GALECTINS AS MODULATORS OF TUMOR PROGRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS

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Abstract: Head and neck squamous cell carcinomas (HNSCCs) remain a significant cause of morbidity worldwide. Biological therapies able to induce and/or upregulate antitumor immune responses could represent a complementary approach to conventional treatments for patients with HNSCC because, despite advances in surgery, radiotherapy, and chemotherapy, the overall survival rates for these patients have not changed over recent decades. Galectins are involved in the control of cell proliferation, cell death, and cell migration and in the modulation of various functions of the immune system. In this context, galectin-1 is known to protect HNSCCs from the immune system. The present review details the involvement of galectins in HNSCC biology and suggests a number of approaches to reduce the levels of expression of galectin-1 in HNSCCs, with the aim of improving the efficiency of HNSCC immunotherapy.

Keywords: galectin; tumor progression; head and neck; cancer

Epidemiology, Treatment, and Prognosis

Head and neck squamous cell carcinomas (HNSCCs) remain a significant cause of morbidity worldwide, with approximately 500,000 new cases diagnosed each year.1,2 HNSCCs constitute a collection of diseases that, although united by location and histology, involve very different types of tumors that differ in pathogenesis, biology, sublocation, treatment, and their impact on the quality of life, including survival.1,2 Patients with HNSCC with low clinical stages (stage I and II) have similar survival rates, with a 5-year period accounting for between 70% and 90% independently of type of the sublocation.3 In contrast, patients with HNSCC with advanced clinical stages (stage III and IV) display totally different survival rates depending on the histological type of tumor and its sublocation.3,4 The treatment of patients with HNSCC with advanced stages of the disease combines surgery, radiation oncology, medical oncology, medical imaging, and clinical pathology.1–4 This type of collaborative medical approach began as early as 1970, when Fletcher and Evers5 reported the first convincing evidence of the benefit of combining radiotherapy with surgery. In this context, cisplatin was investigated in the treatment of HNSCC long ago, as soon as the early 1970s, and from the late 1970s to the early 1990s promising results emerged from the use of...
various combinations of postoperative chemotherapy with radiotherapy in randomized and non-randomized studies. In the early 2000s, the Radiation Therapy Oncology Group and the European Organization for Research and Treatment of Cancer carried out 2 randomized studies to test the relative efficiency of concurrent postoperative cisplatin administration and radiotherapy in the treatment for the disease. These 2 studies demonstrated that the local control of the disease was significantly higher in the combined therapy group than in the group given radiotherapy alone. Unfortunately, these combined treatments were frequently associated with adverse side effects. Although significant progress has been observed after combined treatments, a number of statements currently remain valid concerning HNSCCs. First, almost two thirds of the number of patients have advanced forms (stages III and IV) of the disease at diagnosis. Second, 50% of the patients die of the disease within the 2 years following initial diagnosis. Third, every year 5% of the patients develop additional second primary tumors. Novel approaches are thus required to provide head and neck oncologists with a more effective armamentarium against this challenging disease. The application of modern analytic approaches based on genetics, proteomics, and bioinformatics has facilitated the identification of critical genomic and proteomic changes in HNSCCs, many of which have been linked to clinical outcome. In these conditions, it thus appeared interesting to identify subsets of patients associated with dismal prognoses to apply more selectively some of the combined treatments described earlier.

**GENOMIC ALTERATIONS IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS**

Cancer develops when normal cells undergo specific changes in their genetic information that allow them to overcome normal growth regulating mechanisms, to invade surrounding structures, and to spread to distant anatomical sites. Granis et al recently reviewed the molecular pathway impairments that lead to HNSCC development and progression. Statistical analysis based on the age-specific incidence of head and neck cancer suggests that HNSCCs arise after the accumulation of between 6 and 10 independent genetic events. The model proposed in Figure 1 is the result of the comparison of a variety of preinvasive and invasive lesions by determining the frequency of the genetic alterations at each stage of the neoplastic processes. The most frequent...
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WHAT ARE GALECTINS?

Galectins are members of a phylogenetically conserved family of lectins sharing a consensus sequence of about 130 amino acids and a carbohydrate recognition domain (CRD) responsible for β-galactoside binding. Fifteen mammalian galectins have been identified to date. Some contain one CRD and are biologically active as monomers (galectins -5, -7, -10), as homodimers (galectins-1, -2, -11, -13, -14, -15), or as oligomers that aggregate through their nonlectin domain (galectin-3), whereas others contain 2 CRDs connected by a short linker peptide (galectins-4, -6, -8, -9, -12). Although the CRDs of all the galectins share an affinity for the minimum saccharide ligand N-acetyllactosamine—a common disaccharide found on many cellular glycoproteins—individual galectins can also recognize different modifications to this minimum saccharide ligand, therefore demonstrating the fine specificity of certain galectins for tissue-specific or developmentally specific ligands. It is now clearly established that galectins can segregate into various cellular compartments depending on the cellular status. Although galectins are devoid of a signal sequence, normally required for protein secretion through the usual secretory pathway, some galectins are nevertheless secreted and are found in the extracellular matrix.

