and TP53 coding regions in 90 unrelated probands with strong family history or early-onset breast cancer cases with or without family history, 50 sporadic breast cancer cases and 23 bilateral breast cancer patients without family history of breast and ovarian cancer.

As the observed distribution of mutations favour routine pre-screening using a simple and cost-effective test we used allele specific oligonucleotide PCR-based assays (ASO) followed by reamplification and sequencing on ABI377 automated sequencer.

Results: We found five different disease predisposing mutations in BRCA1 gene (185delAG, 300T/G, 4174delA, 5382insC, 5528del1+IV22-6), four mutation in BRCA2 gene (6174del, 6886del5, 9599A/T, 9631delC) and one mutation in TP53 (1095del8) which is the largest mutation ever detected in this gene. Five mutations detected in our study (9599A/T, 9631delC, 6886del5 in BRCA2; 5528del1+IV22-6 in BRCA1; 1095del8 in TP53) were not reported previously and may be specific to the southern Polish population while the others were recurrent. Mutation prevalence was substantially higher in cases with strong family history. Of the 90 women with family history 33 carried a germ-line mutation in BRCA1 (26 of these cases were 5382insC mutation caries) and 6 carried mutations in BRCA2. We have also identified a family with mutations in both genes BRCA1 and BRCA2 (185delAG BRCA1 and 6174delT BRCA2). Our results are in agreement with the idea that mutations in BRCA1 and BRCA2 significantly contribute to familial breast cancer risk and their contribution to sporadic breast cancer and bilateral breast cancer without family history of breast and ovarian cancer is much lower.

Conclusion: Further studies will be necessary to estimate more accurately the risk of the BRCA1, BRCA2 and TP53 mutations among breast and ovarian cancer patients in Polish population.

Grant KBN No 6 P04B 003 13

645 POSTER

Gene expression profiling of estrogen-responsive genes in human breast cancer: development of a DNA microarray system for monitoring hormone therapy

S. Hayashi[1], A. Inoue[1,5], N. Yoshida[1,2], Y. Omoto[1,3], S. Oguchi[4,5], R. Kiyama[5]. *[1] Saitama Cancer Center, Division of Endocrinology, Saitama, Japan; [2] University of Tokyo School of Medicine, Department of Internal Medicine, Tokyo, Japan; [3] Nagoya City University Medical School, Department of Surgery II, Nagoya, Japan; [4] InfoGenes Co. Ltd, Tsukuba, Japan; [5] National Institute of Advanced Industrial Science and Technology, Molecular and Cell Biology, Tsukuba, Japan*

Purpose: Estrogen plays an important role in carcinogenesis and development of human breast cancer. However, the essential downstream molecular targets of estrogen-signals in cancer have not been clarified hitherto. Moreover, along with the recent improvement of hormone therapy, accurate therapeutic prediction of breast cancer is desired earnestly.

Methods & Results: We first comprehensively analyzed the gene expression profiles for estrogen responsiveness among four estrogen receptor (ER)-positive cancer cell lines, MCF-7, MCF-7c9, T47-D and Ishikawa cells, using large-scale cDNA microarray technique. Approximately 4% of 9000 genes showed significant estrogen responsiveness, classified as the induction or repression types, among these cells. Many of the genes in the induction type were found to be growth-associated or tumor-associated genes, while tumor suppressor-related genes were found in the repression type. Based on the obtained information, a total of 138 genes, which showed high induction or repression in expression by estrogen stimulation, were selected and provided for custom microarrays. The results obtained from the custom microarray analysis of the cell lines were consistent with those from large-scale microarray analysis. The time course study of these 138 genes using the custom microarrays revealed that they were clearly categorized into the early- or late-response types. Furthermore, the custom microarray analysis of ER positive breast cancer tissues also showed similar but not identical profiles to those obtained with the cell lines.

Conclusion: Further analysis of these genes categorized into subgroup by custom microarray may provide new clues for elucidation of estrogen-dependent growth mechanisms of cancer. These observations indicate the usefulness of the custom microarrays for assessment of response by estrogen-agonists and antagonists in the human breast cancer. This custom microarray may be applicable for prediction of effectiveness of anti-hormone therapy for breast cancer.

