EPIGENETICS

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HR-10000 Zagreb

Symposium
Book of Abstracts

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Book of Abstracts of the Symposium organized in the frame of the Installation Research Project „Epigenetic biomarkers of prostate cancer" **epiPro** HRZZ-UIP-2017-05-8138 financed by Croatian Science foundation (HRZZ) and co-financed by School of Medicine University of Zagreb (MEF-UniZg), by the Scientific Group for Research on Epigenetic Biomarkers (**epiMark**).

epiPro

Scientific Group for Research on Epigenetic Biomarkers

Editors
Nino Sinčić, Irena Abramović

Online

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Dear colleagues,

Welcome to the **Epigenetics and Male Reproductive Health Symposium.** We are pleased that our Symposium is being held at the Croatian Physicians Association, HR-10000 Zagreb.

Symposium is organized in the frame of the Installation Research Project „Epigenetic biomarkers of prostate cancer" epiPro HRZZ-UIP-2017-05-8138 financed by Croatian Science foundation (HRZZ) and co-financed by School of Medicine University of Zagreb (MEF-UniZg), by the Scientific Group for Research on Epigenetic Biomarkers (epiMark).

We are pleased that the Symposium is co-organized and supported by Croatian Society of Biochemistry and Molecular Biology, University of Zagreb Faculty of Pharmacy and Biochemistry, Croatian Society of Urology and Croatian Society of Pathology and Forensic Medicine.

The Program is focused on challenges related to reproductive health in men: testicular tumors and prostate cancer. Latest developments in the field of epigenetic biomarkers, especially in liquid biopsies, with the potential of translation into clinical practice will be presented.

The Program is headed by outstanding lecturers, most of which are collaborators on epiMark related scientific projects epiSem (IP-HRZZ), epiPro (UIP-HRZZ) and epiNonSem (Center of Excellence for Reproductive and Regenerative Medicine - CERRM). In addition, eminent EpiMark collaborating scientific groups will be introduced and their work presented.

After a bit longer time with scarce personal contacts, we hope to provide an excellent opportunity to exchange ideas and experiences with colleagues, establish new acquaintances, and renew old ones.

Enjoy a successful and stimulating symposium!

Organizers

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Organizer
Scientific Group for Research on Epigenetic Biomarkers

Co-organized and supported by
Croatian Society of Biochemistry and Molecular Biology (HDBMB)
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Croatian Physicians Association
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Symposium Program

