16TH CFGBC SYMPOSIUM

BOOK OF ABSTRACTS

JUNE 17TH, 2021

LJUBLJANA, SLOVENIA

VIRTUAL

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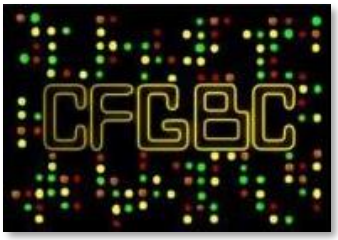
University of Ljubljana
Faculty of Medicine
Institute of Biochemistry and Molecular Genetics



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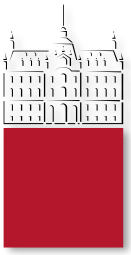

VIRTUAL SYMPOSIUM

SLOVENIA, JUNE 17TH, 2021

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INVITATION

Dear colleagues,

We are pleased to invite you to the **16th CFGBC Scientific Symposium**, taking place on **June 17th 2021**. Researchers and members of the CFGBC consortium will present their recent work through lectures featuring diverse topics. We are also accepting posters for virtual poster session. Symposium is free of admission.

The opening lecture will be presented by **Prof. Dr. Jerzy Adamski**, Professor Emeritus at the Helmholtz Zentrum München, Germany, Visiting Parkway Pantai Professor in Medicine and Healthy Ageing, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, and Visiting Scientist (Professor) at the University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia.

Closing lecture will be presented by **Prof. Dr. Maria Fedorova**, Head of the Center for Biotechnology and Biomedicine, Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, University of Leipzig, Germany.

Welcome!

Assist. Prof. Dr. Tadeja Režen
Chair of Organizing Committee

Assist. Prof. Dr. Toni Petan
Chair of Scientific Committee

CONTENTS

GENERAL INFORMATION

Event was free of charge. Official language was **English**.

Registration was **required** for attending the symposium.

If you have any additional questions, please contact us via cfgbc[at]mf.uni-lj.si.

COMMITTES

ORGANIZING COMMITTEE

Tadeja Režen, chair

Helena Klavžar

Nejc Nadižar

SCIENTIFIC COMMITTEE

Toni Petan, chair

Damjana Rozman

SPONSORS

FINAL PROGRAMME

THURSDAY, JUNE 17TH, 2021

13:00 – 13.10 *Welcome speeches*

- Prof. Dr. **Janja Jan**,Vice-Dean of the Faculty of Medicine, University of Ljubljana
- Prof. Dr. **Marko Goličnik**, Head of the Institute of Biochemistry and Molecular Genetics

CHAIR: TADEJA REŽEN

13.10 – 13.40 **Jerzy Adamski**, Helmholtz Zentrum Munich, Technical University of Munich, Germany

- *Exploring applications of metabolomics in human complex diseases*

CHAIRS: ŠPELA BAEBLER, DAMJANA ROZMAN

13.40 – 14.00 **Anže Zupanič**, National Institute of Biology, Slovenia

- *Analysis of Gene Expression with Causal Networks: cardiotoxicity and neurotoxicity*

14.00 – 14.20 **Cene Skubic**, University of Ljubljana, Faculty of Medicine, Slovenia

- *The impact of disturbed cholesterol synthesis on RORC nuclear receptor*

14.20 – 14.40 **Blaž Vrhovšek**, University of Ljubljana, Biotechnical Faculty, Slovenia

- *QTLspyer: a user-friendly tool for quantitative trait locus detection and visualization*

14.40 – 15.00 **Nik Sušič**, Agricultural Institute of Slovenia, Slovenia

- *Genomic studies of root knot nematodes and their bacterial antagonists*

15.00 – 15.15 *Coffee break(Poster session 3)*

15.15 – 16.15 **VIRTUAL POSTER SESSION (POSTER SESSION 1 & 2)**

16.15 – 16.30 *Coffee break(Poster session 3)*

CHAIRS: MOJCA BENČINA, KATARINA TREBUŠAK PODKRAJŠEK

16.30 – 17.00 **Sašo Džeroski**, Jožef Stefan Institute, Slovenia

- *Artificial Intelligence for Science*

17.00 – 17.20 **Jernej Kovač**, University Medical Centre Ljubljana, Slovenia

- *Rapid SARS-CoV2 variants surveillance by viral genome sequencing*

17.20 – 17.50 **Maria Fedorova**, University of Leipzig, Germany

- *Lipidomics signature of human obesity*

17.50 – 18.00 *Closing remarks*

LECTURE ABSTRACTS

EXPLORING APPLICATIONS OF METABOLOMICS IN HUMAN COMPLEX DISEASES

Jerzy Adamski[1,2,3]

[1]Helmholtz Zentrum Munich, Technical University of Munich, Institute of Experimental Genetics, Germany

[2]Visiting Parkway Pantai Professor in Medicine and Healthy Ageing, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

[3]Visiting Scientist (Professor) at University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

Metabolomics addresses a comprehensive view on metabolites depicting biological and non-enzymatic processes. The holistic view provided by metabolomics reflects intrinsic (e.g. genetic and physiological) and environmental (e.g. nutrition and medication) impact on human metabolism. The metabolomics requests integrative application of epidemiology, analytics and bioinformatics and provides information on specific metabolic signatures in health and disease. Metabolomics was instrumental in describing processes of healthy aging, endocrine homeostasis in transgender individuals and diabetes type 2. Unexpected kinetics in lipid metabolism and cross-talks between urea cycle and TCA were discovered. They represent candidates for diagnostic biomarkers or targets for specific therapies

ANALYSIS OF GENE EXPRESSION WITH CAUSAL NETWORKS: CARDIOTOXICITY AND NEUROTOXICITY

Anže Zupanič[1]

[1]National Institute of Biology, Slovenia

Adverse outcomes are rarely caused by dysregulation of individual proteins; rather, they are often caused by system-level perturbations in networks of molecular events. To fully understand the mechanisms of toxicity, it is necessary to recognize the interactions of molecules, pathways, and biological processes within these networks. The developing brain and heart are prime examples of extremely complex networks, which makes developmental cardio- and neuro-toxicity among the most challenging areas in toxicology. We have developed a systems toxicology approach that uses computable biological networks to represent molecular interactions in zebrafish larvae. The networks are curated from scientific literature and describe interactions between biological processes, signaling pathways, and adverse outcomes associated cardio- and neuro-toxicity. In this talk I will describe the construction of the zebrafish developmental neurotoxicity and cardiotoxicity networks and their validation by integration with publicly available transcriptomic datasets. Our approach can be used for classification of chemicals into neuro and cardiotoxicants, the identification of new biomarkers and for development of new adverse outcome pathways that are becoming one of the vital resources of toxicology in the 21st century.

THE IMPACT OF DISTURBED CHOLESTEROL SYNTHESIS ON RORC NUCLEAR RECEPTOR

Cene Skubic[1], Andrew Walakira[1], Petra Ivanuša[1], Hana Trček[1], Žiga Vičič[1], Tadeja Režen[1], Damjana Rozman[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Slovenia

Cholesterol synthesis is a metabolic pathway with many steps and still open mysteries. Besides being synthesis precursors, sterol intermediates also have different physiological functions. Recent findings suggest that sterols beyond lanosterol can serve as ligands of nuclear receptors, the transcription factor RORC (RAR Related Orphan Receptor C) and in this way affect the expression of genes with RORE (ROR element) sequence in the promoter region. In our work, we focused on the effect of sterol intermediate accumulation on nuclear receptor signalling with a focus on sterol-RORC signalling. To investigate the downstream biological roles of different cholesterol intermediates, we used the CRISPR-Cas9 system on human HepG2 cells. We produced four cell lines, each with a knockout (KO) of a different gene from the late part of cholesterol synthesis (CYP51, DHCR24, SC5DL and HSD17B7). Each of these original cells lines accumulates the upstream sterol intermediates and lack sterol intermediates downstream of the deleted enzyme, which was validated using the new LC-MS method developed in our laboratory (Skubic et al. 2020). Transcriptome changes in KO cell lines were evaluated by Affymetrix microarrays and Oxford Nanopore sequencing and differentially expressed genes and pathways were assessed by KEGG and TF enrichment analysis. We implemented an immunoprecipitation protocol to evaluate which sterol molecules are in our cell models bound to RORC protein. The transcriptome data from microarrays showed 102 common differentially expressed genes, that were changed in all KO cell lines, associated with disturbed cholesterol synthesis and associated metabolic pathways, like Steroid biosynthesis and Complement and coagulation cascades. On the other end, each line has a uniquely expressed set of genes in knockout cell lines (99 in CYP51 KO, 120 in DHCR24 KO, 276 in SC5D KO and 2301 in HSD17B7 KO). We searched also for pathways that could pinpoint a specific role of accumulated sterol molecules. We identified NF-kappa B signalling that changed just in the CYP51 KO, and Cell cycle pathway in HSD17B7 KO. RORC target genes were altered to varying degrees, with the highest number of upregulated genes in SC5D (23) and HSD17B7 (62) KO cells. This suggests a RORC activation through accumulated zymostenol and zymosterol in vivo, with further confirmation in progress using Oxford Nanopore data analysis and immunoprecipitation experiments. In conclusion, we confirmed that the CRISPR-Cas9 generated cell models are functional deletions of the targeted genes from cholesterol synthesis, which resulted in the accumulation of sterols at the blocked step. This lead to the activation of different metabolic pathways, depending on the accumulated sterols. Using transcriptomic data, biochemical techniques and bioinformatic analysis, we aim to further investigate RORC-sterol signalling to decipher new signalling roles of sterol intermediates from this enigmatic part of cholesterol synthesis.

QTLSPYER: A USER-FRIENDLY TOOL FOR QUANTITATIVE TRAIT LOCUS DETECTION AND VISUALIZATION

Blaž Vrhovšek[1], Cene Gostinčar[2], Janez Kokošar[3], Uroš Petrovič[4]

[1]Department of Biotechnology, Biotechnical Faculty, University of Ljubljana, Slovenia
[2]Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia
[3]Genialis d.o.o., Slovenia
[4]Department of Molecular and Biomedical Sciences, Jožef Stefan Institute and Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia

Through genetic engineering we aim to amplify favorable and silence negative traits. Many phenotypes are quantitative traits, meaning that their expression is determined by multiple genes and environmental factors. By combining phenotype and genotype data, we can identify loci which influence a quantitative trait and thus facilitate the manipulation of the desired phenotype. With advances in massively parallel sequencing, whole genome sequencing (WGS) is becoming a viable source of genotype data for an increasing number of studies. However, analysing WGS data remains a complex and computationally demanding process requiring a specific set of skills or specialized tools. To simplify the use of WGS data based on the bulk segregant analysis (BSA) method for detection of quantitative trait loci (QTL), we developed a software tool named QTLspyer [1, 2].

