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.

5028 POSTER

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

Y. Maeda[1], K. Nagano[1], T. Yamashita[1], S. Kanasaki[1], M. Inoue[1], X. Zhao[1], Y. Abe[1], H. Kamada[1], Y. Tsutsumi[1], S. Tsunoda[1]. [1]*National Institute of Biomedical Innovation (NiBio), Laboratory of Biopharmaceutical Research, Osaka, Japan*

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.

5029 POSTER

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.

5030 POSTER

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,

ABCC11, ABCF2 and ABCG1). Finally, SNPs in *ABCB1* gene coding a prototypical anticancer drug efflux pump (rs1128503, rs2032582 and rs1045642) were estimated. The rs1128503 and rs1045642 SNPs were significantly associated with expression of ABCB1 and estrogen receptor in our previous study (Vaclavikova et al. *Pharmacogenet Genomics* 2008;18:263–273).
Conclusions: Our results revealed new candidate genes potentially causing the multidrug resistance of mammary tumour cells. Validation study on upregulated ABC transporters will be performed by absolute quantification in an independent patient cohort. The association of expression profiles with therapy outcome and disease-free survival will also be analyzed. In addition, new HRM method that seems to be rapid, accurate and low-cost as well as time-effective was developed for screening of functional *ABCB1* and analogously another ABC gene(s) SNPs.
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5031 POSTER

Breast Cancer in Young Woman in South of Morocco

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Background: The breast cancer is the first cancer of the woman in the world. it remains infrequent at the young woman. The objective of this study is to identify the epidemiological, clinicopathological and evolutionary features of breast cancer in young women at the oncology department of Mohammed VI University Hospital in Marrakech.
Materials and Methods: This retrospective study involved 154 young patients of 40 years old and less suffering from breast cancer, treated and followed up between January 2003 and December2007.
Results: The mean age was of 35.1 years and 19 patients (12.3%) had familial history of breast cancer. Palpable tumour was found in 96.8% of cases with a clinical average size of 6 cm. Cancers were classified T1 in 17.8% of patient, T2 in 31.8%, T3 in 20.7% and 39.7% with T4. We found an invasive ductal carcinoma in 71.1% of cases, 97% were SBR grade II and III. The study of the operative specimen showed a axillary node invasion in 83 women (68.5%) and Hormone receptors were absent in more than 50%of case. The hormonotherapy by tamoxifene was indicated at 53 patients associated in a castration by radiotherapy to four of them. The average length of the follow-up period was 39.6 months. We noticed that 34% of 103 women having a controlled disease at the end of treatment presented a relapse for an average time of 16.6 months. The global survival rate at 3 years was 66.1%.
Conclusion: With a high rate of relapse in our series, Prognosis appears unfavourable among young women with breast cancer in our region. Our results are consistent with those of the majority of published reports.

5032 POSTER

Clinical Outcome of Central Nervous System Metastases From Breast Cancer: Differences in Survival Depending on Systemic Treatment

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Background: Central nerve system (CNS) metastases are a feared complication of breast cancer and are associated with poor prognosis. The purpose of this study is to investigate the clinical characteristics of CNS metastases and to clarify the prognostic factors after CNS metastases in breast cancer at a single institution over a long time period.
Patients and Methods: We retrospectively reviewed the medical records of breast cancer patients diagnosed at Seoul National University Hospital from 1981 to 2009 and identified the patients who experienced CNS metastases. We collected the data including demographics, clinicopathologic characteristics, dates of diagnosis of original breast cancer and subsequent metastases, date of death and correlated the findings with the clinical outcome.
Results: Total of 400 patients were identified, 17 patients (4.3%) were diagnosed CNS metastases with primary breast cancer concurrently and 383 (95.7%) experienced CNS metastases subsequently after the diagnosis of primary breast cancer. 318 patients (79.5%) had brain parenchymal metastases only, 30 (7.5%) had leptomeningeal metastases only, and 52 (13%) had both. After the diagnosis of CNS metastasis, 170 patients (42.5%) received systemic chemotherapy (CTx) and 143 (35.8%) received CTx after whole brain radiation therapy (WBRT). The patients with good performance status (PS), initial CNS metastasis as recurrence, absence of extracranial metastases, non-visceral extracranial metastases, longer interval from the date of primary breast cancer to the date of CNS metastasis, CTx after WBRT and gamma-knife surgery (GKS) had better outcomes in univariate analyses. In multivariate analysis, good PS, systemic CTx after WBRT, GKS, and longer interval to CNS metastasis, were independent prognostic factors for overall survival after CNS metastases.
Conclusions: Our results suggest that appropriate palliative systemic therapy after WBRT or GKS, adequate palliative treatment via combined modalities are helpful for breast cancer patients, even after the detection of CNS metastases.

5033 POSTER

Re-irradiation Plus Hyperthermia for 415 Patients With Recurrent Breast Cancer in Previously Irradiated Area − the Amsterdam + Tilburg Experience

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Background: Treatment options for patients with locoregional recurrent breast cancer in previously irradiated area are limited. Four hundred and fourteen patients were treated with re-irradiation and hyperthermia (re-RT/HT) in the AMC (n = 301) and the BVI (n = 113) from January 1982 till January 2006. Response, locoregional control and toxicity were analysed as well as prognostic factors.
Patients/methods: All patients received extensive previous treatments, including surgery, chemotherapy and irradiation to a median dose of 50 Gy with or without boost. Median interval between initial treatment and re-RT-HT was 54 months (range 3–469).
The median age was 57 years at start of re-RT/HT. The estimated tumour size was >10 cm in 48% of patients, distant metastases were present in 36% and 74% had experienced 1−13 recurrence episodes, prior to the re-RT-HT. Re-RT consisted typically of 8x4 Gy, twice a week (AMC) or 12x3 Gy, four times per week, (BVI). Superficial hyperthermia was added once/twice a week using 434MHz CFMA antennas. Aim temperature: 41−43ºC for one hour. Fifteen percent of patients received sequential chemotherapy and 30% sequential hormone therapy.
Results: Overall clinical response rate (cCR+cPR) was 84%. The infield 3-year local control (LC) rate was 25%. Tumour size, interval, previous recurrences and distant metastases (DM) were important prognostic factors. For patients with isolated locoregional recurrences $\leqslant$5 cm the 3-year LC rate was 47% (Table 1).
Median overall survival was 17 months. Acute $\geqslant$ grade 3 toxicity occurred in 24% of patients. The actuarial late $\geqslant$ grade 3 toxicity rate was 23% at 3 years.

Table 1

Tumour size	cCR (%)		3-y LC (%)	
	Isolated	With DM	Isolated	With DM
0−5 cm	86	54	47	22
5−10 cm	65	40	29	22
>10 cm	58	36	21	11

Discussion and Conclusion: The combination of re-irradiation and hyperthermia results in high response rates despite extensive disease. Early referral is needed to achieve long term locoregional control. Currently a randomized study of RT-HT versus RT-HT and CisDiamineDichloroPlatinum is performed to further improve results.

5034 POSTER

Baseline Characteristics, Disease Pattern and Outcome of Breast Cancer Patients With Asymptomatic Bone Metastasis

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Background: Nearly 30−70% of all cancer patients develop bone metastasis. There is enough clinical evidence for the role of ionizing radiation in symptomatic bone metastasis. However, nearly 20% of patients