375 The role of A to I RNA editing enzymes in melanoma

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The roles of Adenosine Deaminase Acting on RNA (ADAR) enzymes in carcinogenesis are still mostly unknown, yet aberrant RNA editing patterns and lower ADARs expression have been observed in several human tumours. Here we study for the first time the role of ADAR1 in the biology of melanoma.
ADAR1 knockdown and subsequent rescue cell systems were constructed in the human melanoma cell line 624mel. The effect of altered ADAR1 expression was examined both *in vitro* (proliferation, invasion and cell cycle) and *in vivo* (human xenografts in SCID-NOD mice). In addition, we compared global gene expression of control and ADAR1-knockdown cells. Based on bioinformatic analysis and qRT-PCR of microRNA of two melanoma subclones, the highly aggressive C8161-HAG ($ADAR1^{low}$) and the poorly aggressive C8161-PAG ($ADAR1^{high}$), specific miRs (miR-432*, -17, -20a and -106a) targeting ADAR1−3′UTR were selected and further examined.
Our results demonstrate significant decrease of ADAR1 expression in more than 60% of 30 primary melanoma cultures tested, as compared to normal melanocytes. Importantly, specific knockdown of ADAR1 expression *in vitro* altered cell morphology and revealed a substantial and consistent enhancement in cell proliferation and invasion capabilities. Introducing ADAR1 into the knockdown cells successfully increased ADAR1 expression levels and reversed the effects on proliferation and invasion rates. *In vivo*, ADAR1 knockdown dramatically increased the growth rate of melanoma and caused intense black pigmentation. Moreover, the comparative global gene expression analysis confirmed that the most prominently altered gene clusters were cell growth, proliferation and motility. Over-expression of miR-432* and miR-17 in C8161-PAG melanoma subline significantly decreased ADAR1 expression and increased its net proliferation rate. These combined results strongly support the role of ADAR1 as a central regulator of cancer features in melanoma and imply that microRNAs may regulate its expression.
ADAR1 is ubiquitously expressed and downregulated in many tumours, therefore these findings may represent a general cellular mechanism, not confined to melanoma. Further unraveling of the function and regulation of ADAR1 may lead to the identification of novel diagnostic, prognostic and therapeutic interventions.

376 Regulation of tumour cell invadopodia by hypoxia-induced NHE-1 activation

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Invasive tumours are characterized by an acidic and hypoxic microenvironment that promotes metastasis. The Na+/H+ exchanger (NHE-1) plays an important role in the regulation of pH homeostasis. In tumour cells, it has been demonstrated that NHE-1 is constitutively active, promoting cell invasion, but the mechanisms are not defined. One of the ways that tumour cells are able to promote invasion is by forming invadopodia. Invadopodia are actin-rich structures that secrete matrix-metalloproteases (MMP) and are able to degrade the extracellular matrix. Our lab has shown that hypoxia significantly increases invadopodia formation in human fibrosarcoma cells (HT-1080). Also, it has been demonstrated that MMP matrix-degrading activity is dependent on extracellular acidic pH. Therefore, the aim of our study was to define whether NHE-1 participates in hypoxia-induced invadopodia production.
First, we observed that hypoxic stimulation increased NHE-1 RNA and protein expression. Intracellular pH monitoring by live-cell imaging revealed that NHE-1 activity was also increased under hypoxic conditions. Results using pharmacological inhibitors and shRNA-mediated depletion indicated that NHE-1 participates in invadopodia formation in HT-1080. NHE-1 inhibition also abolished invadopodia-mediated matrix degradation and this correlated with an inhibition of hypoxia-induced MMP-2 activation. Moreover, the ECM degradation areas induced by hypoxia clearly correlated with the presence of discrete puncta of NHE-1 at invadopodial structures.
Alltogether, our results suggest indicated that NHE-1 is necessary for both the formation of the invadopodium structure and the activity of proteinases involed in invadopodia-induced matrix degradation under hypoxia.