09:30–10:00	**Registration**
10:00–10:05	**Opening**
10:05–10:25	**5 years of epiMark** *Assoc. Prof. Nino Sincic, PhD, Department of Biology, University of Zagreb School of Medicine*
10:25–10:45	**Male infertility: can we prevent it?** *Prof. Davor Ježek, PhD, Department of Histology and Embryology, University of Zagreb School of Medicine*
10:45–11:00	**Epidemiology of cancers of male genital organs in Croatia** *Assist. Prof. Mario Šekerija, PhD, Division for Epidemiology and Prevention of Noncommunicable Chronic Diseases, Croatian Institute of Public Health*
	Testicular tumours session
11:00–11:10	**Clinical challenges in the approach to patients with testicular cancer** *Prof. Igor Tomašković, PhD,Clinic for Urology, Sestre milosrdnice University Hospital Center*
11:10–11:20	**Epigenetic mechanisms in development and progression of testicular cancer: an answer to unsolved questions?** *Assist. Prof. Stela Bulimbašić, PhD,Clinical Department of Pathology and Cytology, University Hospital Center Zagreb*
11:20–11:30	**Biology of testicular germ cell tumors from an epigenetic perspective** *Assist. Dajana Krsnik, Department of Biology, University of Zagreb School of Medicine*
11:30–11:45	**Cell-Free DNA Methylation of RASSF1A and PRSS21 in Patients with Testicular Nonseminomas** *Sen. Assist. Jure Krasić, PhD, Croatian Institute for Brain Research, University of Zagreb School of Medicine*
11:45–12:00	**Novel seminoma biomarkers from liquid biopsies** *Sen. Assist. Dora Raos, PhD, Department of Biology, University of Zagreb School of Medicine*
12:00–12:15	**General discussion**
12:15–13:15	**Lunch**
	Prostate cancer session
13:15–13:25	**Challenges in diagnostics and follow up of patients with prostate cancer** *Tomislav Kuliš, PhD, Department of Urology, University Hospital Centre Zagreb*
13:25–13:35	**Prostate cancer – diagnosis, therapy, prognosis – challenges in the new era of pathology** *Assist. Prof. Monika Ulamec, PhD, Ljudevit Jurak Clinical Department of Pathology and Cytology, Sestre milosrdnice University Hospital Center*
13:35–13:45	**Genomic instability of prostate cancer cells: from germline mutation to epigenetic reprogramming** *Assoc. Prof. Ana Katušić Bojanac, PhD, Department of Biology, University of Zagreb School of Medicine*
13:45–14:00	**Potential of cfDNA methylation as minimally-invasive prostate cancer biomarker** *Lucija Škara, PhD, Epigenetic Biomarkers of Prostate Cancer project*
14:00–14:15	**Epigenetic biomarkers of prostate cancer in liquid biopsies: novel approach in discriminating it from benign prostatic hyperplasia** *Sen. Assist. Irena Abramović, PhD, Department of Biology, University of Zagreb School of Medicine*
14:15–14:30	**General discussion**
14:30–15:00	**Coffee break**
	Epigenetic research collaboration session
15:00–15:15	**New perspectives for cholinesterase-based ligands research** *Maja Katalinić, PhD, Biochemistry and Organic Analytical Chemistry Unit, Institute for Medical Research and Occupational Health*
15:15–15:30	**Biomarker potential of N-glycosylation or Sweet side of biomarkers** *Prof. Olga Gornik Kljajić, PhD, Department of Biochemistry and Molecular Biology, University of Zagreb Faculty of Pharmacy and Biochemistry*
15:30–15:45	**Genetic and epigenetic markers as indicators of aggressiveness of differentiated thyroid cancer** *Assoc. Prof. Ivan Šamija, PhD, Department of Oncology and Nuclear Medicine, Sestre milosrdnice University Hospital Center*
15:45–16:00	**Effects of diving with compressed air on markers of function and integrity of the nervous and cardiovascular system, as well as hormonal and immune status and erythropoiesis** *Assist. Prof. Marko Žarak, PhD, Clinical Department of Laboratory Diagnostics, Dubrava University Hospital*
16:00–16:15	**The role of regulatory genomics in human disease** *Anja Barešić, PhD, Division of Electronics, Laboratory for Machine Learning & Knowledge Representation, Ruđer Bošković Institute*
16:15–16:30	**Closing**

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Male infertility: can we prevent it?

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In 2017, a very sharp decline in sperm concentration and total sperm count in North America, Europe, Australia/New Zealand was reported. At that time, too few studies had been published in South/Central America, Asia and Africa to conclude on trends in those continents. A new literature meta-analysis published in 2022 on global trends in total sperm concentration and total sperm count showed a similar drift in the latter continents. The economic and societal burden of male infertility is now widely recognized, as is the unequal burden of male infertility, which falls most heavily on low-income countries. Strong evidence links reduced sperm count and concentration to all-cause mortality and morbidity increases. Causes of sperm decline are numerous but sometimes not well understood. Testicular dysgenesis syndrome, alcohol abuse, estrogen-like substances, obesity, sexually transmitted diseases, prostatitis & prostate tumours, marihuana abuse, lack of sleep and wrong lifestyle are some factors contributing to male infertility. On the contrary, good semen parameters are connected to male longevity, low cardiovascular risk, short period of hospital treatment and healthy offspring.

Scientific Centre of Excellence for Reproductive and Regenerative Medicine at the School of Medicine University of Zagreb, together with collateral projects of Croatian Scientific Foundation (HRZZ) like epiSEM and epiPRO, has significantly influenced awareness of reproductive health in the Croatian population, employing advanced diagnostic and therapeutic tools to address male infertility.

