242 RAS and BRAF oncogenes sensitise colorectal tumours to TRAIL induced apoptosis: from cell and animal models to the clinic

A. Pintzas[1], E. Oikonomou[1], L. Andera[2], G. Zografos[3], G. Kontogeorgos[4], V. Kosmidou[1]. *[1]Institute Biology Resesearch Biotech/National Hellenic Research Foundation, Lab Signal Mediated Gene Expression, Athens, Greece, [2]Institute of Molecular Genetics Czech Acad Scie, Lab Cell Sign and Apoptosis, Prague, Czech Republic, [3]General Hospital of Athens G. Gennimatas, Surgical Deparment, Athens, Greece, [4]General Hospital of Athens G. Gennimatas, Pathology Department, Athens, Greece*

Background: Most data on the therapeutic potential and expression of TRAIL in colorectal cancer has come from *in vitro* studies using tumour cell lines. To gain a clearer understanding about the susceptibility of patient tumours to TRAIL, we derived primary human cancer epithelial cells [1]. Increased apoptosis was observed in both primary PAP60 and MIH55 after treatment with SuperKiller TRAIL. Treating patient tumour xenograft/SCID mouse models with Killer TRAIL *in vivo* for 5 consecutive days suppressed tumour growth, although less efficiently compared to *in vitro* experiments. RAS oncogenes sensitise cells to TRAIL induced apoptosis [2]. We have presented evidence that this effect is usually mediated by TRAIL receptor DR4 and DR5 overexpression and/or redistribution in cell models [4].
Materials and Methods: Primary colorectal tumour cells, colorectal cell lines bearing RAS and BRAF mutant oncogenes, mouse xenographs and colorectal clinical samples were either treated with recombinant TRAIL and/or analysed for the presence of RAS and BRAF oncogenic mutations and DR4, DR5 expression.
Results: We present evidence that BRAF oncogenes sensitise cells to TRAIL induced apoptosis via TRAIL receptor DR5 [3]. We have also shown that DR5 is the most frequently upregulated DR in clinical samples of colon cancer. Furthermore, the presence of K-RAS and BRAF mutations in the tumour may directly or indirectly enhance DR expression [5].
Discussion: Mutations on K-RAS and BRAF oncogenes have been shown in many studies to be associated with resistance to EGFR targeted therapeutics and combinations. TRAIL-based therapeutics, other as mono- or combination therapy could provide a promising alternative for K-RAS/BRAF bearing colorectal tumours.

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243 Platinum-resistance in ovarian cancer is mediated by the IL-6-cIAP-2 axis

S. Cohen[1], I. Bruchim[2], R. Eitan[3], Z. Evron[1], V. Oron-Karni[4], M. Pasmanik-Chor[4], H. Levavi[3], A. Fishman[2], E. Flescher[1]. *[1]Tel Aviv University, Clinical Microbiology and Immunology, Tel Aviv, Israel, [2]Meir Medical Center, Gynecology, Kfar Saba, Israel, [3]Rabin Medical Center, Gynecology, Petah-Tikwa, Israel, [4]Tel Aviv University, Bioinformatics, Tel Aviv, Israel*

Background: Ovarian cancer is the most lethal gynecological malignancy in the Western world. The majority of the patients are initially responsive to platinum-based therapy; however, due to the development of platinum chemoresistance, recurrent disease is often refractory to treatment and is associated with high mortality rates. It is known that large amounts of the cytokine IL-6 are present in the sera and ascites of ovarian cancer patients and their presence is predictive of poor clinical outcome. Our research is focused on IL-6 down-stream targets, and their relation to platinum-resistance. Our ultimate goal, within the realm of translational medicine, is to evaluate the effect of IL-6 axis inhibition, in combination with platinum-based therapy, as a new modality for the treatment of ovarian cancer patients.
Materials and Methods: Our studies were conducted on ovarian cancer cell lines, as well as cells drawn from ovarian cancer patients. Microarray gene expression was performed on ovarian cancer cells upon treatment with cisplatin, and validated at the protein level by ELISA and western blot analysis. Ovarian cancer cells were treated with IL-6 inhibitors: anti-IL-6 antibody and siRNA for IL-6, following cisplatin treatment, and the cytotoxicity rates were evaluated by the XTT cell viability assay.
Results: Our gene array analysis of cisplatin-treated ovarian cancer cells revealed a highly significant increase in mRNA of IL-6 (10 fold) and of an IL-6 target gene, cIAP-2 (12 fold). cIAP-2 is a member of the inhibitor of apoptosis family. Validation of the array results, at the protein level, revealed significantly increased levels of IL-6 and cIAP-2 proteins following cisplatin treatment. Western blot analysis of cisplatin-treated ovarian cancer cells exhibited decreased cIAP-2 expression level following anti-IL-6 antibody addition. Our cytotoxicity assays exhibited the sensitization of cisplatin-resistant ovarian cancer cells to cisplatin following the addition of anti-IL-6 antibody (from 5% to 30% at 10 μM cisplatin) or siRNA (from 4% to 38% at 5 μM cisplatin).
Conclusions: IL-6 inhibitors significantly suppress cIAP-2 expression, and sensitize platinum-resistant ovarian cancer cells to cisplatin. Combining anti-IL-6 inhibitors along with cIAP-2 inhibitors, following cisplatin treatment, should improve the current treatment, and provide new hope for ovarian cancer patients.

