567 NOEY2 gene expression in breast tumour pathogenesis

D.O. Mykytenko[1]. [1]*Academy of Medical Sciences of Ukraine, Laboratory of Molecular Diagnostics, Kyiv, Ukraine*

Background: It is known that disturbances in gene imprinting, defined as gene expression based on the gamete of origin, are implicated in oncogenesis through loss of tumour suppressor gene regulation. The inactivation of expression of NOEY2 gene, an imprinted putative tumour suppressor gene, may contribute to tumours arising in the breast.
The aim of research was to investigate the expression of NOEY2 gene in breast cancer tissue and its relation to clinical and molecular characteristics of cancer cells.
Material and Methods: We studied the biopsy material of breast cancer cases and benign breast tumours (50 samples each). All cases were not BRCA-associated. The expression of NOEY2, ER, Ki67, p53, p21 and mdr1 genes was detected by Real-Time PCR using TaqMan® gene expression assays (Relative quantification, RQ). The expression of GHPDH gene was accepted as endogenous control. The BRCA mutations (c.181T>G, c.4034delA, c.5266dupC, c.68_69delAG of BRCA1 and c.5946delT of BRCA2 gene) were detected using TaqMan® SNP custom assays.
Results: The NOEY2 mRNA was detected in 47 of 50 (94%) noncancerous tissues and in 41 of 50 (82%) breast cancer samples. In 15 of 41 (36.6%) NOEY2 mRNA-positive cancer samples the expression of NOEY2 was substantially reduced (up to 100 times).
The expression of NOEY2 gene was positively correlated with the age of disease manifestation ($r = 0.59$; $P < 0.01$). The expression of NOEY2 was not correlated with ER, Ki67, p53, p21 and mdr1 genes expression.
Conclusions: Thus, the NOEY2 expression may play an important role in abrogation of the breast cancer pathogenesis. The link between epigenetic disturbances and the spreading mechanism of breast cancer may possibly exist.
These experiments were financial supported by private research laboratories.
Conflict of interest: not declared.

568 Synthesis and biological properties of some new thiosemicarbazones

T. Stanojkovic[1], D. Kovala-Demertzi[2], A. Primikiri[2], M. Demertzis[2], J. Antic-Stankovic[3], Z. Juranc[1]. [1]*Institute for Oncology and Radiology of Serbia, Experimental, Belgrade, Serbia,* [2]*University of Ioannina 45110 Ioannina Greece, Inorganic and Analytical Chemistry Department of Chemistry, Ioannina, Greece,* [3]*Faculty of Pharmacy University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia*

Background: Thiosemicarbazones are versatile molecules not only because of their broad profile in pharmacological activity, but also because can act as ligands in coordination chemistry in different ways. It has been demonstrated in previous publications that thiosemicarbazones afford a diverse variety of compounds with different activities. In this work new thiosemicarbazones deriving from 2′-hydroxyacetophenone or salicylaldehyde and containing rings incorporated at N(3)-position have been investigated. These compounds have been prepared and structurally characterized by means of vibrational and NMR (^{1}H and ^{13}C) spectroscopy. These compounds are of considerable interest due to their antibacterial and antitumour activities.
Material and Methods: The compounds have been identified by elementar analysis and spectroscopic techniques (^{1}H-NMR, ^{13}C-NMR, IR, MS and XRF). In vitro cytotoxicity of the thiosemicarbazones was tested by MTT assay against the target cells: human cervix carcinoma (HeLa), chronic myelogenous leukemia (K562), breast carcinoma (MDA-MB-453) and breast adenocarcinoma (MDA-MB-361). Compounds solusions were added to neoplastic cells grown in 96 flat bottomed wells, 20 h after cell seeding. Cell survival was determined 72 h after the continuous agent action. The antibacterial activity of the compounds was evaluating by broth microdilution assay using a panel which included laboratory strains obtained from American Type Culture Collection.
Results: The obtained results showed that both studied compounds expressed significant cytotoxic activity *in vitro* toward malignant HeLa, MDA-MB-361, MDA-MB-453, and K562 cell lines having IC50 values from 0.76 to 7.21 μM, pointing to their similar, or even better antiproliferative action than the action of cis-DDP on the same cell line.
Conclusions: Results obtained indicate the potential of these compounds for the antitumour action, making them particularly interesting for further *in vivo* investigations. In addition, these compounds showed a mild antibacterial activity.

