the function and expression of RORα in normal breast and breast cancer tissue has not been fully understood. In the present study, we examined the relationship between RORα mRNA expression and clinic-pathological findings in human breast cancer tissues.

Methods: Seventy-eight specimens of invasive breast cancer were obtained from Japanese female patients, who underwent surgery at Gunma University Hospital. Expression of RORα mRNA was examined using quantitative real-time RT-PCR.

Results: RORα mRNA was detected in all of the breast cancer specimens, but expression was significantly lower than in the normal tissues surrounding the tumors. A positive correlation was determined between RORα mRNA expression and estrogen and progesterone receptor immunoreactivity, and a negative correlation was found between RORα mRNA and HER2/*neu* immunoreactivity and nuclear grade. No significant association with patient age, tumor size, lymph node metastasis, menopausal status, or vessel invasion status was detected.

Conclusion: We have shown that RORα mRNA expression is lower in human breast cancer tissues than in normal tissues. The reduced expression levels may indicate a tendency toward higher malignancy and thus a poor prognosis for these patients. At the same time, this observation supports the potential of RORα as a novel prognosis factor for breast cancer treatment.

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Circulating Cells in Epithelial Mesenchymal Transition (EMT) Expressing Markers of Hypoxic Stress in Primary and Advanced Breast Cancer

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The MAINTRAC (red cell lysis, immunofluorometric detection and analysis on scanR Olympus) technique as developed by our coauthors from Jena detects more CTC and allows therefore regular follow up and co-analysis. In a first phase (with a 2-colour technique) we investigated coexpression of vimentin on Epcam+ cells. In a second phase with a 3-colour technique we examined coexpression of CD44 on EPCAM+Vimentin+ Cells, or quantification of living (DAPI exclusion) and dead cells coexpressing EPCAM and Vimentin results of first phase s.

Table 1. Percentage of patients showing CTC counts per ml blood as indicated.

2-Colour Analysis EPCAM+	EPCAM+VIM coexpression CTC					
Subgroup	0	50–250	>250	0	50–250	>250
NO (n = 63	11	55	38	3	19	78
N+ (n = 47)	13	41	46	13	14	76
Lum A (n = 58	11	55	34	2	10	88
LumB (n = 30)	12	42	46	8	24	68
TN (n = 11)	9	64	26	0	27	73
Her2+ (n = 15)	10	64	37	0	33	67
met.BC (n = 44)	5	43	52	n.d.a.	n.d.a.	n.d.a.
Controls (n = 237)	67	25	0	n.d.a.	n.d.a.	n.d.a.

In the recently started second phase with 3 colour analysis, the following data were found in early (n = 73) and advanced BC (n = 57) Early: Living EP+VIM+ 0 4%, 50−250 34%, >250 61%. Metastasized BC 0 4%, 50−250 37%, >250 60%. Early: EP+VIM+CD44+ 0 16%, 50−250 14%, 250−500 20%, >500 50%. Advanced: EP+VIM+CD44+ 0 27%, 50−250 4%, 250−500 8%, >250 51%.

These data still are preliminary they show however definitely that more frequently as expected circulating epithelial cells with stemcell characteristics are detectable. Most of these cells are dead. Simultaneous 3 color analysis with ACA 9, showed that cells with high CD44 load mostly expressed ACA 9, indicating hypoxic stress. Further characterization showed that this particular celltype (EP+VIM+CD44high) also coexpresses PARP1 indicating genotoxic stress.in a patient group with liver disease (NAFLD) these cells also can be found, indicating a common stimulus (Hypoxia) turning on an EMT program in these. This phenomenon is used by cancer cells in early stages of metastasis − later on this phenomenon is turned off especially in rapidly aggressive forms, like HER2 + HRneg.

More definitive analysis of this cell type and its behaviour under therapy in advanced an early breast cancer will be presented at the conference.

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The Changes of Molecular Markers Between Before and After Neoadjuvant Chemotherapy in Breast Cancer

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Background: Differences in hormone receptor and HER2 status between primary tumor and corresponding relapsed tumor were observed in breast cancer. This study investigates the changes of molecular markers between before and after neoadjuvant chemotherapy and what factors influence these changes of molecular markers during chemotherapy in breast cancer.

Methods: We set 43 patients underwent neoadjuvant chemotherapy after diagnosis as treatment group and 10 patients who underwent immediate surgery after diagnosis as control group between Jan 2008 and Aug 2011. Immunohistochemical staining was performed for estrogen receptor (ER), progesterone receptor (PR), HER2, Ki67, with diagnostic biopsy tissue and specimen obtained after mastectomy. We analyzed the association of the changes of molecular markers with clinicopathological factors, such as histology, grade, tumor size, nodal status, regimen and duration of chemotherapy, and response to chemotherapy.

Results: Of 43 patients who received neoadjuvant chemotherapy, pathologic complete response occurred at 4 patents (9.3%), partial response did in 19 patients (44.2%) and stable disease did in 20 (46.5%). We were able to obtain 36 paired specimens before and after chemotherapy. ER decreased in 5 (13.9%) and did not increase in any patients. PR decreased in 11 (30.6%) and increased in 2 (5.6%). HER2 increased in 5 (13.9%) and decreased in 1 (2.8%). Ki67 decreased in 24 (66.7%) and did not increase in any patients. There was no significant association between changes of molecular markers and clinicopathological factors. However, three out of 5 patients who increased HER2 were accompanied by PR decrease. In the control group, PR decreased in one (10%), but there were no patients with decreased ER and increased HER2. The changes of molecular markers were not affected by response to chemotherapy, duration of chemotherapy, and regimen used chemotherapy.

Conclusions: Changes of molecular markers has been observed in as many as 30% after neoadjuvant chemotherapy in breast cancer. It would be due to molecular downregulation and development of compensatory pathway. We need to examine molecular markers in tissue obtained by surgery in order to establish a therapeutic strategy in neoadjuvant setting of breast cancer.

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Correlation of CD10 and EGFR Expression in Phyllodes Tumors of the Breast

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Background: Phyllodes tumor of breast is an uncommon disease, with the ability to recur and metastasis. The specific parameters that define the degree of malignancy and predict prognosis still not universally established. The aim of this study is to evaluate the expression of CD10 and epidermal growth factor receptor (EGFR) of phyllodes tumors and to determine whether the degree of their expression is related to the clinical outcome and classification of phyllodes tumors.

Materials and Methods: A total of 82 phyllodes tumors of the female breast were retrieved from our institution between December 1995 and July 2010. This study included 57 benign, 11 borderline and 14 malignant phyllodes tumors for CD10 and EGFR expression using immunohistochemistry (IHC). We investigated the correlation between expression, amplification of CD10 and EGFR, and the degree of malignancy and recurrence. We also we evaluated the relationship between the degree of malignancy and histological features including tumor margin, nuclear pleomorphism, stromal cellularity, stromal overgrowth and other categorical measurements.

Results: All the 82 patients were from women, with the overall age range from 11 to 60 years (mean 36.59±10.81 years). The tumor size ranged from 2.42 to 260 mm (mean 46.93±36.49 mm). Of these, seven patients were recurred. The age of patients was closely related with the degree of malignancy ($p = 0.015$). The correlations of the degree of malignancy with recurrence ($p = 0.022$) and histological parameters such as tumor margin status, stromal cellularity, mitotic activity, nuclear pleomorphism, stromal overgrowth was significant statically ($p < 0.001$). In the expression of CD10, there was a significant difference between benign, borderline and malignant phyllodes tumor ($p = 0.041$ between benign and malignant, $p = 0.017$ between borderline and malignant respectively) except between