GALECTIN-1, -3, AND -7 AND TUMOR REGULATION

The roles played by galectins in cancer biology have been reviewed recently. In the present review, we have focused on galectins-1, -3, and -7 that are clearly involved in HNSCC biology. Galectin-1 is a potent modulator of cancer cell migration. Galectin-3 is involved in various biological phenomena, including cell growth, adhesion, differentiation, angiogenesis, and apoptosis. One of the major biological roles played by galectins relates to their modulatory effects on apoptosis. Many galectins, such as galectin-1, -2, -7, and -9, are characterized by proapoptotic effects whereas galectin-3 seems to act in an antiapoptotic manner in the case of various tumor cell lines. In this respect, it was demonstrated almost a decade ago that galectin-3 inhibits T-cell apoptosis induced by the anti–fatty acid synthase antibody, and other studies have shown that...
Defects in apoptosis constitute one of the major hallmarks of human cancers. Galectin-3 also modifies the levels of migration of cancer cells. The blockage of galectin-3 expression can also lead to a significant decrease in the growth rates of human xenografts in immunocompromized mice.

It seems that the involvement of galectin-3 in tumorigenesis heavily depends on the histological type of tumor. The expression of apoptosis-related genes, such as p53 and bcl-2, is a regulator of differentiation and apoptosis. Galectin-3 expression can be increased in various types of cancers. Galectin-3 also modifies the levels of migration of cancer cells. The blockage of galectin-3 expression can also lead to a significant decrease in the growth rates of human xenografts in immunocompromized mice.

Galectin-7. Galectin-7 contributes to different events associated with the differentiation and development of pluristratified epithelia and is also associated with epithelial cell migration, which plays a crucial role in the re-epithelialization of corneal and epidermal wounds. In addition, recent evidence indicates that galectin-7, which is induced by p53, is a regulator of apoptosis through c-Jun N-terminal kinases activation and mitochondrial cytchrome c release. Defects in apoptosis constitute one of the major hallmarks of human cancers, and galectin-7 can act as either a positive or a negative regulatory factor in tumor development, depending on the histological type of tumor.

The expression of galectin-7 is induced by p53 and functions as a regulator of differentiation and apoptosis, thus helping to control tumor cells. However, galectin-7 can also inhibit tumor growth, without inducing apoptosis. For example, Ueda et al. found that in the absence of apoptosis galectin-7-transfected DLD-1 cells grow significantly more slowly than control transfecants under normal culture conditions, whereas under anchorage-independent cell growth conditions a significantly lower number of colonies are formed by galectin-7-transfected cells as compared with control. Ueda et al. also observed in vivo that galectin-7-transfected DLD-1 cells are associated with lower tumorigenicity than control cells in severe combined immunodeficient mice. The suppressive effect of galectin-7 on in vivo DLD-1 colon cancer growth may relate to the fact that the ectopic expression of galectin-7 suppresses neoangiogenesis in DLD-1 xenografts.

In sharp contrast to the negative roles played by galectin-7 in tumor development, Demers et al. recently reported a study in which they demonstrated the promotion of cancer cell malignancy by galectin-7. They found that the development of experimental thymic lymphomas was accelerated after induction with lymphoma cells overexpressing galectin-7. These authors provide data suggesting that galectin-7 modulates the aggressive behavior of lymphoma cells by controlling the expression of metastatic genes such as matrix metalloproteinases-9.

Galectin-1. In 1996, Gillenwater et al. observed that galectins-1 and -3 were expressed in most of the HNSCC cell lines and in primary tumor specimens that they studied. Since then, galectin-1 has been identified in HNSCCs as a tumor-associated protein. He et al. observed an increase in galectin-1 expression in tongue SCCs as compared with their matched normal mucosa. Galectin-1 is expressed in the invasive compartment of some tumors in relation to their aggressiveness. The level of expression of galectin-1 contributes to the prognosis of recurrence of laryngeal tumors and, to a lesser extent, of pharyngeal tumors after surgery, and to patients’ survival prospects.

