Galectin-1 links tumor hypoxia and radiotherapy

Peiwen Kuo and Quynh-Thu Le
Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA, USA

Received on April 21, 2014; revised on June 17, 2014; accepted on June 21, 2014

Radiation therapy is a main stay in treating solid tumors and plays a significant role in definitive and adjuvant therapy. Unfortunately, local control remains a challenge, in which the success of radiotherapy is largely dictated by tumor hypoxia, DNA damage repair and the antitumor immune response. Extensive efforts have therefore been devoted to targeting the factors that attenuate tumor radiosensitivity, although with limited success. Mounting evidence suggests that tumor and endothelial cells may utilize galectin-1 (Gal-1) for protection against radiation through several mechanisms. Targeting Gal-1 in combination with radiotherapy provides an exciting approach to address several radiotherapy-prohibitive mechanisms.

Keywords: galectin-1 / radiotherapy / tumor hypoxia

Introduction

Despite advances in radiotherapy, disease control in many solid tumors remains a challenge in the clinic. Past approaches to target hypoxia in radiotherapy included modification of tumor oxygen consumption and vasculature as well as development of agents that sensitize hypoxic cancer cells to radiation. These approaches were met with limited success; however, establishing hypoxic markers to properly identify patients best suited for hypoxia-targeted therapies and employing novel therapies that simultaneously address factors affecting tumor radiosensitivity may impart a greater impact on the therapeutic ratio.

Galectin-1 (Gal-1) is the prototype member of the Galectin superfamily, characterized by high affinity binding to β-galactosides through a well-conserved carbohydrate-recognition domain (Barondes et al. 1994). Gal-1 has been shown to regulate a variety of biological processes, including T-cell homeostasis, resolution of inflammatory responses, host–pathogen interactions, selective deletion of specific thymocytes during T-cell development, fetomaternal tolerance and embryogenesis (Perillo et al. 1997; Van den Brule et al. 1997; Rabinovich et al. 1999, 2002; Sotomayor and Rabinovich 2000; Zuniga et al. 2001; Blois et al. 2007).

Gal-1 is overexpressed in most cancers, promotes an aggressive phenotype and is associated with poor survival (Cindolo et al. 1999; Camby et al. 2001; Rorive et al. 2001; van den Brule et al. 2001; Soko et al. 2005; Le et al. 2007; Saussez et al. 2007; Croci et al. 2012; Dalotto-Moreno et al. 2013). Gal-1 is also upregulated in response to both hypoxia (Le et al. 2005; Zhao et al. 2010, 2011) and radiation (Strik et al. 2007; Upreti et al. 2013), in which its enrichment after radiation may boost tumorigenicity. Targeting Gal-1 and, in turn its modulation of DNA damage repair, vasculature normalization and antitumor immune response may address multiple pathways, potentially rendering tumor and endothelial cells (ECs) more radiosensitive (Rubinstien et al. 2004; Huang et al. 2012; Jain 2013; Croci et al. 2014).

Hypoxia impact on tumor radiosensitivity and patient prognosis

Hypoxia, a consequence of rapid cancer cell proliferation with increased oxygen and nutrient demands that cannot be met by the surrounding vasculature, is a common occurrence in solid tumors and is well accepted as a deleterious factor in cancer therapies, compromising radiotherapy and driving malignant progression (Gray et al. 1953; Nordsmark et al. 2005). Radiation-induced DNA damage through the generation of H₂O₂ and hydroxyl radicals requires oxygen (Massie et al. 1972; Lesko et al. 1982; Prise et al. 1989). In turn, the radiation dose required to achieve the same effect as a hypoxic tumor is approximately three times higher than in the presence of normal oxygen levels, described by the oxygen enhancement ratio (Gray et al. 1953; Deschner and Gray 1959).

Studies relating treatment outcomes to direct tumor pO₂ measurements have shown that tumor hypoxia is a major contributor to poor prognosis after radiotherapy (Brizel et al. 1997; Nordsmark and Overgaard 2000). The fraction of tumor pO₂ measurements below 2.5 mm Hg was shown to be an independent predictor for radiation response and locoregional tumor control in a large cohort of HNSCC on multivariate analysis (Nordsmark et al. 2005). A significantly lower 12-month disease-free survival was reported in radiotherapy patients with median tumor pO₂ <10 mmHg vs. those with higher tumor pO₂ (Brizel et al. 1997). The relationship between tumor oxygenation and prognosis has also been demonstrated with endogenous and PET-based hypoxia tracers (Rajendran et al. 2006; Ferreira et al. 2011; Zips et al. 2012).

