

REGULATION OF IGFBP-3 EXPRESSION IN BREAST CANCER CELLS AND UTERUS BY ESTRADIOL AND ANTIESTROGENS: CORRELATIONS WITH EFFECTS ON PROLIFERATION: A REVIEW

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This paper reviews actions of antiestrogens on IGF physiology, and discusses the potential significance of the recent observations that (i) effects of antiestrogens on the uterus are correlated with their effects on uterine IGF-I and IGFBP-3 gene expression, and that (ii) the potent antiestrogen and growth inhibitor ICI 182780 induces autocrine production of IGFBP-3 by estrogen receptor-positive breast cancer cells, while the growth stimulatory action of estradiol is associated with suppression of IGFBP-3 expression.

Keywords: Tamoxifen, estrogen, antiestrogen, uterus, breast, IGFBP-3.

BACKGROUND

In 1990 we observed in the context of a randomized, blinded clinical trial that tamoxifen administration alters the distribution of serum IGF-I levels in breast cancer patients, and lowers mean serum levels by approximately 30% [1]. This observation was subsequently confirmed in several independent studies (reviewed in Ref. [2]). As IGF-I is a potent breast cancer mitogen [3, 4] and tamoxifen is an effective compound in breast cancer treatment [5], this result was consistent with the concept that IGF bioactivity is a determinant of breast cancer behaviour and suggested the hypothesis that actions of tamoxifen on IGF physiology contribute to its antineoplastic action.

We recognized that if the antineoplastic activity of antiestrogens was related in part to effects on IGF physiology, drug-induced changes in tissue IGF bioactivity were likely to be more important than changes in serum IGF-I concentration. However, tissue IGF bioactivity is difficult to measure and is related not only to circulating IGF-I and IGF-II levels, but also to locally produced IGFs acting through autocrine and/or paracrine pathways, and to local concentrations of the IGF binding proteins and binding protein proteases [6]. As a first approach to

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investigating this, we studied the effect of tamoxifen on expression of IGF-I in rat lung and liver, and showed that tamoxifen suppressed IGF-I expression in both organs, and that this suppression was well correlated with suppression of serum IGF-I concentration [7]. It is therefore possible (but not demonstrated) that serum IGF-I level will be useful as a surrogate for tissue IGF-I expression or even for tissue IGF bioactivity. Consistent with this possibility are the observations that the reduction in IGF-I level induced by the combination of the somatostatin analogue octreotide and the antiestrogen tamoxifen is greater than the reduction achievable by either agent alone [8], and that the antineoplastic activity of this combination is greater than that of either single agent [9].

In vivo work using a chronically cannulated rat model [10] and *in vitro* work with pituitary gland cultures [11] provided evidence that tamoxifen reduces pituitary growth hormone output. While this contributes to the suppression of IGF-I expression by tamoxifen, experiments involving tamoxifen administration to hypophysectomized animals treated with growth hormone demonstrated a suppressive effect of the drug on IGF-I expression that is unrelated to suppression of growth hormone, and which may involve a direct inhibitory action on IGF-I gene expression [7].

ESTROGEN AND ANTIESTROGEN EFFECTS ON UTERUS CORRELATE WITH EFFECTS ON UTERINE IGF-I AND UTERINE IGFBP-3 EXPRESSION

We selected uterus for further *in vivo* studies, as antiestrogens are known to have significant trophic effects on this organ. Tamoxifen is regarded as a 'partial antagonist' to the estrogen receptor, as it exhibits varying estrogenic/antiestrogenic activities in an organ-specific and species-specific manner [12]. In humans and in rats, tamoxifen has an estrogenic effect on the uterus. Administration of the drug to post-menopausal women often causes endometrial thickening and has rarely been associated with endometrial neoplasia [5], while in the rat the estrogenic effect can conveniently be estimated by measuring increases in uterine weight. On the contrary, the complete estrogen receptor antagonist ICI 182780 causes uterine involution in rodent models [13] and has not been associated with hypertrophic uterine toxicity in humans. We observed that effects of these compounds on uterine weight were positively correlated with their effect on uterine IGF-I expression, as tamoxifen increased both uterine IGF-I expression and uterine weight, while ICI 182780 decreased uterine IGF-I expression and uterine weight [14].

In preliminary studies of the effect of antiestrogens and estrogens on uterine IGFBP expression, we noted large effects on IGFBP-3, and selected this protein for more detailed studies. We observed that changes in IGFBP-3 expression induced by tamoxifen and ICI were opposite to the changes induced in IGF-I expression: tamoxifen downregulated IGFBP-3 expression, while ICI 182780 increased expression. Thus while IGF-I expression was positively correlated with uterine weight, IGFBP-3 expression was negatively correlated with uterine weight [15]. These data are compatible with the model that IGFBP-3 acts as an inhibitor of cellular proliferation in uterus, either by competing with IGF-I receptors for ligand, or perhaps by the 'direct', IGF-independent inhibitory action seen in certain *in vitro* experimental systems [16].

