1058 POSTER

Adaptor Protein Ruk/CIN85 Expression in Human Tumours

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Background: The characterization of molecular alterations in each single tumour is at the basis of personalized anticancer approaches aimed to give each patient the most appropriate therapy. Adaptor/scaffold proteins are the key components of signalling networks involved in the control of cell physiology. In particular, by binding to numerous effector proteins adaptor/scaffold protein Ruk/CIN85 assembles multimeric complexes implicated in control of receptor tyrosine kinase signalling, neuronal and T cells apoptosis, adhesion and invasion.
Material and Methods: Using Western-blot analysis, samples of uterine cervix, stomach, skin, thyroid, brain tumours and adjacent normal tissues were analyzed.
Results: Increase of Ruk/CIN85 full-length form (p85) expression was revealed in uterine cervix, stomach and skin cancer samples in comparison with corresponding control samples. Down-regulation of p85 was revealed in the majority of thyroid tumour samples in comparison with adjacent normal tissue samples. Using anti-Ruk_S Western-blot analysis, multiple molecular forms of CIN85/Ruk with molecular weight of 140, 130, 85, 70, 56, 50, 40 and 38 kDa were detected. Changes in expression level of some Ruk/CIN85 multiple molecular forms as well as up-regulation of low-molecular mass forms (40 and 34 kDa) were detected in skin cancer samples. Expression level up-regulation of high-molecular (140 and 130 kDa) and low-molecular (40 and 34 kDa) Ruk/CIN85 multiple molecular forms of thyroid tumour, renal tumour and glioblastoma samples in comparison with conditionally normal tissue were revealed. The additional feature of Ruk/CIN85 forms expression patterns in uterine as well as stomach malignancies was the high content of p70, p40 and p34 forms while control tissue samples were characterized by the predominant increase of high molecular forms (p140, p130, p100).
Conclusions: The obtained results suggest that changes in the expression level of multiple molecular forms of CIN85/Ruk in tumour samples can lead to the loss of coordinated control of apoptosis and proliferation in the transformed cells. These data will offer new opportunities for the identification and validation of key molecular tumour targets to be exploited for novel therapeutic approaches.

1059 POSTER

Sensitizing Hemopoietic Malignant Cells to Glucocorticoid Induced Apoptosis by PK Inhibitors

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Glucocorticoids (GCs) are widely used in the therapy of lymphomas and lymphoblastic leukemias owing to their apoptogenic effects on these cancerous cells. A major impediment of GC-based therapy is the gradual acquisition of apoptotic resistance to GC treatment. Also, certain lymphomas and leukemias are *a priori* resistant to GC. Therefore, a desirable goal is to develop therapeutic strategies that confer GC-sensitivity on otherwise GC-resistant cells. We observed that the broad-acting protein kinase (PK) inhibitor Staurosporine (STS) confers GC-sensitivity on several GC-resistant T and B lymphoma cells. GC-resistant T lymphoma cells express elevated levels of the anti-apoptotic proteins Bcl-2 or Bcl-X_L. Transfection with Bcl-2 or Bcl-X_L in sensitive cells confers resistance to GC-induced apoptosis. Surprisingly, STS overcomes the anti-apoptotic properties of Bcl-2 but not of Bcl-X_L. STS acts at several levels. It induces the expression of the pro-apoptotic Nur77 orphan receptor, which overcomes the anti-apoptotic effects of Bcl-2. STS also leads to phosphorylation of Bim by an ERK-dependent mechanism which results in Bim upregulation. In addition, STS inhibits PI3K/Akt, leading to the activation of GSK3. Inhibition of GSK3 by its specific inhibitor SB216763 or by overexpression of a dominant negative GSK3 attenuated the effect of STS. Our study demonstrates a central role for GSK3a, but not GSK3b, in promoting GC-induced apoptosis. We found that GSK3a is sequestered to the glucocorticoid receptor (GR) in the absence of ligand, but dissociates from the GR complex upon exposure to GC to promote apoptosis. GC-resistance in lymphoma cells can be relieved by inhibiting the PI3K-Akt survival pathway, which inactivates GSK3 by its phosphorylation. Notch1, a transcription factor frequently activated in T acute lymphoblastic leukemia(T-ALL), confers GC resistance through activation of Akt. Indeed, inhibition of Akt is effective in sensitizing T-ALL cells to GC induced apoptosis. Our data demonstrate that lymphoma and leukemia therapy can be significantly improved if GCs are combined with PK inhibitors that shift the cell's kinome in favor of apoptosis-prone phenotype.

