Endocrine effects of IGF-I on normal and transformed breast epithelial cells: potential relevance to strategies for breast cancer treatment and prevention

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Summary

Insulin-like growth factors (IGFs) are mitogenic and anti-apoptotic peptides that influence the proliferative behavior of many cell types, including normal and transformed breast epithelial cells. IGF-I has properties of both a tissue growth factor and a systemic hormone: there is evidence that IGF bioactivity in tissues is influenced not only by local factors such as tissue expression of IGFs, IGF binding proteins (IGFBPs), and IGFBP proteases, but also by factors that regulate whole-body IGF physiology and circulating IGF-I levels. Experimental evidence that interventions that reduce circulating IGF-I levels reduce proliferation of breast neoplasms has raised interest in the possibility of developing novel endocrine therapies that target the growth hormone/IGF-I axis. Furthermore, influences of the growth hormone/IGF-I axis on normal breast epithelial cells may underlie recent epidemiological observations that suggest that premenopausal women with high circulating IGF-I level are at increased risk for breast cancer. These studies suggest that the growth hormone/IGF-I axis deserves investigation as a possible target for novel breast cancer prevention strategies.

Background

Knowledge regarding the insulin-like growth factor system is increasing rapidly [1,2]. A number of research groups are working to determine the relevance of this information to the pathophysiology of neoplasia in general, and breast cancer in particular. Recent research has revealed considerable complexity of the IGF system of mitogens at the genetic, cellular, and whole-organism levels. This review will emphasize recent data regarding systemic (as distinct from cellular) IGF physiology potentially related to the regulation of behavior of normal breast epithelial cells and breast cancer, and will also point out areas of potential relevance to breast cancer treatment and prevention.

Tissue IGF bioactivity is determined by both local and systemic factors [1]. Local factors include tissue expression of type I and type II IGF receptors, IGF I and IGF II, the various IGF binding proteins, and binding protein proteases.
Systemic factors regulate circulating levels of IGFs and binding proteins. Pituitary growth hormone secretion, which is controlled by hypothalamic somatostatin and growth hormone releasing hormone, is the key regulator of hepatic IGF-I production, which in turn is the main source of circulating IGF-I [1]. Other systemic factors, including nutritional ones [3], also have important influences on IGF-I physiology.

It is noteworthy that there is evidence that certain factors that lower IGF-I levels systemically have separate influences on local factors that influence tissue IGF bioactivity. These tend to act in a manner that would be expected to further reduce IGF activity, such as down-regulation of type I receptor expression [4] or up-regulation of IGF binding protein expression [5,6]. Conversely, there is evidence that growth hormone, which raises the circulating IGF-I level, also up-regulates IGF-I expression within the mammary gland [7]. This raises the possibility that at least in certain instances, changes in circulating IGF-I level may reflect changes in tissue IGF bioactivity. This is not because circulating IGF levels are necessarily the dominant factor determining tissue IGF bioactivity, but rather because in many cases, there appears to be parallel regulation of systemic IGF-I levels on one hand and key determinants of local IGF bioactivity on the other.

**Systemic IGF-I physiology and breast cancer behavior**

The classic example of an influence of host endocrinology on breast cancer behavior involves estrogen responsiveness. This was demonstrated in classic clinical and experimental studies of ovarian ablation [8]. In view of extensive data suggesting that breast cancer cell lines are responsive to exogenous IGFs [9-12], the possibility that at least a subset of breast cancers is dependent on host-derived IGFs in a manner analogous to dependence on host steroid hormones has been proposed. Obviously, testing this hypothesis by means of ablative surgery is impossible in view of the multiple endocrine effects of hypophysectomy.

