

POSTER PRESENTATIONS

P1: NF- κ B-INDUCED UPREGULATION OF miR-548as-3p INCREASES INVASION OF NSCLC BY TARGETING PTEN

HAKAN AKCA^{1,2}, SAKIR AKGUN^{2,3}, HAKAN KUCUKSAYAN^{2,3}, OSMAN NIDAI OZES⁴

¹ Medical Genetic Department

(e-mails: hakca@pau.edu.tr, hakanakca@yahoo.com)

² ILTEM Cancer Biology Laboratory, Pamukkale University, Kinikli, Denizli, Turkey

³ Medical Biology Department School of Medicine

⁴ Department of Medical Biology and Genetics, Akdeniz University, Antalya, Turkey

Growing number of evidences in past decade suggest that miRNAs play pivotal role in regulation of cell growth, and function as tumor promoter or supressor. In this line, deregulated expression of miRNAs have been shown to play important role in development, progression and metastasis of lung cancer. Although many treatment options have been developed against lung cancer in past 40 years expected 5 year survival have not significantly increased. One of the reason for this is the limitations of subclassification of lung cancer at molecular level, which results in use of generalized or limited treatment options for lung cancer. For better understanding of lung cancer subtypes recent studies have focused on identifying expression profile or transcriptional regulation of miRNAs in of lung cancers. Along this line, several different miRNAs have been identified as biomarkers of different subtypes of lung cancer. In this study, we wanted to determine whether TNF α -induced activation of NF- κ B would induce specific set of miRNAs in non-small cell lung cancer (NSCLC) cell line H1299, and found that NF- κ B significantly upregulates transcription of miR-548as-3p, and this targets 3'-UTR of PTEN mRNA, resulting in further activation of AKT pathway. The miR-548as-3p induced down regulation of PTEN accelerates invasion of this cell line.

P2: FLYWCH1, A NOVEL TRANSCRIPTION FACTOR WITH TUMOR SUPPRESSOR ACTIVITY

SHEEM ALMOZYAN¹, ROYA BABAEI-JADIDI, ABDOLRAHMAN S NATERI

¹ Nottingham 1

(e-mail: shaima-jm@hotmail.com)

Wnt/ β -catenin-signaling pathway is the principal force of intestinal homeostasis and morphogenesis. Aberrant activity of this pathway is highly implicated in cancer development, particularly in colorectal cancer (CRC). Although Wnt/ β -catenin pathway is a well-studied pathway, the precise molecular mechanisms involved in the nuclear interactions of β -catenin with DNA-binding transcription factors (other than TCF4) are still under debates. Human FLYWCH1 has been first characterized and identified in our lab as a novel transcription factor, which interacts with nuclear β -catenin and modulates its transcriptional activity (Muhammed et al., under publication). FLYWCH1 is differentially expressed between normal as well as between different stages of colorectal cancers. However, we have just started exploring the biological functions and mechanisms of FLYWCH1 in colon/intestinal development, homeostasis and tumor formation.

Our findings suggest that FLYWCH1 protein binds to the endogenous catenin protein while negatively regulates the transcription of a specific-subset of β -catenin/TCF target genes, regardless of Wnt signaling status. In this study we also propose FLYWCH1 as a new Wnt/ β -catenin target gene. Our current study provides insights on a novel molecular mechanism mediated by FLYWCH1 and the canonical Wnt/ β catenin pathway in CRC carcinogens. Ongoing research may ultimately offer a new potential approach(s) and implications for future therapeutics.

P3: EFFECTS OF ENDOPLASMIC RETICULUM STRESS IN TREFOIL FACTOR FAMILY 3 PROTEIN DEFICIENT MICE

IVA BAZINA, KATE ŠEŠELJA, MIRELA BAUS LONČAR

*Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: Iva.Bazina@irb.hr)*

Endoplasmic reticulum stress, a cellular condition caused by the accumulation of unfolded proteins inside the ER, has been recognized as a major pathological mechanism in a variety of conditions, including cancer, metabolic and neurodegenerative diseases. Trefoil factor family (TFFs) comprises three small proteins (Tff1, Tff2 and Tff3) that are present in different epithelial organs, blood supply, neural tissues and liver. TFFs play multifunctional role in protection of mucosa through participation in apoptosis, cell migration and immune response, and their expression is deregulated in different tumors. Complete diminishment of liver Tff3 expression is noticed as one of the first events in early phase of diabetes development in multigenic mouse models of diabetes (Tally Ho mice). To elucidate the role of Tff3 in different pathologically relevant pathways we have developed new congenic mouse model Tff3/C57Bl6/N from mixed background strain (C57Bl6/N /SV129) by using speed congenics approach. ER stress was evoked by tunicamycin treatment (3ug/g of body mass) and mice were sacrificed 24 h later. We have monitored expression of different oxidative and ER stress genes and relevant proinflammatory cytokines/chemokines. Most dramatic change was noticed at the level of inflammation related genes, while markers for unfolded protein response and oxidative stress were not significantly affected. ER stress induction in Tff3 deficient mice caused attenuation of gene expression for several proinflammatory cytokines.

P4: THE ROLE OF PROTEINS RHOD AND KIF20B IN TUMOR CELL SENSITIVITY TO ANTICANCER DRUGS

TIHANA DE ZAN¹, ANDREJA AMBRIOVIĆ RISTOV¹, TOSHIMASA ISHIZAKI²

¹ *Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: tjelusic@irb.hr)*

² *Department of Pharmacology, School of Medicine, Faculty of Medicine, University of Oita, Oita, Japan*

RhoD, the atypical Rho GTPase, is the less known member of the Rho protein family. RhoD limits endosomal movement and suppresses migration due to its interactions with actin. It has roles antagonistic to those of RhoA transforming protein, a known oncogen. The potential interacting partner of RhoD, kinesin 6 family protein KIF20B, is overexpressed in several tumor cell lines, especially bladder cancer, and recently has been found to be involved in the process of EMT transition. It has even been considered as a target of oncolytic adenovirus based therapy. Thus far, only one study has shown the influence of RhoD expression on tumor cell resistance to cisplatin, and the topic of KIF20B in tumor cell sensitivity to antitumor drugs has been marginally covered in anticancer drug research. With this work we have aimed to elucidate the potential of both RhoD and KIF20B as possible therapeutic targets by assessing sensitivity of several cancer cell lines (HeLa, HeLa CK, HCT-116, CaCO-2, MDA-MB-231, MDA-MB-468 and MCF-7) to the widely used antitumor drugs cisplatin, paclitaxel and vincristine, after knockdown of RhoD or KIF20B.

By means of the MTT cell proliferation assay, we have found that RhoD knockdown reduces cell proliferation in most of the tested cell lines, and also induces various degrees of sensitivity to cisplatin in tested cell lines. Out of all tested cell lines the most prominent response to KIF20B knockdown was observed in HeLa cells, where KIF20B knockdown induced sensitivity to cisplatin and resistance to paclitaxel and vincristine.

P5: INTEGRIN SWITCHING IN HUMAN TONGUE SQUAMOUS CARCINOMA CELLS CAL27: DE NOVO EXPRESSION OF $\alpha V\beta 3$ RESULTS IN INCREASED EXPRESSION OF INTEGRINS $\alpha V\beta 5$ AND $\alpha 6\beta 4$

ANA DEKANIĆ¹, KRISTINA ČULJAK¹, NIKOLINA STOJANOVIĆ¹, MLADEN PARADŽIK¹, JONATHAN D. HUMPHRIES², MARTIN J. HUMPHRIES², ANDREJA AMBRIOVIĆ-RISTOV¹

¹ Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia

(e-mail: ana.dekanic@irb.hr)

² Wellcome Trust Centre for Cell-Matrix Research, Faculty of Biology, Medicine & Health, University of Manchester, Manchester, United Kingdom

Integrins are heterodimeric transmembrane proteins that lack enzymatic activity, instead, signalling is induced by binding to extracellular matrix (ECM) and forming integrin adhesion complexes, composed of various structural and signalling proteins, referred to as the integrin adhesome. Integrins play key roles in the regulation of tumor cell adhesion, survival, motility and drug sensitivity. We have previously shown in Cal27 tongue squamous carcinoma cells that transfection of integrin $\beta 3$, giving rise to cell clone 2B1, led to de novo integrin $\alpha V\beta 3$ expression and increased expression of integrin $\alpha V\beta 5$. 2B1 cells displayed increased migration and invasion, and were resistant to multiple antitumor drugs as a consequence of integrin $\alpha V\beta 3$ -mediated loss of pSrc(Y418). The goal of this study was to analyse the adhesome composition of Cal27 and 2B1 cells by mass spectrometry (MS)-based proteomics. MS analysis identified 639 proteins including 19 out of the 60 consensus adhesome. Based on DAVID analysis, focal adhesion proteins accounted for 17% of identified proteins, whereas 13.8% were ECM components. Unexpectedly, in 2B1 the integrin $\beta 3$ subunit was not detected, whilst integrin $\beta 5$ and αV subunits were only identified in one out of five sets of replicates. Interestingly, the MS analysis suggested that Cal27 and 2B1 formed focal adhesions through integrin $\alpha 6\beta 4$. Moreover, an integrin switching effect, i.e. upregulation of integrin $\alpha 6\beta 4$ in 2B1 as compared to parental Cal27 cells was observed. Additionally, in focal adhesions isolated from 2B1 cells we found increased abundance of seven focal adhesion proteins (filamin A, filamin B, talin-1, α -actinin-1, Ras GTPase-activating-like protein, tensin-3, vinculin) and eight ECM proteins (trombospondin-1, EGF-like repeat and discoidin I-like domain-containing protein 3, laminin subunit $\alpha 5$, tubulin β chain, perlecan, transforming growth factor beta-induced protein ig-h3 and tenascin C) as compared to Cal27. Of particular interest was the upregulation of both integrin $\alpha 6$ and $\beta 4$ subunits and the $\alpha 6\beta 4$ ligand laminin 5 in the more drug resistant and motile 2B1 cells since the upregulation of these genes was already found to be significant in the squamous cell carcinoma of the head and neck from patient samples. In conclusion, MS-based proteomic analysis of the integrin adhesome may be a valuable tool for identifying key changes in signalling pathways which can be potentially exploited for diagnostic or therapy purposes.

P6: OXIDATIVE STATUS OF PATIENTS WITH THYROID DISEASES

MARKO GERIĆ¹, ANA-MARIJA DOMIJAN², RENATO JANUŠIĆ³, BOŽENA ŠARČEVIĆ³,
VERA GARAJ-VRHOVAC¹

¹ *Institute for Medical Research and Occupational Health, Mutagenesis Unit, Zagreb, Croatia*

² *University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia*
(e-mail: adomijan@pharma.hr)

³ *Clinical Hospital for Tumors, Zagreb, Croatia*

Although thyroid cancer comprises only about 2% of new cancer cases, it is one of the fastest-growing cancer types. In fact, in Croatian population the number of newly diagnosed patients has doubled in the last 15 years. Thyroid cancer is more frequent in female population (about 4-fold compared to male population) and is most usually detected at ages over 50. There is also a negative trend indicating that more people at the age <50 are diagnosed with thyroid cancer.

In our previous studies we observed that patients with thyroid diseases had higher baseline DNA damage levels, high levels of aberrantly expressed proteins (B-Raf and Ret) and altered oxidative status. Therefore, we performed additional analyses investigating enzymatic antioxidative defense – the activity of superoxide dismutase (SOD) and the levels of copper (Cu), essential trace element.

Based on 20 patients (13 female and 7 male; 7 active smokers), aged 52.3±14.4 and matched control group (13 female and 7 male; 7 active smokers) aged 51.1±12.7 we observed lower SOD activity in thyroid patients group (0.80±0.49 U/mL) compared to control group (1.24±0.43 U/mL). At the same time, plasma Cu concentration was higher in thyroid patients (1.62±0.51 µg/mL) compared to control group (1.27±0.50 µg/mL).

Taken together, we observed altered antioxidative defense mechanism and high Cu concentration in patients with thyroid diseases. Although Cu is essential for normal organism functioning, in excess it can have toxic potential, among other for ROS production. These results confirmed our previous findings where we observed more lipid peroxidation products, protein carbonyls, and altered enzymatic and non-enzymatic antioxidative defense. The question still remains is the oxidative stress cause or consequence of thyroid diseases.

P7: HYDROGEN PEROXIDE-INDUCED OXIDATIVE STRESS INCREASES EXPRESSION OF p53 AND p73 PROTEINS IN NEUROBLASTOMA SH-SY5Y CELLS

SNJEŽANA DUNĐER¹, NIKOLINA HANŽIĆ², MAJA JAZVINŠČAK JEMBREK²

¹ *Department of Chemistry, Faculty of Science, Zagreb, Croatia
(e-mail: snjezana.dundjer@gmail.com)*

² *Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia*

Many approaches in tumour therapy are based on the oxidative stress-induced apoptosis by using radiotherapy or chemotherapeutics. The aim of the present study was to examine the effect of hydrogen peroxide (H₂O₂)-provoked oxidative stress on the expression of tumour suppressor proteins p53 and p73 in the neuroblastoma cell line SH-SY5Y. Both proteins are involved in the initiation of apoptosis and are present in cells in various isoforms whose dynamic changes may contribute to apoptotic response. In a concentration-dependent manner H₂O₂ decreased cell survival, intracellular glutathione and ATP levels, and increased accumulation of reactive oxygen species and caspase-3/7 activity. Western blot method revealed an enhancement in the expression of the total p53 protein and its isoform p53 α by using antibodies SAPU, DO-11 and DO-12. Protein detection with KJC8 antibody that recognises all β isoforms of p53 protein indicated reduced expression of isoforms p53 β and Δ 40p53 β in p53-mediated cell death. H₂O₂ also triggered strong induction of p73 expression, particularly the TAp73 α isoform. The obtained results suggest that increased expressions of p53 and p73 proteins greatly contribute to reduced survival of SH-SY5Y cells under oxidative stress conditions. Although further investigations are required to more clearly identify contribution of specific p53 and p73 isoforms to the initiation of death cascade, it seems that certain isoforms may be promising candidates as novel therapeutic targets in the anti-cancer therapy.

P8: 3D TUMOR SPHEROIDS AS A MODEL FOR ANTICANCER ACTIVITY ANALYSIS OF HYPERICUM PERFORATUM EXTRACTS

MARIJA ĐORĐIĆ CRNOGORAC¹, IVANA Z. MATIĆ¹, ANA DAMJANOVIĆ¹, SERCAN ERGÜN², SEMA MISIR³, YÜKSEL ALIYAZICIOĞLU⁴, TATJANA STANOJKOVIĆ¹

¹ Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
(e-mail: marijadjordjic@gmail.com)

² Department of Medical Biology, Faculty of Medicine, Ordu University, Ordu, Turkey

³ Department of Biochemistry, Faculty of Pharmacy, Cumhuriyet University, Sivas, Turkey

⁴ Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

3D tumor models are widely used for examination of efficacy and discovery of new antitumor agents. In this study we use HeLa (human cervical adenocarcinoma cell line) 3D spheroids as a model for examination of anticancer activity of methanol (1), ethyl-acetate (2), and hexane (3) extracts of flowers, and also ethyl-acetate (4), and hexane (5) extracts of branch-body part of Turkish endemic species *Hypericum perforatum*. Spheroids were produced using a hanging drop method. Morphological features of spheroids were analyzed using light microscope. Cell viability of HeLa spheroids treated with extracts at concentrations of 100 µg/mL and 200 µg/mL was assessed by fluorescent microscopy and flow cytometry. Changes in diameters of spheroids after 24h and 48h, and fluorescence intensity after 48h of treatment were analysed by ImageJ software. The distribution of viability of cells in spheroids was calculated as a ratio of fluorescence intensity of live and dead cells, respectively. After 48h of growth without treatment, the average spheroid diameters were about 450 µm. Extract 1 showed the strongest ability to reduce spheroid diameter to a value of 250 µm and 350 µm at applied concentrations. Extract 3 is the only one which increased the diameter of spheroid after 24h at lower concentration, as well as at higher concentration. That effect could be the result of breaking of the cell-cell and cell-extracellular matrix interactions in spheroids, what makes extract 3 the most efficient. After 48h the diameter was decreased. Extract 2, 4, and 5 showed variable effect to spheroid diameters. At concentration of 200 µg/mL, these extracts induced the highest increase of spheroid diameters, 654.7 µm, 652.4 µm and 585.8 µm, respectively. The ratio of fluorescence intensity of live and dead cells in treated spheroids were much lower comparing with the control, untreated spheroid, 8.54. The lowest value was obtained in spheroid treated with extract 1 (200 µg/mL), and it was 0.16. The results showed that the fluorescence intensity of dead cells increased with increasing extracts concentration. Flow cytometry confirmed cytotoxic activity of extracts. All extracts induced death in 50% to 80% of the cells in spheroids. Different changes of diameters indicate to differences in mechanisms of activity and efficacy of studied extracts. The conclusion is that examined extracts have good anticancer activity, but need to be investigated further, especially on 3D cell culture models.