1060 POSTER

The Conserved Length of the C- Terminal Tail is Essential for the Activity of Mdm2 RING Towards p53

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Background: Tumour suppressor p53 play a key role in the regulation of responses to various types of cellular stress. The importance of p53 is highlighted by the fact that p53 is mutated and its cellular activity lost in about half of all human cancers. Another way by which cancer cells inactivate p53 is the overexpression of inhibitory proteins Mdm2 and MdmX, RING domain proteins that are critical negative regulators of p53 in normal cells and during development. Mdm2 exhibits E3 ubiquitin ligase activity and is capable of regulating its own levels and the levels of p53 through proteasome-mediated degradation. Despite a strong similarity with Mdm2, MdmX does not possess the ubiquitin ligase function. However, MdmX is able to contribute to the E3 activity of Mdm2 by forming a stable heterodimer with Mdm2 through their RING domains. The RING finger domains of both Mdm2 and MdmX are located close to the C-terminus of the proteins, with the last cysteine of the RING followed by only 13 amino acids. We have shown previously that this extreme C-terminal tail is essential for the E3 activity of Mdm2, as well as for its ability to dimerize through the RING domain. Analysis of the sequences of the extreme C-terminus of various RING domains showed that the length of the C-terminal tail is highly conserved through evolution and suggested that it could be important for the biological activity of proteins containing the RING domain at their C-terminus.
Materials and Methods: The Mdm2 mutants were created using site-directed mutagenesis. The activity of mutants was tested in p53 degradation, ubiquitylation, immunoprecipitation and immunofluorescence assays. Human U2OS and HEK293T were transfected using Lipofectamine 2000 reagent and analyzed by Western blotting or fluorescence microscopy.
Results: We have created Mdm2 mutants containing different number of extra amino acids at the C-terminus to study the role of the length of the C-terminal tail in Mdm2 activity. Our results indicate that the conserved length of Mdm2 C-terminus is critical for Mdm2 activity towards p53 while not being essential for the ability of Mdm2 RING domain to dimerize. All mutants retained the ability to oligomerize with the related protein MdmX, but lost the ability to ubiquitylate and degrade p53. Interestingly, mutants with C-terminus extended by five extra amino acids were able to degrade p53 as part of a complex with wild-type Mdm2 RING domain. In contrast, mutants extended by more than five amino acids could not be reactivated. Surprisingly, all Mdm2 mutants were reactivated when coexpressed with MdmX, regardless of the length of the C-terminal extension.
Conclusions: Taken together, these results confirm our previous observations indicating that MdmX can cooperate with Mdm2 in the form of heterodimer targeting tumour suppressor p53. Moreover, our new data suggest that the Mdm2 homodimers and Mdm2-MdmX heterodimers may not be fully functionally equivalent to each other.

1061 POSTER

Foxo3a Loss is a Key Event in High-grade Pelvic Serous Carcinogenesis

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Background: Attempts to discover early-detection biomarkers and candidate pathway for targeted therapy are currently based upon genome-wide exploration of expression profiles of the malignant vs. the benign states. Little progress has been achieved in high-grade serous ovarian carcinoma, due to the fact that the cell-of-origin has been elusive until recently. With the identification of the fallopian tube secretory epithelial cells (FTSECs) as the cell-of-origin of most serous carcinomas, the analysis of differences in pathways' expression and activation is now achievable.
Materials and Methods: We developed a set of *hTERT* immortalized FTSEC lines, derived from normal human fallopian tube specimens. We performed expression profiling in comparison to either high-grade serous tumours, or ascites-derived primary serous carcinoma cells.
Results: We detected *FOXO3a*, a transcription factors known to be involved in cell cycle arrest and apoptosis, as being significantly down-regulated during serous carcinogenesis. FOXO3a protein was lost as early

as in the in-situ serous carcinoma of the FT. The down regulation results from hemizygous loss in many of the tumours and from activation of the PI3K/AKT and the Ras/MEK/ERK pathway, which targets FOXO3a for degradation, and in some cases due to up-regulation of miR-182. We managed to restore partial activity of FOXO3a using inhibitors of these pathways.
Conclusions: The immortalized benign FTSEC lines are an important asset for the identification of early-detection biomarkers and 'drugable' pathways in serous carcinoma. FOXO3a loss may be a key event in the progression into an invasive disease. It is possible to rescue FOXO3a activity with currently available experimental drugs.

