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Conventional type 1 dendritic cells in protective antitumor immunity and its potential in hepatocellular carcinoma

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Abstract

Immunotherapy is revolutionizing the clinical management of cancer patients by modulating T cells and natural killer cells. Dendritic cells (DCs) have the capacity to orchestrate the expansion and function of these effector cells both in lymphoid and non-lymphoid tissues of cancer patients. Distinct subtypes of DCs have various capacities to prime and activate different T cell responses. Here, we review conventional type 1 dendritic cells (cDC1s) and their crucial role in protective anti-tumor immunity. Targeting cDC1s as a cancer vaccine against the development of hepatocellular carcinoma will be discussed.

Keywords: Conventional type 1 dendritic cells, antitumor immunity, hepatocellular carcinoma, cancer vaccine

INTRODUCTION

Immunotherapy is now widely considered as an important tool for the treatment of individuals with cancer. Several effective immunotherapy approaches have been developed over the past decade, including adoptive cell transfer (e.g., CAR-T therapy) and immune checkpoint blockade (e.g., anti-PD1/PDL1 antibodies)[1,2]. In solid tumors including hepatocellular carcinoma (HCC), the tumor microenvironment contains a large amount of stromal cells and immune cells, which shape cancer development and impact

upon the response to tumor therapy. The effectiveness of immune-based therapeutic strategies clearly demonstrates the possibility of eradicating cancer through cellular immunity, particularly when active T cells recognize cognate tumor antigens[1,3]. Dendritic cells (DCs) have the capacity to prime naïve T cells by inducing functional polarization, and are in charge of orchestrating the expansion and functions of T cells and natural killer (NK) cells in lymphoid and non-lymphoid tissues of cancer patients. About 25 years ago, some investigators put forward the concept of harnessing DC immunogenicity to induce protective responses in cancer patients[4,5]. Nevertheless, results have been far below expectations. These failures occurred due to the almost exclusive usage of monocyte-derived cells (MoDCs) and tumor-associated antigens. The advancement in basic understanding of the heterogeneity and functional plasticity of different DC subsets suggests that conventional (classical) type 1 DCs (cDC1s) other than MoDCs may be better suited for this purpose.

DENDRITIC CELL SUBSETS

Shortly after the discovery of DCs by Steinman in 1973, van Furth grouped them under the mononuclear phagocyte system. Since then monocytes, macrophages and DCs have been grouped together, and they are distinguished on the basis of their morphology, function and origin[6]. However, lineage-tracing studies by different groups demonstrate that most macrophages in adults are maintained independently of blood monocytes and rely on self-renewal of embryonically derived macrophages under steady-state conditions[7,8]. The circulating monocytes could contribute to the expanding pool of macrophages in the liver under inflammation status[9,10]. DCs arise from a common DC precursor in the bone marrow, and their development depends on the cytokine Flt3L[11,12]. Although monocytes in some inflammation develop to inflammatory DCs, termed IDCs[13], DCs are now generally grouped into conventional (classical) type 1 DCs (cDC1s), conventional type 2 DCs (cDC2s) and plasmacytoid DCs (pDCs)[14].

In mice, cDC1s include murine lymphoid $CD8\alpha^+$ DCs, migratory $CD103^{high}$ DCs, and dermal $CD207^+$ DCs; cDC2s include lymphoid $CD4^+$ DCs and migratory $CD11b^+$ DCs; pDCs are IFN-producing DCs with the cell surface markers B220, PDCA.1 and Ly6C[14]. DCs have been divided into many different subsets based on the expression of surface markers including CD40, CD11c, CD103 (integrin αE), CD11b (integrin αM), F4/80, CD8α, CD24, CD172a (SIRPα and SHPS1), CX_3C-chemokine receptor 1 (CX_3CR1), XC-chemokine receptor 1 (XCR1), CLEC9A (DNGR1), E-cadherin (cadherin 1) and CD64 (FcγRI)[14]. Inflammation further complicates the picture as mononuclear phagocytes in inflamed tissues undergo phenotypical changes. Some researchers consider monocyte-derived cells as MoDCs or macrophages, based on CD11c expression[13-15]. The analysis of gene expression profiles revealed that the gene transcripts in different populations of macrophages are diverse, and some mRNA transcripts and surface proteins were selectively expressed by macrophages but not DCs[16]. Therefore, macrophages, monocytes and DCs are different cell types with distinct ontogeny[7-14,16].

