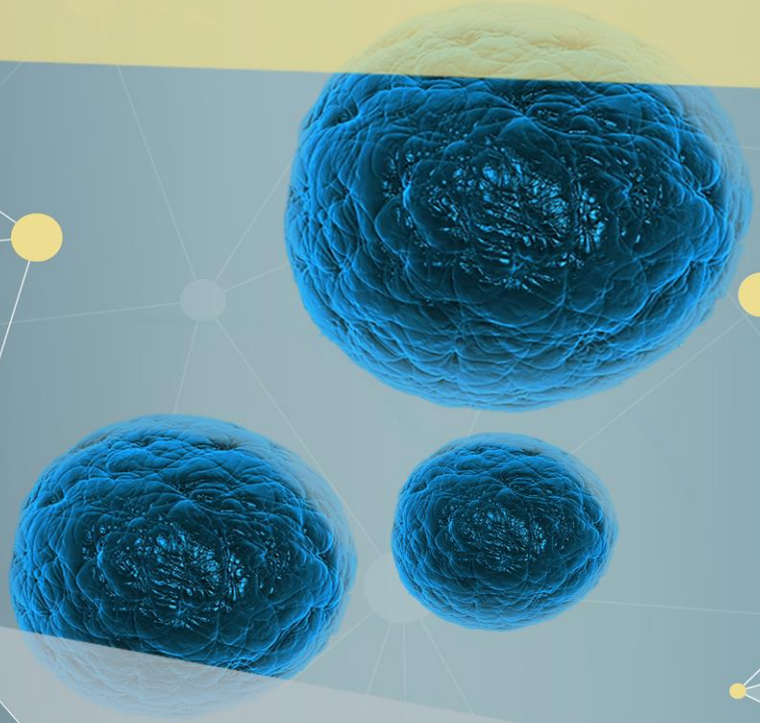
Serbian Association for Cancer Research

5th CONGRESS OF SDIR: TRANSLATIONAL POTENTIAL OF CANCER RESEARCH IN SERBIA

ABSTRACT BOOK



Virtual event
December 3

2021

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5th CONGRESS OF THE SERBIAN ASSOCIATION FOR CANCER RESEARCH

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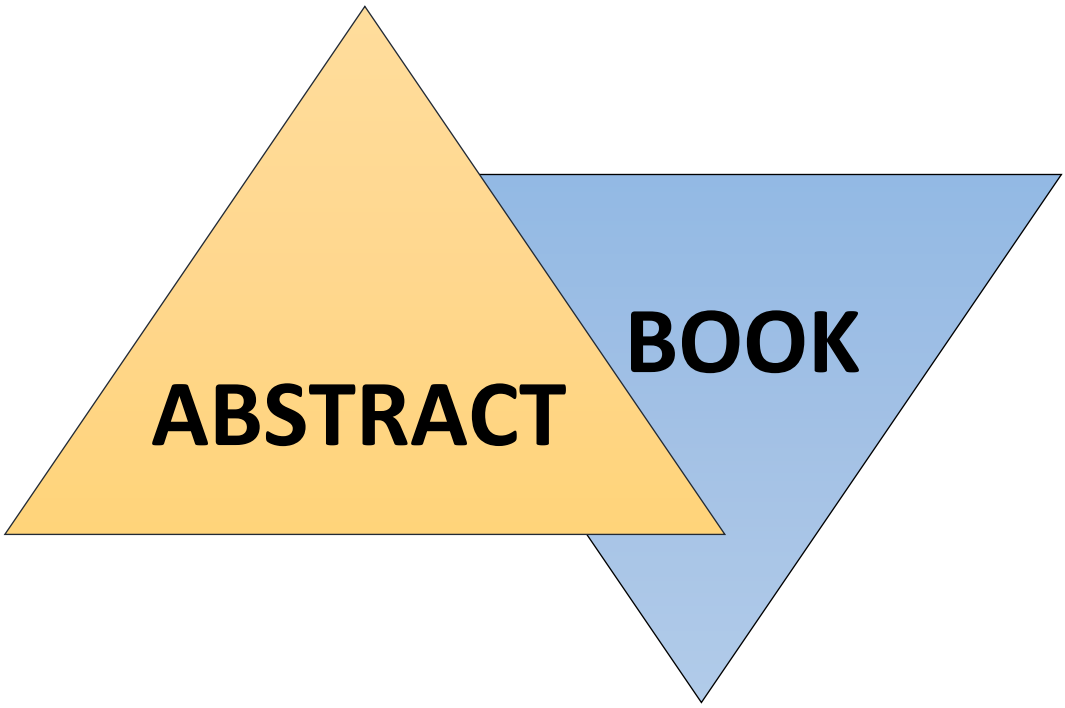

TRANSLATIONAL POTENTIAL OF CANCER RESEARCH IN SERBIA

SDIR – 5

Virtual event, December 3, 2021

THE FIFTH CONGRESS OF THE SERBIAN ASSOCIATION FOR CANCER RESEARCH

with international participation

"TRANSLATIONAL POTENTIAL OF CANCER RESEARCH IN SERBIA "

December 3, 2021, Virtual event

Serbian Association for Cancer Research (SDIR) is a member of the European Association for Cancer Research (EACR).

President of SDIR-5 Congress

dr sc. med. Mirjana Branković-Magić

THE FIFTH CONGRESS OF THE SERBIAN ASSOCIATION FOR CANCER RESEARCH
with international participation "Translational potential of cancer research in Serbia"Virtual event,

December 3, 2021

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LETTER OF WELCOME

Dear colleagues,

We are very pleased to welcome you to the 5th Congress of the Serbian Association for Cancer Research (SDIR) with international participation "Translational potential of cancer research in Serbia" to be held on December 3, 2021 as a virtual event.

During the congress, lectures will be delivered by a distinguished Serbian and international researchers, that will cover the following topics:

- *Liquid biopsies in lung cancer*
- *Advances in solid tumor research*
- *Cancer and metabolism*
- *Radiobiology*
- *Imaging in cancer*

We are pleased to say that our fifth congress is actively supported by the European Association for Cancer Research.

We are delighted to welcome you!

Kind regards,

dr sc. med. Mirjana Branković-Magić,president of SDIR

dr sc. Milena Čavić, president of the Organizing Committee

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The Serbian Association for Cancer Research would like to acknowledge the ongoing support of Ministry of Science, Education and Technological Development of the Republic of Serbia, European Association for Cancer Research, Institute of Oncology and Radiology of Serbia and Institute for Biological Research "Siniša Stanković".

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PROGRAMME

03.12.2021. Virtual event 09.00 – 17.30

09.00 – 09.05	**Congress welcome and opening.** SDIR President Mirjana Branković-Magić
09.05 – 09.30	EACR Plenary lecture – EACR President Caroline Dive. **Liquid biopsies in lung cancer**. *University of Manchester, Manchester, UK*
09.30 - 09.40	Discussion
09.40 – 11.05	**Session: Advances in solid tumor research**
	Chairs: Caroline Dive and Milena Čavić
09.40 – 10.00	Remond J.A. Fijneman. **ctDNA biomarker detection in patients with colorectal cancer**. *The Netherlands Cancer Institute, Amsterdam, Netherlands*
10.00 – 10.20	Gunes Esendagli. **Mesenchymal properties and immune checkpoint pathways in small cell lung cancer (SCLC) stem cells**. *Hacettepe University Cancer Institute, Ankara, Turkey*
10.20 – 10.50	**Short talks selected from SDIR member PIs of The Program for Excellent Projects of Young Researchers (PROMIS) of the Science Fund of the Republic of Serbia**
10.20 – 10.30	Miljana Tanić. **Tracking systemic therapy resistance of lung and colorectal cancer through targeted NGS analysis of genetic and epigenetic variants in liquid biopsies**. *Institute of Oncology and Radiology of Serbia, Serbia*
10.30 – 10.40	Aleksandra Nikolić. **Cancer biosensors based on gene regulatory elements**. *Institute of Molecular Genetics and Genetic Engineering, Serbia*
10.40 – 10.50	Jelena Grahovac. **Drug repurposing in pancreatic ductal adenocarcinoma**. *Institute of Oncology and Radiology of Serbia, Serbia*
10.50 – 11.05	Discussion
11.05 – 11.15	Break
11.15 – 12.40	**Session: Cancer and metabolism**
	Chair: Milica Pešić
11.15 – 11.35	Liang Li. **An antibody drug conjugate-like agent DTLL sensitizes gemcitabine efficacy in pancreatic cancer based on SMAD4 profiles**. *Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China*

11.35 – 11.55 Ljubica Harhaji-Trajković. **Dual targeting of energy metabolism and lysosomes as an anticancer strategy; it is not all about autophagy**. *Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia*

11.55 – 12.23 **Short talks selected from abstracts**

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12.02 – 12.09 Cristina P.R. Xavier. **Chitinase 3-like-1 (CHI3L1) as a potential therapeutic target for pancreatic cancer**. *Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Institute of Molecular Pathology and Immunology, University of Porto, Portugal*

12.09 – 12.16 Jovana Jagodić. **Elemental profile of glioblastomas – analysis of blood, cerebrospinal fluid and brain tissue**. *Faculty of Chemistry, Belgrade, Serbia*

12.16 – 12.23 Stefana Stojanović. **Hsa-miR-222 identifies high-risk PTC patients with classical variant architecture**. *Institute for the Application of Nuclear Energy — INEP, University of Belgrade, Belgrade, Serbia*

12.23 – 12.40 Discussion

12.40 – 12.45 Break

12.45 – 14.30 **Session: Radiobiology**

Chairs: Marina Nikitović and Ivana Matić

12.45 – 13.00 Irina Besu Žižak. **The role of IL6 in radiotherapy-induced toxicity.** *Institute of Oncology and Radiology of Serbia, Serbia*

13.00 – 13.15 Bojana Ilić. **Cellular senescence in ionizing radiation**. *Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia, Serbia*

13.15 – 13.30 Jadranka Antić. **Effects of ionizing radiation on DNA methylation: from experimental biology to clinical applications**. *Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Serbia*

13.30 – 13.45 Sercan Ergün. **The interrelationship between FYN and miR-128/193a-5p/494 in Imatinib resistance in prostate cancer**. *Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey*

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13.52 – 13.59 Jelena Stanić. **Radiation-induced lymphocyte apoptosis as a possible biological predictor of radiotherapy toxicity in prostate cancer patients**. *Institute of Oncology and Radiology of Serbia, Serbia*

13.59 – 14.06 Aleksandar Stepanović. **Can miRNA expression patterns predict radiotoxicity in patients with glioblastoma?** *Institute of Oncology and Radiology of Serbia, Serbia*

14.06 – 14.13 Bojana Kožik. **Potential predictive role of K-ras gene mutation and BCL2 protein expression status in locally advanced rectal cancers treated with neoadjuvant chemoradiotherapy**. *Vinča Institute of Nuclear Sciences, National Institute of Republic of Serbia, University of Belgrade, Serbia*

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Chair: Jelena Grahovac

15.15 – 15.35 Bojana Gligorijević. **Real-time microscopy of invasive cancer cells in the tumor microenvironment context**. *Temple University, USA*

15.35 – 15.55 Jelena Stanisavljević. **Defining and imaging colon cancer heterogeneity.** *Institute of Photonic Sciences, Barcelona Institute for Science and Technology, Spain*

15.55 – 16.15 Giorgio Seano**. Vessel co-option and resistance to therapy in glioblastoma**. *Institut Curie Research Center, Centre Universitaire, France*

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16.22 – 16.29 F. Koutsougianni**. Siramesine, a non-opioid σ2 receptor agonist as a potential agent for the development of novel targeted treatments for pancreatic cancer**. *University of Thessaly, Larisa, Greece*

16.29 – 16.36 Batuhan Mert Kalkan. **The role of Nek2 on centrosome clustering in cancer cells with extra centrosomes**. *Koç University, Graduate School of Health Sciences, Istanbul, Turkey*

16.36 – 16.43 Tijana Martinov. **Sox2-targeted T cell therapy for treating multiple myeloma**. *Fred Hutchinson Cancer Research Center, Seattle, United States of America*

16.43 – 17.00 Discussion

17.00 – 17.30 **Closing remarks and best poster awards.** SDIR President Mirjana Branković-Magić

Contents

EACR Plenary lecture

Liquid biopsies in lung cancer

Professor Caroline Dive CBE, FBPhS, FMedSci.
Institute Director and Director of the Cancer Biomarker Centre, CRUK Manchester Institute, University of Manchester, UK.
Co-Director, CRUK Lung Cancer Centre of Excellence
President, European Association for Cancer Research

In my presentation I will exemplify the multiple uses of circulating tumour cells (CTC) and ctDNA in lung cancer research. CTC number using the 'gold standard' CellSearch platform (that identifies epithelial cells) before treatment in Non Small Cell Lung Cancers (NSCLC) and Small Cell Lung Cancers (SCLC) is prognostic for overall and progression free survival. Cellsearch CTCs are scarce in NSCLC but prevalent in SCLC where they can be used as a pharmacodynamic biomarker for treatment response and to measure predictive biomarkers. In this regard, we developed a single SCLC CTC copy number alteration (CNA) classifier that predicts chemotherapy response that can be used alongside ctDNA analysis for therapy monitoring. Obtaining clinical tumour biopsies to study metastasis in SCLC is particularly challenging. We derived mouse models from patients CTCs (we termed CDX) to explore biology and test new therapies. Our biobank of >45 CDX models, some generated pre- and post-patient therapy, allows analysis of tumour heterogeneity, therapy resistance and biology of progressing disease. CDX cells metastasise to the same organs in mice as commonly observed in patients. Using labelled CDX cells, we developed new workflows to study SCLC metastasis to the liver and the brain. SCLC cells exhibit plasticity and can undergo vasculogenic mimicry (VM) adopting endothelial cell behaviours to form blood vessels, a process that worsens prognosis and supports metastasis. ctDNA is also prevalent in SCLC and I will describe routine monitoring of SCLC with ctDNA CNA and a new liquid biopsy based on ctDNA methylation that increases sensitivity for detection and allows us to subtype a patient's SCLC.

In our NSCLC CTC studies within the UK TRACERx consortium, we showed that CTCs in the pulmonary draining vein of stage I-IIIa patients at surgery with curative intent predicts recurrence risk. In a case study we identified a lethal subclone that gave rise to metastasis 10 months later in the pulmonary vein at surgery. I will also briefly highlight discuss the potential for CTCs within a multimodal liquid biopsy for the earlier detection of NSCLC.

Session: Advances in solid tumor research

LECTURES

ctDNA biomarker detection in patients with colorectal cancer

Remond J.A. Fijneman[1]
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Background: Biomarkers reflect disease biology and have the potential to improve diagnosis, prognosis, prediction and monitoring of treatment response. Cell-free circulating tumor DNA (ctDNA) is an example of a very promising biomarker. Our studies aim to investigate the clinical utility of ctDNA testing to improve disease management of patients with colorectal cancer (CRC). Patients and methods: We make use of multicenter clinical trials and clinical studies organized within the Prospective Dutch CRC cohort PLCRC (www.PLCRC.nl) to arrange informed consent and collect clinical data, tissue and blood samples. Industrial grade ctDNA biomarker assays are developed and provided by private partners. In the COIN project, ctDNA study data is used for early health technology assessment towards scenarios for reimbursement and approval by authorities. Results: We succeeded to establish the efficient logistics for multicenter collection of clinical samples and data. So far, more than 1500 CRC patients were accrued with stage II (PLCRC-MEDOCC and -CrEATE), stage III (PLCRC-PROVENC3) and stage IV (PLCRC-ORCA, CAIRO5, CAIRO6) disease. We compared diagnostic strategies how to apply liquid biopsy ctDNA testing as a predictive biomarker to determine eligibility for anti-EGFR therapy in patients with liver metastases. We currently investigate ctDNA prognostic value in post-surgery blood samples to better stratify stage II and stage III colon cancer patients for adjuvant chemotherapy. Conclusions: Successful translation of disease biology into new diagnostic applications requires solving scientific, ethical, legal, societal, financial and regulatory problems. Coordinated and collaborative (inter)national initiatives to establish a clinical framework for biomarker validation are instrumental to pave the road towards clinical implementation.
Keywords: colorectal cancer; biomarkers; cell-free circulating tumor DNA

Mesenchymal properties and immune checkpoint pathways in small cell lung cancer (SCLC) stem cells

Gunes Esendagli[1]
[1]*Hacettepe University Cancer Institute, Ankara, Turkey*

Small cell lung cancer (SCLC) is an aggressive tumor type with early dissemination and distant metastasis capacity. Even though optimal chemotherapy responses are observed initially in many patients, therapy resistance is almost inevitable. Accordingly, SCLC has been regarded as an archetype for cancer stem cell (CSC) dynamics. Even though the immune regulatory capacities of CSCs are well-acknowledged, their influence on the anti-tumor immunity in SCLC remains to be better elucidated. To determine the immune-modulatory influence of CSC in SCLC, this study focused on the characterization of CD44+CD90+ CSC-like subpopulations in SCLC. These cells displayed mesenchymal properties, differentiated into different lineages and further contributed to CD8+ cytotoxic T lymphocytes (CTL) responses. The interaction between CD44+CD90+ CSC-like cells and T cells led to the upregulation of checkpoint molecules PD-1, CTLA-4, TIM-3, and LAG3. In the patient-derived lymph nodes, CD44+ SCLC metastases were also observed with T cells expressing PD-1, TIM-3, or LAG3. Proliferation and IFN-γ expression capacity of TIM-3 and LAG3 co-expressing CTLs are adversely affected over long-time co-culture with CD44+CD90+ CSC-like cells. Moreover, especially through IFN-γ secreted by the T cells, the CSC-like SCLC cells highly expressed PD-L1 and PD-L2. Upon a second encounter with immune-experienced, IFN-γ-stimulated CSC-like SCLC cells, both cytotoxic and proliferation capacities of T cells were hampered. In conclusion, our data provide evidence for the superior potential of the SCLC cells with stem-like and mesenchymal properties to gain immune regulatory capacities and cope with cytotoxic T cell responses. With their high metastatic and immune-modulatory assets, the CSC subpopulation in SCLC may serve as a preferential target for checkpoint blockade immunotherapy.

Short talks

Targeted BS-seq methods for the study of epigenetic landscape in solid tumors and liquid biopsies

Miljana Tanić[1,2]
[1]*Institute for Oncology and Radiology of Serbia, Experimental Oncology Department, Belgrade, Serbia;*
[2]*University College London, UCL Cancer Institute, London, United Kingdom;*

DNA methylation is a key epigenetic modification in the regulation of cell fate and differentiation, and its analysis is gaining increasing importance in both basic and clinical research. Targeted Bisulfite Sequencing (TBS) has become the method of choice for the cost-effective, targeted analysis of the human methylome at base-pair resolution. Several commercial platforms based on hybridization capture (HC) or reduced representation bilufite sequencig (RRBS) are available. We performed a comprahensive benchmarking study to identify advantages and limitations of available technologies. To study epigenetic landsacepe of non-small-cell lung cancer (NSCLC) within the TRACERx study we applied RRBS and developed new bioinformatic tools for deconvolution of pure methylome and delineation of intratumoral heterogeneity. This allowed us to discover a novel epigenetic mechanism of immune evasion in NSCLC. To detect cancer-specific DNA methylation marks from cfDNA we are developing a novel method based for ultra-senstitve capture and library prep. This method is being applied to monitor the acquision of resistance to systemic therapy in NSCLC and CRC in a prospective longitudinal study at the Institute of Oncology and Radiology of Serbia.

Cancer biosensors based on gene regulatory elements

Aleksandra Nikolić[1]
[1]*Institut of Molecular Genetics and Genetic Engineering, University of Belgrade*

Cancer diagnostics still relies on imaging techniques and traditional tumor biomarkers, which keep failing in detection of early stage tumors and premalignant lesions. Biosensor technologies have emerged as a promising tool for noninvasive and effective early detection of malignant disease, which remains a major unmet need in cancer management. There is growing evidence on aberrant use of multiple promoters in malignant cell and also of the importance of the promoter choice and its precedence over the gene's overall level of transcriptional activity. Transcriptional regulation of gene expression is affected by various intracellular and extracellular factors, which results in context-specific activity of alternative gene promoters, which could serve as sensors of malignancy. The aim of this research is to identify gene regulatory elements differentially active in malignant and non-malignant cells. Gene regulatory elements of interest are selected using open access databases and bioinformatics tools. Structural and functional analysis of gene regulatory elements serves to identify those with potential to be developed into biosensors. The prototype of cancer biosensor combines selected gene regulatory elements as biorecognition components with widely used genes for fluorescent proteins as signal transducers. The diagnostic sensitivity of biosensor prototype is tested using organoid cultures and clinical samples from patients with colorectal cancer, an excellent model of a multistage tumor progression. The major expected output is a molecular diagnostic tool developed for colorectal cancer with potential for further improvement and application across different types of malignant diseases.

