Insulin, insulin-like growth factors and neoplasia

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Over the past decade, dozens of epidemiological studies and laboratory experiments have provided evidence for relationships between insulin-like growth factor (IGF) physiology and neoplasia. Population studies provide evidence for a modestly increased risk of a subsequent cancer diagnosis in subjects with IGF-I levels at the high end of the broad normal range, as compared to those at the low end of the normal range. At the cellular level, IGF-I receptor signalling has been shown to play an important role in facilitating the transforming action of a variety of oncogenes. Reducing receptor function with anti-receptor antibodies or specific tyrosine kinase inhibitors reduces the proliferation of many cancers in vitro and in vivo. At present, clinical relevance of the relationship between circulating IGF-I level and cancer risk is limited, but in terms of experimental therapeutics, many clinical trials have been initiated to investigate the possibility that the paradigm of hormonal treatment of cancer may be extended from targeting gonadal steroids to targeting the growth hormone–IGF-I axis.

Key words: cancer; insulin; IGF-I; targeted therapy; metformin; risk; prevention.

BACKGROUND

Over the past decade there has been substantial progress in understanding key elements of molecular, cellular and whole-organism physiology of insulin and the insulin-like growth factors (IGFs). These general areas have been summarized in recent reviews[1–3], and will not be described here in detail. A few background points of particular relevance deserve mention, however.

Receptors

Insulin-like signalling plays crucial roles in regulating cell proliferation, lifespan, and metabolism in simple organisms such as *Caenorhabditis elegans*.[4] Early in evolutionary

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history, an ancestral insulin-like receptor – rather than a specific insulin receptor or insulin-like growth factor receptor – initiated signalling. In higher organisms, as the need arose to regulate cellular proliferation and survival independently of short-term regulation of cellular uptake of glucose, distinct insulin-like growth factor and insulin receptors and ligands evolved.

It is well recognized that IGF-I receptors are widely distributed in normal and malignant tissues.[1] (So-called IGF-II receptors do not transduce a signal but serve to restrain growth by competing with IGF-I receptors for IGF-II; IGF-II is commonly over-expressed in cancer, and accordingly the gene encoding the IGF-II receptor has the properties of a tumour suppressor gene.)[5–7] Classic insulin-sensitive tissues include muscle, liver, and fat, and these tissues display insulin receptors. Less well studied is the role of the insulin receptor present on normal and transformed epithelial cells.[8] While insulin receptors may be involved in regulation of glucose uptake by epithelial cells, epithelial tissues comprise a small proportion of body weight relative to the total weight of liver, muscle, and fat, so these tissues probably play only a minor role in disposing of circulating glucose.

Most common cancers arise from epithelial cells, and express both the gene encoding the insulin receptor and the gene encoding the IGF-I receptor.[8] This leads to a situation where not only insulin and IGF-I receptors but also hybrid receptors (composed of a 'half insulin receptor' and a 'half IGF-I receptor') are expressed on the cell surface.[9] In general terms, hybrid receptors appear to have higher affinity for IGF-I and IGF-II than insulin. There are important gaps in knowledge concerning the relative expression levels of insulin receptors and IGF-I receptors by cancer cells. Furthermore, the significance of the relative expression levels of the two insulin receptor isoforms requires clarification. The IR-A insulin receptor isoform, which appears to have affinity for IGF-II, could be involved in IGF-II autocrine loops, which are commonly seen in neoplastic tissue, and which were previously thought to involve exclusively the IGF-I receptor.[8,10]

Ligands

The microenvironment of normal cells at risk for transformation and of cancer cells contains insulin, IGF-I, and IGF-II. With rare exceptions[11], insulin is not produced by cancers. In contrast, substantial IGF-I and/or IGF-II is locally produced by neoplastic tissue in an autocrine or paracrine manner, and this provides a source of these ligands supplementary to the classic 'endocrine' production by the liver delivered via the circulation. Also present in the extracellular fluid in the cellular microenvironment are IGF binding proteins and IGF binding protein proteases, which can regulate bioavailability of IGF-I and IGF-II. Classically, IGF binding proteins reduce ligand bioavailability by competing with receptors for ligand.[12,13] This model accounts for some recent findings which associate abnormal IGF-IR activation in cancer with down-regulation of IGFBP-3 production.[14] However, there are complexities, as high levels of expression of certain IGFBPs, particularly IGFBP-2, appear to be associated with accelerated rather than inhibited proliferation[15,16] through mechanisms that remain to be fully clarified.

