient and drug metabolism. In the meantime, the host's immune system must prevent the invasion of both pathogenic and non-pathogenic microbes. Immune cells, including macrophages, phagocytes, and dendritic cells, closely interact with the intestinal microbiome and its metabolites thus maintaining intestinal homeostasis and recognizing bacteria that might be pathogenic (8).

The gut microbiome of each individual is formed early in life, and several factors play a role in its development. These factors include in what way a baby is born (vaginal or cesarean section), childhood diet (breast milk or formula), adult diet (vegan or meat-based), as well as the use of antibiotics or antibiotic-like molecules derived from the environment or the gut's commensal community (7). The gut microbiome of an adult host is relatively stable. Still, it varies from person to person, mainly due to differences in lifestyle, including frequency of physical activity and cultural practices, as well as enterotype, body mass index (BMI), and dietary habits. Alpha and beta diversity are two metrics used to look at microbiome diversity. Alpha diversity measures how many types of species live in a given area in a person or a single sample and beta diversity measures the differences in the microbiome composition between body sites or people (9).

As a result, there is no unique optimal composition of the gut microbiome, as it differs for everyone. However, despite this diverse composition, statistics show that dominant types of gut microbes include *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia*, with *Firmicutes* and *Bacteroidetes* comprising 90% of gut microbes (10, 11).

The gut microbiome and cancer

Microbiota-induced tumors are estimated to represent about 20% of all tumors worldwide (12). In the past decade alone, the number of cancer patients in the USA has increased from 13.8 to 18.1 million, and it is projected to continue rising in the upcoming years. Additionally, a diet high in fat and heavily processed foods is believed to influence the diversity of gut microbiomes. A recent study showed that higher fat consumption in healthy young adults is associated with unfavorable changes in gut microbiomes, which could impact the overall health of the host (13). Numerous studies point out that changes in the gut microbiome can lead to a predisposition to various types of cancer. Moreover, bacteria and their metabolites have been found not only to contribute to cancer development, such as colorectal cancer but also to alter the pharmacodynamics of cancer drugs (14, 15). When the balance in the gut microbiome is disrupted, bacteria can penetrate the intestinal mucosa and surrounding tissues, causing inflammation. As the inflammatory process promotes tumor development and progression, it accelerates the invasion of tumor cells and may eventually lead to metastasis. Increased levels of inflammatory cytokines can directly damage the DNA of epithelial cells, triggering inflammation-associated cancer (16, 17).

For instance, the presence of the bacterium *Helicobacter pylori* can promote an immune response and chronic inflammation, which can cause stomach cancer. Many products from this bacterium disrupt the regulation of normal cell homeostasis, resulting in a build-up of cytokines and other signaling molecules that cause stomach and esophageal cancer. In healthy individuals, the esophagus is densely populated with *Firmicutes* and *Streptococcus* (mostly Gram-positive bacteria). In cases of dysbiosis, Gram-negative bacteria (anaerobic and microaerophilic) replace Gram-positive bacteria, which can later lead to esophagitis or inflammation of the esophagus. Among other gut microbes associated with esophageal cancer are *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (18).

One of the more significant health problems across the world is colorectal cancer. Many studies have proven that several bacterial species play a role in colorectal carcinogenesis, including *Escherichia coli*, *Bacteroides fragilis*, *Fusobacterium spp.*, *Enterococcus faecalis*, *Streptococcus bovis*, *H. pylori* and *Clostridium septicum*. The colon contains ten times more bacteria than the small intestine,

which means that the likelihood of developing colorectal cancer is about 12 times higher in the colon than in the small intestine, emphasizing the role of commensal bacteria (19, 20). Other research has indicated that obesity may be associated with the gut microbiome composition and increased liver cancer. In obese individuals, the epithelium junctions are damaged, allowing gut bacteria to enter the bloodstream and cause systemic infections. Additionally, the gut microbiota––driven COX2 pathway secretes secondary metabolites and other small molecules such as lipoteichoic acid (LTA), lipopolysaccharides (LPS), and bile acids, which cause inactivation of the immune system in the liver, possibly leading to liver cancer (21–24).

The role of the gut microbiome in tumorigenesis

The symbiotic microbiome is recognized for its vital function in upholding human well-being and fortifying the host's immune defenses. Nevertheless, a group of bacteria remains associated, either directly or indirectly, with the advancement and progression of cancer (25). In an imbalanced gut ecosystem, harmful microbes can inflict various damages upon the host's organism in several ways (Figure 1). Research has proven that the intestinal flora can infiltrate deep within bodily tissues, instigating tumor formation in mouse models deficient in IL-10, a critical cytokine pivotal in the host's anti-cancer immunity (26–29).

When there is a microbial imbalance in the gut microbiota, pathogenic bacteria can generate and discharge an array of toxins. These toxins can make the genome unstable by instigating breaks in the host's DNA, triggering the development of tumor in predisposed tissues. One example of such a toxin is cytotoxin-associated gene A (CagA), synthesized by *H. pylori*. Through this cytokine, CagA can degrade the tumor suppressor protein p53 in gastric cells, disrupting the host's serine/threonine kinase pathway which leads to inhibition of cell apoptosis, survival of damaged cells and stomach cancer development (Figure 2). Similarly, E. coli produces colibactin, and CDT+ strains produce a cytotoxic toxin (CDT) with DNase activity that leads to host cell apoptosis. When these toxins are released near the intestinal epithelium, they create double-strand breaks in the host's DNA, culminating in genetic mutations and the development of tumors. By producing enzymes like Virulence A (VirA) and inositol phosphate phosphatases D (IpgD), *Shigella flexneri* can induce degradation of the p53 protein in host cells and disrupt DNA damage and repair pathways (30, 31).

Pathogenic bacteria are capable of indirectly influencing tumorigenesis in host cells through various mechanisms. For instance, they can produce molecules such as different bile acid metabolites that inhibit the host's immune response and enhance inflammation and with this help cancer cells evade the immune system (32). Bacteria can also generate oxidative stress, which subsequently drives genetic mutations in host cells (33, 34). For example, flavoenzyme (spermine oxidase) can be activated in host cells by *Helicobacter pylori* and *Bacteroides fragilis*, creating hydrogen peroxide (H_2O_2) and reactive oxygen species (ROS), which adds to DNA damage accumulation. Bacteria *Enterococcus faecalis* can infiltrate host cells by producing reactive oxygen species, accelerating host DNA damage (35–37). *Fusobacterium nucleatum* can block the cytotoxic

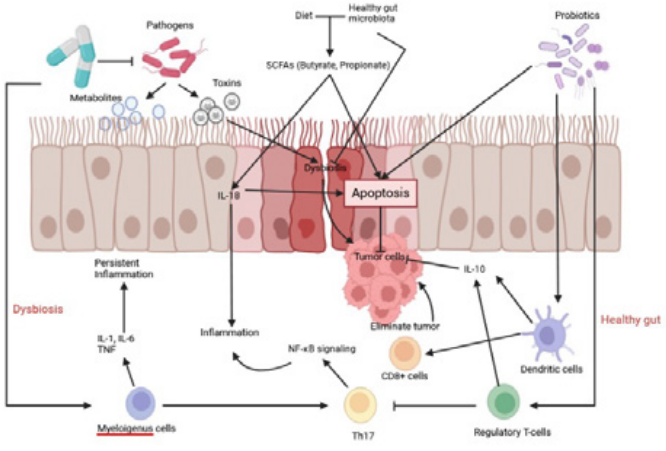


Figure 1: The interaction between the gut microbiota and the immune system. In an imbalanced gut ecosystem, harmful microbes can inflict various damages upon the host's organism and affect local and systemic immune responses. Adapted from (30). The Scheme was created using Biorender.com

Abbreviations: interleukin (IL), short-chain fatty acids (SCFAs), tumor necrosis factor (TNF), nuclear factor kappa B (NF-κB), T helper 17 cell (Th17)

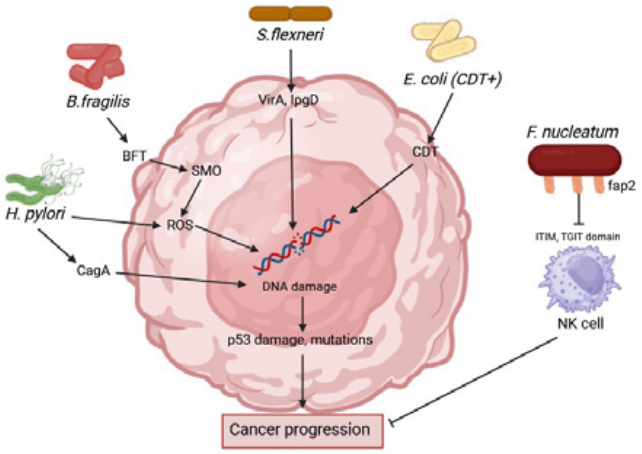


Figure 2: The role of different bacteria in cancer progression. An illustration of how numerous bacteria can contribute to or even outright cause cancer progression and their mechanism of action. The Scheme was created using Biorender.com

Abbreviations: B. fragilis toxin (BFT), cytotoxin-associated gene A (CagA), Cytolethal distending toxins (CDT), fibroblast activation protein-2 (Fap2), inositol phosphate phosphatases D (IpgD), natural killer (NK), reactive oxygen species (ROS), spermine oxidase (SMO), virulence A (VirA)

activity of natural killer cells (NK) by producing a virulence factor (Fap2), which can bind to the NK inhibitory receptor TGIT and ITIM domain, preventing NK cells from attacking cancer cells (25, 38).

Anti-cancer properties of intestinal microbiota

Microbiota plays a crucial role in tumor formation and the outcomes of anticancer therapies (39, 40). The interaction between the host's immune system and gut microbiota enables immune cells to recognize and eliminate opportunistic bacteria before they can invade the host's body. Apart from that, the interaction also impacts food digestion and the elimination of metabolites against gastrointestinal antigens (41). Moreover, the microbiota influences both innate and acquired immune systems systemically. In studies with germ-free mouse models lacking gut microbiota, scientists have observed a lack of the mucus layer, altered immunoglobulin A (IgA) levels, and mesenteric lymphadenitis (41, 42). Additionally, microbiota deficiency has been shown to negatively affect the efficacy of therapeutic interventions (43).

Cyclophosphamide (CTX) is a popular chemotherapeutic drug that stimulates the host's T-cell immune response. It is used as a treatment for different types of cancers, and it works by crossing the small intestine and breaching the epithelial membrane. In this way commensal gut microbiota is transferred to the spleen and mesenteric lymph nodes, thus stimulating helper T cells (T_H17 cells) and further inducing anti-cancer effects against tumor development (25, 44). Studies on mouse models have shown that several bacterial species such as *Branesiella intestinihominis*, *Enterococcus hirae*, and *Lactobacillus johnsonii* enhance the anti-cancer activity of CTX (44, 45). On the other hand, antibiotic-treated and germ-free mouse models have shown reduced immune responses due to the lack of Gram-positive bacteria in their intestines. Based on these studies, it has been found that commensal bacteria can alter the efficacy of immunotherapy and chemotherapy drugs (44).

Bacteria *Bifidobacterium longum* and *B. breve* have been shown to enhance the dendritic cell function. Active dendritic cells can then trigger the recruitment of cytotoxic T cells in the tumor microenvironment. Cytotoxic T cells and NK cells are the main components of the immune system responsible for eliminating cancer cells (25). Numerous studies analyzing 1000 patients with sarcoma have found out that heat-killed bacteria *Serratia* and *Streptococcus pyogenes* can increase the survival rate of patients by approximately 80% over five years. Additionally, heat-killed microorganisms may activate a sustained immune response and potentially exhibit anti-cancer effects against sarcomas. It is presumed that CD8+ cells can effectively infiltrate infected cells or tissues in solid tumors because of the gut microbiota (46). For example, it was shown that CTLA-4 inhibitors have anti-cancer effects and depend on the gut microbiota, especially Gram-negative obligate anaerobic bacteria. The CTLA-4 inhibitor had no anti-cancer effects in germ-free mice, but when Gram-negative obligate anaerobic bacteria were introduced into sterile mice, the inhibitor's anti-cancer efficiency was restored (47).

Molecules or components derived from microorganisms have potent anti-cancer properties. Short-chain fatty acids (SCFA), produced by the gut microbiota, play a crucial role in suppressing tumors/cancer (48–50). Common SCFAs produced by commensal gut bacteria like butyrate and propionate have effective anti-cancer effects (51). They inhibit the histone deacetylases of cancer cells and induce the programmed cell death known as apoptosis. In patients with colorectal cancer there were lower levels of bacteria producing butyrate found. Butyrate activates the GPR109A receptor, which then induces IL-18 production in epithelial cells of the intestinal mucosa, which may trigger the repair mechanisms of the mucous layer (52).

Metabolites obtained from probiotics can initiate an indirect immune response against tumor formation by modifying the host's immune system. For instance, lipopolysaccharides (LPS) can activate Toll-like receptor 4 (TLR 4), which further enhances the T cell immune response against tumor cells (53). Likewise, monophosphoryl lipid A from the bacterium *Salmonella enterica* which has a high efficacy against cervical cancer is used as an adjuvant in vaccine development (54). Some gut bacteria can produce probiotic molecules with anti-tumor effects. For instance, *Lactobacillus casei* produces ferrichrome, which activates the c-Jun N-terminal kinase signaling pathway and ultimately induces programmed cell death in cancer cells (55). *Lactobacilli* are also believed to play a role in an anti-tumor response by stimulating the host's immune system such as dendritic cells, NK cells, and T_H1 cells (38).

Bacteria and viruses in the intestines influence the effects of chemotherapy, immunotherapy, and the immune response. Studies have shown that mice with tumors that do not typically respond to immunotherapy drugs can start responding if they receive specific gut bacteria from mice that have positively responded to the drugs (56). The same phenomenon has been observed in humans, where altering the composition of bacteria in the gut microbiota using fecal transplants can improve the condition of some patients with tumors who did not respond to immunotherapy or drugs. The most well-known donor of his feces is Zion Levy, who was diagnosed with melanoma but showed very good responsiveness to immune treatment with nivolumab. Doctors concluded that with his feces, he could also help other patients who do not respond to immunotherapy as well. This led to the first such research studies at Sheba Medical Center in Israeli study and a study led by scientists at Memorial Sloan Kettering Cancer Center in New York City and scientists at the University of Minnesota Medical School in the USA in which the transfer of microbes from

feces (FMT) improved the response to immunotherapy in patients. The results of these two studies, published in the journal Science (56, 57), were modest - out of 26 people who did not previously respond to immunotherapy, about one in three responded after FMT. However, they attracted a lot of attention. In the Israeli study, ten individuals with advanced melanoma were included, whose cancer had progressed despite treatment with checkpoint inhibitors. Only three out of ten overcame treatment resistance, and only two of them partially. Interestingly, all three responders received FMT from donor Levy. None of the five participants who received material from another donor who also survived cancer responded. Due to encouraging results, there are now at least 30 clinical studies of fecal microbiota transplantation being conducted (58).

The role of probiotics in cancer prevention

Probiotics are generally considered safe live microorganisms and can play a key role in preventing various diseases, including different types of tumors (59). Besides live probiotics, it has also been proven that dead probiotics, such as bacterial components (cell wall), have numerous benefits in managing various diseases, including cancer (60). Although probiotics are generally considered safe, they should be carefully considered before giving them to cancer patients since they are immunocompromised. There may be a risk of antibiotic resistance transfer and the development of opportunistic infections (61). Nonetheless, probiotics have many positive effects in cancer patients, as they can prevent diarrhea and other gastrointestinal issues and increase the populations of beneficial bacteria in the gut, thereby enabling the establishment of a healthy gut microbiota (62). For example, in a study where a patient received the probiotic strains *Lactobacillus johnsonii* and *Bifidobacterium longum*, the strain successfully adhered to the intestinal mucosa and eliminated pathogenic bacteria by triggering a local immune response (63). A similar study showed that *Bifidobacterium longum* and *Lactobacillus acidophilus* significantly reduced severe diarrhea during pelvic radiotherapy indicating their probiotic efficacy (64). Likewise, a blend of ten probiotic strains not only alleviated diarrhea but also demonstrated decreased chemotherapy-induced cytotoxicity in the treatment of certain patients battling metastatic colorectal cancer (65). Probiotic bacteria can indirectly prevent colorectal cancer by attenuating the activity of intestinal enzymes responsible for converting amines and complex aromatic hydrocarbons into active carcinogens (66). Furthermore, research has established that *Bifidobacterium animalis* subspecies *Lactis* and other probiotic strains modulate the host's immune response by activating phagocytic cells, which subsequently target and eliminate cancer cells in their early developmental stages (67–69). The direct consumption of probiotics by colorectal cancer patients has been associated with proapoptotic effects on cancer cells. For example, *Lactobacillus delbrueckii* significantly upregulates the expression of caspase 3, thereby triggering programmed cell death in human colorectal cancer cells. Consequently, probiotic bacteria stand as promising biotherapeutic agents capable of preventing intestinal dysbiosis, enhancing the host's immune response, and eliminating various curable and difficult-to-cure diseases, including different types of cancers (70).

Microbiota of the gut interacts not only with the immune system but also engages gut epithelial cells via inflammasome activation. These inflammasomes, expressed by both intestinal and immune cells, enable the distinction between toxic and non-toxic molecules produced by pathogenic and non-pathogenic microorganisms based on the nucleotide-binding oligomerization domain-like receptors (NOD-like receptors) (71). When homeostasis in the body is disrupted for any reason, inflammasomes become active and mediate a strong immune response. They activate caspase 1, which in turn triggers the secretion of pro-inflammatory cytokines (IL-1β), IL-18, and ultimately leads to apoptosis (72). Dysregulation of inflammasomes is implicated in various diseases, including autoimmune conditions, neurodegenerative and metabolic disorders, and cancer, with the gut microbiome emerging as a pivotal factor in their activation (73).

Comparative studies of microbiome between human and animal models

Scientists have been using animals to study human diseases for over a hundred years. In this regard, mice have been particularly useful, as they share many biological characteristics with humans. Moreover, they share over 80% of their genome with humans. Due to their phylogenetic similarity, physiological resemblance to humans, ease of maintenance and breeding in laboratories, and the availability of numerous inbred strains, domestic mice (*Mus musculus*) have long served as models for human biology and diseases, including cancer (74).

However, despite the anatomical, histological, and physiological similarities between mouse and human intestines, there are significant differences in size, metabolism rate, and dietary habits. The use of mice as model organisms for studying human biology is based on genetic and physiological similarities between them. It is important to be aware that despite their phylogenetic closeness, mice, and humans have evolved and adapted to different environments, leading to significant differences in their characteristics. This is also why mice often respond to experimental interventions in ways that differ considerably from humans. Mice models in the laboratory are 'specific-pathogen-free' (SPF) mice. These differences are also reflected in the development of the gut microbiome compared to humans. Instead of SPF mice, microbially exposed, or ‘dirty’ mice model that better mimics the diverse infectious history that is typical of most humans, can be used (75). For example,

a study done by Sjaastad et al found the potential limitation of exclusive use of SPF mice when testing vaccine efficacy compared with "dirty" mice, which may also be one of the reasons that some new therapies work in experimental mice and then the efficacy is lost when transferred to humans (76).

The anatomy of the gastrointestinal tract plays a crucial role in these differences, with significant variations between the two species. The ratio of the length of the small and large intestines is greater in mice than in humans, mice have a distinct cecum, and they lack an appendix. An important site for microbial fermentation of undigested food in mice is the cecum. Thus, both species provide different environments that support the growth of different gastrointestinal microbiota (77).

An example where the impact of anatomy can be observed is in mice with "cecal lymphoid patches, " which can be synonymous with the human appendix. Here, the flora of these two compartments differs, with *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Fusobacteria* being predominant in the human appendix in terms of abundance, whereas the mouse cecal lymphoid patches consist of Bacteroidetes, *Firmicutes*, *Actinobacteria*, and *Proteobacteria* (78, 79).

Previous studies have shown that human and mouse gut flora share 90% and 89% similarity in species and genera, respectively. However, more recent research has shown that the differences are much larger than previously thought (80). A study from 2021 revealed that more than half of the species in both human and mouse microbiomes belonged to the *Firmicutes_A* species. *Firmicutes_A* and *Bacteroidota* (*Bacteroidetes*) were the most common species in both human and mouse microbiomes. *Firmicutes_B* was more common in mice than in humans, while *Firmicutes_C* was less represented. In general, 16 species were common to both human and mouse microbiomes, with 5 species found only in humans and not in mice. In contrast, species such as *Deferribacterota*, *Thermoto gota* and two species *Chlamydia muridarum* and *Chlamydophila psittaci* were specific to mice. No archaea were reconstructed from the mouse gut metagenome, whereas 0.4% of genomes in the human gut were attributed to this domain. At the family level, humans and mice shared 88 out of 109 taxa, and their average abundances in human and mouse microbiota were strongly correlated. Two families *Lachnospiraceae* and *Oscillospiraceae* which dominate *Firmicutes_A* were highly present in both humans and mice. In mice, the *Muribacula ceae* family was more than 30 times more abundant than in humans, while the *Bacteroidaceae* family was 14 times smaller. While 255 out of 412 taxa were shared at the genus level, the abundance of genera showed a moderate correlation (r = 0.44), consistent with the results of 16S rDNA sequencing (81). Interestingly, the genus *Collinsella* (phylum *Actinobacteria*), associated with atherosclerosis and rheumatoid arthritis, was represented by 579 species in humans but was not found in the mouse metagenome. Surprisingly, out of 1573 CMMG (comprehensive mouse microbiota genome) species, only 170 (10.8%) were identified in the human gut microbiota. Common species, on average, represented 13% of the composition of the mouse gut microbiome. Mapping mouse metagenome samples to the human reference database and vice versa achieved only a 30% mapping rate (82).

While these numbers may initially suggest a high degree of similarity in the gut microbiota, a closer look reveals key deviations, especially in terms of microbial composition and abundance. This demonstrates that mice and the human microbiome are significantly different. These results challenge our analogy between the human and mouse microbiota. Such changes in the microbiome composition can have a significant impact on experimental plans and research approaches to studying the human gut microbiome using mice as intermediaries.

Considering the significant influence that the microbiome can have on the efficacy of various drugs, the differences between the human and mouse gut microbiomes present a considerable challenge. Establishing a humanized gnotobiotic mouse model by transplanting human fecal microbiota into mice without their own microbiota represents an innovative and powerful tool for mimicking the human microbial system in mice. However, creating such a model requires careful consideration of various factors, from aspects related to human donors to the genetic background of the mice, all of which can influence the final research outcomes (83). It is also important to question how much of the human microbiome mice can retain, given the anatomical differences in the gastrointestinal tract between humans and mice.

While mice models are the most prevalent models to study the gut microbiome (84), there are also other animal candidates for these studies. For example, non-human primates have the most similar microbiome to human primates than to any other animal (85), therefore various studies have been conducted using them to explore the influence of different diets, for example, Western and Mediterranean diets on the gut microbiome (86, 87). We need to note here that of course there are differences in abundance of certain bacterial taxa between species. It was found that in non-human primates and in rats there is a higher abundance of *Prevotella* compared to humans and mice (88). As many diseases, disorders, and cancer progression have been linked to an abnormal gut microbiome in humans, research has now expanded to other animal models such as horses (89, 90) and dogs (91) to name a few. Researchers are also trying to create pig models that resemble the human gut microbiome to make future experiments easier and more reliable (92, 93).

For now, using mouse models to study the gut microbiome seems to be the optimal option, and the potential of optimized humanized gnotobiotic mouse models is something to look forward to in the future.

Studying the microbiome using alternative *in vitro* and *ex vivo* models

Despite great progress being made in deciphering the role of the gut microbiota in connection with various diseases using different animal models, especially mouse models, there are still several limitations with those models. They are time-consuming, under ethical considerations, and often fail to copy real human conditions because of inter-species differences and the complexity of the gut microbiome in general (94). Advances in three-dimensional cell biology and bioengineering enabled researchers to come up with alternative *in vitro* and *ex vivo* cellular and tissue models to study the microbiome. These models could decrease the number of animal experiments in the future.

Organoids are gaining more and more attraction since they have been proven to be valuable *in vitro* systems for modeling different human diseases (95). They are 3D self-assembled tissue constructs that contain highly polarized cells that mimic the *in vivo* organization and architecture of the tissue of origin (96). Organoids can be derived either from pluripotent stem cells (PCSs) or adult stem cells (ASCs). In the intestines, ACSs are located at the bottom of the intestinal crypts which can then be grown in extracellular matrix Matrigel to make organoid models which contain fully mature goblets cells, enteroendocrine cells, enterocytes and Paneth cells (97). When using PCSs to form organoids, cells are often either derived from embryonic stem cells or from induced pluripotent stem cells which are then treated with specific growth factors that direct the tissue-specific development of the cells (98). The PCS-derived organoids can contain also mesenchymal cells in comparison to intestinal epithelial cell types seen in ASC-derived organoids (99).

Gastrointestinal organoids either from ASC or PCS-derived cells have the basal membrane displayed outwards and the lumen in the center of the construct. The most popular method to deliver the bacteria or their metabolites to form a relevant microbiome organoid model is through microinjection into the lumen. This method mimics bacteria that normally also infects the host from the lumen but has also downsides since it is a difficult method to perform, and organoid damage often happens during the whole process. There is also a method where organoids in suspensions are mixed with microbes and then cultivated to reform 3D organoids but again this method does not appropriately capture the mechanisms by which microbes infect the cells (100, 101). One very important limitation researchers should be aware of includes the lack of cellular components of the microenvironment particularly in ASC-derived organoids (102). Among cellular components, organoids lack mesenchymal cell heterogeneity and architecture, vasculature, neuronal connections, and interaction with immune cells and the intestinal microbial flora (103).

Because of the emerging connection of microbiome influence on tumor development and progression, scientists also try to use different cancer organoid models to study this correlation. Traditional 2D cell cultures fail to mimic the complex tumor microenvironment and the interaction of tumor and non-tumor cells as well as the interaction with the extracellular matrix. The results so far show that tumor cells in organoids react differently to chemotherapy than 2D cell cultures or just tumor cells embedded in 3D gels, showing a promising future for the use of organoids (104).

Another very promising *in vitro* model is organs-on-chips, which can mimic the physiology, structure, function, and pathology of human organs. Scientists have invented organ chip models of the human intestine which are novel cell culture devices that offer greater control over important biological parameters such as oxygen availability and pH levels using different micro-fluidic channels (105). The chips complexity over the last years has increased and can now even mimic intestinal peristaltic-like motions and flow, using different mechanical forces (106). The advancements also include a variety of channels that are surrounded by commensal microbes, pathogens, immune cells, and human microvascular endothelium and can also enable villus-crypt formation and added mucus layer (105). For example, researchers have made an intestine chip that contains epithelial cells from human intestinal biopsies that successfully mimics real human physiological conditions (107). Apart from primary cells, other types of cells can be grown on such chips for example ASC or PCS-derived organoids (108, 109) or immortalized cell lines (110). In the end, these models could serve as a great option in addition to animal models to study the influence of the gut microbiome as well as being a valuable tool to study different kinds of tissues.

Alternative cell and tissue models in the laboratory will allow us to study the interactions between tissue, tumor, and microbiome by mimicking microbiome-host-cancer interactions as a function of species. This will allow us to understand the molecular mechanisms and develop alternative treatment approaches for various diseases, including cancer.

Gut microbiota in patients with aggressive primary brain tumors

We know that gut microbiota influences tumor growth and progression. Since gliomas are the most aggressive primary brain tumors in adults and are challenging to treat there is ongoing research about the connection to the gut microbiome trying to find new ways to improve current treatment outcomes (111). Researchers have found out that glioma tumor growth leads to dysbiosis in the gut microbiome in mice before weight loss occurs. They found a significant decrease in the *Firmicutes* to *Bacteroides* (F/B) ratio following tumor growth. They observed a decrease in *Firmicutes* and an increase in *Verrucomicrobia phyla*. Interestingly,

after treating the mice with the oral chemotherapy drug temozolomide (TMZ) there was no glioma-induced dysbiosis seen since there was no significant difference in the F/B ratio. They later showed that TMZ administration in healthy mice causes dysbiosis but with no significant change in *Verrucomicrobia*. When they analyzed fecal samples from human glioma patients and healthy controls they found a correlation with their mice results, with similar microbiome changes observed in both species (112).

We mentioned in previous sections the effects of short-chain fatty acids (SCFAs) like butyrate, acetate, and propionate have anti-cancer properties. In a study made by A. Dono, *et al.* they show that the short fatty acids were all decreased in mouse models after glioma growth after an analysis of fecal metabolites. Apart from that, they saw a decrease in important neurotransmitters such as norepinephrine and 5-hydroxyindoleacetic acid (5-HIAA) after tumor development and an increase in serotonin, 3-methyl valerate, caproate, and acetylcholine. This study also found the same increases in the *Verrucomicrobia* phylum and a decrease in *Bacteroidetes* which coincides with the findings of the study mentioned in the previous paragraph since *Bacteroides* belong to the *Bacteroidetes* phylum of Gram-negative bacteria. After treating the mice with TMZ there was no significant change in SCFAs which is consistent with the findings that glioma growth impacts the gut microbiome. Similar data was also obtained from human samples which means that we can draw parallels between the mouse model and actual human samples (113).

Another study found conflicting results with the previously mentioned studies. After the growth of glioma in mouse models they observed a decrease in the abundance of *Bacteroidia* (new name for *Bacteroidetes*) and an increase in the abundance of *Firmicutes*. However, the results still suggest that glioma growth contributed to dysbiosis in the gut. They also found that after administering antibiotics to mice, the glioma progression is worse than in non-antibiotic treated mice. These results confirm the hypothesis that gut dysbiosis can worsen glioma progression. After detecting expression levels of CD8 and Foxp3 in mouse brain tissues in antibiotic and non-antibiotic-treated mice, they found out that CD8 expression levels were not significantly different between the two groups. On the other hand, there was a decrease in Foxp3 expression in antibiotic-treated mice compared to the control group suggesting that gut microbiome dysbiosis downregulated Foxp3 expression in glioma tissue and Foxp3 may act as a tumor suppressor protein (114).

The importance of gut microbiota in glioma development was highlighted in another study where they also found that glioma growth increases in mice treated with antibiotics. They also observed changes in microglia phenotype and a reduction in $CD27^+/CD11b^+$ NK cells that are involved in tumor cell lysis which could explain the tumor size increase in antibiotic-treated mice (115). The *Bifidobacterium* genus was shown to have antitumor effects (116) and in a study where mixtures of *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* were administered into an orthotopic mouse model of glioma via gavage treatment, the tumor volume was reduced, and the lifespan of the mice was prolonged (117). This experiment gives us promising new therapeutic options for future glioma treatments.

