TASTE OF GENOMICS

20 June 2018

70th anniversary of Prof. Dr. Radovan Komel

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TASTE OF GENOMICS

70th anniversary of Prof. Dr. Radovan Komel

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University of Ljubljana, Faculty of Medicine

Ljubljana, 20th June 2018

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Foreword

We welcome you to the symposium Taste of Genomics at the University of Ljubljana, Faculty of Medicine, June 20th 2018. The symposium is organised by the Institute of Biochemistry, Medical Centre for Molecular Biology, UL MF to celebrate the 70th anniversary of prof. Radovan Komel. Prof. Komel dedicated his scientific life to study diverse aspects of life sciences. His research is invariably characterized by original thinking, boldness, determined persistence and pioneering achievements, valuable for the promotion of science at the local and international levels. His successes are the result of his tireless efforts to develop interdisciplinary research thinking among his students and broader scientific community as well as his dedication to secure the continuous funding for various research projects from the private and governmental sectors. For many years he was the head of a research programme, under the auspices of which many young enthusiastic students were educated and trained.

The programme of the symposium is focused on diverse research aspects intertwining his long career. Many of his former students will present their work highlighting Prof. Komel's scientific pathway offering a unique picture of his scientific contributions over the years.

The symposium Taste of Genomics provides a welcome opportunity to meet former and new colleagues in a formal setting combined with exciting renuion after the conclusion of presentations.

We wish you all a fruitful and enjoyable meeting.

Sincerely,

Petra Hudler

Alja Videtič Paska

Chairs of the Scientific Committee

Tadeja Režen

Chair of the Organising Committee

Medicinski center za molekularno biologijo – kmalu bomo obeležili 30 let njegove dejavnosti

Medicinski center za molekularno biologijo (MCMB) se nahaja na Inštitutu za biokemijo Medicinske fakultete Univerze v Ljubljani. Center je bil ustanovljen februarja 1992 na pobudo prof. Dušana Sketa, dr. Janeza Zidarja in prof. Radovana Komela. Ustanovil ga je konzorcij inštitutov Medicinske fakultete in kliničnih ustanov v Ljubljani, da bi usklajeval raziskovalne in pedagoške dejavnosti na področju medicinske molekularne biologije / genetike med inštituti Medicinske fakultete, zainteresiranimi klinikami Univerzitetnega kliničnega centra Ljubljana in Onkološkega inštituta v Ljubljani. Institucije podpisnice (člani MCMB) so bili inštituti Medicinske fakultete – Inštitut za biokemijo (prof. Katja Breskvar), Inštitut za celično biologijo (prof. Nada Pipan), Inštitut za biofiziko (prof. Saša Svetina), Inštitut za patološko fiziologijo (prof. Janez Sketelj), Inštitut za patologijo (prof. Dušan Ferluga), Inštitut za mikrobiologijo in imunologijo (prof. Srečko Koren), Inštitut za sodno medicino (prof. Anton Dolenc) ter klinike Univerzitetnega kliničnega centra – Ginekološka klinika (prof. Srečko Rainer), Pediatrična klinika (prof. Ciril Kržišnik), Klinika za endokrinologijo in presnovne bolezni (prof. Andreja Kocijančič), Inštitut za klinično nevrofiziologijo (dr. Janez Zidar), Psihiatrična klinika (prof. Martina Tomori), in Onkološki inštitut (prof. Zvonimir Rudolf). Filozofija Centra na samem začetku je bila (in je še vedno) odprtost za vse člane z namenom usposabljanja mladih raziskovalcev bodisi z vključevanjem v tekoče projekte Centra bodisi z vzpostavitvijo skupnih raziskovalnih projektov, ki so v interesu partnerskih institucij. Po usposabljanju se v večini primerov raziskovalci vrnejo v domačo ustanovo in začnejo z lastnim laboratorijem za molekularno biologijo, vendar ohranjajo raziskovalne povezave z MCMB.

Osrednja raziskovalna skupina Centra je ena od raziskovalnih skupin Inštituta za biokemijo Medicinske fakultete, v preteklosti imenovana "Laboratorij za molekularno genetiko", ki je bil v obdobju 1984-89 med pionirji uvajanja genske tehnologije (genskega inženirstva) v slovenski biokemiji in biotehnologiji (Komel R. et al., *Vest, Slov. Kem. Druš.*, 1987: 34 (1), 39-52) in v obdobju 1988-90 začetnik molekularno genetske analize v slovenskih medicinskih raziskavah in njene translacije v diagnostično prakso (Gasparini P. et al., *N. Engl J. Med.*, 1990: 323 (1), 62-63).

Na začetku smo raziskave izvajali v dveh glavnih tematskih blokih: (A) BIOTEHNOLOGIJA – biokemijska analiza in gensko kloniranje ter izražanje encimov mikrobnih pretvorb steroidov, in (B) MEDICINSKA MOLEKULARNA BIOLOGIJA / GENETIKA – genetska analiza monogenskih bolezni. V zvezi s sklopom A (Biotehnologija) je bila ideja kloniranje dveh ključnih encimov za bioprodukcijo steroidnih glukokortikoidov, enega iz bakterij (steroidna 1: 2-dehidrogenaza) in enega iz filamentnih gliv (steroidna 11β-hidroksilaza), ter obe genski informaciji združiti in izrazili proteina v enem samem gostiteljskem organizmu. To je bil nekakšen »metabolični inženiring«, znanstvena metodologija pred sodobno »sintetično biologijo«. Namreč, uvedba OH-skupine v položaj 11 steroidne molekule je ključnega

pomena za glukokortikoidno funkcijo steroidnih hormonov, medtem ko uvedba dvojne vezi 1:2 v A-obroč molekule lahko znatno zmanjša neželene stranske mineralokortikoidne učinke. Tako bi povezovanje teh dveh reakcij pomenilo izgradnjo biotehnološko pomembnega mikroorganizma. Leta 1993 je bila 1:2-dehidrogenaza uspešno klonirana, medtem ko kloniranje kompleksne membransko vezane 11β-hidroksilaze, ki pripada encimski družini citokromov P450, ni bilo uspešno. Namesto tega smo se posvetili podrobnim raziskavam družine encimov P450 na ravni razmerja glivni patogen – (rastlinski) gostitelj in leta 2002 je bilo eksperimentiranje v največji meri preneseno z MCMB na Nacionalni inštitut za kemijo (partner skupnega medinstitucionalnega programa Funkcijska genomika in biotehnologija za zdravje, P1-0104, 2004-2014), kjer smo uspešno klonirali glivno benzoatno 4-monoksigenazo (BPH) in nato raziskavo usmerili v preučevanje tega citokroma kot potencialne nove tarče za razvoj protiglivnih zdravil.

V sklopu B (medicinska molekularna biologija/genetika) je bila prva genetska bolezen, ki smo jo raziskovali s pristopi molekularno-genetske analize (1988 - 1994 - 2000), monogenska bolezen cistična fibroza (CF), v sodelovanju z Ginekološko kliniko UKC (prof. Srečko Rainer, prof. Nina Canki Klain) in Pediatrično kliniko UKC (prof. Milan Štrukelj) in z lepo in prijateljsko podporo Medicinske fakultete v Veroni, Italija (prof. Pier-Franco Pignatti). Avgusta 1990 smo se pridružili svetovnemu konzorciju Cystic Fibrosis Genetic Analysis Consortium in v letih, ki so sledila, smo izvedli sistematično mutacijsko analizo slovenske CF populacije, rezultate vzporejali in delili z drugimi evropskimi in širšimi populacijami, molekularno genetska analiza pa je bila uvedena kot prvi molekularno diagnostični postopek v naše klinične ustanove (na ginekološki in pediatrični kliniki UKC). V naslednjih letih smo na enak način preučevali nekatere druge monogenske bolezni kot so hemofilija A, hiperplazija nadledvične žleze, policistična ledvična bolezen, kožne keratinske motnje in osteoporoza, in ugotovitve prenesli v diagnostične postopke, v izobraževanje in v podporo pri izgradnji molekularno diagnostičnih laboratorijev na kliničnih, raziskovalnih in univerzitetnih ustanovah. Poleg tega smo skupaj s Inštitutom za sodno medicino na Medicinski fakulteti tudi uvedli populacijsko genetsko analizo in genotipizacijo za identifikacijo posameznikov (2001-2005), ki se danes uporablja v slovenski sodnomedicinski in kriminološki praksi. Od leta 2000 naprej se je velik del raziskav postopoma začel prenašati na temeljne raziskave molekularnih osnov kompleksnih procesov in bolezni kot so psihiatrične motnje (motnje hranjenja, samomorilno vedenje), regeneracija tkiv (sistemsko izražanje genov pri živalskem modelu, mehiški dvoživki aksolotlu) in rak (limfom, adenokarcinom želodca, HNPCC). Kot nosilec interdisciplinarnega in medinštitutskega programa P1-0104 / P1-0390 Funkcijska genomika in biotehnologija za zdravje, ESRR centra odličnosti Biotehnologija s farmacijo in Slovenske mreže za funkcijsko genomiko je MCMB ob veliki podpori Evropskega sklada za regionalni razvoj in Medicinske fakultete leta 2004 vzpostavil Center za funkcijsko genomiko in biočipe (CFGBC), ki danes deluje kot odprt med/nadinštitutski infrastrukturni center na področju funkcijske genomike. V zadnjem obdobju (od leta 2010) smo pospešeno pričeli uvajati pristope funkcijske genomike, zlasti transkriptomike in proteomike, kot tudi globalne analize SNP, GWAS

in epigenetike, ki jih uporabljamo pri preučevanju molekulskih osnov raka (rak želodca, tumorji možganov) ter eritrocitoze in v študijah genskega ozadja samomorilnega vedenja. V zadnjem času je pomemben del raziskav osredotočen na iskanje proteomskih biomarkerjev rakastih (glioblastomskih) matičnih celic z uporabo reverzne proteomike in nano(proti)teles, z namenom načrtovanja novih diagnostičnih in terapevtskih pristopov, ter v raziskave mehanizmov celjenja ran z uporabo induciranih pluripotentnih matičnih celic in popolnih 3D modelov kože.