However, the *lit* mutation provides a convenient model for studies of influence of the host growth hormone/IGF-I axis on breast cancer behavior[13]. The *lit* mutation inactivates the growth hormone releasing hormone receptor and results in selective ablation of the growth hormone/IGF-I axis [14,15]. Circulating GH and IGF-I levels in animals with the *lit* mutation are ~10% of control values, although other aspects of pituitary function are normal. The *lit* model is, of course, quite distinct from an IGF-I knock-out model: the latter involves complete absence of autocrine, paracrine and endocrine IGF-I, while the former targets “endocrine” IGF-I selectively. Experiments made possible by the availability of immunodeficient IGF-I deficient (*lit/lit*) and IGF-I replete (*lit/+*) mice demonstrated significant attenuation of neoplastic proliferation of human MCF-7 human breast cancer xenografts in the IGF-I deficient animals [13]. In these experiments, the possibility that results are related to lack of estrogen resulting from impaired ovarian production secondary to IGF-I deficiency in *lit/lit* hosts was excluded as all animals received estradiol supplementation.

These results deserve follow-up, as they suggest the possibility that novel treatments that target the GH/IGF-I axis may have antineoplastic activity. On the other hand, it is premature to make general conclusions in this regard, as the proportion of breast cancers that exhibit dependence on host IGFs remains unknown. In particular, it would be predicted that those neoplasms that constitutively produce significant quantities of IGF-II in an autocrine fashion would be minimally influenced by host IGF physiology. Another area for further studies concerns the need to more precisely describe the basis of dependence on the host GH/IGF-I axis in this model. In the *lit* experimental system, the endpoint involves macroscopic growth of tumor xenografts, and such growth requires the participation of normal host cells, in particular stromal and angiogenesis components. Notwithstanding the *in vitro* evidence for a stimulatory effect of IGF-I on
many breast cancer cell lines, the possibility that the results reflect dependence of stromal cells or
cells involved in angiogenesis on the host GH/
IGF-I axis has not been ruled out.

There is considerable variability among normal
individuals with respect to IGF-I levels [16].
Prospective clinical studies to evaluate the possi-
bility that IGF-I levels or some other measure of
activity of the host GH/IGF-I axis are related to
prognosis or clinical behaviour of breast cancer
have not been reported to date. Several such
studies are in progress, including companion
studies to the NSABP B29 and NCIC MA14
protocols. Older clinical studies [17-19] suggest-
ing efficacy of hypophysectomy in the palliative
management of breast cancer, even in the setting
of prior ovariectomy and adrenalectomy, are com-
patible with the hypothesis that ablation of the
growth hormone/IGF-I axis can influence breast
cancer behaviour. However, these studies were
uncontrolled, involved relatively small numbers of
patients, and of course did not quantify circulating
IGF-I levels. Therefore, formal clinical evidence
for or against an effect of host growth hormone/
IGF-I physiology on prognosis of breast cancer
will be available only when ongoing studies are
completed.

The observation that tamoxifen reduces circu-
lating IGF-I levels [20] has been confirmed by
several groups [21-25], but it remains unclear to
what extent, if any, the antineoplastic activity of
tamoxifen is related to this action. This question
has not been resolved by studies of receptor pro-
files in breast tumors, since in human breast
cancers, estrogen receptor levels are positively
 correlated with IGF-I receptor levels [26]. Thus,
neoplasms predicted to be most responsive to
antiestrogens on the basis of high ER level might
also be most dependent on stimulation by exo-
genous IGF-I, and the tamoxifen-induced decline
in circulating IGF-I levels might contribute to the
efficacy of antiestrogens against these tumors, as
might antiestrogen-induced up-regulation of
IGFBP expression [5,6].

The mechanism by which tamoxifen reduces
IGF-I levels has received attention. There is evi-
dence for both an indirect and a direct inhibitory
effect on IGF-I gene expression. The indirect
mechanism involves suppression of pulsatile
growth hormone secretion by antiestrogens [27].
This has been documented in both in vivo models
in which growth hormone output was directly
assayed [27] and in vitro pituitary cell cultures
[28]. Evidence for a pituitary-independent effect of
antiestrogens on IGF-I levels and IGF-I gene
expression comes from studies using hypophysec-
tomized animals that received growth hormone
replacement with or without antiestrogen treat-
ment [29]. Antiestrogen treated animals showed
reduced IGF-I gene expression and serum levels
even when growth hormone levels were held con-
stant by these manipulations. These results
indicate that the reduction of IGF-I levels by
tamoxifen involves both suppression of pituitary
growth hormone output and growth-hormone
independent mechanisms.

