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AUTHOR'S VIEW

Precise identification of immunotherapeutic targets for solid malignancies using clues within the tumor microenvironment—Evidence to turn on the LIGHT

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ABSTRACT
Targeted immunotherapy for solid gastrointestinal malignancies is challenging due to a lack of identified tumor antigens. Therefore, a strategy that supports and expands tumor infiltrating lymphocytes in the tumor microenvironment may allow these tumor-reactive T-cells to incite an antitumor response. Gene expression analysis of colon metastases has identified specific immunotherapeutic targets for this malignancy.

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Colon cancer; immunotherapy; gastrointestinal cancers; LIGHT; metastasis; Tnfsf14; tumor infiltrating lymphocytes; tumor microenvironment

Gastrointestinal cancers carry a poor prognosis accounting for 4 of the top 5 malignancies with the highest 5-year mortality. Surgical resection remains the mainstay of curative treatment for these cancers, however, the challenge occurs when the disease progresses systemically and the biology of disease outpaces the reach of the knife. The majority of patients with colon cancer, the most common gastrointestinal tumor, present with advanced disease, resulting in it being the 2nd leading cause of cancer related deaths in the US. A combination of advances have increased the survival of patients with surgically resectable colorectal liver metastases (CRLM), however, long-term survival is rare even in these highly selected patients and for the vast majority, palliative chemotherapy is the only present option.

The myriad of therapeutic modalities for patients with cancer have greatly expanded in the 21st century, driven by technology that has enabled both a deeper understanding of cumulative and specific gene mutations that control tumor growth and metastases, and the ability to engineer corresponding molecular inhibitors. This model of personalized medicine for cancer remains a promising goal though it currently struggles to address multiple barriers to widespread application, including the ability to manage resistance, that have hindered clinical use for a large number of patients. The newest targeted treatments for CRLM involve targeting growth factors and their receptors, however, these regimens have limited durability and struggle to greatly improve long-term survival, therefore, there is a need to identify additional treatment strategies.

It has been demonstrated that patient survival and tumor biology are accurately governed by the number and location of lymphocytes invading a primary tumor. Specifically, the presence of phenotypically activated and proliferating T-cells within primary colon tumors is associated with improved survival.[1,2] Therefore, the role of the immune system and tumor infiltrating lymphocytes (TIL) in determining the prognosis of patients with metastatic colorectal cancer is of great interest. We have previously demonstrated an association between increased T-cell infiltrates and improved survival in patients with CRLM,[3] and our recent study of 76 patients identified that increased numbers of TIL were associated with improved overall (OS) and recurrence-free survival (RFS) after surgical resection.[4] These data validate the underlying concept that immunotherapy may play a viable role in managing patients with advanced gastrointestinal malignancies, including colon cancer.

The use of immunotherapy for gastrointestinal cancers, however, has met significant challenges. Clinical trials with vaccines have shown an ability to increase circulating tumor specific lymphocytes without the ability to impart significant tumor regressions, even when specific gastrointestinal cancer associated antigens are utilized.[5,6] Checkpoint blockade with antibodies including Ipilimumab, which have displayed great promise in other malignancies,[7,8] have not been successful in advanced colon cancer, and furthermore, act through imprecise activation of the immune system that can result in significant autoimmunity. Although adoptive cell transfer has been used successfully in a subset of patients with melanoma, renal cell cancer, and synovial cell sarcoma, it has been challenging to utilize in colon cancer patients due to self-antigens, and a recent trial using T-cells engineered against the carcinoembryonic antigen demonstrated dose limiting toxicity. Furthermore, though responses are impressive and durable when they occur, the use of adoptive cell transfer is resource intensive, requires ablative chemotherapy, and is patient specific. Some of these limitations can be addressed through chimeric antigen receptor (CAR) expressing T cells, though the main limitation

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to this approach has been the lack of known tumor antigens on solid malignancies, including colon cancer. Until gastrointestinal cancer specific antigens are identified across patients, strategies to enhance TIL activation in the tumor microenvironment are needed. The ideal strategy would focus immunostimulation at the level of the tumor in a very precise fashion. Therefore, we sought to further characterize the immune environment of CRLM by defining their immunologic gene expression profiles to evaluate potential targets for immunotherapy within the tumors.

Microarray analysis provides a direct representation of transcriptional and post-transcriptional regulation occurring in the tumor microenvironment, and the breadth of data generated was organized into defined biologic processes using gene ontology analysis in order to accurately identify patterns of gene expression that correlated with outcomes. It was determined that an intratumoral environment reflective of "T-cell proliferation" and "activation" significantly correlated with survival, and of the genes that defined these biologic processes, LIGHT expression was found to be most significantly associated with both OS and RFS.[4] A separate cohort of patient tumors was stained for LIGHT expression on TIL based on this data and it was found that increased LIGHT$^+$ TIL correlated with both improved OS and RFS. LIGHT (an acronym for homologous to lymphotoxins, shows inducible expression, and competes with herpes simplex glycoprotein D for HVEM, a receptor expressed by T lymphocytes) is a member of the Tumor Necrosis Factor Superfamily (TNFSF14). It is an immune-stimulatory cytokine that has been shown to augment the antitumor immune response.[9] Forced LIGHT over expression in tumors leads to increased levels of cytotoxic T lymphocytes (CTLs) in and around the tumor and can induce tumor regression. LIGHT may allow CTL's to overcome the antigenic barrier formed by host cell stroma around the tumor and to mount an antitumor response. Collectively the findings in CRLM support the possibility that increased LIGHT expression was associated with T-cell proliferation in the tumor microenvironment and patient survival.