The QTL detection process in QTLspyer is divided into two steps. In the first step a Python script is used to call single nucleotide variants (SNVs) between the sequenced data and the reference genome in a variant call format (VCF). Sequence data quality reports are generated by FastQC, reads are trimmed with BBduk and the reads are mapped to the reference by BWA. Variant calling is done with the Genome Analysis Toolkit (GATK). In the second step the probabilities of potential QTL findings are estimated. The significance is estimated based on the G' and QTL-seq approach using a R library called QTLseqr [3].

Custom parameters for each tool involved in the analysis can be specified inside the graphical user interface (GUI), designed using the Shiny R library. Results are presented to the user as data tables of SNVs and QTLs statistical properties and graphically with plots showing QTL probabilities for all genome positions. To increase the ease of use and portability, QTLspyer is fully contained inside a Docker image and can be run on any operating system with Docker support. Furthermore, the user is not required to manually install any software involved in a QTL analysis. The application guides the user through the analysis by providing detailed description of every required step and setting, while also providing sane default values of each parameter. The analysis can be run as a pipeline or step by step. QTLspyer can analyse data from any organism if correct annotation and reference data is provided.

To verify accuracy of QTLspyer, we ran the analysis using the raw sequence data from a QTL study on yeast lipid storage [4]. The analysis successfully identified QTLs discovered by the study.

References:
[1] Michelmore RW, et al. (1991) Proc Natl Acad Sci USA 88(21), pp.9828–32
[2] Oduola A, et al. (2003) Nature Reviews Genetics 4, pp.911–16
[3] Mansfeld BN, et al. (2018) Plant Genome 11(2), p.3853
[4] Pačnik K, et al. (2021) BMC Genomics 22 p.1186

GENOMIC STUDIES OF ROOT KNOT NEMATODES AND THEIR BACTERIAL ANTAGONISTS

Nik Sušič[1], Saša Širca[1], Barbara Gerič Stare[1]

[1]Agricultural Institute of Slovenia, Slovenia

Plant parasitic nematodes have a major impact on global food production, with annual losses estimated at about €110 billion worldwide. Of these, root-knot nematodes (RKN; Meloidogyne spp.) are considered the most important among all nematodes due to their economic threat to agriculture. Species of the tropical RKN group are extremely polyphagous, can parasitize a wide range of host plants, and reproduce asexually by mitotic parthenogenesis. Several genomes of clade I tropical Meloidogyne spp. have been sequenced and showed to be complex allopolyploids with heterozygous duplicated genomic regions and abundant transposable elements. In addition, some tropical RKN species such as Meloidogyne ethiopica, M. inornata and M. luci are very similar at the morphological, biological and genetic levels, making them species of interest for whole genome sequencing. Due to the complex nature of the genomes of parthenogenetic RKN species, the use of short-read next-generation sequencing limits the contiguity of the resulting genome assemblies. We used long-read Pacific Biosciences Sequel and short-read Illumina HiSeqX sequencing data to generate a high-quality M. luci genome assembly. The 209.2 Mb M. luci genome assembly consists of 327 contigs with an N50 of 1.7 Mb. The genome is estimated to be triploid (AAB). The assembly is currently the most contiguous tropical RKN genome assembly publicly available, with an estimated coding space coverage of 95.2% based on Core Eukaryotic Genes Mapping Approach (CEGMA). The polished assembly was 88% complete based on the eukaryote set (n=303) from Benchmarking Universal Single-Copy Orthologs (BUSCO). Assemblies such as this can be used to determine the correct phylogenetic position of the clade, to identify genetic changes associated with the origins of virulence, or in the evolutionary history studies.Antagonistic Bacillus firmus bacteria are used to control plant parasitic nematode infestations of crops in agricultural production. The primary nematode virulence factors in nematicidal B. firmus are thought to be various extracellular proteases. We determined and compared the whole genome sequences of two nematicidal strains: a commercial bionematicidal isolate B. firmus I-1582 and a wild-type Bacillus sp. ZZV12-4809. The resulting genome assemblies of B. firmus I-1582 and Bacillus sp. ZZV12-4809 have a length of 4.6 Mb and 5.3 Mb; and contain 5,048 and 5,671 predicted genes, respectively. Interestingly, 18 and 19 homologs to nematode virulent proteases were found in the genome assemblies of B. firmus I-1582 and Bacillus sp. ZZV12-4809, respectively, and 2 homolos to virulent chitinase genes in ZZV12-4809. Secondary metabolite gene clusters were also found in both genomes, suggesting the genetic capacity for nematode virulence. The results of this bioinformatics study indicate the genetic capacity of B. firmus and related species for nematode virulence through a number of direct and indirect mechanisms.

ARTIFICIAL INTELLIGENCE FOR SCIENCE

Sašo Džeroski[1]

[1]Jožef Stefan Institute, Slovenia

Artificial intelligence (AI) and machine learning (ML) are revolutionizing science by helping to handle the flood of scientific data. The talk will give a very brief introduction to AI and two types of explainable ML, i.e., predicting structured outputs and automated modelling of dynamical systems. It will then discuss several applications of these ML methods in the life sciences and medicine, ranging from gene function prediction through modelling cellular processes and virtual compound screening for drug design and repurposing.

RAPID SARS-COV2 VARIANTS SURVEILLANCE BY VIRAL GENOME SEQUENCING

Jernej Kovač[1]

[1]University Medical Centre Ljubljana, Slovenia

About 15 months ago, in March 2020, first official case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified in Slovenia. As the health care system scrambled to assure proper clinical treatment of infected in first, and especially in second wave of the pandemics, the need to establish rapid diagnostic platforms to identify infected, had to be fulfilled. To keep the central health institution, University Medical Centre Ljubljana (UMC Ljubljana), operative at the peak of the pandemics second wave and to prevent hospital outbreaks and viral intrusions into safe zones, a rapid diagnostic laboratory was established on site. Additionally, with the advent of novel, more invasive variants of SARS-CoV-2 the need of rapid viral genomic surveillance arisen and proper response had to be established. A national network with close cooperation between laboratories of UMC Ljubljana, National Laboratory of Health, Environment and Food (NLZOH) and National Institute of Biology (NIB) was established to trace and report on novel variants of SARS-CoV-2 in Slovenian population. To fulfil the needs of health regulators, protocols of rapid viral identification (less than 24h) on one hand and massive multiplex NGS library preps on the other were developed. More than 7.000 SARS-CoV-2 viral genomes were sequenced in the past 5 months, establishing an efficient surveillance system to support national epidemics response. A journey of resolving and developing technical solutions for viral surveillance, building up sequencing capacity and optimising logistics will be presented in this short lecture.

LIPIDOMCIS SIGNATURE OF HUMAN OBESITY

Maria Fedorova[1,2]

[1]Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig, Germany

[2]Centre for Biotechnology and Biomedicine, Universität Leipzig, Germany

Obesity, characterized by expansion and metabolic dysregulation of white adipose tissue (WAT), has reached pandemic proportions and acts as a primer for a wide range of metabolic disorders. Remodelling of WAT lipidome in obesity and associated comorbidities can explain disease etiology and provide valuable diagnostic and prognostic markers. To support understanding of WAT lipidome remodelling at the molecular level, we performed in-depth lipidomics profiling of human subcutaneous and visceral WAT of lean and obese individuals. Tissue tailored LC-MS/MS lipidomics analysis allowed to reconstruct human WAT reference lipidome (*AdipoAtlas*) and provided an inventory of over 1600 lipid molecular species from 23 lipid (sub)classes fortified by their semi-quantitative values in two WAT depots (subcutaneous and visceral) from lean and obese individuals. *AdipoAtlas* was used as a reference to illustrate the remodeling of WAT lipidome upon development of obesity. We show that ceramides containing the unusual sphingadienine base and TG containing polyunsaturated fatty acid (PUFA) residues are enriched in obese WAT. Moreover, we identified distinct responses of adipose tissue depots to increased metabolic demand by upregulation of depot-specific plasmalogen synthesis. Identified lipidomics signatures were further confirmed by high-throughput screening for WAT lipidome alterations in obese insulin sensitive and resistant individuals (n=180) as well as the corresponding blood plasma samples to illustrate the cross-talk between WAT (mechanism and pharmacological targets) and systemic (biomarkers) lipid metabolism. Furthermore, *AdipoAtlas* provides so far missing scaffold for systems biology integration of lipidomics data via reconstruction of lipid-centric genome scale metabolic models, linking big omics data with identification of disease characteristic metabolic and signaling pathways.

POSTER ABSTRACTS

POSTER SESSION 1

DNA, miRNA AND CELL MODELS

CHAIR: ALJA VIDETIČ PASKA

GENETIC POLYMORPHISMS IN GLUCOCORTICOID PATHWAY INCREASE THE RISK FOR NEW ONSET DIABETES MELLITUS AFTER TRANSPLANTATION

Tanja Blagus[1], Klemen Pahor[2], Blaž Maver[3], Blaž Vončina[1], Katja Goričar[1], Miha Arnol[4], Gregor Mlinšek[4], Vita Dolžan[1]

[1]Pharmacogenetics Laboratory, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana
[2]Department of Urology, University Medical Centre Ljubljana
[3]Department of Otorhinolaryngology and Cervicofacial Surgery, General Hospital Celje
[4]Department of Nephrology, University Medical Centre Ljubljana

BACKGROUND: New onset diabetes mellitus after transplantation (NODAT) is a relatively frequent complication after solid organ transplantation. Kidney transplant recipients with NODAT are at greater risk for cardiovascular events, infections and prone to accelerated graft loss or graft rejection compared with recipients without NODAT. Even though patient's physical condition, comorbidities and treatment with immunosuppressive drugs, such as tacrolimus and steroids, are predominant factors increasing the risk for NODAT development, interindividual variability in response to steroids indicates the important role of genetic factors as well. In this study, we investigated the association between genetic polymorphisms in glucocorticoid signaling and metabolic pathway and NODAT development within the first year after kidney transplantation.

METHODS: In total 283 transplant patients were included in the study. Clinical data on NODAT, which was defined as a need for additional treatment with insulin or oral antidiabetics post transplantation, were obtained from patients' medical records. All patients were genotyped for selected polymorphisms in genes encoding glucocorticoid receptor (NR3C1), p-glycoprotein (ABCB1), glutathione S-transferase P1 (GSTP1) and for GSTM1 and GSTT1 gene deletions. Logistic regression was used in statistical analysis.

