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

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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.
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545 Antiestrogen 4-OHT activity in lung cancer in vitro

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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

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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

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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

of MAPK (PD98059; 40 μM) was used to evaluate the role of this signaling pathway.
Results: Leptin dose-dependently increased cell number in both androgen-resistant cell lines after 24 hrs and 48 hrs of incubation (percent of control; DU145 = 194.6±5.9%, PC-3 = 177.9±6.8%; 100 ng/ml leptin; 48 hrs; $p < 0.001$). Conversely, leptin's proliferative effect on androgen-sensitive cell was less pronounced (percent of control; LNCaP = 112.3±6.1%; 100 ng/ml leptin; 48 hrs). Leptin also caused dose-dependent ERK1/2 phosphorylation in both androgen-resistant cell lines. Further, pre-treatment with PD98059 inhibited these responses and attenuated leptin's mitogenic action.
Conclusions: Data from this in vitro study suggest an association between obesity-associated hyperleptinemia and an increased risk for prostate cancer. Further investigations are necessary to clarify whether these data have a clinical relevance regarding the use as a prognostic marker for predicting the timing of the occurrence of androgen resistency.

548 Role of protein kinase C delta in musculoskeletal tumours

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Background: Protein kinase C delta (PKC δ), one of the isoforms of protein kinase C, has been shown to act as either positive or negative regulator of tumour progression, however its role in musculoskeletal tumours are still unknown. In this study, we investigated the expression of PKC δ in musculoskeletal tumours and the relationship between expression of PKC δ and malignancy. We also determined the role of PKC δ on cell proliferation of human malignant fibrous histiocytoma (MFH) *in vitro*.
Material and Methods: We used 41 human musculoskeletal tumour samples including 32 malignant and 9 benign tumours to analyze the mRNA expression of PKC δ. Seven human cell lines including three osteosarcoma cell lines (KHOS, KTHOS, MG63) and four MFH cell lines (Nara F, Nara H, TNMY1, GBS-1) were also used for *in vitro* studies. We performed quantitative real time PCR with 41 human musculoskeletal tumour samples to evaluate mRNA expression and the expression levels of PKC δ. We also performed immunoblot analysis to analyse protein expression of PKC δ in 7 cell lines. Furthermore, we performed siRNA knockdown of PKC δ with four human MFH cell lines to evaluate whether PKC δ siRNA affects cell proliferation of MFH cells.
Results: Real time PCR analysis with human musculoskeletal tumour samples showed that mRNA expression of PKC δ in malignant tumours was significantly lower than that in benign tumours ($p < 0.05$), and PKC δ expression in high-grade malignant tumours such as osteosarcomas and MFHs was especially low. By immunoblot analysis, protein expression of PKC δ was detected in all cell lines and the expression in osteosarcoma cell lines was weaker than that in MFH cell lines. In MFH cells transfected with PKC δ siRNA, mRNA expression of PKC δ was decreased to 50 to 65% of that with control siRNA and the protein expression of PKC δ was also strongly reduced. Cell proliferation assay revealed that siRNA knockdown of PKC δ significantly activated cell proliferation after 72 hours of transfection ($p < 0.05$).
Conclusions: In this study, we demonstrated that mRNA expression of PKC δ in malignant musculoskeletal tumours was significantly lower than in benign tumours and that siRNA knockdown of PKC δ activated cell proliferation in all MFH cell lines. Taken together, these results suggest that PKC δ may play a role on cell proliferation in human musculoskeletal tumours and that PKC δ may be a therapeutic target in malignant musculoskeletal tumours.