1062 POSTER

Blood and Lymphatic Vessels: Early Crucial Players of Malignancy and Metastasis in Cervical Cancer

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Background: Cervical neoplasia remains one of the most controversial issues for clinicians, pathologists and researchers. Screening programs reduced the incidence of invasive neoplastic lesions but did not change the rate of precursor lesions. Usually, malignant lesions of the uterine cervix are considered more important than precursor lesions.
Angiogenesis and lymphangiogenesis are accepted as important factors favouring tumour growth and metastases. But, questions about (i) startpoint of angiogenesis and lymphangiogenesis in cervical lesions, (ii) proliferative and/or activated status of cervical neovessels or (iii) the origin of lymph vessels and prognostic impact of lymphangiogenesis in precursor lesions of the uterine cervix still remain without a precise response.
Material and Methods: One hundred and twenty eight specimens of benign, premalignant and malignant cervical lesions were included in the present study. Co-localisation of Ki67 proliferation marker with CD105 in blood vessels endothelium and D2−40 in lymphatic endothelium was obtained by applying doublestain method followed by use of two different chromogens (3.3′diaminobenzidine for nuclear brown staining of Ki67 and aetil amino charbazole for cytoplasmic red staining of CD105 and D2−40).
Results: Specimens evaluation of normal, premalignant and malignant lesions of the uterine cervix revealed that activation and proliferation of blood vessels in cervical lesions are distinct processes. Activation of endothelial cells is an early event which predominate in benign and premalignant conditions of the uterine cervix while endothelial cell proliferation is observed in tumour vessels endothelial cell from cervical invasive carcinoma. Lymphangiogenesis is an early event in the pathogenesis of cervical lesions. The highest number of proliferative lymphatic vessels (D2/40+/Ki67+) was significant correlated with low grade intraepithelial lesions (LSIL, $p = 0.009$), high grade intraepithelial lesions (HSIL, $p = 0.044$), and microinvasive carcinoma ($p = 0.002$). The last correlation also persist in invasive carcinoma.
Conclusions: Our data showed that early lymphatic endothelial proliferation in preneoplastic stages of cervical lesions preceed the development of the angiogenic switch. Angiogenic process also begin in preinvasive lesions stages of cervical lesions and had different and distinct mechanisms.

1063 POSTER

Vasohibin-1 and Vasohibin-2 Are Expressed in Both Gastric Cancer Cells and Tumour-associated Macrophages and Play Roles in Anti-Angiogenesis Not Only as Intrinsic Inhibitors

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Background: Recently, Vasohibin-1 and vasohibin-2 are found in endothelial cells and considered as two intrinsic anti-angiogenesis factors. However, So far, we don't know whether they are expressed in cancer cells themselves and tumour-associated macrophages (TAMs) which have been confirmed to contribute to tumour progression.
Materials and Methods: Realtime RT-PCR were used to quantitatively investigate the vasohibin-1 and vasohibin-2 expression in four gastric cancer cell lines including non-metastatic cell line AGS and metatatic cell lines HGC-27, Hs-746T and NCI-N87 with or without co-cultured with TAMs, as well as their expressions in TAMs under normal or hypoxia condition. Furthermore, the correlation between vasohibin-1, vasohibin-2 and VEGF-A expressions were analyzed under different culture condition.
Results: Both vasohibin-1 and vasohibin-2 were expressed in four gastric cancer cell lines and TAMs. Under normal condition, vasohibin-1 and vasohibin-2 expression were up regulated significantly by macrophages in four gastric cancer cell lines. Under hypoxia condition, both vasohibin-1 and vaosohibin-2 expression were decreased significantly in distant metastasis cancer cell line Hs-746T ($P < 0.001$), moreover, the increase induced by macrophages was also down regulated significantly in Hs-746T cell line($P < 0.001$). The regulations for vasohibin-1 and vasohibin-2 expression by macrophages and hypoxia had correlation with VEGF-A expression. In addition, hypoxia induced vasohibin-1 and vasohibin-2 significant up-regulations in TAMs co-cultured with metastatic cancer cell lines ($P < 0.05$).
Conclusions: Both vasohibin-1 and vasohibin-2 was expressed in gastric cancer cells and TAMs, and their expression were regulated by TAMs and hypoxia. Vasohibin-1 and vasohibin-2 might not only be an intrinsic angiogenesis inhibitors in endothelial cells, but also play important roles in anti-angiogenesis as an extrinsic inhibitors mediated by TAMs. Vasohibin-1 and vasohibin-2 might be as a novel anti-angiogenesis target in the treatment of gastric cancer.