Medical centre for molecular biology – close to 30 years of its activities

Medical Centre for Molecular Biology (MCMB) is located at the Institute of Biochemistry of the Faculty of Medicine of the University of Ljubljana. The Centre was established in February 1992, on the initiative of Prof. Dušan Sket, Dr. Janez Zidar and Prof. Radovan Komel. It was funded by a consortium of institutes of the Faculty of Medicine and clinical institutions in Ljubljana, in order to coordinate research and teaching activities in the field of medical molecular biology/genetics among the institutes of the Faculty of Medicine, several clinics of the Clinical Centre Ljubljana, and the Institute of Oncology in Ljubljana. The signatory institutions (members of MCMB) were institutes of the Faculty of Medicine – Institute of Biochemistry (Prof. Katja Breskvar), Institute of Cell Biology (Prof. Nada Pipan), Institute of Biophysics (Prof. Saša Svetina), Institute of Pathological Physiology (Prof. Janez Sketelj), Institute of Pathology (Prof. Dušan Ferluga), Institute of Microbiology and Immunology (Prof. Srečko Koren), Institute of Forensic Medicine (Prof. Anton Dolenc), as well as clinics of the University Clinical Centre – Gynaecological Clinic (Prof. Srečko Rainer), Paediatric Clinic (Prof. Ciril Kržišnik), Clinic of Endocrinology and Metabolic Diseases (Prof. Andreja Kocijančič), Institute of Clinical Neurophysiology (Dr. Janez Zidar), Psychiatric Clinic (Prof. Martina Tomori), and Institute of Oncology (Prof. Zvonimir Rudolf). The philosophy of the Centre at the very beginning was (and still is) openness for all members with the purpose of training young researchers, either by integrating them into the ongoing projects of the Centre, or by establishing joint research projects that are in the interest of partner institutions. After training in most cases, researchers return to their home institution and start their own molecular biology laboratory, but they maintain research links with the MCMB.

The core research group of the Centre is one of the research groups of the Institute of Biochemistry of the Faculty of Medicine, in the past named "Laboratory of Molecular Genetics", which was among the pioneers of introducing gene technology (genetic engineering) in Slovenian biochemistry and biotechnology in the period 1984-89 (Komel R. et al., *Vest, Slov. Kem. Druš.*, 1987: 34 (1), 39-52) and in the period 1988-90 initiated molecular genetic analysis in Slovenian medical research and its translation to diagnostic practice (Gasparini P. et al., *N. Engl J. Med.*, 1990: 323 (1); 62-63).

Initially, the research was carried out in two main thematic blocks: (A) BIOTECHNOLOGY – biochemical analysis and gene cloning and expression of microbial steroid bio-converting enzymes; and (B) MEDICAL MOLECULAR BIOLOGY/GENETICS – genetic analysis of monogenic diseases. Concerning part A (Biotechnology), the idea was to clone two key enzymes for bioproduction of steroid glucocorticoids, one from bacteria (steroid 1:2-dehydrogenase) and one from filamentous fungi (steroid 11β-hydroxylase), and to combine and express both proteins in a single host organism. This was some kind of »metabolic engineering«, scientific methodology preceding the modern »synthetic biology«. Namely,

introduction of OH-group into 11β-position of the steroid molecule is crucial for the glucocorticoid function of steroid hormones, while introduction of a 1:2 double bond into the A-ring of the molecule can significantly reduce the undesired mineralocorticoid side effects. Thus, combining these two reactions would result in building a biotechnologically relevant microorganism. In 1993, the 1:2-dehydrogenase was successfully cloned, while cloning of the complex membrane-bound 11β-hydroxylase belonging to the cytochrome P450 enzyme family failed. Instead, we focused on detailed studies of the P450 enzyme family at the level of the fungal pathogen – (plant) host relationship, and in 2002 experimentation was largely transferred from the MCMB to the National Institute of Chemistry (partner of the joint interinstitutional program Functional Genomics and Biotechnology for Health, P1-0104; 2004-2014), where benzoate 4-monooxygenase (BPH) was cloned from the experimental fungus and the study was then directed into the study of this cytochrome P450 as a potential new target for the development of antifungal drugs.

Within the framework of part B (Medical Molecular Biology/Genetics), first genetic disease studied by molecular genetic analysis (1988 – 1994 – 2000) was monogenic disease cystic fibrosis (CF), in collaboration with UKC Gynaecological Clinic (Prof. Srečko Rainer, Prof. Nina Canki Klain) and UKC Paediatric Clinic (Prof. Milan Štrukelj), and with the nice and friendly support of the Medical Faculty in Verona, Italy (Prof. Pier-Franco Pignatti). In August 1990 we joined the worldwide Cystic Fibrosis Genetic Analysis Consortium and in the years that followed a systematic mutation analysis of the Slovenian CF population was performed, the results compiled and shared with other European and wider populations, and the molecular genetic analysis was introduced as a first molecular diagnostic procedure to our clinical institutions (namely the Gynaecological and Paediatric clinic). In the following years, some other monogenic diseases such as haemophilia A, adrenal hyperplasia, polycystic kidney disease, skin keratin disorders, and osteoporosis were studied in the same way and findings were translated to diagnostic procedures, in education and in support of establishing molecular diagnostic laboratories on clinical, research and university institutions. In addition, together with the Institute of Forensic Medicine at the Faculty of Medicine we have also pioneered population genetic analysis and genotyping for identification of individuals (2001-2005) which is now used in Slovenian forensic and criminology practice. However, since 2000 great part of the research gradually moved to more basic research on the molecular basis of complex processes and diseases such as psychiatric disorders (eating disorders, suicidal behaviour), tissue regeneration (systemic expression of genes in an animal model, Mexican amphibian axolotl) and cancer (lymphoma, gastric adenocarcinoma, HNPCC). As the carrier of the interdisciplinary and interinstitutional program P1-0104/P1-0390 Functional Genomics and Biotechnology for Health, the ERSD Centre of Excellence in Biotechnology with Pharmacy and the Slovenian Functional Genomics Network, the MCMB, with the support of the European Regional Structure Development Fund and the Faculty of Medicine, in 2004 established the Centre for Functional Genomics and Biochips (CFGBC), which today functions as an open / inter-institutional infrastructure centre in the field of functional genomics. In the last

period (from 2010), we have been accelerating the introduction of functional genomic approaches, in particular transcriptomics and proteomics, as well as global SNP analysis, GWAS and epigenetics, used in the study of molecular roots of cancer (gastric cancer, brain tumours) and erythrocytosis, and in studies of the genetic background of suicidal behaviour. Recently, an important part of research has focused on the search for proteomic biomarkers of cancer (glioblastoma) stem cells using the nanobody-based reverse proteomics approach, in order to design new diagnostic and therapeutic approaches, and on the research of wound healing mechanisms using induced pluripotent stem cells and complete 3D skin models.

PROGRAMME, June 20th 2018

12:30–13:00	Registration
13:00–13:10	**Opening of the symposium** Chairs: **Borut Štrukelj** & **Ana Plemenitaš**
13:10–13:55	**Radovan Komel**, University of Ljubljana, Faculty of Medicine, SI: From "synthetic biology" to "nanomedicine" and back
13:55–14:10	**Marina Dermastia**, National Institute of Biology, SI: Young science (to say nothing of the fish)
14:10–14:25	**Ljerka Lah**, Novartis/LEK d.d., SI: Non-clinical and clinical aspects of biosimilar development
14:25–14:40	**Barbara Podobnik**, Novartis/LEK d.d., SI: Breast Cancer and Stromal Cell Co-culture Models for Immuno-conjugate Therapy Optimization
14:40–14:55	**Damjana Rozman**, University of Ljubljana, Faculty of Medicine, SI: Fungal lessons about endocrinology
14:55–15:20	**Coffee break**
15:20–15:35	**Borut Peterlin**, University Medical Centre, SI: From Reverse Genetics to Genomic Medicine
15:35–15:50	**Janja Marc**, University of Ljubljana, Faculty of Pharmacy, SI: Secret OMICs life of skeleton
15:50–16:05	**Irena Zupanič Pajnič**, University of Ljubljana, Faculty of Medicine, SI: Ancient DNA (aDNA) Analyses in Slovenia
16:05–16:20	**Petra Hudler**, University of Ljubljana, Faculty of Medicine, SI: Genetic background of gastric cancer
16:20–16:35	**Tamara Lah Turnšek**, National Institute of Biology, SI: Brain Tumour Microenvironment is Driving Force of Glioblastoma Progression and Therapy Target
16:35–17.05	**Serge Muyldermans**, Vrije University Brussel, B: Camel antibodies in reverse proteomics to identify new glioblastoma biomarkers
17:05–19:00	**Dinner with poster session**

Lectures

Od »sintezne biologije« do »nanomedicine« in nazaj

Radovan Komel

Medicinski center za molekularno biologijo, Inštitut za biokemijo, Medicinska fakulteta, Univerza v Ljubljani

