

# B7x and myeloid-derived suppressor cells in the tumor microenvironment

## A tale of two cities

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A new study demonstrates the tumorigenic functions of B7x and reveals a link between B7x and myeloid-derived suppressor cells (MDSCs) within the tumor microenvironment. We propose that the binding of B7x to a hitherto unidentified receptor on MDSCs may stimulate their proliferation and/or immunosuppressive functions, hence promoting tumor growth.

B7x (also known as B7-H4 or B7S1) is a member of the B7 protein family that inhibits T-cell functions by binding to a hitherto unidentified receptor.<sup>1-3</sup> The levels of the B7x mRNA are much higher in peripheral non-lymphoid organs than in their lymphoid counterparts,<sup>1,4</sup> which is in marked contrast with the levels of mRNAs coding for the conventional B7 proteins B7-1 and B7-2. B7x is hardly detectable on the surface of immune cells<sup>4,5</sup> but is expressed on epithelial cells and other cell types in non-lymphoid including the lung and pancreas.<sup>4,6,7</sup> Therefore, B7x exhibits an unique expression pattern. This has important implications for the biological functions of the B7x signaling pathway.

The literature on B7x expression by human cancers and its links with clinical outcome has grown considerably. B7x is overexpressed by a variety of human neoplasms, including cancers of the brain, esophagus, lung, breast, pancreas, kidney, gut, skin, ovary and prostate.<sup>8</sup> Furthermore, increased B7x expression levels in many cases correlate directly with disease stage, progression or recurrence, and inversely with tumor infiltrate by lymphocytes and patient survival. Taken together, these observations raise the possibility that B7x may underpin a mechanism whereby tumor cells avoid

recognition and destruction by the immune system.

Although a consistent amount of clinical data suggested a strong correlation between B7x expression levels and poor disease outcome in cancer patients, until recently it was unclear whether such an association would be causative or merely correlative. Furthermore, the functions of B7x (be it expressed by malignant or stromal cells) *in vivo*, in the context of oncogenesis, tumor progression and response to therapy had not been elucidated. To address these issues, we took advantage of mice lacking the B7x-coding gene (*Vtcn1*<sup>-/-</sup>) and of 4T1 metastatic breast cancer cells (which do not express B7x) and investigated the effect of host B7x in tumor progression.<sup>9</sup> Strikingly, 18 d after the intravenous injection of 4T1 cells, the average number of tumor nodules in the lungs of the wild-type (WT) mice was 9-fold higher than that of *Vtcn1*<sup>-/-</sup> animals. In addition, *Vtcn1*<sup>-/-</sup> mice exhibited an enhanced survival and memory responses that provided protection against a subsequent challenge with 4T1 cells.<sup>9</sup> B7x is expressed on epithelial, but not on CD45<sup>+</sup> hematopoietic, cells, suggesting that the local amount of B7x is sufficient to promote the growth of metastatic cancer cells in lungs.

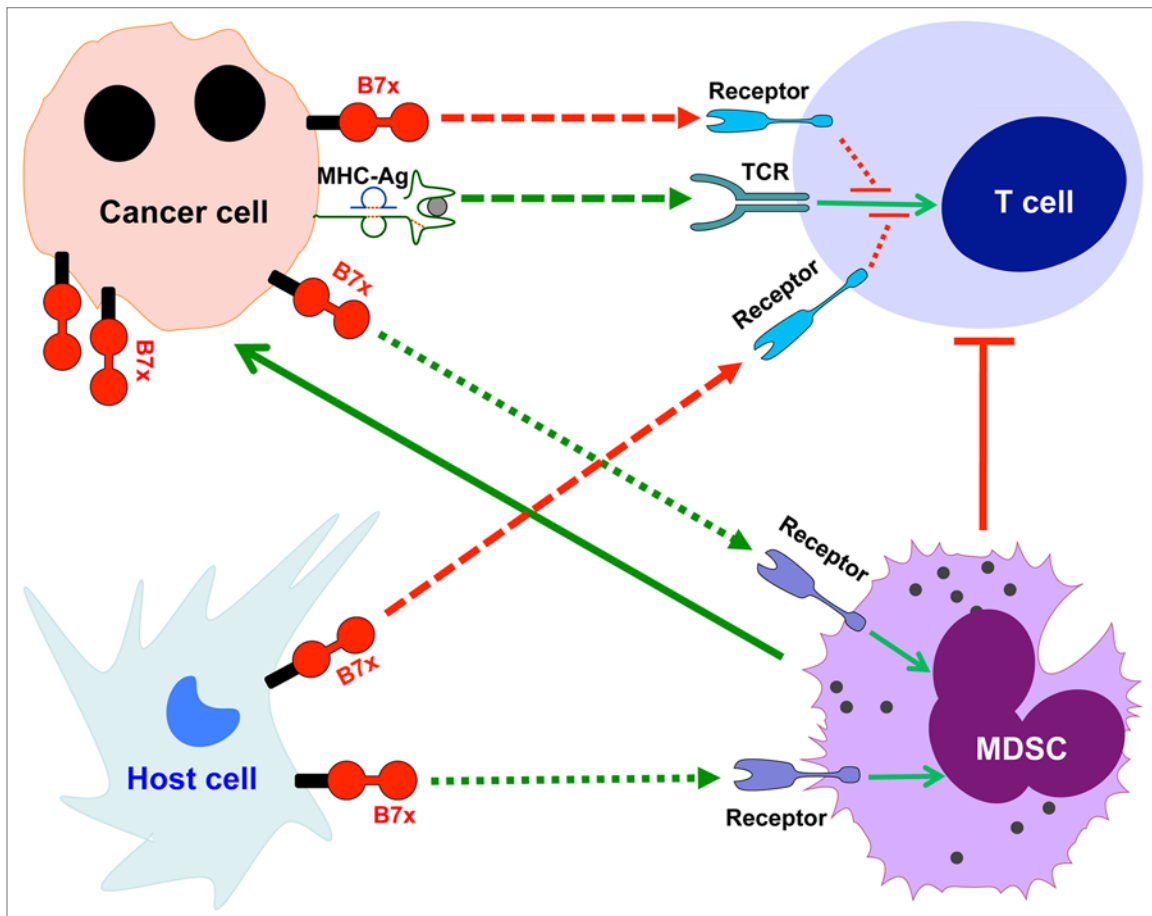
Mechanistic experiments revealed an interesting link between B7x and myeloid-derived suppressor cells (MDSCs). T cells infiltrating neoplastic lesions in *Vtcn1*<sup>-/-</sup> mice were indeed more responsive to malignant cells as compared with those isolated from cancers growing in WT mice. These results are consistent with the notion that B7x functions as a T-cell co-inhibitor. In addition, tumors growing in *Vtcn1*<sup>-/-</sup> mice exhibited a markedly decreased infiltration by immunosuppressive cells, notably CD11b<sup>+</sup>GR-1<sup>+</sup>MDSCs.<sup>9</sup> MDSCs represent a heterogeneous group of myeloid cells and are emerging as a major immunosuppressive force during tumor progression.<sup>10</sup> We therefore took a closer look at the phenotype and functions of MDSCs in our model.

