POSTER PRESENTATIONS

P1: COMPARATIVE CYTOTOXIC AND CYTOPROTECTIVE EFFECTS OF TAUROURSODEOXYCHOLIC ACID ON TUNICAMYCIN TREATED HUMAN LIVER EPITHELIAL THLE-3 CELLS

EBRU KIRAÇ[1], ÖZLEM YILMAZ[2], MUTAY ASLAN[1]

[1] *Department of Medical Biochemistry, Akdeniz University Faculty of Medicine, Antalya, Turkey (e-mail: mutayaslan@akdeniz.edu.tr)*
[2] *Human Gene and Cell Therapy Center, Akdeniz University Faculty of Medicine, Antalya, Turkey*

This study was performed to analyze the effects of tunicamycin (TM) and taurohyodeoxycholic acid (TUDCA) on THLE-3 cells. Cells were treated with TM to induce endoplasmic reticulum (ER) stress and TUDCA was administered as an ER stress inhibitor. Cytotoxicity was evaluated at different times of exposure by incubating cells with increasing concentrations of either TUDCA, TM or both. THLE3 cells were cultured in fibronectin, bovine collagen I and bovine serum albumin coated plates. Cell lines were grown in BEGM media supplemented with epidermal growth factor, phosphoethanolamine, fetal bovine serum, 100 U of penicillin-streptomycin and maintained in a humidified incubator at 37°C and a 5% CO2 atmosphere. Cell viability was measured using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit. Cells were grown to confluence in 96-well plates and incubated with 1 μl/ml DMSO, 1–10 μg/ml TM, 0.1-5 mM TUDCA, or 10 μg/ml TM + 0.1-1 mM TUDCA for 18–48 h. Control cells were prepared in plates containing only medium. At the end of the incubation period, MTT was added to each well and incubation was carried out for 4 h at 37 °C. Formazan production was expressed as a percentage of the values obtained from control cells. At all hours of incubation neither DMSO nor 1 mM TUDCA was cytotoxic. At 24 and 48h incubations 5 mM TUDCA and 10 μg/ml TM + 1 mM TUDCA were significantly cytotoxic compared to control, DMSO and 1 mM TUDCA groups. Treatment of cells with 0.5 mM TUDCA 8h before administrating 10 μg/ml TM significantly decreased the cytotoxic effect of TM. We conclude that TUDCA may show cytotoxic effects at 1 mM concentration when treated with TM. Therefore 0.5 mM of TUDCA, administered 8h before TM treatment should be applied to protect against ER stress.

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P2: DYSREGULATION OF microRNA CLUSTER miR-29 IN CULTURED FIBROCYTES FROM PATIENTS WITH PRIMARY MYELOFIBROSIS

KSENIJA BOŽINOVIĆ[1,2], TAGHI MANSHOURI[1], ZEEV ESTROV[1], DARRELL PILLING[3], LIZA KNEZ[1], KATE J. NEWBERRY[1], SRDAN VERSTOVSEK[1]

[1] *The University of Texas, MD Anderson Cancer Center, Houston, TX, USA*
[2] *Laboratory of Molecular Virology and Bacteriology, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia*
(e-mail: ksenija.bozinovic@irb.hr)
[3] *Texas A&M University, Department of Biology, College Station, TX, USA*

Myeloproliferative neoplasms (MPN) are a class of stem cell–derived hematologic malignancies, characterized by an expansion of one or more myeloid lineages with resulting bone marrow (BM) hypercellularity. A gain-of-function mutation in *JAK2*, detected in most patients with MPN, induces constitutive activation of *JAK2*, stimulation of downstream signaling pathways, and activation of several cytokine and growth factor genes. Recently we have reported that the BM of Primary myelofibrosis (PMF) patients harbors more neoplastic functionally distinct fibrocytes and fewer mesenchymal stromal cells (MSCs) than hematologically normal BM. In addition, we detected an overabundance of fibrocytes in the BM and spleen of an established PMF mouse model and a xenograft mouse model of PMF created using BM-derived low-density cells from patients with PMF. Fibrocytes, spindle-shaped fibroblast-like cells that differentiate from a subpopulation of CD14+ monocytes, are associated with evolving tissue fibrosis, such as pulmonary fibrosis, end-stage liver or kidney disease, heart disease and autoimmune disorders. Here, we show the dysregulation of microRNAs (miR-16, -21, -29a, -29b1, -29b2, -29c, -155, -181a, and -451) in cultured fibrocytes from BM of PMF patients. Bone marrow mononuclear cells (MNC) from twenty eight primary myelofibrosis patients and nine BM normal controls were cultured. The resulting fibrocytes were used for present study. Using qRT-PCR we found that expression levels of miR-16 ($p=0.0122$), and miR-21 were higher in MF patients than in normal controls. Expression level of miR-29-b1 and -b2 levels were significantly lower ($p=0.0007$, $p=0.0001$) in MF patients; however, the miR29-c level was higher ($p=0.0028$). Dysregulation of miR-29 has been associated with coronary artery fibrosis and we conclude that miR-29 might play a role in bone marrow fibrosis and represent a potential therapeutic target.

P3: miR-375 AND miR-192 EXPRESSION LEVELS DISTINGUISH NEUROENDOCRINE FROM NON-NEUROENDOCRINE TUMORS. NOVEL BIOMARKERS FROM RESECTED SAMPLES

VALERIO DEL VESCOVO[1], MARGHERITA GRASSO[1], CHIARA CANTALONI[2], LEONARDO RICCI[3], MATTIA BARBARESCHI[2], MICHELA ALESSANDRA DENTI[1]

[1] *Laboratory of RNA Biology and Biotechnology, Centre for Integrative Biology (CIBIO), University of Trento Trento, Italy*
(e-mail: valerio.delvescovo@unitn.it)
[2] *Unit of Surgical Pathology, S.Chiara Hospital, Trento, Italy*
[3] *Deptartment of Physics, University of Trento, Trento, Italy*

Pulmonary neuroendocrine (NE) tumors form a distinct group of neoplasms that share characteristic morphologic, immunohistochemical, ultrastructural, and molecular features. The clinical spectrum is various, from low-grade typical carcinoid (TC) and intermediate-grade atypical carcinoid (AC) to high-grade large cell NE carcinoma (LCNEC) and small cell lung carcinomas (SCLC). However, they represent a wide spectrum of phenotypically distinct entities, from which pulmonary NE tumors can sometimes be difficult to differentiate, even for an expert pathologist. miRNAs, short non coding RNAs (18-21nt), are a promising new class of cancer biomarkers which may potentially affect all aspects of clinical care from early detection, diagnosis, and prognosis. miR-375 was markedly induced by ASH1 in lung cancer cells where it was sufficient to induce NE differentiation. On the other hand miR-192 is known to show a high variability among solid tumors. In the present study we analyze miR-375 and miR-192 expression in a series of surgically resected NE lung tumors, including TC, ATC, LCNEC, SCLC, ADC and SQCC. The two aims of the study are 1) to verify whether there are differences in the expression levels of these putative markers in NE versus non-NE lung tumors, and 2) to verify the miRNAs differential expression within the different subtypes of NE lung tumors.

P4: MANIPULATION OF BIOELECTRICAL PROPERTIES IN BREAST CANCER STEM CELL MODEL

KATJA ESTER[1], MARKO MARJANOVIĆ[1], IVA GUBEROVIĆ[1], LIDIJA UZELAC[1], ANA-MATEA MIKECIN[1], IRENA MARTIN KLEINER[1], TATJANA ŠUMANOVAC-RAMLJAK[2], KATA MLINARIĆ-MAJERSKI[2], WOLFGANG SCHREIBMAYER[3], MARIJETA KRALJ[1]

[1] *Laboratory of Experimental Therapy, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: kester@irb.hr)*
[2] *Laboratory of Synthetic Organic Chemistry, Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia*
[3] *Institute of Biophysics, Medical University of Graz, Austria*

Bioelectricity represents endogenous electrical signaling via ion channels and pumps at the plasma membrane, which shapes unique patterns of voltage potentials across the membranes. Resting membrane potential (Vm) is a functional determinant of cell behaviour. There is a functional relationship between resting membrane potential (Vm) and cell proliferation and differentiation, which can be seen in many cells including normal stem cells and various tumors. Sundelacruz *et al*. (Tissue Eng Part A 2013) have shown that Vm depolarization promotes maintenance of mesenchymal stem cells in an undifferentiated stage. We postulated that depolarized Vm may also help to maintain a population of cancer stem cells (CSCs) in undifferentiated, but highly invasive state. Cancer stem cells (CSCs) represent a subpopulation of cancer cells responsible for tumor formation, relapse and metastasis. The breast CSC model used in this study consists of cells with up-regulated Twist expression (HMLETwist), which are showing markers of CSCs and have functional CSC properties.

Therefore, Vm was measured by Nystatin-perforated Patch-Clamp method in a breast CSC model. Also, we measured Vm of SUM159 and MCF7, two breast cancer cell lines with high and low percentage of CSCs among whole population, respectively. Furthermore, we used proprietary potassium ionophores, previously shown to be highly cytotoxic for breast CSC model cells, to modulate membrane potential. Membrane potential after treatment with compounds was estimated using the cationic dye DiOC6(3).

