Bre 19

MONOCLONAL ANTIBODIES TO HUMAN PROGESTERONE RECEPTORS
G. Bastert, U. Ketzler-Sasse (a.G.) and S. Kaul (a.G.)

The role of steroid and pituitary hormones in the pathogenesis of human breast cancer is unclear, but the value of measuring hormone receptor levels to evaluate the endocrine dependence is well established. Monoclonal antibodies to human estrophilin have important clinical applications for the analysis of the receptor by ELISA and immunohistochemistry. Consequently, the development of monoclonal antibodies to other hormone receptors, especially the progesterone receptor proteins, would be highly desirable.

T-47D mammary carcinoma cells synthesize extraordinary amounts of progesterone receptor proteins (PR). The total level is 5 pmol/mg protein and account for 0.2-0.5% of the cellular protein. PR was prepared using affinity chromatography and FPLC purification from 1×10^9 T-47D cells, stabilized by adding the synthetic progestin ORG 2058 at a final concentration of 30 nM and used immediately for the immunization of BALB/c-mice. Splenocytes from three high-responder mice were fused to X63Ag8.653 myeloma cells.

Hybridomas (2880) were screened by a solidphase RIA. Mouse Ig was bound to solid phase and incubated with affinity purified PR labeled with 3H-ORG 2058. Positive cultures were cloned and recloned. Monoclonal antibodies used for purification and characterization of the receptor protein from T-47D mammary carcinoma cells.

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Bre 20

ESTROGEN RECEPTORS IN AXILLARY LYMPH NODE METASTASES OF INVASIVE DUCTAL BREAST CANCER
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In the present study axillary lymph node metastases of 34 female patients with invasive ductal breast cancer were evaluated immunohistochemically (ER-ICA; ABBOTT, Wiesbaden) for their estrogen receptor (ER) content. Biochemically, the primary tumors of 23 patients were found to be ER+, while 11 tumors were ER-. Both patient groups were comparable, considering age as well as stage of the disease. One half of the lymph nodes which were dissected from the unfixed axillary fat tissue, had been snap-frozen in liquid nitrogen and stored at -75°C between 1 and 33 months. In addition, the minimal and maximal number of proliferating cells in the metastases was determined immunohistologically using the monoclonal antibody Ki 67.

- In the ER- group 62.2 % out of 188 axillary lymph nodes exhibited metastases while in the ER+ group only 25.8% out of 326 lymph nodes were infiltrated ($p < 0.001$).

- Irrespective of the duration of storage, nearly all lymph node metastases of the ER+ group showed a mostly intensive staining of the tumor cell nuclei. Only in three of these cases the lymph node metastases were ER-.

- Immunohistochemically, the frequency distribution of ER+ tumor cells sometimes varied considerably within the metastases.

- A semiquantitative analysis of our material revealed no statistical correlation between the ER-content and the minimal as well as the maximal proliferation rate of the lymph node metastases.

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Bre 21

TUMOR MARKERS IN DIFFERENTIAL DIAGNOSIS OF SINUS HISTIOCYTOSIS AND AXILLA LYMPH NODE METASTASIS WITH LOBULAR INVASIVE BREAST-CARCINOMA.
C.Tschahargane, I. Breuner and A. Müller

The problem becomes particularly evident if one compares axilla lymph nodes with diffuse sinus histiocytosis and lymph node metastases of highly differentiated lobular carcinoma. In the last 5 years we classified 39 lobular carcinoma in the stage T1-4, No, Mx. From these female patients 15 to 25 lymph nodes proved to be tumor cell-free, by the usual HE-coloring. For the immunohistochemical presentation of micrometastases in the lymph nodes, Epithelial Membran Antigen (EMA) and Ulex-Lectin I (UL) were used. The immunohistochemical marking was done following the PAP method of BURNS. All lymph nodes of 39 cases (n:775) were examined with UL and EMA. Six out of the 775 lymph nodes contained single tumor cells in the margin sinus and showed a definitive positive expression as well with UL as with EMA. This corresponds to 3 out of the investigated cases. These patients were clinically retrospectively examined; they had carcinoma in stage T1 and T2 which were surgically removed in 1980, 1981 and 1984. The so far insufficient observation period was inconspicuous, that means local recidives of tumor metastases have not appeared up to now. The percentage of micrometastases of the lobular carcinoma in all states in relation to the number of the examined lymph nodes is with only 0.77 % minimal. With regard to the number of the examined N-cases one can find such a micrometastasis in 9.6 % of the examined patients. The underlying investigation shows that with the help of EMA a differentiation between sinus histiocytosis and lymph node metastases in formalin fixated lymph nodes is possible.

Bre 22

CYTOLOGY AND HORMONE RECEPTORS (TCC and mARs) IN BREAST CARCINOMA
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745 adequate Pappenheim stained biopsy smears of 923 breast cancer specimens sent for hormone receptor analysis were visually graded at 500x magnification in eight groups. The most important groups are listed below with the number of cases, percentage of receptor positives (PR+ and/or PR+), perc. of PR+, perc. of PR+, perc. of PR+ and PR+ (TTC-Methd.). Group 2: small nuclei n = 37, 97%, 76%, 70%, 49%; Group 3: mostly small nuclei n = 143, 81%, 64%, 57%, 40%; Group 4: mostly large nuclei n = 168, 84%, 68%, 53%, 38%; Group 5: nucleoli rare/small n = 150, 69%, 53%, 47%, 31%; Group 6: prominent nucleoli n = 146, 51%, 34%, 28%, 11%; Group 7: multiple prom. nucleoli n = 63, 37%, 30%, 19%, 13%. Statistically significant differences to complementary subsets were proven for PR- and PR- (receptor negative) versus PR+ and/or PR+ (receptor positive); PR- vs. PR+; PR- vs. PR+; both receptors negative vs. borderline (PR and/or PR 10-19); borderline vs. PR and/or PR over 19; PR 10-49 vs. PR over 49; PR 10-49 vs. PR over 49. Monoclonal antibodies: We were able to apply the PR-ICA (Estrogen Receptor Immunocytochemical Assay, ABBOTT) to breast carcinoma biopsy smears and the PR-FIA (Estrogen Receptor Enzyme-Immuno-Assay, ABBOTT) to breast carcinoma cytospins in 728 cases and compare the results to PR(TTC), PR(TCC) and the cytological grading described above. The visual assessment of the PR-ICA slides showed a subjective component. Cases with high estrogen receptor values as determined by PR-FIA had the best chance of being detected by the PR-ICA method by the observers. There is a relation between the visual nuclear grade from the Pappenheim-stained specimens and PR-ICA and PR-FIA. The 48 cases without nucleoli (groups 1-4) had an average FIA-value of 100 fmol/mg. The 57 cases with nucleoli (groups 5-7) had an average PR-FIA value of 37 fmol/mg. For 28 cases with prominent or multiple prominent nucleoli (groups 6-7) average PR-FIA was 15 fmol/mg. With increasing PR-FIA values we found decreasing average nuclear grades: 31 cases PR-FIA < 10, $\bar{x}$ nucl. grade = 5.4; 34 cases PR-FIA $\geq 10 < 50$, $\bar{x}$ nucl. grade = 4.6; 20 cases PR-FIA $\geq 50 < 100$, $\bar{x}$ nucl. grade = 4.2; 19 cases PR-FIA ≥ 100 , $\bar{x}$ nucl. grade = 3.8. Conclusion: Hormone receptor values decrease with increasing nuclear grade. Our present data show a closer correlation of cytology with PR-FIA than with PR (TTC) and PR (TCC).

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