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.