Galectin-1 and Immune Suppression during Radiotherapy

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Radiotherapy induces galectin-1 (Gal-1) secretion by tumors, which induces CD8⁺ T-cell apoptosis and lymphopenia. These effects are substantially decreased by Gal-1 shRNA. Inhibition of Gal-1 may be an effective strategy for overcoming radiation-induced lymphopenia, which may improve clinical outcomes. Clin Cancer Res; 20(24); 6230–2. ©2014 AACR.

In the November 1 issue of Clinical Cancer Research, Kuo and colleagues (1) provided compelling preclinical evidence that radiotherapy induces the secretion of galectin-1 (Gal-1) by tumors, which results in T-cell apoptosis and systemic lymphopenia. By using wild-type and Gal-1⁻/⁻ mice implanted with Lewis lung carcinoma tumors, they found that the decrease in CD8⁺ T cells associated with elevated Gal-1 expression after radiotherapy depended on tumor Gal-1. They then used an intriguing experimental approach to block Gal-1 expression by using thiogalactoside (TDG) or Gal-1 shRNA, which clearly blunted radiotherapy-associated lymphopenia. Combining radiotherapy with TDG or Gal-1 shRNA in vivo resulted in reduced rates of lung metastasis and increased CD8⁺ T-cell tumor infiltration. Kuo and colleagues (1) further correlated clinical radiotherapy-induced lymphopenia with decreased progression-free and overall survival in 20 patients with non–small cell lung cancer (NSCLC) receiving stereotactic ablative body radiation. Because this group of patients did not have plasma samples drawn, the authors were unable to examine for a relationship between galectin expression and lymphopenia. As an alternative, they investigated this idea in an unrelated group of 24 patients with head and neck cancer. They noted a trend toward higher Gal-1 expression and lower lymphocyte counts in patients who experienced a recurrence, but due to the small number of failures, they were unable to make any correlation with survival. The results from this study suggest relationships among galectin expression, lymphopenia, and ultimate tumor control, leading the authors to propose a mechanism for causation of poorer prognosis linked to radiation-related lymphopenia. These associations do not prove causality but are hypothesis generating.

Galectins are a group of lectins classified by their ability to bind β-galactoside. Gal-1, the prototype member of this superfamily, has already been implicated in the modulation of several processes required for cancer invasion such as cellular adhesion and migration. Gal-1 promotes tumor evasion of the immune response by activating CD8⁺ T-cell apoptosis through Bcl-2 signaling (2). Kuo’s group is one of the first to demonstrate that this effect is augmented after radiotherapy from the secretion of Gal-1 by tumors into peripheral blood. We similarly showed that lymphopenia during chemoradiotherapy for NSCLC was highly associated with clinical outcomes on multivariate analysis (3).

Hypofractionated high doses of radiotherapy can potentially act as an "in situ vaccine" that primes T cells with tumor antigen, allowing cytotoxic T cells not only to attack the local irradiated tumor but also to attack sites of distant metastatic disease. One of the first demonstrations of this idea of radiotherapy as an "in situ vaccine" was performed by Chakravarty and colleagues (4), who showed that combining Flt-3 ligand, which activates dendritic cells, with radiation decreased the incidence of lung metastases in mice using the same model as Kuo and colleagues. Subsequently, Lee and colleagues (5) demonstrated that the ability of ablative radiotherapy to control tumors was dependent on the presence of CD8⁺ T cells. However, radiotherapy-induced changes in the tumor microenvironment can both dampen and activate the immune system. Radiotherapy promotes the proliferation of T-regulatory cells, secretion of TGFβ, and tumor surface expression of PD-L1, which all depress the T-cell response (6). On the other hand, radiotherapy induces tumor expression of MHC-I, death receptors, and chemokines, which promote cytotoxic T-cell destruction of cancer cells (6). Kuo and colleagues (1) add to our knowledge of the tumor environment by elucidating the apoptotic effect of Gal on CD8⁺ T cells during tumor radiotherapy. Nonetheless, it remains to be discovered how radiotherapy promotes Gal-1 secretion by tumors and which components of the apoptotic and angiogenic pathways regulated by Gal-1 are affecting the survival of T cells.

