TRENDO (MSCA-RISE 2021-2025)

Univerza v Ljubljani
Medicinska fakulteta

Workshop

Importance of Biobanking in Biomarker Discovery

Ljubljana, October 19th-20th, 2023

Book of Abstracts

Workshop: Importance of Biobanking in Biomarker Discovery

Ljubljana, October 19th-20th, 2023

Book of Abstracts

Organized by the Program Group P1-0390 of the Faculty of Medicine, University of Ljubljana and the TRENDO Consortium.

Scientific and Organizing Committee at the Faculty of Medicine, University of Ljubljana:

Tea Lanišnik Rižner, Tadeja Režen

Sponsors:

Edited by: Tea Lanišnik Rižner, Tadeja Režen

Technical editors: Tea Lanišnik Rižner, Tadeja Režen

Organized by: University of Ljubljana, Faculty of Medicine and the TRENDO Consortium

Published by: University of Ljubljana, Faculty of Medicine, Ljubljana, October 2023

Electronic version: https://www.mf.uni-lj.si/ibk/dogodki/biobanks-and-biomarkers

Electronic format: Electronic version in PDF format

The publication is free of charge.

Kataložni zapis o publikaciji (CIP) pripravili v Narodni in univerzitetni knjižnici v Ljubljani
COBISS.SI-ID 169953795
ISBN 978-961-267-254-6 (PDF)

Dear Colleagues,

The Workshop on the Importance of Biobanking in Biomarker Discovery is a two-day event organized by the Programme Group *Functional Genomics and Biotechnology for Health* P1-0390 of the Faculty of Medicine, University of Ljubljana and the TRENDO Consortium on 19th and 20th October, 2023 at City Hotel Ljubljana, Slovenia.

The aim of this workshop is to share knowledge on biobanking and omics approaches for biomarker discovery, and to raise awareness of the importance of biobanking in minimising pre-analytical biases that can affect biomarker discovery.

The first session of the workshop entitled "*Importance of Biobanking*" will include lectures by the head of the European infrastructure BBMRI-ERIC Prof. Jens K. Habermann and the national coordinator BBMRI-SI Prof. Dr. Urban Bren, who will introduce European and Slovenian biobanking. This will be followed by lectures by the heads of the European biobanks (Biobank Graz and Unified Biobank Hannover) and presentations of the Slovenian biobanks (Biobank of the National Transfusion Centre, Gliobank of the National Institute of Biology and Biobank of the University of Maribor).

In the second session, "*TRENDO: Translational Research on ENDOmetriosis: from biobanks, biomarkers to treatment*", coordinator Prof. Dr. Martin Götte will present the TRENDO project and this will be followed by lectures of the consortium partners on translational research in endometriosis, from biobanks, biomarkers to models. In the final session, "*Omics approaches for biomarker discovery*" experts in the field of transcriptomics, proteomics, metabolomics and machine learning will present omics approaches and machine learning methods and their use in biomarker discovery. The workshop will conclude with a lecture by Prof. Dr. Rainer Lehmann on the importance of pre-analytical biases in biomarker research.

The workshop will provide ample time for networking and for establishing new contacts and collaborations that will contribute to high-quality biobanking and sound biomarker discovery studies not only in endometriosis but also in other diseases.

We would like to thank all the speakers contributing to the high quality of the workshop, as well as the sponsors and everyone involved in the organisation of this event.

Prof. Dr. Tea Lanišnik Rižner and Assist. Prof. Tadeja Režen

PROGRAMME

Thursday 19th October, 14:00-17:00

13:00-14:00	Arrival and registration	

WELCOME

14:00-14:10	**Damjana Rozman**, head of programme group P1-0390 **(Ljubljana, SI)**	
	Martin Götte, coordinator of the TRENDO project **(Münster, DE)**	

Importance of biobanking

Chairs: Urban Bren, Tadeja Režen

14:10-14:35	**Jens K. Habermann (BBMRI-ERIC)**	recorded
	Presentation of BBMRI-ERIC	
14:35 -15:00	**Monika Valjan (Graz, AT)**	
	Biobank Graz - Presentation of the largest EU biobank	
15:00:15:25	**Norman Klopp (Hannover, DE)**	
	Hannover Unified Biobank	
15:25- 16:00	Tea & coffee break / Networking	
16:00-16:20	**Urban Bren (Maribor, SI)**	
	Current status of biobanking in Slovenia	
16:20- 16:35	**Vladka Čurin Šerbec (Ljubljana, SI)**	
	Biobank at National Transfusion Centre	
16:35-16:50	**Metka Novak (Ljubljana, SI)**	
	Gliobank at National Institute of Biology	
16:50 -17:05	**Uroš Potočnik (Maribor, SI)**	**on line**
	Biobank at University of Maribor	
17:15 - 18:30	Social event/Networking	

Friday 20th October, 9:30- 14:00

TRENDO: Translational Research on ENDOmetriosis from biobanks, biomarkers to treatment

