Basic Science/Translational research

Oral presentations (Thu, 24 Sep, 09:00−11:00) Basic Science/Translational research

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A novel drug Toll-like receptor 9 (TLR9) agonist synergizes with trastuzumab in different trastuzumab-resistant breast tumours via multiple mechanisms of action

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Background: Treatment of breast cancer with anti-ErbB2 MAb trastuzumab is a successfull strategy; however, resistance to trastuzumab is a relevant issue. Several mechanisms and cellular effectors have been implicated in trastuzumab resistance. Recently, increasing evidence have supported a role of tumor microenvironment. Toll Like Receptor 9 (TLR9) agonists are a novel class of agents possessing antitumor activity and ability to potentiate different anticancer agents. We have previously found that a novel TLR9 agonist, termed immune modulatory oligonucleotide (IMO), and currently under clinical investigation, act via EGFR and shows direct antiangiogenic effects cooperating with anti-EGFR or -VEGF drugs, thus interfering with cancer cells signalling and microenvironment. In this study we evaluated the combination IMO plus trastuzumab as a therapeutic option for trastuzumab-resistant breast cancer.
Materials and Methods: We used KPL4 and JIMT1 trastuzumab-resistant breast cancer cells coexpressing EGFR and ErbB2, and evaluated IMO capability to inhibit growth and enhance trastuzumab activity in vivo.
Results: IMO inhibits KPL4 and JIMT1 xenografts growth and potentiates the antitumor effect of trastuzumab, with complete suppression of tumor growth, potent enhancement of trastuzumab-mediated ADCC and strong inhibition of EGFR/ErbB2-related signaling. In KPL4 xenografts IMO alone interferes with ErbB signal transduction, while trastuzumab is totally ineffective. IMO induces an ErbB-dependent signal inhibition also in vitro, therefore we investigated if a functional/structural interaction between TLR9 and ErbB receptors may occur. We demonstrated for the first time that TLR9 is also expressed under the plasmamembrane of KPL4 cells, partially co-localizing with EGFR. Moreover, TLR9 coimmunoprecipitates with both ErbB2 and EGFR and IMO reduces such interaction, particularly with EGFR. Finally, on human endothelial cells, the combination IMO plus trastuzumab produces a cooperative antiangiogenic effect related to a complete suppression of endothelial ErbB-related signaling.
Conclusions: We demonstrated a synergism of IMO plus trastuzumab in different trastuzumab-resistant breast cancers, due to both, IMO direct antitumor and antiangiogenic activity and enhancement of ADCC. Moreover, we provided the first evidence of a TLR9 and ErbB interaction at membrane level as novel mechanism of action. Altogether, we propose IMO plus trastuzumab as an effective therapeutic strategy in trastuzumab-resistant breast cancers.

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Stromal cell gene expression changes by secreted factors from cancer cells

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Background: Tumor microenvironment plays an essential role in cancer initiation and progression by providing both supportive and inhibitory factors. The mechanisms by which stromal cells remodel the tumor extracellular matrix are poorly understood. The aim of this study is to investigate mechanisms regulating the stromal gene expression through cancer stem cells from high-grade malignant tumors.
Material and Methods: Primary culture of stromal cells from non-cancer (NP) and cancer patients (CP) were co-cultured with with cancer cells from the respective patient and as control from the embryonal carcinoma (EC) cell line, NCCIT for 3 days in a transwell format to preclude direct cell contact but allow diffusion of signaling molecules. The cells were harvested and analyzed by gene arrays for microRNA (miRNA, Agilent Human Microarray) and mRNA (Affymetrix HU133 Plus 2.0) expression as a result of intercellular communication.
Results: Gene array analyses showed distinct miRNA and mRNA expression between NP and CP stromal cells. Second, NCCIT-derived factors-induced gene expression alteration in NP stromal cells to resemble that of CP stromal cells as shown by the miRNA pattern, and differential expression of selected miRNA was validated by qPCR. At the same time, the mRNA expression pattern in induced NP stromal cells became similar to that of CP stromal cells including MIRN21, a polyadenylated transcript that encodes has-miR21. Increase in CD90/THY1 correlated with the strong staining of CP stromal cells by CD90 antibody in primary tumors. Some of the differentially expressed genes between NP and CP stromal cells were previously reported by another group (e.g., up-regulation of PSG family members, CCL2, BGN, SFRP1, and down-regulation of IGFBP5). In contrast, NCCIT had no significant effect on the gene expression of CP stromal cells. Third, miRNA expression in NCCIT was not significantly altered upon induction by NP or CP stromal cells, although its mRNA expression was. There are about 50 stem cell-specific genes encoding secreted/extracellular proteins as identified by software tools.
Conclusions: Genome-wide gene expression changes involving both coding mRNA and non-coding miRNA can be induced through intercellular communication via diffusible (protein) factors. Factors from a cancer stem cell type can alter gene expression in stromal cells from high-grade malignant tumors to a state similar to that of tumor-associated stromal cells.

