10th CFGBC Symposium with ISBE and CASyM workshops:
"From Functional Genomics to Systems Biology and Systems Medicine"

&

Hands-on tutorial Systems Biology/Medicine

Book of Abstracts

Electronic version

Faculty of Medicine, University of Ljubljana

Ljubljana, June 30 - July 3, 2015

10th CFGBC Symposium with ISBE and CASyM workshops: "From Functional Genomics to Systems Biology and Systems Medicine" & Hands-on tutorial Systems Biology/Medicine

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Edited by
Jure Ačimovič, Petra Hudler, Rok Košir, Peter Juvan

Technical Editor
Jure Ačimovič

Reviewed by
Vita Dolžan, Marko Goličnik

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The 10th Anniversary of Centre for Functional Genomics and Bio-Chips

Prof. dr. Damjana Rozman, Head of the Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Slovenia. Email: damjana.rozman@mf.uni-lj.si; www: http://cfgbc.mf.uni-lj.si/;

From the Slovenian Consortium for Bio-Chips to Centre for Functional Genomics and Bio-Chips

It has been over 10 years from the initial "Slovenian Network for Functional Genomics". This informal network has been constituted in 2001 at the Medical Centre for Molecular Biology (MCMB), Faculty of Medicine at the University of Ljubljana (UL) with the aim to virtually connect research groups and laboratories that work in Slovenia in the various fields of functional genomics. In December 2001 the initiators of the network called the kick-off meeting of the Slovenian Consortium of Bio-Chips which has been joined by the academic, research and clinical institutions, as well as by pharmaceutical industry. The initiative was triggered by colleagues from different Faculties and Institutions, from UL Faculty of Medicine (R. Komel, D. Rozman), Faculty of Pharmacy (B. Štrukelj) and Biotechnical Faculty (P. Dovč) as well as the National Institute of Biology (T. Lah, M. Ravnikar, K. Gruden). In 2005 Faculty of Medicine renovated 220 m^2 of laboratory space that became home of the Centre for Functional Genomics and Bio-Chips (CFGBC, http://cfgbc.mf.uni-lj.si/). The consortium members in November 2005 signed a formal act "Regulations of the Activities of the Centre for Functional Genomics and Bio-Chips" *("Pravilnik o delovanju Centra za funkcijsko genomiko in bio-čipe"*) that was signed by 12 directors or deans from the collaborating institutions from UL (Faculty of Medicine, Biotechnical Faculty, Faculty of Pharmacy), Lek Pharmaceuticals, National Chemical Institute, Agricultural Institute of Slovenia, National Institute of Biology, Institute of Oncology, University Medical Centre Ljubljana, Jožef Stefan Institute, Blood Transfusion Centre, Institute for Health Protection. The boards of CFGBC, including the Management Board and the Scientific Board, have been established in January 2006. In the same year the members signed the agreement to purchase common equipment *"Pogodba o nakupu skupne raziskovalne opreme"*. This document represented a crucial step in further development of the consortium and its activities. In 2004 we also became a part of the Network of Research Infrastructural Centres of UL (MRIC-UL, http://www.uni-lj.si/research_and_ development/). Of particular importance and help to Head of CFGBC and the entire Consortium were the Heads of the management board, prof. dr. Radovan Komel (2006 – 2009), prof. dr. Simon Horvat (2010 – 2012) and prof. dr. Kristina Gruden (2013 – present).

The Consortium has now 16 members (Figure 1). We still share the equipment, with open access to all partners, and organize common activities. The 1st CFGBC symposium was organized on 14.6.2006 to celebrate the 1st anniversary of CFGBC. It has become a traditional scientific networking event on the post-genome technologies in Slovenia, which is in 2015 held for the 10th time.

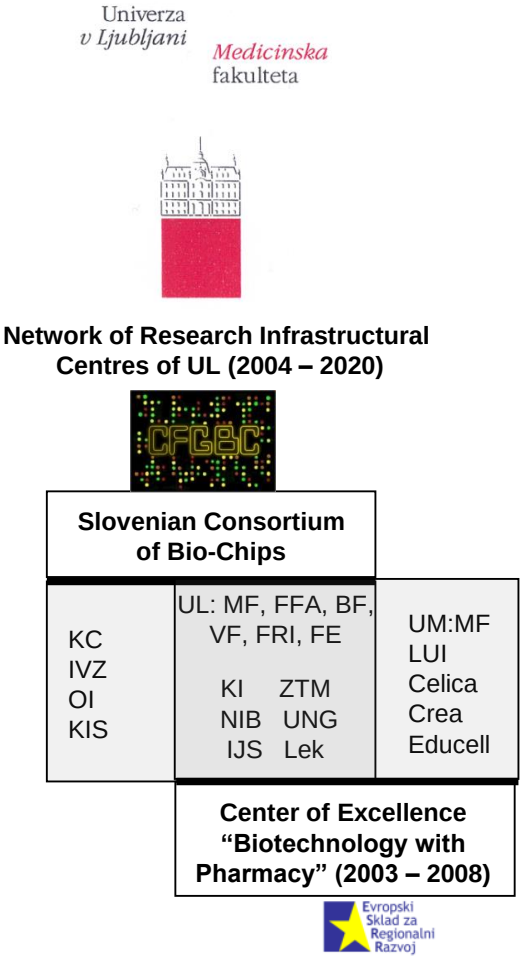


Figure 1: CFGBC members: UL – University of Ljubljana; MF – Faculty of Medicine, FFA – Faculty of Pharmacy; BF – Biotechnical Faculty, VF – Veterinary Faculty; FRI – Faculty of Computer and Information Science; FE – Faculty of Electrical Engineering; KI – National Chemical Institute; ZTM - Blood Transfusion Centre, NIB - National Institute of Biology; IJS - Jožef Stefan Institute; Lek - Lek Pharmaceuticals, UNG – University of Nova Gorica; KC - University Medical Centre Ljubljana, IVZ - Institute of Public Health (currently, National Institute of Public Health, NLZOH), OI – Institute of Oncology, KIS - Agricultural Institute of Slovenia. Within the **Centre of Excellence**, the CFGBC partners were joined by other partners from academia and private sector.

Research Activities of CFGBC - From Functional Genomics to Systems Biology, Systems Medicine and the ESFRI Research Infrastructures

Throughout the time CFGBC hosted multiple national and international functional genomic projects where scientists from the Consortium co-ordinated activities or participated as collaborators, such as the cross-talk of cholesterol homeostasis and drug metabolism in the liver (FP6 project Steroltalk), FP7 Marie Curie FightingDrugFailure, the Interreg project GLIOMA, etc. CFGBC members were active in searching for biomarkers of different types of cancer, of neurodegenerative disorders, in understanding the plant-host interactions, in studies of the yeast interactome, etc. On the national level, the consortium was awarded with the Centre of Excellence "Biotechnology with Pharmacy" (Figure 1) guided by prof. dr. R. Komel from the Faculty of Medicine, UL. Unfor-

tunately, this Centre of Excellence was funded only in 2004 – 2008 which caused a large handicap for the entire functional genomics community in Slovenia. We still experience the consequences of this gap, particularly in being less competitive due to the lack of critical mass of scientists and lagging behind in obtaining the relevant high-throughput equipment for functional genomic studies.

Despite this handicap the Consortium remained dedicated to continue developing the inter-disciplinary approaches since this was (still is) one of the European and also worldwide priority strategies. In past five years several CFGBC groups thus oriented towards the systems approaches, including the systems biology and systems medicine. These novel interdisciplinary research fields join the biological or biomedical sciences with mathematical, technical and physical sciences. Systems approaches apply the quantitative and high-throughput post-genomic experimental and theoretical research towards holistic view on biological processes. It is becoming clear that there is no simple solution for understanding the pathogenesis of complex human disorders, their prognosis and diagnosis, as well as to predict the treatment outcomes, taking into account the inter-individual variations. The wet laboratory and clinical works have to go hand-in-hand with computational approaches, not only with bioinformatics and statistics, but also with different kinds of modelling, towards the prediction models applicable in e.g. the clinical practice.

One of the research strengths of the Slovenian Consortium is the timely notion of the post-genome era challenges. We worked continuously with Slovenian Ministries, especially with Ministry of Science, Education and Sports, presenting the urgent needs for Slovenia to stay in line with the novel developments. These efforts resulted in inclusion of two for CFGBC consortium relevant European ESFRI infrastructures into the "Plan of Research Infrastructure Development 2011 – 2020", which is a formal act of the Government of Republic Slovenia. The first infrastructure is ELIXIR (Infrastructure for Life-Science Information, https://www.elixir-europe.org/) which is coordinated at the Faculty of Medicine, UL. Majority of CFGBC partners participate actively in the Slovenian Elixir node (Figure 2). The second is EATRIS (European Infrastructure for Translational Medicine, http://www.eatris.eu/) which is coordinated at the Faculty of Pharmacy, UL. The infrastructure BBMRI Biobanking and Molecular Resources Research Infrastructure, http://bbmri-eric.eu/) is on the waiting list until the first update of the document. ISBE (Infrastructure Systems Biology Europe, http://project.isbe.eu/) is also of greatest interest for the Slovenian consortium since two of its partners (National Institute of Biology and Faculty of Medicine, UL) participate in the preparatory phase.

CFGBC training of the next generations of biomedical scientists

CFGBC represents an educational unit of national importance not only for Slovenian but also for international students. We practice the cross-disciplinary education of undergraduate and masters students for several programmes at University of Ljubljana (over 300 students per school year). By this we exemplify how to introduce the systems and interdisciplinary education as early as possible in professional careers to make students more competitive in the job market. Systems education and training is available also at the doctoral, post-doctoral levels and lifelong levels, especially for the systems medicine research field. The reason for this focus is the running FP7 project CASyM (Coordinating Action Systems Medicine, https://www.casym.eu/) where Faculty of Medicine, UL coordinates the training activities. The major difference between systems biology and systems medicine seems to be in the scale of the available data. For systems biology majority of the data can be obtained from model organisms or cell cultures while

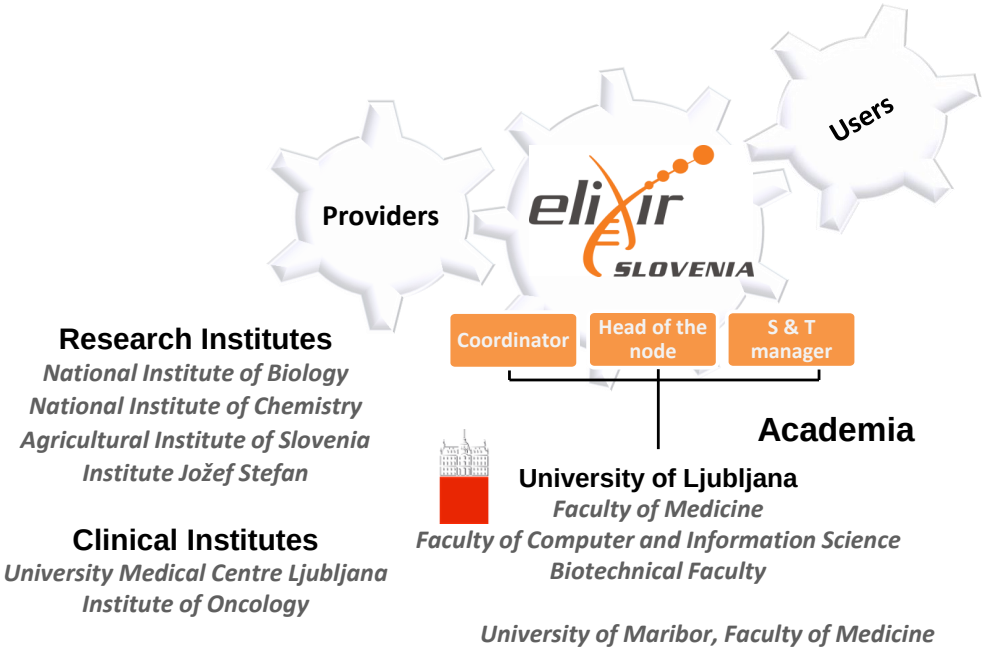


Figure 2: The Slovenian ELIXIR node in 2015 composed of CFGBC members and University of Maribor.

for humans only selected measurements/trials are approved due to ethical constraints. It is, for example, impossible to count on kinetic data from human organs *in vivo*, except if they arise from imaging techniques. Even *ex vivo* studies relying on data from human organs, such as the human liver, are frequently small and difficult to compare with one another. These facts represent the current challenges of systems medicine education. So far we have scattered best practices of introducing the wider systems medicine topics into the medical education at pre-doctoral and doctoral levels (reviewed in Systems Biology for Medicine (eds. U Schmitz, O Wolkenhauer). Series: Methods in Molecular Biology (series ed. J Walker), Springer 2015).
Important activities of CFGBC are related also to dissemination of knowledge and promotion of post-genomic technologies in Slovenia and on the international scale. The peak of these activities is the CFGBC symposium which in the latest years always connects to a meeting from European projects/activities where CFGBC members participate. As Head of the Scientific Committee of the 10th CFGBC symposium with ISBE and CASyM workshops: "From Functional Genomics to Systems Biology and Systems Medicine", I wish you will enjoy this meeting in Ljubljana both scientifically and socially. The brief history of CFGBC and its decade of activities is a memoir and also a lesson. The paths towards our goals were not always straight and we are not yet at the end. And as Ernest Hemingway has said: *"It is good to have an end to journey toward; but it is the journey that matters, in the end"*.