Chairs: Martin Götte, Tea Lanišnik Rižner

9:30-9:55 **Martin Götte (Münster, DE)**

Presentation of the TRENDO project

9:55- 10:15 **Tea Lanišnik Rižner (Ljubljana, SI)**

Biomarker discovery in endometriosis

10:15-10:30 **Heba El-Shorafa (Münster, DE)**

Functional analysis of microRNA miR-29c in endometriosis and infertility

10:30-10:45 **Adrián Seijas-Gamardo (Maastricht, NL)**

In vitro model of innervation in endometriosis

10:45-11:15 Tea & coffee break / Networking

Omics approaches for biomarker discovery

Chairs: Jochen Schwenk, Rainer Lehmann

11:15-11:40 **Jerzy Adamski (Neuherberg, DE; Ljubljana, SI)**

Metabolomics for biomarker discovery

11:40-12:05 **Jochen Schwenk (Solna, SE)**

Proteomics for discovery of circulating biomarkers in biobanks

12:05-12:15 **Tadeja Režen (Ljubljana, SI)**

Transcriptomics for biomarker discovery

12:15-12:25 **Matthias Hackl (Vienna, AT)**

Development of microRNA-based diagnostic tests utilizing biobanks and transcriptomic tools

12:25-12:50 **Rainer Lehmann (Tübingen, DE)**

How to avoid preanalytical bias and assure sample quality for biomarker discovery

12:50-13:10	**Marko Kokol (Maribor, SI)**	**on line**
	Harnessing Machine Learning on Small Sample Sizes for Biomarker Discovery	

CLOSING REMARKS AND END OF THE WORKSHOP

13:10-13:20 Organizers

Importance of biobanking

Biobank Graz

Monika Valjan

Medical University of Graz
e-mail: monika.valjan@medunigraz.at

Biobank Graz is central research infrastructure at the Medical University of Graz and coordinates biobanking in close collaboration with its clinical partners. Biobank Graz provides state-of-the-art logistics and infrastructure for the collection and storage of high quality biological material, while protecting the personal rights of sample donors. The available biological specimens include formalin-fixed paraffin-embedded (FFPE) tissues, cryopreserved tissues and biological fluids. Special attention is paid to sample quality. Depending on the experimental demands, prospective cohorts are collected according to appropriate CEN/ISO standards. Biobank supports researchers in biobanking by taking on all logistical and project-relevant services

Biobank Graz allows access to collected specimens and associated data for scientific research purposes. Today the scientific examination of these specimens in connection with symptoms of disease is one of the most important requirements for a better understanding of the causes and progression of disease. The common goal is the development of new preventative measures, diagnostic procedures and treatment methods based on this understanding.

Hannover Unified Biobank (HUB)

Norman Klopp[1], *Thomas Illig*[1]

[1]Hannover Unified Biobank (HUB), Medical School Hannover (MHH), Carl-Neuberg-Str.1, 30625 Hannover, Germany
e-mail: klopp.norman@mh-hannover.de

Establishing high quality biobanking standards in the clinic requires large and unified biobanks to guarantee a sustainable biobank infrastructure. Since 10 years, Hannover Medical School (MHH) operates the Hannover Unified Biobank (HUB), which organizes standardized sample preparation, storage, and distribution as well as harmonization of sample related data. The HUB is connected by a transport service to the MHH and provides a rapid sample exchange and sample preparation with all clinics, operating rooms, and research institutes. Samples are processed with a high level of automation. Robotics is used for blood fractionation and aliquotting and fully automated DNA extraction ensures high sample quality. 1D/2D codes for permanent sample tracking together with the direct connection of the scanners and robotics to the central LIMS database system guarantee a streamlined data flow. The long-term storage of the biomaterial is realized in liquid nitrogen tanks, which are located in a modern, secured infrastructure. Samples and racks are compiled by an automated -80°C BIOS system. Sample related data, including the complete sample tracking information is documented and securely stored in the LIMS. A quality management system ensures the compliance to standards. Additionally, the HUB is certified according to DIN ISO 9001. Access to samples and data of the biobank and the linked clinical data warehouse of the MHH is organized in standardized procedures. It requires the approval and prioritization of the requesting research projects and the approval of the sample owner. The biobank is also open for national and international collaborations, networks and scientists.

Current Status of Biobanking in Slovenia

Urban Bren

Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova ulica 17, SI-2000 Maribor, Slovenia
e-mail: urban.bren@um.si

Slovenian Biobanking and Biomolecular Resources Research Infrastructure Consortium BBMRI.SI was established in December 2020. Its founding members are all three Slovenian public universities - University of Maribor, University of Ljubljana, and University of Primorska - as well as University Medical Center Maribor. University of Maribor acts as the coordinating institution and Prof. Dr. Urban Bren as the national node coordinator. In 2021 we successfully became full members of the Biobanking and Biomolecular Resources - European Research Infrastructure Consortium BBMRI-ERIC. In 2023, we received two new membership applications from the National Institute of Biology and from the University Clinic Golnik. What lies ahead is the challenging consolidation of the fragmented Slovenian biobanking landscape.

