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

I. Tveteraas[1], K.M. Müller[1], O. Dajani[2], T. Guren[2], M. Aasrum[1], J. Ødegård[1], D. Sandnes[1], T. Christoffersen[1]. [1]*University of Oslo Faculty of Medicine, Department of Pharmacology, Oslo, Norway*, [2]*Oslo University Hospital Ullevål, Department of Oncology, Oslo, Norway*

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

E. Lindholm[1], O. Engebraaten[1], G. Mælandsmo[1]. [1]*Institute for Cancer Research, Tumour Biology, Oslo, Norway*

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

G. Zweig Rocha[1], M.M. Dias[1], E.R. Ropelle[1], F.O. Costa[1], J.B.C. Carvalheira[1]. [1]*University of Campinas, Internal Medicine, Campinas SP, Brazil*

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.
Financial support: FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo.

555 Leptin increases the invasiveness and angiogenesis-mediated but not proliferation in human epithelial ovarian cancer cells

M.A. Cuello[1], S. Kato[1], D. Díaz[1], G.I. Owen[2]. [1]*Faculty of Medicine Pontificia Universidad Catolica de Chile, Obstetrics and Gynecology, Santiago, Chile,* [2]*Faculty of Biological Sciences Pontificia Universidad Catolica de Chile, Physiological Sciences, Santiago, Chile*

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.
Research funding: FONDECYT 1080163 (MC), 1060495 (GO).