569 Synergistic effect of cisplatin combination with indole-3-ethyl isothiocyanate on proliferation of human ovarian cancer cells in vitro

L. Hunakova[1], P. Stehlik[1], H. Paulikova[2], P. Gronesova[1], M. Pastorek[1], D. Cholujova[1], J. Duraj[1], J. Sedlak[1]. [1]*Cancer Research Institute, Laboratory of Tumour Immunology, Bratislava, Slovak Republic,* [2]*Faculty of Chemical and Food Technology Slovak Technical University, Department of Biochemistry and Microbiology, Bratislava, Slovak Republic*

Chemopreventive isothiocyanates found in cruciferous vegetables, consumption of which has been associated with reduced risk of cancer, exhibit also growth-inhibiting and apoptosis-inducing properties in cancer cell lines in vitro. Our study presents a new synthetic ITC derivate indole-3-ethyl isothiocyanate (homoITC) as an inhibitor of cellular proliferation and inducer of apoptosis with potential utility as an anticancer drug or a sensitizer to routinely used chemotherapeutic agent cisplatin (cis-Pt).
We analysed the growth inhibitory effects of homoITC in the human ovarian carcinoma cell line A2780 and its cisplatin-resistant variant A2780/CP using MTT-test and its apoptosis-inducing properties by flow cytometry.
Combination index (CI) values from Calcusyn software were used to characterize the interactions of homoITC and cis-Pt as synergistic ($CI < 1$), additive ($CI = 1$), or antagonistic ($CI > 1$).
Significant synergistic effect in growth inhibition of homoITC (5−15 μM) and cis-Pt (2.5−10 μM) on A2780 parental cell line (CI from 0.42 to 0.85) was also observed on A2780/CP resistant subline (CI from 0.18 to 0.73) for 10−50 μM cis-Pt concentrations and the same concentrations of homoITC. Synergy in growth inhibition correlated with the potential of homoITC to stimulate apoptosis induced by cis-Pt.
Conclusion: homoITC may be worth of further studies assessing its value in the ovarian cancer treatment and elucidating mechanisms of its action.

570 Expression of beta-catenin and MUC1 in malignant, benign and normal breast tissues

M. Croce[1], S. Demichelis[2], M. Isla-Larrian[1], L. Cermignani[1], N. Giacomi[3], A. Segal-Eiras[1]. [1]*CINIBA, Faculty of Medical Sciences, La Plata, Argentina,* [2]*Biological Department, UAJF Kennedy, Buenos Aires, Argentina,* [3]*Site, Faculty of Medical Sciences, La Plata, Argentina*

Background: During cancer progression, MUC1 binds β-catenin resulting in enhanced transformation and metastasis. The purpose of this work was to study the characteristics and correlation of the immunohistochemical expression of β-catenin and MUC1 in malignant, benign and normal breast tissues.
Material and Methods: Immunohistochemical analysis was performed on 60 breast carcinoma samples, 15 benign breast diseases and 10 normal tissues. MUC1 was detected employing HMFG1 MAb and β-catenin with anti-beta-catenin 7D11 MAb (Santa Cruz, USA); Catalyzed Signal Amplification System (Dako, USA) was employed. Immunohistochemistry was performed following standard procedures without antigenic retrieval. Positive area of reaction, intensity and pattern of expression were considered. A reactivity index (RI) was calculated as intensity (I) × 100 + percentage of positive area (A). Statistical correlation analysis was performed employing Pearson correlation.
Results: Malignant samples expressed β-catenin in 51/60 (85%) samples and MUC1 in 54/60 (90%) while breast benign samples in 12/15 (80%) and 15/15 (100%), respectively; finally, normal samples expressed β-catenin in 9/10 (90%) and MUC1 in all samples. In all groups a statistical significant correlation between β-catenin and MUC1 ($p < 0.005$) was found. The pattern of β-catenin expression was different since, in malignant specimens, nuclei staining was frequently found (68%) while nuclei were not reactive in benign and normal samples. The intensity of the reaction was strong in most malignant and benign samples while normal samples showed a low reaction. MUC1 differed in the pattern of expression since malignant samples showed a mixed (cytoplasmic and membrane) non-apical staining while membrane apical pattern was predominantly found in benign and normal specimens.
Conclusions: IHC is an ancillary tool in pathological diagnosis as well as in experimental analysis; employing this methodology we found that β-catenin and MUC1increased their expression and change their pattern in malignant breast samples compared to benign and normal specimens.

