170

EVIDENCE OF HORMONE RECEPTORS IN TESTICULAR TISSUE

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In our study on the involvement of hormones as promoters of tumor growth in testicular cancer (7), the receptors for FSH, LH, PRL, testosterone, and estradiol were investigated immunohistochemically. FSH receptors were found in the surface of Sertoli cells of 5/5 human healthy testicles. Intensive fluorescence was also demonstrated in case of a malignant teratoma with trophoblastic component, a benign Leydig cell tumor, an embryonal carcinoma, a differentiated carcinoma, and two pure seminomas; another one was negative. The evidence of LH receptors was successfully probed in the healthy testicles on the surface of all Leydig cells in varying intensity. In analogy to FSH, the LH receptors were also positive in all testicular tumors, though in varying intensity in the Leydig cell tumor. Prolactin receptors were found in all Leydig cells of healthy testicles as well as on the cell membranes of 2 embryonal carcinomas, 2 seminomas, 1 differentiated teratoma, and 1 immature teratoma with trophoblastic components. The evidence of testosterone receptors was found within the Leydig and Sertoli cells in varying intensity, whereas the cells of the spermiogenesis and all tumor cells did not react. The estrogen receptor was determined intracellularily at different staining intensities in all Sertoli cells as well as in embryonal carcinomas, seminomas, Leydig cell tumors, and in differentiated or immature teratomas.

171

MEDIATION BY ANDROGEN RECEPTOR OF THE STIMULATORY EFFECT OF TESTOSTERONE ON THE GROWTH OF FIBROSARCOMA IN THE RAT.

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The aim of this work was to investigate the effect of testosterone (T) on the growth of a 20 methylcholanthrene-induced fibrosarcoma transplanted in the rat. An assay of androgen receptor (AR) in this tumor has been developped. An antiandrogen, cyproterone acetate (CA) was used to demonstrate the androgen sensibility of this tumor. The tumor growth in male Wistar rats was studied in 2 experiments - **Exp I**: orchidectomized, four groups; G1: (n=10) treated by T heptanoate ; G2: (n=10) identical to G1 plus treatment with (CA); G3 (n=10) control and G4 control not castrated; -**Exp II**: normal, two groups; G5 treated with CA and G6 control . Daily measurement of the tumor was carried out by calliper and the tumors were weighted at sacrifice. T treatment induced a statistically significant increase of tumor growth rate, CA prevented this effect. Castration of animals slows down the growth of the tumor. Nuclear (N) and cytosolic (C) extracts of the tumor were found to bind tritiated methyltrienolone (^{3}H1881). The binding sites were saturable with 20-30 nM of ^{3}H1881. The dissociation constant of the hormone receptor complexes (Kd) was 30-40 nM and the number of binding sites was 80-140 fmole/mg of protein. The hormone receptor complexes sedimented in the region of 3.7S on linear sucrose gradient (5-20%) analysis. **These data strongly suggest that androgen action on the tumor is mediated by androgen receptors.**

172

RESPONSE OF HUMAN OVARIAN CANCER CELLS IN VITRO TO ANTIANDROGENS

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We previously demonstrated that most ovarian cancers contain androgen receptors (AR). We investigated the effect of three antiandrogens (anandron (ANAN), flutamide (FLU) and hydroxy-flutamide (OHF)) on the proliferation of primary cultures initiated from ovarian cancer tissue (n=15) and ascitic fluid of ovarian cancer patients (n=9) *in vitro*. Antiandrogens were added daily to monolayer layer cultures in RPMI 1640 medium, supplemented with fetal calf serum (10 % v/v). After 10 days, cells were harvested and viable cells counted. In the tumor tissue, the AR concentration was determined by saturation analysis.

Overall, the addition of antiandrogens (0.01, 0.1 and 1 μM) resulted in 89.4, 75.4 and 62.6 % survival, compared to untreated controls (survival = 100 %). There was no significant difference in effectiveness of ANAN, FLU and OHF. At a concentration of 1 μM, all three antiandrogens inhibited cell proliferation (survival < 70 %) in about 60 % of the cases. AR were detected in 13 of the 15 tumors. Eleven of the 13 AR-positive were inhibited by antiandrogens (1 μM: % survival = 58.4 ± 12.5: mean ± SD), while none of the two cultures initiated from AR-negative tumors showed such a response (1 μM: % survival = 77.0 ± 5.6).

The present results, showing that the *in vitro* proliferation of cultures initaited from AR-positive tumors can be inhibited by antiandrogens, together with our previous observations on the presence of AR in most ovarian cancers, strenghten the need for a clinical trial on the effect of antiandrogen therapy in ovarian cancer.

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173

SECRETION OF BINDING PROTEINS FOR INSULIN-LIKE GROWTH FACTOR-1 BY OVARIAN CARCINOMA CELLS

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The possible growth regulation of human ovarian carcinomas by hormones or growth factors is largely unknown. In the present study, the mitogenic effects of insulin-like growth factors (IGFs) and the secretion of binding proteins for IGFs (IGF-BPs) were analyzed in cultures of the permanent ovarian carcinoma cell lines EFO-21 and EFO-27.
Proliferative effects of IGF-1 (10 nM) were observed only with one of the two cell lines (EFO-21). However, high affinity binding sites for IGF-1 were detected in cultures of both cell lines investigated, indicating additional mechanisms modulating the action of IGFs. Using a dialysis chamber with a separating membrane of 12-14 kDa exclusion limit and (125)I-IGF-1 as tracer, high amounts of IGF-BPs in the conditioned media (CM) of both cell lines were demonstrated. Incubation of EFO-21 CM with (125)I-IGF-1 followed by gel filtration through Sephadex G-100 Superfine revealed the presence of at least two different specific IGF-BPs. By crosslinking of CM with (125)I-IGF-1 and subsequent SDS-polyacrylamide electrophoresis and autoradiography the IGF-BPs secreted by the cell lines were further characterized. A qualitative and quantitative comparison of the cellular production of IGF-BPs is forwarded.