

UC Irvine

UC Irvine Previously Published Works

Title

Dendritic cells based immunotherapy.

Permalink

<https://escholarship.org/uc/item/6c7752q6>

Journal

American Journal of Cancer Research, 7(10)

ISSN

2156-6976

Authors

Shang, Na

Figini, Matteo

Shangguan, Junjie

et al.

Publication Date

2017

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Review Article

Dendritic cells based immunotherapy

Na Shang¹, Matteo Figini¹, Junjie Shangguan¹, Bin Wang¹, Chong Sun¹, Liang Pan¹, Quanhong Ma¹, Zhuoli Zhang^{1,2}

¹Department of Radiology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; ²Robert H. Lurie Comprehensive Cancer Center, Chicago, IL, USA

Received September 6, 2017; Accepted September 14, 2017; Epub October 1, 2017; Published October 15, 2017

Abstract: Dendritic cells (DCs) are the most potent antigen-presenting cells, and tumor antigen-loaded DCs (DC-vaccines) can activate tumor-specific cytotoxic T lymphocytes (CTLs) in lymphatic tissues. DC vaccination is a newly emerging and potent form of cancer immunotherapy and has clinically relevant mechanisms of action with great potential for the systemic treatment of cancers. However, clinical trials have demonstrated relatively poor therapeutic efficacy. The efficacy of DC-vaccines is strongly influenced by various techniques for the priming antigen loading onto DCs and their ability to migrate to the draining lymph nodes (LNs). Therefore, it is critical to improve DC-vaccines homing to draining LNs after administration in order to optimize DC-based therapy for individual patients. This review underlines 1) appropriate strategy to load tumor antigens onto DCs and 2) to optimize vaccine administration methods to ensure loaded DCs can migrate to LNs, in particular, Intraperitoneal (IP) injection. IP injection of DC-based vaccine may be a potential regimen for gastrointestinal tumors including hepatocellular carcinoma (HCC) and pancreatic adenocarcinoma (PDAC) since huge populations of LNs are present throughout the gastrointestinal track. Which might improve the subsequent migration to LNs.

Keywords: Dendritic cells, cancer vaccine, immunotherapy, hepatocellular carcinoma, pancreatic cancer

Introduction

Dendritic cells (DCs) are a specialized family of professional antigen presenting cells (APCs) with unique ability to initiate and maintain primary immune responses when pulsed with antigens [1-3]. DCs were first observed by Paul Langerhans in 1868, then Ralph Steinman and Zanvil Cohn identified DCs from mouse spleen in 1973 [4]. Starting in the 1990s, protocols for in vitro culture of mouse and human DCs were established which accelerated the study of DCs [5].

DCs are important for inducing cellular and humoral immunity and can also activate natural killer (NK) cells and natural killer T (NKT) cells [6]. DCs play a critical role at the interface between the innate and adaptive arms of the immune system. These properties have driven attempts to develop vaccines containing DCs loaded with tumor antigens, for induction of anti-tumor immune responses in patients with cancer [7, 8]. DCs can be used as preventive

vaccines as well as therapeutic vaccines against cancer. Preventive vaccines are designed to prevent diseases and induce pathogen specific T-cells to establish immune memory, while therapeutic vaccines aimed to raise a specific immune response against existing tumor cells [9, 10]. DC vaccines have become a promising tool for cancer immunotherapy due to considerable advances related to their biology and their role in T-cell activation, which has clearly opened avenues for the development of vastly improved clinical protocols [11-13].

Multiple factors contribute to the decreased effectiveness of DC-induced antitumor responses in tumor-bearing hosts such as: the low number of DCs in the tumor site, poor access of DCs to tumor antigen, the limited capacity of tumor cells to activate intratumoral DCs [14-16], and the secretion of cytokines by the tumor cells that inhibit DC maturation [17, 18]. The purpose of this review is to summarize the current methods in preparing and delivering DCs vaccines, with a special emphasis on DCs vaccination in