Po moji znanstveno raziskovalni adolescenci, ki jo je zaznamovalo raziskovanje mikrobioloških pretvorb steroidov za biotehnološke potrebe, je prišel trenutek srečanja s takrat v Sloveniji še neuveljavljeno gensko tehnologijo oziroma genskim inženirstvom in v sredini osemdesetih let preteklega stoletja sem po podoktorskem usposabljanju na Tehniški univerzi v Gradcu to metodologijo začel uvajati v naše biokemijske in biotehnološke raziskave na Inštitutu za biokemijo Medicinske fakultete Univerze v Ljubljani. Ker smo bili petelini, ki se oglašajo prezgodaj, smo z razmeroma prešibkim znanjem vendar močno ambicijo želeli genetski informaciji dveh različnih mikroorganizmov (bakterije in nitaste glive) združiti v enem samem mikroorganizmu, ki bi s tem postal za biotehnološko proizvodnjo glukokortikoidnih hormonov pomemben biotehnološki organizem. Temu se je takrat reklo »metabolično inženirstvo«, danes pa se taki pristopi slišijo na sodobnejše in bolj zveneče ime »sintezna biologija«. Kakor koli že, prvi encim, mikrobno 1:2-dehidrogenazo, ki v prvi steroidni obroč glukokortikoidov uvaja dvojno vez in mu s tem odvzame neželjene stranske učinke, smo uspešno izrazili v bakteriji *E. coli* leta 1993. Na žalost pa kloniranje 11β-hidroksilaze, encimskega sistema, ki z uvedbo OH-skupine v steroidno jedro vzpostavi njegovo gluko/mineralo-kortikoidno funkcijo, nismo uspeli in do danes tega membransko vezanega encima nitastih gliv še nihče ni kloniral, toliko za malce kislo tolažbo… Medtem sem bil tudi povabljen, da prevzamem vodenje raziskovalne skupine na Kemijskem inštitutu, ki je bila sestavljena iz raziskovalcev kemijskega inštituta in farmacevtske družbe LEK, s katero sem sodeloval že prej, v sedemdesetih in osemdesetih letih, pri raziskavah mikrobne produkcije srčnih spodbujevalcev ergot alkaloidov. V zgodnjih devetdesetih letih smo vzpostavili laboratorije za gensko tehnologijo ter produkcijo in prečiščevanje rekombinantnih proteinov, kot »šolski model« smo izbrali dejavnik tumorske nekroze TNFα, skupina pa je v nadaljevanju izdelala celovit produkcijski sistem za rekombinanten biofarmacevtik, ki je bil nato kot najboljši sprejet v globalno proizvodnjo pri Novartisovi generični družbi Sandoz, novemu lastniku našega farmacevtika LEK. Jaz sem pri tem imel vlogo koordinatorja skupine, so pa številni moji doktorski raziskovalci, ki so delali na tej problematiki, našli zaposlitev v družbi LEK-Sandoz, kjer danes opravljajo vidne zadolžitve v njeni enoti Biofarmacevtika. Raziskovalna skupina Kemijskega inštituta je bila tudi sestavni del medinštitutske programske skupine Funkcijska genomika in biotehnologija za zdravje, ki je pod mojim vodstvom opravljala raziskovalno delo skupaj z Medicinskim centrom za molekularno biologijo na Medicinski fakulteti. Leta 2004 smo na Kemijskem inštitutu uspešno klonirali glivno benzoatno 4-monoksigenazo (BPH) in nato raziskavo usmerili v preučevanje tega citokroma kot potencialne nove tarče za razvoj protiglivnih zdravil. Ker smo ugotovili, da bi encim BPH morda lahko uspešno

blokirali s sintetičnimi analogi obrambnih snovi, ki jih izloča z glivico napadena rastlina, smo del raziskav posvetili akutnemu vprašanju smrekovega lubadarja, katerega invazija je povezana s simbioznim odnosom med žuželko in glivico pomagalko. Prišli smo do obetavne spojine vodnice, dlje pa ni šlo, saj je skupina leta 2011, ko sem v povezavi z nesrečno zgodbo o centrih odličnosti zapustil Kemijski inštitut, prenehala z dejavnostjo na tem področju.

Medtem smo leta 1992 na Medicinski fakulteti ustanovili Medicinski center za molekularno biologijo, katerega namen je bil usposabljati strokovnjake na področju analize genov človekovega genoma, uvajati pristope molekularnogenetske diagnostike in nuditi pomoč pri vzpostavljanju ustreznih laboratorijev v naših raziskovalnih in kliničnih ustanovah. Vse to je temeljilo na pionirski in uspešni uvedbi genske preiskave pri enogenski bolezni cistična fibroza v obdobju 1988-1990, kasneje pa se je nadaljevalo tudi z genskimi preiskavami bolezenskih genov pri drugih enogenskih boleznih kot so hemofilija A, hiperplazija nadledvične žleze, policistična ledvična bolezen, kožne keratinske motnje in osteoporoza, ki pa že pomeni prehod na preučevanje molekularnih osnov kompleksnih procesov, kot je regeneracija tkiv, in kompleksnih večfaktorskih bolezni kot so psihiatrične motnje (motnje hranjenja, samomorilno vedenje) in rak (limfom, adenokarcinom želodca, HNPCC). To je bilo zelo plodno obdobje, saj smo opravili prve mutacijske analize genov človeka v našem prostoru, uvedli genotipizacijo za potrebe sode medicine, usposobili številne odlične strokovnjake ter izsledke in metodologije uspešno prenesli v diagnostične prakse na naših kliničnih ustanovah. Kompleksne bolezni, v katere so vpletene, na prvi pogled večkrat tudi »nevidne« spremembe oziroma različnosti številnih, tudi do nekaj deset, sto ali tisoč različnih genov, so seveda bistveno večji zalogaj za molekularno medicino. Potrebne so dolgoletne, kadrovsko in finančno izdatno podprte temeljne multidisciplinarne raziskave, ki običajno potekajo v okviru konzorcijev večjega števila raziskovalnih ekip, in hitri prenosi v klinične prakse niso samo po sebi umevni. Imamo srečo, da v tem trenutku vodimo mednaroden projekt TRANS-GLIOMA, ki je naslednik predhodnega projekta GLIOMA in v veliki meri izpolnjuje prej navedene pogoje. Naš namen je poiskati proteinske označevalce matičnih celic najbolj maligne oblike tumorjev možganov, glioblastoma, in oblikovati ustrezne strategije za ciljano zdravljenje te vrste rakavega obolenja. Rakave matične celice so namreč hud problem, saj v možganskem tkivu ostanejo tudi po kirurški odstranitvi tumorja, so odporne na radio- in kemo-terapijo in zato odgovorne za hiter povratek bolezni. Za reševanje tega problema smo uvedli metodologijo pridobivanja in uporabe posebne oblike protiteles, tako imenovanih nanoteles, ki so rekombinantno pridobljene variabilne domene posebnih, v kamelidi prisotnih, težkoverižnih protiteles. Lepota pristopa je v tem, da omogoča sočasno identifikacijo za rakave celice specifičnega antigena oz. proteina in obenem njemu odgovarjajočega nanotelesa, ki je takoj na voljo za nadaljnje poskuse ugotavljanja biološke vloge ugotovljenega antigena kot tudi iskanja primernega načina za njegovo dostavo in ciljano terapijo. Prvi poskusi kažejo, da specifična nanotelesa sama po sebi razmeroma uspešno prehajajo krvno-možgansko pregrado in v tarčne celice, kjer pomembno blokirajo neželene proteine, za izboljšanje natančnosti dostave pa v tem trenutku preučujemo tudi prenos z

vezikli kot so umetni liposomi in posebno prilagojeni naravni ekstracelularni vezikli. Časovno malo bolj odmaknjena pa je ideja, da bi, v primeru uspešnih rezultatov poskusov z nanotelesi tako na ravni celičnih linij kot tudi živalskih modelov, v prenosni »voziček« uvedli genetsko informacijo za nekaj vrst nanoteles, ki bi se v tarčnih celicah izražale nadzorovano, v različni časovni dinamiki po sprožitvi ustreznih ukazov. Sestavljenje takega načina multiplega ciljanja onkoproteinov matičnih celic glioblastoma pa seveda zahteva znanja in metode, ki so blizu sodobne »sintetične« ali »sintezne« biologije. Kot vse kaže, smo se tako nekako vrnili na izhodišče iz moje znanstveno-raziskovalne adolescence, vendar tokrat upam, da bodo znanje, ki je morda na nekoliko višji ravni, odlični sodelavci in pomembna sodelovanja skupaj prispevali k srečnim zaključkom, ki se bodo kot droben kamenček lahko vključili v korist razvoja naše biomedicinske znanosti.

From »synthetic biology« to »nanomedicine« and back

Radovan Komel

Medical Centre for Molecular Biology, Institute for Biochemistry, Faculty of Medicine, University of Ljubljana, Slovenia

After the end of my scientific research adolescence, which was marked by the research of microbiological transformations of steroids for biotechnological purposes, the moment of meeting with the then in Slovenia in the unrecognized gene technology or genetic engineering took place, and in the middle of the 1980s, after post-doctoral training at the Technical University in Graz this methodology was introduced into our biochemical and biotechnological research at the Institute of Biochemistry of the Faculty of Medicine of the University of Ljubljana. Because we have been roosters that sing too early in the morning, we wanted with a relatively weak knowledge, but a strong ambition, to combine the genetic information of two different microorganisms (bacteria and filamentous fungi) in a single microorganism which would thus become an important biotechnological organism for the production of glucocorticoid hormones. This was then called "metabolic engineering," but today such approaches are heard on a more recent and more sublime name "synthetic biology". In any case, the first enzyme, a microbial 1:2-dehydrogenase, which introduces a double bond in the first steroid ring of glucocorticoids, thereby depriving it of undesired side effects, was successfully expressed in E. coli in 1993. Unfortunately, the cloning of 11β-hydroxylase, an enzyme system that, with the introduction of the OH group into the steroid core, establishes its gluco/mineralo-corticoid function, did not succeed, and to date no one has cloned this membrane-bound enzyme of filamentous fungi, just for a little bitter comfort ... Meanwhile, I was invited to take charge of a research team at the National Institute of Chemistry, which consisted of researchers from the chemical institute and pharmaceutical company LEK, with which I collaborated in the seventies and eighties in the research of the microbial production of heart stimulating dugs, ergot alkaloids. In the early nineties, we established laboratories for gene technology and the production and purification of recombinant proteins, as a "training model" we selected tumor necrosis factor TNFα, and the group subsequently produced a complete production system for a recombinant biopharmaceutical, which was then selected and accepted in global production at Novartis's generic company Sandoz, the new owner of our pharmacist LEK. I had the role of coordinator of the group, but many of my doctoral researchers who worked on this issue found employment in the company LEK-Sandoz, where today they perform visible tasks in its unit Biopharmaceutica. The research group of the Institute of Chemistry was also an integral part of the interinstitutional research program group Functional Genomics and Biotechnology for Health, which under my leadership carried out research work together with the Medical Centre for Molecular Biology at the Faculty of Medicine. In 2004, at the Institute of Chemistry we have successfully cloned fungal benzoate 4-

monoxygenase (BPH) and then directed our research to the study of this cytochrome as a potential new target for the development of antifungal drugs. Since we found that the BPH enzyme could be successfully blocked with synthetic analogues of defence substances that are secreted by the plants after fungal attack, part of the research was dedicated to the acute issue of the spruce bark beetle whose invasion is related to the symbiotic relationship between the insect and its supporting fungus. We came to the promising lead compound, but research did not go any further, since the group in 2011, when I left the Institute in connection with the unfortunate story of the centres of excellence, ceased its activity in this field.