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Epidemiology of cancers of male genital organs in Croatia

Mario Šekerija[1]

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Malignant neoplasms of male genital organs (C60-C63 according to the ICD-10) are a heterogenous group of cancers comprising three most common sites: penis (C60), prostate (C61) and testis (C62) as well as other and unspecified male genital organs (C63). These cancers vary significantly by risk factors and epidemiology, with average age of onset in Croatia in 2020 being 67 years for penile cancer, 71 years for prostate cancer and 37 years of age for testicular cancer. Since 2016, prostate cancer is the most common newly diagnosed cancer in males in Croatia, and it is constantly the third most common cause of cancer-related death in males (behind only lung and colorectal cancer). There were 2299 new cases of prostate cancer in Croatia in 2020, 200 cases of testicular cancer, 52 cases of penile cancer and 3 cases having C63 diagnosis. 805 men died of prostate cancer in Croatia in 2021. Prostate cancer is also the most common malignant disease in men in most countries of the world. The incidence of prostate cancer has increased considerably in most highly developed countries of the world in recent decades, attributable primarily to more frequent screening for prostate cancer by measuring PSA levels. However, in recent years there has been a stabilization or decline in incidence in many developed countries. This lecture will present the most recent available epidemiological data for Croatia for this group of cancers, the relevant trends and international comparisons.

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Clinical challenges in the approach to patients with testicular cancer

Igor Tomašković[1], Miroslav Tomić[1]

[1] Department of Urology, University Hospital Center "Sestre milosrdnice", Zagreb, Croatia

Although testicular cancers account for only 1 % of all cancers in men and 5 % of all urological malignancies, they are the most common cancers in young men between 15 - 35 years of age. Because of that testicular cancer have important medical and socio-economic importance. The incidence of testicular cancer varies between ethnic groups with a higher incidence in highly developed countries and a lower incidence in less developed countries, but with a stable increase in incidence in the last decade. It is estimated that one in 100 men will be diagnosed with a testicular cancer annually in the three European countries with the highest risk rate, including Croatia. The Republic of Croatia, compared to other European countries, has a medium increase in frequency, but with a constant and rapid increase in frequency and probably with the highest rate of increase in the world. Mortality of testicular cancers in the world shows the opposite trend in relation to incidence, with higher mortality in less and medium developed countries, than in highly developed countries. Clinical challenges in the approach to patient with testicular cancer is that any man with a solid testicular mass, should be considered and treated as cancer until proven otherwise. Radical inguinal orchiectomy is still the gold standard and the treatment of choice in patients with testis mass. Testis-sparing surgery can be option and performed in highly selected patients. Individual and multidisciplinary approach to patients with testicular cancer is also standard of care in our institution, as well as conducting new research in better diagnosis of new patients and in follow up patients with testicular cancer.

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Epigenetic mechanisms in development and progression of testicular cancer: an answer to unsolved questions?

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Testicular cancer accounts for 1% of all cancers in males, and in more than 90% of cases originates from germ cells. Even though the overall incidence of germ cell tumours (GCTs) is low, the fact that the majority of patients are young men, makes this group of tumors important and interesting for study. Due to better understanding of pathogenesis, introduction of newer diagnostic methods, as well as development of modern oncologic protocols, overall prognosis of patients with GCTs improved during the last few decades. However, some unsolved questions still remain. Around 15% of patients with GCTs, mostly from the high risk category, are resistant to conventional chemotherapeutic protocols and will succumb to a disease. For such patients, novel markers for better risk stratification are needed. On the other part of the spectrum, there is also a need for additional markers for primary detection of males with higher risk for development of GCTs. There is a growing body of evidence documenting the importance of epigenetic mechanisms in the development and progression of GCTs. Epigenetic modifications are also described as potential causes of resistance to standard chemotherapeutic agents. Therefore, better understanding of epigenetic modifications might improve not just primary diagnostics, but also can be used as a potential target for development of epigenetic therapeutic agents and treatment of patients with GCTs. Properly prepared samples from primary tumors as well as residual tumors after chemotherapy can be used for additional testing. Therefore, enrollment of pathologists into a multidisciplinary team responsible for management of patients with GCTs is important.