Committees

Organizing Committee:

Jure Ačimovič, chair
Rok Košir, co-chair
Helena Klavžar, treasurer
Žiga Urlep
Anja Korenčič
Petra Hudler
Nataša Debeljak
Peter Juvan

International Scientific Committee:

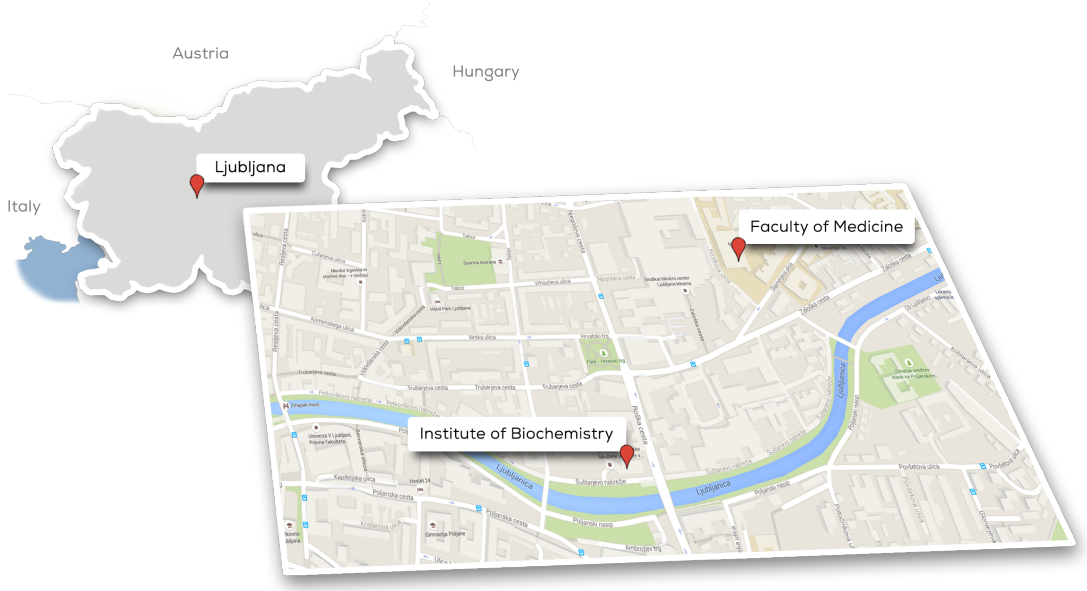
Damjana Rozman, chair (Slovenia)
Kristina Gruden, co-chair (Slovenia)
Francis Levi, MD (UK)
Hans V. Westerhoff, (UK/Netherlands)
Lilia Alberghina, (Italy)
Borut Peterlin, MD (Slovenia)
Jure Ačimovič, (Slovenia)

General Information

CONGRESS VENUE

The workshop will be held at the **Faculty of Medicine**, University of Ljubljana, Korytkova 2, Ljubljana.

The tutorial will be held in the seminar rooms of the **Institute of Biochemistry**, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, Ljubljana.



REGISTRATION AND INFORMATION DESK

Tuesday, June 30, 8:00 – 19:00 in the lobby of the Faculty of Medicine, **Korytkova 2**, Ljubljana.

Wednesday, July 1, 9:00 – 10:00 at the Institute of Biochemistry, **Vrazov trg 2**, Ljubljana.

The certificate of attendance will be issued at the registration desk.

NAME BADGES

All participants will receive name badges upon registration and are kindly requested to wear badges during all sessions and events of the workshop and symposium.

CREDITS

All participants that wish to receive ECTS or CME credits have to sign the appropriate attendance list every day.

PRESENTATION PREVIEW AND DEPOSITION

Presentation preview point where speakers can check and load their presentations will be available in lecture halls. Speakers are kindly requested to bring their presentations in the lecture hall where the talk will be given during breaks before sessions.

POSTER DISPLAY AREA

Poster session will be held in the lobby of the Faculty of Medicine.

Presenters are kindly asked to mount their posters by 11:00 on Tuesday, June 30, and remove them on Tuesday, June 30 by 19:00.

Presenters are responsible for setting and removing the posters. Material for mounting the posters will be available at the venue. Authors are kindly requested to be present at their poster board for the duration of the poster session.

All participants attending tutorials must bring their posters to tutorials from the poster session.

INTERNET ACCESS

EDUROAM will be available during the workshop and tutorial.

COFFEE BREAKS AND LUNCHES

Coffee breaks and lunches will be arranged in the lobby of the Faculty of Medicine during the workshop and in the seminar room during the tutorials.

CURRENCY AND BANKING

Slovenian official currency is Euro.

IMPORTANT PHONE NUMBERS AND EMERGENCY CALLS

For any additional information during the workshop and tutorial, or in case of emergency, please call:

Helena Klavžar: +386 1 543 7590; mobile: +386 31 573348

Jure Ačimovič - mobile: +386 31 323640

Rok Košir - mobile: +386 31 338906

Emergency number: 112

Police: 113

SOCIAL ACTIVITIES FOR ALL REGISTERED PARTICIPANTS

Tuesday, June 30, 2015

19:00 – 21:00 Lobby of the Faculty of Medicine

Get together Reception

Wednesday, July 1, 2015

18:30 – 22:00 Paint-ball and picnic

Thursday, July 2, 2015

19:30 – 22:00 River Ljubljanica Boat trip and dinner

Contents

Programme Outline

Tuesday, June 30, 2015

08:00 - 09:00	**Registration**
09:00 - 09:30	**The 10th Anniversary of Centre for Functional Genomics and Bio-Chips**
09:30 - 13:10	**Functional Genomics and Systems Biology**
13:10 - 14:30	Lunch Buffet & Poster Viewing
14:30 - 18:40	**From Systems Biology to Systems Medicine**
18:40 - 19:00	**Workshop Closing and Tutorials Opening Ceremony**
19:00 - 21:00	Get Together Reception

Wednesday, July 1, 2015

09:00 - 10:00	**Registration**
10:00 - 18:00	**Hands-on tutorial Systems Biology/Medicine** ***day 1***
18:30 - 22:00	Social Activity 1

Thursday, July 2, 2015

09:00 - 19:00	**Hands-on tutorial Systems Biology/Medicine** ***day 2***
19:30 - 22:00	Social Activity 2

Friday, July 3, 2015

08:30 - 18:30	**Hands-on tutorial Systems Biology/Medicine** ***day 3***
18:30 - 19:30	ECTS tests + Questionnaires
19:30	Closing Ceremony, Delivery of Certificates

Detailed Programme

Tuesday, June 30, 2015

08:00 - 09:00	**Registration**
09:00 - 09:30	**The 10th Anniversary of the Centre for Functional Genomics and Bio-Chips**

Functional Genomics and Systems Biology

Chairs: R. Komel, Š. Baebler

09:30 - 10:15	**M. Vanoni**, Italy: Critical cell size control: Systems biology solves a long-standing enigma
10:15 -10:35	**R. Komel**, Slovenia: Brain tumours and proteomic biomarkers
10:35 - 10:55	**P. Dovč**, Slovenia: Functional genomics in mammary gland biology
10:55 - 11:15	**Š. Baebler**, Slovenia: Ecotoxicogenomic analysis of environmental cytostatic exposure in zebrafish
11:15 - 11:30	Coffee Break & Poster Viewing
11:30 - 12:00	**L. Küpfer**, Germany: Using physiologically-based pharmacokinetic modelling in pharmaceutical research and development
12:00 - 12:20	**I. Mlinarič-Raščan**, Slovenia: Thiopurine S-methyltransferase (TPMT) pharmacogenetics: in search for novel modulators of TPMT activity
12:20 - 12:40	**M. Moškon & M. Mraz,** Slovenia: Novel approaches for computational analysis of biological oscillators
12:40 - 13:00	**A. Belič**, Slovenia: Systems biology entering industrial environment
13:00 - 13:10	**N. Nečimer**, Slovenia: Roche Target Enrichment Technologies; commercial presentation
13:10 - 14:30	Lunch Buffet & Poster Viewing

Tuesday, June 30, 2015

From Systems Biology to Systems Medicine

Chairs: D. Rozman, S. Vasudevan

14:30 - 15:00	**C. Auffray**, France: From functional genomics to systems P4 medicine: Impact on health and wellness management
15:00 - 15:30	**S. Vasudevan**, USA: Systems view of Inflammatory Bowel Disease (IBD)
15:30 - 16:00	**C. Bendtsen**, UK: Data driven drug discovery & development - a computational biology perspective
16:00 - 16:30	Coffee Break & Poster Viewing
16:30 - 17:00	**H. Byrne**, UK: The role of Wnt signalling in stem cells and early colorectal cancer
17:00 - 17:30	**F. Lévi**, UK: Personalized systems cancer chronotherapeutics
17:30 - 18:05	**M. Cvijovic**, Sweden: The Infrastructure for Systems Biology Europe (ISBE)
18:05 - 18:40	**M. Kirschner**, Germany: Joint European implementation strategy for systems medicine is published
18:40 - 19:00	**Workshop Closing and Tutorials Opening Ceremony**
19:00 - 21:00	Get Together Reception

Wednesday, July 1, 2015

Hands-on tutorial Systems Biology/Medicine

09:00 - 10:00	**Registration**
10:00 - 12:00	**M. Vanoni**, Italy: Students' oral poster presentations & discussions
12:00 - 13:40	Poster Session and Lunch
13:40 - 15:40	**J. Schuchhardt**, G: Employing Bioinformatics resources for Systems Medicine 1/2 (L + T)
15:40 - 15:55	Coffee Break
15:55 - 18:00	**J. Schuchhardt**, G: Employing Bioinformatics resources for Systems Medicine 2/2 (L + T)
18:30 - 22:00	Social Acivity 1

L - Lecture, T - Tutorial

Thursday, July 2, 2015

09:00 - 10:00	**F. Lévi**, UK: Systems medicine approaches for personalizing cancer chronotherapeutics (L)
10:00 - 10:15	Coffee Break
10:15 - 11:45	**A. Ballesta**, UK: Systems medicine approaches for personalizing cancer chronotherapeutics 1/2 (T)
11:45 - 11:50	Short Break
11:50 - 13:20	**A. Ballesta**, UK: Systems medicine approaches for personalizing cancer chronotherapeutics 2/2 (T)
13:20 - 14:50	Poster Session and Lunch
14:50 - 16:50	**M. Cvijovic**, S: Soft introduction to differential equations and modelling (L)
16:50 - 17:05	Coffee Break
17:05 - 19:00	**M. Cvijovic**, S: Soft introduction to differential equations and modelling (T)
19:30 - 22:00	Social Acivity 2

L - Lecture, T - Tutorial

Friday, July 3, 2015

08:30 - 09:30	**J. B. Nielsen**, Sweden: Genome scale metabolic modelling of human metabolism
09:30 - 10:15	**Borut Peterlin**, SI: Clinical next generation sequencing for diagnosis of rare diseases (L)
10:15 - 10:30	Coffee Break
10:30 - 11:45	**A. Maver**, SI: Clinical next generation sequencing for diagnosis of rare diseases 1/2 (T)
11:45 - 11:50	Short Break
11:50 - 13:20	**A. Maver**, SI: Clinical next generation sequencing for diagnosis of rare diseases 2/2 (T)
13:20 - 14:50	Poster Session and Lunch
14:50 - 15:40	**H. V. Westerhoff**, UK/NL: From personalized to individualized medicine (L)
15:40 - 15:45	Short Break
15:45 - 17:15	**H. V. Westerhoff**, UK/NL; **A. Kolodkin**, LUX: Designing individualized therapies by using systems medicine 1/2 (T)
17:15 - 17:30	Coffee Break
17:30 - 18:45	**H. V. Westerhoff**, UK/NL; **A. Kolodkin**, LUX: Designing individualized therapies by using systems medicine 2/2 (T)
18:30 - 19:30	ECTS tests + Questionnaires
19:30	Closing Ceremony, Delivery of Certificates

L - Lecture, T - Tutorial

Lecture Abstracts

Functional Genomics and Systems Biology

Critical cell size control: Systems biology solves a long-standing enigma

Marco Vanoni, Lilia Alberghina

SYSBIO Centre, and University of Milano-Bicocca, Milan, Italy

A long-standing enigma in cell biology relates to a crucial regulatory function in budding yeast: the requirement of a critical cell size to enter S phase. It is an example of a complex control device for which genetic, genomic and biochemical approaches have not been able, in decades, to propose a satisfactory molecular mechanism.