A reliable immunocompetent model of CRLM in which to study LIGHT expression was thus created. In a murine model of syngeneic CRLM, characterization of the tumor microenvironment revealed that the number of TIL and LIGHT$^+$ T cells infiltrating the tumor were very low.[10] The CD4$^+$ TIL were activated (increased CD4$^+$ CD107a$^+$) compared to normal liver CD4$^+$ cells, and these CD4$^+$ TIL were perhaps driving the increased expression and activation of the CD8$^+$ TIL (CD69$^+$, CD107a$^+$). This was also supported by cytokine analysis of the tumor microenvironment that reflected a Th1 cell-mediated immune response. When evaluated by immunofluorescence confocal microscopy, the majority of TIL were in the peritumoral tissues rather than intratumoral nests, and intratumoral TIL expressed significantly lower levels of LIGHT.[10] Considering that LIGHT can stimulate proliferation and activation of T-cells in tumors, tumor regression, and is associated with improved OS in CRLM, it is possible that increasing LIGHT within CRLM could amplify the existing immune response precisely and result in tumor regressions (Fig. 1).

Solid organ malignancies are difficult to treat due to acquired resistance to many therapeutic interventions and extensive immunoediting. Particularly challenging for gastrointestinal tumors has been the identification of unique tumor antigens, that when targeted, do not result in significant dose-limiting autoimmunity. We have investigated a strategy to broadly map the genetic microenvironment within CRLM in order to identify specific immunologic genes that correlate with survival to determine specific therapeutic targets for

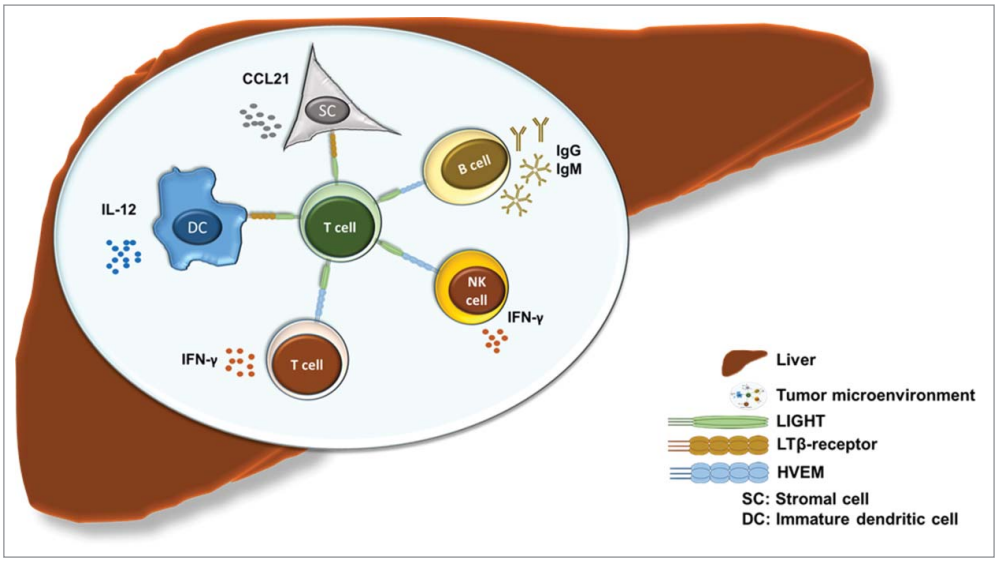

Figure 1. Influence of LIGHT in the Tumor Microenvironment: LIGHT and its cognate receptors are expressed on various tumors and immunocytes. Increased gene and protein expression in the tumor microenvironment, in particular on tumor infiltrating lymphoctyes, correlated with improved survival in patients with surgically resected colorectal liver metastases. A hypothetical model of the downstream effects of T-cell expressed LIGHT in these tumors is demonstrated, including stimulation of stromal cells via LTβR (LIGHT receptor) to upregulate chemokines and adhesion molecules to recruit naive T cells, natural killer (NK) cells, and dendritic cells (DC) into the tumor. LIGHT may additionally function to stimulate T cells, possibly through HVEM (LIGHT receptor), inciting proliferation and activation. Furthermore, LIGHT may promote DC cell maturation and expansion, supporting the presentation of tumor antigens to T cells in-situ. Moreover, LIGHT has the ability to directly activate NK cells, which further enhances T cell activation and cytotoxicity. In addition, LIGHT binding to tumor cell LTβR and HVEM may directly induce tumor cell apoptosis, which can result in the release of tumor antigens and potentially prime antitumor immunity.

immunotherapy. Using targets identified with this strategy, like LIGHT, to stimulate TIL that already recognize tumor and have migrated to the tumor microenvironment holds promise for precision immunotherapy.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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