549 Transcriptomics meets metabolomics − correlating snapshots of breast cancer metabolism

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Background: The aim of this study was to compare metabolite concentrations to gene expression levels in the same breast carcinomas, to study the metabolism of breast cancer.
Material and Methods: Fresh frozen tumour tissue from 34 patients diagnosed with invasive ductal carcinomas (ER and/or PR positive) were used in HR MAS MRS experiments on a Bruker Avance DRX600 spectrometer as previously described [1]. Total RNA from the same tissue (7 samples from neighbouring tissue) was extracted and used in two-colour Agilent microarray experiments. Quantification of 8 tissue metabolites was performed as described in [1]. Spearman correlation tests were performed between each transcript in the normalized microarray data and the quantified metabolites. The correlating genes ($r > 0.4$ and $p < 0.01$) were tested for enriched GO-terms and used to create networks of the shortest paths between the metabolites and their correlated genes using Ingenuity Pathway Analysis (IPA).
Results: For the transcripts that were correlated to glucose, enriched GO-terms are related to immune response, lipid homeostasis and ribosomes. The enriched GO-terms for the transcripts that were correlated to taurine, myo-inositol and choline, are related to the extracellular matrix and collagen, while for the transcripts that were correlated to creatine, phosphocholine (PCho), glycerophosphocholine (GPC) and glycine, enriched GO-terms include generation of precursor metabolites and energy and organelle envelope. IPA networks show indirect associations between gene transcripts and the metabolites that correlate to each other. Many of the correlated genes in a network involving taurine, myo-inositol and choline code for proteins that are associated to the extracellular matrix, while the majority of the correlated genes in a network involving creatine, GPC, PCho and glycine, code for proteins that with a role in the nucleus or cytoplasm.
Conclusions: Combining metabolic and transcriptional profiling of the same breast carcinoma samples using HR MAS MRS and microarrays resulted in hypotheses of the biological activities that are associated to different metabolites.

Reference(s)

[1] B. Sitter, *et al.*, Comparison of HR MAS MR spectroscopic profiles of breast cancer tissue with clinical parameters. NMR Biomed 2006; 19(1): 30.

550 Response to oxidative or genotoxic stress differs in cells representing progressive stages of cutaneous cell carcinomas

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Background: Previous investigations have demonstrated that isogenic cutaneous squamous cell carcinoma cell lines (SCC), isolated from dysplastic skin (PM1), primary invasive SCC of sundamaged skin (MET1) and its lymph node metastasis (MET4), show an increasing resistance to cisplatin-induced apoptosis in the more advanced stages of carcinogenesis.
Material and Methods: To investigate whether the pattern of sensitivity in progressive stages of skin carcinogenesis is dependent on the kind of stress (genotoxic versus oxidative stress), we investigated the sensitivity of these isogenic cell lines to apoptosis in response to a single UVB-dose (120 mJ/cm^2) (genotoxic and oxidative stress), hydrogen peroxide (1 mM) and hypericin-photodynamic treatment (hypericin 90 nM – 24 h incubation time) (oxidative stress).
Results: MET1-cells, followed by the MET4-cells were more sensitive to UVB, which represents a mixture of genotoxic and oxidative stress, resulting in more cell death (detected by trypan blue exclusion- and MTT-assay) and more apoptosis (detected by cleavage of Poly(ADP-Ribose) Polymerase and caspase 3) in comparison with the PM1-cells. A similar pattern of sensitivity was observed when we exposed the SCC-cells to hydrogen peroxide or hypericin-photodynamic treatment, which both mainly exhibit oxidative stress. Hereby the MET1-cells remained the most sensitive.
Conclusions: While more advanced skin cancer cells like MET1- and MET4-cells lose their sensitivity to the genotoxic stressor and chemotherapeuticum cisplatin, they remain sensitive to oxidative stress, which could have further implications for the treatment of advanced skin cancer.

551 High levels of miR-363* inhibit proliferation of oral carcinoma cell line E10

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Background: MicroRNAs (miRNAs) are a group of none-coding genes which are involved in almost every biological process. MicroRNAs are known to have abnormal expression in human diseases and malignancies. The mir-17−92 and mir-106–363 clusters are often associated with carcinogenesis.
Materials and Methods: Oral carcinoma cell line PE/CA-PJ49 clone E10 (ECACC).
miRNeasy Mini Kit (Qiagen) was used for isolating miRNA for miRNA microarrays (Phalanx Biotech) and mirPremier kit (Sigma-Aldrich) for Real-Time PCR quantification with TaqMan MicroRNA Assay (Applied Biosystems).
Transfections with miR-363* mimic and inhibitor (GenePharma) were carried out using INTERFERin (Polyplus-transfection) transfection reagent.
Results: Our data from miRNA microarrays screening showed that both miR-17−92 and miR-106–363 clusters were present in the oral carcinoma cell line E10. miR-363* which is one of the six members from the miR-106–363 cluster, was selected for a more detailed study.
Real-time PCR assays showed that miR-363* is expressed at a low level in cell line E10. Transfection with miR-363* mimic increased levels of miR-363* several 100 fold. This led to a marked decrease in cell numbers of transfected cultures. By contrast, blocking miR-363* with miR-363* inhibitor did not alter the number of cells in the cultures.