Dual roles of TLR7 in the lung cancer microenvironment

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TLR7 agonists are under investigation for their ability to enhance antitumor immune responses. However, these agonists can also stimulate TLR7-expressing tumor cells. High TLR7 expression in the primary tumor confers poor clinical outcome and resistance to chemotherapy in lung cancer patients. This protumorigenic effect of TLR7 has been validated in murine models of lung carcinoma.

Introduction

TLR7, a receptor for single stranded RNA, is expressed on endosomes in immune cells including plasmacytoid and conventional dendritic cells (pDC and cDC), macrophages, B lymphocytes and NK cells. Stimulation of these cells with TLR7 ligands induces their maturation and activation, and the secretion of proinflammatory cytokines. These properties are currently being exploited in pre-clinical studies to improve antitumor therapies. Contrarily, stimulation of TLR7 on tumor cells can lead to tumor progression and resistance to treatment.

Antitumor effects of TLR7

The use of TLR7 agonists such as imiquimod, loxoribine, CL264, ssRNA40, R848, and SM-276 001, either alone or as vaccine adjuvants, induces potent immunity leading to antitumor therapeutic efficacy in several murine models. In line with these observations, it has been demonstrated that systemic TLR7 agonist injection reduces tumor progression and modulates the systemic and intratumoral immune contexture in colon, renal and mammary carcinomas. This has been illustrated by a decrease in intratumoral regulatory T cells, an increase of antigen-specific interferon γ (IFN-γ) producing effector cells in the spleen, an increased number of natural killer (NK), NKT cells and T lymphocytes, and by activation and maturation of pDC able to efficiently stimulate antitumoral responses. These results observed in animal models demonstrate the use of TLR7 agonists as an attractive strategy to treat several tumor types.

The antitumor effects arising from TLR7 stimulation have also been demonstrated in human skin cancers and cervical intraepithelial neoplasia. It has been shown that TLR7 stimulation by imiquimod is a successful treatment for actinic keratosis (intradermal neoplasia), with 27% of patients exhibiting a complete clinical response. It has also been shown that the application of imiquimod on invasive primary melanoma results in local regression of tumor size, which is associated with increased levels of CD4+T and CD8+T lymphocytes both in the skin and in lymph nodes.

Protumorigenic effects of TLR7

Contrary to the therapeutic benefits of TLR7 agonists on the immune cells, several studies have shown that TLR7 stimulation augments tumor progression. Previously we have shown that TLR7 is highly expressed on primary tumor cells from non-small-cell lung carcinoma (NSCLC) patients. Furthermore, stimulation with TLR7 agonists induced a strong in vitro protumorigenic effect and resistance to chemotherapeutic drugs currently used to treat NSCLC patients. We recently verified these effects in both immunodeficient (NOD/SCID) and immunocompetent (C57BL/6) murine models, using subcutaneously grafted lung carcinoma cells. In both models, we showed that repeated administration of CL264, loxoribine or imiquimod leads to increased tumor volume, which was similar to the results we obtained in TLR7-deficient mice. This protumorigenic effect could be mediated either by direct stimulation of TLR7-expressing tumor cells as we observed in experiments in vitro or by increased recruitment and differentiation of immunosuppressive cells in the tumor microenvironment. In support of the second scenario, we observed an increased frequency of myeloid-derived suppressor cells (MDSCs) and a reduction of CD8+T cells in the tumors of mice treated with TLR7 agonists compared to no treatment. Additional experiments are needed to further characterize the role of MDSCs in the protumorigenic effects of TLR7 stimulation. We hypothesize that the recruitment of MDSCs could be induced by cytokines and/or chemokines produced by tumor cells upon TLR7 stimulation (Fig. 1).
Other groups have shown similar results in additional tumor models. For example, in a pancreatic cancer model composed of TLR7-expressing tumor cells, the stimulation of this receptor was shown to induce an acceleration of tumor growth and reduce the expression of several antitumor molecules such as PTEN, p16 and cyclin D1, concomitantly with an increase of pro-tumoral molecules, including p21, p27, p53, c-Myc and cyclin-B1. Similarly, in a study of hepatocellular carcinoma tumor cells were shown to express TLR7, the stimulation of which induced increased proliferation of malignant cells. In addition to effects on tumor progression, TLR stimulation also impacts the efficacy of cancer treatments, particularly in the case of chemotherapy. We first demonstrated in vitro that the addition of TLR7 agonist significantly reduced the effectiveness of different chemotherapeutic drugs (cisplatin, carboplatin, doxorubicin and navelbine), used alone or in combination, to kill human lung adenocarcinoma cells. We later reproduced these effects in murine models of lung carcinoma with the elimination of antitumor efficacy of cisplatin when co-injected with a TLR7 agonist. Finally, we found that among NSCLC patients those who highly express TLR7 on primary tumor cells have a significantly reduced response to chemotherapy. Drug resistance induced by TLR7 stimulation could, conceptually, be mediated by several distinct mechanisms, such as an increase in expression of anti-apoptotic molecules, an upregulation of members of the ABC drug-transporter family, or a decrease in the levels of apoptotic promoting factors. Such mechanisms have been demonstrated in an ovarian carcinoma model in which...
stimulation of another TLR, namely TLR4 induced resistance to paclitaxel treatment through the induction of anti-apoptotic proteins.  

**Conclusions**

We have demonstrated that in opposition to the antitumor enhancement of immune cells, TLR7 stimulation of tumor cells induces tumor progression (summarized in Fig. 1). Multiple parameters could underlie this apparent discrepancy, such as the type of tumor, the level of TLR7 expression, the downstream function of TLR7 signaling in particular tumor cells, or chemotaxis of suppressive cells into the tumor. These major pathways of TLR7 stimulation could act either directly or indirectly on both immune and tumor cells, converging on cancer patient outcome.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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