Key words: biosensor, cancer, gene regulation, molecular diagnostics
Acknowledgement: This research was supported by the Science Fund of the Republic of Serbia, PROMIS, #6052315, SENSOGENE.

Drug repurposing in pancreatic ductal adenocarcinoma

Jelena Grahovac[1], Marijana Pavlović[1], Marija Ostojić[1], Kristina Živić[1], Daniel Galun[2,3], Tatjana Srdić-Rajić[1]
[1]*Institute for Oncology and Radiology of Serbia, Belgrade, Serbia*
[2]*School of Medicine, University of Belgrade, Belgrade, Serbia*
[3] *First Surgical Clinic, Clinical Center of Serbia, Belgrade, Serbia*

The main objective of the REPANCAN project funded by the Science Fund of the Republic of Serbia was to establish a new dedicated research team, that will address the urgent unmet need for new treatment options for patients with pancreatic ductal adenocarcinoma (PDAC). PDAC is one of the most lethal types of cancer in the world, with a 5-year survival rate of less than 10% and an increasing incidence rate. Research on PDAC is limited and progress in patient survival has not been made in the last 40 years. To circumvent the high costs and long development times for new oncological drugs, the REPANCAN project uses the drug repurposing concept to establish a novel use for a class of nischarin (NISCH) receptor agonists for treatment of PDAC. NISCH receptor itself has been shown to be a tumor suppressor in breast cancer and its agonists hold promise for targeting several aspects of the PDAC pathology: cell invasiveness, survival, metabolism, and the microenvironment. The REPANCAN project integrates the basic bioinformatic, molecular and cellular research with the retrospective patient study. In so far, we have examined the expression in PDAC patient tumor samples and in vitro model systems and found that NISCH is expressed in both tumor cells and stroma and that is a prognostic marker. We examined the effects of NISCH agonists on cell viability and migratory potential and found that it can limit PDAC cell survival and invasive potential. Once completed, the REPANCAN project results will lay a ground for clinical evaluation of NISCH agonists in PDAC patients.
Keywords: Pancreatic cancer, drug repurposing, nischarin

Session: Cancer and metabolism

LECTURES

An antibody drug conjugate-like agent DTLL sensitizes gemcitabine efficacy in pancreatic cancer based on SMAD4 profiles

Liang Li[1]

[1]*Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, China*

Chemoresistance to gemcitabine limits its clinical implementation for pancreatic ductal adenocarcinoma (PDAC). We previously generated an antibody drug conjugate (ADC)-like agent, EGFR/HER2 dual-targeting ligand-based lidamycin (DTLL), and studied the effect of DTLL combined with gemcitabine and the potential role of SMAD4 in the chemoresistance of PDAC. On the basis of SMAD4 profiles in *in vitro* and *in vivo* models, we investigated the antitumor effects of DTLL, gemcitabine and their combination, followed with mechanistic characterization. The results suggested that DTLL combination treatment with gemcitabine significantly repressed tumors with remarkably enhanced efficacy as compared to gemcitabine or DTLL alone given in either SMAD4-deficient/gemcitabine-resistant or SMAD4-sufficient/gemcitabinesensitive cell line derived xenograft (CDX) and patient derived xenograft (PDX) tumors, respectively. Functional studies indicated that SMAD4 genetic status is responsible for SMAD4 protein level which determines different cellular susceptibility of PDAC. R100T mutation contributes to loss of SMAD4 protein and function with rapid protein degradation, leading to resistance to gemcitabine in PDAC cells. Moreover, DTLL significantly altered the protein half-life time and level of mutant and wild-type SMAD4 by inhibiting protein degradation at different velocities and distinctly changing the interaction of SMAD4 with TRIM33. Mechanism studies implied that DTLL combinational treatment might not only prevent from neoplastic proliferation via blockage of ATK/mTOR signaling and antiapoptotic proteins (BCL-2 and MCL1) mediated by impaired NF-kB function in SMAD4-sufficient/gemcitabine-sensitive PDAC cells, but also restore the bioactivity of SMAD4 as a tumor suppressor to trigger its downstream NF-kB-regulated signaling of cell apoptosis in SMAD4- deficient/gemcitabine-resistant tumors. In conclusion, SMAD4 is the key central mediator of not only the occurrence and development but also susceptibility in PDAC. DTLL sensitized gemcitabine efficacy via distinct action mechanisms based on SMAD4 profiles in SMAD4-sufficient/gemcitabine-sensitive and SMAD4-deficient/- resistant PDAC, respectively. Our findings provide insight into a rational SMAD4-directed precision treatment strategy and reveal a promising DTLL combination therapy to overcome chemoresistance in gemcitabine-resistant PDAC.

Key words: SMAD4; Pancreatic ductal adenocarcinoma (PDAC), Gemcitabine, Chemoresistance, Dual-targeting ligand-based lidamycin (DTLL), Antibody drug conjugate (ADC)

Dual targeting of energy metabolism and lysosomes as an anticancer strategy; It is not all about autophagy

Ljubica Harhaji-Trajkovic[1], Milica Kosic[2], Verica Paunovic[2], Biljana Ristic[2], Mihajlo Bosnjak[2], Nevena Zogovic[1], Milos Mandic[2], Gordana Tovilovic[1], Kristina Janjetovic[1], Vladimir Trajkovic[2]

[1]*Department of Neurophysiology, Institute for Biological Research "Sinisa Stankovic"-National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia*

[2]*Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia*

Background: Intensive proliferation of tumor cells consumes a lot of energy. In nutrient deficiency substrates for energy metabolism are obtained by lysosomal degradation of unnecessary/dysfunctional intracellular organelles/molecules in the process of autophagy. Leakage of enlarged unstable lysosomes, which characterize tumor cells, causes cell death. We investigated antitumor effect of combined targeting of lysosomes/autophagy and energy metabolism. Material and Methods: Toxicity against U251 human glioma and B16 mouse melanoma cells was measured by viability tests. Type/mechanisms of cell death were determined by flow cytometry, immunoblot, fluorescent/electron microscopy and confirmed by appropriate genetic/pharmacological inhibitors. Therapeutic potential was estimated in B16 melanoma-bearing C57Bl/6 mice. Results: In the first study, lysosomotropic autophagy inhibitor chloroquine (CQ) rapidly killed tumor cells incubated in the absence of serum. CQ-induced lysosomal destabilization triggered: oxidative stress, mitochondrial depolarization, and mixed apoptosis/necrosis of serum-deprived cells. In the second study, lysosomal detergent N-dodecylimidazole (NDI) synergized in antitumor activity with the glycolytic inhibitor 2-deoxy-D-glucose (2DG). NDI-triggered release of lysosomal enzymes into the cytoplasm caused mitochondrial damage and blocked oxidative phosphorylation, which synergized with 2DG-mediated glycolysis block in ATP reduction, oxidative stress, and necrosis. Interestingly, although both serum deprivation and 2DG stimulated autophagy, CQ- and NDI-induced autophagy suppression was irrelevant for their cytotoxicity. Importantly, CQ+food restriction and 2DG+NDI reduced melanoma growth *in vivo*. Conclusion: Autophagy independent antitumor effects of combined energy metabolism suppression and lysosomal destabilization might be exploited in cancer therapy.

Keywords: Autophagy; Combined Therapy; Energy Metabolism; Lysosomes.

Short talks

Genes for competing endogenous RNAs as targets of transcription factors GLI in melanoma cell lines

Nikolina Piteša[1], Nenad Bartoniček[2], Matea Kurtović[1], Vesna Musani[1], Diana Trnski[1], Petar Ozretić[1], Maja Sabol[1]

[1]Laboratory for Hereditary Cancer, Department of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia

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Hedgehog-GLI (HH-GLI) signaling is a conserved signaling pathway reported to be aberrantly activated in various human cancers, including melanoma. Beside its canonical signaling, HH-GLI can also be activated noncanonically through interactions with other signaling pathways like MAPK. Both result in activation of GLI transcription factors. Our previous studies demonstrated that BRAF and NRAS mutated melanoma cell lines have different response following HH-GLI inhibition, which indicates that HH-GLI potentially has a different role in BRAF and NRAS mutated melanoma. To identify GLI transcriptional targets, we performed ChIP-Seq. Firstly, 14 collected melanoma cell lines were sequenced and divided into categories based on their BRAF and NRAS mutation background. Three cell lines with the strongest basal GLI protein expression were selected (A375 BRAFmut, CHL-1 wt and MEL224 NRASmut). Selected target genes were analyzed in silico and validated by qPCR. ChIP-seq analysis revealed 603 potential GLI targets of which 30% have GLI1, 66% GLI2 and 21% GLI3 binding sites. Only 3.8% were mutual for all three GLI proteins. Besides protein coding genes, 20 miRNAs and 29 lncRNAs were identified. miRNA analysis revealed that 50% of identified miRNAs regulate genes related to MAPK signaling. Validation included selected 23 protein coding genes, 9 miRNAs and 3 lncRNAs involved in regulation of MAPK signaling pathway. We validated 4 miRNAs and 2 lncRNAs and constructed a potential GLI-lncRNA-miRNA interactome which will further be examined. Future analysis can potentially bring new insights into miRNA-lncRNA regulatory networks involved in HH-GLI and MAPK interplay.

Chitinase 3-like-1 (CHI3L1) as a potential therapeutic target for pancreatic cancer

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The crosstalk between cancer cells and the tumor microenvironment plays an important role in resistance to therapy. Several studies showed that tumor-associated macrophages, which are highly abundant in the stroma of pancreatic ductal adenocarcinoma (PDAC), influence therapy response. Our previous work demonstrated that extracellular vesicles (EVs) shed by polarized human macrophages decreased BxPC3 PDAC cellular sensitivity to gemcitabine [1]. Proteomic analysis of those EVs identified Chitinase 3-like 1 (CHI3L1) as the most abundant protein [1] and a possible therapeutic target for PDAC treatment. The aim of the present work was to: i) validate CHI3L1 as a novel therapeutic target for PDAC treatment, and 2) identify in silico inhibitors of CHI3L1 that could be used as adjuvants in PDAC treatment.

First, we confirmed that recombinant CHI3L1 caused resistance to gemcitabine and to gemcitabine plus paclitaxel, in PDAC BxPC3 cells (determined by the SRB assay). Interestingly, CHI3L1 was found (by immunohistochemistry analysis) to be expressed in the stroma of PDAC tumor patient samples and to be associated with the presence of macrophages [1]. Then, a structure-based virtual screening was performed, to retrieve known molecules from the DrugBank database that could potentially bind to CHI3L1. One of the identified ligands was pentoxyfilline, a drug inhibitor of CHI3L1. Studies in PDAC cell lines confirmed that pentoxyfilline reverted the CHI3L1-induced gemcitabine resistance [1]. Other top ranked compounds from the molecular docking study are being tested for their potential as chemosensitizers in PDAC cells. One of them is darifenacin, an FDA-approved drug to treat urinary incontinence. Darifenacin increased the sensitivity of PDAC cell lines to gemcitabine treatment.

This work highlights the relevance of CHI3L1 as a therapeutic target for PDAC treatment and the possibility of repurposing drugs, which are inhibitors of CHI3L1, as chemosensitizers to enhance response of PDAC to conventional therapy.

References: [1] Xavier CPR, et al. Cancer Letters 2021, 501, 210-223.

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Elemental profile of glioblastomas – analysis of blood, cerebrospinal fluid and brain tissue

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Background: Glioblastomas are the most common and aggressive type of malignant brain tumors (MBTs) that affect people worldwide. Prolonged exposure to trace elements can contribute to a higher risk of MBTs. Thus, the primary aims of this study were to examine whether trace elements have a role in the pathogenesis of MBTs and if any of examined elements/element ratios could be utilized as a blood marker in MBTs diagnostic evaluation. Data were compared to control groups (CGs). **Material and Methods**: The study involved 70 MBT patients and 70 healthy blood donors. After centrifugation of whole blood, serum was isolated from cell fraction (CF). During a surgical shunt procedure, cerebrospinal fluid (CSF) was obtained, and samples of healthy brain tissue (HBT) and malignant brain tissue (MBT) were acquired after surgery. Microwave digestion was utilized to decompose clinical samples, and ICP-MS was used to determine their concentration. **Results and Conclusion:** For the first time, this study demonstrated that elemental profiles of MBT considerably changed when compared to suitable controls. Higher levels of Mn, Se, and Pb could have a role in the pathogenesis of MBTs. However, increased U content generated the most evident changes in all studied types of samples, demonstrating its tremendous relevance as a strong brain discriminator of the presence/absence of MBTs. Also, the U/Se ratio content could be a suitable blood marker in the diagnosis of MBTs. These findings could help to improve the understanding of the poorly known pathogenesis of MBTs.
Keywords: Glioblastoma; Malignant brain tumor; Trace elements.

Hsa-miR-222 identifies high-risk PTC patients with classical variant architecture

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Background: Finding novel tumor markers useful to recognize high-risk thyroid carcinoma patients is of great importance. We evaluated hsa-microRNA-222 (miR-222) for differential diagnosis of thyroid neoplastic lesions and assessed its prognostic usefulness. Material and Methods: MiR-222 levels were determined in 77 cases of papillary thyroid carcinomas (PTC) of diverse histological variants, 12 cases of follicular thyroid adenoma (FTA), and 99 matched nonmalignant thyroid epithelial tissues (NMT) using RT-qPCR. The results were evaluated in comparison with clinicopathological features of PTC. Results: Relative miR-222 expression is higher in PTC than in FTA and NMT ($p<0.05$). Compared to the levels in paired NMT, miR-222 is up-regulated in PTC, while being down-regulated in FTA (median 2.22 [0.71–8.81] vs. 0.40 [0.09–2.07], $p<0.05$). Althought increased expression of miR-222 could be used as sensitive (76.6%) and specific (75.0%) marker for discriminating PTC from FTA (cut off=0.66, AUC=0.747, $p<0.01$), miR-222 expression depends on PTC subtype. Only PTCs with areas of classical variant architecture tend to have high values of miR-222 relative expression, while folicular variant of PTC and selected rare PTC variants (Warthin-like, tall cell, solid, clear cell, oxyphilic) tend to have lower values ($p<0.05$). Higher miR-222 expression in PTC associate with the presence of metastasis in the regional lymph nodes, the presence of extrathyroidal invasion, degree of tumor infiltration through the gland, higher pT and pTNM stage ($p<0.05$). Conclusions: MiR-222 is a useful marker for PTC detection, particularly its classical variant, and can help in risk stratification of these patients.

Keywords: Diagnosis, Micro RNA, Papillary Thyroid Carcinoma, Prognosis, Tumor Marker

Session: Radiobiology

LECTURES

The role of IL-6 in radiotherapy-induced toxicity

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Background: Prostate cancer is the second most frequently occurring cancer and the fifth leading cause of cancer death among males worldwide. In approximately 50% of all cancer patients, radiotherapy is a widely used anticancer treatment, often used in combination with surgery and chemotherapy. Exposure to radiation initiates a programmed cellular response to promote tissue repair, which involves the induction and regulation of proinflammatory cytokines. Cytokine expression is associated with radiation-related tissue damage and inflammation, and may serve as an indicator of cell and tissue toxicity during prostate cancer radiotherapy. The aim of our researches was to evaluate the possible clinical value of the associations between clinical, physical, and biological factors, and risk for development of acute radiotoxicity in patients with prostate cancer. Patients and Methods: The study involved forty-four patients treated with three-dimensional conformal radiotherapy. The concentrations of IL-1β, IL-2, IL-6, IFN-γ and TGF-β1 were assessed before radiotherapy, after 5th, 15th, 25th radiotherapy fractions and 1 month after the end of radiotherapy. Results: Analysis of circulating cytokine levels during radiotherapy showed that increased serum concentrations of IL-6 were significantly associated with higher grade of acute genitourinary toxicity. Increased level of IL-6 during the radiotherapy was significantly associated with higher grade of acute genitourinary toxicity across treatment. Conclusion: The prediction of individual radiosensitivity might be based on determination of cytokine levels changes at specific time during course of radiotherapy which is only a part of the complex multifactorial puzzle influencing the radiotoxicity.
Keywords: cancer, cytokine, immunology

Cellular senescence in ionizing radiation

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Radiotherapy alone or in combination with surgery or chemotherapy is a treatment of choice for many malignancies, but its efficacy is limited by the dose that can be safely administered as well as the high incidence of tumor radioresistance, recurrence and metastasis. Ionizing radiation acts by causing DNA damage that triggers a complex response in tumor and non-tumor cells and tissues. Senescence induced by ionizing radiation has been considered as a tumor-suppressive mechanism that prevents the proliferation of genetically unstable and damaged cells. However, an increasing number of studies have shown that chronic accumulation of senescent cells can, paradoxically, promote cellular transformation, tumor regrowth and metastasis. Furthermore, there is evidence that suggests that tumor cells can escape senescence and recover proliferative capacity. These antitumorigenic and protumorigenic effects are mediated through acquired senescence-associated secretory phenotype (SASP) and undoubtedly depend on cellular and tissue context. Senescent cells induced by radiation may alter neighbouring microenvironment via autocrine and paracrine signaling and contribute to additional health problems, such as fibrosis and cardiovascular disease. Progress in the understanding of the molecular pathways that regulate senescence in cancer can lead to the discovery of molecular biomarkers and targets for anticancer therapy. Senotherapeutic agents, including senomorphics (small molecules that are able to modulate senescent cells activity by reducing or inhibiting SASP generation) and senolytics (agents that induce apoptosis of senescent cells) have the potential to enhance the efficacy of radiotherapy and/or reduce its side-effects. The clinical application of senotherapeutics as adjuvants in cancer therapy needs further investigation.
Keywords: Radiotherapy; Senescence; Senescence Associated Secretory Phenotype;

Effects of Ionizing Radiation on DNA Methylation: From Experimental Biology to Clinical Applications

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Ionizing radiation (IR) is a ubiquitous environmental factor and a clinically important diagnostic and treatment modality with genotoxic and epigenotoxic capabilities. DNA methylation is a key epigenetic mechanism in the regulation of the proper expression of genetic information. Alterations in DNA methylation may result in changes in gene expression or reactivation of repetitive elements (REs), and may lead to genomic instability and the development of pathological states. Knowledge regarding the effects of IR on DNA methylation is constantly growing. Radiation can cause changes in global DNA methylation and the extent of radiation-induced alterations in DNA methylation is tissue-dependent. Radiation-induced alterations in DNA methylation may differ between species and even among strains of the same species. DNA hypermethylation-induced silencing of tumor-suppressor genes and hypomethylation-induced activation of oncogenes have been described in almost all human cancers and are considered driving mechanisms of carcinogenesis. Alterations in the DNA methylation status of REs often lead to their reactivation and retrotransposition, and are documented in human cancers as well as in response to environmental stressors. In vitro studies reported mostly sex-specific bystander effect – the loss of genomic DNA methylation in both irradiated and bystander regions. The phenomenon of IR-induced alterations in DNA methylation is undeniable, however our understanding on the functional outcomes is very limited. Epigenetic modifications are not limited to DNA only, histone proteins can also undergo those modifications. Recently, of particular interest is the possibility of dietary modulation of the DNA methylation patterns, which may potentially be a promissing direction in radiation oncology. Key words: DNA methylation; Genomic instabillity; Ionizing radiation

The Interrelationship between FYN and miR-128/193a-5p/494 in Imatinib Resistance in Prostate Cancer

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Background: C-KIT is a receptor tyrosine kinase with oncogenic properties overexpressed in PCa cases. Through the use of an alternative promoter, a truncated c-KIT protein (tr-KIT) of 30-50 kDa is generated, lacking the extracellular and transmembrane domain. Tr-KIT promotes the formation of a multi-molecular complex composed by FYN, PLCγ1 and SAM68. Imatinib blocks the activity of full-length c-KIT but has no effect on tr-KIT. LNCaP is the human PCa cell line that shows tr-KIT overexpression and PC3 does not show tr-KIT overexpression. Also, LNCaP and PC3 are regarded as relatively resistant to both radiation-induced clonogenic death and apoptosis. miR-128/193a-5p/494 are miRNAs targeting FYN, PLCγ1 and SAM68 combinatorily and they are related with radiosensitivity in different types of cancer. The question of the study is that: can miR-128/193a-5p/494 be related with imatinib resistance in PCa? Material and Methods: LNCaP and PC3 cells were treated with imatinib in IC50 doses. Before and after imatinib administration, RNA was isolated and cDNA conversion was performed. By qPCR analysis, expression changes of tr-KIT specific pathway elements and miR-128/193a-5p/494 analyzed before and after imatinib administration.
Results: After imatinib administration, miR-128/193a-5p/494 were overexpressed statistically significantly in LNCaP cells while they were downregulated statistically significantly in PC3 cells ($p<0.05$). Also, FYN was upregulated in LNCaP cells ($p<0.05$) but there was no change in PC3 after imatinib administration.
Conclusion: In LNCaP cells having tr-KIT acitivity, especially upregulation of FYN may sponge miR-128/193a-5p/494 and downregulate their transcriptional activity. So, miR-128/193a-5p/494 may have critical role in imatinib resistance via tr-KIT pathway.