In studies where serum levels of ligands are studied in relation to cancer risk or prognosis, an implicit assumption is made that serum levels are valid surrogates for levels in the relevant cellular microenvironment; this is probably valid in the case of insulin, but may represent an oversimplification for the ligands and binding proteins

that are produced locally within the target tissue as well as in the liver. While epidemiological research regarding the influence of insulin on cancer is less hampered by this issue than studies of IGFs, studies of insulin have other challenges related to the imprecision of using random or even fasting or postprandial measurements to estimate the impact of levels that fluctuate throughout the day according to nutrient consumption.

LABORATORY STUDIES

Laboratory studies regarding roles of insulin in neoplasia preceded those concerning roles of the IGFs. Early studies not only showed that insulin at physiologically relevant concentrations stimulates DNA synthesis in breast cancer cells[17], they also provided early evidence that insulin deficiency is associated with less aggressive cancer proliferation in vivo.[18] Until the recent resurgence of interest[19], however, little attention was given to following up on these observations made more than 20 years ago, probably because of the assumption that any attempt to reduce insulin signalling would have grave metabolic consequences.

IGF-I receptor targeting strategies were first proposed over 20 years ago, when IGF-I receptors were detected on human cancers.[20] Many subsequent in-vitro and in-vivo models, when viewed as a whole, provide convincing evidence for a role for the IGF-I receptor in neoplasia. A comprehensive listing of all studies in the literature is beyond the scope of this review, but key examples will be highlighted. Early in-vitro experiments demonstrated dose-dependent increases in neoplastic cell proliferation with increasing IGF-I concentration.[21] In-vivo models made use of naturally occurring mutations associated with low IGF-I levels[22] or genetic manipulations[23,24] to influence ligand levels to show that, in vivo, tumour growth is influenced by host IGF-I physiology. More recently, several drug candidates that target IGF-I signalling were found to have anti-neoplastic activity by using in-vivo models, both as single agents and in combination with currently approved drugs.[25–29] Finally, the influence of host hyperinsulinism on cancer behaviour has been the subject of recent experiments.[30] In general, these results have provided strong (but circumstantial) evidence that hyperinsulinaemia may be a mediator of the adverse effect of obesity or excess caloric intake on cancer prognosis.

POPULATION STUDIES: CANCER RISK

One of the reasons for increasing interest in the role of IGF-I in neoplasia is that, in addition to direct epidemiological and laboratory studies, there is substantial circumstantial clinical evidence that suggests relevance. Key examples include the association of high birth weight with both subsequent cancer risk and high cord blood IGF-I level[31–36]; the association of both height[37,38] and patterns of childhood and adolescent growth[39,40] (both of which are IGF-I-determined) with cancer risk; and also the association of mammographic density, a strong risk factor for breast cancer, with IGF-I serum levels.[41,42]

The relationship between circulating IGF-I levels and cancer risk remains poorly understood. Early, rigorous, prospective studies provided evidence for a relationship between circulating IGF-I levels and cancer risk, which applied to prostate, breast, colorectal, and other cancers[43–49], such that individuals at the high end of the normal range of serum IGF-I concentration had more than double the risk of a subsequent

cancer diagnosis than those at the low end of the normal range. Some of these early reports also described a finding that higher circulating levels of IGFBP-3 were associated with reduced risk, which was interpreted as reflecting an influence of IGFBP-3 as reducing IGF-I bioactivity, in keeping with laboratory studies.[43,44] However, some follow-up studies (for example that of Schernhammer et al)[50] have failed to confirm these reports, or have revealed weaker relationships.