571 Molecular events during cold stress induced cell-death on multidrug resistant leukemic cells

D. Cerezo[1], A.J. Ruiz-Alcaraz[1], M. Lencina[1], C. Bernal[1], M. Cánovas[1], P. García-Peñarrubia[1], E. Martin-Orozco[1]. [1]*University of Murcia, Biochemistry and Molecular Biology B and Immunology, Murcia, Spain*

We have previously shown that acquisition of multidrug resistant phenotype (MDR) by leukemic cells is accompanied by pleiotropic changes that result on reduced tumour capacity to survive under stress conditions such as hypothermia. Furthermore, by using selective inhibitors of individual caspases (caspase 3, 8 or 9) we have reported a reversal on cold stress-induced cell-death in the presence of any of those inhibitors, suggesting that the cell death mechanism is caspase-dependent. Next, our aim was to gain a broader insight

into caspase 3 involvement on this cell-death process and, furthermore, to characterize the role of other apoptogenic molecules such us cytochrome c, p53 or AIF (apoptosis inducing factor) into this phenomenom. On this regard, active caspase 3 levels were studied by western-blot and also by measuring enzymatic activity of this caspase at different time points on leukemic cells incubated below 4 ºC. Additionally, we have studied the timing of cytochrome c release and the expression patterns of p53 and AIF on cytosolic, mitochondrial and nuclear fractions. In summary, there are different expression and/or release patterns of apoptogenic molecules on resistant versus sensitive leukemic cells, which correlates with the cell death time course observed for each one of these leukemic cells. The study of the signalling molecules implicated on cold stress-induced cell-death is fundamental on the design of new approaches that allow a better understanding to eliminate drug-resistant tumours.

572 MDR modulation in adenocarcinoma cell lines: nuclear medicine as an important approach

J. Casalta-Lopes[1], A.M. Abrantes[2], J. Rio[3], M. Laranjo[1], B. Oliveiros[1], C. Gonçalves[4], A.B. Sarmento-Ribeiro[4], M.F. Botelho[5]. *[1]Instituto Biofisica/Biomatematica IBILI, Faculdade de Medicina Coimbra, Coimbra, Portugal, [2]Instituto Biofisica/Biomatematica CIMAGO IBILI, Faculdade de Medicina Coimbra, Coimbra, Portugal, [3]ICNAS Universidade de Coimbra, Instituto Biofisica/Biomatematica FMUC, Coimbra, Portugal, [4]Faculdade de Medicina, Instituto Bioquímica, Coimbra, Portugal, [5]Instituto Biofisica/Biomatematica CIMAGO IBILI-FMUC, ICNAS Universidade de Coimbra, Coimbra, Portugal*