Keywords: Prostate cancer, imatinib resistance, truncated KIT (tr-KIT), FYN, miRNA sponging
Funding: This study has been financed by Scientific Research Projects Commission of Ondokuz Mayıs University (Project number: PYO.TIP.1902.21.001).

Short talks

Gene expression kinetics and pathway analysis of skin fibroblasts irradiated *in vitro*

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Background: Fibroblasts are considered an important factor in the development of radiation-induced subcutaneous fibrosis. The aim was to validate a previous gene expression study [1] and extend the kinetics to 1-6 days after irradiation. Material and Methods: RNA was isolated from the early-passage skin fibroblast strains (GS3, GS4, GS5), 1-6 days after X-irradiation with 4Gy (6MV) and analyzed on Breakthrough 20k human expression microarrays. Biostatistical and bioinformatical analyses were performed with R and JMP Genomics statistical software. Validation of selected genes and timepoints was performed in independent experiments with quantitative real-time PCR (qPCR) and Western blotting. Results: Principal and variant component analysis of the transcriptome pattern showed 42.2% dependence on time-point while 25.7% was influenced by the biological background and 32.1% by residual factors. After adjustment for biological background, Hierarchical Clustering analysis identified two subgroups of irradiated samples: early (1-3 days) and late (4-6 days) after irradiation. After Two-Way ANOVA analysis between three groups (non-irradiated, early and late), 3196 statistically significant differentially expressed genes (DEGs) were identified. 991 genes were early DEGs and 2252 genes were late DEGs relative to unirradiated controls, while 2013 DEGs were differentially regulated between the late and early timepoints. Pathway analysis was performed for each consecutive time point. Radiation-induced downregulation of cell-cycle regulatory processes and upregulation of ECM regulation processes confirmed the previous findings [1]. Conclusion: The present study validates and extends findings from the previous study using a different microarray platform [1]. Ref. [1]: Herskind *et al.*, Front Cell Dev Biol 9:539893 (2021).

Keywords: fibroblasts, gene expression, pathway analysis, radiotherapy, skin fibrosis

Radiation-induced lymphocyte apoptosis as a possible biological predictor of radiotherapy toxicity in prostate cancer patients

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Background: Localized prostate cancer (PC) can be treated with different therapeutic modalities that may have a similar outcome but different toxicity profiles. Individual differences in radiation-induced toxicity, even with the use of advanced radiotherapy (RT) techniques, such as intensity-modulated radiotherapy (IMRT), are observed. Radiation-induced apoptosis of T lymphocytes could be used to identify radiosensitive patients before RT. The aim of ongoing study is to investigate the possible correlations between the levels of CD4+ and CD8+ T lymphocyte apoptosis and RT-induced acute and late adverse reactions in patients with PC. Patients and methods: We have started to recruit patients with localized PC, treated with IMRT, at the Institute of Oncology and Radiology of Serbia, since May 2021. Peripheral blood samples are collected before RT. The samples are irradiated with 8 Gy (1Gy/min). Non-irradiated blood samples are used as control samples. The percentages of CD4+ and CD8+ T lymphocytes in apoptosis are determined by flow cytometry. Results: Irradiation of peripheral blood of patients induced diverse levels of apoptosis of CD4+ and CD8+ T lymphocytes after 48h incubation. The possible associations between low, medium and high apoptosis levels of T lymphocytes and acute and late normal tissue radiotoxicity are examined. Conclusion: Predicting which patients will develop RT toxicity is important for determining the optimal personalized therapeutic approach with the goal of long-term preservation of patient's quality of life. Assessment of individual and clinical parameters in addition to lymphocyte radiosensitivity may be useful for identification of patients with higher risk of developing radiotoxicity.

Keywords: prostate cancer; radiotherapy; radiation toxicity, T-lymphocyte apoptosis

Can miRNA expression patterns predict radiotoxicity in patients with glioblastoma?

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Background: Standard treatment of the glioblastoma patients is implemented with Stupp's protocol since 2005, but 2-years survival of the patients are still at low percentage. Even with implementation of modern radiotherapy techniques, acute radiotoxicity is still observed in these patients. Micro RNAs (miRNAs) expression patterns are introduced in recent years as factors that might predict survival in patients with glioblastoma, as well as cancer treatment related side effects. Material and methods: The data used in this review were conducted from research papers in PUBMED/MEDLINE databases with a special focus on role of miRNAs in cancer and with emphasis on brain tumors correlated to acute radiotoxicity. Results: Inflammation due to oxidative stress via free radicals is one of the mechanisms of radiation injury of irradiated cells and may correlate with toxicity. MiRNAs expression in prostate cancer patients may predict acute radiotoxicity, while miRNAs expression levels that could predict radiation toxicity in glioblastoma are still under investigation. However, it has been shown that miRNA levels like miR-16, miR 21, miR-19a and miR-22 are increased after radiation in glioma, while levels of miR-107, miR-181a are decreased. Conclusion: Recent studies have shown that miRNAs may have role as potent indicator of radiotoxicity in cancer patients. To date, there is no available data about acute brain radiotoxicity and its correlation with expression levels of mRNAs in patients with GB. There is a emerge need for further investigation on role of radioresponsive miRNAs in glioblastoma patients with acute radiotoxicity.

Keywords: acute radiotoxicity, cancer, glioblastoma, miRNA

Potential predictive role of K-ras gene mutation and BCL2 protein expression status in locally advanced rectal cancers treated with neoadjuvant chemoradiotherapy

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Background: Rectal cancer represents approximately 30% of cases of colorectal carcinoma and locally advanced stages of rectal cancers (LARC) remain a great clinical challenge due to chemoresistance and high local recurrence rate. The current management of LARC involves neoadjuvant chemoradiotherapy (neoCRT) before surgery. Since only a subset of patients benefit from this preoperative treatment, the development of reliable molecular biomarkers is required. In this retrospective study, we investigated the mutation status of K-ras proto-oncogene, as well as the expression level of apoptosis regulator protein, BCL2, to evaluate their potential predictive role in LARC. **Patients and Methods:** K-ras gene mutation status was determined by direct sequencing, while BCL2 protein expression was detected immunohistochemically (semi-quantitatively method) in pre-therapeutic and pre-operative biopsy specimens of 61 patients with LARC treated with neoCRT. **Results:** According to the results of this study, K-ras mutation status and BCL2 expression status were mutually independent events. In general, K-ras mutation status did not affect the response to CRT, while in the group of patients with high BCL2 expression was observed a tendency toward a worse response to the same treatment (p=0.098). However, the subgroup of patients with the simultaneous presence of K-ras mutation and high BCL2 expression showed significantly worse response to neoCRT (p=0.022). **Conclusion:** Obtained results strongly suggest that combined analyses of molecular aberrations in K-ras proto-oncogene and BCL2 anti-apoptotic protein expression level could have a potential predictive role and important clinical relevance in the identification of LARC patient subgroups, with a distinct pattern of response to neoCRT.

Keywords: BCL2 proteins, K-ras gene, Neoadjuvant Chemoradiotherapy, Predictive Medicine, Rectal Cancer.

Session: Imaging in cancer
LECTURES

Real-time microscopy of invasive cancer cells in the tumor microenvironment context

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Tumor cell structures that have long been hypothesized as necessary for metastasis are invadopodia, invasive protrusions rich in structural and adhesion proteins, as well as metalloproteases. Using our unique intravital imaging approaches (Perrin et al, 2019 Cancer Rep; Bayarmagnai et al, 2018 Meth Mol Biol.), we previously demonstrated that invadopodia in vivo are necessary for intravasation and consequent lung metastasis (Gligorijevic et al, Plos Bio 2014).

To assemble invadopodia, cells are required to enter, but not complete EMT transition. Cells which do not assemble invadopodia cannot metastasize individually, but only as a part of a cluster with invadopodia-assembling cells (Perrin et al, 2021 BioRxiv). Such clusters require cell-cell adhesions.

In primary breast carcinoma, we found that cells which assemble invadopodia migrate at slow speeds, in perivascular niches where the ECM is cross-linked. Outside of these niches, no invadopodia were observed and cells migrated at high speeds, via contact guidance along collagen fibers. Invadopodia emergence is independent of EMT status and can occur in individually invading cells, or leaders of collectively invading cells. The invadopodia-driven motility can be switched to contact guidance by reducing the ECM cross-linking or by knocking down Tks5, which in turn reduces intravasation and metastasis.

We next deduced that invadopodia-driven motility consists of two oscillating states: i. Invadopodia state, in which a cell is relatively sessile while it assembles invadopodia and degrades ECM; ii. Locomotion state. State balance is regulated by integrin β1 activation levels (Esmaeili et al 2018 Biophys J).

Importantly, the Invadopodia state only occurs in early G1, whereas the Locomotion state can be seen throughout the entire cell cycle, suggesting that the cell cycle controls invadopodia assembly. Using FUCCI markers (Esmaeili et al 2018 APL Bioeng), we next show that Invadopodia state occurs during the G1 phase of the cell cycle (Bayarmagnai et al, 2019 J Cell Sci). A close look at the regulators of G1 revealed that the cell cycle regulator p27kip1 localizes to the sites of invadopodia assembly and overexpression of p27kip1 causes faster turnover of invadopodia and increased ECM degradation, while reducing cell migration efficiency.

Taken together, these findings suggest that invadopodia assembly, which occurs in the perivascular niche, is necessary for lung metastasis and function is controlled by the cell cycle.

Defining and imaging colon cancer heterogeneity

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LGR5 (Leucine-rich repeat-containing G protein-coupled receptor) has been widely used as the marker of cancer stem cells (CSCs) in colorectal cancers (CRCs). However, lineage-tracing studies suggest that only a small portion of the LGR5 pool gives rise to large clones, and recent studies have shown LGR5 negative cells present in primary CRCs act as seeds for metastasis.

We have combined patient-derived organoids, several CRISPR-based techniques, single-cell sequencing analysis, and click-chemistry-based labeling to describe a previously unappreciated heterogeneity in the biosynthetic capacities of CRC cells. In transplantation experiments and intact tumors, most of the ribosomal DNA transcription and protein synthesis in CRCs occurs in a limited subset of tumor cells, and both LGR5+ and LGR5− tumor cells that display elevated biosynthetic features function as CSCs. Cancer cells within the biosynthetic domains are characterized by elevated levels of the RNA polymerase I subunit A (POLR1A). Genetic ablation of POLR1A-high cell population imposes an irreversible growth arrest on CRCs. We show that elevated biosynthesis defines stemness in both LGR5+ and LGR5- tumor cells. Some differentiated (KRT20+) cells can give rise to progeny, but as differentiation and the shutdown of the rDNA machinery progresses, the capacity to return to the previous state is gradually lost. Therefore, a common architecture in CRCs is a simple cell hierarchy based on the differential capacity to transcribe ribosomal DNA and synthesize proteins. Our findings fit well with a model where the properties of cancer cells are defined both by the microenvironment and cell-intrinsic programs dictated by a stem cell hierarchy.

Vessel co-option and resistance to therapy in glioblastoma

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Glioblastoma (GBM) is one of the deadliest types of human cancer. Despite a very aggressive treatment regime – including resection of the tumor, radiation and chemotherapy – its recurrence rate is more than 90%. Recurrence is mostly caused by the regrowth of highly invasive and resistant cells spreading from the tumor bulk, which are not removed by resection. To develop an effective therapeutic approach, we need to better understand the underlying molecular mechanism of chemoradiation resistance and tumor spreading in GBM.

GBM cells may use multiple strategies to spread in the surrounding brain tissue, one of them is vessel co-option, i.e. the movement of tumor cells towards and along the pre-existing vasculature. By using intravital microscopy we visualized and characterized the mechanisms driving vessel co-option in GBM. Interestingly, these spreading and co-opting GBM cells may also acquire a resistant phenotype making them particularly decisive during the GBM recurrence phase. This prompted us to deeply investigate the protective functions of the perivascular niche environment.

Recently, using bulk and single cell RNA-Seq, in vitro and in vivo time-lapse imaging, organotypic cultures and functional assays, we demonstrated that both chemoradiation and perivascular niche induce GBM reprogramming towards a resistant subpopulation of GBM cells. Moreover, these resistant GBM cells are more vessel co-opting allowing homing to the perivascular niche, that in turn induces further resistance to therapy.

We are now investigating and targeting the upstream pathways responsible of reprogramming and vessel co-option in order to reduce the intrinsic and extrinsic resistance to therapy typical of recurrent GBM cells.

Short talks

Characterizing the role of 4E-BP1 in breast cancer metastasis

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Metastasis is the leading cause of death among breast cancer patients. The multi-step process of metastasis is intricate and still poorly understood. However, it is recognized to be a highly inefficient and strenuous process for a cancer cell. mRNA translation, which rapidly and reversibly perturbs the proteome, is thought to play a major role in response to cellular stress during metastatic dissemination. Specifically, eukaryotic translation initiation factor 4E (eIF4E) binding proteins (4E-BPs) play a major role in translational regulation by impeding the assembly of the eIF4F complex that recruits mRNA to the ribosome. In mammals, 4E-BPs are represented by a family of three members, 4E-BP1, 2, and 3. Although literature has supported the role of eIF4E in promoting breast cancer progression and metastasis, the role of the 4E-BPs in this process is less understood. We demonstrate that the germline loss of 4E-BP1/2 significantly reduces lung metastases in a mouse model of metastatic breast cancer, without impacting the growth of the primary tumors. To dissect the mechanism, we reconstituted 4E-BP1 expression in 4E-BP1/2-deficient breast cancer cells. Restoring 4E-BP1 expression in 4E-BP1/2 null cells promoted spontaneous metastasis of breast cancer cells to the lung from the primary tumor site, using an orthotopic injection model. In turn, 4E-BP1 re-expression had no effect on lung colonization and metastatic outgrowth in a tail vein injection model. Our findings suggest that 4E-BP1 is specifically necessary for the early steps of the metastatic cascade, including invasion, intravasation and/or survival in the blood stream.

Keywords: 4E-BP1, breast cancer, metastasis, mRNA translation

Siramesine, a non-opioid σ2 receptor agonist as a potential agent for the development of novel targeted treatments for pancreatic cancer

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Background: We previously reported that the σ2-receptor ligand, siramesine (SRM), demonstrated the best activity among five commonly used sigma ligands screened in vitro for anticancer efficacy, with pancreatic cancer being the most sensitive type of cancer in siramesine's activity.

Aim: To investigate the efficacy of SRM on primary human pancreatic ductal adenocarcinoma (PDAC).

Materials and methods: The levels of σ receptors were studied by western blot analysis in two patient derived ex vivo pancreatic cancer cell populations, isolated and developed in our laboratory. In vitro evaluation of SRM was performed not only by the SRB cytotoxicity method, and the clonogenic assay. Western blot, used to study the mechanism of action (MoA). SRM was further tested for toxicity in zebrafish and in NOD/SCID mice. Finally, SRM was tested for in vivo activity in a new, patient-derived, pancreatic cancer xenograft model (PDAC/PDX) developed in our lab. Results: In vitro studies showed that SRM could kill primary pancreatic tumor cells via both autophagic death and apoptosis induction in a dose and time dependent manner. Also, our data show that siramesine could significantly delay the growth of the PDAC/PDX tumors at non-toxic doses alone or in combination with gemcitabine. Conclusion: Sigma-2 ligand, siramesine, found to exhibit good anticancer activity against a PDAC/PDX developed in our lab either as monotherapy or as a sensitizer to gemcitabine. This is the first study employing a PDAC/PDX model to study SRM's activity. Further studies of SRM are ongoing to optimize its anticancer efficacy and elucidate its underlying MoA.

Keywords: Siramesine, σ2 agonists, autophagy, apoptosis, pancreatic cancer, patient derived xenograft

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The Role of Nek2 on Centrosome Clustering in Cancer Cells with Extra Centrosomes

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Background: Unlike normal cells, cancer cells frequently exhibit extra centrosomes, which tend to form multipolar spindles (MPS), triggering cell death. Nevertheless, cancer cells divide successfully by clustering their extra centrosomes into two poles. Nek2 kinase is a key molecule regulating mitotic processes, including centrosome cycle. In this project, we tested whether Nek2 has a role in centrosome clustering in addition to its role in splitting centrioles, and which of the Nek2 targets might be responsible. This way, we wish to find novel strategies that may selectively kill cancer cells exhibiting supernumerary centrosomes. **Materials-Methods:** Unclustering effect of Nek2 was studied in cells with endogenously supernumerary centrosomes (N1E115), or via induction of extra centrosomes via microtubule inhibitors and PLK4 overexpression (U2OS, MDA-MB-231). Nek2 was overexpressed under a Dox inducible promoter or silenced using siRNA or knockout using gRNAs. Centrosomes were labelled using ɣ-Tubulin. Known Nek2 targets with relevant function were assessed for their involvement in centrosomal unclustering (C-NAP1, Rootletin, Gas2L1, Trf1) using KO or siRNA. Live cell imaging was utilized to determine the duration of metaphase. **Results:** Overexpression of Nek2 induced unclustering of extra centrosomes and lead to MPS, while reduction of Nek2 reclustered the poles, leading bipolar divisions in the cell lines studied. Known Nek2 targets tested have shown that they don't involve in the centrosome clustering mechanism which Nek2 regulates. Nek2 organizes centrosome clustering independent of a known pathway orchestrated by Kifc1 (HSET). Moreover, overexpression of Nek2 abridges the duration of metaphase, which could interfere centrosome clustering events requiring time during metaphase. We are currently studying to elucidate the mechanism of Nek2 regulating centrosome clustering in cancer cells. **Conclusion:** In our studies, we assigned a novel function for Nek2 in centrosome clustering. Understanding the mechanism will provide new translational approaches for cancer-specific treatment.

Keywords: Nek2, centrosome clustering, cancer

Sox2-targeted T cell therapy for treating multiple myeloma

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Background: Multiple myeloma (MM) results from the uncontrolled growth of clonal antibody-producing plasma cells in the bone marrow, and it remains incurable. Adoptive cell therapy (ACT) is emerging as a promising treatment for MM, but it requires further improvement and mechanistic understanding prior to becoming a reproducibly effective standard of care. Recent studies suggest that the transcription factor Sox2 regulates cell proliferation and self-renewal in MM and other cancers. Spontaneous T cell immunity to Sox2 has been associated with slower MM progression, and with a prolonged response to therapy. We hypothesize that adoptively transferred T cells targeting Sox2 will have a clinical benefit in MM. Materials and Methods: We used in silico epitope prediction algorithms and in vitro CD8+ T cell stimulation assays to identify Sox2 epitopes that are immunogenic in the context of the HLA-A*02:01 allele. We generated Sox2-specific CD8+ T cell lines from healthy HLA-A*02:01+ donors, and tested their cytotoxicity using flow cytometry and real-time imaging assays. We sequenced T cell receptors (TCRs) from the responding T cell lines, and selected 12 TCRs with a high affinity for further analysis. Results: We confirmed that Sox2-TCR-transduced T cells can respond to low doses of Sox2 peptide, as well as kill Sox2+ cancer cells in vitro. We are now poised to test the safety and efficacy of our lead Sox2-TCR candidate in a humanized mouse model of MM. Conclusion: Collectively, this work will reveal whether Sox2 is a safe and effective T cell target for treating MM.