In considering these inconsistencies, it is worthwhile reflecting first on the underlying biology and then on methodological issues. Why might circulating IGF-I levels be related to cancer risk in the first place? Two hypotheses are worth considering. One suggests that early in carcinogenesis, as somatic cell mutations lead to accumulating DNA damage in an at-risk cell, the IGF bioactivity in the cellular microenvironment is a critical factor that influences the fate of the cell: will it survive and evolve to a frankly malignant cell lineage, or will it undergo apoptotic death? Given that IGF-I receptor activation activates pro-survival signalling pathways[51], the balance between apoptotic cell death versus survival of damaged cells might be slightly tipped towards survival in a 'high IGF' environment, and this would favour the emergence of a malignant clone. Many other factors also influence this process, but over many years, and recognizing that the fate of millions of DNA-damaged cells is determined every hour, even a modest influence of higher IGF-I level on survival probability might lead to an association of circulating level with cancer risk.

A second hypothesis suggests that the influence of IGF-I level on cancer risk has little to do with early carcinogenesis. This view suggests that higher IGF-I levels simply favour the more rapid proliferation of early cancers to the point at which they are clinically detectable. This hypothesis would predict that if one had a means to detect 1-mm tumours, the number of these lesions would be unaffected by IGF-I levels. Rather, such lesions would be common in all adults, and risk of a clinical cancer diagnosis would reflect the probability of these lesions progressing toward a detectable and clinically significant size, with this latter process being influenced by IGF-I level. Findings in the case of prostate cancer may be consistent with this second hypothesis. First, autopsy studies show that undetected prostate cancers are very common, and present in the majority of adult men.[52] Second, there is evidence that diagnosis of prostate cancer years after a baseline IGF-I level is obtained is more closely associated with this baseline level in a population without PSA screening than one with PSA screening.[46,47] This is consistent with the view that the IGF-I level is more related to the probability of progression of early lesions than to the process of early carcinogenesis. Both hypotheses are plausible. They are not mutually exclusive. There is no definitive mechanistic evidence to support either of them.

Why are there inconsistencies among studies relating cancer risk to IGF-I level? One possibility is that the problem is *technical*. The measurement methodology for circulating IGFs is far from perfect, and some null results may simply be accounted for by inaccurate measurements. In other cases, null conclusions may be the result of *misinterpretation of data*. Some non-prospective studies are in fact not asking whether IGF-I levels are a risk factor, but rather whether these levels can be used as a tumour marker. There is good evidence that this is not the case. For example, if one examines the serum IGF-I level obtained on the day of a prostate biopsy and asks whether it predicts the probability of a positive biopsy (or adds to the predictive value of the PSA level), most studies demonstrate that it does not.[53] This is evidence against serum levels of IGF-I as a tumour marker, but is not evidence against its possible role as a risk factor. This point may be understood by pointing out analogies in cardiology. High serum cholesterol represents a risk factor for myocardial infarction,

but it is not of use in clarifying the cause of chest pain. High cholesterol indicates an environment where cardiac disease is more likely to develop, but does not represent direct evidence of the disease. Similarly, IGF-I levels that are in the high end of the normal range do not represent evidence of the presence of cancer, but rather may reflect a host characteristic that may indicate a relatively favourable environment for carcinogenesis and/or neoplastic progression.

However, it is also plausible that some of the inconsistencies in the literature result from biological factors. Perhaps IGF-I levels are only related to risk in specific subsets of patients, and variation in *modifying factors* that influence the IGF-I risk relation among different populations account for the varying results of different studies. For example, perhaps the strength of the IGF-I-to-cancer risk relationship is diminished in hyperinsulinaemic subjects. This and other possibilities are under investigation by many groups. Finally, we must acknowledge the possibility that the original observations of relationships between cancer risk and IGF-I were simply spurious chance findings. However, many feel that this is unlikely, given the *P*-values associated with those studies, the plausible biological rationale, and the findings (at least for some cancers) of consistent trends in meta-analyses.[54]

It is worth noting that genetic studies[55,56] provide evidence methodologically unrelated to serum assays that implicate IGF-I physiology in cancer risk. There is also an interesting report[57] suggesting that in some individuals high IGF-I levels are in fact associated with reduced IGF-I receptor activation due to subtle IGF-I receptor variants that are deficient in signalling. In this situation, homeostatic control mechanisms raise the serum levels in an attempt to compensate. In such cases, the assumption that higher ligand levels in the serum can be used as a surrogate for higher levels of signalling may be false, and this would obviously serve to attenuate any association between IGF-I serum levels and cancer risk. More work needs to be done to clarify how common in various populations these receptors variants are.