Biobank at National Transfusion Centre

Vladka Čurin Šerbec

Blood Transfusion Centre of Slovenia, Šlajmerjeva 6, Ljubljana
e-mail: vladka.curin@ztm.si

Biobanks are repositories of human biological material that responsibly receive, store and distribute biological samples and relevant clinical data for research, education purposes and for clinical use. They are a complex system of processes through which unique and valuable samples are managed. Biobanks are an important part of development and progress in the medical field and are crucial for the implementation of high-quality clinical studies and research, including the field of personalized medicine. At the beginning of this year, a pilot biobank was established at the Blood Transfusion Centre of Slovenia (BTCS) in the scope of Interreg project C3B. Among the goals of the project were also to check the interest in biological samples of healthy donors for research, education and clinical studies, to arrange and organize the collection, storage and use of samples of blood from healthy donors, to develop a pilot model and to prepare the prerequisites for the establishment and operation of a national biobank of the blood samples of healthy donors, based on our experience and good practices in blood banking and according to the Strategy of the Blood Transfusion Centre of Slovenia. The project C3B was chosen twice as one of the best projects in the programme - in the field of management and in the field of health.

Our model is based on ethical, moral and medical principles that BTCS takes into account when managing the blood bank. We implemented good practices from the field of biobanking, used our own good practices and quality standards, and established a pilot model of a biobank of blood samples from healthy donors. The samples are intended to be used for education, research and clinical studies, approved by the Medical Ethics Committee. The establishment of a pilot model of a biobank of blood samples from healthy donors will represent the integration of the national biobank into already existing protocols at BTCS with an emphasis on the processes by which we monitor the blood sample from the reception of the donor to the release of the blood sample or its components from the biobank to the end users. A quality system is included in the biobanking and the activities are supported by information system which ensures the traceability of the handling of samples and enables the protection of personal data.

GlioBank: Connecting preclinical and clinical data of glioblastoma

Metka Novak[1], Bernarda Majc[1], Anamarija Habič[1,2], Mateja Mlinar[1], Andrej Porčnik[3], Roman Bošnjak[3], Jernej Mlakar[4], Andrej Zupan[4], Alenka Matjašič[4], Marija Vidmar Skoblar[5], Matjaž Hren[6], Ivana Jovčevska[7], Neja Šamec[7], Alja Zottel[7], Radovan Komel[7], Tamara Lah Turnšek[1], Barbara Breznik[1]

[1]Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Slovenia;
[2]Jožef Stefan International Postgraduate School, Ljubljana, Slovenia;
[3]Department of Neurosurgery, University Medical Centre Ljubljana, Ljubljana, Slovenia;
[4]Institute of Pathology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia;
[5]Institute of Oncology, Ljubljana, Slovenia;
[6]Biosistemika d.o.o., Ljubljana, Slovenia;
[7]Medical Centre for Molecular Biology, Institute for Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana,Slovenia
e-mail: barbara.breznik@nib.si, metka.novak@nib.si

Glioblastoma (GB) is one of the most lethal human solid tumors with an average life expectancy of 15 months with treatments. Currently, GB is considered incurable. In contrast to the diagnostic advances, there is a lack of progress in identifying clinically relevant biomarkers and developing effective therapeutic options. Therefore, data generated by large “omics” projects and smaller research groups should be securely stored in repositories so that they are available for future use. Successful completion of such studies requires a large number of samples and the associated clinical and molecular information. The data obtained from these studies are stored in databases. For that purpose, we established a disease-specific research-based tumor tissue bank and repository, named GlioBank, that will help accelerate the field of biomarker discovery for GB, and their consecutive translation into clinical practice. GlioBank is a database of different clinical (KPS, extent of surgical resection, age, gender, survival, medications) and molecular (gene and protein expression data, most common genetic features) characteristics, and corresponding biological material and experimental tumor models (tissues, plasma, blood PBMCs, primary cell lines and organoids) from GB patients. We have primary and recurrent samples, data that allow us to track changes in the tumor over time, as well as samples from different regions of the same patient (tumor rim and core). Collecting such extensive data requires a common storage location and controlled access to sensitive information. GlioBank thus provides a disease-oriented biobank in Slovenia that can facilitate the development of new treatment approaches and the search for new biomarkers, with the ultimate goal of improving outcomes for GB patients.