It was indeed shown that SUM 159 cells are more depolarized than MCF7. Regarding breast CSC model, there was no difference in Vm between HMLEtwist and control HMLE cells, pointing to potential limitations of this model.

Two highly cytotoxic proprietary potassium ionophores modulated membrane potential in breast CSC model. Although related by structure, one depolarized, while other hyperpolaryzed cells. Compound 613 causes severe depolarization in HMLEtwist cells. Electrophysiological measurements pointed that compound at first hyperpolarizes cells, which is followed by depolarization. Mode of action of both compounds is under investigation.

Measurements of resting potential in CSCs with and without modulators will be further investigated.

P5: CAVEOLIN-1 GENE PROMOTER METHYLATION ON PRIMARY GLIOBLASTOMA

MEHMET TAŞPINAR[1], ASLIHAN KURT KIZILDOĞAN[2], YAHYA EFE GÜNER[3], FILIZ TASPINAR[4], HASAN ÇAĞLAR UĞUR[3]

[1] *Department of Medical Biology, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey (e-mail: mtaspinartr@gmail.com)*
[2] *Department of Molecular Biology and Genetics, Faculty of Science, Yuzuncu Yil University, Van, Turkey*
[3] *Department of Neurosurgery, Faculty of Medicine, Ankara University, Sihhiye, Ankara, Turkey*
[4] *Department of Physiology, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey*

Caveolin-1 (CAV1), a structural protein of caveolae, directly interacts with multiple proteins that play role in different physiological signaling. Thus, the genetic and epigenetic pattern of *CAV1* gene could be an important factor for normal and pathological conditions. The expression of *CAV1* gene is quite in different in normal and cancer brain tissues. The regulation mechanism of *CAV1* expression is unknown in brain. Methylation could be regulatory mechanism of *CAV1* gene and *CAV1* methylation status may be an important factor in terms of clinic parameters in Glioblastoma (GB). There is not any published study about *CAV1* methylation and clinical importance.

The aim of this study is to investigate *CAV1* gene promoter methylation pattern in normal and cancer tissues of GB patients.

The tumor and normal tissues from 43 patients with GB were included in this study.

Genomic DNA was isolated from all tissues. After bisulfite modification of gDNA, MS-PCR techniques were performed to identify of the *CAV1* methylation status in tissues. Amplified PCR products were visualized on 2.5% agarose gels. Clinic information and *CAV1* methylation status are compared for the effect on patients' survival, resistance to chemotherapy and recurrence of the tumor.

The rate of unmethylation and methylation of *CAV1* gene promoter were found at 58.1%, and 41.9% in tumor samples, while it was present at 76.5% and 23.5% in normal tissues, respectively. In two patients, while normal samples have UM (hemimethylated) pattern of *CAV1* gene tumor samples have UU (unmethylated) pattern. Likewise, in other two patients, while normal samples have UU pattern, tumor samples have UM pattern. Not detected MM (full methylated) pattern in tumor and normal tissues. There is no any statistical significance between methylation and recurrence, chemotherapeutic resistance in patients ($p>0.05$).

CAV1 is initial protein for several signalling pathway and thus it has an important role in cells. Epigenetic and genetic pattern of *CAV1* may directly affect the cell's destiny. Our study is first report showed that *CAV1* promoter methylation in GB patients. When clinical effect of *CAV1* methylation was assessed, all assays should be studied in both normal and tumour tissues in the same patients because this study showed that GB patients have genetic heterogeneity in terms of *CAV1* methylation. According to our results, the epigenetic effects of *CAV1* gene can be cell type specific.

The epigenetic elements (methylation, miRNAs and acetylation) for *CAV1* gene should be studied in brain tumours. This is the first report that investigated the Caveolin-1 promoter methylation and its clinical effects in GB.

P6: THE ROLE OF *BIRC5* POLYMORPHISMS AND SURVIVIN GENE EXPRESSION IN OVARIAN CANCER

MAJA GREGORIĆ[1], VESNA MUSANI[2], PETAR OZRETIĆ[2], MAJA SABOL[2], DIANA TRNSKI[2], DRŽISLAV KALAFATIĆ[3,4], IVANA MAURAC[3], SLAVKO OREŠKOVIĆ[3,4], SONJA LEVANAT[2]

[1] *Zagreb Health School, Zagreb, Croatia*
(e-mail: majagr79@gmail.com)
[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*
[4] *University of Zagreb, School of Medicine, Zagreb, Croatia*

Survivin, encoded by *BIRC5* gene, belongs to the family of inhibitors of apoptosis (IAP) proteins. Although its expression is usually confined to G2-phase and mitosis, survivin is often expressed throughout the cell cycle in cancer. In healthy organisms it is not expressed in differentiated tissues, while its expression is markedly increased in tumors. Its abundance in tumors correlates with increased resistance to chemotherapy and radiation, treatments lethal to cells through DNA damage and apoptosis induction. *BIRC5* polymorphisms have been previously associated with increased expression, stability and localization of survivin, that all can affect tumor development.

At least 5 different splice variants of the survivin gene have been reported in humans so far (wild type, 2α, 2B, 3B and deltaEx3). All survivin protein isoforms arising from the splice variants share the same N-terminus region, including either part or the entire Baculovirus IAP Repeat (BIR) domain, but they differ in the carboxyl end. The transcript expression levels of various survivin isoforms have been significantly associated with clinico-pathologic characteristics in several cancers.

In this study we investigated the role of *BIRC5* polymorphisms and survivin gene expression in ovarian cancer. Genetic testing of 40 patients and 74 healthy controls was conducted using high resolution melting analysis and Sanger sequencing. Fifteen different polymorphisms were found in ovarian carcinoma samples, 9 common and 6 rare. The distribution of polymorphisms did not differ between healthy controls and ovarian carcinoma samples.

For 22 patients that had RNA of sufficient quality, the expression of five splice variants was determined using qPCR. The highest expression of all splice variants was of survivin 2α, then wild type survivin, followed by survivin deltaEx3 and survivin 3B. The lowest expression was of the splice variant survivin 2B, which was expressed in only 15 of 22 samples analyzed. All splice variants had higher expression in ovarian cancer compared to healthy Fallopian tube tissue.

For three polymorphisms (c.-1547C>T, c.9386T>C and c.10611C>A), homozygous major genotype showed higher expression of wild type, 2α, 2B and 3B isoforms compared to heterozygous genotype. Furthermore, major allele of the same polymorphisms showed higher expression of wild type, 2α and 2B isoforms compared to minor allele.

This was the first study in Croatia which correlated *BIRC5* polymorphisms with the level of its splice variants expression. Our results have also demonstrated a possible role of *BIRC5* polymorphisms in ovarian cancer etiology, and their potential as prognostic biomarkers.

P7: PROPRIETARY CROWN ETHERS SIGNIFICANTLY INHIBIT P-GLYCOPROTEIN ACTIVITY

IVA GUBEROVIĆ[1], MARKO MARJANOVIĆ[1], KATJA ESTER[1], IRENA MARTIN KLEINER[1], TATJANA ŠUMANOVAC-RAMLJAK[2], KATA MLINARIĆ-MAJERSKI[2], MARIJETA KRALJ[1]

[1] *Laboratory of Experimental Therapy, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: iguberov@irb.hr)*
[2] *Laboratory of Synthetic Organic Chemistry, Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia*

Cancer stem cells (CSCs) are regarded as a leading cause of cancer relapse due to their ability to reconstitute tumour's heterogeneity even after its eradication. It has been suggested that CSC resistance to chemo- and radiotherapy could be related to enhanced survival pathways, decreased levels of reactive oxygen species and increased expression of drug efflux pumps. Nevertheless, related molecular mechanisms remain elusive. Recent research has pinpointed salinomycin, a naturally occurring potassium ionophore, as a promising compound for targeting CSCs. Moreover, several studies indicate that one of the mechanisms of action of salinomycin towards CSCs is the inhibition of P-gp drug efflux pump activity.

Pertaining to the mentioned studies and results previously published by our group regarding anticancer activity of proprietary crown ethers that act as K+ ionophores, we hypothesized that these compounds could show selectivity towards CSCs. Therefore, we set about to elucidate their mechanism of action, with the focus on the effect towards the function and activity of drug efflux pumps.

To evaluate the impact of proprietary crown ethers on cancer cells with respect to P-gp expression, multidrug resistance model cell lines A2780 and A2780/Adr were used, latter having overexpressed P-gp. Cytotoxicity evaluated by MTT assay showed increased resistance of A2780/Adr cells to P-gp substrates (e.g. doxorubicine, paclitaxel), as expected. Moreover, in order to assess the functional activity of P-gp, rhodamine efflux assay was performed. Obtained results showed strong inhibition by proprietary compounds, while combined treatment with paclitaxel indicated cell sensitization towards this chemotherapeutic. These results were further confirmed by cell cycle analysis and annexin V assay. Additionally, implications of crown ether molecular mechanisms of action were obtained by analysis of the impact of these compounds on P-gp expression and upstream regulators of this drug efflux pump.