P9: BIOCHEMICAL DIFFERENCES AMONG NATURALLY OCCURRING HPV-16 E6 VARIANTS

ANAMARIA ĐUKIĆ¹, JAYASHREE VIJAY THATTE², KSENIJA BOŽINOVIĆ¹, EMIL DEDIOL³,
MAGDALENA GRCE¹, LAWRENCE BANKS², VJEKOSLAV TOMAIĆ¹

¹ *Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia*
(e-mail: tomaic@irb.hr)

² *International Centre for Genetic Engineering and Biotechnology, Trieste, Italy*

³ *Department of Maxillofacial Surgery, Clinical hospital Dubrava, Zagreb, Croatia*

A number of Human papillomaviruses (HPVs) were shown to be associated with various human cancers. Of these cervical cancer is the most important disease. Whilst the HPV E6 and E7 oncoproteins are responsible for cervical cancer development, it is also clear that there are differences of these two oncoproteins among the viral isolates. Good examples of this are HPV-16 E6 variants found in various geographical locations, shown to differ in their capacities to cause cancer. Interestingly, some of these variants are more frequently detected in cancers than the other variants, suggesting that they exhibit very important differences in their ability to induce malignancy. The aim our studies is investigate these different cancer-causing activities which are still largely poorly understood at the molecular level.

P10: DIAZA-CROWN ETHER KILLS TUMOR CELLS BY CHANGES IN ION HOMEOSTASIS AND DISRUPTION OF CELL MEMBRANES

KATJA ESTER¹, MARIJA MIOČ¹, JELENA GABRILO¹, LIDIJA UZELAC¹, MARKO MARJANOVIĆ¹, PAVEL SPURNY², DANIEL BONHENRY³, TATJANA ŠUMANOVAC RAMLJAK⁴, JOST LUDWIG², KATA MLINARIĆ-MAJERSKI⁴, MARIJETA KRALJ¹

¹ *Laboratory of Experimental Therapy, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: kester@irb.hr)*

² *Laboratory of Bioenergetics and Membrane Physiology, Institute of Microbiology ASCR, Czech Republic*

³ *Laboratory of Structure and Function of Proteins, Institute of Microbiology ASCR, Czech Republic*

⁴ *Laboratory of Synthetic Organic Chemistry, Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia*

Majority of cancer therapeutics are designed to interact with nucleic acids and proteins. The increasing knowledge of membrane lipid composition and its alterations in cancer could provide novel therapeutic targets. Crown ethers represent small molecules that hold promising antitumor potential. Among other modes of action, crown ethers can act as ionophores, i.e. disturbing ion homeostasis and disrupting biological membranes. We showed that crown ethers inhibit P-glycoprotein and reverse multidrug resistance in cancer cells. Based on the previous in vitro screening, we identified the proprietary diaza-crown ether compound as a potential antitumor drug. Inhibition of tumor growth was confirmed on the panel of breast tumor cells, and in an in vivo mouse model. To unravel the mechanism of action, we choose an approach based on compound structure: we postulated that diaza-crown ether could: 1) potentially bind and move ions through the membrane and change the ion homeostasis, and 2) cause perturbations of the lipid membranes throughout the cell due to its hydrophobicity. We found that compound induces K⁺ and Na⁺ fluxes and modulates resting potential of cells. Physical disturbance of lipid membranes throughout the cell was demonstrated by increase in plasma membrane permeability and impairment of mitochondrial functions. The importance and/or interplay of the two mechanisms responsible for antitumor effects still remain to be elucidated.

P11: CaCo-2 3D SPHEROIDS ARE AFFECTED BY FRESH ROYAL JELLY (F-RY) (M) AND HuIFN- α N3

BRATKO FILIPIČ¹, KLEMEN RIHAR², ADRIANA PEREYRA³, DUNJA EXEL GREGORIČ², HRVOJE MAZIJA¹

¹ Croatian Institute for Experimental and Translational Oncology (CIETO), Zagreb, Croatia
(e-mail: bratko.filipic@gmail.com)

² Ljubljana, Slovenia

³ MEDEX d.o.o., Ljubljana, Slovenia

The interest in using 3-D in vitro models is increasing. They are designed for quick screening of formulations and sensitivity with the possibility for employment in in vivo condition. Spheroid cultures possess a complex network of cell-cell contacts and advanced extra cellular matrix development, as well as pH, oxygen, metabolic and proliferate conditions analogous to the vascular and vascular regions of solid tumours. They really mimic in vivo micro tumours. The presented experiments were aimed to analyse the sensitivity of CaCo-2 3D stellate Spheroids for Fresh Royal Jelly (F-RY) (M) and HuIFN- α N3 for volume, growth, apoptosis and cytotoxicity.

The Fresh Royal Jelly (10 mg/ml) (F-RY) (M) and HuIFN- α N3 (5000 I.U. /ml) were used. The spheroids were first treated with HuIFN- α N3 and followed with the F-RJ (M). CaCo-2 3D stellate spheroid volume was plotted over a 12-day period following a 24 h incubation with HuIFN- α N3 following with RJ. The growth of 3D spheroid was completely impeded after incubation with 5000 I.U. /ml of HuIFN- α N3 following with F-RJ (M) (10 mg/ml). Interestingly, following re-treatment on day 7 demonstrated greater inhibition of spheroid growth. The changes in the 3D stellate Spheroids volume were: from 70 μ m³ to 12 – 15 μ m³ after seven days and one retreatment. The cytotoxicity level after the treatment was measured. The 3D stellate CaCo-2 spheroid viability declined to 20 – 25%. Additionally, the apoptosis level was determined. The increase of apoptosis of the viable 3D stellate CaCo-2 spheroid in the level of 35 – 48% was observed.

P12: THE ROLE OF TOPOISOMERASE II-A (TOPO IIA) AS A PREDICTIVE FACTOR FOR RESPONSE TO NEOADJUVANT ANTHRACYCLINES BASED CHEMOTHERAPY IN LOCALLY ADVANCED BREAST CANCER

MOHAMED M. GAMEA¹, ASHRAF F. BARAKAT², FATMA Z. HUSSEIN²

¹ *Gharbia Cancer Society, Tanta, Egypt*
(e-mail: mohammed.game3@hotmail.com)

² *Clinical Oncology Department, Tanta University Hospital, Tanta, Egypt*

Topoisomerase II- α is a molecular target of anthracyclines; several studies have suggested that topoisomerase II- α expression is related to response to anthracycline treatment. The objective of this study was to evaluate if topoisomerase II- α overexpression predicts response to anthracycline treatment in locally advanced breast cancer patients.

This prospective study included 50 patients with primary non metastatic locally advanced breast cancer according to American Joint Committee For Cancer Staging (T3-4; N0-3) were treated between January 2012 and June 2012 at Clinical Oncology Department, Tanta University Hospital.

Topoisomerase II- α , HER2, estrogen receptor (ER), progesterone receptor (PR) expression and KI-67 were evaluated by immunohistochemistry in formalin-fixed, paraffin-embedded breast tumors from 50 patients presenting with locally advanced breast cancer.

Tumors from 50 patients, 45 (90%) showed topoisomerase II- α overexpression, patients 34 (68%) for ER positive, 32 (64%) for PR positive and 10 (20%) for HER2 overexpression and 16 (32%) for high KI 67. Significant correlation was found between clinical and pathological response with topo IIA ($p \leq 0.001$), HER2 ($p = 0.005$) and KI-67 ($p = 0.015$).

1-Responders : Clinical (CR): 3 patients had co-expression of topo II and HER2, hormonal receptor negative and high KI-67. Clinical (PR): 43 patients majority of them had topo IIA overexpression.

2-Non responders : 4 (8%) patients all had negative (TOPOII/HER2), low KI-67 and 2 had hormonal receptor positive and another 2 had hormonal receptor negative.

Our data support a correlation between topoisomerase II- α expression in locally advanced breast cancer patients and improved clinical benefit with neoadjuvant anthracyclines based therapy.

P13: CYTO/GENOTOXIC AND OXIDATIVE STATUS OF ANTICANCER DRUG IMATINIB MESYLATE IN NON-TARGET HUMAN CELLS

MARKO GERIĆ¹, ANA-MARIJA DOMIJAN², VERA GARAJ-VRHOVAC¹, GORAN GAJSKI¹

¹ *Mutagenesis Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia
(e-mail: mgeric@imi.hr)*

² *Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia*

Imatinib mesylate (IM), a protein kinase inhibitor developed for targeted chemotherapy and sold under the brand name Gleevec/Glivec, is one of the most consumed anticancer drugs. Although there are indications that IM possesses cyto/genotoxic activities against normal non-target cells as well, there is a lack of information regarding the underlying mechanism involved in these actions. The aim of the present study was to evaluate cyto/genotoxic potential and oxidative stress responses in human peripheral blood lymphocytes (HPBLs) after IM treatment (0.001 – 10 µg/mL) in vitro. According to the results, cell viability was significantly affected in dose- and time-dependent manner. Exposure to IM did not induce DNA strand breaks based on the alkaline comet assay results. On the contrary, an increase in the number of micronuclei originating from broken or lagging chromosomes as well as an increase of nucleoplasmic bridges and nuclear buds was observed suggesting that IM induces genomic instability. Nuclear buds represent the elimination of amplified DNA, DNA repair complexes, and excess chromosomes from aneuploid cells, while nucleoplasmic bridges are considered to originate from dicentric chromosomes, which can occur due to misrepair of DNA breaks and telomere end-fusion, and might also be observed during defective separation of sister chromatids at anaphase. IM also had influence on oxidative stress parameters tested. After the treatment we observed increase in the malondialdehyde concentration suggesting lipid peroxidation. IM reduced glutathione level indicating impairment in oxidative stress defence. Observed changes coincided with formation of oxidative purines detected by formamidopyrimidine-DNA glycosylase (Fpg) modified comet assay suggesting IM-induced oxidative DNA lesions. The obtained results provide new evidence and clarification of mechanism of action of IM-induced cyto/genotoxicity, suggesting that in addition to cancer cells IM can also affect normal non-target human cells leading to the observed adverse effects that are related to induction of oxidative stress. The concentrations that caused adverse effects in selected cells are higher than those that can be expected in the environment. However, considering that IM exerts genotoxic activity, an increased risk for human health at chronic exposure to low concentrations cannot be completely excluded.

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P14: IN VITRO EVALUATION OF ANTI-MIGRATION AND ANTI-ANGIOGENIC EFFECT OF FUCUS VIRSOIDES SEAWEED AND INFLUENCE OF SEASONAL VARIATIONS

NADA GROZDANIĆ¹, VESNA MAČIĆ², GORDANA ZDUNIĆ³, KATARINA ŠAVIKIN³,
IVANA MATIĆ¹, ZORAN KLJAJIĆ², TATJANA P. STANOJKOVIĆ¹

¹ Institute for Oncology and Radiology of Serbia, Belgrade, Serbia
(e-mail: nadjagrozdanic@gmail.com)

² Institute of Marine Biology, Kotor, Montenegro

³ Institute for Medical Plant Research "dr. Josif Pančić", Belgrade, Serbia

Seaweed species from *Fucus* genus are well known for their anticancer properties and they may possess nutritional value and several health beneficial properties. Literature data about biological activity of endemic species *Fucus virsoides* are in scarce. The aim of this study was to examine anti-migration and anti-angiogenic activities of extract and fractions of *F. virsoides* and influence of seasonal variations. Material and Methods: *F. virsoides* was collected from Adriatic Sea Boka Kotorska bay in October and July. Dichloromethane-methanol extract, petrol-ether, ethyl-acetate and butanol fractions were made. They were tested for their anti-migration and anti-angiogenic properties (subtoxic IC20 concentrations) against human endothelium-derived permanent EA.hy926 cell line using scratch assay and tube formation assay. Results: We observed significant difference between activities of two *F. virsoides* samples taken in different season. The best anti-migration effect showed autumn picked sample and its ethyl-acetate fraction with scratch gap reduction of $9.80 \pm 0.49\%$ after 24 hours and $23.61 \pm 2.39\%$ after 48 hours exposure, followed by petrol-ether ($15.17 \pm 2.16\%$, $26.62 \pm 1.84\%$) dichloromethane-methanol extract ($19.55 \pm 3.93\%$, $40.28 \pm 6.16\%$), while butanol fraction had poorest effect ($45.65 \pm 8.89\%$, $72.66 \pm 13.28\%$). Summer sample showed less potential for migration inhibition; gap reduction in cell samples treated with dichloromethane-methanol extract was $38.68 \pm 2.55\%$ after 24 hours and $54.37 \pm 2.31\%$ after 48 hours, followed by samples treated with petrol-ether fraction ($41.36 \pm 9.56\%$, $45.60 \pm 6.34\%$), ethyl-acetate ($49.84 \pm 8.78\%$, $87.65 \pm 1.37\%$) and butanol fraction ($85.37 \pm 7.65\%$, $99.98 \pm 0.02\%$). Angiogenic assessment demonstrated that ethyl-acetate fraction of both samples exerted significant anti-angiogenic effect and prevented any tube formation or cell connections, followed by autumn samples of whole extract and petrol-ether fraction where we sporadically found little cell to cell interaction. All other samples and control EA.hy 926 cells showed polygonal structures, complex meshes and large vessel structures. Conclusion: Our research showed that there was significant seasonal variation in anti-migration and anti-angiogenic activity of extracts and that some fractions compared to the whole extract exerted better activity. Further analysis of *Fucus virsoides* extract and fractions is needed to determine the most powerful component that can stop migration and angiogenesis.