646 POSTER

Shifts in phenotype of dendritic cells (DC) in healthy BRCA1 mutation carriers and in patients with early breast cancer

C. Kichler-Lakomy[1], A.C. Budinsky[1,5], R. Möslinger[3], T. Wagner[3], R.M. Wolfram[1], C. Wiltschke[1], T. Brodowicz[1], H. Viernstein[4], C.C. Zielinski[1,2,5]. *[1] Clinical Division of Oncology, Departement of Medicine I, Vienna, Austria; [2] Chair of Medical Experimental Oncology, Departement of Medicine I, Vienna, Austria; [3] Clinical Division of Special Gynaecology, Departement of Obstetrics and Gynecology, Vienna, Austria; [4] Departement of pharmaceutical Technology, Faculty of Pharmaceutical Developments, Vienna, Austria; [5] Ludwig Boltzmann Institute, Clinical Experimental Oncology, Vienna, Austria*

Rationale: Investigations originating from our laboratory have reported deficiencies in antigen presentation and functionality of monocytes derived from patients with early breast cancer (EBC), but not from healthy women with germline mutations of BRCA1.We have now expanded our investigations to dendritic cells (DC), only insufficiently studied in patients with EBC in general and in BRCA1 mutant carriers in particular.

Objective: DC derived from patients with EBC, healthy women with BRCA1 germline mutations as well as EBC patients with BRCA1 mutations were analysed for the expression of CD1a, CD11c, CD83, CD80, CD86, CD54 and CD14. Antigen presentation by DC was evaluated by a T-cell proliferation assay.

Methods: Peripheral blood was obtained from 36 patients with EBC, 7 healthy women with germline mutations of BRCA1, 4 patients with EBC with BRCA1 mutations and 26 healthy age-matched control persons. PBMC were prepared for ex vivo DC generation using GM-CSF, IL4 and TNF-alpha according to standard procedures. DC phenotype was examined by FACS.T cell proliferation in response to TTX-pulsed DC was measured by (3H)thymidine assay.

Results: Phenotypically, DC derived from patients with EBC presented with a significantly reduced expression of CD1a, CD83, CD80, CD86, CD54, compared to DC from healthy control females. Moreover, DC derived from healthy women with BRCA1 germline mutations showed a significant increase in CD54 and CD80 antigens, as compared to DC derived from healthy control persons. Finally, DC derived from EBC patients with germline mutations of BRCA1 showed not only a higher expression of CD1a and CD83, but also of CD86 and CD54,compared to DC from patients with EBC without BRCA1 mutations.

Functionally, T cell-proliferation in response to TTX-pulsed autologous DC was significantly decreased in patients with EBC.

Conclusions: DC derived from patients with EBC had not only an immature phenotype, but also a functional impairment in antigen presentation. Our results showed, furthermore, that differences in DC phenotype preceded the clinical manifestation of breast cancer and shifts in antigen expression were also present in BRCA1 germline mutation carriers persisting throughout the development of EBC.These results suggested the presence of a characteristic phenotype in individuals with BRCA1 germline mutations.

647 POSTER

Comparative genomic hybridization in phyllodes tumors of the breast

K.J. Jee, G. Gong, S. Ahn, J. Park, S. Knuutila. *[1] Haartman Institute, University of Helsinki, Medical Genetics, Helsinki, Finland; [2] University of Ulsan, College of Medicine, ASAN Medical Center, Pathology, Seoul, South Korea; [3] University of Ulsan, College of Medicine, ASAN Medical Center, General Surgery, Seoul, South Korea; [4] University of Ulsan, College of Medicine, ASAN Medical Center, Diagnostic Radiology, Seoul, South Korea*

Purpose: Phyllodes tumors are a rare neoplasm of the breast and it is not easy to predict their clinical behavior, for instance, recurrence and metastasis. The aim of this study was to search for specific alterations of genes associated with various phyllodes tumor grades.

Methods: To analyse genetic alterations, we used the comparative genomic hybridization (CGH) method in 25 cases of archival paraffin embedded materials which were classified into 3 histological categories as benign, borderline and malignant.

