I. The IGF / IGF-I receptor signalling pathway a) in breast cancer cells

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The type I insulin-like growth factor receptor (IGF-IR) mediates the mitogenic, transforming, differentiating, and antiapoptotic actions of the IGF ligands, IGF-1 and IGF-2. In the mammary gland the IGF-IR has an important role in the promotion of normal breast growth and development, and in the initiation of breast cancer. We have recently reported that the IGF-IR and its substrate IRS-1 are highly expressed in early stages of primary breast cancer. Moreover, the expression of both proteins correlates with the expression of the estrogen receptor (ER), suggesting a functional link between the ER and IGF systems. At late malignant stages of breast cancer, IGF-IR and IRS-1, as well as ER, were found to be significantly decreased [Schnarr et al., Int. J. Cancer (Pred. Oncol.) 89, (2000) 506-513]. These findings suggest that the IGF-IR is required for initiation of breast cancer, whereas the acquisition of the malignant phenotype results from decreased IGF-IR gene expression and action. In vitro studies in estrogen-dependent breast cancer cells have shown that the expression of IGF-IR and IRS-1 is controlled by estradiol (E2) [1].

In our attempt to unravel the molecular mechanisms involved in the control of IGF-IR and IRS-1 expression by E2 we started to investigate the effect of E2-treatment of estrogen-dependent breast cancer cells on the phosphorylation stage and activity of proteins of the IGF-IR pathway. Stimulation of the IGF-IR by IGF results in autophosphorylation on tyrosine residues which, in turn, activates the intrinsic receptor tyrosine kinase activity towards other substrates. IRS-1 is the major substrate of the IGF-IR and is tyrosine phosphorylated after IGF-IR stimulation. The phosphorylated IRS-1 represents a docking protein for molecules mediating the activation of the phosphatidylinositol-3-kinase (PI3K)/AKT/PKB pathway. The serine/threonine kinase AKT is a central component of the pathway which controls the activity of numerous downstream molecules. Activation of AKT results in inhibition of apoptosis and inactivation of proteins that inhibit cell cycle progression. In vitro studies suggest that E2 interacts with this signalling pathway at different levels. While IGF-IR and IRS-1 are not phosphorylated by short-term E2-treatment, AKT/PKB is phosphorylated and activated in estrogen-dependent breast cancer cells under the influence of E2. Using appropriate inhibitors (e.g. wortmannin, an inhibitor of PI3K) it could be shown that PI3K is involved in E2-stimulation of AKT-phosphorylation. The (enzymatic) reaction that activates PI3K has not yet been clearly identified. It is possible that activation of the tyrosine kinase c-src which is tyrosine-phosphorylated after stimulation of MCF-7 cells with E2 is involved in activation of PI3K. Inhibition of Src by a specific inhibitor resulted in a 30% inhibition of E2-induced cell proliferation. This agrees with find-
ins on estradiol-dependent gene expression which was inhibited by 30% in cells stably transfected with a dominant negative src-mutant [2] or with a dominant negative AKT mutant. It is concluded that E2-treatment of estrogen-dependent breast cancer cells may activate the PI3K-AKT pathway.

b) in prostate cancer cells

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Insulin-like growth factors are important growth factors in the development of prostate cancer and its progression. The biological activity of IGFs is mediated via the IGF-1 receptor signalling pathway, which involves activation of the protein kinase AKT/PKB, and is regulated by inhibitory IGF-binding proteins (IGFBP). Overexpression of related proteins or changes which raise the balance of IGF/IGF-IR activity versus IGFBP function can potentially contribute to prostate carcinogenesis.