P15: FUNCTIONAL INTERPLAY BETWEEN p53 AND p53/p73, NME AND GLI PROTEIN FAMILIES IN HUMAN MELANOMA

NIKOLINA HANŽIĆ¹, BASTIEN PROUST¹, PETAR OZRETIĆ², MARTINA RADIĆ¹, ANĐELA HORVAT¹, IVAN MILAS³, ZVONIMIR PULJIZ³, MAJA HERAK BOSNAR¹, SONJA LEVANAT², NEDA SLADE¹

¹ *Laboratory for Protein Dynamics, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: nhanzic@irb.hr)*

² *Laboratory for Hereditary Cancer, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia*

³ *“Sestre milosrdnice” University Hospital Center, University Hospital for Tumors, Zagreb, Croatia*

In metastatic melanoma tumor suppressor protein p53 is not functional, although it is rarely mutated (less than 10%). Despite the low rate of mutations, p53 fails to function as tumor suppressor and tumor cells continue to proliferate and spread. Therefore, the question arises what in the metastatic melanoma prevents the wild type p53 protein to function. Since p53 interactions play a significant role in tumorigenesis, we have investigated whether the p53 protein function can be altered by interactions with p53 low molecular weight isoforms, p73 isoforms, and NME and GLI families of proteins. Therefore, we studied the protein expression profile of p53 and its potential interaction partners (p73/NME/GLI) in metastatic melanoma and in adjacent healthy skin tissue. In the study on 38 patients with metastatic melanoma, the expression of p53, p73, NME and GLI protein families was determined by western blot analysis. Protein expression analysis showed elevated expression of $\Delta 133p53\alpha$, $\Delta 160p53\alpha$, $\Delta Np73\alpha$, NME1, NME2, GLI1_160 kDa, GLI1_130 kDa and GLI2_133 kDa in tumor samples compared with healthy tissue samples. Strong correlations were detected only between TAp73 β and p53 α , and GLI3R and p53 α . The remaining correlations were mostly moderate (p53 α with $\Delta 160p53\alpha$, TAp73 α , $\Delta Np73\alpha$, GLI1_118 and GLI1_160). Interestingly, $\Delta 133p53\beta$ was negatively correlated with p53 α . We have shown that p53 forms complexes with p53 and p73 isoforms hence inhibiting its transcriptional and apoptotic activity. Although NME and GLI did not form complexes with p53, they were able to modify its activity. Defining the interactions between protein p53 and other proteins could contribute to better understanding of the molecular basis of melanoma and finally lead to the development of new approaches in melanoma treatment.

P16: STAT3 SIGNALING IS ESSENTIAL FOR BLADDER CANCER PROGRESSION

JELENA KORAC PRLIC¹, MARINA DEGORICIJA¹, KATARINA VILOVIC², BENEDIKT HAUPT¹, LUCIJA FRANKOVIC¹, TONCI IVANISEVIC¹, IVANA KARAMAN², SERGEI GRIVENNIKOV³, JANOS TERZIC¹

¹ School of Medicine, University of Split, Split, Croatia
(e-mail: Benedikt-Haupt@gmx.net)

² Department of Pathology, Split University Hospital Centre, Split, Croatia

³ Cancer Prevention and Control Program, Fox Chase Cancer Center, Philadelphia, USA

Proper immune response is an important defense mechanism, which deters cancer development and progression. However, chronic inflammation can facilitate tumorigenesis. The role of inflammation in urinary bladder cancer (BC) pathogenesis is poorly understood. It is well documented that one of the main molecular links between inflammation and cancer are interleukin-6 (IL6) and its downstream transcription factor – Stat3. Here, by using N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN)-induced bladder cancer mouse model, we tested the role of IL6 and Stat3 in bladder cancer development and progression. In our experiments we used genetic IL6 deficient mice (IL6 KO) model and mice with urothelial specific conditional deletion of Stat3 as well as chemical inhibitor of Stat3. We employed histopathological, immunohistochemical approaches and gene expression profiling to demonstrate an essential role of IL6 and Stat3 in BC development. Results show that inhibition of Stat3 activation slows down progression and invasiveness of bladder cancer in BBN-induced mouse model. Interestingly, Stat3 activation in BC occurred largely independently of IL6 signaling, as IL6 deficient mice still developed BC tumors with marked Stat3 activation. Taken together, our study demonstrates an important role of Stat3 signaling in bladder cancer and creates a rationale to test therapeutic potential of Stat3 inhibitors in patients with bladder cancer.

P17: CONTRIBUTION OF EPIGENETIC MODIFYING ENZYMES TO CHEMORESISTANCE IN CANCER

WEN-CHUN HUNG, MING-CHUAN HSU

*National Institute of Cancer Research, National Health Research Institutes, Tainan, Taiwan
(e-mail: hung1228@nhri.org.tw)*

Gemcitabine (GEM) is a chemotherapeutic drug and the standard treatment option for pancreatic cancer patients. Although some patients respond well in the treatment firstly, development of resistance is commonly observed in clinic. To dissect the mechanism underlying GEM resistance, we screened for the epigenetic modifying enzymes that are increased in GEM-resistant pancreatic cancer cells and identified 21 upregulated enzymes in resistant cells. We first addressed the functional role of protein arginine methyltransferase 3 (PRMT3) in chemoresistance. We found the upregulation of PRMT3 in GEM-resistant pancreatic cancer cells. By using proteomics approach, we identified a number of PRMT3-interacting proteins. One candidate gene possibly involves in the chemoresistance is the transporter ATP binding cassette sub-family G member 2 (ABCG2). In this study, we will introduce how PRMT3 modulates the expression of ABCG2 via a novel post-transcriptional mechanism. Our results suggest that PRMT3 could be a potential target to overcome GEM resistance.

P18: NEUROTENSIN REGULATES PANCREATIC CANCER PERINEURAL INVASION AND IS TARGETABLE BY PI3K INHIBITOR

YU-HSUAN HUNG, LI-TZONG CHEN

*National Institute of Cancer Research, National Health Research Institute, Taiwan (R.O.C.)
(e-mail: paganpoetry2005@gmail.com)*

Pancreatic adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in the U.S. and in Taiwan. Due to its low 5-year survival rate as 5%, identification of novel mechanisms and therapeutics for PDAC is needed. One of the characteristics of PDAC is perineural invasion (PNI), in which neuron serves as a source of protumorigenic factors and a route for cancer metastasis. To investigate the impact of neurogenesis on PDAC formation and prognosis as well as its counteracting therapeutics, we analyzed the prognosis prediction power of neural factors *in silico* and found neurotensin (NTS) and its receptor 1 (NTSR1) predicted poor PDAC overall survival. *In vitro* we found NTS as synthetic peptide or from conditioned medium of either pancreatic neuroendocrine tumor (PNET) or differentiated neuron increased slightly the proliferation and anchorage-dependent growth of human and mouse PDAC cell lines, while increased mainly the migration and invasion of above cell lines. Besides, knockdown of NTSR1 in human and mouse PDAC decreased their response to neurotensin during migration and invasion, and knock-down of NTS in differentiated neuron decreased its induction of PDAC migration and invasion. Moreover, *in silico* analysis revealed that PI3K inhibitor targeted NTS- and NTSR1-high PDAC in human patients, and *in vitro* result validated the suppression by PI3K inhibitor wortmannin and/or GDC-0941 on NTS-induced migration and invasion in human and mouse PDAC. In mouse tissue we found spontaneous pancreatic cancer mouse models LSL-KrasG12D/+; p53Loxp/+;Pdx-1-Cre (KPC) and the one with additional APC mutation (KPA) displayed increased NTS and NTSR1 expression compared to that of wildtype C57BL/6, and NTS colocalized with neuron marker glial fibrillary acidic protein (GFAP). To sum up, present study identified *in silico* neural factor NTS and its receptor 1 predicted poor PDAC survival and validated *in vitro* and in mouse tissue the contribution of NTS-NTSR1-PI3K axis to PDAC formation and neurogenesis.

P19: THE EFFECTS OF BLENDED MEDICINAL MUSHROOM EXTRACTS WITH OR WITHOUT 5-FLUOROURACIL ON SURVIVAL AND VARIOUS ANTITUMOR PARAMETERS IN MOUSE CT26.WT COLON CANCER

BORIS JAKOPOVIĆ¹, NADA ORŠOLIĆ², SANDRA KRALJEVIĆ PAVELIĆ³, IVAN JAKOPOVICH¹

¹ *Dr Myko San – Health from Mushrooms Co, Zagreb, Croatia
(e-mail: borisjakopovic@yahoo.com)*

² *Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*

³ *Department of Biotechnology, University of Rijeka, Rijeka, Croatia*

Medicinal mushrooms are for millennia widely appreciated as natural sources of bioactive substances and are recognized as significant immunomodulatory and antitumor agents. In the last 50 years there has been an increased interest in the use of fungi in the (traditional) medicine as well as in determining their therapeutic and medicinal properties, resulting in exponential growth of the number of scientific research papers on their impact on animal and human organism. This paper describes the effects of two commercial preparations from medicinal mushrooms, tableted preparation AGARIKON.1® and liquid preparation AGARIKON PLUS® (manufacturer: Dr Myko San – Health from Mushrooms). These extract preparations from various mushroom species with proven antitumor effects were compared in various combinations, with or without 5-fluorouracil (5-FU), which is a common antimetabolite class drug used in treating colon cancer. An important hypothesis throughout is that the blend of different medicinal mushroom substances of the same class of compounds achieved synergistic effects, and consequently significantly stronger cytotoxic and other effects. The preparations AGARIKON.1® and AGARIKON PLUS® differ significantly in their polysaccharide to polyphenol contents ratio which differentiates their immunological and antimetastatic effects. We have simulated an advanced disease model by starting with the therapy late in the course of the disease. Two models of experimental design in studying survival were used: curative and preventative. Besides observed effects on survival, we also recorded the effects of mushroom preparations with or without cytostatic drug on angiogenesis and tumor invasiveness by measuring MMP-2, MMP-9 and VEGF levels, as well as immunological parameters such as macrophage polarization by measuring NO, arginase levels, as well as by using Th1/Th2/Th17 cytokine panels. Additionally, we have observed direct cytotoxic effects of mushroom preparations on human colorectal cell lines using MTT assay and proved their apoptosis inducing properties by flow cytometry. The results show significant beneficial effects on various parameters related to tumor suppression and show that medicinal mushrooms preparations alone and in combination with standard therapies have a major role in this in vivo model of colorectal cancer, which should be further explored in the clinic.

P20: ANTIPROLIFERATIVE EFFECT OF NOVEL COUMARIN-1, 2, 3-TRIAZOLE HYBRIDS AGAINST TUMOR CELLS IN VITRO

MARIJANA JUKIĆ¹, TEUTA OPAČAK-BERNARDI¹, ZORISLAVA ŽIVKOVIĆ¹,
MATEA MATIĆ¹, ANDREA BISTROVIĆ², NIKOLINA STIPANIČEV², SILVANA RAIĆ-MALIĆ²,
LJUBICA GLAVAŠ-OBROVAC¹

¹ Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine,
Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
(e-mail: marjukic@mefos.hr)

² Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb,
Zagreb, Croatia.

Coumarins constitute a large family of oxygenated heterocyclic secondary metabolites widely distributed in plants. Natural and synthetic coumarins have attracted interest of scientist because of their broad spectrum of pharmacological activities among which are acting as an antioxidants and antitumor agents. Novel of coumarin-1, 2, 3-triazole hybrids were designed to evaluate their antiproliferative effect against carcinoma cells (HeLa, CaCo-2, SW620 and HT29), leukemia cells (K562 and CCRF-CEM), lymphoma cells (Raji and HuT-78), and MDCK as a normal cell line. Antiproliferative effect on coumarins hybrids was estimated after 72h by MTT test. Membrane integrity, as an indicator of cytotoxicity of selected hybrids was determined after 3, 6, 12 and 24 h by LDH release assay. The antioxidant capacity of new coumarine hybrids was determined by DPPH scavenging during 1h. Flow cytometry was used to determine cell cycle, as well as detection of ROS and apoptosis in K562 cells. Among tested coumarin hybrids, 6,7 – dihydroxycoumarins showed the highest antioxidative activity with meaningful cell growth inhibition of tumor cells. Disrupted cell membrane integrity is apparent after 3h of incubation with >25% of LDH released. Tested hybrids caused accumulation of cells mostly in G1 and G2/M phases in cell cycle. One of tested dihydroxycoumarin showed reduction of ROS accumulation in K562 cells. Induction of apoptosis in K562 cells was not detected. Obtained results suggest that further biological evaluation of new synthesized coumarines hybrids is required.

P21: PI3K δ IS A NEW THERAPEUTIC TARGET IN HEPATOCELLULAR CARCINOMA

EUNKYONG KO, GUHUNG JUNG

*Department of Biological Sciences, College of Natural Sciences, Seoul National University, Gwanak-gu, Republic of Korea
(e-mail: drjung@snu.ac.kr)*

Class I phosphoinositide 3-kinase (PI3K) signaling is a major pathway in human cancer development and progression. Amongst the four PI3K isoforms, PI3K α and PI3K β are ubiquitously expressed while PI3K γ and PI3K δ are mainly found in leukocytes. Until now, PI3K targeting in solid tumors has focused on inhibiting PI3K α - and PI3K β -mediated cancer-cell-intrinsic PI3K activity. The role of PI3K δ in solid tumors is unknown. Here, we evaluated the effects of PI3K δ using established hepatocellular carcinoma (HCC) cells, malignant hepatocytes derived from patients with advanced HCC, murine models, and HCC tissues using RNA-sequencing, quantitative PCR, immunoblotting, immunofluorescence, microarray, LC/MS-MS, and kinase assay. We established a chemical carcinogenesis model of liver malignancy, which reflects the malignant phenotype and the in vivo environment of advanced HCC. In this in vivo advanced HCC-mimic system using HCC-cells treated with H₂O₂, we showed that H₂O₂ selectively increases PI3K δ activity while decreasing that of other class I PI3Ks. Blocking PI3K δ activity with a PI3K δ inhibitor or siRNA-mediated PI3K δ gene silencing inhibited HCC-cell proliferation and dampened key features of malignant HCC, including the upregulation of telomerase reverse transcriptase (TERT). Mechanistically, H₂O₂ induced oxidative modification of the serpin peptidase inhibitor, SERPINA3, blocking its ubiquitin-dependent degradation and enhancing its activity as a transcriptional activator of PI3K δ and TERT. High PI3K δ levels in HCC were found to correlate with poor survival rates, with human advanced HCC showing positive correlations between the protein levels of oxidized-SERPINA3, PI3K δ , and TERT. Thus, PI3K δ plays significant roles in malignant liver tumors.

P22: SALINOMYCIN AFFECTS SECRETORY PATHWAY AND PROTEIN GLYCOSYLATION IN CELLS THAT HAVE UNDERGONE AN EPITHELIAL TO MESENCHYMAL TRANSITION

MARKO MARJANOVIĆ¹, ANA-MATEA MIKECIN DRAŽIĆ¹, FILIP KLIČEK², MISLAV NOVOKMET², GORDAN LAUC², MARIJETA KRALJ¹

¹ *Laboratory for Experimental Therapy, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: marijeta.kralj@irb.hr)*

² *Glycoscience Research Laboratory, Genos Ltd., Zagreb, Croatia*

Cancer cells that undergo an epithelial to mesenchymal transition (EMT) become invasive and drug-resistant. Also, such cells form primary and metastatic tumors, making them functionally indistinguishable from cancer stem cells (CSC). EMT can be induced experimentally by overexpression of transcription factors, such as Snail or Twist. Thus, a breast EMT/CSC model was established by Twist overexpression in immortalized human mammary epithelial cells (HMLE). Using this model, salinomycin was identified to be selectively toxic towards CSCs. Salinomycin is a K⁺/H⁺ exchanger that can affect cation transport across different cell membranes. However, the exact mechanism of the observed selectivity remains largely unknown.

We have shown that salinomycin affects postranslational modifications of membrane proteins in the aforementioned cell model: HMLE-Twist (EMT) and HMLE-pBp (control) cell lines. We demonstrate that salinomycin induces ER and Golgi apparatus stress leading to disturbances in secretory pathway which may represent a key vulnerability of EMT cells. Besides, modifications of N-glycans in the Golgi apparatus are pH dependent and a disruption of pH homeostasis in Golgi is visible in change of N-glycan structures. Therefore, we also investigated the effect of salinomycin on N-glyco profile of the CSC model. We correlated its effect with monensin, another ionophore, which is a well described inhibitor of Golgi function. The results show that the N-glycome profile of secreted proteins changed on both cell lines after the treatment with salinomycin. The most evident difference in profiles after treatment, is the loss of highly branched complex glycan structures and the relative increase of simpler glycans. Importantly, the effect of salinomycin is similar to monensin and is more prominent on HMLE-Twist than on HMLE-pBp cell line.