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Biology of testicular germ cell tumors from an epigenetic perspective

Dajana Krsnik[1]

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Testicular cancers are the most common malignancy in adolescents and young men, and their incidence has risen over the past two decades in Western countries. Despite the high cure rate of >90% in patients with testicular cancer, some patients become refractory to chemotherapy or have a late relapse. An improved understanding of the molecular determinants underlying tumor sensitivity and resistance may lead to the development of new therapeutic strategies for these patients. Most testicular cancers are testicular germ cell tumors (TGCTs), which are classified as seminomas (SGCTs) and non-seminomatous testicular germ cell tumors (NSGCTs). During their development, primordial germ cells (PGCs) undergo epigenetic modifications and any disturbances in their pattern might lead to cancer development. Both inherited genetic and environmental risk factors emerge as important contributors to TGCT susceptibility. Current data support the model that human TGCTs are rarely caused by somatic driver mutations but arise through failure to control the latent developmental potential of their cells of origin, resulting in their reprogramming. This elegantly explains the role of both genetic susceptibility as well as environmental factors in the pathogenesis, referred to as 'genvironment'. This lecture provides insight into the biology of TGCTs and epigenetic mechanisms associated with their susceptibility, initiation, progression, and response to chemotherapy. Another important purpose is to highlight the recent investigations regarding the identification and development of epigenetic biomarkers as powerful tools for the diagnostic, prognostic, and especially for the epigenetic-based treatment of resistant disease.

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Cell-Free DNA Methylation of RASSF1A and PRSS21 in Patients with Nonseminomatous Testicular Germ Cell Tumors

Jure Krasić[2,3,9], Lucija Škara[1,2,3], Ana Katušić Bojanac[1,3], Marijana Ćorić[3,4], Monika Ulamec[2,3,5,6], Davor Ježek[3,7], Tomislav Kuliš[2,3,8], Nino Sinčić[1,2,3]

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Testicular germ cell tumors (TGCT) are the most common malignancy among young males. TGCT are subdivided into seminomas and nonseminomas (NSE). Confirmation of a TGCT diagnosis always includes radical orchidectomy, making a reliable noninvasive diagnostic method required for earlier diagnosis. Analysis of circulating cell-free DNA (cfDNA) from body liquids is a perspective noninvasive procedure for diagnosis of oncological patients. Genomic DNA methylation (gDNA) of RASSF1A and PRSS21 genes in the tissue of TGCT has been shown as a potential biomarker. The aim of this study was to assess the potential of cfDNA methylation of genes RASSF1A and PRSS21 in the blood and ejaculate as biomarkers for patients with NSE. Pyrosequencing was used to analyze cfDNA methylation of genes RASSF1A and PRSS21 in blood and ejaculate of patients with a confirmed diagnosis of NSE and healthy donors with no prior diagnosis of TGCT as well as in gDNA from the tumor tissue and the surrounding healthy tissue of patients with NSE. RASSF1A was hypermethylated in gDNA of tumor tissue. No difference in gDNA methylation levels was found in PRSS21. In NSE patients hypermethylated RASSF1A was detected in cfDNA isolated from the blood, while hypermethylated PRSS21 was detected in cfDNA isolated from the ejaculate. Methylation of RASSF1A and PRSS21 cfDNA shows higher sensitivity and specificity than the currently used clinical biomarkers. This study confirms the potential of methylation of RASSF1A in cfDNA from blood and discovers the methylation of PRSS21 in cfDNA from the ejaculate as diagnostic biomarkers of patients with NSE.

