Mini-Review

Insulin-Like Growth Factor System and Sporadic Malignant Melanoma

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Insulin and insulin-like growth factors (IGFs) are important regulators of energy metabolism and growth. Several findings have outlined an important role played by this family of molecules in both tumor maintenance and development. Despite the established contribution of the IGF system in carcinogenesis, little and contrasting data have been reported concerning the intertwined relationships between melanoma and this family of molecules. The present minireview aims to summarize the main topics and evidence concerning this malignant skin cancer, with a focus on the following: i) melanoma and cell proliferation effects induced by the IGF system, ii) in vitro and in vivo experimental data, and iii) targeting studies. Because of consistent findings regarding the role of the IGF-1 receptor in the modulation of IGF-1 activity, possible therapeutic strategies combining the use of antisense oligonucleotides against IGF-1 receptor mRNA could be applied in the future. (Am J Pathol 2011, 178:26–31; DOI: 10.1016/j.ajpath.2010.11.004)

Melanoma is a malignant tumor originating from melanocytes, the melanin-producing cells.1,2 During fetal development, melanocytes migrate to different body areas, such as the skin, uvea, leptomeninges, and mucous membranes (eg, upper esophagus, vulva, and anus).2 Although melanoma may arise in all these sites, cutaneous melanoma is by far the most frequent type of melanoma, representing approximately 5% to 7% of all skin malignancies and the most lethal skin cancer (approximately 75% of all deaths from skin tumors).2,3 The following melanoma risk factors have been extensively described: i) sun exposure, ii) presence of dysplastic nevi, iii) age, iv) ethnicity, v) personal or family history of melanoma, vi) phototype, vii) presence of xeroderma pigmentosum, viii) Li-Fraumeni syndrome, ix) hereditary retinoblastoma history, and x) immunosuppressive conditions.4 In addition to environmental and individual risk factors, the genetic pedigree may also represent a further risk factor for melanoma development; indeed, multiple differences between melanoma cell genomes and those of normal melanocytes were identified in genomewide analysis studies. In particular, point mutations, deletions, gene amplifications and translocations, and/or epigenetic modifications (eg, promoter hypermethylation) appear to be associated with a significant growth advantage, as opposed to normal skin cells.5 Furthermore, many molecular changes have been reported in advanced stages of melanoma, as opposed to normal melanocytes. The most common mutations are as follows: i) protocogene serine/threonine kinase B-Raf-activating mutations,6 ii) E-cadherin expression silencing,7 and iii) telomerase activity acquisition,8 followed by hundreds more, identified by comparative gene expression profiling of melanomas with various American Joint Committee on Cancer stages.1,9,10

A recent article by Bennet1 provided a detailed list of possible genes involved in melanoma spreading and development.

Along with all pathways and molecules studied in different cancers, including melanoma itself, the study of the insulin-like growth factor (IGF) network of ligands, cell surface receptors, and IGF–binding proteins (IGFBPs; eg, the IGF-1 system) have attracted considerable interest because these pathways play important roles at multiple levels, from cellular and organ to organism.11–13 The IGF system mediates growth, differentiation, and developmental processes; it is involved in a variety of metabolic activities.13 Deregulation of IGF system expression and action is linked to several pathological features, ranging from growth deficits to cancer development. Tar-
Table 1. The IGF System Complex*

<table>
<thead>
<tr>
<th>IGF ligands</th>
<th>IGF receptors</th>
<th>IGF-binding proteins</th>
<th>Main effectors</th>
</tr>
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<tbody>
<tr>
<td>Insulin</td>
<td>Insulin receptor</td>
<td>IGFBP1-IGFBP6</td>
<td>IRS-1</td>
</tr>
<tr>
<td>IGF-1</td>
<td>IGF-1 receptors A and B</td>
<td>IGFBP-related proteins</td>
<td>JCV-T</td>
</tr>
<tr>
<td>IGF-2</td>
<td>IGF-2/mannose-6-phosphate receptor</td>
<td>IGFBP proteases</td>
<td>MAPKs</td>
</tr>
<tr>
<td>ALS</td>
<td>Insulin receptor–related receptor</td>
<td>insulin/IGF-1 hybrid receptor</td>
<td>PI3K/Akt</td>
</tr>
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</table>