Glioblastoma is the most aggressive type of glioma with a median survival of 12-15 months (118). The most effective treatment so far is the combination of surgical removal of the tumor, radiotherapy, and chemotherapy with TMZ. Immunotherapy is successful only in preclinical mouse models however, it is not effective in humans. The reason could be in the difference between the gut microbiome in humans and mouse models since studies report that 85% of bacterial genera found in the mouse gut microbiota are not present in human (77). A study using humanized mouse models, where they transplanted the human gut microbiome into mice, found that mice with different human gut donors responded differently to immunotherapy using the checkpoint inhibitor anti-PD-1 drug. Out of five tested human mouse models only 2 responded to the treatment and displayed a significant increase in survival compared to the control groups. The two responsive mouse models had an abundance of *Bacteroides cellulosilyticus*, while the non-responsive models had an abundance of *Bacteroides intestinalis* and *Bacteroides uniformis*. They also found an increase in cytotoxic $CD8^+$ and $CD4^+$ T-cells producing IFN-γ after anti-PD-1 treatment in a humanized mouse model that was responsive to immunotherapy. The same results were not observed in the humanized mouse model that did not respond to immunotherapy (119). A recent study comparing healthy individuals with glioblastoma brain tumor (GBM) patients has shown that the GBM patients had a higher gut microbial diversity compared to the healthy individuals. The GBM group had a decrease in *Firmicutes* and an increase in the Proteobacteria phylum (120).

The gut microbiota also plays a role in the metabolism of various amino acids, which has different effects on the progression of gliomas. For example, the gut microbiome plays an important role in tryptophan metabolism, the product of which are AHR agonists. AHR stands for the aryl hydrocarbon receptor, a transcription factor that is activated by various ligands and is involved in cell proliferation, differentiation, cell death, and cell adhesion (121). The receptor is expressed in gliomas with the highest expression seen in glioblastomas (122). The AHR agonists produced by gut bacteria can activate the AHR receptor which then increases $FoxP3^+$ regulatory T cells through different mechanisms. It also regulates other T cell function as well as the differentiation and function of dendritic cells (123). In the case of arginine metabolism, the gut microbiota can turn dietary arginine into polyamines and nitric oxide which arrive to the brain through the blood-brain barrier (124, 125). Polyamine can affect tumor growth by up-regulating the expression of

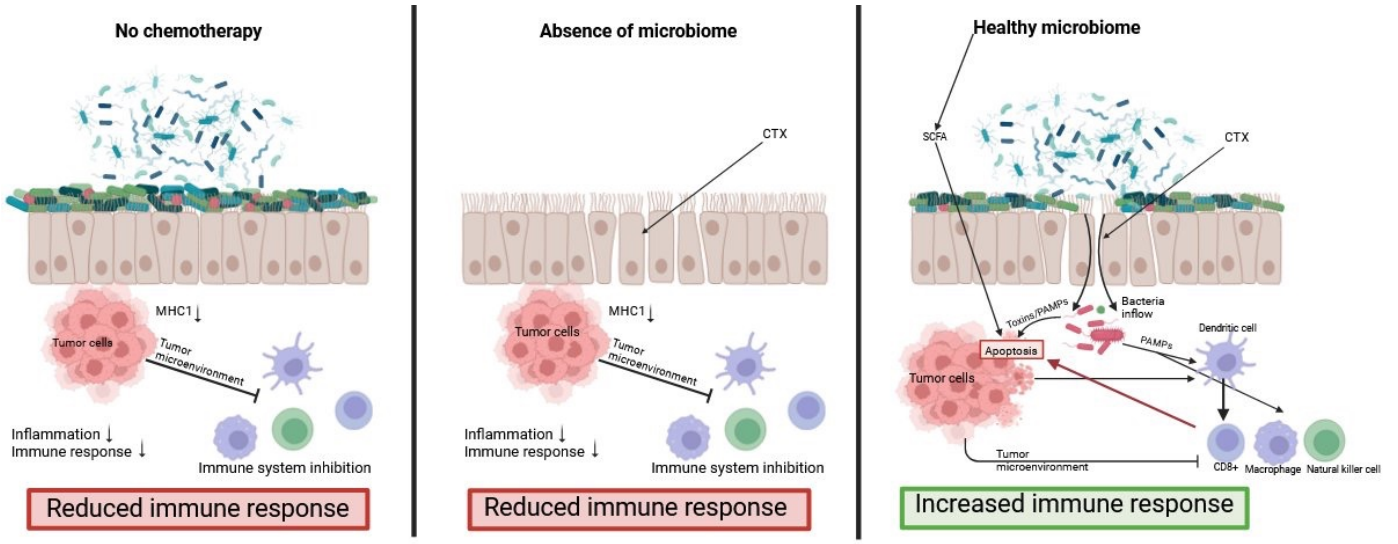


Figure 3: The influence of a healthy gut microbiome on chemotherapy treatment. On the left we can see that without chemotherapy the tumor microenvironment can suppress the immune system and hence hindering adequate immune response to eliminate the tumor. In the middle we can see that the absence of a gut microbiome, in gnotobiotic mice for instance, after administration of a chemotherapeutic drug CTX we can see no improvement in immune response. On the right where we have a healthy gut microbiome, the administration of CTX increases immune response and therefore impacts the natural immune system and increases the anti-tumor immune response. The Scheme was created using Biorender.com

Abbreviations: major histocompatibility complex 1 (MHC1), short-chain fatty acids (SCFAs), cyclophosphamide (CTX), pathogen-associated molecular pattern molecules (PAMPs).

ornithine decarboxylase, spermidine, spermine acetyltransferase, and Akt1 which can induce tumor cell proliferation and metastasis (126). The effects of nitric oxide on glioma are still not fully known but since nitric oxide can interfere with T cell function by promoting T cell apoptosis it can be speculated that it could promote glioma development (127, 128).

These studies suggest that the gut microbiome is also crucial for distant tumors, such as primary brain tumors, and that the microbiome is also found in the microenvironment of brain tumors (129), where its role remains to be explored. In this regard, the microbiome should be exploited to increase the efficacy of current therapies and/or to develop new, more efficient treatments.

Future outlook and conclusions

The gut microbiome has been shown to influence the success of various cancer therapies (Figure 3). Some bacteria have a tumor-inhibiting effect and help the individual fight cancer, while others have the opposite effect and worsen the state of health. Ideally, it would be desirable to eliminate the harmful bacteria while promoting the proliferation of the beneficial ones. However, current antibiotics have a broad spectrum of action that affects both types of bacteria. For this reason, research is being carried out into the synthesis of specific antibiotics that only eliminate the bad bacteria and leave the good bacteria untouched. Another possible solution would be the use of bacteriophages, which only infect and destroy certain bacteria, thus creating a microbiome that benefits the patient. Research on fecal microbiome transfer seems promising, but scientists have yet to determine why some donors' feces are more successful in increasing the effectiveness of immunotherapy than others. Nonetheless, this area of research is gaining increasing attention and could prove to be a crucial approach to cancer treatment in the future.

The gut microbiome is a dynamic and influential component of human health. While it offers many benefits, disturbances in its balance can contribute to the development and progression of cancer. It plays an important role in the anti-tumor response, as demonstrated by successful cancer remission after fecal transplantation in mice and humans. Understanding the intricate relationship between the gut microbiome and cancer is crucial for the development of new strategies to prevent and treat this complex disease. Further research is needed to explore the full extent of these interactions and their potential therapeutic implications so that we can improve treatment outcomes for difficult malignancies such as gliomas in the future. Because mouse models have genetic and physiological similarities to humans, they are invaluable for biomedical research, including research into human disease. However, it is important to recognize the microbiome-related differences between these two species, as this is the only way we can achieve more relevant results. New in vitro models such as organoids on chip organs offer a promising alternative to animal models to study the gut microbiome in a more controlled and species-specific way. This emerging field of research could help us fight cancer and other diseases in a novel way that could significantly improve our overall well-being.

Acknowledgement

This work was supported by the Slovenian Research and Innovation Agency (Programme and research grants P1-0245, J3-4504, J3-2526 and NC-0023) and by the European Union's Horizon project Twinning for excellence to strategically advance research in carcinogenesis and cancer (CutCancer; 101079113)

References

1. Schwabe RF, Jobin C. The microbiome and cancer. Nat Rev Cancer 2013; 13(11): 800–12. doi: 10.1038/nrc3610
2. Gomaa EZ. Human gut microbiota/microbiome in health and diseases: a review. Antonie Van Leeuwenhoek 2020; 113(12): 2019–40. doi: 10.1007/s10482-020-01474-7
3. Frank DN, St. Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA 2007; 104(34): 13780–5. doi: 10.1073/pnas.0706625104
4. Bielanski A, Haber J. Structure, function and diversity of the healthy human microbiome. Nature 2012;486(7402): 207–14. doi: 10.1038/nature11234
5. Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol 2011 ;9(4): 244–53. doi: 10.1038/nrmicro2537
6. Chen D, Jin D, Huang S, Wu J, Xu M, Liu T, et al. Clostridium butyricum, a butyrate-producing probiotic, inhibits intestinal tumor development through modulating Wnt signaling and gut microbiota. Cancer Lett 2020; 469: 456–67. doi: 10.1016/j.canlet.2019.11.019
7. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. Role of the normal gut microbiota. World J Gastroenterol 2015; 21(29): 8787–803. doi: 10.3748/wjg.v21.i29.8787
8. Jiao Y, Wu L, Huntington ND, Zhang X. Crosstalk Between Gut Microbiota and Innate Immunity and Its Implication in Autoimmune Diseases. Front Immunol 2020; 11: 282. doi: 10.3389/fimmu.2020.00282
9. Rosas-Plaza S, Hernández-Terán A, Navarro-Díaz M, Escalante AE, Morales-Espinosa R, Cerritos R. Human gut microbiome across different lifestyles: from hunter-gatherers to urban populations. Front Microbiol 2022; 13: 843170. doi: 10.3389/fmicb.2022.843170
10. Shu YZ, Arcuri M, Kozlowski M, et al. Haloemodins, a new class of endothelin-1 type B (ETB) receptor binding inhibitors. J Antibiot 1994;47(11):1328–32.
11. Kim S, Covington A, Pamer EG. The intestinal microbiota: Antibiotics, colonization resistance, and enteric pathogens. Immunol Rev 2017; 279(1): 90–105. doi: 10.1111/imr.12563
12. De Martel C, Ferlay J, Franceschi S, et al. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol 2012; 13(6): 607–15. doi: 10.1016/S1470-2045(12)70137-7
13. Wan Y, Wang F, Yuan J, et al. Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. Gut Microbiota 2019; 68(8): 1417–29. doi:10.1136/gutjnl-2018-317609
14. Lam W, Bussom S, Guan F, et al. The four-herb Chinese medicine PHY906 reduces chemotherapy-induced gastrointestinal toxicity. Sci Transl Med 2010; 2(45): 45ra59. doi: 10.1126/scitranslmed.3001270
15. Wallace BD, Wang H, Lane KT, et al. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. Science 2010; 330(6005): 831–5. doi: 10.1126/science.1191175
16. Hattori N, Ushijima T. Epigenetic impact of infection on carcinogenesis: mechanisms and applications. Genome Med 2016; 8(1): 10. doi: 10.1186/s13073-016-0267-2
17. Meng C, Bai C, Brown TD, Hood LE, Tian Q. Human gut microbiota and gastrointestinal cancer. Genomics Proteomics Bioinformatics 2018; 16(1): 33–49. doi: 10.1016/j.gpb.2017.06.002
18. Nasrollahzadeh D, Malekzadeh R, Ploner A, et al. Variations of gastric corpus microbiota are associated with early esophageal squamous cell carcinoma and squamous dysplasia. Sci Rep 2015; 5: 8820. doi: 10.1038/srep08820
19. Proctor LM. The human microbiome project in 2011 and beyond. Cell Host Microbe 2011; 10(4): 287–91. doi: 10.1016/j.chom.2011.10.001
20. Gagnière J, Raisch J, Veziant J, et al. Gut microbiota imbalance and colorectal cancer. World J Gastroenterol 2016; 22(2): 501–18. doi: 10.3748/wjg.v22.i2.501
21. Edwards PT, Kashyap PC, Preidis GA. Microbiota on biotics: Probiotics, prebiotics, and synbiotics to optimize growth and metabolism. Am J Physiol Gastrointest Liver Physiol. 2020;319(3):G382–90.
22. Yoshimoto S, Loo TM, Atarashi K, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature 2013; 499(7456): 97–101. doi: 10.1038/nature12347
23. Loo TM, Kamachi F, Watanabe Y, et al. Gut microbiota promotes obesity-associated liver cancer through PGE2-mediated suppression of antitumor immunity. Cancer Discov 2017; 7(5): 522–38. doi: 10.1158/2159-8290.CD-16-0932
24. Ma C, Han M, Heinrich B, et al. Gut microbiome–mediated bile acid metabolism regulates liver cancer via NKT cells. Science 2018; 360(6391): eaan5931. doi: 10.1126/science.aan5931
25. Cheng WY, Wu CY, Yu J. The role of gut microbiota in cancer treatment: friend or foe? Gut 2020; 69(10): 1867–76. doi: 10.1136/gutjnl-2020-321153
26. Nougayrède JP, Homburg S, Taieb F, et al. Escherichia coli induces DNA double-strand breaks in eukaryotic cells. Science 2006; 313(5788): 848–51. doi: 10.1126/science.1127059
27. Arthur JC, Perez-Chanona E, Mühlbauer M, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 2012; 338(6103): 120–3. doi: 10.1126/science.1224820
28. Bultman SJ. Emerging roles of the microbiome in cancer. Carcinogenesis 2014; 35(2): 249–55. doi: 10.1093/carcin/bgt392
29. Dennis KL, Blatner NR, Gounari F, Khazaie K. Current status of interleukin-10 and regulatory T-cells in cancer. Curr Opin Oncol 2013; 25(6): 637–45. doi: 10.1097/CCO.0000000000000006
30. Akbar N, Khan NA, Muhammad JS, Siddiqui R. The role of gut microbiome in cancer genesis and cancer prevention. Health Sci Rev 2022; 2(3): 100010. 10.1016/j.hsr.2021.100010
31. Bergounioux J, Elisee R, Prunier AL, et al. Calpain activation by the Shigella flexneri effector VirA regulates key steps in the formation and life of the bacterium's epithelial niche. Cell Host Microbe 2012; 11(3): 240–52. doi: 10.1016/j.chom.2012.01.013
32. Fomby P, Cherlin AJ. Role of microbiota in immunity and inflammation. National Institute of Health 2011;72(2): 181–204.
33. Wada Y, Takemura K, Tummala P, et al. Helicobacter pylori induces somatic mutations in TP53 via overexpression of CHAC1 in infected gastric epithelial cells. FEBS Open Bio 2018; 8(4): 671–9. doi: 10.1002/2211-5463.12402

34. Ding SZ, Minohara Y, Xue JF, et al. Helicobacter pylori infection induces oxidative stress and programmed cell death in human gastric epithelial cells. Infect Immun 2007; 75(8): 4030–9. doi: 10.1128/IAI.00172-07

35. Huycke MM, Moore D, Joyce W, et al. Extracellular superoxide production by Enterococcus faecalis requires demethylmenaquinone and is attenuated by functional terminal quinol oxidases. Mol Microbiol 2001; 42(3): 729–40. doi: 10.1046/j.1365-2958.2001.02638.x

36. Chaturvedi R, Asim M, Romero-Gallo J, et al. Spermine oxidase mediates the gastric cancer risk associated with Helicobacter pylori CagA. Gastroenterology 2011; 141(5): 1696-1708.e1–2. doi: 10.1053/j.gastro.2011.07.045

37. Goodwin AC, Destefano Shields CE, Wu, et al. Polyamine catabolism contributes to enterotoxigenic Bacteroides fragilis-induced colon tumorigenesis. Proc Natl Acad Sci U S A 2011; 108(37): 15354–9. doi: 10.1073/pnas.1010203108

38. Vivarelli S, Salemi R, Candido S, et al. Gut Microbiota and cancer: from pathogenesis to therapy. Cancers (Basel) 2019; 11(1): 38. doi: 10.3390/cancers11010038

39. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol 2016; 14(8): e1002533. doi: 10.1371/journal.pbio.1002533

40. Morgan XC, Huttenhower C. Chapter 12: Human microbiome analysis. PLoS Comput Biol 2012; 8(12): e1002808. doi: 10.1371/journal.pcbi.1002808

41. Johansson MEV, Jakobsson HE, Holmén-Larsson J, et al. Normalization of host intestinal mucus layers requires long-term microbial colonization. Cell Host Microbe 2015; 18(5): 582–92. doi: 10.1016/j.chom.2015.10.007

42. Spiljar M, Merkler D, Trajkovski M. The immune system bridges the gut microbiota with systemic energy homeostasis: focus on TLRs, mucosal barrier, and SCFAs. Front Immunol 2017; 8: 1353. doi: 10.3389/fimmu.2017.01353

43. Kamada N, Chen GY, Inohara N, Núñez G. Control of pathogens and pathobionts by the gut microbiota. Nat Immunol 2013; 14(7): 685–90. doi: 10.1038/ni.2608

44. Viaud S, Saccheri F, Mignot G, et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 2013; 342(6161): 971–6. doi: 10.1126/science.1240537

45. Daillère R, Vétizou M, Waldschmitt N, et al. Enterococcus hirae and Barnesiella intestinihominis Facilitate Cyclophosphamide-Induced therapeutic immunomodulatory effects. Immunity 2016; 45(4): 931–43. doi: 10.1016/j.immuni.2016.09.009

46. Lin C, Cai X, Zhang J, et al. Role of gut microbiota in the development and treatment of colorectal cancer. Digestion 2019; 100(1): 72–8. doi: 10.1159/000494052

47. Vétizou M, Pitt JM, Daillère R, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015; 350(6264): 1079–84. doi: 10.1126/science.aad1329

48. Jan G, Belzacq AS, Haouzi D, et al. Propionibacteria induce apoptosis of colorectal carcinoma cells via short-chain fatty acids acting on mitochondria. Cell Death Differ 2002; 9(2): 179–88. doi: 10.1038/sj.cdd.4400935

49. Wei W, Sun W, Yu S, Yang Y, Ai L. Butyrate production from high-fiber diet protects against lymphoma tumor. Leuk Lymphoma 2016; 57(10): 2401–8. doi: 103109/1042819420161144879.

50. Zhang J, Xia Y, Sun J. Breast and gut microbiome in health and cancer. Genes Dis 2020; 8(5): 581–9. doi: 10.1016/j.gendis.2020.08.002

51. Sánchez-Alcoholado L, Ramos-Molina B, Otero A, et al. The role of the gut microbiome in colorectal cancer development and therapy response. Cancers (Basel) 2020; 12(6): 1406. doi: 10.3390/cancers12061406

52. Salcedo R, Worschech A, Cardone M, et al. MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. J Exp Med 2010; 207(8): 1625–36. doi: 10.1084/jem.20100199

53. Paulos CM, Wrzesinski C, Kaiser A, et al. Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8+ T cells via TLR4 signaling. J Clin Invest 2007; 117(8): 2197–204. doi: 10.1172/JCI32205

54. Paavonen J, Naud P, Salmerón J, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 2009; 374(9686): 301–14. doi: 10.1016/S0140-6736(09)61248-4

55. Konishi H, Fujiya M, Tanaka H, Ueno N, Moriichi K, Sasajima J, et al. Probiotic-derived ferrichrome inhibits colon cancer progression via JNK-mediated apoptosis. Nature Communications 2016; 7: 12365. doi: 10.1038/ncomms12365

56. Davar D, Dzutsev AK, McCulloch JA, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. Science 2021; 371(6529): 595–602. doi: 10.1126/science.abf3363

57. Baruch EN, Youngster I, Ben-Betzalel G, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science 2021; 371(6529): 602–9. doi: 10.1126/science.abb5920

58. Erdmann J. How gut bacteria could boost cancer treatments. Nature 2022; 607(7919): 436–9. doi: 10.1038/d41586-022-01959-7

59. Legesse Bedada T, Feto TK, Awoke KS, Garedew AD, Yifat FT, Birri DJ. Probiotics for cancer alternative prevention and treatment. Biomed Pharmacother 2020; 129: 110409. doi: 10.1016/j.biopha.2020.110409

60. Rasouli BS, Ghadimi-Darsajini A, Nekouian R, Iragian GR. In vitro activity of probiotic Lactobacillus reuteri against gastric cancer progression by downregulation of urokinase plasminogen activator/urokinase plasminogen activator receptor gene expression. J Cancer Res Ther 2017; 13(2): 246–51. doi: 10.4103/0973-1482.204897

61. Redman MG, Ward EJ, Phillips RS. The efficacy and safety of probiotics in people with cancer: a systematic review. Ann Oncol 2014; 25(10): 1919–29. doi: 10.1093/annonc/mdu106

62. Mego M, Holec V, Drgona L, Hainova K, Ciernikova S, Zajac V. Probiotic bacteria in cancer patients undergoing chemotherapy and radiation therapy. Complement Ther Med 2013; 21(6): 712–23. doi: 10.1016/j.ctim.2013.08.018

63. Gianotti L, Morelli L, Galbiati F, et al. A randomized double-blind trial on perioperative administration of probiotics in colorectal cancer patients. World J Gastroenterol 2010; 16(2): 167–75. doi: 10.3748/wjg.v16.i2.167

64. Demers M, Dagnault A, Desjardins J. A randomized double-blind controlled trial: impact of probiotics on diarrhea in patients treated with pelvic radiation. Clin Nutr 2014; 33(5): 761–7. doi: 10.1016/j.clnu.2013.10.015

65. Mego M, Chovanec J, Vochyanova-Andrezalova I, et al. Prevention of irinotecan induced diarrhea by probiotics: a randomized double blind, placebo controlled pilot study. Complement Ther Med 2015; 23(3): 356–62. doi: 10.1016/j.ctim.2015.03.008

66. Hatakka K, Saxelin M, Mutanen M, et al. Lactobacillus rhamnosus LC705 Together with Propionibacterium freudenreichii ssp shermanii JS administered in capsules is ineffective in lowering serum lipids. 2013; 27(4): 441–7. doi: 101080/07315724200810719723

67. Gopalakrishnan V, Spencer CN, Nezi L, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018; 359(6371): 97–103. doi: 10.1126/science.aan4236

68. Matson V, Fessler J, Bao R, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 2018; 359(6371): 104–8. doi: 10.1126/science.aao3290

69. Chen Q, Wang C, Chen G, Hu Q, Gu Z. Delivery Strategies for Immune Checkpoint Blockade. Adv Healthc Mater 2018; 7(20): e1800424. doi: 10.1002/adhm.201800424

70. Chaput N, Lepage P, Coutzac C, et al. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. Ann Oncol 2017; 28(6): 1368–79. doi: 10.1093/annonc/mdx108

71. Levy M, Thaiss CA, Katz MN, Suez J, Elinav E. Inflammasomes and the microbiota—partners in the preservation of mucosal homeostasis. Semin Immunopathol 2015; 37(1): 39–46. doi: 10.1007/s00281-014-0451-7

72. Franchi L, Eigenbrod T, Muñoz-Planillo R, Nuñez G. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nature Immunol 2009; 10(3): 241–7. doi: 10.1038/ni.1703

73. Zitvogel L, Kepp O, Galluzzi L, Kroemer G. Inflammasomes in carcinogenesis and anticancer immune responses. Nature Immunol 2012; 13(4): 343–51. doi: 10.1038/ni.2224

74. Morse HC. Building a better mouse: one hundred years of genetics and biology. In: Fox JG, eds. The mouse in biomedical research: history, wild mice, and genetics. 2nd ed. Amsterdam: Elsevier, 2006.

75. Li Y, Baldridge MT. Modelling human immune responses using microbial exposures in rodents. Nat Microbiol 2023; 8(3): 363–6. doi: 10.1038/s41564-023-01334-w

76. Sjaastad FV, Huggins MA, Lucas ED, et al. Reduced T cell priming in microbially experienced "dirty" mice results from limited il-27 production by xcr1+ dendritic cells. J Immunol 2022; 209(11): 2149–59.

77. Nguyen TLA, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? Dis Model Mech. 2015 Jan 1;8(1):1–16.

78. Guinane CM, Tadrous A, Fouhy F, et al. Microbial composition of human appendices from patients following appendectomy. mBio 2013; 4(1): e00366–12. doi: 10.1128/mBio.00366-12

79. Alkadhi S, Kunde D, Cheluvappa R, Randall-Demllo S, Eri R. The murine appendiceal microbiome is altered in spontaneous colitis and its pathological progression. Gut Pathog 2014; 6: 25. doi: 10.1186/1757-4749-6-25

80. Krych L, Hansen CHF, Hansen AK, van den Berg FWJ, Nielsen DS. Quantitatively different, yet qualitatively alike: a meta-analysis of the mouse core gut microbiome with a view towards the human gut microbiome. PLoS One 2013; 8(5): e62578. doi: 10.1371/journal.pone.0062578

81. Almeida A, Nayfach S, Boland M, et al. A unified catalog of 204, 938 reference genomes from the human gut microbiome. Nat Biotechnol 2021; 39(1): 105–14. doi: 10.1038/s41587-020-0603-3

82. Kieser S, Zdobnov EM, Trajkovski M. Comprehensive mouse microbiota genome catalog reveals major difference to its human counterpart. PLoS Comput Biol 2022; 18(3): e1009947. doi: 10.1371/journal.pcbi.1009947

83. Park JC, Im SH. Of men in mice: the development and application of a humanized gnotobiotic mouse model for microbiome therapeutics. Exp Mol Med 2020; 52(9): 1383–96. doi: 10.1038/s12276-020-0473-2

84. Kennedy EA, King KY, Baldridge MT. Mouse microbiota models: comparing germ-free mice and antibiotics treatment as tools for modifying gut bacteria. Front Physiol 2018; 9: 1534. doi: 10.3389/fphys.2018.01534

85. Ley RE, Hamady M, Lozupone C, et al. Evolution of mammals and their gut microbes. Science 2008; 320(5883): 1647–51. doi: 10.1126/science.1155725

86. Amato KR, Yeoman CJ, Cerda G, et al. Variable responses of human and non-human primate gut microbiomes to a Western diet. Microbiome 2015; 3(1): 53. doi: 10.1186/s40168-015-0120-7

87. Nagpal R, Shively CA, Appt SA, et al. Gut microbiome composition in non-human primates consuming a western or mediterranean diet. Front Nutr 2018; 5: 28. doi: 10.3389/fnut.2018.00028

88. Nagpal R, Wang S, Solberg Woods LC, et al. Comparative microbiome signatures and short-chain fatty acids in mouse, rat, non-human primate, and human feces. Front Microbiol 2018; 9: 2897. doi: 10.3389/fmicb.2018.02897

89. Garrett LA, Brown R, Poxton IR. A comparative study of the intestinal microbiota of healthy horses and those suffering from equine grass sickness. Vet Microbiol 2002; 87(1): 81–8. doi: 10.1016/s0378-1135(02)00018-4

90. Costa MC, Arroyo LG, Allen-Vercoe E, et al. Comparison of the fecal microbiota of healthy horses and horses with colitis by high throughput sequencing of the v3-v5 region of the 16s rrna gene. PLoS One 2012; 7(7): e41484. doi: 10.1371/journal.pone.0041484

91. Rostaher A, Morsy Y, Favrot C, Unterer S, Schnyder M, Scharl M, et al. Comparison of the gut microbiome between atopic and healthy dogs—preliminary data. Animals (Basel) 2022; 12(18): 2377. doi: 10.3390/ani12182377

92. Heinritz SN, Mosenthin R, Weiss E. Use of pigs as a potential model for research into dietary modulation of the human gut microbiota. Nutr Res Rev 2013; 26(2): 191–209. doi: 10.1017/S0954422413000152

93. Zhang Q, Widmer G, Tzipori S. A pig model of the human gastrointestinal tract. Gut Microbes 2013; 4(3): 193–200. doi: 10.4161/gmic.23867

94. Paul W, Marta C, Tom V de W. Resolving host–microbe interactions in the gut: the promise of in vitro models to complement in vivo research. Curr Opin Microbiol 2018; 44: 28–33. doi: 10.1016/j.mib.2018.07.001

95. Schweiger PJ, Jensen KB. Modeling human disease using organotypic cultures. Curr Opin Cell Biol 2016; 43: 22–9. doi: 10.1016/j.ceb.2016.07.003

96. Hill DR, Spence JR. Gastrointestinal organoids: understanding the molecular basis of the host–microbe interface. Cell Mol Gastroenterol Hepatol 2017; 3(2): 138–49. doi: 10.1016/j.jcmgh.2016.11.007

97. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009; 459(7244): 262–5. doi: 10.1038/nature07935

98. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 2011; 470(7332): 105–9. doi: 10.1038/nature09691

99. Forbester JL, Goulding D, Vallier L, et al. Interaction of Salmonella enterica serovar Typhimurium with intestinal organoids derived from human induced pluripotent stem cells. Infect Immun 2015; 83(7): 2926–34. doi: 10.1128/IAI.00161-15

100. Williamson IA, Arnold JW, Samsa LA, et al. A high-throughput organoid microinjection platform to study gastrointestinal microbiota and luminal physiology. Cell Mol Gastroenterol Hepatol 2018; 6(3): 301–19. doi: 10.1016/j.jcmgh.2018.05.004

101. Dutta D, Heo I, Clevers H. Disease modeling in stem cell-derived 3d organoid systems. Trends Mol Med 2017; 23(5): 393–410. doi: 10.1016/j.molmed.2017.02.007

102. Günther C, Winner B, Neurath MF, Stappenbeck TS. Organoids in gastrointestinal diseases: from experimental models to clinical translation. Gut 2022; 71(9): 1892–908. doi: 10.1136/gutjnl-2021-326560

103. Taelman J, Diaz M, Guiu J. Human intestinal organoids: promise and challenge. Front Cell Dev Biol 2022; 10: 854740. doi: 10.3389/fcell.2022.854740

104. Shelkey E, Oommen D, Stirling ER, et al. Immuno-reactive cancer organoid model to assess effects of the microbiome on cancer immunotherapy. Sci Rep 2022; 12(1): 9983. doi: 10.1038/s41598-022-13930-7

105. Bein A, Shin W, Jalili-Firoozinezhad S, et al. Microfluidic organ-on-a-chip models of human intestine. Cell Mol Gastroenterol Hepatol 2018; 5(4): 659–68. doi: 10.1016/j.jcmgh.2017.12.010

106. Kim HJ, Huh D, Hamilton G, Ingber DE. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab Chip 2012; 12(12): 2165–74. doi: 10.1039/c2lc40074j

107. Kasendra M, Tovaglieri A, Sontheimer-Phelps A, et al. Development of a primary human small intestine-on-a-chip using biopsy-derived organoids. Sci Rep 2018; 8(1): 2871. doi: 10.1038/s41598-018-21201-7

108. Nikolaev M, Mitrofanova O, Broguiere N, et al. Homeostatic mini-intestines through scaffold-guided organoid morphogenesis. Nature 2020; 585(7826): 574–8. doi: 10.1038/s41586-020-2724-8

109. Naumovska E, Aalderink G, Valencia CW, et al. Direct On-Chip Differentiation of Intestinal Tubules from Induced Pluripotent Stem Cells. Int J Mol Sci 2020; 21(14): 4964. doi: 10.3390/ijms21144964

110. Beaurivage C, Naumovska E, Chang YX, et al. Development of a gut-on-a-chip model for high throughput disease modeling and drug discovery. Int J Mol Sci. 2019; 20(22): 5661. doi: 10.3390/ijms20225661

111. Bush NAO, Chang SM, Berger MS. Current and future strategies for treatment of glioma. Neurosurg Rev 2017; 40(1): 1–14. doi: 10.1007/s10143-016-0709-8

112. Patrizz A, Dono A, Zorofchian S, et al. Glioma and temozolomide induced alterations in gut microbiome. Sci Rep 2020; 10(1): 21002. doi: 10.1038/s41598-020-77919-w

113. Dono A, Patrizz A, McCormack RM, et al. Glioma induced alterations in fecal short-chain fatty acids and neurotransmitters. CNS Oncol 2020; 9(2): CNS57. doi: 10.2217/cns-2020-0007

114. Fan Y, Su Q, Chen J, Wang Y, He S. Gut Microbiome alterations affect glioma development and foxp3 expression in tumor microenvironment in mice. Front Oncol 2022; 12: 836953. doi: 10.3389/fonc.2022.836953

115. D'Alessandro G, Antonangeli F, Marrocco F, et al. Gut microbiota alterations affect glioma growth and innate immune cells involved in tumor immunosurveillance in mice. Eur J Immunol 2020; 50(5): 705–11. doi: 10.1002/eji.201948354

116. Wei H, Chen L, Lian G, et al. Antitumor mechanisms of bifidobacteria . Oncol Lett 2018; 16(1): 3–8. doi: 10.3892/ol.2018.8692

117. Fan H, Wang Y, Han M, et al. Multi-omics-based investigation of Bifidobacterium's inhibitory effect on glioma: regulation of tumor and gut microbiota, and MEK/ERK cascade. Front Microbiol 2024; 15: 1344284. doi: 10.3389/fmicb.2024.1344284

118. Dunn GP, Rinne ML, Wykosky, et al. Emerging insights into the molecular and cellular basis of glioblastoma. Gen Dev 2012; 26(8): 756–84. doi: 10.1101/gad.187922.112

119. Dees KJ, Koo H, Humphreys JF, et al. Human gut microbial communities dictate efficacy of anti-PD-1 therapy in a humanized microbiome mouse model of glioma. Neurooncol Adv 2021; 3(1): vdab023. doi: 10.1093/noajnl/vdab023

120. Ishaq HM, Yasin R, Mohammad IS, et al. The gut-brain-axis: a positive relationship between gut microbial dysbiosis and glioblastoma brain tumour. Heliyon 2024; 10(9): e30494. doi: 10.1016/j.heliyon.2024.e30494

121. Zaragoza-Ojeda M, Apatiga-Vega E, Arenas-Huertero F. Role of aryl hydrocarbon receptor in central nervous system tumors: biological and therapeutic implications . Oncol Lett 2021; 21(6): 460. doi: 10.3892/ol.2021.12721

122. Jin UH, Michelhaugh SK, Polin LA, Shrestha R, Mittal S, Safe S. Omeprazole inhibits glioblastoma cell invasion and tumor growth. Cancers 2020; 12(8): 2097. doi: 10.3390/cancers12082097

123. Gutiérrez-Vázquez C, Quintana FJ. Regulation of the immune response by the aryl hydrocarbon receptor. Immunity 2018; 48(1): 19–33. doi: 10.1016/j.immuni.2017.12.012

124. Dai Z, Wu Z, Hang S, Zhu W, Wu G. Amino acid metabolism in intestinal bacteria and its potential implications for mammalian reproduction. Mol Hum Reprod 2015; 21(5): 389–409. doi: 10.1093/molehr/gav003

125. Kao CC, Cope JL, Hsu JW, et al. The microbiome, intestinal function, and arginine metabolism of healthy indian women are different from those of american and jamaican women. J Nutr 2015; 146(4): 706–13. doi: 10.3945/jn.115.227579

126. Dai F, Yu W, Song J, Li Q, Wang C, Xie S. Extracellular polyamines-induced proliferation and migration of cancer cells by ODC, SSAT, and Akt1-mediated pathway. Anticancer Drugs 2017; 28(4): 457–64. doi: 10.1097/CAD.0000000000000465

127. Rivoltini L, Carrabba M, Huber V, et al. Immunity to cancer: attack and escape in T lymphocyte–tumor cell interaction. Immunol Rev 2002; 188(1): 97–113. doi: 10.1034/j.1600-065x.2002.18809.x

128. Harari O, Liao JK. Inhibition of MHC II Gene Transcription by Nitric Oxide and Antioxidants. Curr Pharm Des 2004; 10(8): 893–8. doi: 10.2174/1381612043452893

129. Nejman D, Livyatan I, Fuks G, et al. The human tumor microbiome is composed of tumor type-specific intracellular bacteria. Science 2020; 368(6494): 973–80. doi: 10.1126/science.aay9189

Črevesni mikrobiom pri raku: naslednja velika priložnost za boljši izid zdravljenja?