IGF-II also deserves mention. As IGF-II is a ligand for the IGF-I receptor, and is present in serum at concentrations that are generally higher than IGF-I, why have no studies identified IGF-II as a risk factor? The most plausible explanation is that serum IGF-II concentration is not a surrogate for IGF-II bioactivity in the cellular microenvironment, because of the important influence of the IGF-II receptor, which is widely expressed and serves as a 'sink' for IGF-II that does not transduce a biological signal and in fact has the characteristics of a tumour suppressor.[5]

POPULATION STUDIES: CANCER PROGNOSIS

A topic of increasing interest concerns the influence of IGF-I and insulin on cancer *prognosis*, as distinct from risk. Available evidence[58–62] suggests that measures of hyperinsulinaemia (c-peptide, fasting insulin levels, postprandial insulin levels) are associated with worse outcome, while IGF-I levels are less important as prognostic factors.

The hypothesis is that for insulin-resistant, hyperinsulinaemic cancer patients, the cancer remains insulin-sensitive, and in fact is stimulated by the abnormally high insulin levels present. This is consistent with reports of insulin receptors on neoplastic tissue.[63] In fact, however, carcinoma cells usually express insulin receptors, IGF-I receptors, and hybrid receptors, so this remains an area of active research.

There is potential clinical relevance because of the possibility that correction of hyperinsulinaemia in the substantial proportion of cancer patients with this metabolic abnormality – either by lifestyle modification or by the use of drugs such

as metformin[64–67] – might be beneficial. This area is under intense investigation by many groups. Obesity is associated with excess cancer mortality[68], and this may be mediated at least in part by obesity-associated hyperinsulinism, so this topic has potential public health relevance.

CLINICAL IMPLICATIONS

Cancer risk

The detection of a relationship between circulating IGF-I levels and risk of a subsequent diagnosis of certain common cancers is intriguing, but does not have major clinical relevance at present. The increased risk associated with high-normal as compared to low-normal IGF-I levels is very much less than the risks associated with smoking or with inherited cancer predisposition syndromes. Furthermore, there is no obvious specific prevention strategy to offer to those with IGF-I levels in the high-normal range. It is occasionally stated that reduction of caloric intake and/or increased exercise might be particularly beneficial for those with high IGF-I levels, but this is speculation rather than evidence-based advice. There is a possibility that future research will show that attempts to devise global cancer risk assessment tools will include IGF-I levels as one of the predictive variables, and there is also considerable interest in the possibility that IGF-I serum levels may interact with or modify the impact of genetic risk, such as *BRCA1* mutation. However, these topics remain in the research domain at present.

Does the accumulated evidence have implications for growth hormone or IGF-I replacement therapy? This is an area of controversy[69], but it is rational to speculate that achieving levels of IGF-I in excess of age-specific norms, particularly if maintained indefinitely, might stimulate growth of any existing cancers. This can lead to a clinical recommendation to avoid GH therapy in the setting of a diagnosed cancer. However, as most cancers are believed to have a long latency period before becoming clinically detectable, and since careful autopsy studies of subjects who have died of non-cancer causes have demonstrated a high incidence of undiagnosed malignancies[52], caution should be exercised even when treating GH or IGF-I deficiency in a patient without cancer. We would advise striving to achieve no higher than mean age-specific norms. The paediatric and adolescent settings are areas where gaps in knowledge are particularly challenging. Short- and medium-term follow-up studies have not documented increased cancer risk among patients treated with growth hormone; this is reassuring but not definitive. An impressive study linked patterns of peri-pubertal growth (likely IGF-I-mediated) to subsequent cancer risk decades later.[40] Thus, complete reassurance of safety of GH replacement will require long-term follow-up. It is known that for breast cancer, adolescence is a key period that influences life-long cancer risk.[70] There is no evidence whatsoever that GH or IGF-I are carcinogens, but the possibility of interactions between IGF-I-stimulated survival and proliferation signalling in the developing breast and sensitivity to environmental carcinogens has not been examined.