I am going to report on a work done in my laboratory (Palumbo, Vanoni et al, 2015, submitted), in which we present the construction of a mathematical model of the molecular events controlling the G1/S transition, together with the mating/mitosis switch. The model is centred on the phosphorylation of Whi5, a transcriptional inhibitor of the G1/S specific transcription. It accounts for a large set of independently published data sets, that were not considered for its design and parametrization. New findings in agreement with model predictions are presented. The significance of these results will be discussed under a systems biology perspective.

Brain tumours and proteomic biomarkers

Radovan Komel[1], Ivana Jovčevska[1], Neja Zupanec[1], Daniela Ceselli[2], Clara Limbaeck Stokin[1], Andrej Vranič[3], Boštjan Matos[3], Uroš Smrdel[4], Serge Muyldermans[5], Mike Myers[6], Tamara Lah Turnšek[7]

[1]Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [2]Department of Medical and Biological Sciences, University of Udine, Udine, Italy, [3]Neurosurgery Clinic, University Medical Centre, Ljubljana, Slovenia, [4]Institute of Oncology, Ljubljana, Slovenia, [5]Cellular and Molecular Immunology, VUB, Brussels, Belgium, [6]International Centre for Genetic Engineering and Biotechnology, Trieste, Italy, [7]Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Slovenia

The term "brain tumours" refers to a mixed group of neoplasms originating from intracranial tissues and the meninges, with degrees of malignancy ranging from benign to aggresive. The overall annual incidence of primary brain tumours ranges from 14 to 25 per 100,000 population, and approximately one-third of tumours are malignant and the reminder are benign or borderline malignant. Malignant tumours of the brain belong to the group of »rare diseases« accounting for approximately 2 % of all cancers in adults. Primary brain tumours occur in people of all ages, but are more frequent in children and older adults. In adults, gliomas and meningiomas are most common. Gliomas arise from glial cells such as astrocytes (astrocytomas, anaplastic astrocytomas and glioblastomas), oligodendrocytes (oligodendrogliomas), and epyndymal cells (ependymomas). Glioblastoma multiforme (GBM) is the most frequent and most malignant primary brain tumour in adults, with the incidence of 3–5 cases per 100,000 EU inhabitants. Its localization to the brain, invasive behavior and extremely poor prognosis make it one the most lethal forms of cancer. Despite improvement of surgical removal, chemotherapy and radiotherapy, the median survival of patients with multimodal treatment approaches is approximately 15 months, with only 3-5 % of patients surviving longer than 36 months. The presence of disseminating tumour stem-like cells (GSCs) that support tumour self-renewal and are particularly resistant to chemo- and radio-therapy, is the major factor in preventing complete surgical resection and causing tumour recurrence. Although several studies in the course of the last few years have found some markers that may be informative towards the brain tumour stem cell identification, specific markers that may be reliably used to distinguish normal stem cells from a cancer stem cell, as well as a stem cell from a progenitor, are still unknown. Biomarkers, which due to their overexpression in GSCs were selected as potential targets for new therapies, are often expressed also in normal stem cells or even normal adult tissue cells. For this reason the discovery of new, more specific GSC markers remains one of priorities in the research on how to effectively battle this type of cancer. For identification and validation of new biomarkers specific to GSCs we applied an advanced differential proteomic approach based on camelid heavy-chain-antibody-(HcAb)-derived variable domains (VHHs) referred to as Nanobodies. VHHs are the smallest naturally occurring fragments capable of binding an antigen. They possess good specificity and affinity for the antigen, because the VHH, cloned from an immunised llama, has been maturated in vivo against the antigen. An additional advantage is their ability to recognize novel epitopes on antigens, which are not accessible by conventional antibodies. Following immunization of llama with GSC-like cells we produced a phage-displayed VHH library and after a number of immunoaffinity selections we identified two new potential biomarkers, specific to GBM stem-like cells. The advantage of the approach is the simultaneous acquisition of both antigen and corresponding nanobody that can be used for further development of diagnostic and/or targeting strategies.

Functional genomics in mammary gland biology

Peter Dovč[1], Jernej Ogorevc[1], Sonja Prpar[1,2], Tanja Kunej[1], Eva Čeh[1,3]

[1]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia, [2]Jožef Stefan Institute, Ljubljana, Slovenia, [3]Lek d.d., Ljubljana, Slovenia

odern methods in functional genomics revolutionized virtually all fields of biological research. Also biology of lactation as very complex research area has benefited from the possibility to follow complex expression patterns in mammary gland through different stages of lactation. In addition to quantitative aspect of lacto protein gene expression, the modulation of immune response and regulation of cell cycle gained significantly on importance. Our research group entered the field of lactation biology some 20 years ago, mainly because of its economic importance in farm animals. At this stage the effects of lacto protein variants on milk production traits were in the centre of our interest. Later we expanded our research on gene expression profiling in mammary epithelial cell cultures in different lactation stages and during infection. The holistic approach using RNA Seq strategy enabled us to identify characteristics of gene expression during early infection. In collaboration with other research groups we started to develop bioinformatics tools for integration of different types of data sets in a specialized, genome map based tool, which could help researchers to prioritize relevant genomic regions associated with mammary gland production- and health traits. Recently, we entered the field of mammary gland stem cells and developed different assays to trace epithelial cells with developmental and regenerative potential. We also established heterologous mouse/goat *in vivo* system to prove developmental potential of enriched mammary epithelial precursor cells. This exciting field of research opens new avenues for applications in agriculture, but offers also an experimental model for mammary cancer research.

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Ecotoxicogenomic analysis of environmental cytostatic exposure in zebrafish

Špela Baebler

National Institute of Biology, Ljubljana, Slovenia

Cytostatics are, due to their mechanism of action, classified as carcinogenic, mutagenic, carcinogenic and/or toxic to reproductive systems and it can be assumed that they can elicit these effects in exposed non-target aquatic organisms. Transcriptome can be related to either adaptive processes or are the indicator for toxic effects and can indicate harmful impacts of chemicals even in cases where classical toxicological endpoints show no obvious adverse effects therefore transcriptome analysis is a valuable tools for testing for potential adverse effects in water systems. In the framework of the Cytothreat project we have established a methodology for transcriptome analysis and obtained transcript signatures of most consumed cytostatic drugs in zebrafish (Danio rerio) *in vitro* and *in vivo* experimental systems. The transcriptome response to short treatment with 5-fluorouracil (5-FU) and imatinib mesylate in *in vitro* systems (ZFL cell line, embryos and larvae) was relatively weak and gene expression markers that could predict adverse effects could not be identified.

On the other hand, continuous exposure of zebrafish to environmentally relevant concentrations (0.01 µg/L and 1 µg/L) of 5-FU caused dramatic gene expression changes in the liver of F1 generation of treated fish. Genes involved in the regulation of cell growth, signalling and response to stress were up-regulated while genes involved in developmental processes were down-regulated. Along with the primary effects of the cytostatic, such as inhibition of protein synthesis, we observed deregulation of a number of genes involved in DNA damage response and oncogenesis. The observed gene expression changes can be linked to the DNA damage, micronuclei formation and histopathological alterations in liver and kidney that was observed in the same experiment [1] leading to conclusion that chronic exposure to environmental residual 5-FU can affect water ecosystems.

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Using physiologically-based pharmacokinetic modelling in pharmaceutical research and development

L. Küpfer

Computational Systems Biology, Bayer Technology Services GmbH, Leverkusen

Physiology-based pharmacokinetic (PBPK) models describe adsorption, distribution, metabolisation and excretion (ADME) of drugs in the human body based on a large amount of prior anatomical and physiological knowledge. In contrast to compartmental pharmacokinetic modelling which uses rather phenomenological model structures, PBPK models aim for a detailed mechanistic representation of processes underlying drug pharmacokinetics within the body. Organs in PBPK are usually subdivided in plasma, interstitial space, intracellular space and red blood cells. Mass transfer between the different compartments is quantified by means of so-called distribution models which are parameterized based-upon the physicochemical properties of the drug which is investigated. These properties include amongst others lipophilicity or the molecular weight of the compound. Additional physiological information ranging from the whole body level (e.g. organ volumes, blood flow rates, tissue composition) to relative tissue-specific protein abundance is explicitly provided in the software structure. PBPK models are nowadays routinely used to analyse pharmacokinetics in drug development. Due to the mechanistic information which is provided in the models, they are in particular used for pediatric scaling, investigation of pharmacogenomics or analysis of drug-drug-interactions. In the presentation, the general concepts in PBPK modelling will be introduced and different examples for applications in drug development will be given.

Thiopurine S-methyltransferase (TPMT) pharmacogenetics: in search for novel modulators of TPMT activity

Irena Mlinarič-Raščan[1], Alenka Smid[1], Riin Tamm[2], Andres Metspalu[2]

[1]Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia, [2]Estonian Genome Center and Institute of Molecular and Cell Biology, University of Tartu, Estonia

Thiopurine S-methyltransferase (TPMT) is a genetically polymorphic enzyme that deactivates thiopurine drugs such as 6-mercaptopurine (6-MP) and is a major pharmacogenomics predictor of thiopurine-induced toxicity in paediatric patients with acute lymphoblastic leukemia (ALL). Due to incomplete genotype-to-phenotype correlation, identification of new factors influencing TPMT activity is essential in order to improve the prediction of thiopurine therapy outcome.

The aim of our study was to evaluate the influence of S-adenosylmethionine (SAM) on TPMT activity in vivo on human subjects and to identify novel genetic factors influencing TPMT activity using innovative data mining approaches combining different data sets collected from donors of the Estonian genome centre, Estonia. Blood samples of 1017 donors have been collected. DNA was analysed on DNA microarrays (Illumina OmniExpress). RNA was analysed using whole-genome expression microarrays (Illumina HumanHT-12 Beadchip). Routine biochemical and haematological analysis was performed on whole-blood samples and TPMT activity and concentrations of SAM were measured in the haemolysates (HPLC). The demographic and objective data were extracted from the Biobank database.

Using simple statistical methods, we have first analysed the influence of TPMT genotype, SAM levels and 41 other factors (objective, demographic, biochemical and haematological values) on TPMT activity. After adjustment in the multiple regression model and correction for multiple testing, only TPMT genotype ($p = 1\times10^{-13}$) and SAM levels ($p = 1\times10^{-13}$) were found to significantly influence TPMT activity. With this finding we have confirmed the relevance of our previous in vitro studies in cultured cells showing that SAM is the metabolite responsible for direct post-translational TPMT stabilization resulting in decreased susceptibility to proteasome degradation and increased enzyme activity [1]. For the identification of novel genetic factors affecting TPMT activity, we have used data fusion approach with penalized matrix tri-factorization [2] that simultaneously factorizes data matrices to reveal hidden associations. The approach can directly consider any data sets that can be expressed in a matrix, including those from attribute-based representations, ontologies, associations and networks. Combining the collected transcriptomic, genomic and biochemical data with gene ontologies and protein-protein interactions database, we have identified a set of new genes and polymorphisms, which will now be further validated in *in vitro* as well as clinical studies to confirm their effects on TPMT activity and possible influence on thiopurine therapy outcome.