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TGF-β-activated kinase 1 (TAK1) is an in vivo druggable target for reverting pancreatic cancer chemoresistance

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Background: Resistance to chemotherapeutic drugs poses one of the greatest challenges in pancreatic cancer (PC) research. TAK1 is a MAP3K essential for the activation of NFkB and AP1 transcription factors. cIAP2 is a NFkB and AP1 target gene that regulates apoptosis by direct inhibition of caspases. We hypothesized that TAK1 is responsible for PC chemoresistance by regulating NFkB- and AP1-mediated transcription of cIAP2.
Materials and Methods: The expression of TAK1 in PC cell lines was studied by Western blot. TAK1 expression was silenced by shRNA in AsPC1, Panc1, and Panc28 cell lines. NFkB and AP1 activation was analyzed by EMSA. Apoptosis was quantified using cleavage of caspase-3 and PARP1 and DNA fragmentation. MTT assays were used to assess the in vitro chemopotentiation of gemcitabine (GEM), oxaliplatin (OX), and SN-38. TAK1 kinase activity was targeted using an orally available small molecule selective inhibitor (K_i in enzymatic assay: TAK1 = 13 nM; p38 >20 μM; IKKβ >20 μM) provided by Eli Lilly Research Labs. In vivo activity of the TAK1 inhibitor alone and in combination with GEM was evaluated in an orthotopic nude mouse model with luciferase-expressing AsPC1 PC cells.
Results: TAK1 protein was overexpressed in all PC cell lines studied but not in normal pancreatic ductal epithelial cells. shRNA knockdown of TAK1 completely suppressed both NFkB and AP1 DNA binding activities. As a result, cIAP2 expression was completely suppressed, inducing a proapoptotic phenotype as demonstrated by higher levels of cleaved caspase-3 and PARP1 and by DNA fragmentation. shRNA silencing of TAK1 in AsPC1, Panc1, and Panc28 cell lines resulted in significantly higher in vitro sensitivity to GEM, OX, and SN-38, compared to the respective control cell lines. In vitro, the TAK1 inhibitor alone demonstrated potent cytotoxic activity (IC_{50} 5−39 nM) and suppressed NFkB DNA binding activity in all three PC cell lines studied. In combination, the TAK1 inhibitor strongly potentiated the cytotoxic activities of GEM, OX, or SN-38 in all three PC cell lines. In nude mice, oral administration of the TAK1 inhibitor plus GEM significantly reduced tumor burden and prolonged survival.
Conclusion: Our study is the first to demonstrate that genetic silencing or inhibition of TAK1 activity is a valid approach to revert in vivo the intrinsic chemoresistance of PC. The TAK1 inhibitor used in this study is an exciting drug that warrants further development for the treatment of PC.

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Identification of the albumin-binding domain and the angiogenic domain of SPARC

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Background: SPARC (Secreted Protein Acidic and Rich in Cysteine) is overexpressed in many cancers including breast, prostate, lung, brain,