While the effect of antiestrogens on lowering
serum IGF-I level has been confirmed in several
studies, it is clear that this effect is modest in
magnitude, as most reports suggest reduction to
about 75% of pretreatment levels [20]. There is
limited evidence suggesting that this suppression
can last for years during tamoxifen therapy [22].
A variety of measures have been proposed to
more profoundly lower systemic IGF-I bioactivity.
These include the use of recombinant IGF binding
proteins [30], the use of somatostatin analogues
[21,31,32], or the use of growth hormone releas-
ing hormone antagonists [33].

The first approach is challenging at a practical
level, as it would necessitate chronic administra-
tion of large amounts of a fairly large recombi-
nant protein. On the other hand, somatostatin
analogues have been used clinically for many
years in the treatment of acromegaly and carcino-
oid syndrome, and are known to be well toler-
ated. These compounds dramatically lower IGF-I
levels in some acromegalic patients with markedly
elevated growth hormone and IGF-I levels [34],
but have relatively modest IGF-I suppressing
activity in non-acromegalic individuals. This is
likely due to the fact that there is no counter-
regulation to oppose reduction of acromegalic IGF-I levels to the normal range, but in non-acromegalic individuals, the effects of somatostatin analogues on the pituitary are likely attenuated by compensatory mechanisms such as up-regulation of growth hormone releasing hormone secretion or down-regulation of somatostatin receptor expression by somatotrophs.

The combination of tamoxifen and the somatostatin analogue octreotide has been shown both clinically [35] and experimentally [36] to be more potent than either agent alone in suppressing circulating IGF-I levels, achieving serum levels near 50% of pretreatment levels. Experimentally, this combination was noted in the DMBA model to be more potent than either single agent in antineoplastic activity [37]. However, significantly enhanced antineoplastic activity of the combination was detected only in low tumor burden models [37]. Furthermore, the mechanism underlying this enhanced activity remains unclear; while it may be related to enhanced suppression of the growth hormone/IGF-I axis, it is also possible that a direct action of octreotide on somatostatin receptors present on breast cancer cells is involved [31,32]. These two proposed mechanisms of action are not mutually exclusive.

The clinical relevance of the preclinical data concerning the octreotide/tamoxifen combination is now being evaluated in two randomized adjuvant trials (NSABP B29 and NCIC MA14). Execution of these large trials was made practical by the development of a monthly depot formulation of octreotide, which previously had to be administered subcutaneously several times per day to achieve stable IGF-I suppression. These trials differ in entry criteria and duration of therapy, but both involve randomization between tamoxifen and the tamoxifen-octreotide combination. Both involve companion studies of effects of treatments on the growth hormone/IGF-I axis and of somatostatin receptor expression on neoplasms, and therefore should provide useful data concerning relationship of systemic IGF-I physiology to breast cancer behaviour.

Other approaches to reduce IGF-I bioactivity have also been proposed, but are less advanced in their development. These include growth hormone antagonists [38] and growth hormone releasing hormone antagonists [33]. Just as there are many pharmaceutical strategies to exploit estrogen responsivity, it is likely that it will be possible to develop many strategies to pharmacologically target the growth hormone/IGF-I axis. At present, the major questions relate not so much to the challenges of optimizing pharmaceutical approaches to suppression of the growth hormone/IGF-I axis, as to the uncertainties as to the proportion of human breast cancers that are indeed dependent on host IGF-I bioactivity.