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Novel approaches for computational analysis of biological oscillators

Miha Moškon, Miha Mraz

Faculty of Computer and Information Science, University of Ljubljana, Ljubljana, Slovenia

The work of our research group has been recently directed towards the analysis of biological oscillators. Two main questions arise. What is the mechanism behind the oscillatory response, i.e. how do oscillations occur, and why is the oscillatory response triggered. In this talk we will mainly address the first question and also discuss the second one. The topologies of regulatory motifs that may exhibit oscillatory behaviour are mostly known and well described. Oscillations are conditioned with a delayed negative feedback loop, which can be achieved in different ways. However, various parameters that additionally define the oscillatory behaviour, such as kinetic rates, usually need to be determined additionally and analysed precisely, whether studying existing or designing a novel biological oscillator. Parameter ranges for which a given topology exhibits oscillatory behaviour present an important aspect regarding oscillator's robustness and its sensitivity to environmental changes. Firstly, we will describe an analytical approach for the analysis of oscillatory behaviour in biological systems based on the projection of mathematical models describing system under study to a classical mechanics system. Presented approach allows us to analyse the response of an oscillator topology given the parameter values without expensive numerical simulations. It may be used to determine the parameter ranges for which a system exhibits oscillations and additionally the periods of obtained oscillations. Secondly, we will present an analysis of effects that clustered DNA binding sites might have on the oscillatory response and its robustness. We will briefly present the modelling and analysis techniques that might be applied to such systems. The analysis will be demonstrated on the case study of an amplified negative feedback genetic oscillator and NF-$\varkappa$B transcription factor oscillatory response. We will conclude with the discussion about our future work in the context of cellular decoding of oscillatory behaviour and its importance in biological systems from information processing perspective.

Systems biology entering industrial environment

Aleš Belič

Lek Pharmaceuticals d.d., Mengeš, Slovenia

Systems biology emerged as combination of mathematical modelling and biology with aim to describe the fundamental processes of metabolic and regulatory networks within the cell and to extrapolate the macro properties of the organisms from these basic rules. However, the problem of scales and extremely complex organisation of relatively simple chemical reactions still presents a huge obstacle for the extrapolation of functional properties of cells onto entire organism. On the other hand, unicellular organisms can be relatively well described with mathematical models. A huge variety of different behaviours can be observed even in the simplest organisms, emerging from regulatory and redundant cellular systems that enable survival of the organism in the widest possible variety of environmental conditions. However, in industrial environment the environmental parameters are well controlled in a narrow band of acceptable values. This reduces the modelling and simulation problems to a manageable level. Some industrial applications of genome scale models have been published already (yeast – fermentation, *E. coli* – lysine biosynthesis). The biggest challenge represents understanding of control loops that control the cell processes in a highly interactive mode and often cause opposite results from the model prediction. Therefore, it is essential to include a simplified version of the regulatory system into the model for any industrial application. Mammalian cells that are often used for drug biosynthesis are far more complicated that unicellular organisms, however, first attempts of model applications are already seen. To illustrate the complexity and how the complexity can create new quality a system of cholesterol biosynthesis can be used. Cholesterol biosynthesis is highly regulated cellular process that strongly interacts with other processes in the cell to generate a homeostatic environment suitable for functioning of the cell. Any disturbance of the metabolic pathway or its regulatory mechanism results in global response, since the biosynthesis must find a new homeostasis to eliminate the disturbance. Unique character of the regulatory system enables very precise cholesterol regulation, unless saturation of the material fluxes of reactions is achieved. Massive simplifications of the model can be made without significant loss of descriptive realism of the model which enables clearer insight into cell functioning and more realistic predictions which is a prerequisite for industrial applications. The combination of systems biology with simplification approaches borrowed from engineering show promising results also in industrial environment and may soon result in much more efficient biotechnology.

From Systems Biology to Systems Medicine

From functional genomics to systems P4 medicine: Impact on health and wellness management

Charles Auffray

European Institute for Systems Biology & Medicine, Lyon, France, CNRS-ENS-UCBL – Campus Charles Mérieux – Université de Lyon

Systems biology approaches are combining high-dimensional functional genomics data with biological, clinical, environmental and lifestyle assessments through iterative statistical analyses, computational modelling and experimental validation. They are transforming biomedical research and clinical practice, triggering the transition from a reactive to a proactive practice of medicine [1-2]. The effective development of predictive, preventive, personalized and participatory systems (P4) medicine requires harmonization of experimental and computational methods for data, information and knowledge collection, storage and sharing. In order to address the associated ethical, legal and social issues, the active participation of all stakeholders including researchers and clinicians in academy and industry, regulatory and funding bodies, individuals and patient organizations is essential. The success of this collective endeavour will be demonstrated through the reversal of the escalating costs of drug and diagnostic development in industry, and of patient management in hospital and community practice, providing the basis for a more cost–]efficient and sustainable integrated healthcare system. This is revolutionizing how medicine will be practiced in the 21st century [3-5].

Two examples of individuals who have managed to anticipate the occurrence of disease and take preventive measures through a regular assessment of their exposome (environmental and occupational exposures, nutrition, sleep, exercise, stress), clinicome (biological and clinical features), and integrome (metabolomics, proteomic, transcriptomic, epigenomic, genomic and genetic features) provide the basis for effective implementation of systems P4 medicine [6-7]. The computational infrastructure required for the collection, storage, analysis and sharing of translational research information and knowledge on a big data scale is being developed using high-performance cloud computing infrastructures ensuring data security and compliance with personal data protection regulations. Pilot studies such as Vistera initiated by EISBM in Grenoble and Lyon in partnership with ISB have been designed to scale up the monitoring of wellness, health and disease through the collection of billions of data points for increasing numbers of individuals who are healthy, at risk of developing disease, or in the course of disease development, with the active participation of individuals through social networks [8]. The EISBM-ISB pilot studies form the basis for the development of a network of systems medicine centres in the context of the new framework programme of the European Union Horizon 2020. Through its worldwide extension, this network will catalyse the transformation of healthcare delivery and the transition toward emphasis on management of wellness for millions then billions of individuals in the next generation.

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Systems view of Inflammatory Bowel Disease (IBD)

Sona Vasudevan

Department of Biochemistry, Georgetown University, Washington DC, USA

Introduction: IBD is a chronic inflammation of all or part of the digestive tract [1]. The two most common and distinct form of IBD include Crohn's disease (CD) and Ulcerative Colitis (UC). Inflammation affects the entire digestive tract in CD while only the large intestine in UC. Both diseases are characterized by an abnormal immune response which has formed the focus of current treatments [2]. Research in the last decade has focused on understanding the causes of these debilitating diseases. While we have come a long way, the aetiology and pathogenesis of CD and CD still remain largely unknown. With the explosion of –omic data, systems medicine approaches may lead to identification of biomarkers and newer drug-targets for finding cures for these diseases. We have taken such an approach for understanding CD and UC by mining GWAS data, UniprotKB data, expression profiles, pathways, and miRNA data. In addition, we are applying a novel strategy of looking at evolutionary aspects of these diseases by using the Phylomics tool [3].

Results: Mining the GWAS data that contained 2000 diseases, 9000 genes and about 15000 SNPs, we found 92 genes implicated in CD that corresponded to about 58 mapped diseases. On the other hand UC corresponded to about 38 mapped diseases. We also found distinct pathways that distinguished between CD and UC. An evolutionary analysis carried out on expression data indicated that UC is a mitochondrial disease. In addition, based on the expression data we found that gender plays a very important role in the etiology of these diseases. We found about 50 genes implicated in CD. Results of all of the multi-omic analysis that clearly distinguish between CD and UC will be presented. We have also created a comprehensive database on all the collected data on CD and UC.

Conclusions: Systems medicine is powerful and will be successful in identifying biomarkers and new targets. We are able to distinguish the commonalities and major differences in the aetiology of these two diseases.

Acknowledgements: We would like to thank the contributions of Abu-Asab MS & Amri H for introduction to the Phylomics tool. We would also like to thank Ramyasree Madupuri for the creating GDSome and UDSome data. We thank Ritika Kundra for creating a preliminary version of the database.

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Data driven drug discovery & development - a computational biology perspective

Claus Bendtsen

AstraZeneca, UK

The pharmaceutical industry is challenged by high cost and attrition rates. To break this cycle we must improve our ability to use available information. I will illustrate this by taking a climb to the top from early drug discovery through development. Examples from computational biology will be used along the journey: D1 – phenotypic screening is the primary source for first in class NMEs but poses the challenge of deconvolution. Historical bioactivity data can be used to help solve this puzzle. D2 – high throughput screening is the workhorse for hit identification but poor on content. A workflow, cherry picking positive wells for high content imaging together with automated image analysis, allows identification of artefacts and improves the ability to make decisions in HTS. D3 – the DDR pathway provides attractive oncology targets. A mechanistic cell cycle model can explain *in vitro* behaviour and *in vivo* observations of combination treatment to inform clinical design. D4 – safety concerns were raised when a clinical study noticed drop in neutrophil counts. A multiscale model was developed and led to the conclusion that neutrophil blood count is expected to saturate at high dosages. D5 – gamma secretase inhibitors for Alzheimer's disease have been observed not to work – or even make patients worse. This conundrum can be explained by a tale of three secretases. This boosts the confidence in current AZ programs. Together these examples show why the quantitative use of data is a must in drug discovery and development.

The role of Wnt signalling in stem cells and early colorectal cancer

Helen Byrne

Mathematical Institute, University of Oxford, Oxford, UK

Wnt signalling plays a key regulatory role in many biological processes including cell proliferation, migration and cell fate specification. It is active during development and also during adulthood when it assists in the maintenance of homeostasis. Dysregulation of the Wnt signalling pathway is a hallmark of several developmental disorders, a number of degenerative diseases and a variety of different cancers. As such, it is an obvious target for therapeutic intervention. However, its complexity and cross talk with other subcellular and cellular processes make it difficult to understand the consequences of abnormal Wnt signalling and to predict (and compare) the impact of different therapeutic approaches.

Motivated in part by these considerations, a variety of mathematical models of the Wnt signalling pathway have now been developed. The models are typically formulated as systems of ordinary differential equations that describe how the subcellular concentrations of proteins such as b-catenin, APC and Axin change over time and in response to Wnt stimulation. Recent models account for the localisation of these Wnt proteins in different subcellular compartments (e.g. the cytoplasm, nucleus and membrane), their transport between the various pools and, to a limited extent, their cross talk with the Delta-Notch and ERK signalling pathways.

In this talk I will review existing subcellular and multiscale models of Wnt signalling, with particular focus on the intestinal crypt, stem cells and the early stages of colorectal cancer.

Personalized systems cancer chronotherapeutics

Francis Lévi, Annabelle Ballesta

Warwick Medical School, Warwick University, Coventry, United Kingdom

Chronotherapeutics aim at improving treatment outcomes through the delivery of medicines according to the Circadian Timing System (CTS), a complex hierarchical and dynamic network system involving all cells in the body. As a result, circadian timing modifies up to 10-fold the tolerability of anticancer medications in experimental models and in cancer patients [1]. However, sex, circadian disruption and tumor protein expressions are independent determinants of the optimal chronotherapeutic schedule, in international studies involving large number of patients with metastatic colorectal cancer [2-3]. Such clinical data have driven experimental confirmation studies in mice. Moreover, human cancer chronotherapeutics constitute a unique paradigm for cancer therapy, where "the lesser the toxicity, the better the efficacy", based on several landmark analyses of a randomized clinical trial involving 564 patients [4-6]. Stochastic and deterministic mathematical models help analyze the dynamic interactions between circadian clocks, cell cycle and drug pharmacodynamics from single cell to whole organism. Biosimulation leads to the design of model-based optimal chronotherapeutic schedules, through the exploration of a wide range of parameter values, as shown for irinotecan [7-8; Dulong et al. Mol Cancer Ther 2015, in revision]. The systems approach to chronopharmacokinetics-chronopharmacodynamics modeling further reveals that optimal chronotherapeutics require circadian entrainment to be robust in healthy cells and disrupted in cancer cells [9]. Moreover, optimal chemotherapy timing can vary by up to 8 h according to genotype and sex in mice, a finding similar to that observed in cancer patients. The liver circadian expression patterns of Rev-erb? and Bmal1, two clock genes that regulate each other, were used as inputs into a mathematical model that was designed and enabled the identification of optimal circadian timing of irinotecan irrespective of genotype and sex [10]. In practice, non invasive reliable circadian biomarkers are critical for modeling CTS dynamics [11-12], as well as for increasing CTS robustness through intervention measures, and effectively personalizing circadian drug delivery schedules.