Results indicate stronger P-gp inhibition obtained by proprietary crown ether treatment than by the commercially available P-gp inhibitor, verapamil. Additionally, plausible molecular mechanisms of action of crown ether compounds will be presented.

P8: SYNTHESIS AND APPLICATION OF GOLD NANOPARTICLES TO BREAST CANCER CELLS

NIKOLINA HANŽIĆ[1], ANĐELA HORVAT[1], INGA MARIJANOVIĆ[2], NEDA SLADE[1], MARIJAN GOTIĆ[3]

[1] *Laboratory for Protein Dynamics, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: nhanzic@irb.hr)*
[2] *University of Zagreb, Faculty of Science, Zagreb, Croatia*
[3] *Center of Excellence for Advanced Materials and Sensing Devices, Ruđer Bošković Institute, Zagreb, Croatia*

Nanoparticle-based therapies to treat cancer are widely evolving. Gold nanoparticles (GNPs) are small units that are inert and non-toxic to cells. The aim of this work was to study the radiosensitization effect of three types of GNPs to MDA-MB-231 breast cancer cells in vitro. Citrate, glutathione and amino-dextran were used to modify GNPs surface and to increase their accumulation in cancer cells. All GNPs were well characterized with respect to their particle size, shape and colloidal stability in aqueous solution and cell media. Transmission electron microscopy demonstrated accumulation of GNPs in cytoplasmic vesicles, while the mass spectroscopy demonstrated the highest uptake of glutathione modified GNPs. GNPs alone show no significant effect on cell cycle demonstrated by flow cytometry and western blot analysis. As well, cells treated with GNPs in combination with x-ray irradiation show no significant effect on cell cycle. Nevertheless, long-term overall survival of cells treated with GNPs in combination with irradiation was reduced. In conclusion, we showed that GNPs have certain biological effect, but still the precise mechanism of their activity remains to be explored.

P9: THE INTERPLAY BETWEEN sFRP3 AND DVL3 IN GLIOBLASTOMA

ANJA KAFKA[1], ANJA BUKOVAC[2], DAVOR TOMAS[3], MIRNA LECHPAMMER[4], TEA GABUD[5], NIVES PEĆINA-ŠLAUS[1,2]

[1] Laboratory of Neurooncology, Croatian Institute for Brain Research, School of Medicine University of Zagreb, Zagreb, Croatia
(e-mail: anja.kafka@mef.hr)
[2] Department of Biology, School of Medicine, University of Zagreb, Zagreb, Croatia
[3] Department of Pathology, School of Medicine, University of Zagreb, Zagreb, Croatia
[4] University Hospital Center Sestre milosrdnice, Zagreb, Croatia
[5] Department of Pathology & Laboratory Medicine, School of Medicine, University of California Davis, Sacramento, CA, USA

In the present study the expression patterns of critical molecular components of Wnt signaling pathway – sFRP3 and DVL3 protein were investigated in 34 glioblastoma patients.

Immunostaining and Image J analysis revealed the quantity and subcellular localization of the proteins. The protein expression levels in tumor tissue were evaluated by the semiquantitative method in the 3-stage signal strength and immunoreactivity score (IRS). Majority of glioblastomas had moderate levels of expression for both DVL3 (52.4%) and sFRP3 (52.3%). Strong expression levels of DVL3 and sFRP3 proteins were observed in 23.1% and 36.0% of samples, respectively. DVL3 was localized in cytoplasm in 97% of glioblastoma, of which 44% coexpressed the protein in the nucleus. The analysis of sFRP3 protein's subcellular distribution showed that it was localized in the cytoplasm in 94% of cases. Colocalization in the cytoplasm and nucleus was observed in 50% of samples. No significant correlation between DVL3 and sFRP3 mutual expression was established, nor were signal strengths correlated with epidemiological parameters. Wilcox test indicated that the domination of the strong signal in cells is in connection with simultaneous localization of DVL3 protein in the cytoplasm and the nucleus. Patients with strong expression of DVL3 will significantly more often have the protein in the nucleus ($P=6.33x10^{-5}$). Strong signal for sFRP3 did not show such an association.

Our study shows that dynamic changes in the expression levels and localizations of the studied proteins may influence the activtion of Wnt signaling in glioblastoma. These findings may contribute to better understanding of glioblastoma molecular profile.

P10: CANCER RELATED GENES AND THEIR POSTTRANSCRIPTIONAL REGULATION IN ARISTOLOCHIAC ACID ASSOCIATED UROTHELIAL CANCERS

SANDRA KARANOVIĆ[1], KARLA TOMIĆ[2], DAMIR DITTRICH[2], KREŠIMIR KARLOVIĆ[2], IVANA VUKOVIĆ[1], ŽELIMIR STIPANČIĆ[3], FRAN BOROVEČKI[4], JIŘÍ ZAVADIL[5], BOJAN JELAKOVIĆ[1]

[1] *Department for Nephrology, University Hospital Center Zagreb, University of Zagreb, School of Medicine, Zagreb, Croatia*
(e-mail: sandra_k910@yahoo.com)
[2] *General Hospital Slavonski Brod, Slavonski Brod, Croatia*
[3] *Dialysis Unit Odžak, General Hospital Orašje, Odžak, Bosnia and Herzegovina*
[4] *Department for Functional Genomics, University of Zagreb, School of Medicine, Zagreb, Croatia*
[5] *Molecular Mechanisms and Biomarkers Group, International Agency for Research on Cancer, WHO, Lyon, France*

Upper urinary tract urothelial cancers (UUC) are rare in general population but occur in almost 50% of patients with endemic nephropathy (EN)/aristolochic acid nephropathy (AAN). Aristolochic acid (AA) was proven as the etiological factor both for EN/AAN and UUC. The goal of this study was to identify tumor suppressor genes and oncogenes involved in AA induced carcinogenesis and their posttranscriptional regulation mehanisms by microRNAs.

Paired samples of tumors and adjacent normal urothelial tissues of 13 patients from Croatian and Bosnian endemic regions were analyzed. MiRNA profiling was performed by high-capacity quantitative PCR, while mRNA expression profiling of the same RNAs was done using microarray technology. Immunohistochemistry was performed on tissue microarrays.

The broad involvement of known cancer genes in the process of UUC tumorigenesis was revealed by expression profiling analysis of the tumors, implicating on many oncogenes (35 up-regulated; 54 down-modulated), and known tumor suppressor genes (17 up-regulated; 3 down-modulated). We studied whether the upregulation of specific oncogenes (*MYC, FGFR3, HRAS* and *KRAS*) and down regulation of tumor suppressor genes (*PTEN* and *PTCH1*) might be augmented in UUC by coordinated action of miRNAs. Our results showed a correlative network implicating numerous down-modulated miRNA regulators of these oncogenes, including tumor suppressor miRNAs miR-23b, miR-143 and miR-145, and also a broad inhibition of *PTCH1* by increased oncogenic miRNAs including miR-21, miR-18a and miR-9, and of *PTEN* by oncogenic miRNAs of the miR-17-92 family. Both *PTEN* and *PTCH1* appear commonly inhibited by essentially all the members of the miR-200 family. The elevated *MYC* then represents a central node in these processes, functioning as the activator of miR-17, miR-20 and miR-9 and inhibitor of miR-23b, the repressor of *FGFR3* and *KRAS*, and the tumor-suppressor miRNA let-7c. By means of immunohistochemistry a trend of lower expression of PTEN protein was observed in tumor versus the normal tissue.

AAN-associated UTUC carcinogenesis is characterized by a major deregulation of oncogenes and tumor suppressor genes, whose activity may be broadly modulated by coordinate action of miRNAs ultimately affecting the UUC-specific protein levels.

P11: SONIC HEDGEHOG PROTEINS ARE OVEREXPRESSED IN HIGH-GRADE SEROUS OVARIAN CARCINOMAS

VALENTINA KARIN[1], LUKA KAKARIGI[1], ANITA SKRTIC[2], TAMARA NIKUSEVA MARTIC[1], FRANE PAIC[1], NERMINA IBISEVIC[3], FARUK SKENDERI[3], SEMIR VRANIC[3,4], LJILJANA SERMAN[1]

[1] *Department of Biology, University of Zagreb, School of Medicine, Zagreb, Croatia (e-mail: valentina.karin@mef.hr)*
[2] *Department of Pathology, University of Zagreb, School of Medicine, Zagreb, Croatia*
[3] *Department of Pathology, Clinical Center, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*
[4] *School of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*

Sonic Hedgehog (SHH) protein, along with Indian Hedgehog (IHH) and Desert Hedgehog (DHH), is a ligand that activates Hedgehog signaling pathway (HH). It is an evolutionary conserved pathway that is necessary for normal embryonal development of diverse organisms, from fruit flies to humans. Whereas in adult human cells HH is largely inactive, it may become active following various tissue injuries that require reparation and regeneration of damaged cellular structures. Disruption in activation of this signaling pathway has been found in many types of human cancers indicating its important role in carcinogenesis. Since SHH is one of the activators of HH signaling pathway, the aim of this study was to define the pattern of SHH protein expression. This is essential for better understanding of proliferative, migratory and invasive potential of cells of human ovarian high-grade serous carcinoma and human ovarian benign serous tumors. Thus, expression of SHH ligands was analyzed by immunohistochemistry and quantified using stereological method of tissue volume density (Vv; mm0) estimation. We found statistically higher expression of SHH ligands in ovarian high-grade serous carcinoma (0.1122±0.01590) compared to ovarian benign serous tumors (0.02917±0.003564) (Mann-Whitney test; p=0.0002), which indicates an increased activation of Hedgehog signaling pathway in carcinomas with high malignant potential. This result, i.e. an association between increased expression of SHH proteins and development of aggressive ovarian malignancy, points to potential important role of HH signaling pathway in ovarian tumorigenesis.