On the basis of current knowledge, it is plausible that GH deficiency states might actually be associated with cancer risk lower than that of the general population, and that physiological (as distinct from supra-physiological) replacement would increase risk, but only to 'normal' levels. In many clinical circumstances, the morbidity of GH deficiency would justify appropriate replacement therapy. On the other hand, long-term therapy that achieves supraphysiological IGF-I levels would be harder to justify.

Cancer treatment

As a result of the evolving consensus for a role of IGF signalling in neoplasia[1], the pharmaceutical industry has undertaken many drug development projects to develop agents that target this pathway. These include anti-ligand and anti- receptor approaches.

Anti-ligand approaches

The earliest anti-ligand approach involved efforts to reduce IGF-I levels by the use of somatostatin analogues.[71] This approach has now been shown to be flawed. Despite evidence for preclinical activity[72], it was shown in a long-term clinical trial that in non-acromegalic subjects, tolerance develops to the GH- and IGF-I-suppressing properties of the somatostatin analogue octreotide, so the lack of an important influence on cancer endpoints[71] should not come as a surprise. More recently, anti-ligand antibodies that cross-react with IGF-I and IGF-II have been developed, and these show impressive activity in preclinical cancer models[25], but these have not been evaluated in the clinic.

Anti-receptor antibodies

There is major interest in targeting IGF-I receptors with anti-receptor antibodies, and many of these drug candidates have been found to be active in model systems, subsequently found to have acceptable safety in phase-I trials, and now are undergoing evaluation for efficacy. The Merck anti-IGF-I receptor antibody[73] has been shown to down-regulate the signalling pathway downstream of the IGF-I receptor in sequential tumour biopsy specimens. The Pfizer antibody has been evaluated more extensively than the others at this time.[74–80] There is early evidence that it improves the response to chemotherapy for lung cancer[76], and large phase-III studies are under way to examine this agent in more detail. Remarkably, several different IGF-I-receptor-targeting agents have been found in phase-I studies to have significant single-agent activity against Ewing's sarcoma, even in patients who were refractory to conventional chemotherapy. The mechanism underlying the sensitivity of certain (but not all) cases of Ewing's sarcoma is under intense study.

It is interesting that IGF-I targeting appears to result in compensatory increases in GH secretion, with substantial increases in circulating IGF-I (in the setting of blocked or down-regulated IGF-I receptors). This is reminiscent of the increase in oestrogen levels observed many years ago in pre-menopausal breast cancer patients treated with tamoxifen. The high levels of growth hormone may lead to peripheral insulin resistance which in turn may lead to hyperglycaemia and hyperinsulinaemia noted in some treated patients.[78] While there is no evidence that these effects represent major short-term toxicities that require cessation of treatment, there is a question whether any hyperinsulinaemia might attenuate efficacy.

Small molecule receptor inhibitors

Tyrosine kinase inhibitors against the insulin and IGF-I receptor represent an alternate approach. While some effort was made to create small-molecule receptor inhibitors that were IGF-I-receptor-specific[81], it is now questionable whether this is achievable given the high degree of homology between insulin and IGF-I receptor. At least two small-molecule receptor inhibitors are under clinical investigation, from OSI[27] and

BMS.[26] Clinical trials of these agents are at an earlier stage than those of the IGF-I receptor antibodies. There is an obvious concern that these agents may be associated with more serious metabolic adverse effects than the antibodies, as they may reduce insulin receptor as well as IGF receptor signalling. On the other hand, given (a) the emerging evidence that insulin receptors are commonly expressed on cancers[8,63]; (b) the evidence that hyperinsulinism is associated with aggressive cancer behaviour[30,59,62]; and (c) a recent report of insulin-receptor-mediated resistance to IGF-I-receptor-targeting therapy[82], it is possible that the broader spectrum of inhibition of the kinase inhibitors will result in anti-neoplastic activity superior to that of the antibodies. The results of clinical trials are therefore of considerable interest.