377 Oncogenic role of neuropilin-2

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Background: Neuropilins (NRPs) are transmembrane non-tyrosine kinase glycoproteins originally described in the nervous system. Initially characterized as neuronal receptors, NRPs were also found to be expressed in endothelial cells and subsequently were shown to play a role in the development of the vascular system. The multiple functions of NRPs were recently highlighted by the identification of NRP role in oncogenesis. In this study, we first confirmed the role of NRP2 in tumour progession in vitro and in vivo. We also extended the understanding of NRP2 oncogenic functions by investigating the ability of NRP2 to orchestrate epithelial-mesenchymal transition (EMT) in colorectal cancer cells.
Methods: We first sought to examine the expression of NRP2 glycoprotein in various cancer cell lines and tumoural tissues by immunofluorescence analysis and immunohistochemical staining. Using specific siRNA to target NRP2 expression, or NRP2 gene transfer we studied the influence of NRP2 expression on proliferation and tumour formation in vitro by MTT and soft agar assays and then in vivo using xenografts experiments. NRP2 induced-EMT was then investigated by flow cytometry, immunohistochemical (IHC) staining and quantitative real-time PCR.
Results: Immunofluorescence analysis confirmed that NRP2 is expressed at the membrane of several human cancer cell lines. Moreover, IHC staining showed that NRP2 is expressed at the membrane of human colon carcinoma and breast carcinoma while it is not expressed in non malignant tissues. Our results first confirmed the role of NRP2 in cancer proliferation *in vitro* and xenograft formation *in vivo*. Analysis of NRP2 transfected cell lines and NRP2 expressing xenografts established that NRP2-expressing tumour cells displayed an immunohistochemical phenotype of EMT characterized by the loss of E-Cadherin and an increase of vimentin expression. Moreover, the expression of NRP2 on colon cancer cell lines was shown to promote transforming-growth factor-β1 (TGF-β1) signaling, leading to an increased phosphorylation of the Smad2/3 complex in colorectal cancer cell lines. Specific NRP2 inhibition using siRNA prevented the promoter effect of TGF-β1 on colony formation.
Conclusions: Our results suggest a direct role of NRP2 in EMT and highlight a cross-talk between NRP2 and TGF-β1 signaling to promote cancer progression. These results suggest that NRP2 fulfill all the criteria of a therapeutic target to disrupt multiple oncogenic functions in solid tumours.

378 Aberrant phosphatidylcholine metabolism as source of biomarkers and therapeutic targets in human ovarian cancer

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Background: The detection and characterization by MRS of aberrant phosphatidylcholine (PC) metabolism in tumours fostered new areas of investigation in cancer cell biology and allowed identification of indicators of in vivo tumour progression by of choline-based MRS and PET.. We showed a 2- to 5-fold increases in total choline containing metabolites (tCho), with parallel 3- to 8-fold increases in phosphocholine (PCho), in human epithelial ovarian cancer (EOC)cells compared to non-tumoural cells (EONT) (Iorio et al, Cancer Res 2005).
Purposes of this study was to investigate the molecular mechanisms underlying aberrant choline metabolism in preclinical models of ovary cancer.
Methods: Human EOC cell lines were established from ascitic fluid or from primary tumours; EONT cells were either isolated from normal ovary surface epithelium or its immortalized variants. MRS analyses were performed on cell extracts at 16.4 or 9.4 T. Microarray-based gene expression was evaluated by Gene Set Enrichment Analysis on EOC and EONT data sets. Western blot experiments were performed using polyclonal rabbit anti-human Chok antibody [Glunde et al, Cancer Res 2005] and polyclonal rabbit anti-B. cereus antibodies (Spadaro et al, Cancer Res 2008). MRS/MRI were performed in human EOC xenografts at 4.7T.
Results: The increase in PCho content in cancer cells was associated with activation of enzymes involved in both biosynthetic and catabolic PC pathways. Choline Kinase (ChoK) was over-expressed at protein level (about 3×) and activated (9−25 ×) in EOC cells. Moreover, the mRNA level of ChoKα isoform was constitutively over-expressed in cancer cells, in presence of unaltered levels of the other Kennedy pathway enzymes. PC-specific phospholipase C (PC-plc) protein was also over-expressed (ca 3×) and activated (up to 17-fold) in EOC cells (Iorio et al, Cancer Res March 2010). Exposure of EOC cells to a D609 (inhibitor of PC-plc) led a drop in PCho content and reduced cell proliferation, in the absence of apoptosis. Furthermore, evidence in our laboratory also showed that: (a) activation of PC-plc was associated in EOC cells with accumulation of this enzyme on the external plasma membrane; and (b) D609 induced long-lasting block in the recovery of both PC-plc activity and S-phase fraction in receptor re-stimulated cancer cells (Spadaro et al, Cancer res 2008).
The availability of intra-peritoneal and sub-cutanous EOC xenotransplant models, allowed the identification of the tCho signal as the highest resonance in the in vivo spectra of xenografts, opening the possibility of preliminary clinical