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The hedgehog's trick for escaping immunosurveillance

The molecular mechanisms driving myeloid-derived suppressor cell recruitment in hedgehog signaling-dependent tumors

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Abbreviations: CCL2, chemokine (C-C motif) ligand 2; CCR2, chemokine (C-C motif) receptor 2; GEM, genetically engineered mouse; MDSC, myeloid-derived suppressor cell; SmoM2, constitutively active mutation of Smoothened; TME, tumor microenvironment

Myeloid-derived suppressor cells (MDSCs) are an important means by which tumor cells evade immunosurveillance. Here, we set out to determine how MDSCs are recruited to tumors in genetically engineered mouse cancer models. Expression of oncogenic and constitutively active SmoM2, a key hedgehog-signaling regulatory protein, revealed that MDSC recruitment to the tumor microenvironment is mediated by the CCL2/CCR2 axis in a TGFβ dependent fashion.

In addition to eliminating foreign pathogens such as viruses, the immune system also detects and eliminates neoplastic cells by recognizing tumor-specific surface antigens.[1] Malignant cells, on the other hand, develop several mechanisms to escape the immunosurveillance system. For example, cancer cells may reduce their expression of tumor-specific antigens or secrete T-cell inhibitory factors. Another important avoidance mechanism is the accumulation of lymphocytes with immune suppressive functions, such as myeloid-derived suppressor cells (MDSCs) and T regulatory cells, within the tumor milieu.[2,3] MDSCs can reduce T-cell accumulation by preventing T-cell activation and by directly killing T cells. Numerous studies using xenograft models have elucidated the molecular mechanisms by which MDSCs are produced, recruited, and activated during tumor growth. However, the mechanisms employed in genetically engineered mouse (GEM) models of cancer are not well understood.[4]

We used GEM models with inducible expression of oncogenic Smoothened (SmoM2), to investigate the molecular mechanisms responsible for MDSC accumulation in basal cell carcinomas and rhabdomyosarcomas.[5] Hedgehog signaling-dependent tumors developed in mice after induction of SmoM2 expression by tamoxifen using a keratin 14 promoter-driven creER (K14creER)/Rosa26 promoter for basal cell carcinomas, and chicken actin promoter driven-creER (CAG-creER)/Rosa26 promoter for rhabdomyosarcoma .[6,7] In both mouse models, we did not detect cells expressing cluster of differentiation 11b (CD11b) and granulocyte receptor 1 (Gr1) until visible tumors were present, suggesting that MDSCs were recruited to the tumor site by tumor-secreted factors. We also found that $CD11b^+Gr1^+$ cells at the tumor site were more active in suppressing T-cell activation than those from spleen. These results indicate that $CD11b^+Gr1^+$ cells in the spleen are likely to be immature myeloid cells whereas $CD11b^+Gr1^+$ cells at the tumor site are MDSCs. Because our system did not have activated hedgehog signaling in myeloid cells, the MDSCs were not directly regulated by hedgehog signaling.

Through a series of molecular analyses, we obtained evidence supporting the importance of transforming growth factor β (TGFβ) signaling in the tumor microenvironment (TME) for hedgehog signaling-mediated tumor development.[5,7] First, activation of hedgehog signaling results in elevated phosphorylation of Sma and Mad related family members SMAD2 and SMAD3, markers for TGFβ signaling activation in a number of cell types in the tumor microenvironment (TME). Second, the TGFβ signaling inhibitor

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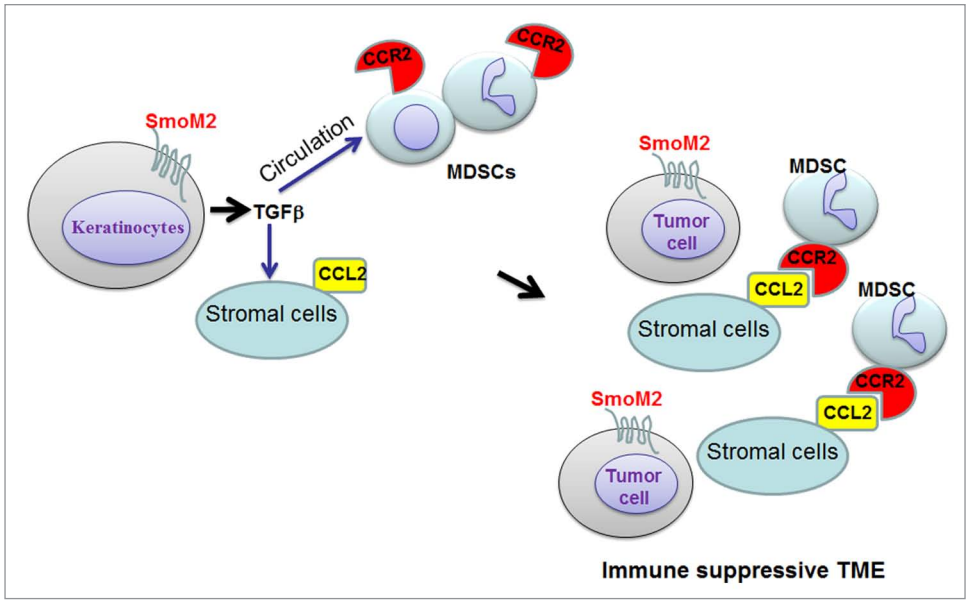

