P38. THE INTERACTION OF EpCAM AND CLAUDIN-7 – STRUCTURAL CHARACTERIZATION AND FUNCTIONAL RELEVANCE

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Background: The homotypic cell–cell adhesion molecule EpCAM is a transmembrane protein expressed on the surface of nearly all epithelia. Originally it was identified as a tumor specific antigen overexpressed in various types of carcinomas. Claudin-7 is a transmembrane protein known as one of the major components of tight junctions. We recently demonstrated a direct interaction between EpCAM and Claudin-7 on several carcinomas and non-transformed tissue of the gastrointestinal tract that was not restricted to tight junctions.
Methods: The ectopic expression of EpCAM and Claudin-7 in HEK293T cells was used to characterize the interaction of both proteins and to analyze the influence of Claudin-7 on different known biological properties of EpCAM like oligomerisation or association of EpCAM with glycolipid-enriched membrane microdomains (GEM).
Results: The binding site of EpCAM for Claudin-7 could be restricted to the extracellular cystein-poor region or the transmembrane domain of EpCAM. Interestingly the oligomerisation of EpCAM is decreased in the presence of Claudin-7. Furthermore, both EpCAM and Claudin-7 are recovered from GEMs enriched in tetraspanins, whereby Claudin-7 is required for the EpCAM recruitment into GEMs.
Conclusions: Though the functional consequences of the interaction of EpCAM with Claudin-7 remain to be explored both observations offer starting points for an understanding of the functional relevance of this complex. First, by interfering with the oligomeric state of EpCAM Claudin-7 could well influence EpCAM mediated cell–cell-adhesion properties. Second, by recruiting of EpCAM to GEM associated signaling platforms Claudin-7 might facilitate the induction of downstream signaling cascades, yet to be identified.

doi:10.1016/j.ejcsup.2006.04.098

P39. Chk1 ACCUMULATES AT THE CENTROSOME IN RESPONSE TO DNA DAMAGE

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Background: One of the most common properties of cancer is genomic instability. Possible causes include centrosome aberrations and defects of the DNA damage response (DDR). ATR-Seckel syndrome and ataxia-teleangiectasia are autosomal recessive disorders associated with hematologic malignancies, which share the feature of genomic instability and are caused by DDR defects, namely by a hypomorphic ATR mutation and inactivating ATM mutations, respectively. A downstream substrate of both ATM and ATR is the nuclear checkpoint kinase Chk1, which we have recently shown to localize to interphase centrosomes and thereby negatively regulate entry into mitosis by preventing premature activation of cyclin B-Cdk1 [*Nat Cell Biol* 2004;**6**:884–91].
Methods: We assessed centrosomal Chk1 by immunofluorescence in different fibroblast cell lines with and without DNA-damaging treatment.
Results: DNA damage by ultraviolet radiation or hydroxyurea enhanced the centrosomal localization of Chk1. In fibroblasts derived from ATR-Seckel or ataxia-teleangiectasia patients, this DNA damage-dependent centrosomal accumulation of Chk1 was more pronounced as compared to control fibroblasts. Accordingly, this phenotype is not dependent on ATR or ATM.
Conclusion: Centrosomal accumulation of Chk1 in response to DNA damage leading to G_2/M arrest may contribute a novel regulatory mechanism to the DDR armamentarium.

doi:10.1016/j.ejcsup.2006.04.099

P40. THE HUMAN IIIb-mRNA SPLICE-VARIANT OF THE FGF-RECEPTOR-1(FGFR1) REDUCES THE TRANSFORMING POTENTIAL OF PANCREATIC CARCINOMA CELL LINES IN VITRO AND IN VIVO

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Background: Fibroblast growth factors (FGFs) and their specific receptors (FGFRs) are involved in several biological processes like angiogenesis and cell proliferation. We showed that the IIIc-variant of the human FGFR1 is able to induce the transforming potential in TAKA-1 ductal pancreatic cells. We also demonstrated that the now completely cloned human FGFR1-IIIb-variant is a functional FGFR, which reduces growth of TAKA-1 ductal and PANK-1 pancreatic carcinoma cells. The aim of this study was to investigate the influence of FGFR1-IIIb-expression on transforming potential of pancreatic carcinoma cells.
Methods: Colony-forming rate, cell mobility in real-time-microscopy and ability of invasion in a Boyden-chamber were assessed in vitro. Tumor formation rate of either PANC-1 control clones (PN5, PN6) or FGFR1-IIIb expressing clones was monitored after establishment in nude mice.
Results: FGFR1-IIIb expressing clones showed minor growth and lower colony forming rate of >70% compared to control clones and a significant reduction of cell mobility in vitro. Ability of invasion was lowered to >75%. In vivo 14 of 16 (PF4) and 16 of 24 (PF40) FGFR1-IIIb-clone injected areas showed solid tumors within 6 weeks. By comparison almost all areas being injected with control clones formed solid tumors (8 of 8 for PN5, 22 of 24 for PN6). Total amount of solid tumors as well as volume increase of the FGFR1-IIIb expressing tumors was reduced.