Dendritic cells based immunotherapy

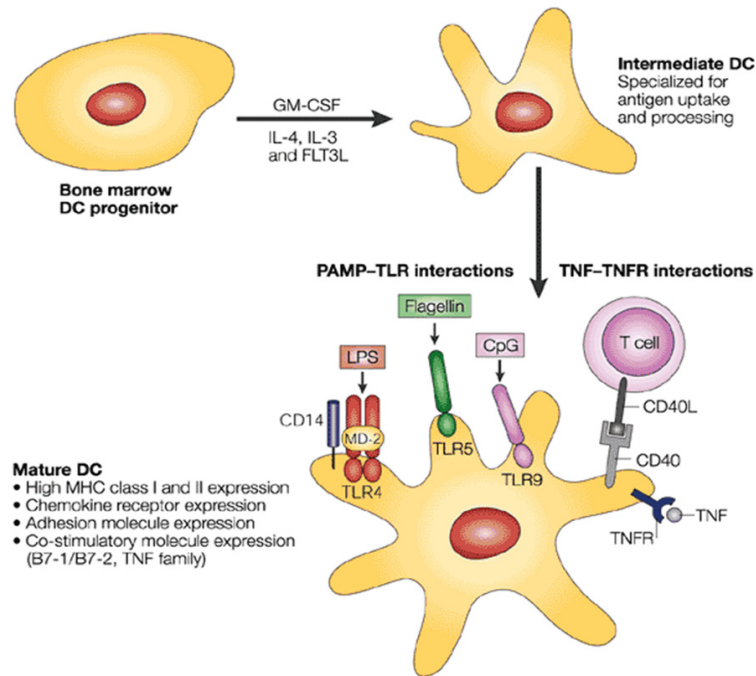


Figure 1. The diagram shows the process of DCs maturation. DC progenitors originate from bone marrow. Certain ‘maturation’ cytokines including GM-CSF, FLT3, interleukin-3 (IL-3) and/or IL-4 can help DC progenitors differentiate to intermediate DCs. The immature DCs transform to mature DCs in response to pathogen-associated molecular pattern molecules (PAMPs) or signals of TNF family. Reproduced from http://www.nature.com/nri/journal/v2/n4/fig_tab/nri774_F1.html.

HCC and PDAC, and to draw attention to their current and future roles in HCC and PDAC immunotherapies.

Current approaches in DC vaccine preparation

DC vaccines can be prepared *ex vivo* and *in vivo*. *Ex vivo* DCs are mainly generated from bone marrow progenitor cells in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4 or IL-13 [19-21]. *In vivo* targeting allows for vaccines to be produced on a larger scale and for direct stimulation and activation of natural DC subsets at multiple sites. It is especially advantageous compared to the *ex vivo* DC generation process which is expensive, labor-intensive, and often difficult to standardize and scale up in *ex vivo* DC generation [22]. DCs are activated by multi-step pathways (Figure 1). Upon infection or inflammation, bone marrow progenitor cells respond to signals including GM-CSF, IL-4 and other cytokines that induce an intermediate stage of DC differentiation; then immature

DCs differentiate in response to maturation signals. This has been reviewed in detail previously [23].

Fundamental issues regarding optimization of the dendritic cells for tumor vaccination include: (1) selecting proper tumor antigens and choosing the appropriate strategy to load tumor antigens onto DCs, and (2) determining the optimal vaccine administration methods to ensure loaded DCs can migrate to lymph nodes (LNs), which is critical for inducing immune responses. Each of these aspects of DC vaccine production will be discussed below.

Selecting proper tumor antigens and choosing the appropriate strategy to load tumor antigens into DCs

In cancer immunotherapy, a tumor vaccine is defined as one that increases specific immune responses to tumor antigens

[24]. An ideal antigenic target for cancer vaccines is uniquely expressed in the cancer cell, important for maintenance of the malignant phenotype, expressed on the cell surface, and immunogenic. To enhance the loading of DCs with tumor-associated antigen (TAA) *in vitro* and further increase the efficacy of DC vaccines, various techniques for delivery of the priming antigen have been tested (Figure 2), including: 1) pulsing DCs with known tumor antigens [25, 26], 2) transfection with DNA [27] or RNA [28] that contains the gene for the antigen of the protein of interest, viral vector-mediated transduction [29-31], 3) incubation with lysates of autologous or allogeneic whole tumors or tumor cell lines [32-36] and 4) fusion of DCs and tumor cells [37].

DCs are typically labeled *ex vivo*. DCs derive their potency from the prominent expression of MHC class I and II, costimulatory receptors (CD80 and CD86), and adhesion molecules that provide secondary signals for the activation of naive CD4⁺ and CD8⁺ T cells [2]. Inaba