P12: THE OVEREXPRESSION OF Δ133p53 CORRELATES WITH POOR CLINICAL OUTCOME IN PATIENTS WITH RENAL CELL CARCINOMA

MARIJANA KNEZOVIĆ[1], PETAR OZRETIĆ[2], MARO BUJAK[3], ŽELJKO KAŠTELAN[4], TVRTKO HUDOLIN[4], NEDA SLADE[5]

[1] *University Hospital Center Sestre milosrdnice, Zagreb, Croatia (e-mail: mknezovic@gmail.com)*
[2] *Laboratory for Hereditary Cancer, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia*
[3] *Laboratory for Biotechnology in Aquaculture, Division of Materials Chemistry, Ruđer Bošković Institute, Zagreb, Croatia*
[4] *Department of Urology, University Hospital Centre Zagreb, Zagreb, Croatia*
[5] *Laboratory for Protein Dynamics, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia*

Renal cell carcinoma (RCC) represents 2-3% of all cancers, with the highest incidence in Western countries. Over the last two decades the incidence of RCC is increased by 2%, mostly due to increased detection of tumors by ultrasound and computed tomography. It is accepted that prognosis of RCC differs according to the histological type, nuclear grade and tumor stage. In some cases these prognostic factors are not sufficient to predict clinical outcome of RCC. New molecular prognostic factors are being evaluated and, accordingly, the prognostic value of *TP53* tumor supressor gene has been investigated. The p53 tumor suppressor is classified as "guardian of the genome" since it is critical in the control of cell growth and the maintenance of genomic stability. *TP53* encodes 12 different isoforms and the sum of their activities determines the p53-mediated cell response. The expression of p53 isoforms has been shown to be dysregulated in several human cancer types. In this study we have analyzed mRNA expression of p53, Δ40p53 and Δ133p53 isoforms in 42 renal cell cancers and matched normal adjacent tissues by real-time PCR, and analyzed their expression in relationship to clinical features and outcome.

The overexpression of Δ133p53 was observed in the RCC tissues, and it was expressed at levels approximately two times higher than the median expression of p53 and Δ40p53. Moreover, the high expression of Δ133p53 correlated with short overall survival (P=0.027). Relative expression of each isoform in the tumors highly correlated with one another. In tumors, Δ40p53 mRNA and Δ133p53 mRNA were expressed at levels significantly higher (2.3-times Δ40p53, 3.5-times Δ133p53) then the median expression of matched normal adjacent tissues. We have also determined that Δ40p53 expression was negatively associated with invasion of kidney capsule and positively associated with disease free survival, suggesting that high levels of Δ40p53 are protective.

In conclusion, Δ133p53 is overexpressed in RCC tissue and the high expression of Δ133p53 is correlated with short overall survival. Future clinical studies are needed to investigate function of p53 isofoms and their relationship to clinical features and outcome in patients with renal cell carcinoma.

P13: "OMICS"-APPROACH TO INVESTIGATE CANCER ASSOCIATED PHENOTYPIC CHANGES IN HEP G2 CELLS AFTER TARGETED SILENCING OF AHCY HYDROLASE

LUCIJA KOVAČEVIĆ[1], ADRIANA LEPUR[1], IVANA GRBEŠA[2], ROBERT BELUŽIĆ[1], OLIVER VUGREK[1]

[1] *Laboratory for Advanced Genomics, Division of Molecular Medicine, Ruđer Bosković Institute, Zagreb, Croatia (e-mail: lucija.kovacevic@gmail.com)*
[2] *Ofir Hakim's lab, Bar-Ilan University, Ramat-Gan, Israel*

Most methylating enzymes use AdoMet as methyl group donor to methylate their substrate. AdoHyc is additional product of transmethylation reactions and is also proven to be one of the strongest competitive inhibitors of methyltransferases. Proper activity of AHCY hydrolase is therefore essential for fast removal of AdoHyc in order to avoid global alterations of cell methylation. Recently, it has been reported that level of AHCY hydrolase expression might have impact on changes in cell characteristics usually associated with so called cancer phenotype: cell cycle regulation, cell proliferation and migration. To investigate possible changes, targeted silencing of AHCY hydrolase in hepatocellular carcinoma cell line was performed using shRNA. Libraries for transcriptome sequencing were prepared automatically on NeoPrep Library System with TruSeq Stranded mRNA Library Prep Kit and sequenced on NextSeq desktop with integrated Basespace Sequence Hub platform (Illumina) for automatic data streaming, demultiplexing and filtering. Comparative proteomics was performed by Stable Isotope Labeling by Amino acids in Cell culture (SILAC) and liquid chromatography – mass spectrometry (Orbitrap Velos Pro). All datasets were analyzed and compared using IPA software (QIAGEN) to investigate potential biomarkers and assess changes in cellular mechanisms, pathways and functions. Additionally, metabolic cell activity was measured by MTT viability test and Neutral red uptake assay to analyze differences in cell proliferation, while transwell chamber assay was used to study changes in cell migration. We found that metabolic activity tests show decrease and indicate reduced cell proliferation. Also, slower cell migration is seen using transwell assay. IPA analysis confirmed these results and predicted that cell cycle is affected through several checkpoints leading to possible cell cycle arrest. Progression to cancer phenotype usually starts with alterations of cell cycle since its control is necessary for preventing unrestrained cellular growth. Our research indicates complex changes in regulation of cell cycle after AHCY hydrolase silencing with competing contribution of various pathways. Inhibitors of AHCY are currently being investigated as potential cancer treatments, thus understanding their effect on cell cycle is critical to predict possible treatment outcomes as well as for effective mixed drug therapies.

P14: *BRAF* V600E AND V600K MUTATIONS IN CROATIAN PATIENTS WITH MELANOMA

SANJA KOŽAJ[1], MONIKA ULAMEC[2], MAJDA VUČIĆ[2], NINA DABELIĆ[1], JASMINA MARIĆ-BROZIĆ[1], SILVIJA HARAMUSTEK[1], TANJA MILETA[1], ŽELJKA BUKOVEC MEGLA[1], ZVONKO KUSIĆ[1], IVAN ŠAMIJA[1]

[1] *Department of Oncology and Nuclear Medicine, University Hospital Center Sestre milosrdnice, Zagreb, Croatia (e-mail: sanjakozaj@gmail.com)*
[2] *Department of Pathology, University Hospital Center Sestre milosrdnice, Zagreb, Croatia*

BRAF mutations, which are found in 40–60% of all melanomas, are predictive marker for targeted therapy with BRAF-inhibitors in patients with metastatic melanoma. 80–90% of all *BRAF* mutations in melanoma are V600E and next in frequency (10-20%) is V600K. The aim of this study was to determine the frequency of V600E and V600K *BRAF* mutations and association between gender, age and type of sample material (primary melanoma or metastasis) with presence and type of *BRAF* mutation in melanomas from patients treated at University Hospital Sestre milosrdnice, Croatia. To our knowledge this will be the first such study for Croatian population.

DNA was isolated from archived formalin-fixed paraffin-embedded (FFPE) melanoma tissue and assayed by real-time PCR for *BRAF* V600E and *BRAF* V600K mutations using specific TaqMan probes. For statistical analysis Mann-Whitney U test or $\chi 2$ test were used, depending on type of variable.

A total of 189 samples (57 primary melanomas, 132 metastases) from 177 patients (97 male, 80 female; age 21-89 years, median 62 years) were analysed. Among 189 samples, 54% had *BRAF* mutation (11% V600K and 43% V600E), 41% did not have *BRAF* mutation, and 9 (5%) were inadequate for the analysis. In 8 patients two different lesions (primary melanoma and metastasis or 2 different metastases) were analysed and in one of these patients results were discordant, primary melanoma had V600E mutation and metastasis did not have *BRAF* mutations. Patients with *BRAF* mutations were significantly younger than patients without *BRAF* mutation (median age 59 and 67 years, p=0.0005). Among patients with *BRAF* mutation, the ones with V600E mutation were significantly younger than the ones with V600K mutation (median age 56 and 71 years, p=0.0001). Frequency of *BRAF* mutations was significantly higher in male patients than in female patients (p=0.001). Frequency of *BRAF* mutations was significantly higher in samples from metastases than in samples from primary melanoma (p=0.032).

Frequency of *BRAF* V600E and V600K mutations and association with age shown in our patients is concordant with previously published results in other populations of melanoma patients. Discrepancy in *BRAF* mutation status between primary melanoma and metastasis shown in one patient warrants that inter-lesional heterogeneity should be taken into account while interpreting *BRAF* mutation analysis results.

