To study the effect of DUSP-1 in angiogenesis, movement of human umbilical endothelial cells (HUVEC) was assessed using Boyden chamber assay. In this assay, HUVEC cells showed an increased migration when media was obtained from H460 rather than H460-siDUSP1. The effect of DUSP-1 in migration was tested by the wounding healing technique. In this case, only H460 cells closed up the wound at 24 hours of post-wounding. H460-siDUSP1 showed lower invasivity potential through the matrigel than H460 cell line. To analyze the role of DUSP-1 in tumorogenesis, 16 nu_/nu_ mice were inoculated with H460 or H460-siDUSP1 cell lines. The last one induced less number of tumours with a slower growth rate than H460 wt.

All together these results indicate that the interference of DUSP1 in H460 cells reduce angiogenesis, cell migration, invasivity, and tumorogenicity, suggesting a main role of DUSP-1 in lung cancer progression.

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Telomere function and p16/RB and p53-mediated senescence pathways in human cancer

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According to telomere hypothesis, telomere shortening prevents somatic cells from dividing and status senescence. However, cell may escape from the senescence barrier if key tumour suppressor genes, especially p53 and/or p16/RB lose their function. Previous results from our group in colorectal cancer (CRC) and non-small cell lung cancer (NSCLC) indicated a differential clinical implication for combined telomerase activity and p16 expression analyses. In this work, our main aim consists of evaluating telomere function in relation to p16/RB and p53-mediated senescence mechanisms in different types of human tumours.

We analyzed telomere function by evaluating telomerase activity and telomere length in a series of CRC and NSCLC tumours obtained from patients who suffered potentially curative surgery. p16/RB and p53-mediated senescence pathways were investigated performing expression assays with oligonucleotide arrays containing 113 genes related to each one of the two senescence pathways. Also, prognosis studies were established.

In NSCLC our data indicated a protective effect for p16 expression in patients showing tumours with significant telomere attrition ($P < 0.05$). However, in CRC it seems more relevant the effect of p53-mediated senescence pathway. Thus, p53 positive expression was a protective parameter in patients with tumours underlying alterations in telomere function. In order to better investigate different roles of these senescence pathways in CRC and NSCLC, following we performed expression studies by arrays. As result, a number of genes from the two pathways showed different expression profiles in relation to telomerase activity and/or telomere length in the two tumour populations considered in this work.

In conclusion, our results suggest a differential impact for p16/RB and p53-mediated senescence pathways in CRC and NSCLC, in relation to telomere function.

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The vasoactive intestinal peptide-receptor system is involved in human glioblastoma cell migration

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Background: Glioblastoma multiforme (GBM) is the most aggressive form of primary brain tumor in adults. This cancer has a highly proliferative and invasive nature and is characterized by inter- and intratumoral heterogeneity. The neuropeptides of the vasoactive intestinal peptide (VIP) family and their receptors (VIP-receptor system) play an important role in the regulation of normal neuronal development, in growth rate of numerous cancer cell lines and also in migration in prostatic and colonic cancer cell lines. Little is known about the involvement of this system in proliferation or migration of GBM cells. Materials and methods: Expression of the VIP-receptor system was studied by RT-PCR, western immunoblotting and binding experiments in two human glioblastoma cell lines, M059K and M059J, established from different regions of a same tumor. The effects of neuropeptides or receptor antagonists of the VIP-system on proliferation or migration of these cells were tested by MTS proliferation and wound healing assays, respectively. The rearrangement of the actin cytoskeleton was visualized by immunofluorescence. Results: The VIP-receptor system was less expressed in M059J cells than in M059K cells. Compared to M059K cells, M059J cells expressed only 20% of VPAC1 receptors, one of the receptors of the system. No effect on proliferation was observed in both cell lines, but differences in migration were found. M059J cells which express less the VIP-receptor system than M059K cells migrated faster. Migration was decreased in neuropeptide-treated M059J cells and was increased in VPAC1 receptor antagonist-treated M059K cells. In agreement with stimulation of migration, a reorganization of the actin cytoskeleton in filopodia was observed in the M059K cells treated with antagonists. Conclusions: The VIP-receptor system is expressed differentially in M059J and M059K cell lines, reflecting intratumoral heterogeneity, and is involved in migration of these cells.

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Marrow-derived mesenchymal stem cells (MSCs) stimulate breast cancer cell secretion and expression of chemokines

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Background: Breast cancer related bone metastasis remains a devastating progression of disease for which no curative therapy exists. Mesenchymal Stem Cells (MSCs) within the bone marrow stroma are postulated to play a role in developing a pre-metastatic niche to support the engraftment and progression of disseminating breast cancer cells. The potential role of MSCs in tumorigenesis is thought to be as a result of their ability to secrete a range of chemokines. The aim of this study was to investigate the effect of direct co-culture with MSCs on breast cancer cell chemokine secretion and gene expression.

Materials and Methods: MSCs were isolated from bone marrow aspirates of healthy volunteers, and their ability to differentiate along connective tissue lineages confirmed. Breast Cancer Cell lines, MDA-MB-231 and T47D, were cultured individually and also on a confluent monolayer of MSCs. Conditioned medium was harvested at 48 and 72 hours from cells cultured individually or in co-culture, and the concentration of chemokines, Stromal Cell-Derived Factor-1α (SDF-1α) and Monocyte Chemotactic Protein-1 (MCP-1), were quantified by ELISA. Epithelial cell specific beads were used to retrieve breast cancer cells following co-culture with MSCs, for RNA extraction. Expression of MCP-1 and SDF-1α was quantified in retrieved tumour cells by RQ-PCR.

Results: Breast cancer cells cultured alone secreted low levels of MCP-1 (48 ± 21pg/ml - Mean ± SEM) while MSCs secreted relatively high levels (1266±141pg/ml). Following 72hrs co-culture, a synergistic effect was observed with MCP-1 levels significantly higher than those seen in the individual populations (MDA-MB-231 + MSC: 7175 ± 1732 pg/ml $p<0.05$, T47D + MSC: 4853 ± 1295 pg/ml, $p<0.05$). In contrast, following 72hrs in co-culture conditions, there was a net decrease in SDF-1α detected, compared to levels secreted by the individual populations (range 27%- 63% decrease in SDF-1α).RQ-PCR analysis of RNA from breast cancer cells retrieved following co-culture with MSCs revealed upregulation of MCP-1 expression in both breast cancer populations, while SDF-1α expression remained virtually unchanged.

Conclusion: MSCs alter the secretion and expression of MCP-1 and SDF-1α in breast cancer cells following direct co-culture. Considering the potential role of these chemokines in developing and cultivating the tumour microenvironment, these interactions may play an important role in the development of bone metastases.

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A potential role for vascular endothelial growth factor-D as an autocrine factor for human gastric carcinoma cells

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Background: Vascular endothelial growth factor (VEGF)-D induces lymphangiogenesis by activating VEGF receptor (VEGFR)-3, which is expressed mainly by lymphatic endothelial cells. VEGFR-3 has also been detected in several types of malignant cells, including lung, colorectal, and prostate carcinoma cells, but the significance of VEGFR-3 expression by malignant cells remains unclear. We have reported the expression and role of the VEGF-C/VEGFR-3 axis in human gastric carcinoma, but a role of VEGF-D in gastric carcinoma has not been characterized. In this study, we examined the expression and function of VEGF-D/VEGFR-3 in human gastric carcinoma cells.

Materials and Methods: We examined the expression of VEGF-D and VEGFR-3 in four human gastric carcinoma cell lines by reverse transcription-polymerase chain reaction. We also used cDNA microarrays