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Impact of targeting insulin-like growth factor signaling in head and neck cancers

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Abstract

The IGF system has been shown to have either negative or negligible impact on clinical outcomes of tumor development depending on specific tumor sites or stages. This review focuses on the clinical impact of IGF signaling in head and neck cancer, effects of IGF targeted therapies, and the multi-dimensional role of IRS 1/2 signaling as a potential mechanism in resistance to targeted therapies. Similar to other tumor sites, both negative and positive correlations between levels of IGF-1/IGF-1-R and clinical outcomes in head and neck cancer have been reported. In addition, utilization of IGF targeted therapies has not demonstrated significant clinical benefit; therefore the prognostic impact of the IGF system on head and neck cancer remains uncertain.

Insulin-like growth factor signaling

The insulin-like growth factor (IGF) pathway is involved in growth and development of normal tissue at both cellular and organism levels (1). IGF ligands and its superfamily of receptors are ubiquitously expressed in higher eukaryotes and are among the first signaling factors secreted by the liver in the developing embryo (1). Insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-1 receptor (IGF-1-R) are required for cell cycle progression and have been shown to play a role in proliferation, differentiation, cell survival, transformation, tumor invasion, metastasis and inhibition of apoptosis (2–9). IGF-1-R has tyrosine kinase activity and upon activation by ligand (IGF-1 or IGF-2) it leads to the phosphorylation of insulin receptor substrate (IRS) proteins and the activation of numerous signaling cascades including MAPK, AKT, and mTOR (1). There are six distinct IRS proteins (IRS-1-6) with IRS-1 and IRS-2 having the broadest tissue distribution and mediating most of the signaling downstream of IGF-1 (10;11). Following ligand stimulation, IRS-1 levels have been shown to gradually decline beginning around 4 hours and this process is dependent on phosphatidylinositol 3-kinase (PI3K) and proteasome activities (12). Interestingly, addition of epidermal growth factor (EGF) to IGF-1 stimulation has been demonstrated to prevent IRS-1 degradation (12). Regulation of IRS-1 is also accomplished through phosphorylation, with tyrosine sites promoting downstream signaling and serine sites facilitating negative feedback loops to terminate signaling (figure 1). Receptor activation leading to phosphorylation of tyrosine⁸⁹⁶ on IRS-1 promotes binding of Grb2 and

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subsequent activation of MAPK signaling. Phosphorylation of tyrosine⁶¹² on IRS-1 promotes binding of PI3K and subsequent activation of PKB/Akt signaling. mTor signaling leads to phosphorylation of IRS-1 on serine⁶³⁶, which serves as a negative feedback loop to decrease activation of the PI3K/Akt pathway (13;14). Treatment with rapamycin reduces mTor/S6K signaling and alleviates this inhibitory phosphorylation leading to increased Akt phosphorylation (13;15). Metformin treatment activates AMPK which phosphorylates IRS-1 on serine⁷⁸⁹. While both rapamycin and metformin are able to reduce mTor activation, metformin treatment does not lead to Akt activation since the inhibitory phosphorylation of IRS-1 remains (16). Activation of IGF signaling is primarily regulated by at least six insulin-like growth factor binding proteins (IGFBPs), among which IGFBP-3 binds to >95% of IGF in circulation thus reducing its bioavailability (3;5;17;18). IGFBP-3 has also been shown to inhibit cell growth independent of IGF-1 (5;18).

Negative impact of IGF signaling on clinical outcomes of tumor development

Over the last few decades, several studies have searched for a connection between the IGF system and its potential role in cancer development. In general, it has been hypothesized that high levels of IGF-1 correlate with elevated risk of developing cancer due to its mitogenic function (2;9;19;20). A study on colorectal cancer (21) reported that the role of IGF-1 was to contribute to a more aggressive malignant phenotype in a subset of colorectal cancers. This study also found a correlation between elevated expression of IGF-1-R and more advanced stages of cancer. Interestingly, a different study (22) found high levels of IGF-1 to be positively correlated with the presence of colorectal adenomas; however, after the adenomas were removed, serum levels of IGF-1 were inversely correlated with adenoma recurrence. In a small case-control study on ovarian cancer (6), serum IGF-1 levels in cancer patients did not correlate in a significant manner compared to controls; however, after normalizing to age, IGF-1 levels strongly correlated with higher risk of ovarian cancer in patients younger than 55 years. In this study IGFBP-3 showed no relation to risk of developing ovarian cancer. Collectively, these studies suggest IGFs promote an environment where tumor development at specific sites is successful.