J. Povšin, T. Sotošek, M. Novak, B. Breznik

Izvleček: Črevesni mikrobiom, raznolika skupnost mikroorganizmov v človeškem telesu, igra pomembno vlogo pri ohranjanju zdravja in vpliva na različne telesne procese. Edinstven črevesni mikrobiom posameznika, ki ga oblikujejo dejavniki, kot so način rojstva, prehrana, vnos antibiotikov in življenjski slog, prispeva k različnim telesnim funkcijam. Te funkcije so presnova hranil, metabolizem zdravil in uravnavanje imunskega sistema. Spremembe v črevesnem mikrobiomu so povezane s predispozicijo za nastanek raka in lahko vplivajo na učinkovitost njegovega zdravljenja. Porušeno črevesno ravnovesje oziroma disbioza v črevesnem mikrobiomu lahko vodi do vnetja, razvoja tumorjev in metastaz, kar poudarja njegov pomen v raziskavah raka. Črevesna mikrobiota pomembno vpliva na razvoj raka in rezultate zdravljenja. Nekatere bakterije povečajo učinke terapij, kot je ciklofosfamid, in prispevajo k boljšemu imunskemu odzivu proti raku. Mikroorganizmi proizvajajo protirakave molekule in probiotične spojine, ki so pomembno orodje pri preprečevanju in zdravljenju raka. Z nadaljnjimi raziskavami si znanstveniki želijo razviti ciljne antibiotike in raziskati prenos fekalne mikrobiote za selektivno manipulacijo mikrobiote. Zaradi genetskih in fizioloških podobnosti so mišji modeli neprecenljivi v biomedicinskih raziskavah, vendar pa zaradi razlik v črevesnem mikrobiomu ljudi in miši ter sestavi tumorskega mikrookolja neposredna primerjava med tema dvema modeloma lahko predstavlja izziv. Premostitev teh vrzeli je ključna za primerjalno medicino zlasti pri raziskavah raka, kjer mikrobiom igra pomembno vlogo pri izidih zdravljenja. Pri možganskih tumorjih gliomih lahko črevesni mikrobiom izkoristimo za potencialne nove možnosti zdravljenja. Dolgoročnega učinkovitega zdravljenja za to vrsto raka še ni, vendar raziskave na mišjih modelih kažejo povezavo med napredovanjem tumorja in odzivom na zdravljenje ter spremembami v črevesnem mikrobiomu. Črevesni mikrobiom in njegova modulacija predstavljata priložnost za učinkovitejše zdravljenje raka v prihodnosti.

Ključne besede: črevesni mikrobiom; rak; izid zdravljenja; tumorski modeli; gliom

Slov Vet Res

DOI 10.26873/SVR-2151-2024
UDC 001.814:616-006:616-063.22:616-091.8:636.7.09(497.4)
Pages: 27–39

Original Research Article

Incidence and types of canine tumours in Slovenia (2000-2020): A Retrospective study

Key words

dog;
tumour;
incidence;
age;
breed;
Slovenia

Teodora Nikodinovska, Mitja Gombač, Tamara Dolenšek, Kristina Tekavec, Sabina Šturm, Marko Cvetko, Katarina Pavlin, Domen Maj Fras, Tanja Švara*

Institute of Pathology, Wild Animals, Fish and Bees, University of Ljubljana Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia

***Corresponding author:** tanja.svara@vf.uni-lj.si

Abstract: We conducted a large retrospective study to establish a registry of canine tumours diagnosed in Slovenia over a 20-year period and to analyse their incidence rate and some epidemiological characteristics. In the study, we analysed the results of histopathological examinations of biopsies and samples from the necropsies of dogs submitted to the Institute of Pathology, Wild Animals, Fish and Bees of the Veterinary Faculty, University of Ljubljana between 2000 and 2020. Malignant tumours predominated (58.81%), followed by benign tumours (37.17%); 4.02% of tumours were of unspecified biological behaviour. Tumours were most frequently found in the skin and subcutaneous tissue (57.83% of all tumours), the mammary gland (14.07%) and the haemolymphatic system (6.63%). The most frequently diagnosed tumours were mast cell tumour (14.17%), mammary (adeno)carcinoma (10.02%), cutaneous histiocytoma (7.34%) and cutaneous/subcutaneous lipoma (6.92%). 51.69% of dogs with tumours were female, and 48.17% were male. Tumours were most common in Golden Retrievers (4.86%), Boxers (4.72%), German Shepherds (4.66%) and Labradors (4.27%), and were the most common between age of 8 and 11 years. In 151 dogs (2.03%) multiple tumours of different types were detected at the same time. The results of our study are mostly comparable with the results of other similar studies.

Received: 6 December 2024
Accepted: 23 December 2024

Introduction

Neoplasia is one of the most common diagnoses in dogs today and is the main cause of mortality in dogs. The Veterinary Cancer Society estimates that 25% of dogs will be diagnosed with cancer during their lifespan (1-3). Cancer epidemiology is a growing field of research and emerges as a crucial and expanding area of investigation in veterinary medicine. The limited information available on the incidence, type, location, and behaviour of neoplasms in canine populations underscores the significance of advancing research in this field (1). Effective tracking of cancers in animals is an essential prerequisite for scientific evidence that demonstrates the potential of companion animals to serve as valuable models for human cancer studies and for cancer prevention and control in general (4). Analysis data on the incidence rate of animal tumours is crucial for predicting disease progression and outcome, as well as for identifying possible mechanisms involved in the development of certain tumour types. Such early discoveries can lead to development of more appropriate therapy for patients. Companion animals and humans share the same household and living environment, exposing them to similar risk factors. This close cohabitation makes companion animals valuable indicators for the identification of environmental pollutants that may contribute to oncogenesis, making such studies beneficial for both veterinary and human medicine. Dogs have been shown to be effective sentinels for environmental hazards such as asbestos, dyes, passive smoking, and insecticides (6).

In the field of veterinary medicine, there are only a limited number of (in)active cancer registries that collect information on the incidence of tumours in animals. The California Animal Neoplasm Registry (CANR) is one of the most frequently cited veterinary cancer registries. It began collecting data in 1963 and within three years had collected records from a clearly defined study region in which more

than 30,000 cases of malignant tumours were recorded. Each of these cases was subjected to a histopathological examination (7). In Europe, for example, a study from Switzerland presented by the Swiss Canine Cancer Registry contains data on 121,963 dogs that were histopathologically examined between 1955 and 2008 (8). In a study in Italy, a tumour registry was established for dogs and cats in two provinces of the Veneto region, in which the incidence of tumours was recorded over a three-year period (9). In another study in Italy, all tumour cases in Genoa between 1985 and 2002 were reviewed (10). A study in Croatia presented data on canine tumours diagnosed between 2006 and 2009 (2).

In the past, researchers in Slovenia have conducted several retrospective studies on the occurrence of some specific tumour types in different animal species, e.g. a retrospective study on testicular tumours in dogs (11), a retrospective study on spontaneous tumours and non-neoplastic proliferative lesions in pet degus (12) and a retrospective study on the occurrence of tumours in sheep in Slovenia (13).

Storing and analysing more data over longer periods of time can have a positive impact on the scientific conclusions and estimates of the data, as well as on the reliability of the results, as shown by McAfee et al. (14).

Therefore, we conducted a large retrospective study to create a registry of canine tumours diagnosed in Slovenia over 20 years (period 2000 to 2020) and to analyse the data on the incidence of tumours and some epidemiological characteristics.

Materials and methods

The retrospective study was done at the Institute of Pathology, Wild Animals, Fish and Bees of Veterinary Faculty, University of Ljubljana. The study analysed data on canine tumours diagnosed in over 20 years (from 1st January 2000 to 31st December 2020). The samples analysed included biopsies sent in by veterinarians and samples collected at the routine necropsies performed at the institute upon the request of pet owners or veterinarians.

Samples were routinely prepared for histopathological examination, fixed in 10% buffered formalin, embedded in paraffin, sectioned at a thickness of 4 µm and stained with haematoxylin and eosin. For poorly differentiated tumours, when the owner or veterinarian has agreed, additional histochemical and immunohistochemical staining was performed according to the manufacturer's instructions and in accordance with the protocols validated by the Institute.

In order to collect the data from the archive, a data collection form was created with the following information: sex, age, breed, region of residence in Slovenia, organ system in which the tumour was diagnosed, biological behaviour of the tumour and specific type of tumour.

To evaluate the distribution of tumours by age of the dog, we divided the dogs into different age groups of two years as follows: 0-1.99 years, 2-3.99 years, 4-5.99 years, etc.

For the classification of breeds, The Fédération Cynologique Internationale (FCI) classification was used.

To determine the incidence rate of tumours in dogs, we used data from the Central Pet Animals Database of the Administration for Food Safety, Veterinary Sector, and Plant Protection. The dataset included information on registered dogs from 2008 (when the registry was established) to 2020, with the calculation of the incidence rate based on the number of active (live) dogs on June 1st of each year.

The registry provides also data on the number of registered dogs by sex, breed and municipality of residence.

To calculate the incidence rate of tumours, we used the following formula:

$$\text{Incidence rate of tumours} = \frac{\text{Number of diagnosed tumours in X year}}{\text{Population of dogs in X year}} \times 100{,}000$$

This formula was used to calculate the overall incidence rate of tumours in dogs as well as the incidence of tumours in males and females, the incidence of benign and malignant tumours, and the incidence of tumours in selected dog breeds.

In order to evaluate the influence of the place of residence on the occurrence of tumours, the dogs were divided into 12 statistical regions of Slovenia: Mura, Drava, Carinthia, Savinja, Central Sava, Lower Sava, Southeast Slovenia, Littoral–Inner Carniola, Central Slovenia, Upper Carniola, Gorizia and Coastal–Karst region (15).

The tumours were assigned to one of the following organ systems: 1) skin and subcutaneous tissue (including perianal glands), 2) mammary gland, 3) genital system, 4) haemolymphatic system (including thymus, lymph nodes, spleen and bone marrow), 5) endocrine and exocrine glands (including anal sacs), 6) bones and joints, 7) respiratory system, 8) alimentary system (including oral cavity), 9) sensory system (including eyes and ears), 10) urinary system, 11) hepatobiliary system, 12) cardiovascular system, 13) nervous system, and 14) muscular system.

In addition, we categorised the dogs according to the number of tumours diagnosed at the same time. The category: "solitary tumour" was applied to dogs with one tumour and "multiple tumours" to dogs with two or more tumours of different histological types. If biologically identical tumours were found at different anatomical sites in the same dog, the case was classified as a solitary tumour.

The biological behaviour of the tumours was indicated as benign, malignant, or tumour of unspecified biological behaviour (granulosa cell tumour, theca cell tumour, Leydig cell tumour, seminoma and Sertoli tumour).

The tumours were classified according to the World Health Organization's classifications of tumours of domestic animals.

The collected data were analysed using Microsoft Office Excel and Python. The Matplotlib and Plotly packages were used for visualisation, while the SciPy package was used for statistics. The Chi-square test, with $\alpha = 0.05$, was used to evaluate the relationships between tumour type and the sex of the dogs, tumour type and dog breed, and tumour type and the age group of the dogs.

Results

Detection rate and incidence of tumours

In the period from 1st January 2000 to 31st December 2020, a total of 15,584 submissions were accepted at the Institute and 7,574 tumours were diagnosed in 7,423 dogs, representing 48.60% of the samples submitted (Figure 1).

Between 2008 and 2020, the average incidence rate of tumours was 197 cases per 100,000 dogs per year. The incidence rate was relatively stable until 2014, when it began to rise sharply, peaked in 2017, and then declined slightly (Figure 2).

Biological behaviour of the tumours

Of all tumours, malignant tumours were in the majority with 4454 (58.81%) of all tumours, benign tumours were diagnosed in 2815 (37.17%) samples, and the remaining 305 tumours (4.02%) were classified as tumours of unspecified biological behaviour. The percentage of malignant tumours was statistically significantly higher than the percentage of benign tumours and tumours of unspecified biological behaviour ($p < 0.01$).

Benign tumours were reported with an incidence rate of 75 cases per 100,000 dogs per year, while malignant tumours were more common with an incidence rate of 115 cases per 100,000 dogs per year. Tumours of unspecified biological behaviour had an incidence rate of 34 cases per 100,000 dogs per year.

Tumour types

The most common tumour was the mast cell tumour (1073 cases; 14.17% of all tumours), followed by mammary (adeno)carcinoma (759 cases; 10.02%), cutaneous histiocytoma (556 cases; 7.34%), cutaneous/subcutaneous lipoma (524 cases; 6.92%), lymphoma (456 cases; 6.02%),

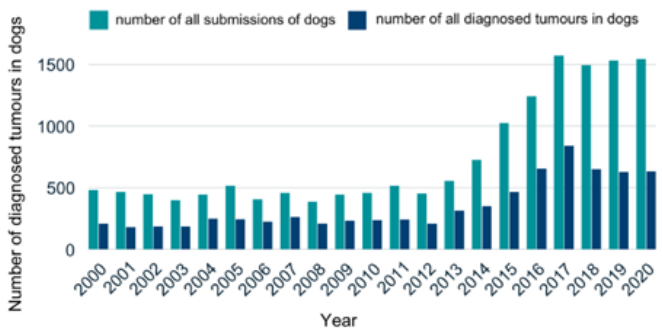


Figure 1: Data on the number of all submissions of dogs in the period 2000-2020 and the number of diagnosed tumours in dogs

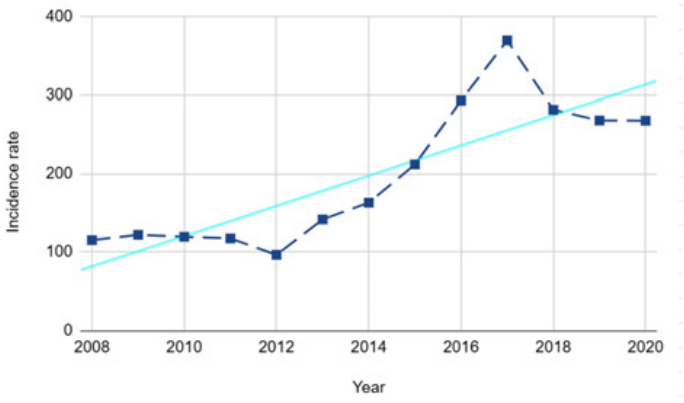


Figure 2: The incidence rate shows the number of tumours per 100,000 dogs per year for the years 2008 to 2020 (blue dashed line). The light blue line represents the linear trend for the incidence of canine tumours in Slovenia

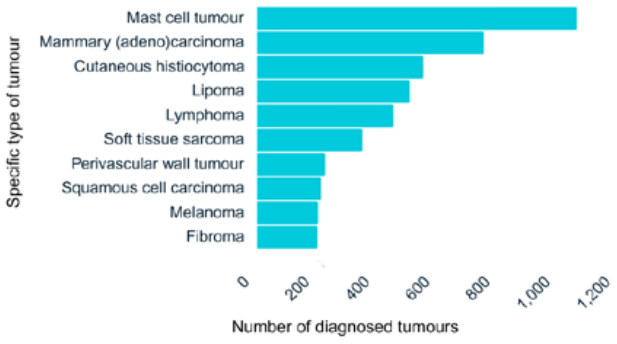


Figure 3: The most frequently diagnosed canine tumours in Slovenia in the period 2000-2020

soft tissue sarcoma (363 cases; 4.79%), perivascular wall tumour (229 cases; 3.02%), squamous cell carcinoma (213 cases; 2.81%), melanoma (208 cases; 2.75%) and fibroma (204 cases; 2.69%) (Figure 3).

Organ systems with the tumours

The three most common organ systems with tumours were the skin and subcutaneous tissue, the mammary glands and the haemolymphatic system. Tumours at these sites accounted 78.41% of all diagnosed tumour cases. The skin and subcutaneous tissue accounted for 4371 cases

(57.71%), followed by the mammary gland (1066 cases; 14.07%) and the haemolymphatic system (502 cases; 6.63%). The least frequent tumours were those of the cardiovascular system (34 cases; 0.46%), nervous system (3 cases; 0.04%) and muscular system (1 case; 0.01%).

The five most common tumour types of the skin and subcutaneous tissue were mast cell tumours (1049 cases; 24.00% of all skin and subcutaneous tumours), cutaneous histiocytomas (537 cases; 12.29%), cutaneous/subcutaneous lipomas (505 cases; 11.55%), soft tissue sarcomas (264 cases; 6.04%) and perivascular wall tumours (225 cases; 5.15%).

The five most common tumour types of the mammary gland were mammary (adeno)carcinoma (758 cases; 71.11% of all mammary tumours), mammary adenoma (151 cases; 14.17%), mammary mixed tumour (78 cases; 7.32%), fibroadenoma (16 cases; 1.50%) and squamous cell carcinoma (12 cases; 1.14%).

The most common tumour of the haemolymphatic system was lymphoma, which accounted for three quarters of all tumours of this system (371 cases; 73.90% of all haemolymphatic tumours), followed by hemangiosarcoma (80 cases; 15.94%), while the other tumour types were much rarer (Figure 4).

Figure 4: The frequency of tumour occurrence in different organ systems and the most common tumour types for each organ system

The sex of dogs with tumours

Information on sex was available for 7413 dogs (99.87%). Tumours were diagnosed in 3576 (48.17%) male dogs and 3837 (51.69%) female dogs.

In terms of sex differences, the incidence rate was 155 cases per 100,000 in male dogs and 262 cases per 100,000 in female dogs.

In female dogs mammary (adeno)carcinomas were most frequently diagnosed (745 cases; 19.42% of all female dog tumours), followed by mast cell tumours (592 cases; 15.43%), cutaneous/subcutaneous lipomas (327 cases; 8.52%), lymphomas (219 cases; 5.71%) and cutaneous histiocytomas (213 cases; 5.55%). Mammary (adeno)carcinoma, mast cell tumour, lipoma and mammary gland adenoma were statistically significantly more frequent in females than in males ($p < 0.01$) (Figure 5).

The most frequently diagnosed tumours in males were mast cell tumours (480 cases; 13.42% of all male dog tumours), cutaneous histiocytomas (340 cases; 9.51%), lymphomas (237 cases; 6.63%), cutaneous/subcutaneous lipomas (197 cases; 5.51%), and soft tissue sarcomas (195 cases; 5.45%) (Figure 6). The statistical analysis showed that cutaneous histiocytomas, adenomas and (adeno)carcinomas of the perianal gland and melanomas occurred statistically significantly more often in male dogs ($p< 0.01$).

The organ systems most frequently affected by tumours in female dogs included the skin and subcutaneous tissue (1994 cases; 51.97% of all female dog tumours), followed by the mammary gland (1049 cases; 27.34%), the alimentary system (315 cases; 8.21%), the haemolymphatic system (235 cases; 6.12%) and genital system (86 cases; 2.24%).

The organ system most frequently affected by tumours in male dogs is the skin and subcutaneous tissue (2371 cases; 66.30% of all male dog tumours), followed by the alimentary system (376 cases; 10.51%), the reproductive system (302 cases; 8.45%), the haemolymphatic system (264 cases; 7.38%) and the sensory system (105 cases; 2.94%).

Breeds of dogs

Information on the dog breed was available for 5460 (73.56%). Tumours were diagnosed in 459 different dog breeds, being most common in Golden Retrievers (361 dogs; 4.86%), Boxers (350 dogs; 4.72%), German Shepherds (346 dogs; 4.66%), Labrador Retrievers (317 dogs; 4.27%), Cocker Spaniels (293 dogs; 3.95%), Crossbreeds (234 dogs; 3.15%), Bernese Mountain Dogs (171 dogs; 2.30%), Maltese (141 dogs; 1.90%), American Staffordshire Terriers (123 dogs; 1.66%) and French Bulldogs (113 dogs; 1.52%) (Figure 7).

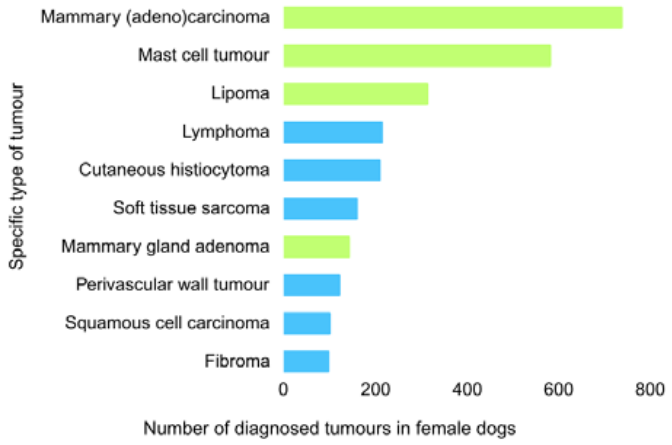


Figure 5: The most frequently diagnosed tumours in female dogs. Green coloured bars indicate a statistically significant association between the sex and the tumour type ($p< 0.01$)

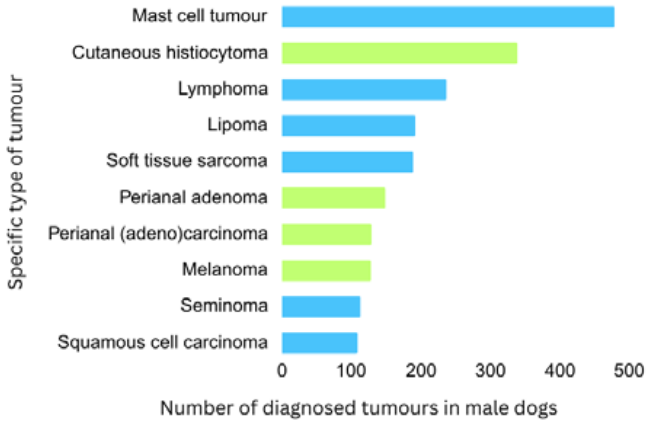


Figure 6: The most frequently diagnosed tumours in male dogs. Green coloured bars indicate a statistically significant association between the sex and the tumour type ($p< 0.01$)

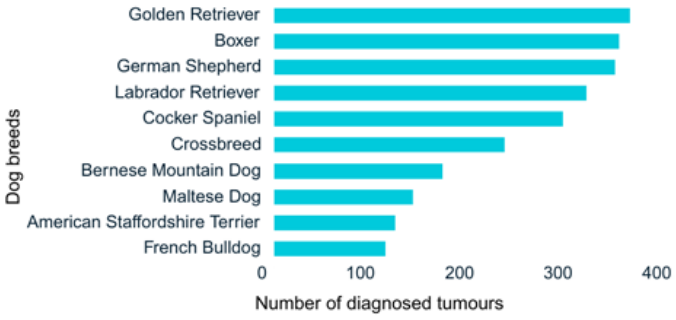


Figure 7: Number of cases of canine tumours in the dog breeds in which the tumours were most frequently diagnosed

The incidence rates of diagnosed tumours were calculated for the ten most common dog breeds. Boxers had the highest incidence rate (924 per 100,000 dogs), followed by French Bulldogs (544), English Cocker Spaniels (479), American Staffordshire Terriers (414), Golden Retrievers (303), Labrador Retrievers (281), Bernese Mountain Dogs

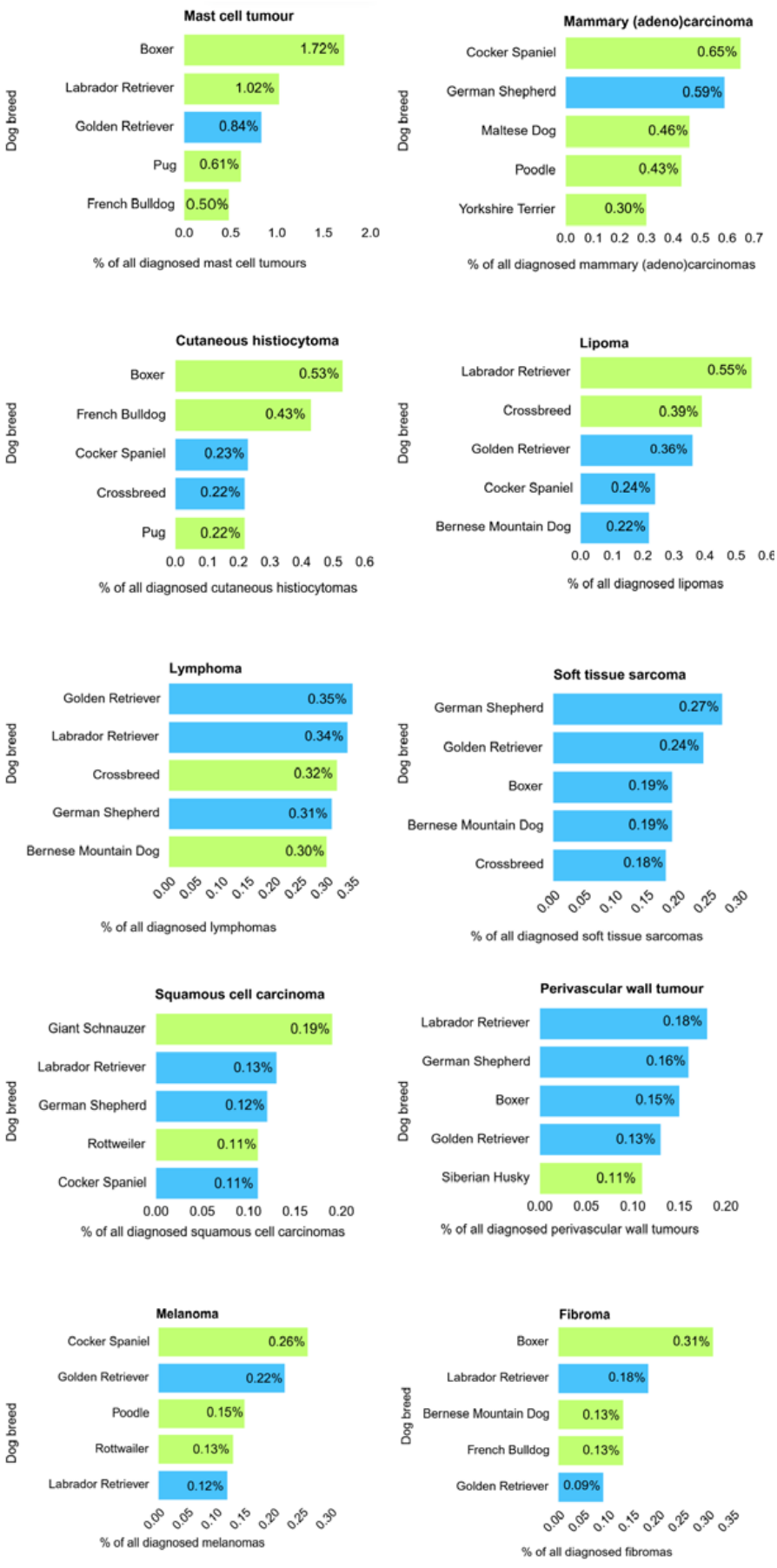


Figure 8: The most common dog breeds diagnosed with the 10 most common tumours. Green coloured bars indicate a statistically significant association between the occurrence of the tumour and the dog breed (p=0.05).

