

preliminary data suggest that lowering TAM dose by 1/2 or even by 1/4 still allows maintenance of the full effect on total cholesterol.

28

POSTER

ALL-trans-Retinoic (ATRA) acid induces cell cycle perturbations and apoptosis in human breast cancer

R. Mangiarotti¹, M. Danova², R. Alberici³, P. Pugliese², C. Pelliciani¹.
¹Dept. Animal Biology, ²Histochemistry Study Center, C.N.R., Univ. of Pavia, ³Medical Oncology, Univ. and IRCCS S. Matteo, Pavia, Italy

Purpose: The antiproliferative effect of ATRA on MCF-7 human breast cancer cells was correlated with the specific effects on the cell cycle progression and apoptosis.

Methods: These effects of ATRA were investigated using DNA content evaluation and dual parameter flow cytometry (FCM) of bromodeoxyuridine incorporation and of the expression of cell cycle-related proteins (Ki-67 as proliferation marker and Statin as quiescence marker) vs DNA content. Apoptosis was studied by FCM of both DNA content and labelling of phosphatidylserine residues by Annexin V.

Results: After 4-days of ATRA treatment, the % of S-phase cells decreased significantly and cells accumulated in the G0/G1 range of DNA content. FCM analysis showed a decrease in the % of Ki-67+ve cells with a simultaneous increase of the 57% of Statin +ve cells. From 5 days of treatments onwards, apoptosis was found to occur.

Conclusions: ATRA-induced inhibition of MCF-7 cell growth is related with the block of cell proliferation mostly in a pre-DNA synthetic phase and the induction of apoptosis. This should be taken into account in the attempt to associate ATRA with other antiproliferative drugs.

29

PUBLICATION

Presence of germline BRCA2 mutations in sporadic breast cancer: Clinical correlation

B. Gomendio, J.M. Silva, M. Provencio, E. Garcia-Patiño, J.M. Garcia, S. San Martín¹, T. Rivera², R. Cubedo, P. España, F. Bonilla. Department of Medical Oncology, Clínica Puerta de Hierro; ¹Department of Gynecology; ²Department of Pathology, H. Santa Cristina, Madrid, Spain

Purpose: Breast cancer is the most common malignancy in women. It occurs in hereditary and sporadic forms. At present, the sporadic breast cancer accounts for 90% of all breast cancers. Germline BRCA2 gene mutations have been identified in families prone to breast cancer. We designed the present study, now under way, to detect the presence of germline mutations in BRCA2 gene in sporadic breast cancer.

Methods: Our series consists of 93 patients diagnosed of breast cancer, without family history of breast and ovarian cancer. The mean age was 55 years, and the median age 53. The following clinical parameters were analyzing: Birth and diagnosis date, family history, menopausal status, histology of tumors, pathological stage, hormonal receptors, survival and vital status. The mutational study was performed by PCR-SSCP in peripheral blood lymphocytes DNA of the patients.

Results: We observed in 4 patients (4.3%) presence of aberrant fragment migration (now under sequencing process), 3 of them located at the same fragment (exon 11.19). The comparison of the 9 clinical parameters, between the two subgroups of patients, with and without mutation, did not show any significant difference.

Conclusion: The prevalence rate of mutations in sporadic breast cancer, considering their age, is higher than expected. It is possible that we detected a founder mutation. No implication of germline BRCA2 mutations as prognostic factor.

30

PUBLICATION

Analysis of bcl-2 and p53 genes in patients with breast cancer

A. Dueñas^{1,2}, J.J. Cruz², R. Salazar-Saez^{1,2}, M.M. Abad³, R. González-Sarmiento¹, C.A. Rodríguez², E. Fonseca², A. Gómez², G. Martín², P. Sánchez². ¹Dept. Genética Molecular; ²Dept. Oncología; ³Dept. A. Patológica. University Hospital. Salamanca, Spain

Methods: 46 primary nonmetastatic breast carcinomas. Immunohistochemical expression of bcl-2 and p53 proteins was analyzed in formalin-fixed paraffin-embedded sections. Exons 5 to 9 of the p53 gene were analyzed by PCR-SSCP in 40 of the 46 tumors. Genomic organization of bcl-2 was

analyzed by southern blot from tumor tissues and from the peripheral blood of 11 patients (pt).

