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

benign and borderline. While the difference in the IHC expression of EGFR between benign and malignant were significant (p = 0.027) and also between borderline and malignant were significant (p = 0.014), there was no different relation between benign and borderline cases.

Conclusions: The results of this study provide evidence that immunohistochemical CD10 and EGFR over-expression is significantly related with the degree of malignancy and pathogenesis of phyllodes tumors.

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Flow Cytometry Analysis of Circulating Endothelial Cells in Women with Breast Cancer. Preliminary Results

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Background: Despite advances in diagnosis and treatment, breast cancer (BC) remains one of the main causes of cancer death in women. Currently, all relevant prognostic information should be obtained integrating traditional clinicopathological parameters with the molecular classification of BC, and measurement of different tumor-specific markers. Angiogenesis is crucial for tumor growth, and it has been shown that mature and immature endothelial cells, such as circulating endothelial cells (CECs) or endothelial progenitor cells, are present in the blood. CECs are extremely rare in normal peripheral blood, but their number increases significantly in cancer patients. Different cell surface markers have been used to detect these cells, and flow cytometry is a method well suited for their detection and quantitation. The aim of this study was to assess the levels of the microenvironmental cell marker $CD146^+CD45^-$ phenotype, which is linked to angiogenesis, vessel damage, and disease progression, in patients with metastatic and localized BC.

Patients and Methods: Blood samples from 34 women (median age 48 years, range 33−76 years) with primary invasive ductal carcinoma were collected for analysis of CECs. Patients with distant metastases (M+) have been excluded, as well as those who underwent adjuvant chemotherapy. All samples were stained with antihuman CD146-PE and CD45-FITC, and immunophenotyping was obtained using a Beckman Coulter XL-MCL flow cytometer to assess levels of $CD146^+CD45^-$ CECs, using a 600 s acquisition time for each sample. Using a post-hoc criteria, two age-matched groups of patients were obtained: Group A (18 patients) with local disease and negative (N0) axillary lymph nodes (AN), and Group B (16 patients) with positive (N+) AN. Overall, in this group, 329 AN (median 19, range 15−26 per patient) have been removed, of which 34 (10.3%) were N+.

Results: Patients with localized BC (Group A) had a level of $CD146^+/CD45^-$ cells significantly increased (451±357 *vs.* 87±72 CECs, $p < 0.0001$) in respect to those with metastatic disease (Group B). No correlation (R = 0.11, $p = 0.16$) was found between number of the N+ and level of putative CECs.

Conclusions: Although the rare nature of CECs ($<10^{-4}$ elements per mononuclear blood cell) and their not yet completely established phenotype represent a technical challenge, our preliminary data suggest a possible increased angiogenic activity in patients with metastatic BC, which can be demonstrated using flow cytometry analysis of CECs. However, with the aim of increasing sensitivity (note the very high standard deviation), further endothelial cell markers, alone or in combination, should be studied.

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The Reference of Immunodeficiency and Molecule-Genes Characteristics of Breast Cancer (BC)

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Immunological disorders have variability and can change hormone answers of cancer treatment, it mainly concerns Luminal A (LA) BC.

The aim of the research was to compare immunological characteristics of common variable immunodeficiency (CVID) and of serious combination immunodeficiency (SCID) on different molecule-genes forms of BC. 33 patients of the USMA department of oncology have been included in the research since 2007 with complex treatment of BC ($T_{1-4}N_{0-2}M_0$), on LA, Luminal B (LB) and triple-negative (TN) forms of BC of different genes of San-Gallen consensus 2011, at the age of 50.2±10.9 years. All the patients were divided into three groups. Group I were LABC (n = 15), Group II were LBBC (n = 10) and Group III were TNBC (n = 8). The division in groups was in cases of decrease of any index of immunoglobulin (A, M, G) smaller than standard with B-cells less than $0.2*10^9$/l − it was CVID. The SCID was a mixture with decrease CD4+ less than $0.5*10^9$/l cells by D. Mail classification 2007. We compared indexes of leucocells, limfocells, CD3+, CD4+, CD8+. The Coefficient Reaction was index “Cr” (Cr = nCD3+stimulation/nCD3+wild where n = count CD3+ that can synthesize cytokines TNF-α, IL-2, IFN-γ in the tests after stimulation and in wild). The CVID and the SCID were typical compare points for groups I and III, with the reliability index X^2 >2.

