743 Lymphatic microvessel density and mast cells in molecular types of breast cancer

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Background: The importance of lymphangiogenesis in the natural evolution of breast cancer is well known, but the mechanisms that stimulate this process are not fully understood. Although the contribution of mast cells to tumour angiogenesis is well documented, their involvement in lymphangiogenesis is virtually unknown. In the present work we show a relationship between lymphatic microvessel density and mast cells in HER2 and luminal types of breast cancer.
Material and Methods: There were investigated 55 patients with ductal invasive breast carcinoma and from these, 26 had lymph node metastases. Patients were stratified according the molecular classification, based on the immunohistochemical expression of estrogen receptor, progesterone receptor, HER2 protein, cytokeratin 5, p53, epidermal growth factor receptor, and Bcl-2. Double staining for D2−40 and mast cell tryptase was performed to evaluate the number of lymphatic vessels (LVs) and mast cells (MCs). Counting of LVs and MCs was performed in the same fields, at magnification $\times$200. Results were statistically analyzed, taking into account the clinico-pathological factors of prognosis and the molecular type of carcinoma.
Results: We found basal-like carcinoma in 8 cases (14.54%), luminal A in 26 (47.27%), luminal B in 7 (12.72%), HER2 in 10 (18.18%), and unclassified tumours in 4 cases (7.27%). LVs were found in the peritumoural stroma in all cases (0.6–15.3/x200) and in the intratumoural area in 39 cases (range 0 to 6.6). MCs were found in both intra-/peritumoural area (3 to 123.33, and 7 to 61.6, respectively). No correlation was found between LVs/MCs, and stage and grade of the tumour. A strong positive correlation was found between LVs/MCs and lymph node metastasis in HER2 and luminal types of breast cancer. A significant negative correlation was found between LVs/MCs and basal-like carcinoma.
Conclusion: Our data suggest that MCs could be involved in breast cancer-associated lymphangiogenesis, and LVs/MCs count is a useful predictor of lymph node metastasis.

744 WWOX tumour suppressor gene is involved in Wilms tumour cancerogenesis in a haploinsufficiency way

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WWOX gene (*locus* 16q23.3–24.1) is known to play crucial role in suppression of tumourigenesis in different cancers. It encompasses common chromosomal fragile site FRA16D characterized with frequent LOH and chromosomal rearrangements observed in many cancers along with Wilms tumour (*nephroblastoma*). Despite of available molecular data on childhood *nephroblastoma* tumourigenesis, along with LOH and methylation level alterations of 16q chromosomal fragment, detailed modifications of *WWOX* gene associated with this tumour type lesions still remain unclear. The main aim of our study was to investigate epigenetic alterations of *WWOX* gene associated with *nephroblastoma* cancerogenesis and to assess this gene function in progression of this tumour.
We analyzed 24 Wilms tumour samples stored at −80ºC till RNA extraction. To evaluate LOH frequency of FRA16D we performed genomic analysis of three highly polymorphic microsatellite markers: D16S518, D16S3096 and D16S504 residing inside *WWOX* gene. Meanwhile, to assess methylation level of two *WWOX* promoter regions we performed MethylScreen assay. By comparative quantitation analysis we assessed expression level of *WWOX* gene and in addition other genes potentially associated with tumourigenesis (*BCL2*, *BAX, CCND, CCNE1, KI67, CDH1*, *TP73, EGFR, ERBB2* and *ERBB4*). To analyze WWOX protein expression we performed Western-blot assay with anti-WWOX rabbit primary polyclonal antibodies.
We identified for the first time in Wilms tumour relatively high LOH frequency of FRA16D markers with the lowest value for D16S518 marker. Hemizygosity was observed at frequency of 16.33%, 49% and 46.83% for D16S518, D16S3096 and D16S504 markers, respectively. In analyzed tumours the level of *WWOX* gene expression was positively correlated with *BCL2* expression ($p < 0.0001$) and *BCL2*/*BAX* ratio ($p = 0.035$), *CDH1* ($p = 0.001$) and *ERBB4* expression ($p = 0.003$). Our results showed also association of *WWOX* gene expression with methylation of its promoter.
Loss of heterozygosity observed in FRA16D region demonstrate involvement of *WWOX* gene in kidney tumourigenesis in a haploinsufficiency fashion. Additionally, positive correlation of *WWOX* gene expression level with lowered apoptosis driven by elevated expression of anti-apoptotic marker − *BCL2* gene, association with expression of other tumour suppressor gene − *CDH1*, coexpression with membranous ERBB4 receptor and association with clinical data confirm tumour suppressor function of investigated gene in Wilms tumour.

