544 Aberrant choline metabolism in epithelial ovarian cancer: relevance of choline kinase activity and expression

A. Granata[1], M.T. Comito[1], E. Iorio[2], A. Ricci[2], M.E. Pisanu[2], Z. Bhujwalla[3], F. Podo[2], S. Canevari[1], D. Mezzanzanica[1], M. Bagnoli[1]. [1]*Istituto Nazionale Tumouri, Experimental Oncology, Molecular Therapies Unit, Milan, Italy*, [2]*Istituto Superiore Sanità, Department Cell Biology and Neurosciences, Rome, Italy,* [3]*John Hopkins University, John Hopkins University, Baltimore (MA), USA*

Background: Epithelial Ovarian Cancer (EOC) remains a highly lethal malignancy due to late diagnosis and early relapse in association with development of resistance to conventional chemotherapeutic treatment. Detection and characterization by magnetic resonance spectroscopy of altered phosphatidylcholine (PC) metabolism in EOC could provide choline-based approaches as powerful tools to improve diagnosis and identify new therapeutic targets.
We recently reported alterations of the choline-metabolites spectral profile in EOC cells characterized by the increase in the major choline-containing metabolite phosphocholine (PCho) as compared to the normal counterpart. Biochemical, protein, and mRNA expression analyses showed that the most relevant changes in EOC cells were activation of Choline Kinase (ChoK, the enzyme responsible for PCho production in the biosynthetic pathway), in association with higher protein content and increased ChoKα (but not ChoKβ) mRNA expression levels. Since Chokα has been found to be constitutively activated in different tumour types and also to act as a prognostic factor, we aimed to assess the biological relevance of Chok expression and activity in EOC.
Material and Methods: To investigate the role of ChoKα in EOC growth and progression we specifically silenced CHKA gene expression by transient RNA interference in two EOC cell lines and we evaluated the main biological effects related to metabolic profiles, cell cycle regulation, proliferation, and alterations of global gene expression.
Results: Inhibition of ChoKα mRNA expression was associated with significant reduction of ChoK protein expression and a drop of about 70% in PCho accumulation in both cell lines. We observed a 20% inhibition of cell growth together with a comparable increase of cells blocked in the G1-phase of cell cycle. Comparative evaluation of the global transcriptome, showed 440 genes differentially expressed (FDR < 0.25, $p < 0.05$) in CHKA silenced cells as compared to controls, equally distributed among induced and repressed genes. Interestingly, among the most relevant co-repressed genes we found CyclinA1, IL6 and IL8, whose biological validation is currently ongoing.
Conclusions: Our observations, confirming a main role for ChoKα in deregulated choline metabolism in EOC tumours, warrant further investigations on the upstream and downstream signaling and metabolic alterations associated to ChoK enhanced activity and suggest this molecule as a promising target for alternative therapeutic approaches.
Partially supported by AIRC.

545 Antiestrogen 4-OHT activity in lung cancer in vitro

F. Pancotti[1], L. Roncuzzi[2], A. Gasperi-Campani[3]. [1]*University of Bologna, Experimental Pathology, Bologna, Italy*, [2]*Istituto Ortopedico Rizzoli, Pathophysiology and Regenerative Medicine in the Department Exp. Pathol. University of Bologna, Bologna, Italy,* [3]*University of Bologna, Experimental Pathology and Interdepartmental Center for the Research on Cancer, Bologna, Italy*

Background: Lung cancer is the leading cause of cancer death in the industrialized world, with a mortality rate of nearly 90% and a median survival of about 12 months.
Several studies on sex differences in lung cancer risk and disease presentation suggest that estrogen-signalling pathways may play a key role in the genesis and in controlling the growth of lung cancer. The cellular response to estrogen is mediated by estrogen receptor α (ERα) and β (ERβ). Recently, the orphan receptor GPR30 (G protein-coupled receptor-30) has been implicated in rapid and specific estrogens binding in mediating the action of several estrogenic compounds.
In this work, we analyzed the ER pathway including GPR30, in human metastatic lung cancer with respect to the activity of a selective estrogen receptor modulator, 4-hydroxytamoxifen (4-OHT).
Material and Methods: The human cell lines RAL (NSCLC) and SCLC-R1 were obtained from metastatic lesions of lung adenocarcinoma and of small cell lung carcinoma respectively. Each cell line was grown in H/H medium supplemented with 10% FBS. Growth inhibition was evaluated by tripan blue dye exclusion assay and protein expression by Western Blotting analysis.
Results: The study started with the evaluation of the ER α/β and GPR30 expression, in RAL and SCLC-R1 cell lines. Each cell line expressed GPR30 and ERβ and not ERα. To establish whether estrogens could modulate cell proliferation, cells were exposed to estradiol (E_2). Results indicate a significant increase in cellular growth in both cell lines in the range of concentrations tested [1 μM-10 nM]. . We next explored the effects of 4-OHT at therapeutic doses in each cell line and demonstrated that this antiestrogen causes a significant dose- and time-dependent growth inhibition in the range of concentrations used [1−10 μM].
Discussion: The present data indicate for the first time that lung RAL and SCLC-R1 cell lines express ERβ and GPR30 and not ERα. This is in line with previous data indicating opposite roles for ERα and GPR30 in the control of cell proliferation in most cancers. In addition, results show that the growth of these cell lines is sensitive to estrogen stimulation. Since the antiestrogen 4-OHT is effective here in inhibiting the cell growth, it may warrant attention for future utilization in anti-lung cancer therapy.

