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Immunomodulation of the tumor microenvironment by Toll-like receptor-3 (TLR3) ligands

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Abbreviations: HCC, hepatocellular carcinoma; MDSC, myeloid-derived suppressor cell; TLR, Toll-like receptor; polyA:U, polyadenylic-polyuridylic acid; polyI:C, polyinosinic-polycytidylic acid

In hepatocellular carcinoma (HCC) patients, the intratumoral expression of Toll-like receptor-3 (TLR3) correlates with prolonged survival. We demonstrated that TLR3 ligands can operate through three independent mechanisms: by directly killing TLR3-expressing cancer cells, by inducing T- and natural killer (NK)-cell infiltration and by activating TLR3-expressing NK cells.

While the importance of cancer cell-intrinsic genetic alterations in oncogenesis is well established, the role of the tumor microenvironment in tumor progression is being increasingly recognized. In many types of cancer, the nature and abundance of the immune cells that infiltrate the primary tumor is an independent predictor of patient survival. In most studies, primary tumor infiltration by $CD8^+$, T_H1 or natural killer (NK) cells is associated with a favorable disease outcome, while infiltration by regulatory T cells (Tregs), macrophages or myeloid-derived suppressor cells (MDSCs) correlated with accelerated tumor growth and metastatic disease. The transcriptomic analysis of neoplastic lesions has led to the identification and validation of gene signatures with prognostic and predictive values. Some of the most potent signatures derive from the tumor stroma or from tumor-infiltrating immune cells. Therefore, an attractive strategy to improve the survival of cancer patients would be to modify the tumor stroma and make it permissive for infiltration by antitumor immune cells. Such approaches could also potentiate the efficacy of anticancer vaccines, which are known to induce circulating effector immune cells in the vast majority of the patients, but promote clinical responses only in a minority of them. Knowing the crucial role of chemokines in cell trafficking, it is tempting to speculate that chemokines expressed within neoplastic lesions may determine which immune cells are recruited. Identifying signals that control the intratumoral expression of chemokines could help to develop strategies aimed at modifying the tumor immune microenvironment to promote antitumor responses.

By analyzing immune genes whose expression correlates with HCC patient survival, we became interested in Toll-like receptor 3 (TLR3).[1,2] TLR3 is a pattern recognition receptor binding double-stranded RNA and activating innate immunity upon infection by certain types of viruses. TLR3 is expressed by many cells, including immune, epithelial and endothelial cells, as well as some cancer cells. We found that HCC patients whose tumor expresses high levels of TLR3 have a 17-fold longer median survival than patients exhibiting low intratumoral TLR3 expression levels. To understand how the expression of TLR3 leads to a more favorable prognosis, we first determined which cells within the tumor express it. TLR3 turned out to be expressed by both parenchymal tumor cells (malignant hepatocytes) and by tumor-infiltrating natural killer (NK) cells. Through a series of in vitro experiments with HCC cell lines and animal studies based on two models of HCC, we showed that TLR3 ligands operate through three mechanisms (**Fig. 1**). First, TLR3 ligands directly induce cancer cell death. This is not unique to HCC cells but also applies to breast and melanoma cell lines. Second, TLR3 ligands induce the expression of CXCR3 ligands (CXCL9 and CXCL10) and CCL5, which are known to attract T_H1, $CD8^+$ and NK cells. The intratumoral expression of these chemokines has also been associated with prolonged patient survival in colorectal cancer, Ewing's sarcoma and melanoma.[3-5] Third, TLR3 ligands activate NK cells. NK cells treated with polyinosinic-polycytidylic acid (polyI:C) or polyadenylic-polyuridylic acid (polyA:U), two TLR3 agonists, express increased levels of CD69, produce high amounts of interferon γ (IFNγ) and exert cytotoxic functions against HCC cells.