1064 POSTER

Adaptive Exploitation of Stromal Cell Metabolism by Tumour Cells

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Background: Tumour cells secrete factors to recruit and activate stromal cells in the tumour microenvironment (TME) leading to reciprocal paracrine support of tumour growth by stroma-derived growth factors. This is an important means by which tumours adapt their microenvironment to facilitate their growth. Indeed, breast cancer development and metastatic progression is highly dependent on stromal support, particularly from carcinoma associated fibroblasts (CAFs). As a result of aerobic glycolysis, tumour cells produce and secrete high levels of lactate, thought to be a toxic byproduct that needs to be extruded into the tumour milieu.
Using *in vitro*-generated CAFs, we investigated the role of lactate in CAF-mediated support of tumour growth. In addition to extruding lactate as a byproduct of glycolysis, we suggest that tumour cells secrete it to recruit and subsequently exploit stromal cells to recycle lactate into utilizable metabolites, such as pyruvate, to fulfill metabolic demands of tumour cells.
Materials and Methods: We used a lactate analyzer (Roche Diagnostics) to quantify lactate in media; transwell migration assays were used to measure lactate-induced *in vitro* migration; RT-PCR was used to determine expression of genes involved in lactate transport; ^{13}C NMR spectroscopy was used to track the metabolic fate of lactate; luciferase assays were used to monitor growth of tumour cells.
Results: We find that MDA-MB-231 breast cancer cells (MDAs) secrete significantly higher levels of lactate under hypoxia, and that lactate recruits mesenchymal stem cells (MSCs), the precursors of CAFs. Lactate is transported by monocarboxylate transporters (MCTs); cells take up lactate via MCT1 and efflux it via MCT4. Expectedly, MDAs display low expression of MCT1 while exhibiting high expression of MCT4. However, CAFs show high expression of MCT1 while displaying low expression of MCT4, indicating that lactate extruded by the tumour cells is taken up by stromal cells, in a source-sink manner. NMR analyses indicate that ^{13}C-lactate is metabolized via the Krebs cycle in stromal cells. Finally, pyruvate-mediated tumour cell growth assays indicate that CAFs may serve to evacuate lactate from the TME, thereby reducing lactate-mediated inhibition of stroma-derived pyruvate influx into tumour cells.
Conclusions: Thus, stromal cells in the TME (1) have the capacity to take up tumour-secreted lactate and use it as an energy source, and (2) may provide subsequent/surplus metabolites, such as pyruvate, to tumour cells as a secondary source of energetic and biosynthetic precursors. To our knowledge this is the first *in vitro* model system demonstrating tumour/stroma metabolic coupling by which tumour cells exploit stromal cells. A better understanding of the molecular mechanisms governing metabolic cooperation within the tumour milieu will potentially identify new targets for therapeutic intervention.

1065 POSTER

An in Vitro Comparative Study of Fulvestrant and Tamoxifen in Breast Cancer Cells

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Aim: In the current study, two selective antagonists of the estrogen receptor (ER), fulvestrant which suppresses and degrades the ER (SERD, selective estrogen receptor down regulator) and tamoxifen which modifies the function of the ER (SERM, selective estrogen receptor modulator) were