*IGF, insulin-like growth factor; IGFBP, IGF-binding protein; IRS, insulin receptor substrate; JCV-T, human polyomavirus JCV protein T; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; Akt, antiapoptotic protein family; ALS, acid-labile subunit.

As reported in Table 1, the main IGF ligands are IGF-1, IGF-2, insulin, and several nonclassic ligands with controversial biological roles. Insulin-like growth factor-1 is a cellular progression factor required by the cell to advance through the various phases of the cell cycle. Serum or plasma circulating IGF-1 levels are mainly dependent on production by the liver, which is tightly controlled by growth hormone. At the organ level, IGFs display paracrine and autocrine pathways, often interacting with vitamin D, inflammatory molecules, or locally produced factors (eg, suppressor of cytokine signaling, transforming growth factor-β, and steroid hormones; Figure 1). Insulin-like growth factors act through their receptors, which include the insulin receptor (IR), IGF-1R, IGF-2R, and several atypical receptors (eg, the IR-related receptor and the IGF-1R hybrid receptor), inducing mitogenic, antiapoptotic, and transforming activities. In particular, IGF-1R is a cell surface heterotetrameric tyrosine kinase receptor coupled to numerous intracellular second messenger pathways, including Ral/Ras–mitogen-activated protein kinase and phosphatidylinositol-3 kinase signaling cascades; for these reasons, IGF-1R is crucial for cell survival, whereas IGF-2 appears to have a tumor suppressor role because mutated or deleted forms have been reported in several cancers. In contrast, some reports did not confirm a role for IGF-2 in intracellular signaling, its bioactivity being mediated via a particular isoform of the IGF-1R (ie, IGF-1R-A) derived from an alternative splicing of the IR gene.

IGF System and Cancer

Insulin-like growth factor-1 is a multifunctional peptide that shares high structural homology with proinsulin. Along with the liver, other tissues are capable of producing this mediator on the basis of its local concentration. As opposed to IGF-2, which is considered a more active form during prenatal life, several IGFBPs are involved in the regulation or bioavailability of IGFs, the most abundant of which is IGFBP-3. This molecule regulates IGF-1 signaling by acting as a competitive inhibitor for IGF-1 by protecting it from accelerated degradation in circulation and facilitating its transport to target organs. Insulin-like growth factor–BP-3 is also believed to have an IGF-independent inhibitory effect on cell growth that is mediated through a specific cell membrane receptor. In addition, in specific contexts, overexpression of IGFBPs (ie, IGFBP-2 and IGFBP-5) is associated with increased, rather than decreased, IGF action; adverse effects on cancer prognosis; and loss of the phosphatase and tensin homolog function. The mechanisms regarding the involvement of IGFBPs and IGF axis remain uncovered, although some hypotheses were reported by Pollak in his recently published review. On the other hand, some early (although not confirmed) reports described that higher circulating levels of IGF-1 were associated with increased risk of prostate cancer, whereas higher IGFBP-3 serum levels were related to reduced cancer risk, suggesting a possible influence of IGFBP-3 in reducing IGF-1 bioactivity, in keeping with laboratory studies. Furthermore, growing evidence suggests that IGF-1 is involved in the pathogenesis of various types of human neoplasias, including breast, prostate, colon, and skin carcinogenesis by modulating IGF-1 levels or its bioavailability. In addition, high circulating ALS levels reduce the concentration of free IGF-1 in circulation.
and lung cancers. The most appealing among these findings is that both IGF-1 and IGF-2 peptides are frequently expressed within neoplastic tissues, possibly influencing the previously mentioned cancers through autocrine, paracrine, or endocrine mechanisms. In addition, more recent large studies have suggested that particularly high circulating IGF-1 levels and/or low IGFBP-3 levels are associated with increased risk of several cancers, supporting a possible paracrine role of the IGF system in carcinogenesis. Furthermore, the negative correlation between IGFBP-3 levels and cancer risk is consistent with a protective role of IGFBP-3 (eg, high IGFBP-3 concentrations may lead to reduced free IGF-1 values). Insulin-like growth factor-1 circulating levels could also influence the cancer progression as follows: i) enhancing the proliferation of cell clones accumulating mutations, ii) modulating IGFBP serum or local concentrations, and iii) modulating IGF-1R activity or cell membrane density.