MDSCs can be divided into 2 cell subsets based on the expression of Ly6C and Ly6G: CD11b<sup>+</sup>Ly6G<sup>+</sup>Ly6C<sup>low</sup> granulocytic MDSCs (g-MDSCs) and CD11b<sup>+</sup>Ly6G<sup>-</sup>Ly6C<sup>hi</sup> monocytic MDSCs (m-MDSCs). We observed that g-MDSCs account for the majority of MDSCs infiltrating 4T1 lung metastases. To further characterize these g-MDSCs, we isolated them by FACS and found that they exhibit morphological features that are typical of neutrophils, including

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**Figure 1.** B7x promotes tumor progression through interactions not only with immune effector cells but also with immunosuppressive cells. B7x is highly expressed on tumor cells but not on hematopoietic cells. B7x binds to a hitherto unidentified receptor on activated T cells, hence exerting inhibitory effect. Furthermore, B7x can bind to a receptor on MDSCs that may stimulate their proliferation and/or their immunosuppressive functions. Globally, B7x exerts therefore robust immunosuppressive functions and hence supports tumor growth.

ring-shaped and/or segmented nuclei. Therefore, the g-MDSCs that infiltrate 4T1 lung metastases are mostly tumor-associated neutrophils (TANs).<sup>9</sup> Of note, g-MDSCs represent the largest population (60%) of CD45<sup>+</sup> hematopoietic cells infiltrating tumor-bearing WT lungs, whereas they account for only 20% of the CD45<sup>+</sup> cell infiltrate in tumors growing in *Vicn1*<sup>-/-</sup> mice.<sup>9</sup> These results suggest that B7x may promote the expansion of MDSCs within neoplastic lesions. We next developed an antigen-independent system in which normal T cells are activated by plate-bound anti-CD3 antibodies in the presence of MDSCs, to examine whether g-MDSCs (or TANs) are indeed capable of suppressing T-cell function. We found that g-MDSCs from

the metastatic lungs of both WT and *Vicn1*<sup>-/-</sup> mice significantly inhibit CD4<sup>+</sup> and CD8<sup>+</sup> T-cell proliferation. Finally, we turned our attention to the hematopoietic cell infiltrate, to characterize any cell type that might express the hitherto unidentified receptor(s) for B7x, as these cells do not express B7x. Surprisingly, we found that B7x strongly binds to g-MDSCs,<sup>9</sup> suggesting that these immunosuppressive myeloid cells express the receptor(s) for B7x. Therefore, in addition to activated T cells, MDSCs also express B7x receptor(s). Interestingly enough, B7x binds MDSCs more potently than activated T cells, indicating that these two cell types may express different B7x receptors or the same receptor at highly distinct levels.

In summary, we have shown for the first time that B7x promotes cancer growth in vivo and that MDSCs express B7x receptor(s). Although the mechanisms whereby B7x functions as a pro-tumorigenic factor and the precise identity of B7x receptor(s) remain to be elucidated, it is likely that B7x enables cancer cells to escape antitumor immunity by binding not only to immune effector cells (such as CD4<sup>+</sup> and CD8<sup>+</sup> T cells) but also to immunosuppressive cells (such as MDSCs) (Fig. 1). Therefore, targeting the B7x signaling pathway holds great promise for anticancer immunotherapy.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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