Results: Chromosomal aberration was identified in 11 cases (44%) but it was difficult to identify a sequential DNA copy number change associated with histological type. However, the most interesting finding was the gain of the long arm in chromosome 1 that was shown in 9 cases. Gain of 1q was significantly associated with recurrences. Other changes were the loss of 4q, 6q, 9p and 13q and the gain of chromosomes 17,19, and 20.

Conclusion: The DNA copy number changes and tumor stage in the phyllodes tumor did not correlate. However, 1q regions may play an important role in relapse. Therefore, more detailed molecular characterization of 1q amplification is needed in order to identify the target genes.

648 POSTER

The clinical significance of thymidine kinase 1 measurement in patients with breast cancer using anti-TK1 antibody

Q. He[1], J.P. Wu[2], R.Y. Mao[2], N. Wang[3], L.X. He[2], S. Skog[1]. [1] *Department of Oncology, KFC, Huddinge University Hospital, Stockholm;* [3] *Department of Oncology and Pathology, Section Medical Radiobiology, Karolinska Institute, Stockholm, Sweden;* [2] *Deptartment of Pathology, Hubei Cancer Hospital, China*

Cytosolic thymidine kinase (TK1) is of considerable interest because its intracellular level is highly dependent upon the growth stage of the cells. The level of TK1 in human tumor is proportional to their proliferating rate and it is also related to the degree of remission. Both of mono- and polyclonal antibodies against TK1 have been developed and were characterized in our group. TK1 in serum (STK1) of patients with breast cancer were previously studied (He et al., Inter Biol. Markers. 15: 139–144, 2000). Results indicated that TK1 should be a good tumor marker for monitoring both progression of the tumor and therapy response.

In the present study

Purpose: To explore the expression of TK1 in the same specimens of patients with breast cancers as compared to the expression of the proliferating cell nuclear antigen (PCNA).

Methods: Immunohistochemical staining was used to detect the expression of TK1 and PCNA in 52 breast malignant lesions. 20 breast benign lesions and 16 normal breast tissues were used as controls.

Results: The TK1-labelling index (LI) was 78.9% and the PCNA-labeling index (LI) was 64.5% in malignant lesions. The TK1-LI and PCNA-(PANA-LI) were significant higher in malignant lesions than non-malignant lesions ($p < 0.0001$ and $p < 0.0001$, respectively. No significant difference was found for TK1-LI and PCNA-LI between benign lesions and normal tissues. Concerning the tumor stage and the tumor grade, TK1-LI showed a significant correlation with the increased tumor stages ($P = 0.012$) and tumor grades ($p = 0.009$). However, PCNA-LI was neither significantly different in tumor stages ($p = 0.062$) nor in tumor grades ($P = 0.073$).

Conclusion: That TK1 will be a more accuracy marker than PCNA for estimation of cell proliferation and the malignant potentials in breast carcinomas.

649 POSTER

BRCA1 and BRCA2 mutations in breast and breast/ovarian cancer families from Galicia (NW Spain)

A. Vega, M. Torres, C. Ruiz-Ponte, A. Carracedo, F. Barros. *Unit of Molecular Medicine, University Santiago de Compostela, Santiago de Compostela, Spain*

Purpose: Germline mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 predispose carriers to early-onset breast and breast-ovarian cancer, and it is estimated that 5% of all breast cancer cases are caused by inherited mutations in dominant disease genes. To date, several hundred pathogenic mutations in these two genes have been reported. The mutations are distributed throughout both genes and generally, no hot spots mutations are present. However, within defined ethnic groups, specific and relatively frequent mutations have been identified.

Methods: Thirty breast and breast/ovarian cancers in Spanish families (29 from Galicia, NW Spain, and one from Catalonia, NE Spain) with at least two cases of breast or breast/ovarian cancer under age 50, were screened for mutations in the BRCA1 and BRCA2 genes. The analysis of these genes was carried out by SSCP for shorter exons and direct sequence in the case of longer ones.

Results: Mutations were found in 8 of the 30 families studied (26.66%). It is important to note that all mutations were detected within the BRCA1 gene: 3958del5ins4, 910delGTTC, 5530 T>A, 2121 C>T; and 330 A>G. The BRCA1 330 A>G mutation was found in four unrelated families and accounted for 50% of all identified mutations.

