proteins activity is broadly controlled by phosphorylation, but little is known about cellular signals that regulate their phosphorylation status as well as the specific kinases involved in these effects. Furthermore, other post-translational modifications that affect SC35 functions have not been described to date.
Material and Methods: SC35 acetylation was analyzed "*in vitro*" by performing acetylation assays of recombinant proteins and "*in vivo*" by immunoprecipitation of acetylated proteins. Overexpression and knock-down experiments were carried out in several lung carcinoma cell lines that were treated or not with histone deacetylase inhibitors or DNA damaging agents, in order to investigate the biological consequences of SC35 acetylation on its expression, its phosphorylation level and its activity. Protein or mRNA expression level was analyzed by western-blotting or RT-QPCR, respectively.
Results: We demonstrate that SC35 can be acetylated by the acetyltransferase Tip60 on its lysine K52 residue inside the RNA-binding domain. In addition, we show that Tip60 negatively controls SC35 protein expression by promoting its proteasomal degradation. Importantly, such degradation is prevented by the deacetylase HDAC6. These data provide the first evidence that acetylation/deacetylation pathways control SC35 turn-over. Moreover, we demonstrate that Tip60 negatively affects the phosphorylation status of SC35 by modifying the sub-cellular localization of the kinases SRPK1 and SRPK2, thereby identifying a dialogue between phosphorylation and acetylation networks. Finally, we provide evidence that these networks are required for SC35-mediated apoptosis in response to cisplatin. Indeed, we show that SC35 accumulates in a hypoacetylated and phosphorylated form in cisplatin-treated cells, concomitant with a drastic decrease of Tip60 expression and a nuclear accumulation of both SRPK1 and SRPK2. In this context, we demonstrate that SRPK-mediated SC35 phosphorylation governs cell fate decision (apoptosis versus G2/M arrest) in response to cisplatin treatment and that both SRPK1 and SRPK2 proteins do not act in the same way.
Conclusion: Overall, these results underscore an acetylation/phosphorylation signalling network that controls the turn-over and activity of the splicing factor SC35 in response to genotoxic stress.

413 Oncogenic properties of twist are regulated through its antioxidant activity

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Tumourigenesis results from a series of genetic and epigenetic alterations that promote the malignant transformation of the cell by disrupting key processes involved in normal growth control and tissue homeostasis. These alterations, often identified as DNA lesions can provoke the activation of proto-oncogenes and the inactivation of tumour suppressor genes. This leads to the inhibition of cellular safeguard programs such as apoptosis or senescence, which is a general prerequisite for malignant conversion.
The two functionally related and highly conserved Twist genes (Twist-1 and Twist-2), belong to the super family of bHLH transcription factors. Similarly to other genes controlling embryonic development, Twist-1 and -2 are also involved in tumourigenesis and have been reported to display multiple pro-oncogenic activities. Their oncogenic potential is thought to arise from the combination of multiple properties. First of all, by promoting the epithelial to mesenchymal transition transdifferentiation process, Twist proteins promote invasiness. Moreover by disrupting both Rb- and p53-dependent pathways, Twist proteins additionally override the two main oncogene-induced failsafe programs, senescence and apoptosis, thereby promoting the malignant conversion.
Through a functional study of both members, we made a serie of unexpected observations that led us to identify a new function of Twist. We found indeed that Twist displays an antioxidant activity in primary cells. We demonstrate that these factors are able to inhibit the accumulation of reactive oxygen species in several cell type. Moreover, we show that this activity is involved in inhibition of both apoptosis and senescence and that Twist protects cells from oncogene and oxidative stress induced DNA damage through this new activity.
This discovery should better help understanding the function of Twist and more generally how oncogenes regulate tumour progression.