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The Infrastructure for Systems Biology in Europe (ISBE)

Marija Cvijovic

Department of Mathematical Sciences, University of Gothenburg, Sweden

The Infrastructure for Systems Biology in Europe (ISBE) programme comprises an infrastructure that is designed to meet the needs of European systems biology, in terms of development, applications and training. ISBE aims at connecting the existing expertise for systems biology, resources and services at a national and European level and link them to other pan-European research infrastructures to develop a coherent infrastructure. Through ISBE, the European life sciences research community will come together to provide a range of integrated research 'toolkits' that allow all life scientists to implement computational modelling and systems approaches in their research. This will be accompanied by the development of software to facilitate modelling of complex systems and development of standards and interoperability, making research more efficient. The ISBE training strategy will focus on the development and dissemination of best practice in systems biology training and education at the Masters, PhD and postdoctoral level.

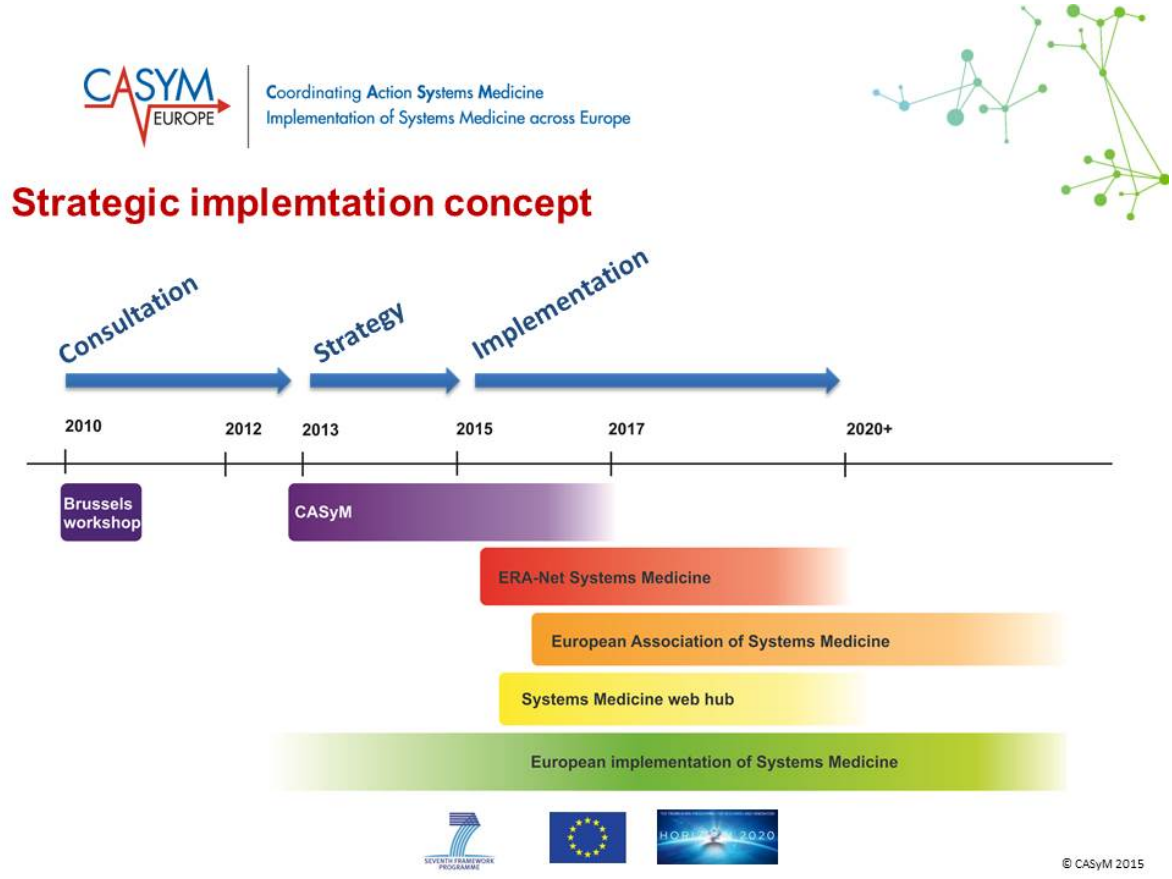
ISBE operates in three phases: the Preparatory Phase of ISBE will finish in July 2015 with the primary output of delivering a comprehensive ISBE Business Plan that describes the ISBE infrastructure. After this ISBE will enter Interim Phase and is anticipated to enter Operational Phase in 2017.

Joint European implementation strategy for systems medicine is published

Marc Kirschner

Forschungszentrum Jülich GmbH. Jülich, Germany

After a two year cross-disciplinary consultation process the Coordinating Action Systems Medicine (CASyM) published its European implementation strategy (roadmap) for Systems Medicine. The vision of this roadmap is to develop Systems Medicine into a practical framework that assists clinical decision making and the design of personalised prevention and treatment plans. Central to this is a systems approach that addresses clinical questions and provides solutions to the most pressing clinical challenges. The CASyM roadmap process was the result of a broad stakeholder consultation and reviewing process across many disciplines that included clinicians, scientists, funding bodies, Industry/SME as well as regulators and patient organizations with the aim to draft the first strategic implementation plan for a European Systems Medicine. The roadmap identifies four core priority actions and asserts that (i) investment in proof of concept and demonstrator projects is needed to help to precipitate a paradigm shift in the way medicine is practiced. This shift will be supported by (ii) a strong Systems Medicine community, (iii) new multidisciplinary training programmes and (iv) the development of new European-wide practices in clinical data access, sharing and standardisation. These actions are outlined along with ten cross-cutting key areas and specific recommendations over a period of two, five and ten years.



Genome scale metabolic modelling of human metabolism

Jens Nielsen[1,2,3]

[1]*Chalmers University of Technology, Gothenburg, Sweden,* [2]*Technical University of Denmark, Lyngby, Denmark,* [3]*Royal Institute of Technology, Stockholm, Sweden*

Metabolism is highly complex involving a very large number of chemical reactions. These reactions are traditionally grouped into pathways with dedicated functions, but recent analysis of metabolism has shown that there is a high degree of connectivity between these pathways due to common sharing of co-factors and key metabolites. It is therefore necessary to study metabolism in its whole, and this can be done through the use of the so-called genome scale metabolic models (GEMs). These models represent comprehensive reconstruction of all known metabolic reactions operating in a cell, and these GEMs represent knowledge databases that link genes, proteins, enzymes, metabolic reactions and metabolites. We have recently reconstructed the to date most comprehensive GEM for human metabolism, HMR2.0. This reconstruction represents human metabolism in general and includes 3,765 genes, 3,160 metabolites, and 8,181 metabolic reactions. In order to study specific cell types and their associated diseases we developed a computational platform, INIT, that enables reconstruction of cell type specific GEMs from HMR2.0 using different types of omics data, e.g. proteomics data or RNAseq data. In this presentation HMR2.0 and INIT will be presented. It will further be demonstrated how high quality models for adipocytes, hepatocytes and myocytes could be used for studies of how these cell types respond to obesity, NAFLD, NASH and type-2-diabetes. By combining these cell type specific GEMs with high-throughput experimental data, in particular RNAseq data, it will be demonstrated how metabolic reprogramming is occurring in these cell types in response to disease development. It will further be shown how HMR2.0 and INIT can be used for reconstruction of functional metabolic networks in cancer cells, and how analysis of these metabolic networks can be used to gain insight into metabolic reprogramming occuring in connection with cancer progression, exemplified for clear cell renal carcinoma. It will also be shown how patient specific proteomics data can be used for reconstruction of personalized GEMs for hepatocellular carcinoma, and that these personalized GEMs offer opportunity for improved therapy compared with the use of a generic model.

Poster Abstracts

GenCover: NGS Clinical Coverage Report Tool

Miha Štajdohar[1], Miha Škalič[1], Domen Blenkuš[1], Luka Jeran[1], Luka Ausec[1], Nejc Škoberne[1], Mark Cowley[2], Marcel Dinger[2]

[1]Genialis d.o.o., Ljubljana, Slovenia, [2]Garvan Institute of Medical Research, Sydney, Australia

Exome sequencing has low coverage over certain exons and genes. This is important for clinical application, as we need to be confident that a gene with no found variant is a true negative rather than a result of a chance event. The most important predictor of this is coverage, but there are others such as quality, mappability, GC content etc. GenCover is a web tool based on the GenCloud platform that takes multiple inputs: a mapping file (BAM), a gene list, specific variants of interest (VCF) and a minimum coverage to pass. It produces an interactive output summarizing the coverage of genes, exons and variants. Moreover, the nucleotide level coverage is visualized by an interactive genome browser showing multiple predictor tracks. The tool has been validated in a research environment with WES and WGS data. The tool will be improved by additional computations of the likelihood of identifying a variant in the given gene list, gene/transcript or exon, based either on the mapping file or other data obtained from a collection of mapping files.

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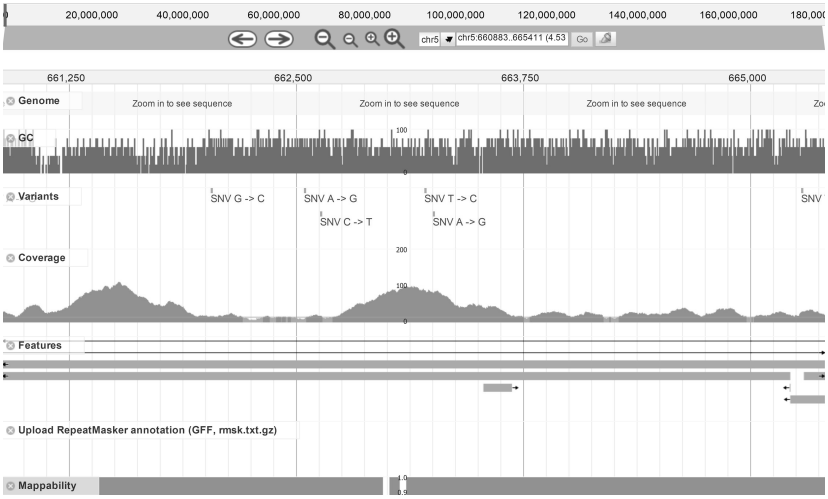

Figure 1: Visualization of coverage for variants of interested on nucleotide level. JBrowse genome browser is used to show multiple predictor tracks. Hg19 and b37 coordinates are currently supported, but other coordinate systems can be added easily.

Explicit modelling of binding site clusters in NF-κB oscillatory response

Manca Bizjak, Miha Mraz, Nikolaj Zimic, Miha Moškon

Faculty of Computer and Information Science, University of Ljubljana, Ljubljana, Slovenia

Introduction: Recent progress in the analysis of transcription factor NF-κB has shown that many of its target genes contain clustered DNA binding sites with negligible cooperativity. These also include NFKBIA, gene encoding NF-κB inhibitor IκBa [1]. It has been previously shown that the NF-κB – IκBα negative feedback interaction may exhibit oscillatory system response [2,3]. The activation of NFKBIA transcription is triggered by the presence of the IκB kinase (IKK), which phosphorylates IκBα, leading to its saturated degradation [4]. Constant IKK concentration may result in a persistent delayed negative feedback loop and oscillatory behaviour of the described system.

Results: We present an extension of existent computational models in the sense of explicit modelling of binding site clusters located in the regulatory region of the NFKBIA gene. This allows us to observe how the size of binding site clusters affects the oscillatory dynamics. Occupancy of binding sites with transcription factors may affect the promoter activity in different ways. We additionally tested different rules that define promoter activity in dependence on binding sites occupancy, i.e. promoter is active when at least one binding site is occupied, promoter is active only when all binding sites are occupied and promoter activity linearly increases with the number of occupied binding sites. Our results indicate that different rules result in different dynamics in dependence on the observed binding site cluster size.

Conclusions: Explicit modelling of clustered DNA binding sites allows us describe the oscillatory dynamics of NF-κB – IκBα interaction more accurately. Our model allows us to predict the system response in dependence on the size of binding site clusters and in dependence on the rules governing the promoter activity. Accurate characterization of dynamics caused by different scenarios is still a subject of our current research.

