Dendritic cell (DC)-based immunotherapy has great promise for cancer treatment. We have recently demonstrated that dectin-1-activated DCs trigger potent antitumor Th9 cells in vivo. Dectin-1-activated DC-induced antitumor responses rely on the induced Th9/IL-9. These findings offer new strategies for the development of more effective DC vaccines in tumor immunotherapy.

Dendritic cell (DC)-based immunotherapy is a promising approach for the treatment of cancer. DC-based immunotherapies have been tested in cancer patients for more than a decade and the recent approval of the first DC-based vaccine (Provenge, sipuleucel-T) for the treatment of prostate cancer by the Food and Drug Administration further stimulated the interests of using DC-based vaccines to fight cancer. Though these previous clinical studies proved that DC-based cancer vaccines are safe and non-toxic, their clinical benefits for patients were rare. In cancer, some immunosuppressive mechanisms in the tumor microenvironment impair DC functions and block the development of antitumor immunity, which may be the reason for their low clinical benefits in cancer therapy. Therefore, strategies to generate more effective DCs for cancer therapy will be crucial for increasing the antitumor benefits of DC-based immunotherapy.

DCs exert the antitumor effects through the induction of effector T cells, which include CD4<sup>+</sup> Th helper (Th) cells and CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs). There is an increasing list of Th cell subsets, and among these, Th1, Th2, Th9, and Th17 cells are involved in antitumor immunity. Generally, Th1 cells are traditional antitumor Th cells, whereas Th2/17 cells may also play a role in antitumor immunity. We and others have shown that IL-9-producing Th9 cells mediate potent antitumor effects, much better than other Th cells. We demonstrated that Th9 and IL-9 could not directly kill the tumor cells, but rather through the stimulation of tumor-specific CTL responses in vivo. Considering the potent antitumor capacity of Th9/IL-9, we were curious as to whether we could generate some types of DCs that preferentially induced Th9/IL-9 and we speculated that these kinds of DCs will potently improve the antitumor clinical benefits of DC-based cancer immunotherapy. Dectin-1 is a major β-glucan receptor on DCs, macrophages, and neutrophils. We recently used dectin-1 agonist curdlan or Scleroglucan to activate mouse bone marrow (BM)-derived immature DCs (CurDCs or ScIDCs) and used these DCs to prime naive CD4<sup>+</sup> T cells under Th9-polarizing conditions. We found that dectin-1-activated DCs potently promoted the development of Th9 cells, with upregulated expression of IL-9- and Th9-related transcription factor IRF4, but not other Th cell-related cytokines and transcription factors. Importantly, in the in vivo tests, dectin-1-activated DCs but not regular BMDCs stimulated the powerful production of Th9 cells and IL-9. Since Th9 cells are potent antitumor effector cells, dectin-1-activated DCs may be manipulated for clinical uses in cancer immunotherapy.

Th cell differentiation depends on antigenic, costimulatory, and cytokine-mediated signals provided by DCs. To gain mechanistic insights into how dectin-1-activated DCs may induce Th9 cells, we performed microarrays to analyze gene expression profiles by dectin-1-activated DCs. We found that 42 of cytokine, chemokine, and costimulatory molecule genes were upregulated in dectin-1-activated DCs, including the TNF family members TNF-α, TNFSF15, OX40L, and TNFSF8 and low IL-12. OX40/OX40L axis was reported to inhibit the production of regulatory T cell and Th17 cells and drive Th9 cell induction. TNFSF15 was recently shown to potentially promote Th9 differentiation. These observations suggested that dectin-1 stimulates DC production of a specific profile of cytokines, chemokines, and costimulatory molecules, especially TNFSF15 and OX40L, which favor Th9 cell induction in vivo. Indeed, in the functional tests, blocking OX40/OX40L axis or TNFSF15 by antibodies or OX40-deficiency in T cells inhibited dectin-1-activated DC-induced Th9 differentiation, demonstrating the important role of OX40L and TNFSF15 in dectin-1-activated DC-induced Th9 differentiation.

We demonstrated that dectin-1-activated DC-based cancer vaccines induced potent therapeutic antitumor effects in B16-OVA melanoma OT-II and MPC-11 myeloma Balb/c mouse models. As about the mechanisms of dectin-1-activated
DC-induced antitumor activities, we found that dectin-1-activated DC vaccination potently promoted the production of Th9 cells and IL-9 in vivo, whereas dectin-1-activated DCs moderately increased the induction of Th1 and Th17 cells. In B16-OVA melanoma OT-II mouse model, administration of IL-9 neutralization Abs inhibited the antitumor response against established melanoma tumors, indicating that dectin-1-activated DCs induced antitumor effects in a Th9/IL-9-dependent manner. However, since Th1 and Th17 cells are traditional and potential antitumor effector cells, respectively, Th1 and Th17 cells may also be involved in dectin-1-activated DC-induced antitumor effects in vivo. We found that dectin-1-activated DCs induced potent tumor-specific CTL responses in MPC-11 myeloma Balb/c mouse model, and administration of an IL-9-neutralizing Ab inhibited CTL responses induced by dectin-1-activated DCs, indicating that the induction of Th9/IL-9 by dectin-1-activated DCs in vivo resulted in increased antitumor CTL responses and suggesting that tumor-specific CTLs may be related to the antitumor effects induced by dectin-1-activated DCs. However, considering that OT-II mice used in this study largely lack CD8+ T cells (i.e., no CD8+ CTLs), Th9/IL-9 may mediate the antitumor effects induced by dectin-1-activated DCs through some other unknown mechanisms that need further investigations and clarification. Fig. 1 depicts the potential mechanisms underlying dectin-1-activated DC-induced antitumor effects in vivo.

We demonstrated that dectin-1 signaling stimulates DC production of a specific profile of cytokines, chemokines, and costimulatory molecules that favor the induction of antitumor Th9 cells in mouse BM-derived DC model. Further investigations are needed to validate these findings by using human monocyte-derived DCs, the major source of DCs for DC-based immunotherapy against human cancers. We also demonstrated the potent antitumor effects of dectin-1-activated DCs in mouse models. Further clinical studies are needed to determine the potential and potency of dectin-1-activated DC-based vaccines in fighting human cancers.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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