Biobank at University of Maribor

Uroš Potočnik,[1,2,3], *Anja Bizjak*[1], *Boris Gole*[1]

[1]University of Maribor, Faculty of Medicine, Centre for Human Molecular Genetics and Pharmacogenomics, Maribor, Slovenia
[2]University of Maribor, Faculty for Chemistry and Chemical Engineering, Laboratory for Biochemistry, Molecular Biology and Genomics, Maribor, Slovenia
[3]University Medical Centre Maribor, Department for Science and Research, Maribor, Slovenia
e-mail:uros.potocnik@um.si

Biobank at University of Maribor (BUM), located within Center for human genetics and pharmacogenomics (CHMGF) at Faculty of Medicine, was developed since 2005 as a collection of biological samples from patients enrolled into several genetic studies conducted in collaboration between CHMGF and University Medical Center Maribor. BUM hosts now more than 30 different disease-oriented studies with well-defined scientific goals and by 2023 has enrolled more than 7000 patients for more than 20 different diseases, including chronic immune diseases (Inflammatory bowel disease (Crohn´s disease)(4 studies), asthma (3 studies), rheumatoid arthritis, multiple sclerosis), Cancer (breast cancer, head and neck cancer), chronic kidney disease, rare hereditary diseases (Mendelian).The common aim of all studies is understanding of molecular pathways involved in pathogenesis, discovery of biomarkers and development of predictive models for better diagnosis, disease monitoring and personalized therapy. More recently, we use systems (bio)medicine approach and multi-omics technology (genomics, transcriptomics, proteomics, epigenomics, metabolomics) to better understand molecular mechanisms of non-response to biological drugs in chronic immune diseases, to discover biomarkers for patient classification based on molecular endotypes that will aid in personalized medicine and to discover molecular targets for novel drugs. The newly discovered biomarkers are functionally evaluated using in vitro cell models based on patients' primary cells, 3D organoid models and finally, organ-on-a-chip systems. To enable such studies, several clinical biological samples are taken from the same patient: blood, tissues, saliva, urine, stool, nasal swabs…) and several biomolecules are isolated simultaneously from the same clinical sample (DNA, RNA, proteins, metabolites, microbiom). For many studies, samples are taken before and during therapy where treatment response is measured at each time point. So far, more than 50 000 different samples are included in our biobank. Biobank is developed based on highest international standards and SOPs for sample acquisition, analysis, collection, data management, distribution, preparation, preservation, and testing, developed through our comprehensive collaboration within international genetic consortia, such as PERMEABLE »“PERsonalized MEdicine Approach for asthma and allergy Biologicals seLEction”and IIBDGC-International Inflammatory bowel disease Genetics consortium. Since 2023 BUM is enrolled in BBMRI-ERIC network. The future goal is in full biobank automatization and digitalization supported with LIMS that could be directly linked to digital platform we are currently developing for integration of clinical data and multi-omics data that will all improve development of predictive models using machine learning algorithms and artificial intelligence.

TRENDO

Translational Research on ENDOmetriosis from biobanks, biomarkers to treatment

Presentation of the TRENDO project

Martin Götte

Department of Gynecology and Obstetrics, Münster University Hospital, Vesaliusweg 2-4, D-48149 Münster, Germany
e-mail: Martin.Goette@ukmuenster.de

Endometriosis is a severe disease affecting 10% of reproductive age women, characterized by the presence of endometrial-like tissue outside of uterus. Due to the associated pain, inertility and a small, but increased risk for developing ovarian cancer, its socioeconomic impacts are considerable, and have a profound effect on women's mental and physical health. Endometriosis is characterized by a diagnostic delay of a decade, as its diagnosis is surgery-based, and all attempts to identify the non-invasive biomarkers have not been progressed beyond the discovery. Moreover, current treatments include only surgery or hormonal medications with significant side effects. In view of the above, the endometriosis care is challenging, and is hampered by the considerable heterogeneity of clinical manifestations. The TRENDO consortium addresses the main problems in endometriosis diagnosis and therapy in a multidisciplinary and trans-sectoral effort, bringing together the clinical and translational science experts, and industry partners from six European and South American countries. We develop non-invasive biomarkers and testing, which shorten the diagnostic delay, and reduce the unequal access to expensive healthcare. These biomarkers are tested for the suitability as drug targets, to monitor treatment response, and to predict the personalized clinical recommendations. By combining the strengths of our partners, we provide novel tools addressing the most urgent priorities in endometriosis and to successfully translate our findings into novel therapeutic approaches. This challenging scientific task provides an ideal career-enhancing setting for young scientists, providing them with transdisciplinary and trans-domain training, and with essential transferable skills. The training program provides a new generation of researchers with a strong competitive advantage on the job market and increase European academic and industry competence in one of the under-investigated clinical domains.
https://cordis.europa.eu/project/id/101008193
https://www.medizin.uni-muenster.de/trendo/herzlich-willkommen.html

Biomarker discovery in endometriosis

Tea Lanišnik Rižner

Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana,
Ljubljana, Slovenia
e-mail: tea.lanisnik-rizner@mf.uni-lj.si