Negligible impact of IGF signaling on clinical outcomes of tumor development

Conversely to the previously mentioned studies, a case-control study conducted within a cancer prevention study (23), it was reported that low concentrations of IGF-1 were correlated with higher risk of developing glioma, while IGFBP-3 showed no correlation. Another case control study within the European Prospective Investigation into Cancer and Nutrition cohort reported no association between circulating IGF-1 or IGFBP3 levels and pancreatic cancer risk (24). Similarly, several reports have shown conflicting results when trying to establish a relationship between IGF-1, IGF-1-R and IGFBP-3 with risk of developing prostate cancer. While some of these studies found no association between IGF-1 or IGFBP-3 and risk of prostate cancer (25;26), another study reported that elevated levels of IGFBP-3 were related to a modest decrease in risk (27), although no statistical significance was shown. In an effort to clarify this uncertainty, a prospective case control analysis within the ProtecT trial was initiated to determine the association between IGFs and prostate cancer in 6,000 men (3,000 cases and 3,000 controls) who underwent PSA screening (28). This report did not find a positive association between IGF-1 and PSA detected prostate cancer. Furthermore, the authors conducted a meta analysis of all citations evaluating IGF-1 and PSA detected prostate cancer which also did not find any positive associations. The authors concluded that IGF-1 may not have a key role in the development

of early stage cancers; however it cannot be concluded to have no role in progression of clinically identified prostate cancers (28). Collectively, these studies suggest IGF levels have little impact on the development of certain tumor types.

Impact of IGF signaling on head and neck cancers

Head and neck cancer encompasses tumors that develop in the pharynx, oral cavity, tongue, larynx, nasal cavity and salivary gland with a majority of cases in the first three listed sites (29). Typical genetic alterations observed in these cancers include mutations of p53 and overexpression of epidermal growth factor receptor (EGFR) (30); however more recent work has investigated the impact of the IGF system. Intratumoral expression of IGF-1-R does not affect time to progression in head and neck squamous cell carcinoma (HNSCC); however elevated expression of IGFBP-3 was associated with a shorter time to progression (31). In addition, the combination of elevated IGF-1-R and IGFBP-3 had the shortest time to progression in these patients (31). Elevated levels of IGFBP-3 have also been reported in metastatic melanoma and targeted silencing of IGFBP-3 expression through siRNA lead to a reduction in proliferation (32). This suggests that elevated IGFBP-3 contributes to increased proliferation in late stages of cancer development. Serum levels of IGFBP-2 have also been reported to be elevated in head and neck cancer patients when compared to healthy controls (33). Conversely, this same study reported no differences in serum levels of IGF-1, IGF-2 and IGFBP-3 between these two populations (33). In oral cavity squamous cell carcinoma (OCSCC), elevated intratumoral expression of IGF-1-R was significantly higher in patients with grade II-III when compared to patients with grade I disease (34). However, IGF-1-R expression was not predictive of tumor treatment response across all grades of OCSCC disease (34). In patients with advanced OCSCC (grade III-IV), elevated IGF-1-R expression was correlated with poorer survival (34). Evaluation of patients with head and neck cancer that developed second primary tumors has demonstrated that high levels of IGF-1 and extremely low or high levels of IGFBP-3 represent positive risk factors (35). This suggests IGFBP-3 levels have a U shaped curve effect, with the greatest inhibition of IGF signaling at intermediate concentrations. Salivary gland tumors are a small subset of the total head and neck cancer cases and contain a heterogeneous histopathology and considerable variability in the molecular signatures of these cancers (36). Due to the rarity of these tumors clinically, the importance of IGF signaling in salivary tumor biology has largely been studied in model systems. Transgenic mice bearing an inducible IGF-1-R fusion receptor spontaneously developed invasive parotid adenocarcinomas with sporadic squamous differentiation. The xenografts and cell lines derived from these tumors were sensitive to BMS-536924, a small molecule inhibitor (ATP competitor) of IGF-1-R kinase activity (37). Importantly, the converse also appears to be true in that the deletion of either IGF-1 or IGF-1-R dramatically reduced the rates of several cancers in murine models (38;39). Currently, the prognostic impact of the IGF system on head and neck cancer is uncertain probably due to the diversity of tumor types within this category.