(269), Crossbreeds (242), Maltese Dogs (165), and German Shepherds (81).

Mast cell tumours occurred most frequently in the Boxers (128 cases; 12.04% of all mast cell tumours), followed by Labrador Retriever (76 cases; 7.15% of all mast cell tumours) and Golden Retriever (62 cases; 5.83% of all mast cell tumours).

Mammary (adeno)carcinomas occurred most frequently in Cocker Spaniels (48 cases; 6.37% of all mammary (adeno) carcinomas), German Shepherds (44 cases; 5.84%) and Maltese (34 cases; 4.51%).

Cutaneous histiocytomas were most frequently observed in Boxers (39 cases; 7.04% of all cutaneous histiocytomas), Bulldogs (30 cases; 5.42%) and Cocker Spaniels (17 cases; 3.07%). There was a significant association between some breeds and certain tumour types (p=0.05) (Figure 8).

The age of dogs with tumours

Information on the age of the dogs with tumours was available for 6723 dogs (90.57%). The age of the dogs with tumours ranged from 2 months to 22 years. Most tumours were diagnosed between 8 and 11 years (3118 cases; 42.00%), less frequently between 6 and 7 years (1221 cases; 16.45%) and between 12 and 13 years (789 cases; 10.63%). The average age of a dog with the tumour was 8.26 ± 3.28 years (Figure 9).

In dogs up to and including 1 year of age, cutaneous histiocytomas were by far the most common, accounting for 65.74% of all tumours, while the most common tumours in dogs aged 8 to 11 years were mammary (adeno)carcinomas and mast cell tumours, which accounted for 12.40% to 14.75% of tumours diagnosed in these age groups (Table 1).

There was a statistically significant association between the age group of the dog and certain tumour types (p<0.01). In dogs under 4 years old, cutaneous histiocytomas and papillomas were statistically significantly more common, mast cell tumours were common between 4 and 9.99 years, lipomas between 6 and 9.99 years of age, and lymphomas between 4 and 5.99 years. Mammary (adeno)carcinomas occurred statistically significantly more frequently in dogs aged 8 to 11.99 years (p<0.01) (Table 1).

The age at which the tumours occurred in males and females was similar. The average age of the male dogs with tumours was 8.17 ± 3.38 years, which was only 2.16 months lower than the average age of the female dogs (8.35 ± 3.18 years).

Table 1. The most frequently diagnosed tumours by age group and sex

Age group (years)	The sex of dogs (number of cases; %)	The most frequently diagnosed tumour types	Number of cases of all specific tumour type diagnosed (% of all tumours in the age group)
0-1.99	Male (154; 55.00%) Female (125; 44.64%)	cutaneous histiocytoma* papilloma* mast cell tumour	184 (65.74%) 20 (7.14%) 13 (4.64%)
2-3.99	Male (196; 56.81%) Female (149; 50.35 %)	cutaneous histiocytoma* mast cell tumour papilloma*	115 (33.33%) 54 (15.65%) 20 (8.41%)
4-5.99	Male (356; 49.51%) Female (362; 50.35%)	mast cell tumour* cutaneous histiocytoma lymphoma*	148 (20.58%) 85 (11.82%) 79 (10.99%)
6-7.99	Male (542; 44.39%) Female (678; 55.53%)	mast cell tumour* mammary (adeno)carcinoma lipoma*	248 (20.31%) 109 (8.93%) 104 (8.52%)
8-9.99	Male (718; 46.44%) Female (827; 53.49%)	mast cell tumour* mammary (adeno)carcinoma* lipoma*	228 (14.75%) 192 (12.42%) 149 (9.64%)
10-11.99	Male (764; 48.60%) Female (806; 51.27%)	mammary (adeno)carcinoma* mast cell tumour lipoma	203 (12.91%) 195 (12.40%) 108 (6.87%)
12-13.99	Male (366; 46.39%) Female (423; 53.61%)	mammary (adeno)carcinoma mast cell tumour melanoma	96 (12.17%) 68 (8.62%) 53 (6.72%)

* Statistically significant association between the age of the dog and tumours type

The average age of male dogs with benign tumours was 7.07 ± 3.62 years, while the average age of female dogs with tumours was 7.50 ± 3.45 years.

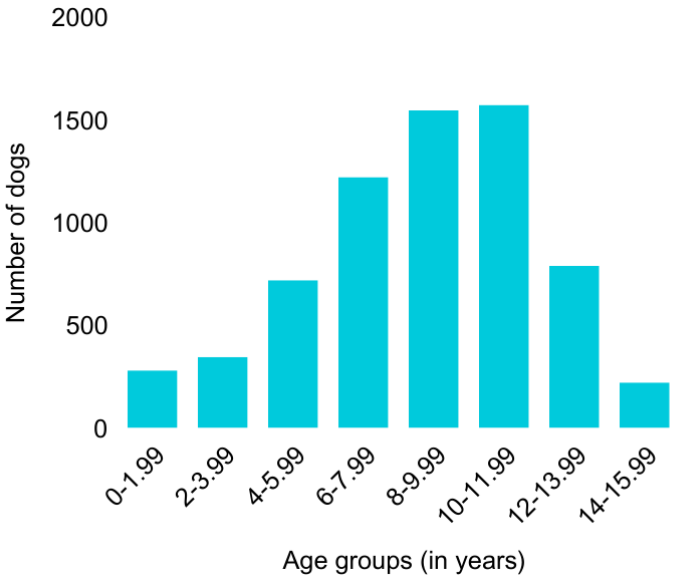


Figure 9: The number of cases of canine tumours according to age groups

The age at which malignant tumours occurred was slightly higher than the age at which benign tumours occurred. The average age of male dogs with malignant tumours was 8.68 ± 3.04 years, while the average age of female dogs was 8.84 ± 2.91 years. The tumours with unspecified biological behaviour occurred later in life in both sexes. The average age of the female dogs with tumours of unspecified biological behaviour was 10.17 ± 2.63 years, while the average age of the male dogs was 7.87 ± 3.29 years.

Region of residence of dogs with tumours

The dogs with tumours came from all 12 statistical regions of Slovenia. The data about residence were not available for 968 (13.04%) dogs. Most dogs, namely 2907 (39.16%), were from the Central Slovenia region, followed by the Drava region with 897 (12.08%) dogs. The regions of Upper Carniola (671; 9.04%), Savinja (471; 6.35%), Gorizia (376; 5.07%), Coastal- Karst region (353; 4.76%) and Southeast Slovenia (253; 3.41%) followed closely behind. Less than 200 dogs were diagnosed with tumours in the Central Sava, Lower Sava region and in the Littoral-Inner Carnolia region, while less than 100 dogs with tumours were recorded in the Mura and Carinthia region (Figure 10).

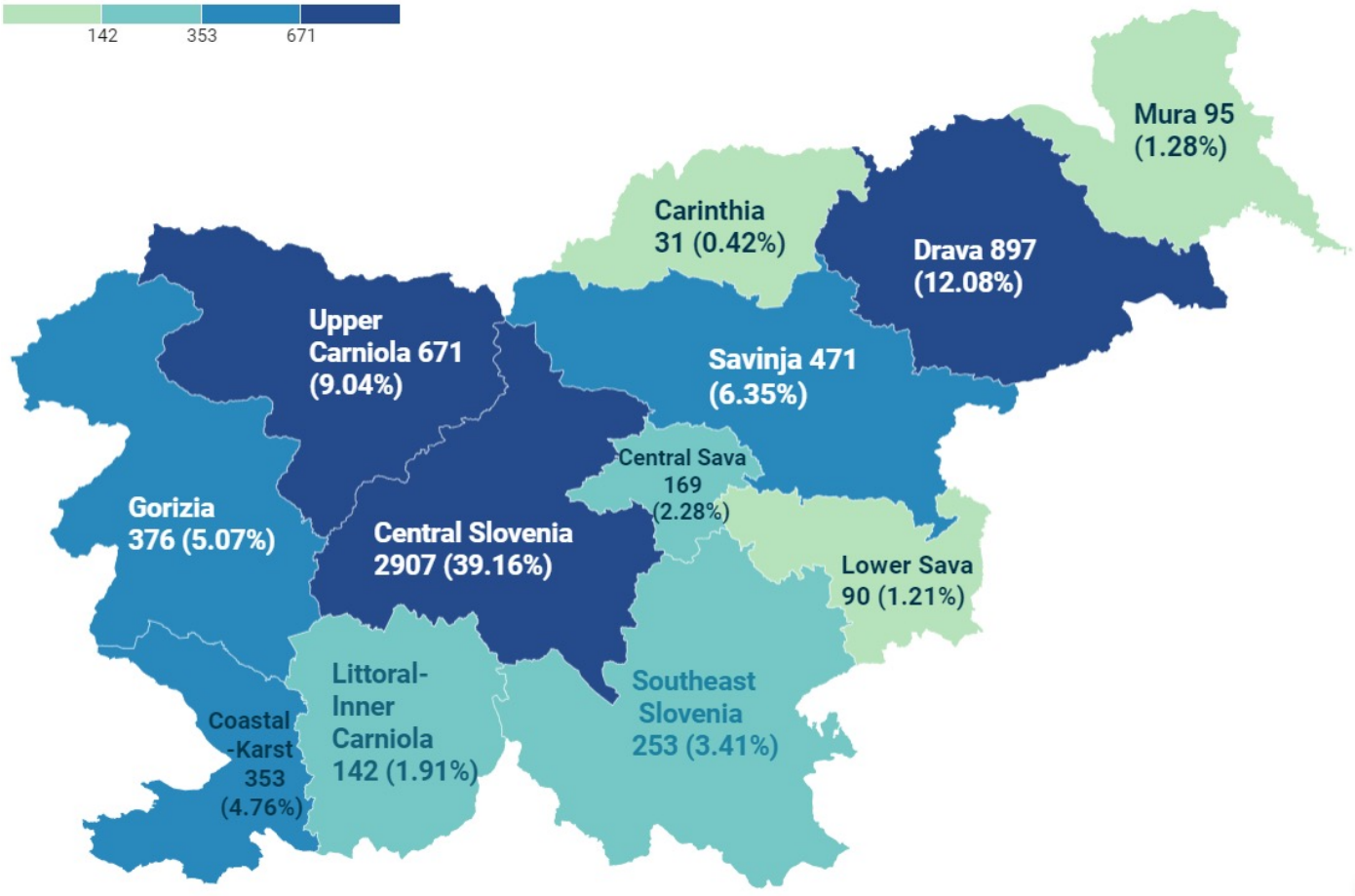


Figure 10: Distribution of tumour cases by region of residence

Temporal trend in the occurrence of the tumours

In the first five years of the observation period, mammary (adeno)carcinoma was consistently the most common tumour, accounting for around 15.00% of all cases per year. In 2005, however, there was a notable shift: the mast cell tumour showed an upward trend, while mammary (adeno) carcinoma declined. In 2008, mammary (adeno)carcinoma recorded a significant increase in cases again, while mast cell tumours continued to rise until 2016 and then reached a plateau. Cutaneous/subcutaneous lipomas and cutaneous histiocytomas also increased steadily between 2008 and 2015 (Figure 11).

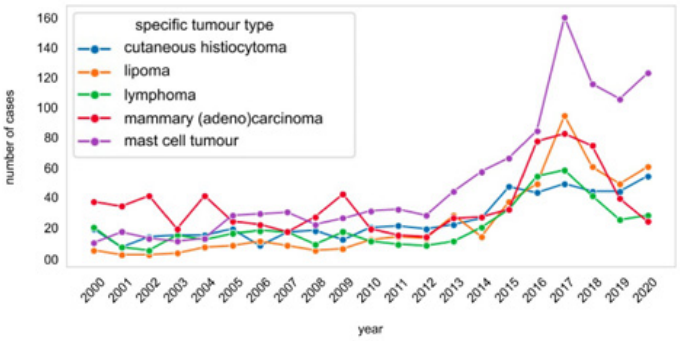


Figure 11: The temporal trends in the occurrence of the five most common canine tumours

Synchronous occurrence of multiple tumours

Multiple tumours within the same organ system or in different organ systems were diagnosed in 151 dogs (2.03% of all dogs). These were 76 female dogs (50.33% of dogs with multiple tumours) and 74 (49.00%) male dogs, while one dog (0.67%) had no sex information. The average age of the dogs with multiple synchronous tumours was 9.28 ± 3.64 years. The most common combinations of multiple tumours were melanoma and soft tissue sarcoma (6 dogs; 0.08% of all dogs with tumours) and seminoma and Leydig cell tumour (6 dogs; 0.08%), followed by mast cell tumour and lipoma (5 dogs; 0.07%) and seminoma and Sertoli cell tumour (5 dogs; 0.07%) (Figure 12).

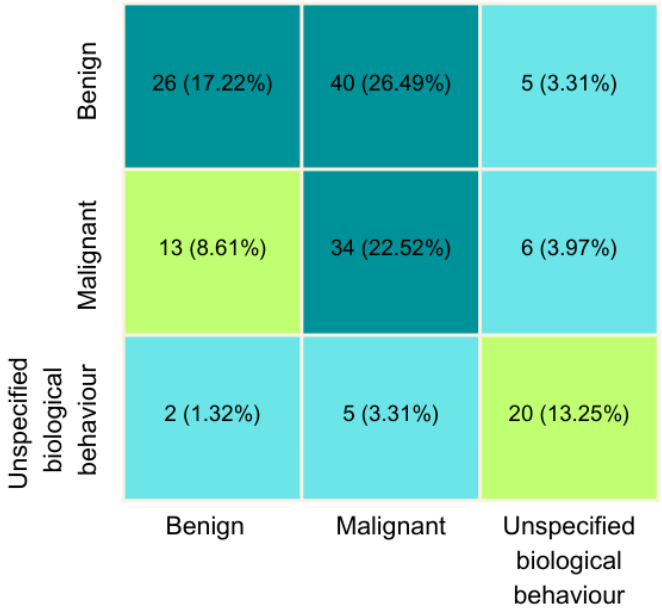


Figure 12: Number and frequency of different combinations of the biological behaviour of multiple synchronous tumours

Discussion

During the study period, 15,584 submissions for necropsy and histopathological examination were accepted at the Institute, and 48.60% of the submissions were diagnosed as tumours. The number of samples submitted, and tumours diagnosed was roughly the same until 2013, after which the number of samples received increased rapidly within a few years, almost doubling. We attribute this sudden increase in submissions to changes in the work of the histopathology laboratory, where we began to proactively approach veterinarians with changes in reporting, faster results, regular biennial training on the importance of histopathology, cytopathology and immunohistochemistry, a strong emphasis on teaching students, and our publications on these topics.

Merlo et al. (10) reported a stable number of samples during the 18-year study period, while Grüntzig et al. (8) described that the incidence rate increased from 13 cases per 100,000 dogs in 1955 to 695 cases in 2008. The increase was attributed to several causes - the constant growth of the dog population, the availability of diagnostic methods, longer life expectancy, the changing role of dogs in society, a higher standard of living, a greater number of veterinarians and possibly environmental factors (8) - all factors that probably also contributed to the increase in cases in Slovenia. Other authors investigated the incidence of tumours over a shorter period. However, Vascellari et al. (9) reported an increasing number of submissions during a three-year pilot project to establish a tumour registry for dogs and cats, confirming our findings.

Over a 12-year period, the average annual incidence rate of tumours in canine populations was calculated to be 197 cases per 100,000 dogs. The highest incidence rate was recorded in 2017 with 369 cases per 100,000 dogs per year, and the lowest was documented in 2008 with 115 cases per 100,000 dogs per year.

Other countries have reported different incidence rates. In northern Italy, the incidence was 282 cases per 100,000 dogs (9), similar to ours, but a much higher incidence was reported - 500 cases per 100,000 dogs per year in Sweden (17) and about 748 cases per 100,000 dogs in the UK (18). The lowest incidence rate in 2008 can be attributed to the establishment of a Central Pet Animals Database during that year. The establishment of such a registry may have

led to under-reporting, as comprehensive data collection typically requires several years (19).

In terms of biological behaviour, malignant tumours predominated in Slovenia, found in 58.81% of the samples. Similar results were also found by other authors (2,5), while in Swiss Registry (8) and Registry of Genoa (Italy) (10), the malignant tumours represented close to half of the tumours. We believe that the higher proportion of malignant tumours in our study is probably due to the individual decisions of the veterinarians to perform a histopathological examination when malignancy is suspected and not to send the excision for examination if they consider it to be benign.

The most frequently diagnosed tumours in Slovenia were mast cell tumours (14.17%), mammary (adeno)carcinoma (10.02%) and cutaneous histiocytomas (7.34%). Our results differ slightly from those of other studies in which mammary carcinoma was reported as the most common specific tumour type (2,16,20). In Germany, the most frequently diagnosed tumours were mammary tumours, mast cell tumours and histiocytomas (20), while in Poland the most common skin tumours were mast cell tumours, histiocytomas, and lipomas (5).

Mast cell tumours, the most common tumours in dogs in our study, occur with varying incidence worldwide, and account for between 7% and 27% of skin neoplasms in dogs (21). Mast cell tumours are more common in certain dog breeds, such as the Boxer, Labrador, Golden Retriever (22). In Slovenia, according to Central Pet Animals Database, the number of listed dog breeds has been increasing, so the increase in the number of cases of mast cell tumours in dogs can be partially attributed to a larger population of dogs predisposed to this tumour.

Differences in the practice of spaying bitches across Europe are associated with a higher incidence of mammary gland tumours in countries and regions where spaying is less common, such as southern Europe and Scandinavia. In contrast, a significant reduction in mammary tumours was observed in the United States due to the widespread early practice of ovariohysterectomy (23,24). Unfortunately, our research lacked data on the spaying status of bitches, so we cannot assume any correlation between the number of cases of (adeno)carcinomas and this procedure (24-27).

The organ systems with the most tumours were the skin and subcutaneous tissue (57.71%), the mammary gland (14.07%) and the haemolymphatic system (6.63%). Two of the most common locations with similar representation were also described by most authors of the retrospective studies (2,5,8,9,10,28). However, in all these cited publications the third most frequent location was the genital system (2,9,10,28), while Ciaputa et al. (5) reported the lymphatic system as the third most frequent location.

The highest number of cases of skin/subcutaneous tumours can be explained by the fact that it is a location where the changes can be easily noticed by the owner himself and that these lesions are usually more accessible for sampling than lesions in other locations, e.g. in the thoracic or cranial cavity or in internal organs.

The tumours were diagnosed with similar frequency in male dogs (48.17%) and female dogs (51.69%). The results are similar to the findings of Šoštarić-Zuckermann et al. (2) and Kimura et al. (16). In contrast to the above results, Merlo et al. (10) described an almost threefold higher incidence of all types of cancer in female dogs, which is mainly explained by the high incidence of mammary cancer in female dogs.

Tumours were found most frequently in Golden Retrievers (4.86%), Boxers (4.72%) and German Shepherds (4.66%). Considering the number of each dog breed in Slovenia, our analysis revealed that Boxers had the highest tumour incidence, with 924 tumours per 100,000 dogs per year, followed by French Bulldogs and Cocker Spaniels. These results are consistent with previous studies (2,18) reporting similar findings. A study conducted in Germany indicated an increased risk of neoplasia in certain breeds, namely Beagles, Magyar Vizslas, Boxers, Schnauzers, Spaniels, French Bulldogs and Golden Retrievers (20). Contrary, Ciaputa et al. (5) described that tumours in Poland were most common in Crossbreeds (30.64% of all tumours), followed by Labradors and German Shepherds. Vascellari et al. (9) which found the opposite, that purebred dogs have a higher risk of developing malignant tumours than crossbreeds.

In our study, mast cell tumours occurred most frequently in Boxers (12.04%), Labradors (7.15%) and Golden Retrievers (5.83%). More frequent occurrence in these three breeds was also shown by data from Croatia (2), and Brazil where the highest prevalence of mast cell tumours was described in Boxers (16).

Aupperle-Lellbach et al. (20) found a higher risk of cutaneous histiocytomas occurrence in French Bulldogs, Boxers and Pugs than in Crossbreed dogs. In our study, Boxers had the highest occurrence of cutaneous histiocytomas (7.04%), however crossbreeds with this specific tumour type were relatively common (0.22%). Yet, we must emphasise that the reported numbers of tumour cases are very low.

In one of the most recent retrospective studies on tumours in dogs, an increased risk of developing mammary tumours was described in Yorkshire Terriers, Chihuahuas, and Spaniels (20). In Slovenia, mammary (adeno)carcinoma was most common in Cocker Spaniels (6.37% of all mammary gland tumours), which is in accordance with data on breed predispositions described in other studies (29,30). We believe that the results regarding the frequency of occurrence of tumours or certain tumour types between

different authors may differ due to several factors. One of them is that the popularity of different breeds varies greatly between countries and changes over time, and there are also different proportions of crossbreeds and purebred dogs in different regions and countries.

Most tumours were diagnosed between 8 and 11 years (42.00%), 6 and 7 years (16.45%) and between 12 and 13 years (10.63%). In other studies (5,8,9,10,16), the authors used different age groups for the analysis, however, the results are similar and align with the findings on tumour occurrence in dogs in Slovenia.

In dogs up to and including one year of age, cutaneous histiocytomas were by far the most common, accounting for 65.74% of all tumours in this age group. Cutaneous histiocytomas were statistically significantly more common between 2 and 4 years of age. The most common tumours in dogs aged 8 to 11 years were mammary (adeno)carcinomas and mast cell tumours. According to data from the literature, the average age of dogs with mammary gland carcinomas is between 8 and 10 years (24), and mast cell tumours most often occur in middle-aged and older dogs, with an average age of 9 years (22).

In a small number of dogs (2.03%) we have found two or more types of tumours at the same time. The question raised by the finding of multiple tumours is whether the presence of a specific type of tumour implies a predisposition to another type of tumour. While literature suggests significant associations, contradictions also exist. It is not known for certain that the patterns of occurrence of these multiple tumours are governed by anything than coincidence (31). In our analysis, 17.22% of cases revealed two or more benign tumours, while 22.52% exhibited multiple malignant tumours, and 26.49% were combinations of malignant and benign tumours. Similarly, a study conducted in Poland (5) by Ciaputa et al., reported comparable findings, 25.71% of cases involved two or more malignant tumours, and 23.72% were all benign tumours.

In the first five years of the observation, the most common tumour was mammary (adeno)carcinoma. A notable shift occurred in 2005, when the number of mammary (adeno) carcinomas occurrences fell. We believe that spaying has been performed more frequently in the last ten years than in the past, which has influenced the occurrence of mammary gland tumours. In our research, unfortunately, we did not have available data on the spaying status of bitches, so we could not analyse the number of cases of mammary (adeno)carcinomas and the time trend of their occurrence in connection with spaying.

Most dogs with diagnosed tumours were from the Central Slovenia (39.16%). The results can be explained by the fact that this region has the largest number of inhabitants, and according to data from Central Pet Animals Database, also has the largest number of registered dogs.

This study has several potential limitations, mainly due to the retrospective nature of the study and the limited data sources. The number of tumour cases submitted and diagnosed only provides an estimate of the actual incidence of this pathology in Slovenia. Some of the tumour cases remain untreated by the veterinarians, while others are excised but not examined histopathologically. In some cases, only cytopathological analysis is performed or samples are sent to foreign laboratories, which further limits the amount of data. In addition, necropsies are only performed in a limited number of cases, mostly at the request of animal owners or veterinarians and especially when the diagnosis is unclear. Cases with a confirmed diagnosis prior to death or euthanasia are not usually submitted for necropsy. These factors limit the completeness and representativeness of the results of the study. The accompanying data on sex, age, breed, region of residence in Slovenia and organ system in which the tumour was diagnosed are often insufficient, which reduces the input data available for the analyses or made certain analyses, such as the correlation between the occurrence of certain tumours and spaying status, impossible.

Future work should focus on prospective studies with more comprehensive and standardised data collection, including detailed information on breed, breed size and potential environmental risk factors, to gain a deeper understanding of the epidemiology of canine tumours and improve prevention, diagnosis and treatment strategies.

In conclusion, we would like to emphasize the importance of establishing tumour registries for companion animals. Although rodent models are useful for preclinical studies (32), companion animals, because they share several naturally occurring tumours with humans, often allow faster translation from the laboratory to the bedside.

Acknowledgments

The authors would like to thank Dr. Urša Kos and Maja Vilfan, M. Sc. from the Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection for data from the Central pet animals database.

This study was conducted as part of a bilateral project BI-ME/21-22-017 entitled "Incidence and tumour types of domestic, exotic and wild animals in Slovenia and Montenegro" and funded by the Slovenian Research Agency under grant number P4-0092.

References

1. Bronden LB, Flagstad A, Kristensen AT. Veterinary cancer registry in companion animal cancer; a review. Vet Comp Oncol 2007; 5(3): 133–44. doi: 10.1111/j.1476-5829.2007.00126.x

2. Šoštarić-Zuckermann IC, Severin K, Hohšteter M, et al. Incidence and types of canine tumours in Croatia. Vet Arh 2013; 83(1): 31–45.

3. Sarver AL, Makielski KM, DePauw TA, Schulte AJ, Modiano JF. Increased risk of cancer in dogs and humans: A consequence of recent extension of lifespan beyond evolutionarily determined limitations? Aging Cancer 2022; 3(1): 3–19. doi: 10.1002/aac2.12046

4. Pinello K, Pires I, Castro AF, et al. Cross Species analysis and comparison of tumors in dogs and cats, by age, sex, topography and main morphologies. Data from Vet-OncoNet. Vet Sci 2022; 9(4): 167. doi: 10.3390/vetsci9040167

5. Ciaputa R, Gach J, Baranowski K, et al. Prevalence of tumours and tumour-like lesions in domestic and exotic animals from Lower Silesia and its surrounds in Poland in 2014–2017. J Vet Res 2022; 66(3): 427–34. doi: 10.2478/jvetres-2022-0036

6. Misdorp W. Veterinary cancer epidemiology. Vet Q 1996; 18(1): 32–6. doi: 10.1080/01652176.1996.9694610

7. Dorn CR, Taylor DO, Schneider R, Hibbard HH, Klauber MR. Survey of animal neoplasms in Alameda and Contra Costa Counties, California. II. Cancer morbidity in dogs and cats from Alameda County. J Natl Cancer Inst 1968; 40(2): 307–18.

8. Grüntzig K, Graf R, Hässig M, et al. The Swiss canine cancer registry: a retrospective study on the occurrence of tumours in dogs in Switzerland from 1955 to 2008. J Comp Pathol 2015; 152(2/3): 161–71. doi: 10.1016/j.jcpa.2015.02.005

9. Vascellari M, Baioni E, Ru G, Carminato A, Mutinelli F. Animal tumour registry of two provinces in northern Italy: incidence of spontaneous tumours in dogs and cats. BMC Vet Res 2009; 5: 39. doi: 10.1186/1746-6148-5-39

10. Merlo DF, Rossi L, Pellegrino C, et al. Cancer incidence in pet dogs: findings of the animal tumour registry of Genoa, Italy. J Vet Intern Med 2008; 22(4): 976–84. doi: 10.1111/j.1939-1676.2008.0133.x

11. Švara T, Gombač M, Pogorevc E, Plavec T, Zrimšek P, Pogačnik M. A retrospective study of canine testicular tumours in Slovenia. Slov Vet Res 2014; 51(2): 81–8.

12. Švara T, Gombač M, Poli A, Račnik J, Zadravec M. Spontaneous tumours and non-neoplastic proliferative lesions in pet degus (Octodon degus). Vet Sci 2020; 7(1): 32. doi: 10.3390/vetsci7010032

13. Švara T, Gombač M, Pogačnik M. Retrospective study on occurrence of tumours in sheep in Slovenia. Slov Vet Res 2011; 48(Suppl. 13): 293–5.

14. McAfee A, Brynjolfsson E, Davenport TH, Patil D J, Barton D. Big data: the management revolution. Harvard Bus Rev 2012; 90(10): 61–7.

15. SI-STAT Podatkovna baza. Ljubljana: Statistični urad Republike Slovenije, 2024. https://www.stat.si/ StatWeb/news/Index/7323 (20. 12. 2024).

16. Kimura KC, Gárate AP, Dagli MLZ. Retrospective study of neoplasms in domestic animals: A survey between 1993 and 2002 of the service of animal pathology, department of pathology, school of veterinary medicine and animal science, University of Sao Paulo, Southeast Brazil. Braz J Vet Pathol 2012; 5(2): 60–9.

17. Egenvall A, Nødtvedt A, Roepstorff L, Bonnett B. Integrating databases for research on health and performance in small animals and horses in the Nordic countries. Acta Vet Scand 2011; 53(Suppl. 1): S4. doi: 10.1186/1751-0147-53-S1-S4

18. Dobson JM, Samuel S, Milstein H, Rogers K, Wood JLN. Canine neoplasia in the UK: estimates of incidence rates from a population of insured dogs. J Small Anim Pract 2002; 43(6): 240–6. doi: 10.1111/j.1748-5827.2002.tb00066.x

19. Jensen OM, Whelan S. Planning a cancer registry. IARC Sci Publ 1991; 95: 22–8.

20. Aupperle-Lellbach H, Grassinger J M, Floren A, et al. Tumour incidence in dogs in Germany: a retrospective analysis of 109,616 histopathological diagnoses (2014–2019). J Comp Pathol 2022; 198: 33–55. doi: 10.1016/j.jcpa.2022.07.009

21. Artuković B, Medven L, Hohšteter M, et al. Prevalence of cutaneous mast cell sarcoma in dogs in Croatia. Vet Arh 2014; 86(6): 601–14.