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Novel seminoma biomarkers from liquid biopsies

Dora Raos[1,2,3], Davor Oršolić[4], Jure Krasić[1,2,3], Irena Abramović[1,2,3], Miroslav Tomić[3,5], Ana Katušić Bojanac[1,3], Anja Barešić[4], Monika Ulamec[2,3,6,7], Davor Ježek[3,8], Nino Sinčić[1,2,3]

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Seminoma (SE) is the most frequent type of testicular tumor. Diagnosis of seminoma is a multistep process that also includes checking tumor biomarkers from blood. However, these biomarkers are not specific for SE and to conclude definite diagnosis of seminoma immunohistochemical analysis is needed, which requires the removal of a whole or partial testicle. Therefore, there is a need for novel, noninvasive biomarkers. Liquid biopsy represents source of novel biomarkers for non-invasive cancer diagnostics and patient management. cfDNA methylation and CNV represent the most prominent ones. Therefore, cfDNA methylation of six genes and CNV of four genes were assessed in tumor tissue, as well as in blood and ejaculate from patient and healthy volunteers' samples. CfDNA methylation was assessed by pyrosequencing, while for the CNV analysis, digital droplet PCR was used. Regarding cfDNA methylation, detailed analysis revealed specific CpGs as possible seminoma biomarkers, but receiver operating characteristic curve analysis showed modest diagnostic performance. In an analysis of panels of statistically significant CpGs, two DNA methylation panels emerged as potential seminoma screening panels, one in blood CpG8/CpG9/CpG10 (KITLG) and the other in seminal plasma CpG1(MAGEC2)/CpG1(OCT3/4). In case of CNV, CNV hotspot in gDNA from SE tissue was detected for the first time in all analyzed genes, and for two genes, NANOG and KITLG it was reflected in cfDNA from seminal plasma. Although clinical value is yet to be determined, presented data emphasize the potential use of CNV, as well as cfDNA methylation as a potential SE biomarker from a liquid biopsy.

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Challenges in diagnostics and follow-up of patients with prostate cancer

Tomislav Kuliš[1,2], Jerko Anđelić[1], Luka Penezić[1], Toni Zekulić[1], Hrvoje Saić[1], Ilija Jurić[1], Tvrtko Hudolin[1], Željko Kaštelan[1,2]

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Prostate cancer is one of the most common cancers in men. Early diagnosis of the disease results in better clinical outcomes. Application of serum level of Prostate specific antigen (PSA) facilitates early diagnosis and today it is widely used as a screening tool. However, more than half of patients have a negative prostate biopsy when we use only PSA. The development of imaging technologies, like micro-ultrasound and multiparametric magnetic resonance help to achieve better sensitivity and specificity and it furthermore provides the possibility for targeted prostate biopsies. Once prostate cancer is diagnosed, it is important to determine the malignant potential and the stage of the disease. For staging, we usually use standard imaging methods i.e., bone scintigraphy and computerized tomography while grading of the disease is determined according to the Gleason score. The development of radionuclide tracers provided an opportunity to use PET/CT scan with choline or prostate-specific membrane antigen (PSMA) that are more sensitive than standard methods for staging the disease, and this can even visualise micrometastases in lymph nodes of normal size. Furthermore, there are challenges to predicting clinical outcomes after the initial treatment and the challenge of dividing patients into risk groups. Using just the level of PSA and Gleason score as parameters for risk and later for follow-up proved to be usually inadequate. We are always in a race to find better, more sensitive and more specific tests to drive diagnostics, prognostics and follow-up. Applying genetics, epigenetics and liquid biopsy is promising to facilitate this race.

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Prostate cancer – diagnosis, therapy, prognosis – challenges in the new era of pathology

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Incidence of prostate cancer is increasing, which is a significant burden on the healthcare system, and it has led to different approaches in diagnosis and treatment. The treatment of prostate cancer is becoming more complex for early and late stages of the disease, which requires additional efforts of the pathologist, in establishing a diagnosis, grading, etermining the stage, as well as in the expected information related to the biology of the tumor, predictive factors and guidance of therapy. Due to the well-known limitations of prostate-specific antigen (PSA) and biopsy as a diagnostic tool, new emerging biomarkers, as well as new methods indicating different abilities to detect cancer and predict its behavior are used. Only a few such markers and technologies have been approved in the routine workflow, but the potential and future possibilities are obvious. In this presentation I'm addressing pathologist issues and challenges in the current state of prostate cancer diagnostic tools, such as artificial intelligence-based software in the processing of the tissue, as well as in the diagnostic algorithm. There is a challenge of new and emerging platforms for diagnostic and prognostic biomarkers and different technics to increase predictive and diagnostic accuracy, as well as guided personalized therapy.