© The Author 2014. Published by Oxford University Press. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com
The impact of hypoxia on radiosensitivity is also attributed to proteomic and genomic changes that increase tumor cell proliferation while decreasing their apoptotic potential (Loeb 1991; Graeber et al. 1996; Vaupel 2004). The adaptive responses to match oxygen supply with metabolic and bioenergetics demands under hypoxia involve various cellular pathways including gene regulation by hypoxia-inducible factors (HIFs). Therefore, targeting HIF downstream effectors, such as Gal-1, may render the tumor microenvironment more vulnerable to radiation.

**Gal-1: a hypoxia-responsive protein**

Gal-1 was first shown to be a hypoxia-responsive protein by Le and colleagues using SELDI-TOF-MS to identify hypoxia marker secreted under hypoxia in a head and neck cancer cell line (Le et al. 2005). Their analysis revealed that a 15 kDa protein that reliably increased with hypoxia treatment in vitro was a B-galactoside-binding protein, Gal-1. This induction was also observed in hormone (Scc4) and head and neck (SQB20) squamous cell carcinomas, pancreatic adenocarcinoma (Panc1) and an immortalized B-cell line (V2P3). Increased circulating Gal-1 was observed in HNC tumor bearing mice after breathing 10% oxygen, which effectively increased tumor hypoxia (Le et al. 2005). Gal-1 expression also correlated with the hypoxia marker, carbonic anhydrase IX (CAIX) (Le et al. 2007). Gal-1 upregulation by hypoxia has since been documented in colorectal and prostate cancer cells as well as Kaposi’s sarcoma and mature myeloid leukemia cells (Zhao et al. 2010, 2011; Croci et al. 2012; Laderach et al. 2013).

Hypoxia driven Gal-1 expression occurs through two HREs 441 and 423 base pairs upstream of the transcriptional start site. Overexpression of HIF1α ectopically potently increases Gal-1 expression while its inhibition attenuates hypoxia induction of Gal-1 (Zhao et al. 2010) in colorectal cell lines. CoCl2 treatment, which mimics hypoxia by stabilizing HIF1α, also increased Gal-1 expression. Gal-1 is also regulated by an HIF interacting transcription factor for hematopoietic cell differentiation (C/EPBα), which works in synergism with HIF1α, enhancing its transcriptional activity in acute myeloid leukemia (Zhao et al. 2011).

Hypoxia induction of Gal-1 has also been shown to occur through an NFKB-dependent and HIF-independent mechanism in Kaposi’s sarcoma; Inhibition of HIF1α and HIF2α in these tumor cells did not prevent Gal-1 upregulation by hypoxia (Croci et al. 2012). Human LGALS1 contains several putative NFKB consensus sites and the expression of a super-repressor (IkB-α-SR) or pharmacological inhibitor (BA Y-117802) prevented hypoxia induction of Gal-1. Furthermore, NFKB-mediated Gal-1 expression under hypoxia was activated by ROS and N-acetylcysteine scavenging of ROS inhibited hypoxia induction of Gal-1 (Croci et al. 2012).

**Gal-1 upregulation by radiation and modulation of tumor radiation response**

In addition to hypoxia, radiation is also a potent inducer of Gal-1 in glioma and ECs in vitro (Strik et al. 2007; Upreti et al. 2013). Mounting evidence suggests that tumor and ECs may utilize Gal-1 to attenuate tumor radiation response (Figure 1). Anginex, which recognizes Gal-1 overexpressed by the tumor neovasculature, is a 33 amino acid antiangiogenic peptide that was designed based on known features of endogenous inhibitors of angiogenesis (Griffioen et al. 2001; Thijssen et al. 2006; Upreti et al. 2013). Anginex infusion combined with suboptimal radiation dose caused human ovarian tumor xenografts to regress and combined Anginex and a single radiation dose of 25 Gy reduced SCK mammary tumor growth synergistically compared with either therapy alone (Dings et al. 2005). Anginex improvement of radiation response was also observed in syngeneic SCCVII squamous cell carcinoma and focal multiple myeloma SCID-rab mouse models (Amano et al. 2007; Jia et al. 2010). In a microbeam radiation therapy study, Anginex radiosensitized tumors when combined with wider beam spacing and lower radiation doses that did not affect tumor growth alone. Beam geometries and doses capable of slowing tumor growth were also more effective when combined with Anginex (Griffin et al. 2012).

Antiangiogenic agents have been shown to transiently normalize tumor vasculature, which can alleviate hypoxia, increase drug and antitumor immune cell delivery and improve response with various therapies (Jain 2013). Croci and colleagues found that mAb inhibition of Gal-1, similar to other antiangiogenic agents, resulted in transient vessel normalization as evidenced by vasculature remodeling, increased pericyte coverage of vessels and T-cell infiltration, as well as reduced tumor hypoxia (Croci et al. 2014). Interestingly, hypoxia influences the EC surface glycome by increasing B-6GlcNAc-branched N-glycans and poly-LacNAc structures, resulting in enhanced Gal-1 to EC binding and proangiogenic signaling, which may contribute to hypoxia-mediated angiogenic rescue programs (Potente et al. 2011; Croci et al. 2014). Targeting Gal-1 to transiently normalize tumor vasculature may create a window of tumor vulnerability to radiation and enhance therapeutic response.