The idea that radiotherapy can modulate immune function to provoke systemic responses has led to a heightened interest in combining radiotherapy with immunomodulatory drugs that are currently gaining widespread acceptance.
in the treatment of various tumors. The ability of cancer to evade the immune system prompted the search for immunomodulation targets to block as a means to enhance systemic immunity, a search that resulted in the recent discovery of immune checkpoint inhibitors. The first immune checkpoint inhibitor to be discovered was CTLA-4, by the from the laboratory of James Allison (The University of Texas MD Anderson Cancer Center, Houston, TX), who pioneered the idea that blockade of this checkpoint could enhance tumor immunity. Since that discovery, other immune-suppressing (PD-L1 and PD-1) and immune-stimulating ligands (CD-137 and OX-40) have been discovered (7).

Humanized antibody therapies targeting these molecules (anti–CTLA-4, anti–PD-L1, and anti–PD-1) have produced compelling responses in various solid tumors, confirming the importance of immunomodulation in cancer treatment (8, 9). Clinical trials exploring combinations of immunotherapy and radiotherapy are currently ongoing. One case report showed that ablative radiotherapy of a spine lesion in a patient with ipilimumab-refractory metastatic melanoma produced complete regression of two nonirradiated sites (10). This finding underscores the concept of radiotherapy acting as an in situ vaccine, in that it can prime T cells with tumor antigen to combat distant disease (6). Because lymphopenia during radiotherapy is associated with inferior outcomes, an alternative therapeutic approach may be preradiation infusion of autologous T cells during treatment. Dudley and colleagues (11) investigated the use of myeloablative chemoradiotherapy with autologous tumor-infiltrating lymphocytes in a trial of 50 patients with refractory melanoma. Objective response rates in that trial ranged from 50% to 70%, with regression of several visceral sites, including the brain (11). These results, in combination with those from Kuo and colleagues (1) demonstrating that Gal-1 inhibition promotes systemic CD8⁺ T-cell stimulation, support further study of Gal-1 inhibitors with radiotherapy.

With the emergence of immunotherapy, the development of associated new biomarkers is imperative, which would allow for the stratification of cancer patients into specific subgroups according to their predicted systemic immune response to radiotherapy. For those with a highly immunogenic phenotype (including high lymphocyte counts), ablative radiotherapy alone may be the preferred option. Patients with a weak immunogenic phenotype or severe lymphopenia may benefit more from other local therapies. For intermediate immunogenic phenotypes, the addition of immune-modifying agents to radiotherapy might further boost a patient’s endogenous immune response (Fig. 1). With the recent FDA approval of anti–CTLA-4 and anti–PD-1 therapies, one solution could be treatment with a cocktail of immune-enhancing drugs. In support of this notion, Wolchok and colleagues (12) recently combined ipilimumab and nivolumab (anti–PD-1) as a regimen for patients with advanced melanoma and found that the combination produced higher rates of objective response than prior studies of either agent as monotherapy.

The success of dual therapy such as this most likely results from the fact that these targets block different pathways in T-cell signaling, which also raises the issue of immunotherapy resistance. Perhaps those patients who did not achieve an objective response in the Wolchok trial had developed resistance to ipilimumab and nivolumab via activation of

Figure 1. Temporal relation for combining various immunotherapies and radiotherapy. 1, CD8⁺ T-cell activity could potentially be enhanced through (a) autologous T-cell infusion, (b) Gal-1 inhibition, or (c) PD-1/CTLA-4 blockade. 2, CD8⁺ T cells secrete TNFα, which inhibits myeloid-derived suppressor cell (MDSC) frequency. MDSC inhibition prevents tumor escape from systemic immunity. 3, activated CD8⁺ T cells circulate back into the locally irradiated tumor, providing enhanced local control. 4, activated CD8⁺ T cells circulate systemically through the body to treat and/or prevent the spread of diffuse metastatic disease.
the Gal-1 pathway. Adding a Gal-1 inhibitor to ipilimumab and nivolumab during radiotherapy may significantly boost the number of active cytotoxic T cells, which could result in enhanced disease responses. The findings of Kuo and colleagues (1) are thought provoking, as they shed light on the role of Gal-1 in the tumor microenvironment, but they require prospective validation in an independent dataset. With the goal of boosting the immune system of patients to facilitate systemic responses, Gal-1 inhibition could be an important way to enhance immune stimulation after radiotherapy.

Disclosure of Potential Conflicts of Interest
J.W. Welsh has ownership interest (including patents) in Healios Oncology and MolecularMatch, and is a consultant/advisory board member for MolecularMatch and RefleXion Medical. A. Maity and S.M. Hahn report receiving commercial research support from Merck. No potential conflicts of interest were disclosed by the other authors.

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