RESULTS: Interim analysis was performed in a subset of 227 patients (66.1% male and 33.9% female), with a median age of 50.7 years, of whom 80.5% were receiving methylprednisolone. NODAT developed in 43 (19%) patients. NR3C1, ABCB1, GSTP1 rs1695 polymorphisms and GSTT1 deletion were not associated with the risk for NODAT. GSTP1 rs1138272 was associated with an increased risk for NODAT in dominant genetic model in univariable analysis and after adjustment for age and concurrent treatment with methylprednisolone in multivariable analysis (OR = 2.24, 95% CI = 1.02-4.89, P = 0.044 and OR = 2.26, 95% CI = 1.00-5.09, P = 0.050, respectively). GSTM1 deletion was also associated with an increased risk for NODAT in univariable and multivariable analysis (OR = 2.02, 95% CI = 1.01-4.02, P = 0.047 and OR = 2.09, 95% CI = 1.02-4.25, P = 0.043, respectively).

CONCLUSION: GSTP1 rs1138272 polymorphism and GSTM1 gene deletion were significantly associated with increased risk for NODAT in the first year after kidney transplantation.

DISTINCT SNP PROFILES IN HYPOXIA-INDUCIBLE FACTOR 3 ALPHA SUBUNIT (HIF3A) GENE BETWEEN DIVERGENT MOUSE MODELS FOR BODY FAT CONTENT

Martin Šimon[1], Špela Mikec[1], Nicholas Morton[2], Janez Konc[3], Peter Dovč[1], Simon Horvat[1], Tanja Kunej[1]

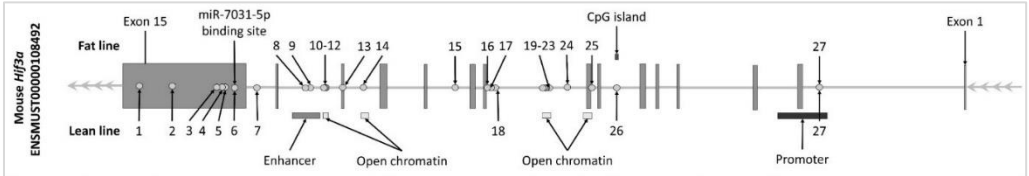
[1]Biotechnical Faculty, University of Ljubljana
[2]The Queen's Medical Research Institute, University of Edinburgh
[3]Laboratory for Molecular Modeling, National Institute of Chemistry

BACKGROUND: Adipose tissue hypoxia has been directly linked to its dysfunction and obesity; however, the mechanism remains poorly characterized. Adaptation and responses to hypoxia are mediated by hypoxia-inducible transcription factors alpha (HIFA): HIF1a, EPAS1 (HIF2a), and a less studied HIF3a that acts as a positive or negative regulator. Here we examined the presence of genetic variability in Hif3a gene of two divergent mouse models for obesity (fat line, F) and leanness (lean line, L).

METHODS AND RESULTS: Genetic variability in mouse Hif3a was extracted from the results of our whole genome sequencing (WGS) data, literature and bioinformatics databases to reveal potential regulatory variants. Together, 89 SNPs that differed between F (n = 39 SNPs) and L (n = 50 SNPs) lines were detected, mainly in the intronic regions of the F line and in the 3' UTR of transcript ENSMUST00000108492 of the L line. Twenty-six SNPs either locate in Hif3a regulatory elements and/or cause loss or gain of CpG dinucleotides (Figure 1).

CONCLUSION: Analysing genetic variability between mouse strains can provide clues for their phenotypic divergence. The Hif3a variations could be related to obesity, potentially due to alternative utilization of Hif3a transcripts, post-transcriptional pre-mRNA and mRNA processing, and ultimately the abundance of HIF3a protein isoforms. However, additional bioinformatics and functional analyses are needed to decipher how variability in Hif3a affects body fat content.

ACKNOWLEDGMENTS: The authors acknowledge the study was financially supported by the Slovenian Research Agency under the postgraduate research program Young researchers, J4-2548 research project and P4-0220 research program.



Number	Line	SNP	Location	Consequence	Regulatory type
1	LEAN	rs50769915	7:17032362	3' UTR variant (Exon 15)	CpG > CpA
2	LEAN	rs49164232	7:17033659	3' UTR variant (Exon 15)	CpG > TpG
3	LEAN	rs249866502	7:17035207	3' UTR variant (Exon 15)	CpG > CpA
4	LEAN	rs213275509	7:17035424	3' UTR variant (Exon 15)	CpG > CPA
5	LEAN	rs254059618	7:17035539	3' UTR variant (Exon 15)	CpG > CpA
6	LEAN	rs31624146	7:17035853	3' UTR variant (Exon 15)	miR-7031-5p
7	LEAN	rs50767825	7:17036031	intron variant (Intron 14)	TpG > CpG
8	FAT	rs51281810	7:17038429	intron variant (Intron 13)	Enhancer
9	FAT	rs52514580	7:17038598	intron variant (Intron 13)	Enhancer, TpG > CpG
10	FAT	rs49665660	7:17039171	intron variant (Intron 13)	Open chromatin
11	FAT	rs50347975	7:17039214	intron variant (Intron 13)	Open chromatin, CpA > CpG
12	FAT	rs45746789	7:17039218	intron variant (Intron 13)	Open chromatin, CpA > CpG
13	FAT	rs37739792	7:17039615	synonymous variant (Exon 13)	CpG > CpT
14	FAT	rs49346424	7:17040562	intron variant (Intron 12)	Open chromatin
15	FAT	rs48130871	7:17043897	intron variant (Intron 10)	CpG > TpG
16	FAT	rs49356896	7:17045030	intron variant (Intron 8)	CpG > ApG
17	FAT	rs248490880	7:17045192	intron variant (Intron 8)	CpG > GpG
18	LEAN	rs37518309	7:17045355	intron variant (Intron 8)	CpC > CpG
19	FAT	rs32206454	7:17047124	intron variant (Intron 8)	Open chromatin
20	FAT	rs51928031	7:17047128	intron variant (Intron 8)	Open chromatin
21	FAT	rs50312843	7:17047202	intron variant (Intron 8)	Open chromatin
22	FAT	rs31707356	7:17047221	intron variant (Intron 8)	Open chromatin
23	FAT	rs32322492	7:17047280	intron variant (Intron 8)	Open chromatin
24	FAT	rs49273709	7:17047967	intron variant (Intron 8)	CpG > TpG
25	FAT	rs36950082	7:17048848	intron variant (Intron 7)	Open chromatin
26	LEAN	7_17049768_C/T	7:17049768	intron variant (Intron 6)	CpG island, CpG > CpA
27	BOTH	rs47681146	7:17057107	upstream gene variant	Promoter

Figure 1: SNPs within Hif3a of F and L mouse line overlapping regulatory elements and/or causing loss or gain of CpG dinucleotides.

GENETIC VARIABILITY OF *JAK2* GENE IN ERYTHROCYTOSIS

Monika Banfi[1], Aleša Kristan[2], Tanja Kunej[3], Nataša Debeljak[2]

[1]Master study Programme of Biotechnology, Biotehnical faculty, University of Ljubljana
[2]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana
[3]Department of Animal Science, Biotechnical faculty, University of Ljubljana

BACKGROUND: Erythrocytosis is a condition with an increased mass of erythrocytes in the body. It is reflected by increased haematocrit, haemoglobin concentration and number of erythrocytes in the blood. Mutations in JAK2 gene altered gene expression and may result in the loss of certain protein functions or a reduction in individual protein functions. Somatic JAK2 variants lead to polycythemia vera (PV), an acquired type of erythrocytosis that is well recognized. While the role of JAK2 gene in the development of Congenital erythrocytosis (CE), inherited disorder with a diverse genetic background is still poorly understood1. Several new JAK2 variants with unknown pathogenicity were discovered in a preliminary NGS analysis of patients with idiopathic erythrocytosis 2. The aim of the present study was to overview and select regions of JAK2 gene, potentially associated with erythrocytosis and analyse their genetic variability in selected cohort of patients.

METHODS: Potentially causal JAK2 variants for erythrocytosis development (PV and CE) were reviewed with the literature mining in PubMed database and in parallel, the data from Ensembl browser was used to allocate those variants on specific gene regions. Based on the collected data, we chose regions of JAK2 gene, selected suitable primers and optimized PCR conditions for Sanger sequencing analysis on selected biological samples.

RESULTS: Based on the data we managed to collect with bioinformatic tools and databases, variants in exon 12 (rs1166634241, rs121912473), exon 13 (rs753281669), exon 14 (rs77375493, rs375442615), exon 19 (rs150221602) and exon 24 (rs41316003) are causative for PV. While one variant in exon 19 (rs1230493711) was associated with development of CE. Therefore, we selected JAK2 gene exons 12-14, 19 and 24 for further sequencing analysis. To this point, we have successfully optimized the analysis of the exons 12-14. With the following Sanger sequencing analysis, we try to confirm the variants previously discovered upon NGS analysis2 and find some additional ones on selected cohort of patients.

CONCLUSION: Results of the analysis revealed that variants of the JAK2 gene sequence show potential for further functional analysis based on their location in previously reported regulatory region. Discovering novel variants might assist clinicians in diagnosing patients with CE erythrocytosis and integrate diagnosis of PV.

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ALZHEIMER'S DISEASE AND CANDIDATE GENE ANALYSIS OF DNA METHYLATION

Matea Nikolac Perković[1], Katarina Kouter[2], Dubravka Švob Štrac[1], Mojca Katrašnik[2], Suzana Uzun[3,4], Oliver Kozumplik[3,4], Ninoslav Mimica[3,4], Nela Pivac[1], Alja Videtič Paska[2]

[1]Laboratory for Molecular Neuropsychiatry, Division of Molecular Medicine, Ruder Boskovic Institute
[2]Medical Center for Molecular Biology, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana
[3]University Psychiatric Hospital Vrapce
[4]School of Medicine, Josip Juraj Strossmayer University of Osijek

Alzheimer's disease (AD) is slow, irreversible, but progressive, complex and multifactorial disorder. It is characterized by gradual memory loss and cognitive impairments, behavioral changes, and consequently death. It represents the most common cause of dementia in older population, and a major health problem worldwide. Due to the accelerated aging of human population, it is assumed that the number of AD patients will quadruple by the year 2050. The risk of developing AD significantly increases after 65 years of age, and it reaches up to 31% for individuals beyond age 85. The treatment of AD was not significantly changed or improved in the last decade: it includes acetylcholine esterase inhibitors donepezil, galantamine and rivastigmine, and NMDA receptor antagonist memantine.

Main risk factors for AD are older age, genetic predisposition, gender, cardiovascular factors and presence of the mild cognitive impairment (MCI). MCI is characterized by the slight cognitive changes and disruptions that occur as mild changes in memory, altered ability to think and to remember. MCI might lead to AD, since a great percent of subjects with MCI (50 % - 65 %) later develop some form of dementia, especially AD. At present, clinical diagnosis of (probable) AD is established thorough a detection of clinical symptoms, combined with cognitive screening tests, detailed neuropsychological testing and imaging techniques. Tests based on molecular-genetic biology analysis are only entering the routine clinical practice, but, as other clinical tests, they are relevant only after the disease has already made considerable progress.