P15: INHIBITION OF MOUSE TERATOMA DEVELOPMENT BY EPIGENETIC AGENTS *IN VITRO*

JURE KRASIĆ[1], ROBERT BULJUBAŠIĆ[2], MAJA BULJUBAŠIĆ[3], MAJA VLAHOVIĆ[1], ANA KATUŠIĆ BOJANAC[1], FLORIANA BULIĆ-JAKUŠ[1], MONIKA ULAMEC[4,5], NINO SINČIĆ[1]

[1] *Laboratory for Epigenetics and Molecular Medicine, Department of Medical Biology, University of Zagreb, School of Medicine, Zagreb, Croatia*
(e-mail: jure.krasic@gmail.com)
[2] *Clinical Department of Surgery, Clinical Hospital Dubrava, Zagreb, Croatia*
[3] *Community Health Centre, Zagreb East, Zagreb, Croatia*
[4] *Department of Clinical Pathology "Ljudevit Jurak", University Hospital Center Sestre milosrdnice, Zagreb, Croatia*
[5] *Department of Pathology, University of Zagreb, School of Medicine, Zagreb, Croatia*

Testicular Germ Cell Tumours (TGCT), although rare, are the most frequent malignancies in young male population and believed to be initiated by epimutations, i.e. aberrant epigenetics, already in utero. Among various, teratoma is the most differentiated TGCT type encompassing all three germ layer derived tissues. Mouse teratoma is a well-established in vitro model which may be obtained by cultivating 7,5-days-old C3H mouse embryos and represent an ideal system to investigate the effect of the most prominent epigenetic drugs and agents.

After embryo isolation, they were treated for two hours with 5-azacytidine, Trichostatin A, Valproat, esiNanog, esiOct3/4 and esiTrrap, respectively. Embryos/teratomas treated with esiGFP served as a negative control. The embryos/teratomas were measured on day 0 and for the consequent 7 days of culturing, after which teratomas were scrapped, Sainte-Marie fixed and paraffin embedded for IHC analyses.

Epigenetic drugs and agents reduced significantly teratoma growth, all except esiNanog and esiTrrap. Most prominent decrease in growth was determined in 5-azaC and esiOct3/4 treated embryos/teratomas.

IHC analysis of proliferative activity showed significant rise in Ki-67 signal in esiNanog and esiTrrap, but in 5azaC treated as well, compared to control. Apoptotic activity showed no significant change in any treatment.

This preliminary data notify that epigenetic drugs and agents may have a significant effect on embryo/teratoma growth. It seems that teratoma growth is inhibited by necrotic activity rather than apoptosis which could consequently induce a rise in proliferation as a tissue reaction.

P16: SALINOMYCIN AFFECTS GOLGI APPARATUS FUNCTION IN CANCER STEM-LIKE CELLS

ANA-MATEA MIKECIN, MARKO MARJANOVIĆ, IVA GUBEROVIĆ, MARIJETA KRALJ

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

Cancer stem cells (CSC) are an immortal tumor-initiating population that can selfrenew and has pluripotent capacity. Despite their small number, CSCs are believed to be critical for tumor initiation, progression and metastasis, as well as for the treatment failure and disease relapse. CSCs have been confirmed to exist in various types of tumors which include leukemia as well as solid cancers. There are several obstacles involved with CSC research among which are their low number within tumor cell population and their relative instability in culture. Recently, a breast CSC model was established by experimental induction of epithelial to mesenchymal transition (EMT) in immortalized human mammary epithelial cells (HMLE). Using this model salinomycin was identified to be selectively toxic towards epithelial CSCs. Salinomycin is a K+/H+ exchanger that can affect cation transport across different membranes present in the cell. However the exact mechanism of the observed selectivity remains largely unknown. We have noticed that salinomycin affects potranslational modifications of membrane proteins in the fore mentioned CSC model. This led us to the hypothesis that salinomycin induces Golgi apparatus stress that affects secretory pathway which may represent a key vulnerability of EMT cells. Of note, monensin, another ionophore with chemical structure similar to that of salinomycin, is a well described inhibitor of Golgi function.

P17: PROGNOSTIC AND PREDICTIVE RELEVANCE OF METASTASIS ASSOCIATED COLON CANCER 1 (MACC1) IN BREAST CANCER

JASMINKA MUJIĆ[1], TEA BEĆIREVIĆ[2], MIRZA SULJAGIĆ[1], JOKO ĆORIĆ[3], DARIA LER[2]

[1] *Genetics and Bioengineering, International University of Sarajevo, Sarajevo, Bosnia and Herzegovina (e-mail: jasminka.mujic@gmail.com)*
[2] *Laboratory for Molecular Diagnostics, PZU 'Moja Klinika', General Hospital Sarajevo, Sarajevo, Bosnia and Herzegovina*
[3] *Department of Clinical Chemistry, Clinical University Center Sarajevo, Sarajevo, Bosnia and Herzegovina*

Breast cancer is the most common cancer in women worldwide and the incidence continues to rise in many countries. Due to potential of recurrence of this disease, it is important to find a molecular marker helping in prediction of late recurrence. Metastasis associated in colon cancer 1 (MACC1) was first identified to be over-expressed in primary and metastatic tumor specimens of colon cancer. MACC1 has also been found to stimulate proliferation, motility and invasion in colon cancer cells. To our knowledge limited information regarding MACC1 function in late recurrence of breast cancer. Western blot analysis of MACC1 was conducted in 125 protein extracts of mammary carcinoma and evaluated by densitometry. In addition, ten formalin fixed and paraffin embedded samples of breast- and ovarian cancer were analyzed for in situ localization of MACC1 by immunohistochemistry (IHC). Obtained results showed no statistical significance of MACC1 expression in the entire tested patient cohort. However, within the lymph node negative ($p<0.05$) and estrogen receptor positive ($p<0.05$) subgroups, a significant association between high MACC1 expression and shorter disease-free survival was observed. Furthermore, no significant statistical correlation between MACC1 and c-MET could be detected in tested patients. IHC results showed a positive MACC1 immunodetection. In conclusion, these results indicate a potential predictive/prognostic role of MACC1 in tested subgroups of ER (+) and lymph node negative breast cancer patients in later recurrence since these two subgroups are associated with favorable prognosis.

P18: DELETION OF EXONS 4-6 OF *BRCA1* GENE: THE FIRST CASE OF A LARGE REARRANGEMENT IN CROATIA

VESNA MUSANI[1], ILONA SUŠAC[2], PETAR OZRETIĆ[1], DOMAGOJ ELJUGA[3], SONJA LEVANAT[1]

[1] *Laboratory for Hereditary Cancer, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: vmusani@irb.hr)*
[2] *Eljuga Polyclinic, Zagreb, Croatia*
[3] *Department for Oncoplastic and Reconstructive Surgery, University Hospital for Tumors, University Clinical Hospital Centre Sisters of Mercy, Zagreb, Croatia*

Breast cancer is the most common cancer in women worldwide, and it is the leading cause of cancer related deaths in Croatia. Ovarian cancer is in the fifth place, both in incidence and mortality. About 5-10% of all breast and/or ovarian cancer cases are hereditary, and heterozygous germline BRCA1/2 mutations are responsible for the majority of hereditary breast and/or ovarian cancers. In the most cases, the mutations are small nucleotide alterations that lead to the truncation of BRCA1 or BRCA2 proteins or affect amino acids that are critical for its structure or function. Large rearrangements of *BRCA1* gene and less often, *BRCA2* gene have been described in recent years, but haven't been found in Croatia so far.

Here we describe a case of a Croatian breast cancer patient with no apparent family history of cancer, who developed Her-2 positive breast cancer at the age of 29, and triple negative breast cancer at the age of 33. Due to the young age of onset and the triple negative status (known to be connected with *BRCA1* mutations), she was referred for *BRCA1* gene testing. No mutation was found by HRM or sequencing, but the deletion of exons 4-6 of the *BRCA1* gene was determined with the Quantitative Multiplex PCR method and confirmed with MLPA analysis. Long range PCR and subsequent sequencing showed the mutation to be 10,257 bp long (NG_005905.2:g.107648_117905del10257).

To determine if the mutation was de novo, patients' family was contacted. Mother and sister do not share the mutation, but the father died from the heart attack at the age of 47. On the father's side there was a history of cancer (lymphoma, uterine, colon and lung cancer), but not breast and/or ovarian cancer. No family member from the father's side of the family was available for testing.

This deletion has been reported six times in the literature. In three cases the deletion size was not determined (Italy, Slovenia and USA) and in the other three cases (Denmark, Germany and Spain), the deletions had different breakpoints than in this case. Since the reported deletions are different then here, it cannot be excluded that this is a case of a de novo mutation, but since there is a history of cancer on the father's side of the family it is far more likely that it is not the case, and the lack of breast and ovarian cancers is just a coincidence. The fact that there were several cases of uterine cancer in the family, and it is known to be caused by *BRCA1* mutations also contributes to this conclusion. It is also worth to mention that mutation in our case is more than twice in size than any other deletion of exons 4-6 of *BRCA1* gene yet.