Keywords: adoptive cellular immunotherapy, T-cell therapy, multiple myeloma

POSTER SESSION

P1

Pan-cancer analysis of the role of flap endonuclease 1 (FEN1) in human various tumors

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Background: Flap endonuclease 1 (FEN1) involves in DNA replication, long-patch excision repair, and telomere maintenance. FEN1 overexpression has been reported to be associated with the different types of cancers and it might be a predictive biomarker and therapeutic target in some cancers. But still there is no pan-cancer analysis available now. Materials and methods: Through analysis of 33 types of tumors based on the datasets of TCGA and GEO, we conduct the FEN1 gene expression analysis through TIMER2, then based on the CPTAC dataset, we analyzed the total protein expression level of FEN1 between normal tissue and primary tissue of breast cancer, ovarian cancer, colon cancer, clear cell RCC and UCEC. FEN1 genetic alteration evaluated by using cBioPortal web and survival status analyzed with GEPIA2. Results: Fen1 is highly expressed in most cancers (Fig.1,2). Highly expressed FEN1 was linked to poor prognosis of overall Survival for cancers of LIHC (p=0.007), KICH(p=0.039), ACC(p=0.007), PAAD(p=0.014), MESO(p=0.002), LGG(p=0.019), UVM(p=0.014) and LUAD(p=0.007), while low expression is poor prognosis only in THYM(p=0.04) within the TCGA (Fig.3). The genetic alteration status of FEN1 in different tumor samples of the TCGA cohorts were observed. The highest alteration frequency of FEN1 (> 8%) appeared with primary Cholangiocarcinoma with "CNA amplification" and nearly 5,26% in uterine carcinosarcomas. The "mutation" type was the primary Uterine Corpus Endometrial Carcinomas, which show an alteration frequency of ~3,51% (Fig.4). Conclusion: It seems FEN1 play a common molecular pathway in the pathogenesis of different tumors. But still remains to be answered. Our pan-cancer study provides a relatively comprehensive understanding of the roles of FEN1 in different tumorigenesis.

P2

In Vitro Investigations of miR-33a Expression in Estrogen Receptor-Targeting Therapies in Breast Cancer Cells

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Background: Elevated levels of fatty acid production promote breast cancer's aggressive character and reduces treatment efficacy. Regulatory microRNAs (miRNAs) on lipid production pathways, such as miR-33a, may be able to enlighten the mechanism. In the current study, we aimed to elucidate the role of miR-33a in MCF-7 and MDA-MB-231 breast cancer cells related to fatty acid mechanism in the treatment of estrogen receptor (ER) activator (estradiol-17β, E2) and anti-estrogens (ICI 182,780, Fulvestrant, FUL). Results: Treatment of MCF-7 cells with E2 and FUL did not cause a significant cell viability decrease at low concentrations, whereas higher concentrations showed a suppressive effect on cell survival. We determined miR-33a expression levels in MCF-7 and MDA-MB-231 breast cancer cells following E2 and FUL treatment and observed that FUL treatment enhanced the miR-33a expression both in MCF-7 and MDA-MB-231 cells. Considering the key regulator role of miR-33a on cholesterol mechanism, we investigated the expression profile of fatty acid synthesis pathway members in miR-33a mimics or anti-miR-33a treated-breast cancer cells. Conclusion: According to our results, the cellular expression level of miR-33a is critical to understanding differential responses of breast cancer cells.

P3

Gene expression profiling of MSI and EMAST colorectal cancers

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Background: Sporadic colorectal cancer (CRC) occurs via progressive accumulation of genetic alterations, genomic instability, or deficiency in DNA mismatch repair (MMR). Even though etiology of both microsatellite instability (MSI) and microsatellite alterations at selected tetranucleotide repeats (EMAST) is due to DNA MMR deficiency, they represent distinct subset of CRCs. EMAST CRCs have been poorly studied, however, they were linked to a chronic inflammation and overall poor prognosis. Contrarily, MSI CRCs have been shown to represents a unique subset of CRC that is associated with high immune cell infiltration, better prognoses, and good response to checkpoint inhibitors.
EMAST development has been brought into connection with overexpression of IL-6 and the dysfunction of MSH3 whereas MSI development with loss of expression of MSH2 and MLH1. Therefore, we decided to investigate mRNA expression of MMR genes and IL-6 as well as IL-1β as one of the most important proinflammatory cytokines in tumorigenesis. Material and methods: CRC patients were divided into three groups based on their MSI-H and/or EMAST-H status. From each group twenty samples of tumor and normal adjacent tissues were taken and mRNA was isolated to evaluate the expression of MSH2, MLH1, MSH3, IL-6 and IL-1β with real-time qPCR. Results: When different groups were compared analysis showed that MLH1 expression was significantly reduced, and MSH2 and IL-1β significantly increased in MSI-H tumor tissues compared to other groups. There were no differences in the expression of MSH3, and IL-6 between different groups. Conclusions: Our findings confirmed already established role of MLH-1 in development of MSI-H tumors. In addition, increased expression of MSH-2 and IL-1β suggests a role in MSI-H CRC tumorigenesis.
This study is supported by Croatian Science Foundation Grant IP-2016-06-1430.

P4

Expression and diagnostic potential of genes involved in PI3K/AKT/mTOR pathway in endometrial cancer

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Background: Endometrial cancer is the fourth most common cancer in Serbian women. Changes in genes whose protein products are involved in the PI3K/AKT/mTOR pathway are found to be crucial in the development of this disease. We aimed to investigate expression of *PIK3CA*, *PTEN*, and *ARID1A* genes in endometrial cancer and to evaluate their potential diagnostic value in this disease. Materials and methods: The cohort was comprised of 54 women with the diagnosis of endometrial cancer whose surgery was performed at IORS during 2016 and 2017. Tumor and normal-adjacent to tumor tissue (NAT), which was used as a control, were reviewed by a pathologist after the surgery. The RNA was isolated from fresh frozen tissue samples by TRIzol extraction, converted to cDNA by reverse transcription and quantified by qPCR. Results: Significantly higher expression of *PTEN* was detected in NAT, compared to tumor tissue (p=0.0168). No difference was found for *PIK3CA* (p=0.188) and *ARID1A* (p=0.124). ROC curve analysis showed that *PTEN* expression has a discriminatory potential (AUC=0.74, Cut-off 1.14, sensitivity 0.65, specificity 0.84). *ARID1A* expression could also serve as a discriminant (AUC=0.74, Cut-off 0.94, sensitivity 0.81, specificity 0.68). *PIK3CA* expression does not have a discriminatory potential (AUC=0.55, Cut-off 0.89, sensitivity 0.31, specificity 0.91). Conclusions: Higher expression of *PTEN* in tumor tissue compared to one in the NAT highlights the need for elucidation of its role as a tumor suppressor in endometrial cancer. Expression of *PTEN* and *ARID1A* higher than established threshold in the tumor tissue could serve as a potential diagnostic biomarker of endometrial cancer.

Keywords: gene expression, endometrial cancer, PI3K/AKT/mTOR pathway

P5

Intratumor heterogeneity of microsatellite instability in sporadic colorectal cancer

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Background: Microsatellite instability (MSI) status has become an important factor in colorectal cancer (CRC) therapeutic decisions due to novel targeted therapies including immune checkpoint inhibitors. Recently, a new form of microsatellite instability, elevated microsatellite instability at selected tetranucleotides (EMAST) has been described leading to possible speculations about its role in CRC development and progression. Intratumor heterogeneity (ITH) is a phenomenon that arises from evolutionary dynamic of tumor progression leading to the clonal expansion of different clones within the tumor with different responses to antitumor therapy. Previous CRC heterogeneity studies have mainly focused on major mutations such as KRAS and TP53 while the data regarding the microsatellite instability is scarce. Materials and methods: In this study we have examined the possible ITH of MSI/EMAST status in 30 sporadic CRCs. In four tumor sections and corresponding normal mucous tissue MSI status was examined using the conventional Bethesda panel while EMAST status was established using five tetranucleotide microsatellite markers (MYCL1, D2S82, D2S85, D8S321 and D9S252). Targeted loci were amplified by PCR and analyzed either by polyacrylamide gel electrophoresis or GeneScan analysis. Results: In MSI and EMAST positive tumors instability was present in all tumor specimens, but with different instability profiles in one tumor. Tumors positive only for EMAST presented with more uniform pattern. The most unstable markers were D17S250, MYCL1 and D20S82. Conclusion: In this preliminary study we have shown that MSI affects the ITH profile of MSI/EMAST positive tumors.
Keywords: colorectal cancer, microsatellite instability, MSI, EMAST, tumor heterogeneity

P6

Multi-omic profiling of cancer cells, exosomes, and cell-free DNA isolated from the cerebrospinal fluid of pediatric brain cancer patients

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Background: Clinical management of pediatric brain tumors is challenging since they often exhibit low responsiveness to standard treatment resulting in poor survival. Molecular characterization of tumor tissues may guide novel therapies and monitor their effectiveness. However, brain cancer biopsies are often difficult to obtain. Therefore, we established a workflow allowing multi-omics analysis of disseminated cancer cells (DCCs), exosomes, and cell-free-DNA (cfDNA), isolated from cerebrospinal fluid (CSF) aspirates of pediatric brain cancer patients. Patients and Methods: We used this workflow on CSF samples of two patients with medulloblastoma and pineal-anlage tumor, respectively. Using centrifugation we separated cells from the rest of CSF. Since this workflow is used for multiple tumor types, we stained the cells for CD45, and both $CD45^+$ (immune cells) and $CD45^-$ (putative cancer cells) cells were isolated. cfDNA and exosomes were isolated from the liquid fraction of CSF. We used PCR or RNA-seq to analyze gene expression in cDNA samples, and DNA-seq to analyze copy number alterations (CNAs) and single nucleotide variants (SNVs) in genomic DNA. Results: Transcriptome analysis showed that neural lineage markers were almost exclusively expressed in $CD45^-$ cells. $CD45^+$ cells harbored no CNAs. In contrast, all $CD45^-$ cells had CNAs, confirming that they are DCCs. Interestingly, sequencing of cfDNA and exoDNA revealed similar CNA profile as observed in genomes of DCCs. SNV analysis showed that genomes of DCCs exhibited heterogeneous profiles of oncogenic mutations. Conclusion: This proof-of-concept study demonstrates establishment of workflow allowing identification, isolation, and comprehensive molecular characterization of CSF-derived DCCs, cfDNA and exosomes.

Keywords: Cell-Free Nucleic Acids; Gene Expression; Brain Neoplasms; DNA Copy Number Variations; Cerebrospinal Fluid; Mutation

P7

The effect of osteogenic differentiation on oral cancer stem cells' miR-21 and miR-133 expression

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Background: Cancer stem cells (CSCs) are frequently associated with initiation and progression of various tumors, including oral squamous cell carcinoma (OSCC). CD44 has been documented as oral CSC surface marker and has been used for the detection and isolation of oral CSCs. CSCs possess the potential to transdifferentiate into different cell lineages, as do normal adult stem cells. Such plasticity of CSCs is considered to be a promising therapeutic tool. Recent studies suggest that microRNAs (miRNAs) can regulate CSCs at a molecular level and are associated with different cancer properties. This study focused on the most common oncogenic microRNA miR-21, frequently upregulated in a variety of cancers and miR-133a which acts as a tumor suppressor. The aim of this study was to examine levels of miR-21 and miR-133 after osteogenic differentiation of $CD44^+$ cells isolated from OSCC cell line SCC-25. Materials and methods: $CD44^+$ cells were magnetically separated using magnetic-activated cell sorting system. $CD44^+$ cells were seeded onto 24-well culture plates (8×10^4 per well) and cultured in osteogenic differentiation medium for 21 days. Cells were cultivated under standard conditions in humidified atmosphere with 5% CO_2 at 37°C. After 21 days, total RNA was extracted from the culture cells with TRIzol Reagent. Reverse transcription of microRNA was performed using TaqMan MicroRNA Reverse Transcription Kit in accordance with the manufacturer's instructions. MiRNA expression was normalized to RNU6. Results: After osteogenic differentiation, expression levels of miR-21 were significantly lower compared to uninduced cells. The levels of miR-133, on the opposite were significantly higher compared to uninduced cells. Conclusion: This study suggests that osteogenic differentiation of oral CSCs significantly down-regulates the oncogenic miR-21 and up-regulates the tumor-suppressor miR-133, which are both favorable effects in terms of potential cancer treatment.

Keywords: oral squamous cell carcinoma, cancer stem cells, miR-21, miR-133, osteogenic differentiation.

P8

Evaluation of differential transcriptional regulator binding to alternative CD81 gene promoters

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Background: Understanding alternative transcription initiation could lead to better understanding of carcinogenesis, and may serve to discover novel biomarkers and therapeutic targets in cancer. Gene CD81 encodes for a member of the tetraspanin protein family with multiple biological activities implicated in cancer. Transcription of CD81 is regulated by two alternative promoters indicated by *in silico* analyses to be differentially active in colon and rectal cancer tissue. This study aimed to investigate transcriptional regulator binding to alternative CD81 gene promoters *in silico*. Material and Methods: The nucleotide sequences of promoters of interest were analyzed using GPMiner and the Eukaryotic Promoter Database. Six bioinformatics tools were used to predict the binding of transcriptional regulators to alternative CD81 gene promoters: Alggen, AliBaba, CiiiDER, MATCH, TFBIND, Tfsitescan. For each transcriptional regulator, only the positive findings obtained with at least two software were taken into consideration. Results: The analysis of characteristic promoter elements in alternative CD81 gene promoters indicate atypical structure in the promoter A and typical in the promoter B. According to the software prediction, 14 proteins predominantly involved in cell cycle regulation and development bind to the promoter A, while 8 proteins mostly responsive to stimuli bind to the promoter B. Conclusion: The profile of predicted transcriptional regulators binding to alternative CD81 promoters may explain their differential regulation in colorectal cancer. These results indicate that alternative CD81 transcripts may serve as cancer biomarkers, while proteins binding to the promoter A can further be investigated as therapeutic targets.

Keywords: alternative transcription initiation, CD81, promoter, transcriptional regulator
Acknowledgement: This research was supported by the Science Fund of the Republic of Serbia, PROMIS, #6052315, SENSOGENE.

P9

Methylation status of *MGMT* promoter in glioblastoma in Serbian patients: valuable marker or not?

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Background: Newly diagnosed glioblastomas (GBM) express remarkable genetic and morphological heterogeneity, which is often encountered during a histopathological examination. The development of reliable molecular markers is essential for further advancement in therapeutic and diagnostic procedures in GBM patients. This study aimed to evaluate the prognostic properties of *MGMT* (O^6-methylguanine-DNA methyltransferase) promoter methylation status in a Serbian population of GBM patients. Material and Methods: Patients (N=34) operated on the Neurosurgery Clinic (The University Clinical Centre of Niš, Serbia) between 2013 and 2019 were included in this study. To confirm primary GBM diagnosis, the presence of isocitrate dehydrogenase 1 (*IDH1-R132H)* mutation in samples was determined by Sanger direct sequencing. Methylation-specific polymerase chain reaction method (MSP) was used for MGMT methylation status evaluation. Results: Age (hazard ratio 1.0689, p = 0.0007), the extent of tumor resection (hazard ratio 0.4093, p = 0.0004), and type of adjuvant chemotherapy (hazard ratio 0.1639, p = 0.0001) were recognized as independent prognostic factors. Semi-quantitative MSP approach resulted in improved detection sensitivity of *MGMT* methylation status compared to the qualitative MSP method. Among *IDH-wt* homogenous cohort of GBM patients older than 50 years with complete/partial resection of the tumor, overall survival of patients harboring hypermethylated *MGMT* was significantly longer (11±6.68 months) in comparison with the unmethylated group (5.2±3.9 months) (KW-H(1,14)=3.4328, p=0.06).

Conclusion: In this study, hypermethylation of the *MGMT* promoter region was correlated with longer OS within Serbian population of GBM patients. Given the relatively small cohort group, this correlation should be further investigated in more comprehensive research.

Keywords: biomarkers, epigenomics, glioblastoma, methylation, prognosis

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P10

The prognostic significance of interleukin-6 in hormonally dependent breast cancer

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Background: Interleukin-6 (IL-6) is a pleiotropic cytokine with both pro- and anti-inflammatory properties. This study aimed to investigate the prognostic significance of IL-6 and its association with the established breast cancer parameters ER and HER2. Patients and Methods: The study included 79 premenopausal women with early and locally advanced estrogen-dependent (ER+) breast cancer. All patients received adjuvant hormonal therapy: tamoxifen alone (56/79) or combination with goserelin (23/79). The median follow-up period was 85 months. IL-6 serum protein concentrations were measured by ELISA. Cox proportional hazards regression analysis was implemented for prognostic evaluation of the data categorized based on metastasis outcome. Results: IL-6 median serum concentrations were indicative of their possible association with the actual metastasis outcome, as these values differed consistently between patient groups with metastasis and without metastasis. Medians for IL-6 were 1.3, 1.7, 1.1, 1.5 and 2.1 pg/mL for the patients with metastasis on bones, brain, liver, lungs and without metastasis, respectively. In the whole group of patients, IL-6 associated with good disease outcome (p=0.001, HR=0.05). The median for IL-6 was 1.7 pg/mL in the group prognosticated as high-risk and 2.2 pg/mL in the group prognosticated as low-risk. Multivariate analysis highlighted IL-6 as the independent prognostic factor (p=0.001, HR=0.0007). IL-6 lost its prognostic significance in ER^{low} and ER^{high} subgroups but IL-6 remained prognostically significant in both HER2− and HER2+ subgroups. Conclusion: Serum IL-6 is indicated as a biomarker of favorable disease outcome. Clinical applicability of the study is based on its relevance for the breast cancer immunotherapy research.
Keywords: breast cancer, inflammation, interleukin-6, metastasis, prognosis.

P11

Role of *TP53* and *PTEN* tumor suppressor genes alterations in breast cancer response to therapy

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Background: Breast cancer (BC) is the most frequent type of malignancy and the leading cause of cancer related death among women worldwide. Multiple interconnected factors determine BC response to therapy and clinical outcome. *TP53* and *PTEN* are the most frequently altered tumor suppressor genes (TSGs) in human cancers. Material and methods: To determine the potential influence of TSGs on the response to therapy we analyzed alterations of *TP53* and *PTEN* in 90 BC specimens. The specimens were stratified based on systemic adjuvant therapy (hormonal therapy only (HT), HT and chemotherapy (HT/CHT), HT/CHT and biological therapy (HT/CHT/H). Functional inactivation of *TP53* by mutations and/or loss of heterozygosity (LOH) and *PTEN* by LOH and/or promoter hypermethylation, were tested using single-strand conformational polymorphism (SSCP) analysis, gene sequencing, fragment analysis and methylation-specific PCR (MS-PCR) methods respectively. Results: Altered *TP53* was found in 63/90 specimens (70%) while 54/90 (60%) had inactivated *PTEN*. Inactivation of *PTEN* was more frequent in tumors with altered *TP53*. Patients with altered *TP53*, lived shorter (p=0.0007) compared to those with wild type (wt) gene. The survival of patients with both TSGs altered was shorter compared to wt genes (p=0.024). Patients with wt*TP53* treated with HT had longer survival (p=0.000001) when compared to all other groups. Women with both TSGs altered who received tamoxifen lived shorter than those on HT with both/one TSGs intact (p = 0.03). Conclusion: Patients with wt*TP53* showed significantly better therapy response regardless of type of therapy, compared to carriers of altered *TP53*.

Key words: *PTEN*, *TP53*, therapy response, survival, breast cancer

P12

A novel triple negative lipid rich breast cancer (TN/LRBC) patient derived xenograft (PDX)

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Background: Lipid-rich breast cancer (LRBC) is a rare subtype of breast cancer, highly metastatic and with poor prognosis. It is reported to generally be negative for ER/PR receptors but highly positive for HER2 expression with triple negative (TN) cancers to be even rarer. Noteworthy, there are no models available for studies on this rare type of cancer so far. Our aim was to establish and characterize a PDX from a patient with TN/LRBC. Materials and methods: The model was developed in immunocompromised mice after direct engraftment of tumor fragments surgically excised from the patient. The PDX was further evaluated pharmacologically following patient's schedule. Histological, karyotypic and NGS analysis were performed for the first time for this type of cancer. Results: Pharmacological characterization revealed that the xenograft responded well to cyclophosphamide and docetaxel, as was expected, but doxorubicin was found to be highly toxic. As an alternative Caelyx® (stealth liposomal doxorubicin) was for the first time tested on this type of breast cancer and found to be highly efficient with lower toxicity. Karyotyping revealed polyploidy, while NGS analysis the presence of a pathogenic mutation in the MSH2 gene (c.482T> A, p. Val161Asp) in both the patient and the xenograft. Data suggest that this mutation may be a driver mutation. Conclusion: This is the first report on the development of a PDX for TN/ LRBC, a model that we anticipate will be an extremely valuable tool towards developing novel treatments and understanding the biology of this rare type of breast cancer.