Contributing aspect of obesity

Clinical studies evaluating the prognostic relevance of components of the IGF signaling pathway need to be cognizant of the contribution of adiposity within overweight and obese populations. These patients are at an elevated risk for a number of cancers including colon cancer, postmenopausal breast cancer, and esophageal adenocarcinoma (40). Both positive and no effect correlations have been reported on the effect of obesity on pancreatic and prostate cancer (40). Based on the growing appreciation that adipose tissue has important endocrine functions, attempts have been made to associate insulin and IGF-1 signaling as part of the mechanism of elevated cancer risk in overweight/obese patients. A recent study evaluating esophageal adenocarcinoma reported elevated serum IGF-1 in patients with high

visceral fat area while IGF-1 had no association with body mass index (BMI) (41). Critics contend that esophageal adenocarcinoma is positively associated with a broad range of BMI values; therefore the impact of IGF-1 is complex and incompletely identifies the population at elevated risk (42). While insulin and IGF action have been included as potential mechanisms of increased cancer risk in obese/overweight populations, the role of hormones (estrogens and androgens) and chronic low levels of inflammation may also play significant potentiating roles (40). With the increasing prevalence of obesity, especially in Western societies, it is clear that more work needs to be done on the interplay or independence between these potential mechanisms.

Targeting the IGF pathway in tumor therapy

Selective targeting of the IGF signaling network has proven to be extremely challenging due, in part, to the fact that insulin, IGF-1 and IGF-2 can all activate IGF-1-R and/or IGF-2R, as well as the insulin receptor (Figure 2). An important distinction is the change in affinity when these different ligands bind to a hybrid receptor. For example, insulin binds to hybrid receptors consisting of insulin and IGF-1 receptor components with a lower affinity compared to binding to insulin receptor homodimers (1). Therefore, elevated levels of ligand can induce signaling from canonical as well as hybrid receptors. Ganitumab is a fully humanized anti-IGF-1-R monoclonal antibody that interferes with the binding of IGF-1 and IGF-2 and does not cross react with the insulin receptor (IR) that has received a great deal of attention recently. The anticancer properties of ganitumab have been studied in a variety of advanced human solid tumors, including HNSCC, alone and in combination with myriad monoclonal antibodies and chemotherapeutics (43–47). Initial *in vitro* data were very promising and demonstrated the necessary specificity for IGF-1/2, and efficacy in tumor cell cultures, to warrant *in vivo* studies and eventually clinical trials. Although the drug has pharmacokinetic and safety profiles within the necessary range for human therapeutics, large clinical trials in prostate, lung, pancreatic, and head and neck cancers have suffered early termination, as patients receiving the drug did not demonstrate improvement at intermediate analysis (48–51). These therapeutic setbacks highlight the need for further understanding of the specific IGF signaling cascade activated in specific cancers and the ability to utilize this information to segregate the population into cohorts that could benefit from the therapy and those that would not.

