mutations in BRCA1/2 are point mutations and small insertion/deletions, but recently increasing number of large genomic rearrangements in BRCA genes have been reported in different populations with various prevalence. However, little is known about the prevalence and types of genomic rearrangements of BRCA genes in the Korean population. In this study, we have analyzed for the presence of BRCA1/2 large genomic rearrangements in Korean breast cancer patients.

Methods: Multiplex ligation-dependent probe amplification (MLPA) was used to screen BRCA1/2 large genomic rearrangements in Korean breast cancer patients (249 for BRCA1 and 215 for BRCA2) at a priori risk of BRCA1/2 mutations due to known risk factors. The patients have been comprehensively analyzed for germline mutation in the entire regions of the BRCA1 and BRCA2 genes, using using a combination of fluorescent-conformation sensitive capillary electrophoresis (F-CSCE) and direct sequencing, and were found negative. Positive MLPA result was confirmed and located by long-range PCR and sequencing.

Results: We identified one large deletion in BRCA1, deleting exon 13−15, in one patient with family history of breast cancer. Breakpoints of this deletion are novel. However, we could not find any large deletion in BRCA2.

Conclusion: Our results suggest that the large genomic rearrangements in BRCA1/2 genes are not a major cause for increased breast cancer susceptibility in Korean population.

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Ki67 in Breast Cancer Patients and Its Correlation with Clinico Pathological Factors

A. Syed[1], P.S. Giridhar[2], K. Sandhu[3], S. Jader[2], S. Al-Sam[2], V. Sundaresan[2], J. Singer[2], S. Jenkins[2], H.A. Bradpiece[2], A. Patel[2].
[1]Princess Alexandra Hospital, Breast Surgery, Essex, United Kingdom; [2]Princess Alexandra Hospital, Pathology, Essex, United Kingdom; [3]Princess Alexandra Hospital, General Surgery, Essex, United Kingdom

Background: Ki-67 is a nuclear protein universally expressed in all proliferative tissues. Large number of studies have confirmed the potential use of Ki-67 as a prognostic indicator and in predicting response to treatment in early breast cancer. However, due to variation in analytical practice, measurement of Ki-67 is still not recommended for management of early breast cancer patients. The aim is to study our initial results of measurement of Ki67 in breast cancer patients and correlate its significance with known prognostic factors.

Table 1. Clinico pathologic indicators and the Ki-67 index in primary breast cancer patients

Ki-67 index	<20%	20−50%	>50%
Mean tumour size, in cm	2.7	3.4	3.0
Mean age, in years	60.2	59.9	58.1
Breast Cancer Subtypes			
Luminal A (%)	80.9	10.7	8.4
Luminal B (%)	64.3	21.4	14.3
HER 2 type (%)	55.6	22.2	22.2
Triple Negative (%)	44.4	0	55.6
Total (%)	74.1	12.1	13.8
Positive Nodes (%)			
0	67.2	20.9	11.9
1−3	30.8	38.5	30.8
⩾ 4	40.0	30.0	30.0
Nuclear Grade (%)			
1	100	0	0
2	84.8	13.0	2.2
3	43.5	26.1	30.4
Estrogen receptor (%)			
Positive	78.2	12.9	8.9
Negative	44.4	16.7	38.9
Progesterone receptor (%)			
Positive	83.1	14.5	2.4
Negative	50.0	11.1	38.9
HER2 (%)			
1+	75	10	15
2+	74.6	13.4	11.9
3+	54.5	27.3	18.9

Materials and Methods: The Ki67 index was measured in all patients with early breast cancer in our breast unit over a period of six months. Prognostic factors such as Estrogen Receptor, Progesterone Receptor and HER-2 Receptors were simultaneously measured. Ki 67 index was categorised based on median value of 20% and analysed in accordance with breast cancer subtypes.

Results: Ki67 index was measured in 119 patients from February 2011 to July 2011. Age ranges from 29 to 89 with the mean tumour diameter of 2.8 cm (range from 0.6 to 11 cm). The median value of Ki-67 index was 20% and of the subtypes were Luminal A 12.5%, Luminal B 20%, HER 2 type 30% and Triple Negative tumours was 70%. Table 1 shows the relationship between the different groups of Ki67 index (<20%, 20−50% and >50%) and the clinic-pathological indicators of primary breast cancer.

Conclusion: The Ki-67 index ranged from 1−98% and the median was 20%, similar to other studies. The median Ki-67 values were different among subtypes, being low for the good prognostic Luminal A type tumours and high for the triple negative tumours. A higher Ki-67 index correlated with negative ER/PR, higher nuclear grade, younger age, larger tumour and positive HER 2. These data suggest that patients with a higher Ki-67 index have a poorer prognosis. It is therefore important to measure Ki-67 index and to be considered in the treatment and follow up of breast cancer patients.