P23: TESTICULAR TERATOCARCINOMA OF MAN AND EXPERIMENTAL MOUSE TERATOCARCINOMA: COMPARATIVE ANALYSIS OF APOPTOTIC AND PROLIFERATIVE ACTIVITY

JURE KRASIC^{1,2}, NEBOJSA VUJNOVIC^{1,2}, ROBERT TERLEVIC³, ANA KATUSIC BOJANAC^{1,2}, MONIKA ULAMEC^{4,5}, FLORIJANA BULIC-JAKUS^{1,2}, BOZO KRUSLIN^{4,5}, DAVOR JEZEK^{2,6}, NINO SINCIC^{1,2}

¹ Department of Medical Biology, University of Zagreb School of Medicine, Zagreb, Croatia

² Centre of Excellence for Reproductive and Regenerative Medicine, University of Zagreb School of Medicine, Zagreb, Croatia
(e-mail: jure.krasic@mef.hr)

³ Department of Pathology, General Hospital Pula, Pula, Croatia

⁴ Ljudevit Jurak Clinical Department of Pathology and Cytology, University Clinical Hospital Center Sestre milosrdnice, Zagreb, Croatia

⁵ Department of Pathology, University of Zagreb, School of Medicine, Zagreb, Croatia

⁶ Department of Histology, University of Zagreb School of Medicine, Zagreb, Croatia

Testicular Germ Cell Tumours (TGCT) are the most frequent malignancies in young male population and believed to be initiated by epimutations, i.e. aberrant epigenetics. Teratocarcinoma is a mixed TGCT merging teratoma and embryonal carcinoma in a single neoplastic entity. Teratoma is the most differentiated TGCT type encompassing all three germ layer derived tissues. EC cells constitute a pluripotent “core” of teratocarcinomas, are highly similar to embryonic stem cells and express same pluripotent markers: OCT4, SOX2 and NANOG. EC cells may differentiate into teratoma tissue or retain their pluripotency. The well-known and established experimental mouse model of teratocarcinoma, at Biology Department of Zagreb’s School of Medicine, has been defined based on a histological/histochemical basis and its molecular signature has not yet been comprehensively studied. The aim of this study was to compare the rate of apoptotic and proliferative activity in the experimental mouse model to human teratocarcinomas.

Formalin-fixed paraffin-embedded tissue from 10 human testicular teratocarcinoma and 10 animal model tumors was used for immunohistochemical detection of Caspase-3 and PCNA expression. Slides were analysed semi-quantitatively, at the area of strongest reaction (“hot-spot”), by pathologist, graded from 0-3, depending on the percentage of reactive cells. Data was analysed in GraphPad Prism using Mann-Whitney test.

Results have shown difference in rate of apoptosis between human and experimental mouse teratocarcinomas, with mouse showing higher rate (>25% of positive cells) in 64% of tumors compared to 30% of human with highest reaction. PCNA quantification has shown comparable levels of PCNA expression in EC regions of experimental mouse model and human teratocarcinomas, while in the teratoma regions mouse model has a higher proliferative activity defined with PCNA staining.

Some of differences could be attributed to the fact that this study was a pilot with a relatively small sample pool, intragroup difference in biological development and “age” between human teratocarcinomas as well as intergroup difference in biological development between human and mouse model teratocarcinomas. Further analysis of expression between comparable regions should be done to account for these differences.

P24: PUMA AND CELLULAR DEATH OF FAS-MEDIATED APOPTOSIS IN LIVER CANCER

RUSDINA BTE LADJU^{1,2}, MUH. NASRUM MASSI², CLAUDIO TIRIBELLI¹, CAECILIA HC SUKOWATI¹

¹ *Fondazione Italiana Fegato, AREA Science Park, Trieste, Italy*

² *Faculty of Medicine, University of Hasanuddin, Makassar, Indonesia
(e-mail: rbteladju@fegato.it)*

FAS/CD95 is a member of the death receptor superfamily that can induce apoptosis when binds to its cognate ligand FASL/CD95L and it acts as the key component of the extrinsic death pathway. PUMA is a pro-apoptotic protein that involved in p53-dependent and independent apoptosis. This study reports on a relevant function of PUMA after an extrinsic FAS-apoptosis induction in liver cancer (hepatocellular carcinoma; HCC) cells. For the in vivo study, the mRNA analysis of FAS, FASL, and PUMA were collected from 111 patients undergoing liver resection without any prior treatments (39 HCC, 30 peri-HCC, 31 distal/cirrhosis); liver donors were used as control (11 normal). For the in vitro study, human immortalized hepatocyte cell line IHH and HCC cell line HepG2 were used. Apoptosis-induction was performed by using anti-FAS (DX2) at concentrations of 250 ng/ml and 500 ng/ml for 24 hours. Flow cytometry was performed for FAS/FASL positivity and Annexin-V- PI apoptosis test, quantitative real-time PCR for mRNA expressions, and growth curve test for cells viability. The expressions of FAS and FASL mRNA were significantly increased in HCC as compared to normal tissues ($p < 0.05$). PUMA mRNA was positive in 13/32 (41%) HCC tissue samples, 19/27 (70%) in peri-HCC and 24/31 (77%) cirrhosis tissues samples. For in vitro study, anti-FAS treatment did not affect cells viability up to 13 days. After 24 hours treatment, PUMA mRNA was up regulated only in IHH where the transcription factors p53 and c-Myc were up-regulated, while NF- κ B was unchanged. In contrast, all these three factors were slightly down-regulated in HepG2 while TNF- α was significantly up-regulated. In both models, the importance of PUMA and FAS/FASL in HCC pathologies was demonstrated. Acute treatment by anti-FAS dysregulated transcription factors p53, c-Myc and NF- κ B suggesting their correlation with the PUMA. The differential pattern of expression between hepatocyte and HCC cell lines suggests a less-effective FAS-induction apoptosis in HCC.

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P25: ANTICANCER EFFECTS OF THE EXTRACTS OF TURKISH ENDEMIC SPECIES *HYPERICUM PERFORATUM*

IVANA Z. MATIĆ^{1*}, SERCAN ERGÜN^{2*}, MARIJA ĐORĐIĆ CRNOGORAC¹, NINA PETROVIĆ^{1,3}, ANA DAMJANOVIĆ¹, SEMA MISIR⁴, YÜKSEL ALIYAZICIOĞLU⁵, TATJANA STANOJKOVIĆ¹

*These authors contributed equally to this work.

¹ *Institute of Oncology and Radiology of Serbia, Belgrade, Serbia*
(e-mail: ivanamatic@sbb.rs)

² *Department of Medical Biology, Faculty of Medicine, Ordu University, Ordu, Turkey*

³ *Vinča Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia*

⁴ *Department of Biochemistry, Faculty of Pharmacy, Cumhuriyet University, Sivas, Turkey*

⁵ *Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey*

Six extracts were prepared from *Hypericum perforatum*, an endemic plant species which grows in Turkey: methanol (1), ethyl-acetate (2), and hexane (3) extracts of branch-body part, as well as methanol (4), ethyl-acetate (5), and hexane (6) extracts of flowers. The aim of this research was to evaluate the anti-cancer properties of these extracts. The cytotoxic activity of the extracts was examined against three human cancer cell lines: cervical adenocarcinoma HeLa, chronic myelogenous leukemia K562, and lung carcinoma A549, as well as against normal human lung fibroblasts MRC-5. Changes in the cell cycle of treated HeLa cells were analyzed by flow cytometry. Measurement of mRNA expression levels in HeLa cells was done by quantitative real time PCR. Five examined extracts (2-6) showed selective concentration-dependent cytotoxic effects on HeLa, K562 and A549 cancer cells. Extract 1 exhibited weak cytotoxicity. Among tested cancer cell lines, HeLa cells were the most sensitive to the cytotoxic activities of the extracts 6, 5 and 3 with IC₅₀ values of 6.76, 8.37, and 10.91 µg/mL respectively. In addition, the extracts 4 and 2 exerted pronounced cytotoxicity against HeLa cells with IC₅₀ values of 23.74 and 34.67 µg/mL. The mechanisms of the anticancer effects of the extracts 2-6 in HeLa cells were further examined. The investigated extracts applied at 2IC₅₀ concentrations for 24 h caused increase in the percentage of HeLa cells within subG1 cell cycle phase when compared with untreated, control cell samples. These extracts demonstrated the ability to trigger apoptosis in HeLa cells through activation of caspase-3, the main effector caspase. To explore the possible antimetastatic and anti-angiogenic properties of *Hypericum perforatum* extracts, their effects on gene expression levels of matrix-metalloproteinase-2 (MMP2), matrix-metalloproteinase-9 (MMP9), matrix-metalloproteinase inhibitor 3 (TIMP3), and vascular endothelial growth factor A (VEGFA) in HeLa cells were determined. All tested extracts remarkably decreased the expression levels of each of the four examined genes in HeLa cells after 24 h incubation. In conclusion, results of our research may suggest the promising anticancer potential of extracts obtained from *Hypericum perforatum*, plant species endemic to Turkey.

P26: IQGAP-RELATED PROTEIN IqgC SUPPRESSES Ras SIGNALING DURING MACROPINOCYTOSIS

MAJA MARINOVIĆ¹, LUCIJA MIJANOVIĆ¹, MARKO ŠOŠTAR¹, MATEJ VIZOVIŠEK²,
ALEXANDER JUNEMANN³, MARKO FONOVIĆ^{2,4}, BORIS TURK^{2,4,5}, IGOR WEBER¹,
JAN FAIX³, VEDRANA FILIĆ¹

¹ Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: Lucija.Mijanovic@irb.hr)

² Department of Biochemistry and Molecular and Structural Biology, Jožef Stefan Institute, Ljubljana, Slovenia

³ Institute for Biophysical Chemistry, Hannover Medical School, Hannover, Germany

⁴ Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins, Ljubljana, Slovenia

⁵ Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

Macropinocytosis is a highly conserved large-scale endocytic process utilized by eukaryotic cells to internalize extracellular fluid. It is an actin cytoskeleton-driven process, regulated by Ras proteins. On early endosomes, Ras signaling needs to be precisely regulated and key factors responsible for the termination of Ras signaling in timely manner are GTPase activating proteins (GAPs), which inactivate Ras by promoting the hydrolysis of the GTP bound to active Ras into GDP. In our work, we characterized Dictyostelium discoideum RasGAP protein involved in macropinocytosis regulation, an IQGAP-related protein IqgC, which we showed to be a genuine RasGAP. Using mass spectrometry, yeast two-hybrid and bimolecular fluorescence complementation assays, we showed that IqgC directly binds active RasG, one of the main positive regulators of macropinocytosis in Dictyostelium. RasGAP activity of IqgC towards RasG and human H-Ras was demonstrated using luminescence based GAP assay. Confocal microscopy confirmed that IqgC colocalizes with RasG and its localization is almost exclusively confined to macropinosomes. Phenotypic assays showed that IqgC negatively regulates macropinocytosis and restrains the size of macropinosomes. The iqqC- cells have a mild cytokinesis defect but no growth defects when grown in shaken suspension. Taken together, our results show that IqgC is a RasGAP that negatively regulates RasG signaling in Dictyostelium discoideum specifically during macropinocytosis. In higher eukaryotes, owing to the development of multicellularity, macropinocytosis is no longer used for acquiring nutrients. Instead, it is used for a range of other physiological processes; for example, dendritic cells use constitutive macropinocytosis to internalize soluble antigens. However, in recent years it has become apparent that in Ras-driven tumors cancer cells upregulate the macropinocytic pathway and utilize it in a similar way to amoebae, as a means of nutrient uptake. More precisely, it has been shown that they use it to internalize ATP and serum albumin as a source of glutamine to sustain cell proliferation, which indicates possible alternative strategies for approaching anti-cancer therapies and emphasizes the need to understand the process of macropinocytosis in depth.

P27: PROPRIETARY CROWN ETHERS REVERSE MULTIDRUG RESISTANCE AND AFFECT MITOCHONDRIAL FUNCTION IN CANCER CELLS

MARIJA MIOČ, MARKO MARJANOVIĆ, IVA GUBEROVIĆ, MARIJETA KRALJ

*Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: marijacovic1990@gmail.com)*

Multidrug resistance (MDR) is a known characteristic of many cancers and is a major cause of failure of chemotherapy treatments. Various cellular pathways are responsible for this phenomenon, including overexpression of ATP binding cassette (ABC) transporters called ABCB1 (MDR1/P-glycoprotein) and ABCG2 (BCRP) by cancer cells, which makes this group of proteins a valuable target for cancer therapy by reversal of MDR. Salinomycin, naturally occurring potassium (K⁺) ionophore, was shown to inhibit P-gp transporter and to reverse MDR effectively. Salinomycin is a K⁺/H⁺ exchanger that can affect cation transport across different cellular membranes and thus impact on membranes' bioenergetic performance, including mitochondrial membrane polarization and function.

Based on the above-mentioned studies and anticancer activity of crown-ethers that act as K⁺ ionophores (previously published by our group), we showed that these compounds exhibit inhibitory effect towards ABC transporters, disrupt the potassium transport and modulate mitochondrial membrane transport.

In this study, we showed the effect of adamantane-substituted monoaza- and diaza-18-crown-6 ether compounds on MDR reversal and their effect on mitochondrial membrane function, depending on their lipophilicity as well as on the chemical structure of the linker to adamantane moiety. Compounds that showed as the most potent P-gp and ABCG2 inhibitors were able to sensitize cancer cells to conventional chemotherapeutics paclitaxel and mitoxantrone, the substrates of P-gp and ABCG2 transporters, respectively. These data are promising and demonstrate a potential use of crown ethers in cancer therapy, by their ability to reverse MDR.

P28: SURVIVIN ISOFORM EXPRESION IN GLI1-3 KNOCK-OUT OVARIAN CARCINOMA CELL LINES

MAJA GREGORIĆ¹, DIANA TRNSKI², MAJA SABOL², PETAR OZRETIĆ², SONJA LEVANAT², NIKOLINA RINČIĆ², DRŽISLAV KALAFATIĆ³, VESNA MUSANI¹

¹ Zagreb Health School, Zagreb, Croatia
(e-mail: vmusani@irb.hr)

² Laboratory for Hereditary Cancer, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia

³ Department of Obstetrics and Gynecology, University Hospital Centre Zagreb, Zagreb, Croatia

Ovarian cancer is the eighth most common tumor type in women worldwide, and the most lethal female gynecological malignancy with almost no difference in mortality between developed and developing countries. Because it remains asymptomatic for long time it usually gets diagnosed in late stages when treatment options are limited and overall survival remains poor.

Hedgehog-GLI (HH-GLI) signaling pathway is one of the major developmental pathways and is responsible for the formation of various tissues and organs, including ovaries. In the adult organism it is involved in the stem cell maintenance, tissue homeostasis and in the immune response.

Survivin is an inhibitor of apoptosis protein (IAP) that plays a role in multiple processes, including proliferation and cell survival. It is widely expressed during development, but rarely expressed in adult tissues. Survivin expression in tumors has been associated with aggressive behavior, resistance to radiation and chemotherapy and poor survival. Survivin has at least 5 different splice variants (wild type, 2 α , 2B, 3B and deltaEx3). The expression levels of various survivin isoforms have been associated with clinic-pathologic characteristics in some cancers. Survivin has been recently shown to be a direct target of HH/GLI signaling pathway and has several binding sites for GLI transcription factors in its promoter region.

The aim of this study was to investigate the expression of survivin isoforms in SKOV-3 ovarian carcinoma cell line with GLI1-3 knock-outs (KO) treated with GANT-61 pathway inhibitor.

GLI1 and GLI2 KO lines show downregulation of survivin isoforms, while GLI3 KO has no effect on expression of survivin isoforms. GLI1 KO regulates expression of all five analyzed isoforms, while GLI2 affects only 3B and deltaEx3 isoforms. Combined treatment of KO cell lines with GANT-61 shows additional inhibitory effect of GANT-61 on wild type, 2 α and 2B isoforms, suggesting that these isoforms are regulated by both GLI1 and GLI2. On the other hand, 3B and deltaEx3 isoforms are not additionally down-regulated by GANT-61 treatment, suggesting a different regulatory mechanism for these two isoforms.

