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

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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)

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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

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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

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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.