DC-BASED CANCER VACCINES

DC-based cancer vaccines typically culture DCs with various tumor associated antigens *ex vivo*, such as pulsing with peptides, whole proteins, tumor lysates, or fusion of DCs with entire tumor cells[1,4,5]. Most DCs were generated from peripheral blood monocular cells (PBMCs)-derived $CD14^+$ monocytes or $CD34^+$ hematopoietic stem and progenitor cells via culturing with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin 4 (IL-4). The efficacy of such vaccine formulations is suboptimal since the macrophages were generated in culture with GM-CSF and its migratory capacity limited to lymph nodes[1,5].

To overcome these tedious processes and the uncertainty of quality, approaches that home antigens directly to DCs *in vivo* via DC receptors were developed, such as antibodies against DEC-205, Clec9A and Clec12A[1,4,17,18]. By using model antigens, i.e., OVA, or antigens from microbial products, direct targeting

strategies showed some protective effects on tumor growth[4,18,19]. However, these effects were suboptimal in clinical trials when the antibody against DEC-205, which is expressed in different subsets of DCs in human, was conjugated with the tumor antigen NY-ESO[20]. Therefore, when contemplating DCs for *in vivo* targeting for tumor vaccination, it is important to consider the differences of targeting DC receptors and their function[1,5].

THE CDC1S AND THEIR ROLES AGAINST CANCERS

The "cross-presentation" by DCs of tumor antigens that are expressed in solid tumors is crucial for generating effective $CD8^+$ cytotoxic T lymphocytes (CTLs)[1,21]. Accumulated experimental evidence revealed that distinct subtypes of DCs show various capacities to prime and activate different T cells[1,5]. Mouse cDC1s were identified as the most efficient cells in cross-presenting cellular-associated antigens to initiate $CD8^+$ T cells[22-25]. The following transcription factors *ICSBP (Irf8)*[26], *Id2*[27], *Batf3*[28], *Nfil3*[29] were reported to control the development of cDC1s. Further epistasis analysis after single-cell RNA sequencing revealed a genetic hierarchy. The *Nfil3* induces a transition from common DC precursors to express high levels of *Id2* and low levels of *Zeb2*. Upon *Id2* induction, *Batf3* expression is increased and *Zeb2* is repressed. Meanwhile, *Id2* extinguished E-protein activity at the +41-kb *Irf8* enhancer, and the expressed BATF3 acted at the +32-kb *Irf8* enhancer to maintain *Irf8* activation for the commitment of cDC1 clonogenic progenitors[30,31]. Currently, the homolog and functional equivalent of mouse cDC1s have been identified as $CD141\ (BDCA3)^+ XCR1^+$ DCs in humans[14,32,33]. Both mouse and human cDC1s selectively express the chemokine receptor XCR1 and C-type lectin endocytic receptor CLEC9A[4,14,34,35].

The role of cDC1s in antitumor immunity was investigated in different animal models. In the Batf3-/- mouse, the rejection of highly immunogenic syngeneic tumors was impaired[28]. Using 2-photon intravital imaging, the spatial organization of DCs and macrophages within the tumor and their dynamics with T cells were analyzed. It was found that macrophage populations were preferentially marginating tightly on tumoral lesions, DC populations were typically in separate collagen-rich zones distal to tumor lesions. Stable T cell interactions were largely confined to tumor margins, where macrophage populations dominated and DC numbers were little[36]. Analyzing the different types of human tumors in TCGA data revealed that patients with a high ratio of the $CD103^+/CD103^-$ gene showed better survival compared to those with a low ratio[37]. In mouse melanoma models, it was found that $CD103^+$ DCs were the only antigen-presenting cells to transport intact antigens to lymph nodes for priming tumor-specific $CD8^+$ T cells. When $CD103^+$ DC progenitors in the tumor were expanded and activated, the effects of anti-checkpoint inhibitor were enhanced for anti-tumor responses[38].

In the context of cancer, there are many different cell populations. In addition to cDC1s, several myeloid cell populations, including tumor-associated macrophages, are also able to acquire tumor material. However, cDC1s have been demonstrated to be superior to other cells in stimulating T cell activation and proliferation[36-38]. The stimulatory function of cDC1s in tumors is not restricted to T cells only. IL-12 production by these cells support IFN-γ production from NK cells for eradicating established tumor cells[39,40]. More importantly, activated NK cells can generate the chemokines CCL5 and XCL1 to recruit cDC1s into the tumor microenvironment and promote cancer immune control[25]. In melanoma patients, it was found that the abundance of cDC1s is associated with intra-tumor gene expression of the cytokine FLT3LG, which is predominantly produced by NK cells in tumors. The numbers of cDC1 is correlated with NK frequency and the patients' response to anti-PD1 immunotherapy[41]. It was found that cDC1s might be the main source of CXCL9 and CXCL10, and these chemokines are able to recruit $CXCR3^+$ effector T cells[24,40,42,43]. Therefore, the cDC1s in tumors and their interactions with NK and effector T cells establish local anti-tumor immunity to control and eventually, eradicate established tumors[24,40].