P29: DEVELOPMENT OF RESISTANCE TO ANTITELOMERASE THERAPY IN BREAST CANCER CELLS SUBMITTED TO LONG-TERM EXPOSITION TO MST-312

DIEGO MADUREIRA DE OLIVEIRA¹, KAROLLYNE DA SILVA MORAIS¹,
ANA FLÁVIA REIS GUIMARÃES¹, GISELLE PINTO DE FARIA LOPES²

¹ *Multidisciplinary Laboratory of Human Health, University of Brasilia, Federal District, Brazil*
(e-mail: dmadureira@unb.br)

² *National Cancer Institute of Brazil, Rio de Janeiro, Brazil*

Telomerase is an enzyme responsible for telomere maintenance in almost all human cancer cells (80-90%) but generally not expressed in somatic ones. Therefore, antitelomerase therapy is a potential revolutionary therapeutic strategy, and antitumor activity of telomerase inhibitors has been extensively studied recently. However, the effects expected from treatment with telomerase inhibitors, such as breakage-fusion-bridge cycle or cell senescence, will appear only after many cell divisions. In this work, the consequences of long-term exposure of human breast cancer cells to telomerase inhibitor MST-312 were investigated. This compound presented cytotoxic action and promoted telomere erosion, senescence and chromosome aberrations, but in a small proportion. As main effect, the chronic exposition caused cell adaptation by different mechanisms and development of resistance to antitelomerase action of MST-312. In MDA-MB-231 cells, selection of clones with very long telomeres was responsible for the new phenotype. In the other hand, MCF7 cells overexpressed telomerase in response to the inhibitor. Classical multi-drug resistance mechanisms (MDR) and alternative lengthening of telomeres (ALT) had no relevant role in the development of resistance. Both of these mechanisms are potential causes of therapeutic failure, and have to be evaluated when considering antitelomerase therapy for breast cancer.

P30: MODELLING NON CANONICAL NOTCH INTERACTION OF dnMAML WITH p53 IN BREAST CANCER

TEUTA OPAČAK-BERNARDI¹, HRVOJE BRKIĆ², MARIJANA JUKIĆ¹, LJUBICA GLAVAŠ-OBROVAC¹

¹ *Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
(e-mail: tbernardi@mefos.hr)*

² *Department of Biophysics and Radiology, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia*

Notch signaling is one of the most important developmental pathways present in all organisms. Canonical branch of the signaling cascade affects target genes and regulates cell metabolism through regulation of expression. Non-canonical branch represents a complicated network of signaling crosstalk. The basis for non-canonical signaling is direct interaction with effectors from different signaling cascades, including but not limited to Akt, Hif, NFκB, mTOR and p53. This branch is still relatively undiscovered in spite of great impact it can have on cellular development. One of the possibilities of interaction is between p53 and MAML.

The region of MAML responsible for interaction with p53 is located on the N-terminal portion of MAML, in the section that was identified as dnMAML due to its ability to inhibit canonical Notch. To test the hypothesis of dnMAML affecting p53 availability in the cell dnMAML was incorporated into a carrier molecule to enable cellular delivery and tested on three breast cancer cells lines (MCF7, MDA-MB 231 and MDA-MB 468). The cells were chosen due to their difference in p53 status. Cells were treated with dnMAML and empty carrier to determine if there is a change in p53 quantity after treatment with dnMAML. p53 was identified using Western blot and its quantity in different samples determined by total protein evaluation. The treated samples showed decreased levels of p53 when dnMAML was present compared to both untreated controls and samples treated with only the carrier protein.

The interaction was then modeled in silico using structure information from the PDB database. The models were rendered using AutoDock software to find the best binding solution for the two molecules. The model can be used to further determine changes in binding profiles when different p53 mutations are taken into account.

The presented results need to be further confirmed using direct interaction methods like mammalian two hybrid method, but show that there is still a great deal to be discovered about crosstalk between signaling cascades and even more about the role Notch pathway had in development and regulation of cellular fates in cancer.

P31: INTERACTION OF microRNA MOLECULES AND HEDGEHOG-GLI SIGNALING PATHWAY GENES IN HIGH-GRADE SEROUS OVARIAN CANCER

PETAR OZRETIĆ¹, MAJA SABOL¹, HUBERT HACKL², CHRISTINA PLATTNER², DIANA TRNSKI¹, DRŽISLAV KALAFATIĆ³, VESNA MUSANI¹, ROBERT ZEILINGER³, SONJA LEVANAT²

¹ Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: pozretic@irb.hr)

² Division of Bioinformatics, Biocenter, Medical University of Innsbruck, Innsbruck, Austria

³ Department of Obstetrics and Gynaecology, University Hospital Centre Zagreb, Zagreb, Croatia

⁴ Department of Obstetrics and Gynaecology, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria

Ovarian cancer is the leading cause of death from gynecological malignancies in the Western world. Its high death rate is a result of the fact that >60% patients are diagnosed in advanced stage of the disease. Hedgehog-GLI (HH-GLI) signaling pathway is involved in embryonal ovarian development, but its atypical activation can lead to different types of ovarian tumors. Our previous studies showed aberrant HH-GLI activity in some ovarian tumor types and hypermethylation in the promoter of tumor suppressor gene PTCH1 (Ozretić et al. *Int J Oncol.* 2017; Musani et al. *Gene.* 2013; Sabol et al. *Int J Oncol.* 2012; Maurac et al. *Int J Gynecol Pathol.* 2012; Cretnik et al. *Int J Mol Med.* 2007). We hypothesize that changes in the expression of miRNA molecules related to the HH-GLI signaling pathway genes contribute to the development of high-grade serous ovarian cancer (HGSOC), the most malignant type and most difficult to detect at an earlier stage.

We conducted miRNA and gene expression profiling of 16 HGSOC and 8 healthy Fallopian tube fresh frozen tissue samples as a control (Reade et al. *J Obstet Gynaecol Can.* 2014) with Agilent SurePrint G3 Human miRNA 8x60K Microarray Kit and Agilent SurePrint G3 Human Gene Expression v3 8x60K Microarray Kit, respectively. Microarray data was analyzed using R/Bioconductor packages AgiMicroRna and limma. Online tools DianaMicroT, miRDB, RNA22-HSA and TargetScan were used to predict target genes of discovered differentially expressed miRNAs. Expression of selected miRNAs and genes was verified using TaqMan and SYBR Green qPCR, respectively, on an extended set of 10 Fallopian tube and 47 HGSOC samples.

Data filtration gave a list of 55 miRNAs differentially expressed in HGSOC: 32 up- and 23 down-regulated. In addition, 1,090 genes were significantly over- and 1,692 were under-expressed in HGSOC. By comparing a list of predicted miRNA target genes and differentially expressed HH-GLI pathway genes we revealed a couple of combinations of miRNAs and their target genes: GRK3 / hsa-miR-513a-5p; BCL2 / hsa-miR-96-5p and hsa-miR-21-5p; IHH / hsa-miR-103a-3p, hsa-miR-107 and hsa-miR-16-5p; and GLI3 / hsa-miR-200b-3p. Co-expression of all those target genes / miRNAs has been confirmed in an extended set of samples. Additional in vitro verification will be continued with in vivo approach.

Our results highlighted several candidate miRNAs potentially targeting HH-GLI pathway genes. When additionally verified, they could be used as potential diagnostic and prognostic markers or even therapeutic targets for HGSOC.

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P32: MITF-miR211 AXIS IS A NOVEL AUTOPHAGY AMPLIFIER SYSTEM DURING CELLULAR STRESS

DENIZ GULFEM OZTURK¹, MUHAMMED KOCAK¹, DEVRIM GOZUACIK^{1,2}

¹ Sabanci University, Faculty of Engineering and Natural Sciences, Molecular Biology, Genetics and Bioengineering Program, Orhanli-Tuzla, Istanbul, Turkey
(e-mail: dgulfemozturk@sabanciuniv.edu)

² Sabanci University Nanotechnology Research and Application Center (SUNUM), Sabanci University, Orhanli-Tuzla, Istanbul, Turkey

Macroautophagy (autophagy) is an evolutionarily conserved recycling and stress response mechanism. Active at basal levels in eukaryotes, autophagy is upregulated under stress providing cells with building blocks such as amino acids. A lysosome-integrated sensor system composed of RAG GTPases and MTOR complex 1 (MTORC1) regulates lysosome biogenesis and autophagy in response to amino acid availability. Stress-mediated inhibition of MTORC1 results in the dephosphorylation and nuclear translocation of the TFE/MITF family of transcriptional factors, and triggers an autophagy- and lysosomal-related gene transcription program. The role of family members TFEB and TFE3 have been studied in detail, but the importance of MITF proteins in autophagy regulation is not clear so far. Here we introduce for the first time a specific role for MITF in autophagy control that involves upregulation of MIR211. We show that, under stress conditions including starvation and MTOR inhibition, a MITF-MIR211 axis constitutes a novel feed-forward loop that controls autophagic activity in cells. Direct targeting of the MTORC2 component RICTOR by MIR211 led to the inhibition of the MTORC1 pathway, further stimulating MITF translocation to the nucleus and completing an autophagy amplification loop. In line with a ubiquitous function, MITF and MIR211 were co-expressed in all tested cell lines and human tissues, and the effects on autophagy were observed in a cell-type independent manner. Thus, our study provides direct evidence that MITF has rate-limiting and specific functions in autophagy regulation. Collectively, the MITF-MIR211 axis constitutes a novel and universal autophagy amplification system that sustains autophagic activity under stress conditions.

P33: CONTACT DERMATITIS AS A RESULT OF COMPLEX PATHOGENETIC PATHWAYS

GABY NOVAK-BILIĆ¹, LIBORIJA LUGOVIĆ-MIHIC^{2,3}, NIVES PAP², MAJDA VUČIĆ^{3,4},
SANDRA STANIĆ-DUKTAJ¹

¹ *Dermatology and Venerology Unit, Zabok General Hospital, Zabok, Croatia*

² *Clinical Department of Dermatovenereology, Sestre milosrdnice University Hospital Center, Zagreb, Croatia*
(e-mail: nives.pap@gmail.com)

³ *School of Dental Medicine, University of Zagreb, Zagreb, Croatia*

⁴ *Department of Pathology, University Hospital Center Sestre milosrdnice, Zagreb, Croatia.*

Contact dermatitis (CD) is caused by various substances that exert irritant (toxic) effects or induce immune responses (allergic reactions) and may manifest as Irritant CD and Allergic CD and. Irritant CD is a nonspecific skin response to direct chemical skin damage and/or with releasing inflammatory mediators, while Allergic CD is a delayed hypersensitivity reaction (type IV) to allergens which includes immunological responses (due to the interaction of T cells and cytokines). Thereby, contact skin lesions may be the consequences of contact with various irritants or allergens, or due to other factors (e.g., UV radiation, microbials), intrinsic factors (e.g. in autoimmune responses) or even by a combination of both. Impaired barrier function also participates by promoting bacterial biofilms and creating an environment favoring sensitization. The development of Allergic CD skin lesions includes complex immunological pathways and inflammatory mediators, influenced by both genetic (predominantly filaggrin mutations) and environmental triggers. In the pathogenesis of Allergic CD the role of antimicrobial peptides (AMP) is prominent; but also other factors such as alarmins, proteases, immunoproteomics, lipids, natural moisturizing factors, tight junctions, smoking, etc. However, based on many current investigations, future perspectives may reveal new pathogenetic factors and scientific data important for the work and treatment of CD patients.

P34: HOW TO MANAGE PATIENTS WITH CUTANEOUS PSEUDOLYMPHOMA?

GABY NOVAK-BILIĆ¹, SANDRA STANIĆ DUKTAJ¹, NIVES PAP^{2,3}, MIRNA BRADAMANTE², LIBORIJA LUGOVIĆ-MIHIĆ^{3,4}

¹ *Dermatology and Venereology Unit, Zabok General Hospital, Zabok, Croatia*

² *Zagreb University School of Medicine, University Department of Dermatology and Venereology, Zagreb University Hospital, Zagreb, Croatia*

³ *Clinical Department of Dermatovenereology, Sestre milosrdnice University Hospital Center, Zagreb, Croatia (e-mail: nives.pap@gmail.com)*

⁴ *School of Dental Medicine, University of Zagreb, Zagreb, Croatia*

The term cutaneous pseudolymphoma refers to a heterogeneous group of benign skin disorders that simulate cutaneous lymphomas histologically and sometimes clinically. The diagnosis of B cell pseudolymphoma requires a representative biopsy (excisional biopsy) which should be evaluated for morphology, growth pattern, and immunophenotype. We treated a female patient, aged 33, presented with the painful, erythematous, radiant tumor formation at skin in the temporal region. The patient had enlarged lymph nodes on the right side of the neck before the appearance of that tumor formation. The dermatoscopic finding was nonspecific. After the tumor biopsy was done, histologically the diagnosis of reactive lymphatic proliferation – pseudolymphoma or cutaneous lymphoma of B-cell immunophenotyp was set. After we had completely excised the change and had sent it to the immunohistochemical analysis, the finding hinted at florid skin lymphocyte hyperplasia of B- and T- lymphocytes. The results of other findings were normal (serologic test on *Borelia Burgdorferi*, ultrasound scan of the neck's lymph nodes, supraclavicular, and of the axillary nodes, nodes in inguinal canal and abdomen. Finally, the etiology remains unknown. In conclusion, the diagnosis of B cell pseudolymphoma requires a skin biopsy for histopathologic evaluation and immunophenotyping. In areas endemic for Lyme disease, patients with cutaneous B cell pseudolymphomas should undergo laboratory investigations for *Borelia burgdorferi* infection. B cell pseudolymphomas may resolve spontaneously over time. In clinical practice, lesions are often initially treated with topical or intralesional corticosteroids, but refractory lesions may be treated with surgical excision or radiotherapy.

P35: CHARACTERISATION OF INTEGRIN α V-DEPENDENT ADHESOME IN MELANOMA CELL LINE MDA-MB-435S

MLADEN PARADŽIK¹, JONATHAN D. HUMPHRIES², DAVOR NESTIĆ¹, DRAGOMIRA MAJHEN¹, ANA DEKANIĆ¹, NIKOLINA STOJANOVIĆ¹, DELPHINE SEDDA¹, IVANA SAMARŽIJA¹, IGOR WEBER³, MARTIN J. HUMPHRIES², ANDREJA AMBRIOVIĆ-RISTOV¹

¹ Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia

(e-mail: Mladen.Paradzic@irb.hr)

² Wellcome Trust Centre for Cell-Matrix Research, Faculty of Biology, Medicine & Health, University of Manchester, Manchester, United Kingdom

³ Laboratory for Cell Biophysics, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia

Integrins are heterodimeric glycoproteins that bind cells to extracellular matrix proteins. Upon integrin clustering, multimolecular integrin adhesion complexes (IACs) are formed, facilitating the linkage between integrins and the actin cytoskeleton and permitting bidirectional signalling. The α V integrin is expressed in most tumour cells, where it regulates an array of cellular functions and plays a role in anti-tumour drug resistance. The aim of this work was to assess α V-dependent changes in IAC composition in MDA-MB-435S melanoma cells in order to better understand the increased sensitivity to paclitaxel and vincristine upon integrin α V knockdown. Integrin α V-specific shRNA was cloned into pSUPER.puro, transfected into MDA-MB-435S cells using Lipofectamine, and cell clones were selected using puromycin. The sensitivity of cells to antitumor drugs was determined using an MTT assay. Cell migration was monitored using a Transwell assay. IACs were isolated following crosslinking and their molecular composition analysed using mass spectrometry (MS)-based proteomics. In two MDA-MB-435S-derived cell clones with decreased expression of integrin α V, expressing 15% (2 α V) or 5% (3 α V) of the control cells amount, increased sensitivity to paclitaxel and vincristine, decreased sensitivity to cisplatin, and decreased migration were observed. This data is consistent with previous results obtained following transient transfection with integrin α V siRNA. Cell clones 2 α V and 3 α V were smaller than the control cells and had lower number of focal adhesions as observed by interference reflection microscopy and immunofluorescence detection of phospho-paxillin, vinculin, talin and phospho-Src. MS analysis of isolated IACs from control MDA-MB-435S, 2 α V and 3 α V cells identified 282 proteins, including 36 out of 60 consensus adhesome proteins. As expected, in clones 2 α V and 3 α V, integrins α V, β 3 and β 5 were detected at much lower levels compared to control cells. In addition, lower levels of alpha-actinin-1 and -4, AHNAK, filamin-A and -B, HSP-70, liprin β 1, plectin, talin-1, tensin-3, vimentin, and vinculin were detected. These data will enable follow-up analyses of signalling by integrins α V β 3/ β 5 and therefore represent a valuable resource to improve our understanding of the mechanisms involved adhesion control of cell sensitivity to antitumor drugs and metastatic potential.