In the meantime, at the Medical Faculty in 1992, we established the Medical Centre for Molecular Biology, which aimed to train experts in the field of gene analysis of the human genome, to introduce approaches of molecular genetic diagnostics and to provide assistance in establishing appropriate laboratories in our research and clinical institutions. All this was based on the pioneering and successful introduction of a genetic analysis of the monogenic disease cystic fibrosis in the 1988-1990 period, and which later continued with genetic analysis of disease genes in other monogenic diseases such as haemophilia A, hyperplasia of the adrenal gland, polycystic kidney disease, skin keratin disorders and osteoporosis, which already means a transition to the study of molecular basis of complex processes such as tissue regeneration and complex multifactorial diseases such as psychiatric disorders (eating disorders, suicidal behaviour) and cancer (lymphoma, gastric adenocarcinoma, HNPCC). This was a very productive period because we performed the first mutation analyses of human genes in our area, introduced genotyping for the needs of forensic medicine, trained many excellent experts and successfully translated the results and methodology into diagnostic practices at our clinical establishments. The complex diseases which can harbour changes or variations of many, even up to a few dozen, hundreds or thousands of different genes, that at the first glance are often "invisible, of course, represent a significantly larger challenge of molecular medicine. Human resource and financially supported long-term basic multidisciplinary research is required, which usually takes place within the consortia of a large number of research teams, and rapid transmissions into clinical practices are not always self-evident. We are fortunate to run an international project TRANS-GLIOMA, which is the successor to the previous GLIOMA project and to a great extent meets the above criteria. Our intention is to find protein markers of stem cells of the most malignant form of brain tumours, glioblastoma, and to formulate appropriate strategies for the targeted treatment of this type of cancer. Cancerous stem cells are a serious problem because they remain in the brain tissue even after surgical removal of the tumour, they are resistant to radio- and chemo-therapy and are therefore responsible for the rapid relapse of the disease. In order to solve this problem, we introduced a methodology for the production and use of a specific form of antibodies, so-called nanobodies, which are recombinantly derived variable domains of the particular heavy chain antibodies, present in camels and llamas. The beauty of the approach is that it enables simultaneous identification of specific antigens or proteins of cancer cells and at the same time the corresponding nanobodies, which

are immediately available for further attempts to determine the biological role of the antigens found, as well as the search for an appropriate route for their delivery and targeted therapy. The first attempts indicate that specific nanobodies themselves are relatively successful in transcending the blood-brain barrier and in targeting cells where significantly blocking unwanted proteins; however, for the purpose of improving the accuracy of delivery, we are also studying their transfer with vesicles such as artificial liposomes and specially adapted natural extracellular vesicles. A bit more distant idea, however, is the development, in the case of the successful results of experiments with nanotechnologies, both at the level of cell lines and in animal models, of the transporting vehicle, in which genetic information would be introduced for several types of nanobodies, which would be expressed in the target cells in a controlled, selected time dynamics after being triggered by the corresponding signals. The formation of such a method of multiple targeting of oncoproteins of glioblastoma stem cells, of course, requires knowledge and methods that are close to modern "synthetic" biology. As it seems, we have somehow returned to the starting point of my scientific-research adolescence, but this time I hope that knowledge, which may be at a somewhat higher level, excellent co-workers and important collaborations will together contribute to the happy conclusions that will be like a tiny pebbles to be incorporated in favour of the development of our biomedical science.

New genes for old hearts

Mauro Giacca

International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

There is an impelling need to develop novel therapeutics to combat tissue degeneration and ageing. In this perspective, we wish to identify effective growth factors and small RNAs, to be used as innovative drugs, by functional screenings. On the one hand, we have generated an AAV library coding for the mouse secretome (1200+ viral vectors) and developed a procedure for the direct, in vivo screening of this library for factors protecting against myocardial infarction, diabetes and other degenerative conditions. On the other hand, we took advantage on *ex vivo* high throughput robotic screenings to search for microRNAs inducing cell proliferation and organ regeneration. The results so far obtained have revealed a few factors protecting from heart failure by inducing autophagy and several microRNAs that stimulate cardiomyocyte and senescent cell proliferation; a few of the identified microRNAs induce cardiac regeneration and tissue repair during aging in vivo.

Young science (to say nothing of the fish)

Marina Dermastia

National Institute of Biology, Ljubljana, Slovenia

My mutual history with Professor Radovan Komel has lasted for more than 30 years. He was my first mentor, but *vice versa*, I was also his first student in the then new Slovenian research scheme known as young researchers. I feel very privileged to be involved in many of his great ideas about establishing the field of molecular biology and biotechnology on Slovenian ground. Many of today's eminent scientists gathered in his laboratory, not only to do science but also to learn simple things, such as making an electrophoresis tank or a chromatographic column from a glass pipette. Although it sounds quite odd now, that was the reality of the eighties; and not only in Slovenia. However, Professor Komel was, even at that time, a cosmopolitan scientist. He worked in France, Austria, Germany, Italy, and brought new ideas to Ljubljana. He also encouraged us to go abroad and to publish scientific papers, which is a standard now, but was exceptional at that time. With a lot of enthusiasm, he helped us bring great science to the industry and solve genetically linked diseases with the help of molecular biology. These today sound as big modern inventions and everyone talks about them, but in fact, Professor Komel paved the road for them decades ago. As time passed, our scientific paths parted, and my research work literally returned to the roots. However, more recently, we met again in combining our passion for teaching and communicating science, this resulting in an important European award.

Non-clinical and clinical aspects of biosimilar development

Ljerka Lah

Novartis/LEK d.d., Mengeš, Slovenia

The presentation will cover a general overview of Biosimilars development, which includes (i) the definition of biosimilars development goals and target; (ii) target-directed development of product quality attributes, which can be influenced at all stages of cell line and process development; (iii) biosimilarity confirmation and the regulatory basis of biosimilars approval and the (iv) principles of Quality-by-Design (QbD) development for biosimilars.

Breast cancer and stromal cell co-culture models for immuno-conjugate therapy optimization

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Antibody drug conjugates (ADC) are promising biological therapeutics against various cancers. In the subgroup of HER2 positive breast carcinoma (BCa), the patients have been shown to benefit from treatment with Kadcyla®, a HER2 monoclonal antibody trastuzumab, conjugated with a toxic microtubulin inhibitor mayatansinoid (1). However, despite the effectiveness of Kadcyla®, a significant population of patients still progress to a later stage of the disease (2-4). The underlying mechanisms could be attributed to the type of $HER2^+$ BCa tumour and cancer cell heterogeneity, and on the other hand also to poor penetration of Kadcyla® into the multicellular tumour mass, induction of resistance mechanisms against the toxin, and other properties of this drug (5).

Due to their complex activity antibody drug conjugates need to be carefully designed for their optimal performance. The aim of our study was to establish reliable *in vitro* models to evaluate these parameters and utilize the findings for further improvement of aHer2 ADCs

To prepare 3D spheroids of mono- and co-cultured models, we selected the SkBr3 cells (originating from pleural effusion) expressing highest HER2 protein levels, and lower HER2+ HCC1569 cells (from solid tumours) out of a panel of BCa lines.

First, we found that HCC1569 cells were more drug resistant than SkBr3. In contrast to Kadcyla®, the treatment with the "control" cancer drug 5'Fluoro uracil (5FU) seemed to be efficient on both types of spheroids. This indicates that different morphology of BCa cells associated with spheroid shape and compaction as well as differences in HER2 expression levels, were relevant for Kadcyla® response. Therefore, cell surface targeting by trastuzumab may be more dependent on cells' membrane plasticity, spheroid formation capacity and compactness compared to small drugs inhibitors of cell proliferation /metabolic rate.

Secondly, to reach the tumour cells in vascularized tumours, Kadcyla® should penetrate the layers of endothelial cells, mimicked by coated and mixed spheroids models of BCa: endothelial cells (HUVEC) co-cultures. Better compaction was observed in HUVEC mixed spheroids compared to monoculture spheroid models. However, the toxicity testing with HUVEC cells was less consistent, as HUVEC cells tend to overgrow BCa cells during the assays, indicating angiogenic growth stimulation by the BCa tumour cells.

BCa heterogeneity also entails the presence of various levels of stromal cells, which possibly protect tumour cells from Kadcyla®. Most relevant type of stromal cells are myoepithelial cells. In the model of BCa: MCF10A spheroid co-cultures, we found impaired delivery of these drugs to tumour cells compared to monocultures, in particular in HCC1569 cells. Here, we partially resolved the downstream effects of Kadcyla® on induction of immunogenic cell death (ICD) by testing its ability to induce calreticulin and HMGB1 translocation, ATP release and enhanced activity of effector caspases 3 and 7.