Systemic IGF-I physiology and the normal breast

Several lines of investigation support the view that the growth hormone/IGF-I axis plays an important regulatory role in controlling growth and development of glandular structures in the breast (reviewed in [39]). While it is clear that steroid hormones play key roles in regulating breast development, the fact that the stimulatory actions of estradiol on breast development are greatly attenuated in hypophysectomized animals indicates a role for other pituitary dependent factors in breast epithelial cells [40]. Recent studies have demonstrated that growth hormone is the key pituitary factor required to allow steroid-induced development of the breast to proceed [7,39,41]. There is evidence that growth hormone acts in this respect not only by raising circulating IGF-I levels, but also by up-regulating IGF-I gene expression within the breast [7].

In contrast to the relatively well studied influences of the growth hormone/IGF-I axis on regulation of lactation [42,43] and on the postnatal development of the mammary gland [39], there is relatively little information available on the role of this axis in regulating epithelial cell proliferation and cell turnover in the mature breast. This is a potentially important issue, as
there is evidence that in certain epithelial cell populations, risk of neoplastic transformation is positively correlated with proliferation rate [44, 45].

It is of interest that there is evidence that mice with higher IGF-I levels appear to have advantages in reproduction, as assessed by weight of offspring [46]. A variety of factors may underlie this observation, but it is possible that enhanced lactation performance is involved. Thus, there may be selective pressure favouring higher IGF-I levels, notwithstanding the possibility that such selection may predispose to neoplasia later in life.

In view of the well documented person-to-person variability in IGF-I levels [16] and data suggesting that activity of the growth hormone/IGF-I axis may be a determinant of epithelial cell turnover in the breast, is it possible that individuals with higher levels of IGF-I have increased risk of breast cancer? Until recently, only circumstantial evidence, such as the three examples that follow, has been available. First, in rodent models, energy-restricted diets, which suppress IGF-I [3], are well known to decrease cancer incidence [47-49]. Second, the positive correlation between birthweight and breast cancer risk [50] is compatible with the hypothesis if individuals stay true to their birth centile of IGF-I level, as low IGF-I level has been associated with low birthweight [51,52]. Finally, height, which is positively associated with breast cancer risk in most epidemiologic studies [53-59], is positively correlated with IGF-I levels [16].

Recent laboratory studies provide more direct data compatible with the hypothesis that the growth hormone/IGF-I axis stimulates proliferation of normal breast epithelial cells, and that higher rates of proliferation, over time, increase risk of transformation. For example, it has been shown that transgenic mice that overexpress growth hormone and consequently have high levels of circulating IGF-I exhibit a high frequency of breast cancer [60,61]. They also exhibit morphological evidence of mammary gland epithelial cell hyperplasia [62] Mice exposed to exogenously administered growth hormone also show histological evidence of mammary gland epithelial cell hyperplasia [62,63]. Such histological changes have been associated with increased breast cancer risk in humans [64]. On the other hand, transgenic animals engineered to overexpress a growth hormone antagonist [65] show ductal hypoplasia [66]. In addition, experiments involving overexpression of IGF-I receptor agonists within the mammary gland showed that such manipulations increased mammary cancer and resulted in incomplete mammary gland involution [67,68]. Finally, there is evidence from older studies in mice of a positive correlation between strain-specific evidence of breast cancer and activity of the GH/IGF-I axis [69,70]. This remains an area of active investigation, and further studies to evaluate the physiological role of the GH/IGF-I axis in the adult breast are required.

It is well known that there is considerable heterogeneity between individuals with respect to circulating levels of IGF-I, IGF binding protein 3, and activity of the growth hormone/IGF-I axis [16,1]. Although laboratory evidence for a role of this axis in stimulating proliferation of epithelial cells in the mature breast is suggestive rather than conclusive, the hypothesis that person-to-person variability in function of the growth hormone/IGF-I axis may be related to variability in breast cancer risk has already been studied clinically. Two case-control studies have been carried out to evaluate the possibility that there is a relationship between circulating IGF-I level and breast cancer risk [72,73]. Both found significantly higher IGF-I levels in women with breast cancer than in controls. In the larger of the two studies (with 109 cases) [72], the relationship observed was strongest among premenopausal women.