22. Meuten DJ, ed. Tumours in domestic animals. 5th ed. Ames: Wiley, 2017.

23. Polton G. Mammary tumours in dogs. Irish Vet J 2009; 62(1): 50–6.

24. Vazquez E, Lipovka Y, Cervantes-Arias A, et al. Canine mammary cancer: state of the art and future perspectives. Animals (Basel) 2023; 13(19): 3147. doi: 10.3390/ani13193147

25. Urfer SR, Kaeberlein M. Desexing dogs: a review of the current literature. Animals (Basel) 2019; 9(12): 1086. doi: 10.3390/ani9121086

26. Lim HY, Im KS, Kim NH, et al. Effects of obesity and obesity-related molecules on canine mammary gland tumors. Vet Pathol 2015; 52(6): 1045–51. doi: 10.1177/0300985815579994

27. Alenza DP, Rutteman GR, Peña L, Beynen AC, Cuesta P. Relation between habitual diet and canine mammary tumors in a case-control study. J Vet Intern Med 1998; 12(3): 132–9. doi: 10.1111/j.1939-1676.1998.tb02108.x

28. Tesi M, Millanta F, Poli A, et al. Role of body condition score and adiponectin expression in the progression of canine mammary carcinomas. Vet Med Sci 2020; 6(3): 265–71. doi: 10.1002/vms3.238

29. Egenvall A, Bonnett BN, Öhagen P, Olson P, Hedhammar Å, von Euler H. Incidence of and survival after mammary tumors in a population of over 80,000 insured female dogs in Sweden from 1995 to 2002. Prev Vet Med 2005; 69(1/2): 109–27. doi: 10.1016/j.prevetmed.2005.01.014

30. Salas Y, Márquez A, Diaz D, Romero L. Epidemiological study of mammary tumors in female dogs diagnosed during the period 2002-2012: a growing animal health problem. PloS One 2015; 10(5): e0127381. doi: 10.1371/journal.pone.0127381

31. Zatloukal J, Lorenzova J, Tichý F, Nečas A, Kecova H, Kohout P. Breed and age as risk factors for canine mammary tumours. Acta Vet Brno 2005; 74(1): 103–9. doi: 10.2754/avb200574010103

32. Theilen GH, eds. Veterinary cancer medicine. Philadelphia: Lea and Febiger, 1979.

Incidenca in tipi tumorjev pri psih v Sloveniji v obdobju 2000-2020: retrospektivna raziskava

T. Nikodinovska, M. Gombač, T. Dolenšek, K. Tekavec, S. Šturm, M. Cvetko, K. Pavlin, D. M. Fras, T. Švara

Izvleček: Namen retrospektivne raziskave je vzpostaviti register tumorjev, diagnosticiranih pri psih v Sloveniji v obdobju 20 let, ter analizirati incidenco tumorjev in nekatere njihove epidemiološke značilnosti. V raziskavi, ki smo jo opravili na Inštitutu za patologijo, divjad, ribe in čebele Veterinarske fakultete Univerze v Ljubljani med letoma 2000 in 2020, smo analizirali rezultate histopatoloških preiskav bioptov psov, ki so jih poslali kliniki, in tumorjev, odvzetih med raztelesbo psov. Malignih tumorjev je bilo več kot benignih (58,1 % vs. 37,17 %), 4,02 % tumorjev je bilo nespecificiranega biološkega obnašanja. Najpogostejši so bili tumorji kože in podkožja (57,83 % vseh tumorjev), mlečne žleze (14,07 % vseh tumorjev) ter hematopoetičnega in limfatičnega sistema (6,63 % vseh tumorjev). Najpogosteje smo diagnosticirali mastocitom (14,17 %), mamarni (adeno)karcinom (10,02 %), kožni histiocitom (7,34 %) in kožni/podkožni lipom (6,92 %). Pri 2,03 % psov smo ugotovili multiple tumorje različnih tipov. 51,69 % psov s tumorji je bilo samic in 48,17 % samcev. Tumorji so bili najpogostejši pri zlatih prinašalcih (4,86 %), bokserjih (4,72 %), nemških ovčarjih (4,66 %) in labradorcih (4,27 %), najpogosteje so bili ugotovljeni med 8. in 11. letom starosti. Rezultati naše raziskave so pretežno primerljivi z rezultati drugih podobnih raziskav.

Ključne besede: pes; tumor; incidenca; starost; pasma; Slovenija

Slov Vet Res
DOI 10.26873/SVR-1797-2024
UDC 575:591.4:616-006.6:623.454.4:636.7
Pages: 41–52

Original Research Article

Transcriptome Analysis and Bioinformatics Characterization of Canine Hemangiosarcoma: Potential Therapeutic Targets

Key words

angiosarcoma;
spleen;
heart;
liver;
comparative oncology;
transcriptomic profiling

Özge Özmen[1]*, Berna Kaya[1], Kardelen Karaman[2]

[1]Ankara University, Faculty of Veterinary Medicine, Department of Genetics, 06110, Altındag, Ankara, Türkiye, [2]Kırıkkale University, Faculty of Veterinary Medicine, Department of Animal Breeding, Prof. Dr. Beşir Atalay Campus, 71450, Yahşihan, Kırıkkale, Türkiye

***Corresponding author:** ozgeozmen@ankara.edu.tr

Abstract: Canine hemangiosarcoma is an aggressive cancer with a poor prognosis. It originates in the cells that line blood vessels and affects various organs, including the spleen, heart, and liver. Despite its rarity, canine hemangiosarcoma presents significant diagnostic and therapeutic challenges. Certain breeds, such as Golden Retrievers, Boxers, and German Shepherds, have a higher susceptibility to Hemangiosarcoma (HSA), suggesting a possible genetic basis for disease susceptibility. However, the exact molecular mechanisms underlying the predisposition of these breeds to HSA are not fully understood. This study aimed to improve our understanding of the molecular mechanisms underlying canine hemangiosarcoma by re-analyzing publicly available RNA sequencing data using bioinformatic techniques in dogs. Our results suggest that the genes *ALB*, *TNNT2*, *VIM* and *CA9* have the potential to be used as novel biomarkers for spleen, heart, and liver HSA in the Golden Retriever breed. Based on our findings, we propose that *STAT3*, *TP53*, *PPARG*, *ATF3*, *CCND1*, and miR-21-5p, miR-92a-3p, and miR-155-5p have the potential to serve as biomarkers for hepatic HSA in Golden Retrievers. In addition, our analysis of splenic HSA datasets from six different dog breeds reveals the expression of breed-specific genes in canine splenic HSA. The identification of these biomarkers enhances our understanding of the molecular mechanisms involved in AS and provides potential targets for therapeutic intervention.

Received: 19 June 2024
Accepted: 2 August 2024

Introduction

Human Angiosarcoma (AS) is a highly aggressive type of cancer that originates from the cells responsible for blood vessel formation. It is characterized by its aggressive behavior and generally poor prognosis. However, there have been few large-scale genomic studies of the disease in humans due to its rarity, accounting for only 0.01% of all cancers. It is challenging to conduct large clinical trials due to their rarity and genetic heterogeneity to establish treatment guidelines for existing regimens, and the development of novel therapies has also proven to be difficult (1). However, hemangiosarcoma (HSA) is a common type of cancer in dogs that affects endothelial cells, which form blood vessels, and shares many similarities with human AS (2). Therefore, it has been suggested as a relevant model for studying the pathophysiology of the disease. In particular, certain dog breeds, such as golden retrievers, are prone to developing HSA, with up to 20% of cases occurring in this breed, providing a large sample size for studying the disease that would otherwise be inaccessible using only human data. HSA is often aggressive and fatal within a few months of diagnosis, resulting in a need for new diagnostic and therapeutic options for patients (2).

Visceral canine HSA is particularly hazardous, with a high probability of recurrence and a poor prognosis due to the advanced stage of the disease at diagnosis, resulting from multifocal growth and metastases. Histopathologically, human AS and canine HSA are identical and emerge from the

same cell of origin. HSA accounts for up to 7% of all malignant tumors in dogs and typically affects the spleen, liver, and the right atrium/auricle of the heart (3, 4). The prognosis for dogs with splenic HSA is extremely unfavorable, with a survival period typically ranging from 10 to 86 days following surgical removal of the tumor without additional treatment. Even when chemotherapy is combined with surgical resection, the survival period extends to only 141 to 179 days (5). Certain breeds, such as Golden Retrievers, Boxers, and German Shepherds, are more prone to HSA, indicating that genetic factors may contribute to the development of the disease (6). Visceral HSA commonly metastasizes to the lungs, liver, mesentery, and omentum and can often present concurrently in the spleen and heart. Cutaneous HSA, which occurs in non-pigmented or light-haired skin, is associated with exposure to UV radiation. Compared to cutaneous HSA, visceral HSA has a poorer prognosis due to local infiltration, primary tumor rupture, and/or metastases (7). Overall, the identification of genetic mutations associated with HSA represents a significant step in understanding and treating this disease. Continued research in this area is necessary to fully comprehend the role of genetics in HSA and to develop effective treatment options.

Certain breeds, such as Golden Retrievers, Boxers, and German Shepherds, have a higher susceptibility to HSA, suggesting a possible genetic basis for disease susceptibility. However, the exact molecular mechanisms underlying the predisposition of these breeds to HSA are not fully understood. The aim of this study is to gain a better understanding of the underlying molecular mechanism of canine HSA at the transcriptomic level. In this study, a bioinformatics approach was used to identify differentially expressed genes (DEGs) in tissue samples obtained from the spleen, liver, and heart of Golden Retriever dogs with HSA. Furthermore, DEG analysis was performed to compare different dog breeds with splenic HSA. Our results suggest that the genes *ALB*, *TNNT2*, *VIM* and *CA9* have the potential to be used as novel biomarkers for splenic, cardiac, and liver HSA in the Golden Retriever breed. In addition, our analysis of splenic HSA datasets from six different dog breeds reveals the expression of breed-specific genes in canine splenic HSA.

Material and methods

RNA-Seq Data Sources

This retrospective study was based on cohort data that included 23 samples of canine HSA. The Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) is the most widely used public functional genomics data repository for high-throughput gene expression data. The gene expression profile dataset, BioProject PRJNA562916, and BioSamples SAMN12659339 - SAMN12659361, generated by Megquier et al. (2), were downloaded from the GEO database and used for bioinformatics analysis. The SRR10355666-SRR10355688 dataset contains 23 samples of canine HSA, and it was used in a bioinformatics analysis to identify differentially expressed genes associated with the disease (Table 1). The data was produced using the Illumina HiSeq 2500.

Bioinformatics Analysis

The FastQ files of the obtained sequence reads were analyzed using a local Galaxy installation (8). Initially, the raw reads underwent quality control checks, and then the adapter was trimmed using Trimmomatic (9). All fragmented reads were mapped to the reference genome (CanFam3.1/canFam3) from UCSC (https://genome.ucsc.edu/index.html) using the HISAT2 tool. The alignments were then assembled into full-length transcripts using the StringTie tool (10). Differential expression analysis was performed using DESeq2 (11).

First, we compared RNA-seq data from Golden Retrievers with three types of HSA tissues (spleen, heart, and liver). The following contrasts were used to compare the experimental groups for the Golden Retriever dog breed: splenic HSA versus liver HSA (SPLNvsLVR), splenic HSA versus heart HSA (SPLNvsHRT), and heart HSA versus liver HSA (HRTvsLVR). Second, splenic HSA RNA-Seq data were compared between six dog breeds: Golden Retriever, German Shepherd, Portuguese Water Dog, American Staffordshire Terrier, Parsons Russell Terrier, and Mix (Labrador Retriever). Table 2 lists the groups created to compare the experimental groups and their abbreviations.

Differentially expressed genes were identified using fold change and adjusted p-values (FDR). Genes with FDR <0.05 were assigned as differentially expressed. For up-regulated genes, a log2FC>1 and for downregulated genes, a log2FC<1 was considered statistically significant, along with an FDR <0.05. A volcano plot was constructed using the ggplot2 package in the R language.

Gene Ontology and pathway enrichment analysis of DEGs

We used the KOBAS (12) online analysis database to conduct Gene Ontology (GO) and KEGG pathway enrichment analysis on the DEGs. The enriched terms for GO and pathways for Reactome and KEGG were selected with an adjusted P-value of <0.05.

To confirm the function enrichment analysis, web tools such as DAVID (https://david.ncifcrf.gov/), g:Profiler (https://biit.cs.ut.ee/gprofiler/gost), WebGestalt (http://www.webgestalt.org/), and GeneCodis (https://genecodis.genyo.es/) were used to analyze each list of DEGs. Additionally, GOView (http://www.webgestalt.org/2017/GOView/) was utilized to obtain a more meaningful view and compare GO enrichment results.

PPI Network and Identification of Hub Genes

We performed protein-protein interaction (PPI) network analysis using STRING (13) and Cytoscape (14). The proteins were first mapped to the STRING database, and PPIs with the highest confidence score (≥0.9) were selected. Subsequently, these PPIs were analyzed using Cytoscape. To gain a better understanding of biological systems, it is important to comprehend the modular structures of biological networks since these systems are composed of modules. Seed growth clustering algorithms, of which MCODE is a typical one, can be used to detect functional modules based on the density of protein interaction networks (15). Interactions were visualized and evaluated using Cytoscape, and the hub gene in the functional network was identified. The next step involved applying the Molecular Complex Detection (MCODE) algorithm (16) to identify densely connected protein neighborhoods in the network. To visualize these modules, the MCODE app in Cytoscape was used with specific parameters, including a node score

Table 1: The characteristics of sample information and accession numbers

Accession	Breed	Gender	SRA Study	Tissue
SRR10355666	Golden Retriever	male	SRP227237	Spleen
SRR10355667	Golden Retriever	female	SRP227237	Heart
SRR10355668	Mix (Labrador Retriever)	female	SRP227237	Liver
SRR10355669	Golden Retriever	female	SRP227237	Spleen
SRR10355670	Portuguese Water Dog	female	SRP227237	Spleen
SRR10355671	Golden Retriever	male	SRP227237	Spleen
SRR10355672	Golden Retriever	female	SRP227237	Heart
SRR10355673	American Staffordshire Terrier	female	SRP227237	Spleen
SRR10355674	German Shepherd Dog	male	SRP227237	Spleen
SRR10355675	German Shepherd Dog	male	SRP227237	Spleen
SRR10355676	Golden Retriever	male	SRP227237	Liver
SRR10355677	Golden Retriever	male	SRP227237	Spleen
SRR10355678	Golden Retriever	female	SRP227237	Spleen
SRR10355679	Parsons Russell Terrier	male	SRP227237	Spleen
SRR10355680	Golden Retriever	male	SRP227237	Spleen
SRR10355681	Mix (Labrador Retriever)	male	SRP227237	Spleen
SRR10355682	Mix	female	SRP227237	Spleen
SRR10355683	Golden Retriever	female	SRP227237	Heart
SRR10355684	German Shepherd Dog	male	SRP227237	Spleen
SRR10355685	Portuguese Water Dog	female	SRP227237	Spleen
SRR10355686	Golden Retriever	female	SRP227237	Heart
SRR10355687	Golden Retriever	male	SRP227237	Spleen
SRR10355688	Golden Retriever	female	SRP227237	Spleen

Table 2: The established groups for the purpose of comparing to the splenic hemangiosarcoma between six dog breeds

Comparison groups	Abbreviations	Animal number in each comparison group
Golden Retriever versus German Shepherd Dog	GLDNvsGRMN	11
Golden Retriever versus Portuguese Water Dog	GLDNvsPORTG	10
Golden Retriever versus Parsons Russell Terrier	GLDNvsRUSTRR	9
Golden Retriever versus American Staffordshire Terrier	GLDNvsSTFTRR	9
Golden Retriever versus Mix	GLDNvsMIX	10
Portuguese Water Dog versus Parsons Russell Terrier	PORTGvsRUSTRR	3
Portuguese Water Dog versus American Staffordshire Terrier	PORTGvsSTFTRR	3
German Shepherd Dog versus Portuguese Water Dog	GRMNvsPORTG	5
German Shepherd Dog versus Parsons Russell Terrier	GRMNvsRUSTRR	4
German Shepherd Dog versus American Staffordshire Terrier	GRMNvsSTFTRR	4
Parsons Russell Terrier versus American Staffordshire Terrier	RUSTRRvsSTFTRR	2

Table 3: Differentially expressed genes in splenic hemangiosarcoma among six breeds of dogs

GLDNvsMIX				
Gene name	**Log2(FC)**	**P-value**	**P-adj**	**Chromosome**
ADAM9	-4,37E+14	4,50E+09	0.009	chr16
SPEG	-2,25E+14	9,55E+09	0.015	chr37
SLPI	3,43E+14	0.0006	0.04	chr24
PORTGvsRUSTRR				
Gene name	**Log2(FC)**	**P-value**	**P-adj**	**Chromosome**
TYROBP	9,59E+14	1,52E-14	1,40E-12	chr1
PEG10	-1,02E+14	8,53E-13	5,24E-09	chr14
CD40LG	5,95E+14	5,09E+05	1,00E-05	chrX
SLPI	3,03E+14	7,73E+09	0.004	chr24
GOT2	3,03E+14	0.0002	0.009	chr2
KRT222	-2,57E+14	0.0017	0.044	chr9
PORTGvsSTFTRR				
Gene name	**Log2(FC)**	**P-value**	**P-adj**	**Chromosome**
PEG10	-1,11E+14	1,57E-13	5,95E-11	chr14
TYROBP	1,00E+14	1,02E-11	1,75E-08	chr1

SLPI	8,28E+14	9,46E-08	1,37E-04	chr24
NOX5	-8,32E+14	4,50E+00	1,33E-04	chr30
MADCAM1	-7,05E+14	1,05E+04	1,00E-04	chr20
TRIB1	-4,39E+14	8,54E+04	1,19E-04	chr13
GOT2	4,07E+14	1,72E+08	0.00011	chr2
TACR1	-4,50E+14	3,42E+05	0.00019	chr17
SNRPC	3,09E+14	9,42E+09	0.0033	chr12
KRT222	3,64E+14	0.0008	0.02	chr9
RPL26	-2,20E+14	0.002	0.04	chr5

GRMNvsPORTG

Gene name	Log2(FC)	P-value	P-adj	Chromosome
TYROBP	-1,05E+14	1,97E-38	4,00953E-34	chr1
CEACAM23	-3,73E+11	1,13E+08	0.0003	chr1
VIM	1,85E+14	0.0002	0.01	chr2
TRIB1	2,57E+13	0.0009	0.04	chr13

GRMNvsRUSTRR

Gene name	Log2(FC)	P-value	P-adj	Chromosome
GPR83	3,27E+14	2,00E+09	0.004	chr21
KRT222	-3,31E+14	3,15E+09	0.006	chr9
CD40LG	2,99E+13	0.0001	0.01	chrX

GRMNvsSTFTRR

Gene name	Log2(FC)	P-value	P-adj	Chromosome
NOX5	-8,75E+12	2,09E-02	1,35E-07	chr30
MSMO1	-2,96E+14	9,21E+05	1,32E-05	chr15
GPR83	5,54E+14	1,13E+07	1,00E-05	chr21
BRCA2	2,03E+14	3,17E+09	0.001	chr25
XRCC1	1,97E+14	0.0002	0.008	chr1
SNRPC	2,56E+14	0.0007	0.02	chr12
DES	2,47E+14	0.001	0.02	chr37
TACR1	-3,90E+13	0.001	0.03	chr17
SLC37A4	-3,20E+14	0.001	0.03	chr5
MAP2K1	-3,35E+13	0.001	0.03	chr30
CD40	1,75E+14	0.001	0.03	chr24

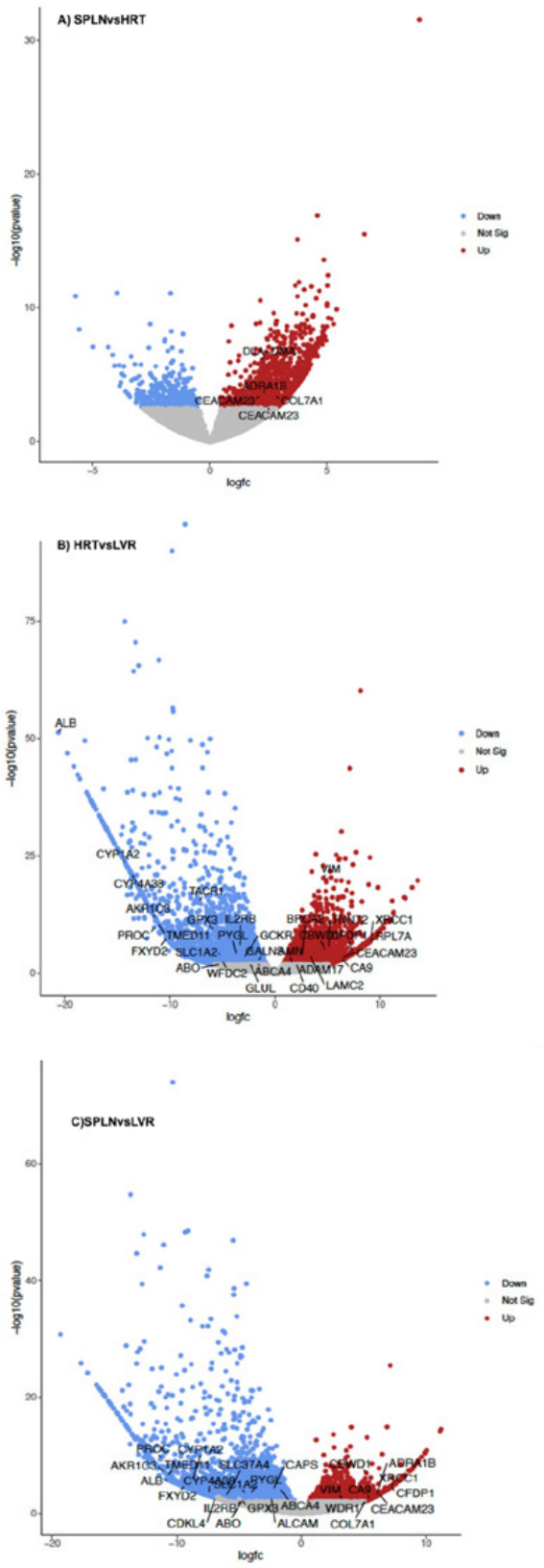


Figure 1: Volcano plots of differentially expressed genes from Golden Retriever dog breed with three hemangiosarcoma tissues. A) SPLNvsHRT, B) HRTvsLVR, and C) SPLNvsLVR, respectively. Red dots represented down-regulated significant genes, and red dots represented up-regulated significant genes. SPLNvsLVR: Splenic Hemangiosarcoma versus Liver Hemangiosarcoma, SPLNvsHRT: Splenic Hemangiosarcoma versus Heart Hemangiosarcoma, and HRTvsLVR: Heart Hemangiosarcoma versus Liver Hemangiosarcoma

cutoff of 0.2, k-core of 2, max depth from the seed of 100, and degree cutoff of 2. Additionally, the Maximal Clique Centrality (MCC) algorithm of cytoHubba (17) was utilized to identify the hub genes.

Transcription factors and miRNAs regulatory network

The miRNet online database (18) was used to identify the transcription factors and microRNAs that target the DEGs. The mirTarbase (19) and DIANA TarBase V.8 (20) tools were used for the prediction of experimentally validated microRNAs. The miRNet database was also used to identify tissue-specific microRNAs. These microRNAs were validated by experiments in the mirTarbase and Tarbase v.8 tools. The 3'UTR sequence of the target gene was obtained from the ENSEMBL website. The miRNA-mRNA targets were validated using the RNA22v2 (21) and RNAhybrid (22) tools.

Results

Identification of DEGs

The spleen is the most common tissue involved in canine HSA, followed by the right atrium/auricle of the heart and the liver. While this type of cancer can affect dogs of any breed, Golden Retrievers have been identified as a breed with an increased genetic predisposition to develop HSA. Gene expression data sets were used to compare altered gene expression in three tissue types of HSA in the Golden Retriever dog breed. The sets used for comparison were SPLNvsLVR, SPLNvsHRT, and HRTvsLVR. A total of four genes were found to be statistically significantly up-regulated in the SPLNvsHRT group. Among the 26 DEGs in the SPLNvsLVR group, 9 genes were up-regulated, and 17 genes were down-regulated. Furthermore, among the 31 DEGs in the HRTvsLVR group, 13 genes were up-regulated, and 18 genes were down-regulated (Figure 1, Supplementary File 1). Interestingly, *CEACAM23* was found to be common in all three groups (Figure 2). A total of five genes, which are CAPS, *SLC37A4*, *CDKL4*, *ALCAM*, and *WDR1*, were only found in the SPLNvsLVR group. On the other hand, the *DLA-DMA* gene was only determined in the SPLNvsHRT group.

Specific breeds like Golden Retrievers, Boxers, and German Shepherds exhibit a higher susceptibility to HSA, indicating a possible genetic basis for disease susceptibility. However, the exact molecular mechanisms underlying the predisposition of these breeds to HSA are not fully understood. In addition, we compared the splenic HSA data from six breeds of dogs to understand the molecular mechanisms underlying this breed predisposition. In the GLDNvsGRMN group, no differentially expressed genes were identified. However, in the GLDNvsPORTG, GLDNvsRUSTRR, and GLDNvsSTFTRR groups, only one gene was found to be differentially expressed in each group: *IL2RB*, *CD40LG*, and *NOX5*, respectively. In the comparison results of

the following groups: GLDNvsMIX, PORTGvsRUSTRR, PORTGvsSTFTRR, GRMNvsPORTG, GRMNvsRUSTRR, and GRMNvsSTFTRR we identified 3, 6, 11, 4, 3, and 11 differentially expressed genes as statistically significant, respectively (Table 3, Supplementary File 2).

Functional enrichment analysis of DEGs

The KEGG pathway analysis revealed that the DEGs in the comparison between spleen and liver HSA tissues in the Golden Retriever breed were significantly enriched in Arachidonic acid metabolism and Metabolic pathway. The arachidonic acid pathway is known to have a significant impact on cardiovascular biology and carcinogenesis (23). Moreover, the GO BP enrichment analysis showed that the DEGs group in SPLNvsLVR was significantly enriched in positive regulation of single-strand break repair, positive regulation of DNA ligase activity, and positive regulation of heart rate by epinephrine-norepinephrine. The main

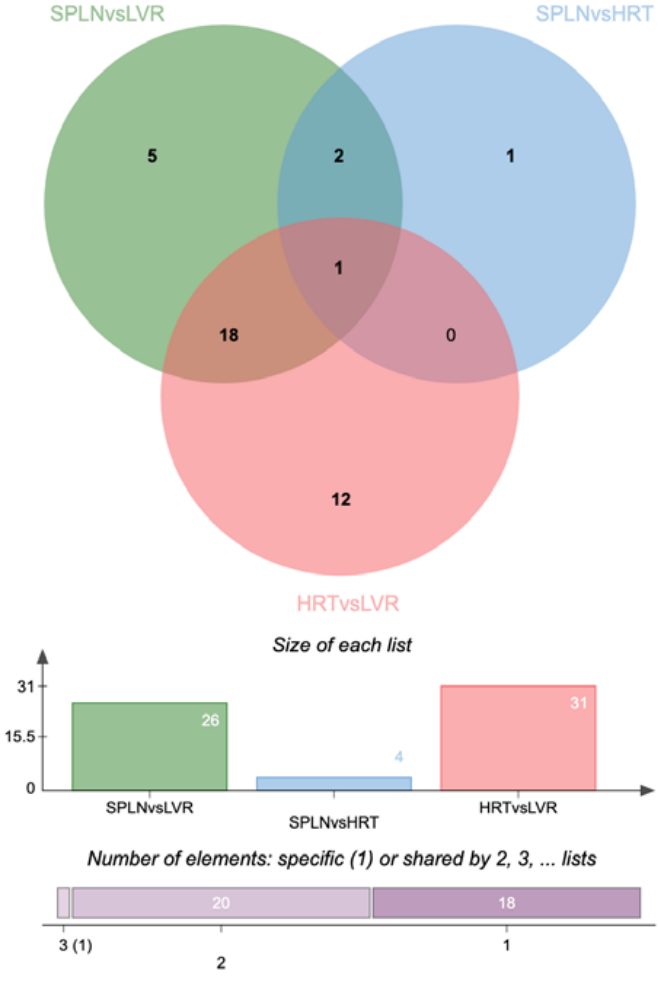


Figure 2: Identification of common differentially expressed genes (DEGs) between SPLNvsHRT, HRTvsLVR, SPLNvsLVR determined from Golden Retriever dog breed. Venn diagram showing 1 DEG (CEACAM23) common to both datasets. SPLNvsHRT: Splenic hemangiosarcoma versus Heart hemangiosarcoma; HRTvsLVR: heart hemangiosarcoma versus liver hemangiosarcoma; SPLNvsLVR: splenic hemangiosarcoma versus liver hemangiosarcoma

enriched GO CC terms were found to be the ERCC4-ERCC1 complex and immunological synapse. The GO MF analysis was significantly enriched in interleukin-15 and interleukin-2 receptor activity. (Supplementary File 3).

The HRTvsLVR group of differentially expressed genes was found to be significantly enriched in several pathways based on KEGG pathway analysis. These pathways included Metabolic pathways and Cardiac muscle contraction. The GO BP enrichment analysis revealed significant enrichment of negative regulation of glucokinase activity and negative regulation of apoptotic process in the HRTvsLVR group, with specific genes implicated in each process. More details can be found in Supplementary File 4.

The genes *DLA-DMA*, *ADRA1B*, *COL7A1*, and *CEACAM23* showed statistically significant differential expression in the comparison between spleen and heart groups, with *DLA-DMA* being uniquely differentially expressed in this group. Meanwhile, *ADRA1B* and *COL7A1* were differentially expressed in both the SPLNvsHRT and spleen and liver comparisons. In the GO BP enrichment analysis, *ADRA1B* was significantly enriched in pathways related to the positive regulation of heart rate by epinephrine-norepinephrine and regulation of cardiac muscle contraction.

According to the results of the comparison of splenic HSA data among six dog breeds, splenic HSA tissues of PORTGvsRUSTRR were significantly enriched in GO BP analysis. *CD40LG* was significantly enriched in pathways related to positive regulation of endothelial cell apoptotic process, and *TYROBP* was significantly enriched in the positive regulation of natural killer cell activation, macrophage activation involved in immune response, and the apoptotic signaling pathway.

In the PORTGvsSTFTRR DEGs group, the GO BP analysis showed significant enrichment in the positive regulation of lymphocyte migration and integrin-mediated signaling pathway. Enrichment analysis indicated that the genes in PORTGvsSTFTRR DEGs were significantly associated with the metabolism of RNA, eukaryotic translation initiation, and mRNA splicing at the Reactome pathway terms. Conversely, the enriched categories for GRMNvsPORTG DEGs were significantly enriched with microRNAs in cancer (cfa05206; *VIM*) in the KEGG pathway terms.

Notably, *MSMO1*, *BRCA2*, *XRCC1*, *DES*, *SLC37A4*, *MAP2K1*, and *CD40* were identified exclusively in the GRMNvsSTFTRR group, whereas *IL2RB* was found only in the GLDNvsPORTG group. In addition, MADCAM1 and *RPL26* genes were unique to the PORTGvsSTFTRR group, and *CEACAM23* and *VIM* genes were identified exclusively in the GRMNvsPORTG group. In addition, *ADAM9* and *SPEG* genes were observed exclusively in the GLDNvsMIX group (Figure 3). These results demonstrate that breed-specific genes are expressed in canine splenic HSA.

