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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.

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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.

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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.

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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