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I.3 An Epigenetic Mechanism for Tamoxifen-associated Uterine Carcinogenesis?

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Tamoxifen is a potent anti-oestrogenic drug effective in treating human breast cancer but is associated with an increase in endometrial tumours in some women. Although tamoxifen is an oestrogen antagonist in the breast it possesses partial agonist activity in the human uterus. We hypothesise that tamoxifen's carcinogenic activity in the endometrium involves dysregulation of the transforming growth factor-beta (TGFβ) activation and signalling pathway. Consequently, we are screening human biopsies for alterations in these TGFβ pathways. Altered responsiveness to TGFβ signalling could form the basis of an epigenetic mechanism for endometrial carcinogenesis with tamoxifen. © **1998 Elsevier Science Ltd. All rights reserved.**

TAMOXIFEN IS a drug of immense value in the treatment of breast cancer but epidemiological evidence has linked its usage with endometrial cancer in some women, resulting in such damning but confusing indictments as the 1996 IARC ruling that "tamoxifen is carcinogenic to humans (Group 1) and there is conclusive evidence that tamoxifen reduces the risk of contra-lateral breast cancer". In the risk versus benefit equation, the majority of clinicians would agree that the small risk to the endometrium from tamoxifen use is vastly outweighed by the benefit to the patient with breast cancer, but it is in the prophylactic trial setting that the equation is perhaps more complex. With tamoxifen, we also have an unusual situation in that the human clinical data outweigh the experimental toxicology and hence the role of the toxicologist studying tamoxifen may not necessarily be to attempt risk/benefit decisions but rather to contribute the best possible mechanistic evidence which can factor-in to these considerations. To date, the genetic toxicology of tamoxifen has focused on what is really a classical tale of chemical carcinogenicity in that in the rat, tamoxifen is metabolised to a reactive electrophile which binds covalently to DNA, forming what are known as DNA adducts. Through such a 'genotoxic' mechanism, tamoxifen is a potent rat hepatocarcinogen. However, rodent and human metabolism of tamoxifen is quite different and evidence to support a genotoxic mechanism in the human endometrium is very limited [1, 2]. Indeed, we speculate that such a mechanism of carcinogenicity may not be responsible for the pathology seen in the uterus of some women treated with tamoxifen. Our current approach is to investigate an epigenetic (non-genotoxic) mechanism of carcinogenicity, i.e. the dysregulation of transforming growth factor-beta (TGFβ) signalling in the human uterus.

TGFβ is a term which describes a family of cytokines (I, II and III in mammals) which act as multifunctional growth factors with a role in chemotaxis, angiogenesis and the regulation of cell growth and differentiation. In particular, TGFβ can stimulate the growth of cells of mesenchymal origin, yet is a potent inhibitor of epithelial cell growth. However, TGFβ is secreted as an inactive latent complex and activation is facilitated by binding to the mannose-6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2r). Following activation, TGFβ binds to its cognate signalling receptors designated TGFβI, II and IIIr. Due to the obligate role of these receptors in TGFβ function, the M6P/IGF2r and TGFβIIr have been reported as tumour suppressors [3]. The rationale for investigating TGFβ dysfunction comes from the knowledge that tamoxifen exerts some very beneficial effects on transforming growth factors in the breast as a component of its anti-oestrogenic activity, including the ability to down-regulate the expression of the mitogen TGF and enhance the expression of TGFβ with its negative growth factor influence [4]. Hence, with regard to the known pro-oestrogenic component of tamoxifen in the human uterus, a working hypothesis of uterine TGFβ dysregulation appears plausible. Our approach in these investigations is to focus upon human tissues rather than rodent models and to explore the TGFβ milieu in the endometrium of tamoxifen- and toremifene-treated patients as compared to 'control' patients, especially those receiving hormone replacement therapy

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(HRT). Through the preparation of sequential cryotone sections we are diagnostically grading the pathologies/histology present in a large number of biopsies, scoring for percentage tissue involvement and relating pathology with TGFβ status by histomorphometry using a range of TGFβ-specific antibodies and probes. In addition, we are using laser capture microdissection to determine the expression of TGFβI, II and III and their cognate receptors using RNA protection assays. Furthermore, we are exploring the TGFβ activation pathway and investigating the endometrial expression of M6P/IGF2r. We hypothesise that tamoxifen causes dysregulation of the TGFβ signalling pathway in preneoplastic and normal cells creating an environment which selects, preferentially, for cells with genetic alterations in the TGFβ signalling pathway, i.e. M6P/IGF2r, TGFβIIr. Cells with mutations in this pathway become non-responsive to normal cellular mito-inhibitory signals and develop into end stage neoplasms.

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I.4 Effects of Anti-oestrogens on Insulin-like Growth Factor (IGF-I) Physiology Systemically and in the Uterus

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Insulin-like growth factor-I (IGF-I) is a potent mitogen for normal and neoplastic breast epithelial cells. It has been shown that anti-oestrogens decrease IGF-I levels and gene expression, but it is not clear to what extent this contributes to their antineoplastic activity. In the uterus IGF-I is also a mitogen and has been shown to be an important mediator of the uterotrophic effect of oestrogens. The effect of 'anti-oestrogens' on the expression of IGF-I in the uterus is closely related to their uterotrophic action. For example, tamoxifen induces uterine hypertrophy and upregulates uterine IGF-I expression, while ICI 182780 causes uterine involution and is associated with suppression of uterine IGF-I expression. In studies of novel oestrogen receptor ligands, it will be of interest to determine their effect on IGF-I expression both systemically and in the uterus.

IN 1990, WE published the first randomised, blinded study that demonstrated that anti-oestrogens reduce circulating levels of insulin-like growth factor-I (IGF-I), a potent mitogen for both normal and neoplastic breast epithelial cells [1]. The degree of suppression of IGF-I associated with tamoxifen use was modest (approximately 30%) but was statistically significant and reproducible in many subsequent studies for example, [2].

We subsequently showed that tamoxifen has inhibitory effects *in vitro* [3] and *in vivo* [4] on growth hormone (GH) secretion, which likely accounts for at least a portion of the suppression of IGF-I levels. However, we also showed [5] that even in hypophysectomised animals with GH levels maintained constant by recombinant GH administration, tamoxifen suppressed IGF-I levels, suggesting a separate, pituitary-independent mechanism for suppression of IGF-I gene expression.

Interestingly, tamoxifen was seen to suppress IGF-I gene expression in several target organs for breast cancer metastasis [5], a result which may be relevant to the activity of the compound in adjuvant treatment of breast cancer, as such an action would be expected to make the organ a less fertile 'soil' for metastasis to progress. The co-administration of a somatostatin analogue such as octreotide with tamoxifen has been shown experimentally to enhance both the antineoplastic activity [6] and the IGF-I suppressive actions [7] of tamoxifen and this contributes to the rationale for major adjuvant clinical trials (NCIC MA14 and NSABP B29) that are comparing tamoxifen to the combination of tamoxifen and octreotide in adjuvant breast cancer treatment. Apart from its