Conclusion: Human FGFR1-IIIb-variant was shown to reduce tumor growth in vitro and in vivo and prolong overall survival.

doi:10.1016/j.ejcsup.2006.04.100

P41. RELEVANCE OF THE PTEN AND p27^{KIP1} EXPRESSION IN PROSTATE CANCER AFTER SHORT TERM ANTIHORMONAL THERAPY

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Background: PTEN is an important phosphatase, suppressing the phosphatidylinositol-3-kinase/Akt pathway which induces apoptosis. p27^{kip1} binds to cyclin-E/Cdk2 and prevents mitosis. The inactivation of the tumor suppressor genes has been associated with many different types of cancer including the prostatic carcinoma (PCa). In this study we investigated the influence of an antihormonal treatment on the expression of PTEN and p27^{kip1} in PCa.
Methods: 82 prostate cancer patients treated with antiandrogens or LH-RH analogs or a combination therapy between 1 and 77 weeks (mean 9.7 ± 1.2) were included in this study. The expression of PTEN and p27^{kip1} were analyzed by immunhistochemistry and an immunreactive score. The results were compared with 183 untreated cases of a previous study.
Results: PTEN expression levels correlated with the duration of antihormonal therapy: significantly more PTEN positive cases were found after 3 weeks antihormonal therapy ($p = 0.0003$). PTEN-expression compared to untreated tumors showed a significantly stronger PTEN-expression in treated tumors ($p = 0.03$). A nuclear expression of p27^{kip1} was more frequent in tumors after 4 weeks of treatment compared to tumors treated less than 4 weeks ($p = 0.015$).
Conclusions: Both PTEN and p27^{kip1} expression was increased after 4 weeks of antihormonal treatment. This fact might indicate an early stress reaction of the tumor cells due to the androgen-deprivation therapy.

doi:10.1016/j.ejcsup.2006.04.101

P42. GLYCOGEN SYNTHASE KINASE 3BETA (GSK3BETA) AS A KEY COMPONENT OF ESTRADIOL SIGNALLING PATHWAY

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Background: GSK3beta is involved in the control of gene expression via the regulation of transcription factors, including estrogen receptor alpha (ERalpha). Recently, we discovered involvement of GSK3beta in estrogen-independent and estrogen-dependent activation of ERalpha, respectively.[1,2] While phosphorylation of ERalpha appears to be crucial for its activation, the impact of GSK3beta on the estrogen-dependent regulation of ERalpha function and activity remains to be clarified.
Methods: Phosphorylation of ERalpha by GSK3beta was analysed by in vitro kinase assays. Thereafter, the effects of GSK3 inhibitors on ERalpha phosphorylation and activation were analysed in breast cancer cells using Western blot and luciferase reporter assays. Further experiments using siRNA technology and transfection of cells with GSK3beta mutants were performed to investigate the effects of GSK3beta regarding ERalpha signalling pathway.
Results: In vitro kinase assays first depicted that GSK3beta phosphorylated ERalpha at Ser-118. Moreover, the addition of a GSK3 inhibitor (LiCl) on MCF-7 cells in culture stimulated with estradiol (E2) led to a decrease in Ser-118 phosphorylation and to an inhibition of ERalpha-controlled luciferase activity. In agreement with the previous observations, the knock-down of GSK3 by use of siRNA resulted in decreased basal and E2-induced ERalpha phosphorylation at Ser-118 as well as in reduced luciferase activity.
Conclusion: We suggest that GSK3beta plays an important role in the estrogen-dependent regulation of ERalpha function and activity.

doi:10.1016/j.ejcsup.2006.04.102

P43. ErbB-SIGNALLING IN MULTIPLE MYELOMA – FROM THE IDENTIFICATION AS POTENTIAL THERAPEUTIC TARGET BY GENE EXPRESSION ANALYSIS AND FUNCTIONAL TESTING TO CLINICAL TRIALS

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Background: ErbB-receptors/ligands are involved in several cancers. Plasma cells expressing the heparin-sulphate proteoglycan (HSPG) syndecan-1 (CD138) attach heparin-binding growth factors.[1] The aim of this study is to identify new targets in the therapy of multiple myeloma (MM).
Methods: Samples of 65 patients (CD138-purified MM-cells (MMC) and bone-marrow-microenvironment (BMME)), 7 normal bone-marrow-plasma-cell-samples (BMPC) and 20 human-myeloma-cell-lines were studied. The expression of the 4 ErbB-receptors/ligands on MMC and BMME will be assessed by real-time RT-PCR and Affymetrix U133 A+B DNA-microarrays. BMME-cells from MM-patients were exposed to PD169540 (a pan-ErbB-inhibitor) and IRESSA (ErbB1-specific).
Results: ErbB1 and ErbB2 are expressed by BMPC, MMC and the BMME. ErbB3 and ErbB4 are expressed by a subgroup of MMC. 7/10 ErbB-ligands are expressed by MMC and/or the BBME. Myeloma cell growth is stimulated by the 3 ErbB-ligands that are able to attach HSPG (i.e amphiregulin, HB-EGF and neuregulin-1) via binding to syndecan-1. PD169540 and IRESSA induced apoptosis of primary MMC from 10/14 and 4/14 patients in vitro, respectively.[1,2]