Background: One of the major setbacks to chemotherapy is multidrug resistance (MDR), characterized by cross-resistance to several drugs. It can occur due to overexpression of efflux pumps such as P-glycoprotein (Pgp), multiple resistance-related protein 1 (MRP1) and lung resistance-related protein (LRP). They have different extrusion mechanisms but confer resistance to similar substrates. L-buthionine-sulfoximine (BSO) inhibits glutathione synthesis and can be used as blocker for MRP1. Verapamil is a known substrate for Pgp, modulating its activity. In this study we aim to compare transport kinetics for sensitive and resistant human colorectal adenocarcinoma cell lines, in the presence and absence of verapamil and BSO, through 99mTc-MIBI.
Methods: Pgp, MRP1 and LRP expression was evaluated in resistant (LS1034) and sensitive (WiDr) human colorectal adenocarcinoma cell lines using flow-cytometry. Pgp expression was also analyzed using western blotting techniques. Cellular transport kinetics was analyzed in the presence and absence of verapamil and BSO. Retention studies were performed after cell incubation with those drugs, for different time intervals (10 and 60 minutes) and concentrations (10, 25, 50 and 100 μM) with 99mTc-MIBI. Cells were incubated for 60 minutes, washed after and resuspended in fresh medium. Samples were collected and cell metabolism stopped at different time-points in order to obtain retention percentage, measured by gamma-counting adjusted for 140 keV. Retention studies were also performed using LigandTracer® Yellow (Ridgeview Instruments AB, Uppsala-Sweden), an equipment that enables real-time measurements and obtains continuous retention curves. Data was analyzed using appropriate software.
Results: Pgp and MRP1 expression was significantly higher ($p < 0.05$) in resistant cells when compared to the sensitive ones, although LRP was also expressed. Western blotting analysis confirmed flow-cytometry results. 99mTc-MIBI retention percentage was significantly higher ($p < 0.05$) in the resistant cell line when compared with the sensitive one for all time-points. In resistant cells incubated with MDR modulators there were no statistically significant differences ($p > 0.05$) when all points of the retention curves are considered; however there are differences among the MDR modulators used, for the first minutes.
Conclusions: The data obtained until now suggest that these modulators must be used immediately before the cytotoxic drug is administrated.

573 Does GLUTs expression influence 18F-FDG uptake? Study in breast cancer cell lines

A.M. Abrantes[1], M. Martins[2], M.C. Rodrigues[3], S. Tavares[4], C. Mamede[5], C. Gonçalves[6], B. Oliveiros[7], A.B. Sarmento-Ribeiro[6], A. Rodrigues[3], M.F. Botelho[8]. *[1]Instituto Biofisica/Biomatematica CIMAGO IBILI, Faculdade de Medicina Coimbra, Coimbra, Portugal, [2]Serviço Medicina Nuclear HUC, Instituto Biofisica/Biomatematica IBILI-FMUC, Coimbra, Portugal, [3]ICNAS, Universidade de Coimbra, Coimbra, Portugal, [4]Instituto Biofisica/Biomatematica IBILI-FMUC FCTUC, Universidade de Coimbra, Coimbra, Portugal, [5]Instituto Biofisica/Biomatematica IBILI-FM Universidade de Coimbra, Universidade da Beira Interior, Coimbra, Portugal, [6]Instituto Bioqímica, Faculdade Medicina Coimbra, Coimbra, Portugal, [7]Instituto Biofisica/Biomatematica IBILI, Faculdade Medicina Coimbra, Coimbra, Portugal, [8]Instituto Biofisica/Biomatematica CIMAGO IBILI-FMUC, ICNAS Universidade de Coimbra, Coimbra, Portugal*

Background: Positron emission tomography (PET) uses the radiolabeled glucose analogue ^{18}F-FDG to detect glycolysis in cancer cells. ^{18}F-FDG uptake by cancer cells showed high value which allows diagnosis, staging, and detection of recurrence and evaluation of response to therapy in several malignancies.
Breast cancer is the most common malignancy among women with an increasing prevalence and is potentially curable when diagnosed early and the treatment is optimized. For the appropriate hormonal therapy the expression of estrogen and progesterone receptors is essential. The presence of the receptor HER2/neu was recently introduced as a new predictive marker of prognosis.
Breast cancer is of considerable variability in the uptake of ^{18}F-FDG, which results in different sensitivity and specificity, which in turn interferes on evaluation. The ^{18}F-FDG enters in cells through the same mechanisms of membrane transport of glucose, the glucose transporters (GLUT). Among the GLUT isoforms, the GLUT-1 and GLUT-3 overexpression is one of the mechanisms responsible for the increased utilization of glucose by tumour cells.
Aims: In this context, the main objective of this study is to determine the ^{18}F-FDG uptake in two cell lines of breast cancer with different expression of hormonal receptors and overrexpression of HER2 gene and setting eventual correlation with the expression of GLUT-1 and GLUT-3.
Material and Methods: Two different cell lines of human breast cancer, MCF-7 (estrogen and progesterone receptors positive) and HCC1806 (triple negative) were used. ^{18}F-FDG uptake for both cell lines was obtained for different times. The expression of GLUT-1 and GLUT-3 were analyzed by flow cytometry for two cell lines.
Results: When analyzed GLUT-1 and GLUT-3 expression by flow cytometry, it was found that HCC1806 cell line had higher expression than MCF-7. The ^{18}F-FDG uptake was significantly higher in MCF-7 cell line than HCC1806.
Conclusions: Despite the expression of GLUT-1 and GLUT-3 isoforms be responsible for ^{18}F-FDG uptake we verified a negative correlation between expression of glucose transporters and ^{18}F-FDG uptake.

