develop the familial form of the disease may be calculated by identifying mutations in BRCA1 or BRCA2 genes.

From the screening of 1100 medical records of patients subjected to mastectomy in S. Chiara Hospital in Pisa during the period 1990–1994, the patients with a referred familial recurrence of breast or ovarian cancer were selected for interview. 81 patients belonging to 75 families fitted the BRCA1 eligibility criteria.

37 patients belonging to different families were analyzed for BRCA1 germline mutation. All 37 families are Caucasian and principally reside in Tuscany. Mutation screening was done by ASO (allelic specific oligo hybridization) for three common mutations and by direct sequencing of each of the coding exons. The analysis is under way and approximately 50% of the coding region has been screened up to now. We have so far identified 7 mutations of which 5 are different. Three are frameshift and 2 missense. Only one of the mutations found has been reported previously (5382insC). The insertion of an A has been reported in three different families, the aplotype analysis has been performed to ascertain if a common ancestor exist for those three families. No evident correlation between phenotype and genotype has been detected.

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450 ORAL

SQUAMOUS CELL CARCINOMA CELLS BECOME SENSITIVE TO DNA-DAMAGING AGENTS AFTER INFECTION WITH THE ADENOVIRUS E1A

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Squamous cell carcinomas are very common tumors that may show different histologic patterns and oncogenic changes. Clinical behavior is often unpredictable. We reported that adenovirus E1a gene may induce sensitivity to DNA-damaging agents. To determine whether E1a gene expression increases sensitivity to said agents in malignant keratinocytes derived from highly tumorigenic cell lines (HACA-4, A5), we infected them with a retro-virus carrying the E1a region of adenovirus 5. Wild type and mutant p53 protein were assessed by immunoprecipitation with antibodies Pab240 and Pab246. Cell viability after exposure to cisplatin (CDDP), doxorubicin, and gamma irradiation (RX) was studied by the crystal violet method, thymidine uptake and flow-cytometry. Cell lines expressing E1a were about 4 and 10-fold more sensitive to CDDP and RX, respectively, than controls. Thus, we conclude that E1a expression may confer great sensitivity to DNA-damaging agents in squamous cell carcinoma cells regardless of the p53 protein status.

451a POSTER

A TUMOR SUPPRESSOR GENE ON CHROMOSOME 1P32-PTER CONTROLS THE AMPLIFICATION OF *MYC* FAMILY GENES IN BREAST CANCER

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To investigate the possibility of collaboration between telomeric deletion on the short arm of chromosome 1 and genetic amplification, similar to that described in human neuroblastoma, 122 human primary breast tumors were examined by restriction fragment length polymorphism analysis for loss of heterozygosity on 1p32-pter and for the three most frequently amplified genetic regions in breast carcinomas (*MYC* and *ERBB2* protooncogenes and the chromosomal region 11q13). Allelic losses at one or more loci on the telomeric part of the short arm of chromosome 1 was observed in 57 (47%) of 122 informative tumors. *MYC*, *ERBB2* and the 11q13 region were amplified in 23, 20 and 21% of breast tumors, respectively. A correlation was found between loss of heterozygosity on chromosome 1p32-pter and amplification of the *MYC* (formerly c-myc) protooncogene ($P = 0.003$), suggesting that these two genetic events may collaborate during tumor progression in human breast cancer.

These results, together with those obtained in human neuroblastoma, suggest that the distal part of the short arm of chromosome 1 harbors (a) unidentified tumor suppressor gene(s), whose inactivation may be involved in *MYC* family gene amplification (an example of genetic instability) in tumors of various cellular origins.

451b POSTER

INT-2/FGF3 AMPLIFICATION IS A BETTER INDEPENDENT PREDICTOR OF RELAPSE THAN *C-MYC* AND *C-ERBB-2/NEU* AMPLIFICATIONS IN PRIMARY HUMAN BREAST CANCER

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The prognostic significance of the three genes most frequently amplified in breast tumors was investigated by multivariate analysis in a retrospective study of 112 primary human breast cancers. These three genes, c-myc, int-2/FGF3 (a marker for the 11q13 amplicon) and c-erbB-2/neu, were amplified in 37, 14 and 10% of breast tumors studied respectively. Amplification of the c-myc gene was not related to metastasis-free survival (MFS) in the total population, but was a discriminant prognostic indicator in premenopausal patients. Int-2/FGF3 gene amplications were good indicators of prognosis, especially in premenopausal patients, and also in lymph-node-positive and steroid receptor-negative patients. Int-2/FGF3 amplification and progesterone receptor (PgR) status together proved to be the only independent variable predictive of MFS. The risk of relapse in the sub-group of PgR-negative patients was five times greater for those with int-2/FGF3 amplification than patients without this alteration. These results suggest that the combined use of classical prognostic factors and molecular markers may improve prognostic value and be applicable to patients with specific tumor phenotypes.

452 POSTER

LOSS OF HETEROZYGOSITY ON 7Q31 OCCURS EARLY DURING BREAST TUMORIGENESIS

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Although human breast tumorigenesis is associated with the accumulation of mutations both in oncogenes and in tumor suppressor genes, the identity of the genetic alterations that are critical in the early stages of the breast tumorigenic process remains obscure. A high frequency (27–41%) of LOH occurrence has been shown at the *MET* locus on chromosome band 7q31 and this specific alteration is associated with poorer survival. Here, we report that RFLP analysis on 221 informative (heterozygous) primary breast tumors and 57 informative relapses (13 local recurrences and 44 distant metastases) revealed a similar frequency of 7q31 LOH as tumors progress from primary cancer to relapse, in marked contrast to other changes such as 11p15.5 LOH. This finding suggests that inactivation of a putative tumor suppressor gene located in 7q31 is a very early event in breast tumorigenesis. Our results also show that metastatic potential is an induced phenomenon that occurs at a relatively early stage, rather than a marker of tumor progression.

453 POSTER

LOSS OF HETEROZYGOSITY AT 7Q31 IS A FREQUENT AND EARLY EVENT IN PROSTATE CANCER

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Recent studies have identified regions on chromosome arms 8p, 10q, 16q and 18q that are frequently deleted in human prostate cancer. We have previously described a loss of heterozygosity (LOH) at the MET locus on chromosome band 7q31, in a study of 20 localized prostate tumors. To determine whether a region on the 7q arm is important in the initiation and/or progression of prostate cancer, prostate tissue from 13 patients with confined prostate tumors, 17 with local extracapsular extention and 13 with metastatic forms were analysed for LOH, using a DNA probe for restriction fragment length polymorphism (RFLP, pMetH) and eight CA microsatellite repeats (seven on 7q21-q33, one on 7p). Twenty of the 43 cases studied (47%) showed LOH at one or more 7q loci.

The most frequently deleted region was chromosome 7q31.1-7q31.2, while the centromeric locus on 7q21 was generally conserved. The percentage of LOH was normally distributed around the D7S480 locus. Moreover, the rate of LOH in the 7q31 region was lower in metastatic tumors than in localized tumors. These results strongly suggest the presence of a tumor suppressor gene on the chromosome band 7q31 with an important role in the early stages of prostate cancer.