In conclusion: Different resistance to cytotoxic drugs observed between the treated types of mono and mixed spheroids indicate that the stromal part of the tumour microenvironment may play a crucial role in tumour resistance to ADCs, such as Kadcyla®.

1. Kadcyla® is a trademark © of Genentech, Inc. 2016

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Fungal lessons about endocrinology and genomics

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The story starts with 10-year research in the microbial world under supervision of prof. dr. Radovan Komel who was my M.Sci and Ph.D. mentor. Initially we worked on fungi from the genus *Claviceps* where we aimed to increase the production of ergotalkaloids in collaboration with Chemical institute. The biotechnological path continued with aims to clone the first fungal cytochrome P450 enzyme that performs 11-beta hydroxylation of steroids in *Cochliobolus lunatus* – on the way towards pharmaceutically important corticosteroids. We never found the gene but I learned my first steps of genomics – isolating DNA, separating fungal chromosomes, transforming protoplasts with foreign DNA and thinking about cloning genes when PCR was not yet around. I also got excited about steroid hormones and endocrinology. We were reading mostly about mammalian enzymes that enable synthesis of steroid hormones since nothing was known about the fungal systems at that time. Looking back I certainly was lucky. Prof. Komel was a mentor who supported my early creativity. He let me leave the lab with my own ideas about what I want to do in my future career. And indeed my research stayed in the broader areas of functional genomics and endocrinology. I was isolating DNA from mouse, rat. pig and human aimed at cloning the first mammalian cytochrome P450 from cholesterol synthesis (when PCR was already around), mapping CYP51 genes on human chromosomes, transforming cells with foreign DNA to monitor transcriptional activity and more – just as the fungi had taught me.

From reverse genetics to genomic medicine

Borut Peterlin

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In the past 30 years, we faced an enormous advance in human genetics. Positional cloning, a hypothesis-free approach to identification of human genes, enabled identification of genes for frequent genetic diseases such as cystic fibrosis, Duchenne muscular dystrophy, and spinal muscular atrophy and led to the development of genetic diagnostics.

Efficacy of testing depended on correct diagnostic hypothesis and molecular pathology of given disease.

New genomic technologies, especially next-generation sequencing have revolutionized not only identification of new genes for human disorders but also diagnostics of rare, genetic diseases. Systematic application of exome sequencing in Slovene health system had a significant impact on the access of patients to genetic testing and efficacy of genetic diagnostics and provided an opportunity to identify new genes while providing "routine" diagnostic testing. Moreover, it gives us an insight into genomic variability in Slovene population and potential for implementation of genomic testing in the context of personalized medicine.

Secret OMICs life of skeleton

Janja Marc

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With the ageing of population the incidence of age-related diseases including osteoporosis increases. The major focus of bone research is to identify better predictors of future fractures and novel drug targets for (if possible) anabolic treatment of osteoporosis. In recent years, the lower costs of arrays and sequencing technologies, and data from genome-wide association studies have led to more reports on genetic factors that are associated with bone health. Genome-wide association studies (GWAS) revealed dozens of novel genetic loci that are associated with bone mineral density (BMD). Some of these targets have already been functionally characterized, although the vast majority have not. Hits that were significantly associated with BMD in different studies represent likely candidates (e.g. SOST, WNT16, ESR1, RANKL) for functional characterization and development of osteoporosis treatments. In two decades of our bone research, which we started with prof. Komel, we identified and/or evaluated over 50 genetic variations in nearly 20 candidate genes and we are pioneers in osteoporosis pharmacogenetics and also epigenetics. Namely, epigenetic mechanisms, which include microRNAs are key regulators of gene expression and we performed one of the first studies to identify osteoporosis related microRNAs. As the partner of GEFOS/GENOMOS consortia we entered "bone OMICs world" by GWAS studies and transcriptome analyses of primary osteoblasts. We recently showed, that adrenergic receptor α2A (α2A-AR) is significantly upregulated in osteoporotic bone osteoblasts and ADRA2A is involved in neuro-endocrine regulation of bone resorption. In the next few years, sophisticated functional studies on model organisms and mesenchymal stem cells through sophisticated computational analyses that can integrate '-omics' data will provide novel more significant genetic loci important for regulation of bone remodelling.

Ancient DNA (aDNA) analyses in Slovenia

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For extracting genomic DNA from Second World War (WWII) victim's remains and skeletal remains from archaeological sites, a highly efficient extraction protocol was developed in Slovenia. Procedures for processing the bone and tooth samples, DNA extraction, quantification and typing (for some of aDNA analyses massive parallel sequencing technology was already used) will be shown, and the measures for preventing contamination in the DNA laboratory will be discussed. The characteristics of aDNA and the environmental factors that affect its preservation will be described. Since over 100,000 victims of post-war killings are still buried in hidden mass graves all over Slovenia and remain unidentified, some examples of identification of the WWII mass grave victims will be presented, focused on 88 victims from Konfin I mass grave where 32 victims were identified, followed by identification of Ksenija and Rado Hribar (the spouse Hribar came from well-known Slovenian families, who were part of the pre-war elite in Slovenia), and identification of victims of the biggest family killing happened in Slovenia where 10 members of the same family were killed, and seven of them buried in a hidden mass grave Babna gora. Molecular genetic analyses of skeletons from archaeological sites will be presented as well, including badly preserved skeletons from the Auersperg tomb that were excavated in 2009 at the open market place in Ljubljana (the first archaeogenetic research performed in Slovenia), and two recent studies of aDNA extracted from 15th century femurs presumably belonged to counts of Celje, and some ancient animal bones.

Genetic background of gastric cancer

Petra Hudler

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Prof. Komel led me into the world of molecular research as my MSc and PhD supervisor, encouraging me to dive into the abyss of genetic studies of gastric cancer. Gastric cancer is a major health problem worldwide. It is mostly diagnosed at an advanced stage when the prognosis is poor. Despite advances in treatment modalities, it remains the second leading cause of cancer-related death in the world. Molecular mechanisms of its development are complex and still largely unclear. In my MSc studies, I initially focused on mutational analyses of mismatch repair genes in tumour and non-tumour samples, obtained from Slovenian patients. We discovered several genetic variants, which were later further explored using yeast two hybrid system. Additionally, I was engaged in various other projects, involving the studies of molecular mechanisms of other diseases.

The complexity of gastric cancer led me further into the explorations of gene expression patterns. Screening for differentially expressed genes is a straightforward approach to elucidate molecular basis of a disease and comparing molecular signatures of changes that occur in tumour tissues at different stages of development holds a great promise to advance our understanding and treatment of cancerous diseases. I identified gene expression profiles in tissues of gastric cancer and in biopsies of gastric precancerous lesions by constructing a cDNA library using suppressive subtraction hybridization (SSH). Data analyses of differentially expressed genes showed differences in gene expression in tumour tissues compared to corresponding normal mucosa and also differences between precancerous lesions compared to gastric tumour tissues. I also identified differences in gene expression between individual patients, which confirmed the heterogeneous nature of this disease. Despite these differences, the analyses showed that the consequence of these changes is deregulation of several signalling pathways controlling the homeostasis of gastric mucosa, including immune response, cell cycle and cell growth regulation, cell adhesion, regulation of cytoskeleton, DNA repair genes, and so on. One of these, MAPK signalling pathway, was found to be affected through changes in expression of different genes in all patients. Therefore, we, as well as other researchers in the field, identified critical signalling pathways involved in the development of gastric cancer, which could be modified by appropriately selected targeted treatments.

To conclude, working under the supervision of Prof. Komel helped me to develop valuable skills, critical thinking and inspired me to continue the studies in this field.

Brain tumour microenvironment is driving force of glioblastoma progression and therapy target

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The brain tumor microenvironment is emerging as a critical regulator of cancer progression in primary and relapsed brain malignancies. It is comprised of stromal cells, embedded into brain–specific extracellular matrix. The unique properties of this organ require a specific framework for designing the microenvironment-targeted interventions. Here we will discuss two types of brain-resident cell types, the endothelial cells and infiltrating mesenchymal stem cells (MSC). The endothelial cells are of great interest as brain tumour regulators, due to the formation of blood-brain barrier and they also invade into the tumour mass during the process of angiogenesis. The most relevant is the fact that in the arterioles, these cells are crucial for glioblastoma stem cells' niche formation. Mesenchymal stem cells got most attention due to their tricky, differential interactions with various glioblastoma cell subtypes involving immune- related cytokines signaling and bradykinin induced Ca++ signalling. The cross-talk among these heterogeneous cell types represents new targets, being less resistant to therapeutic modalities that are currently used for glioblastoma treatment. Only comprehensive understanding of the complex and interconnected micro environmental landscape of brain malignancies will enable to expand the range of therapeutic strategies available to target these deadly diseases.

Camel antibodies in reverse proteomics to identify new glioblastoma biomarkers.

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Camelids possess IgG antibodies comprising only heavy chains and no light chains Therefore, the antigen is recognised by these HCAbs by virtue of one single domain, the VHH. The VHH has a size in the low nanometer range and when it is expressed recombinantly it is referred to as Nanobody (Nb).

In a “reverse proteomics” effort we first immunize a camelid with a total proteome extract (or a subproteome fraction (e.g. membrane proteins or nuclear protein extract) to elicit HCAbs against the various proteins. After cloning the VHH and retrieving with phage display the Nbs against proteome targets that are only present in e.g. diseased tissue but not in healthy tissue. Once a Nb is identified, that binds differentially to contents of diseased tissue and healthy tissue, the most difficult part is to identify the cognate antigen. This is achieved by producing the Nb protein and using it to capture the target from the proteome mixture followed by its MS characterization. In principle, this “reverse proteomics” strategy allows to identify new and unknown targets (neo-targets) that are not the result of an increased or decreased transcription, but for example from an altered glycosylation. The proof of principle will be shown during the lecture, together with the collaboration on glioblastoma with the group of Professor Radovan Komel.

