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.

Reference(s)

[1] Oikonomou, E., Kothonidis, K., Taoufik, E., Probert, L., Zografos, G., Nasioulas, G., Andera, L., and Pintzas, A. (2007). Newly Established Tumourigenic Primary Human Colon Cancer Cell Lines are Sensitive to TRAIL Induced Apoptosis in vitro and in vivo. *Br. J. Cancer*. 97, 73−84.
[2] Drosopoulos, K. Roberts, M., Cermak, L., Sasazuki, T., Shirasawa, S., Andera L. and Pintzas, A. (2005). Oncogenic Ras transformation sensitizes human colon cancer cells to TRAIL induced apoptosis by upregulating DR4 and DR5 receptors through a MEK dependent pathway. *J. Biol. Chem*. 280, 22856–22867.
[3] Oikonomou E., Koc M., Sourkova, V., Andera, L., and Pintzas A. (2010). BRAFV600E an Hsp90 Client; Sensitizes Human Colon Cancer Cells to TRAIL-Induced Apoptosis via DR5. Under review.
[4] Psahoulia, F. H., Drosopoulos K. G., Doubravska, L., Andera, L. and Pintzas, A. (2007). Quercetin enhances TRAIL-mediated apoptosis in colon cancer cells by inducing the accumulation of death receptors in lipid rafts. *Mol. Cancer. Ther* 6, 2591–2599.
[5] Oikonomou, E., Kosmidou, V., Katseli, A., Kothonidis, K., Mourtzoukou, D., Kontogeorgos, G., Andera, L., Zografos, G., and Pintzas, A. (2009). TRAIL Receptor Upregulation Correlates to KRAS/BRAF Mutations in Human Colon Cancer Tumours and Respective Normal Tissue. *Int. J. Cancer* 125, 2127–2135

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