

and ER-positive patients. This observation does not confirm previously reported results (3). However, we observed a significant negative correlation between both the androsterone-aetiocholanolone (A/Ae) ratio ($r = -0.60$, $P < 0.01$) and the allo-tetrahydrocortisol-tetrahydrocortisol (aTHF/THF) ratio ($r = -0.56$, $P < 0.01$), and the logarithm of the ER-concentration. This correlation was especially good for the 5 α -reductase products androsterone ($r = -0.36$, $P < 0.1$) and allo-tetrahydrocortisol ($r = -0.61$, $P < 0.005$). Contrarywise no significant correlation was found between the ER-concentration and the 5 β -reduced metabolites. On the other hand there was no significant difference in A and 5 α -THF values between ER-negative and ER-positive patients. These observations suggest an inverse relationship of 5 α -reductase activity with the ER-concentration in breast cancer.

References

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 41. Estradiol receptor activity (ER) in right or left sided breast cancer, L. BEECH, A. KOENDERS, G. PIETERS, A. SMALS, J. HENDRIKX, Th. BENRAAD, P. KLOPPENBORG, Departments of Medicine and Radiology, University of Nijmegen, Nijmegen, The Netherlands
- In 145 patients with advanced breast cancer the primary tumor was located in the right breast in 76 cases and in the left breast in 63 cases, whereas in 6 patients both breasts were affected (left/right ratio L/R 63/76). Most patients detected their tumors by self-palpation. In the patients, premenopausal at time of initial diagnosis, L/R was 29/42. In this group the L/R for women with estradiol receptor positive tumors (ER +, dextran coated charcoal assay and Scatchard plot analysis) was 22/18, and for ER negative (ER-) patients 7/24 ($\chi^2 = 6.314$, $P < 0.02$). In patients postmenopausal at the time of initial diagnosis, L/R for ER+ patients was 20/22 and for ER-patients 14/12 ($\chi^2 = 0.052$, $P < 0.1$).
- Conclusion: in patients with advanced breast cancer who developed their primary tumor premenopausally, ER-tumors were predominantly located in the right breast.
42. Estrogen receptors in normal and malignant human breast tissues, J. DE BOEVER, K. DE GEEST, G. VAN MAELE¹ and D. VANDEKERCKHOVE, Department of Obstetrics and Gynecology and ¹Medical Information
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- Cytoplasmic estrogen receptors (ER) were measured in extracts of normal breast tissue and of the mammary tumour specimens of 180 breast cancer patients - 142 patients with early breast cancer and 33 with advanced breast cancer. Receptors were measured with a Dextran-coated charcoal technique employing ³H-estradiol and diethylstilbestrol. Steroid receptor (ER) binding parameters were calculated from Scatchard plots. 58% of all breast cancer tissues were estrogen receptor positive (ER+) with binding capacities of 3.1 to 2545 fmol of ER/mg protein and with dissociation constants (Kd) between 0.1 and 7×10^{-10} mol/l. 41% of the breast cancer tissues did not contain measurable quantities of ER and were considered to be negative (ER-). 42 normal breast tissue samples were examined for ER content and all were found to be negative (ER-). Both ways of expressing the ER content of tissues: fmol/mg protein of fmol/g wet tissue were found to be equally suitable and reliable (correlation $r = 0.99$; $P < 0.001$). Two more good correlations were found: between Kd and the binding index (BI) and between ER levels and BI.
- In the early breast cancer group ER+ tumours were found in 65% of the postmenopausal patients and in 50% of the premenopausal patients. Postmenopausal patients had significantly higher ($P < 0.01$) ER levels.
- Multivariate analysis revealed no significant differences between ER+ and ER- primary tumours of pre- and postmenopausal patients in relation to known prognostic factors: histology, tumour volume, tumour location in the breast, clinical stage and axillary nodal status. In addition there was no correlation between these factors and ER levels.
- In the group with advanced breast cancer 64% of the tumours were ER+ for both pre- and postmenopausal categories.
43. Immunofluorescent observation of prolactin receptors in cultured mammary carcinoma cells, H. TAKIKAWA¹, R. HORIUCHI¹, S. TANAKA¹ and K. TARUSAWA², ¹Institute of Endocrinology, Gunma University, Maebashi, and ²Japan Immunoresearch Laboratories, Takasaki, Japan
- The presence of prolactin receptors in mammary carcinoma has been reported by a number of investigators. It is accepted that prolactin plays an important role in carcinogenesis of mammary tumours. Data will be presented on the immunofluorescent observation of the receptor in DMBA induced rat mammary carcinoma. Tumours were induced in female Sprague-Dawley rats with a single dose of DMBA in cotton seed oil given orally. Tumours were rapidly excised and after removal of necrotic parts cut into small pieces. The tissue pieces were incubated with collagenase dissolved in Dulbecco's modified Eagle's medium supplemented with foetal calf serum. After filtration, the cell suspension was centrifuged and the cell pellet suspended in the medium,

which contained antibiotics, HEPES and TES, and inoculated into plastic dishes. After 2 or 3 passages, cloning was done using micro plates. Anti-prolactin rabbit serum was labelled with FITC. The cultured cells on the micro plates were incubated with prolactin and washed with PBS. Then they were incubated with FITC-labelled anti-prolactin. Immediately after removal of excess antibody by washing with PBS immunofluorescence was observed by fluorescence microscopy. Prolactin receptors could be observed in the cultured carcinoma cells.