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Genomic instability of prostate cancer cells: from germline mutation to epigenetic reprogramming

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Prostate cancer (PCa) is the fifth leading cause of cancer-related death among men nowadays. Extensive clinical and research studies based on sequencing methods have been performed to describe the phenotypic changes occurring during malignant cell transformation in the prostate. Several mechanisms involved in prostate tumorigenesis such as de novo epigenetic changes, alternative splicing, and the presence of somatic gene mutations, are possible novel biomarkers with potential clinical utility. However, their emergence must be correlated with the germline mutations, especially those regarding the genome stability, as for example, germline pathogenic variants in DNA repair genes have been reported in up to 10 % of men with advanced or metastatic prostate cancer. Here, novel epigenetic changes and germline gene variants are critically analyzed according to their capability to specify new molecular subtypes of prostate cancer and classify patients according to the most appropriate and effective therapy to increase the efficacy of treatment and reduce unnecessary interventions that have no effect.

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Potential of cfDNA methylation as minimally-invasive prostate cancer biomarker

Lucija Škara[10], Tonći Vodopić[3], Ivan Pezelj[4], Irena Abramović[1,2,5], Borna Vrhovec[4], Alen Vrtarić[6], Davor Tomas[3,7], Stela Bulimbašić[7,8], Tomislav Kulišić[1,2,9], Nino Sinčić[1,2,5], Monika Ulamec[1,2,3,7]

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High prevalence and mortality of prostate cancer (PCa) are well-known global health issues. Prostate-specific antigen (PSA) shows low specificity in screening and diagnostics, leading to unnecessary biopsies and health costs. In need of a new biomarker, we assessed potential of RASSF1 and CAV1 gene methylation of cfDNA from blood and seminal plasma as a biomarker to distinguish between PCa and benign prostatic hyperplasia (BPH). Forty patients with PCa and forty patients with BPH were included in the study. RASSF1 methylation was similar between groups. Although CAV1 methylation in blood plasma did not differ between PCa and BPH patients, methylation in seminal plasma showed better PCa biomarker performances than tPSA (AUC 0.63 vs. AUC 0.52). Discrimination of BPH and Gleason grade group 1 PCa patients from patients with higher Gleason grade groups revealed very good performance as well (AUC 0.72). CAV1 methylation is a useful biomarker with potential for further seminal plasma cfDNA research, but its diagnostic accuracy should be improved, as well as general knowledge about cfDNA in seminal plasma. CAV1 has an important role in the regulation of cellular cholesterol homeostasis. Cholesterol metabolism in PCa is deregulated and impacts cancer progression. An overview of how commonly deregulated signalling pathways in PCa are linked with cholesterol homeostasis regulation will be presented.

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Epigenetic biomarkers of prostate cancer in liquid biopsies: novel approach in discriminating it from benign prostatic hyperplasia

Irena Abramović[1,2,3], Dora Raos[1,2,3], Lucija Škara[1,2,3], Ivan Pezelj[4], Tomislav Kulиš[2,3,5], Stela Bulimbašić[6,7], Monika Ulamec[2,3,7,8], Nino Sinčić[1,2,3]