TARGETING CDC1S FOR HCC INTERVENTION

Some patients with a variety of cancers, including HCC, benefit from immune checkpoint inhibitors[44,45]. Since not all HCC patients are sensitive to this therapy, research has found that WNT activation correlated with T cell exclusion and resistance to anti-PD1 therapy[46,47]. By using a murine autochthonous liver cancer model based on hydrodynamic tail vein delivery of different genetic elements, it was found that WNT activation led to defective DC recruitment, mainly cDC1s. As a consequence, the anti-tumor immune response was impaired. The impaired immunity in HCC from WNT activation might be due to decreased CCL5 secretion by the tumor[48]. These investigations pointed to the importance of cDC1 recruitment in HCC immunotherapy.

HCCs arising in cirrhosis are usually preceded by the appearance of malignant precancerous nodules[49]. The liver of a cirrhotic patient may harbor either a single, benign or precancerous malignant nodule, or even both. HCC progression could be interfered with if the malignant progenitors in cirrhosis were eliminated. At this stage, it is difficult to treat with conventional means such as surgery, radiation, and chemotherapy. However, it may still be controllable by stimulating the immune response through cancer vaccines. Tumor-associated antigens, which are re-expressed in tumor and not/lowly expressed in normal tissues, might potentially be appropriate targets[50]. Previous studies have documented that inducing the generation of specific T cells to alpha-fetoprotein (AFP), which is re-expressed in most HCCs, led to tumor regression[50] and prevented carcinogen-induced murine autochthonous HCC[51]. However, in regenerating mouse liver significant hepatocyte damage was observed[52]. Two decades ago, glypican-3 (GPC3) was identified as a new HCC-associated antigen[53]. Different from AFP, it is undetectable in cirrhotic livers, or even in benign hepatic lesions. Tissue expression of GPC3 was used to discriminate the nature of a < 2 cm hepatocellular lesion lacking HCC radiological features detected in a cirrhotic patient under surveillance. Up to 60% of early HCCs showed immunoreactivity to GPC3, either as membranous and/or cytoplasmic staining in biopsy material[53]. We therefore hypothesized that eliciting the host's own specific T cell immunity against GPC3 could interfere with disease progression in cirrhosis patients.

With the growing importance of cDC1s in initiating effective anti-tumor immunity, and the selective expression of XCR1 on these cDC1s, the XCL1-GPC3 fusion protein may be an efficient cancer vaccine. We have linked the XCL1 chemokine to GPC3 for *in vivo* targeting to induce GPC3-specific CTLs for eliminating GPC3-positive HCC. Our results showed that the expressed XCL1-GPC3 chemoattracted murine $XCR1^+CD8\alpha^+$DCs and human $XCR1^+CD141^+$DCs *in vitro* and promoted IL-12 production. After the *mXCL1-GPC3* plasmid was injected subcutaneously, the expressed mXCL1-GPC3 protein was detected mainly in $CD8\alpha^+$DCs of mice draining lymph nodes. XCL1-GPC3-targeted DCs enhanced antigen-specific $CD8^+$ T cell proliferation and induced the *de novo* generation of GPC3-specific $CD8^+$ T cells, which abolished GPC3-expressing tumor cells both in the murine and human systems. In a murine autochthonous liver cancer model of AlBlHBV mice, the 3-dose (30-μg in total) immunization suspended tumor development with significantly reduced tumor incidence and tumor load compared with *GPC3*-immunized mice. Notably, the mouse livers have increased infiltration of GPC3-specific $CD8^+$ T cells, activated NK cells and NKT cells after *mXCL1-GPC3* immunization. The antitumor effects of these cells were further enhanced by the administration of anti-PD-1[40]. Therefore, the XCL1-GPC3, which targets cDC1s, might be a promising cancer vaccine to compensate for the deficiency of checkpoint blockades in HCC immunotherapy.

SUMMARY

The presence of cDC1s in the tumor microenvironment is often associated with a good prognosis in cancer patients and better response to immune checkpoint inhibitors. This cell population is an attractive target for delivering HCC tumor antigens to induce antitumor specific T cell responses. The combination of cDC1-

targeting cancer vaccines and checkpoint inhibitors will improve the efficacy of immunotherapy. Murine models have proven the effect of HCC cancer vaccines in compensating for anti-PD-1 by targeting cDC1s. However, clinical trials of such cancer vaccine are still required. It is also necessary to understand how cDC1s regulate anti-tumor immunity.

DECLARATIONS

Authors' contributions

Manuscript draft and finalization: Qu C
Participated in drafting of the manuscript: Chen K, Cheng SY

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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