model: B16F10 and B16Bl6 cells can both induce primary tumours after subcutaneous implantation of cells, but pulmonary metastasis are only found in the mice bearing a B16Bl6 tumour. We performed comparative proteomic studies on these two cell lines, using both cultured cells and subcutaneous tumours. 7 and 12 differentially expressed proteins respectively were identified by mass spectrometry. Especially, annexin A1 (ANXA1) was increased 1,5 to 2 fold in B16Bl6 cells as compared to B16F10 cells, in vivo and in vitro. In an attempt to characterize its role in melanoma B16 spreading, we showed that reducing ANXA1 protein level by siRNA in B16Bl6 cells decreased their in vitro invasion properties on Matrigel® coated chambers. This should be associated with the presence of formyl peptide receptors (FPR), which have been shown to activate invasion in an epithelial cell line SKCO-15 (Babbin et al, 2006). Indeed, we demonstrated by RT-PCR the presence of transcripts encoding for two FPR isoforms (FPR1 and FPRrs) in the two B16 lines without any reliable quantitative difference. These receptors seemed to be functional since B16Bl6 cells incubation with the FPR agonists (fMLP) or antagonists (tBOC) respectively enhanced or decreased Matrigel coated chamber invasion. Furthermore, preliminary data suggested that incubation of B16Bl6 cells with fMLP and tBOC lead to an increase or decrease of ANXA1 steady state level. In conclusion, our results showed that increased ANXA1 expression could be associated at least in vitro with an increased invasion capacity, that might be mediated by the FPR receptors.

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Expression of alpha(1,6)fucosyltransferase in the early tumorigenesis of human colorectal cancer

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An elevated level of fucose content in glycoproteins is one of the cancer-related alterations found in patients with malignant diseases. In a recent study we have demonstrated that α(1,6)fucosyltransferase [α(1,6)FT], the enzyme which catalyzes the core fucosylation in these glycoproteins, was enhanced in tumoral colon when compared to healthy adjacent tissues from colorectal carcinoma (CRC) patients. Most CRC arise from neoplastic adenomatous polyps thus we developed the present work in order to determine weather this increase is an early event or it only occurs lately in tumorigenesis.

We investigated the α(1,6)FT expression by means of immuno-histochemistry in 81 adenomas, 13 inflammatory lesions and 9 healthy tissues of free-CRC patients. Tissue sections were stained using anti-human α(1,6)FT MAb and visualized with DAB (3,3´-diaminobenzidine). Negative controls were performed using PBS instead of primary antibody.

After the immunohistochemical assay, no positive expression was found in the healthy and inflammatory tissues. In the case of adenomas, 13 of the 81 polyps analysed (16%) were positive for α(1,6)FT expression whereas the percentage of positive expression in tumor tissues (that we previous described) was the 61.3%. After the statistical analysis, we found significant differences for tumour vs. adenomas, inflammatory lesions and control healthy tissues ($p<0.01$). We also analysed the possible association between the α(1,6)FT expression in the neoplastic polyps and the histological type or the grade of dysplasia in this polyps. A significant increase of expression was observed in the cases without dysplasia with respect to the dysplasic ones ($p=0.05$), whereas a correlation between the expression and the histology was not found.

In conclusion, the absence of α(1,6)FT immunohistochemical expression in the inflammatory and healthy tissues of patients without CRC clearly suggests that the alteration of the enzyme expression is specific of the malignant transformation and is not related to the inflammatory process normally associated to the cancer. On the other hand, the low rate of positive expression obtained in pre-cancerous lesions indicates that the α(1,6)FT expression increase is a late event in the tumorigenesis strongly associated with a total cell transformation of the colorectal tumor.