We have previously shown that the patterns of galectin expression in laryngeal and hypopharyngeal cancers are quite different. Le et al. recently demonstrated that galectin-1 is a hypoxia-regulated protein in HNSCCs. The microenvironment of solid tumors, including HNSCCs, possesses hypoxic regions that are not found in normal tissues. Hypoxia confers cellular resist-
ance to conventional chemotherapy and acceler-
ates malignant progression.\textsuperscript{32} Thus, the study by
Le et al\textsuperscript{32} presents a new mechanism on how hy-
poxia can affect the malignant progression and therapeu-
tic response of solid tumors, such as
HNSCCs, by regulating the secretion of proteins,
including galectin-1, which modulate immune
 privilege.

\textbf{Galectin-3.} Several studies have demonstrated
the close association of galectin-3 expression with
biological aggressiveness in various types of
HNSCCs.

Honjo et al\textsuperscript{47} analyzed galectin-3 expression in
77 tongue specimens (including 54 squamous cell
carcinomas and 23 specimens of normal mucosa)
and observed that the levels of nuclear expression
of galectin-3 decreased noticeably during the pro-
gression from normal to cancerous states,
whereas cytoplasmic expression increased. The
enhanced expression of galectin-3 in the cyto-
plasm was associated with a reduced level of dis-
ease-free survival in the case of these patients
with tongue cancer.\textsuperscript{47} Multivariate analysis has
identified the enhanced expression of cytoplasmic
galectin-3 as an independent predictor of disease
recurrence for patients with tongue cancer.\textsuperscript{47}

Piantelli et al\textsuperscript{56} evaluated the prognostic value
of galectin-3 expression in 73 node-negative laryn-
geal squamous cell carcinomas and observed that
42 (58\%) of 73 patients expressed galectin-3. A
significant correlation was found between galectin-3
tumor positivity and longer relapse-free periods
and overall survival.\textsuperscript{56} In univariate analysis,
high-grade (grade 3 or 4) tumors, nonkeratinizing
tumors, and galectin-3–negative tumors showed a
significantly increased risk of relapse and death.\textsuperscript{56}
In multivariate analysis, only galectin-3 expres-
sion retained an independent prognostic signifi-
cance for both relapse-free and overall survival,
therefore demonstrating that the absence of galec-
tin-3 expression is an independent negative prog-
nostic marker in patients with laryngeal squamous
cell carcinoma.\textsuperscript{56}

Plzak et al\textsuperscript{57} showed that decreased levels
of galectin-3 expression are associated with unfavor-
able prognoses in oropharyngeal and laryngeal
cancer specimens. In high-stage hypopharyngeal
squamous cell carcinomas, we observed that
galectin-3 expression is not related to prognosis.\textsuperscript{48}

Wu et al\textsuperscript{58} analyzed the secreted proteomes
of 2 nasopharyngeal cancer cell lines, with 23 pro-
teins being found in cultured media from both
lines. Of these proteins, fibronectin, galectin-3,
and the plasminogen activator inhibitor 1 were
highly expressed in nasopharyngeal cancer biop-
sies, but only weakly, if at all, in normal nasopha-
ryngeal tissue.\textsuperscript{58}

The involvement of galectins as differentiation
markers was also demonstrated in HNSCCs.\textsuperscript{31}
In normal mucosa, galectin-1 is detected in the (least
differentiated) basal layer, whereas galectin-3
tends to concentrate in the more superficial (more
differentiated) layers (Figure 2).\textsuperscript{31} In HNSCCs,
Galectin-3 staining is greater in the more differentiated cells (the tumor cells nearest the keratin pearl), whereas galectin-1 staining is more intense in the less differentiated cells (Figure 2). The loss of galectin-3 expression in HNSCCs during the processes of dedifferentiation is a common feature in nearly all HNSCC histological types. Galectin-3 binding sites are colocated with 2 desmosomal proteins (desmoplakin-1 and desmoglein), a fact that suggests a potential role played by galectin-3 in the mediation of desmosomal-type intercellular contacts in HNSCCs.

Galectin-7. Galectin-7 is overexpressed in buccal squamous cell carcinomas as compared with normal buccal epithelia and is involved in the control of corneal epithelial cell migration. These observations suggest that galectin-7 could play pivotal roles in cancer cell migration, in particular for cancers arising from pluristratified epithelia, including, for example, some types of HNSCCs. Indeed, we determined the immunohistochemical expression of galectins-1, -3, and -7 in a series of 81 stage IV hypopharyngeal SCCs and observed that high levels of galectin-7 expression are associated with rapid recurrence rates and dismal prognoses, a feature not observed with galectin-3 and weakly, if at all, with galectin-1. These data suggest that the immunohistochemical determination of galectin-7 expression in the case of high-risk hypopharyngeal cancers is a meaningful tool to identify those patients who should benefit from aggressive postsurgical adjuvant therapy.