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Prostate cancer (PCa) is a malignancy with the highest prevalence among men worldwide. The clinical challenge is to differentiate localized PCa from benign prostate hyperplasia (BPH) due to the low specificity of routinely used biomarker PSA. Research is directed towards liquid biopsies as a source of potential PCa biomarkers, especially microRNAs (miRNAs) and DNA methylation. Men scheduled for prostate biopsy due to clinical suspicion of PCa were included in the study. Blood and ejaculate samples were obtained before prostate biopsy, and processed into the blood and seminal plasma, followed by miRNA and cfDNA isolation. According to the histopathology report, patients were subsequently divided into two groups i.e. 65 with early-stage PCa, and 58 with BPH. The analysis of the absolute expression of miR-375-3p, miR-182-5p, miR-21-5p, and miR-148a-3p was performed by digital droplet PCR, while cfDNA methylation by pyrosequencing. The higher expression of miR-182-5p and miR-375-3p in the blood plasma of PCa patients was statistically significant as compared to BPH. Their combination achieved a specificity of 90.2 % for predicting positive or negative biopsy results, while a PSA cut-off of 4 µg/L had specificity of 1.7 %. Statistically significant higher cfDNA methylation of the LGALS3 gene in seminal plasma of BPH than in PCa patients was detected by pyrosequencing. ROC curve analysis showed that it could distinguish PCa and BPH patients with 56.4% sensitivity and 70.4% specificity, while PSA did not differ between the two patient groups. CfDNA methylation of APC and GSTP1 genes displayed no discriminating power in liquid biopsies.

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New perspectives for cholinesterase-based ligands research

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Compounds interacting reversibly with acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8), two important enzymes in the neurotransmission, have been designed and synthesised for the last 70 years. The main purpose of such compounds is either to reversibly inhibit these enzymes as a therapy for several nerve-related disorders and conditions (like Alzheimer's disease and myasthenia gravis), or to reactivate their activity upon covalent inhibition by deadly organophosphorus (OP) agents. Combination of the specific structural requirements needed to fit the cholinesterases' active site, together with the motifs ensuring desired biochemical properties, created an outstanding compounds and scaffolds library unfortunately unexplored beyond the main scope of action. During the studies within the project CellToxTargets financed by the Croatian Science Foundation, we have observed that selected cholinesterase-based ligands have a cytotoxic effect on different cell types. Results showed that some compounds caused time-dependent toxicity accompanied by increased induction of ROS and mitochondrial membrane potential dysfunction, which leads to disruption of oxidative phosphorylation chain that maintains adenosine triphosphate (ATP) energy levels. Also, some compounds activated specific caspase 8 and 9 enzymes responsible for initiating the apoptosis process, either through the extrinsic or intrinsic pathway. This led us to conclude that such effects come either from interactions with one of the cell-surface receptors or by a direct binding to some intracellular target, perhaps on mitochondria, upon entering the cell by any transport mechanism. According to the results, compounds that were toxic to neuroblastoma (SH-SY5Y) and hepatocytoma (HepG2) cells were also toxic to breast cancer cells (MDA-MB-231) and prostate cancer cells (PC3). This may suggest that their putative mechanism of action is the same, which indicates potential antitumour activity of these compounds worth further investigation.

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EPIGENETICS
& MALE REPRODUCTIVE HEALTH

Biomarker potential of N-glycosylation or *Sweet side of biomarkers*

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Although neglected for many years due to their structural complexity and the demand for highly advanced laboratory technologies, oligosaccharide structures attached to human proteins, called glycans, are now rapidly emerging as potential biomarkers of different pathological conditions. Glycans are also one of the main regulators of antibody effector function and other features of the immune system. Changes in N-glycosylation have been described in various diseases as associated with disease risk, course, and therapy outcome. For instance, glycans show great potential in diabetes differentiation as well as risk assessment of future diabetes development. At the Faculty of Pharmacy and Biochemistry we possess skills and facilities to perform high-throughput analysis of glycans from different biological samples and we are constantly assessing their biomarker potential. Currently, we are conducting studies on N-glycosylation in type 1 diabetes where we revealed changes in glycosylation in children at the onset of the disease, as well as identified responsible genes. We are also collaborating with the group of Prof. Sinčić from the School of Medicine University of Zagreb in evaluating biomarker potential of glycans in prostate cancer.