Multi-dimensional role of IRS 1/2 signaling

The differential outcomes from clinical IGF-1 levels could be related to specific downstream signaling cascades mediated by IRS-1 or IRS-2. In a study of 195 primary breast tumors, elevated total levels of IRS-1 correlated with decreased disease free survival (52). Genetically engineered mouse models with disruptions in IRS-1 or IRS-2 have provided powerful insight into the relative roles of these two proteins. Nagle et al, crossed these mice with the established PyV-MT mammary tumor model and evaluated primary tumor growth and lung metastasis (53). Loss of IRS-2 had no effect on primary tumor initiation or growth; however there were significantly fewer lung metastases when compared to wildtype controls. In contrast, loss of IRS-1 resulted in significantly more invasive cells as determined by Matrigel invasion assay. This suggests that IRS-2 may positively influence metastasis while IRS-1 prevents invasion (figure 2). In support of this conclusion, Byron et al utilized T47D breast cancer cells which lack IRS-1 and IRS-2 expression. Stable transfection of IRS-1 was associated with IGF-1 induced proliferation while stable transfection of IRS-2 was associated with IGF-1 induced cell migration and metastasis (54). It is unclear whether this phenotypic differentiation between IRS-1 and IRS-2 is translatable to humans since one study has reported that high levels of IRS-1 correlate with worse disease free survival in breast cancer (55). The role of IRS signaling has been sparingly

investigated in head and neck cancer. Luo et al (56) reported a negative correlation between IRS-1 expression and tumor metastasis in clinical samples of HNSCC, which corroborates the results reported by Nagle in the PyV-MT mammary tumor model.

Another aspect of IGF/IGF-1-R signaling that is likely driving the limited clinical success of current strategies is that IGF-1-R survival signals can be transduced following heterodimerization with its own family of receptors, or with epidermal growth factor receptor (EGFR). Receptor “crosstalk”, in this fashion, can activate EGFR signaling, IGF-1-R signaling or both (57). While canonical signaling pathways depict IRS 1/2 binding to the insulin or IGF-1 receptors, evidence suggests that IRS-1 can also bind to other growth factor receptors (58). In particular, it has been demonstrated that both the EGF and erbB3 receptors can bind to IRS-1 and promote downstream signaling in a number of cancer cell lines (59;60). Heregulin stimulation of the erbB3 receptor resulted in enhanced binding of IRS-1 to erbB3 and consequently reduced IGF-1-R and IRS-1 interaction (59). The multi-faceted binding capability of IRS-1 is hypothesized to be involved in resistance to EGFR or IGF-1-R targeted therapies. In breast cancer cell lines, the growth inhibitory effects of IGF-1-R inhibitors require elevated IGF-1-R and IRS-1 expression (54;61). However, treatment with IGF-1-R inhibitors can result in enhanced binding of IRS-1 to erbB3 and increased activation of Akt and ERK pathways (59). In addition, the growth inhibitory effects induced by ABDP (IGF-1-R inhibitor) were completely reversed when heregulin was added (59). Targeting the EGFR utilizing Gefitinib altered the phosphorylation profile of IRS-1 and decreased the amount of IGF-2 needed to activate Akt (60). Guix et al determined that the Gefitinib-induced increase in IGF-1/IRS-1/Akt signaling lead to reduced expression of IGF binding proteins (62). These studies suggest that resistance to single receptor inhibitor therapies (EGFR or IGF-1-R) could be mediated by IRS-1; therefore combinatorial strategies may need to be considered.

Effects of combining IGF inhibition with other agents in tumor therapy

Although the exact reasons for the clinical failure of specific IGF signaling inhibitors are not yet clear, it is very likely that an under-appreciation for the complexity and redundancy built into the IGF signaling web in malignancy is key. In particular, alternative pathway activation has created a huge clinical challenge for the use of monoclonal antibodies or small molecule inhibitors when treating salivary or HNSCC patients. Synergistic effects on tumor cell death observed by combining IGF inhibition with other treatments have been demonstrated in a variety of *in vitro* and *in vivo* models. In addition to EGFR inhibitors, improved results have been reported when combining IGF-1-R antibodies or kinase inhibitors with histone deacetylase (HDAC) inhibitors (63), radiation (44;64–66), rapamycin (44), gemcitabine (45). In head and neck squamous cell carcinoma patients, IGF-1-R targeted therapies combined with EGFR antagonists, such as cetuximab or panitumumab resulted in increased sensitivity of HNSCC to chemotherapy (43) and radiation therapy (44). Efforts to address the challenges faced by single target monoclonal antibodies in the clinic, have led to the development of a novel bi-specific antibody, dubbed MM-141, by Merrimack Pharmaceuticals for co-targeting IGF-1-R and ErbB3 (67;68). This compound interferes with IGF-1 and IGF-2 binding to IGF-1-R, and heregulin binding to ErbB3, leading to a marked decrease in receptor expression. Importantly, the insulin receptor is not recognized by the antibody which, to some extent, ameliorates the risk of metabolic side effects. Another important feature is that MM-141 also inhibits IGF-1-R and ErbB3 phosphorylation, which short-circuits the activation of PI3K/Akt survival pathway, which is presumed to be a critical mediator of single-target antibody failure. Xenograft experiments demonstrated potency in the presence of single or multiple ligands, over a broad range of receptor profiles in pancreatic cancer and prostate cancer; and promising preclinical data

