of ILEI in EpH4 and EpRas cells caused EMT, tumor growth and metastasis. RNAi-mediated knock-down of ILEI in EpRas cells before and after EMT (EpRasXT) prevented and reverted TGFbeta-dependent EMT, also abrogating metastasis formation.

ILEI (FAM3C) belongs to the FAM3 family of secreted cytokines. Thus, the simplest explanation for the effects of ILEI overexpression in epithelial cells might be an autocrine action of the secreted protein. However, it was difficult so far to show this with purified recombinant ILEI. Our aim is to understand the way of ILEI action and find possibilities for potential therapeutic interference with the pathway.

Initially, Western blot analysis showed that the secreted form of the ILEI protein is smaller in size than intracellular ILEI. Mass Spec data confirmed the lack of 17 amino acids at the N-terminus in addition to the signal peptide sequence, giving a strong indication for additional proteolytic processing of ILEI. The cleaved form was not detectable in whole cell extracts. In ILEI cleavage assays using purified full length protein we found, that ILEI was cleaved extracellularly, mostly by serum proteases.

To investigate the role of ILEI processing, we generated a series of mutant ILEI forms with the hope being defective in proteolytic cleavage. Using these mutants in overexpression studies we could identify essential amino acids for proteolytic cleavage and secretion. Some mutants were defective in proteolytic processing but not in secretion and we found one mutant which was neither cleaved nor secreted. Surprisingly, all overexpressed non-cleavable ILEI forms were able to induce EMT, including the non-secretable form.

These findings show first, that proteolytic cleavage is not essential for ILEI secretion, providing additional support for extracellular processing of the protein. Secondly, these data indicate that proteolytic processing is not required for ILEI action, raising the question, if full length ILEI might have higher biological activity than the cleaved form. Finally and most unexpectedly, these data show that a sole intracellular action of ILEI can induce EMT in vitro. Currently, we are investigating the capacity of these mutant ILEI forms for metastasis induction, to reveal if autocrine or paracrine functions of this cytokine are required for tumor progression.

556 Poster

Overactivation of STAT3 by interferon-alpha may negatively influence disease outcome in melanoma patients

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Background: Malignant melanoma is one of the most chemo- and radiotherapy resistant tumours. Few years ago, interferon-α (IFN) has been introduced as an adjuvant treatment of this disease. It stimulates immune defence mechanisms and possesses antiproliferative and proapoptotic activity. However, clinical experiences showed that the response rate was in some patients less than expected to be. Impaired function of some signalling proteins may negatively affect treatment response. Of those, the frequent cancer-associated perturbations were described in two members of STAT family (Signal Transducer and Activators of Transcription) i.e. STAT1 and STAT3. While the former protein behaves as a tumour suppressor, the latter acts as an oncogene. IFN-α activates these proteins by phosphorylation of tyrosine and serine residues. Since STAT 3 transactivates growth-promoting and anti-apoptotic genes we have hypothesized that hyperactivation and/or overexpression of STAT3 induced by IFN-α may negatively affect disease outcome and interfere with the therapeutical effect of this cytokine. No valid data about the association of STAT3 abnormal expression and activation with the clinical parameters of malignant melanoma are available. In this study we investigated the activation response of STAT3 to IFN-α in melanoma cells derived from node metastases and evaluated the possible connection of phosphorylation responses with the course of disease within 5 years follow up.

Material and methods: Melanoma short-term cultures were established from lymph node metastases of 24 patients. Malignant cells as well as normal melanocytes were treated with IFN-α and phosphorylation profiles of STAT3 were determined by Western blot using specific antibodies. STAT 3 phosphorylation responses of individual patients were correlated with disease evolution and statistically analysed.