P19: EPIGENETIC (DYS)REGULATION OF HEDGEHOG-GLI SIGNALING PATHWAY IN OVARIAN CANCER BY microRNA MOLECULES

PETAR OZRETIĆ[1], HUBERT HACKL[2], ROBERT BELUŽIĆ[3], MAJA SABOL[1], DIANA TRNSKI[1], VESNA MUSANI[1], DRŽISLAV KALAFATIĆ[4,5], IVANA MAURAC[4], SLAVKO OREŠKOVIĆ[4,5], SONJA LEVANAT[1]

[1] *Laboratory for Hereditary Cancer, 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] *Laboratory for Advanced Genomics, Division of Molecular Medicine, Ruđer Bosković Institute, Zagreb, Croatia*
[4] *Department of Obstetrics and Gynecology, University Hospital Centre Zagreb, Zagreb, Croatia*
[5] *University of Zagreb, School of Medicine, Zagreb, Croatia*

Ovarian carcinoma is the leading cause of death from gynecological malignancies in the Western world. Its high death rate is a result of the fact that most patients (>60%) are diagnosed in advanced stage of the disease. Our experience with ovarian cancer so far came out from the research on genetic (mutations and polymorphisms) and epigenetic (promoter methylation) mechanisms of Hedghog-Gli signaling pathway in the development of various types of ovarian malignancies. The purpose of this study was to find out which Hedgehog-Gli (Hh-Gli) signaling pathway genes could be potential targets of microRNA molecules (miRNAs) differentially expressed in high-grade serous ovarian cancer (HGSOC), which is the most malignant type and most difficult to detect at an earlier stage.

We conducted a miRNA profiling on 8 fresh-frozen high-grade serous ovarian cancer tissue samples using Agilent SurePrint Human miRNA Microarray Kit 8x60K which contains probes for 2,549 human miRNAs represented in miRBase database (Release 21.0). In addition, 8 healthy Fallopian tube samples were used as a control tissue to find differentially expressed miRNAs, since this type of tissue is presumed origin of HGSOC (Reade *et al.*, J Obstet Gynaecol Can 2014). Acquired miRNA expression data were analyzed using LIMMA and AgiMicroRna tools from R/Bioconductor software package. Resulting p-values were adjusted for multiple hypothesis testing based on false discovery rate by Benjamini-Hochberg method. Furthermore, on-line DIANA Tools (diana.imis.athena-innovation.gr) were used to find which Hh-Gli pathway genes are experimentally proven or potential targets of observed differentially expressed miRNAs.

Data filtration (IQR > 0.1, FDR < 0.1, logFC > 0.58 and < -0.58) gave us a list of 55 miRNAs: 32 were up- and 23 were down-regulated in HGSOC. Out of 47 genes which are involved in Hh-Gli pathway in humans (according to the KEGG Pathway hsa04340), 35 are known targets for 27 over-expressed observed miRNAs, while 22 genes are known targets for 16 under-expressed miRNAs. In addition, 28 genes are potential targets for 26 up-regulated miRNAs while 24 are potential targets for 19 down-regulated miRNAs in HGSOC.

Our results highlighted several candidate miRNAs which we intend to verify and functionally connect to Hh-Gli pathway genes in our future research. Enlightening the interplay between miRNAs and Hh-Gli signaling pathway genes in serous ovarian carcinoma pathogenesis could give us new information for better therapeutic approaches and early prevention programs.

P20: ORGANOMETALLIC RUTHENIUM COMPLEXES WITH TRIPHENYLPHOSPHANE AMINO ACID BIOCONJUGATES AS POSSIBLE ANTICANCER COMPOUNDS

MARGARETA PERNAR[1], ZORAN KOKAN[2], MARIJA MATKOVIĆ[3], IVO PIANTANIDA[3], DENIS POLANČEC[4], IZTOK TUREL[5], SREĆKO I. KIRIN[2], ANAMARIA BROZOVIĆ[1]

[1] *Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia*
(e-mail: megi.pernar@gmail.com)
[2] *Laboratory for Solid State and Complex Compounds Chemistry, Division of Materials Chemistry, Ruđer Bošković Institute, Zagreb, Croatia*
[3] *Laboratory for Biomolecular Interactions and Spectroscopy, Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia*
[4] *Children's Hospital Srebrnjak, Department for Translational Medicine, Zagreb, Croatia*
[5] *Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia*

Platinum-based drugs have been predominantly used in chemotherapy for decades. However, due to their high general toxicity and development of tumour drug resistance, there is a constant need to synthesize new compounds with reduced side effects. Organometallic ruthenium complexes have already shown promising anticancer activity. In this research, we explored biological characteristics of newly synthesized (p-cymene)-ruthenium complexes 1 and 2, bearing phosphane ligands substituted with chiral or non-chiral amino acid esters. Interestingly, unlike most of the previously synthesized complexes that are described as "platinum mimicking", we showed that these ruthenium complexes bind to proteins instead of DNA, although their possible specific target remains unknown. Cytotoxic activity was investigated on human cervix carcinoma cell line (HeLa) using MTT assay. Four (2pG, 2pA, 2mG and 2mA) out of six synthesized metallated biconjugates showed significant toxicity, with IC50 values of 5 to 30 μM. 2mG compound with average value of IC50 16 μM was selected for further biological characterisation. The higher level of toxicity towards tumour compared to normal cell lines indicates its selective activity, important characteristic for potential medical use. Further, using flow cytometry and propidium iodide staining, it was detected that 2mG caused dose-dependent increase of SubG1 cell population, suggesting its ability to induce apoptosis. Decreased survival of HeLa cells pretreated with specific inhibitor of glutathione (GSH) synthesis, buthionine sulfoximine, and opposite effect obtained by pretreatment of cells with GSH precursor N-acetyl-cysteine, indicates important role of GSH in HeLa cells response to investigated organometallic ruthenium complexes. Since 2mG did not induce reactive oxygen species, the decreased survival of HeLa cells pretreated with the inhibitor of glutathione-S-transferase, ethacrynic acid, and inhibitor of multidrug resistance protein family, probenecid, emphasizes involvement of GSH in detoxification of 2mG compound. We speculate that ruthenium complexes bind protein-based biomolecules triggering further cell death. Based on the gained knowledge, the synthesis and development of next generation of tumour-specific ruthenium-based complexes as potential anticancer drugs is expected.

P21: ASSESSING THE PROGNOSTIC SIGNIFICANCE OF p53 ISOFORMS IN SOFT TISSUE SARCOMA

NEDA SLADE[1], ARIJANA ZORIĆ[1], MARO BUJAK[2], ANĐELA HORVAT[1], MLADEN JOKIĆ[1], IVAN MILAS[3], PETAR OZRETIĆ[4], BOŽENA ŠARČEVIĆ[5]

[1] *Laboratory for Protein Dynamics, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: slade@irb.hr)*
[2] *Laboratory for Biotechnology in Aquaculture, Division of Materials Chemistry, Ruđer Bošković Institute, Zagreb, Croatia*
[3] *Department for Oncoplastic and Reconstructive Surgery, University Hospital for Tumors, University Hospital Center Sestre milosrdnice, Zagreb, Croatia*
[4] *Laboratory for Hereditary Cancer, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia*
[5] *Department of Pathology, University Hospital for Tumours, Sestre milosrdnice University Hospital Center, Zagreb, Croatia*

Soft tissue sarcoma (STS) encompasses a plenitude of malignant tumors of the connective tissue. Most of these tumors are very invasive and the response to chemotherapy and radiation is very limited. The mechanism of soft tissue sarcoma tumorigenesis is still unclear; therefore, they represent a major therapeutic challenge. Mutation of p53 is a common hallmark of cancer. It is mutated in more than 50% of human tumors. However, knowledge about the prognostic relevance of p53 mutations in STS is not comprehensive, and, according to our data, seems to be rare. Therefore, other mechanisms of p53 inactivation might be involved. As is already well accepted, p53 is expressed not only as full length, but also as isoforms, some of which inhibit wild-type p53. There are no studies that have examined the relative expression of p53 isoforms in STS so far.

Here, we have analyzed the relative mRNA expression of the p53, Δ40p53, Δ133p53 (C-terminal isoforms), p53α, p53β and p53γ (N-terminal isoforms) in a panel of 5 STS cell lines, 26 STS specimens and 15 matched normal adjacent tissues using real-time PCR. Further we have analyzed their relationship to the outcome of disease. We have found that almost all isoforms were expressed equally, only p53γ was expressed at a higher level compared to p53. Further, we have observed that all three N-terminal isoforms are positively correlated to each other, compared to C-terminal ones which were not correlated. Additionally, higher p53β expression was significantly associated with longer overall survival (OS) of patients, while there was a trend toward significant association of higher Δ40p53 expression with longer OS. However, the higher Δ40p53 expression was positively correlated to reappearance of tumor with marginal statistical significance. These results have shown the implications of p53 isoforms in development of STS and might provide the elucidation for deregulated p53 function in STS.

