## 98

UPREGULATION OF EGF-RECEPTOR INDUCED BY ALPHA-INTERFERON IN HUMAN A431 CELLS

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The availability of recombinant human Interferon (IFN) has paralleled their increasing use in the treatment of human cancer: however the biological bases of their antitumor activity have not been completely elucidated. Modulation of growth factor receptors by physiological agents,like IFNs, in human tumor cells,could be of potential clinical interest. We have studied the possible relationships between growth inhibition induced by r-alpha-IFN and the expression of EGF-R in the human cancer A431 cells. 500 UI/ml of IFN induced 50% growth inhibition after 48 hrs of treatment without cell toxicity and in the absence of cell cycle perturbations,as assessed with Flow Cytometric analysis. EGF binding evaluated 48 hrs from beginning of treatment showed an upregulation of EGF-R expression. The overall increase in EGF binding sites was not an early event, was dose-dependent and was due to an increase of low affinity receptors number ($3.6x10^6$ sites/cell in controls, $5.04x10^6$ sites/cell in IFN-treated cells). The affinity of the receptor for EGF was unaffected by IFN as well as the number of high affinity receptors. These results of radiobinding were confirmed by immunoelectron microscopic analysis and quantitation of the gold labeled receptors on the cell plasma membranes. No clear morphological changes were observed in IFN-treated cells. (Supported by A.I.R.C.)

## 99

**ANTIPROLIFERATIVE POTENCY OF THE NEW NONSTEROIDAL ANTIESTROGEN ZK 119.010 IN ESTROGEN-DEPENDENT TARGET ORGANS AND MAMMARY TUMORS**

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2-(4-Hydroxyphenyl)-5-hydroxy-3-methyl-1-[6-(1-pyrrolidino) hexyl]-indole (**ZK 119.010**) was shown to be a potent and pure antiestrogen in the immature mouse uterus as well as a strong inhibitor of the MCF-7 breast cancer cell line (von Angerer et al., J. Cancer Res. Clin. Oncol. 1989, 115, S56). In this study, a comparison of ZK 119.010 with ICI 164.384 and tamoxifen in antiuterotrophic effects and antiproliferative activity on the normal mammary gland and on hormone-dependent mammary tumors was made. ZK 119.010 was superior to ICI 164.384 and tamoxifen in antiuterotrophic potency in immature mice. In rats, ZK 119.010 was more potent than ICI 164.384 and, unlike tamoxifen, both were able to induce almost complete inhibition of uterine growth. The estrogenic activity of ZK 119.010 was much below that of tamoxifen, but slightly above that of ICI 164.384. ZK 119.010 as well as tamoxifen was able to significantly inhibit the development of tubular alveolar buds in the normal mammary gland of ovariectomized, hormone-substituted rats, whereas ICI 164.384 was ineffective. ZK 119.010 and ICI 164.384 - in contrast to tamoxifen - had no marked effect on growth of the hormone-dependent MXT mammary tumor grown either in intact or in estrogen-substituted, ovariectomized mice, whereas both compounds strongly inhibited uterine weight in the same assays. However, in the NMU-induced mammary carcinoma of the rat, ZK 119.010 (%change of tumor area: 171 %; control: 455 %) as well as ICI 164.384 (168%) caused a significant inhibition of tumor growth though lower than that after ovariectomy (8%).

## 100

**THE PROGESTERONE ANTAGONIST ONAPRISTONE INDUCES CELL CYCLE BLOCKADE AND CELL DEATH BY APOPTOSIS IN EXPERIMENTAL MAMMARY TUMORS**

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The progesterone antagonists, ONAPRISTONE and ZK 112.993 (Schering Berlin, West Germany), proved to possess tumor inhibitory effects in several progesterone receptor positive experimental mammary tumors. Their antitumor activity is as strong or even better than that of tamoxifen or ovariectomy in the MXT-mammary tumor of the mouse and the DMBA- and MNU-induced mammary tumors of the rat. Interestingly, antitumor activity was found in spite of elevated levels of ovarian and pituitary hormones. To elucidate the mechanism of action of progesterone antagonists we evaluated their effects on growth, ultrastructure and cell cycle kinetics in the MNU and MXT tumor model. Light and electron microscopical examination of MXT, MNU and DMBA tumors lead us to propose that the growth inhibitory effects of progesterone receptor antagonists result from the induction of terminal differentiation, leading to cell death and the appearance of nuclear fragmentation by apoptosis. Analysis of nuclear DNA content in such tumors using flow cytometry showed that the progesterone antagonists were able to block progression of tumor cells in $G_0$ $G_1$ phase of the cell cycle and reduced the fraction of cells in the S and $G_2$ M phases. In contrast, use of the conventional therapies with tamoxifen or DES as well as ovariectomy displayed no changes in the distribution of cells within the cell cycle, indicating that progesterone receptor antagonists represent a fundamentally innovative endocrine therapy of breast cancer.

## 101

The effects of antiprogestins on the proliferation of breast cancer cells

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In this study the in vitro effects of the antiprogestins ZK 98.734 and ZK 98.299 on proliferation of the human breast cancer cell line MCF-7 were investigated. The experimental cultivations were performed with 10 % charcoal-stripped FCS. Proliferation of MCF-7 cells is stimulated by 10 nM 17β-Estradiol ($E_2$) (> 200 % versus control after 7 days). The progestin R5020 (100 nM to 1µM) antagonizes stimulated cell proliferation.

The antiprogestin ZK 98.299 stimulates slightly cell proliferation, whereas it is not altered by ZK 98.734. Stimulation of cell proliferation by 10 nM $E_2$ is inhibited by ZK 98.734, but not by ZK 98.299. The growth inhibitory potential of 100 nM R5020 can be antagonized only by 1µM ZK 98.734 but not by ZK 98.299. 1 µM R5020 cannot be antagonized by either of the substanzes.

The results show the diversity of the effects of different antiprogestins on breast cancer cells in vitro, which have to be investigated in more detail before a clinical application of these substanzes.