Endometriosis is a chronic gynaecological disease that affects women of reproductive age and significantly affects their quality of life. Diagnosis of peritoneal endometriosis currently relies only on laparoscopy, thus there is an urgent need for a non-invasive diagnostic test. Biomarkers are needed as a replacement test for diagnostic surgery or as a triage test to select patients who need surgery. Although a large number of candidate biomarkers have been identified, there are no clinically validated biomarkers yet. In collaboration with the Department of Gynaecology at the University Medical Centre Ljubljana, we have investigated diagnostic biomarkers using metabolomics, proteomics and transcriptomics. Over the past 12 years, we have enrolled more than 600 patients with symptoms suggestive of endometriosis and divided them into case and control groups based on laparoscopy and histology. Peritoneal fluid and blood samples were collected according to a strict SOP and clinical, demographic and lifestyle data were collected with an extensive questionnaire. Together with Helmholtz Zentrum Muenchen we performed the first metabolomics study in endometriosis (1) and have since created and validated several diagnostic models. Next, we conducted a targeted proteomics study using antibody arrays (Sciomics®) that captured 1360 different proteins with more than 1830 antibodies. Biomarker discovery identified 16 proteins in peritoneal fluid (2) and three proteins in plasma samples (3). Validation in plasma samples by ELISA assay gave the best results for transforming growth factor beta induced (TGFBI) in peritoneal endometriosis with an AUC of 0.76, sensitivity of 58%, and specificity of 89%. A support vector machine model combining TGFBI and CA-125 showed even higher AUC values in peritoneal endometriosis combined with ovarian endometriosis (AUC of 0.94), deep endometriosis (AUC of 0.83) and both ovarian and deep endometriosis (AUC of 0.98)(4). TGFBI thus represents a candidate biomarker that needs to be validated in a multicenter study. Recently, we performed non-targeted whole blood transcriptomics study. In the discovery phase, 7 DEGs and more than 400 DETs were identified in the proliferative phase and 379 DEGs and 457 DETs in the secretory phase, and PCA showed a clear separation between patients with peritoneal endometriosis and the control group (5). Validation of the selected DEGs is ongoing. In the future we aim to create models based on the combination of available omics data using artificial intelligence and machine learning methods. Supported by J3-1755 and P1-0390, both from the Slovenian Research and Innovation Agency, and the EU-H2020-MSCA-RISE project TRENDO (grant 10100819).

1. Vouk K. et al. Hum. Reprod., 2012, Vol. 27, 2955-2965
2. Janša V. et al., Sci. Rep., 2021, Vol. 11, 20870
3. Pušić Novak M. et al. J. Pers. Med. 2022, Vol. 12, 1-16
4. Janša V. and Pušić Novak M. et al. Hum. Reprod. 2023, Vol. 38, 1284–1296
5. Pušić Novak M. and Režen T. et al. unpublished data

Functional analysis of miR-29c in endometriosis and infertility

Heba M. El-Shorafa[1,*], *Theresa Wentges*[1,*], Kadi Tilk[2], Jessica Oyie Sousa Onyeisi[1], Maire Peters[2], Andres Salumets[2], Martin Götte[1]

[1]Department of Gynecology and Obstetrics, Münster University Hospital, Vesaliusweg 2-4, D-48149 Münster, Germany. [2] Competence Centre on Health Technologies, Tartu, Estonia.
e-mail: hm.shorafa@alaqsa.edu.ps
*Authors contributed equally

Unreceptive endometrium and disrupted fetal-maternal interaction may lead to infertility, pregnancy loss, and many other pregnancy complications. Due to ethical consideration, an in-vivo model of human blastocyst implantation is challenging. Therefore, we have established in-vitro 3D endometrium model to provide an alternative research tool to study the role of miR-29c-3p in embryo implantation which is dysregulated in endometriosis.

The endometrial epithelial cell line (12Z) and primary endometrial stromal cells (OP5) were transfected with pre-miR-29c-3p followed by migration, invasion and viability assays. The 3D model of endometrial epithelial cells (Ishikawa and RL-95) cultured with immortalized St-T1b endometrial stroma cells was established. HTR-8/SVneo spheroids were used as an embryo surrogate to measure their adhesion. First, the epithelial cells were transfected with pre-miR-29c-3p followed by transcriptomic comparison between miR-29c-3p-transfected and non-transfected RL-95 cells. qPCR, Western blot and Immunofluorescence staining was then performed as confirmatory test of RNA sequencing results. The transfected epithelial cells were then included in the 3D model followed by adhesion assay.

Transfecting 12Z cells with pre-miR-29c-3p reduced their invasion without any effect on their migration. However, the miR-29c-3p reduced OP5 and 12Z viability. A total of 30 messenger RNAs (mRNA) were found to be differently expressed between transfected and non-transfected RL-95 cells. The expression of STAT-3, CDK6, COL4A2, and VEGF was significantly downregulated in the transfected cells assessed by qPCR. At the protein level, STAT-3 was downregulated in the transfected cells. The 3D model of pre-miR-29c-3p transfected Ishikawa as well as RL-95 cells had a significant decreased adhesion of HTR-8/SVneo spheroids compared to controls.