HEAD AND NECK SQUAMOUS CELL CARCINOMAS, GALECTIN-1, AND THE IMMUNE SYSTEM

Although effective antitumor immune responses probably involve many elements of the immune system, T lymphocytes continue to be considered as the critical immune cells involved in antitumor immunity. The development of HNSCCs is strongly influenced by the host immune system. Recent evidence suggests that antitumor responses of patients with HNSCC were compromised in the presence of functional defects or apoptosis of T cells, both circulating or tumor-infiltrating. Tumor-derived factors or factors produced by normal cells in a local microenvironment favor tumors and disable tumor-infiltrating lymphocytes (TILs). In fact, TILs look like activated T cells, but these cells are functionally compromised. Functional assays with TILs isolated from the tumor bed have identified a number of defects including the following: (1) the absence of (or a low) expression of the ζ chain, which is the key signaling molecule in the T cell receptor pathway; (2) the decrease in proliferation in response to mitogens or IL-2; (3) the inability to kill tumor cell targets; (4) the imbalance in the cytokine profile, with the striking absence of interleukin-2 (IL-2) and/or interferon-gamma production, and (5) the evidence for pronounced apoptotic features in a considerable proportion of TILs. Moreover, immune cell dysfunction in patients with HNSCC appears to extend far beyond the tumor microenvironment because both functional defects and massive lymphocyte death have also been observed in the peripheral circulation of patients with advanced HNSCCs. In addition, it has been demonstrated that HNSCC cells autonomously producing proinflammatory cytokines are endowed with an advantage in terms of survival and growth advantage. 

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cytokines. In fact, galectin-1 suppresses the secretion of the proinflammatory cytokine IL-2 and favors the secretion of the anti-inflammatory cytokine interleukin-10 (IL-10). It has recently been very elegantly demonstrated by Rubinstein et al. that galectin-1 confers immune privilege to experimental melanomas by the modulation of the survival of T cells. The targeted inhibition of galectin-1 expression in vivo renders mice resistant to tumor challenge, a process requiring an intact CD4+ and CD8+ T-cell response. Le et al. recently observed an inverse relationship between the expression of galectin-1 in HNSCCs and T-cell expression as evaluated by a pan-T cell marker (CD3). These data suggest that the increased expression of the galectin-1 protein by hypoxia can lead to enhanced T-cell apoptosis and a decrease in the overall level of tumor-infiltrating T cells in HNSCCs (Figure 3). Interestingly enough, recent evidence indicates that the amount of galectin-1 produced by different cell types in the extracellular matrix is sufficient to kill T cells. The cytocidal effects of galectin-1 on immune cells probably results from the binding of galectin-1 and its cross-linking on T-cell-surface glycoproteins, including CD2, CD3, CD7, CD43, and CD45. The signal transduction events that lead to cell death induced by galectin-1 in activated T cells involve a number of intracellular mediators, including the induction of specific transcription factors (ie, nuclear factor of activated T cells, activator protein-1), the activation of the LSTRA cell kinase/zeta chain associated protein-70 kilodaltons/mitogen-activated protein kinase (Lck/ZAP-70/MAPK) signaling pathway, the modulation of B-cell lymphoma-2 (Bcl-2) protein production, the depolarization of the mitochondrial membrane potential and cytochrome c release, the activation of caspases, and the participation of the ceramide pathway.

FIGURE 3. Galectin-1 and cancer progression. (A, B) The increased expression of galectin-1 in head and neck squamous cell carcinoma by hypoxia can lead to enhanced T-cell apoptosis and a decrease in the overall level of tumor-infiltrating T cells. (C) Different galectins (-1, -3, -9) can promote the apoptosis of activated T cells. (D) The interactions of galectin-1 with T cells and activated macrophages can alter or modulate their functions. Galectin-1 inhibits T-cell effector functioning by promoting T-cell apoptosis, blocking T-cell activation and inhibiting the secretion of proinflammatory cytokines. Galectin-1 inhibits the standard NO pathway production in activated macrophages. High levels of NO may be cytostatic or cytotoxic for tumor cells, whereas low levels can have the opposite effect and promote tumor growth.

COULD GALECTINS BE TARGETED TO TREAT HEAD AND NECK SQUAMOUS CELL CARCINOMAS?