These results extend the work of Murphy and Ghahary [17] on estrogen effects on uterine IGF physiology and are significant not only because they provide evidence consistent with the view that estrogens act as uterotrophic agents at least in part by altering expression of genes in a way that upregulates IGF bioactivity, but also because they demonstrate that the direction (hypertrophy vs. involution) of the trophic effect on uterus of drugs that acts as ligands for the estrogen receptor can be related to their effect on uterine IGF-I and IGFBP-3 gene expression. This suggests a molecular mechanism underlying uterine toxicity of tamoxifen and is of potential clinical relevance given the association of uterine hyperplasia and rarely uterine neoplasia with long-term tamoxifen usage.

Apart from its role as a useful agent in the palliative management of metastatic breast cancer, tamoxifen is widely used as an 'adjuvant' therapy [5]. Adjuvant therapy refers to post-operative treatment in apparently healthy women who are at risk of developing clinically apparent metastatic disease from occult micrometastatic disease. Adjuvant therapy with tamoxifen has been demonstrated in large clinical trials to have a modest but definite beneficial effect on disease-free survival, and therefore is prescribed to millions of breast cancer patients worldwide [5]. The success of tamoxifen as an adjuvant treatment provided a foundation for current trials of this drug in breast cancer prevention [5, 18]. One of the reasons that long-term tamoxifen therapy is practical relates to the fortuitous tissue-specific nature of the agonist vs. antagonist effects of the drug. In humans, tamoxifen acts as an estrogen receptor antagonist in breast, but as a mild estrogen in uterus, bone, and in lipid metabolism. If tamoxifen functioned as antagonist universally, it might be unsuitable for long-term adjuvant treatment in relatively healthy women because such activity would be expected to increase osteoporosis and/or increase cardiovascular morbidity. While the profile of tamoxifen is favourable for long-term use, given its estrogenic effects on serum lipids and bone density together with its antiestrogenic antineoplastic effect on breast epithelial cells, it is not optimum, as its estrogenic action in the uterus can lead to uterotrophic toxicity [5], and as it has been proposed that stronger antagonist activity in the breast would result in additional antineoplastic activity [19, 20].

Despite its clinical importance, the molecular basis for the tissue-specific action of tamoxifen has not been well characterized. The correlation between the direction of effect of ICI 182780 and tamoxifen on uterine weight and their effects on uterine IGFBP-3 gene expression may provide a strategy for further investigation of this issue through characterization of the molecular basis for the opposite consequences of ICI 182780 vs. tamoxifen binding to uterine estrogen receptors on uterine IGFBP-3 regulation. The effects of estrogen receptor ligands on IGF-I and IGFBP-3 expression may provide a strategy for screening novel compounds proposed for post-menopausal hormone replacement therapy. An ideal compound would display weak estrogen-like activity on IGF-I and IGFBP-3 expression in bone, strong antiestrogenic activity (that is, induction of IGFBP-3 expression) in breast, but no estrogenic activity on uterus.

EFFECTS OF ANTIESTROGENS ON IGFBP-3 EXPRESSION IN MCF-7 HUMAN BREAST CANCER CELLS [21]

The observation that the uterine antiestrogenic action of ICI 182780 is associated with increased uterine IGFBP-3 expression prompted us to examine the possibility

that the antineoplastic action of this compound on human estrogen receptor positive breast cancer cells is also associated with upregulation of IGFBP-3 expression. To examine this possibility, we used the MCF-7 human breast cancer cell line. Despite a prior report that these cells do not secrete IGFBP-3 [22], we observed that this protein is in fact secreted, but only in the absence of estradiol-stimulated cellular proliferation. The antiestrogen ICI 182780 strongly stimulated IGFBP-3 gene expression and accumulation in conditioned medium. Tamoxifen had similar effects, but was much less potent, and maximal induction of IGFBP-3 expression was only ~10% of the maximal induction achievable with ICI 182780. We found no evidence for estrogen or antiestrogen regulation of IGFBP-3 proteolytic activity in MCF-7 cell conditioned media, consistent with a previous report [23]. Dose-response studies carried out by varying concentrations of ICI 182780 or estradiol revealed a highly significant negative correlation between proliferation rate and IGFBP-3 expression. In order to determine if the effects on IGFBP-3 mediate or merely are correlated with the effects of estrogens and antiestrogens on proliferation, we carried out experiments with an antisense oligodeoxynucleotide (oligo) complementary to IGFBP-3 mRNA. This work showed that the antiproliferative effect of ICI 182780 was abolished in the presence of 5 $\mu\text{g ml}^{-1}$ IGFBP-3 antisense oligo, but not by the same concentration of sense oligo, which served as a control. This implies that in our experimental system, the induction of IGFBP-3 expression actually mediates that antiproliferative action of antiestrogens. Further support for this conclusion comes from the demonstration that recombinant IGFBP-3 can attenuate the growth stimulating action of estradiol on MCF-7 cells *in vitro*. [24]. However, caution is required before extrapolating these results to *in vivo* physiology: other mechanisms of action of antiestrogens have been described, and our assays measure early (<48 h) as compared with later actions of estrogens and antiestrogens.

