is known that inhibitors of HDACs (HDACi) induce cell cycle arrest and apoptosis of pancreatic cancer cells, the contribution of the individual HDACs to the carcinogenesis of pancreatic cancer is unknown so far.

Materials and methods: Expression of HDAC2 in pancreatic ductal adenocarcinomas was investigated using immunohistochemistry of tissue microarrays. Function of HDAC2 was analyzed using RNA interference in pancreatic cancer cell lines. Results were reproduced using the selective class I HDACi valproic acid. Proliferation and viability was measured using BrdU and MTT assays, respectively. Apoptosis was analyzed using Hoechst stains and PARP western blots.

Results: Immunohistochemistry and scoring reveals for the first time the overexpression of nuclear HDAC2, especially in undifferentiated pancreatic ductal adenocarcinomas. The knockdown of HDAC2 neither impaired proliferation nor reduced viability of pancreatic cancer cell lines. Instead we observed a sensitization towards death receptor (TRAIL)- and chemotherapy (etoposide)-induced apoptosis. In line, the selective class I HDACi valproic acid sensitizes pancreatic cancer cells towards death receptor (TRAIL)- and chemotherapy (etoposide)-induced apoptosis in a time- and dose-dependent fashion, without change of proliferation and viability.

Conclusions: Taken together, these data suggest a pivotal role of HDAC2 in regulating anti-apoptotic signaling and therapeutic resistance in pancreatic ductal adenocarcinoma. Therefore, targeting HDAC2 could be a promising future approach for the treatment of this dismal disease.

269 Poster

Kallikrein-related peptidase 6 overexpression promotes non-small cell lung cancer cell proliferation and is associated with poor patient outcome

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Background: The human kallikrein-related peptidases (KLK) are a family of serine proteases that are often aberrantly expressed in common human malignancies and contribute to neoplastic progression through multifaceted roles.

Materials and Methods: We evaluated KLK6 expression in the tumoral and normal adjacent lung tissue of 56 patients with Non-Small Cell Lung Cancer (NSCLC) by real-time RT-PCR and immunohistochemistry. To determine the impact of KLK6 overexpression on the growth of lung cancer cells, we integrated the cDNA encoding the complete sequence of KLK6, through homolog recombination, in a NSCLC line (A549 Flp-In) and determined the growth rate of two independent clones. Progression of the KLK6- and parental cells inside the cell cycle was assessed by flow cytometry following synchronization of cells at the end of the G1 phase with starvation and hydroxyurea treatments. Key regulator proteins of the cell cycle were analyzed by Western blot in synchronized and unsynchronized cells.

Results: We found KLK6 transcript up-regulation in tumor tissues from patients with NSCLC and association of KLK6 status with low patient survival. KLK6 immunoreactivity was restricted to epithelial cells of normal bronchi and detected in most of cancer samples, in which KLK6 signal intensity correlated with well differentiated tumors. Ectopic expression of KLK6 dramatically enhanced NSCLC cell growth. Analysis of cell cycle progression revealed that promotion of cell growth caused by KLK6 results from an acceleration of cell cycle progression through G1/S transition, which was accompanied with a marked increase of cyclin E and repression of p21. In addition, expression of KLK6 in NSCLC cells was associated with an increase of c-Myc that is well-know to promote cell-cycle progression via regulation of cyclin D/E activation and down-regulation of p21.

Conclusion: In conclusion, ectopic expression of KLK6 facilitates cell cycle progression, certainly through alteration of c-Myc and downstream key regulators, and thus promotes cell proliferation. Moreover, KLK6 is overexpressed in NSCLC and associated with poor prognosis. Altogether, those findings suggest that KLK6 might play a central role in NSCLC development and progression.