In our study we used most contemporary methods (next generation sequencing, droplet digital PCR, qPCR) to determine methylation status and single nucleotide polymorphisms of AD candidate genes, catechol-o-methyl transferase (COMT) and brain-derived neurotrophic factor (BDNF), in blood based liquid biopsy samples and cell-free DNA (cfDNA) from plasma in clinically well-defined AD patients and subjects with MCI. Differences in DNA methylation between AD and MCI subjects were the highest for *BDNF*.

The introduction of additional epigenetic markers in a set of clinical tests that are currently in use in the diagnosis of AD would enable a more accurate diagnosis in the early stages of the disease, reducing the number of false-positive and false-negative diagnoses. Determining the differences of white blood cells methylation status and methylation cfDNA as a pool of DNA from distant tissues could confirm existence of the so-called 'mirror effect' where the peripheral markers reflect the status of unobtainable tissue for molecular studies. The identification of integrative biomarkers would mean a large step forward for the early diagnosis of AD and for the prediction of its progression, but also for the preclinical stages like MCI, and through a translation to clinical practice.

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CHARACTERIZATION OF URINARY EXTRACELLULAR VESICLES AND THEIR MICRORNA CARGO IN PATIENTS WITH FABRY DISEASE

Teo Mlinšek[1], Tina Levstek[1], Marija Holcar[1], Katja Goričar[1], Metka Lenassi[1], Vita Dolžan[1], Bojan Vujkovac[2], Katarina Trebušak Podkrajšek[1,3]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana
[2]Centre for Fabry Disease, General Hospital Slovenj Gradec
[3]Clinical Institute for Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana

Fabry disease (FD) is a rare X-linked genetic disorder characterised by a deficiency in α-galactosidase A activity. This leads to an accumulation of glycosphingolipids in various cells throughout the body, such as cardiac, neural, and renal cells. Fabry nephropathy has an important impact on morbidity and mortality in Fabry patients. Current biomarkers are associated with late signs of renal damage and do not predict the progression of Fabry nephropathy. Urinary extracellular vesicles (uEVs) are secreted by the cells lining the urinary tract and therefore may reflect early changes in renal function. We conducted a proof-of-concept study to investigate the characteristics of uEVs and the expression of uEVs-derived microRNAs (miRNAs) as potential biomarkers of Fabry nephropathy.

Small uEVs were isolated from patients with genetically confirmed FD (n = 20) by size exclusion chromatography from chronological samples obtained approximately 5 years apart over the past 10 years. Patients were divided into two cohorts depending on the presence of Fabry nephropathy. Nanoparticle tracking analysis was used to determine the size and concentration of uEVs. The expression of seven uEVs miRNAs was analysed using miRCURY LNA miRNA PCR Assays, two of which were used for normalisation.

Patients with Fabry nephropathy had significantly higher urinary-protein-to-creatinine ratio, urinary-albumin-to-creatinine ratio and lower estimated glomerular filtration rate compared to the patients with no signs of renal impairment. uEVs concentration, size, and expression of miR-200a-3p, miR-29a-3p, miR-30b-5p, miR-23a-3p, and miR-34a-5p were not significantly different between patients with and without Fabry nephropathy at last follow-up. However, the expression of miR-29a-3p and miR-200-3p increased in a 5-year period in patients with Fabry nephropathy, which may be a mechanism to ameliorate the progression of nephropathy, as these miRNAs have an antifibrotic function. In a 10-year period, the expression of miR-29a-3p and miR-200a-3p increased in patients with and without Fabry nephropathy. Moreover, the expression of miR-30b-5p increased over 10 years in patients without nephropathy, which may indicate a possible protective role in the development of nephropathy, as this miRNA has a protective role in podocyte injury and endothelial-to-mesenchymal transition.

To our knowledge, the present study was the first to investigate uEVs in patients with FD and we showed altered expression of some miRNAs. Next, we plan to study the expression of a larger number of miRNAs that could be used as biomarkers for the development and progression of Fabry nephropathy along with other clinical and biochemical parameters.

SELECTING AND TESTING miRNAs FROM BRAIN TISSUE OF SUICIDE VICTIMS

Iris Šalamon[1], Urban Alič[1], Katarina Kouter[1], Tomaž Zupanc[2], Alja Videtič Paska[1]

[1]Medical Centre for Molecular Biology, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana
[2]Institute of Forensic Medicine, Faculty of Medicine, University of Ljubljana

Suicide is a serious public health problem worldwide. With more than 800,000 deaths every year it is one of the leading causes of death. Suicide is an important public health problem also in Slovenia. Over the past years, numbers of suicides in Slovenia have been declining, but with around 400 suicides every year, Slovenia still ranks among the countries with the highest suicide rate (number of suicide victims/100,000 citizens) in Europe. Besides social and economic factors, genetic and epigenetic factors importantly contribute to suicidal behavior. The term epigenetics include different mechanisms, DNA methylation, posttranslational modifications of histone tails, and noncoding RNAs. The first two alter the accessibility of genes to the regulatory elements and the last regulate transcribed RNAs. Differences at the level of epigenetics are happening all the time and are reflecting external stimuli. Micro RNAs (miRNAs), one of the noncoding RNAs, complementary bind to messenger RNA (mRNA) and in that way regulate gene transcript. Around 70 % of known mature miRNAs transcribe in the central nervous system, where they regulate gene expression during neurogenesis and neuroplasticity. The aim of our study was to research if downregulated genes associated with suicidal behavior (brain-derived neurotrophic factor and genes of the serotonergic system) are regulated by miRNAs. The selection of miRNAs was made with target prediction analysis and expression analysis. For target prediction analysis, we designed an algorithm that included data from five different miRNA prediction databases (DIANA microT, miRDB, miRmap, miRWalk, and TargetScan). MiRNAs that occurred in more miRNA prediction databases are more likely to be the ones that regulate selected genes and had a higher score. With expression analysis, we checked if selected miRNAs are (highly) transcribed in human brain tissue. We tested ten miRNAs with a high score and high expression in brain tissue with quantitative PCR. Results were normalized and statistically processed.

ESCHERICHIA COLI AFFECTS EXPRESSION OF CIRCADIAN CLOCK GENES IN HUMAN HEPATOMA CELLS

Lucija Raspor Dall'Olio[1], Urša Kovač[1], Zala Žužek[2], Katka Pohar[3], Alojz Ihan[3], Miha Moškon[4], Marjanca Starčič Erjavec[3], Damjana Rozman[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana
[2]Biotechnical Faculty, University of Ljubljana
[3]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana
[4]Faculty of Computer and Information Science, University of Ljubljana

Recent research indicated that dysbiosis of the gut microbiota can lead to an altered circadian clock of the mammalian host. Herein we developed an original system that allows real-time circadian studies of human HepG2 hepatoma cells co-cultured with bacteria. The HepG2 cells with stably integrated firefly luciferase reporter under control of Period2 promoter were co-cultured with E. coli strains isolated from human faecal samples from healthy individuals. The two E. coli strains differ in the phylogenetic group and the number of ExPEC virulence-associated genes: *BJ17* has only two, and *BJ23* has 15 of 23 tested. In the first 24 h the E. coli *BJ17* affects the HepG2 circadian clock more than *BJ23*. Cosinor analysis shows a statistically significant change in the amplitude of *PER1* and *2*, and the phase advance of *PER3*. A high percentage of necrotic and apoptotic cells occurred at 72 h when a correlation between the number of ExPEC genes and the influence on the HepG2 core clock gene expression was observed. Our study reveals that the E. coli genetic background is important for the effect on the mammalian circadian clock genes, indicating possible future use of probiotic E. coli strains to influence the host circadian clock.

MIGRATION ASSAY OPTIMIZATION FOR MKN45 AND MCF-10A CELL LINES

Vesna Bricman[1,2], Pia Pužar Dominkuš[2], Petra Hudler[2]

[1]Faculty of Pharmacy, University of Ljubljana
[2]Medical Centre for Molecular Biology, Institute of Biochemistry and Molecular Genetics, , Faculty of Medicine, University of Ljubljana

INTRODUCTION: Stomach cancer is a heterogeneous disease often detected at an advanced stage. Better understanding of molecular mechanisms in stomach cancer is necessary for more efficient diagnosis and therapy approaches. Extracellular vesicles (EVs) carry biomolecules and therefore have an important role in communication between cells. EVs are also involved in carcinogenesis. Hormone gastrin promotes stomach carcinogenesis by affecting cell proliferation, invasion and migration. Gastrin-induced EVs may also play a role in promoting cell proliferation and migration. To investigate this, we will perform migration assays on cells exposed to gastrin-induced EVs.

PURPOSE: Optimization of migration assays for studying the effects of gastrin-induced EVs on migration of naive cancer and normal cells.

METHODS: We used gastric adenocarcinoma cell line MKN45 and normal breast epithelial cell line MCF-10A. MCF-10A is commonly used in vitro model for normal epithelial cells. Cells were prepared at the following starting concentrations, MKN45: 5*105 cells/mL, 8*105 cells/mL, and 10*105 cells/mL; MCF-10A: 2*105 cells/mL, 4*105 cells/mL, and 6*105 cells/mL. We cultured the cells at these seeding densities on a 24-well plate, using Ibidi migration assay inserts. We added 70 µL of cell suspension per insert in duplicates. After 24 h we removed the inserts and washed the cells with a vesicle-free medium to remove any detached cells. We added 500 µL of vesicle-free medium to each well. Using inCellis cell imager, we took two images per well at 10x magnification at 0 h, 6 h, 24 h, 30 h, 48 h, 54 h. We used Image J software for wound closure analysis. Percent of wound closure was calculated using the following formula: % wound closure = (free area at 0 h – free area at X h)/(free area at 0 h)

RESULTS:

MKN45: At seeding concentration 5*105 cells/mL, the cell confluence at 0 h was 75-80 %, at 8*105 cells/mL the confluence was 80 % and at 10*105 cells/mL the confluence was 90 %. There were clusters of cells in each well at all concentrations. The gap was closing over time, however some cells migrated towards each other to form a monolayer and not towards the gap. At maximum seeding density 10*105 cells/mL there was 14,6 % wound closure at 24 h and 38,1% at 54 h.

MCF-10A: The images at 0 h showed that cells grew in different patterns - in clusters or in a layer with big gaps in between at all concentrations. The gap was closing, but not as uniformly as with MKN45 cells and wound closure could not have been measured using Image J software.

CONCLUSION: Monolayer was not reached in either cell line after 24 h. From this we concluded that optimization of seeding would be necessary, e.g. seeding at higher starting densities, 15*105 cells/mL for MKN45 cell line and 10*105 cells/mL for MCF-10A cell line. We will also consider analyzing the percentage of wound closure by measuring cell confluence for MCF-10A cell line, as they do not migrate as a front.

POSTER ABSTRACTS

POSTER SESSION 2

TRANSCRIPTOME AND METABOLITES

CHAIR: TADEJA REŽEN

SUBTYPING PATIENTS WITH IMMUNOGLOBULIN A VASCULITIS USING NGS APPROACH

Matija Bajželj[1,2], Katja Lakota[1,3], Snežna Sodin-Šemrl[1,3], Alojzija Hočevar[1,4]

[1]Department of Rheumatology, University Medical Centre Ljubljana
[2]Faculty of Medicine, University of Ljubljana
[3]Natural Sciences and Information Technologies, Faculty of Mathematics, University of Primorska
[4]Internal Medicine, Faculty of Medicine, University of Ljubljana

Immunoglobulin A vasculitis (IgAV) is a small vessel leukocytoclastic vasculitis, characterized by vascular IgA deposits. Skin involvement in the form of palpable purpura and/or petechiae is a prerequisite for diagnosis, while a broad range of organs might be involved. Renal involvement can be found in up to 84% of patients and is associated with an increased risk of progression to chronic kidney failure. An invasive procedure of skin biopsy is still the golden standard for diagnosis. Currently used markers for assessing IgAV renal involvement inaccurately estimate the risks and poorly guide clinicians in clinical management of IgAV patients leading to undertreated patients that progress to dialysis and have increased risk of morbidity and mortality. We hypothesize that blood/skin biomarkers at the RNA or protein levels could distinguish IgAV patients with or without renal involvement.

Examining databanks of "–omics" data (NCBI-GEO and Array Express) returned no result for IgAV samples. Our aim is to identify differentially expressed genes and dysregulated molecular pathways in IgAV patients with renal involvement as compared to patients with skin involvement only and healthy controls.

Skin biopsies and peripheral blood leukocytes were collected from treatment naïve IgAV patients at time of diagnosis: 1) with renal complications (n=3), 2) with skin limited disease (n=3), and age- and sex-matched healthy controls (n=3) at UMCL. RNA was isolated from leukocytes and skin and will be sequenced on Illumina4000 platform (40 million reads). Reads will be mapped onto human genome (GRCh38). Bioinformatic analysis for differentially expressed (DE) genes will be performed and data clustered using unsupervised hierarchical clustering analysis. To determine possible functional interactions of DE expressed genes, STRING database will be used. Gene set enrichment analysis (GSEA) will be done and DE genes in important pathways specifically visualized in Reactome, KEGG and Biocharta. Deconvolution is planned with program DeconRNASeq. Key differentially expressed genes will be confirmed on a validation cohort by qPCR and at the protein levels in sera using ELISA and Luminex.

Based on differentially expressed genes we will be able to discriminate between IgAV patients with renal involvement and those with skin involvement only, as well as healthy controls. Gene expression analysis of leukocytes and skin will determine usefulness of less invasive PBMC samples for diagnosis and prognosis of IgAV. Deconvolution will be used to investigate the relationship between deregulated immune profiles and stromal cells in IgAV. GSEA may enable insight into major pathogenic processes and might result in discovering targets for new therapeutic approaches.

TACKLING METABOLIC ASSOCIATED LIVER DISEASES PATIENT'S STRATIFICATION WITH TRANSCRIPTOME PROFILES

Eva Kočar[1], Tadeja Režen[1], Silvia Palmisano[2], Pablo J. Giraudi[2], Andrew Walakira[1], Peter Juvan[1], Claudio Tiribelli[2], Natalia Rosso[2], Damjana Rozman[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana
[2]Fondazione Italiana Fegato –ONLUS Area Science Park Basovizza

The incidence of the most prevalent form of chronic liver disease in the developed world, Metabolic Associated Fatty Liver Disease (MAFLD), is still expanding. Patients with advanced stages of the disease possess a high risk for co-morbidities, such as cardiovascular diseases and sleep disorders (obstructive sleep apnea). Growing evidence show that the latter increase the susceptibility for SARS-CoV-2 infection and worsen the outcomes of COVID-19. Prediction of MAFLD progression is unfortunately still limited due to a gap in the knowledge about biochemical mechanisms and pathways defining a particular stage of MAFLD. Therefore, the search of sensitive biomarkers, that would upgrade the current diagnostic approaches based on histological evaluation after tissue biopsies, is pivotal. In order to propose novel target genes that would better describe particular stages of MAFLD, we have applied transcriptome analysis and statistical modelling on the histologically determined MAFLD stages of patients. According to Kleiner classification system, liver samples from an Italian cohort of morbidly obese patients who underwent bariatric surgery were stratified into different MAFLD stages of fibrosis (F0-F4). Due to the low quality of RNA isolated from frozen liver biopsy samples, we performed global gene expression profiling using Affymetrix microarrays. A biclustering algorithm, Factor Analysis for Bicluster Acquisition (FABIA) was iteratively applied on normalised data (5000 iterations). Samples that were selected at least 1000 times in the first bicluster were considered for downstream analysis. Principal component analysis was done and two main clusters were identified. We then tested for differentially expressed genes between the two clusters using LIMMA. Logistic regression was applied to test for the association of clinical variables with the clusters. A 5% level of significance was considered and multiplicity correction was done where appropriate. Initial data show no patient stratification by fibrosis stage. However, further unsupervised machine learning methods and statistical modelling are currently being used to decipher differentially expressed genes and enriched pathways by KEGG and Reactome and transcription factors using TRANSFAC database. Furthermore, collecting new clinical samples is in progress to better the experimental work and consequently the results.

MOLECULAR SIGNATURE OF ENDOMETRIAL RECEPTIVITY IN ADENOMYOSIS

Erika Prašnikar[1], Jure Knez[2], Mario Gorenjak[3], Uroš Potočnik[3,4], Borut Kovačič[1], Tanja Kunej[5]

[1]Department of Reproductive Medicine and Gynaecological Endocrinology, University Medical Centre Maribor
[2]Department of Gynaecology, University Medical Centre Maribor
[3]Centre for Human Molecular Genetics and Pharmacogenomics, Faculty of Medicine, University of Maribor
[4]Molecular Biology and Genomics, Laboratory for Biochemistry, Faculty of Chemistry and Chemical Engineering, University of Maribor
[5]Department of Animal Science, Biotechnical Faculty, University of Ljubljana

Uterine adenomyosis is a common pathology in infertile women defined as endometrial tissue persistence in the muscular wall. Adenomyosis is associated with reduced pregnancy rate, but molecular knowledge of endometrial receptivity for embryo implantation is scarce. Therefore, we performed transcriptome sequencing of receptive endometrial samples and compared acquired data between women with and without adenomyosis and identified 382 differentially expressed genes (DEGs). To understand their role in the context of endometrial biology in adenomyosis, we integrated 382 DEGs with reported genes from genome-wide studies on endometrial receptivity in women with healthy uterus and women with similar but better studied endometriosis. Gene lists for adenomyosis (A=382), endometriosis (E=173) and healthy uterus (H=151) were used for the enrichment analysis using Cytoscape app ClueGO. Identified pathways were sorted in 11 network groups (Figure 1) based on their common biological role and associated genes. Six unspecific groups (e.g. ECM organisation and regulation of reproductive process) characterized by mapped genes from all 3 lists could indicate interference of pathologies with required processes for normal endometrial receptivity. Five groups with majority of mapped genes from A (e.g. expression of IFN-induced genes) or E (e.g. interleukin-10 signalling) lists could indicate pathophysiological effect of adenomyosis as well as endometriosis on endometrial molecular organisation.

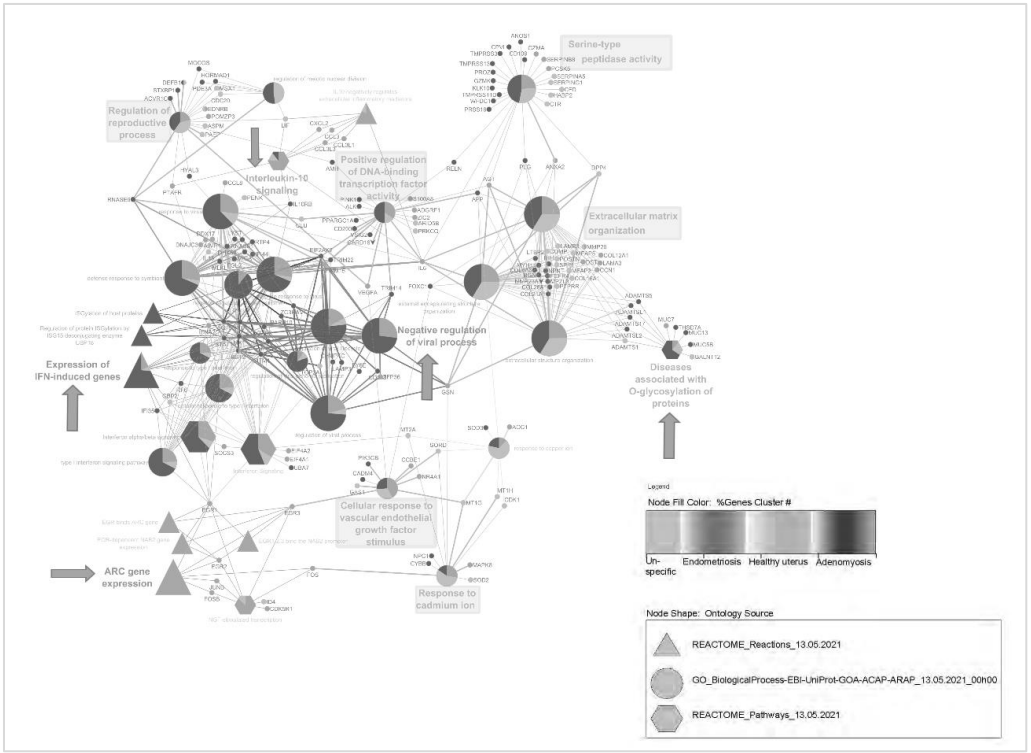

Figure 1: Network groups of integrated gene lists associated with endometrial receptivity in adenomyosis (A_dark grey), endometriosis (E_medium grey) and healthy uterus (H_light grey). Only pathways with corrected p-value < 0.05 according to the Bonferroni step down test were considered.

TRANSCRIPTOMIC CHANGES UPON THE PERTURBATION OF A NOVEL ONCOGENIC CIRCRNA IN HCC

Rok Razpotnik[1], Tadeja Režen[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana

BACKGROUND AND AIMS: By using publicly available datasets we have identified differentially expressed circular RNA (circRNA) in hepatocellular carcinoma (HCC). Furthermore, we have identified a novel oncogenic circRNA influencing cell proliferation, migration, colony formation and invasion in HCC model cell lines. Molecular mechanisms and binding partners of the identified circRNA are unknown. The aim of the study was to investigate transcriptomic changes upon overexpression or knockdown of a candidate circRNA in HCC model cell lines.