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Role of StERF transcription factor in potato – PVY interaction

Anna Coll, Ana Lazar, David Dobnik, Špela Baebler, Kristina Gruden

Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia

Potato (*Solanum tuberosum L.*) is the world's most widely grown tuber crop and potato virus Y (PVY) is one of the major potato pathogen causing severe crop loss in different areas worldwide. To better understand the potato defence response against PVY we studied the role of *ethylene response* factor (ERF) genes from group IX since they have been related to plant defence response and defined as important elements on hormone crosstalk. Potato ERF-IX genes were identified and classified in this study. Among them, *StERF* was selected for further analyses based on previous transcriptomics experiments performed in our group. Expression patterns of the gene in hypersensitive resistance (HR) potato cultivar infected with PVY pointed to its importance as a signalling component in potato defence response. Using virus-induced gene silencing (VIGS) we demonstrated that PVY systemic spread is delayed in *StERF* silenced plants. We further examined the potential hormonal signalling involved in the expression of *StERF* and demonstrated that our gene integrates several signalling pathways. Getting more insights into the regulation of the gene, localisation studies showed that *StERF* strongly accumulated in cell nucleus after PVY infection. Taken together our results suggested the importance of *StERF* in potato-PVY interaction. Therefore the data contributes to better understand the complex network of plant defence signalling pathways.

Validation of SteatoNet for prediction the network disorder in liver in order of personalized medicine

Tanja Cvitanović[1], Miha Moškon[2], Miha Mraz[2], Aleš Belič[3,4], Damjana Rozman[1]

[1]Centre for Functional Genomics and Bio-Chips, Faculty of Medicine, University of Ljubljana, Slovenia, [2]Faculty of Computer and Information Science, University of Ljubljana, Slovenia, [3]Faculty of Electrical Engineering, University of Ljubljana, Slovenia, [4]Lek Pharmaceuticals d.d., Mengeš, Slovenia

Non-alcoholic fatty liver disease (NAFLD) is a poorly understood complex disorder that occurs at a high frequency in western populations with unhealthy dietary and lifestyle habits. *SteatoNet* has been developed representing a total of 194 reactions that may provide further insight into the multi-dimensional nature of NAFLD. *SteatoNet* is a dynamic semi-quantitative model of metabolic and signalling pathways, their interaction with extra-hepatic tissues and hierarchical feedback regulation at the gene expression and signal transduction level. It is based on a steady-state analysis of ordinary differential equations that do not require kinetic parameters and can uniquely identify a solution by just defining the reversibility of reactions, the distribution of fluxes at pathway branch- points and the substrate influx into the network. It is a good *in silico* platform to test biological hypotheses prior to experimental testing [1]. Lanosterol 14α -demethylase (CYP51) is a key regulatory enzyme in the late stage of cholesterol synthesis. Demethylation of lanosterol caused by CYP51 is regarded as the initial checkpoint in the transformation of lanosterol to cholesterol. Cholesterol metabolism and its transcription regulators are known as major steatosis factors. The *Cyp51* liver conditional knockout (LKO) mouse developed hepatomegaly with oval cell proliferation, fibrosis and inflammation, but without steatosis [2].

When we adjust the model with expanded functional cholesterol synthesis (at genes and protein level) in hepatocyte, parameters of the model will be validated with experimental results gained from LKO mouse. Applying *SteatoNet* for simulations of *Cyp51* knockout in hepatocytes should predict a network disturbance in adipose tissue, which is a good starting point for further experimental testing. We will measure the effects of liver *Cyp51* knockout on transcriptional and protein level in adipose tissue by evaluating expression of candidate genes suggested by the simulations. Adipose tissue will be collected from *Cyp51* LKO mouse. Successful validation and improving the efficacy of *SteatoNet* is excellent base for even better comprehension of the links between the liver and other organs in the sense of understanding NAFLD and for adapting the model to other liver-related diseases like alcoholic liver disease. The improved model opens the door to adapting the model to various new applications for the purpose of personalized medicine.

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Modulation of tamoxifen response via erythropoietin receptor

Nina Trošt[1,2], Tea Lanišnik Rižner[1], Radovan Komel[1], Peter Solár[3], Nataša Debeljak[1]

[1]Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Slovenia, [2]Immunology Frontier Research Center (IFReC), Osaka University, Japan, [3]Department of Cell Biology, Institute of Biology and Ecology, Faculty of Sciences, P.J. Šafárik University, Košice, Slovakia

Introduction: High expression of erythropoietin receptor (EPOR) in breast cancer cells carrying estrogen receptor (ESR) was related with impaired response to tamoxifen treatment. Correlations between EPOR and membrane estrogen receptor, G-protein coupled receptor (GPR30) were indicated, linking EPOR and ESR signalling pathways. The aim of this study is to elucidate mechanisms of EPOR involvement in response to ESR antagonist tamoxifen.

Results: To assess expression of EPOR, ESR, PGR and GPR30 qPCR and western blot analysis were performed. Gene expression analysis in breast cancer cell lines revealed correlations in the expression of EPOR to GPR30 and GPR30 to ESR. Low expression of GPR30 was confirmed in T-47D cells and high in MCF-7. ESR positive and stable EPOR overexpressing cells (T-47D^{EPOR+} and MCF-7^{EPOR+}) were prepared via transfections of parental cells with wild-type EPOR. T-47D and MCF-7 cell lines were chosen as working model, since they represent hormone-dependent breast cancer but differentiate in the expression of GPR30. Wild-type and transformed cells were exposed to tamoxifen and analysed for their viability. T-47D^{EPOR+} cells showed increased survival after tamoxifen treatment.

Conclusions: EPOR may be implicated in impaired cell response to tamoxifen via GPER30 and ESR, since strong EPOR to GPR30 and GPR30 to ESR correlations were confirmed. The analysis of T-47D^{EPOR+} and MCF-7^{EPOR+} cells is in progress in aim to indicate mechanisms of receptor interactions involved in tamoxifen response.

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Polymorphisms in DNA repair mechanisms and glutathione-S-transferase genes affect survival in osteosarcoma

Katja Goričar[1], Viljem Kovač[2], Janez Jazbec[3], Branko Zakotnik[2], Janez Lamovec[2], Vita Dolžan[1]

[1]Pharmacogenetics Laboratory, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [2]Institute of Oncology Ljubljana, Ljubljana, Slovenia, [3]Oncology and Haematology Unit, University Children's Hospital, University Medical Centre, Ljubljana, Slovenia

Introduction: Osteosarcoma is a bone cancer, most common in children and adolescents. Patients are usually treated with cisplatin-based preoperative and postoperative chemotherapy. Cisplatin forms both intrastrand and interstrand DNA-crosslinks, inhibiting DNA replication. Different DNA repair mechanisms are involved in the repair of cisplatin-induced lesions, while glutathione-S-transferases (GSTs) participate in cisplatin detoxification. Our aim was to investigate the influence of genetic variability of DNA repair mechanisms and GSTs on outcome of cisplatin-based chemotherapy in osteosarcoma patients. Methods: In total, 66 osteosarcoma patients were genotyped for *ERCC1*, *ERCC2*, *NBN*, *RAD51*, *XRCC3*, and *GSTP1* single nucleotide polymorphisms, and *GSTM1* and *GSTT1* gene deletion. We determined the influence of polymorphisms on survival and treatment outcome using Cox and logistic regression. Benjamini-Hochberg false discovery rate was used to account for multiple comparisons.

Results: Patients with at least one polymorphic *ERCC2* rs1799793 allele had longer event-free survival (EFS) (P=0.006; hazard ratio (HR)=0.28; 95 % confidence interval (CI)=0.11-0.70). Polymorphic *GSTP1* rs1138272 allele was associated with both shorter EFS (P=0.005; HR=3.67; 95 %CI=1.47-9.16) and overall survival (P=0.004; HR=3.52; 95 %CI=1.51-8.22). Compared to the reference *NBN* CAA haplotype, *NBN* CGA haplotype was associated with shorter EFS (P=0.001; HR=4.12; 95 %CI=1.77-9.56). In the multivariable survival analysis that included all important clinical and genetic factors, both *ERCC2* rs1799793 and GSTP1 rs1138272 remained significantly associated with EFS (HR=0.22; 95 %CI=0.08-0.58; P=0.002 and HR=4.95; 95 %CI=1.81-13.54; P=0.002, respectively).

Conclusions: Our results suggest that DNA repair and GST polymorphisms, particularly *ERCC2* rs1799793 and *GSTP1* rs1138272, could be used as predictive factors for response to cisplatin-based chemotherapy in osteosarcoma and could contribute to treatment personalization.

Cholesterol homeostasis, drug metabolism and the liver clock interplay

Anja Korenčič[1], Rok Košir[1], Hanspeter Herzel[2], Damjana Rozman[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Slovenia, [2]Charité Universitätsmedizin, Berlin, Germany; and Institute for Theoretical Biology, Berlin, Germany

Circadian clocks are endogenous transcription-translation feedback loop oscillators driving daily rhythms in physiology. Over 3000 genes of the liver are expressed in circadian manner, including genes from cholesterol homeostasis and drug metabolism. Transcriptome studies show that different organs feature different sets of clock controlled genes with different peak phase distributions. Based on expression profiles and known cis-regulatory sites we developed a core clock model for the mouse liver and adrenal gland [1]. Most of the phase variability from transcriptome data was traced back to E-box and ROR-element regulations. ROR-elements and the REV-ERB/ROR systems represent a link between the circadian clock and lipid metabolism and are inter-connected to BMAL1 regulation. REV-ERBs have heme as their natural ligand, and cholesterol and other oxidized sterols bind to the activation modulator ROR. In contrast to lipid metabolism, the link of the ROR/REV-ERB system to drug metabolism is not well understood. We evaluated phase I, phase II and drug transporter genes for their circadian rhythmicity and potential regulation by REV-ERB and BMAL1. As expected, few genes are targets of BMAL1, and more of REV-ERB. The rhythmically expressed genes show a two-phase profile, with Phase II enzymes and transporters in antiphase. Model simulations of phase distributions of ROR-element regulated genes support the conclusion that REV-ERB directly regulates the drug metabolism components.

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Characterization of erythropoietin and estrogen receptor proteins in breast cancer cell lines

Sara Košenina[1], Urška Pevec[1], Nina Trošt[1,2], Neli Hevir[1], Tea Lanišnik Rižner[1], Radovan Komel[1], Nataša Debeljak[1]

[1]Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [2]Immunology Frontier Research Center (IFReC), Osaka University, Osaka, Japan

Introduction: Basic role of erythropoietin (EPO) in its receptor (EPOR) is enhanced proliferation, differentiation and survival of red blood cells. EPOR was found in normal as well as cancer tissues, including breasts cancer [1]. Tamoxifen, estrogen receptor antagonist, is a treatment of choice for hormone depended breast cancer. Some studies showed correlation between level of EPO/EPOR expression and estrogen (ESR), progesterone (PR) and androgen receptors (AR). A research on breast cancer patients showed correlation between high EPOR expression and worse response to tamoxifen treatment. Impact of EPOR and ESR (nuclear or membrane) co-expression in breast cancer as well as it impact on sensitivity to tamoxifen is not explained yet [1].

Expression of receptors involved in signal transduction of erythropoietin and estrogen in different breast cancer cell lines has already been defined on gene level [2]. The aim of our study is to confirm and quantify the presence of receptors on protein level.

Results: Total proteins were isolated from various breast cancer cell lines (human MCF-7, T-47D, MDA-MB 231, SKBR3, Hs578T and rat RAMA 37, RAMA 37-28). The receptors involved in signal transduction of erythropoietin (erythropoietin receptor (EPOR), ephrin type-B receptor 4 (EPHB4), beta-common receptor (CSF2RB)) and estrogen (estrogen receptor 1 (ESR1), estrogen receptor 2 (ESR2), membrane G protein-coupled estrogen receptor (GPR30)) were analysed and quantified by Western blot analysis. The expression of EPOR protein was proven in all analyzed cell lines. The expression of EPOR protein in RAMA 37-28 cell line was the strongest, while the expression of EPOR in SKBR3 and Hs578T cell lines was weak. The detection of ESR2 in GPR30 proteins in rat cell lines RAMA 37 and RAMA 37-28 was confirmed.

Conclusions: The expression of EPOR on protein level was shown to correlate with mRNA. The correlation of expression of other proteins is in progress. Our goal is to identify the model cell line suitable for correlation study between EPOR and ESR in aim to elucidate the mechanisms of tamoxifen resistance.

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The influence of mouse genetic background on circadian gene expression patterns in peripheral tissues.