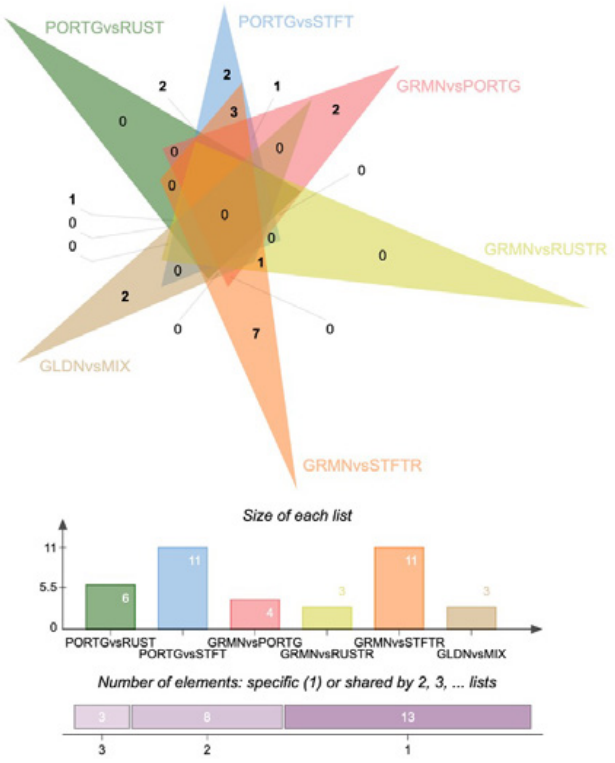


Figure 3: The identification of common and unique differentially expressed genes in splenic hemangiosarcoma among six breeds of dogs. PORTGvsRUSTRR: Portuguese Water Dog versus American Staffordshire Terrier; PORTGvsSTFTRR: Portuguese Water Dog versus American Staffordshire Terrier; GRMNvsPORTG: German Shepherd Dog versus Portuguese Water Dog; GRMNvsRUSTRR: German Shepherd Dog versus Parsons Russell Terrier; GRMNvsSTFTRR: German Shepherd Dog versus American Staffordshire Terrier; GLDNvsMIX: Golden Retriever versus Mix

Identification of PPI Networks and Hub Genes

The comparison between spleen and liver HSA tissues in Golden Retrievers revealed a protein-protein interaction (PPI) network consisting of 8 edges and 11 nodes (Figure 4A). The most highly connected hub genes in this network, as determined by MCC and Cytohubba Degree value, were *ALB*, *CYP1A2*, and *CA9* (Figure 4C). Similarly, in the HRTvsLVR group of Golden Retrievers, the PPI network of DEGs comprised 14 edges and 15 nodes (Figure 4B). The hub genes with the highest connectivity, based on MCC and Cytohubba Degree value, were identified as *ALB*, *TNNT2*, *CYP1A2*, *VIM*, and *CA9* (Figure 4D). Among the other analyzed groups, no protein-protein interaction network was identified for the differentially expressed genes. These findings provide insights into the key genes and their interactions within the PPI networks associated with the different comparisons, shedding light on the molecular mechanisms underlying spleen and liver HSA in Golden Retrievers.

Prediction of miRNAs and Transcription factors

The miRNet database was utilized to identify the miRNAs and Transcription Factors (TF) targeting the *ALB*, *TNNT2*, *CYP1A2*, *VIM*, and *CA9* genes (Figure 5). Functional enrichment analysis was also conducted to determine the associated miRNAs and TFs. The results revealed 10 miRNAs targeting *ALB*, 17 miRNAs targeting *CA9*, 18 miRNAs targeting *CYP1A2*, and two miRNAs (hsa-mir-335-5p and hsa-mir-101-3p) targeting *TNNT2*. Additionally, 117 miRNAs were found to target *VIM*. The determined transcription factors for the *ALB*, *TNNT2*, *CYP1A2*, *VIM*, and *CA9* genes can be found in Supplementary Table 5. Specifically, 12 transcription factors were identified for *ALB*, 13 for *CA9*, 7 for *CYP1A2*, 12 for *TNNT2*, and 43 for *VIM*. The functional enrichment analysis of miRNAs revealed their involvement in the cell cycle, angiogenesis, and onco-miRNAs. In terms of disease enrichment, miRNAs were significantly enriched in pancreatic neoplasms, malignant neoplasms, cardiovascular diseases, and cardiomyopathy ($p<0.05$). Furthermore, 24 miRNAs were found to be specific to liver tissue, while one miRNA (hsa-let-7b) was specific to heart tissue. (Supplementary Table 6).

Functional enrichment analysis was also conducted for the identified TFs. The disease enrichment analysis revealed that these genes were statistically significantly enriched in HSA and liver neoplasms (Figure 5B,5C). In addition, acute coronary syndrome, heart disease, cardiovascular disease, and liver failure were significantly enriched for *ALB*, *TNNT2*, and *VIM*. (Supplementary Table 7).

Discussion

The One Health or One Medicine interdisciplinary approach aims to enhance human and animal health by examining health concepts at the individual, population, and ecosystem levels. Here, we have demonstrated that by employing dogs as models for human diseases within comparative medicine, particularly focusing on the 400 inherited diseases (2) they share with humans and their spontaneous development of cancer with similar molecular profiles, we can significantly enhance our understanding of the molecular basis of cancer including rare types like AS and HAS and contribute to the development of new treatments.

This research may help us to develop new diagnostic and therapeutic options for AS patients. First, we identified genes with altered expression in splenic, heart, and liver HSA tissues in Golden Retriever dogs. The most highly connected hub genes between the spleen and the liver in HSA tissues of Golden Retrievers were identified as *ALB*, *CYP1A2*, and *CA9*. Similarly, in the HRTvsLVR group of Golden Retrievers, the hub genes were identified as *ALB*, *TNNT2*, *CYP1A2*, *VIM*, and *CA9*.

In our study, we successfully identified the miRNAs and TFs predicted for the *ALB* gene. Conducting a functional enrichment analysis, we discovered that miR-107, miR-155-5p, miR-203-3p, and miR-27a-5p, which are specifically associated with the *ALB* gene, are considered to be oncomiRs. Furthermore, our investigation revealed that miR-1-3p,

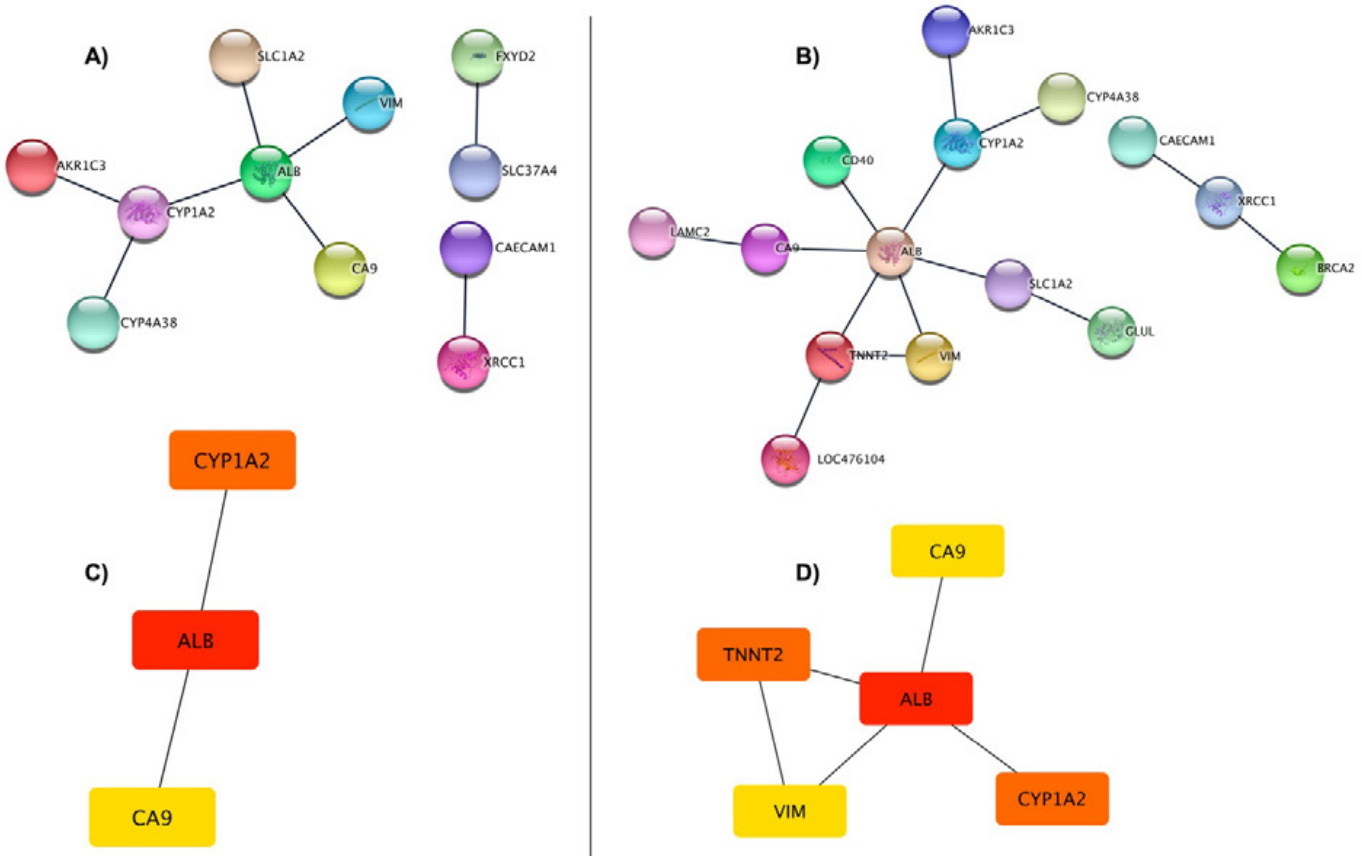


Figure 4: The protein-protein interaction network for the differentially expressed genes in the SPLNvsLVR (A) and HRTvsLVR group (B). Visualization of the networks of the top three hub genes in the SPLNvsLVR (C) and HRTvsLVR group (D). SPLNvsHRT: Splenic hemangiosarcoma versus Heart hemangiosarcoma; HRTvsLVR: heart hemangiosarcoma versus liver hemangiosarcoma; SPLNvsLVR: splenic hemangiosarcoma versus liver hemangiosarcoma

miR-124-3p, and miR-155-5p have specificity to liver tissue. In addition, we performed a functional enrichment analysis for the TFs identified for the *ALB* gene, specifically *STAT3*, which showed statistically significant enrichment in liver neoplasms. *STAT3* has previously been detected in a number of canine malignancies, consistent with our findings. The TFs are proteins responsible for regulating gene expression by binding to specific DNA sequences within promoter regions. They modulate gene expression by pre-transcriptionally activating or repressing downstream genes (25). Our results showed that the *ALB* gene was downregulated in both the SPLNvsLVR and HRTvsLVR groups. Based on this observation, we speculated that *ALB* may play a role in the regulation of liver function during hepatic AS. However, some of the DEGs between HSA in different tissues appear to be tissue-specific genes. This may reflect differences in tissue composition between organs rather than the cancer genes themselves. As a result, we propose that *STAT3*, miR-107, miR-155-5p, miR-203-3p, and miR-27a-5p may serve as potential biomarkers for HSA. Further research is needed to confirm these findings and to investigate the mechanisms by which *ALB* may affect liver HSA progression.

Vimentin (*VIM*), categorized as a type III intermediate filament (IF) protein, holds a prominent position among the IF protein family and has been extensively investigated. Studies have demonstrated the potential role of vimentin (*VIM*) as a significant biomarker in specific histological variants of canine liver cancer, such as moderately differentiated hepatocellular carcinoma, mixed carcinoma, and poorly differentiated carcinoma (26). The research conducted by Sawa et al. (27) provided support for the diagnostic sensitivity of rapid immunocytochemistry (ICC) in detecting vimentin in the neoplastic tissues of dogs. Further support for the significance of vimentin in canine neoplastic conditions was provided by the observations made by Shiga and Shirota (28). Their study revealed that vimentin exhibited co-expression with bile duct-type cytokeratin in well-differentiated hepatocellular carcinoma in a dog. These findings highlighted the role of vimentin as an important marker for determining the cellular origin of tumors in the liver. In accordance with the above study, our results show that *VIM* may be important in liver function and cancer progression. In our study, we conducted an analysis and identified a total of 117 miRNAs that are predicted to target the *VIM* gene and also 43 transcription factors for *VIM*. Among these miRNAs, several oncomiRs were identified.

These miRNAs have been associated with oncogenic properties and are implicated in various cancer-related processes. Furthermore, our analysis revealed that miR-21-5p, miR-92a-3p, and miR-155-5p exhibit both liver tissue

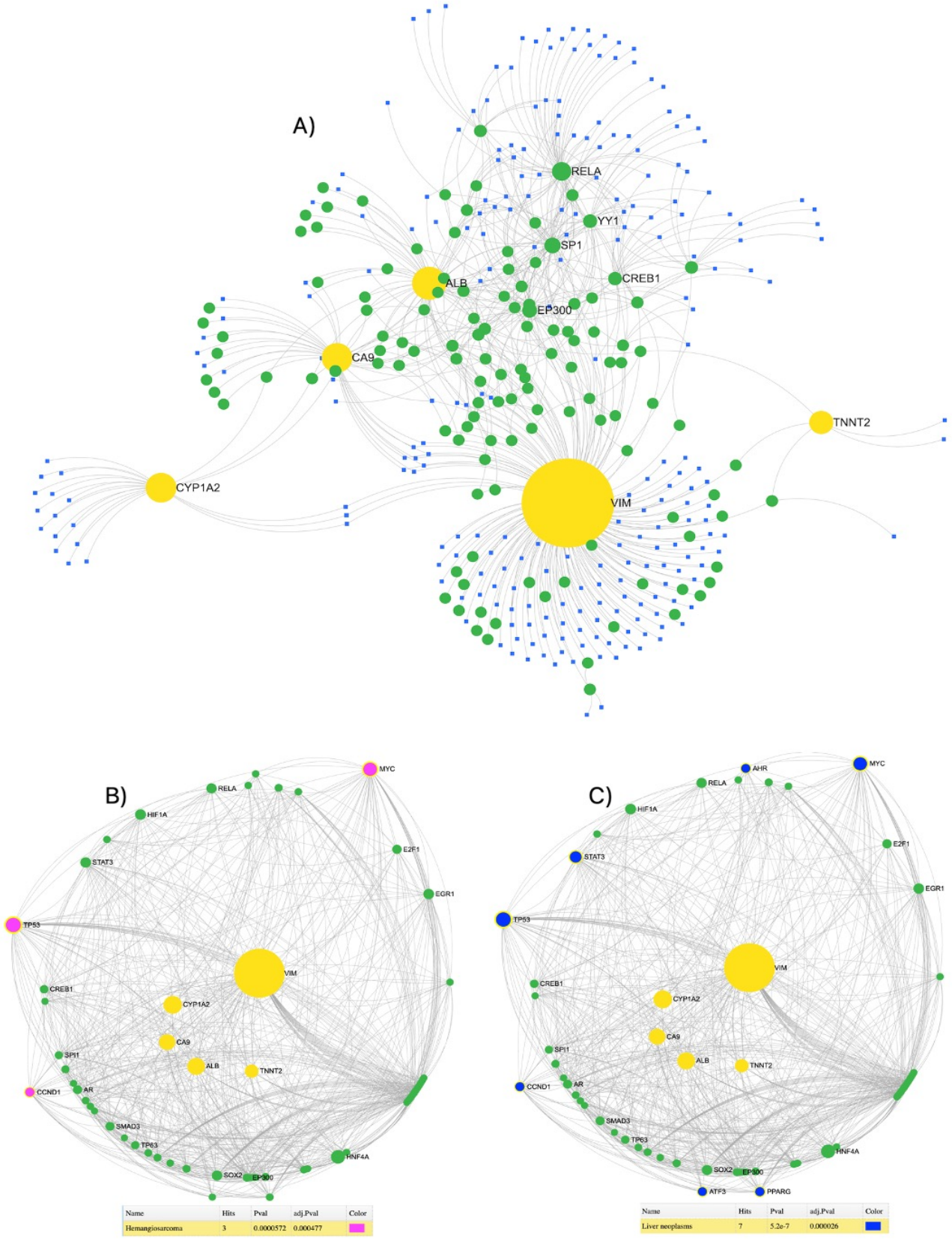


Figure 5. Target gene-miRNA-transcription factors (TF) interaction network between *ALB, TNNT2, CYP1A2, VIM*, and *CA9* genes, the TF, and miRNAs. Green nodes represent TFs, yellow nodes represent genes, and blue nodes represent miRNAs (A). Visualization of the functional enrichment results. The disease enrichment analysis revealed that these genes were statistically significantly enriched in (B) hemangiosarcoma (*MYC, TP53, CCND1*) and (C) liver neoplasms (*AHR, STAT3, MYC, TP53, PPARG, ATF3, CCND1*)

specificity and oncomiR characteristics. This indicates that these miRNAs may play a dual role in liver tissue and contribute to the development and progression of liver-related cancers.

Based on our findings, we propose that *STAT3*, *TP53*, *PPARG*, *ATF3*, *CCND1*, and miR-21-5p, miR-92a-3p, and miR-155-5p have the potential to serve as biomarkers for hepatics HSA in Golden Retrievers. These molecules have demonstrated significant associations with the *VIM* gene and show relevance to liver neoplasms. By further investigating their expression patterns and functional roles, these biomarkers may contribute to the early detection, diagnosis, and prognosis of HSA in golden retrievers. However, additional research is needed to validate and establish the clinical utility of these biomarkers in the context of HSA.

Yoshikawa et al. (5) discovered that miR-214 5AE is a promising novel chemotherapeutic agent for the treatment of canine HSA. In a study by Grimes et al. (29) comparing healthy dogs with a splenic mass, the researchers identified five specific miRNAs (miR-214-3p, miR-452, miR-494-3p, miR-497-5p, and miR-543) in the circulation. These five miRNAs were found to be up-regulated in dogs with HSA or hematoma compared to healthy control dogs. In this study, several transcription factors, including *CUX1*, *EGR1*, *HIF1A*, *SRF*, and *STAT3*, were predicted to be targeted by miR-214. Specifically, *CUX1* was found to regulate the *VIM* gene, *EGR1* was associated with the *ALB* and *TNNT2* genes, *STAT3* was linked to the *ALB*, *TNNT2*, and *VIM* genes, *HIF1A* was implicated in the regulation of the *CA9* gene, and *SRF* was found to modulate the *TNNT2* gene. Both miRNAs and TFs can work together, exerting their regulatory functions on shared target genes. Moreover, they can also influence each other's expression (25). Therefore, based on our findings and consistent with previous studies, we propose that miR-214 has the potential to serve as a biomarker for HSA.

In conclusion, our study suggests that the genes *ALB*, *TNNT2*, *VIM*, and *CA9* have the potential to be used as novel biomarkers for splenic, cardiac, and liver HSA in the golden retriever dog breed. Additionally, our analysis of splenic HSA datasets across six different dog breeds reveals the expression of breed-specific genes in canine splenic HSA. Our findings have the potential to contribute to the development of novel diagnostic and therapeutic approaches for patients with AS. However, it is important to validate these findings with additional clinical case data. The identification of these biomarkers enhances our understanding of the molecular mechanisms involved in AS and provides potential targets for therapeutic interventions.

Acknowledgements

One of the authors, Kardelen Karaman, would like to thank the Council of Higher Education of Turkey for the 100/2000 CoHE Doctoral Scholarship.

Funding information: not applicable. Originality statement: All content is original and not published elsewhere.

Declarations

Ethical Approval: not applicable. Consent to Participate: all authors contributed to this project.

Consent for Publication: all authors are agreed.

Conflict of Interest: the authors declare no competing interests.

Author contributions: Özge Özmen contributed to conceptualization, methodology design, data collection, visualization of data, selection, or development of software tools, writing the original draft, validation of results, acquiring resources, supervising the study's progress, and obtaining funding. Berna Kaya was involved in developing the theoretical framework and manuscript preparation. Kardelen Karaman contributed to developing the theoretical framework, drafting the manuscript, and arranging the figures. All authors participated in discussions on the results and provided comments on the manuscript.

References

1. Florou V, Wilky BA. Current and future directions for angiosarcoma therapy. Curr Treat Options Oncol 2018; 19: 14. doi: 10.1007/s11864-018-0531-3
2. Megquier K, Turner-Maier J, Swofford R, et al. Comparative genomics reveals shared mutational landscape in canine hemangiosarcoma and human angiosarcoma. Mol Cancer Res 2019; 17: 2410–21. doi: 10.1158/1541-7786.MCR-19-0221
3. Vail DM, MacEwen EG. Spontaneously occurring tumors of companion animals as models for human cancer. Cancer Invest 2000; 18: 781–92. doi: 10.3109/07357900009012210
4. Wong K, Ludwig L, Krijgsman O, Adams DJ, Wood GA, van der Weyden L. Comparison of the oncogenomic landscape of canine and feline hemangiosarcoma shows novel parallels with human angiosarcoma. Dis Model Mech 2021; 14(7): dmm049044. doi: 10.1242/dmm.049044
5. Yoshikawa R, Maeda A, Ueno Y, et al. Intraperitoneal administration of synthetic microRNA-214 elicits tumor suppression in an intraperitoneal dissemination mouse model of canine hemangiosarcoma. Vet Res Commun 2022; 46: 447–57. doi: 10.1007/s11259-021-09869-1
6. Tamburini BA, Trapp S, Phang TL, Schappa JT, Hunter LE, Modiano JF. Gene expression profiles of sporadic canine hemangiosarcoma are uniquely associated with breed. PLoS One 2009; 4(5): e5549. doi: 10.1371/journal.pone.0005549
7. Schultheiss PC. A retrospective study of visceral and nonvisceral hemangiosarcoma and hemangiomas in domestic animals. J Vet Diagn Invest 2004; 16(6): 522–6. doi: 10.1177/104063870401600 60
8. Giardine B, Riemer C, Hardison RC, et al. Galaxy: a platform for interactive large-scale genome analysis. Genome Res 2005; 15(10): 1451-5. doi: 10.1101/gr.4086505.

9. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014; 30: 2114–20. doi: 10.1093/bioinformatics/btu170

10. Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Nat Protoc 2016; 11(9): 1650–67. doi: 10.1038/nprot.2016.095

11. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014; 15(1): 550. doi: 10.1186/s13059-014-0550-8

12. Xie C, Mao X, Huang J, et al. KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res 2011; 39: W316–22. doi: 10.1093/nar/gkr483

13. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019; 47(D1): D607–D613. doi: 10.1093/nar/gky1131

14. Su G, Morris JH, Demchak B, Bader GD. Biological network exploration with Cytoscape 3. Curr Protoc Bioinformatics 2014; 47: 8.13.1–24. doi: 10.1002/0471250953.bi0813s47

15. Meng X, Li W, Peng X, Li Y, Li M. Protein interaction networks: centrality, modularity, dynamics, and applications. Front Comput Sci 2021; 15(6): 156902. doi: 10.1007/s11704-020-8179-0

16. Bader GD, Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics 2003; 4: 2. doi: 10.1186/1471-2105-4-2

17. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 2014; 8 Suppl 4: S11. doi: 10.1186/1752-0509-8-S4-S11

18. Chang L, Zhou G, Soufan O, Xia J. miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology. Nucleic Acids Res 2020; 48(W1): W244–W51. doi: 10.1093/nar/gkaa467

19. Hsu SD, Lin FM, Wu WY, et al. miRTarBase: a database curates experimentally validated microRNA-target interactions. Nucleic Acids Res 2011; 39: D163–9. doi: 10.1093/nar/gkq1107

20. Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, et al. DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA-gene interactions. Nucleic Acids Res 2018; 46: D239–D245. doi: 10.1093/nar/gkx1141

21. Miranda KC, Huynh T, Tay Y, et al. A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. Cell 2006; 126(6): 1203–17. doi: 10.1016/j.cell.2006.07.031

22. Rehmsmeier M, Steffen P, Hochsmann M, Giegerich R. Fast and effective prediction of microRNA/target duplexes. RNA 2004; 10(10): 1507–17. doi: 10.1261/rna.5248604.

23. Wang B, Wu L, Chen J, et al. Metabolism pathways of arachidonic acids: mechanisms and potential therapeutic targets. Signal Transduct Target Ther 2021; 6(1): 94. doi: 10.1038/s41392-020-00443-w

24. Chiarella E, Aloisio A, Scicchitano S, Bond HM, Mesuraca M. Regulatory role of microRNAs targeting the transcription co-factor ZNF521 in normal tissues and cancers. Int J Mol Sci 2021; 22(16): 8461. doi: 10.3390/ijms22168461

25. Martín de las Mulas J, Gómez-Villamandos JC, Pérez J, Mozos E, Estrado M, Méndez A. Immunohistochemical evaluation of canine primary liver carcinomas: distribution of alpha-fetoprotein, carcinoembryonic antigen, keratins and vimentin. Res Vet Sci 1995; 59(2): 124–7. doi: 10.1016/0034-5288(95)90045-4

26. Sawa M, Inoue M, Yabuki A, et al. Rapid immunocytochemistry for the detection of cytokeratin and vimentin: assessment of its diagnostic value in neoplastic diseases of dogs. Vet Clin Pathol 2017; 46(1): 172–8. doi: 10.1111/vcp.12462

27. Shiga A, Shirota K. Vimentin/cytokeratin co-expression foci in a well-differentiated canine hepatocellular carcinoma. J Vet Med Sci 2000; 62(2): 199–202. doi: 10.1292/jvms.62.199

28. Grimes JA, Robinson KR, Bullington AM, Schmiedt JM. Identification of serum microRNAs with differential expression between dogs with splenic masses and healthy dogs with histologically normal spleens. Am J Vet Res 2021; 82(8): 659–66. doi: 10.2460/ajvr.82.8.659

Analiza transkriptoma in bioinformacijska karakterizacija hemangiosarkoma pri psih: potencialne terapevtske tarče

Ö. Özmen, B. Kaya, K. Karaman

Izvleček: Pasji hemangiosarkom (HSA) je agresiven rak s slabo prognozo. Nastane v celicah, ki obdajajo krvne žile, in prizadene različne organe, vključno z vranico, srcem in jetri. Kljub redki pojavnosti predstavlja velike diagnostične in terapevtske izzive. Nekatere pasme, kot so zlati prinašalci, bokserji in nemški ovčarji, so dovzetnejše za hemangiosarkom, kar kaže na možno genetsko podlago dovzetnosti za bolezen. Vendar pa natančni molekularni mehanizmi, ki določajo nagnjenost teh pasem k HSA, še niso povsem pojasnjeni. Namen te študije je bil izboljšati naše razumevanje molekularnih mehanizmov za določanje hemangiosarkoma pri psih, in sicer s ponovno analizo javno dostopnih podatkov o sekvenciranju RNA z uporabo bioinformacijskih tehnik pri psih. Naši rezultati kažejo, da bi se geni ALB, TNNT2, VIM in CA9 lahko uporabili kot novi biomarkerji za HSA vranice, srca in jeter pri pasmi zlati prinašalec. Na podlagi naših ugotovitev predlagamo, da bi STAT3, TP53, PPARG, ATF3, CCND1 ter miR-21-5p, miR-92a-3p in miR-155-5p služili kot biomarkerji za jetrni HSA pri zlatih prinašalcih. Poleg tega naša analiza nabora podatkov HSA vranice šestih različnih pasem psov razkriva izražanje pasemsko značilnih genov v HSA vranice psov. Identifikacija teh biomarkerjev krepi naše razumevanje molekularnih mehanizmov angiosarkoma (AS) in predlaga potencialne tarče za zdravljenje.

Ključne besede: angiosarkom; vranica; srce; jetra; primerjalna onkologija; transkriptomsko profiliranje

Slov Vet Res
DOI 10.26873/SVR-1769-2023
UDC 591.424:612.21:616-006.6:616-033.2:636.32/.38
Pages: 53–9

Original Research Article

Intrathoracic and Extrathoracic Metastases in Pırlak Ewes With Ovine Pulmonary Adenocarcinoma (Jaagsiekte)

Key words

extrathoracic metastasis;
intrathoracic metastasis;
jaagsiekte sheep retrovirus;
Pırlak ewe;
ovine pulmonary adenocarcinoma

Received: 18 May 2023
Accepted: 26 November 2023

Belma Dayı[1]*, Marcelo De las Heras[2], Alper Sevimli[1]

[1]Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Pathology, Afyonkarahisar, Turkey, [2]Zaragoza University, Faculty of Veterinary Medicine, Department of Pathology, Zaragoza, Spain

***Corresponding author:** belmadayi@aku.edu.tr

Abstract: Ovine pulmonary adenocarcinoma (OPA) is a contagious neoplastic disease caused by jaagsiekte sheep retrovirus (JSRV) and is characterized by chronic respiratory clinical signs. In this study we describe intrathoracic and extrathoracic metastases in Pırlak ewes naturally infected with JSRV. Two Pırlak sheep flocks brought from two different provinces had progressive respiratory distress, nasal discharge, cough, and emaciation, and nine ewes from these two flocks were necropsied at the request of the owners. Gross findings revealed purple colored and consolidated cranioventral lung lobes with scattered white nodules of various sizes. One ewe had metastasis in the mediastinal lymph node, and another had metastasis in the left kidney. Histopathological examinations of the tumors in the lungs and metastases showed papillary and acinary growth patterns. Immunohistochemically, strong JSRV-Env expression was observed in neoplastic cells.

Introduction

Ovine pulmonary adenocarcinoma (OPA, Jaagsiekte), caused by jaagsiekte sheep retrovirus (JSRV), is an infectious, neoplastic disease characterized by chronic respiratory clinical signs (1). Progressive cases that cause neoplastic pulmonary lesions cause economic losses (decreased milk, meat, and wool production) in sheep-raising in many countries (2). JSRV is an exogenous beta-retrovirus that induces neoplastic transformation of secretory epithelial cells of the respiratory tract (1, 3). JSRV, mainly transmitted by inhalation, can also occur experimentally by intratracheal inoculation with lung secretions (4). Perinatal transmission to lambs through colostrum and milk has also been reported (5). The incidence of OPA in affected flocks is usually around 2-5% effective in the average age group of 2–4 years after a long incubation period (6). Replication of JSRV occurs in epithelial cells of the respiratory system, bronchiolar cells, type II pneumocytes, and Club cells (7).

Classical and atypical OPA forms have been defined in the literature. In classical OPA, the neoplastic lesions occur generally in the cranioventral lobes. There is copious amount of lung fluid in the bronchi and bronchioles. In atypical OPA, focal to multifocal coalescing white nodules are observed in the lungs (4, 6). The classical form is invasive, while the atypical form is more easily circumscribed. There may not be visible changes in the lymph nodes, or there may sometimes be small metastases in the mediastinal lymph nodes (4). Renal, cardiac (8), liver, spleen, skeletal muscle, and adrenal gland (9) metastases have been reported in different studies. Microscopically, papillary and acinary or myxoid growth patterns have been described in both forms (4). Mornex et al (2003), suggested that these pathological findings observed in OPA are similar to a subtype of lung adenocarcinoma found in humans (10). Due to some clinical and pathological similarities, OPA is considered as a useful animal model for understanding human lung adenocarcinomas (11-13).

Clinical signs of OPA are tachypnea or dyspnea, progressive pneumonia, unresponsiveness to antibiotic treatment, emaciation, and exercise intolerance (4). The wheelbarrow test in affected animals is used as an in vivo diagnostic tool that

facilitates diagnosis, but it has possible consequences for animal welfare and may result in euthanasia (14). Although PCR and ELISA techniques are also used in the diagnosis, no reliable farm-level antemortem diagnostic test exists (3, 4). Therefore, necropsy followed by histopathological examination is the convenient method of diagnosis (15).

In this study, we describe intrathoracic and extrathoracic metastases in Pırlak ewes naturally affected by OPA. The present work reveals that it is essential to evaluate regional lymph nodes and distant organs for metastasis along with the lungs in sheep with OPA.