Poster session presenters

Yeasts & extracellular vesicles & pore forming proteins

Apolonija Bedina Zavec

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The beginning of my research path has begun 20 years ago at the National Institute of Chemistry. The research work was led by Prof. Dr. Radovan Komel, the head of the Department for biosynthesis and biotransformations, and mentor of my master's and doctoral thesis. My work was led by researcher Dr. Aleksandra Comino, a former student of Prof. Komel. We have studied the cell cycle in the budding yeast Saccharomyces cerevisiae and we have used Yeast two hybrid systems for detecting protein-protein interactions in vivo. We have also collaborated with Dr. Carlo Bruschi who was working on chromosome translocations. Our research has been published in a prestigious PNAS magazine. Later, in collaboration with Dr. Veronika Kralj-Iglič, I have started to work on extracellular vesicles. The initial work on extracellular vesicles in clinical samples has led to the development of a new method for bioprocess monitoring. The method was developed in cooperation with pharmaceutical company Lek. A few years ago, Dr. Gregor Anderluh has come to the National Institute of Chemistry. He works on pore forming proteins, which play a key role in virulence and immune defence. Together with Dr. Marjetka Podobnik, the co-leader of our research group, we were able to publish our results in a prestigious Science magazine. My work was influenced by outstanding researchers, and I am grateful to them, especially to Prof. Komel who has mentored me at the start of my research career.

New steroid 5alpha-reductase type I (SRD5A1) homologous sequences on human chromosomes 6 and 8

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To date, two genes encoding 5alpha-reductase isoenzymes are known (type I, type II), and one type I pseudogene. The divergent localization of these genes and the still not fully understood function of the encoded enzymes as well as the perplexing results we obtained after sequencing PCR-amplified SRD5A1 gene fragments (out of genomic DNA), made us assume that, in addition to the known SRD5A1 gene, one or more different human 5alpha-reductase type I coding genes may exist. Our research provides the first evidence for the existence of two new SRD5A1 related, previously unidentified sequences in the human genome. These sequences which were localized to chromosomes 6 and 8 are highly homologous (> 99%) to SRD5A1, and also do not contain any deletions or insertions that are otherwise a characteristic of the SRD5API pseudogene. Our results imply that these sequences may be either coding parts of yet unknown, active SRD5A1 genes, and/or of previously unidentified pseudogenes. These findings additionally support data of Chen *et al*. who confirmed the existence of various SRD5A1 proteins in cultured human skin cells.

Nanobody

Damjana Kastelic

Department of International and Scientific Affairs, Centre for Genomic Regulation, Barcelona, Spain

Sanjski protein vsakega molekularnega biologa,
ki v zavetju laboratorija upa in čaka,
da bo njegov eluat visoko koncentriran in neprecipitiran,
da ne bo sticky, da ne bo moten, ampak bo visoko topen.

Nanobody vsem si nam v veselje,
stabilen in robusten, da se te lahko melje.
Tudi če te pozabimo na laboratorijskem pultu nam odpustiš,
in svoje specifičnosti ne izgubiš.

Čeprav nekajkrat manjši kot IgG tvoj veliki brat,
se svoje velikosti nimaš kaj sramovat.
Označen nam pokažeš pot,
zlahka se prebiješ do metastaz in drugih zarot.

V imunoterapiji si nepogrešljiv,
in v biotehnologiji nenadomestljiv:
označiš biomarkerje, blokiraš encime,
nevtraliziraš viruse in kristaliziraš proteine.

Pred več milijoni let si se pojavil,
v krvi kamel, dromedarjev in lam.
Verjetno se že sprašujete: ´´Le zakaj?´´
No, tudi tokrat je Darwin imel prav.

Gastric cancer proteomics

Nina Kočevar Britovšek

Lek d.d., DS BioProduction Mengeš, Slovenia

Gastric cancer (GC) is in the top five of the most commonly diagnosed malignancies and one of the most common causes of cancer-related deaths worldwide. The majority of patients are diagnosed at a late stage, when the prognosis is poor. Therefore it is a challenge for medical doctors as well as for scientists to find new biomarkers for early diagnosis and drug targets for effective treatment.

Professor Radovan Komel has enabled me to become a part of this effort tackling the gastric cancer challenge: using two-dimensional gel electrophoresis (2-DE) in combination with mass spectrometry (MS) we investigated tissue samples of gastric adenocarcinoma, the most common form of GC, and adjacent non-tumour tissues on the protein level.

First we performed 2-DE in the acidic pH range (4-7) on twelve pairs of GC tissue samples. After computational comparison of tumour and non-tumour samples, 32 differentially expressed spots were analyzed with MS. We identified 30 unique proteins involved in various biological processes, e.g., metabolism, response to stress cell cycle. Eight proteins were chosen for further validation by immunoblotting. Our results showed that gastrokine-1 (GKN1), 39S ribosomal protein L12 (mitochondrial precursor, MRPL12), plasma cell-induced resident endoplasmic reticulum protein (PACAP), and glutathione S-transferase mu 3 (GSTM3) were statistically significantly under-expressed in GC. On the other hand, septin-2 (SEPT2), ubiquitin-conjugating enzyme E2 N (UBE2N), and transaldolase (TALDO1) were statistically significantly over-expressed. Translationally controlled tumour protein (TPT1) was shown to be differentially expressed only in patients with cancer of the gastric cardia/oesophageal border.

While acidic and wide pH ranges have been widely investigated, alkaline pH range was a bit more challenging, so we initially optimized 2-DE in pH range 7-11 for gastric tissue samples and then analysed twelve pooled non-tumour and pooled tumour samples for proteins with altered abundance. We identified 38 spots as 24 different proteins. Four of these were chosen for investigation with immunoblotting on individual paired samples to determine whether the observed changes represent the overall abundance of the protein or possibly only a single form. While mitochondrial trifunctional protein (MTP) subunits were decreased in 2-DE gels, immunoblotting identified their overall abundance as being differently dysregulated: in GC, MTP-α subunit (HADHA) was under-expressed, and MTP-β subunit (HADHB) was over-expressed. On the other hand, heterogeneous nuclear ribonucleoprotein M (HNRNPM) and galectin-4 (LGALS4) were over-expressed in GC both in 2-DE and immunoblotting.

The work described above contributes to the efforts of understanding gastric cancer carcinogenesis and possibly also to the biomarker hunt. Furthermore, it helped shape me into the researcher I am today and it wouldn't have been possible without my PhD mentor. Happy birthday, Professor!

Introduction of molecular biology methods in diagnostics of lymphomas

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Background. Clonality of lymphoid proliferations has important diagnostic value for distinguishing between neoplastic and reactive lesions. The first studies using the PCR-based techniques for clonality analysis were published in the early nineties of the last century. The aim of our study was to implement the PCR-based methods to improve the final diagnosis of patients with lymphoproliferative disorders. The study was performed in collaboration of the Medical Centre for Molecular Biology, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana and the Department of Pathology, Institute of Oncology Ljubljana.

Materials and methods. With this purpose, 50 formalin-fixed, paraffin-embedded tissue specimens from patients with non-Hodgkin lymphomas were retrospectively analysed. Clonality testing was performed using the "in-house" methods which were implemented according to Slack DN *et al*. and Trainor KJ *et al*. Methods are based on PCR amplification of the rearranged IGH and TCRG genes and electrophoretic separation of amplified products.

Results. Monoclonal IGH rearrangements consistent with the presence of monoclonal B-cell populations were detected in 13 of 20 (65%) analysed B-cell lymphomas. Monoclonal TCRG rearrangements consistent with the presence of monoclonal T-cell populations were detected in 20 of 30 (66.7%) analysed T-cell lymphomas. Detection rates of both methods were in agreement with the published results.

Conclusions. We have implemented PCR-based methods for the detection of clonality in a routine diagnostical setting of non-Hodgkin lymphomas. "In house" methods have been successfully performed at the Institute of Oncology Ljubljana from 1997 to 2009, when they have been replaced by the standardized BIOMED-2 assays.

Prolonged effects of neonatal and pubertal stress on adult behaviour and some physiological parameters

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Stress is a natural, beneficial response of living organisms to adverse environment and threats. Yet prolonged stress could have deleterious effects for health and numerous studies have shown that prolonged stress in adult life affects mental health, and could cause increased risk for cardiovascular diseases and obesity. Some studies in recent years have shown that exposure to stress mediating glucocorticoid hormones in neonatal period could have long lasting effects for health in adult life of individuals, exposed to stress during neonatal period. In our studies, we examined the effect of prenatal stress, caused by injection of pregnant mice, and effects of social isolation stress during puberty, on behaviour and some physiological parameters in adult life in laboratory mice. Results have shown that injections of pregnant mice cause strong alterations in aggressive, but not sexual behaviour, in adult male offspring of stressed mothers. Furthermore, such stress caused reduced levels of testosterone, diminished daily sperm production and increased body weight in male mice exposed to stress through their mothers. Social isolation stress during puberty causes alterations in female sexual behaviour, presumably through the regulation of oestrogen receptor alpha expression in the brain, as we found changes in the expression of oestrogen receptor alpha in several parts of the brain, connected with the regulation of female sexual behaviour. We have also performed translational studies, using population born around 10 days war for Slovenia as population presumably exposed to increased stress in neonatal period. Although both psychological tests performed on male volunteers and andrological examinations suggest some perturbations in psychological testing and in morphology of sperms in ejaculates, the number of volunteers recruited was unfortunately too small to allow firm conclusions.

From cytochromes p450 from blue-stain fungi to the world of a glioblastoma

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Cytochromes P450 belong to a superfamily of haemoproteins found in all kingdoms of life. Their unique significance lies in capabilities to chemically modify various substrates. In fungi, they are key enzymes in general and specialized metabolism, and xenobiotic detoxification. The bark beetle-associated fungus *Grosmannia clavigera* participates in the large-scale destruction of pine forests. In the tree, it must tolerate saturating levels of toxic conifer defence chemicals (e.g. monoterpenes). The fungus can metabolize some of these compounds through the ß-oxidation pathway and use them as a source of carbon. Fungus also uses carbon from pine triglycerides, where oleic acid is the most common fatty acid.