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Endo180 expression by tumour cells with an invasive phenotype correlates with prostate cancer progression

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The purpose of this study was to investigate whether stromal or epithelial expression of Endo180 (CD280; MRC2; urokinase-type plasminogen activator receptor-associated protein, uPARAP) can contribute to metastatic prostate cancer. The prognostic and functional roles of Endo180 were characterised using tissue microarray (TMA) and immunofluorescent costaining with pan-cytokeratin (pCk) to allow quantitative analysis of stromal (pCk-) and epithelial (pCk+) cellular expression. Expression of Endo180 and its function-associated partners in collagen remodeling and tumour cell migration and chemotaxis: membrane type-1 matrix metalloproteinase (MT1-MMP) and urokinase-type plasminogen activator (uPA)-uPA receptor (uPAR) respectively; were quantified in human prostate tissue clinically graded as benign prostate hyperplasia (BPH) (n=29) or with good (n=26), intermediate (n=96) or poor (n=18) tissue differentiation. Significant differences or correlations between categorical variables, including serum prostate specific antigen (PSA), were determined using two-sided statistical tests and 95% confidence intervals. Increases in Endo180+/pCk- and Endo180+/pCk+ cells confirmed both stromal and epithelial upregulation of Endo180 respectively. The increase in epithelial expression of Endo180 displayed linear correlation with advanced clinical grade and greater prognostic capability than serum PSA. The differential patterns of stromal and epithelial Endo180 coexpression with MT1-MMP and uPAR-uPA with increased clinical grade revealed the potential for a distinct sequence of their molecular and functional interplay during the different stages of prostate cancer progression. Taken together these data support the use of Endo180 as a potential biomarker for the prognosis of prostate cancer tissue biopsies and as a target to prevent tumour cell migration, chemotaxis, invasion and tissue remodelling during prostate cancer metastasis.

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Candidate genes for the recurrence of glioblastoma multiforme identified by microarray

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Background: Glioblastoma multiforme (GBM) is the most aggressive and most lethal primary malignant brain tumor, correlated with survival rates of less than one year from the time of diagnosis. Current surgical procedure attempts to remove the bulk of the tumor mass, whereas GBM frequently recurs within 1-3cm from the primary tumor resection site. Molecular mechanisms involved in the recurrence of the tumor are still poorly understood. The aim of the study was to define the molecular signature of GBM surrounding white matter (WM) in order to better understand the molecular mechanisms involved with tumor relapse.

Material & Methods: Human GBM tumor bulk and surrounding tissue (1-3cm from the border of the tumor) were obtained from five patients who underwent total tumour resection, while normal white matter was harvested from patients who underwent surgical procedure for nonmalignant pathologies. Samples were processed for hybridization on the Affymetrix Human U133A arrays and data were examined with the GeneSpring analysis software. A subset of interesting genes was further validated by RT-PCR.

Results: Gene expression analysis of the samples was done in 2 independent steps. First, molecular profiling comparison of GBM surrounding WM and normal WM resulted in 59 genes differentially expressed between both tissues. Among these, numerous genes expressed by mature neural cells were down-regulated in GBM surrounding WM. Moreover, KLRC1, a specific natural killer receptor naturally involved in the activation of antitumoral cells was drastically repressed in GBM surrounding WM, suggesting that the antitumoral immune surveillance is compromised in this tissue.

Second, we focused our study on genes specifically regulated in GBM periphery respectively to GBM core.

The highest up-regulated gene in GBM surrounding tissue codes for DTX4, a regulator of NOTCH signalling pathway described for its key role in maintaining neural progenitors in an uncommitted state.

Conclusion: This study revealed unique molecular characteristics of GBM surrounding tissue, showing the dysregulation of genes involved in immune surveillance along with genes associated to stemness maintenance. All together, these data may help to understand the molecular mechanisms associated with GBM recurrence.