indicated that MM-141 had a favorable pharmacokinetic profile supporting its advancement to clinical trials.

Summary

In January 2010 there were approximately 60 ongoing U.S. clinical trials involving the use of IGF1R-modulating compounds (69). As of the submission of this manuscript, www.clinicaltrials.gov registered nearly 100 prospective IGF1R clinical trials, at various stages of completion, for over 30 different cancers. The dramatic increase in the number and diversity of current clinical trials likely reflects a broad initiative to determine which patients, drug combinations and cancers will best respond to IGF targeted therapy. Although a recent immunohistochemical analysis of 113 archived biopsies taken from oral and oropharyngeal cancer patients prior to treatment revealed that nearly all specimens expressed significant levels IGF1R (none were negative) (70) there is only one trial listed on the clinical trials website focused specifically for head and neck cancer patients that is actively recruiting. There are trials for general neoplasms and recurrent or refractory cancers that head and neck patients might be enrolled; however, many head and neck focused trials have been withdrawn or suspended. Although redundant signaling pathways and the activation of multiple transcription factors have made the interpretation of clinical trials involving IGF-1 targeted agents difficult, it is clear that certain patient sub-populations and cancers respond better than others. There is general agreement amongst researchers and clinicians that a paucity of validated biomarkers is driving the spotty clinical success (71;72). It is clear, as in the case of oral cancers, highly expressed IGF1R alone is insufficient, and as mentioned previously, depending on tumor site and stage, serum levels of IGF1 and other IGF1R ligands has also not been consistently correlated with positive patient outcomes. On the heels of clinical failures such as Amgen's IGF1 monoclonal antibody ganitumab (73) and Bristol-Myers Squibb's abandonment of the IGF1R tyrosine kinase inhibitor (TKI) BMS-754807 it is critical now more than ever to identify predictive biomarkers and how other pathway inhibitors (e.g. mTOR) might improve the utility of the IGF1R class of compounds. Ultimately, with currently very limited clinical studies with IGF1R inhibitors for oral cancer patients, the burden of proof for these compounds as a productive therapy will be borne (or not) from the myriad clinical trials underway for other cancers. Hopefully these studies that target multiple receptors, use IGF1R class compounds in combination with other therapies, and those that successfully identify *bona fide* biomarkers predictive of success will translate into more personalized therapies for those who suffer from oral cancer.

Abbreviations

IGF	Insulin-like growth factor
IGF-1-R	insulin-like growth factor-1 receptor
IRS	insulin receptor substrate
IGFBP	insulin-like growth factor binding protein
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
MAPK	Mitogen-activated protein kinases
AMPK	AMP-activated protein kinase
mTOR	mammalian target of rapamycin

HNSCC	head and neck squamous cell carcinoma
PKB	Akt (protein kinase B)
OCSCC	oral cavity squamous cell carcinoma
PI3K	phosphatidylinositol 3-kinase

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Figure 1.
Select IRS-1 phosphorylation sites and activation or inhibition of downstream pathways

Figure 2. Binding capability of IGF-1, IGF-2 and insulin to IGF-1-R, IGF-2R, insulin receptor (IR) or hybrid receptors. Dotted lines downstream of IRS-1/IRS-2 represent proposed mechanisms from studies on breast cancer.