EPIGENETICS
& MALE REPRODUCTIVE HEALTH

Genetic and epigenetic markers as indicators of aggressiveness of differentiated thyroid cancer

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In this presentation research project 'Genetic and epigenetic markers as indicators of aggressiveness of differentiated thyroid cancer' founded by Croatian Science Foundation and collaboration with Scientific Group for Research on Epigenetic Biomarkers (epiMark) will be presented.
Thyroid carcinoma, despite its predominantly indolent course, represents a significant burden on the health system with its high incidence. Recent publications emphasize the need for advancement in molecular diagnostics for the purpose of detecting a new markers of prognostic significance for the aggressiveness of the disease that is characterized by recurrence or persistence of the disease, the development of regional and distant metastases or the resistance on today's accepted radioiodine therapy. This project seeks to explore the benefit of BRAF and TERT mutation analysis and the epigenetic changes in the TERT promoter in patients in relation to the aggressiveness of differentiated thyroid carcinoma. The field of epigenetic modifications in the disease pathophysiology is poorly investigated and the analysis of epigenetic changes is one of the important advantages of this project. By confirming the usefulness of these markers, routine analysis of said markers in patients with differentiated thyroid carcinoma would be introduced in clinical practice in order to provide early selection of patients requiring further treatment, diagnosis and monitoring and possibly for selection of patients suitable for therapy with B-RAF inhibitors.

EPIGENETICS
& MALE REPRODUCTIVE HEALTH

Effects of diving with compressed air on markers of function and integrity of the nervous and cardiovascular system, as well as hormonal and immune status and erythropoiesis

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The project "Effects of diving with compressed air on markers of function and integrity of the nervous and cardiovascular system as well as hormonal and immune status and erythropoiesis" is the product of the collaboration of 3 teaching and healthcare institutions and brings together employees of the: (1) University of Zagreb Faculty of Pharmacy and Biochemistry (prof. Jerka Dumić and assist. prof. Sandra Šupraha Goreta), (2) Dubrovnik General Hospital (Antonija Perović, Ph.D. and Marina Njire Bratičević), (3) and Dubrava University Hospital (assist. prof. Marko Žarak). The outcomes of the project so far are: 2 doctoral dissertations, 8 scientific papers and 6 poster abstracts, and another doctoral dissertation is currently being prepared. The results of this project were also presented several times at international congresses where the project participants were invited to give lectures. In one of the last phases of the research, a successful collaboration was achieved with the employees of the epiMark research group, especially Irena Abramović, Ph.D. and prof. Nino Sinčić. Their technology for the detection and quantification of cell-free DNA greatly helped us in understanding (patho)physiological mechanisms that occur during and after diving with compressed air (SCUBA diving). As a result of a successful collaboration a scientific paper has been recently published in the prestigious international journal Frontiers in Cardiovascular Medicine. The results were also presented as a poster abstract at The Biochemistry Global Summit (25th IUBMB, 46th FEBS, 15th PABMB Congress) which was held in Lisbon, Portugal last year.

EPIGENETICS
& MALE REPRODUCTIVE HEALTH

The role of regulatory genomics in human disease

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Our group focuses on utilization of available (epi)genomics data, often combined with genomes of other vertebrate genomes to elucidate novel mechanisms of human disease. While a lot is known on the phenotypic effects of variants in the coding part of the genome, the remaining 98% of genomic sequence, termed "genomic dark matter" bears a wealth of information on human variation, many of which are disease-associated but difficult to interpret in terms of function, mechanism of action and ultimately its causality to investigated phenotype. We use the genomic regulatory block model, defining large domains of genome maintained in conserved synteny often across entire vertebrate evolution in order to keep a key gene and its entire regulatory landscape in the same genomic vicinity. By combining it with information on common disease-associated variants, we provide a superior inference of putative target genes through which regulatory variants exert their molecular phenotypes leading to complex diseases like schizophrenia, obesity, developmental diseases, etc. We apply various machine learning methods to model evolution of human genome and combinatorial landscape of long-range regulation of transcription. Other applications of our work span across interpretation of various epigenomic signals using advanced statistics, to provide mechanistic insights into various disease. In the case of testicular seminoma, we helped identify candidate CpGs bearing predictive power, alone and in combination, as cell-free DNA biomarkers in blood and seminal plasma of seminoma patients as well as prostate cancer patients.