Results: Our results demonstrated that patients disclosed as activation responders to IFN-α, i.e. whose ex vivo metastatic melanoma cells showed IFN-α-induced STAT3 phosphorylation at Tyr705, exhibited significantly shorter disease-free survival (5 vs. 34,9 months; p=0,049), shorter progression-free interval (26,1 vs. 62,3 months; p=0,041) and shorter overall survival (26,5 vs. 78,4 months; p=0,039) as compared to the non-responder group.

Conclusions: Our data provide evidence that activation of STAT3 at Tyr705 by IFN-α negatively correlates with disease outcome.

557 Poster

Modulation of cell cycle by extracellular p27

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p27Kip is a cell cycle regulator that, when abundant, binds and inhibits kinase activity of cyclin/cdk complexes necessary for G1/S transition. It has also been proven that p27 is able to induce apoptosis. Through the cell cycle, p27 expression level is maintained by transcriptional, translational and posttranslational mechanisms. Apparently, the most important mechanism of reducing p27 level is ubiquitin-mediated proteolisis. Deregulation in signaling pathway for ubiquitination of p27 is believed to be important for development of cancer in numerous tissues.

In this study the influence of extracellular p27 on proliferation and apoptosis of different cell lines was examined. For that purpose TAT fusion proteins: TAT-p27 (wt), TAT-ptp27 (point mutation) and TAT-N' p27 (truncated form) were transduced in RKO and Raji cell lines as well as in MCF7, which is caspasis 3 negative. The influence of examined proteins on proliferation was monitored by MTT or WST test. Additionally, effect of extracellular p27 on cell cycle and apoptosis was measured using flow cytometry. The expression of different cell cycle and apoptosis regulatory proteins was determined by Western blot.

After the transduction of p27 variants in the investigated cell lines, halt in the proliferation was detected with MTT and WST test. Flow cytometry has shown the elevation of the amount of both, the cells in G1/G0 phase of the cell cycle, as well as dead cells, after the treatment with wt and mutated p27. In RKO and Raji cells treated with p27 wt and p27 mut caspasis 3 activity was found to be raised. On the other hand, in caspasis 3 negative MCF7 cells, treated with p27 wt and p27 mut the expression of proteins in caspasis 3 independent pathway was found to be changed compared to non treated cells. These results show that the influence of extracellular p27 depends on the type of cells and transduced protein.

The extracellular p27 lead to apoptosis in examined cell lines. It seems that in different cell lines, apoptosis was induced by different pathways. According to these results, modulation of p27 expression could be a good candidate for targeted tumor therapy.

558 Poster

Implication of MAPK signalling pathways on cold stress-induced apoptosis in a multidrug resistant leukaemic cell line

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We have shown that acquisition of Multidrug-resistant (MDR) phenotype by leukaemic cells is accompanied by pleiotropic changes that result on reduced tumour capacity to survive under stress conditions such as hypothermia. Thus, the study of the signalling pathways implicated on it, are fundamental on the design of new approaches to eliminate drug-resistant tumours. For this purpose, we have studied expression and activation of signalling molecules involved on the fate of the cells (survival or cell-death) like Akt/PKB and p38, ERK1/2 and JNK1/2 MAP kinases. We have found that leukaemic cells with MDR phenotype show a different activation profile for Akt/PKB and MAPK signalling molecules versus their sensitive counterparts when exposed to low temperatures. Furthermore, the use of different inhibitors show that Akt or p38 are not involved in cold-stress induced cell death. However, the use of the ERK inhibitor PD98059 and JNK Inhibitor II, partially counteract hypothermia-induced cell-death on resistant cells. Together, these findings indicate the existence of a collateral sensitivity of MDR leukaemic cells to extreme low temperatures due to alterations of the signal transduction pathways involved on regulation of cell death and survival after treatment with anti-neoplasic drugs.

559 Poster

The proteolysis of ERalpha induced by thiazolidinediones in breast cancer cell lines is a PPAR-independent event

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The aim of this study was to identify the mechanism leading to ERalpha degradation in breast cancer cell lines exposed to ligands of Peroxisome