Materials and methods

Pırlak sheep from two different flocks from two geographically close provinces had severe progressive respiratory distress along with nasal discharge, cough, and emaciation despite a good appetite. The animals were unresponsive to antibiotic treatment, and death followed. The anamnesis stated that similar clinical signs were observed in most of the sheep in these flocks and that they had continued over two years. The first flock was previously diagnosed as pasteurellosis by a veterinarian, and a wheelbarrow test demonstrated severe seromucous nasal discharge in the second flock.

At the request of the owners, nine four-year-old ewes, which were brought to our laboratory as dead at different times, were necropsied. Tissue samples were taken for histopathologic evaluation and fixed in 10% formalin solution. After routine processing, samples were embedded in paraffin, cut 4 µm thickness, and stained with hematoxylin & eosin (H&E). In addition, the slides of the ewes with the most severe lesions (case 1) and with metastasis (cases 6 and 7) were stained with JSRV-Env antiserum (kindly supplied by Dr. Dusty Miller) by routine immunohistochemistry technique. Briefly, hydrated tissue sections were exposed to tris-buffered saline for 3x10 minutes (TBS; 0.05 M tris HCl, 0.15 M NaCl, pH 7.4-7.6). Sections were incubated with Bloxall™ endogenous peroxidase and alkaline phosphatase blocking solution (Vector Laboratories Inc, Burlingame, California, USA) for 10 minutes to inactivate endogenous peroxidase activity. Slides were then washed in TBS for 3x10 minutes. The slides were then treated for 30 minutes with 2.5% horse serum (Vector Laboratories Inc, 30022) to remove nonspecific tissue antigens. After removing the excess solution, the slides were incubated at 4 °C overnight with primary antibody against JSRV-Env (mouse monoclonal diluted 1:500 in TBS). Diluted normal rabbit serum and TBS replaced primary antibodies for nonspecific reactions and endogenous peroxidase activity. After 3x10 minutes of TBS washes after incubation, slides were coated with ImmPRESS polymer peroxidase (Vector Laboratories Inc, MP-7500) for 30 minutes, then washed for 3x10 minutes with TBS. Sections were then covered with ImmPACT DAB peroxidase substrate (Vector Laboratories Inc, SK-4105) solution and incubated for 3 minutes. Finally, these sections were counterstained with Carrazi's hematoxylin for 2 minutes, and then were washed with tap water, dehydrated, and mounted with DPX.

Results

Necropsy Findings

In flock I, we noted the classical form of OPA in four animals and the atypical form in one animal (Table 1). Gross examinations of the lungs demonstrated that several lobes (mostly the cranial lobes) were purple in color and were consolidated. In the atypical form, the lung lobes contained white nodules about 0.5 cm in size distributed throughout the lung surface (Fig. 1A). Caudal lobules were more extensively affected, and the cut surface was granular and white in color (Fig. 1B). A purple thin area sharply separated these areas from normal lung tissue. Frothy fluid oozed from the trachea and also from the airways on the cut surface in all ewes. Affected lung lobes were heavier and larger compared to healthy lung lobes.

In flock II, we detected the classical form of OPA in four ewes (Table 1). In one animal, extrathoracic metastasis was found in the left kidney. This was a well-demarcated white nodule approximately 2.5 cm in size (Fig. 1C and 1D). The rest of the kidney showed pale or hemorrhagic areas. In another animal, metastasis was observed as a single well-demarcated white nodule approximately 1 cm in size in the mediastinal lymph node. Additionally, it was observed that there was an abscess in the rest of the mediastinal lymph node. It was noted that the nodules were embedded in the surrounding tissue in both organs.

Histopathological Findings

Histopathological examination revealed neoplastic proliferations of epithelial cells in alveolar and bronchiolar areas (Fig. 2A). These proliferations were comprised of a simple cuboidal to columnar epithelium on a connective tissue stroma, forming acinary, papillary, lepidic, or solid structures. Also, masses formed by neoplastic cells in alveolar lumens were notable. These masses had compressed adjacent alveoli. Similar growth patterns were also present in the bronchi. In some areas, solid growths were observed. The tumor stroma comprised connective tissue and severe lymphocytic infiltration. Also, mild intraalveolar macrophage infiltrations were observed in these areas. Fibrinous bronchopneumonia and nonpurulent interstitial pneumonia were other significant findings.

Kidney and mediastinal lymph node metastases were confirmed by histopathological examination in two cases. In the kidney, the neoplastic cells were cuboidal and exhibited lepidic to papillary growth in interstitial areas (Fig. 2B). Besides, the neoplastic proliferations had expanded

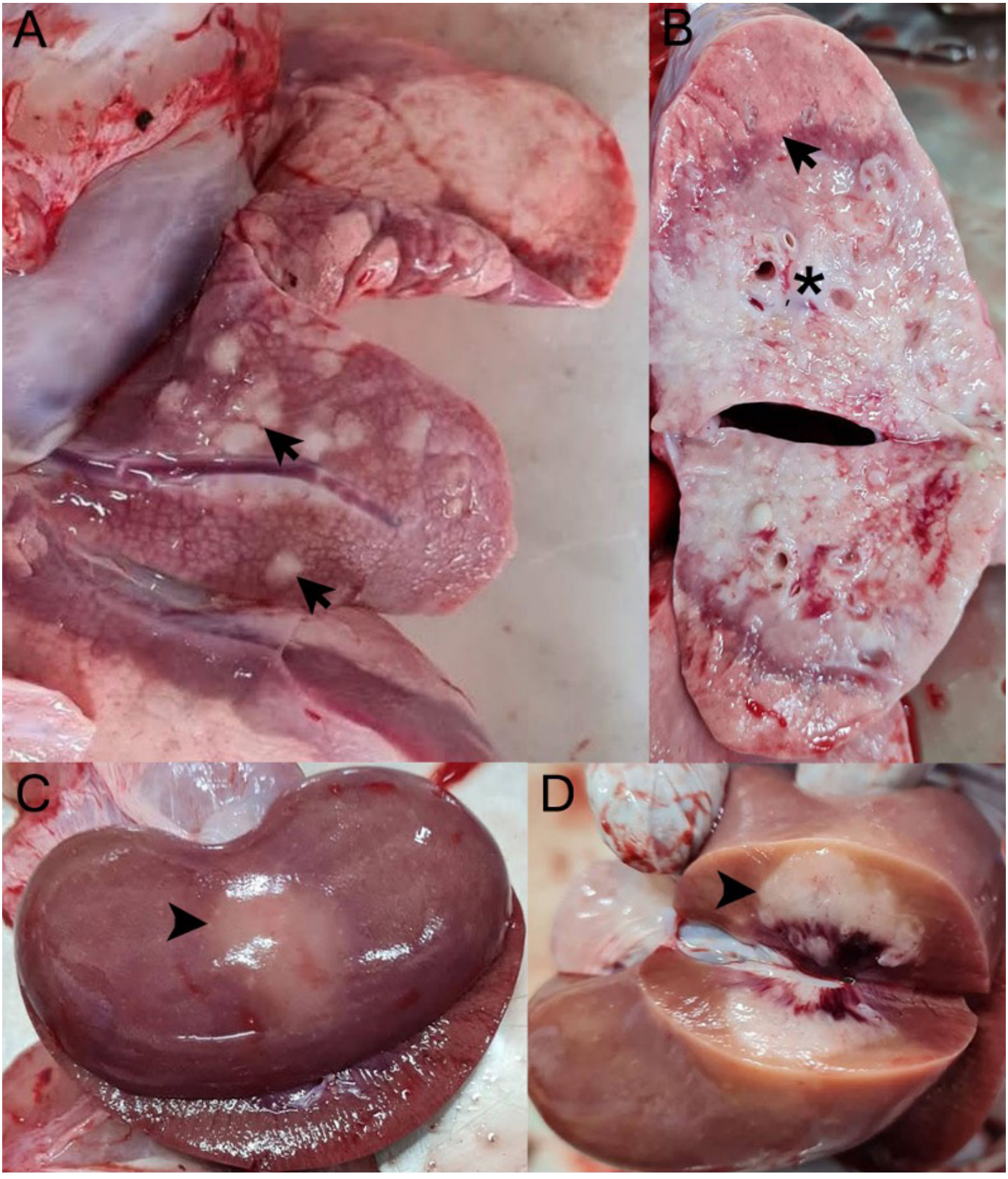


Figure 1: Gross appearance of OPA lesions and extrathoracic metastasis. (A) Multiple white nodules of various sizes on the right lung cranioventral lobe (arrows). (B) Right cranial lung cut surface showing the junction (arrow) between the normal and tumor tissue (star). (C) Neoplastic white nodule (arrowhead) in left kidney. (D) A neoplastic nodule (arrowhead) embedded in the cut surface of the left kidney

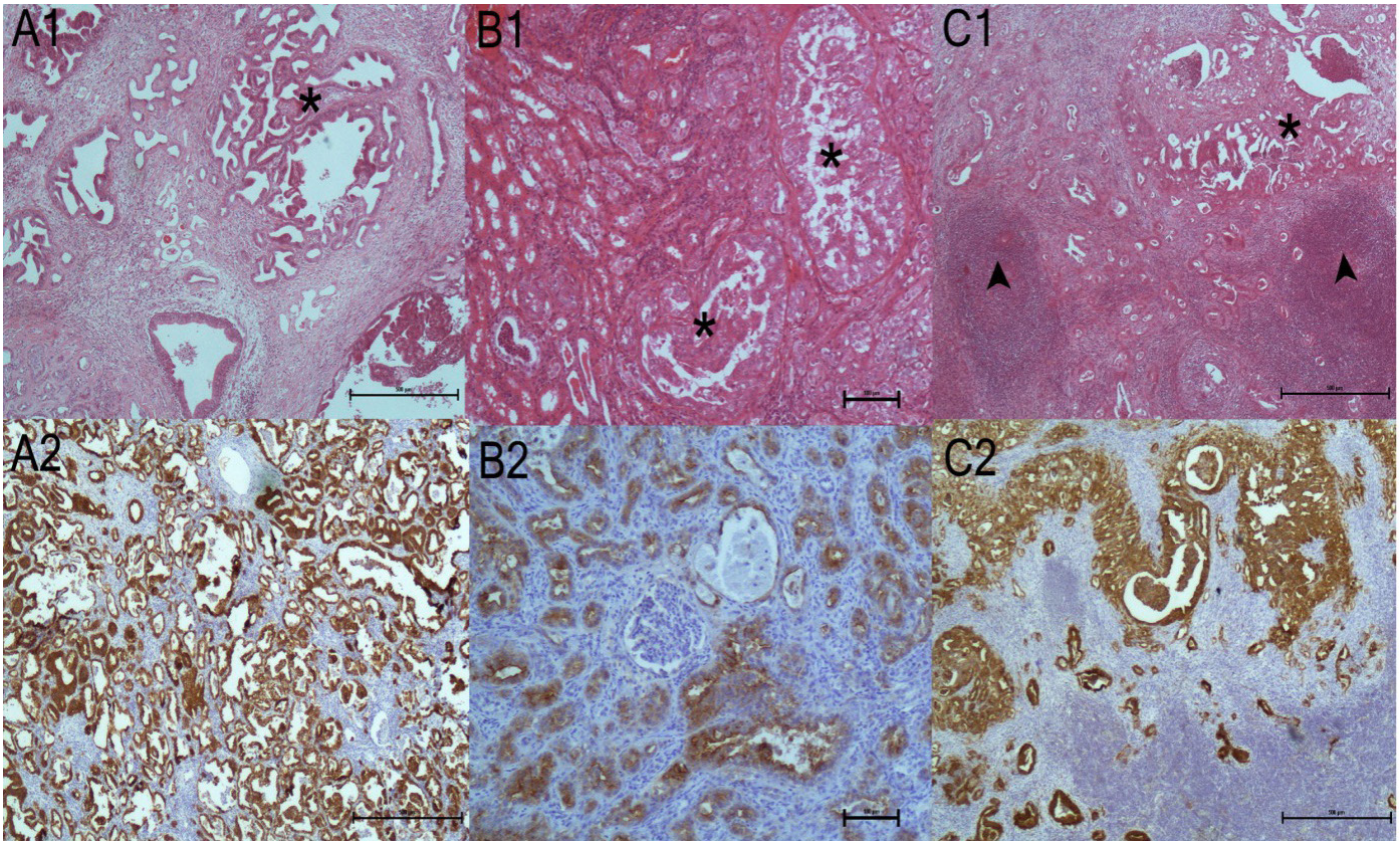


Figure 2: Histopathological appearance and immunopositive reactions of OPA. (A1) Lung, neoplastic proliferations in the alveoli and bronchi (star), H&E, bar=500µm. (A2) Expression of JSRV-Env protein in the cytoplasm of neoplastic cells in the lungs, bar=500µm. (B1) Metastasis in the kidney. Neoplastic proliferations (stars), degenerative and necrotic changes in the tubules, H&E, bar=100 µm. (B2) Moderate expression of JSRV-Env in the kidney, bar=100 µm. (C1) Metastasis in the mediastinal lymph node (star), lymphoid follicles (arrowheads), H&E, bar=500 µm. (C2) Severe positive reaction against JSRV-Env antibody in the lymph node, bar=500µm

Table 1: Details of flocks and OPA forms

Flock	Total number of sheep	Total cases	Age/sex range of affected sheep	Pathologic form	Total intrathoracic metastasis	Total extrathoracic metastasis
Flock I (Afyonkarahisar Province)	133	5	4 year old/female	4 classical 1 atypical	-	-
Flock II (Denizli Province)	200	4	4 year old/female	4 classical	1 mediastinal lymph node (case 7)	1 kidney (case 6)

into adjacent structures. The tumor stroma consisted of connective tissue and moderate lymphocytic infiltration. Degenerative and necrotic changes were also observed. In the lymph node, acinar growths were dominant in some areas, while papillary growths were prominent in others (Fig. 2C). Moreover, depletion of cortical lymphoid follicles and necrosis with numerous neutrophilic leukocytes in the abscess was noted.

IHC Findings

Immunohistochemically, strong positive expression of JSRV-Env was characterized by granular dark brown staining in the membranes and cytoplasm of neoplastic cells. Moderate staining of tumor cells were observed in the kidney (Fig. 2E), while the tumor cells in the mediastinal lymph node showed severe positive reaction consistent with the presence of intracytoplasmic JSRV antigen (Fig. 2F).

Discussion

OPA is contagious lung cancer caused by JSRV in sheep (1, 3). According to the World Organisation for Animal Health (OIE), it is considered a significant disease in the international sheep trade (16, 17). The disease, reported in many regions with sheep-raising worldwide, causes significant economic losses due to difficulties in control and

eradication, lack of vaccine, and difficulties in determining preclinical stages (6, 18). The determination of the JSRV by molecular methods is limited (19). ELISA or PCR can support the diagnosis, however, it is difficult to identify infected sheep without circulating JSRV-specific antibodies at the preclinical stage (2), and there are no routine tests used for diagnosis at this stage (20). Definitive diagnosis is only possible with postmortem and histopathological examinations made in line with the anamnesis and clinical findings (20). Immunohistochemically identifying neoplastic cells expressing JSRV-related antigens is also of great diagnostic value in the case of OPA (21-24).

JSRV is a slow infection virus with a prolonged incubation period and most infected sheep do not show clinical signs (30). Therefore the cause of the disease is generally not entirely investigated, and the sheep are slaughtered before they are diagnosed. For this reason, the exact number of the animals affected with OPA is unknown (24, 31). Abass and Khudhair (2022), suggested an association between JSRV infection and flock size groups (29). In our study, OPA was detected in five ewes in the first flock of 133 sheep and in four ewes in a flock of 200 sheep. However, it is unknown whether other sheep in the flocks were infected because they were sent to slaughter by the flock owners.

OPA occurs mainly in 2–4 years old animals (27, 28) and the ewes in our study were four years old. Abass and Khudhair (2022), reported no breed and sex susceptibility for OPA (29), whereas Toma et al. (2020), noted that all diagnosed sheep were adult females, ranging from 2 to 6 years (28). In the current study, all nine sheep were of the local Pırlak breed and were female. Pırlak ewes are one of Turkey's most widely grown breeds, and further studies are needed to identify whether female Pırlak sheep are more sensitive to JSRV infection.

In the current study, postmortem examinations of nine ewes in two flocks revealed characteristic macroscopic and histopathologic findings of OPA. Papillary and acinary growths were consistent with the definition of OPA, an adenocarcinoma with different proliferation patterns (25). In addition, the cranioventral lobes were significantly affected, and there was a large amount of fluid in the bronchi and bronchioli. As stated in the literature, these pathological findings were identical to the presentation of classical OPA (4, 6). Besides these pathological findings, multifocal white nodules on the lung surface observed in two cases were similar to atypical OPA. Besides necropsy findings and histopathological evaluations, JSRV-Env expression in the lungs, lymph node, and kidney helped confirm the diagnosis. These expressions were in the membranes and cytoplasm of neoplastic cells. However, we could not use molecular techniques to determine viral DNA.

OPA is generally known to metastasize to mediastinal lymph nodes. It rarely metastasizes to distant organs such as the heart, liver, and kidney (4, 6). Minguijon et al (2013), intrathoracic metastasis in 0.3-25% of cases (9). In the current study, metastasis to mediastinal lymph node and kidney were observed in two animals. The microscopical features of the metastatic cells in the mediastinal lymph node and kidney were similar to the characteristics of tumor cells in lungs. These findings were consistent with previous studies (9, 26).

Maedi disease is important in the differential diagnosis (32, 33) of OPA because, chronic progressive respiratory problems are observed similarly in Maedi infection (25, 33). OPA can be complicated by respiratory disorders such as secondary bacterial infections, lung abscessation, or other lung lesions (31). Neutrophils in the lungs have been frequently reported in OPA and evaluated as resulting from secondary bacterial infections (4, 34). It should be noted that OPA can cause chronic respiratory problems in sheep and secondary bacterial infections may complicate them (35). In this study, in one case fibrinous pneumonia suggested pasteurellosis/manheimiosis. Besides, in another case the abscess observed in the mediastinal lymph node suggested caseous lymphadenitis. Toma et al (2020), also observed abscesses due to caseous lymphadenitis in their study (28). However, in our cases microbiological examinations could not be performed and the etiology could not be revealed.

Our study provides valuable data about OPA's pathological evaluation and metastasis features. The results have revealed the importance of necropsy findings for the definitive diagnosis of OPA, which to date does not have an effective intravital diagnostic method nor prophylactic tools in live animals. Our study once more showed that OPA can cause distant metastases and for this reason the lungs, regional lymph nodes, and distant organs should be evaluated carefully.

Acknowledgements

The authors are grateful to Dr. Dusty Miller for generously providing the JSRV-Env protein and to Prof. Dr. Massimo Palmarini and Assoc. Prof. Dr. İ. Taci Cangül for his scientific help'.

References

1. DeMartini JC, Bishop JV, Allen TE, et al. Jaagsiekte sheep retrovirus proviral clone JSRV (JS7), derived from the JS7 lung tumor cell line, induces ovine pulmonary carcinoma and is integrated into the surfactant protein A gene. J Virol 2001; 75(9): 4239–46. doi: 10.1128/JVI.75.9.4239-4246.2001

2. Lee AM, Wolfe A, Cassidy JP, et al. First confirmation by PCR of Jaagsiekte sheep retrovirus in Ireland and prevalence of ovine pulmonary adenocarcinoma in adult sheep at slaughter. Ir Vet J 2017; 70: 33. doi: 10.1186/s13620-017-0111-z

3. Palmarini M, Sharp JM, De las Heras M, Fan H. Jaagsiekte sheep retrovirus is necessary and sufficient to induce a contagious lung cancer in sheep. J. Virol 1999;73(8): 6964–72. doi: 10.1128/JVI.73.8.6964-6972

4. De las Heras M, Gonzalez L, Sharp JM. Pathology of ovine pulmonary adenocarcinoma. Curr Top Microbiol Immunol 2003; 275: 25–54. doi: 10.1007/978-3-642-55638-8_2

5. Grego E, De Meneghi D, Alvarez V, et al. Colostrum and milk can transmit jaagsiekte retrovirus to lambs. Vet Microbiol 2008; 130(3/4): 247–57. doi: 10.1016/j.vetmic.2008.01.011

6. Griffiths DJ, Martineau HM, Cousens C. Pathology and pathogenesis of ovine pulmonary adenocarcinoma. J Comp Pathol 2010; 142(4): 260–83. doi: 10.1016/j.jcpa.2009.12.013

7. Monot M, Archer F, Gomes M, Mornex JF, Leroux C. Advances in the study of transmissible respiratory tumours in small ruminants. Vet Microbiol 2015; 181(1/2) :170–7. doi: 10.1016/j.vetmic.2015.08.008

8. Al-Dubaib MA. Renal and cardiac metastases of Jaagsiekte-like tumour in a goat. Small Rumin Res 2005; 58(1): 75–8. doi: 10.1016/j.smallrumres.2004.09.009

9. Minguijon E, Gonzalez L, De las Heras M, et al. Pathological and aetiological studies in sheep exhibiting extrathoracic metastasis of ovine pulmonary adenocarcinoma (Jaagsiekte). J Comp Pathol 2013; 148(2/3): 139–47. doi: 10.1016/j.jcpa.2012.06.003

10. Mornex JF, Thivolet F, De las Heras M, Leroux C. Pathology of human bronchioloalveolar carcinoma and its relationship to the ovine disease. Curr Top Microbiol Immunol 2003; 275: 225–48. doi: 10.1007/978-3-642-55638-8_9

11. Youssef G, Wallace WAH, Dagleish MP, Cousens C, Griffiths DJ. Ovine pulmonary adenocarcinoma: a large animal model for human lung cancer. ILAR J 2015; 56(1): 99–115. doi: 10.1093/ilar/ilv014

12. Gray ME, Meehan J, Sullivan P, et al. Ovine pulmonary adenocarcinoma: a unique model to improve lung cancer research. Front Oncol 2019; 9: 335. doi: 10.3389/fonc.2019.00335

13. Özkan C, Yıldırım S, Huyut Z, Özbek MA . Selected tumour biomarker levels in sheep with pulmonary adenomatosis. J Vet Res 2020; 64(1): 39–44. doi: 10.2478/jvetres-2020-0017

14. Cousens C, Thonur L, Imlach S, Crawford J, Sales J, Griffiths DJ. Jaagsiekte sheep retrovirus is present at high concentration in lung fluid produced by ovine pulmonary adenocarcinoma-affected sheep and can survive for several weeks at ambient temperatures. Res Vet Sci 2009; 87(1):154–6. doi: 10.1016/j.rvsc.2008.11.007

15. Radad K, Khalil S. Natural ovine pulmonary adenocarcinoma in an Egyptian sheep farm. Eurasian J Vet Sci 2014; 30(1): 39–43.

16. Zhang K, Kong H, Liu Y, Shang Y, Wu B, Liu X. Diagnosis and phylogenetic analysis of ovine pulmonary adenocarcinoma in China. Virus Genes 2014; 48(1): 64–73. doi: 10.1007/s11262-013-0988-x

17. Jörger A, Acevedo C, Busley D, Ganter M, Schmiedl A, Humann-Ziehank E. Sterological and biophysical characteristics of the ovine surfactant system and its changes caused by ovine pulmonary adenocarcinoma. Res Vet Sci 2017; 114: 332–40. doi: 10.1016/j.rvsc.2017.06.008

18. Ortin A, De las Heras M, Borobia M, Ramo MA, Ortega M, Ruíz de Arcaute M. Ovine pulmonary adenocarcinoma: a transmissible lung cancer of sheep, difficult to control. Small Rumin Res 2019; 176: 37–41. doi: 10.1016/j.smallrumres.2019.05.014

19. Liu Y, Zhang YF, Sun XL, Liu SY. Detection of Jaagsiekte sheep retrovirus in the peripheral blood during the pre-clinical period of ovine pulmonary adenomatosis. Genet Mol Res 2016; 15(3): 15038521. doi: 10.4238/gmr.15038521

20. Sonawane GG, Tripathi BN, Kumar R, Kumar J. Diagnosis and prevalence of ovine pulmonary adenocarcinoma in lung tissues of naturally infected farm sheep. Vet World 2016; 9(4): 365–70. doi: 10.14202/vetworld.2016.365-370

21. De las Heras M, Barsky SH, Hasleton P, et al. Evidence for a protein related immunologically to the jaagsiekte sheep retrovirus in some human lung tumours. Eur Respir J 2000; 16(2): 330–2. doi: 10.1034/j.1399-3003.2000.16b23.x

22. De las Heras M, De Martino A, Borobia M, et al. Solitary tumours associated with jaagsiekte retrovirus in sheep are heterogeneous and contain cells expressing markers identifying progenitor cells in lung repair. J Comp Pathol 2014; 150(2/3): 138–47. doi: 10.1016/j.jcpa.2013.09.001

23. Borobia M, De las Heras M, Ramos JJ, et al. Jaagsiekte sheep retrovirus can reach peyer's patches and mesenteric lymph nodes of lambs nursed by infected mothers. Vet Pathol 2016; 53(6): 1172–9. doi: 10.1177/0300985816641993

24. Quintas H, Pires I, Garces A, Prada J, Silva F, Alegria N. The diagnostic challenges of ovine pulmonary adenocarcinoma. Ruminants 2021; 1(1): 58–71. doi: 10.3390/ruminants1010005

25. Caswell JL, Williams KJ. Respiratory System. In: Maxie GM, ed. Jubb, Kennedy, and Palmer's pathology of domestic animals. Vol. 2. Missouri: Elsevier, 2016: 560–1.

26. Beytut E, Sözmen M, Erginsoy S. Immunohistochemical detection of pulmonary surfactant proteins and retroviral antigens in the lungs of sheep with pulmonary adenomatosis. J Comp Pathol 2009; 140(1): 43–53. doi: 10.1016/j.jcpa.2008.10.003

27. Jassim A, Al-Husseiny SH, Mansour KA, Kshash QH. First molecular diagnosis of ovine pulmonary adenocarcinoma in Awassi sheep in Iraq. Al Qadisiyah J Vet Med Sci 2017; 16(1): 112–7. doi: 10.29079/vol16iss1art46

28. Toma C, Balteanu VA, Tripon S, et al. Exogenous Jaagsiekte sheep retrovirus type 2 (exJSRV2) related to ovine pulmonary adenocarcinoma (OPS) in Romania: prevalence, anatomical forms, pathological description, immunophenotyping and virus identification. BMC Vet Res 2020; 16(1): 296. doi: 10.1186/s12917-020-02521-1

29. Abass FA, Khudhair YI. Clinical, molecular, and pathological investigations of ovine pulmonary adenocarcinoma in the middle of Iraq. Open Vet J 2022; 12(2): 264–72. doi: 10.5455/OVJ.2022.v12.i2.15

30. Shi W, Jia S, Guan X, et al. A survey of jaagsiekte sheep retrovirus (JSRV) infection in sheep in the three northeastern provinces of China. Arch Virol 2021; 166(3): 831–40. doi: 10.1007/s00705-020-04919-6

31. Lewis FI, Brulisauer F, Cousens C, McKendrick IJ, Gunn GJ. Diagnostic accuracy of PCR for Jaagsiekte sheep retrovirus using field data from 125 Scottish sheep flocks. Vet J 2011; 187(1): 104–8. doi: 10.1016/j.tvjl.2009.10.024

32. Sigurdsson B. Adenomatosis of sheep lungs: experimental transmission. Arch Gesamte Virusforsch 1958; 8: 51–8.

33. Preziuso S, Renzoni G, Allen TE, et al. Colostral transmission of maedi visna virus: sites of viral entry in lambs born from experimentally infected ewes. Vet Microbiol 2004; 104(3/4): 157–64. doi: 10.1016/j.vetmic.2004.09.010

34. Summers C, Benito A, Ortin A, et al. The distribution of immune cells in the lungs of classical and atypical ovine pulmonary adenocarcinoma. Vet Immunol Immunopathol 2012; 146(1): 1–7. doi: 10.1016/j.vetimm.2012.01.007

35. Weikel J. Double infection with maedi virus and adenomatosis virus in Merino sheep. Tierarztl Prax 1991; 19(6): 596–8.

Intratorakalne in ekstratorakalne metastaze pri ovcah Pırlak z ovčjim pljučnim adenokarcinomom (jaagsiekte)

B. Dayı, M. De las Heras, A. Sevimli

Izvleček: Pljučni adenokarcinom pri ovcah (OPA) je nalezljiva neoplastična bolezen, ki jo povzroča retrovirus ovc jaagsiekte (JSRV) in za katero so značilni kronični klinični znaki na dihalih. V tej študiji opisujemo intratorakalne in ekstratorakalne metastaze pri ovcah Pırlak, naravno okuženih z JSRV. Pri dveh čredah ovc pasme Pırlak, pripeljanih iz dveh različnih pokrajin, so se pojavili progresivna dihalna stiska, izcedek iz nosu, kašelj in izčrpanost, devet ovc iz teh dveh čred pa je bilo na zahtevo lastnikov evtanaziranih. Grobe ugotovitve so pokazale vijolično obarvane in konsolidirane kranioventralne pljučne lobe z razpršenimi belimi noduli različnih velikosti. Ena ovca je imela metastaze v mediastinalni bezgavki, druga pa v levi ledvici. Histopatološke preiskave tumorjev v pljučih in metastaz so pokazale papilarne in acinarne vzorce rasti. Imunohistokemično je bilo v neoplastičnih celicah ugotovljeno močno izražanje JSRV-Env.

Ključne besede: ekstratorakalne metastaze; intratorakalne metastaze; ovčji retrovirus jaagsiekte; ovca Pırlak; ovčji pljučni adenokarcinom

Slov Vet Res
DOI 10.26873/SVR-1670-2024
UDC 591.8:616-006.6:616-091.8:636.7
Pages: 61–7

Case Report

Undifferentiated Embryonal Rhabdomyosarcoma in a German Shepherd Dog: Macroscopic, Histopathologic and Immunohistochemical Features

Key words

embryonal rhabdomyosarcoma;
dog;
histopathology;
immunohistochemistry

Fatih Hatipoglu[1,2]*, Abuzer Tas[3,4], Mehmet Fatih Bozkurt[5], Ayperi Aitmyrza Kyzy[4], Nariste Kadiralieva[6]

[1]Selcuk University, Faculty of Veterinary Medicine, Department of Pathology, 42130, Konya, Turkiye, [2]Kyrgyz-Turkish Manas University, Faculty of Veterinary Medicine, Department of Pathology, 720044, Bishkek, Kyrgyzstan, [3]Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Surgery, Van, Turkiye, [4]Kyrgyz-Turkish Manas University, Faculty of Veterinary Medicine, Department of Surgery, Bishkek, Kyrgyzstan, [5]Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Pathology, Ayfonkarahisar, Turkiye, [6]Kyrgyz-Turkish Manas University, Faculty of Veterinary Medicine, Department of Histology, Bishkek, Kyrgyzstan

***Corresponding authors:** fhatip@selcuk.edu.tr, fatih.hatipoglu@manas.edu.kg

Abstract: This case report describes an 8-year-old female German Shepherd dog with undifferentiated embryonal rhabdomyosarcoma. The mass measured 13 x 12 x 9 cm, weighed 900 grams, and had an elastic consistency. Histopathologic examination revealed a large necrosis area in the center of the mass. We determined cells with spindle-oval and rounded morphology, hyperchromatic nuclei, unclear cytoplasmic borders, tightly arranged atypia, and mitosis around the necrosis. We noted long cells with nuclei arranged in a row and wreath-like multinucleated giant cells among these cells. In the immunohistochemical examination, neoplastic cells were stained with vimentin, desmin, skeletal muscle myosin, sarcomeric actin, and SMA positively, while Iba1, HLA-DR, pancytokeratin, S100B, SOX10, and GFAP were stained negatively. Myogenin was intranuclearly positive in approximately half of the cells. The case was diagnosed as RMS and was classified as undifferanted varyant of embryonal type on the basis of histopathologic and immunohistochemical findings. The original morphological and immunophenotyping structure of the tumor, along with the intriguing structure of the giant cells, led us to believe that sharing the case would be beneficial. This case will contribute to the pathomorphological knowledge of canine striated muscle tumors for studies in the field of veterinary oncology.