270 Poster

Antiproliferative effect of GNRH-III and GNRH-II peptide derivatives on MCF-7, T47-d and HT-29 cells

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The hypothalamic decapeptide gonadotropin-releasing hormone (GnRH-I; <EHWSYGLRPG-NH_2) is the central regulator of reproductive system through the stimulation of the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Some kinds of tumour cells (e.g. breast, prostate, colon) produce GnRH and express its receptors. GnRH-II (<EHWSHGWYPG-NH_2) is also expressed in humans and it has more inhibitory effect to tumour cell replication than GnRH-I. GnRH-III (<EHWSHDWKPG-NH_2) is a weak GnRH agonist. It has antiproliferative effect without significant endocrine effect therefore it could be used as a selective antitumour agent in the cancer therapy. We have found that symmetric dimmer derivatives of GnRH-III were more potent for the inhibition of tumour growth in vitro and in vivo, but their endocrine effect was even lower than the monomer had.

Our aim was to synthesise new asymmetric dimer derivatives of GnRH-I or GnRH-II and GnRH-III which increased receptor binding activity and can enhance the antiproliferative effect of GnRH-III (20%).

GnRH-derivatives were synthesized by solid phase synthesis using mixed Fmoc and Boc strategies. Different natural GnRH-derivatives were coupled to directly or through a spacer each other forming asymmetric dimers. The following dimers were produced in solution via thioeter linkage: GnRH-I-GnRH-III([VD-1]; [VD-12]; [VD-3]; [VD-31]) and GnRH-II-GnRH-III ([VD-2]; [VD-22]).

The receptor binding assay was used for determination of LH secretion of different GnRH derivatives on rat pituitary cells. We found that the asymmetric dimers retained the endocrine activity of natural GnRH-I or GnRH-II. The coupling of GnRH-III to the modified GnRH-I or GnRH-II did not disorder this effect.. Dimers VD-2 and VD-22 evoked LH release only in the higher concentration, but dimers VD-1 and VD-12 were more potent in the lower concentration. VD-31 dimer had the highest endocrin activity.

The in vitro cytostatic and antiproliferative effect of GnRH-derivatives were studied on MCF-7 and T-47D human breast cancer, HT-29, human colon carcinoma cell lines. We found that no cytostatic effect of the asymmetric dimers was observed on MCF-7 and HT-29 cell lines in the studied concentration range.

The asymmetric dimers had different antiproliferative effect on MCF-7, T-47D and HT-29.The most sensitive cell was T-47D for these asymmetric dimers. Dimer [VD-12] was the most potent (80%) on T-47D cells.

271 Poster

Identification of DUSP1/MKP1 mediated pathways in lung cancer progression

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Mitogen-activated protein kinase (MAPK) signalling pathways are activated in response to a diverse array of extracellular stimuli, cell proliferation, differentiation or transformation. MAPK and JNK/SAPK activation requires phosphorylation of both threonine and tyrosine residues, that are dephosphorylated by protein phosphatases, resulting in inactivation of MAP kinases. The enzymes involved in this inactivation are the dual specific family of protein phosphatases (DUSP). DUSP1 was the first one DUSP to be identified and is encoded by an immediate early gene that has been shown to be stimulated under conditions of inflammation and stress, oxidative stress or growth factors. In addition, we have shown previously that DUSP1inhibition decreases tumor growth and sensitizes cancer cells to conventional chemotherapy, resulting in a NSCLC tumor size arrest.

The aim of this work is to gain insight into the cellular pathways involving DUSP-1 actions by using a double strategy that combines siRNA and microarray technologies. This strategy will provide a differential expression profile of genes regarding functionality of DUSP1.

The present study is based on a comparative analysis of RNA expression of the NSCLC H460 and H460-siDUSP1 cell lines. Total RNA from both cell lines was extracted, reverse-transcribed and hybridized into an array platform containing the whole human genome (affymetrix Human Genome U133 Plus 2.0). After data normalization, we selected 136 genes at least 3-fold up and down regulated comparing the interfered versus wild type cell lines (H460 and H460-siDUSP1). Posterior gene ontology analysis identified some of specific biological pathways related to angiogenesis, MAP kinase phosphatase activity, cell-cell signalling and growth factor activity. We validated the gene expression by real time PCR and pathways obtained by the gene ontology study were confirmed by the next complementary assays: