I. Hepatocarcinogenesis by the Steroid Hormone Dehydroepiandrosterone

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Dehydroepiandrosterone, a steroid of the adrenal gland and a precursor in the biosynthesis of potent estrogens and androgens induces liver cancer in rats [1-5]. The tumour incidence is higher in females than in males which indicates a gender specific hormonal effect of DHEA related to hepatocarcinogenesis. During the past years we have studied the pathogenesis of hepatocellular carcinomas and biochemical alterations induced by DHEA in rat liver [3,6]. A number of different mechanisms associated with DHEA-induced hepatocarcinogenesis could be elucidated. In this report only a few aspects on DHEA function will be briefly discussed. Other results are described in the final report of the Division of Cell Pathology (C0100).

Alterations in cell cycle control by DHEA.

DHEA acts as a mitogen in liver, particularly of female rats. This could be shown by BrdU-incorporation and by immunohistochemical detection of proteins of the G1-S phase of the cell cycle such as cyclins or proliferating cell nuclear antigen (PCNA). It also causes a significant increase in the size of hepatocytes [7] which is not due to polyploidy.

Lipid peroxidation und protein carbonylation.

DHEA induces a strong lipid peroxidation and, as a consequence, an increase in protein carbonylation in cellular membranes, particularly in mitochondria and microsomes. [1,3,8]. This increase in lipid peroxidation may be the consequence of induction by DHEA of microsomal cytochrome P450-dependent monoxygenases and of enzymes of the mitochondrial respiratory chain which are both sources of reactive oxygen species formation. Increased production of reactive oxygen species, increased lipid peroxidation and resulting hepatotoxic effects may be related to the carcinogenic insult of DHEA.

Proliferation of peroxisomes and mitochondria in rat liver.

Livers of DHEA-treated rats show a strong increase in the number of peroxisomes and mitochondria [2]. It has been known for a long time that peroxisome proliferators act as hepatocarcinogens in rodents. However, our studies have clearly shown that hepatocellular carcinomas do not arise from liver areas with the strongest peroxisome proliferation but from focal lesions with abundant mitochondria [1,2,6] which questions the hypothesis that peroxisome proliferation is the cause for induction of hepatocarcinogenesis. Histochemical studies of enzymes of energy metabolism in the mitochondria-rich preneoplastic lesions and biochem-
cal studies of DHEA-treated liver revealed a characteristic pattern of metabolic alterations indicating a thyroid hormone-like effect of DHEA on the liver of both genders. The molecular mechanism underlying this thrymimetic effect of a steroid hormone remains to be clarified. It may be noteworthy in this context that the thyroid hormone receptor belongs to the steroid hormone receptor family and may form heterodimers with estrogen and androgen receptors.

Phenotypic modulation of preneoplastic liver lesions by DHEA.

Administration of DHEA to rats previously treated with a nitrosamine to induce hepatocarcinogenesis results in an increase of the number of neoplasms in the liver (tumor promotion). Surprisingly, it also results in the modulation of the lesions to a less malignant phenotype [1-6]. This modulation is accompanied by the down-regulation of proteins of the insulin / IGF-I signal transduction pathway, such as insulin receptor substrate-1 (IRS-1) [9]. The reduction in the expression of proteins of the insulin signalling pathway by DHEA may be explained by an interaction of peptide and steroid hormone signalling pathways.

Androgenic effect of DHEA.

While a thrymimetic effect of DHEA was observed in the livers of both genders, an androgenic effect was observed only in females. This androgenic effect was detected when the expression and distribution of glutamine synthetase was studied. This enzyme is expressed exclusively in peri-vanular hepatocytes. In male rats glutamine synthetase is detected immunohistochemically in about three cell layers around the central terminal vein and in one cell layer in females [10]. During DHEA-treatment the expression pattern in the liver of female rats changed significantly and became similar to that observed in males as determined by combination of morphometric and immunohistochemical methods. In males no effect of DHEA on glutamine synthetase expression pattern was observed. In this context it may be of interest that DHEA induces a significantly higher incidence of hepatocellular carcinomas in female rats as compared to males [1]. It remains to be clarified whether the hepatocarcinogenic effect of DHEA is due to its androgenic properties.

II. The Insulin / IGF-IR Signalling Pathway in Human Breast Cancer

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The ligands, receptors and related signalling proteins of the insulin-like growth factor family are involved in the regulation of breast cancer cell growth. Human breast carcinomas have been shown to produce IGF-I and IGF-II which were suggested to stimulate the proliferation of the cancer cells in a paracrine or autocrine manner. We investigated the expression pattern of insulin-like growth factor-I receptor (IGF-IR), insulin receptor (IR) and insulin receptor substrate-1 (IRS-1), a core downstream signalling protein, in 69 primary breast cancer specimens of different grades and in 21 control tissues by immunohistochemistry. In addition, cell proliferation (percentage of Ki67+ nuclei) and estrogen receptor (ER) expression were determined. IGF-IR, IRS-1 and IR were found expressed mainly in epithelial cells. IRS-1 and IGF-IR were expressed at high levels in control tissues and in well and moderately differentiated carcinomas but at low levels in poorly differentiated breast cancers. IR expression did not show a significant correlation with the differentiation grade of the tissues investigated. Statistical analysis demonstrated that down-regulation of IGF-IR and IRS-1 correlated better with tumour progression than reduction of ER expression or increase in cell proliferation, IGF-IR showing the best correlation followed by IRS-1 and, less significant, ER or Ki67. Our findings clearly show that progression of breast cancer is accompanied by a reduction of IGF-IR/ IRS-1 expression and that IGF-IR/ IRS-1 expression inversely correlates with high proliferation rate in dedifferentiated breast cancers. The strong correlation of IGF-IR and IRS-1 down-regulation with tumour progression suggests the use of IGF-IR and IRS-1 as a novel set of marker proteins for tumour grading [11].

III. DHEA- effect on Breast Cancer Cells

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DHEA serves as precursor for the biosynthesis of estrogens and androgens in peripheral organs in vivo. It has been reported from epidemiological studies that post-menopausal women with high DHEA blood-levels have an increased risk for breast cancer. The compound is accumulated in breast tissue and breast cancer from the blood. We were interested to clarify whether DHEA is a mitogen for estrogen-dependent breast cancer cells, and if so whether it required conversion to estrogens in order to stimulate cell proliferation and estrogen-dependent gene expression. After incubation of cells with 100 nM DHEA for four days, estradiol was present in the medium at a concentration of ~200 pM. Other compounds identified were testosterone and estrone. Significant stimulation of cell proliferation by 1 nM estradiol and 100 nM DHEA was observed after 38 h and 4 days of incubation, respectively, indicating the necessity of DHEA conversion. DHEA doses ≥10 nM induced estrogen-dependent gene expression in MCF-7 cells transfected with a luciferase reporter gene under the control of the estradiol reporter element. DHEA-dependent stimulation of proliferation and luciferase induction could be inhibited by the anti-estrogens IC182,780 and tamoxifen, respectively, and by the aromatase inhibitor 4-hydroxyandrostenedione. An androgenic effect of DHEA on proliferation and gene expression of MCF-7 cells was not observed. We conclude that DHEA is converted to estrogens, particularly estradiol, in breast cancer cells, and that this conversion is required to exert a mitogenic response [12,13].
Publications (* = external coauthor)