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MYC inhibition of p27-induced erythroid differentiation is mediated by the repression of erythroid master genes and uncoupled from its cell cycle promoting activity

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Inhibition of differentiation has been proposed as an important mechanism for the tumorigenesis mediated by MYC, but the mechanisms involved are

unclear. Induction of p27 in human K562 cells results in erythroid differentiation as well as CDK inhibition, RB hypo-phosphorylation and G1 arrest. We have studied the involvement of MYC in the p27-induced differentiation using K562 cells with conditional expression of p27 (inducible by zinc cations) and MYC (activable by 4-hydroxy-tamoxifen). In this model, activation of MYC inhibits the p27-mediated erythroid differentiation without reversing the cell cycle arrest imposed by p27. Microarray analysis revealed that, in the presence of p27, MYC blocked the up-regulation of several erythroid-specific genes, including GATA1 and NFE2, two transcriptions factors with a pivotal role in erythropoiesis. Co-transfection experiments show that MYC inhibits p27-induced differentiation, at least in part, through GATA1 down-regulation. In conclusion, we demonstrate a mechanism for MYC-mediated inhibition of differentiation depending on specific gene regulation and that can be separated from cell cycle effects. We hypothesize that this proliferation-independent differentiation inhibitory activity may be important for MYC-induced tumorigenesis.

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The modulation of protein synthesis by T3 in Caco-2 colorectal cancer cells - the role of nuclear targeting of TR receptors

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Introduction: Thyroid hormones, mainly T3, regulate growth, development, differentiation and metabolic processes in target tissues including intestinal mucosa. The actions of T3 are exerted through thyroid hormone receptors (TR), which belong to the nuclear steroid hormone receptor family. We have recently found decreased expression of TRβ1 in colorectal cancer. The effect of thyroid hormones on the growth on intestinal epithelial cells has not been studied.

Materials and methods: The effect of various concentrations of T3 on Caco-2 cell growth was studied. Cell growth was assessed with leucine incorporation, three-dimensional (3D) culture and by quantifying cell proliferation and apoptosis levels. TRα1, TRβ1 and β-catenin expression in nuclear and cytoplasmic fractions was assessed by Western blot analysis completed with immunohistochemistry and immunofluorescence.

Results: T3 limited cell growth and decreased protein synthesis in Caco-2 cells, induced nuclear targeting of TRα1 and TRβ1, but had no effect on β-catenin localization, except weaker membranous immunoreaction was observed in areas of evident nuclear translocation of TRα1 and TRβ1. In 3D culture, T3 induced differentiation of Caco-2 cells grown in type I collagen gel.

Conclusions: T3 induces nuclear translocation of its receptors TRα1 and Trβ1, and thereby mediates proliferation, differentiation and apoptosis in Caco-2 cells. Loss of membranous β-catenin associated with nuclear localization of TRα1 and Trβ1 suggests a link between Wnt and TR signaling pathways. All these findings suggest that disturbances in T3 signaling pathways could be involved in colorectal carcinogenesis.

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Expression of Aurora kinases in clear cell renal carcinoma

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Chromosome aberration is a hallmark of cancer cells. During mitosis, replicated chromosomes need to be equally distributed between the two daughter cells since errors at this stage can lead to aneuploïd cells and initiate cancerous transformation. Aurora protein kinases (A, B and C) have an important role in the progression of mitosis. They are involved in the formation and stability of the mitotic spindle and in cytokinesis. These kinases were described over-expressed in different cancers, leading to the development of inhibitors for these kinases.

A study of the transcriptional and translational expression of the Aurora kinases was initiated in clear cell renal carcinomas (CCRC). These tumours have been shown to be frequently mutated or inactivated for the suppressor of tumour Von-Hippel Lindau gene (VHL). Identification of new potential targets in CCRC which are resistant to most current treatments would allow the development of new anticancerous strategies.

The expression of the Aurora kinases was studied by RT-PCR, Western-blot and immunohistochemistry in tumour versus normal tissues. Aurora A and B transcripts were detected in tumour and normal tissues. In contrast, the Aurora C kinase was not expressed in normal nor in tumour tissues. High levels of Aurora-A kinase transcripts in the tumour were correlated with a bad prognosis and the presence of metastases. However, the amount of Aurora-A protein was always higher in normal kidney tissues than in the corresponding tumours, independently of the tumour stage. Discrepancies between the transcriptional and translational expression of the Aurora-A kinase in CCRC will be discussed.