Ionomizing radiation induces cell death through apoptosis, mitotic catastrophe, autophagy and senescence when radiation-induced lesions, such as DNA single- and double-strand breaks, base alterations and DNA-to-DNA and DNA-to-protein cross-linking, are left unrepaired. Interestingly, Gal-1 overexpression reduced DNA damage after C33A irradiation, while shGal-1 HeLa cells exhibited greater DNA damage (Huang et al. 2012). DNA damage repair has been shown to occur through Raf-1 signaling (Golding et al. 2007) and Gal-1 interacts with H-ras and its overexpression enhanced Raf-1 and ERK1 phosphorylation to promote repair of radiation-induced DNA damage. Radiation stimulation of tumor Gal-1 expression can theoretically increase the efficiency of DNA damage repair, promoting tumor and EC survival through H-ras signaling (Huang et al. 2012).

Interestingly, Chen and colleagues reported that an interaction exists between H-ras and HIF1, providing a possible HIF1/Gal-1-positive feedback loop where HIF1 signaling under hypoxia can enhance Gal-1, which acts through H-ras to further promote HIF1 transcriptional activity (Chen et al. 2001; Huang et al. 2012). Tumors may utilize this positive feedback loop to maintain elevated Gal-1 expression and HIF1 signaling to drive radioreistance and aggressive tumor phenotypes.
Another avenue in which Gal-1 promotes tumor radioresistance involves its immunomodulatory functions. Radiotherapy can enhance the antitumor immune response, increasing the peptide repertoire to promote the antigen presentation pathway and recruit cytotoxic T lymphocytes to lyse tumor cells (Lugade et al. 2005; Liang et al. 2013). However, radiotherapy does not always result in protective immunity as relapse occurs. Gal-1 potently induces activated T-cell apoptosis and enforces a Th2 cytokine profile to block immune effector functions while promoting IL-10-producing T regulatory cells to create an immune privileged site at the tumor (Rubinstein et al. 2004; Toscano et al. 2006). The enhancement of Gal-1 expression after radiation may promote tumor immune evasion, limiting therapeutic response.

Patient selection for hypoxia-targeted therapies: hypoxia markers with prognostic value

Aside from targeting hypoxia through its downstream effectors, significant effort has also been devoted to developing tools to select patients who may benefit the most from hypoxia-targeted therapies. In 2007, Le and colleagues sought out to establish a panel of hypoxia markers having prognostic value in head and neck squamous cell carcinoma (HNSCC) treated with concurrent chemoradiation or adjuvant radiation therapy to fulfill this need. In a cohort of 101 HNSCC patients, Gal-1 stained strongly in approximately half of the tumors and significantly correlated with the CAIX hypoxia marker. Furthermore, tumors with strong Gal-1 staining showed a trend for increasing tumor pO2 measurements <5 mgHg, although the correlation was not statistically significant. Most notably, Gal-1 tumor staining achieved a statistically significant difference for overall survival (Le et al. 2007).

Huang et al. (2013) recently published Gal-1 as an independent prognostic factor for local recurrence uterine cervix squamous cell carcinoma treated with definitive radiotherapy. Local recurrence rates were higher in tumors staining higher for Gal-1 and tumor-specific Gal-1 was also associated with reduced cancer-specific survival rates (Huang et al. 2013).

Conclusions

In summary, Gal-1 has been shown to be highly expressed in tumors that are particularly radioresistant, including prostate cancer, glioma and melanoma, which require large radiation doses to establish control (Rorive et al. 2001; Mathieu et al. 2012; Laderach et al. 2013). In contrast, Gal-1 level is not detectable in nodular lymphocyte-predominant Hodgkin’s lymphoma, which is a group of radioresponsive Hodgkin’s lymphoma with better prognosis (Gandhi et al. 2007). Preclinical and clinical studies suggest that Gal-1 expression in many cancer cells can be upregulated by hypoxia, which has been intimately linked to radiation resistance and tumor aggressiveness. Mechanistically, Gal-1 affects the tumor vasculature, DNA damage repair and antitumor immunity, all of which have been implicated in modulation of radiation-induced cell kill and tumor growth. Based on these data, targeting Gal-1 with radiotherapy provides a compelling multifaceted approach to
increasing radiation effectiveness, which in turn will result in a higher cure rate for certain solid tumors.

**Abbreviations**

CAIX, carbonic anhydrase IX; Gal-1, galectin-1; HIFs, hypoxia-inducible factors; HNSCC, head and neck squamous cell carcinoma

**Conflict of interest statement**

None declared.

**Funding**

This work was supported by grant from the National Institutes of Health (R01 CA161585-02A1).

**References**