P36: PROTEOMIC ANALYSIS OF INTEGRIN α V-DEPENDENT ADHESION COMPLEXES IN TRIPLE NEGATIVE BREAST CANCER CELL LINE MDA-MB-231

MLADEN PARADŽIK¹, ZRINKA VITKOVIĆ¹, JONATHAN D. HUMPHRIES², ANA DEKANIĆ¹, NIKOLINA STOJANOVIĆ¹, MARTIN J. HUMPHRIES², ANDREJA AMBRIOVIĆ-RISTOV¹

¹ Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia

(e-mail: Mladen.Paradzic@irb.hr)

² Wellcome Trust Centre for Cell-Matrix Research, Faculty of Biology, Medicine & Health, University of Manchester, Manchester, United Kingdom

Integrins are heterodimeric receptors that mediate signalling across the plasma membrane from the extracellular environment to the actin cytoskeleton. Integrin binding to the the extracellular matrix induces formation of integrin adhesion complexes (IACs), large protein aggregates on the cytoplasmic tails of integrins. The composition of IACs has been named the adhesome, and some adhesion proteins have been identified as promising druggable candidates in cancer therapy. Our recently published data in triple negative breast cancer cell line MDA-MB-231 has shown that transient transfection with integrin α V-specific siRNA increased sensitivity to paclitaxel, vincristine and cisplatin, and decreased migration and invasion. Transient knockdown of integrin subunit β 5, but not β 3, has shown the same effect. The aim of this work was to assess integrin α V-dependent changes in IAC composition. Plasmid pSUPER.puro or pSUPER.puro containing integrin α V-specific shRNA were transfected into MDA-MB-231 cells using Lipofectamine, and two stably transfected cell clones 231sh (control plasmid) and 231sh α V (plasmid containing integrin α V-specific shRNA) were selected using puromycin. Decreased expression of integrin subunit α V and integrin heterodimers α V β 3 and α V β 5 was confirmed using flow cytometry and the sensitivity of cells to antitumor drugs was determined by using MTT assay. Surprisingly, cell clone 231sh α V was shown to be less sensitive to paclitaxel, vincristine and cisplatin as compared to 231sh which is not in line with the results obtained by transient transfection experiments. IACs were isolated following crosslinking and their molecular composition analysed using mass spectrometry (MS)-based proteomics. MS analysis of isolated IACs from control 231sh and 231sh α V cells identified 426 proteins, including 27 out of 60 consensus adhesome proteins. As expected, in 231sh α V clone, integrin α V was detected at much lower levels compared to 231sh. In addition, lower levels of various adhesion proteins such as actinin alpha (1 and 4), AHNK, filamin (A, B, C), HSP70, myosin 9, plectin, IQGAP1, talin-1, tensin-3, vimentin and vinculin were detected. These data will potentiate further analyses of integrin-related signalling. Also, the biological basis for the contradictory results of sensitivity to antitumor drugs for transient versus stable knock-down warrants further exploration.

P37: BIOLOGICAL ACTIVITY OF NOVEL RUTHENIUM(II)-ARENE COMPLEXES CONTAINING INTERCALATING LIGANDS IN MELANOMA CELLS

MARIJANA PAVLOVIĆ¹, STEFAN NIKOLIĆ², NEVENKA GLIGORIJEVIĆ¹, BILJANA DOJČINOVIĆ³, SANDRA ARANĐELOVIĆ¹, SANJA GRGURIĆ-ŠIPKA², SINIŠA RADULOVIĆ¹

¹ Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia
(e-mail: marijana.kajzerberger@gmail.com)

² Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

³ Centre of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Belgrade, Serbia

During the last decades in the field of metallothrapeutics development, ruthenium(II)-arene complexes with intercalating ligands, have attracted significant attention, due to their unique chemical and biological properties. Three newly synthesized ruthenium(II)-arene complexes: C1 ([η^6 -toluene)RuLCl]PF₆), C2 ([η^6 -p-cymene)RuLCl]PF₆) and C3 ([η^6 -benzene)RuLCl]PF₆; L=pyrido[2',3':5,6]pyrazino[2,3-f][1,10]phenanthroline) have been evaluated for in vitro cytotoxic activity on three human neoplastic cell lines (A549, A375, LS 174T) and on one human non-tumor cell line (MRC-5), by the MTT assay, in comparison to cisplatin as a referent compound. Complexes C1-C3 showed IC₅₀ values in the micromolar range below 100 μ M on all tested cell lines. Complex C2 displayed cytoselectivity against melanoma A375 cells (IC₅₀=15.78 μ M), observed in four times lower activity in MRC-5 cells and two times lower cytotoxicity than cisplatin, and it has been selected for further analyses of its biological effects. Flow cytometry analyses revealed that complex C2 induce neither substantial alterations in cell cycle phase distribution nor triggers apoptosis or necrosis in A375 cells, indicating distinguish and more specific type of action by which it manifests its high activity. Drug-accumulation study performed by ICP-MS showed that after 24 h of treatment with equimolar concentrations (10 μ M), complex C2 entered the cells less efficiently (1.55 ng Ru/106 cells) in comparison to cisplatin (28.58 ng Pt/106 cells) and distributed approximately equally in the cytosol and membrane/organelle fraction of A375 cells. Investigations in the 3D model of A375 cells, disclosed different effects of complex C2 and cisplatin on growth of multicellular tumor spheroids (MCTSs). While the size of cisplatin-treated MCTSs decreased with time, MCTSs treated with C2 continued to growth. Calcein-AM/PI dual staining for live/dead analysis of A375 MCTSs revealed preserved compactness of C2-treated MCTSs and appearance of dark necrotic core with predominantly live peripheral edge of cells, in contrast to cisplatin-induced dispersed spheroids with majority of dead cells. These results pointed out completely different mechanism of action of this type of ruthenium(II)-arene complexes versus cisplatin in A375 malignant melanoma cells, and necessity for additional biological studies for its potential application as anti-cancer drug candidate.

P38: PHYSICAL INTERACTIONS OF MAMMARY TUMOR CELLS MODULATE PHAGOCYTIC AND MICROBICIDAL CAPACITY OF RAW 264.7 MACROPHAGES

DÉBORA SILVESTRO MARES, JOSÉ RENILDO CARVALHO, THIAGO ALBUQUERQUE VIRAÇÃO, FABIANA TOSHIE CAMARGO KONNO, ANUSKA MARCELINO ALVARES-SARAIVA, VANESSA XAVIER, MARIA ANETE LALLO, ELIZABETH CRISTINA PEREZ

*Graduate Program in Environmental and Experimental Pathology, Paulista University, São Paulo, Brazil
(e-mail: elicristin@hotmail.com)*

Macrophages are the major populations of immune system cells found in the tumor microenvironment. Considering that tumor microenvironmental signals can affect macrophages functions, we investigate the influence of mammary tumor cells on phagocytic activity of murine macrophages in an in vitro model. For this, Raw 264.7 macrophages were cultivated alone or with 4T1 mammary tumor cells or supernatants of 4T1 mammary tumor cells for 48 hours on glass coverslips in a 24 well plates. The heat inactivated bread yeast particles were added at a 1:10 effector target ratio and incubated for two hours. After removed non-ingested yeast particles, coverslips were collected at 2 and 24 hours and submitted to Giemsa stained. Phagocytic index was calculated considering % of macrophages that internalized at least one yeast X the average number of fungal cells in these macrophages. In addition, production of nitric oxide and hydrogen peroxide were evaluated. Results show that macrophages cultivated with tumor cells presented higher phagocytic index, lower production of nitric oxide and unaltered levels of hydrogen peroxide when compared with macrophages cultured alone. In contrast, macrophages cultivated with mammary tumor cells supernatants demonstrated lower phagocytic index and similar levels of nitric oxide and hydrogen peroxide when compared with macrophages cultured alone. In conclusion, these results indicate that soluble factors and mainly physical interactions of mammary tumor cells modulate phagocytic and microbicidal activity of murine macrophages in vitro.

P39: DE NOVO EXPRESSION OF TRANSFECTED SIRTUIN 3 ENHANCES SUSCEPTIBILITY OF HUMAN MCF-7 BREAST CANCER CELLS TO HYPEROXIA TREATMENT

MARIJA PINTERIĆ¹, IVA I. PODGORSKI¹, SANDRA SOBOČANEC¹, MARIJANA POPOVIĆ HADŽIJA¹, MLADEN PARADŽIK², ANA DEKANIĆ², MAJA MARINOVIĆ², MIRNA HALASZ², ROBERT BELUŽIĆ¹, GRAZIA DAVIDOVIĆ¹, ANDREJA AMBRIOVIĆ-RISTOV², TIHOMIR BALOG¹

¹ Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: Marija.Pinteric@irb.hr)

² Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia

Sirtuin 3 (Sirt3) is the only member of sirtuin family linked to longevity in humans. In addition, important cellular and mitochondrial processes, including reactive oxygen species (ROS) generation are integrated through Sirt3. Furthermore, Sirt3 has a promising role in cancer tumorigenesis and treatment, but there have been controversies about its role as oncogene or tumor suppressor in different types of cancer. Breast cancer is the most frequent cancer among women, and ranks as the fifth cause of cancer death worldwide. Since breast cancer cells have strikingly reduced Sirt3 level, and even 20% of them have almost no detectable Sirt3 protein we wanted to examine the effect of de novo Sirt3 expression upon treatment with 95% O₂ (hyperoxia) in human MCF-7 breast cancer cells model. Since hypoxia is a hallmark of various tumors, we hypothesized that hyperoxia would have negative impact on cancer cells, thus providing therapeutic strategy against their tumorigenic properties. Therefore, in this study we have developed human MCF-7 breast cancer cells transfectants expressing the Sirt3 protein in order to test its potential in affecting the response of these cells upon the hyperoxic treatment, i.e. to clarify whether it sensitizes or makes these cancer cells more resistant to oxidative stress. De novo expression of Sirt3 decreased metabolic activity and cellular growth of MCF-7 cells, induced metabolic switch from glycolysis to oxidative phosphorylation, and decreased abundance of senescent cells. These effects were enhanced upon hyperoxic treatment: induction of DNA damage and upregulation of p53, with increase of ROS levels followed by mitochondrial and antioxidant dysfunction, resulted in additional reduction of metabolic activity and inhibition of cellular growth and survival. The mitigation of tumorigenic properties and enhancement of the susceptibility of the MCF-7 breast cancer cells to the hyperoxic treatment upon de novo Sirt3 expression, indicates that the impact of these factors, individually and in combination, should be further explored in vitro and particularly in vivo in breast cancer malignancies.

P40: THE HUMAN NME6: EXPRESSION AND SUBCELLULAR LOCALIZATION IN TUMOR CELL LINES

BASTIEN PROUST¹, MARTINA RADIĆ¹, NIKOLINA ŠKROBOT VIDAČEK¹, LUCIJA AČKAR¹, HELENA ČETKOVIĆ², MAJA HERAK BOSNAR¹

¹ *Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia*
(e-mail: bproust@irb.hr)

² *Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia*

Nucleoside diphosphate kinase (NDPK/NME/Nm23) family of enzymes catalyze the transfer of gamma phosphate from nucleoside triphosphates to nucleoside diphosphates. After the discovery of tumor metastasis regulation activity of NME1, this protein family sparked considerable interest in the scientific community. The Group I members (NME1-NME4) are highly conserved in their amino acid sequence and exhibit NDPK activity, while Group II (NME5-NME9) members display less homology and seem to lack NDPK activity, with a possible exception of NME6. Although little is known about Group II members, those evolutionary very old genes are presumed to participate in one or more basic cellular process. Therefore, we focused our studies on revealing the subcellular localization, quaternary structure and function of Group II human NME6 protein. The expression of NME6 was screened in several human tumor cell lines by Western blot, using specific anti-NME6 antibodies. Subcellular localization has been addressed using immunofluorescence coupled with confocal microscopy and confirmed by cell fractionation followed by Western blot analysis. CRISPR/Cas9 genome editing system was used for generating NME6 “knock-out” clones.

All human tumor cell lines studied express significant amounts of NME6 protein. Immunofluorescence revealed the colocalization of NME6 predominantly with mitochondria. The cell fractionation confirmed the presence of NME6 in the mitochondrial fraction although human NME6 protein does not possess the mitochondrial targeting sequence. Only monoallelic NME6 “knock-out” clones were produced, indicating that NME6 plays a significant role in cell survival. Further studies will be conducted to detect the NME6 potential NDPK activity, quaternary structure, its function in cellular processes and potential role in cancer.

P41: PROTEIN INTERACTIONS OF WILD-TYPE p53 IN HUMAN MELANOMA

MARTINA RADIĆ, NIKOLINA HANŽIĆ, MAJA HERAK BOSNAR, NEDA SLADE

*Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: Martina.Radic@irb.hr)*

The tumor suppressor protein p53 has been described as “the guardian of the genome” because of its role in conserving genome stability. p53 is mutated in more than 50% of human cancers increasing the oncogenic potential. In metastatic melanoma, p53 is rarely mutated, but, nevertheless fails to execute its tumor suppressor activity. We hypothesize that in malignant melanoma p53 function might be impaired by interactions with yet unknown proteins. Therefore, we are testing possible interactions of p53 with its family members, namely p53 and p73 isoforms and with members of the nm23/NDPK protein family, nm23-H1 and nm23-H2.

Experiments were performed with and without the induction of DNA damage, assuming that certain interactions occur only in specific cellular events such as genotoxic stress. Co-immunoprecipitation experiments in cell lines expressing wild-type p53 show interactions of endogenous p53 with several p73 isoforms. DNA damage treatment has enhanced p53- Δ Np73 binding affinity. The subcellular dynamics and co-localization of interacting partners were determined using live cell imaging. The results show that p53 mainly localizes in the nucleus, but occasionally enters the cytosol, p73 isoforms remain in the nucleus while Nm23 proteins, mainly localize in the cytosol but translocate to the nucleus upon DNA damage.

Our future plans will involve advanced confocal microscopy techniques, FRET/FLIM, in order to quantify and localize the detected protein interactions. We hope that determination of the nature and localization of p53 protein interactions will lead to a better understanding of its behavior in melanoma.