METHODS: To investigate the transcriptomic changes upon the perturbation of a candidate circRNA we have used the Huh-7 cell line with transient overexpression of circRNA and the SNU-449 cell line with a stable knockdown. Total RNA was isolated by using Trizol and transcriptomic changes were investigated by performing microarray analysis on a Clariom S Human assay. Transcriptome Analysis Console (TAC) Software was used to identify differentially expressed genes. Enrichment of gene ontologies, pathways and transcription factors binding motifs were performed by using Enrichr, g:Profiler and Gene Set Enrichment Analysis (GSEA).

RESULTS: We have identified 1717 upregulated and 1546 downregulated genes in SNU-449 cell line with knocked down expression (FDR ≤ 0.05) of a candidate circRNA and 620 upregulated and 398 downregulated (p-value ≤ 0.05) genes in Huh-7 with overexpressed circRNA. General processes such as cell cycle, migration and adhesion were found to be enriched by systemic analyses. Pathway analyses revealed the enrichment of specific pathways such as TGF-β and MAPK signaling pathways and interestingly also circadian cycle and steroid biosynthesis. The family of E2F transcription factors has been consistently linked to transcriptomic changes in our cell models.

CONCLUSION: We have uncovered the transcriptomic changes in HCC model cell lines with a perturbed expression of a candidate circRNA. These changes pointed us towards systemic changes commonly found in cancer and confirmed the results of functional assays in HCC model cell lines. Signaling pathways such as TGF-β and MAPK have already been connected to the pathology of HCC. The family of E2F transcription factors is often perturbed in cancer and is implicated in the regulation of the cell cycle.

THE LONG NONCODING RNA HOTTIP REGULATES CELL CYCLE AND INFLAMMATORY RESPONSE IN HAND SYNOVIAL FIBROBLASTS

Tadeja Kuret[1], Mojca Frank-Bertoncelj[2], Ana Zupanič[2], Snežna Sodin-Šemrl[3], Oliver Distler[2], Caroline Ospelt[2]

[1]Institute of Cell Biology, Faculty of Medicine, University of Ljubljana
[2]Center of Experimental Rheumatology, Department of Rheumatology, University Hospital Zurich
[3]Department of Rheumatology, University Medical Center Ljubljana

BACKGROUND: Previously we showed that the long noncoding RNA (lncRNA) HOTTIP is exclusively expressed in synovial fibroblasts (SF) from distal joints, such as small joints of hands and feet, which exhibit prominent proliferative and chemotactic activities (1). Our objective was to explore the role of HOTTIP in shaping the function of hand SF in arthritis.

METHODS: We silenced the lncRNA HOTTIP in hand SF using LNA GapmeRs (100 nM, 48h). We conducted RNA-sequencing (n=2) and confirmed RNA-sequencing data with qPCR in a larger cohort of hand SF from RA patients (n=6). Protein-protein interaction analysis of RNA-sequencing data was performed using STRING. Protein levels of IL8 (n=4) and MMP3 (n=6) in cell supernatants were measured by ELISA. Cyclin dependent kinase inhibitor p21 (n=4) was detected by Western blot. Proliferation (n=3) was measured in vitro using the BrdU assay. Apoptosis and necrosis (n=6) were determined with Real time-Glo annexin V apoptosis and necrosis assay. Significance was defined as $p<0.05$ measured by one-sample t-test or paired t-test.

RESULTS: STRING analysis of RNA sequencing data showed changes in cell cycle, inflammatory response and integrin pathways after silencing of HOTTIP in hand SF. We confirmed the significant downregulation of transcripts involved in mitotic cell cycle (NCAPG, TUBGCP5, TADA3, ASPM, ZWILCH, CDC27, BUB1, GPSM2 and CDK6), significantly increased transcript and protein expression of the cyclin-dependent kinase inhibitor p21 and significantly less proliferation in HOTTIP silenced hand SF. No difference in apoptosis and necrosis was observed between HOTTIP and control silenced hand SF. Furthermore, silencing of HOTTIP resulted in significant upregulation of transcripts involved in inflammatory and immune response pathways (IL8, IL12A, IL17C, CXCL3, MMP3 and TNFAIP3) and significantly increased protein levels of IL8 and MMP3. Silencing of HOTTIP also significantly altered gene expression of different types of integrins (ITGA3, ITGB1, ITGB5, ITGB7 and ITGA2B) that play a role in adhesion and organization of newly synthesized extracellular matrix. Stimulation of hand SF with TNFα and IL6/IL6R resulted in significant decrease of HOTTIP expression.

CONCLUSION: Distal-specific expression of HOTTIP could support enhanced proliferative properties in hand SF. In inflammatory conditions, reduced levels of HOTTIP might shape a location-specific inflammatory response with joint-specific changes in cytokine and chemokine expression, cell adhesion and cell-to-extracellular matrix interactions.

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INCORPORATION OF MOTIF INFORMATION IN THE GENE REGULATORY NETWORK INFERENCE

Žiga Pušnik[1], Miha Mraz[1], Nikolaj Zimic[1], Miha Moškon[1]

[1]Faculty of Computer and Information Science, University of Ljubljana

Gene regulatory networks (GRNs) inference is often employed to uncover underlying structure and dynamics from gene expression data. Nonetheless, this is still a challenging task due to the noise component and disproportionate ratio between the size of a system and the ammount of available data [1]. To avoid over-fitting researchers utilized regularization in the regression problem, where every gene is expressed as a linear combination of all other genes. An example of this approach is a partial-correlation-based method SPACE [2]. We impose structural constraints on the inferred gene regulatory network based on prior knowledge from the structure of reference GRNs.

Motifs are patterns that occur significantly more frequently than expected in randomized networks [3] and introduce the evolutionary advantage due to their capability to execute various functions and process information. The same types of motifs emerged in organisms that are not related. Furthermore, motifs govern the general structure of GRNs. One can easily see why incorporating this prior knowledge into the network inference can be beneficial. Here, we present a gene regulatory inference approach based on SPACE, that can incorporate prior knowledge about hub genes, motifs, and sparsity of GRNs [4]. We impose these constraints by modifying the weights of genes contributing to a joint loss function in the regression problem. We modify weights iteratively with gradient descent. By extracting the expected number of regulatory genes, gene degree distribution, and motifs from the reference networks we improved inference accuracy, precision, and F1 rate in the inference of GRNs derived from GRN of bacteria E. coli for a few percentage points.

In further work, we will explore the sensitivity of our approach to perturbations of reference networks. In addition, we will focus on inference methods based on heuristic algorithms and multi-objective optimization.

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COMBINED LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR QUANTIFICATION OF ANDROGENS AND 11-OXYANDROGENS

Marija Gjorgoska[1], Maša Sinreih[1], Tea Lanišnik Rižner[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana

Androgens are steroid hormones with a multitude of roles in sex-specific and sex non specific physiological processes. Unlike traditional androgens, their 11 oxygenated metabolites do not demonstrate an age-dependent decline, and some of them have equal potencies as the parent androgen. The 11 oxyandrogens have been associated with several disorders of androgen excess, such as polycystic ovary syndrome and castration-resistant prostate cancer. Moreover, a potential role of androgens and their metabolites in the pathogenesis of endometrial (EC) and ovarian cancer (OC), where local androgen synthesis might take place, has been suggested.Objective: To develop a robust liquid chromatography-tandem mass spectrometry (LC MS/MS) method for simultaneous detection and quantification of a panel of endogenous androgens, namely, androstenedione (A4), testosterone (T), 5α-dihydrotestosterone (5α DHT), dehydroepiandrosterone (DHEA), and its sulfate, DHEA S, and their 11-oxygenated metabolites, namely, 11β-hydroxyandrostenedione (11OHA4), 11β hydroxytestosterone (11OHT), 11 ketoandrostenedione (11KA4), 11 ketotestosterone (11KT), 11 ketodyhydrotestosterone (11KDHT).Methods: Androgen standards were prepared in 70% methanol/0.2 mM ammonium fluoride and analyzed on a Sciex 3500 triple quadruple mass spectrometer coupled to a Shimadzu ultra-high performance chromatography (UHPLC) system. MS/MS analysis was performed under constant electrospray ionization conditions and polarity switching. Two MS/MS transitions for each analyte of interest were evaluated using the Scheduled MRM algorithm. The method linearity was assessed by calibration curves ranging from 5 to 50.000 pg/ml. Quantification was based on linear regression analysis using 13C3-T as an internal standard. The limit of quantification (LOQ) was defined as the lowest concentration where the calculated concentration is ± 20% of the nominal concentration. Low, medium and high quality control samples were analyzed to estimate method precision.Results: The developed LC-MS/MS method is linear from 5 to 50.000 pg/ml, with a regression coefficients (R2) greater than 0.99 for each analyte. The estimated LOQ is 10 pg/ml for A4, T, 5α DHT, and DHEA-S, 25 pg/ml for 11OHA4, 11OHT, 11KA4, 11KT, 11KDHT, and 250 pg/ml for DHEA. The method has excellent selectivity and no carryover.Conclusions: The described LC-MS/MS method can reliably determine a panel of endogenous androgens and 11-oxyandrogens at picomolar levels. The method has high sensitivity and specificity, good linearity over a wide concentration range, and excellent precision. Moreover, it requires small sample volumes and minimal sample preparation, without the need for derivatization. We intend to apply the method for assessment of the androgen metabolism in model cell lines of EC and OC as well as to biological specimens. This work was supported by the Slovenian Reseach Agency. Grant number: J3-2535.

THE STEROL CRYSTALLIZATION AND LIPID DROPLETS BIOGENESIS IN DYSREGULATED CHOLESTEROL SYNTHESIS ARE INCIDENTAL TO METABOLIC ASSOCIATED LIVER DISEASE PROGRESSION IN MICE

Kaja Blagotinšek Cokan[1,2], Petra Ivanuša[1,2], Cene Skubic[1], Martina Perše[3], Karmen Wechtersbach[4], Nika Kojc[4], Jera Jeruc[4], Eva Jarc Jovičić[5], Toni Petan[5], Željko Debeljak[6,7], Marija Heffer[8], Madlen Matz-Soja[9,10], Damjana Rozman[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana
[2]Institute for Biostatistics and Medical Informatics (ELIXIR-SI), Faculty of Medicine, University of Ljubljana
[3]Medical Experimental Centre, Institute of Pathology, Faculty of Medicine, University of Ljubljana
[4]Institute of Pathology, Faculty of Medicine, University of Ljubljana
[5]Department of Molecular and Biomedical Sciences, Jožef Stefan Institute
[6]Clinical Institute of Laboratory Diagnostics, Osijek University Hospital
[7]Department of Pharmacology, Faculty of Medicine, JJ Strossmayer University of Osijek
[8]Department of Medical Biology and Genetics, Faculty of Medicine, JJ Strossmayer University of Osijek
[9]Rudol-Schönheimer-Institute of Biochemistry, Divison of General Biochemistry, Faculty of Medicine, University of Leipzig
[10]Section of Hepatology, Clinic and Polyclinic for Gastroenterology, Hepatology, Infectious disease, Pneumology, University Clinic Leipzig

BACKGROUND AND AIMS: Hepatic lipid metabolic reprogramming has been identified as a contributing factor to hepatocellular carcinoma (HCC). Current evidence suggests that lanosterol 14α-demethylase (Cyp51) from postsqualene part of cholesterol synthesis is crucial for the normal liver function and diminished activity of Cyp51 in the hepatocytes leads to metabolism associated liver disease, with ductular reaction and fibrosis, ending in HCC (Blagotinšek Cokan K.; Cancers, 2020). The purpose of this study is to identify the initial processes that are most affected in the hepatocytes and their communication link with other cells towards progressive liver pathologies.

