

The Clinical Impact of the Dendritic Cell-based Cancer Vaccine: the Role in the Inflammatory Tumor Micro-environment

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As a novel therapeutic module, the dendritic cell (DC) based cancer vaccine has been recognized with great hope in eliminating cancers, including minimal residual cells, without harming normal tissue. A key factor in initiating and operating the immune system against foreign bodies including tumor cells, the DC has been regarded as the next possible breakthrough in new cancer therapy. However, the results of more than 15 years of clinical studies with DC vaccine revealed the difficulties fulfilling this expectation. Evidence has disclosed that the DC activation required for proper tumor-specific effector CD4⁺ and CD8⁺ T cell stimulation is inhibited in the micro-environment of cancer. Studies have further reported that DC phenotypes in cancer tissue and draining lymph nodes are mostly immature, which results in regulatory immune responses. Also, the existence of myeloid derived suppressor cells (MDSCs) and tumor associated macrophages (TAMs) adversely affect both DC function and immune suppression in the cancer-environment. In this review, the impact of an inflammatory micro-environment induced by cancer on the effect of DC-based cancer immunotherapy and the possibility of a clinical efficacy improvement are discussed.

Key Words: *Cancer immunotherapy, DC-based cancer vaccine, tumor micro-environment*

Introduction

The role of DC is at the center of the immune system by initiating, progressing and regulating the responses against pathogens including tumors. After the first successful clinical achievement in DC-based immunotherapy trials in follicular lymphoma and melanoma in the mid-1990s,^{1,2} the DC vaccine has been successfully used to treat patients with melanoma, lymphoma and renal cell carcinoma.³⁻⁷ However clinical expectations have not been fulfilled due to an overall clinical response rates of under 10~15%,

the usual response rates observed in various types of immunotherapies.⁶⁻¹¹ Although the clinical expectation has not been satisfied, the outcomes of many clinical trials with tumor antigen-loaded conventional DCs have provided proof that therapeutic immunity can be elicited.¹²⁻¹⁴ The clinical data has helped to establish a standard for properly activated DCs with appropriate form and doses of loading antigens. These activated DCs can migrate to the lymph nodes which then initiate and expand tumor-specific CD4⁺ and CD8⁺ T cell responses and later induce meaningful therapeutic responses in patients.

Several mechanisms are involved in unsatisfactory anti-tumor responses of DC vaccine in the clinic. Mechanisms include; the presence of keep leukocytes like MDSCs, TAMs with or without the presence of constitutive

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p-STAT3 signaling, immunoediting, abnormal tumor vasculature inhibiting effector T cell entry or tumor cell interaction with the stromal environment.¹⁵⁻¹⁹ On the other hand, in order to improve the DC vaccine clinical efficacy, it is critical to control the therapeutic DC quality and standardize the vaccine design and protocol. Looking at this very view, several investigators have analyzed DC vaccine problems in their publications.^{4,6,12,14,20-22} Thus, without further discussing about the DC vaccine quality, cancer or host side hindering factors and the possibility of improving antitumor immune-therapeutic efficacy will be discussed in this review.

DCs in cancer patients

DCs are lymphocytes in the immune system which control overall immunity by interacting with other immune cells, including T cell, B cell and NK cells.^{6,23-24} DCs themselves are a complicated system consisting of various anatomic localizations, subsets and functions that are correlated with one another. DCs control the immune system, not only in stimulatory but also in regulatory immunity as professional APC.^{22,24} In cancer tissues or cancer-draining lymph nodes, DCs are found as resting, non-activated and immature cells.²⁵⁻²⁹ Cancer-induced immunosuppressive milieu generally causes a decrease in the numbers of conventional myeloid DCs in patients.²⁵ In rodent models, immature myeloid DCs promote the expansion of regulatory T cells (T_{reg}) in tumor-draining lymph nodes, which are associated with tumor progression in a TGF- β dependent fashion. Immunosuppressive factors, mostly pro-inflammatory molecules from the cancer micro-environment, target endogenous DCs in patients resulting in dysfunction and impaired development of tumor-specific effector lymphocytes.³⁰⁻³¹ Typical inflammatory mediators of tumor-induced DC dysfunctions include; IL-10, TGF- β , VEGF, IL-6 and prostanoids such as PGE_{2,6}.³²⁻³⁵ These mediators are produced from either the cancer itself or the infiltrated host factors including MDSCs and TAMs. In this milieu, DCs are having trouble maturing, expressing

