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ALTERATIONS OF THE POSITIVE AND NEGATIVE GROWTH FACTOR BINDINGS IN BLOOD PLASMA IS SUFFICIENT FOR MODIFICATION THEIR BIOACTIVITY IN MALIGNANT GROWTH

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RIA,ELISA,RRA, ultrafiltration and HPLC for studies the binding of growth factors in blood plasma(BP)were used.The binding of multipotent(epidermal growth factor-EGF) and organo-specific(gastrin -G; vasopressin -V)positive growth factors in BP from tumor bearers 1.7-1.6 - and 1.9-fold respectively are decreased.Contrary to this,the binding of multipotent negative growth factors(interferon-I; somatostatin-S) 1.5- and 4.8-fold respectively are enhanced.These alterations in peptide-protein binding are polyamine-dependent and are significant for the modification of the growth factors bioactivity since 1)spermidine and/or tumor -associated polyamine-containing peptides(TAPCP) decrease the in vitro binding of EGF, G, V in BP and enhance the binding of I and S; 2)in vivo treatment with the inhibitor of ornitinedecarboxylase(DFMO)brings about enhance binding of EGF and decreased binding of S in BP from tumor bearing animals; 3) in vitro influence of polyamines and/or TAPCP on the binding of S,V and EGF; diurnal alteration in the BP content of polyamines and TAPCP; circadian rhythms in biological activity of S, EGF and V in vivo; diurnal alterations in their binding in BP are in time correlation with each other; 4) BP from tumor bearers became unable(vs normal one)to inhibit the mitogenic effect of EGF on target cells and specific binding of EGF to the receptors is over 100-fold enhanced in the presence of BP from tumor bearers; 5) specific binding of S and enhanced high affinity binding of I is present in BP from tumor bearers. The given mechanism activates the action of positive growth factors and inhibits negative growth factors in malignant growth.

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A NEW PEPTIDE GROWTH FACTOR FROM HUMAN PLACENTA: BIOCHEMICAL & IMMUNOLOGICAL CHARACTERIZATION

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Various hormones, growth factors, growth factor receptors and oncogene products are found in placenta, and they appear in a developmentally regulated manner during pregnancy. Recently, we isolated a 34000 Da protein from the trophoblastic brush border membranes of human placenta by nondenaturing gel electrophoresis. In its biochemical and immunological specificity, this growth factor is different from several well characterized growth factors (e.g. EGF, PDGF, IGF, etc.). Binding of ^{125}I-34 kDa mitogen to target fibroblasts was specific and chemical cross-linking studies suggest that the binding is mediated through a 50kDa surface protein. A monospecific rabbit antibody made against the mitogen did not cross-react to any other growth factors and hormones tested. Immunoperoxidase staining of placental tissue slices showed (a) cytoplasmic localization of 34kDa protein in cytotrophoblasts and (b) in the brush border membranes of syncytiotrophoblasts. Moreover, tissue sections obtained from first trimester placenta consistently displayed stronger staining pattern in placenta. Biosynthesis followed by immunoprecipitation with ^{35}S-methionine labelled cytotrophoblasts showed that this peptide is synthesised in these cells. Interestingly, metabolic labelling studies carried out with some carcinoma cell lines (e.g. A431 and SKBR-5) demonstrated the production of 34kDa mitogen. Thus the presence of this membrane associated growth factor in trophoblasts and in some carcinoma cell lines probably suggest an autocrine mechanism of stimulation.

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ANDROGEN RECEPTOR FORMS IN AND THE EFFECT OF ANDROGENS ON THE RELEASE OF PROTEIN(S) BY A HUMAN PROSTATE CANCER CELL LINE.

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The rates of differentation and proliferation of human prostate carcinoma are dependent upon androgen stimuli. The hormonal effects are mediated by intracellular receptors. In search for a possible autocrine regulatory function in androgen dependent tumors, we have studied synthesis and release of androgen stimulated protein(s) in androgen sensitive LNCaP cells.

The nuclear extract of the LNCaP cells contained 17,000±2,500 KCl-extractable androgen receptor sites/cell (n=5). Sucrose gradient (high salt) centrifugation revealed two receptor forms sedimenting at 4.5±0.2 S (n=11) and 2.8±0.3 S (n=9) with corresponding Mw of 91 kD and 33 kD respectively (n=2). Only the 4.5 S form bound to DNA-cellulose. Estrogen and progesterone receptors were not detectable in the nuclear extract nor in the cytosol. Cells grown in media containing charcoal treated fetal calf serum released lower amounts of several (35S)-labeled proteins, especially of a protein with a Mw of 40±2 kD (n=6). The release of this protein could be restored in cells cultured in the presence of dihydrotestosterone (DHT, 0.1-1uM) or R1881 (0.1nM-0.1uM) whereas estrogens or corticoids and progesterone had no effect. The high concentrations of DHT needed to restore the 40 kD protein is probably related to extensive metabolism of DHT by the LNCaP cells. Anti-androgens, which inhibit cell growth also exerted inhibitory effects on the release of the 40 kD protein.

The observed correlation between the effects of (anti)-androgens on the growth of the LNCaP cells and the release of the Mw 40 kD protein may be related to the regulation of malignant prostate cell growth.

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HORMONAL REGULATION OF AN OSTEOBLAST STIMULATING SUBSTANCE SECRETED BY HUMAN BREAST TUMOR CELL LINES

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Bone metastases of breast tumors are able to induce osteolytic and osteosclerotic lesions. We have previously shown that human breast tumor cell lines (MCF-7) produced an osteoblast stimulating substance (OSS) which is secreted in conditioned medium (CM) and stimulates the alcaline phosphatase activity (APA) of osteoblast like cells. The APA has been evaluated on a murine osteosarcoma cell line (ROS 17/2.8) and on normal human osteoblasts derived from bone biopsies. We have investigated the potential hormonal modulation of the production of this OSS by MCF-7, and the basal activity of several cell lines (ZR-45, BT-20, MDA.MB 231).

CM obtained from MCF-7, ZR-75, MDA.MB 231 and BT-20 cells grown under hormone free conditions elicited an increase of the APA of ROS cells representing 180, 114, 115, 90% of the effect of control medium.

CM obtained from MCF-7 grown in the presence of hormone induced additional increase of APA expressed in % of CM:

DHT	DEX	Pg	E2	OH.TAM	hGH	hPL
(10^{-7}M)	(10^{-7}M)	(10^{-7}M)	(10^{-9}M)	(10^{-6}M)	(10^{-8}M)	(1 µgml)
188%	198%	187%	150%	140%	236%	174%

A similar response was obtained when CM was tested on ROS 17/2.8 or on human osteoblasts.

These experiments indicate that 1/ human breast tumor cell lines produce a substance having a neo-osteoblastic activity 2/ various hormones increase the production of this substance whereas their activities have been characterized as antagonists on other biological parameters.