Keywords: Triple negative lipid rich breast cancer, patient derived xenograft, liposomal doxorubicin

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P13

Quality of life in patients surgically treated for oral carcinoma

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Background: Most oral planocellular carcinomas are diagnosed in the late stages, which significantly reduces the chances of survival and impairs quality of life. This study examined quality of life in patients who were surgically treated. **Patients and Methods:** The study included patients surgically treated over a 3-year period (2014-2016). Data on patients, tumor type and localization, TNM classification, type of surgical intervention, and time since surgery were collected from the medical records. Post-surgery functional and aesthetic results were evaluated using the adapted University of Washington Quality of Life questionnaire. **Results:** Forty patients were included in the study. Male patients were more prevalent (27 vs 13) (χ^2=4.225p<0.05). Ratio of planocellular vs adenocarcinoma was 7:1 (χ^2=11.404, p=0.0007). Osteotomy was performed in 52.5% of patients, and surgical intervention in the soft tissue in 47.5%. Compared to patients evaluated <1 year after surgery, patients who had been recovering >1 year showed better mood (p=0.036), functions of speech (p=0.008) and chewing (p=0.04), as well as patients who had soft tissue surgery (chewing: p=0.016; speech: p=0.043). Patients with T1 stage tumors considered their looks less disfigured and had fewer problems in appearing in public, compared with patients with T3 and T4 stage (CI – 95%). Interest in sex was significantly diminished in patients older than 30 years (p=0.013). **Conclusion:** The stage of disease, range of resection and success of reconstruction were decisive parameters for postoperative quality of life. Early detection of disease is of utmost importance for both survival and quality of life of patients with carcinoma.

Keywords: Oral cancer, quality of life, postoperative period

P14

Genetic analysis of *SMAD7* 3'UTR in human colorectal cancer

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Background: SMAD7 is a pleiotropic regulator with an inhibitory role pivotal for the control of the TGF-β pathway. Several *SMAD7* variants have been associated with colorectal cancer (CRC) risk and proven to disrupt the SMAD7-mediated regulation of the TGF-β cascade involved in malignant cell processes. This study aimed to analyze *SMAD7* 3'UTR variants in CRC tumor tissue, considering their relevance for the regulation of gene expression. **Patients and Methods:** Primary tumor tissue samples obtained from 50 colorectal cancer patients who underwent surgical resection without preoperative chemoradiotherapy were used in this study. The 1126 nt long genomic region of *SMAD7* 3'UTR (48,920,135–48,921,260; Homo sapiens GRCh38.p13 Primary Assembly) was analyzed by polymerase chain reaction (PCR) using two primer sets followed by direct DNA sequencing. **Results:** Two single nucleotide polymorphisms (SNPs) were detected in 5 CRC cases, rs16950113 (T>C) in 4 patients and rs1050799536 (G>A) in one patient. **Conclusion:** Detected variants should be further functionally annotated since variation in potential miRNA-binding sites in the 3'UTR of *SMAD7* may modulate expression and increase susceptibility to CRC. Furthermore, *in silico* analysis using the PolymiRTS Database 3.0 (available at https://compbio.uthsc.edu/miRSNP/) predicted that the derived allele of rs16950113 disrupts conserved and creates a new miRNA site, indicating its functional impact.

Keywords: Colorectal Cancer, SMAD7, SNP, 3'UTR

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P15

The expression of MicroRNA-30a-3p and Estrogen Receptor β in Papillary Thyroid Cancer

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Background: A number of studies point to a significant role of microRNAs (miRNAs) in papillary thyroid cancer (PTC), where specific miRNA expression profiles associate with distinct clinical and biological phenotypes of the lesion. One of the microRNAs deregulated in PTC is miR-30a-3p. Evidence suggests that estrogen receptor β (ERβ), also found to be deregulated in PTCs, may directly regulate microRNA-30a-3p biogenesis and expression. Considering the possibility that ERβ might influence PTC cell behavior via miRNAs, in particular, miR-30a-3p, we have investigated their expression and correlation in PTCs with different clinico-pathological characteristics. **Patients and Methods:** Quantitative PCR was used to determine the relative miR-30a-3p and ERβ expression levels in 37 pairs of PTCs and matched non-tumor thyroid tissues. **Results:** The expression levels of miR-30a and ERβ were significantly altered in tumors compared with non-tumor tissues. A negative correlation between miR-30 and ERβ was detected in tumors with pT4 category (P=0.038, r = - 0.738) and capsular invasion (only in women) (P=0.041. r= -0.552) compared to positive correlations (or trends) found in tumors with lower pT categories (pT1+pT2) (P=0.061, r=0.463) and tumors with no capsular invasion (P=0.019, r=0.618). Similar trend was found in tumors with classic papillary pattern in the group of women (P=0.09, r= - 0.432) while in women with histovariants other than classic there was a trend towards positive correlation (P=0.066, r=0.486). **Conclusion:** The results suggest that in some PTCs, ERβ might negatively regulate miR-30a expression, and the opposite roles they may play are associated with more aggressive tumor features.

Keywords: ERbeta, MicroRNA-30a-3p, Papillary Thyroid Carcinoma

P16

Differential Expression of *VHL* mRNA in Parathyroid Carcinoma and Adenoma

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Background: Parathyroid cancer (PTC) is an exceedingly rare endocrine malignancy, and the knowledge about the underlying molecular mechanisms of its pathogenesis is scarce. Being characterized by a spectrum of clinical phenotypes and histological heterogeneity that make it difficult to preoperatively distinguish malignant from benign tumors, PTC represents a significant clinical and therapeutic challenge. Broadening the knowledge of molecular signatures that characterize different parathyroid tumor subtypes is a key step to identify potential diagnostic biomarkers able to distinguish among different parathyroid neoplastic types, as well as provide novel therapeutic targets and strategies for these neoplasms. Aberrant expression of VHL (von Hippel-Lindau) tumor suppressor has been detected in various types of cancer including thyroid cancers, but no studies so far investigated the biological role or clinical relevance of VHL in neoplastic parathyroid lesions. In order to shed more light on the potential involvement of VHL in PTC, we investigated the expression levels of *VHL* mRNA in parathyroid tumors and their association with clinical and pathological parameters. **Patients and Methods:** Quantitative PCR was used to determine the relative *VHL* mRNA expression levels in 10 parathyroid carcinoma and 14 parathyroid adenoma. **Results:** The expression level of *VHL* was significantly higher in PTCs compared to parathyroid adenoma (P=0.021, Mann-Whitney test). However, there was no association with clinico-pathological characteristics such as tumor size, vascular invasion, invasion of the adjacent tissue and preoperative serum PTH levels.

Conclusion: The results suggest that VHL might be involved in the pathogenesis of PTC but its role/significance remain to be clarified.

Keywords: Expression, Parathyroid Neoplasms, Von Hippel-Lindau Tumor Suppressor

P17

uPA/PAI-1, MMP-2, -9, IL-8 and VEGF as markers of progression in early breast cancer patients

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Background: Cancer progression and metastasis are complex processes, dependent of molecules involved in inflammation, degradation and invasion. Interleukin-8 (IL-8) has a role in inflammation, urokinase plasminogen activator (uPA), plasminogen activator inhibitor type-1 (PAI-1) and matrix metalloproteinase-2, -9 have a decisive part in the process of degradation and invasion, while vascular endothelial growth factor (VEGF) is consequential for angiogenesis. This study aimed to evaluate the relations between IL-8, uPA, PAI-1, MMP-2, MMP-9 and VEGF, as well as their prognostic significance in terms of recurrence free survival (RFS). Patients and Methods: The study included 91 adjuvantly untreated patients with lymph node negative primary breast cancer. The follow-up period was 30 months. Biomarker protein concentrations were determined in primary tumor tissue homogenates by ELISA. Survival curves for RFS were constructed according to the Kaplan-Meier method and compared with the log-rank test, using an optimal cut-off point and the minimal p-value approach. Results: Patients with higher concentrations of uPA (>0.286 ng/mg), PAI-1 (>6.601 ng/mg), IL-8 (>99.27 pg/mg) or MMP-2 (>20.54 ng/mg) had significantly shorter RFS (Log rank test). Positive correlations were found between uPA and PAI-1 (Spearman's rank order test, $p<0.001$), uPA and MMP-9 ($p=0.02$), PAI-1 and IL-8 ($p=0.03$), MMP-9 and IL-8 ($p<0.001$), MMP-9 and VEGF ($p=0.02$), IL-8 and VEGF ($p=0.01$). Conclusion: Higher concentrations of uPA, PAI-1, IL-8 and MMP2 are indicators of aggressive tumor behavior and poor prognosis. Since they all have the role in tumor progression and could have related expression pattern (positive correlation), they are perhaps controlled by the same regulators.
Keywords: biomarkers, breast cancer, interleukin 8, matrix metalloproteinases, plasminogen activators, prognosis.

P18

The role of TLR4 in sporadic colorectal cancer displaying microsatellite instability MSI and EMAST

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Background: Sporadic colorectal cancer remains a significant health problem worldwide. Microsatellite instability (MSI) is recognized as one of the three main pathways that drive sporadic CRC tumorigenesis and is frequently associated with specific immune response and better prognosis in early-stage CRC. Another form of microsatellite instability that affects tetranucleotide repeats – EMAST, seems to be involved in the progression of sporadic CRC by modulating biological behavior of CRC towards metastasis and poor survival in patients. Current evidence implicates pro-inflammatory environment as a major trigger for EMAST. Inflammation in colon is frequently the result of prolonged immune response to dysbiosis where TLR4 receptor plays a major role as a regulator of intestinal homeostasis. Yet, aberrant TLR4 signaling might lead to the induction of proliferative and anti-apoptotic pathways, contributing to the tumor development.
Material and Methods: Patients were stratified into groups according to MSI and EMAST status by VNTR analysis. Tumors were further examined with regard to TLR4 mRNA expression by qPCR and the expression of TLR4 protein by immunohistochemistry and western blot. Results: Analysis showed that EMAST was present in majority of MSI-H sporadic CRC tumors. Compared to normal mucosae, tumor tissue showed increased overall expression of TLR4 mRNA. TLR4 protein analysis revealed concordance with findings of mRNA expression, whereas microsatellite-stable (MSS) CRC tumors showed significantly higher levels of TLR4 protein than tumors with high microsatellite instability. Conclusion: Our findings suggest that TLR4 is involved in development of sporadic CRC, but plays a more prominent role in sporadic CRCs with intact mismatch-repair system.
Keywords: colorectal cancer, microsatellite instability, toll-like receptor 4
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P19

Triple negative breast cancer and anoikis

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Background: Estrogen, progesterone and HER2 expression negative-triple negative breast cancer (TNBC) with poor prognosis, constitutes 10-15% of all breast cancer cases. Chemotherapy is currently the only systemic treatment option for TNBC. Anoikis is the induction of apoptosis in cells upon loss of connection with the extracellular matrix (ECM) and neighboring cells. Anoikis can regulate the survival of ECM-independent cells. **Material and methods**: In this review, TNBC and anoikis-related articles were evaluated. **Results:** Changes in gene expressions of S100a7, MnSOD, TGF-β1, AMPK and Hypoxia/reoxygenation signaling in metastatic cells growing independently of anchorage play a role in anoikis resistance through nuclear factor NF-κB, ensures cell survival. In healthy cells, Snail and FAK expression are suppressed when the cell-cell connection is disrupted. In metastatic cells, the expression of these proteins is increased by NF-kB. In addition, the sensitivity to anoikis increases with inhibition of TDO2 expressed. The role of anoikis is seen in metabolism related studies aimed at preventing proliferation and invasion in TNBC. The miRNA200 family, which is prominent in prognostic and diagnostic aspects for TNBC, has been shown to play a role in anoikis regulation in TNBC and different cancer types. Resistance to anoikis develops as the highly expressed nerve growth factor receptor suppresses pro-apoptotic genes in TNBC. Proteins or pathways that can be biomarkers in determining poor prognosis and metastatic potential play a role in the development of resistance to anoikis. **Conclusion**: The development of targeted therapy should be focused on effective ways in the anoikis process in TNBC.

Keywords: TNBC, anoikis, metastasis

P20

Diagnostic and prognostic potential of *miR-146* gene expression in oral carcinoma

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Background: Micro non-coding RNA, *miR-146a*, has not fully elucidated role in oral carcinoma. There are still conflicting results in the literature regarding its oncogenic or tumor suppressor role. The potential of using *miR-146a* as a sensitive discriminator of cancerous from non-cancerous tissue as well as prognostic biomarker is not studied so far. We aimed to test whether *miR-146a* acts as an oncogene or tumor suppressor, and analyze its diagnostic and prognostic potential in oral carcinoma. Material and Methods: Clinical samples of oral carcinoma and its normal tissue counterparts were obtained from 35 patients. Relative expression of *miR-146a* was performed by Real Time PCR. *RNU6B* was used for normalization. Results were calculated by $2^{-\Delta Ct}$ method. Results: There were no significant differences in *miR-146a* expression in cancer and non-cancerous tissue (p=0.272, Wilcoxon signed test). *miR-146a* is not good discriminatory biomarker of oral cancer from non-cancerous counterparts according to receiver operating curve analysis (AUC=0.560, 95%CI=0.424-0.696, p=0.388). None of demographic and clinicopathological characteristics were associated with *miR-146a* expression. There was no difference in survival among oral carcinoma patients with high and low expressed *miR-146a* (p=0.362, log-rank test). Results of cox regression analysis revealed that *miR-146a* cannot be considered as a prognostic biomarker of oral carcinoma (HR=0.460, 95%CI=0.077-2.755, p=0.395). Conclusion: According to our findings, it is still inconclusive whether *miR-146a* is an oncogene or tumor suppressor. Our results showed that *miR-146a* is not satisfactory discriminatory biomarker of oral cancer from non-cancerous tissue. *miR-146a* can not be used as a prognostic biomarker of oral carcinoma.
Keywords: biomarker, expression, *miR-146a*

P21

Analysis of alpha-1 antitrypsin expression in multidrug resistant cell lines

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Background: Identification of the signature molecular factors and transcriptional profiles involved in therapy resistance is of vital importance in developing new strategies for treatments and disease monitoring. Tumour secretome is a set of macromolecules secreted by tumour cells into the extracellular space as a response to changes in tumour environment that at the same time shapes the microenvironment further promoting specific phenotypes and contributing to cellular plasticity in tumour. Protein alpha-1 antitrypsin (AAT, encoded by SERPINA1 gene) is an acute phase protein that has emerged as one of the key components in tumour secretome involved in crucial stages of tumour development and progression, with recent data also implicating it in therapeutic resistance. However, what exactly leads to SERPINA1 upregulation during development of therapy resistance, as well as its biological significance in this process, is still unclear. Our aim was to analyse SERPINA1 expression in multidrug resistant cell lines and 3D cellular models. Expression of IL-6 was also analysed, as AAT is an acute phase reactant and its levels increase in response to inflammatory cytokines. Patients and methods: We analysed SERPINA1 and IL-6 expression in three different cell lines - human glioblastoma U87, non-small cell lung carcinoma NCI-H460 and colorectal carcinoma DLD1 as well as their multidrug resistant counterparts U87-TxR, NCI-H460/R and DLD1-TxR, respectively. In addition, expression analysis was performed in long-term 3D glioblastoma model of U87 cells cultured in alginate microfibers, and compared to long-term 2D cell culture of U87. Quantitative RT-PCR was performed using Taqman gene expression assays and data were normalized to GAPDH. Results: We found that SERPINA1 expression is significantly upregulated in all the multidrug resistant cell lines analysed compared to their sensitive counterparts. Expression of IL-6 was significantly upregulated in U87-TxR and DLD1-TxR compared to their parental lines, however NCI-H460/R cell line had lower IL-6 expression compared to NCI-H460. In 3D glioblastoma model of U87 cells, previously found to exhibit increased therapy resistance compared to 2D cell culture, both SERPINA1 and IL-6 expression were significantly upregulated. Conclusion: Our results indicate that SERPINA1 expression correlates with therapy resistance in analysed cell lines and 3D model of glioblastoma, revealing the potential of utilizing this molecule as a biomarker of therapy resistance. However, transcriptional profiles connected to its expression in therapy resistance still remain to be determined.
Key words: multidrug resistance, alpha-1 antitrypsin, biomarkers

P22

Identification and validation of mechanism responsible for leukemia cell death treated with bis-(salicylaldehyde)thiocarbohydrazone (BTCH1)

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Background: Methyl-2-pyridyl keton thiocarbohydrazone (MTCH1) and Salicylaldehyde thiocarbohydrazone (MTCH2), with their two corresponding symmetrical bis-counterparts Bis-(salicylaldehyde)thiocarbohydrazone (BTCH1) and Bis-(methyl-2-pyridyl ketone)thiocarbohydrazone (BTCH2) were assessed for activity on acute human monocytic leukemia THP1 cells. Material and methods: Response of THP1 cells to applied treatments was evaluated by Annexin V/PI staining, cell cycle distribution, caspase-8 and -9 activation, and mitochondrial superoxide radicals (MSR) generation. Relaxation assay (RA), decatenation assay (DA) on kinetoplast DNA and plasmid DNA cleavage assay (pDNAcA) were utilized to test compounds´ ability to inhibit topoisomerase II-alpha (topo-IIα) activity. Results: All four compounds were revealed as strong inducers of cell death through the activation of caspase-8 in cells treated with MTCHs, and caspase-9 in cells treated with BTCHs. While both MTCHs and BTCH2 induced accumulation of cells at the G1-to-S phase, a striking arrest at the S-to-G2 transition point was seen only in cells subjected to BTCH1. According to MSR assay, reactive oxygen species are not responsible for DNA damage observed in BTCH1-treated cells. RA showed that BTCH1 should be further evaluated as topo-IIα inhibitor. DA demonstrated that BTCH1 is stronger topo-IIα inhibitor than etoposide, while the pDNAcA confirmed BTCH1 as a catalytic topo-IIα inhibitor. Conclusion: The results of this study provide insights into the mechanism of BTCH1 activity in leukemia model. Additional investigations are necessary to find out whether catalytic inhibition of topo-IIα activity by BTCH1 is ATP-dependent, as well as to determine selectivity of BTCH1 toward topo-IIα enzyme.

Keywords: topoisomerase II-alpha; thicarbohydrazone; catalytic topo II- alpha inhibitor; acute human monocytic leukemia

P23

Investigation of the molecular effects of palbociclib and celastrol combination treatment in pancreatic cancer cells

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Background: Pancreatic cancer is the seventh leading cause cancer-associated deaths worldwide. Drug resistance is a major problem associated with the loss of regulation of the cell cycle in pancreatic cancer treatment. Therefore, therapeutic approaches targeting the cell cycle gains importance in pancreatic cancer therapy. Palbociclib is a potent CDK4/6 inhibitor that blocks Rb phosphorylation and inhibits E2F release, leading to G1 phase arrest and suppression of tumor growth. Considering the better outcomes of combination therapy in pancreatic cancer, a potent leptin sensitizer Celastrol is used in this study to enhance the efficiency of palbociclib. Celastrol functions in the inhibition of the malignant progression of cancer, maintaining the homeostasis of lipid metabolism and triggering apoptosis by regulating genes involved in lipid synthesis and catabolism. The current study aims to elucidate the potential roles of palbociclib and celastrol combination treatment in Panc-1, MiaPaCa-2, Capan-2, Aspc-1 pancreatic cancer cells related to fatty acid metabolism. Results: Our results showed that individual treatment of celastrol or palbociclib reduced cell viability and proliferation of cells in dose-dependent manner. Combinational treatment of palbociclib and celastrol enhanced the cell viability decrease. In addition, PI3K/Akt and epithelial-mesenchymal transition (EMT) signaling were activated by suppressing fatty acid metabolism. Conclusion: According to our results, co-treatment of palbociclib and celastrol is a novel insight into pancreatic cancer therapy related to elucidating the association of fatty acid and apoptotic mechanisms.