There are more than 20 ongoing clinical trials of various IGF-I-receptor-targeting agents, making this area one of the more active areas in cancer drug development today. It is premature to reach any conclusions regarding efficacy, but it is clear that unlike many oncology drug development projects, interest has steadily increased as data from laboratory models and early clinical trials have accumulated. However, there are challenges associated with drug development in this area. As the molecular target is widely expressed by many different cancers, and as there are many rational drug combinations (with EGF-receptor-targeting agents[14,83,84] or mTOR inhibitors[85,86], for example), prioritizing among different worthwhile clinical trials is difficult. In addition, no clear molecular markers for response or resistance to therapy have so far emerged, and this area of investigation is proceeding in parallel with studies of efficacy.

Metformin

This agent has two properties of interest with respect to cancer treatment. First, it is now established that it acts directly on cancer cells not as an insulin sensitizer, but rather as an AMP-kinase-dependent growth inhibitor.[67] This has been shown to actually attenuate insulin- and IGF-I-stimulated proliferation and protein synthesis.[66,67] Second, by lowering hepatic glucose output (also by its action on the LKB1-AMP kinase pathway)[87], metformin reduces circulating insulin levels of hyperinsulinaemic subjects, which might be of therapeutic value given recent results suggesting that hyperinsulinaemia is associated with aggressive cancer behaviour and poor prognosis.[30,58,59,62] Metformin has been used successfully to control the hyperglycaemia associated with anti-IGF-I receptor antibody therapy, but the possibility that it might also enhance efficacy has not yet been examined. There are early hypothesis-generating reports of reduced cancer risk or improved outcome among subjects on metformin[88–90], but substantial additional research is required in this area, especially as AMPK activation has in certain contexts increased VEGF production.[91]

Pegvisamant

The use of this growth hormone receptor antagonist as a way of reducing IGF-I levels, thereby reducing proliferation of IGF-I-dependent cancers, has received attention.[92] However, single-agent therapy with this class of agent would not be expected to block IGF-I or IGF-II produced in an autocrine or paracrine fashion; therefore, an important topic concerning GH antagonists in cancer therapy is their potential value in combination with IGF-I-receptor-targeting agents, where they might attenuate the GH-induced insulin resistance, hyperinsulinaemia, and hyperglycaemia described above.

Practice points

- growth hormone and IGF-I are not carcinogens; nevertheless, in situations where there is a clinical indication for their use in the treatment of deficiency states, the goal should be to achieve replacement levels no higher than physiological
- the use of growth hormone or IGF-I is not recommended for patients with cancer
- although there is evidence for a modest increase in cancer risk among subjects with higher circulating IGF-I levels, pharmacological reduction of GH or IGF-I levels for the purpose of cancer risk reduction has not been the subject of clinical trials and is not currently recommended.

Research agenda

- more than a dozen new drugs designed to reduce signal transduction through the IGF-I receptor (and/or the insulin receptor) are now being evaluated to determine whether they have significant anti-neoplastic activity for various different cancers, either alone or in combination with other drugs; this area of research has become one of the most active research areas at the interface between oncology and endocrinology
- although there is considerable circumstantial evidence that implicates hyperinsulinaemia as a mediator of the adverse effect of obesity on cancer prognosis, this remains to be formally demonstrated; more studies on the relationship of the influence of the 'metabolic syndrome' on cancer risk and prognosis are needed
- one specific area of interest concerns prostate cancer, where androgen-deprivation therapies result in hyperinsulinaemia: does this secondary endocrine effect contribute to the subsequent development of androgen-independent behaviour?

SUMMARY

Taken together, laboratory and epidemiological findings provide convincing evidence that insulin and IGF-I physiology are relevant to neoplasia. Higher IGF-I levels in the circulation have been associated with moderately increased risk of a subsequent diagnosis of several common cancers, but there is limited clinical application of this information at present. In contrast, the potential clinical relevance of evidence that IGF-I signalling in cancer cells contributes to neoplastic behaviour is now being evaluated by over 20 clinical trials involving several drug candidates. Furthermore, there is increasing interest in the evidence that hyperinsulinism leads to adverse prognosis

among cancer patients; this has led to ongoing investigations of the concept that drugs such as metformin may be of value as adjunctive treatment in the substantial subpopulation of cancer patients who are hyperinsulinaemic.

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