The evidence indicating that antiproliferative action of antiestrogens is related to active induction of growth inhibitory signal transduction pathways rather than merely being a consequence of passive interference with estrogen action is consistent with the often overlooked fact that antiestrogens generally exhibit antiproliferative effects on estrogen-receptor positive cells even when these are cultured in the absence of estrogenic stimulation [21, 25].

IGFBPs are thought to modulate bioactivity of IGFs by competing with cell surface receptors for ligands [6]. Recent evidence suggests that IGFBP-3 may have a separate, direct (that is, IGF-independent) growth inhibitory activity [16]. As we have observed that recombinant IGFBP-3 or ICI 182780 can inhibit proliferation in the absence of serum and exogenous IGFs under conditions where autocrine production of IGF-I and IGF-II cannot be detected, our results are consistent with a 'direct' growth inhibitory action of IGFBP-3. However, the proposed direct and IGF-competitive actions of IGFBP-3 are not mutually exclusive. *In vivo*, IGFs are clearly present and there is evidence that antiestrogens upregulate TGF β expression [26] and that TGF β upregulates expression of IGFBP-3 in fibroblasts [27]. Thus antiestrogens may increase IGFBP-3 concentration in the microenvironment of tumor cells by both autocrine and paracrine mechanisms, and this IGFBP-3 may influence proliferation by 'direct' and/or 'indirect' mechanisms.

In the natural history of breast cancer; it is common for neoplasms to change from an estrogen-receptor positive, antiestrogen responsive phenotype to a more rapidly proliferating antiestrogen-resistant phenotype, which may be associated

with either presence or absence of estrogen receptors [28–30]. We have observed that estrogen receptor negative breast cancer cell lines generally constitutively express IGFBP-3 as distinct from estrogen receptor positive cell lines, in which expression is suppressed by estradiol. This constitutive expression may be a consequence of absence of function of the as yet poorly characterized signal transduction pathway that makes suppression of IGFBP-3 expression a consequence of estradiol binding to the estrogen receptor. The relatively rapid proliferation of estrogen receptor negative cells in the presence of constitutive IGFBP-3 expression is paradoxical in the context of the negative correlation between IGFBP-3 expression and proliferation reported for MCF-7 cells. This may be a consequence of unrelated aspects of neoplastic progression that render cells resistant to the direct growth inhibitory action of IGFBP-3, or more generally permit rapid proliferation independent of exogenous peptide mitogens.

The molecular basis of the pathway linking the estrogen receptor to IGFBP-3 expression remains to be elucidated. The characterization of this pathway may provide important clues related to several aspects of estrogen and antiestrogen action, and to the development of antiestrogen resistance. The fact that the available sequence data for the human IGFBP-3 promoter reveal no estrogen receptor response element suggests that the mechanism by which estradiol suppresses IGFBP-3 expression may be indirect, involving, as a first step, changes in expression of an estrogen-responsive regulatory gene which, in turn, controls IGFBP-3 expression. Our data suggest that expression of the putative regulatory gene is oppositely influenced by ICI 192780-estrogen receptor complexes and estradiol-estrogen receptor complexes in all tissues. The tissue-specific nature of the effect of tamoxifen on IGFBP-3 expression (downregulation in uterus, upregulation in breast) suggests that tissue-specific transcription factors may be involved.

It is of interest that recent reports provide evidence that $TGF\beta$ [27, 31] and retinoids [32] also inhibit breast cancer cell proliferation and upregulate accumulation of IGFBP-3 in conditioned media. ICI 182780 is both a more potent inhibitor of proliferation and a more potent inducer of IGFBP-3 expression than these compounds. These results raise the possibility that IGFBP-3 induction is a common factor involved in the action of a class of inhibitors of breast cancer proliferation, and that candidate novel growth inhibitors could be screened by examining their effects on IGFBP-3 expression. This remains a hypothesis, however, as some investigators favour a different model whereby IGFBP-3 acts to enhance the mitogenic activity of IGFs, and view enhanced expression of IGFBP-3 as a futile attempt to 'escape' from the effects inhibitors of proliferation [32, 33].

The results from our laboratory reviewed here suggest that antiestrogens act at multiple levels to reduce IGF bioactivity. These include downregulation of IGF-I expression, downregulation of type I IGF receptor expression (Pollak M., in preparation) and upregulation of IGFBP-3 expression. These data raise the possibility that in general, the action of gonadal steroids involves modulation of IGF physiology, both at the level of the entire organism and at the level of specific target organs for gonadal steroid action.

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