Our newly established 3D model can be used to study the biological factors that influence the physiology and pathology of the human endometrium. miR-29c-3p reduces endometrial epithelial cells viability and invasion. It decreased the trophoblastic spheroid adhesion to 3D model endometrium. Such data suggest miR-29c-3p as a potential biomarker for endometrium receptivity.

Modelling the innervation in endometriosis: A biofabrication strategy for generating organized complex 3D *in vitro* models

Adrian Seijas-Gamardo[1], A. Schulten[1], Victor de la Rosa[2], Richard Hoogenboom[3], A. Romano[4], L.Moroni[1] and P. Wieringa[1]

[1]MERLN Institute, Maastricht University, The Netherlands
[2]Avroxa BV, Ghent, Belgium
[3]Supramolecular Chemistry department, Ghent University, Belgium
[4]GROW, School for Oncology and Reproduction, Maastricht University, The Netherlands
e-mail: a.seijasgamardo@maastrichtuniversity.nl

Endometriosis is a chronic condition characterized by the ectopic growth of endometrial-like tissue outside the endometrial lining. Its symptoms are diverse, including common manifestations like inflammation and pain, and in severe cases, infertility. This condition is estimated to affect roughly 10% of women of reproductive age. The precise etiology of endometriosis remains elusive. The disease's inherent heterogeneity, combined with a historical lack of research, has limited our understanding of the disease, having just theories regarding its origin and constraining the efficacy of available treatments. While the exact cause of endometriosis is not understood, there is evidence suggesting that the extracellular matrix (ECM) at the lesion site may play a role in the development and progression of the disease. Changes in the ECM composition may influence the adhesion of endometrial cells, facilitating their implantation and growth. Moreover, they can influence nerve growth and sensitivity in the lesions area, potentially explaining the pain suffered by patients. Additionally, hormones play a role in the development and progression of endometriosis.
Here we present the development of a 3D *in vitro* model for the study of endometriosis using a novel sacrificial templating strategy to organize and give structure to soft hydrogel coculture systems. Using this technology, we generated a hydrogel platform that better emulates the spatial organization of different cell types and ECM compositions that are relevant for the disease. The sacrificial template is fabricated from a novel thermoresponsive polymer that is stable at cell culture conditions, but it quickly dissolves at standard refrigerator temperatures. With this we were able to generate high resolution microchannels between two mini-wells within a hydrogel. We seeded iPSC-derived nociceptive neurons in one mini-well and patient-derived endometrial organoids in another, directing axonal outgrowth towards the endometrial tissue. By increasing the concentration of collagen in the endometrial mini-well, we can emulate an increasingly fibrotic microenvironment around the lesion. We validated this mimetic fibrotic environment through comparison with histological samples from patients suffering from endometriosis. When culturing the organoids in this higher collagen concentration hydrogel we observed an effect in the morphology of the organoids, demonstrating an effect derived from the ECM composition. To mimic the estrogen disbalance of the disease, hormones can also be added into the culture system. The effects of this hormonal treatment and ECM composition were assessed quantifying the proliferation, viability and gene expression of the endometrial organoids and the outgrowth of the nociceptor neurons. The development of this *in vitro* model provides an essential tool for understanding endometriosis and test new treatments that can alleviate the burden of the patients suffering from it.

OMICS APPROACHES FOR BIOMARKER DISCOVERY

Metabolomics for Biomarker Discovery

Jerzy Adamski [1,2,3,4]

[1] Institute of Experimental Genetics, Helmholtz Zentrum München, Neuherberg, Germany
[2] Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[3] Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[4] Metaron Diagnostics i.G., Neuherberg, Germany
e-mail: info.adamski@gmx.org

Metabolomics as a holistic analysis of metabolites provides functional insights into biological systems. Metabolomics provides dynamic descriptive signatures useful for risk stratification, early diagnosis, therapy monitoring, or theranostics. In both health and disease, metabolomics goes beyond genetic coding to also consider the effects of lifestyle, environment, and interventions on metabolic pathways.
In our unbiased search for diagnostic biomarkers for endometriosis, we have used metabolomics. Non-invasive measurement of metabolite signatures was based on absolute quantification in human plasma with targeted metabolomics using a tandem mass spectrometry platform. We analysed the concentrations of metabolites (lysophosphatidylcholines, phosphatidylcholines, sphingomyelins, acylcarnitines, amino acids, biogenic amines, and hexoses) in plasma samples from two multicenter Europoid cohorts. The data were further processed using machine learning methods, and finally, generalised linear models were built based on metabolite concentrations and their ratios. Using this approach, we were able to discover unique signatures specific for the presence of endometriosis and also specific for different types of endometriosis, e.g., peritoneal, ovarian, or deep infiltrating. The advantage of the metabolomics approach was also demonstrated by the fact that the disease-specific signatures were independent of menstrual cycle, BMI, or diet. The signatures also allow for faster diagnosis and show better AUC for diagnosis compared to other invasive methods currently in use.