P24

MiR-93-5p expression in response to the systemic, targeted, and combinational therapy for metastatic colorectal cancer and therapy resistance: *in vitro* analysis

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Background: Addition of a targeted agent bevacizumab to the systemic chemotherapy (5-fluorouracil (5-FU) combined with oxaliplatin) improves the survival of metastatic colorectal cancer (mCRC) patients. However, half of them do not respond to therapy, so a challenge remains to identify treatment response biomarkers. The expression of microRNAs has been associated with the therapeutic response and resistance in CRC. The aim of this study was to analyze the miR-93-5p expression in response to the systemic, targeted, and combinational therapy for mCRC and its potential role in therapy resistance. Material and Methods: Human metastatic colon adenocarcinoma SW620 cells and colonic epithelial progenitor HCEC-1CT cells were treated with FOX (21.4 µM 5-FU + 85 µM oxaliplatin) and/or 25, 85 and 250 µg/mL bevacizumab for 72 h. Cell response was evaluated through cell viability using MTT assay, while miR-93-5p expression was analyzed by quantitative reverse transcription polymerase chain reaction. Expression of hsa-miR-93-5p was also analyzed in 5-FU-resistant SW620 cells. Results: FOX and FOX/250 µg/mL bevacizumab treatments significantly reduced viability of SW620 and HCEC-1CT cells. Increasing concentrations of bevacizumab alone or in combination with FOX reduced the viability of HCEC-1CT cells. FOX and FOX/250 µg/mL bevacizumab combination significantly decreased miR-93-5p expression in HCEC-1CT cells, but not in SW620 cells. Expression of miR-93-5p was similar in SW620 cells compared to the 5-FU-resistant SW620 cells. Conclusion: Combination of targeted and systemic treatment for mCRC affects SW620 viability, but not miR-93-5p expression. MiR-93-5p was not associated with 5-FU resistance. Thus, miR-93-5p does not have a role in therapy response or resistance in mCRC.

Keywords: metastatic colorectal cancer, chemotherapy, bevacizumab, hsa-miR-93-5p

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P25

Overcoming paclitaxel-induced multidrug resistance in glioblastoma cells by using a combination of metformin and bafilomycin A1

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Background: Glioblastoma (GBM) is the most frequent and aggressive malignant brain tumor, and is associated with poor patient survival. Conventional GBM treatment includes surgery, radiotherapy and chemotherapy, however drug resistance and relapse continue to occur which highlights the need for alternative approaches. The use of autophagy modulators in combination with chemotherapeutic drugs has potential therapeutic value. Material and methods: The effects of bafilomycin A1, an inhibitor of lysosomal degradation, and metformin, a drug commonly used for type 2 diabetes treatment, that induces autophagy through mTOR inhibition, were studied in GBM cell line U87 and its multidrug resistant counterpart U87-TxR. The effects of bafilomycin A1 and metformin on autophagy, cell death, and cell growth were evaluated by western blot, flow cytometry and SRB assay. Results: U87-TxR cells responded differently to autophagy modulation, in comparison to sensitive parental U87 cells. Metformin induced cell death in U87-TxR cells but not in U87, while bafilomycin A1 further enhanced metformin-induced cell death of multidrug resistant cells. Furthermore, a co-treatment with metformin and bafilomycin A1 reversed paclitaxel-induced resistance in multidrug resistant cells. Conclusion: These results suggest that metformin and bafilomycin A1 could be used to enhance the cytotoxicity of classic chemotherapeutics and support further research into compound combinations as a therapeutic approach that helps to overcome multidrug resistance.

Keywords: bafilomycin A1; glioblastoma; metformin; multidrug resistance; paclitaxel

P26

Anti-obesity drug Orlistat (Xenical®) induces antiangiogenic potential in breast cancer cell lines

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Background: Obesity is one of the risk factors for development of breast cancer among women. Human breast cancer is a heterogeneous group of invasive tumors with a specific response to therapy, so treating and preventing this disease is difficult. The modern lifestyle imposes daily unhealthy habits which may contribute to the development of obesity-associated breast cancer. A great effort has been made to investigate non-standard drugs to improve treatment protocols. Orlistat as US Food and Drug Administration-approved drug for bodyweight loss has been demonstrated to exhibit antitumor properties towards breast cancer cells by its ability to induce cytotoxic, proapoptotic, antiangiogenic, and antimetastatic effects. Material and Methods: The immunocytochemistry staining method was used to evaluate the protein expression of CXCR-4, $VEGF_{165}b$, MMP-9 as angiogenic markers in triple-negative breast cancer cell lines (MDA-MB-231 and MDA-MB-468) under the influence of Orlistat in two doses (1 and 25 µg/ml). Results: Angiogenic factors are highly expressed in obesity-linked breast cancer, maintaining tumor progression and angiogenesis, so their suppression may result in a positive outcome in patients' therapy. Our results indicate that treatment whit Orlistat induces a significant dose-dependent suppression of monitored parameters in breast cancer cell lines concerning control values. The MDA-MB-468 cells were more sensitive to the applied drug, which is linked to poor aggressiveness, compared whit highly aggressive MDA-MB-231 cells originated from same organ. Conclusion: Based on our results Orlistat can be considered as a novel inhibitor of CXCR-4, $VEGF_{165}b$, and MMP-9 which are strongly linked to cancer progression, angiogenesis, and metastasis.

Keywords: angiogenesis, breast cancer, CXCR-4, MMP-9, orlistat, $VEGF_{165}b$

P27

Molecular mechanisms of nanoparticle-mediated biological effects in doxorubicin treated cells

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Background: Engineered nanomaterials are at the leading edge of the rapidly advancing domain of nanomedicine, particularly in cancer research. Cytoprotective properties of nanoparticles due to their ROS scavenging capacity may significantly increase the therapeutic potential of cytotoxic drugs. Therefore, the main aim of our *in vitro* study was to investigate the expression of genes in fullerenol (FNM) treated cells in order to evaluate its therapeutic potential. Material and methods: Expression of genes involved in key cellular functions and processes, such as proliferation, apoptosis, redox regulation, DNA damage and repair was measured in K562 cells by the means of quantitative real-time PCR. RNA for cDNA synthesis was isolated from doxorubicin (DOX) treated erythroleukemia K562 cells, from FNM and from FNM+DOX treated cells. Gene expression was analyzed using comparative Ct method, and statistical analysis was performed using one-way Anova and Tuckey's post-hoc test. Results: Expression analysis of genes involved in antioxidative cell defense showed that FNM significantly suppresses DOX-induced inhibition of *MnSOD*, *GR* and *gGCS*. DOX-induced block in proliferation, as shown by decreased Ki67 expression was maintained also in FNM+DOX group of cells. FMN alone induced down-regulation of pro-apoptotic *BAX*, which is mediated by FMN-induced over-expression of BAX-inhibitor. Up-regulated BAX-inhibitor diminishes ER-stress-induced ROS accumulation and it is involved in induction of *HMOX*, *gGCS* and *GST* in K562 cells. Expression of *hOGG1* coding for base-excision repair enzyme was inhibited in FNM+DOX group, indicating that fullerenol contributes to increased K562 sensitivity to DOX. Conclusion: Fullerenol modulates redox status of DOX-treated K562 cells, it synergistically contributes to the proliferation block induced by DOX and exerts important ROS scavenging and apoptosis-modulating properties in erythroleukemia cells.
Key words: oxidative stress, nanoparticle, doxorubicin, apoptosis.
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P28

Effect of lipid raft disruption on AQP3, AQP5, and EFGR pathway

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Background: Breast cancer is the leading cause of morbidity and mortality of cancer in women. Therefore, there is a need for understanding modifications in biological processes and signaling pathways that contribute to breast cancer malignancy. Oxidative stress is certainly an important factor that encourages tumor growth by supporting several hallmarks of cancer. Peroxiporins are aquaporins that channel hydrogen peroxide and could, therefore, play a role in regulating intracellular levels of oxidative stress, consequently regulating important signaling pathways. Materials and methods: In this study, the effect of lipid raft disruption on AQP3, AQP5, and EFGR pathway was explored. Lipid rafts were disrupted by cholesterol depletion using methyl-β- cyclodextin. The effect of depletion was measured by MTT and BrdU incorporation assay, and the effects on EGFR signaling pathway was assayed by Western blot levels of pathway components. Results: Our results indicate that EGFR signaling pathway is affected by cholesterol depletion. Conclusions: We have managed to disrupt lipid rafts to interfere with EGFR signaling and are currently studying the consequences on signaling pathway components and levels of aquaporin 3 and aquaporin 5.
Keywords: aquaporin, breast cancer, EGFR, lipid raft disruption.

P29

Studying the ability of tumor multidrug-resistant cells and drug-sensitive counterparts to release and capture extracellular vesicles

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Background: Multidrug resistance (MDR) is one of the main challenges for cancer treatment efficacy. MDR is a phenomenon by which tumor cells develop cross-resistance to several unrelated drugs and is frequently caused by an overexpression of drug-efflux pumps, particularly P-glycoprotein (P-gp). Interestingly, in different tumor models, Extracellular Vesicles (EVs) carrying P-gp in their cargo were found to promote the horizontal transfer or MDR phenotype between MDR cells and drug-sensitive cells. The aim of the present work was to unravel the mechanisms involved in the release of EVs by MDR cells and their uptake by drug-sensitive counterparts. Material and methods: To achieve this, two pairs of cell lines from two different tumor models [chronic myeloid leukemia (CML) and non-small cell lung cancer (NSCLC)] were used, consisting of a drug-sensitive cell line and its MDR (P-gp overexpressing) counterpart. EVs released by those pairs of cell lines were isolated by ultracentrifugation and properly characterized by some of us. Results: Our results showed that MDR cells released more EVs than their drug-sensitive counterparts and also that drug-sensitive cells captured more EVs than their MDR counterparts. This difference (in the release and capture of EVs by drug-sensitive and MDR cells) may be associated with differences in the endocytic pathway. Importantly, manipulation of the recycling pathway influenced the response of drug-sensitive cells to doxorubicin treatment. Conclusion: Increasing knowledge about the players involved may contribute to identifying targets to overcome the horizontal transfer of MDR.

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P30

I1-imidazoline receptor ligand inhibits P-glycoprotein efflux in pancreatic ductal adenocarcinoma cells

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Background: One of the most common causes of treatment failure in cancer is overexpression of drug efflux pumps, which leads to decreased intracellular drug accumulation and chemotherapy efficacy. P-glycoprotein (P-gp) is one of the most studied membrane drug efflux transporters, frequently expressed in pancreatic ductal adenocarcinoma (PDAC) and contributing to its poor 5-year survival rate of just 6%. The aim of this study was to examine the effects of I1-imidazoline receptor ligands clonidine, moxonidine, rilmenidine, and efaroxan on the P-gp levels and function in PDAC cells *in vitro*. These drugs contain an imidazoline ring within their structure that may inhibit the activity of adenosine triphosphate (ATP) - sensitive channels, such is P-gp. Material and Methods: The effect of tested compounds on Calcein AM efflux was examined using flow cytometry for adherent Panc-1 and MiaPaCa-2 cells, and fluorescent microscopy for same cells grown in 3D culture. P-gp surface levels in PDAC cells were analyzed by flow cytometry after 48h of treatment with 100µM rilmenidine. Results: Out of the tested compounds, only rilmenidine inhibited Calcein AM efflux, and it did so in a dose dependent manner in both 2D and 3D cultures. Importantly, treatment with 100µM rilmenidine did not have significant effects on the P-gp expression in PDAC cells. Conclusion: Our preliminary findings indicate that rilmenidine has a potential to help overcome multidrug resistance in PDAC. Given that rilmenidine can target several other aspects of the PDAC pathology (cell invasiveness, survival, metabolism, and the microenvironment), it holds a great promise for developing more effective PDAC treatment.
Keywords: pancreatic ductal adenocarcinoma, P-glycoprotein, rilmenidine

P31

Combination of sirtuin 3 and hyperoxia diminishes tumorigenic properties of MDA-MB-231 cells

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Background: Since the role of the major mitochondrial NAD+-dependent deacetylase, sirtuin 3 (Sirt3), is differential in cancer, opposite to the well-known tumor-suppressing effect of hyperoxia, this study aimed to investigate the role of Sirt3 in triple-negative breast cancer (TNBC) cell line MDA-MB-231 upon hyperoxic (95% O2) conditions. Material and methods: MDA-MB-231 cells were stably transfected with Flag-tagged Sirt-3 or empty plasmid. Western blot and real-time PCR were used to monitor the expression of proteins or genes involved in mitochondrial biogenesis, metabolic regulation and antioxidant defense. Immunocytochemistry and confocal microscopy were used to confirm the cellular localization and abundance of proteins. Flow cytometry was used to analyze mitochondrial mass, potential and ROS production, and MTT test as a measure of metabolic activity. Mitotic index analysis, colony-forming unit assay, DNA damage and Annexin V-FITC analyses were used to assess the differences in the growth and apoptosis rate. Results: Although Sirt3 seemed to improve mitochondrial properties by increasing mitochondrial mass and potential, metabolic activity (Warburg effect) and antioxidative defence (SOD2, Cat), it also increased mitochondrial ROS, induced DNA damage, *timp-1* expression, formation of multinucleated cells and apoptosis, and finally markedly reduced the proliferation of MDA-MB-231 cells. All these effects were even more evident upon the hyperoxic treatment, thus pointing towards combined negative effect of Sirt3 and hyperoxia on MDA-MB-231 cells. Conclusion: Both Sirt3 and hyperoxia, alone or in combination, have the potential to negatively affect the malignant properties of the MDA-MB-231 cells and should be further explored as a possible therapy for TNBC.

Keywords: breast cancer, hyperoxia, MDA-MB-231, oxidative stress, sirtuin 3

P32

Ursodeoxycholic acid influences antioxidative capacity in human breast adenocarcinoma cell line through Nrf2-dependent axis

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Background: The transcription factor, nuclear respiratory factor-2 (NRF2) is one of the main orchestrators of the cellular antioxidant response. The main objective of this *in vitro* study is to analyze the effects of ursodeoxycholic acid on the cytotoxic activity of doxorubicin, and the expression of NRF2 and antioxidant enzymes at the transcription level. Material and methods: MCF-7 cells were incubated for 24 h in a medium containing doxorubicin alone (0.25μM) or in combination with ursodeoxycholic acid (50μM). Cytotoxicity was determined using the MTT assay, whereas gene expression was determined using RT-qPCR method with beta-actin as a reference gene. Gene expression was analyzed using comparative Ct method, and statistical analysis was performed using one-way Anova and Tuckey's post-hoc test. Results: Incubation of MCF-7 cells with doxorubicin and ursodeoxycholic acid resulted in an increase in cytotoxicity compared to the group of cells incubated with doxorubicin only ($p=0.005$). Treatment of cells with doxorubicin significantly reduced the expression of *NRF2*, *SOD*, and *GR* compared to the control group ($p<0.001$; $p<0.001$; $p=0.002$, respectively). In the co-treated cells, the expression of *NRF2* was also significantly reduced ($p=0.004$), however, the expression of *SOD* and *GR* was highly statistically significantly reduced ($p<0.001$) compared to the group of cells incubated with doxorubicin only. Conclusion: Ursodeoxycholic acid and doxorubicin synergistically increased cytotoxicity in the MCF-7 cell line, by reducing the expression of *NRF2,* and its downstream targets, *SOD* and *GR* at the transcription level, aggravating redox homeostasis in malignant cells.

Key words: oxidative stress, bile acid, breast adenocarcinoma, doxorubicin, apoptosis

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P33

Association between *TGFB1* C-509T polymorphism and acute toxicity after radiotherapy for prostate cancer

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Background: Radiotherapy (RT) for prostate cancer (PCa) is associated with a spectrum of side effects (toxicity) in the surrounding normal tissues. TGFB1 is a key cytokine associated with inflammation and fibrosis, but its role in acute toxicity is unclear. The presence of T allele at −509 bp of the promoter region of *TGFB1* gene is related to higher concentrations of TGFB1 than C allele. We aimed to investigate association between C-509T polymorphism (rs1800469) and RT-induced acute toxicity. Patients and methods: Eighty six patients who had a localized or locally advanced PCa were treated with radical (72 Gy)(n=49) or postoperative/salvage (66 Gy)(n=37) RT without previous hormonal therapy. *TGFB1* C-509T was determined by PCR-RFLP analysis on DNA from PBMC. The differences in the distribution of genotypes between patients with or without acute genitourinary (GU) or gastrointestinal (GI) toxicity were tested by $\chi 2$ and Fisher's exact test. P values ≤0.05 were considered statistically significant. The genotype-specific associations with toxicity were estimated as odds ratios (OR) with 95% confidence intervals (CIs) for dominant, recessive, codominant and overdominant genetic model. Results: Heterozygote carriers of *TGFB1* C-509T had statistically significant lower rate of acute GU and GI toxicity then homozygotes (CC *plus* TT) (p=0.048; p=0.047). Additionally, the OR indicated lower risk for acute toxicity development in heterozygote than homozygote patients (OR (95%CI) were: 0.12 (0.01- 1.11) for GU and 0.19 (0.03- 1.02) for GI). Conclusion: The obtained data indicate that CT genotype of *TGFB1* C-509T could be potential biomarkers of lower risk for acute RT-induced toxicity.

Keywords: polymorphism, prostate cancer, radiotherapy, toxicity

P34

Impact of *TGFB1* Leu10Pro polymorphism on acute radiotherapy-induced toxicity in prostate cancer patients

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Background: Radiotherapy (RT)-induced acute toxicity associated with bladder and bowel injury has great impact the quality of life for prostate cancer (PCa) patients. TGFB1 is a key proinflammatory and profibrotic cytokine, but its role in acute toxicity is still unclear. *TGFB1* T>C transition at codon 10 results in leucine to proline substitution and increased TGFB1 protein levels. The aim of this study was to examine impact of *TGFB1* Leu10Pro (rs1800470) polymorphism on RT-induced acute toxicity in PCa patients. Patients and methods: Eighty two patients who had a localized or locally advanced PCa were treated with radical (72 Gy)(n=47) or postoperative/salvage (66 Gy)(n=35) RT without previous hormonal therapy. *TGFB1* Leu10Pro was determined by PCR-RFLP analysis on DNA from PBMC. The differences in the distribution of genotypes for dominant, recessive, codominant and overdominant genetic model between patients with or without acute genitourinary (GU) or gastrointestinal (GI) toxicity as well as different grade of toxicity were tested by $\chi 2$ and Fisher's exact test. P values ≤ 0.05 were considered statistically significant. Results: Heterozygote PCa patients had lower rate of acute GU and GI toxicity then homozygotes (LeuLeu, LeuPro, ProPro were: 100%, 90.7%, 100% for GU and 92.0%, 88.4%, 100%, respectively for GI). The frequency of toxicity grade ≥ 2 were higher in LeuPro then both homozygote carriers (41% vs. 28.2% for GU and 26.3% vs. 21.6% for GI acute toxicity). The differences were not statistically significant. Conclusion: The present study did not establish impact of *TGFB1* Leu10Pro polymorphism on RT-induced acute toxicity in PCa patients.

Keywords: polymorphism, prostate cancer, radiotherapy, toxicity

P35

Organ preservation approach for distant located rectal cancer

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Background: Standard treatment for locally advanced rectal carcinoma (LARC) is neoadjuvant chemoradiotherapy (CRT) followed by surgery. Complete clinical (cCR) or pathologic response is registered in up to 30% of patients, using standard fractionation and total dose (TD) of 45-50.4 Gy. The aim of this study was to evaluate the optimal time for assessment of response in order to identify candidates for watch and wait approach (WW). Patients and Methods: Patients with LARC were treated with long-course CRT. Radiotherapy was delivered using new approach, volumetric modulated arc therapy-simultaneous integrated boost with TD of 54 Gy. Concomitant chemotherapy (5FU, Leucovorine) was given during first and fifth week of RT. Tumor response was assessed in eighth week after CRT with MRI scan and proctoscopy. Patients with cCR (mrTRG1)/near cCR (mrTRG2) and distant located tumor were suggested no immediate radical surgery, and were enrolled in WW with the aim of sphincter preservation. Results: Between June 2020 and January 2021 thirty patients were included. cCR/near cCR according to MRI was detected in 37% of patients. Four patients were enrolled in WW. In three of them, control MRI was categorized as near cCR in combination with negative proctoscopy examination. In these patients we postponed surgery longer and did one more MRI 4 weeks after, which was categorized as mrTRG1. Conclusion: For patients with distant located tumors with cCR after CRT, where sphincter preservation isn't optional, WW would be beneficial. Further studies with identification of prognostic biomarkers could be crucial to identify the best candidates for WW.

