386 The role of DUSP1 in angiogenesis and lung cancer metastases

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MAPK constitute a family of proteins that control cell proliferation, cell migration and apoptosis. Their activation requires phosphorylation of the threonine and tyrosine residues, while dephosphorylation is mediated by a dual specific family of protein phosphatases (DUSPs). The first member identified in this family was DUSP1 and its expression is stimulated by inflammation, oxidative stress or growth factors.

The aim of this study is to gain insight into the cellular pathways involving *DUSP1* actions using a double strategy that combines siRNA and microarray technologies. Specifically, gene expression profiles of H460v (empty vector) and H460cri (DUSP1 siRNA) cells were compared using an array platform. After data analysis, gene expression was validated by qPCR and functional assays were performed. Thus, angiogenesis was tested both *in vitro* and *in vivo* using H460v or H460cri conditioned media, and *cell migration and invasion* assays were carried out using the wounding healing technique and invasiveness, through Boyden chambers. For the *tumourogenesis and metastasis* analysis, nu^-/nu^- mice were inoculated with both cell lines. Finally, we validated our results on human non small cell lung cancer (NCSLC) specimens.

Results showed that some of statistically significant genes were involved in angiogenesis and tumour progression. Angiogenesis studies revealed in H460cri conditioned media a decrease in the migration of HMVEC cells and *in vivo* assays performed with this media in matrigel plugs, showed lower vessel density. This was also observed in solid tumours derived from H460cri cells, which correlated with the decrease observed on VEGFC expression in tumours. On the other hand, we observed less migration and invasive capacity of H460cri compared to H460v cells. When cells were inoculated in nu^-/nu^- mice, H460cri cells induced fewer tumours and metastasis. Metastasis induced by H460cri cells were only found in lungs. However, the animals inoculated with H460v cells developed tumours in several tissues and lower median survival time was observed. Finally, in human NSCLC specimens we found a significant correlation between DUSP1 and VEGFC expression.

All together, our results suggest that DUSP1 targeting in NSCLC, would be a good strategy to inhibit tumour angiogenesis and progression and therefore DUSP1 might represent a good biomaker for combined treatment with chemotherapeutic drugs and angiogenic inhibitors.

Supported by FIS project numbers: PI08/1485 and PS09/00472.

387 An internal domain of dyskerin as a gene therapy approach in X-linked dyskeratosis congenita

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Dyskeratosis congenita is an inheritable syndrome, which is characterised by mucocutaneous abnormalities, cancer predisposition and bone marrow failure. These symptoms are consequence of mutations, which affect different members of the telomerase or shelterin complex, and as result, the length of telomeres is progressively reduced. We have described that GSE24.2, an internal domain of DKC1 gene (dyskerin, a component of telomerase complex) reactivates telomerase activity in X-linked dyskeratosis congenita (X-DC) patient cells.

As we are interested in exploring the therapeutic use of GSE24.2, we have cloned it in a lentiviral vector under the control of a weak promoter (PGK), which also has the EGFP reporter gene. As a control, we have used an EGFP lentiviral vector. We have demonstrated by quantitative-PCR, that transfection of GSE24.2 lentiviral vector in X-DC fibroblasts slightly increases the levels of c-myc versus cells transfect with control vector, as we have previously described. Moreover, in these fibroblasts, expressing GSE24.2, the telomerase activity is increased. We have produced viruses by transfecting this lentiviral vector with packaging vectors in 293T cells, and with use them to infect patient X-DC fibroblasts to stable express GSE24.2. We have observed that the proliferation level of these GSE24.2 expressing fibroblasts is increased versus control fibroblasts. Furthermore, we have concentrated lentivirus by ultracentrifugation to obtain viruses with high titer to infect patient X-DC lymphoblasts with high efficiency and observed that GSE24.2 expression increases telomerase activity in X-DC lymphoblasts. Finally, we have also infected mouse bone marrow stem cells with high efficiency, to be implanted in the bone marrow of a dyskeratosis congenita mouse model. We are currently studying the ability of X-DC stem cells infected with GSE24.2 lentivirus to repopulate the bone marrow and to maintain other tissues affected by this disease.

Supported by FIS project numbers: PI081485 and CIBER de Enfermedades Raras.

