468 Role of the p14ARF tumour suppressor in EGFR-mediated growth control of lung cancer cells

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Background: Tyrosine Kinase Inhibitors (TKIs) that specifically block activity of the Epidermal Growth Factor Receptor (EGFR) are currently used in the therapy of lung cancer. Although responses to TKIs strongly depend on the presence of EGFR mutations (L858R, Del19), complete clinical response is rarely achieved. Having observed that expression of the p14ARF tumour suppressor is altered in human lung tumours with mutated EGFR (Mounawar et al, 2007, Cancer Res.), we postulated that p14ARF expression could counteract the growth properties of mutated EGFR lung tumour cells and play a role in TKIs response.
Material and Methods: Lung tumour cell lines with wild type or mutated EGFR (L858R) were transfected with p14ARF and/or mutant EGFR (L858R) encoding vectors. Cell growth was analyzed by methylene blue staining or cell counting. Apoptosis was evaluated after active caspase 3 staining (FACS). Expression of genes of interest was studied by western blotting and/or by quantitative PCR. Co-immunoprecipitation experiments were used to detect interactions between proteins.
Results: We show that p14ARF overexpression inhibits the growth of cells with constitutive or transfected mutant EGFR (L858R) by inducing apoptosis. We also provide evidence that p14ARF uses the STAT3 pathway to exert its negative control on cell growth, and induces by this way the downregulation of the anti-apoptotic protein Bcl2, a known target of STAT3. Preliminary results indicate that the histone acetyl transferase Tip60 could be involved in p14ARF-mediated Bcl2 downregulation. Moreover, we show that apoptosis induced by TKI exposure (Gefitinib) correlates with the accumulation of the p14ARF protein as well as with the downregulation of Bcl2 expression in EGFR-mutated (L858R) cells.
Conclusions: These results indicate that p14ARF protects cells from the antiapoptotic signals induced by mutated EGFR (L858R). They also suggest that p14ARF could play a role in the response to TKI.

469 Vitamin D signalling and metabolic pathways expression in breast cancer progression

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Background: Breast cancer is a heterogeneous disease associated with different patient prognosis and responses to therapy. Vitamin D has been emerging as a potential treatment for cancer, as it has been demonstrated that it modulates proliferation, apoptosis, invasion and metastasis, among others. It acts mostly through the Vitamin D receptor (VDR) and the synthesis and degradation of this hormone are regulated by the enzymes CYP27B1 and CYP24A1, respectively. We aimed to study the expression of these three proteins by immunohistochemistry in a series of breast lesions.
Materials and Methods: We have used a cohort of 947 samples, comprising normal breast, benign mammary lesions, ductal carcinomas *in situ* and invasive carcinomas and we have assessed the expression of the VDR, CYP27B1 and CYP24A1 by immunohistochemistry.
Results: The results that we have obtained show that all proteins are expressed in the various breast tissues, although at different amounts. The VDR was frequently expressed in benign lesions (93.5%) and its levels of expression were diminished in invasive tumours (56.2%). Additionally, the VDR was strongly associated with the oestrogen receptor positivity in breast carcinomas. CYP27B1 expression is slightly lower in invasive carcinomas (44.6%) than in benign lesions (55.8%). In contrast, CYP24A1 expression was augmented in carcinomas (56.0% in *in situ* and 53.7% in invasive carcinomas) when compared with that in benign lesions (19.0%).
Conclusions: From this study, we conclude that there is a deregulation of the Vitamin D signalling and metabolic pathways in breast cancer, favouring tumour progression. Thus, during mammary malignant transformation, tumour cells lose their ability to synthesize the active form of Vitamin D and respond to VDR-mediated Vitamin D effects, while increasing their ability to degrade this hormone.

470 Proteomic analysis of microvesicles released by the prostate cancer cell line PC-3

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Background: Prostate cancer is one of the most frequent cancer types in men with 679,000 diagnoses and 220,000 deaths worldwide each year [1]. There is a need for new strategies for prostate cancer diagnosis and treatment, and a strategy that has recently received some attention is based on microvesicles. It has been known for three decades that epithelial cells of the prostate gland release vesicles, so-called prostasomes [2]. Interestingly, prostasomes contain molecules acting on immunosupression, invasion, angiogenesis and malignant conversion and have recently been implicated in prostate cancer [3]. Moreover, molecules in prostasomes might be used as cancer biomarkers.
Material and Methods: We have performed a proteomic analysis of microvesicles released by the metastatic prostate cancer cell line PC-3 looking for proteins that may have this function.
Results: 435 proteins were identified by liquid chromatography/tandem mass spectrometry (LC-MSMS), 226 of these proteins were identified with more than 1 peptide. Several of the proteins identified in our study belong to the list of proteins commonly found in microvesicles [4], but other proteins seem to be specific to the vesicular population released by PC-3 cells. After Gene Ontology annotations, GO-cellular component, 23% of the proteins were classified as extracellular. Intracellular proteins were annotated in a variety of cellular compartments: cytosol, endosomes, nucleus, mitochondria, Golgi apparatus and endoplasmic reticulum. 30% of the proteins were annotated in the category cytoskeleton. After GO-Biological Process, the main annotations found were: transport, metabolic processes, cell communication, cell organization and biogenesis and regulation of biological processes.
Conclusions: Further characterization of the proteins found in prostasomes released by PC-3 cells is required to determine whether any of these proteins can be used as prostate cancer biomarker or can help us to understand the role of prostasomes in prostate cancer.

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471 Neurobiological studies of tumour progression

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Tumour progression represents very complex process. Cancer cells actively interact with their environment e.g. surrounding stroma and especially with the immune cells. Moreover, there are accumulated evidences that progression of cancer and development of metastasis is under tight influence of the nervous system. The system may monitor and modulate cancer growth directly and/or indirectly, via the immune system. Direct interactions between the cancer cells and nervous system is proved by several facts: (i) some types of tumour tissues are actively innervated; (ii) neurotransmitters may modulate certain steps of tumourigenesis; (iii) interruption or stimulation of nervous pathways influences cancer progression; (iv) cancer induces specific changes in activity of the brain of tumour bearing animals, as well as, cancer patients.
In the study, we investigated transmission of information between BP6 fibrosarcoma cells and the brain during various circumstances in experimental animals. Firstly, we analyzed the effect of chemical sympathectomy on survival of BP6 tumour-bearing rats. Moreover, we followed the effect of BP6 cells progression on the activity of selected brain areas.
We found that chemical sympathectomy, induced by application of neurotoxin 6-OH dopamine, prolonged survival of the BP6 tumour bearing rats. Moreover, we detected increased activity in certain brainstem and hypothalamic areas, including nucleus of the solitary tract, parabrachial nucleus and paraventricular hypothalamic nucleus in the rats administered intraperitoneally the BP6 cells.
Our data indicate that autonomic nervous system modulates progression of tumour growth and that information related to tumour progression is transmitted to the brain. These findings support neurobiological view of cancer based on assumption that the brain monitors, as well as, modulates cancer progression and development of metastasis.
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472 Interaction of ephrinB2 with its receptors EphB4 and EphB6 − potential impact on tumour-associated inflammation in human melanoma

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Background: Tumour-associated inflammatory cells (TAIC) are a major component of the tumour microenvironment and can contribute to both tumour progression and metastasis for instance by direct cell-cell interaction via membrane-bound proteins. Tumour cells show varying expression of Eph receptors and their ephrin ligands, which both are receptor tyrosine kinases.