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MECHANISMS OF GROWTH INHIBITION BY ANTIESTROGENS AND PROGESTINS IN HUMAN BREAST AND ENDOMETRIAL CANCER CELLS

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Summary—Marked changes in both growth factor and proto-oncogene expression occur due to treatment of hormonally-responsive human cancers with progestins and antiestrogens. In human endometrial cancer cell lines the antiproliferative effects of progestins and antiestrogens in a particular cell line appear to be associated with similar effects on growth factor and/or proto-oncogene expression. This suggests that although these compounds initially interact with different steroid hormone receptors, the molecular mechanisms of their growth inhibition may be essentially similar. In the case of human breast cancer cell lines, however, the effects of progestins and antiestrogens on gene regulation are often different, suggesting that the molecular mechanisms of progestin and antiestrogen growth inhibition may be essentially dissimilar.

Antiestrogens and progestins inhibit the growth of hormone-responsive human breast and endometrial cancer cells *in vitro* [1–4]. This may be one mechanism which explains the efficacy of these agents as antitumor agents *in vivo* [5, 6]. As with most drugs used in cancer therapy the development of resistance to antiestrogens and progestins is a major problem which limits their usefulness. However, understanding the molecular mechanisms by which these agents inhibit growth may provide us with insights into how resistance to these agents develops in human breast and endometrial cancer.

We have approached such an investigation from two aspects: firstly, we have studied the regulation of expression of putative autocrine/paracrine growth factors by these antiproliferative agents and secondly, we have studied the regulation of expression of specific proto-oncogenes, thought to have important roles in normal growth and differentiation.

REGULATION OF EPIDERMAL GROWTH FACTOR (EGF) AND TRANSFORMING GROWTH FACTORS (TGFs) IN HUMAN BREAST CANCER CELLS

The expression of putative autocrine/paracrine growth modulating factors by both normal and neoplastic cells is thought to play a role in their growth.

Growth promoting factors such as EGF, TGF-α, IGF-II, IGF-I-like, FGF-like as well as growth inhibiting factors such as members of the TGF-β family [7–11] have been found to be expressed by human breast cancers. Interestingly, regulation of some of these factors by estrogens and antiestrogens has been implicated at least in part in mediating the growth modulating effects of these agents [8, 10]. Moreover, its been shown many times that the addition of exogenous growth factors such as EGF, TGF-α, IGF-I and insulin to the medium of cells in culture can decrease the sensitivity of the cells to growth inhibition by progestins as well as antiestrogens [12–15]. These data support the hypothesis that growth factors may play an important role in growth modulation of human breast cancer cells by hormonal agents.

T-47D human breast cancer cells have previously been shown to be growth inhibited in a dose- and time-dependent fashion by progestins such as medroxyprogesterone acetate and antiestrogens such as monohydroxytamoxifen [13]. Therefore, initially we investigated the possibility that regulation of endogenous growth factors and/or their receptors in T-47D cells may be associated with the antiproliferative actions of progestins and antiestrogens. Both

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EGF and TGF-α mRNA levels are specifically increased by progestin treatment in these cells while monohydroxytamoxifen has no effect on EGF expression [7, 13, 16]. However, as has been shown previously [8] antiestrogen decreased the expression of TGF-α. Furthermore, progestins also increased the expression of the EGF receptor in these cells [17, 18]. Although the antiprogestin RU 486 is also growth inhibitory for these cells [19], this agent had little if any effect alone although it specifically inhibited the progestin induced effects on growth factor gene expression [7, 16].

TGF-β_1 mRNA levels were decreased by progestin treatment. This effect was specifically inhibited by the antiprogestin RU 486 [16]. As has been found previously in MCF-7 cells [10], the level of TGF-β_1 mRNA was unaffected by antiestrogen treatment. Although bioactive TGF-β was increased significantly in the conditioned medium of antiestrogen treated MCF-7 cells [10], it is not known at this time whether antiestrogens effect the production of bioactive TGF-β in T-47D cells.

These data show that in human breast cancer cells, effects of progestins and antiestrogens on growth factor gene regulation are different. Whilst the effects of monohydroxytamoxifen on TGF-α and TGF-β expression may explain, at least in part, the growth inhibitory effect of this agent, the regulation of these growth factors is unlikely to be responsible for the growth inhibitory activity of progestins in human breast cancer cells. Furthermore, regulation of the TGF-like genes in human breast cancer cells is unlikely to be a common mechanism of growth inhibition by hormonal agents, since antiestrogens, progestins and antiprogestins are all antiproliferative but have different effects on the expression of these genes.

Interestingly, the T-47D cells which express EGF and high levels of TGF-α mRNA, are much less sensitive to the antiproliferative effects of progestins and antiestrogens than the T-47D-5 cells, a variant cell line which does not express EGF and has very low levels of TGF-α mRNA [13]. These data together with the observations that exogenously added growth factors [12–15] decrease the sensitivity of breast cancer cells to growth inhibition by progestins and antiestrogens, suggest that increased growth factor gene expression or the expression of multiple growth factors may be one way in which breast cancer cells decrease their sensitivity or increase their resistance to antiproliferative agents such as progestins and antiestrogens. Indeed increased TGF-α expression has been found to be associated with breast cancer cells which are resistant to the antiproliferative effects of progestins [20].

