Poster Presentations (Sun, 25 Sep, 14:00–16:30)
Breast Cancer – Advanced Disease

5027 POSTER

Binding of Trastuzumab to ErbB2 is Inhibited by a High Local Density of Hyaluronan

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Background: Overexpression of ErbB2 in breast tumours is associated with poor prognosis and is a target of receptor-oriented cancer therapy. Trastuzumab (Herceptin) is an anti-ErbB2 antibody used in the treatment of ErbB2-overexpressing breast cancer, but the development of resistance is currently inevitable. We have previously shown in cell cultures and mouse xenograft experiments that masking of ErbB2 by the cell surface mucin MUC-4 or by hyaluronan leads to diminished binding of trastuzumab and consequent trastuzumab resistance. However, such correlations have not been established for human tumour samples. In the current work we investigated ErbB2-overexpressing breast cancer tissue samples and correlated the binding of trastuzumab to ErbB2 with the local density of hyaluronan.
Methods: ErbB2 in frozen tissue sections were dual stained with a fluorescent antibody against the intracellular domain (OP15) and with trastuzumab. Hyaluronan was visualized by labeling with HABC (hyaluronic acid binding complex). Immunofluorescence images were taken by confocal microscopy.
Results: We calculated the relative binding of trastuzumab by normalizing the fluorescence of trastuzumab with that of OP15. We found that the relative binding of trastuzumab showed a negative correlation with the local density of hyaluronan. Analysis of the relationship between clinical trastuzumab resistance, patient survival and hyaluronan-mediated masking of ErbB2 is in progress.
Conclusions: Although hyaluronan is by no means the only molecule contributing to trastuzumab resistance and ErbB2 masking, our results confirming its role in inhibiting trastuzumab binding in human tissue samples has both diagnostic and therapeutic implications.

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Functional Analysis of a Novel Breast Cancer Related Protein, Ephrin Receptor A10

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Background: Recently, the interaction between Eph receptors and ephrins has become a major topic in cancer research. Specifically, this interaction is reported to correlate with some vital functions such as cancer cell invasion and regulation of tumour angiogenesis/lymphogenesis. We previously reported that ephrin receptor A10 (EphA10), one of the ephrin receptor family, is highly expressed in breast cancers based on proteomic analysis between normal and cancerous mammary cells. However, the function of EphA10 was not fully-clarified. In this study, we attempted to analyze the function of EphA10 in breast cancer.
Material and Methods: *Interaction analysis between EphA10 and the ephrin A family*: The interaction between EphA10 and the ephrin A family (ephrin A1-A5) was analyzed by SPR. EphA10-Fc chimera protein was immobilized on a sensor chip. Kinetic parameter was measured by addition of various concentrations of the ephrin A family (ephrin A1-A5) onto the sensor chip using BIAcore instrument.
Evaluation of Proliferating activity by EphA10 signaling: The proliferating activity was evaluated by WST-8 assay. MDA-MB-468, a breast cancer cell line expressing EphA10, was incubated with ligand candidates or anti-EphA10 antibody. The WST-8 assay was performed 24 hr after incubation.
Expression profile analysis of EphA10 and its ligand candidates in breast cancer tissue: Expression profile of EphA10 and its ligand candidates was analyzed by immunostaining paraffin-embedded breast cancer tissues with each antibody.
Results: SPR analysis showed that EphA10 interacts with ephrin A3, A4 and A5, but not ephrin A1 and A2. Next, we examined the effect of this interaction on MDA-MB-468 cell proliferation. Our results show that the cells proliferate upon addition of ephrin A3, A4 and A5 in a dose-dependent manner. Moreover, the proliferative activity is inhibited by the addition of anti-EphA10 antibody. Finally, we evaluated the distribution of EphA10 and its ligand candidates in breast cancer tissue in order to elucidate the mechanism of interaction. Immunohistochemical analysis indicated that all molecules were expressed in breast cancer tissue.
Conclusions: Our results suggest that ephrin A3, A4 and A5 are ligands of EphA10 and that their interaction is correlated with the proliferation of breast cancer cells by cell-cell contact. We are currently attempting to analyze the further function of EphA10 in breast cancer.

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Possibility of Ephrin Receptor A10 as a Drug Target in Triple Negative Breast Cancer

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Background: Triple negative breast cancers (TNBC) are generally unresponsive to many common anticancer drugs, such as anti-Her2 antibody and estrogen inhibitors, because the tumour cells lack Her2, estrogen receptors and progesterone receptors. Thus, effective molecular targets for TNBC are urgently needed. In a previous study, we searched for new therapeutic targets using a proteomics approach and identified Ephrin receptor A10 (EphA10), which is highly expressed in breast cancer cells, as a promising candidate. Here, we evaluate the usefulness of EphA10 as a new therapeutic target for TNBC in terms of expression profile and function.
Material and Methods: *Expression profile in TNBC and normal tissues*: The expression profile of EphA10 in TNBC and normal tissues was analyzed by immunostaining with anti-EphA10 antibody using tissue microarray (TMA) slides. The TMA slides were mounted with breast cancer tissues derived from each patient and various kinds of normal tissue.
Invasion assay: MDA-MB-231, a TNBC cell line expressing EphA10, was labelled with calcein-AM. EphA10-siRNA transfected with MDA-MB-231 were seeded to the upper chamber of a basal matrix extract (BME). After 72 hr incubation, the number of cells invading into BME was evaluated by measuring fluorescence intensity in the bottom chamber.
Results: Expression profile analysis using TMA showed that EphA10 was expressed in 67% of TNBC cases while it was expressed specifically in testis among 32 normal tissues. In order to reveal the involvement of EphA10 in cancer malignancy, invasion of TNBC cells was examined. As a result, invasive cell ratio decreased significantly in EphA10-siRNA transfected group compared to the control group.
Conclusions: Our results suggest that EphA10 is a promising target for cases of TNBC because this molecule is highly expressed in TNBC and is associated with invasion. We are currently analyzing the usefulness of EphA10 as a drug target in detail and developing novel therapeutic agents for TNBC cases.

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Expression Profile of ABC Transporter Genes in Breast Carcinoma

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Background: Worldwide, breast cancer comprises the fifth most common cause of cancer-related deaths in women. Chemotherapeutic treatment is limited by the interindividual variability in drug response and by the development of resistance of cancer cells. ATP-binding cassette (ABC) transporters belong to a family of transporter proteins that contribute to drug resistance via ATP-dependent drug efflux pumps, e.g. P-glycoprotein. We followed the expression and variability of ABC transporter genes and intended to evaluate their associations with clinico-pathological data including therapy outcome of individual patients.
Material and Methods: Expression profile of all known human ABC transporter genes (49) was evaluated in postoperative tissue samples from 28 breast cancer patients treated by FAC, FEC or taxane-based neoadjuvant chemotherapy regimens. Gene expression was assessed using real-time PCR with relative quantification. High Resolution Melting Analysis (HRM) was developed for the study of single nucleotide polymorphisms (SNPs) in *ABCB1*.
Results: ABC transporters were expressed in the majority of samples (tumours and paired adjacent non-neoplastic tissues) with striking inter-individual variability. Twelve ABC transporters were significantly downregulated in tumour tissues. On the other side, fifteen ABC transporters were significantly upregulated in tumours (ABCA2, ABCA3, ABCA7, ABCA12, ABCB2, ABCB8, ABCB9, ABCB10, ABCC1, ABCC4, ABCC5, ABCC10,