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)

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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.

Conclusion: High expression of microRNA miR-363 seemed to retard proliferation of the carcinoma cell line E10. The mechanism of actions is being investigated.

552 Mechanisms of prostaglandin E2-induced transactivation of the EGF receptor in MH1C1 hepatoma cells

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Background: The epidermal growth factor (EGF) receptor (EGFR) mediates strong stimulation of hepatocyte proliferation and may play a role in hepatoma development. Several prostaglandins (PGs), including PGE_2, act as co-mitogens in hepatocytes, and much evidence implicates PGs (and COX-2) in oncogenesis in many tissues. We have examined mechanisms that integrate signalling from EGFR and PG receptors. Previous work indicated that in the hepatoma cell line MH_1C_1, unlike normal hepatocytes, PGE_2 transactivated EGFR. In the present study we have explored these mechanisms further.
Methods: The Morris hepatoma-derived cell line MH_1C_1 was used. The cells were cultured and exposed to various agonists and antagonists. Levels and phosphorylation of proteins in signalling pathways were assessed by Western blotting. The specificity of agents acting at prostaglandin receptors was assessed by determination of cAMP and inositol phosphates.
Results: PGE_2 induced phosphorylation of the EGFR in the MH_1C_1 cells. This effect of PGE_2 was delayed and more prolonged as compared to the phosphorylation elicited by EGF or TGFa, consistent with an indirect mechanism. PGE_2 stimulation of these cells also elicited phosphorylation of Erk and Akt, which was inhibited by the EGFR-tyrosine kinase inhibitor gefitinib, the Src-inhibitor CGP77675, and the matrix metalloproteinase (MMP) inhibitor GM6001. Furthermore, studies with prostaglandin receptor agonists and antagonists showed that the stimulation by PGE_2 of Erk and Akt phosphorylation was mimicked by fluprostenol (FP/EP3 agonist) and sulprostone (EP3/EP1/FP agonist), while effects of these agonists were inhibited by AL8810 (FP antagonist), but not affected by SC51322 (EP1/EP3 antagonist).
Conclusion: The results suggest that in MH_1C_1 hepatoma cells, PGE_2 activates the pathways to Erk and Akt by transactivating EGFR, through mechanisms involving FP prostaglandin receptors, kinase(s) of the Src family, and metalloproteinase-mediated release of EGFR ligand(s).

553 Molecular effects of anti-angiogenic therapy in breast cancer xenografts

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Neoangiogenesis (stimulation of new blood vessel formation) is a fundamental step in the transition of tumours from a dormant state to a malignant state, and for tumour growth. VEGF (Vascular Endothelial Growth Factor) is secreted by oxygen-deprived cells, including malignant cells, and stimulates new blood vessel formation by binding to receptors on nearby endothelial cells.
Bevacizumab (Avastin) is an antibody recognizing VEGF-A and may thus bind to and inhibit the formation of new blood vessels. Although the effect of bevacizumab is well documented, its molecular effects in breast cancer are not completely exploited, particular in combination with endocrine therapy and chemotherapy.
In this work we have utilized the PamChip kinome profiling system to investigate the changes in protein phosphorylation in two breast cancer xenografts (one luminal – and one basal-like) after anti-angiogenic therapy, chemotherapy and endocrine therapy, either separately or in combination. The results revealed different phosphorylated kinase substrates in the two xenograft models, suggesting that different signalling pathways are activated upon treatment. We also showed that the luminal – and basal-like xenograft models respond differently, the basal-like being a better responder than the luminal-like to anti-angiogenic therapy in combination with chemotherapy.
The PamChip results are currently being confirmed through western blotting, and will also be analysed by Reverse Phase Protein Array (RPPA).
Since tumour vasculature is an important factor influencing anticancer therapy, extensive characterization of the vascularisation profiles in the two xenograft models will be carried out using Immunohistochemistry (IHC) in these tumour models.
By isolating organs and blood from xenograft bearing mice, we are also at present evaluating the extent of distant metastasis and the level of circulating tumour cells (CTCs) after different treatment regimens. The results from these studies will provide further information about changes in tumour aggressiveness after anti-angiogenic therapy.
In conclusion, the results from these analyses may provide valuable information on tumour changes upon anti-angiogenic therapy, and suggestions regarding molecular targets that may be further exploited for utilization in personalized therapy protocols for breast cancer patients.