Rok Košir, Jure Ačimovič, Uršula Prosenc Zmrzljak, Anja Korenčič, Martina Perše, Damjana Rozman

Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Introduction: The biological clock is an important component in body homeostasis. However, it is not clear how the genetic background of each individual influences circadian expression and regulation. We addressed this question by investigating the liver and adrenal gland gene expression patterns of inbreed mouse strains 129S2/SvPasCrlf × C57BL/6JRj and C57BL6J/OlaHsd in complete darkness (DD) and 12h light 12h dark conditions (LD). Whole exome sequencing was applied to understand how genotype can affect expression of selected core clock and metabolic genes.

Methods: 24 h gene expression profiles of 25 core clock and metabolic genes were analysed by cosinor analysis to determine circadian amplitudes and phases. Whole exome sequencing (WES) was performed with Agilent SureSelect exome capture protocol on Illumina Hiseq2000 to determine genomic differences between strains that could influence the differences observed in expression profiles.

Results: Our study in mice shows that amplitudes and phases of core clock and metabolic genes depend on genetic background (genotype). In the liver the majority of differences were observed in amplitudes of core clock genes in DD and LD. Phase shifts were observed in DD for the three core clock genes *Arntl*, *Dec1* and *Cry1* while the selected metabolic genes including cytochromes P450 and nuclear receptors displayed similar circadian expression pattern in livers of the observed mouse strains. The adrenal gland is known to have direct neural and humoral connections to the SCN and is known to respond to light changes in the environment. In accordance to that the adrenal phases (44 % of genes) and amplitudes (88 % of genes) were influenced by the genotype mainly in LD (core clock genes *Per2*, *Dec1*, *Cry1*, *Reverba* and metabolic genes *Cyp51*, *Cyp17* and *Cyp39*). Whole genome sequencing discovered 5770 and 85224 single nucleotide variations (SNV's) in C57BL6/OlaHsd and 129S2/SvPasCrlf × C57BL/6JRj strains respectively compared to the NCBI37 reference mouse strain. Among these were SNVs in intron and exon regions of ten genes with genotype-dependent expression profiles (phases and/or amplitudes).

Conclusions: The differences observed at the level of gene expression between strains can to some extent be explained by the large number of SNVs that were discovered with whole exome sequencing. Especially in adrenal glands under LD condition the SNVs found likely influence the pathways which enable these two strains to transmit light information from the SCN to the adrenal glands. These and similar findings have important implications for understanding the genetic bases of the circadian rhythm differences in human individuals and their susceptibility to develop the clock-based diseases.

PNPLA3 as a potential marker for non-alcoholic fatty liver disease in children with familial hypercholesterolemia?

Urša Kovač[1], Rok Košir[1], Natalija Fužir[1], Monika Lewinska[1], Jernej Kovač[2], Gašper Klančar[3], Katarina Trebušak Podkrajšek[2,4], Primož Kotnik[3,5], Tadej Battelino[3,5], Damjana Rozman[1]

[1]Center for Functional Genomics and Bio-Chips, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, [2]Unit for Special Laboratory Diagnostics, University Children's Hospital, UMC Ljubljana, [3]Department of Endocrinology, Diabetes and Metabolic Diseases, University Children's Hospital, UMC Ljubljana, [4]Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, [5]Department of Pediatrics, Faculty of Medicine, University of Ljubljana; Ljubljana, Slovenia

Introduction: Non-alcoholic fatty liver disease (NAFLD) is a complex and widely prevalent liver disorder characterized by pathological changes from simple steatosis to non-alcoholic steatohepatitis (NASH) that can progress to cirrhosis and hepatocellular carcinoma. Susceptibility to and progression of NAFLD is associated with well-established risk factors, such as insulin resistance, diabetes, obesity and hypercholesterolemia. Polymorphisms in *PNPLA3* and *TM6SF2* both having roles in lipid metabolism were recently identified as perspective markers for NAFLD in adults. The aim of our study is to evaluate the significance of these markers in children with familial hypercholesterolemia (FH).

Methods: To study polymorphisms in *PNPLA3* in children with FH, we applied TaqMan genotyping on collection of DNA isolated from blood of 121 FH children (boys only). The children were referred to the University Children's Hospital through Slovenian national screening of preschool children for hypercholesterolaemia. The study was approved by the Slovenian National Medical Ethics Committee.

Results and conclusions: The frequency of *PNPLA3 rs738409 G* allele in Slovenian children with familial hypercholesterolemia is 0.29 while in control population (male European individuals from 1000 Genomes and European individuals from the Exome Variant Server (EVS)) is approximately 0.22. A correlation between the *PNPLA3 rs738409* allele and hypercholesterolemia has been identified in our samples if compared to male European individuals from 1000 Genomes project (OR=1.451, 95 % CI=1.093-1.926, $p<0.01$) and European Americans from the EVS collection (OR=1.491, 95 % CI=1.075-2.097, $p<0.05$).

Future perspective: In addition to the *PNPLA3* we need to assess the *TM6SF2* genotypes and apply them to a larger group, including females and Slovenian controls. This could give more reliable results and improve understanding of the role of *PNPLA3* and *TM6SF2* polymorphisms in relation with the NAFLD in children with FH.

Bioinformatic analysis of transcription factor MYC2 promoters

Tjaša Lukan, Anna Coll, Špela Baebler, Kristina Gruden

Department Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia

Plant hormones are crucial signalling molecules that coordinate all aspects of plant growth, development, reproduction and defence. Three hormones are especially important for plant immune response. The SA, JA and ET signalling pathways represent the backbone of the defence signalling network, with other hormonal signalling pathways feeding into it. The importance of salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) as dominant primary signals in local and systemic induced defence signalling has been well documented. However, the way these signal molecules function in a complex network of interacting pathways is less well understood and the majority of research has been done on model plant species and very little on potato. Our aim is to upgrade our current understanding of the roles of SA, JA and ET in the plant's immune system and crosstalk between defence hormone signalling pathways, with a focus on promoter analysis of chosen genes from defence signalling network.

In order to understand transcriptional network of signalling components in potato following PVY infection we decided to analyse the promoters of some genes that are crucial components in plant defence signalling pathways. One of them is transcription factor MYC2 from jasmonic acid pathway that regulates PCPI expression.

Promoters obtained from different potato cultivars were sequenced, compared to the available model genome sequence and analysed with TRANSFAC and PlantCARE. The results showed that promoter sequences of the same gene differ between cultivars. Furthermore, each gene can have different promoter sequences within the same cultivar. Using TRANSFAC and PlantCARE we managed to characterized transcription factor binding sites and established how transcription factor binding sites vary between promoters of the same gene within each cultivar.

A parallel algorithm for stochastic multiscale simulation of gene regulatory networks with multiple binding sites

Mattia Petroni, Nikolaj Zimic, Miha Mraz, Miha Moškon

Faculty of Computer and Information Science, University of Ljubljana, Ljubljana, Slovenia

It has recently been discovered that several important genes contain regulatory regions that consist of multiple non-cooperative transcription factors binding sites. Stochastic multiscale simulation algorithm (multiscale SSA) adapted to gene regulatory networks (GRNs) with regions containing multiple regulatory binding sites has already been described in Petroni et al. [1]. Repetitions of simulation responses obtained with stochastic methods often exhibit relatively large deviations. Several iterations of the same simulation therefore have to be performed to obtain quantitatively relevant results. Simulation repetitions may present computational demanding task, especially when we are dealing with multiple non-cooperative binding sites that have to be regarded explicitly. We present a parallelization of multiscale SSA adapted to GRNs with multiple repetitions of DNA binding sites in order to reduce the time needed to obtain quantitatively relevant simulation results. The parallelization is performed on a multithreading graphic processing unit, which is available on most modern personal computer and additionally extends the applicability of presented approach.

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The effect of lipoproteins and western diet on myocardium

Tadeja Režen[1], Jera Jeruc[2], Mauro Giacca[3], Damjana Rozman[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [2]Institute of Pathology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [3]International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

Introduction: Elevated blood cholesterol level is a risk factor for development of cardiovascular diseases. The effect of lipoproteins on macrophages and endothelium and their role in the development of atherosclerotic plaque is well studied. However, recent studies indicate that elevated lipoprotein levels also affect the heart itself. In particular, animal models on high-fat diet indicate that the diet induces pathological changes in the myocardium. Even short-term diet aggravates damage after myocardial infarction. Aim of this project is to study the effect of cholesterol on cardiac function, through feeding mice western diet and treating cardiomyocytes with different lipoproteins also in conditions of oxidative stress.

Results: Feeding wild type and heterozygous *Cyp51* knockout mice [1] with western diet consisting of 20% of lipids and 1.25% of cholesterol (versus normal diet with 10% of lipids) leads to adaptive changes in expression of key genes in lipid/cholesterol homeostasis. In left ventricle we measured down-regulation of lipoprotein lipase and up-regulation of cholesterol transporters *Abca1* and *Abcg1*. We observed no pathological changes in myocardial histology (cell area and interstitial connective tissue). Treatment of the HL-1 cell line with VLDLs induced massive enlargement of lipid droplets in low serum conditions. Gene expression studies indicated that two regulatory pathways, the SREBF and LXR/PPAR signalling pathways, were differentially modulated in response to VLDL treatment. Interestingly, VLDLR expression was found to be not controlled by the level of lipids/cholesterol but to be upregulated by VLDL treatment itself. Further analyses of HL-1 response to a combination of lipoproteins and oxidative stress will be presented.

Conclusions: The myocardium/cardiomyocytes respond to changes in cholesterol/lipoproteins in their environment (blood, cell medium) by modulating expression of genes involved in cholesterol homeostasis. VLDLR is known to be involved in lipid infiltration in different tissues and its unresponsiveness to cellular lipids further consolidates its role in pathological processes.

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Constructing a library of domain knowledge for process-based modeling of neurons using the Hodgkin-Huxley formalism

Nikola Simidjievski[1,2], Ljupčo Todorovski[3], Sašo Džeroski[1,2]

[1]Department of Knowledge Technologies, Jozef Stefan Institute, Ljubljana, Slovenia, [2]Jozef Stefan International Postgraduate School, Ljubljana, Slovenia, [3]Faculty of Administration, University of Ljubljana, Slovenia

Equation discovery is a sub-field of machine learning, which aims at developing methods for automated learning of quantitative laws, expressed in the form of equations. It studies methods for learning the model structure and parameter values from collections of observed data. In the context of modeling dynamic systems, the observations are time-series and the models take form of ordinary differential equations (ODEs). The state-of-the-art equation discovery methods for modeling dynamic systems, referred to as process-based modeling, integrate domain-specific modeling knowledge and data into explanatory models of the observed systems. Using process-based domain-specific knowledge formulated in a library, and observed data from the system at hand, these methods construct process-based models (PBMs) - an accurate, understandable and modular representation of the observed system. Such models relate to the processes that govern the dynamics of the observed system, and to the entities involved. While providing a higher-level representation of knowledge, PBMs are commonly translated into ODEs, offering a quantitative way of expressing the models' behavior over time. Conceptual mathematical modeling of single neurons consists of a considerable amount of knowledge represented with a variety of different models that can be found in literature. However, formalization of these concepts in forms of processes and entities has not been considered. This process-based formalization of knowledge would provide better understanding of the observed systems and would allow for exchange of knowledge between experts from different backgrounds, e.g., neuroscience, nonlinear dynamics and biology. Moreover, it will serve as a basis for computational methods to simulate, estimate the parameters of model structures, and induce process-based neuron models. Using the Hodgkin-Huxley formalism which includes independent gating particles and assumes unconstrained voltage dependencies of the firing rate coefficients we formalize the different components in terms of different entities and processes (ex. the membrane potential, ion pumps and the gating variables are defined as entities, whereas the ion and leak channels as processes). This approach allows for defining and learning a variety of different single neuron models in a process-based formalism. We illustrate the generality of the knowledge in the library through reconstruction of a well-known single neuron models of different complexity, such as Integrate-and-Fire, Integrate-and-Fire-or-burst, Morris-Lecar, Hodgkin-Huxley etc.

Process-based design of synthetic biological systems

Jovan Tanevski[1,2], Ljupčo Todorovski[3],Džeroski[1,2]

[1]Jožef Stefan Institute, Ljubljana, Slovenia, [2]Jožef Stefan International Postgraduate School, Ljubljana, Slovenia, [3]University of Ljubljana, Ljubljana, Slovenia

Introduction: The systems-based approaches to biology lead to better understanding of the interactions at different biological organizational levels. These interactions give rise to different dynamical behaviours which can be analysed in detail. Beside analysis, the systems-level understanding can be deployed in the context of control of existing and design of novel systems that have desired behaviours. We address here the task of design, which we base on the approach of process-based modelling (PBM). PBM integrates formalized knowledge and measured data in the process of automated identification of the structure and estimation of the parameters of dynamical systems. We transform the PBM approach to a process-based design (PBD) approach and apply it to the task of designing synthetic biological systems.