PROSTATE CANCER

44. Characterization of a transplantable androgen-dependent human prostatic carcinoma (PC 82), J.C. ROMIJN, G.J. v. STEENBRUGGE, K. OISHI, J. BOLT-DE-VRIES, W. HÖHN and F.H. SCHRÖDER, Erasmus University, Rotterdam, Dr. Molewaterplein 40, Rotterdam, The Netherlands

Recently a human prostatic tumor line (PC 82), growing on athymic (nude) mice by serial transplantation, has been established in our laboratory. Characterization of this tumor line was carried out to establish whether the PC 82 line might serve as an appropriate model system for the investigation of human prostatic cancer.

The histological characteristics of the PC 82 tumor closely resemble those of the original tumor, a moderately differentiated adenocarcinoma, even after two years of growth in nude mice. Doubling times were estimated from a large number of growth curves; in the exponential phase of growth a doubling time of 2-3 weeks was measured. The presence of large amounts of (human) prostatic acid phosphatase, in the original tumor as well as in the PC 82 line, was demonstrated by means of an immunohistochemical technique. The PC 82 tumor did not grow on female nude mice, whereas in male nude mice the take rate was around 75%. Furthermore, castration of a tumor-bearing male resulted in regression of the tumor. The presence of an androgen receptor was demonstrated.

The results mentioned above (maintenance of original histology, relatively slow growth rate, presence of acid phosphatase, androgen dependence) suggest that PC 82 might be considered as a useful system for the study of human prostatic cancer.

45. In vitro metabolism of androgen by rat prostate, J.C. PLASSE¹, A. REVOL¹ and B.P. LISBOA², ¹Hospices Civils de Lyon, Hôpital Jules Courmont-Sainte Eugénie, 69230 Saint Genis Laval, France, and ²Universitäts-Frauenklinik Eppendorf, 2000 Hamburg 20, West Germany

In order to investigate the metabolism of some physiological androgen steroids by rat prostate, testosterone, androstenedione, 5 α -

androstenedione, 5 α -androstane-3 β ,17 β -diol, 5 α -androstane-3 α ,17 β -diol, epiandrosterone and androsterone were separately incubated with a ventral rat prostate homogenate in a medium supplemented with a NADPH regenerating system at 37°C under air. All incubations were carried out simultaneously with a portion of the same rat prostate homogenate. Identification and evaluation of metabolites was done by liquid and radio-gas chromatography. In vitro metabolism of testosterone by rat prostate can follow two main pathways of similar quantitative intensity, through either 5 α -dihydrotestosterone or 4-androstenedione. The relationship between both the 17-keto and the 17 β -hydroxy-pathway exists although the 17 β -hydroxysteroid oxidoreductase activity is weak towards 17-ketosteroids. Both 17-keto and 17 β -hydroxysteroid are good substrates for 3 α -hydroxysteroid oxidoreductase and thus an equilibrium is reached between 3 α -hydroxy and 3-keto-metabolites. On the contrary equilibrium between 3-keto and 3 β -hydroxy-metabolites is in favour of the 3 β -hydroxysteroid formation. Some hydroxylated metabolites such as 6 α - and 6 β -hydroxysteroid were identified.

46. Characterization of androgen receptors in a rat prostate adenocarcinoma, O.A. LEA and F.S. FRENCH, Hormone Laboratory, University of Bergen, Norway, and Department of Pediatrics, University of North Carolina, Chapel Hill NC 27514, U.S.A.

Androgen receptors present in cytosols prepared from transplants of the Dunning H 3327 adenocarcinoma were characterized following labelling in vitro with ³H-dihydrotestosterone and ³H-testosterone. Apparent dissociation constants measured at 0°C were 3·10⁻⁹ M for both steroids. Dissociation half-lives were 87 h and > 200 for testosterone and DHT, respectively. In buffers of low ionic strength the receptor showed a sedimentation coefficient of 8 S. A shift to approximately 5 S was observed when ionic strength was increased to 0.5. Two forms of androgen receptor were recovered when cytosol was fractionated on phosphocellulose columns. A predominant form eluted from the cation exchanger at 0.15-0.20 M KCl. Further characterization in the presence of 0.5 M KCl revealed a sedimentation coefficient of 5.0 S, a gel filtration radius of 53 Å, a molecular weight of 110-120,000 Daltons and a frictional ratio of 1.7. A smaller form of the androgen receptor was eluted from the phosphocellulose columns at a higher salt concentration. It has a sedimentation coefficient of 3.6, a gel filtration radius of 35 Å, a molecular weight of 55-60,000 Daltons and a frictional ratio of 1.4. Heating of tumor cytosol at 25-37°C for periods up to 30 min did not produce an inter-conversion of the two receptor forms. Androgen receptors with similar size, charge and symmetry were present in cytosols derived from testis; epididymis, ventral and dorsal prostate and seminal vesicles.