Received: 26 December 2022
Accepted: 17 October 2024

Introduction

Rhabdomyosarcoma (RMS) is arised in skeletal muscle, where they could be derived from the resting myoblasts, or satellite cells, they can arise in any part of the body, including sites that normally lack skeletal muscle. The cell of origin of RMS is still controversial and may differ for different subtypes. It has been suggested that in some cases they arise from primitive mesenchymal cells capable of differentiation into skeletal muscle cells (1). RMS in humans and dogs can resemble undifferentiated myoblasts or early embryonic myotubes. The term embryonal is a descriptive term for neoplasms exhibiting a range of cellular morphologies that resemble various developmental intermediates and often have a myxomatous stroma as seen in developing muscle (1-3). According to the international

classification system, human RMS is subdivided into embryonal, botryoid, alveolar, and pleomorphic (anaplastic) subcategories (1, 2). Classification of canine RMS closely parallels classification schemes in human medicine (1). The accurate classification of these tumors in humans is prognostically important, with the best outcomes associated with botryoid rhabdomyosarcoma and the worst with alveolar rhabdomyosarcoma. Embryonal forms have an intermediate prognosis. There is presently insufficient information on clinical outcome to make such prognostic predictions in animals, but development of such information will depend on accurate and consistent classification (2). RMSs in domestic animals are classified based on histopathological findings as embryonal, botryoid, alveolar and pleomorphic RMS (1, 2). Embryonal RMS includes three variants: myotubular, rhabdomyoblastic and spindyloid. Histology of myotubular variant consists of presence of characteristic multinucleated "strap cells", which form myutubes. The rhabdomyoblastic variant consist on histology of frequent round to polygonal cells with abundant eosinophilic cytoplasm. The spindle-cell variant of RMS is rare and a relatively new category. Histological aspect consists of thin spindyloid myoblast cells, usually with formation of bundles and myxoid stroma (1, 3). Botryoid RMS is considered a variant of embryonal RMS in both human and veterinary medicine. Macroscopically, it appears as a polypoid, grape-like mass and is encountered most commonly in the urinary bladder, where it can be seen protruding from the mucosa. Histological examination reveals many undifferentiated rhabdomyoblasts and/or strap cells suspended in a myxoid matrix, these being characteristic (1-3). Alveolar RMS is histologically subdivided in classic and solid variants. The classic variant is characterized by aggregates of small, poorly differentiated round cells. The solid variant in dogs consists of sheets of small round neoplastic cells divided by thin fibrous septa. This pattern is not always present, making the histologic architecture similar to rhabdomyoblastic embryonal RMS, thus the diagnosis is very difficult. Molecular genetic analysis has been proven efficient in this matter (1, 3). Pleomorphic RMS marks the least common variant in human medicine. In dogs, like in humans, is diagnosed typically in adults and is extremely rare in young patients. The tumour rises almost exclusively within skeletal muscle of the limbs. Typically, this variant occurs in large muscles of the limbs and histologically contains a very pleomorphic cell population (1-3).

RMS are relatively rare domestic animal neoplasms with a variety of gross morphologies, histologic variations, and cellular phenotypic variations. In veterinary medicine, the frequency of nonlaryngeal or noncardiac canine RMS is low, with 65 total case reports published (2). RMS cases have been most commonly reported at dogs (2-9), less so in other species; cows (10), cats (11), horses (12) and sheep (13). In dogs, the most common sites involved the skeletal muscle, tongue, larynx, lip, myocardium, urinary bladder and ovarium (2-4, 7, 8, 14). Cooper and Valentine (2) reported in a 20-year retrospective registry study at Cornell University that 58 of the approximately 83,000-neoplasia cases were diagnosed with rhabdomyoma or RMS. They noted that only 16 of the 58 cases could be confirmed by contemporary methods.

In this case report, it was aimed at defining undifferentiated embryonal RMS, which was diagnosed in an 8-year-old female German Shepherd dog by macroscopic, histopathological, and immunohistochemical methods.

Case Presentation

The animal owner provided informed consent. An 8-year-old female German Shepherd dog, with the complaint of the formation of a round mass, approximately 12 cm in diameter, within the borders of the ventro-caudal region of the scapula, sternum, and neck and closer to the left side, was admitted to the clinics of the Faculty of Veterinary Medicine, Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan. The owner stated that the mass reached this size for approximately 6 months and did not cause any functional disorders in the animal. The dog was in good general condition, and the appetite was unchanged. Radiographs were taken, and blood analyses were performed. The radiographs revealed that the mass had no connection to deeper tissue. Thoracic radiographs did not show any metastatic lesions. The results of the blood analysis were within normal limits (Table 1).

As a result of the evaluations, it was decided to remove the mass by surgery. For the surgery, the area was shaved and disinfected (Figure 1A). Anesthesia was administered with

Table 1: The dog's blood test data

Parameters	Value	Units
WBC	9.1	x 10^9/ L
Lymph	1.7	x 10^9/ L
Gran	6.5	x 10^9/ L
HGB	195	g/L
RBC	7.51	x 10^{12}/ L
HCT	49.0	%
MCV	65.3	fL
MCH	25.9	Pg
MCHC	35,7	g/dL
PLT	376	x 10^9/ L
MPV	9.1	fL

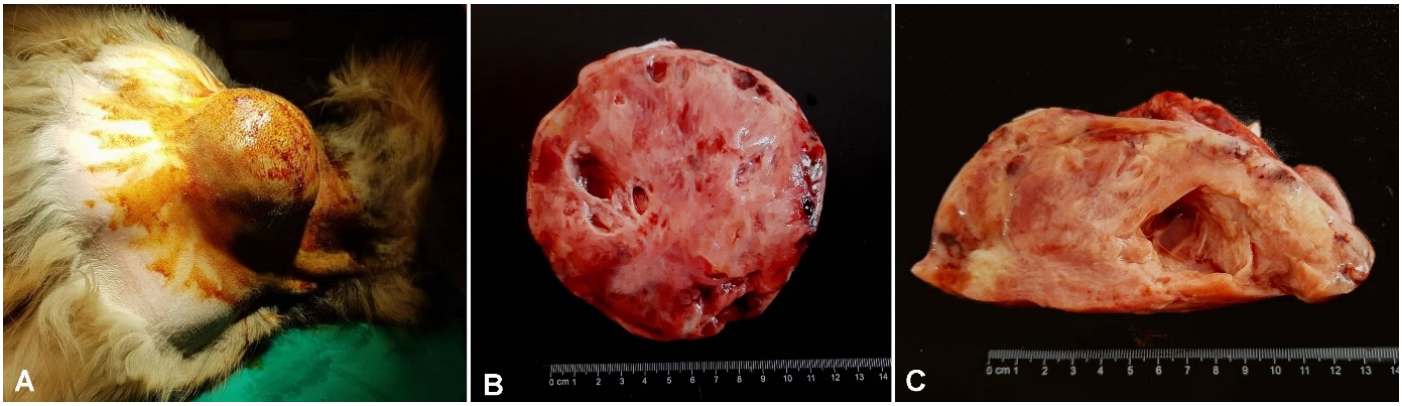


Figure 1: A. Preoperatif appearence of round mass, B- On cut-surface, in the middle of the mass, yellowish-gray colored, occasionally hard areas and slightly red colored structures in gelatinous-pelmetic structure. Melting areas of different sizes and irregular cavernous structures, C- The areas close to the wall of the mass harder consistency and dark red nodular structures with a diameter of 1-2 cm in these areas. A grayish-white thin fibrous capsule outside the mass

xylazine hydrochloride (Vetaxyl Vetaş, Istanbul, Turkey) (1 ml/10 kg) and ketamine hydrochloride (Ketamidor Vetaş, Istanbul, Turkey) (10 mg/kg). An oval incision was made into the skin. The mass was resected with at least 3 cm lateral margins and completely removed. Later, the muscles were closed with continuous sutures and the skin with simple separate sutures. The area was put into protective dressing. Postoperatively, ceftriaxone sodium (Novosef 1 g, Sanofi Istanbul, Turkey) was used for 7 days against secondary infections.

The surgically removed mass was sent to the pathology laboratory for diagnostic examinations. The mass was 13x12x9 cm in size, weighed 900 grams and had an elastic consistency. When the mass was sectioned, a yellowish coloured, viscous serous fluid was observed. Yellowish-gray coloured hard areas and red coloured jelly-like structures were seen in the middle of the mass. Melting areas of different sizes (5-40 mm) and irregular cavernous structures were noted in these areas (Figure 1B). It was noted that the areas close to the wall of the mass had a harder consistency and dark red nodular structures with a diameter of 1-2 cm in these areas. A grayish-white thin fibrous capsule was observed outside the mass (Figure 1C). According to the information obtained from the owner of the patient after the operation, it was learned that no new tumour

Table 2: For IHC staining primary antibodies

Primary Antibody	Company, product code	Dilution, incubation time /temperature
Vimentin	Abcam, ab28028	1/200, 2 hours/room temperature
Iba1	Wako, 019-19741	1/500, 18 hours/+4 °C
Desmin	DAKO, M0760	1/20, 2 hours/room temperature
SMA	DAKO, M085	1/100, 2 hours/room temperature
Anti-pan-Cytokeratin	Santa Cruz, SC-58830	1/50, 2 hours/37 °C
SOX-10	Santa Cruz, SC-365692	1/50, 2 hours/room temperature
S100B	DAKO, Z0311	1/500, 2 hours/room temperature
GFAP	Thermo Scientific, RB-087	1/50, 2 hours/room temperature
Sarcomeric actin	DAKO, M-0874	1/200, 2 hours/room temperature
Myogenin	Santa Cruz, SC-12732	1/50, 2 hours/room temperature
Skeletal Muscle Myosin	Santa Cruz, SC-32732	1/50, 2 hours/room temperature
HLA-DR	Santa Cruz, SC-53319	1/50, 2 hours/room temperature

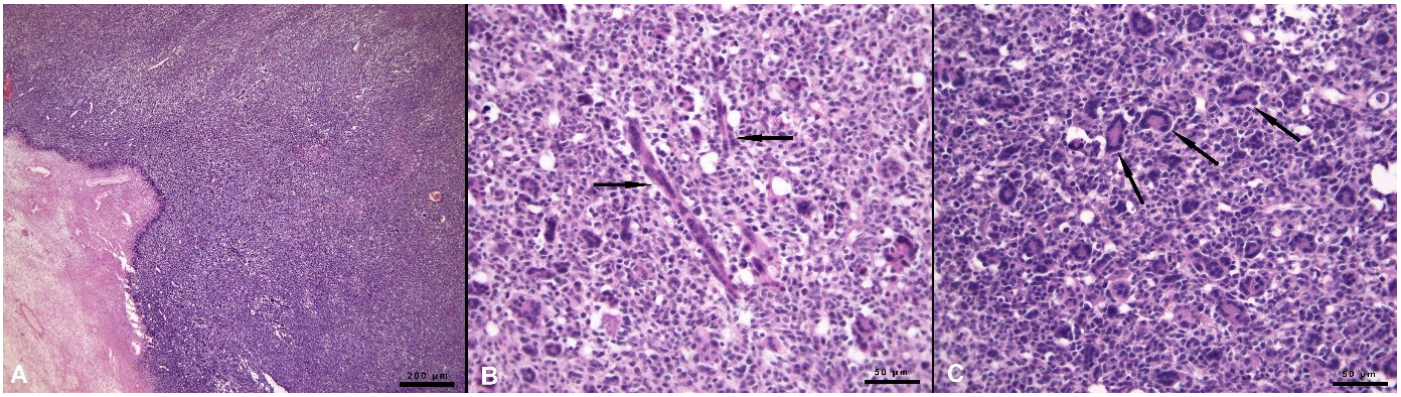


Figure 2: Microscopic view of neoplastic mass at low-power magnification; cell-rich neoplastic area around the necrotic center, H&E. Bar, 200 µm (A). The view of these neoplasic cells at high-power magnification: areas of spindle-oval-shaped cells with hyperchromatic nuclei and giant cells with nuclei lined up in a row (arrows) H&E. Bar, 50 µm (B), wreath-like multinucleated giant cells (arrows) at high-power magnification H&E. Bar, 200 µm (C)

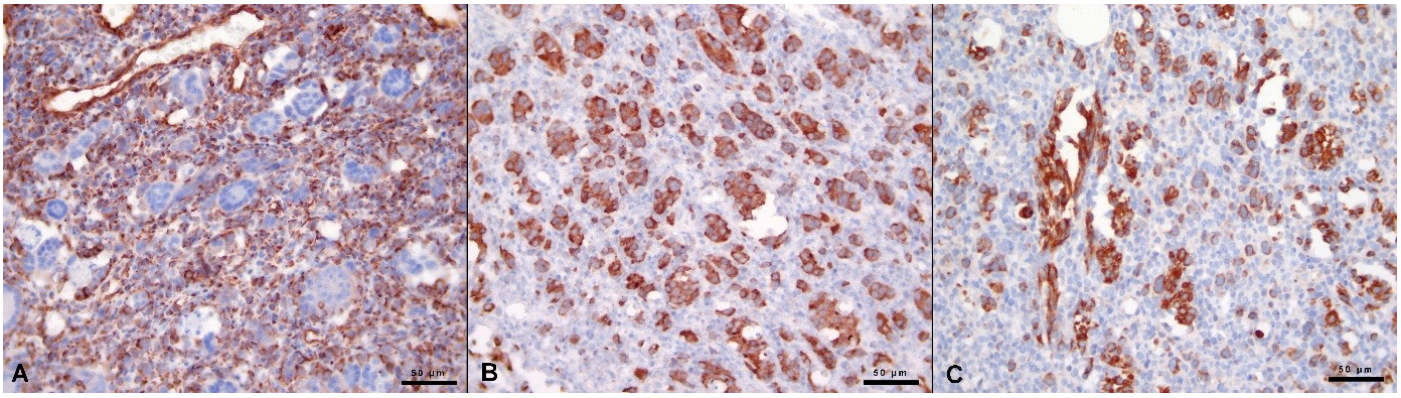


Figure 3: In immunohistochemical staining: vimentin (A), desmin (B) and skeletal muscle specific myosin (C) are positively stained. In wreath-like multinucleated giant cells vimentin is negative while desmin (A) and skeletal muscle specific myosin are positive (C). AEC chromogen, Gill's hematoxylin. Bars, 50 µm

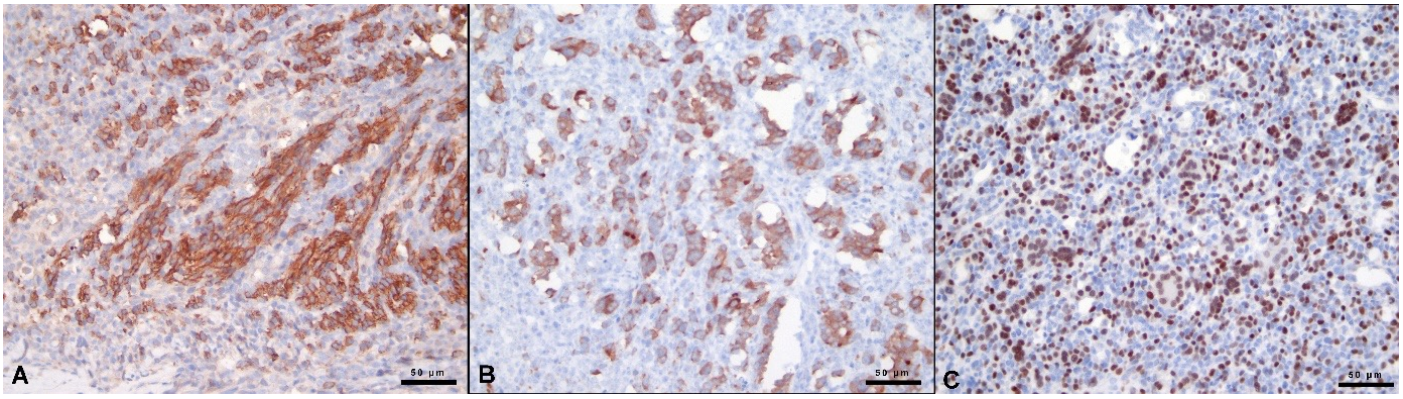


Figure 4: Sarcomeric actin (A), SMA (B) and myogenin (C) positivity in immunohistochemical staining. AEC chromogen, Gill's hematoxylin, Bars, 50 µm

or infection occurred in the area where the mass was removed. Likewise, it was confirmed that there was no mass formation in any other part of the body.

Tissue samples taken from different areas of the mass (close to the capsule, middle area, around the melting areas, hard and nodular structures) taken by the operation were fixed in 10% neutral buffered formalin solution. Fixed tissues were processed routinely and blocked in paraffin. Then, 4-5 micron thick sections were taken from the samples in paraffin blocks with a microtome to normal and silane-coated slides. Sections were stained with hematoxylin-eosin (H&E) and immunohistochemically. All stained sections were examined under a light microscope (Olympus BX 51, Japan).

Primary antibodies given in the Table 2 were used for IHC staining. For IHC staining method, Avidin-Biotin-Peroxidase Complex (ABC) method was used (15). ABC KIT (VECTASTAIN® Elite® ABC-HRP Kit, Peroxidase, PK-6100) were applied according to the user guide of the ABC-KIT. Anti-rabbit/mouse biotinized antibodies (1/100 dilution,

Boster bio- BA1007, BA1003) were dripped onto control sections instead of primary and secondary antibodies. 3-amino-9-ethylcarbazole (AEC) chromogen (TA-060-HA, AEC Substrate System, LabVision/ThermoScientific) which is a substrate of horse radish peroxidase enzyme, was used for 30 min. After that, non-alcoholic, 20% Gill's (III) hematoxylin was used for the background staining for 60 sec. The slides were covered with a coverslip by aqueous adhesive.

On histopathologic examination, there was a large necrosis area in the center of the mass. Around the necrosis, cells with spindle-oval and rounded morphology, hyperchromatic nuclei, unclear cytoplasmic borders, tightly arranged, atypia and mitosis were determined (Figure 2A). Among these cells, long cells with nuclei lined up in a row (Figure 2B) and wreath-like multinucleated giant cells (Figure 2C) were noted.

In the immunohistochemical examination, neoplastic cells were stained with vimentin (Figure 3A), desmin (Figure 3B), skeletal muscle myosin (Figure 3C), sarcomeric actin (Figure 4A), and SMA (Figure 4B) positively while Iba1, HLA-DR, Pancytokeratin, S100B, SOX10 and GFAP were stained negatively. Myogenin (Figure 4C) was intranuclear positive in approximately half of the cells. On the other hand, while the giant cells were negative with vimentin (Figure 3A), they were strongly stained cytoplasmically with desmin (Figure 3B), skeletal muscle myosin (Figure 3C), sarcomeric actin (Figure 4A), and SMA (Figure 4B).

The case was diagnosed as RMS, with wreath-like multinucleated giant cells, long cells with nuclei lined up in a row in the histopathology and immunohistochemistry findings.

Discussion

In the presented case, the tumor mass diagnosed was located in the neck region, which was reported the common localizations of the RMSs. The regions where RMS is most frequently observed in dogs have been reported as follows; skeletal muscle, tongue, larynx, lip, myocardium, urinary bladder and ovary (2-5, 7, 8, 14). In the study in which RMSs were evaluated (1), information about the localization of the tumor was stated as follows; the urogenital tract was the most common location (n=32/65; 49%), the head, neck, and face were common locations (n=24/65; 37%), less common locations included the limbs (n=5/65; 8%), in the hip and spine (n= /65; 3%), in the skin and mammary glands (n=2/65; 3%).

In the differential diagnosis, RMS, histiocytic sarcoma, and undifferentiated pleomorphic sarcoma are tumors compatible with morphology. Malignant melanoma, malignant peripheral nerve sheath tumor, and perivascular wall tumor should also be considered in the differential diagnosis, although they are less likely (16). Because SOX-10 and S100B were negative, we eliminated amelanotic malignant melanoma and malignant peripheral nerve sheath tumors, and Iba1 and HLA-DR negativity eliminated histiocytic sarcoma despite the presence of giant cells (16, 17). Desmin and, more specifically, sarcomeric actin, myogenin, and skeletal muscle myosin positivity distinguish this tumor from perivascular wall tumors (PWTs) (16, 18). Desmin and SMA can be positive in myopericytomas, which is one of the PWTs. But myosin is negative. Myosin (skeletal muscle-specific myosin) positivity in the case, as well as sarcomeric actin and myogenin positivity, distinguish it from this tumor (16). At the same time, Tuohy et al. (6) reported that myogenin positivity strengthens the diagnosis of RMS.

The histopathology and immunohistochemistry findings identified the present case as RMS, characterized by wreath-like multinucleated giant cells and long cells with nuclei lined up in a row. The wreath-like multinucleated giant cells, which are conspicuous in histopathology and positive with antibodies staining striated muscle, consisting of nuclei circumscribed like a wreath under the cytoplasmic membrane, were found interesting. These wreath-like multinucleated giant cells are similar to giant cells previously identified in a heifer (10) and some human rhabdomyosarcomas (19, 20). However, the available sources do not contain any reports of these giant cells in canine RMSs.

The myotubular variant of embryonal RMS is dominated by multinucleated "strap" cells forming myotubes, whereas the rhabdomyoblastic variant is dominated by round to polygonal cells with abundant eosinophilic cytoplasm. The spindyloid embryonal RMS, as the name implies, is composed of thin spindyloid myoblast cells forming bundles within a myxoid stroma (1). The mass's center displayed a large necrosis area. Around the necrosis, we identified tumor cells with spindle-oval and rounded morphology, hyperchromatic nuclei, unclear cytoplasmic borders, tightly arranged atypia, and mitosis. Long cells with nuclei lined up in a row and wreath-like giant cells were also noted among these cells.

Conclusions

The presented case is original in terms of giant cells, morphological appearance and immunophenotyping. The rhabdomyosarcoma in the present case was classified as undifferanted varyant of embryonal type on the basis of histopathologic and immunohistochemical findings. Important histopathological findings for this classification can be as follows; wreath-like multinucleated giant cells, spindle-oval and rounded morphology, hyperchromatic nuclei, unclear cytoplasmic borders, tightly arranged, atypia and mitosis, long cells with nuclei lined up in a row. In addition, crucial immunohistochemical findings for this classification listed as follows; neoplastic cells were stained with vimentin, desmin, skeletal muscle myosin, sarcomeric actin, and SMA positively while Iba1, HLA-DR, Pancytokeratin, S100B, SOX10 and GFAP were stained negatively. While the

giant cells were negative with vimentin, they were strongly stained cytoplasmically with desmin, skeletal muscle myosin, sarcomeric actin, and SMA. In veterinary pathology, striated muscle tissue tumors are not frequently encountered. According to the available literature, the morphology and even immunophenotyping of the tumor is not fully established. Examining the aforementioned cases and retroscopic studies reveals that the majority of the described striated muscle tumors exhibit distinct characteristics. The reviewed literature does not contain any similar cases exhibiting these findings. This case will contribute to the pathomorphological knowledge of canine striated muscle tumors for studies in the field of veterinary oncology.

Acknowledgements

Author contributions. All authors contributed to the study conception and design. FH: Conceptualization, Methodology, Validation, Writing- Original draft preparation, Writing- Reviewing and Editing. AT: Resources, Writing-Original draft preparation. MFB: Methodology, Visualization, Writing- Original draft preparation, Validation. AAK: Resources, Visualization. NK: Methodology, Visualization.

Ethical approval. Informed consent was obtained from the animal owner.

Funding. This research did not receive any specific grant from funding agencies in the public,commercial, or not-for-profit sectors

Conflict of Interest Statement. The authors declared that there is no conflict of interest.

References

1. Caserto BG. A comparative review of canine and human rhabdomyosarcoma with emphasis on classification and pathogenesis. Vet Pathol 2013; 50(5): 806–26. doi: 10.1177/0300985813476069
2. Cooper BJ, Valentine BA. Tumors of muscle. In: Meuten DJ ed. Tumors in Domestic animals. 5th ed. Ames: Wiley Blackwell, 2017: 425–66.
3. Pirvu AM, Nicolae GL, Militaru M. Canine rhabdomyosarcoma - literature review. Sci Work Ser C Vet Med 2023; 69(1): 130–36.
4. Dias FGG, Cintra PP, Calazans SG, et al. Laryngeal rhabdomyosarcoma in a dog: case report. Arq Bras Med Vet Zootec 2018; 70 (5): 1423–6. doi: 10.1590/1678-4162-9911
5. Dias FGG, Santilli J, Magalhães GM, et al. Clinical, pathological and therapeutic findings on lingual rhabdomyosarcoma in a dog - case report. Arq Bras Med Vet Zootec 2019; 71 (6): 1815–20. doi: 10.1590/1678-4162-9979
6. Tuohy JL, Byer BJ, Royer S, et al. Evaluation of myogenin and MyoD1 as immunohistochemical markers of canine rhabdomyosarcoma. Vet Pathol 2021; 58 (3): 516–26. doi: 10.1177/0300985820988146
7. Szaluś-Jordanow O, Czopowicz M, Moroz-Fik A. et al. A primary multiple pleomorphic rhabdomyosarcoma of the heart in an adult dog. BMC Vet Res 2023; 19(1): 137. doi: 10.1186/s12917-023-03701-5
8. Pierini A, Criscuolo MC, Caccamo R, et al. A case series of urinary bladder rhabdomyosarcoma in seven dogs. Open Vet J 2023;13(11): 1498–503. doi: 10.5455/OVJ.2023.v13.i11.15.
9. Shi J, Gao R, Zhang J, et al. Invasive spindle-cell rhabdomyosarcoma with osteolysis in a dog: case report and literature review. J Vet Diag Invest 2023; 35(2): 168–172. doi:10.1177/10406387221147319
10. Hatai H, Nagai K, Tanaka Y, Miyoshi N. Primary pharyngeal alveolar rhabdomyosarcoma in an adolescent Japanese black heifer. J Vet Med Sci 2020; 82(8): 1146-50. doi: 10.1292/jvms.20-0305
11. Vazir N , Sasani F, Vazir B, Kabir F. Pleomorphic rhabdomyosarcoma on maxillary region in a persian cat. Eltiam 2020; 6(1): 81–7.
12. Clegg PD, Coumbe A. Alveolar rhabdomyosarcoma: an unusual cause of lameness in a pony. Equine Vet J 1993; 25: 547–49. doi: 10.1111/j.2042-3306.1993.tb03012.x
13. Yener Z. Alveolar rhabdomyosarcoma of the limb in a sheep. Dtsch Tierarztl Wochenschr 2001; 108(7): 315-6.
14. Cappelleri A, Bertola L, Caniatti M, Recordati C. Diagnostic challenge in veterinary pathology: Disseminated tumor in a young dog. Vet Pathol 2022; 59(3): 394–6. doi: 10.1177/03009858211067464.
15. Ramos-Vara JA, Borst LB. Immunohistochemistry: fundamentals and applications in oncology. In: Meuten DJ ed. Tumors in domestic animals. 5th ed. Ames: Wiley Blackwell, 2017: 44–87.
16. Mattie JH. Mesenchymal tumors of the skin and soft tissues. In: Meuten DJ ed. Tumors in domestic animals. 5th ed. Ames: Wiley Blackwell, 2017: 142–76.
17. Schulman FY, Johnson TO, Facemire PR, Fanburg-Smit JC. Feline peripheral nerve sheath tumors: histologic, immunohistochemical, and clinicopathologic correlation (59 tumors in 53 cats). Vet Pathol 2009; 46(6): 1166–80. doi: 10.1354/vp.08-VP-0327-S-FL.
18. Avallone G, Helmbold P, Caniatti M, Stefanello D, Nayak RC, Roccabianca P. The spectrum of canine cutaneous perivascular wall tumors: morphologic, phenotypic and clinical characterization. Vet Pathol 2007; 44(5): 607–20. doi: 10.1354/vp.44-5-607.
19. Kumar A, Singh M, Sharma MC, Bakshi S, Sharma BS. Pediatric sclerosing rhabdomyosarcomas: a review. ISRN Oncol 2014; 640195. doi: 10.1155/2014/640195
20. Taran SJ, Taran R, Prakash A, Kumar R, Singh S. Neonatal rhabdomyosarcoma: an unusual presentation. Transl Pediatr 2014; 3(4): 10–31. doi: 10.3978/j.issn.2224-4336.2014.07.04

Nediferenciran embrionalni rabdomiosarkom pri nemškem ovčarju: makroskopske, histopatološke in imunohistokemijske značilnosti

F. Hatipoglu, A. Tas, M. F. Bozkurt, A. A. Kyzy, N. Kadiralieva

Izvleček: V tem poročilu primera je opisan primer osemletne samice nemškega ovčarja z nediferenciranim embrionalnim rabdomiosarkomom. Tumor je meril 13 x 12 x 9 cm, tehtal 900 gramov in imel elastično konsistenco. Histopatološki pregled je pokazal veliko območje nekroze v središču mase. Okoli nekroze smo določili celice z vretenasto ovalno in okroglo morfologijo, hiperkromatičnimi jedri, nejasnimi citoplazemskimi mejami, tesno razporejenimi atipijami in mitozo. Med temi celicami smo opazili dolge celice z jedri, razporejenimi v vrsto, in vencu podobne večjedrne orjaške celice. Pri imunohistokemijskem pregledu so se neoplastične celice pozitivno obarvale z vimentinom, dezminom, miozinom skeletnih mišic, sarkomernim aktinom in SMA, negativno pa z Iba1, HLA-DR, pancitokeratinom, S100B, SOX10 in GFAP. Miogenin je bil intranuklearno pozitiven v približno polovici celic. Primer je bil diagnosticiran kot RMS in na podlagi histopatoloških in imunohistokemijskih izvidov razvrščen kot nediferenciran varietetni embrionalni tip tumorja. Izvirna morfološka in imunofenotipska struktura tumorja, skupaj z zanimivo strukturo orjaških celic, nas je napeljala na misel, da bi bila objava tega primera koristna, saj bi lahko prispevala k patomorfološkemu znanju o tumorjih progastih mišic pri psih za študije na področju veterinarske onkologije.

Ključne besede: embrionalni rabdomiosarkom; pes; histopatologija; imunohistokemija

Table of Content