Method: PBM uses domain knowledge to specify a set of candidate model structures appropriate for a given modelling scenario. The knowledge is specified in a form of a set of entities representing candidate constituents of the system and a set of processes describing the possible (alternative) interactions between them. To describe the interactions, we introduce a mid-level formalism based on reaction equations which is more flexible and easier to understand within the domain of life sciences than a formalism based on fragments of differential equations. The mid-level formalism allows for multiple interpretations: deterministic and stochastic. An important issue in the task of design is the lack of measured data. Within the PBD approach, the data is replaced by a specification of number of desired model properties. The properties are commonly based on the simulated model behaviour. Following, the PBD approach requires optimization of multiple possibly conflicting properties. Within PBD we include a step of multi-objective parameter estimation replacing the current single-objective. The model parameters are optimized towards the desired behaviour. As a result we obtain a set of models ranked by their ability to achieve a behaviour with the desired properties.

Results: We implement the modifications within the process-based modelling tool ProB-MoT and apply it to the problem of designing stochastic genetic toggle switch without cooperation. We are able to reconstruct results reported in the related studies. In contrast to them, we encode the domain knowledge in a formal manner that enables re-usability and scalability. Using the knowledge for automated design, we are able to consider larger number of different structures. Finally, our PBD approach suggests new potential designs.

Conclusions: The PBD approach allows for consideration of multiple competing structures, it allows for different interpretations of models and deals with the issue of multiple desired properties of a behaviour. The results show that the approach performs successfully, the obtained model structures and parameters are able to achieve a desired behaviour.

γKlotho is a novel marker and cell survival factor in a subset of triple negative breast cancers

Nuša Trošt[1,2], Jurij Stojan[1], Patrick Ryan Potts[3], Klementina Fon Tacer[3], Elisabeth Martinez[2,4]

[1]Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Slovenia, [2]Department of Pharmacology, UT Southwestern Dallas Medical Center, Dallas, TX, USA, [3]Department of Physiology, UT Southwestern Dallas Medical Center, Dallas, TX, USA, [4]Hamon Center for Therapeutic Oncology Research, UT Southwestern Dallas Medical Center, Dallas, TX, USA

Klotho proteins are single-pass transmembrane glycoproteins with extracellular region that contains glycosidase-like domains. The family consists of three members, Klotho, βKlotho and γKlotho, that are evolutionary conserved in vertebrates. Klotho and βKlotho function as co-receptors for endocrine fibroblast growth factors (FGFs) while the role of γKlotho in FGF signaling was not determined yet. Klotho serves as a co-receptor for endocrine fibroblast growth factor 23 (FGF-23), controlling phosphate homeostasis. Additionally, it participates in processes such as regulation of ion channels and suppression of oxidative stress and growth factor signalling in cells. Klotho is epigenetically silenced in breast and several other cancers and is generally accepted as tumour-suppressor gene. βKlotho is required as a co-receptor for FGF-19 and FGF-21 and possesses tumour suppressor activity in hepatocellular carcinoma. We investigated the role of Klotho proteins, and specially γKlotho, in breast cancer tumorigenesis. Our results showed that γKlotho is significantly up-regulated in breast cancer, specifically in triple-negative breast tumours (TNBC) compared to benign tissue. On the other hand, Klotho and βKlotho are down-regulated in breast tumours what is consistent with literature data. Further, we found that γKlotho is expressed in a subset of TNBC cell lines. Its overexpression in MDA-MB-231 cells promoted cell growth, while γKlotho depletion in HCC1395 cells provoked cell cycle arrest and led cells into apoptosis. We suggest γKlotho acts as potential oncogene for TNBC.

The aim of the current study was to determine mechanistic background of γKlotho oncogenic activity. For this purpose, we conducted a microarray gene expression analysis on HCC1395 cells treated with γKlotho or corresponding control siRNA. Results revealed several differentially expressed genes of which many are known to be involved in cancer pathogenesis. Genes participating in reactive oxygen species (ROS) homeostasis presented one of the most altered groups; oxidative-stress responsive genes were extensively induced while genes conferring protection against ROS were mostly down-regulated after γKlotho knockdown. Further, we observed that depletion of γKlotho in TNBC cells causes persistent high activation of ERK. Sustained ERK activation is detrimental to cells and is usually observed in the presence of ROS. Increased levels of ROS in γKlotho depleted HCC1395 cells were confirmed using flow cytometry. Altogether, our results suggest that γKlotho may be involved in protection of cancer cells against increased oxidative stress and thus facilitating their rapid growth. γKlotho might be a potential marker for patients that would benefit from oxidative cancer therapy and even a novel drug target for TNBC.

Male prevalence and features of advanced NASH with cirrhosis in the liver knockout of *Cyp51* from cholesterol synthesis

Žiga Urlep[1], Gregor Lorbek[1], Peter Juvan[1], Martina Perše[2], Jera Jeruc[2], Ingmar Björkhem[3], Rolf Gebhardt[4], Damjana Rozman[1]

[1]Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [2]Medical Experimental Centre, Institute of Pathology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [3]Department of Laboratory Medicine, Division of Clinical Chemistry, Karolinska Institute, Karolinska University Hospital, Huddinge, Sweden, [4]Institute of Biochemistry, Medical Faculty, University of Leipzig, Leipzig, Germany

Background: Accumulation of free cholesterol in the liver promotes hepatocyte apoptosis and fibrosis, and is associated with NASH pathogenesis. Lanosterol 14α-demethylase (CYP51) presents a rate-limiting step in the latter part of cholesterol synthesis. Applying the liver conditional knockout of *Cyp51* (LKO), we show that blocking hepatic cholesterol synthesis in adult mice causes NASH-like liver changes (Lorbek et al., in review). Herein we describe the male predominant most severe phenotypes of the *Cyp51* LKO mice that stopped their development prior to adulthood.

Methods: From 376 mice (WT and LKO) that survived to the weaning period, 19 (4 female, 15 male) were underdeveloped, with jaundice and hepatomegaly (runts) and were euthanized at 5-10 weeks of age. All runts were of the LKO genotype. After initial histochemical evaluation and analysis of sterol metabolites we applied expression profiling by Affymetrix microarrays to search for molecular signatures that would differentiate runts from the rest of *Cyp51* LKOs.

Results: The runt livers were severely damaged, resembling NASH with cirrhosis, with frequent apoptoses and mitoses, though without the accumulation of lipids. Sirius red staining showed bridging fibrosis of the portal areas. This was accompanied by moderate to severe oval cell response and immune cell infiltration. Microarray analysis of runts compared to age-matched non-cachectic LKOs gave 5983 differentially expressed genes. Among significantly elevated genes in runts are *Spp1* (osteopontin), *Cxcl10* and *c-Jun*, all linked to NASH pathogenesis. Gene set enrichment analysis of KEGG pathways showed enrichment of TGFβ signalling (involved in apoptotic and fibrotic processes), apoptosis, signalling with growth factors (VEGF, PDGF, ErbB) and cancer related pathways. Gene ontology term enrichment listed the unfolded protein response (integrated stress response) as significantly deregulated, which is in line with our in-house hypothesis that changes in cholesterol balance result in ER stress. On the metabolite level, runts compared to non-cahectic LKOs exhibit further elevation in hepatic lanosterol and 24,25-dihydrolanosterol, both substrates of CYP51, and significantly elevated free cholesterol.

Conclusions: We present a novel model where disrupted cholesterol synthesis results in NASH-like symptoms without the presence of steatosis. The severity of liver damage depends on the cholesterol balance. The male-predominance in the most severe *Cyp51* LKO phenotype is under investigation.

Cell communication network analysis of glioblastoma stem cell marker candidates β-actin, CD9, FTL, S100A9 and TRIM28

Marko Vidak, Ivana Jovčevska, Neja Zupanec, Radovan Komel

Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Gliomas are malignant tumours of glia, the brain connective tissue. Glioblastoma multiforme (GBM) is the most dangerous type of this malignancy. The median survival time of GBM patients after diagnosis is only 15 months despite surgical treatment combined with aggressive chemotherapy. GBM is so fatal because of glioblastoma stem cells (GSCs) within the tumour. They cannot be totally removed by tumour resection because of their location in perivascular niches on tumour edges. After a resection leftover GSCs represent the focal point of cancer relapse. Targeted destruction of GSCs is a promising approach to GBM therapy since it would prevent tumour recurrence. To enable such therapy, GSCs markers need to be discovered and a search is ongoing for new marker candidates. We have focused on five proteins recently proposed as GSCs markers, namely CD9, FTL, S100A9 [1], β-actin and TRIM28 [2]. There are substantial differences among the five proposed candidates in their cellular locations, functions, connections with other malignancies and experimental methods by which these proteins have been linked to GSCs. Our aims are to analyse cell communication networks of each of the five proposed candidates, to find possible connections between the networks, to clarify the role of the candidates in GBM carcinogenesis and possibly to discover new, better GSCs marker candidates.

By using system biology approaches, we intend to create, for each candidate respectively, cell communication networks for various malignancies in which a particular candidate is involved and where its role has already been explained. We will compare these networks to find similarities which will then be used to create, by analogy, a network model for the candidate's role in GBM carcinogenesis. These models will then be compared to see if they have common points indicating connections among the marker candidates. Such common points may represent (or lead us to) new marker candidates which would be screened with the same approach as the original five. Candidates deemed most promising after the screening will be experimentally validated.

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Mutation analysis in patient with elevated red blood cells number: A case report, review of the literature and genomic databases

Danijela Vočanec[1,2], Tinkara Šilc[1,2], Tadej Pajič[3], Martina Fink[3], Irena Preložnik Zupan[3], Peter Černelč[3], Radovan Komel[1], Tanja Kunej[2], Nataša Debeljak[1]

[1]Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [2]Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia, [3]Clinical Department of Haematology, University Medical Center Ljubljana, Ljubljana, Slovenia

Introduction: Erythrocytosis is an increase in the number of red blood cells (RBC). There are primary and secondary causes for erythrocytosis. Common secondary causes include smoking, hypoxia, and diuretics while primary cause is known as polycythaemia vera (PV), a haematological disease. The mutation in a Janus kinase 2 gene (*JAK2*) is indicative for PV, while erythropoietin receptor gene (*EPOR*) mutation is the indicator for primary familial erythrocytosis. The gain-of-function *JAK2* V617F mutation in exon 14 was detected in 95 %, while 3 % of patients with PV may have *JAK2* gene mutations in exon 12 [1]. Secondary erythrocytosis syndromes are typically associated with a defect in various genes included in oxygen sensing pathway that leads to the increased erythropoietin production [1]. The hormone erythropoietin (EPO) and its receptor are the main regulator of RBC production in the bone marrow.
The aim of our study was to determine the cause of erythrocytosis in one patient with increased RBC mass, elevated RBC count, haemoglobin and haematocrit values. Ensembl database was searched for candidate variations in *EPO* and *EPOR* genes for association with erythrocytosis, followed by confirmation by sequencing analysis.

Results: The level of patient erythropoietin in serum was in the reference intervals. The mutation analysis for *JAK2* V617F and *JAK2* exon 12 mutations turned negative, indicating exclusion of PV. To confirm the primary familial erythrocytosis the *EPOR* mutation analysis is proposed. Ensembl database and literature search was performed in aim to detect the most common mutations in *EPO* and *EPOR* linked with previously described clinical cases of familial erythrocytosis. According to the Ensembl genome browser release 80 there are 540 genomic variations residing within the *EPO* but no known variation is associated with erythrocytosis. Erythropoietin role in erythrocytosis is indirect, there are some known mutations in genes involved in oxygen sensing pathway which are important in *EPO* regulation. Same release of Ensembl includes 801 mutations in the longest, protein coding, EPOR-001 transcript. All of mutations associated with erythrocytosis in *EPOR* are located in exon 8, which encodes the C-terminal negative regulatory domain of the protein. They are leading to cytoplasmic truncation of the receptor and loss of the C-terminal negative regulatory domain [2].

Conclusions: The mutation analysis for *JAK2* V617F and *JAK2* exon 12 mutations turned negative excluding the PV. *EPO* and *EPOR* mutation analysis in association of primary familial erythrocytosis is in progress.

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