P42: SOX2 CANNOT BE USED AS A PREDICTIVE MARKER FOR RADIORESISTANCE OF COLORECTAL CANCER

VELDA SMAJLBEGOVIĆ¹, SNJEŽANA RAMIĆ², MELITA PERIĆ BALJA², IVA KIRAC²,
ANDRINA KOPJAR³, ELIZABETA HORVATIĆ⁴, DANKO VELIMIR VRDOLJAK⁵

¹ Oncology Clinic, Clinical Centre of Sarajevo University, Bosnia and Herzegovina
(e-mail: snjezana.ramic@zg.t-com.hr)

² Department of Genetic Counseling, University Hospital for Tumors, Sestre milosrdnice University Hospital Center, Zagreb, Croatia

³ Department of Pathology, General Hospital Varaždin, Varaždin, Croatia

⁴ Department of Pathology, Dr. T. Bardek General Hospital Koprivnica, Koprivnica, Croatia

⁵ Department of Surgical Oncology, University Hospital for Tumors, Sestre milosrdnice University Hospital Center, Zagreb, Croatia

Neoadjuvant long course chemoradiotherapy (LCCRT) in patients with locally advanced rectal cancer reduces the tumour mass and local recurrence, downstages the pathologic extent of the tumor and enables sphincter preservation surgery. Patients treated with LCCRT but have tumors resistant to such therapy have prolonged waiting time to surgical resection without adequate therapy, which can lead to tumor progression during that period¹. Selection of markers that can predict tumor radiosensitivity is necessary. There are several known mechanisms of radioresistance, and generation of cancer stem cells (CSC) is one of them². CSC has self-renewal ability and multilineage differentiation potential, and is more resistant to various therapeutic treatments². SOX2 was recently recognized as a gene associated with CSC and epithelial-mesenchymal transition that promotes resistance to chemotherapy in ovarian cancer³.

The research was conducted on the assumption that the determination of SOX2 in samples before the neoadjuvant chemoradiotherapy could predict tumor resistance to therapy. We evaluated the immunohistochemical expression of SOX2 protein in 62 colorectal carcinoma samples taken from a biopsy before LCCRT (Group 1) and matched samples after LCCRT (Group 2). We did not find a significant difference in SOX2 expression between groups (33.9% vs. 41.8%) ($\chi^2=0.78$; $P=0.376$). Twenty-one patients had good pathological response to LCCRT and only 19% of tumors showed SOX2 nuclear staining while tumors with poor pathological response to therapy expressed SOX2 in 46.3% of cases ($\chi^2=4.43$; $P=0.035$). Of the 22 patients who demonstrated progression of the disease after LCCRT and surgery, SOX2 was positive in 59% of tumors compared to 25% of SOX2 positive tumors in patients without disease progression ($\chi^2=7.07$; $P=0.008$). We noticed a discrepancy in SOX2 expression between groups. Thirteen tumors gained SOX2 expression after LCCRT compared to match samples before LCCRT whereas eleven tumors lost the SOX2 expression after chemoradiotherapy. Based on our results, nuclear staining of SOX2 is in correlation with poor response to therapy and worse prognosis but cannot be used as predictive marker of radioresistance of the colorectal cancer.

P43: COMPARISON OF EPIDEMIOLOGICAL DATA IN A PATIENT WITH TESTICULAR TUMORS AND HEALTHY VOLUNTEERS

DORA RAOS¹, MILAN MILOŠEVIĆ², JURE KRASIĆ^{1,3}, MIROSLAV TOMIĆ^{3,4}, TOMISLAV KULIŠ^{3,5}, ANA KATUŠIĆ BOJANAC^{1,3}, IGOR TOMAŠKOVIĆ⁵, FLORIANA BULIĆ-JAKUŠ^{1,3}, DAVOR JEŽEK^{3,6}, MONIKA ULAMEC^{3,4,7}, NINO SINČIĆ^{1,3}

¹ Department of Medical Biology, University of Zagreb School of Medicine, Zagreb, Croatia
(e-mail: dora.raos@gmail.com)

² Department of Environmental and Occupational Health and Sports, University of Zagreb School of Medicine, Zagreb, Croatia;

³ Centre of Excellence for Reproductive and Regenerative Medicine, University of Zagreb School of Medicine, Zagreb, Croatia

⁴ Ljudevit Jurak Clinical Department of Pathology and Cytology, University Clinical Hospital Center Sestre milosrdnice, Zagreb, Croatia

⁵ Department of Urology, University Hospital Centre Zagreb, Zagreb, Croatia

⁶ Department of Histology, University of Zagreb School of Medicine, Zagreb, Croatia

⁷ Department of Pathology, University of Zagreb, School of Medicine, Zagreb, Croatia

Testicular tumors represents 1% of all tumors, but it is public health problem while affecting male population in reproductive age. Approximately 95 % of all testicular neoplasms are germ cell tumors (TGCT) which are classified as pure seminomas (which compose about 55% of all TGCT) and non-seminoma group. Important role in their development have (micro)environmental and (epi)genetic factors. DNA methylation epimutations are recognized as potential tumor biomarkers because of their high frequency and extreme chemical and biological stability, giving them possible advantage in non-invasive diagnostics. Seminoma and non-seminoma TGCT differ in DNA methylation pattern.

In this paper results from epidemiological questionnaire collected in studies concerning pure seminoma tumors and their relationship with epigenetic changes are presented.

The study is conducted on two groups of examinees; control group (37 healthy volunteers) and patients (25 patients with diagnosis of pure seminoma). The participants were informed about the study and asked to fill in the epidemiological questionnaire. Five groups of questions were asked and targeted information about the examinee's marriage status, present and past environmental factors, life habits and life habits of their parents were collected. Statistical SPSS program was used to analyze and compare data from the questionnaire.

Average age of healthy volunteers was 27 and of the patients was 35. According to the results, 83.8% of volunteers and 52.0% of patients have a hobby, of which 73.0% of volunteers and 40.0 % of patients, refers to sport. Moreover, positive answer about current smoking gave 24.3% of healthy people and the average number of smoked cigarettes per day amounts for 7. On the other hand, 52.2% of the patients said that they smoke and the average number of cigarettes per day is 20. Also, consumption off vegetables and fruits per day among patients is almost 50% lower than among volunteers.

As expected, differences in lifestyle were observed between healthy control group and patients. Healthy volunteers live healthier, eat healthier food, smoke less and are more active, while patients smoke more and are not so active.

P44: ROYAL JELLY AND HUMAN INTERFERON- α N3 AFFECTS THE GLUTATHIONE LEVEL AND LIPID PEROXIDATION IN HUMAN COLORECTAL ADENOCARCINOMA CELLS *IN VITRO*

KLEMEN RIHAR¹, ADRIANA PEREYRA², BRATKO FILIPIČ³

¹ *Ljubljana, Slovenia*

(*e-mail: klemen.rihar@gmail.com*)

² *MEDEX d.o.o., Ljubljana, Slovenia*

³ *Croatian Institute for Experimental and Translational Oncology (CIETO), Zagreb, Croatia*

As a part of Royal jelly's (RJ) biological activity, investigations of its possible antitumour activity as well as possible interaction with human interferon-alpha (HuIFN- α N3) were performed. The purpose of this study was to investigate the influence of RJ on HuIFN- α N3 induced inhibition of Human colorectal adenocarcinoma cells (CaCo-2) proliferation and their effect on intracellular glutathione (GSH) level and lipid peroxidation. The anti proliferative (AP) activity of RJ (0.1 g/10 ml phosphate buffer saline (PBS), HuIFN- α N3, (1.000 I.U./ml), 10-hidroxy-2-decenoic acid (10-HDA) (100 μ M/ml) and different combinations between them in ratio 1:1, 1:2 and 2:1 on CaCo-2 cells were determined. Their influence on GSH level was measured by glutathione assay kit. The lipid peroxidation was measured by Malondialdehyde (MDA) assay. RJ alone exhibits a low AP activity: 2.0 (0.5 mg/ml). HuIFN- α N3 has AP activity of 2.5 (208.33 I.U. / ml) and 10-HDA has the AP activity 1.5 (37.5 μ M/ml). In the combination between RJ and HuIFN- α N3 (2:1), the AP activity was 3.8. In that combination the level of GSH was 24, 9 $\pm$ 2, 4 nM/mg of proteins (70.2 $\pm$ 3.2 nM/mg in Control) and the level of MDA was 72.3 $\pm$ 3.1 nM/mg (23.6 $\pm$ 9.1 nM/mg in Control). The 10-HDA as the main component of the RJ is responsible for its influence on HuIFN- α N3 inhibition of CaCo-2 cells proliferation in vitro. RJ and HuIFN- α N3 in combination 2:1 decrease the level of GSH and significantly increase the lipid peroxidation via MDA in CaCo-2 cells.

P45: RESPONSIVENESS TO GANT-61 INHIBITOR OF GLI1, GLI2 AND GLI3 KNOCK-OUT OVARIAN CANCER CELL LINES

NIKOLINA RINČIĆ, MAJA SABOL, DIANA TRNSKI, VESNA MUSANI, PETAR OZRETIĆ, SONJA LEVANAT

*Laboratory for Hereditary Cancer, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: nikolina.rincic@gmail.com)*

Hedgehog signaling pathway is a developmental signaling pathway which is dormant in most adult differentiated tissues, but aberrantly activated in various tumors. In ovarian tumors it can be activated in a canonical way, by the SHH ligand, or the non-canonical way, by upregulation of the GLI transcription factors. Usually, only GLI1 expression was associated with tumor progression, but GLI2 and GLI3 also play a role by modifying the activity of GLI1 and transcription of their common transcriptional targets. In ovarian cancer, GLI3 protein is expressed in the full-length activator form, and not the shortened repressor form which is the predominant form for GLI3 protein. To study the roles of the three GLI proteins in detail, we used the CRISPR/Cas9 guided gene editing to generate knock-out lines for each of the three GLI proteins. The knock-out was confirmed by Western blot. The modified cell lines showed differences in cell morphology compared to the original SKOV3 cell line immediately after expansion from the single cell, as well as different response to GANT-61 inhibition on cell cycle, apoptosis and colony forming capabilities of the cell lines. This demonstrates that all three GLI proteins are important for survival of ovarian cancer, and not only GLI1 as was considered previously.

P46: A META-ANALYSIS AND A COHORT STUDY INDICATE ASSOCIATION BETWEEN RASSF1A PROMOTER METHYLATION AND TESTICULAR GERM CELL TUMOR

DORA MARKULIN¹, ALEKSANDAR VOJTA¹, IVANA SAMARŽIJA¹, MARIJA GAMULIN²,
IVONA BEČEHELI³, IRENA JUKIĆ⁴, ČEDOMIR MAGLOV⁴, VLATKA ZOLDOŠ¹,
ALEKSANDRA FUČIĆ⁵

¹ University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Zagreb, Croatia
(e-mail: ivana.samarzija@irb.hr)

² University Hospital Centre Zagreb, Department of Oncology, Zagreb, Croatia

³ Genos Glycoscience Research Laboratory, Zagreb, Croatia

⁴ Croatian Institute of Transfusion Medicine, Zagreb, Croatia

⁵ Institute for Medical Research and Occupational Health, Zagreb, Croatia

The RAS association domain family protein 1a (RASSF1A) is a prominent tumor suppressor similar to RAS effector proteins. It shows altered promoter methylation in many different cancer types including testicular germ cell tumors (TGCT). TGCT are the most common malignancy in young men and they account for approximately 1% of all human malignancies. RASSF1A promoter hypermethylation might represent an early event in TGCT tumorigenesis. In addition to epigenetic studies on tumor tissue, in the last decade, there have been several reports showing correlation between peripheral blood DNA methylation and tumor status. Given the fact that peripheral blood is easily accessible, it is clear that its DNA methylation analysis holds a promise in finding suitable cancer markers. We investigated whether the RASSF1A promoter methylation in peripheral blood of TGCT patients can be associated with testicular cancer risk. Following a meta-analysis, we performed a cohort study including 32 testicular cancer patients and 32 healthy controls. Promoter methylation of the RASSF1A and O6-methylguanine-DNA-methyltransferase (MGMT) genes was analyzed using bisulfite pyrosequencing of DNA from peripheral blood. Meta-analysis showed an odds ratio (OR) of 7.69 for RASSF1A promoter methylation as a risk factor for TGCT. Cohort study found altered methylation of the RASSF1A promoter in blood of TGCT patients. Methylation was higher in TGCT patients before BEP (bleomycin, etoposide, cisplatin) chemotherapy. The meta-analysis indicates a role of the RASSF1A promoter hypermethylation from peripheral blood in TGCT. We confirmed the findings of meta-analysis (encompassing mainly tumor tissue) in our cohort study, which represents the first report of changed RASSF1A promoter methylation in peripheral blood TGCT. We envision that further studies including larger number of patients will bring more light into the potential of using RASSF1A peripheral blood methylation status as a TGCT marker.

P47: DIFFERENTIAL EFFECTS OF INTEGRIN α V KNOCKDOWN AND CILENGITIDE ON SENSITISATION OF TRIPLE-NEGATIVE BREAST CANCER AND MELANOMA CELLS TO MICROTUBULE POISONS

NIKOLINA STOJANOVIĆ^{1*}, ANA DEKANIĆ^{1*}, MLADEN PARADŽIK¹, DRAGOMIRA MAJHEN¹, KREŠIMIR FERENČAK¹, JELENA RUŠČIĆ¹, IRENA BARDAK¹, CHRISTINE SUPINA¹, MAJA T. TOMICIC², MARKUS CHRISTMANN², MAJA OSMAC¹, ANDREJA AMBRIOVIĆ-RISTOV¹
^{*}These authors contributed equally to this work.

¹ Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia
 (e-mail: Nikolina.Stojanovic@irb.hr)

² Department of Toxicology, University Medical Center Mainz, Mainz, Germany

Low survival rates of patients with metastatic triple negative breast cancer (TNBC) and melanoma, in which current therapies are ineffective, emphasize the need for new therapeutic approaches. Integrin signaling regulates proliferation, migration, metastasis and cell death and has been suggested as a possible target in antitumor therapy. Although seen as a promising target when combined with chemotherapy, recent data on integrin β 1 have shown that its inactivation increases metastatic potential due to the compensatory upregulation of other integrin subunits. Therefore, the aim of this study was to investigate the potential of integrin subunits α v, α 3 or α 4 knockdown in increasing sensitivity in seven TNBC (MDA-MB-231, -468 and -436) and melanoma (MDA-MB-435S, RPMI-7951, MeWo and A375) cell lines to cisplatin, microtubule poisons paclitaxel (PTX) and vincristine (VCR), and mitigating metastatic potential. Experiments performed in integrin α v β 1 negative melanoma cell line MDA-MB-435S identified integrin subunit α v as a target whose knockdown increases sensitivity to vincristine or paclitaxel, and decreases migration and invasion. In MDA-MB-435S cell line we also identified a phenomenon in which change in expression of one integrin subunit changes the expression of other integrin/s, leading to an unpredictable influence on sensitivity to anticancer drugs and cell migration referred to as the integrin switching effect. The contribution of integrins α v versus integrins α v β 3/ β 5 was also assessed in a panel of six TNBC and melanoma cell lines by the combined action of α v-specific siRNA or α v β 3/ β 5 inhibitor cilengitide with paclitaxel. Knockdown of integrin α v in combination with VCR and PTX resulted in beneficial effect in all three TNBC cell lines, while in melanoma cell lines we observed beneficial effect in only one cell line out of three. Interestingly, cilengitide-driven inactivation of integrin heterodimers α v β 3 and α v β 5 showed beneficial effect in combination with PTX in five out of seven TNBC and melanoma cell lines, although in a different set of cells as compared to integrin α v knockdown. Our results suggest that for TNBC the knockdown of integrin α v in combination with paclitaxel presents a better therapeutic option than cilengitide, and simultaneously decreases migration and invasion. However, in melanoma neither of these combinations is advisable because decreased sensitivity to paclitaxel was observed.