Expression of AKT, IGF1, IGF2 and IGFBP3 proteins, investigated by immunohistochemistry in human prostate carcinoma specimens, was correlated with clinicopathological parameters [Gleason score, pathological tumour stage (pT) and preoperative serum level of prostate specific antigen (PSA)]. The immunoreaction was investigated separately in benign prostate tissue, PIN lesions (prostatic intraepithelial neoplasia) and prostate cancer tissue according to intensity, fraction of positive cells and score of the immunoreactivity. The expression of IGF2 in prostate cancer, with respect to fraction of positive cells and score of the immunoreactivity, was associated with tumour progression as indicated by Gleason score. IGFBP3 expression was not correlated with any of the clinicopathological parameters. It is concluded that the IGF/IGFBP balance is altered in prostate cancer (Fig. 1). This alteration is characterized by a significant overexpression of IGF1 and IGF2, whereas IGFBP3 expression is unchanged in tumour compared to benign tissue with a clear increase from benign tissue to PIN lesions to tumour and with tumour progression, whereas IGFBP3 expression was similar in all tissue types. The expression of AKT in prostate cancer, regarding the intensity, and the expression of IGF1 and IGF2, regarding the intensity, fraction of positive cells and score of the immunoreactivity were positively correlated to high preoperative PSA serum levels. Moreover, high expression of IGF2 in prostate cancer, with respect to fraction of positive cells and score of the immunoreactivity, was associated with tumour progression as indicated by Gleason score. IGFBP3 expression was not correlated with any of the clinicopathological parameters. It is concluded that the IGF/IGFBP balance is altered in prostate cancer. These data provide evidence that the IGF signalling pathway plays an important role in the initiation and progression of human prostate cancer. Further investigations on this pathway may illustrate the implications in prognosis and treatment of prostate cancer [Liao, Y. Thesis, 2003].

II. Dehydroepiandrosterone effects on cell proliferation

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a) in liver

Dehydroepiandrosterone (DHEA), the main adrenal steroid in humans and a precursor in the biosynthesis of potent androgens and estrogens, acts as a hepatocarcinogen in rats [3, 4]. Neoplasms emerge from a glycogenotic / amphophilic preneoplastic cell lineage (Fig. 2). A higher female tumour incidence suggests a relevant influence of sex hormones. DHEA enhances hepatocarcinogenesis induced by N-nitrosomorpholine (NNM) which is characterised by the glycogenotic / basophilic cell lineage. The tumour promoting effect is related to an additional amphophilic / basophilic preneoplastic lesion sequence and to faster proliferation of the basophilic preneoplastic lesions. Nevertheless, hepatocellular carcinomas provided under DHEA treatment are growing more slowly and seem to have a less malignant phenotype compared to tumours induced by NNM only. Further, DHEA treatment reduces growth and generation of glycogen storage foci (GSF) in initial NNM treated rats. Thus, DHEA treatment results in both, a growth stimulation (promotion) of the late baso-
DHEA inhibits not only growth of NNM-induced glyco-
genetic liver lesions and neoplasias but also the growth of physiologically proliferating liver tissue, e.g. compensatory proliferation after partial hepatectomy [6]. This might be explained by a DHEA related cellular metabolism [7; Mayer et al., Int. J. Cancer (Pred. Oncol.), 79 (1998) 232-240], which is characterized by significant energy consumption. Additionally, DHEA treatment of rats resulted in alterations of cytokine and growth factor levels, e.g. of IL-6 and IGF-1 in rat liver, that might contribute to this growth inhibition as well [5, 6].

**b) in breast cancer cells**

DHEA serum levels in humans are high in young adults and decrease continuously with age. DHEA replacement in the elderly has been reported to result in increased well-being. For this reason DHEA has been suggested for use as anti-aging drug.

Epidemiological studies have shown that postmenopausal women with high DHEA-plasma levels have an increased risk to develop breast cancer. In *vivo*, DHEA can be accumulated in normal breast tissue and in breast cancer from the blood. In *vivo*, DHEA stimulates proliferation of MCF-7 cells, an estrogen-dependent cell line derived from a human breast carcinoma. We could show that growth stimulation is not a direct effect of DHEA but due to the conversion of DHEA to significant amounts of estradiol in MCF-7 cells. Inhibition of aromatase activity prevented growth stimulation by DHEA. It could further be documented that DHEA acts exclusively as estrogen in MCF-7 cells. The cells did not respond to androgenic DHEA-derivatives [8].

We conclude from our results that DHEA acts as an estrogen and represents a mitogen to estrogen-dependent breast cancer cells. Therefore, DHEA replacement in postmenopausal women should be avoided because of potential increase in breast cancer risk.

*Publications (\* = external co-author)*