554 Obesity-induced abnormal inflammatory response drives accelerated growth in prostate cancer xenografts

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Obesity is associated with increased predisposition to some cancers, aggressiveness of others, insulin resistance/hyperinsulinemia as well as a state of abnormal inflammatory response. Recent study focusing on prostate cancer has shown that obesity is an important adverse prognostic factor. However, the molecular mechanisms involved in the increased aggressiveness of prostate cancer in obese individuals are still unknown. In order to investigate the effects of inflammation and hyperinsulinemia induced by high-fat diet (HFD) on prostate cancer growth, SCID mice fed a control or HFD for eight weeks were injected subcutaneously with PTEN positive (DU145) and PTEN negative (PC-3) prostate cancer cell lines. Here, we show that obese mice experienced a higher tumour growth of both DU145 and PC-3 xenografts compared to the control group. Xenografts of mice fed a HFD show an increase in IκB kinase complex and c-Jun NH_2 terminal kinase activity, which is prevented by blocking TNF-α. Interestingly, pharmacological blockade of TNF-α in HFD mice was effective to reduce tumour growth induced by HFD to control levels of both DU145 and PC-3 xenografts. In addition, we show that DU145, when grown as tumour xenografts in mice, are sensitive to the reduction of hyperinsulinemia induced by octreotide treatment, whereas PC-3 cells, that presents a constitutive activation of PI3K, are resistant. Thus, the present study documents that low grade inflammatory response observed in obesity, in an insulin sensitivity independent manner, drives the growth of prostate cancer xenografts.
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555 Leptin increases the invasiveness and angiogenesis-mediated but not proliferation in human epithelial ovarian cancer cells

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Background: Epidemiological studies have established to the obesity as a potential risk factor in the development of epithelial ovarian cancer. This entity constitutes the most lethal malignancy among gynecological cancers. This lethality is due to late diagnosis usually at advanced stages (75% of cases). Despite using ultra radical surgery to achieve optimal debulking of disease followed by adjuvant chemotherapy (usually paclitaxel combined with a platinum derivate) the overall survival is lower than 40% at 5-year follow-up. This poor outcome is also due to acquisition or development of resistance to the different chemotherapeutic schemes. So far is not well known how the obesity can lead to or drive ovarian carcinogenesis including its aggressive behavior. One of the hypothesis postulates that leptin, a peptidic hormone mainly produced by adyposites and playing a key role in regulating energy intake and energy expenditure (including appetite and metabolism), could be involved in ovarian carcinogenesis. In fact, it has been shown that leptin regulates proliferation in other both benign and malignant tissues.
Objectives: To investigate the effects of leptin in proliferation, invasiveness and angiogenesis in human epithelial ovarian cancer cells.
Material and Methods: mRNA and protein expression levels of leptin receptors were measured under basal condition in A2780, UCI 101 and SKOV3 human ovarian cancer cells through RT-PCR and Western Blot (W-B), respectively. Leptin dose/response (0–1000 uM) and time (up to 72 hr exposure) curves were built and cell viability (MTS assays), proliferation (DNA histogram, FACS analysis) and invasion (matrigel invasion assays) were measured upon treatment with each condition. To study the leptin effect in angiogenesis, the endothelial EAHy cells were incubated with conditioned medium extracted from A2780 cells upon leptin treatment (100 ng/ml).
Results: In the three studied cancer cell lines, constitutive mRNA and protein levels of leptin receptors were detected by RT-PCR and W-B. No increase in cell viability or proliferation was detected upon treatment with different doses and time exposure of leptin in A2780 and SKOV3 cells. A significant increase in the number of A2780 cells crossing the matrigel barrier was observed after leptin treatment (100 ug/ml for 48 hrs) compared with the mock treatment (control). Finally, formation of capillary tube-like structures was observed in EAHy cells when treated with conditioned medium obtained from leptin treated A2780 cells.
Conclusions: Here we demonstrate that ovarian cancer cells express leptin receptors and response to its exposure. Leptin increases the invasiveness of human epithelial ovarian cancer cells and stimulates them to release angiogenic factors. High levels of leptin in obese women could mediate the negative impact of obesity in ovarian cancer progression.
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