Proteomics for the Discovery of Circulating Biomarkers in Biobanks

Jochen M Schwenk[1,2]

[1] Department of Protein Science, KTH Royal Institute of Technology, Stockholm, Sweden
[2] Affinity Proteomics, Science for Life Laboratory, Stockholm, Sweden
e-mail: jochen.schwenk@scilifelab.se

Blood-derived samples contain a complex mixture of proteins and other biomolecules, which offers a valuable and accessible source to measure and monitor systemic changes. Proteins circulating our blood are crucial in understanding disease mechanisms, enabling early diagnosis, and guiding personalized treatment strategies. In recent years, proteomic technologies have emerged as a powerful tool for biomarker identification and validation at a much greater depth, precision, and scale than ever before.

However, identifying and validating reliable biomarkers in plasma samples remains challenging. This is attributed to a combination of factors, such as (i) the high dynamic range of protein concentrations, demanding different degrees of sensitivity and specificity from the analysis systems; (ii) the inter-individual variability, requiring to admit that heterogeneity is a prerequisite and not an excuse; (iii) the pre-analytical variables and physiology-related confounders, asking to collect more data right from the blood-draw; (iv) the expected differences in health and disease status at sampling, dictating the necessity to perform longitudinal profiling in as many phenotypes and individuals as possible; and (v) the impact of prior health history and use of medication, calling for need to collect about past and current events.

Beyond this, integrating proteomics with other omics data, such as genetics, has provided an even more comprehensive view of human health and lifted the potential of multi-modal biomarkers. These approaches can unravel complex disease mechanisms and improve diagnostic accuracy, while validating these (artificially intelligent) signatures in practice might remain difficult. My presentation will summarize recent advancements in proteomics biomarker discovery in blood-derived samples to exemplify how new concepts and large-scale analysis can provide more reliable insights into human health and disease pathogenesis.

Transcriptomics for biomarker discovery

Tadeja Režen[1]

[1]Centre for functional genomics and bio-chips, Institute of biochemistry and molecular genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
e-mail: tadeja.rezen@mf.uni-lj.si

Transcriptomics represents a collection of methods that allow the measurement of the expression of thousands of genes in different tissues and body fluids under different physiological and pathophysiological conditions. It has been extensively used for discovery of molecular gene signatures and RNA biomarkers. Transcriptomics can also be used in biomarker discovery at the genomic level, as genetic and epigenetic changes can be detected at the RNA level. Recent advances in technology have enabled analyses at the single-cell and spatial levels. However, the transcriptome is very dynamic and its role is to rapidly adapt and respond to different signals from the internal and external environment. Therefore, different pre-analytical and analytical factors are known to influence the transcriptome expression and depend on the source of the tissue and the type of RNA molecule analyzed.

The use of transcriptomics in biomarker discovery requires careful experimental design even before sample collection begins. The selection of the source tissue for RNA extraction, the type of RNA molecules analyzed, and the technology selected for RNA analysis must be consistent with the study objectives. In most studies, liquid biopsies, mainly blood-derived samples, have been used for RNA biomarker discovery. However, other body fluids, such as urine, saliva and cerebrospinal fluid, can and have been used for biomarker discovery. The extracellular vesicles found in body fluids are also a good source of RNA biomarkers. RNA biomarkers are versatile RNA molecules ranging from mRNA, long non-coding RNA, circular RNA and the most studied miRNA. Different types of RNA molecules require targeted selection of methods for sample collection, preparation and analysis. Several technologies are available for transcriptome analysis that can be easily tailored to a targeted approach. Our studies using transcriptomics for biomarker discovery will be represented.

Development of microRNA-based diagnostic tests utilizing biobanks and transcriptomic tools

Matthias Hackl[1], *Susanna Skalicky*[1], *Andreas Diendorfer*[1], *Marianne Pultar*[1], *Patrick Starlinger*[2]

[1] TAmiRNA GmbH, Vienna, Austria
[2] Mayo Clinic, Rochester, United States
e-mail: matthias.hackl@tamirna.com

MicroRNAs (miRNAs) are considered promising biomarker candidates based on their biological relevance, tissue-specificity, cross-species conservation, and stable presence in biofluids enabling minimal-invasive detection. Despite the high interest in miRNA biomarkers, few candidates have been successfully validated and implemented in diagnostic routines. Common technical challenges on the path to validation of miRNA biomarkers are pre-analytical variability, analytical bias, and bridging from discovery assays to targeted assay formats.

We and others have identified the liver as an organ with high potential for miRNA-based diagnostics due to its unique miRNA profile and the high levels of liver-specific miRNAs detectable in plasma. Liver cancer is associated with high mortality. Surgical removal of liver tumors (hepatectomy) improves patient outcomes such as overall survival (OS) but is associated with an increased risk of post-hepatectomy liver failure (PHLF). There is an urgent need for a preoperative test to predict the risk of PHLF, specifically as current markers are expensive, time consuming, and invasive.