METHOD: The new Cyp51LC knockout mouse model with tissue- and time-specific deletion of Cyp51 in hepatocytes was applied and primary hepatocytes from adult livers were isolated. The livers were evaluated by histology, transmission electron microscopy and MALDI TOF MSI. To examine the primary hepatocytes we performed western blot, RT-qPCR, sterol analysis by LC/MS and FACS analysis of cellular lipid droplet content.

RESULTS: In primary Cyp51 knockout hepatocytes, we detected high levels of lanosterol and 24,25-dihydrolanosterol, which promote hepatocyte injuries at the ultracellular level: abnormal nucleus, swollen mitochondria and dilatated endoplasmic reticulum (ER). The perturbation of ER function was indicated with upregulated Atf4 and Atf6 targets of unfolded protein response signaling. Unexpectedly, the crystals and acicular clefts were observed in hepatocytes and even more in activated Kupffer cells. The high level of hepatocyte sterols proposes them as the main components of the crystals. In addition, higher apoptotic activity was observed in the liver parenchyma. Since lipid metabolism has an important role in the cellular stress response, we showed activated lipolysis coupled with upregulated Pnpla2 and Abdh5, which resulted in changes of lipid droplet contents.

CONCLUSION: Our findings represent a novel insight into hepatic lipid droplet biogenesis and its interaction with the ER as a crucial step to buffer the toxic levels of sterols. The excess sterols, which are stored in the liver primarily within hepatocytes can crystallize and promote different cellular injuries. The sterol crystals showed high proinflammatory capabilities, which may contribute to ˝sterile inflammation˝ in advanced metabolic-related liver pathologies.

POSTER ABSTRACTS

POSTER SESSION 3

LIFE SCIENCES

ASSOCIATION BETWEEN GENETIC VARIABILITY IN SEGREGATION KINASES AND GASTRIC CANCER SUSCEPTIBILITY

Denis Babič[1], Pia Pužar Dominkuš[2], Petra Hudler[2]

[1]Biotechnical Faculty, University of Ljubljana
[2]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana

INTRODUCTION: Gastric cancer is the 8th most common cancer in Slovenia. The incidence around the world and in Slovenia has been declining over the past 100 years due to the improvement of living standards and hygiene, but is now stabilizing. The average age of patients is over 60 years. Gastric cancer is caused by a combination of environmental and genetic factors, such as genetic changes in DNA or changes in mRNA expression. Single nucleotide polymorphisms can cause chromosomal instability, leading to the accumulation of many genetic changes in a short period of time. Defects in chromosomal segregation often lead to aneuploidy, which is characteristic for malignant cells.

METHODS: Using bioinformatics tools, we identified the candidate gene *NEK6* (NIMA related kinase), which carries the transcript for segregation kinase, an enzyme involved in mitosis, responsible for proper establishment of the mitotic spindle. Improper functioning of this protein can trigger cell cycle arrest and can lead to mitotic spindle defects, inaccurate chromosome separation, and cell apoptosis. We identified two polymorphisms that met the selected criteria; rs2416, which lies in the 3'-untranslated region, and rs2065221, located in the intron region of *NEK6*. Genotyping was performed using TaqMan Genotyping Assays on DNA, isolated from tissue samples of patients with gastric cancer and blood samples of healthy individuals. The distribution of genotypes of selected polymorphisms in patients and in control group as well as the distribution according to gender were evaluated by statistical analysis. The polymorphisms were analysed according to genetic models. We also analysed the association between selected polymorphisms and their influence on histopathological characteristics of tumours. Kaplan-Meier analysis and log rank test were used to identify the correlation between survival and histopathological characteristics as well as genotypes. We also investigated the influence that polymorphisms have on several transcription factors, important in the cell division mechanism, and on the binding of miRNA using in silico approaches.

RESULTS: We found that women with the rs2416 GG genotype were more likely to have poorly differentiated tumours, which could indicate a greater likelihood of developing distant metastases. Also, men with the rs2065221 AG genotype were more likely to have tumours in the area of the cardia or oesophageal border. The rs2065221 AA genotype was more common in the population of women with gastric cancer. Rs2416, which is located in the 3'-untranslated region, was the target of several miRNA molecules, implicating that the presence of the polymorphism alters the binding power of miRNA to the gene sequence, thus impairing the precise control over the regulation of *NEK6* translation.

CONCLUSION: In conclusion, our results indicated that genetic variations in mitotic kinase gene *NEK6* could influence the progression of gastric cancer tumours.

THE ROLE OF NON-CODING REGULATORY REGIONS IN DEVELOPMENT OF FAMILIAL ERYTHROCYTOSIS

Tina Turel[1], Aleša Kristan[2], Nataša Debeljak[2]

[1]Faculty of Chemistry and Chemical Technology, University of Ljubljana
[2]Medical Centre for Molecular Biology, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana

BACKGROUND: Familial erythrocytosis is a rare inherited disease characterized by elevated red blood cell counts. It is reflected by increased hematocrit, hemoglobin concentration and erythrocyte count in the blood [1]. Despite sequential analysis of the coding regions of the genes involved in familial erythrocytosis, 70 % of patients with suspected familial erythrocytosis remain undetermined. In a recent study, variants in the VHL crypt exon were detected in seven families with familial erythrocytosis [2].

AIM OF THE STUDY: Within our study, we hypothesized that several additional non-coding regions of erythrocytosis-causing genes must be involved in the disease development. We explored non-coding variants of genes associated with erythrocytosis and compered them with variants identified within the NGS analysis of erythrocytosis patients [1].

METHODS AND RESULTS: We used different databases (NCBI, Ensembl, Encode, UCSC) and tools (MethPrimer, Human Splicing Finder, dbSNV and IGV) to analyze non-coding regions of nine genes previously associated with erythrocytosis (*VHL, EGLN1, EPAS1, EPO, HBB, HBB1, HBB2, BPGM,* and *EPOR),* including *SH2B3* and *JAK2*. We determined the locations of the noncoding regions and reviewed the theoretical background. With data mining, we determined the locations of the CpG islands in the promoters and the first intron. We systematically reviewed and compared the results obtained during our study within variants identified NGS analysis [1] within the project Genetic Basis of Erythrocytosis in Slovenia. The set of variants was narrowed by eliminating variants that did not match with the locations of promoters or areas of CpG islands. Selected variants were further analyzed using several tools to determine the frequency of variants in the population and among patients and the coincidence of the location with the location of the *VHL* cryptic exon [2] and pathogenicity predictions by Combine Annotation Dependent Depletion (CADD) score. We could not confirm any non-coding pathogenic variants in selected group of patients.

CONCLUSION: As no variant met the pathogenic criteria, the future analysis will be extended to 3'UTR regions.

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ACTN3 p.R577X VARIANT IS ASSOCIATED WITH FREQUENCY OF INJURIES IN SLOVENIAN FEMALE FOOTBALL PLAYERS

Inge Sotlar[1], Tisa Podkrajšek[1], Tina Levstek[1], Katja Goričar[1], Vita Dolžan[1], Katarina Trebušak Podkrajšek[1,2]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana
[2]Clinical Institute for Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana

Genetic testing in sport is in high demand, as the informed decision to direct children to a sport or sports discipline has become very attractive to parents and coaches. One of the most researched genes, associated with sports performance, is the ACTN3 gene. In humans, ACTN3 encodes for the α-actinin-3 (ACTN3), a structural component of the Z-disk in sarcomeres, specifically expressed in fast-twitch (type II) muscle fibres. A common ACTN3 gene variant NM_001104.4:c.1729C>T (p.R577X, rs1815739) changes arginine codon (R) to the termination codon (X), and in a homozygous state it reduces strength, muscle mass, and fast-twitch fibre diameter. Furthermore, it was associated with a higher risk of sports injuries and longer exercise recovery. We aimed to evaluate the association of the ACTN3 p.R577X polymorphism with injuries and player position in Slovenian female football players. The study group included 43 participants, with a median age of 16 (13–28) years, who had been actively training football for at least four years. ACTN3 p.R577X genotyping was performed with PCR and DdeI restriction analysis on saliva DNA and subsequent Sanger sequencing when needed. p.R577X in the homozygous or heterozygous state was present in 58.1 % of the players, while 41.9 % had normal genotype, which is roughly comparable to the data in the global and/or related populations. 53.7 % of participants had a four years history of at least one sports injury (eg. sprains, fractures or muscle injuries). In our study, no statistically significant association was found between ACTN3 p.R577X genotypes and the incidence of injuries ($p = 0.309$), nor between the genotypes and player position ($p = 0.830$) or age-dependent category team ($p = 0.427$). However, we confirmed the association between the incidence of injuries and the frequencies of each allele ($p = 0.037$), with the X allele being associated with a higher incidence of injuries. Nevertheless, this data needs to be confirmed in a larger cohort, since the limitation of our research was the small number of female football players involved, and not all of them were professional players. Additionally, the participants were relatively young, therefore the playing positions in some were not settled, and the number of injuries might not be representative.

ANALYSIS OF CANNABINOID RECEPTORS IN BREAST CANCER CELL LINES

Karmen Mlinar[1], Sandra Ropret[2], Nataša Debeljak[3]

[1]Faculty of Chemistry and Chemical Technology, University of Ljubljana
[2]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana
[3]Medical Centre for Molecular Biology, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana

INTRODUCTION: Breast cancer (BC) is the most common form of malignancy in women. Based on the expression of the hormone receptors (HR) and human epidermal growth factor receptor 2 (HER2), three types of BC are distinguished: HR-positive, HER2-positive, and triple-negative BC1.

