Basic Science/Translational research

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

1000 ORAL

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.

1002 ORAL

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.

1003 ORAL

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,

head-and-neck, and kidney. We have previously shown that SPARC expression appeared to correlate with response to nanoparticle albumin-bound (*nab*) paclitaxel (Abraxane) in head-and-neck as well as pancreatic cancer patients, and increases in SPARC suggested improved response to *nab*-paclitaxel in tumor models. To further define the role of SPARC, recombinant human SPARC (rhSPARC) was characterized to identify its angiogenic domain and its albumin-binding domain.
Materials and Methods: Recombinant human SPARC and genetically engineered variants were expressed and purified using HEK 293 cells maintained in hollow fiber bio-reactors. The albumin binding site on SPARC was defined using a solid phase albumin binding assay with Alexa 488-labeled BSA binding to rhSPARC immobilized onto PVDF in presence of increasing concentration of various SPARC derived peptides. The angiogenic activity of rhSPARC and its variants was evaluated using a HUVEC tube formation assay.
Results: The SPARC albumin binding assay revealed a pattern of saturable and specific binding with an estimated Kd of 700 mM, very near the known plasma concentration of albumin (600 μM). Cathepsin K-digested SPARC and competitive binding with SPARC-derived peptides identified the albumin-binding domain to be at aa209–223 of the SPARC C-terminus. In the HUVEC tube formation assay, wild type SPARC was angiogenic at 1 and 10 μg/ml and anti-angiogenic at 100 μg/ml. A proteolytic degradation product of SPARC (SPARC-d) lacking the C-terminus of SPARC was anti-angiogenic at 1, 10, and 100 μg/ml, demonstrating the angiogenic domain was located to the SPARC C-terminus.
Conclusions: SPARC exhibited an affinity for albumin at physiologic concentrations, potentially leading to the targeting of nanoparticle albumin-bound drugs *in vivo*. The identification of SPARC angiogenic domain supports the role of SPARC in tumor angiogenesis and invasiveness. This finding provides useful information in the use of SPARC as a therapeutic target.

1004 ORAL

SPARC may be a predictive biomarker of response to nab-paclitaxel

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Background: *nab*-Paclitaxel (Abraxane®), an albumin-bound nanoparticle form of paclitaxel, increases tumor accumulation of paclitaxel through binding of albumin to SPARC. Recently we have shown that SPARC overexpression may correlate with response to *nab*-paclitaxel monotherapy in head and neck cancer (Desai et al. 2009, *Trans Onc* **2**, 59−64). Here we described the further development of SPARC IHC as a companion biomarker test for *nab*-paclitaxel using data from three different clinical trials: 1) CA-040: phase I/II metastatic pancreatic cancer trial of gemcitabine plus *nab*-paclitaxel; 2) N057E: phase II unresectable stage IV melanoma trial of carboplatin and *nab*-paclitaxel; and 3) BRE73: phase II neoadjuvant breast cancer trial of gemcitabine, epirubicin, and *nab*-paclitaxel.
Materials and Methods: A panel of antibodies against SPARC were evaluated by IHC in a CLIA approved laboratory and scored by an independent pathologist. The data were analyzed to evaluate a correlation between therapeutic responses and SPARC positivity. Level 3 SPARC staining by immunohistochemistry was considered SPARC+.
Results: For pancreatic cancer, 63 pts received treatment. In 27 pts with both tumoral SPARC staining and evaluable response data, SPARC+ pts (8/27, 30%) were more likely to be responders (6/8, 75%) than SPARC- pts (5/19, 26%), $P = 0.03$, Fisher's exact test. Median progression-free survival (PFS) increased from 4.8 months for SPARC- pts (22 pts) to 6.2 months for SPARC+ pts (9 pts). For melanoma, 76 pts received treatment with 41 pts having SPARC staining. Of these 83% (34/41) pts were positive for tumoral SPARC, with some evidence that OS may be longer with tumoral SPARC positivity (10.0 vs 12.8 mo; SPARC- vs SPARC+). For neoadjuvant breast cancer, 123 pts received treatment with 72 pts having SPARC staining. A breakdown of SPARC staining revealed that the improvement in PFS was associated with tumoral SPARC (25% failure at 601 vs. >950 days, SPARC- vs. SPARC+).
Conclusions: Preliminary data from three different clinical trials including melanoma, pancreatic, and neoadjuvant breast cancer are supportive of the hypothesis that SPARC may be a predictive biomarker of response to *nab*-paclitaxel. Further validation of this hypothesis is being conducted in phase III clinical studies.

