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ACTIVATION OF CELL-DEATH GENES IN TUMORIGENIC STEM CELLS OF ANDROGEN-DEPENDENT MALIGNANCIES

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Complete remissions of the androgen-dependent Shionogi mouse mammary carcinoma are observed after androgen withdrawal but invariably the disease recurs and is refractory to further hormonal manipulations. To determine the proportions of androgen-dependent (AD) and -independent (AI) tumorigenic stem cells in parent and recurrent tumors an in vivo limiting dilution assay was developed. There was a marked enrichment of stem cells in the recurrent tumors (1/200 tumor cells) relative to the parent tumors (1/4,000 tumor cells) when assayed in male hosts. By assaying tumor takes in female mice, the proportion of AI stem cells was found to be 1/370,000 tumor cells in the parent versus 1/800 tumor cells in the recurrent carcinoma; a 500-fold increase in AI stem cells resulting from androgen-withdrawal. Unexpectedly, no enrichment of AI stem cells was evident in regressing parent tumors; rather, the proportion of such cells was very small (1/2,200,000 tumor cells). This finding implies that the AI cells which survive androgen withdrawal may result from the ability of small number of initially AD stem cells to adapt to an altered hormonal environment. This adaptive process was further defined in terms of the disappearance of androgen receptors from the nucleus and the expression of androgen-repressed genes including the proto-oncogenes, c-fos and c-myc, and the cell death gene, TRPM-2; all of which are constitutively active in recurrent AI tumor cells. Overall, our results indicate: (1) the tumor mass consists mainly of differentiated cells; (2) stem cells initially are AD but at most the killing effect of androgen-withdrawal will be limited to 2-3 logarithms before compensatory adaptive mechanisms supervene; (3) progression of stem cells to an AI state, in which they are resistant to the killing effects of cell death genes, might be prevented by the inhibition of androgen-repressed adaptive mechanisms which come into play when androgens are withdrawn.

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ANDROGEN AND EGF RECEPTORS IN PROSTATE CANCER

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LNCaP cells are human prostate tumor cells that show androgen responsive growth in vitro. Firstly, the mitogenic activity of several growth factors was studied in 6 day cultures. Addition of 1ng EGF/ml, 10ng TGFα /ml or 20ng basic FGF/ml to the culture medium stimulated cell proliferation 2- to 3-fold (expressed as DNA content per culture versus control cultures). Addition of acidic FGF (≤50 ng/ml), TGFβ (≤5 ng/ml) insulin (≤1 μg/ml), IGF-I (≤10 ng/ml) or PDGF (≤30 ng/ml) to the culture medium did not affect cell proliferation. TGFβ (0.02 ng/ml) inhibited the stimulatory effect of EGF and TGFα. Secondly, the production of growth factor-like activity by LNCaP cells was studied. The presence of autocrine growth factor activity in serum free medium conditioned by LNCaP cells (cultured either in the presence or absence of androgen) could not be demonstrated. However, immunoreactive TGFα was present in LNCaP cells. We used an anti-TGFα antibody to detect TGFα by an immunohistochemical approach. This antibody (MF9) recognizes TGFα but not EGF (Kobrin et al ,1986, JBC 261: 14414). We have shown previously that LNCaP cells contain EGF receptors and that treatment of LNCaP cells with androgens increases the number of EGF receptors per cell 3-fold. In summary: LNCaP cells contain TGFα , express EGF receptors and respond to TGFα when added to the culture medium.

Estrogen and progestagen receptors are not detectable in LNCaP cells. However, like androgens (0.1 nM R1881, a synthetic androgen), both progesterone (1 nM) and estradiol (10 nM) can stimulate cell growth and EGF receptor levels. Competitive binding studies using (3H)R1881 demonstrate that estrogens and progestagens compete with androgens for binding to androgen receptors. Furthermore, antiandrogens do not inhibit androgen responsive growth of LNCaP cells. Like androgens, both cyproterone acetate (10 nM) and anandron (100 nM, RU23908) have striking growth stimulatory effects, increase EGF receptor levels and stimulate the secretion of prostate specific acid phosphatase in the culture fluid. We conclude that LNCaP cells contain a modified androgen receptor system both with respect to steroid binding specificity and antiandrogen sensitivity.