REGULATION OF EGF AND TGFs IN HUMAN ENDOMETRIAL CANCER CELLS

Progestins inhibit the growth of both Ishikawa and HEC-50 human endometrial cells while the antiestrogen monohydroxytamoxifen inhibits the proliferation of Ishikawa cells only [3, 4, 21, 22]. Both these cell lines express TGF-α and TGF-β mRNA [21]. In Ishikawa cells, under conditions where they inhibited growth, progestins and antiestrogen both decrease the expression of TGF-α. Addition of exogenous TGF-α to the medium of these cells partially reversed the growth inhibitory effects of progestins and antiestrogens as well as stimulating the growth of these cells in the absence of any growth inhibitor. Furthermore, addition of antihuman EGF-receptor antibody to Ishikawa cells also inhibited the growth of these cells [21]. The data strongly support the hypothesis that the growth inhibitory effects of progestins and antiestrogens in Ishikawa cells are mediated, at least in part, by decreased expression of TGF-α. However, since progestins induce similar growth inhibitory effects on HEC-50 cells without any effects on TGF-α expression in this cell, other mechanisms are likely to be also involved in the antiproliferative effects of progestins in human endometrial cancer [21].

Little if any effect of antiestrogens, on TGF-β_1 mRNA was found in Ishikawa cells under conditions of growth inhibition [22]. However, progestins cause a decrease in TGF-β_1 mRNA levels in both Ishikawa and HEC-50 cells [21]. The effect in Ishikawa cells was of small magnitude, variable and only observed after prolonged exposure to the progestin. Interestingly, exogenously added TGF-β_1 inhibited the growth of the Ishikawa cells but stimulated the growth of HEC-50 cells [23], therefore the inhibition of TGF-β in HEC-50 cells may be part of the mechanism by which progestins inhibit the growth of these cells [21]

REGULATION OF PROTO-ONCOGENE EXPRESSION IN HUMAN BREAST CANCER CELLS

Progestins and antiestrogens have definite cell cycle effects [24, 25] and the c-*myc* gene product

is thought to have important roles in movement in and out of the cell cycle [26, 27]. In order to investigate further the mechanisms associated with growth inhibition of human breast cancer cells by progestins and nonsteroidal antiestrogens, the effect of these agents on c-*myc* gene expression in T-47D-5 and T-47D cells has been examined. C-*myc* mRNA levels were differentially regulated by the synthetic progestin, medroxyprogesterone acetate and the nonsteroidal antiestrogen, monohydroxytamoxifen in both cell lines. Antiestrogen treatment caused a persistent decrease in c-*myc* mRNA levels [28] a result similar to that obtained in MCF-7 cells [29]. However, the progestin caused a more complex response. Initially, c-*myc* mRNA levels increased approx. 2-fold, followed by a decrease and then partial recovery. The end result, however, of either antiestrogen or progestin treatment in these cells, is decreased cell number.

AP-1 complexes have been shown to play important roles in both cell growth and differentiation [30, 31]. Furthermore, in some cases AP-1 complexes are involved in steroid hormone regulation of gene expression [32]. Therefore, we have studied the effect of both progestins and antiestrogens on c-*jun* and c-*fos* expression. Progestins were found to increase the levels of c-*jun* and c-*fos* in T-47D cells in a time- and dose-dependent fashion [33]. A similar but more prolonged effect of progestins on c-*jun* mRNA levels was found in T-47D-5 cells. Interestingly, progestins decreased the level of c-*fos* mRNA in T-47D-5 cells which in our hands are more sensitive to the growth inhibitory effects of progestins than T-47D cells [34]. This difference seen in regulation of c-*fos* expression by progestins between the cell lines may in part account for the different sensitivities of these two cell lines to the antiproliferative effects of progestins. Interestingly, in preliminary studies in T-47D-5 cells the antiestrogen ICI 134,684 decreased c-*fos* mRNA levels but increased c-*jun* mRNA levels [34]. Although the biological significance of these observations is as yet unclear it is evident that the antiproliferative effects of these hormonal agents are accompanied by significant changes in the expression of genes which comprise AP-1 complexes. Moreover, altered expression of these genes may be associated with resistance of human breast cancer cells to the antiproliferative effects of antiestrogens and progestins.

REGULATION OF PROTO-ONCOGENE EXPRESSION IN HUMAN ENDOMETRIAL CANCER CELLS

In contrast to the more complex situation in human breast cancer cells, c-*myc* mRNA levels are markedly decreased by both progestins and antiestrogens in Ishikawa human endometrial cancer cells [35]. Furthermore, c-*jun* mRNA levels are decreased by both progestins and antiestrogens in this cell line [35].

CONCLUSIONS

It is obvious from these data that marked changes in both growth factor and proto-oncogene expression can occur due to treatment of hormonally-responsive human cancers with progestins and antiestrogens. In human endometrial cancer, although multiple mechanisms are involved, the antiproliferative effects of progestins and antiestrogens in a particular cell line appear to be associated with similar effects on growth factor and/or proto-oncogene expression. This suggests that although they initially interact with different steroid hormone receptors, the molecular mechanisms of their growth inhibition may be essentially similar. In the case of human breast cancer, however, the effects of progestins and antiestrogens on gene regulation are often different, suggesting that the molecular mechanisms of progestin and antiestrogen growth inhibition may be essentially dissimilar.

Although it is tempting to explain the growth inhibitory effects of progestins and antiestrogens in terms of their effects on growth factor and oncogene expression, these responses may be secondary phenoma related to the growth inhibition itself. Furthermore, many cells produce a variety of growth factor activities and to attempt to explain gross perturbations in growth rate in terms of a single class of growth factor may be naive. Alternatively, the differences we have observed may be due to different mechanisms by which cell number i.e. the end-point measured, is decreased. A decrease in cell number could be achieved in a number of different ways for example apoptosis, cytostasis, cytotoxicity, or differentiation. Certainly with respect to two of these possibilities i.e. apoptosis and differentiation, marked alteration in gene expression would be involved [36].

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