388 Positive inter-regulation between beta-catenin and endothelin signaling: critical role of beta-arrestin-1 in the epigenetic regulation of gene transcription in ovarian cancer cells

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Activation of the endothelin A receptor (ET_AR) by endothelin-1 (ET-1) has a critical role in ovarian cancer progression. We previously demonstrated that β-arrestin is recruited to ET_AR to form molecular signaling complexes activating β-catenin/TCF4 transcriptional activity and epithelial to mesenchymal transition. However, the mechanism of β-arrestin-dependent mediated β-catenin transcriptional activation remains poor understood. Here, we show that in ovarian cancer cells, ET_AR promotes epigenetic modification through β-arrestin-1. Thus, as shown by immunoprecipitation, ET-1 promotes the interaction between endogenous β-arrestin-1 and β-catenin in the cytoplasm and in the nucleus. In these cells, ET-1 is a target gene of β-catenin, as shown by effects of gain/loss function of β-catenin on ET-1 expression and activity. Chromatin immunoprecipitation (ChIP) and reporter gene assays revealed that in the nucleus, β-arrestin-1 is selectively enriched at specific ET-1 promoter, and is directly involved in β-catenin transcriptional activity. Moreover, β-arrestin-1 silencing induces the loss of ET-1 mRNA expression and ET-1 secretion, as well as a significant inhibition of ET-1 promoter activity, thus controlling the positive inter-regulation between β-catenin and ET-1/ET_AR autocrine loop in ovarian cancer cells. Moreover, ET-1 promotes the nuclear association between histone acetyltransferase p300 and β-arrestin and the recruitment of p300 on the ET-1 promoter, resulting in H3 and H4 histone acetylation and enhanced ET-1 transcription. Besides ET-1, β-arrestin-1 represents a platform for achieving signal specificity that converges on β-catenin-mediated transcription of defined genes, such as cyclin D1 and matrix metalloprotease (MMP)-2, but not MMP-9. ET_AR blockade with the specific ET_AR antagonist, ZD4054, abrogates the engagement of β-arrestin in the interplay between ET_AR and the β-catenin pathway in controlling gene transcription. In an i.p. model of ovarian cancer metastasis, HEY cells expressing the mutant β-arrestin-1, or treated with ZD4054, metastasized at a reduced rate associated with loss of β-catenin expression, highlighting the importance of β-arrestin-mediated signaling in metastasis formation. Altogether these results reveal a novel function for b-arrestin-1 as a nuclear messenger in ET_AR signalling driving ovarian cancer progression, underpinned by an epigenetic mechanism controlling β-catenin-mediated ET-1 transcription.

Supported by AIRC, AstraZeneca

389 Transition of murine leukemic cells to epithelial-like cells under different growth conditions

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Epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) are basic biological processes during embryogenesis. Recently, it was recognized that EMT/MET are associated with the metastasis of solid malignant tumours to distant sites.

We report that murine leukemic cells of mesenchymal origin acquire an epithelial-like appearance and epithelial cell surface markers upon transfer from in vitro culture to subcutaneous sites in mice. Specifically, C-1498 Acute Myeloid Leukemia (AML) cells of C-57Bl mice origin (NCI Friedrick Institute, MD, USA) inserted subcutaneously in C-57Bl mice form local tumours consisting mainly of cuboidal cells, as observed by light microscopy. (1) The Western blot analysis of tumour samples indicates expression of the epithelial marker, Epidermal Growth Factor Receptor (EGFR), the mesenchymal markers, Vimentin (VIM) and Matrix metalloproteinase 2 (MMP2), as well as Hypoxia Inducible Factor 1a (HIF1a), indicating tumour hypoxia. Thus, the tumour cells express both epithelial and mesenchymal markers. (2) In contrast, cultured AML cells do not express epithelial markers and HIF1a, whereas they do express VIM but not MMP2. Expression of MMP2 in tumours but not in AML cells suggests that stromal cells within tumours contribute to the marker expression. (3) A control murine epithelial tumour cell line expresses EGFR and E-cadherin (E-cad), and lacks VIM, MMP2 and HIF1a. Of note, the subcutaneous tumours do not express E-cad.

We conclude that the tumour cells derived from C-1498 AML cells are not genuine epithelial cells despite EGFR expression. Conversion of AML cells