The data that we reviewed in the previous sections suggest that there are increases in galectin-1 and galectin-7 expression that parallel unfavorable prognoses in various types of HNSCCs, whereas the reverse is true for galectin-3. In the present section, we illustrate the point that there are several methods that could be used to decrease the levels of...
expression of galectin-1 and galectin-7 in HNSCCs, with a major potential impact for such a decrease in galectin-1 expression in the field of immunotherapy. The first therapeutic benefit that could be obtained from decreasing the levels of expression of galectins in HNSCCs involves chemoprevention. Indeed, oral cancer often develops following the prolonged exposure of the epithelial surface to repeated insults contributed by carcinogens, among which are tobacco and alcohol. Chemopreventive treatments could delay, or even reverse, the carcinogenetic process in these insulted epithelia before it leads to invasive cancer. Lotan reports that retinoids suppress premalignant oral lesions and decrease the incidence of second primary tumors in patients with head and neck cancer. Gillenwater et al has shown that in HNSCC cell lines, retinoic acid treatment, which is known to inhibit differentiation, decreases the expression of galectin-1 levels in several HNSCC cell lines. Thus, retinoic acid treatment could offer a way to antagonize the increasing levels of expression of galectin-1, which parallel the increasing levels of aggressiveness in several types of HNSCCs.

Another method of enhancing the therapeutic benefit for patients with HNSCC by decreasing the levels of expression of galectin-1 relies on the reinforcement of the immune system in its attack against HNSCCs. In the previous 2 sections we detail (1) how galectin-1 reduces the virulence of attack by cells from the immune system on cancer cells in general, and in human HNSCCs in particular, and (2) how HNSCC cells defend themselves against attacks launched by the immune system. Thus, decreasing the levels of expression of galectin-1 in HNSCC cells could reinforce the efficiency of immunotherapy-based approaches aiming to counter HNSCC development. It should be emphasized that biological therapies able to induce and/or upregulate antitumor immune responses could constitute a complementary approach to conventional types of treatment for patients with HNSCC because, despite advances in surgery, radiotherapy, and chemotherapy, the overall survival rates for patients with HNSCC have not changed over recent decades.

Various approaches could be employed to decrease the levels of galectin-1 expression in HNSCCs. The first of these would be to use a small interference RNA (siRNA)-related method, and the preliminary data that we have obtained show that a selective and specific siRNA directed against galectin-1 improves the survival, as compared with control (mock and wild-type), of mice bearing orthotopic xenografts of human gliomas. This siRNA-induced decrease in galectin-1 expression also significantly improved the in vivo response to the cytotoxic drug temozolomide in the human U373 orthotopic xenograft model. A second type of approach would involve the use of various types of chemicals (reviewed by Ingrassia et al) that bind more or less selectively to galectins, and antagonize their biological activity. Using the human U373 orthotopic glioma model, we observed that a monosubstituted β-D-lactosyl-steroid significantly increased the survival of mice grafted with this orthotopic glioblastoma model to an extent that was similar to that observed with temozolomide, the reference compound used to treat patients with glioblastoma.

It must be borne in mind that glioblastomas are associated with prognoses that are as dismal (and even worse) than those associated with high-stage HNSCC. The monosubstituted β-D-lactosyl-steroid that we recently synthesized was also a source of pronounced therapeutic benefit in the case of a preclinical model of a subcutaneous murine lymphoma model markedly metastasizing to the liver. In addition, this antigalectin compound increased the antitumor effectiveness of cisplatin, a cytotoxic proapoptotic drug, on this same murine metastatic lymphoma model. Other lactose derivatives, which also act as galectin ligands, display selective binding to galectin-1, -3, and -7. For example, 1-phenyl thiogalactoside, O-nitrophenyl lactoside, and naphtylsulfonyl lactoside. These compounds have a 20 times greater affinity for galectins than galactose or lactose, which are the natural ligands for galectins.

Currently, our objective is to compare the relative activity of siRNA methodology and of galectin-binding molecules in terms of therapeutic benefits for various HNSCC preclinical models. This approach, mainly targeting galectin-1 (thus its cytocidal effects for activated T cells), should be combined with various types of immunotherapies in the case of orthotopic murine HNSCC models grafted into immunocompetent mice.

**CONCLUSIONS**

Galectins-1, -3, and -7 are deeply involved in the biology of HNSCCs, including cell proliferation, cell death, and cell migration. Galectin-1 in partic-
ular confers to some cancers like HNSCCs a resistance to the immune system by inducing major apoptotic processes in activated T cells. Thus, reducing the levels of expression of galectin-1 in galectin-1-secreting HNSCCs could improve the efficiency of T-cell–based immunotherapy against HNSCCs. Various approaches based on the use of siRNA directed against galectin-1 or anti-galectin-1 chemicals could be used for this purpose.

**REFERENCES**


