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

F. Bonnet-Dorion*, I. Hostein, S. de Resende, S. Mautalen, C. Berthier, B. Duboué, R. Lidereau, D. Birnbaum, M. Longy. *Department of Molecular Oncology, Institut Bergonié, 33076 Bordeaux, France*

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

A. Ahmad* [1], S. Mitchell [1], P. Anglard [2], P. Basset [2], I.R. Hart [1]. [1] *St. Thomas' Hospital, London, UK;* [2] *IGBMC, Strasbourg, France*

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

J.-Ph. Peyrat*, L. Vanlemmens, J. Fournier, M.M. Louchez, J. Bonneterre. *Centre Oscar Lambret, BP 307, 59020 Lille Cédex, France*

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

R. Kaaresen, E. Borgen [1], C. Beiske [1], H. Quist [2], I. Guldvaag, E. Schlicting, G. Kvalheim [3], B. Naume [3]. *Department of Surgery, Ullevaal Hospital, Oslo, Norway;* [1] *Department of Pathology, The Norwegian Radium Hospital, Oslo, Norway;* [2] *Department of Surgery, The Norwegian Radium Hospital, Oslo, Norway;* [3] *Clinical Stem Cell Laboratory, The Norwegian Radium Hospital, Oslo, Norway*

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

S. Koscielny, A. Daver, J. Wafflart, J. Goussard, G. Ricolleau, A. Rallet, Ph. Terrier, J.C. Delarue*. *Tissue Biopathology Group of the FNCLCC, 75013 Paris*

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

= 0.31, p = 0.0001) and with results obtained by immunohistochemistry (n = 115, R = 0.53, p = 0.0001). By using the sensitivity and specificity curves, with amplification as reference, a cut-off value of 200 arbitrary units was chosen to appreciate overexpressed cases. With this cut-off, 34% (361/1065) of the cases were overexpressed. So, this kit appears as a good tool to quantitatively determine c.erbB.2 protein and our populations will be followed up to appreciate the prognostic value of this parameter.

PP-1-6 Expression of BCL-2 in Node-Negative Breast Cancer is Associated with Various Prognostic Factors, but does not Predict Response to Peri-Operative Chemotherapy

H.-J. van Slooten*[1], P.C. Clahsen[1], J.H. van Dierendonck[1], Ch. Duval[3], C. Pallud[3], A.-M. Mandard[3], A. Delobelle-Deroide[3], C.J.H. van de Velde[1], M.J. van de Vijver[2]. [1] *Department of Surgery, [2] Department of Pathology, Leiden University Hospital, PO Box 9600, 2300 RC Leiden, The Netherlands;* [3] *Member Institutions of the EORTC Breast Cancer Cooperative Group*

Bcl-2 expression may be related to response to chemotherapy and a number of pathologic and biologic tumor parameters in premenopausal, lymph node-negative (N^-) breast cancer (BC) patients. Expression of Bcl-2 was determined using immunohistochemistry on paraffin-embedded sections in a series of 441 premenopausal, N^- BC's from patients randomized to receive peri-operative chemotherapy (PeCT) (5-fluorouracil, doxorubicin, cyclophosphamide) or no PeCT in EORTC Trial 10854. Strong positive correlations were found between high Bcl-2 expression and estrogen and progesterone receptor positivity and low tumor-grade, whereas high Bcl-2 expression was negatively correlated with p53 and c-erb*B*-2 positivity, high Ki-67 index, mitotic index and large tumor-size. Patients with tumors expressing high levels of Bcl-2 had a significantly better disease-free (p = 0.004) and overall (p = 0.009) survival. However, in a multivariate model this association no longer remained significant. There was a trend for an effect of PeCT on disease-free survival both for patients with Bcl-2 positive (HR = 0.61, 95% C.I. 0.35–1.06, p = 0.07) and negative (HR = 0.55, 95% C.I. 0.27–1.12, p = 0.09) BC's at a median follow-up of 49 months. *Conclusions:* The level of Bcl-2 expression does not seem to predict response to PeCT in premenopausal, N^- BC patients. High levels of Bcl-2 are preferentially expressed in well-differentiated tumors and associated with favorable prognosis.

PP-1-7 Accumulation of TP53 as Predictor of Response to Chemotherapy of Recurrent Breast Cancer

P.M.J.J. Berns, J.A. Foekens*, H.H. de Witte, W.L.J. van Putten, M.P. Look, K. Willman, M.E. Meijervan Gelder, Th.J. Benraad, J.G.M. Klijn. *Rotterdam Cancer Center (Dr Daniel den Hoed Kliniek)/Academic Hospital Rotterdam, the Netherlands; St Radboud Hospital, Nijmegen, the Netherlands; Sangtec Medical, Bromma, Sweden*

We have shown that TP53 protein accumulation predicts a poor response to hormonal therapy in recurrent breast cancer. To evaluate whether TP53 protein accumulation can predict the response to chemotherapy in patients with recurrent breast cancer, TP53 protein levels were measured in routinely prepared cytosols from primary breast tumors, using a quantitative luminometric immunoassay (Sangtec Medical). Patients who developed recurrent disease received either first-line chemotherapy (n = 92; 48% premenopausal, 30% ER/PgR-positive, 60% with a disease-free interval [DFI] > 12 months), or first-line hormonal therapy followed by chemotherapy (n = 180; 27% premenopausal, 67% ER/PgR-positive, 67% with a DFI > 12 months). In univariate analysis, TP53 protein accumulation does not predict response to first-line chemotherapy. With respect to chemotherapy after tamoxifen therapy, TP53 protein accumulation only showed a relation with progression free-survival when analyzed as a dichotomized (cut-off value 1.6 ng/mg protein) variable (p = 0.02), but not as a continuous variable, with a relative hazard rate (95% confidence limits) of 1.5 (1.1–2.2). In conclusion: patients with high TP53 protein levels, as measured by LIA, respond poorly to chemotherapy only after failure to tamoxifen therapy.

