PP-1. Biology (September 11)

ORAL PRESENTATIONS

PP-1-1 Refinement of the Two Regions on the Long Arm of Chromosome 16 Involved in Breast Cancer

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Allelic imbalance on 16q is the most frequent genetic alterations involved in 60 to 65% of invasive breast cancer [1]. Many studies led to the conclusive evidence of at least two separate regions on 16q that are candidates for containing a tumor suppressor gene involved in breast cancer progression [1]. In the aim of the refinement of these two regions we performed a detailed 16q allelic imbalance mapping for 27 invasive breast carcinomas (45%) showing partial genetic alterations on chromosome 16q.

Thirty-two microsatellite markers were analyzed including 10 markers located on 16q22.1 and 4 markers mapping to 16q24.3-qter. The higher incidences of allelic imbalance were observed for D16S397 and D16S301 on 16q22.1 and for D16S413 and D16S3023 on 16q24.3. Our data allow to bracket the smallest regions of overlap (SRO) by markers D16S318 and D16S496 and by markers D16S413 and D16S3023 respectively. The present study that further narrow the SRO allow to begin searching for candidate genes involved in breast cancer.

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[1] Doggett et al. Cytogenet. and Cell Genet. Report of the fourth international workshop on human chromosome 16 mapping. In press.

PP-1-2 Modulation of Human Stromelysin-3 (ST3) Promoter Activity by Human Breast Cancer Cell Conditioned Medium

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ST3 is a matrix metalloproteinase specifically expressed in fibroblasts surrounding invasive foci of human breast cancers (Basset et al. Nature 1990; (348) 699–704). 0.46 kB (ST3-1) and 3.4 kB (ST3-2) of the 5'flanking sequence of the human ST3 gene were subcloned into expressionless chloramphenicol-acetyl transferase (CAT) and luciferase reporter vectors (pBLCAT6 and pGL3Basic respectively) for use in transient transfection assays in NIH3T3 fibroblasts using calcium phosphate co-precipitation. Results were related to the positive control SV40-CAT/Luciferase activity. The addition of various cytokines (phorbol ester 30 ng/ml, tumour necrosis factor 10 ng/ml, basic fibroblast growth factor 20 ng/ml, epidermal growth factor 20 ng/ml) had no effect on reporter gene activity regulated by either ST3 promoter. However the addition of serum-free, conditioned medium from the human breast cancer cell lines MCF-7 and MDA-MB231 resulted consistently in a 1.7–2 fold increase in ST3-2 reporter gene activity whereas conditioned medium from an SV40-immortalized human mammary epithelial cell line (MTSV 1–7) had no effect on ST3-2 promoter activity. These data suggest that a soluble factor released specifically by breast cancer cells can upregulate ST3 promoter activity and provides an explanation for the localization of the enzyme.

PP-1-3 Luminometric Immunoassays of P53 and uPA in 600 Node-Negative Breast Cancers; First Evaluation of the Prognostic Values

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P53 and uPA are potentially useful parameters for evaluating breast cancer prognosis. We detected these two parameters in a population of 600 node-negative primary breast cancers. P53 and uPA were assayed in cytosols prepared for estradiol receptor (ER) and progesterone receptor (PgR) assays and conserved at −80 C. The assay kits were purchased from Sangtec Medical (LIA-mat p53 and LIA-mat uPA, Laboratoires Byk, France). We showed that 13% of the tumours were p53 positive (> 4 ng/ml) and that 22% were uPA positive (> 0.5 ng/ml). P53 was related (X^2) to histoprognostic grading (HPG), ER and PgR. UPA was related to ER, PgR, HPG and tumor size. P53 and uPA were positively correlated (Spearman test) ($p = 0.0001$). In prognostic studies, 313 patients who underwent operations between 1989 and 1992 were included. The mean duration of follow-up of living patients was 4 years. In overall studies, Cox univariate analyses demonstrated a prognostic value of PgR, HPG, tumor size, uPA ($p = 0.037$) and p53 ($p = 0.018$). In Cox multivariate analyses, none of these parameters was shown to be more (or less) important than the others. In relapse free survival studies, Cox univariate and multivariate analyses demonstrated prognostic values of uPA ($p = 0.0004$) and of age. This study confirms that p53 and uPA have prognostic values in node-negative breast cancer and that uPA (with age) strongly predicts relapse.

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PP-1-4 Detection of Isolated Tumor Cells in Peripheral Blood and Bone Marrow in Stage I and II Breast Cancer

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The primary goal of this project is to examine the presence of carcinoma cells in bone marrow (BM) and peripheral blood (PB) in 1000 patients with stage I and II breast cancer and to determine the prognostic significance of positive findings. Mononuclear cell suspensions are made from PB- and BM samples and analyzed for tumor cells by two techniques: a) Immuncytochemical analysis of cytospin preparations using antibodies to cytokeratin. b) Immunomagnetic separation of tumor cells with Dynabeads coated with mAb against surface antigens specific to epithelial cells. The rosetted tumor cells are then visualized with immuncytochemistry. Peripheral blood and bone marrow have been collected from a total of 201 patients from May 1995 through February 1996. So far, 75 patients have been examined for the presence of tumor cells. Of 46 stage-I-patients 17.4 percent had detectable tumor cells in bone marrow and of 29 stage-II-patients 37.9 percent showed bone marrow positivity. Further results and details of the methodology will be presented.

PP-1-5 Quantitative Determination of c.erbB.2 Oncoprotein in 1065 Human Breast Tumours by an Enzymoimmunoassay. Comparison with the Clinico-Biological Parameters: A Multicentric Study

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Overexpression of the c.erbB.2 protooncogen has been shown to correlate with relapse and poor prognosis in human breast adenocarcinomas. This parameter was tested in a prospective study with a commercial kit (Ciba Corning/Triton Diagnostics) in 1065 operable human breast tumours from six anticancer centers: Angers (n = 336), Bordeaux (n = 222), Caen (n = 161), Nantes (n = 150), Villejuif (n = 120), Reims (n = 76). C.erbB.2 protein was determined by an enzymoimmunoassay. Results were expressed in arbitrary units/mg membrane proteins after adjustment taking into account the anticancer Center. A significant correlation was found between median c.erbB.2 value and histoprognostic grading (grade I: 125, grade II: 154, grade III: 196, $p = 0.003$), estrogen receptors (ER^-: 191, ER^+: 151, $p = 0.01$) and progesterone receptors (PgR^-: 233, PgR^+: 136, $p < 0.0001$) in the whole series as well as in infiltrating ductal carcinomas (IDC). Furthermore, there was an excellent correlation with DNA amplification (n = 374, R