the co-stimulatory molecules needed for T cell activation, and producing the cytokines needed to support tumor specific effector T cell activation and survival.³⁶⁻³⁹ Cancer-related malfunctions of DCs are noted in patients with ovarian, breast, melanoma, renal cell, prostate carcinoma,⁴⁰⁻⁴³ and in the blood of head & neck, lung and breast cancer patients.^{36,44} The major intracellular signaling pathway required for DC activation and final maturation in the immunosuppressive milieu of the cancer micro-environment is STAT3.⁴⁵ Oncogene or cytokine-induced over-expression of the STAT3 protein in cancer cells up-regulates the expression of several immunosuppressive cytokines, including IL-10 and TGF- β , and suppresses Th₁ cell immune responses.^{15,46-47} STAT3 expression from cancer cells leads to STAT3 production by a variety of leukocytes, including DCs. STAT3 expression in tumor-associated DCs causes reduced expression of co-stimulatory and MHC II molecules, and correlates with an accumulation of immature DCs, which may induce T_{reg} ,⁴⁸ an inhibitor of effector T cell function. Anti-tumor effects of the STAT3 inhibitor, Cucurbitacin I was observed in mice, human cancer cell lines, and in vivo mouse tumor models.⁴⁹⁻⁵⁰ Although dysfunctional tumor-associated DCs may support immune suppression and promote oncogenesis, it may be possible to evoke therapeutic antitumor activity in these DCs by molecularly defined triggers of DC maturation, causing induction of tumor-specific effector T cells.

Inflammatory nature of tumor micro-environment

The development of about 15~20% of malignancies worldwide are known to be related to chronic inflammation, including esophageal, gastric, hepatic, pancreatic, and colorectal cancer.⁵¹ Inflammatory mediators produced by the cancer cell can create an inflammatory micro-environment and cause both leukocyte recruitment and angiogenesis.⁵²⁻⁵⁴ Also, these inflammatory milieus can help tumor cell survival, motility and chemotaxis. For example, breast cancer cells are known to produce the inflammatory

chemokines CCL2 and CCL5, which are poorly expressed in normal breast cells. These chemokines recruit TAMs and inhibit potential antitumor effector T cells.⁵⁵ In other words, the immunosuppressive tumor micro-environment is created by the inflammatory nature of tumors and an infiltration of assorted leukocytes, in particular MDSCs and TAMs. This infiltration leads to the suppression of the DC-induced effectors, CD4⁺ and CD8⁺ T cell responses and the induction of T_{reg}.²⁵

MDSCs in the tumor micro-environment

The mechanisms by which chronic inflammation promotes the onset and development of tumors are classified into non-immunological and immunological ways.⁵⁶ The non-immune mechanisms include 1) the production of reactive oxygen species which cause DNA mutation, 2) the production of pro-angiogenic factors, like VEGF which promote tumor neo-vascularization, 3) the production of matrix metalloproteases which facilitate invasion and metastasis.⁵⁷⁻⁵⁹ The predominant immune mechanism is the disturbance of myelopoiesis and hemopoiesis, which causes a deficiency in APCs and in dysfunctional cell-mediated antitumor immunity. A key molecule in this deficiency is MDSC.²⁵ In individuals with an established cancer, MDSCs are a major factor in preventing the efficacy of cancer vaccines that require an immune-competent host.⁶⁰ In most cancer patients and experimental mice tumor settings, the accumulation of MDSCs in the blood, lymph nodes, bone marrow and tumor sites is observed. These cells are known to inhibit both adoptive and innate immunity. MDSC induction and recruitment into the tumor site is mediated by tumor-secreted and host-secreted factors, many of which are pro-inflammatory molecules. Thus it may be said that inflammation promotes the accumulation of MDSCs, which down-regulate immune surveillance and antitumor immunity, thereby facilitating tumor growth.⁵⁶