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CLINICAL SIGNIFICANCE OF ONCOGENES AND GROWTH FACTORS IN OVARIAN CARCINOMAS

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The activation of oncogenes and the inappropriate production of growth factors were found in different types of malignant tumors influencing obviously the malignant phenotype. In this study we have investigated the expression of TGFa, EGF receptor (EGF-R, erb B1) and c-myc in specimens of gynecologic carcinomas using molecular, biochemical and immunohistochemical methods. The EGF-R state was correlated with the clinical follow-up of ovarian carcinomas. The frequencies of EGF-R+ cases (EGF-R assay and immunostaining) were about 45% in adenocarcinomas (ovary, endometrial, breast) and 85% in squamous cell carcinomas. EGF-R northern blotting shows a specific signal in nearly all tumor specimens, however only increased mRNA banding intensities correlate with the biochemical and immunohistochemical data. Whereas in adenocarcinomas EGF-R+ and EGF-R- clones were detected, a homogeneous immunostaining existed in squamous cell carcinomas. The TGFa immunostaining demonstrates a widely distributed expression in epithelial and endothelial normal cells, stromal and hematopoetic cells are TGFa-. Also, carcinomas express different amounts of TGFa and sarcomas or fibromas are TGFa-. TGFa immunostaining intensities correlate with TGFa mRNA amount or the biochemical quantification. The simultaneous analysis of c-myc in the same specimens indicates a correlation with TGFa but not with EGF-R expression. Clinically, the EGF-R state is associated with the remission rate but probably also with an early recurrence rate or fast development of drug resistance in ovarian carcinomas. At this moment we belief on the existence of TGFa/EGF sensitive and insensitive ovarian carcinomas differing in malignant phenotypes and that further transmembrane mitogenic pathways (erb B2 ?) are of tumor biological relevance.

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INSULIN-LIKE GROWTH FACTOR 1 RECEPTORS (IGF1-R) AND IGF1 IN HUMAN BREAST TUMORS

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To appreciate the IGF1 sensitivity of breast tumors we detected IGF1-R with a biochemical assay (RRA). We then localized and quantified IGF1-R on frozen tissue sections by histo-autoradiographic analysis (HAA). In some cases, the IGF1 and IGF1-R mRNA expression were studied by Northern blot analysis. We also studied the IGF1 plasma concentration in primary breast cancers compared to controls. IGF1-R (RRA) were found in 87% (n = 297) of the breast cancers. The mean geometric value was 3.87 % (specific binding as percentage of total radioactivity); we found a highly significant correlation between IGF1-R and ER on the one hand ($p = 0.0001$) and PgR on the other ($p = 0.0001$) (Spearman test). The presence of IGF1-R was associated with a better prognosis, either on relapse free survival (actuarial analysis : $p = 0.004$; Cox analysis $p = 0.005$) or overall survival (respectively $p = 0.003$; $p = 0.005$). The median duration of follow-up was 30 months. By Cox analysis IGF1-R was a better prognostic factor than ER and PgR. In a series of 77 cases of benign breast disease only 47 % (36/77) were positive ; the mean geometric level was 1.8 %. The HAA IGF1-R quantification in 20 breast carcinomas and 12 cases of benign breast disease confirmed the RRA results and demonstrated that the labeling was localized on the epithelial component. In four breast cancers, we did not detect IGF1 mRNA ; IGF1-R probe demonstrated two major mRNAs of 11 and 7 Kb. Finally we found that IGF1 plasma level was higher in breast cancer patients than in healthy controls of the same age. These results show that IGF1 is implicated in breast cancer growth and suggest that anti-IGF1 treatment might be useful in human breast cancer : for this reason, we and others carried out a phase II clinical trial with somatostatin.