Recently published data showed a strong influence of acid-labile subunit of the IGF-1 system (Figure 1), which is crucial in preventing the degradation of circulating IGF-1 and IGFBP-3 and in determining the IGF-1 availability to tissues. Data from a twin study indicated that 38% of the differences in IGF-1 and 60% of the differences in IGFBP-3 levels originate from genetic variations. Polymorphisms that are located in important regulatory regions, such as the promoter region or functionally active domains, may affect the function of the IGF-1 protein. The measure of IGF serum levels is critical because it depends on the type of kit used and reflects a single or random point before or at diagnosis, when the interaction between IGFs and other cancer pathways has already occurred. The assay result in this particular patient could be considered an epiphenomenon.

Melanoma and Cell Proliferation Effects by IGF-1

Clear effects exerted by IGF-1 on melanoma cells have already been reported. Proliferative effects on melanoma cells have been observed in vitro; in particular, cell migration (possibly mediated by interleukin-8 induction) was observed. These effects may be further increased by synergism with basic fibroblast growth factor and transforming growth factor-β1, two mediators known to be induced by IGF-1. In addition, although melanoma cells were capable of expressing IGF-1 molecules in some studies, other reports did not confirm these findings. Further genetic factors, such as IGF-1 microsatellites, may account for possible effects on melanoma risk or its aggressiveness. A strong association between melanoma severity (expressed as Breslow index) and the IGF-1/IGFBP-3 repeat frequency was previously found. Concomitantly, serum IGF-1 concentrations were mainly reduced in indi-
workers showed that IGF-1R expression is correlated with melanoma cells, Kanter-Lewensohn and co-workers’ research indicates that silenced expression both locally and systemically. Dar et al concluded that silencing of IGF-1 expression in human melanoma samples compared with non-malignant nevi. Indeed, Dar et al indicated that silencing of IGF-1 expression in human melanoma samples compared with non-malignant nevi. Dar et al’s findings support the idea that IGF-1R plays a crucial role in normal development and in establishing and maintaining the malignant phenotype; its function has been critical for the proliferation and survival of cancer cells in several experimental settings. The effect on survival is mediated by multiple signaling intermediates, especially the phosphatidylinositol-3 kinase–antiapoptotic protein family pathway and the Ras/Raf–mitogen-activated protein kinase pathway.

Moreover, because IGF-1R is overexpressed in malignant cutaneous melanoma but not in benign nevi, it is an attractive target for melanoma treatment. Inhibitors of IGF-1R have been widely studied for their ability to enhance the killing of some types of malignant cells and metastatic melanoma cells; however, whether IGF-1R signaling differentially protects the host and cancer cells against chemotherapy has yet to be established. In fact, a recently published article showed that healthy cells and mice could be protected against chemotherapy-dependent damage by reducing circulating IGF-1 levels via a mechanism that involves down-regulation of proto-oncogene signals. Moreover, antisense oligonucleotides against IGF-1R mRNA were associated with reduced IGF-1R levels along with inhibition of IGF signaling pathways in several cancer types, including breast, prostate, lung, central nervous system, and bladder neoplasias.