The relative contribution of these three mechanisms to HCC patient survival is

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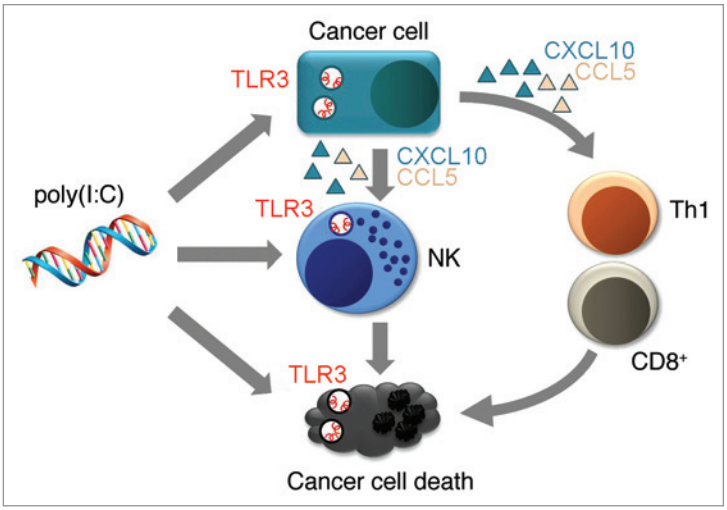

Figure 1. Proposed modes of action of TLR3 ligands. Toll-like receptor 3 (TLR3) ligands operate by (1) directly inducing the apoptotic demise of TLR3-expressing cancer cells, (2) by activating TLR3-expressing natural killer (NK) cells by (3) triggering the secretion of CXCL10 and CCL5 secretion by cancer cells. These chemokines recruit T_H1, $CD8^+$ and NK cells to the tumor bed.

unknown. However, the induction of chemokines is likely to be of prime importance: in HCC samples, TLR3 expression correlates not only with patient survival but also with chemokine production and with the intratumoral density of T and NK cells. Even if not all cancer cells in a given tumor express TLR3, this mechanism is likely to promote the bystander killing of cancer cells that have lost TLR3 expression, thereby preventing the selection of escape variants. The expression of CXCR3 ligands and CCL5 has also been associated with clinical responses to chemotherapy in melanoma patients.[6] In addition, TLR3 agonists have been shown to promote the recruitment of cytotoxic T lymphocytes (CTLs) in transplantable tumor models.[7] By working with colorectal carcinoma samples ex vivo, Kalinski et al. showed that TLR3 ligands in combination with IFNα and a an inhibitor of cyclooxygenase 2 (COX2), trigger the expression of CXCR3-ligands and CCL5.[8] CXCR3 ligands and CCL5 in turn have been shown to attract effector T cells in a variety of cancers (reviewed in ref. 9).

TLR3 agonists are attractive as candidate drugs for anticancer therapy and have been or are currently being tested in clinical trials (reviewed in ref. 9). PolyA:U was used as early as 40 years ago with no obvious toxicity. No convincing evidence of clinical efficacy was obtained at that time but retrospective analysis of a trial conducted between 1982 and 1986 in 517 breast cancer patients showed that patients whose tumor expressed TLR3 manifested a decreased risk of metastatic relapse (hazard ratio: 2) upon adjuvant treatment with poly(A:U) and locoregional irradiation, as compared with patients treated with chemotherapy (cyclophosphamide, methotrexate and fluorouracil).[7] More recently, Ampligen (polyI:C) and Hiltonol (polyICLC) were tested in patients affected by a variety of malignancies, demonstrating encouraging signs of efficacy. To our knowledge, no clinical trials using TLR3 agonists in HCC patients have been reported so far.

TLR3 is also its own target, as triggering the TLR3 pathway increases TLR3 expression levels. Therefore, the elevated expression of TLR3 in long-term cancer survivors may reflect TLR3 activation. Chronic or transient viral infections may provide TLR3 agonists, or alternatively, these may derived from danger signals released by necrotic cells, some of which operate as a TLR3 agonists.[10] We observed that TLR3 expression levels correlates with cancer cell death in HCC samples.

Taken together, these studies and our findings demonstrate the feasibility of modifying the tumor microenvironment by manipulating the expression of chemokines that are known to attract antitumor immune cells. A systematic search for drugs that are able to promote tumor infiltration by antitumor immune cells may help to improve current anticancer therapies including (but not limited to) the immunotherapy of HCC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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