Cannabinoid receptor type 1 (CB1R, gene *CNR1*) and type 2 (CB2R, gene *CNR2*), together with endocannabinoids and proteins responsible for their synthesis, transport, and degradation, form the endocannabinoid system. CB1R and CB2R are G-protein coupled receptors that bind cannabinoids in neural and immune cells, respectively. Their expression has been confirmed also in BC cells and several studies have shown opposing effects of cannabinoids on tumor biology2.

AIMS OF OUR STUDY WERE TO:
- overview known transcripts and isoforms of the genes *CNR1* and *CNR2*,
- characterise the RNA expression of cannabinoid receptors on BC cell lines.

METHODS AND RESULTS: With analysis of the UniProt database we identified three isoforms of CB1R (CB1, CB1a, and CB1b) and two isoforms of CB2R (CB2A and CB2B). By overviewing the RefSeq database at NCBI, we found several CBR transcripts; 11 of isoform CB1, 1 of isoform CB1b, 1 of isoform CB2B and no transcripts encoding CB1a and CB2A.

In aim to determine the expression of CNR1 and CNR2 in BC cells, we first isolated RNA from different BC cell lines that differ in expression of HER2 and HR (MCF7, T-47D, SK-BR-3, MDA-MB-231, MDA-MB-361) and two control samples (SH-SY5Y, brain tissue), followed by reverse transcription into cDNA. We performed quantitative analysis of RNA expression with selected TaqMan assays for genes CNR1, CNR2, reference genes RPLP0 and HPRT1 and used the relative methods for quantification. Contrary to our expectations, we were unable to detect CNR1 gene expression in any analysed BC cell line. We could not determine the expression level of gene CNR2 due to lack of positive control.

CONCLUSION: The research will be extended to several BC cell lines and characterization on RNA and protein level to fully confirm the expression of *CNR1/CNR2* gene in BC cell lines. Appropriate positive control will be selected.

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CONNECTION BETWEEN CHOLESTEROL SYNTHESIS AND THE WNT SIGNALLING PATHWAY

Hana Trček[1], Cene Skubic[1], Damjana Rozman[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana

Cholesterol is probably the best-known steroid, as it plays important biochemical roles as a precursor and is at the same time crucial for the structure and function of vertebrate cell membranes. Given its important functions, the maintenance of its homeostasis is essential. Cholesterol synthesis pathway is downstream of WNT/β-catenin signalisation, an evolutionary conserved pathway among metazoan animals. The WNT/β-catenin signalisation is associated with several biological processes, such as cell fate determination, migration and polarity, neural networks, and organogenesis during embryonic development. Crucial players are the WNT proteins, endogenous agonists of FZD ("Frizzled") receptors, which by binding cause a cascade that leads to β-catenin accumulation and its translocation into the nucleus.

We have studied the connection between cholesterol synthesis and WNT signalling pathway on human HepG2 cell lines with different gene knockouts (*CYP51, DHCR24, HSD17B7, SC5D*) from the late part of cholesterol synthesis. Our hypothesis is that different intermediates of cholesterol synthesis that accumulate in these cell lines modulate the WNT/β-catenin signalling, directly or indirectly. By Affymetrix microarrays we evaluated the transcriptome of mentioned knockout cell lines. Analysis showed 3 differentially expressed genes (*DKK1*, *DKK4* and *TLE4*), involved in WNT pathway, in all knockout cell lines compared to control and 10 differentially expressed genes in either one, two or three knockouts (*CTNNB1, FZD1,4,6, GSK3B, LEF1, PORCN, TLE1,3,5*). Furthermore, we quantified the expression level for selected genes on qPCR, where 8 out of 11 genes (*DKK4, FZD1,6, GSK3B, LEF1, PORCN* and *TLE3,4*) turned out to be differentially expressed. Upregulated expression, with 17-fold change in *CYP51*, 4.4-fold change in *DHCR24* and 33-fold change in *HSD17B7* knockout cell line, was most pronounced in *LEF1* gene. These results show that disrupted cholesterol synthesis affects the WNT signalling pathway. Since *LEF1* is a DNA-binding transcription factor and well-established oncogene, our future research will focus on *LEF1* and its target genes.

COMPARING GENE EXPRESSION ANALYSIS BY OXFORD NANOPORE SEQUENCING AND DNA-MICROARRAYS ON HEPATOMA HEPG2 CELLS WITH *CYP51* KNOCKOUT

Žiga Vičič[1], Cene Skubic[1], Andrew Walakira[1], Nejc Nadižar[1], Tadeja Režen[1], Damjana Rozman[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana

Oxford Nanopore sequencing measures changes in current caused by a single-stranded polynucleotide being driven through protein nanopores. Different current-blocking properties of individual nucleobases reflect in the measured current changes and enable base calling. Compared to DNA-microarrays, Oxford Nanopore sequencing enables measurements of absolute transcript abundance while keeping the transcripts intact. As such it can detect all expressed transcripts as opposed to capturing signal intensities of fluorescently labelled sequences that hybridize to probes present on a DNA-microarray. Our hypothesis is that Oxford Nanopore sequencing will measure a wider range of differentially expressed genes compared to DNA-microarrays, of which the expression of a selected few will further be validated by qPCR.

For Oxford Nanopore sequencing, RNA was isolated from HepG2 cells with and without the CRISPR-Cas9 induced CYP51 knockout. After isolation, RNA was reverse-transcribed and amplified by PCR following ONT cDNA-PCR sequencing protocol. Sequencing was performed on Oxford Nanopore MinION Flow cell using GridION. Sequencing data were aligned to the human genome assembly ninety-fourth release of GRCh38 from Ensembl using minimap2. Differential gene expression was evaluated using DESeq2 and edgeR. Significantly expressed genes were compared to previously analysed data obtained on Affymetrix microarrays.

The DESeq2 model determined 122 genes to be differentially expressed (DE) using FDR adjusted p-values ($p.adj<0.05$). The edgeR model was used to determine 136 DE genes ($p.adj<0.05$). Previously, the limma package was used to determine 410 significantly DE genes ($p.adj<0.05$) using Affymetrix microarrays. Interestingly, among the significantly DE genes, 93 and 134 fit the criteria of more than two-fold change in expression using DESeq2 and edgeR respectively on Oxford Nanopore sequencing data, while 66 genes that fit the criteria were reported by limma on Affymetrix microarray data.

As Oxford Nanopore sequencing pipelines for data analysis are still being developed, different tools need to be tested to determine the results of the experiment. Considering the statistical relevance of the expressed genes and the fold change of the expression in comparison to the control samples, we confirmed the hypothesis that Oxford Nanopore sequencing measures a wider range of genes that fit the criteria. Using the DESeq2 model 51 genes and using the edgeR model 61 genes were detected that were not previously detected using the Affymetrix microarray. To further validate these results additional experiments using qPCR will need to be carried out by measuring selected transcript expression.

DESIGN OF 3D IN VITRO MODEL OF PERITONEAL ENDOMETRIOSIS USING BIOPRINTING TECHNIQUE

Maja Pušić[1], Tea Lanišnik Rižner[1]

[1]Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana

Endometriosis is a common female gynaecological disorder defined with the presence of endometrial tissue outside the uterus cavity. It affects around 190 million women worldwide and is associated with high health care cost. Major problems connected with endometriosis are delayed diagnosis and lack of appropriate treatment. To achieve innovation in discovery of novel diagnostic and treatment options for this debilitating disease, it is essential to establish advanced experimental model of endometriosis. Here, we present a study design for development of novel 3D bioprinted in vitro model of peritoneal endometriosis. To establish novel 3D in vitro model of peritoneal endometriosis, cell suspensions of the 12-Z, 22-B (epithelial and stromal cell lines of peritoneal endometriosis) and MD (macrophage cell line) will be mixed with an appropriate bioinks (Cellink Laminink+/Cellink Fibrin) and 3D spheroids will be printed using BIO X 3D bioprinter (Cellink AB Gothenburg, Sweden). For comparison, 2D in vitro model will include the same combinations of cell lines cultured in direct contact and in transwell chambers. Established models will be characterized and compared. Characterization will include assessment of cell morphology, determination of cell proliferation, migration and invasion rate, determination of specific gene, protein and metabolic profile associated with 2D and 3D culture conditions. Furthermore, established 3D in vitro model will be used for testing the impact of potential therapeutics on cell proliferation, migration, apoptosis activity, inflammation and fibrosis development. The designed novel 3D in vitro model of peritoneal endometriosis includes the following innovative aspects: 1) addition of the main cell types found in the peritoneal endometriosis, 2) representation of inflammatory and fibrotic nature of the disease, 3) use of 3D bioprinting technique that will assure precision, accuracy and reproducibility between 3D spheroids replicates and consequently accelerate screening and discovery of novel therapeutic or diagnostic options for endometriosis. In addition, this study will be the first to compare different 2D and 3D co-culture models of peritoneal endometriosis at several levels (RNA, protein and metabolic). We have designed the study with an aim to develop novel 3D in vitro model of peritoneal endometriosis for the purpose of testing novel potential therapeutics and/or diagnostic agents.

IN VIVO IMAGING OF LISTERIA INNOCUA BIOFILM BY SELECTIVE STAINING OF SURFACE COMPONENTS

Nika Janež[1], Aleksandar Sebastijanović[2], Tanja Zupan[1], Stane Pajk[2], Janez Štrancar[2], Jerica Sabotič[1]

[1]Department of Biotechnology, Jožef Stefan Institute
[2]Condensed matter department, Jožef Stefan Institute
[3]Faculty of Pharmacy, University of Ljubljana

Listeria monocytogenes is the causative agent of listeriosis, which is an important public health problem, because it is associated with high rates of hospitalization and mortality. Biofilms have been suggested to play an important role in surviving the food processing environment in which Listeria spp. persist for months or even years. For the survival and adaptation under various environments Listeria probably relies on its rich repertoire of surface structures. Bacterial surface consists of a range of proteins and carbohydrate polymers in addition to the peptidoglycan. L. innocua and L. monocytogenes share genetic and physiological similarities, but L. innocua lacks virulence factors and is considered non-pathogenic. Therefore, it is being tested as a surrogate organism for L. monocytogenes, particularly to determine the efficacy of antimicrobial and antibiofilm strategies.

Studies of biofilms usually track biofilm biomass, viability or metabolic activity using various dyes or viability assays. Fluorescence microscopy provides a good complementary method to describe biofilm systems by visualization of bacteria and components of the extracellular matrix. Here, we evaluated fluorescent probes targeting surface components using in vitro assays for in vivo imaging of Listeria innocua biofilm. Genomic data of the model strain were used for elucidation of potential probe targets. Despite the abundance of potential surface proteins and primary and secondary carbohydrate polymers, the labelling was successful only for a limited number of probes. Membrane seems to be unavailable for labelling probably due to the thick peptidoglycan layer.

This work paves the way for further research of direct, non-invasive biofilm imaging that is of enormous importance for reliable biofilm research advancement as well as biofilm contamination detection.