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ONCOGENES AND GROWTH FACTOR RECEPTORS IN ADVANCED HUMAN OVARIAN CANCER.

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Both oncogene amplification or rearrangement and/or overexpression of growth factor receptors appear to correlate with biological behaviour and patient outcome in several solid tumours.

Using Southern blotting and autoradiographical techniques, we have studied growth factor receptor status and (onco)-gene amplification in human ovarian cancer. Frozen tissue sections derived from tumour tissues (n=50) showed a higher expression (2+ to 4+) of insulin like growth factor-1 receptors (IGF-1-R) when compared to normal (n=10) ovarian tissues (1+ to 2+). High activity of IGF-1-R was predominantly associated with epithelial tumour cells whereas surrounding connective tissue was negative. About 40-50% of the ovarian tumours showed elevated levels of epidermal growth factor receptors (EGF-R), when compared to normal ovaries. High expression of EGF-R and IGF-1-R was associated with tumour necrosis, in few samples. Southern blot analysis of 20 ovarian cancers revealed no amplification of IGF-1-R gene nor the EGF-R gene or the HER2/neu gene. An amplification of the int-2 gene was observed in 2, both ER and PgR positive, endometrioid tumours. In addition, Msp-1 polymorfisms in the Ha-ras gene were observed in 2 out of 6 tumours, which may result from methylation of 5' cytosines within the ras gene.

In conclusion, ovarian adenocarcinomas show increased expression of EGF-R, 40-50%, and IGF-1-R, in all cases, when compared to normal ovarian tissue, an amplification of the int-2 gene in 2 out of 20 cases and ras polymorfisms in 2 out of 6 cases. The clinical significance of these events awaits further study.

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EPIDERMAL GROWTH FACTOR RECEPTOR (EGF-R) IN ADVANCED OVARIAN CARCINOMA; LACK OF CORRELATION BETWEEN IMMUNOHISTOCHEMISTRY AND BIOCHEMISTRY

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Evidence exists that the presence of EGF-R in tumor cells may be of importance with respect to the clinical outcome of several malignancies. In ovarian carcinomas data on the EGF-R status are limited. It is unknown whether or not EGF-R numbers are enhanced in ovarian carcinomas, when compared to normal ovaries.

We have examined both ovarian carcinoma tissues and normal ovarian tissues for the presence of EGF-R. Frozen tissue sections were cut (5 µm), and subsequently incubated with monoclonal antibody 2E9 (EGF-R, extracellular domain (Dr. L.H.K. Defize, Hubrecht lab, Utrecht)). Specific binding of the antibody was visualized using an indirect immunoperoxidase technique. Positive and negative controls were included. EGF-R assays were performed by Scatchard analysis following incubation with ^{125}I-EGF. Immunohistochemically approximately 55% (21/38) of the carcinoma tissues showed an increased positivity for the presence of EGF-R, varying from + (weak) to +++ (strong) when compared to normal ovarian tissues. In the binding studies EGF-R was present in 76% (29/38) of the adenocarcinoma tissues (B max: median 13.0, range 0-87.3 fmol/mg protein (n=38); Kd: median 1.0, range 0.4-1.4 nM (n=27)). These EGF-R values were higher than those found in normal ovarian tissues (median 0, range 0-39 fmol/mg protein; n=9). There was no significant correlation between EGF-R measured by immunohistochemistry and by Scatchard analysis (p=0.25).

It is concluded that, compared to normal ovarian tissue, ovarian carcinomas contain higher levels of EGF-R. The lack of correlation between immunohistochemistry and biochemistry may be caused by heterogeneity of ovarian tumor tissues and needs further study (see also abstract C.J. Rodenburg et al.).

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THE PROGNOSTIC SIGNIFICANCE OF EPIDERMAL GROWTH FACTOR RECEPTORS (EGF-R) - DETERMINED IMMUNOHISTOCHEMICALLY AND BY SCATCHARD ANALYSIS - IN (ADVANCED) OVARIAN CANCER.

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In advanced ovarian cancer, EGF-R -as determined by means of Scatchard analysis- proved to be of prognostic significance
(Bauknecht et al, Med.Oncol. and Tumor Pharmacother. 1989;6:121-127).

We have studied the prognostic value of EGF-R by both immunohistochemistry (n=35) and Scatchard analysis (n=55) in patients (pts.) with (advanced) ovarian cancer.

All pts. had been treated with cyclophosphamide and cisplatin.

For immunohistochemistry, frozen tissue sections (5 micrometer thickness) were incubated with monoclonal antibody 2E9 (EGF-R, extracellular domain, dr.L H K Defize, Hubrecht Lab, Utrecht). Scatchard analysis was performed following incubation with 125 I-EGF.

Immunohistochemistry: 12 pts. had an early stage disease (5 EGF-R negative, 7 EGF-R positive) and all of them are alive and with no evidence of disease (NED). 23 pts. had advanced disease, 14 of whom have relapsed or developed progressive disease (PD) and 9 out of 23 pts. are NED. Of the 14 pts. with a relapse or PD, 12 were EGF-R positive and only 2 were EGF-R negative. In contrast, only 1 out of the 9 pts. with NED was EGF-R positive ($p<0.05$).

Scatchard analysis: 15 pts. had an early stage (mean EGF-R 14 fmol/mg protein) and 40 had advanced disease (mean EGF-R 16 fmol/mg protein). Of the 40 pts. with advanced disease, 25 relapsed or developed PD (mean EGF-R: 18 fmol/mg protein) and 15 are NED (mean EGF-R: 14 fmol/mg protein) (differences without significance).

It is concluded that using immunohistochemistry, EGF-R may be an important prognostic factor.However, measurement by Scatchard analysis showed different results.

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HUMAN GRANULOSA CELL TUMOR: GONADOTROPINS STIMULATE STEROIDOGENESIS IN VITRO

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A 59 year-old previously oophorectomized woman underwent surgery for a recurrent malignant granulosa cell tumor. Specimens and dispersed cells from the tumor tissue were incubated for 2 h and cultured for 48 h resp., with and without gonadotropins. Steroids and cAMP concentrations were measured.

Incubated specimens from the tumor tissue released measurable amounts of cAMP, progesterone (P) and estradiol into the medium. Human FSH, 1 µg/ml, significantly stimulated the formation of cAMP and both steroids. Human LH, 1 µg/ml, stimulated cAMP and P but not estradiol release. Human CG, 10 µg/ml, stimulated cAMP and P formation in tumor tissue but was totally devoid of effect on estradiol release.

In tissue culture experiments P and estradiol were formed in considerable amounts, with a higher capacity for P than for estradiol. P formation was stimulated by FSH and hCG, while estradiol release was stimulated only by hCG. The addition of testosterone significantly enhanced estradiol formation in both incubation and culture experiments.

It is concluded that the steroidogenesis of this granulosa cell tumor is sensitive to gonadotropins.

We have started a long term treatment of the patient with long acting GnRH analog with the hope that, if residual tumor tissue is still prevailing, the suppression of endogenous gonadotropins may decrease its metabolism and perhaps also its proliferation.