244 Tamoxifen as a potential inhibitor of the chemotherapy resistance in non-small cell lung cancer

T.A. Bogush[1], E.A. Dudko[1], E.A. Bogush[1], M.V. Tikchomirov[1], R.J. Ramanayskaite[1], K.K. Laktionov[2], B.E. Polotsky[2], M.I. Davydov[2]. *[1]N.N. Bokhin Russian Cancer Research Center of the Russian Academy of Medical Sciences, Laboratory of Medical Chemistry, Moscow, Russian Federation, [2]N.N. Bokhin Russian Cancer Research Center of the Russian Academy of Medical Sciences, Surgical Department, Moscow, Russian Federation*

Background: Tamoxifen (Tam) is an effective antiestrogen in therapy of breast cancer. But there are other important activities of Tam, one of them is overcoming of chemotherapy resistance by Tam in various tumours including non-small-cell lung cancer (NSCLC). In all cases Tam was used in anticancer drug therapy associated with multidrug resistance mechanism (MDR) that is determined by extruding of MDR-drugs out of the cells. That is why we have supposed Tam interaction with MDR-transporters, which may prevent further MDR-drug(s)' binding to these transporters and thereby inhibit main MDR mechanism: anticancer drug transport out of the cells.
Materials and Methods: Tumour cells obtained from surgical biopsy specimens of NSCLC were studied. Fluorescence of specific monoclonal antibodies (mAb) bound to Pgp, MRP1 and LRP as well as the isotypic antibodies was estimated by flowcytometry. Mean fluo-rescence of mAb-labelled cells as well as the number of mAb-labelled cells were calculated over fluorescence area of isotypic controls.
Results: 1. Incubation of the cells with mAbs increased significantly their fluorescence intensity compared to the isotypic controls. In some tumours it was shown for MRP1 and LRP mAbs only, in the other one – for MRP1, LRP and Pgp. 2. It was not any influence of Tam on isotypic antibodies binding to the cells. 3. Incubation of the cells with Tam changed interaction of mAbs with the MDR-markers investigated. The mean specific cell fluorescence intensity as well as the number of mAb-labeled cells was changed but with different manner for different MDR-markers. Under Tam action the indexes for MRP1 and LRP mAbs decreased in about 2 times. Tam effect on mAb interaction to Pgp was different in living cells and after 0.5% Tween 20 cell permeabilization. For the first one, Tam increased the mean specific cell fluorescence intensity as well as the number of mAb-labeled cells in about 4 times. For the second one, the indexes decreased up to more than 2 times under Tam action.
Conclusion: These data are direct evidence for the Tam interaction with the Pgp, MRP1 and LRP in NSCLC cells. It should decrease further binding of anticancer drugs with the MDR-markers and thereby decrease of the MDR-drug transport out of the cells. It means that Tam interaction with Pgp, MRP1 and LRP may be one of the reasons for overcoming the MDR-drug chemotherapy resistance of NSCLC under Tam action. Supported by Russian Foundation for Basic Research (Grant N09−04–13560).

245 Identification and evaluation of tumour lymphatic endothelial cell-specific proteins by antibody proteomics technology

S. Tsunoda[1], K. Nagano[1], T. Yamashita[1], S. Imai[1], Y. Abe[1], T. Yoshikawa[2], Y. Yoshioka[3], H. Kamada[1], Y. Tsutsumi[2]. *[1]National Institute of Biomedical Innovation, Laboratory of Pharmaceutical Proteomics, Osaka, Japan, [2]Osaka University, Graduate School of Pharmaceutical Sciences, Osaka, Japan, [3]Osaka University, The Center for Advanced Medical Engineering and Informatics, Osaka, Japan*

Background: Lymph node metastasis of tumours represents a vitally important prognostic factor. Indeed, many researchers are attempting to develop diagnostic and preventive methods for such pathologies. As is the case for tumour blood vessels, specific proteins may be expressed on the lymphatic endothelial cells (LECs) that migrate with the tumour. Such proteins could

be novel therapeutic or diagnostic targets for lymphatic metastasis. Recently, we have established "antibody proteomics technology" which accelerates the discovery of proteins useful for diagnosis and therapies of cancer. In this study, we applied this technology for seeking proteins specifically expressed on tumour LECs and related to lymph node metastasis.