P22: OPTIMIZATION OF IHC-BASED TEST FOR DETECTION OF METASTASIS ASSOCIATED COLON CACER 1 MACC1 IN PATIENTS WITH MELANOMA

MARIJA SPASOJEVIĆ DULETIĆ[1,2], IZET EMINOVIĆ[1], TEA BEĆIREVIĆ[2], DARIA LER[2]

[1] *Department of Biology, Faculty of Natural Sciences and Mathematics, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*
[2] *Laboratory for Molecular Diagnostics, PZU "Moja Klinika", General Hospital Sarajevo, Sarajevo, Bosnia and Herzegovina*
(e-mail: mspasojevic@mojaklinika.ba)

Among all skin malignancies, melanomas represent a serious worldwide health problem. Early detection and diagnosis are the most important steps in successful treatment of all cancers. In the past few decades diagnosis for melanoma has greatly changed. At present, for treatment and prognosis are important combinations of clinical and histopathological parameters. Current, tumor markers are based on the method of determination of antigens and generally exhibit reduced specificity and sensitivity. For this purpose, new tumor markers are necessary to enhance and improve the diagnosis and prognosis of diseases, and enable targeted therapy. Aim of this study was to localize and identify Metastasis Associated Colon Cancer 1 (MACC1) as a potential molecular marker for human melanoma samples and correlate the MACC1 expression in patients diagnosed with colorectal cancer, where MACC1 has already been demonstrated as a potential molecular marker. For the purpose of this study formalin-fixed and paraffin embedded specimens of melanoma and colorectal cancer obtained from 20 patients were used. Obtained results indicated successful in situ localization and identification of the expression of a targeted protein, indicating that the MACC1 is equally expressed in melanoma and samples of colorectal carcinoma. In addition, these results suggest the potential and important role of this particular biomarker in early diagnosis of melanoma cancer and possible development of targeted therapy for melanoma patients. In conclusion, positive immunodetection of MACC1 in analyzed melanoma samples was generally more associated with poor prognosis in patients, and therefore could be used as a potential predictive marker for progressive forms of melanoma.

P23: POLYMORPHISMS OF *PON1* GENE IN PATIENTS WITH PREMALIGNANT LESION OF THE CERVIX

ANA MARIJA STOJIĆ[1], MARIJA GRDIĆ RAJKOVIĆ[2], IVANA ĆELAP[3], ANDREA HULINA[2], DAMIR ELJUGA[1], DRAŽAN BUTORAC[1]

[1] *Department of Gynecology and Obstetrics, University Hospital Center Sestre milosrdnice, Zagreb, Croatia (e-mail: am.vrkljan@gmail.com)*
[2] *Department of Medical Biochemistry and Hematology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia*
[3] *Clinical Institute of Chemistry, University Hospital Center Sestre milosrdnice, Zagreb, Croatia*

Increased lipid peroxidation and changes in antioxidative system in patients with premalignant lesions of the cervix indicates the importance of oxidative stress in early phase of carcinogenesis. Paraoxonase 1 (PON1) is a calcium-dependent esterase, synthesized in the liver and secreted into the plasma where it is associated with HDL. PON1 possess antioxidant/antiatherogenic activity, and it removes carcinogenic radicals of lipid peroxidation in human body. Polymorphisms in coding (Q192R and L55M) and promoter (-108 C>T) regions of *PON1* gene affect Paraoxonase 1 activity. We carried out this study in order to determine frequencies of Q192R, L55M and -108C>T polymorphisms in patients with premalignant lesions of the cervix.

Study included 65womens [37 (29-42) years] with a biopsy confirmed diagnosis of cervical intraepitelial neoplasia (CIN). CIN1 had 18 patients, 23 had CIN2 and 24 had CIN3. 109 healthy volunteers [38 (32-46) years] without any cervical or other disease were enrolled in the study as a control group. DNA was isolated from whole blood and polymorphisms were determined by PCR-RFLP procedure.

The analysis of *PON1* gene polymorphisms in patient group showed following frequencies: 51% QQ, 44% QR, and 5% RR for Q192R genotype; 43% LL, 43% LM, and 14% MM for L55M genotype; 35% CC, 42% CT, and 23% TT for -108 C>T genotype. In the control group we found rather similar frequencies: 59% QQ, 33% QR, 8% RR for Q192R genotype; 40% LL, 42% LM, and 18% MM for L55M genotype; 30% CC, 45% CT, and 25% TT for -108 C>T genotype. No significant differences was found concerning examined polymorphisms between patients and healthy volunteers (Q192R, P=0.258; L55M, P=0.728; -108C>T, P=0.784).

Our results suggest that Q192R, L55M and -108C>T polymorphisms of pon1 gene is not a potential risk factors for cervical intraepithelial neoplasia. However this finding must be confirmed by study conducted on bigger sample size.

P24: REDUCED PROMOTER METHYLATION OF *MYD88* AND *ASC/TMS1* GENES IN TUMOR TISSUE OF PATIENTS WITH LUNG CARCINOMA

MAJA ŠUTIĆ[1], ANTJE FITZNER[2], GORDANA BUBANOVIĆ[3], MATTHIAS LINKE[2], ADRIANA LEPUR[1], LUCIJA KOVAČEVIĆ[1], LUKA BRČIĆ[4], SVEN SEIWERTH[4], MIROSLAV SAMARŽIJA[3], OLIVER VUGREK[1], MARKO JAKOPOVIĆ[3], ULRICH ZECHNER[2], JELENA KNEŽEVIĆ[1]

[1] *Laboratory for Advanced Genomics, Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia (e-mail: Maja.Sutic@irb.hr)*
[2] *Institut for Human Genetics, Johannes Gutenberg-University Mainz, Mainz, Germany*
[3] *Department for Respiratory Diseases, University Hospital Center Zagreb, University of Zagreb, School of Medicine, Zagreb, Croatia*
[4] *Department of Pathology, University of Zagreb, School of Medicine, Zagreb, Croatia*

Aberrant DNA methylation of promoter region CpG islands is associated with gene silencing and serves as an alternative to mutation-induced inactivation of tumor suppressor genes in human cancers. Chronic inflammation and infection have been recognized among major risk factors for the most common types of cancer. Several lines of evidences are linking cancer, inflammation and infection. Transcription factor nuclear factor-κB (NF-κB), major inducer of inflammation is activated by many cancer risk factors (cigarettes smoke) and is constitutively active in most cancer. Activation and controlling mechanisms of inflammation and infection are regulated by NF-κB and almost exclusively relied on receptors of innate immunity, known as Pattern Recognition Receptors (PRR). Thus, suppression of these proinflammatory pathways may provide opportunities for both prevention and treatment of cancer. The aim of presented study was to evaluate methylation status of *ASC/TMS1* (Apoptosis-associated speck-like protein containing a CARD/Target of methylation-induced silencing-1) and *MYD88* genes (Myeloid differentiation primary response 88), key adaptor molecules in innate immunity signalling. Here we found that both *MYD88* and *ASC/TMS1* exhibit reduced methylation status of promoter regions in tumor tissues from patients diagnosed with lung cancer, comparing to healthy tissue. These results were also confirmed on protein level. Reduced methylation status in tumor tissue, of both *MYD88* and *ASC/TMS1*, correlate with higher protein expression and vice versa. To confirm our findings on mRNA level we performed pathway-focused gene expression analysis of 84 genes involved in TLR and NOD signalling pathway using RT-qPCR approach (RT2 ProfilerTM PCR Array Human Inflammasomes). We found that expression of *MYD88* mRNA in tumor tissue is unchanged compared to healthy lung tissue, while mRNA level of *ASC*, gene involved in inflammasome formation, is up-regulated in tumor compared to healthy lung tissue. Also, we detected tumor specific cytokine profile on mRNA level: up-regulation of cytokines as *IL18* and *IL1B* and down-regulation of cytokines as *IL12A*, *IL33* and *IL6*.

P25: CHEMOSENSITISATION TO MICROTUBULE POISONS AND DECREASED MIGRATION OF HUMAN MELANOMA CELL LINES BY SILENCING OF INTEGRIN αV

ANA DEKANIĆ[1], MLADEN PARADŽIK[1], NIKOLINA STOJANOVIĆ[1], IVANA STEINER[1], MAJA T. TOMIČIĆ[2], MAJA OSMAK[1], ANDREJA AMBRIOVIĆ-RISTOV[1]

[1] *Laboratory for Cell Biology and Signalling, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia*
(e-mail: adekanic@irb.hr)
[2] *Department of Toxicology, University Medical Center Mainz, Mainz, Germany*