Keywords: chemoradiotherapy, complete clinical response, rectal carcinoma.

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NF-κB as common target gene of miRNAs related to oxidative stress and prostate cancer radiotherapy response

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Background: Between 50% and 60% of cancer patients receive radiotherapy (RT). Oxidative stress (OS), DNA repair, and hypoxia in combination with individual genetic/epigenetic background are major events influencing the individual response to radiation exposure. Deleterious effects of irradiation (IR) are strongly associated with OS. Oxidative stress occurs when cells fail to maintain balance between the production and scavenging of reactive oxygen species (ROS). Overproduction of ROS is associated with side effects of RT. MicroRNAs are small non-codding RNA involved in a wide variety of cellular processes and radiation response amongst others. Our aim was to investigate common target gene silenced by the highest number of miRNAs involved in oxidative stress during RT. Material and methods: Two approaches have been used to elucidate miRNA network and common target genes-literature survey and bioinformatics analysis with miRNet online software. Based on literature survey results, we performed multiple combinatorial queries for hsa-miR-17-3p/21/34a/96/122/146a/155/193a/200a/206/210 involved in oxidative stress in response to IR/RT in PCa. Results: Common target of the highest number of miRNAs (4)-miR-21/34a/146a/155 was nuclear factor-κB (NF-kB). According to "Function Explorer search" of miRNet, KEGG pathway listed molecules belonging to signaling pathway involved in PCa apoptosis regulation. Conclusion: The most promising miRNAs which should be investigated further in response to RT and oxidative stress in PCa are miR-21/34a/146a/155, and especially its interaction with NF-kB. Transcription factor NF-κB is considered as crucial link between inflammation and cancer and can be activated by ROS and IR. Modulating NF-κB signaling in PCa cells may influence on response to RT.

Keywords: MicroRNA; Oxidative stress; Prostate cancer; Radiotherapy

P37

In silico analysis of predictive biomarkers for neoadjuvant chemoradiotherapy in locally advanced rectal cancer

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Background: Standard treatment for locally advanced rectal cancer (LARC) is neoadjuvant chemoradiotherapy (nCRT). Response to therapy varies among patients and there is a huge need for discovering biomarkers that would enable better prediction of the response. The aim of this study was to evaluate predictive markers for CRT in search for LARC patients that might benefit from no immediate radical surgery, but rather an enrollment in a watch and wait approach. Material and methods: We searched the publicly available NCBI database Gene Expression Omnibus (GEO). Gene Set Enrichment Analysis (GSEA) was performed on selected data sets using keywords rectal cancer, CRT and response. LARC patients were divided into groups according to the pathohistological Mandard tumor regression grading (TRG) system as Responders (TRG 1-2) and Non-responders (TRG 3-5). Hallmark, KEGG, and Reactome gene sets were used to compare expression levels between the two groups. Results: Gene expression sets of inflammatory response-related signaling pathways were found to significantly correlate with good response to CRT ($p<0.05$; FDR<0.25) in patients with LARC. Overlapping the results the following genes were discovered as predictors of favorable response: CXCL10, IDO1, CXCL9, CYBB, TGFB2, PLAU, PDGFRB, INHBA, IL24, HGF, and IL6. Conclusion: A set of inflammatory response-related genes were found to correlate with favorable response to CRT. Validation of these *in silico* discovered biomarkers is under way in a cohort of 94 LARC patients and might lead to a better selection of patients who could benefit from a wait and watch approach thus increasing their quality of life.

Keywords: GSEA, nCRT, rectal cancer, inflammatory response.

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Clinopodium nepeta (L.) Knutze essential oil *in-vitro* anti-proliferative activity on PC-3, Du145 and LS174 human cancer cell lines

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Background: Due to the diversity of chemical structures of natural products, it has been observed that some components often individually or in synergy with others have a toxic effect on malignant cells. Plants of the genus *Clinopodium* are known in folk medicine for their contribution to human health. Material and methods: Essential oils of wild *Clinopodium nepeta* (L.) Knutze from southern Bosnia and Herzegovina, from various different habitats (Pirići, Lokve, Blagaj and Počitelj), were the subject of this research. Using a modified Clevenger apparatus essential oils (EOs) samples were obtained by hydrodistillation. Qualitative and quantitative analysis was performed by GC-MS. EOS effect on PC-3, Du145, and LS174 cancer cell survival was determined calorimetrically using 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide dye (MTT assay). Human cancer cell lines used were obtained from the American Type Culture Collection (Mansas, VA, USA). Results: Chemical characterization revealed *trans*-piperitenone oxide in samples from Lokve and Počitelj (60,03 and 51,66%) and pulegone in samples from Pirići and Blagaj (23,17 and 43,86%) as predominant compounds. The results of cells proliferation measurments by MTT assay, expressed as IC50 (EOs concentration in µg/mL inhibiting cell survival by 50%), indicated high toxicity to cancer cells. The EOs of *C. nepeta* showed strong antiproliferative activity (IC50=1.55±0.27 to 17.42±0.81 µg/mL) against all three cell lines. In general, all four tested EOs samples showed the best activity against LS174 cells, especially the samples from Lokve and Počitelj (1.71±0.58 and 1.55±0.27 µg/mL). Conclusions: Presented dates can be interpreted by the existence of a positive correlation between the presence of certain components in EOs and their antiproliferative activity. To the best of our knowledge, no other work has produced results that show the effects of clinopodium nepeta EOS on the mentioned human cancer cell lines. Therefore, this research can serve as a good preliminary guideline for planning of *in vivo* studies on the same and other cancer cells.

Keywords: Lamiaceae, *Clinopodium nepeta* (L.) Knutze, essential oil, chemical composition, anti-proliferative activity, MTT assay.

P39

An Adapted One-dimensional Computational Approach for Irregular ROI Analysis Improves Osteosarcoma Classification

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Background: The analysis of irregularly shaped tumour ROIs is hindered by the fact that most image analysis methods apart from first-order statistics are compatible only with rectangular ROIs. We thus aimed for the first implementation and testing of the 1-D MRI image analysis method that is fully compatible with irregular ROIs. Material and methods: The retrospective prediction model of osteosarcoma chemoresponsiveness included T2-weighted MRI scans obtained before OsteoSa MAP neoadjuvant cytotoxic chemotherapy. Osteosarcoma morphology was quantified by calculating the one- and two-dimensional (1-D, 2-D) Higuchi dimensions (Dh), directionally and non-directionally. Results: The non-directional 1-D Dh reached a predictive AUC of 0.88, while the directional 1-D analysis along 180 radial lines robustly improved the predictive performance, reaching an AUC of 0.95, $P<0.001$ that is widely considered as nearly ideal. The optimal directional range was between 90^{o} and 97^{o}. Conclusions: We report the first validity testing of the 1-D analysis approach that is fully compatible with irregular ROIs. Such analytical adaptation to ROI shape in MRI has enhanced the osteosarcoma prediction performance over the previously reported standard 2-D analyses. The clinical importance of the early chemoresponsiveness prediction rests on its potential to prolong the survival of chemoresistant patients through personalised treatment adjustments.

P40

Evaluation of the Potential Effects of *Cimcifuga racemosa* Extract and Natural Compounds on Different Cancers

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Background: Conventionally, different approaches are used for the treatment of different cancer types, especially chemotherapy, radiotherapy, biological therapy, immunotherapy and surgical methods. However, observing the negative effects of these treatment methods increases the importance of new generation drug development strategies. Natural compounds have been reported to be effective in the prevention and treatment of various cancer subtypes Drugs designed by isolating the secondary metabolites of plants are of great importance and 25% of the drugs used in modern medicine are natural compounds. The *Cimcifuga* species grown in Far East Asian countries has been used in the treatment of gynecologic disorders and menopausal symptoms and has been reported to have potential in anti-cancer therapy. Material and methods: In this study, a literature review of studies exploring the medicinal use of *Cimcifuga racemosa* is presented. Results: *Cimcifuga racemosa* extract have been reporte to inhibit the proliferation of estrogen receptor-positive and negative human breast carcinoma cell lines by induction of apoptosis. Its use has also been explored for the management of postmenopausal symptoms in breast cancer survivors. Studies using prostate cancer cells have reported that by inhibiting prostate specific antigen (PSA), the growth of cancer cells is blocked thus exhibiting an antiproliferative effect. Additionally, *Cimcifuga racemosa* extracr has been reported to be able to prevent benign prostatic hyperplasia through its 5-reductase inhibitors. Conclusions: *Cimcifuga racemosa* plant extract has the potential to be used in cancer treatment but further studies evaluating its activity *in vivo* are necessary.

Keywords: anti-cancer therapy, *Cimcifuga racemosa,* natural products.

P41

Screening of the cytotoxic, antibacterial and antifungal activities of *Saccorhiza polyschides* algae extract

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Background: Marine macroalgae are well known for their anticancer and antimicrobial potentials as well as a benefit to overall human health after consumption. They are rich in bioactive compounds such as polysaccharides, fatty acids, pigments, terpenes and phenolic compounds. Material and Methods: *Saccorhiza polyschides* was collected from the Atlantic coast of Morocco (Sidi Bouzid coast). Dichloromethane-methanol extract of the algae was tested for *in vitro* cytotoxicity by MTT assay against human cervical adenocarcinoma (HeLa), colorectal adenocarcinoma (LS-174T), lung carcinoma (A549), and chronic myelogenous leukemia (K562) cell lines. Antibacterial and antifungal activities were analyzed using micro-dilution method to determine minimal inhibitory concentration (MIC). Bacterial species used were: *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli* and *Proteus mirabilis*. Fungal species used were: *Mucor mucedo*, *Trichophyton mentagrophytes*, *Aspergilus niger*, *Candida albicans* and *Penicillium italicum*. Results: Extract showed high cytotoxic activity for natural products *in vitro* toward malignant cell lines. The highest cytotoxic activity of *S. polyschides* extract was exerted toward K562 (48.45±0.72 µg/ml) and HeLa (67.87.45±4.42 µg/ml) cell lines. Antibacterial activity was most pronounced against *B. subtilis* and *B. cereus* (3.45 mg/ml) bacteria. Antifungal activity of the extract was the highest toward *C. albicans* (3.45 mg/ml) fungus. Conclusion: Results showed promising cytotoxic antibacterial and antifungal activities of the *S. polyschides* extract, however further tests are needed to confirm these actions and to determine active components that are responsible for these actions.

Keywords: algae, cytotoxic, drug, antimicrobial, *in vitro*, anticancer

P42

Antineuroblastoma potential of polyoxopalladate(II)

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Background: Polyoxometalates are a class of anionic, polynuclear metal-oxo clusters reported as promising *in vitro* and *in vivo* antitumor agents for several decades. The aim of this study was to investigate the antineuroblastoma potential of the polyoxopalladate(II) nanocube $Na8[Pd_{13}As_8O_{34}(OH)_6]\cdot 42H2O$ (Pd_{13}).

Material and methods: All experiments were performed on human neuroblastoma cell line, SH-SY5Y. The number of viable cells after the treatment with Pd_{13} was assessed using an acid phosphatase viability assay. The level of superoxide ion, mitochondrial membrane potential, pan-caspase activity, acidic intracellular vesicles content, and the cell cycle was determined by flow cytometry. Results: The obtained results suggest that Pd_{13} caused a significant decrease in cell viability with IC50 values of 7.7 µM (24 h) and 4.4 µM (48 h). Pd_{13} induced depolarization of mitochondrial membrane (2 h), followed by ~ 30% increase in the production of the superoxide ion (O_2-) 4 h after treatment. An increase (~ 30%) in pancaspase activation and disturbance of neuroblastma cell cycle were observed after 24 h treatment. Namely, Pd_{13} caused an increase (14.4%) in the number of cells with fragmented nuclear DNA ($SubG_0$), a decrease (%) of cells in the G_1 phase, and an increase (%) in the S phase, all suggestive of cell cycle arrest. Finally, Pd_{13} increased the orange to green fluorescence ratio for ~ 45% 24 h after treatment, supporting intracellular acidification.

Conclusion: The polyoxopalladate, Pd_{13} can be regarded as a promising antineuroblastoma agent which induces oxidative stress, and causes pan-caspase activation, DNA fragmentation and cell cycle arrest, which are all hallmarks of apoptotic neuroblastoma cell death.

Keywords: polyoxopalladates, antitumor, neuroblastoma, apoptosis

P43

Selected polyoxopalladates as potential antitumor drug candidates

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Background: Polyoxo-noble-metalates, a class of molecular noble metal-oxo nanoclusters that combine features of both polyoxometalates and noble metals, are a promising platform for the development of next-generation antitumor metallodrugs. The aim of this study was to evaluate the antineuroblastoma potential of three novel polyoxopalladates. **Material and methods:** All experiments were performed on human neuroblastoma cell line, SHSY5Y. The three polyoxo-noble-palladates $Na_4[SrPd_{12}O_6(OH)_3(PhAsO_3)_6(OAc)_3]$ ▪ $2NaOAc$ ▪ $32H_2O$ ($SrPd_{12}$), $Na_{12}[Sn^{IV}O_8Pd_{12}(PO_4)_8]\cdot 43H_2O$ ($SnPd_{12}$) and $Na_{12}[Pb^{IV}O_8Pd_{12}(PO_4)_8]\cdot 38H_2O$ ($PbPd_{12}$) were investigated in our study. The viability of neuroblastoma cells after 24h treatment was assessed using an acid phosphatase assay. The level of superoxide ion, mitochondrial membrane potential, pan-caspase activity, cell cycle analysis and acidic vesicles content were determined by flow cytometry using appropriate fluorochromes. **Results:** Calculated IC_{50} (μM; 24h) values were 75.8 ± 6.7 ($SrPd_{12}$) and >>100 ($SnPd_{12}$ and $PbPd_{12}$), selecting $SrPd_{12}$ as the most efficient. $SrPd_{12}$ did not affect the mitochondrial membrane potential and superoxide production in neuroblastoma cells after short (2 h and 4 h) exposure. Also, it did not induce an increase in the number of neuroblastoma cells with fragmented DNA content, but displayed the cell cycle arrest: the ~ 23% reduction of neuroblastoma cells in G_0/G_1 phase and the ~ 17% increase in S phase. The treatment with $SrPd_{12}$ did not increase the level of acidic vesicles but it increased the activity of caspases five-fold. **Conclusion:** Only $SrPd_{12}$ exhibited a satisfactory antineuroblastoma action by inducing caspase activation and neuroblastoma cell cycle arrest.

Keywords: polyoxopalladates, neuroblastoma, antitumor

P44

Potential of Mesoporous Silica Nanoparticles for Applications in Targeted Treatment of Cancer

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Background: Mesoporous silica nanoparticles (MSN) are being vastly demonstrated in the literature as efficient nanocarriers for cancer therapeutics. Their high surface area (ca. 1000 m^2/g), well-structured porosity, versatile possibilities for obtaining nanocarriers of different morphologies, pore diameters and surface characteristics, allow construction of different nanosystems for efficient cancer targeting. **Methods:** In this study, the potential of mesoporous silica nanoparticles for applications in targeted treatment of cancer is presented. **Results:** Our team focuses on construction and surface functionalization of MSN with different moieties for active cancer-targeting. The drug release process can be also controlled through different externally applicable stimuli (eg. light irradiation, magnetic field), or upon exposure to intratumoral conditions such as weakly acidic environment, the elevated concentration of glutathione or other cancer biomarkers. In addition, MSN can be decorated with specific moieties to enable imaging of cancer (eg. magnetic resonance imaging (MRI)), which would enable simultaneous cancer therapy and diagnostics (theranostics). **Conclusions:** Mesoporous silica nanoparticles have substantial potentials for enhancing the efficacy and precision of cancer treatment.

Keywords: mesoporous silica nanoparticles, nanocarriers, targeted anti-cancer drug delivery.

P45

Anticancer effects of sclareol and its derivatives in glioblastoma cells

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Background: Glioblastoma is the most common, aggressive and lethal brain tumor in adults with high proliferation rate, infiltrating nature and presence of multidrug resistance (MDR). Sclareol (SC) is a naturally occurring labdane type diterpene, derived from *Salvia sclarea*. We examined cell growth inhibition effect of SC and its derivatives (PAS and TNT groups of compounds) - hybrid (chimeric) molecules. Sclareol was covalently bonded to [1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine scaffold, and different diamines were used as linkers. We also studied SC potential to reverse DOX resistance and its accumulation. The combination of SC with DOX has been earlier described to potentiate DOX cytotoxicity if simultaneously delivered in nanoparticles. Material and Methods: SC in combination with DOX as well as SC derivatives were tested on human glioma cell line U87, and its MDR counterpart - U87-TxR. MTT assay was used to examine inhibition of cell growth. Accumulation of DOX was measured by flow cytometry. Results: Thirteen out of nineteen TNT derivatives and three out of six PAS derivatives showed stronger anti-glioma effect than SC. Simultaneous treatment of SC with DOX demonstrated potential of SC to reverse DOX resistance. Even more, SC significantly increased DOX accumulation in both glioblastoma cell lines. Conclusion: Results obtained in this study showed a considerable synergy of SC and DOX in glioma cells. Better results observed with SC derivatives make them good candidates for further testing.

Keywords: chemotherapy, doxorubicin, glioblastoma, MDR, sclareol

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P46

Development and validation of a simple and reliable UV-coupled HPLC assay for the determination of gemcitabine in serum: application in pharmacokinetic analysis

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Background: Gemcitabine (gem) is an important chemotherapeutic drug used for the treatment of pancreatic, and other cancers either as monotherapy or in combination with other medicines.
Aim: The development of a simple and reliable HPLC UV-coupled method for determining gemcitabine in serum. Material and methods: Sample preparation consisted of a single protein precipitation step with perchloric acid. Analysis was accomplished by a reversed-phase column eluted isocratically by sodium phosphate buffer (pH 6.6) and methanol (97/3, v/v) at flow-rate 1mL/min, detection wavelength at 267nm and column temperature at 40ºC. 1,7-dimethyluric acid was used as an internal standard. For the pharmacokinetic study NOD/SCID mice (n= 5/group) were used and the drug was administered. Mice received a single dose of gem at 100mg/kg either subcutaneous (sc) or intraperitoneal (ip). Blood samples were collected at 5, 15, 30min and 1, 2, 4 and 6h post gem administration. Results: Duration of analysis was ~12.5min. Calibration curve was linear with r^2= 0.999 over the range 1-400μM, coefficient of variation was <6.52% and bias <-7.77 %. Mean recovery of gem was 96.53% and the limit of detection was 0.17μM. T1/2, Tmax, Cmax and AUC_{0-t} were 1.03h, 0.083h, 272.14μmol/L and 135.99μmol/L*h for sc while the respective values for ip administration were 0.85 h, 0.083 h, 291.542 μmol/L and 121.227μmol/L*h. Conclusion: We developed a simple, valid and inexpensive HPLC method coupled to UV detection of gemcitabine for the determination of the drug in serum which may also be suitable for clinical practice.
Keywords: gemcitabine, HPLC, pharmacokinetics
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P47

Ruthenium (II) complexes as promising candidates for cancer therapy

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Background: Acting as single compounds, both Ru(II) complexes and phenothiazines are considered promising anticancer drugs with inhibitory effects on cancer cell growth and differentiation. The complexes synthesized by a combination of Ru(II) with N-alkylphenothiazines (chlorpromazine hydrochloride (1), thioridazine hydrochloride (2) and trifluoperazine (3)) are reported to reduce the cell viability of some cancer cell lines. This study explored whether the selected complexes affect the redox homeostasis and genome integrity of normal human blood cells and induce inhibition of membrane-bound enzymes at pharmacologically relevant doses. Material and Methods: To evaluate the genotoxic potential of complexes, the incidences of micronuclei and cell proliferation index were investigated in cultured human peripheral blood lymphocytes. The redox modulating effects were examined by measuring the catalase activity and malondialdehyde level as a measure of oxidative stress. The influence of complexes on enzymes Na^+/K^+-ATPase and AChE bound to the cell membrane was also analyzed. Results: The selected complexes did not affect the activity of Na+/K+-ATPase, while AChE activity was inhibited in a dose-dependent manner. Furthermore, the results have shown that complexes 1 and 2 displayed cytotoxic and prooxidant action. Conversely, complex 3 disturbed the viability and redox homeostasis of the normal cells only at the highest concentration applied. Conclusion: According to our data, all investigated complexes have the potential for use in anticancer therapy. Complex 3 has shown the most promising effects and should be further examined as part of the novel therapeutic strategy to develop a more effective and less toxic therapeutic agent.