We followed a systematic approach to NGS-based discovery and RT-qPCR-based validation of a liver-function biomarker to predict PHLF (hepatomiR®): 1) we assessed the impact of sample collection protocols on miRNA detection and selected platelet-poor citrate/CTAD plasma as the best matrix. 2) we applied an optimized small RNA-sequencing assay (miND®) to 48 plasma samples to select the lead miRNA biomarker candidates, miR-122, miR-151a, and miR-192. 3) we re-analyzed the samples by RT-qPCR to complete assay bridging and developed an algorithm that converts miRNA raw data into an actionable Probability-Score ("P-score").

After analytical validation, the test was applied to 86 patients with end-stage liver disease and preoperative plasma of 333 patients undergoing hepatic resection. Statistics were based on non-parametric tests, receiver operating characteristics (ROC) analysis, Cox-regression, and log-rank tests.

P-score showed moderate correlations with other liver function tests (GOT: r=0.528, GPT r=0.388, $p<0.001$), but a high predictive potential for PHLF with an area under the curve (AUC) of 0.774, which was superior to indocyanine green clearance (ICG) testing (PDR: AUC=0.569, R15: AUC=0.618), LiMAx (AUC=0.564), and HVPG (AUC=0.664). P-score was significantly associated with OS upon Cox-regression (hazard ratio [HR]=4.34, 95% confidence interval [CI]: 1.90-9.98, p=0.001), which remained significant in a multivariable model adjusting for age, sex, tumor entity, and extent of resection.

These data were used to prepare the technical documentation for the authorization of hepatomiR® as a CE-IVD test in Europe. The test can become vital for personalized treatment of patients subjected to hepatic resection and to reduce PHLF and postoperative mortality in this cohort.

How to avoid preanalytical bias and assure sample quality for biomarker discovery

Rainer Lehmann

Institute for Clinical Chemistry and Pathobiochemistry, Department of Diagnostic Laboratory Medicine, University Hospital Tuebingen, Germany
and
Department of Molecular Diabetology and Core Facility DZD Clinical Chemistry Laboratory, Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Centre Munich at the University of Tuebingen, German Center for Diabetes Research (DZD), Germany
e-mail: Rainer.Lehmann@med.uni-tuebingen.de

Abstract – Biobanking is one of the key elements for successful biomarker research in body fluids. Currently, omics analysis are the most promising and most applied tool for biomarker discovery, but neither sophisticated biobanking nor high-end, high resolution omics analyses can prevent disappointing outcomes, when the sample quality is poor. To avoid this issue, the processes of body fluid collection, handling and storage are in general tightly controlled by detailed protocols or strict standard operating procedures (SOP). But pre-analytical errors, either systematically or accidentally, cannot completely be avoided, particularly at the site of sample collection inside the hospitals. This talk will shed light on the preanalytical phase, specifically the pitfalls and unforeseen obstacles occurring during body fluid collection, handling and processing with a special focus on blood as sample material. Suggestions for the detection and prevention of potential preanalytical bias are provided. Aside, examples of effects on the metabolome and lipidome will be demonstrated, and recommendation to achieve high body fluid sample quality for biomarker discovery are provided. Furthermore, for already stored biobank samples a strategy will be discussed to assess whether or not the quality is acceptable for the intended use. Although body fluid collection is entitled as the easy part of complex biomedical studies, it can be a key step on the whole way via biobanking to omics analyses to successful biomarker discovery and finally to new therapeutics or treatment strategies.

Harnessing Machine Learning on Small Sample Sizes for Biomarker Discovery: An Overview

Marko Kokol

Faculty of Electrical Engineering and Computer Science, University of Maribor, Maribor, Slovenia
e-mail: marko@kokol.si

The field of biomarker discovery holds the potential for significant strides in diagnostic and prognostic advancements. A particular challenge in this realm is the frequent encounter with small datasets, which demands specialised machine learning (ML) strategies to extract meaningful insights.
This presentation delves into implementing ML methodologies, drawing from prior research on utilising ML methods on small sample sizes and concrete applications for biomarker discovery. Among others, we will present how using strategies such as dimension reduction, data augmentation, and judicious model selection enabled the achievement of relatively good models with AUC > 0.8 amidst data size limitations in the BioEndoCar project.
We will show how these methodologies can be extended to the context of endometriosis, explore the challenges and unveil promising approaches for biomarker identification, emphasising the significance of tailored ML strategies in overcoming data size constraints. The presentation highlights the implications and the encouraging prospects of employing ML in biomarker discovery within the endometriosis domain. Through this, we seek to foster a discussion on identifying the ML techniques conducive to enhanced diagnostic accuracy and prognostic value in healthcare scenarios, often characterised by limited dataset sizes.