Identification of MDSCs in cancer patients and experimental mice were analyzed by the activity in T cell suppression. In mice, MDSCs are characterized as Gr1⁺CD11b⁺

expressing cells. Gr1 includes Ly6C, a macrophage marker and Ly6G, a neutrophil marker. CD11b is the characteristic marker of macrophage.⁵⁶ In some subsets of MDSCs, several markers are ascribed, including the IL-4 and IL-13 receptor alpha chain (IL-4Ra),^{61,62} F4/80, a macrophage marker,^{61,63,64} *c-fms* (CD115),⁶⁴ and CD80.⁶⁵ Among the MDSCs, mononuclear cells are defined as "monocytic" CD11b⁺Ly6G⁺/-Ly6C^{high}, whereas "granulocytic/neutrophil-like" multilobed nuclei possessing cells are characterized by CD11b⁺Ly6G⁺Ly6C^{low}.^{63,66,67} Immunosuppressive substances produced from MDSCs include arginase, inducible NO synthase, and/or ROS.⁶⁸⁻⁷² Unlike mice, MDSC characterization in cancer patients is complicated but typically characterized by the phenotype CD11b⁺CD33⁺CD34⁺CD14⁻HLA-DR⁻ with various expressions of CD15 and other markers. Recent findings have identified CD14⁺HLA-DR^{-/low} as a new MDSC subtype in melanoma and hepatoma patients.⁷³⁻⁷⁶ It is known that different tumors induce different subtypes of MDSCs in cancer patients.^{73,74} Along with heterogeneity characterized by the surface phenotype, internal markers, morphology and suppressive substances in both mice and humans, MDSCs suppressed multiple immune effectors include; inhibition of CD4⁺ and CD8⁺ T cell functions,⁷⁷⁻⁸⁰ induction of T_{reg} by secreting TGF- β , IL-10 or arginase,⁶⁴ interaction with NKT cells to enhance tumor growth by suppressing antitumor immunity.⁸¹

Improvement of clinical efficacy of the DC vaccine

Considering the inflammatory tumor micro-environment and dysfunctional DCs with suppressed-immunity in cancer patients, it is not surprising to see recent reports indicating that the cancer vaccine induced tumor-specific T cells is not necessarily associated with the induction of functional cytotoxic T lymphocytes, but instead leading to the undesirable activation and expansion of regulatory T cells.¹⁴ Tumor antigen-induced immune responses are weak or ineffective, because unlike infectious pathogens, tumors do

not induce the strong enough inflammatory responses for the optimal activation of DCs. Thus, the primary purpose of a cancer vaccine is to overcome this defect by educating DCs with a stronger antigenic signal and providing optimal conditions for the maturation into potent immune-stimulatory APCs.²² In the immunosuppressive milieu in cancer patients, sufficient numbers of properly activated tumor-specific Th₁ cells and CTLs are not generated despite ample expression of tumor-associated antigens in cancers. The effects of therapeutic cancer vaccines, including DC based therapy, can be expected to enhance by combination with the methods that overcome the immune-suppression associated with cancer. Such therapies include; 1) administration of STAT3 inhibitors, 2) local or systemic treatment with molecularly defined triggers of DC activation, such as TLR ligands and CD40 agonistic antibodies 3) treatment with monoclonal antibodies that block inhibitory co-stimulation pathways, CTLA-4, and PD-1 4) antibodies that enhance the T cell effector function, including OX40.¹³ Another therapeutic vaccination protocol can combine improved DC vaccine with chemotherapy to exploit immunogenic chemotherapy regimens.⁸² Conclusively, correcting the immunological balance in the cancer micro-environment from suppression to a tumor-rejecting condition may be the key factor in succeeding with a DC vaccine clinical trial.

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