745 The PPARgamma-independent antiproliferative effects of thiazolidinediones in breast cancer cells are partially mediated by an ER-stress-related induction of EGR1

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Background: The treatment of human breast cancer cell lines with agonists of peroxisome proliferator-activated receptor gamma (PPARγ) inhibits proliferation. However, these antiproliferative effects seem mainly to be the result of a PPARγ-independent action. In the present study, we aimed at characterizing the PPARγ-independent signaling pathway of PPARγ agonists in the hormone-dependent breast cancer cell line MCF7.
Material and Methods: MCF7 cells were exposed to various PPARγ agonists: Rosiglitazone (RGZ), Ciglitazone (CGZ), Troglitazone (TGZ) and the prostaglandin 15d-PGJ_2. A derivative of TGZ (Δ2-TGZ) devoid of PPARγ agonist activity was also used. Gene expressions were studied by RT-PCR and microarray. Proteins were analyzed by western blotting and immunolocalization. The involvement of signaling pathways was demonstrated using pharmacological agents and RNA interference. Calcium was measured by fluorescent imaging.
Results: EGR1 (Early Growth Response gene 1) mRNA and protein levels peaked after 3 hours of incubation with 25 μM TGZ, CGZ or 15d-PGJ_2 and then gradually decreased. RGZ, the most potent activator of PPARγ, did not show this effect. The PPARγ antagonist GW 9662 did not block EGR1 mRNA induction which also still occurred in case of PPARγ silencing as well as in case of treatment with the PPAR?-inactive compound Δ2-TGZ. Moreover, the MEK/ERK inhibitor U0126 abolished the EGR1 mRNA induction triggered by Δ2-TGZ, TGZ, CGZ and 15d-PGJ2. ERK1/2 phosphorylation and EGR1 mRNA induction were not blocked by EGF Receptor (EGFR) inhibitors (AG1478 and PD153035) whereas these events were prevented by calcium chelation suggesting an increase in cytosolic calcium. Indeed, Δ2-TGZ triggered a rise in intracellular calcium that was associated with endoplasmic reticulum (ER) stress as suggested by ER enlargements and demonstrated by several markers including activated PERK, phosphorylation of eIF2α splicing of XBP-1 and expression of DDIT3. Finally, siRNA targeting EGR1 demonstrated that the early induction of this gene is involved in the antiproliferative effects of Δ2-TGZ.
Conclusions: Taken together, our results show that in MCF7 breast cancer cells, the expression of EGR1 is triggered in a PPARγ-independent manner and is involved in the antiproliferative action of thiazolidinediones. The development of compounds able to disturb calcium homeostasis and to trigger ER stress could be interesting for breast cancer treatment.

746 Combination of Smac-mimetics and TNFalpha induce apoptosis in glioma cell lines

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Smac/DIABLO, through its AVPI motif, is able to antagonize the anti-apoptotic function of XIAP and to induce cIAP1−2 degradation. XIAP, cIAP1−2 belong to a class of central apoptosis regulators called Inhibitor of Apoptosis Proteins (IAPs) and are upregulated in different tumours. For these reasons, many efforts have been made to design molecules that mimic the activity of the endogenous Smac/DIABLO (Smac-mimetics), because they could represent a new class of anticancer drugs able to overcome the resistance to apoptosis of cancer cells. Two types of Smac-mimetics have been developed. Monovalent molecules mimic the binding of a single AVPI binding motif to one BIR domain of IAP proteins, while bivalent compounds, containing two AVPI binding motifs tethered together through a linker, can bind to two BIR domains. Recently, it has been demonstrated that Smac-mimetics can potentiate TRAIL and TNFα mediated cell death in tumour cell lines [1].
Astrocytoma is the most common glioma and can differentiate in glioblastoma multiforme (GBM), one of the most malignant cancers. GBM is marked by high resistance to chemo- and radiotherapeutics and by a median survival of less then 1 year. The initial therapy produces only palliative effects, the recurrence of the tumour is leading to rapid death [2]. Since novel therapies for malignant gliomas are desperately needed, we examined whether newly synthesized Smac-mimetic compounds [3], could sensitize astrocytoma and glioblastoma cell lines to apoptosis. To this purpose, we assessed the viability of T98G, U87MG and CCF-STTG1 cell lines using MTT assay. The cells have been treated with several Smac-mimetic compounds, alone or in combination with TNFα.
We have identified a dimeric Smac-mimetic compound (Smac-083) that, in combination with low doses of TNFα (0.01 ng/mL), significantly sensitizes T98G cell lines to apoptosis already at nanomolar concentrations. Moreover,