Luminal A BC with CVID and SCVD has differenced by the index of leucocells that was ($6.2\pm1.5\times10^9$l and $3.5\pm0.7\times10^9$l), the count of limphocells was ($1.9\pm0.2\times10^9$l and $0.9\pm0.2\times10^9$l), CD3+ ($1.3\pm0.4\times10^9$l and $0.6\pm0.1\times10^9$l), CD4+ ($0.8\pm0.3\times10^9$l and $0.3\pm0.05\times10^9$l), CD8+ ($0.5\pm0.1\times10^9$l and $0.3\pm0.1\times10^9$). LB of BC with CVID and SCID has differenced by CD4+ ($0.7\pm0.2\times10^9$l and $0.4\pm0.06\times10^9$l). TN has defined in groups with CVID and SCVD of indexes of leucocells ($6.7\pm0.7\times10^9$l and $5.2\pm0.9\times10^9$l), CD4+ ($0.7\pm0.06\times10^9$l and $0.3\pm0.07\times10^9$l). “Cr” in groups I and III was different in CVID for TNF-α (30.9±12.0 and 123.5±24.3, X^2 >2) and in SCID for IFN-γ (35,9,1±40.3 and 138.5±82.9, X^2 >2).

As a conclusion, the difference between CVID and SCID can be the core prognostics problem for LA, LB and TN of BC. The prognosis of LA of BC can be the worst for SCID. And the prognosis of aggressive TNBC may be better if patients have only CVID. This difference can be a good way of immunology correction during hormone therapy for LA or chemotherapy of TNBC among the women of the Ural region. The “Cr” may be an interesting aim for individual correction of TNF-α and IFN-γ.

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Inhibition of Breast Cancer Angiogenesis and Metastasis by P16 Gene Therapy − Downregualting VEGF Expression Via Viral-mediated P16is Interaction with HIF-1a

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Background: One effective approach to suppress malignant breast cancer (BCa) progression is to block tumor angiogenesis. Vascular endothelial growth factor (VEGF) plays a pivotal role in tumor angiogenesis. Because the degree of tumor malignancy directly correlates with the expression of VEGF, but inversely correlates with the expression of tumor suppressor gene p16, we examined whether restoration of p16 in BCa cells would modulate VEGF expression and consequently suppress BCa angiogenesis and metastasis.

Materials and Methods: To facilitate induction of p16 expression, a recombinant adenovirus expressing p16 (AdRSVp16) was generated and used to transduce BCa cells. The p16 effects on BCa angiogenesis and metastasis were examined by a series of assays including the dorsal air sac model and spontaneous metastasis animal model. The mechanism of p16-mediated modulation of VEGF expression was further analyzed by studying p16’s effect on hypoxia inducible factor-1a (HIF-1a), the transcriptional factor of VEGF gene promoter, by both co-immunoprecipitation and colocalization assays.

Results: We found that adenoviral-mediated p16 expression down-regulated VEGF gene expression in breast cancer cells, inhibited BCa cell-induced angiogenesis and suppressed breast tumor metastasis in a spontaneous metastasis animal model. Moreover, p16 appears to bind directly to HIF-1a, and consequently translocates cellular location of HIF-1a from the nucleus to cytoplasm in BCa cells.

Conclusions: These results demonstrated that p16 modulates VEGF expression and inhibits tumor-induced angiogenesis and metastasis. The binding between p16 and HIF-1a protein appears to alter HIF-1a’s cellular localization and HIF-1a’s ability to transactivate VEGF expression. This study reveals a novel function of p16, namely, p16’s anti-angiogenesis function by its interaction with HIF-1a and downregulation of VEGF gene expression. The dual function of p16’s anti-angiogenesis and its well-known anti-proliferation should warrant p16 gene transfer as an effective therapeutic strategy for clinical treatment of BCa patients.