Material and Methods: Tumour LEC model; Human primary LECs were cultured in conditioned media (CM) prepared from the cultures of low-metastatic human lung cancer cell line (RERF-LC-MS), high-metastatic cell line (RERF-LC-KJ) or normal bronchial epithelial cells (NHBE) and used as the lymphatic vessel model in tumour tissues or in normal tissues.

In vitro invasion assay; LECs of each model were seeded to the upper chamber of an invasion assay plate and incubated for 72 hr. The invasive cells were stained with calcein-AM and measured by fluorescence.

2D-DIGE analysis; Lysates of each model LECs were labelled with Cy-dyes and analyzed by 2D-DIGE according to the manufacture's protocol. Proteins of interest were extracted from the gel and subjected to protein identification by mass spectrometry analysis.

Tumour tissue microarray (TMA) analysis; Expression profiling of candidate proteins was performed by immunostaining of tumour TMAs.

Results and Conclusions: Results from the invasion assay show that RERF-LC-KJ-CM treated LECs possess a more invasive character than RERF-LC-MS-CM. Therefore, CM-treated LECs were considered to gain one of the metastasis related characters. The differential proteomes were then analyzed by 2D-DIGE in order to identify metastasis-related proteins on LECs. MS analysis identified fourteen proteins over-expressed in the RERF-LC-KJ-treated LECs. These candidate proteins were then validated by TMA analysis. Thus, proteins expressed in lung tumour LECs and tumour cells but not in LECs or normal lung were identified. Although further analysis is required, the identified proteins are potentially useful drug targets for lymphatic metastasis.

246 Utilisation of the DNA repair enzyme, N-Methylpurine DNA glycosylase, as a biomarker of oesophageal Cancer

Z. Zair[1], A.P. Griffiths[2], S.H. Doak[1], G.E. Johnson[1], G.J. Jenkins[1]. [1]*Swansea University, School of Medicine, Swansea, United Kingdom,* [2]*Morriston Hospital, Department of Histopathology, Swansea, United Kingdom*

Background: Barrett's Oesophagus (BO) is an inflammatory disease where continuous exposure of the distal oesophagus to stomach acids can lead to uncontrolled cellular division and ultimately oesophageal adenocarcinoma (OAC). Such uncontrolled cellular division is multifaceted, including the loss of DNA fidelity and genomic miss-repair. Altered expression of the DNA repair enzyme *N*-Methylpurine DNA Glycosylase (MPG), for instance, has previously been associated with breast and colon cancer. Where disruption of DNA repair may well prove deleterious in a normal cell, execution of DNA repair in a tumour cell conversely becomes counterproductive during chemotherapy. Use of a biomarker that will both allow for detection of early stage oesophageal carcinoma as well as predict a patient's response to chemotherapy would thus be most invaluable.

Aim: To explore the role of MPG as a predictive and therapeutic marker of oesophageal cancer.

Material and Methods: Levels of MPG mRNA expression was determined from control and cancer tissues derived from a cDNA panel (Origene, USA). qRT-PCR was carried out using TaqMan probes for MPG (Applied Biosystems, UK) and data normalised to the expression of the endogenous genes B-actin and RPLO. MPG mouse monoclonal antibody (Sigma-Aldrich) was used for protein detection and immunohistochemistry was carried out using the Ventana automated system. H-score was used for semi-quantification of MPG protein levels.

Results: Gene expression studies revealed high levels of MPG mRNA in OAC tissue relative to healthy tissue. Further analysis showed a tumour grade dependent increase in MPG expression at both mRNA and protein levels, signifying MPG as a promising diagnostic marker for the detection of MPG. Relative to cancer tissues derived from the enterohepatic system, altered expression of MPG mRNA was specific to the oesophagus. Indeed with amenable access to the oesophagus, focussed targeting of cancer cell DNA repair, via MPG modulation, could be used as a means to enhanced therapeutic treatment.

Conclusion: Production of a clinically useful biomarker that is capable of detecting oesophageal carcinoma at the earlier stages of tumour development could allow for early intervention of the disease resulting in increased survival. This study highlights the potential use of the DNA repair enzyme, MPG, as a diagnostic marker for the early detection of oesophageal cancer and a therapeutic marker for enhanced chemotherapy.