Figure 1. Model for MDSC recruitment in SmoM2-dependent tumors. Activation of hedgehog signaling in keratinocytes via expression of a constitutively activated, mutant smoothened (SmoM2) results in increased transforming growth factor β (TGFβ) signaling, primarily via TGFβ2. Activation of TGFβ signaling is observed in many cell types, including fibroblasts, $CD11b^{+}Gr1^{+}$ cells, and T cells. Since $CD11b^{+}Gr1^{+}$ cells are not present in skin tissues before tumor formation, secreted TGFβ2 presumably travels through the peripheral blood, possibly facilitated by tumor-derived exosomes. As a result of TGFβ signaling activation, the expression of chemokine (C-C) motif ligand 2 (CCL2) is increased in the tumor microenvironment (TME), whereas chemokine (C-C) motif receptor 2 (CCR2) expression is increased in myeloid derived suppressor cells (MDSC). Circulating MDSCs migrate toward the CCL2-enriched TME and remain to foster an immunosuppressive TME.

SD208 reduces the tumor size in K14-creER/R26-SmoM2 mice, which is consistent with an increase in the number of lymphocytes within the tumor milieu.[6] More importantly, we demonstrated that defective TGFβ signaling as a result of TGFβ receptor 2 (*Tgfbr2*) gene knockout in bone marrow cells prevents SmoM2-mediated tumor formation.[5] Furthermore, we found that *Tgfbr2* gene knockout in bone marrow-derived cells also reduces growth of established tumors in a basal cell carcinoma model (K14-creER/R26-SmoM2) or a B16 xenograft mouse model.[5] Similar results have been reported in other model systems[8,9] and together these data support a tumor-promoting function of TGFβ signaling in the TME.

Recruitment of MDSCs to the tumor site is likely achieved through chemokines and their receptors.[2] Our analyses of candidate chemokines, cytokines, and their receptors in the skin cancer model revealed chemokine (C-C motif) ligand 2 (CCL2)/ chemokine (C-C motif) receptor 2 (CCR2) as the best candidates.[5] We found that CCL2 was highly expressed in the tumor, with an especially high level of expression in non-lymphocyte cells. On the other hand, CCR2 was highly expressed in MDSCs. In fact, we detected a gradient of CCL2 protein, with a high level at the tumor site and a low level in the peripheral blood. The functional significance of this signaling axis for recruitment of MDSCs was demonstrated in Boyden chamber analysis. We found that addition of CCL2 alone to the bottom chamber was sufficient to induce migration of MDSCs toward CCL2 whereas incubation with CCR2 inhibitor with MDSCs prevented their migration. The functional significance of the CCL2/CCR2 axis was further demonstrated using mouse models, in which the CCR2 inhibitor RS102895 decreased tumor development in tamoxifen-treated K14-creER/Rosa26-SmoM2 mice.[5] Further analysis indicated that CCR2 inhibitor reduced the level of MDSCs at the tumor site, but had little effect on MDSC populations in the peripheral blood or spleen. These results demonstrate that secreted CCL2 protein at the tumor site is an important chemokine for the recruitment of CCR2-expressing MDSCs to the tumor.

Taking all the above data together, we propose a model for the mechanism by which MDSCs are regulated in hedgehog signaling-dependent tumors (**Fig. 1**). Activated hedgehog signaling in keratinocytes induces TGFβ signaling in the TME, which is followed by increased secretion of CCL2. The CCL2 gradient (high at the tumor site but low in the peripheral blood) helps to recruit MDSCs to the tumor site, resulting in an immunosuppressive TME.

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