These studies, while provocative, were potentially limited by their retrospective design. In each, plasma IGF-I levels were evaluated after diagnosis in the cases, so that an effect of the breast cancer on IGF-I levels was not ruled out. Furthermore, the control group may not have been completely comparable to the case group. A recent prospective study carried out on the Nurses
Health Study cohort [74] minimizes these concerns.

This study, which involved 397 women with breast cancer and 620 age-matched controls, was carried out in a formal blinded fashion, using prospectively acquired blood samples. The study confirmed that there is considerable heterogeneity among women with respect to IGF-I level. No relationship between IGF-I level measured on a sample obtained after menopause and subsequent breast cancer incidence was seen. In contrast, among premenopausal women less than 50 years of age, those in the top tertile of IGF-I level had a relative risk of breast cancer of 4.58 (95% confidence limits 1.75 – 12.0) compared to those in the bottom tertile. With adjustment for circulating IGFBP-3 level, which was also measured, the relative risk was 7.28 (95% confidence limits 2.4 – 22.0).

These data add substantial direct evidence to support the hypothesis that activity of the growth hormone/IGF-I axis is related to risk of premenopausal breast cancer. If confirmed, they will support the view that reduction of activity of the growth hormone/IGF-I axis deserves study in the context of breast cancer prevention, particularly for women in higher centiles of circulating IGF-I level. However, studies involving larger numbers of premenopausal women will be needed to provide more precise quantification of the relationship between risk and IGF-I levels. While the data demonstrate conclusively the absence of a relationship between postmenopausal IGF-I level and postmenopausal breast cancer risk, the 95% confidence limits for the relative risk related to IGF-I level in postmenopausal women are large, with a lower bound of relative risk at 1.75. This is due to the fact that most women in the study were older.

Another area for epidemiological studies concerns evaluation of the possibility that premenopausal IGF-I level is related to risk of postmenopausal breast cancer. This issue deserves investigation, as there is evidence that in general, breast cancer risk later in life can be influenced by premenopausal factors. As IGF-I levels decline non-specifically with increasing age, it is possible that levels in older women are not correlated with levels during adolescence and early adulthood, which may be the critical periods in determination of risk.

If a relationship between IGF-I level and risk is established, somatostatin analogues, growth hormone releasing hormone antagonists, or growth hormone antagonists would deserve evaluation as candidate risk-reducing agents, specifically for those women at increased risk on the basis of their IGF-I level. Furthermore, IGF-I level would deserve evaluation as a candidate intermediate endpoint for prevention studies. Finally, in view of the fact that tamoxifen suppresses IGF-I levels [20], demonstration of an association between IGF-I level and risk would suggest that IGF-I level might be a variable of importance in the interpretation of tamoxifen breast cancer prevention trials. For example, it is possible that risk reduction might be greater in those with highest IGF-I levels prior to chemopreventive intervention. Presently available data from both clinical and laboratory studies are strong enough to justify further research in these areas.

Conclusion

There is now clear evidence that the IGF system strongly influences epithelial cells of the breast. Although 'local' aspects of IGF physiology are important, evidence for the requirement of an intact pituitary growth hormone/IGF-I axis for postnatal breast development indicates the relevance of systemic components of the IGF system. The possibility of therapeutically exploiting dependence of breast neoplasms on host IGFs is under investigation both clinically and experimentally, but the proportion of human breast cancers that depend on host IGFs is unknown. More recent laboratory studies and epidemiological data have brought into focus the hypothesis that the growth hormone/IGF-I axis may be related to breast cancer risk, perhaps
by an influence on rate of breast epithelial cell turnover. This hypothesis deserves study because if it is valid, it suggests novel approaches to breast cancer prevention.

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