PP-1-8 Cyclin D1 Expression and Response to Tamoxifen Treatment for Metastatic Breast Cancer

D.M. Barnes, C.E. Gillett, P. Smith, R.D. Rubens. *ICRF Clinical Oncology Unit, Guy's Hospital, London*

Cyclin D1 is a cell cycle associated protein active in the G1 phase of the cell cycle. Amplification of the gene is found in approximately 20% of mammary carcinomas and immunohisto-chemistry has revealed that overexpression of cyclin D1 protein occurs more frequently. It is present in 40–50% of breast cancers suggesting oncogenic activity which could be associated with poor clinical outcome. Surprisingly, we found the reverse to be true; in primary breast cancer the highest levels of cyclin D1 expression occur in well differentiated ER positive tumours, usually associated with a good prognosis. We have also investigated the relationship between cyclin D1 protein expression and response to first line tamoxifen treatment for metastatic disease in 149 women. Response to treatment was assessed in a standard manner, according to UICC criteria and was available on all patients. Women whose response was unassessable were excluded from the study. 95 (64%) of cases overexpressed cyclin D1, 78 (82%) were ER positive. Response (complete/partial) was seen in 55 (71%) of these double positive tumours. Conversely tumours which were negative for both proteins had only an 8% chance of responding. Tumours which were positive for only one of the proteins had an intermediate response rate ($x^2 = 31.97$, $p < 0.0001$). Suggesting that immuno-histochemical staining for cyclin D1 could be a useful adjunct to the measurement in ER in identifying women who are likely to respond to endocrine treatment. Furthermore, these results pose interesting questions concerning the role of cyclin D1 in the biology of breast cancer.

POSTER PRESENTATIONS

PP-1-9 Tumor-Associated Lymphomonocytes from Neoplastic Effusions of Patients with Different Primary Tumors Including Breast Cancers are Able to Release Cytokines

G. Mantovani*[1], A. Macciò[1,3], R. Versace[2], M. Pisano[2], P. Lai[1], S. Esu[1], M. Ghiani[1], D. Dessì[1], R. Cherchi[2], G.B. Melis[3], G.S. Del Giacco[1]. [1] *Dept. of Medical Oncology, University of Cagliari, 09124 Cagliari, Italy;* [2] *Thoracic Surgery Division, "R. Binaghi" Hospital, Cagliari, Italy;* [3] *Dept. of Obstectris and Gynecology, University of Cagliari, Cagliari, Italy*

We studied several "in vitro" activities of tumor-associated lymphomonocytes (TALM) and the levels of IL-1α, IL-1β, IL-2, IL-6, TNFα and soluble IL-2 receptor (sIL-2R) in neoplastic effusions and in the serum of advanced stage cancer patients. Comparisons were made with the behavior of autologous peripheral blood mononuclear cells (PBMC) using PBMC from normal subjects as controls. TALM were collected from 12 peritoneal and 15 pleural neoplastic effusions. The peritoneal effusions were mainly secondary to primary ovarian cancers and included 1 breast cancer as primary. The pleural effusions were secondary to primary lung and breast cancers. The blastic response to PHA and anti-CD3 monoclonal antibody (mAb) of TALM was lower than that of autologous PBMC, whereas proliferative response to recombinant IL-2 of both TALM and autologous PBMC was in the same range. Blastic responses of patient PBMC were lower than those of control PBMC. No significant differences were found for the expression of IL-2R subunities after PHA or anti-CD3 mAb stimulation between TALM and autologous PBMC, which, in both cases, was lower than that of control PBMC. After PHA stimulation, the levels of IL-1α, IL-1β and TNFα in culture media of TALM were lower than those of autologous PBMC, whereas, IL-2 and IL-6 levels were significantly higher. The cytokine production from patient PBMC was always lower than that of control PBMC. The levels of IL-6, TNFα and sIL-2R in neoplastic effusions were significantly higher than those of autologous serum. The levels of all cytokines were higher in patient than in control sera. Our data seem to suggest that a general impairment of the immune function is present in cancer patients with advanced disease, such as those with neoplastic effusions, involving not only TALM but also autologous PBMC.

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PP-1-10 Cytogenetic Analysis in Short Term Culture of Breast Cancer in Korea Women

J.W. Bae*, E.S. Lee, B.H. Koo, S.H. Park, Y.H. Chun. *Korea University Hospital, Korea University, Seoul, Korea*

This study was to analyze the cytogenetic characteristics of 20 primary human breast cancer cells. Different growth media and procedures for tissue aggregation and culturing were tested with regarding to cell attachment, the type of cells in outgrowth, and the emergence of cytogenetically abnormal clones. We found out that optimal tissue disaggregation was obtained by combined mechanical and enzymatic treatment of the tumor samples. Use of the plastic flask coated with Vitrogen 100 and the serum free growth