546 hMena overexpression cooperates with HER2 signalling in breast cancer

F. Di Modugno[1], M. Mottolese[1], P. Trono[1], P. Iapicca[1], L. DeMonte[2], M. Balsamo[3], A. Santoni[4], P. Nisticò[1]. [1]*Regina Elena Cancer Institute, Experimental Oncology, Rome, Italy*, [2]*San Raffaele Scientific Institute, Tumour Immunology, Milan, Italy,* [3]*Massachusetts Institute of Technology, Koch Institute for Integrative Cancer Research, Cambridge, USA,* [4]*University 'Sapienza', Department Clinical and Molecular Medicine, Rome, Italy*

Background: hMena and the epithelial specific isoform hMena^{+11a} are cytoskeleton regulatory proteins belonging to the Ena/VASP family. EGF treatment of breast cancer cell lines upregulates hMena/hMena^{+11a} expression and phosphorylates hMena^{+11a}, suggesting that hMena/hMena^{+11a} couple tyrosine kinase receptors to actin cytoskeleton.
Aim of this study was to determine whether a cross-talk between HER-2 activity and hMena/hMena^{+11a} occurs in breast cancer, affecting the HER2 mitogenic activity and whether hMena/hMena^{+11a} overexpression could be considered as a novel prognostic indicator in HER2 overexpressing breast cancer patient.
Material and Methods: hMena and hMena^{+11a} expression has been evaluated by Real time PCR and Western blot with specific antibodies on breast cancer cell lines overexpressing HER2 or on MCF7 cells transfected with HER2 either in untreated or in EGF and NRG1 treated conditions. hMena^{+11a} phosphorylation has been evaluated by 2D WB. MCF7-HER2 cells has been silenced by siRNA for hMena/hMena^{+11a} and the HER2 expression and phosphorylation has been assessed by WB; cell proliferation rate was evaluated by 3H-thymidine incorporation assay. hMena, HER2, P-MAPK, P-AKT and Ki67 were evaluated by immunohistochemistry in a series of 286 breast tumour tissues of different molecular subtypes from breast cancer patients subjected to breast surgery at the Regina Elena Cancer Institute (Rome, Italy). The disease-free survival (DFS) curves were estimated by the Kaplan-Meier product-limit method: the log-rank test was used to assess differences between subgroups of hMena positive and negative, HER2 overexpressing tumours.
Results: HER2 transfection in MCF7 cells increased hMena/hMena^{+11a} expression and hMena^{+11a} phosphorylation. hMena/hMena^{+11a} knock-down inhibits HER2 phosphorylation and reduces p42/44 MAPK and AKT phosphorylation, induced by EGF and NRG1 treatment. Of functional significance, hMena/hMena^{+11a} knock-down associates with reduction in the mitogenic activity of EGF and NRG1. In a large cohort of primary breast cancer tissues, higher frequency of hMena overexpressing tumours was found in the HER2 subtype and a significant correlation among hMena, proliferation index (Ki67 high), and phosphorylated MAPK and AKT was also found. Finally, concomitant overexpression of HER2 and hMena identify a subgroup of breast cancer patients showing the worst prognosis indicating that hMena overexpression adds prognostic information in HER2 overexpressing tumours.
Conclusions: Collectively these data provide new insights in the cross-talk between HER2 signalling pathway and the actin cytoskeleton, pointing out on the relevance of hMena and hMena^{+11a} as downstream effectors of HER2 activity. hMena/hMena^{+11a} expression may represent a novel prognostic indicator in breast cancer.

547 Obesity as a risk factor for prostate cancer: a role for adipocytokines and involvement of tyrosine kinase pathway

M.R. Hoda[1], D. Galayda[1], K. Fischer[1], S. Wagner[1], H. Heynemann[1], P. Fornara[1]. [1]*Martin-Luther University Halle/Wittenberg, Urology, Haale/Saale, Germany*

Background: Obesity is purposed to be a risk factor for prostate cancer. Mitogenic actions of leptin, an adipocyte-derived hormone in a variety of cancer cell types have been indentified. We investigated proliferative effects of leptin on human prostate cancer cells and assessed the role of tyrosine kinase signaling in mediating theses actions.
Materials and Methods: Two human androgen-resistant prostate cancer cell lines and one androgen-sensitive human prostate adenocarcinoma cell line were treated with leptin (5–100 ng/ml) for up to 48 hours. Under serum-free conditions, cell proliferation was measured using enzyme-linked colorimetric assay. Further, phosphorylation of a downstream component of MAPK (ERK1/2) was detected by Western blotting and a specific inhibitor