Melanoma is the most aggressive form of skin cancer. Integrin signalling regulates numerous cellular vital processes in tumour cells. The goal of this study was to investigate the impact of integrin αv knockdown on sensitivity to antitumor drugs, cell migration and invasion. Three human metastatic melanoma cell lines A375, RPMI-7951 and MeWo were used. Flow cytometry analysis using integrin-specific antibodies showed that all three cell lines express comparable amounts of integrin subunit αv and heterodimer αvβ5. However, they differ in expression of integrin αvβ3 which is highly expressed in A375 and RPMI-7951 but almost absent in MeWo cells. The siRNA-directed integrin αv knockdown increased sensitivity of RPMI-7951 and MeWo cells to the microtubule-directed antitumor drugs paclitaxel and vincristine, decreased sensitivity of MeWo cells to cisplatin, and neither changed sensitivity of RPMI-7951 cells to cisplatin nor of A375 cells to any of the abovementioned drugs. In vitro transwell migration and invasion assays conducted in the RPMI-7951 cell line upon αv silencing resulted in a drastic inhibition of both, cell migration and invasion. Using the same assay migration could not be assessed in MeWo cells, which is in accordance with literature data showing their low metastatic potential. We have also analysed the involvement of pERK1/2 signalling pathway in integrin αv sensitisation to paclitaxel. The integrin αv knockdown, as compared to control-siRNA transfected cells moderately decreased the total amount of pERK1/2. The lower amount was maintained during 72 hours of paclitaxel exposure. Approximately 60–70% of malignant melanomas have a *BRAF* mutation that constitutively activates the B-Raf/MEK/ERK1/2 pathway. They are treated with the BRAF inhibitor vemurafenib. The BRAF V600E mutated RPMI-7951 cell line is, however, vemurafenib-resistant due to the overexpression of COT which activates ERK1/2 independent of BRAF. Therefore, we analysed the combined effect of integrin αv knockdown and vemurafenib, and showed that integrin αv knockdown increased sensitivity of RPMI-7951 cells to vemurafenib. Our results identify integrin αv as a potential target for melanomas not responding to vemurafenib therapy such as BRAF V600E mutated but vemurafenib-resistant (RPMI-7951) and BRAF wild type (MeWo) melanoma cell lines to paclitaxel. Also the integrin αv knockdown decreases metastatic potential in the highly metastatic cell model of RPMI-7951 cells.

P26: TROUBLESHOOTING OF RNA ISOLATION METHODS IN PAPANICOLAOU HPV INFECTED SMEARS

TEA BEĆIREVIĆ[1], MIRSADA HUKIĆ[2], JASMINKA MUJIĆ[1], DARIA LER[3]

[1] *Genetics and Bioengineering, International University of Sarajevo, Sarajevo, Bosnia and Herzegovina (e-mail: becirevic.tea@gmail.com)*
[2] *Department of Microbiology, Institute for Biomedical Diagnostic and Research "NALAZ", Sarajevo, Bosnia and Herzegovina*
[3] *Laboratory for Molecular Diagnostics, PZU "Moja Klinika", General Hospital Sarajevo, Sarajevo, Bosnia and Herzegovina*

Aim of this study was to extract total cellular mRNA from cytobrush-collected healthy and Human Papilloma Virus (HPV) infected cervical epithelial cells and analyze whether the samples obtained by regular Papanicolaou test or commonly known as Pap test can be used for gene expression analysis. Pap test represents a non-invasive way to obtain biological material from selected group of patients. A total of 40 cervical specimens were previously tested for HPV infection. Following HPV testing, samples were submitted to extraction and purification of total cellular RNA. Products after RNA extraction methods used in this particularly study could be visualized using 2% agarose gel electrophoresis; however right after the RNA purification step no visible bands were detected. The RNA extraction from a cohort (n=40) of cytobrush-collected cervical epithelial cells, used in this particular study, couldn't be accomplished with either of the two RNA extraction methods used. In conclusion RNA purification represents a necessary step for assurance of high quality extracted RNA in all gene expression analysis studies. Reliance on commercial kits only or misinterpretation of the results can lead to serious errors in the implementation of final conclusions and/or to false positive test results, affecting the efficacy of particular testing. According to obtained results, the sample type used in this study was not suitable for the gene expression analysis.

P27: IMPACT OF WEIGHT REDUCTION PROGRAM ON THE LEVEL OF LIPID PEROXIDATION

MAJA ORTNER HADŽIABDIĆ[1], ANA-MARIJA DOMIJAN[1], JASNA JABLAN[1], SUZANA INIĆ[1], DARIO RAHELIĆ[2], VELIMIR BOŽIKOV[2]

[1] *Department of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia*
(e-mail: adomijan@pharma.hr)
[2] *Department of Endocrinology, Diabetes and Metabolism disorders, Dubrava University Hospital, Zagreb, Croatia*

Obesity is strictly linked to changes in redox state; oxidative stress mainly results from positive energy balance that leads to excessive fat accumulation in non-adipose tissues, with subsequent development of obesity co-morbidities (Savini *et al.*, Int J Mol Sci 2013). The aim of this study was to explore the impact of the weight reduction program on the level of malondialdehide (MDA) well established marker of lipid peroxidation and oxidative stress. Patients at the outpatient Clinic of the Department of Endocrinology, Diabetes and Metabolism disorders at the Dubrava University Hospital were enrolled in the weight reduction program involving diet and exercise. Participants received counseling and education during the initial week and were invited for the follow-up visits after one, three, six and twelve months. The plasma samples were collected at the initial visit and in one year period and were stored at -80 ºC. In plasma samples MDA level was assessed by the use of HPLC. Thirty five obese patients enrolled in the weight reduction program participated in this preliminary study (age 47.9±12.4 (range 21 – 69); body weight 109.2±15.3 kg (range 88.5 – 150 kg); 77.1% were woman). At the beginning of the study MDA level was 4.08±1.7 μM (mean±SD). In one year follow-up, MDA level in the same group of patients was 2.71±0.9 μM, which was statistically lower from the level at the beginning of the study ($p<0.05$). Significant decrease in a year period was also observed for the body weight ($p<0.001$) with the mean difference between initial and final measurement being 5.5±6.5 kg. The results indicate that weight reduction program involving diet and exercise can reduce level of oxidative stress and consequently lead to healthier life.

P28: IN SEARCH OF NOVEL TRANS-ACTIVATORS OF X CHROMOSOME INACTIVATION

ADNA OMERAGIC, CHERYL MADURO, JOOST GRIBNAU

Department of Developmental Biology - Erasmus Medical Center, Rotterdam, The Netherlands (e-mail: adna.omeragic@gmail.com)

Dosage differences in X-linked gene expression between XY males and XX females are compensated in embryonic development of female mammals by the process of X chromosome inactivation (XCI). Prior to onset of XCI, determines the number of active X chromosomes and initiates XCI when more than one X chromosome is present per diploid genome. The master regulator region controlling XCI is the X inactivation center (Xic) that codes for a number of regulators of XCI that can have cis- or trans- mode of regulation. Xist is a long non coding RNA (lncRNA) located at the Xic, is upregulated on the future inactive X, coating the X in cis, thereby recruiting a number of factors needed for silencing. X-linked, trans-acting regulators are expressed in a dosage dependent manner and enable female specific XCI initiation. Previous work in our lab has identified a trans-acting activator, Rnf12, and obtained insights in its involvement in XCI. However, there is evidence that Rnf12 is not the sole activator of XCI since its deletion on one allele of the X chromosome in female cells, does not abolish XCI completely. In this project we sought to identify other putative activators of XCI. A 50Mb of X chromosomal region, located in the vicinity of the Xic locus, telomeric to Xist was deleted using the CRISPR/Cas9 system. Smaller deletions of 10Mb and 20Mb inside this 50Mb region were also performed to refine this 50Mb region. Targetings were performed in a reporter cell line that has GFP and mCHERRY transgenes under control of the endogenous Xist and Tsix promoters, respectively. This way we have a reporter system to study the effect and activity of Rnf12 and newly identified regulators throughout the XCI process. A successful deletion of the 50Mb region in the total cell pool, after targeting was detected. In addition, a smaller 10Mb deletion inside the 50Mb region was also detected in the cell pool. By generating deletions in the vicinity of the Xic locus we can get closer to finding the genomic regions where remaining activators of the XCI initiation are precisely located. Following the generated deletions we can proceed with isolating the desired single cell clones to establish the desired ES cell lines. The impact of the deletions on the XCI process is still to be investigated.

P29: TELOMERE Q-PNA FISH: INSIGHT INTO SENESCENCE AND AGING

NIKOLINA ŠKROBOT VIDAČEK, LUCIA NANIĆ, SANDA RAVLIĆ, IVICA RUBELJ

Laboratory for Molecular and Cellular Biology, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia
(e-mail: nskrobot@irb.hr)

Telomeres are specialized structures found at the ends of eukaryotic chromosomes serving as guardians of genome stability. Normal mammalian cells demonstrate limited growth capacity. Due to the DNA end replication problem and additional 5′-exonuclease activity, their telomeres are shortening during each subsequent division. When telomeres are critically shortened cell proliferation is permanently stopped, a phenomenon known as cellular senescence. While telomere shortening is a main molecular mechanism that limits growth of normal cells, telomere elongation is crucial for the maintenance of immortalization in tumor cells. Thus, structural and functional analysis of telomeres is very important for understanding of cell growth control, cellular senescence and processes of immortalization. It is very important to monitor a behavior of telomere sister chromatids as indicator of molecular processes on telomeres. Therefore, we monitored these processes during cell senescence using quantitative PNA-FISH method. Observed differences between sister telomeres are larger and more frequent than this could be attributed to creation of -3′ single stranded overhangs after the replication of DNA. Sister telomere signals demonstrate high regularity in telomere variations which ensure reproducibility of results using this method. We consider that deficient hybridization and/or complexity of telomere DNA suprastructure result in observed differences in telomere strands labeling.