Extensive transcriptomic data for terpene-treated mycelia revealed that expression of many cytochromes P450 (CYPs), ABC transporters and genes involved in melanin biosynthesis were induced. We address the gap with characterization of biochemical functions of five selected CYPs (CYP630B18, CYP65BJ1, CYP530A13, CYP53A27, CYP529A3) with their unique Fe2+ CO Soret spectra, and two redox partners CPR1 and CPR2. CYP530A13 was, transforming (+/-)-limonene into trans/cis-limonene epoxide (limonene-1,2-oxide), indicating its involvement in the initial steps of the limonene degradation pathway, whose derivatives are then processed further and channelled into the β-oxidation pathway. CYP630B18, which is clustered in the genome with CPR2, supports the substrate specificity, converting oleic acid into 18-hydroxyoleic acid and taking part in the oxidative degradation of fatty acids. With this results we yield further insights into the biochemical mechanisms, by which blue-stain fungi overcomes tree defence compounds, which is one of the challenges in understanding this tripartite symbiosis between the beetle / fungus / forest system.

Scientific career led me from the research field of invasive fungal infections and discovering more powerful antifungal targets, to the field of the most aggressive, invasive, and lethal brain tumour, glioblastoma (GBM). Our research is focusing on introduction of "precision medicine" into glioma field, with identification of new GBM biomarkers, genomic profile of the tumour microenvironment and on a slow proliferating, highly radioresistant glioblastoma patient stem cells (GSC). Using primary GBM and GSC cell lines, established from a set of patient GBM tumours of different subtypes, from a joint bio-bank of TRANS-GLIOMA project partners (Slovenia-Italy), we are addressing the stemness potential of GBM cells, how to reduce the radioresistance of GSC and potentially how to improve treatment of individual GBM patient, using a new, specific medicinal products, which are specific for certain subtype of the disease on the individual level.

Molecular monitoring in chronic myeloid leukaemia patients

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Chronic myeloid leukaemia (CML) results from the Philadelphia chromosome (Ph) translocation (t(9;22)(q34.1;q11.2)) and expression of its fusion oncoprotein BCR-ABL1. Targeted drugs, BCR-ABL1 tyrosine kinase inhibitors (TKI), has changed the treatment strategy, improved quality of life and survival of CML patients. Majority of the patients develop stable and durable deep molecular response. Survival among CML patients is today depended on comorbidities rather than from CML.

Regular molecular monitoring of patients on TKI therapy is basic rule of current recommendations using BCR-ABL1 RT-qPCR method standardized to the International Scale (IS) to early identify nonadherence, treatment resistance, or treatment failure after 3, 6 and 12 months and thereafter every three or six months from the start of TKI therapy. However, much effort was needed to implement it into the recommendations and every day clinical practice.

Background knowledge was the International Randomized Study of Interferon versus STI571 (IRIS) trial where the vast majority of CML patients treated with imatinib achieved a complete cytogenetic response. A 3-logarithm reduction of the BCR-ABL1 level (corresponds to the so-called major molecular response, MMR or 0.1% IS) from the standardized baseline level (defined as 100% IS) after 12 to 18 months of treatment with imatinib was associated with prolonged subsequent remission. In addition, the degree of molecular response at early time points has been shown to be predictive of overall survival. Furthermore, the increased proportion of patients with CML are able to achieve deep molecular response (> MR 4.0) on second generation TKIs (nilotinib, dasatinib). Sustained deep molecular response is an important eligibility criterion for attempting to stop TKI therapy in the context of treatment-free remission clinical trials or practice. All of them require sensitive, reproducible and standardized evaluations of BCR-ABL1 transcript, which was mainly achieved through The EUropean Treatment Outcome Study (EUTOS) program, initiated by the European LeukemiaNet (ELN) group and establishment of the primary (WHO) and secondary standards.

Participating in the EUTOS program, we were/are able to improve our BCR-ABL1 RT-qPCR test. We also obtained EUTOS certificate for deep molecular response monitoring (MR 4.5). Today, we can offer the optimal molecular monitoring for the approximately 250 CML patients in Slovenia.

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Non-classical inclusion bodies produced in bacteria *Escherichia coli*

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Production of pharmaceutically interesting recombinant proteins in bacteria *Escherichia coli* is relatively fast and easy. Therefore, despite some disadvantages, *E. coli* is still one of the most commonly used host organisms. Over-expression of recombinant proteins often leads to the formation of inclusion bodies. They were until recently recognised as insoluble aggregates of unfolded, biologically inactive proteins representing bottleneck in protein production. Isolation of biologically active proteins from such inclusion bodies is lasting, expensive and often inefficient. Therefore, production of properly folded and biologically active proteins in *E. coli* even now represents a challenge for protein biochemistry and biopharmacy.

In the Department for Biosynthesis and Biotransformation at the National Institute of Chemistry a new type of inclusion bodies from protein granulocyte colony stimulating factor (G-CSF) were prepared. They are composed of high proportion of properly folded protein precursor and have some so far undescribed new properties, thus we designated them "non-classical inclusion bodies" (ncIBs).

An important achievement of our work was ascertainment, that with optimisation of the *E. coli* growth conditions, non-classical inclusion bodies can be formed from several structurally different proteins.

ncIBs are soluble in detergents usually used for washing the inclusion bodies. In non-denaturing conditions properly folded and biologically active protein can be extracted from ncIBs.

ncIBs are also very fragile. This should be taken into consideration during their isolation from bacterial cells. Sonication, method usually used for their isolation was found to be unsuitable. During sonication IBs start to decompose and properly folded proteins inside IBs alter their conformation and denaturate.

Inclusion bodies have a porous structure. Therefore they have very large surface area, where the proteins are exposed to the surrounding medium that can be well explored when IBs are used as active nanoparticles.

Another important finding of our work is that IBs contract when buffer pH is changed from neutral to acidic. Contraction is irreversible and is characteristic to all IBs. During their contraction pores size also reduces, thus proteins can no longer enter nor exit the IBs.

Solubility of the IBs as well as protein extractability in non-denaturing conditions is reduced. Based on the properties of IBs a model of their internal structure as well as a model of their formation was proposed. New findings of IBs structure, properties

and their formation led to new, improved, more effective and economically interesting biotechnological process for G-CSF production.

These findings could also help understanding of human conformational diseases.

Špela Peternel: Formation and properties of non-classical inclusion bodies in bacteria Escherichia coli, DOCTORAL THESIS, Medical Faculty, University of Ljubljana, 2008

Selection of candidate polymorphisms for genotyping of gastric cancer patients in Slovenian population

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INTRODUCTION: Although the incidence of gastric cancer is in decline worldwide, this type of cancer is still a major disease burden in Slovenia as well as globally. Non-specific symptoms lead to late diagnosis, when the disease is already at advanced stage. The outcome is poor, with the overall five-year survival rate being approximately 30%. Gastric cancer is a multifactorial disease, where the combination of genetic and environmental factors contributes to the disease development and progress. The environmental risk factors include increased intake of salty, savoury and smoked foods, obesity, smoking and *Helicobacter pylori* infection. On the molecular level gastric tumours show microsatellite and chromosomal instability, with the latter being more frequent. In addition to driver mutations, other genomic features, such as single nucleotide polymorphisms (SNPs) may contribute to gastric cancer susceptibility. Our aim was to investigate the contribution of SNPs to the development of gastric cancer in Slovenian patients. These low penetrating changes may accumulate over time and in combination with other factors contribute to disease development.

METHODS: We selected four candidate genes according to their cell function and their previous connection to different types of cancers in the literature. We focused on genes involved in mitosis and DNA repair. We selected the polymorphisms based on the following criteria: minor allele frequency was equal or more than 10% in Utah residents with Northern and Western European ancestry (CEU) population according to the 1000 Genomes Project Phase 3. We used Ensemble, dbSNP and UCSC genome browser databases for SNP selection. We used online tools ALGENE to evaluate the impact of variants on the transcription factor binding site. With HaploReg application we additionally annotated variants by their effect on changes in chromatin state, DNA methylation, histone methylation and acetylation, and DNAse tracks.

RESULTS: We have selected gene *CDC20*, which is a part of anaphase promoting complex and is involved in the separation of chromatids, *PLK2* and *PLK3*, which are involved in the mitotic check point, spindle assembly and also act as tumour suppressors mediating response to DNA stress. The last candidate gene *ATM* has a well-established role in DNA damage and stress response. We have selected eight SNPs: rs710251 in *CDC20*, rs963615 and rs15009 in *PLK2*, rs17883304 and rs12404160 in PLK3 and rs228589, rs189037 along with rs4585 in the *ATM* gene. Selected SNPs correspond to intron, 3' and 5' UTR regions of the DNA.

FUTURE PLANS: We will use TaqMan allelic discrimination assays for SNP genotyping and determine if genotypes of selected variants increase the risk of gastric cancer in Slovenian patients in comparison to control group.

Genetics and epigenetics of suicide in Slovenia

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Suicide is a well-defined public health problem of global proportions. Despite a decrease in number of suicides for the past decade, Slovenia is still ranked as one of the leading European countries regarding suicide rate. Suicidal behaviour is a result of interplay between hereditary and environmental factors, tied together by epigenetics. So far, many neurotransmitter systems have been studied on a genetic and biochemical level.

Many studies on suicide in Slovenia have been conducted at Medical Centre for Molecular Biology (MCMB) in the past decade, focusing mainly on serotonergic system and neurotrophic factors. Studies focused on single nucleotide polymorphisms and other functional polymorphisms. Differences between studied subject groups were observed in monoamine oxidase A (*MAOA*), serotonin receptor 1A and 2C (*HTR1A* and *HTR2C*), tryptophan hydroxylase 2 (*TPH2*), catechol-O-methyl-transferase (*COMT*), serotonin transporter (*5-HTT*), reelin (*RELN*) and brain-derived neurothropic factor (*BDNF*) genes.