Keywords: Ruthenium (II), N-alkyl phenothiazine, cytotoxicity, redox homeostasis, anticancer agents

P48

Bee venom and melittin induce apoptosis in colon cancer cell lines by Caspase 8 activation

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Background: Animal venoms and their constituents shows ability to induce apoptosis, like a favorable way of tumor cells elimination due to anticancer therapy. Aim of this study is to evaluate mechanism of proapoptotic action of bee venom and melittin by determining Caspase 8 gene expression, as well as its activity in colon cancer cell lines (HCT-116, SW-480, and HT-29). Material and Methods: Caspase 8 activity was determined by colorimetric method (Caspase 8 colorimetric kit, RD Systems). Gene expression of Caspase 8 was monitored by RT qPCR. Results: The results show that both treatments cause a significant increase in the activity of Caspase 8 in colon cancer cell lines HCT-116, SW-480, and HT-29 (compared to untreated cells). The gene expression of Caspase 8 under the influence of bee venom was increased in all tested cell lines, while melittin causes its significant increase only in SW-480 and HT-29 cells. Conclusion: Based on all results, activation of Caspase 8 indicates that in SW-480 and HT-29 cells apoptosis was initiated by external death receptor-mediated pathway. Increasing of Caspase 8 gene expression in HCT-116 cells was less pronounced compared to the other two. This correlates with results of other authors which confirms that these cells require additional signals from the mitochondria for apoptosis activation.

Keywords: cancer, cell lines, colon, drug, venom, Caspase 8

P49

The role of PLAG1 oncogene and miR-26a/miR-26b in the pathogenesis of benign salivary gland tumors

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Background: Pleomorphic adenoma (PA) and Warthin's tumor (WT) are two common benign salivary gland tumors (SGTs) that can undergo malignant transformation. Due to their histological diversity and unpredictable behavior, they represent both diagnostic and therapeutic challenges and their pathogenesis is still unclear. The aim of the present study was to assess in PA and WT the levels of PLAG1 oncogene mRNA and two microRNAs (miR-26a and -26b) known to interact with PLAG1 and to exhibit either oncogenic or tumor suppressive behavior, depending on the tumor type. **Material and Methods:** Relative gene expression levels of PLAG1, miR-26a and miR-26b were determined using quantitative real-time PCR analyses in 17 PAs and 24 WTs of the parotid glands and 7 normal salivary glands (NSGs). **Results:** PLAG1 was significantly overexpressed in both PAs and WTs compared to NSGs (P=0.028 and P=0.008, respectively). Levels of miR-26a were increased in both neoplasms, compared to NSGs, suggesting a potential oncogenic role, but without statistical significance. On the other hand, miR-26b was downregulated in both tumor types, suggesting its potential tumor suppressive role, but again without reaching statistical significance (P>0.05). Additionally, no correlation was observed between PLAG1 expression and relative miR-26a/ miR-26b levels in neither of the examined patient groups. No association was found between markers' expression and clinical/epidemiological parameters of PA and WT patients. **Conclusion:** PLAG1 role has been confirmed in both neoplasms, yet from the present study it does not appear that the oncogene is a target of miR-26a/miR-26b.

Keywords: Salivary Gland Neoplasms; PLAG1; miR-26a; miR-26b; Pleomorphic Adenoma; Warthin Tumor

P50

Evaluation of the Potential Effect of *Helichrysum arenarium* Extract and Natural Compounds on Cancers Triggered by Obesity Mediated Inflammation

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Background: The *Helichrysum spp.* is represented by 27 taxa, 15 of which are endemic, in the flora of Turkey and is widely observed in Anatolia. *Helichrysum arenarium* (L.) Moench from the *Helichrysum spp.*, is a perennial plant defined as "Sandy Everlasting". In Anatolia, it is widely used for its diuretic, anti-inflammatory and detoxifying properties, and is also thought to be effective in the treatment of cystitis, arthritis, rheumatism, gastric secretion stimulation and gallbladder disorders. Material and methods: In this study, the medicinal use of *Helichrysum arenarium* was explored by literature review. Results: *Helichrysum arenarium* is reported to have anti-inflammatory, anti-microbial, anti-thrombotic, anti-allergenic, lipid-reducing, anti-stress, anti-hyperglycemia, vasodilator, detoxification and immune-stimulating effects. It has been shown that there are rich groups of phenolic compounds including flavonoids, chalcones, phenolic acids, phthalides, coumarins and pyrones from the extracts obtained from the flowers, and the presence of other compounds such as polyphenols, sterols and glycosides of aromatic compounds. The intracellular signaling mechanism is activated as a result of the anti-inflammatory effect mediated by the inhibition of c-Jun NH2-terminal kinases (JNK2) and p38 activities, especially by reducing the expression of C Reactive Protein (CRP), and also by suppressing the mitogen-activated protein kinase (MAPK) pathway. Moreover, methanol extract from *Helichrysum arenarium* flowers has been shown to inhibit tumor necrosis factor-α (TNF-α)-induced cytotoxicity in L929 cells. Other studies report that *Helichrysum arenarium* extracts might reduce blood lipid levels and suppress obesity. Conclusions: *Helichrysum arenarium* extracts and its natural compounds may be effective on some cancer subtypes triggered by obesity-induced inflammation.
Keywords: anti-cancer therapy, *Helichrysum arenarium,* obesity.

P51

The effect of CDK4/6 inhibition on cancer stem like-properties-induced Panc-1 and MiaPaCa-2 pancreatic cancer cells

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Background: Pancreatic cancer is one of the most aggressive tumor types and has remarkable resistance mechanism to treatment due to heterogeneity of tumor cells. Another factor that causes resistance mechanism is cancer stem cells. Leptin is an adipokine which stimulate cell proliferation, drug resistance through an increase of Notch signaling in cancer cells. In this study, it was aimed to understand the role of CDK4/6 inhibition in Panc-1 and MiaPaCa-2 cells, which have increased cancer stem cell-like properties due to leptin administration.

Material and methods: Panc-1 and MiaPaCa-2 cells were exposed to leptin treatment (100 ng/ml) for 24 h. Then, leptin-treated (leptin+) and leptin-untreated (leptin-) cells were treated by 3 µM CDK4/6 inhibitor, palbociclib. The effect of treatments on cell viability and colony formation was observed. The response of CDK4/6 inhibition on the stimulation of cell aggressiveness by leptin treatment was determined by measuring CD44, CD133 and CD24 levels on flow cytometry. The effect of palbociclib on Wnt/Notch signaling was analyzed by immunoblotting in leptin +/- Panc-1 and MiaPaCa-2 cells. Results: Leptin treatment stimulated the cell proliferation of both Panc-1 and MiaPaCa-2 cells. Leptin-treated cells had higher colony formation rate and the anchorage-independent growth was higher than leptin untreated cells. Although CDK4/6 inhibition reduced the colony formation, it was obvious that leptin+ cells were more resistant to treatment. The CD44 and CD24 levels significantly increased by leptin treatment but, inhibition of CDK4/6 led to downregulation of both CD44 and CD24 levels in Panc-1 cells. The expression levels of mesenchymal members, N-cadherin, β-catenin, Notch, Akt, were significantly increased in leptin+ cells. Palbociclib treatment had remarkable effect on dowregulation of Notch levels in leptin+ Panc-1 and MiaPaCa-2 cells. However, there was no significant effect of palbociclib on the expression levels of Akt and β-catenin in leptin+ cells. Conclusion: CDK4/6 inhibition had significant therapeutic potential on the regulation of cell proliferation abilities and aggressiveness of Panc-1 and MiaPaCa-2 cells with cancer stem cell characteristics.

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P52

Inhibition of cancer growth with NF-kB suppressor nitroglycerin can be reversed by NF-kB stimulation in hamster fibrosarcoma

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Background: We investigated possible role of the NF-kB in the anticancer effect of nitroglycerin with metformin on experimental BHK-21/C13-induced fibrosarcoma in hamsters. Material and Methods: Peroral treatment of tumor-bearing hamsters carried out with a pure combination of NF-kB suppressors nitroglycerin 25 mg/kg and metformin 500 mg/kg daily and with additional rescue doses of NF-kB stimulator mebendazole 460 mg/kg daily, via a gastric probe after tumor inoculation. After animal sacrification, blood samples were collected for hematological and biochemical analyses, the tumors were excised and weighed, and their diameters and volumes were measured. The tumor samples were pathohistologically and immunohistochemically assessed for proliferation marker protein Ki-67, proliferating cell nuclear antigen PCNA, hematopoietic progenitor cell antigen CD34, cluster of differentiation 31 (CD31), cytochrome *c* oxidase subunit 4 (COX4), mitochondria marker Cytochrome C, glucose transporter 1 (GLUT1) and inducible nitric oxide synthase (iNOS), and the main organs were toxicologically tested. The Ki-67 and PCNA positivity and the cytoplasmic marker (CD34, CD31, COX4, Cytochrome C, GLUT1, iNOS) immunoexpression in the tumor samples were quantified. Results: The combination of NF-kB suppressors nitroglycerin and metformin significantly inhibited fibrosarcoma growth in hamsters without toxicity, compared to control. Co-treatment with NF-kB stimulator mebendazole inhibited anticancer activity of the NF-kB suppressors nitroglycerin and metformin combination. NF-kB stimulator mebendazole rescued tumor progression inhibited by the NF-kB suppressors nitroglycerin and metformin. Conclusion: Anticancer effect of nitroglycerin with metformin might be through NF-kB suppression and might be an effective and safe approach in novel nontoxic adjuvant and relapse prevention oncological treatment.

Keywords: nitroglycerin, metformin, mebendazole, hamsters, BHK-21/C13, fibrosarcoma

Acknowledgements: This study was supported by the Republic of Serbia, Autonomous Province of Vojvodina, Provincial Secretariat for High Education and Scientific Research [Project title: Discovery of effective non-toxic anticancer drug combinations on experimental fibrosarcomas, grant no. 142-451-2498/2021-03 (Project leader Dušica Popović)] and Republic of Serbia, Ministry of Science [grant no. 172013 (DM)].

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NF-kB inactivation is important for disulfiram suppression of fibrosarcoma which can be rescued by NF-kB stimulator mebendazole in hamster model

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Background: We investigated involvement of NF-kB in the anticancer effect of disulfiram and metformin combination on fibrosarcoma in hamsters. Material and Methods: Hamsters (both sexes; ~ 70 g) were randomly allocated to control and experimental groups (8 animals per group). In all groups, 2×10^6 BHK-21/C13 cells in 1 ml were injected subcutaneously into the animals' backs. Peroral treatments were carried out with combination of NF-kB suppressors disulfiram (50 mg/kg/day) and metformin (500 mg/kg/day) and by the combination with addition of rescue daily doses of NF-κB stimulator mebendazole 460 mg/kg, via a gastric probe after tumor inoculation. After 19 days all animals were sacrificed. Blood samples were collected for hematological and biochemical analyses, the tumors were excised and weighed, and their diameters and volumes were measured. The tumor samples were pathohistologically and immunohistochemically assessed (Ki-67, PCNA, CD34, CD31, COX4, Cytochrome C, GLUT1, iNOS), and the main organs were toxicologically tested. Results: The combination of NF-kB suppressors disulfiram and metformin significantly inhibited fibrosarcoma growth in hamsters without toxicity, compared to control. Co-treatment with NF-kB stimulator mebendazole completely blocked anticancer activity of the NF-kB suppressors disulfiram and metformin combination, most likely by NF-κB stimulation. Conclusion: Anticancer effect of the combination of NF-kB suppressors disulfiram and metformin may be through NF-kB suppression and the combination may be used as an effective and safe candidate for novel nontoxic adjuvant and relapse prevention oncological therapy.

Keywords: disulfiram; metformin; mebendazole; hamsters; fibrosarcoma

Acknowledgements: This study was supported by the Republic of Serbia, Autonomous Province of Vojvodina, Provincial Secretariat for High Education and Scientific Research [Project title: Discovery of effective non-toxic anticancer drug combinations on experimental fibrosarcomas, grant no. 142-451-2498/2021-03 (Project leader Dušica Popović)] and Republic of Serbia, Ministry of Science [grant no. 172013 (DM)].

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The effects of Salinomycin on melanoma cell lines of different invasiveness

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Background: Salinomycin belongs to the group of natural polyether antibiotics known as ionophores, isolated from the bacteria *Streptomyces albus*, and exhibits a broad range of antibacterial, antifungal, and antiviral activity. Furthermore, its anti-tumoral effects were investigated on a few cancer cell lines, and it has been shown that Salinomycin inhibits division and metastatic potential and induce tumor cell death. On the other hand, its effects on melanoma cell lines have not been investigated yet. Base on that, our goal was to investigate the effect of Salinomycin on melanoma cell lines of the different stages of differentiation and accordingly, level of aggressiveness. Material and methods: To find the correlation between Salinomycin activity and aggressiveness of melanoma phenotype, viability, proliferation rate, and ROS production were measured in highly invasive anaplastic A375 cells of human origin and two clones of B16 mouse melanoma cells, first F1 isolated from a solid tumor, and the second F10 isolated from lung metastases. Having in mind that one of the most important environmental factors for tumor progression is the lack of oxygen, all tests were performed comparably in normoxic (37^0C, 5%CO_2, 20%O_2) and hypoxic (37^0C, 5%CO_2, 6%O_2) conditions in humidified chambers. Results: The results have shown that Salinomycin strongly downregulated cell viability and inhibited proliferation of all melanoma cell lines. Its efficacy was more profound in highly aggressive phenotype under both, hypoxic and normoxic conditions. Moreover, its overall potential was amplified in a hypoxic environment for all tested cell lines. Both effects were tightly related to the level of ROS production. Conclusion: Considering that Salinomycin potential was enhanced in metastatic cell lines under hypoxia, this naturally occurring agent is a promising tool for the treatment of advanced melanomas. Baring that in mind, further experiments should be done to prove the hypothesis and light up this mechanism in detail.

Keywords: Salinomycin, melanoma, cell lines, culture, normoxia, hypoxia
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Antitumor features of dual COX-2 and 5-LOX inhibitors on melanoma and colon cancer cell lines *in vitro*

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Background: Chronic inflammation inside brain, kidney, liver, melanoma, and colon tumors is in tight connection with their growth and progression. In that context expression of the enzyme cyclooxygenase, COX-2, takes a pivotal role as a link between inflammation and cancerogenesis promoting cell proliferation at the same time. Furthermore, lipooxygenase-5 (5-LOX) also provokes cell division, and viability of many inflammation-related cancers. Since both enzymes are linked to arachidonic acid metabolism, dual inhibition of COX-2/5-LOX can be a promising approach in supression of tumor growth. Material and methods: We have therefore examined the effect of the synthesized COX-2/5-LOX dual inhibitors $C_{13}H_{25}B_{10}O_2NS$ (U), $C_{16}H_{30}B_{10}O_2S$ (V), and $C_{18}H_{27}B_{10}IO_2S$ (W) on the viability of melanoma (B16-F1, B16-F10, A375) as well as colon cancer lines (HCT116, SW480, CT26). All cells were treated in a dose-dependent manner (dose range 100µM to 1.5µM) with each dual inhibitor. Cell viability was measured by MTT and CV. Results: The results show that compound W was the most selective against CT26 cells with an IC50 value of 17.9µM which is an 8.7 times lower dose than on mouse peritoneal macrophages (155.7µM). Cytofluorimetric analysis revealed that compound W slightly inhibits proliferation of CT26 cells in culture, and causes apoptosis independent from phosphatidyl serine (PS) inversion, as well as caspase activity. Furthermore, treatment with compound W strongly upregulates production of reactive oxygen/nitrogen species by CT26 cells. Conclusion: Taken together, the inhibition of both enzymes COX-2 and 5-LOX in CT26 cell line leads to limited tumor cell growth, and might also have a beneficial influence on the tumor microenvironment.

Keywords: *Inflammation, cancer cells, COX-2, 5-LOX, dual inhibitor*

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Effects of vegf on molecular profile and invasiveness of human prostate cancer cells *in vitro*

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Background: Prostate cancer (PCa) is one of the most common malignant neoplasms among men worldwide. The main problem arising from PCa is its propensity to metastasize, in particular to the bones. At the same time, the mechanisms of metastasis development associated with the influence of exogenous factors, such as growth factors, remain unknown. We aimed to investigate the effects of exogenous VEGF on the invasiveness and molecular profile of human prostate cancer cells with varying degrees of malignancy. **Material and methods:** Human PCa cell lines (LNCaP and DU-145) were cultured with VEGF (recombinant human VEGF-A, Abcam, UK). The invasive activity of the cells was examined using a standard invasive test according to the manufacturer's instructions. The expression levels of osteopontin, osteonectin, E-cadherin, N- cadherin, matrix metalloproteinase 2 (MMP-2), and MMP-9 were monitored by immunocytochemical analysis. **Results:** We established that cultivation of human PCa cell lines with VEGF increased invasive activity - by 42,5% ($p<0,05$) and 34,5% ($p<0,05$) in LNCaP and DU-145 cell lines, accordingly. We found that under the action of VEGF there was an increase in the level of expression of N-cadherin and matrix metallopeptidases (MMP-2 and -9). Furthermore, we observed the decrease of expression of osteopontin in LNCaP cell lines and osteonectin in DU-145 cell lines. **Conclusion:** These results suggest that exogenous VEGF stimulates the increase of the metastatic potential of PCa cells regardless of their malignancy degree.

Key words: Prostate cancer, VEGF, invasive activity, osteopontin, osteonectin

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Investigation the role of hippo signaling in metformin-induced apoptosis and autophagy mechanisms in MDA-MB-231 breast cancer cells

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Background: Metformin is a biguanide-class antidiabetic drug administered as first-line therapy in patients with type 2 diabetes. Since it has been shown that metformin was associated with the decreased risk of developing estrogen receptor-positive breast cancer, the tumor suppressive and anti-proliferative effects of metformin were investigated in breast cancer. In this study, we aimed to investigate the role of metformin-induced hippo signaling-regulated apoptosis in MDA-MB-231 breast cancer cells. Material and methods: MDA-MB-231 breast cancer cells were treated with various concentrations of metformin (1-10 µM). The effect of metformin on cell survival was analyzed by the cell viability and colony formation assays, DiOC6 and PI fluorescence stainin. The apoptosis, autophagy and hippo signaling-associated signaling members were analyzed by immunoblotting. Results: Metformin treatment has been shown to suppress proliferation with a decrease in colony formation. The control of cell proliferation by hippo signaling was induced by metformin through increasing the rate of p-YAP (S127) which led to prevention of nuclear translocation of YAP to increase transcription of cell viability-related genes. Moreover, metformin induced apoptotic cell death due to increase in the expression of apoptotic markers in MDA-MB-231 cells in a dose-dependent manner. Moreover, the autophagy mechanism was triggered by metformin treatment and inhibition of autophagy reduced the tumor growth inhibitory effect of metformin in MDA-MB-231 breast cancer cells Conclusion: Metformin significantly increased the autophagy and apoptosis through induction of hippo signaling pathway in breast cancer cells. Therefore, regulation of hippo signaling might be potential therapeutic target in metformin treatment of breast cancer cells.

Keyword: Metformin, breast cancer, apoptosis, autophagy, hippo pathway

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Zašto je BRCA testiranje važno?

ODREĐIVANJE STATUSA BRCA MUTACIJA

~22% žena kojim je dijagnostikovan rak jajnika ima **BRCA mutaciju.**

MOŽE NAPRAVITI RAZLIKU

Vodiči preporučuju testiranje svih žena sa karcinomom jajnika visokog gradusa na mutacije BRCA gena **pri postavljanju dijagnoze.**

Reference:
Konstantinopoulos PA, et al. Cancer Discov. 2015, 5 (11): 1137-54
ESMO = Europian Society for Medical Oncology Recommendations
N. Colombo & J.A. Ledermann, on behalf of the ESMO Guidelines Committeee, July 2021

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