414 ETV5 transcription factor is upregulated in ovarian cancer and has a role in tumour progression

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Background: Epithelial ovarian cancer is the most lethal gynecological malignancy and the fifth leading cause of cancer deaths in women in the Western world. Largely asymptomatic, over 70% of the patients are already at an advanced stage at initial diagnosis. Five year survival rate for women with advanced stage disease is less than 20%. In contrast, the cure rate is almost 90% when women are diagnosed at an early stage. Ets transcription factors have been implicated in the regulation of gene expression during a variety of biological processes including cell growth and differentiation. In particular, Ets transcription factors are able to activate the transcription of proteases, MMPs and TIMPs, which is central to both tumour invasion and angiogenesis.
Material and Methods: In the present study we have investigated the role of the Ets transcription factor ETV5 in epithelial ovarian cancer. We have analysed ETV5 expression in ovarian tumour samples by quantitative RT-PCR and immunohystochemestry. Knockdown of ETV5 expression in OV90 ovarian cancer cells was achieved using lentiviral siRNA constructs. The phenotype and the biological effects of inhibiting ETV5 expression were analysed by using immunofluorescence and Western blot of cell adhesion markers, and proliferation, migration and adhesion assays. Spheroid cell cultures were used to examine apoptosis under anchorage independent conditions.
Results: We found ETV5 upregulated in ovarian tumour samples compared to ovarian control tissue. In vitro inhibition of ETV5 decreased cell proliferation in serum deprived conditions, induced EMT and enhanced cell migration, decreased cell adhesion to different extracellular matrix components. ETV5 inhibition also decreased cell-to-cell adhesion and induced apoptosis in anchorage independent conditions. Moreover, ETV5 upregulation in a second ovarian cancer cell line induced expression of cell adhesion molecules and enhanced cell survival when cells were grown in an spheroid model.
Conclusions: We propose that upregulation of ETV5 in ovarian tumours would contribute to ovarian cancer cell proliferation in a tumour microenviroment with lack of nutrients. In addition, ETV5 upregulation would play a role in ovarian cancer cell dissemination and metastasis into the peritoneal cavity by protecting ovarian cancer cells from apoptosis and by increasing the adhesion of ovarian cancer cells to the peritoneal wall both through the regulation of cell adhesion molecules.

415 Identification of chemokine CXCR5-CXCL13 cross-talk between malignant neurobalstoma cells and schwannian stromal cells suggests a role in the inhibition of metastatic dissemination

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Background: Among neuroblastic tumours (NTs), the most common and aggressive subtype is neuroblastoma stroma-poor (NB-SP). NB-SP is composed of small undifferentiated Neuroblastic cells (Nbc) and scarce Schwannian Stromal cells (SSc). Most of NB-SP is a metastatic disease, mainly involving the bone marrow. In contrast, ganglioneuroblastoma stroma-rich (GNB-SR) is characterized by abundance of SSc and usually onsets as a localized tumour. SSc are supposed to control tumour growth by secreting soluble factors influencing cell proliferation, differentiation and angiogenesis. Chemokines are a family of secreted cytokines involved in chemotaxis, proliferation and cell-cell interactions and play an important role in tumour growth and dissemination. Recently, we observed that *CXCL13* mRNA was more expressed in microdissected SSc with respect to Nbc by analyzing gene expression profile of NTs. Our result suggests that CXCL13 might have a functional role in the relationship between SSc and Nbc.
Material and Methods: *CXCL13* and *CXCR5* mRNA expression was detected by Real-Time RT-qPCR in 14 NB-SP, 14 GNB-SR, 11 NB cell lines and in Nbc and SSc isolated by Laser Capture Microdissection. Detection of CXCR5 and CXCL13 protein expression in NB cell lines and in GNB-SR sections was performed by immunofluorescence, FACS and immunohistochemistry, respectively. Cell migration of CXCR5+ NB cells was performed by chemotaxis assay. The effects of CXCL13 treatments on NB cells were investigated by MTT proliferation assay.
Results: We have found that *CXCR5* mRNA is more expressed in NB-SP than in GNB-SR and *CXCL13 vice-versa*. Nbc express *CXCR5* whereas SSc express *CXCL13*. NB cell lines show a variegated CXCR5 and CXCL13 mRNA and protein expression but several lines express both *CXCR5* and *CXCL13* suggesting an autocrine loop. In GNB-SR sections, SSc show CXCL13 protein expression but not CXCR5. Furthermore, we observed that CXCR5+ NB cells are able to migrate towards rhCXCL13 and that CXCL13 represses NB cells proliferation.
Conclusions: Our data suggest that the CXCR5-CXCL13 axis could mediate a cross-talk between Nbc and SSc by creating a tumour environment in which malignant neuroblasts are entrapped and inhibited to grow. This mechanism could affect the ability of Nbc to migrate and give distant metastasis and hence it might explain why GNB-SR tumour does not show malignant cells dissemination.