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