Results: There was a significant direct correlation between Estrogen and Progesterone receptor expression and bcl-2 protein expression ($p = 0.01$ and $p < 0.0001$). Significant correlation with tumor size was obtained ($p < 0.0001$) but not with lymph node invasion. We did not detect any molecular abnormality in the genomic organization of bcl-2. None of the 11 pt in whom peripheral blood was analyzed showed loss of heterozygosity at bcl-2 locus. The immunohistochemical expression of bcl-2 was inversely related to p53 protein expression, ($p = 0.05$), however the analysis of the correlation between p53 gene mutations and bcl-2 protein expression did not show significant correlation ($p = 0.79$). p53 gene mutations were present in 12 tumors (30% of the cases). (We detected p53 protein expression in 8 of the 12 tumors carrying p53 mutation. p53 protein staining was also detected in 14 cases without mutations at exons 5 to 9).

Conclusion: We have not found any correlation between bcl-2 immunohistochemical expression and p53 gene mutations. Bcl-2 is expressed in tumors with wild-type p53 as well as in those carrying a p53 mutation. This lack of correlation may reflect that regulation of bcl-2 expression is independent of p53.

Cancer genetics

31

ORAL

DNA mismatch repair deficient tumors exhibit length variability of repetitive DNA sequences in diverse promoter regions

C. Sutter, J. Gebert, P. Bischoff, D. Kube, C. Herfarth, M.v. Knebel Doeberitz. *Sektion Molekulare Diagnostik und Therapie, Chirurgische Univ.-Klinik, Im Neuenheimer Feld 116, D-69120 Heidelberg, Germany*

Hereditary Non-Polyposis Colorectal Cancer (HNPCC) is a cancer predisposing trait characterized by germline mutations in DNA mismatch repair (MMR) genes. Loss of MMR function in tumors of HNPCC patients results in length variability (LV) of DNA repeats within microsatellites, of coding and non-coding sequences. DNA repeats are also present within promoter regions, but have not been analyzed in HNPCC-associated cancer so far. Therefore, we analyzed HNPCC tumors for genetic instability of DNA repeats present in different human promoter regions.

Promoter repeats of the human Interleukin-10 (IL10, [CA]₂₁), Retinoblastoma (Rb, [A]₂₀), WAF1/CIP/p21 [A]₁₂, CyclinB1 ([A]₁₁), PhospholipaseA2 (PLA2, [CA]₁₃), Glucokinase (GLK, [GT]₁₅), and Collagenase type IV (COL4, [CA]₂₁) genes were amplified and analyzed on an A.L.F. DNA sequencer.

11/14 HNPCC tumors showed LV within the IL10, 8/12 in the Rb, 8/14 in the CyclinB1, 9/21 in the WAF1, and 3/14 in the COL4 promoter repeat. In contrast, LV of the PLA2 and GLK promoter repeats was absent in 11 and 3 HNPCC tumors, respectively.

Our data reveal a high frequency of LV of DNA repeats within different promoter regions in tumors with DNA MMR deficiency. Case alterations might affect regulatory properties of the promoter elements and subsequently modify the gene expression profile. This new type of 'promoter instability' (PIN) might represent a common mechanism contributing to the pathogenesis of HNPCC.

32

ORAL

Genetic analysis of familial adenomatous polyposis (FAP) families: Lessons and Implications

J. Gebert, C. Dupon, M. Kadmon, A. Tandara, Ch. Herfarth, M. von Knebel Doeberitz. *Sektion Molekulare Diagnostik und Therapie, Chirurgische Klinik, Im Neuenheimer Feld 116, 69120 Heidelberg, Germany*

Aim: Germline mutations in the APC gene cause FAP. Correlations between the site of the APC mutation and the manifestation of the disease have been established. The detection of a germline mutation in the APC gene in an affected family member permits the identification of carriers and non-carriers of the mutant APC allele among relatives at risk. Non-carriers may be excluded from further endoscopic screening.

Methods: Clinical parameters were considered before mutation analysis was initiated. The APC coding region was screened for germline mutation using a non-radioactive protein truncation test (PTT). Direct sequence