247 The medicinal and antitumour mushroom, agaricus blazei murill, activates NF-KB via TLR2 in monocytic cells and induces expression of cell surface markers and production of cytokines in human monocyte-derived dendritic cells (MDDC) in vitro

G. Hetland[1], A.M.A. Tryggestad[1], T. Espevik[2], L. Ryan[2], J.M. Tangen[3], E. Johnson[4], G. Kvalheim[1]. [1]*Oslo University Hospital Radiumhospitalet, Cellular Therapy, Oslo, Norway*, [2]*Norwegian University of Science and Technology, Cancer Research and Molecular Medicine, Trondheim, Norway,* [3]*Oslo University Hospital Ulleval, Hematology, Oslo, Norway,* [4]*Oslo University Hospital Ulleval, Gastroenterological Surgery, Oslo, Norway*

The edible *Basidiomycetes* mushroom *Agaricus* bM (AbM) of Brazilian rain forest origin is used in traditional medicine against cancer and various diseases. It is rich in immunomodulating substances like beta-glucans, and is previously shown to induce apoptosis in cancer cells and inhibit tumours in mouse models. Activation of transcription factor NF-kB, which is linked to cytokine production and cell cyclus regulation in cancer defence, was determined by a luciferase assay system as translocation of NF-kB from cytosol to nucleus of cells stimulated with AbM extract. Surface markers on the cells were studied by flow cytometry, and cytokine release from them to the cell culture supernatnants, were measured in by a 17-plex Luminex system. Activation of NF-kB was examined in promonocytic THP-1 cells and in HEK 293 cells, transfected with CD14/MD2 and TLR2 or TLR4, after the AbM stimulation. The mushroom extract induced NF-kB activation via TLR2, but not TLR4, in both cell lines. Then we studied effects on MDDC cultures of the AbM-based (82%) extract AndoSan^{TM}, also containing *Basidiomycetes* mushrooms *Hericium erinaceum* and *Grifola frondosa*. After 24 h there was down-regulated CD11c, *de novo* CD69 and enhanced CD86 expression on the cells, as well as dose-dependent increased levels of IL-1beta, IL-6, IL-8, MIP-1beta, G-SCF, and TNFalfa in the cell culture supernatants. Whereas synthesis of IL-2, IL-8 and IFNgamma was similar for 10% of AndoSan^{TM} and 0.5 mg/ml of *E. coli* LPS, the AbM-based extract induced a 2- to 10-fold higher production of IL-1beta, TNFalfa and G-SCF, respectively, than did LPS. We conclude that AbM activates transcription factor NF-kB via TLR2 in monocytes and most probably also in MDDC. In MDDC an AbM-based extract induced differential expression of cell surface markers for cell adhesion, activation and antigen presentation, and increased production of proinflammatory, chemotactic and some Th1-type cytokines *in vitro*. This may explain some of the known antitumour properties of AbM. The antitumour effect of the AbM extract is currently being tested in a tumour stem cell model in PVG rats.

248 Histone deacetylase inhibitor vorinostat suppresses the growth of uterine sarcomas in vitro and in vivo

A. Hrzenjak[1], F. Moinfar[2], M.L. Kremser[2], B. Strohmeier[2], E. Petru[3], K. Zatloukal[2], H. Denk[2]. [1]*Medical University of Graz Institute of Internal Medicine, Clinical Department of Pulmonology, Graz, Austria,* [2]*Medical University of Graz, Department of Pathology, Graz, Austria,* [3]*Medical University of Graz, Department of Obstetrics and Gynecology, Graz, Austria*

Background: Uterine sarcomas are very rare malignancies with no approved chemotherapy protocols. Histone deacetylase (HDAC) inhibitors belong to the most promising groups of compounds for molecular targeting therapy. Here, we described the antitumour effects of suberoylanilide hydroxamic acid (SAHA; vorinostat) on MES-SA uterine sarcoma cells *in vitro* and *in vivo*.

Material and Methods: We investigated effects of vorinostat on growth and colony forming ability by using uterine sarcoma MES-SA cells. We analyzed the influence of vorinostat on expression of different HDACs, p21^{WAF1} and activation of apoptosis. Finally, we examined the antitumour effects of vorinostat on uterine sarcoma *in vivo*.

Results: Vorinostat efficiently suppressed MES-SA cell growth at a low dosage (3 μM) already after 24 hours treatment. Decrease of cell survival was even more pronounced after prolonged treatment and reached 9% and 2% after 48 and 72 hours of treatment, respectively. Colony forming capability of MES-SA cells treated with 3 μM vorinostat for 24 and 48 hours was significantly diminished and blocked after 72 hours. HDACs class I (HDAC2 and 3) as well as class II (HDAC7) were preferentially affected by this treatment. Vorinostat significantly increased p21^{WAF1} expression and apoptosis. Nude mice injected with 5×10^6 MES-SA cells were treated for 21 days with vorinostat (50 mg/kg/day) and, in comparison to placebo group, a tumour growth reduction of more than 50% was observed. Results obtained by light- and electron-microscopy suggested pronounced activation of apoptosis in tumours isolated from vorinostat-treated mice.

Conclusions: Our data strongly indicate the high therapeutic potential of vorinostat in uterine sarcomas.