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Investigating the roles of gelsolin in the malignant progression of colorectal tumor cells

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Purpose of study: To investigate the role of gelsolin in the malignant progression of colorectal tumor cells.

Materials and Methods: A metastatic cell line (E1) was previously derived from the poorly metastatic human colorectal cancer cell line, HCT116, and exhibits a mesenchymal phenotype with enhanced migration. We studied the influence of gelsolin in progressive transformation of colorectal tumour cells using the E1/HCT116 in vitro model, as well as matched tumor samples from 13 patients.

Results: Compared to HCT116, gelsolin is upregulated in metastatic E1 cells. Knock down of gelsolin expression in E1 cells by siRNA to levels lower than in HCT116 increased fibronectin expression compared to control E1 and HCT116 cells. Immunohistochemical studies on samples derived from 13 colorectal cancer patients with liver metastases showed that gelsolin was reduced in the majority of both primary and metastatic tumours. However, gelsolin appeared to be upregulated at the invasive front of these tumors, consistent with the increased expression observed in metastatic E1 cells. Overexpression of gelsolin in pancreatic cancer cells increases cell motility (Thompson et al, 2007).

Conclusion: We postulate that gelsolin possibly enhances invasion and metastasis by affecting tumor cell migration, possibly by complex regulation of several migration-associated proteins.

Reference: Thompson, C.C., Ashcroft, F.J., Patel, S., Saraga, G., Vimalachandran, D., Prime, W., Campbell, F., Dodson, A., Jenkins, R.E., Lemoine, N.R., Crnogorac-Jurcevic, T., Yin, H.L., Costello, E. Pancreatic cancer cells overexpress gelsolin family-capping proteins, which contribute to their cell motility (2007). Guy 56:95-106.

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Estrogen receptor beta and the progression of prostate cancer - role of 5alpha-androstane-3beta,17beta-diol (3beta-Adiol)

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Introduction: Prostate cancer (PC) develops in response to an abnormal activation of androgen receptor by circulating androgens and it is pharmacologically controlled by androgen blockade. However, androgen-ablation therapy often correlates with the growth of androgen-independent PC and increased invasiveness. Recently, we found that the testosterone derivative dihydrotestosterone (DHT) inhibits PC cell migration through the conversion to its metabolite 5alpha-androstane-3beta,17beta-diol (3beta-Adiol), which is unable to bind AR, but interacts with the estrogen receptor beta (ERbeta).

Methods and Results: To further investigate the role of 3beta-Adiol in PC progression, we performed in vitro growth and invasion studies on human PC3 cells prostate cancer cells. Thymidine incorporation experiments demonstrated that a dose-dependent decrease in cell proliferation was observed in PC3 treated with 3beta-Adiol for 48 h; on the contrary, estradiol treatment was ineffective, suggesting the existence of different pathways for ERbeta activation in PC3 cells. A 3beta-Adiol treatment of PC3 cells seeded on laminin for 48 h leaded to a significant decrease in cell detachment with respect to untreated cells. Moreover, 3beta-Adiol-treated PC3 cells showed a significant decrease in invasive capacity as measured by the invasion of reconstituted basement membrane (Matrigel).

Finally, we performed in vivo experiments by using a PC-3 orthotopic model with bioluminescence imaging as an end point. PC-3 cells stably expressing the luciferase gene were surgically implanted into the prostates of male nude mice. Mice were given subcutaneous doses of 3beta-Adiol (75 mg/kg) for 15 days. Mice were then imaged twice a week for 2 weeks with a Xenogen system. A significant decrease of prostate tumours was observed in the orthotopic animal model treated with 3beta-Adiol with respect to untreated animals.

Conclusions: These data show that 3beta-Adiol is an effective agent against human prostate cancer development and that the estrogenic effect of testosterone derivatives (ERbeta-dependent) inhibits not only cell migration, but also invasion and may be protective against PC invasion and metastasis.