to hypoxia or serum deprivation. Co-IP and ezrin phosphorylation negative mutants demonstrate that ezrin plays a a fundamental role together PKA and phospho-RhoA in driving these alterations.
Conclusions: Altogether, these data lead to the recognition of a synergistic, positive-feedback interaction between the tumour cell and both the metabolic and stromal microenvironments in tumours that can lead to transient changes in the biochemistry and physiology of the tumour cells and elicit further changes in these microenvironments that support invasion. An important aspect for further research will be to determine the signaling systems that integrate the interplay of these various tumour microenvironmental compartments in driving invadopodial proteolysis.

409 Role of reactive oxygen species and autophagy in the generation of neoplastic cells from senescent keratinocytes

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Senescence is a non-proliferative state that occurs in response to telomere shortening or reactive oxygen species (ROS) accumulation. Using normal human keratinocytes, we recently reported that some rare senescent cells can spontaneously reactivate a mitotic process that generate so-called emergent cells which are transformed and able to form skin hyperplasias in nude mice. Several data suggest that the oxidative DNA damage occurring in senescent cells would be the mutagenic motor of this emergence [Cancer Res, 2009. 69, 7917−25]. In parallel, we have shown that most of the senescent cells end-up in programmed cell death through over-activation of (macro)autophagy [Am J Pathol, 2009. 174, 423−35]. We investigated here the relationships between oxidative stress, emergence and autophagy.
Young keratinocytes treated with H_2O_2 underwent premature senescence followed by massive autophagic cell death. Conversely, a catalase treatment, that degrades H_2O_2, delayed senescence and decreased autophagic cell death, hence evidencing the role of oxidative stress in inducing autophagic senescent-cell death. Inhibiting the initiation of the autophagic process with 1 mM 3-methyladenine increased the emergence frequency, suggesting that emergence requires an escape from autophagic cell death. However, a higher drug concentration (5 mM) almost completely abolishes the emergence process, indicating that a minimal level of housekeeping autophagy remains necessary to senescent cells for reinitiating their mitotic program. To determine the more prone to emerge senescent cell subpopulation, we sorted senescent cells according to their Lysotracker® staining as an indicator of their autophagic activity, or to their H_2-DCFDA staining as an indicator of their ROS level, and then monitored for emergence. The results indicated that the more prone to emerge are the senescent cells displaying a moderate autophagic activity, and a moderate level of ROS.
Taken together, these results indicate that the outcome of a senescent cell is dictated by its ROS level. A high ROS level induces a high and lethal autophagic activity. At a lower ROS level, the cell induces a housekeeping autophagic activity that clears up the oxidized components and avoids cell death, and by the way becomes permissive for neoplastic evolution consecutively to the putative oxidative alteration of some oncogenes, tumour suppressor genes or other crucial cell regulators.

410 HER2 as a relevant molecule in tumour initiating cells

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Background: Recent studies on breast cancer cell lines over expressing HER2 have suggested that tumour initiating cells (TICs) cultured as spheres have greater levels of HER2 as compared to the parental counterpart and the therapeutic activity of Trastuzumab seems to be related to its ability to target not only the bulk tumour but also the tumour initiating cells in HER2 amplified tumours.
We investigated whether HER2 is expressed at higher levels in TICs derived from other carcinoma than breast expressing low HER2 levels in comparison with parental cell lines and this peculiar expression can drive TICs more sensible to anti-HER2 therapies.
Materials and Methods: Human cancer cell lines obtained from prostatic (DU-145), vulvar (A-431), head and neck (Cal-27), and pancreatic (PACA44, GER) tumours characterized by low levels of HER2 were used in our experiments in vitro and in vivo. Sphere forming assays were performed and the activity of Aldheyde Dehydrogenase (ALDH) enzyme, the expression levels and the percentage of CD133, CD44v6, ALDH and HER2 positive cells were evaluated using flow cytometry in spheres and in the parental cell lines. Cells were also treated with Trastuzumab and Lapatinib and sphere forming efficiency (SFE) was evaluated. Experiments *in vivo* were performed on nude mice. Animals were injected subcutaneously with tumour fragments and treated with Trastuzumab or saline. At the end of schedule of treatment, tumours were excised and desegregated to obtain a cellular suspension; tumour sphere assays and serial transplantability of cells were assessed.
Results: Spheres were enriched in cells positive for ALDH, CD133 and CD44v6 in comparison with the parental counterpart (1.2 to 8-fold increase), showed higher HER2 levels and higher percentages of CD133/HER2, CD44v6/HER2 and ALDH/HER2 double-positive cells as compared to the parental cell lines (2 to 3.4-fold increase and 1.5 to 7-fold increase, respectively). The SFE of cells treated *in vitro* with Trastuzumab or Lapatinib was significantly lower than in untreated cells ($p = 0.0043$).
Cells isolated from Trastuzumab-treated xenograft tumours showed a decrease up to 4-fold of SFE and the loss of serial trasplantability in comparison with cells from saline-treated xenograft tumours.
Conclusion: Our results provide evidence that HER2 is expressed at higher levels in TICs of solid tumours than in the correspondent parental cell lines suggesting the use of anti-HER2 therapies for the destabilization of tumour stem cell niche.

411 Epithelial plasticity during Epithelial-Mesenchymal Transition (EMT) is associated with alterations of histone H3 modifications

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Background: During cancer progression epigenetic events, like alteration of histone modification markers, co-exist with genetic events and affect cell properties line cell migration and invasion. We analysed the role of global histone modifications, how this modifications may be affected by pathways activated by oncogenes and their association with epithelial plasticity.
Materials and Methods: We generated oncogene-transformed colon cell lines by RASV12, BRAFV600E oncoproteins. Notably, the phenotype of the H-RASV12 oncoprotein-transformed cells (Caco-H) is associated with Epithelial-Mesenchymal Transition (EMT) characteristics [3]. We have shown that E-cadherin is regulated by TAF12 transcription factors [4].
Results: A global histone modification analysis revealed a general de-regulation of histone modification markers, in particular H3K27me3 by H-RAS. Variations of methyl- and acetyl-transferase enzymes as EZH2, JMJD3, PCAF GNC5 and HDACs are associated with appearance of aggressive tumour properties. ChIP analysis has been used to follow histone markers on the promoter of two selected genes Cyclin D1 and the EMT marker gene E-cadherin. Interestingly, Cyclin D1 and E-cadherin genes demonstrate inverse histone repression patterns on their promoter, associated to their inverse expression levels. Furthermore, we verified the dependence of histone modification marker by MER-ERK signalling pathways [5].
Discussion: We show that (a) Cyclin D1 and E-cadherin promoters are regulated by histone modifications in a RAS-dependent manner. (b) EMT associated E-cadherin expression correlates with existence of H3 histone methylation markers on the promoter (c) global histone modification changes and/or their histone modifiers can be proven reliable tumour markers.

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412 An acetylation/phosphorylation signalling network governs turn-over and activity of the splicing factor SC35 in response to cisplatin

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Background: SC35 belongs to the family of serine/arginine-rich (SR) proteins that are crucial regulators of pre-mRNA splicing. It is well established that SR

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.