Conclusions: In the present study only BRCA1 mutations and no BRCA2 mutations were detected in all the families analysed. Since all the families carrying the mutation 330 A>G in Spain are from Galicia, we propose the BRCA1 A>C being a founder mutation of Galician origin.

650 POSTER

Detection of Epstein-barr virus in breast cancer by polymerase chain reaction

G. Lima, R. Medeiros, C. Palmeira, D. Pereira, A. Vasconcelos, S. Carrilho, P. Ferreira, C. Lopes. [1] *Instituto Português de Oncologia-Porto, Porto, Portugal*

Purpose: Epstein-Barr virus (EBV) is a human herpes virus responsible for the infectious mononucleosis, capable of in vivo infection of B and T-lymphocytes and epithelial cells, and it has been associated with the development of a still-growing number of malignancies. Breast cancer is a multistep disease that includes genetic and environmental factors. Recent studies suggest a possible association of EBV with breast cancer. In this study, the presence of EBV genome in human breast carcinomas was investigated.

Methods: DNA extracted from 68 breast carcinomas was amplified by polymerase chain reaction (PCR), with primers covering two different regions of EBV genome: EBV-encoded small RNA 2 (EBER-2) and Bam HI N Leftward Frame 1 (BNLF1).

Results: The EBV genome was detected in 33 (49%) of the 68 carcinomas. No association was found between EBV detection and the clinicopathological data. We found that the mean age at diagnosis was 55.4 ±12.6 for EBV negative cases and was 61.8 ±10.4 for EBV positive breast cancer cases. This difference was statistically significant ($p=0.048$).

Conclusions: In this study, the EBV genome was detected in 49% of the carcinomas analyzed. These results are in concordance with some prior studies. The association of the presence of EBV genome and older age at diagnosis may result in some new questions on the real meaning of this virus on the onset and/or progression of breast cancer.

Breast cancer pathology and predictive factors

651 POSTER

Choline kinase as a putative tumour marker in breast cancer

A. Ramírez de Molina[1], J.M. Silva[2], F. Bonilla[2], J. Sánchez[3], J.C. Lacal[1]. [1] *Biomedical Research Institute, Molecular and Celular Biology of Cancer, Madrid, Spain;* [2] *Puerta de Hierro Hospital, Medical Oncology, Madrid, Spain;* [3] *Autonoma University, Faculty of Medicine, Madrid, Spain*

Purpose: In the past years several studies have demonstrated an important role of Choline Kinase (ChoK) and its product, phosphocholine (Pcho), in the generation of tumours in humans, and the inhibition of this enzyme has been shown to be an efficient antitumor strategy in vivo in the nude mice system. The aim of this study was to assess if ChoK is involved in the generation of breast cancer, and if there was any relationship between the regulation of ChoK and clinical features in patients with breast carcinomas in order to provide a new antitumoral strategy in the adjuvant setting for these patients. Methods: Normal and tumoral tissues from each patient were extracted of a total of 61 patients with breast carcinomas and some clinical parmeters were analyzed. Statistical analysis was performed using SPSS software, (all reported P-values are two-sided). Choline kinase essays were performed using homogenized tissues as source of ChoK and the physiological Cho concentration as substrate in presence of methyl [14C]-choline chloride. Western blot analysis of the different tissue lysates were performed using hChoK anti-serum and a-tubulin antibody as loading control. Results: We have found an increase in ChoK activity in 72% of the tumoral tissues analysed with respect to the normal ones, being a linear correlation between Choline Kinase activity and histologic tumor grade ($p=0.008$). As well, there is significant correlation between higher ChoK activity and ER-negative breast carcinomas. In addition, we have found an incidence of ChoK overexpression of 20% corresponding with tumoral tissues that display the highest increase in ChoK activity ($p=0.001$), suggesting two different mechanisms of ChoK disregulation under aggressive tumoral conditions. As expected, there is a statistical significant correlation between ChoK overexpression and both high histologic tumor grade ($p=0.01$) and ER-negative tumors ($p=0.003$). Conclusion: ChoK activation is playing a role in the development of breast cancer, suggesting ChoK could be used as a tumoral marker. In addition, a correlation between ChoK disregulation and parameters indicators of poor prognosis like lost of ER regulation and higher histologic grade has been found, suggesting this study might constitute the basis of the development of a new antitumoral strategy for these patients.