1005 ORAL

High resolution microarray copy number analysis (array CGH) suggests that determination of HER2 amplification by FISH (FISH+) is inaccurate in human breast cancer specimens that are HER2 2+ by immunohistochemistry (IHC2+)

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Background: Although 20−30% of human breast cancers are HER2 "positive" by IHC and FISH, these methods have well-documented limitations. Furthermore, clinical studies evaluating HER2 status as a predictor of sensitivity to HER2-targeted, anthracycline-containing, and taxane-containing regimens have been inconsistent, perhaps because of inaccuracies in HER2 measurement. For this reason, we compared HER2 status by array CGH with FISH in 99 archived, formalin-fixed, paraffin-embedded primary breast cancer specimens that were previously determined to be IHC2+. The advantages of array CGH include its ability to distinguish broad (such as whole-arm duplication) from local amplification events, and to distinguish true HER2 amplification from loss of chromosome 17 centromeres as an etiology for altered HER2/CEP ratios.
Methods: The specimens were evaluated by IHC and by FISH at Memorial Sloan-Kettering Cancer Center and by array CGH on Agilent 244K feature arrays at the Cold Spring Harbor Laboratory in a double-blinded manner. FISH+ was defined as a HER2: chromosome 17 centromere signal $\geqslant 2.0$.
Results: Array CGH and FISH demonstrated a high degree of concordance in specimens without HER2 amplification by FISH (Table). However, only 10 of the 22 FISH+ specimens were also HER2 amplified by array CGH. Furthermore, none of the 5 specimens with FISH scores of 1.8 to 2.2 demonstrated amplification by array CGH.
Conclusions: Our results suggest that when examined by the higher resolution technique of array CGH the assessment of HER2 amplification by FISH is inaccurate in IHC 2+ breast cancer. Improved HER2 assessment should permit more efficient use of clinical resources, enhanced development of newer targeting agents, and decreased uncertainty and anxiety on the part of both clinicians and patients. A larger series and correlation of HER2 status by array CGH with trastuzumab response are underway.

Table: HER2 status by ROMA versus FISH in HER2 2+ by IHC human breast cancer specimens

HER2 by FISH (N = 99)	HER2 by array CGH (N = 99)	
	Amplified	Non-amplified
Amplified	10	12
Non-amplified	2	75

1006 ORAL

The anti-IGF-IR antibody figitumumab (CP-751,871) is active in patients with lung adenocarcinoma undergoing epithelial-to-mesenchymal transition

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Background: Invasiveness is a hallmark of carcinomas mediated by Epithelial-to-Mesenchymal Transition (EMT). Growth factor receptors have been implicated in the regulation of EMT in NSCLC. Figitumumab (F), a specific IGF-IR inhibitor, has shown phase 2 activity in NSCLC in some histologies, i.e. squamous cell (SqCC) and adenocarcinoma (AD), but not others, i.e. large cell/NOS (LC/NOS). This ancillary study was undertaken to investigate these findings and inform the design of phase 3 trials of F in advanced NSCLC.
Methods: Tissues samples were obtained from 217 NSCLC pts with known outcome, including 52 pts treated in F trials. A total of 536 serial blood samples from 159 F pts were analyzed. Gene expression profiling was conducted in 35 NSCLC cell lines. Protein expression was assessed using an automated quantitative analysis (AQUA®) system. Plasma marker levels were determined by ELISA.