Results: From both mutation carriers and controls, three RT-PCR products were obtained: one corresponding to the full length transcript with the expected size (214 base pairs (bp)), another with 192bp corresponding to a deletion of 22bp of exon 5 (BRCA1-Δ22ntex5), and a third with 134bp corresponding to the in frame skip of exon 5 (BRCA1-Δex5). Semi-quantitative fragment analysis showed a relative amount of BRCA1-Δ22ntex5 more than eight-fold higher in patients and only the wild type allele was present in the full length transcript. The haplotype identified in the three Portuguese families and in the Galician family is compatible with a common origin of this mutation. The mutation segregates with the disease in the family with two affected members. Of the three breast cancers, one was an atypical medullary carcinoma and two were invasive ductal carcinomas with medullar features. All breast carcinomas were grade III and two of them were hormone receptor negative (data not available from the third case). No LOH was detected.

Conclusions: We conclude that disruption of alternative transcript ratios is the mechanism causing hereditary breast/ovarian cancer associated with the BRCA1 R71G mutation, and segregation and histopathologic data are consistent with its pathogenicity. Furthermore, our findings indicate a common ancestry of the Portuguese and Galician families sharing this mutation.

133 Poster

Heat Shock Protein 60kDa in breast cancer tissue and cell lines

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Background: Breast cancer has been reported as the most common cancer of women in U.S.A., Western Europe and Korea. Breast cancer is curable with an early diagnosis, and many researchers have made efforts to find a marker for this malady. Heat shock protein (HSP) consists of 6 groups, it is highly preserved throughout both the prokaryotic and eukaryotic cells and it acts as a molecular chaperone that is involved in protein folding. HSPs have been recently reported to be related with breast cancer. In this study, we investigated the changes of expression of HSP60 in breast cancer tissues and cancer cell lines.

Materials and Methods: We obtained breast cancer tissues and normal tissues from twenty breast cancer patients, and we purchased several cancer cell lines from ATCC. We treated the human breast cancer tissues and cancer cell lines with heat shock protein. Proteins and mRNAs were isolated from the tissues and the cancer cell lines and then we performed Western bloting, RT-PCR and FACS on them.

Results: On Western blot, HSP60 was more overexpressed in the cancer tissue and the cancer cell lines than in the normal breast tissue and in the normal cell lines. The Expression of HSP60 showed 2 types of molecular weight differences in both the breast cancer tissues and the cancer cell lines, and specifically, low HSP60 was over-expressed in the cancer tissues. There was no difference between the expression of HSP60 protein and mRNA according to the treatment with heat shock protein in both the breast cancer tissue and the normal cell lines. Also, there was no relationship between phosphorylation and the structural difference of HSP60 protein according to HSP60 protein molecular weight.

Conclusion: We conclude that HSP60 may be used as a diagnostic marker for breast cancer. Detailed investigation of the usefulness and significance of the HSP60 expression as a prognostic factor is required in further studies.

134 Poster

Is triple negative a prognostic factor in breast cancer?

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Background: Breast cancer is characterized by hormone dependency and endocrine therapy is a key treatment in breast cancer. Recently, targeted therapies such as Trastuzumab treatment for HER2 positive breast cancer has been important. Triple negative breast cancer is characterized by lack of expression of estrogen receptor (ER) and progesterone receptor (PgR), and the absence of HER2 protein overexpression, and so there is no targeted therapy for this subtype. In this study, we examined the biological and prognostic characteristics in triple negative breast cancer.

Patients and Methods: Between January 1998 and September 2006, 1552 patients with primary breast cancer were investigated retrospectively in this study and ER, PgR and HER2 status were evaluated in all cases. Furthermore, p53 overexpression and Ki67 values were examined immunohistochemically.

Results: Patient distribution according to ER, PgR or HER2 status were as follows; ER and PgR positive: 57.9% and ER and PgR negative: 25.1%. With regards to the HER2 status, HER2 positive was 23.3%, and triple negative (TN) was 14.0%. TN breast cancer has a high proliferation rate, high nuclear grade and frequent p53 overexpression. Patients with TN tumors had a significantly poorer disease-free survival (DFS) than those with non-TN tumors. After recurrence the overall survival (OS) rate in TN cases were significantly lower than that of the non-TN cases. Multivariate analysis revealed that TN was a significant factor for DFS and OS after recurrence.

Conclusion: TN breast cancer is a rare subtype and has a high proliferation rate, a high nuclear grade. p53 overexpression, and lower DFS/OS. To improve the prognosis of TN breast cancer, a new effective strategy needs to be developed.

135 Poster

Simultaneous analysis of HER-2/neu gene amplification and protein overexpression in single cells of pleural and ascitic effusions from patients with breast and ovarian cancer

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Background: HER2/neu protein overexpression is found in 20−30% of breast cancers and correlates with poor clinical outcome. Patients are selected for anti-HER2/neu-therapy by examination of tumour specimens by IHC (immunohistochemistry) and FISH (fluorescence-in-situ-hybridisation). Good correlation between both methods has been found for score 1+ and 3+ samples, but not for score 2+ samples. Combined approaches using FISH and immunofluorescence on the same tumour specimen have been described. In this study we examined pleural and ascitic effusions with a method allowing simultaneous analysis of protein expression by IHC and gene amplification by FISH. We regarded the following aspects: 1. the frequency of HER2/neu protein expression and gene amplification in effusions. 2. the correlation between protein expression, gene amplification and chromosome 17 polyploidy.

Methods: We examined 35 effusions from patients with breast cancer (n = 31) and ovarian cancer (n = 4). The same cytospins were analysed by IHC using two anti-HER2/neu antibodies and by FISH with HER2/neu/CEP17 probes. Amplification was defined as: 1. HER2/neu gene copy number of >4 and 2. HER2/neu/CEP17 ratio $\geqslant$2.0.

Results: 35 tumour-cell-positive effusion specimens were examined. 25 of them were scored HER2/neu positive (score 2+, 3+). All of them contained cells with heterogeneous protein scores. Single cells were analysed for HER-2/neu gene amplification and chromosome 17 ploidy with regard to their scores. 9 of these 25 samples showed mean HER2/neu copy numbers of >4 in cells with a 2+ and 3+ score, but only 12% (n = 3) of these samples were amplified according to HER2/neu/CEP17-ratio. 32% (n = 8) were polyploid (mean CEP17 >4). In some samples we found tumour cells with gene amplification but without protein overexpression (score 1+) and cells without gene amplification but strong protein expression.

Conclusion: The combination of IHC and FISH allows a differentiated analysis of single cells, which is especially important for effusions that often contain heterogeneous cells. In this study only few samples showed HER2/neu amplified cells. Protein overexpression was not always correlated with gene amplification. For the selection of patients for an anti-HER2/neu-therapy protein overexpression might be more important since it might sometimes be caused by CEP17 polyploidy rather than by gene amplification.

136 Poster

Clinical features of BRCA1/BRCA2 positive hereditary breast cancers

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Background: BRCA1 and BRCA2 mutations cause hereditary breast cancer (BC). Patients (pts) who carry this type of mutations have a significantly cumulative lifetime risk of developing breast and ovarian cancer. The authors review all cases of BRCA1/BRCA2 positive BC in their Institution.

Material and Methods: Retrospective analysis of consecutive pts with BRCA1/BRCA2 positive BC followed at the Portuguese Institute of Oncology, Porto. Clinical data were obtained from medical records. Data were analyzed using the statistical package SPSS 13.0. Survival curves were calculated by the Kaplan–Meier method.

Results: A total number of 30 pts were evaluated.