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.

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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.

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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.