P48: THE ROLE OF NUCLEOTIDE EXCISION REPAIR PATHWAY IN THE REPAIR OF DNA-PROTEIN CROSSLINKS

CHRISTINE SUPINA, MARTA POPOVIĆ

*Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: csupina@irb.hr)*

DNA-protein crosslinks (DPCs) are DNA lesions formed by a protein irreversibly covalently bound to DNA. DPCs are common lesions which present a physical blockage to all DNA transactions. If DPCs are left unrepaired, they cause genomic instability, premature aging and liver cancer in mice and humans. Recently, several groups have identified proteases Wss1 and SPRTN which initiate the removal of DPCs through the proteolytic digestion of crosslinked proteins. These discoveries led to recognition of DNA-protein crosslink repair (DPCR) as a separate DNA damage repair pathway. After SPRTN proteolysis of DPCs, peptide remnant of unknown size remains crosslinked to the DNA backbone, and is subsequently removed by unknown factors. Few in vitro studies have shown that small DPCs (below 8-16 kDa) can be removed by Nucleotide Excision Repair (NER), and therefore NER remains one of the possible candidates for peptide excision downstream of SPRTN proteolysis. However, little is known about the role of NER on the organismal level. The goal of our study was to determine if and under which conditions is NER involved in DPC repair in vivo using zebrafish (*Danio rerio*) and CRISPR/Cas9 gene manipulations. Among the NER components, we have chosen to target XPA (Xeroderma pigmentosum complementation group A) for gene knock-out (KO) because: A) other NER factors are involved in other cellular processes while XPA is considered to be specifically involved in NER, and B) XPA KO is not embryonic lethal. Our study is still in progress and will reveal actual contribution of NER pathway to the DPC removal on the organismal level.

P49: SUPPRESSION OF EXPERIMENTAL GERM CELL TUMOR DEVELOPMENT BY EPIGENETIC MODULATORS *IN VITRO*

LUCIJA ŠKARA¹, JURE KRASIĆ^{1,6}, ROBERT BULJUBAŠIĆ^{2,6}, MAJA BULJUBAŠIĆ^{3,6},
MAJA VLAHOVIĆ^{1,6}, ANA KATUŠIĆ BOJANAC^{1,6}, FLORIANA BULIĆ-JAKUŠ^{1,6},
MONIKA ULAMEC^{4,5,6}, NINO SINČIĆ^{1,6}

¹ Department of Medical Biology, School of Medicine, University of Zagreb, Zagreb, Croatia
(e-mail: skara.lucija@gmail.com)

² Clinical Department of Surgery, Clinical Hospital Dubrava, Zagreb, Croatia

³ Community Health Centre, Zagreb East, Croatia

⁴ Department of Clinical Pathology and Cytology "Ljudevit Jurak", University Clinical Hospital
Sestre Milosrdnice, Zagreb

⁵ Pathology Department, University of Zagreb, School of Medicine, University of Zagreb, Zagreb, Croatia

⁶ Centre of Excellence for Reproductive and Regenerative Medicine, School of Medicine, University of Zagreb,
Zagreb, Croatia

Testicular germ cell tumors (TGCT) are the most prevalent malignancies in young men. They arise from primordial germ cells that had impaired maturation to prespermatogonia and spermatogonia. In earliest stages of TGCT development, somatic mutations are believed to be uncommon and it is therefore likely that epimutations, that are reversible, are drivers in their development. Experimental mouse teratoma is a well-established *in vitro* model for studying TGCT development. Namely, a week long cultivation of embryonic shield results in developing teratoma-like structures. We isolated embryonic shield from 7,5 days old mouse embryos, treated them for two hours with epigenetic modulators that influence methylation (5-azacytidine), histone acetylation (Trichostatin A, Valproate) and translation of mRNA (esiNanog, esiOct3/4 and esiTrrap) and then cultured them in MEM enriched with rat serum. Growth of embryonic shields/teratomas was followed by measuring their size at each day of cultivation. On day 7 teratomas were collected. To investigate effect of used epigenetic modulators on cell stemness, DNA methylation status of promoter regions of Nanog, Oct3/4 and Sox2 genes as well as quantification of mRNA of these genes was performed. Furthermore, the expressions of endoderm (Sox17, Gata4), mesoderm (Brachyury, Eomes), neuroectoderm markers (Nestin, Sox2, Six3), marker of primitive ectoderm (Fgf5) and marker of myogenic commitment (MyoD) were determined. Epigenetic agents significantly reduced embryonic shields/teratomas growth. The strongest decrease was detected in esiOct3/4 treated group followed by 5-azacytidine, Valproate and Trichostatin A treated groups. 5-azacytidine decreased methylation in promoter regions of stemness genes. The highest increment of DNA methylation was achieved by esiNanog for Nanog gene and by esiOct3/4 for Oct3/4 gene. Expression of analysed stemness markers was significantly reduced by Valproate which also reduced expression of mesodermal, neuroectodermal markers and marker of myogenic commitment. EsiOct3/4 treated group showed the most prominent effect on mesoderm and primitive ectoderm marker expression. In conclusion, it seems that various epigenetic modulators impact experimental germ cell tumor development *in vitro* via methylation alternations in promoters of stemness and differentiation genes and their expression. Epigenetic modulators have potential for reverting epimutations and therefore restoring appropriate gene expression.

P50: EXPRESSION OF GLI3 AND PTCH1 PROTEINS IN PROSTATE CANCER

IVAN ŠTEFANAC¹, MILANKA MRČELA^{2,3}, DIANA TRNSKI⁴, MAJA SABOL⁴, VESNA MUSANI⁴, PETAR OZRETIĆ⁴, SONJA LEVANAT⁴

¹ Primary Health Care Center Osijek, Osijek, Croatia

(e-mail: ivan.stefanac@gmail.com)

² Department of Pathology, Clinical Hospital Center Osijek, Osijek, Croatia

³ School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

⁴ Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia

The Hedgehog-GLI (HH-GLI) signaling pathway is primarily associated with embryonic development but in the last two decades its role in cancer development has become intensely studied. The HH-GLI pathway is associated with one third of cancer related deaths, which makes it an interesting new therapeutic target. Recent research indicates that the HH-GLI pathway could be a key player in prostate cancer development and progression, as well as in the development of resistance to therapeutics. The aim of this study is to investigate the activity of the HH-GLI pathway in prostate cancer tissue samples in comparison with healthy prostate tissue samples and samples of prostate inflammation.

Around 30 formalin-fixed paraffin-embedded prostate tissue samples per group were collected from prostate cancer patients (Grade Group I-V) and two controls groups (benign prostate tissue and prostate inflammation). Expression of GLI3 and PTCH1 proteins was determined immunohistochemically. The level of protein staining was expressed by multiplying percentage of positive stained cells and staining intensity (histoscore), separately for prostate epithelium and stroma. Localization of protein staining (nuclear and/or cytoplasmic) was also determined.

Both GLI3 and PTCH1 histoscores were significantly higher in epithelial prostate cancer cells ($P < 0.0001$ for both proteins) compared to controls, but not in prostate stroma ($P = 0.835$ for GLI3 and $P = 0.175$ for PTCH1). GLI3 and PTCH1 expressions were positively correlated ($\rho = 0.48$, $P < 0.0001$). Localization of GLI3 in epithelium was generally mostly diffuse (both nuclear and cytoplasmic), while in prostate cancer stroma cytoplasmic localization of GLI3 was negatively associated with higher Grade Group ($P < 0.0001$). The same was observed for PTCH1 localization in prostate cancer epithelium and stroma ($P < 0.0001$ for both). In control samples of prostate inflammation prevails cytoplasmic PTCH1 expression, while in benign prostate tissue it is mostly diffuse. The expression of all HH-GLI pathway components has been found in the androgen-dependent prostate cancer cell line LNCaP. Interestingly, after exposure of these cells to androgen-deprived conditions, the expression of GLI3 was significantly upregulated.

Our study has determined an increased activity of HH-GLI pathway in prostate cancer, and a potential role for GLI3 in androgen-independent growth. However, its relation to prostate cancer progression and mechanisms of sustaining androgen independent growth have yet to be determined. Our results showing higher nuclear localization of GLI3 in higher Grade Group could indicate an increase in paracrine HH-GLI signaling, from the tumor cells toward the stroma.

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P51: ASSOCIATION OF BIRC5 POLYMORPHISMS AND SURVIVIN EXPRESSION WITH CLINICOPATHOLOGICAL CHARACTERISTICS OF BREAST CANCER PATIENTS

ILONA SUŠAČ¹, PETAR OZRETIĆ², MAJA GREGORIĆ³, MIRELA LEVAČIĆ CVOK⁴, MAJA SABOL², DIANA TRNSKI², DOMAGOJ ELJUGA^{1,5}, SVEN SEIWERTH^{6,7}, GORANA ARALICA^{6,8}, MLADEN STANEC⁵, SONJA LEVANAT², VESNA MUSANI²

¹ Eljuga Polyclinic, Zagreb, Croatia
(e-mail: ilona1603@gmail.com)

² Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia

³ Zagreb Health School, Zagreb, Croatia

⁴ Elementary School "Kardinal Alojzije Stepinac", Krašić, Croatia

⁵ Department for Oncoplastic and Reconstructive Surgery, University Hospital for Tumors, Zagreb, Croatia

⁶ Institute of Pathology, University of Zagreb School of Medicine, Zagreb, Croatia

⁷ Clinical Department of Pathology and Cytology, University Hospital Centre Zagreb, Zagreb, Croatia

⁸ Department of Pathology and Cytology, Clinical Hospital Dubrava, Zagreb, Croatia

Breast cancer (BC) is the most common malignancy in women. Common risk factors are age, hormonal factors, reproductive and menstrual history, exposure to radiation, obesity and genetic background. Genetic variation can affect both susceptibility and prognosis. Survivin, encoded by BIRC5 gene (baculoviral IAP repeat containing 5), belongs to the family of inhibitors of apoptosis proteins (IAPs). In mammalian cells it participates in the control of mitosis, apoptosis regulation and cellular stress response. Its expression is increased in almost all cancer types. The aim of this study was to investigate the role of BIRC5 polymorphisms in BC and to correlate survivin expression with various clinicopathological characteristics including age of onset, time since operation, histological grade, tumor type and size, lymph node status, estrogen, progesterone, Her2 and Ki67 status.

Blood and FFPE tumor tissue samples were collected from 26 Croatian BC patients. Majority had invasive ductal carcinoma (81.5%). Survivin expression was determined immunohistochemically and scored between 1 and 3 taking into account percentage of positive cells and staining intensity. BIRC5 promoter, coding region and 3'UTR were genotyped using high resolution melting analysis and Sanger sequencing. Genomic DNA from 74 healthy women was used as control.

Numbers of samples with survivin expression with a score 1, 2 and 3 were 9 (33.3%), 11 (40.7%) and 7 (25.9%), respectively. Most patients had nuclear survivin staining (92.6%). High survivin expression was associated with negative ER status ($p=0.007$) and positive Ki67 expression ($p=0.032$). Ki67 expression was also positively associated with histological grade ($p=0.0009$). Fourteen polymorphisms were found in BC samples, located mostly in promoter and 3'UTR. There were no significant differences in polymorphisms' frequencies between BC and control samples. However, age of onset was associated with five BIRC5 polymorphisms (c.-1547C>T, c.-644T>C, c.-241C>T, c.9386T>C and c.9809T>C).

This was the first study investigating the possible role of BIRC5 polymorphisms in breast cancer etiology conducted in Croatia. Although no association of any of BIRC5 polymorphism with BC or level of survivin expression was observed, several polymorphisms were associated with the age of onset. Further research with a larger sample size is needed to assess the significance of BIRC5 polymorphisms and survivin expression as predictive/prognostic biomarkers for BC.

P52: NISCHARIN - A POTENTIAL TUMOR-SUPPRESSOR IN PANCREATIC DUCTAL ADENOCARCINOMA

MARIJA VIDOSAVLJEVIĆ, SINIŠA RADULOVIĆ, JELENA GRAHOVAC

*Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia
(e-mail: vidosavljevic.marija@gmail.com)*

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers. Despite significant effort in developing novel detection and therapeutic strategies in the last decades, the five-year survival rate remains just around 5–7%. It is projected that by 2030 PDAC will become the second leading cause of cancer-related death. New therapeutic targets in PDAC are urgently needed. In this study, we examined the expression of nischarin in PDAC, an integrin binding protein that is in humans encoded by the NISCH gene. Nischarin's potential tumor-suppressive effect has been shown in breast and ovarian cancer. The expression of NISCH is significantly reduced in human breast cancer tissue compared to normal. NISCH overexpression can induce apoptosis, inhibit cell migration and invasion of breast cancer cells in vitro, and reduce tumor growth and metastasis in in vivo models of breast cancer. Nischarin effects in other types of cancer are unknown. The aim of our study was to examine the expression pattern of nischarin in PDAC and its potential role in progression of the disease. UALCAN, the interactive web resource for analyzing cancer transcriptome data from TCGA database, was used to analyze the association between NISCH expression levels and PDAC sample characteristics. Analysis indicated that NISCH expression was decreased in stage II (n=146, median=57.851, P=0.0743) and stage III (n=4, median=39.546, P=0.0631) PDAC tumors compared with the normal tissue (n=4, median=80.72). For survival analysis, the patients from TCGA PDAC database (n=174) were divided into two groups based on the mean of nischarin expression of the entire population. Patients with low-expressing nischarin (below the mean, n=99 patients) had poor prognosis, while patients with high-expressing nischarin tumors (above the mean, n=75 patients) showed statistically significant increase in overall survival (ratio of survival=1.268, 95% CI=0.8245 to 1.95, log-rank P=0.0177). These results imply that nischarin may be used as a prognostic marker for patients with PDAC. As the role of nischarin in cancer is not fully elucidated, our future study will focus on NISCH role in PDAC and its potential as a druggable target for more effective PDAC treatment.

P53: STROMAL IL-6 AS A DISEASE PROGRESSION MARKER FOR HEAD AND NECK SQUAMOUS CELL CARCINOMA

CEDNA TOMASOVIC LONCARIC¹, ALEKSANDRA FUCIC², VESNA BISOF^{3,4}, ZORAN RAKUSIC³, JELENA KOVACIC¹, MATEJ ANDABAK¹, VANJA VUCICEVIC BORAS⁵

¹ *Clinical Hospital Dubrava, Zagreb, Croatia*

² *Institute for Medical Research and Occupational Health, Zagreb, Croatia*

³ *Clinical Hospital Centre Zagreb, Zagreb, Croatia*

⁴ *Medical Faculty, University of Osijek, Osijek, Croatia*

⁵ *Department of Oral Medicine, School of Dentistry, University of Zagreb, Zagreb, Croatia*
(e-mail: borasvanja@yahoo.com)

Patients suffering from head and neck squamous cell cancer (HNSCC) still have low survival rate due to late diagnosis, insufficient knowledge on chemo- and radioresistance and lack of reliable prognostic and predictive biomarkers. As IL-6 defines the tumorigenic capacity and motility of cancer stem cells there is raised interest for its inclusion as a marker of cancer aggressiveness and concomitant decision upon therapy. The aim of this study was to investigate stromal and epithelial IL-6 levels in HNSCC.

Analysis of IL-6 in 51 HNSCC (24-non-metastatic and 26 metastatic cancers) patients who underwent radical surgery was performed by immunohistochemistry in the tumour epithelium and in the tumour stroma. Mean age of patients was 63±12 years.

IL-6 values in the epithelium and stroma positively correlated (Spearman's rho 0.69, p-value < 0.001). IL-6 was higher in patients with metastases than in non-metastatic patients, and this difference was more pronounced in the stroma (median 18.00 with interquartile range [10.00, 57.50], compared to 8.00 [1.00, 18.50] in patients without metastases) than in the epithelium (20.00 [10.00, 37.50] versus 11.50 [5.00, 30.00]).

Our results suggest that (a) stromal IL-6 might be the marker of neoplastic progression, (b) need for both stromal and epithelial IL-6 analysis in patients with HNSCC. Stromal production of IL-6 may contribute in the future profiling of tumor aggressiveness, poor response to the chemoradiotherapy and regarding decision on immunotherapy.