574 Cellular prion-heat-shock organizing protein interaction as a new therapeutic target for glioblastomas

N.G.T. Queiroz-Hazarbassanov[1], M.H. Lopes[1], I.W. Cunha[2], S.M. Oba-Shinjo[3], S.K.N. Marie[3], M.D. Inda[4], V.R. Martins[1]. *[1]Hospital A. C. Camargo and Ludwig Institute for Cancer Research, Cellular and Molecular Biology Laboratory, São Paulo, SP, Brazil, [2]Hospital A. C. Camargo, Pathology Department, São Paulo I SP, Brazil, [3]School of Medicine of the University of São Paulo, Department of Neurology, São Paulo, SP, Brazil, [4]Ludwig Institute for Cancer Research, Laboratory of Tumour Biology, San Diego, CA, USA*

Malignant gliomas are the most common primary brain tumours and are nearly uniformly fatal, without yet an effective therapy. Previous data from our group have shown that cellular prion protein (PrP^C) and secreted STI1/Hop (Stress induced phosphoprotein 1/hsp70-hsp90 organizing protein) interaction induces glioblastoma proliferation. Additionally, real time PCR and immunohistochemistry of clinical specimens from glioblastoma patients demonstrated that STI1/Hop mRNA and protein expression is significantly higher in these tumours than in normal tissues (Mann-Whitney test, $p < 0.05$ and $p < 0.0001$, respectively). The purpose of this study was to block glioblastoma proliferation using an $STI1/Hop_{230-245}$ peptide, which mimics the STI1/Hop binding site at PrP^C to compete PrP^C-STI1/Hop interaction. Human glioblastoma cell line (U87MG) proliferation was evaluated *in vitro* by bromodeoxyuridine (BrdU) incorporation, anti-BrdU based immunofluorescence and total/BrdU positive nuclei counting. Proliferation of U87MG induced by STI1/Hop treatment was abolished by $STI1/Hop_{230-245}$ (ANOVA-Tukey, $p < 0.05$), while a control peptide from STI1/Hop ($STI1/Hop_{61-76}$) had no effect. Furthermore, intratumoural infusion of three different concentrations $STI1/Hop_{230-245}$ in pre-established U87MG orthotopic xenograft tumours in nude mice delayed tumour growth, compared to saline and control peptide (ANOVA-Kruskal-Wallis, Dunns post test, $p < 0.01$). Immunofluorescence analysis of xenografts using anti-Ki67, anti-caspase 3 and anti-CD31 antibodies revealed that $STI1/Hop_{230-245}$ treatment decreased tumour proliferation and increased apoptosis *in vivo* ($STI1/Hop_{230-245}$ vs. control peptide, t test, $p < 0.0001$ and $p = 0.0002$, respectively), although no change was observed in angiogenesis. Thus, we suggest PrP^C-STI1/Hop as a novel molecular target for glioblastomas and $STI1/Hop_{230-245}$ as a promising candidate for cancer therapy.

575 Alpha-secretase and neprilysin enzyme activities are decreased in renal cell carcinoma

N. Erin[1], T. Ipekçi[2], B.K. Akkaya[3], M. Baykara[2]. *[1]Akdeniz University School of Medicine, Department of Clinical Pharmacology and Health Sciences Laboratories, Antalya, Turkey, [2]Akdeniz University School of Medicine, Department of Urology, Antalya, Turkey, [3]Akdeniz University School of Medicine, Department of Pathology, Antalya, Turkey*

Background: Renal cell carcinoma (RCC) is a malignancy which does not response well to conventional chemotherapy and radiotherapy. Hence, the identification of molecules involved in the development and progression of