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The NF-kappa B Signalling Pathway and the Response to Doxorubicin in Hormone-resistant Breast Cancer Cells

A. Scherbakov[1], Y. Lobanova[2], O. Andreeva[2], V. Shatskaya[2], M. Krasil'nikov[2]. *[1]Cancer Research Center, Laboratory of Clinical Biochemistry, Moscow, Russian Federation; [2]Cancer Research Center, Laboratory of Molecular Endocrinology, Moscow, Russian Federation*

Background: Oestrogen is an intrinsic regulator of the breast cancer growth and progression. Many breast cancers are initially sensitive to estrogen and antiestrogen treatment. The acquired tolerance of breast cancers to growth stimulating estrogen action may provokes the paradoxical tumor sensitization to estrogen apoptotic action. The phenomenon of the cell sensitization to estrogen-induced apoptosis has been demonstrated for the breast cancer cells undergoing long-term estrogen withdrawal or antiestrogen treatment. NF-kB is a transcriptional factor that controls apoptosis and cell responses to stress. Earlier studies have shown that estradiol suppress NF-kB, demonstrating the possible NF-kB involvement in the estrogen apoptotic action. The goal of this work is to study the influence of estrogens on the sensitivity of the resistant breast tumors to doxorubicin, and to evaluate the role of NF-kB signaling in the regulation of the survival of the resistant breast cancer cells.

Material and Methods: MCF-7/LS subline was developed by long-term cultivation of the parental cell line MCF-7 in steroid-free medium. The transcriptional activity of NF-kB and estrogen receptor was determined using luciferase reporter gene assay. The apoptosis level was evaluated by flow cytometry using staining with propidium iodide.

Results: It has been demonstrated that estradiol enhances the apoptotic action of doxorubicin in the resistant MCF-7/LS breast cancer cells. The proapoptotic estrogen action is mediated by NF-kB suppression when NF-kB knock-down sensitizes the resistant cells to both estrogen and doxorubicin.

Conclusions: Thus estrogen-induced NF-kB suppression in the resistant breast cancers results in a imbalance between pro- and anti-apoptotic pathways and cell sensitization to anti-tumor drugs. Additional inhibition of NF-kB by siRNA increases the apoptotic action of estrogen and doxorubicin, demonstrating that NF-kB may be considered as a potential target in the therapy of the resistant breast cancers.

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Quantitative Analysis of RORalpha mRNA Expression in Human Breast Cancer

H.O. Odawara[1], J.H. Horighchi[2], T.I. Iwasaki[3], N.R. Rokutanda[2], R.N. Nagaoka[2], H.T. Tokiniwa[2], A.S. Sato[2], Y.K. Koibuchi[1], N.K. Koibuchi[3], I.T. Takeyoshi[2]. *[1]National Hospital Organization Takasaki General Medical Center, Breast and Endocrine Surgery, Takasaki, Japan; [2]Gunma University Graduate School of Medicine, Department of Thoracic and Visceral Organ Surgery, Maebashi, Japan; [3]Gunma University Graduate School of Medicine, Department of Integrative Physiology, Maebashi, Japan*

Objectives: The retinoic acid receptor-related orphan receptor (ROR) α is a member of the steroid/thyroid hormone nuclear receptor superfamily, which plays an important role on growth and differentiation of many organs by regulating transcription of target genes. We previously reported that RORα directly activated aromatase expression in breast cancer cells through a newly-identified ROR response element located within the promoter region of the aromatase gene. According to previous studies including ours, RORα mRNA is expressed in breast cancer cell lines, such as MCF7. However,

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

L. Habets[1], W. Körber[1], B. Frenken[1], I. El Ghali[1], M. Danaei[2], M. Kusche[2], U. Peisker[3], K. Pachmann[4], T. Kroll[4]. *[1]Metares e.V, CTC, Aachen, Germany; [2]Brustzentrum Aachen Kreis Heinsberg, Senologie, Aachen, Germany; [3]Brustzentrum Aachen Kreis Heinsberg, Senologie, Erkelenz, Germany; [4]Universitätsklinik Jena, KIM II, Jena, Germany*

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

T.H. Kim[1], H.M. Yoo[1], J.Y. Choi[2]. *[1]Busan Paik hospital Inje University, General Surgery, Busan, South Korea; [2]Seoul National University College of Medicine, Medicine, Seoul, South Korea*

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

J. Kim[1], R. Kim[1], K. Kim[1], Y. Jung[1], E. Soh[1], H. Yim[2], M. Chun[3]. *[1]Ajou University School of Medicine, Department of Surgery, Suwon, Korea; [2]Ajou University School of Medicine, Department of Pathology, Suwon, Korea; [3]Ajou University School of Medicine, Department of Radiation-oncology, Suwon, Korea*

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