However, the identification of activating Ras/Raf mutations raises doubts about the efficacy of targeting IGF-1R because of its upstream location in the signaling cascade in human melanoma. Nevertheless, recent studies showed that this effect is independent of B-Raf mutation status, suggesting that IGF-1R targeting may be an effective therapeutic approach for the treatment of melanoma and other tumors carrying activating mutations in the Ras/Raf–mitogen-activated protein kinase signaling pathway. The same was true when antiapoptotic protein family and extracellular signal–regulated kinase signaling was analyzed. Last, in the human melanoma cell line SK-MEL-2, IGF-1R expression was regulated by cyclooxygenase-2 expression, vitamin C, and IGF-2 via a feedback loop for amplification of IGF-1R expression by IGF-2. In particular, vitamin C down-regulation of IGF-1R in the human melanoma cell line SK-MEL-2 determines the inhibition of cell proliferation. Furthermore, vitamin C suppresses SK-MEL-2 proliferation through the inhibition of cyclooxygenase-2 expression and the modulation of IGF-2 production.

IGF-1 System and Uveal Melanoma
An increasing amount of experimental data has been published concerning the strong correlation between the IGF system and uveal melanoma. In particular, the following interesting results were published: i) along with EGF, IGF-1 was involved in the development and progression of uveal melanoma; ii) IGF-1R appears to play a central role in that it is crucial for growth and survival and for invasion and metastasis of uveal melanoma cells; iii) by multivariate statistical analysis, Economou et al. found that 34% of patients with low tumor IGF-1R expression died of metastatic disease, as opposed to 57% in the group with high IGF-1R expression; and iv) IGF-1R expression in uveal melanoma lesions may represent an important prognostic factor. Based on these findings, these researchers also suggested that target-
ing this receptor may represent a strategy to treat this incurable disease and a strategy to prevent development of metastases in patients with primary disease.

**Conclusions and Perspectives**

Insulin-like growth factor and its family members have been extensively studied in some cancer types. The main results obtained by different groups on different types of neoplasias (eg, cutaneous, metastatic, and uveal melanomas) confirmed IGF's potential and often crucial role in tumor transformation, maintenance of malignant phenotype, promotion of cell growth, and prevention of apoptosis. In view of this, IGF-1R represents an attractive therapeutic target because its negative regulation would determine selective apoptosis and growth inhibition of tumor cells.

Although the previously mentioned mechanisms have been deeply analyzed by some researchers, the following questions remain unanswered: i) Are the molecular mechanisms played by IGF-1 on the induction of some molecules (eg, Bcl-2 and survivin) directly or indirectly exerted? ii) Are other pivotal molecules involved in the availability of IGFs at the tumor level? iii) Do the measurements of the IGF system molecules depict a peculiar pathophysiological mechanism of tumor progression? iv) Does the inhibition of IGF-1R synergize with traditional chemotherapeutic agents toward disease regression? and v) Are the in vitro conditions always superimposable to the in vivo conditions? These questions are complicated by the complexity of the IGF pathway. In fact, genetic and biochemical data regarding IGFs are often limited by heterogeneity among studied patients and experimental methods used.

Nevertheless, the central issue in this field is unraveling the mechanisms involved in the modulation of IGF-1R by its soluble IGF molecules in the context of an even more complex scheme, both at the tumor level and at systemic levels. A critical topic is represented by IGF-1R target therapy, as demonstrated by the increased articles published in the past 3 years. Although targeting therapy proved efficient in vitro, some generalized toxic adverse effects were reported despite the high targeting/receptor specificity of anti–IGF-1R targeting agents. This explains the broad range of IGF system targeting molecules and strategies still being investigated for the development of more efficient therapies, especially for melanoma.62 I believe that a better understanding of the complex machinery involved in the regulation of the IGF system in melanomas is required to provide new effective treatment strategies for those conditions in which the IGF system is directly involved.

**References**

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