In recent years, focus shifted towards research in the field of epigenetics, which includes DNA methylation as the most studied epigenetic modification in mammals. One of the first studies that investigated DNA methylation in suicide was carried out on Slovenian subjects. In suicide victims, DNA methylation of *BDNF* in Wernicke area was elevated. This hypermethylation was reflected in downregulation of *BDNF* expression in suicide victims. Recent studies on suicide related genes showed a significantly lower level of methylation of *BDNF* promoter in blood of suicide victims and a decrease in methylation levels of membrane bound *COMT* and soluble *COMT* in hippocampus of suicide victims. Currently we are looking at genome-wide methylation. Our results have shown some differences in methylation between suicide victims and controls in hippocampus and BA9.

Our studies, conducted on a population with high suicide risk, contribute to existing knowledge of suicidal behaviour. The development of new technologies has enabled the study of epigenetic changes, which together with the better elucidated genetic background forms a clearer picture of hereditary background of suicidal behaviour.

Cytochromes P450 as a possible model for enzyme evolution

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For thousands of years, philosophers and naturalists have been on a quest to find an answer to the intriguing question 'How did life begin?' The question on the origin of life is perhaps as old as mankind. Today modern technology offers various scientific approaches to this question. In 1924, Aleksander Oparin introduced his thesis, which states that, in its initial phase, the development of living creatures should have been the subject of a purely chemical evolution. Assuming the hypothesis, that during the emergence of life evolution had to first involve autocatalytic systems which only subsequently acquired the capacity of genetic heredity (metabolism first hypothesis), we propose and discuss on the basis of published literature possible mechanisms, basic aspects of the emergence and subsequent molecular evolution of the enzymes as we know them today.

Cytochrome P450s are haem-thiolate enzymes. For the purpose of our discussion, we are considering three important structural parts of the cytochrome P450 enzyme molecule and the interactions between them. These are: iron sulphide, protoporphyrin IX and apoprotein. Fe-S is at the core of the catalytic activity. As the iron sulphide is considered a major component of the hatcheries of pre-cellular life, the Fe-S bond is here proposed as a remnant or relict from the Iron Sulphur World. Further, we can name the acquisition of the protoporphyrin IX prosthetic group around an Fe atom as the next step in the accretion like evolution of cytochromes P450. Additionally there are proposals in literature that abiotically synthesised peptides would soon stabilize and begin to optimize the metal-cofactor-based catalysts as well as introduce substrate-specificity, which might present the next step in the accretion like the evolutionary history of cytochromes P450. It may be also assumed that the catalytic activity and specificity of sole coenzymes and mineral ions were enhanced by binding to the RNA oligomer portion, which in turn means that by harnessing a functional repertoire of cofactors, metal ions, prosthetic groups, organometallic compounds such as the various vitamins and haem, a broader repertoire of chemical functionalities of RNA ensued, otherwise the functionality would have been rather poor compared to the one of proteins. Indeed, Sen and Poon (2011) showed that RNA can enhance the redox activity of iron protoporphyrin IX, which is a constituent of modern cytochrome P450 enzymes. The proposed accretion model of cytochromes P450 can therefore be supplemented with the RNA–peptide co-evolutionary step placed in between the acquisition of protoporphyrin IX around Fe ion and obtaining an apoprotein protein portion around haem, whereupon, in evolutionary time, proteins would entirely displace the obsolete RNA portion. The proposed mechanism might be extended to basic aspects of the

emergence and subsequent molecular evolution of translation, ribosomes and as well as enzymes as we know them today.

Genetic and epigenetic changes in the DNA mismatch repair genes and their impact on hereditary and sporadic carcinogenesis

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Lynch syndrome (LS) is the most common cause of inherited colorectal cancer and occurs mainly due to pathogenic germline mutations in the DNA mismatch repair (MMR) genes. Identification of inherited MMR mutations and diagnosis of LS can help reduce colorectal cancer incidence and mortality in LS patients. However, for a considerable proportion of identified MMR gene variants, clinical significance is uncertain, representing a major problem in the diagnosis and management of LS. To accurately evaluate functional impact of such variants, Prof Komel et al have developed a novel *in vivo* yeast-based approach, by replacing yeast MLH1 and PMS1 genes with human orthologues, MLH1 and PMS2, directly on yeast chromosomes. The resulting yeast strain co-expressing human MLH1 and PMS2 allowed functional characterization of MLH1 variants, which were identified in unrelated Slovenian patients with MMR-deficient gastric carcinomas.

Loss of MMR activity due to epigenetic silencing of MMR genes or somatic mutation is also known to be associated with a variety of sporadic tumours. Therefore, in collaboration with the International Centre for Genetic Engineering and Biotechnology in Cape Town, we have further examined the effects of MMR polymorphisms and tobacco smoking in association with oesophageal cancer risk among South Africans. In a large case-control study, several MMR-polymorphisms and interactions between them were identified to be associated with increased risk of developing oesophageal cancer. Moreover, our results imply that pathogenesis of common polymorphisms in MMR genes is influenced by exposure to first-hand tobacco smoke. In another study, we have also shown that methylation of MMR genes together with exposure to tobacco smoke is involved in oesophageal carcinogenesis. Due to the active role of MMR proteins in modulating chemosensitivity of cells, methylation of MMR should be further examined in association with the outcome of oesophageal cancer treatment using anticancer drugs.

From basic glioblastoma proteomics to promising diagnostic and therapeutic tools

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The WHO grade IV astrocytoma – glioblastoma multiforme is a uniformly fatal brain malignancy. As a result of unspecific symptoms (headache, confusion, and memory loss) glioblastoma is usually diagnosed at an advanced stage. Using standard clinical care consisting of surgery, radiation and chemotherapy, the majority of the patients succumb to the disease in 12-18 months after diagnosis or 6 months in the cases of recurrent disease. Targeting glioblastoma is difficult due to the lack of specific biomarker. The constant efforts of the scientific community for understanding glioblastoma pathophysiology have not yielded significant results yet, so alternative diagnostic and therapeutic tools are extensively explored.

Our work is based on an original llama heavy-chain antibody-derived nanobody method for selection of glioblastoma-specific proteins with altered expression. Using nanobodies, we have developed a reverse-proteomics approach and identified nine proteins from cell/tissue protein lysate with differential expression (PMID: 25419715, PMID: 28498803, PMID: 29707108). Transcriptomic analysis revealed potential roles of selected genes in discriminating between different WHO glioma grades. With immunohistochemical approach we identified potential biomarkers that could differentiate between glioblastoma and low grade glioma and/or normal brain tissue. Using qPCR, ELISA, immunoblotting and immunocytochemistry we determined the level of mRNA and protein expression of antigens in glioblastoma cell lines and astrocytes. Nanobody specificity towards their corresponding antigens was confirmed with co-immunocytochemistry using commercial monoclonal antibodies. Moreover, functional analysis revealed the cytotoxic effect of a few nanobodies on various glioblastoma cell lines, and also their inhibitory effect on glioblastoma cells migratory potential. We also determined whether specific nanobodies cause apoptosis or necrosis on different cell lines. Furthermore, with data mining we selected two additional genes which show differential expression in mature glioblastoma cell lines (PMID: 29734672). Bioinformatic findings were also experimentally confirmed on proteomic and transcriptomic levels.

We present here the reverse-nanobody technology as a suitable method for selection of disease-specific proteins. So far, we have discovered potential antigens that could discriminate between glioblastoma and low grade glioma and/or normal brain tissue as well. Our experiments suggest potential role of nanobodies as inhibitory agents of glioblastoma cell growth and migration. At last, further development of nanobodies against glioblastoma membrane proteins will open a new opportunity for their development as targeted diagnostic or therapeutic agents.

Molecular and regenerative medicine of the cytoskeleton related diseases

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The cytoskeleton is a highly dynamic system that controls some of the very fundamental processes in cells, such as proliferation, migration, stress response, cell metabolism, organelle positioning, gene expression etc. Mutations in keratin genes cause over 30 phenotypically different disorders that affect the skin and skin appendages (hair and nail), the intestine, liver, and severely affect the health and quality of life of patients. We started our work in 1993 in collaboration with prof. Aleksej Kansky on palmoplantar keratodermas and have extended it since on bullous congenital disorders, their causes, genotype/phenotype correlations and the biology behind it. After over 20 years of active work in this field, our work and publications have contributed to the better understanding of the function of certain cytoskeletal components in these processes, the signalling pathways involved and we also identified novel routes for therapy. We routinely use as model systems primary and immortalized human and animal cells (both in normal and human disease situations), and 2D and 3D model tissue culture systems. We have recently extended this also to the biology of a number of mesenchymal stem cell types and their differentiation potential in vitro, and are also using iPS cells with the aim to develop new therapy approaches for a variety of genetic diseases. We have collaborators on all continents and these include some of the best epithelial biology and stem cell research scientists in the world.

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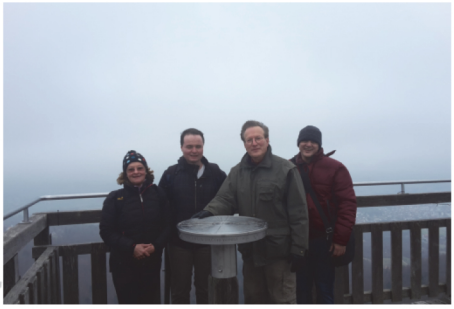
Moments in life of Prof. Komel

Once upon a time ...

The many happy moments ....



The talks ...

Are we lost??!!!

Nope, never ...

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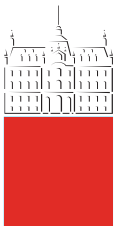

Labena

mediline

mikro+polo
vaš partner za laboratorij

Omega d.o.o.

CHEMASS
MERILNI SISTEMI