Dendritic cells based immunotherapy

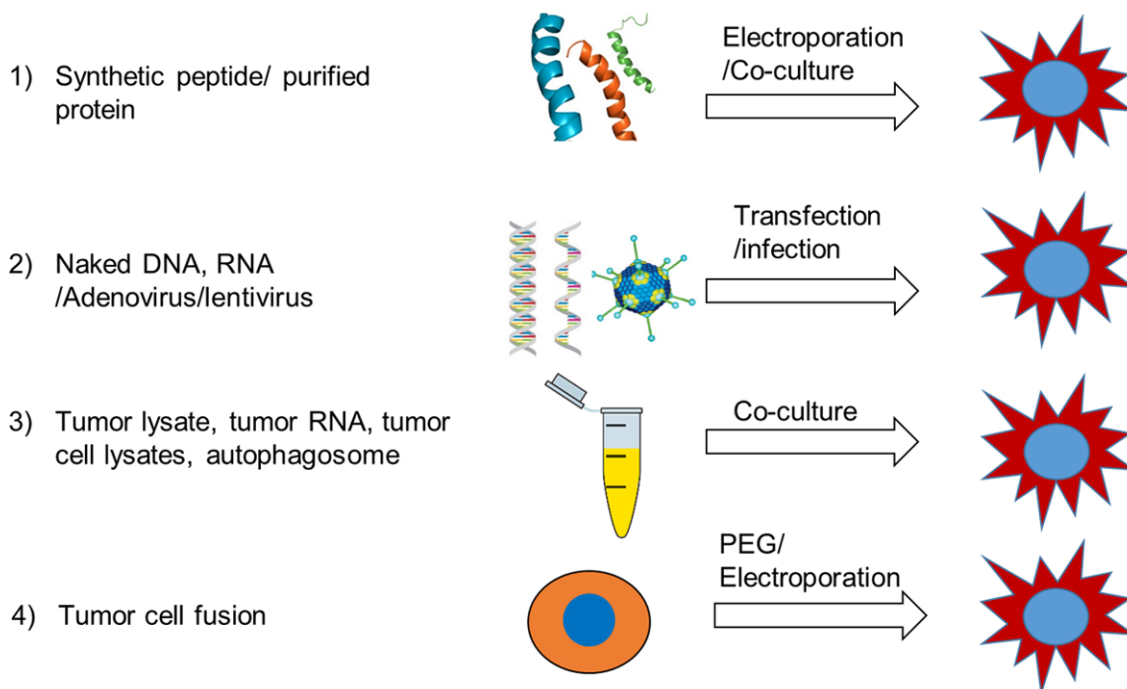


Figure 2. Several strategies have been used to load DC with tumor antigen for antitumor immunity. 1) Synthetic peptide or purified proteins can be pulsed onto the DC surface. 2) DC can be engineered with plasmid DNA, RNA, or viruses to express specific gene products. 3) Tumor lysate, tumor RNA, tumor cell lysates, and auto phagosomes can be mixed with immature DC so that the DC will process and present multiple peptides. 4) DC can be fused with entire tumor cells via PEG or electroporation.

and coworkers first demonstrated that the injection of DCs charged with antigen *ex vivo* could sensitize normal mice to protein antigens [38]. Subsequently, numerous studies in mice showed that DCs loaded with tumor antigens are able to induce protective antitumor responses and therapeutic immunity against established tumors [34, 39-41].

DCs pulsed with peptides or whole proteins: As early as 1990s, scientists have demonstrated that DCs pulsed with protein antigens administered to naive mice can induce proliferation of antigen-responsive T cells in the draining lymphoid tissue [38]. DCs have been pulsed with known tumor antigens such as α -fetoprotein (AFP) [25, 26, 42, 43], glypican-3 (GPC-3) [44], and melanoma-associated antigen 1 (MAGE-1) [45]. In one study, DCs were pulsed with AFP peptides at 10 $\mu\text{g}/\text{mL}$ in serum-free RPMI 1640 at room temperature for 2 h *in vitro* and *in vivo*. *Xenograft* HCC tumor models showed that AFP-specific T cells could markedly suppress HCC tumor formation and morbidity in tumor-bearing nude mice, as well as regulate serum levels of related cytokines and antitu-

mor molecules [43]. The advantage of using peptides is they are easy to manufacture and easy for immune monitoring as peptides and proteins contain few T cell epitopes. However, using peptides or proteins for DC pulsing have several intrinsic disadvantages. This approach is limited to the tumors in which TAA have been identified. To overcome such limitations, other approaches like using tumor lysates to pulse DCs have been used.

DCs pulsed with DNA constructs: Another type of DC vaccine relies on the administration of DNA constructs encoding one or multiple TAAs. Naked or vectored by non-pathogenic viruses, such as adenovirus have been used. In one study, a DNA-based immunization strategy was used, where 1×10^5 immature dendritic cells were transfected via electroporation with the pLAMP/gag DNA plasmid. Transfected DC vaccines were used to immunize mice, and a second immunization with the naked pLAMP/gag DNA plasmid was used as a booster. This method resulted in an increased apparent avidity of peptide in the ELISpot assay [46]. In another study, DCs were transduced with the

GPC3 gene (DCs-GPC3) by electroporation and co-cultured with autologous cytokine-induced killer cells (CIKs). It was reported that DCs-GPC3-CIKs significantly enhanced the cytotoxic activity against GPC3-expressing HepG2 cells and caused significant inhibition of tumor growth in nude mice [47]. In a recent study, a recombinant adeno-associated virus carrying the AFP gene was used to pulse antigen-presenting DCs to stimulate a cytotoxic T lymphocyte (CTL) response against HCC. DCs infected with the AFP gene or the HCC-related antigen (HBsAg) gene induced CTLs cytotoxic activity against the HBV-expressing cell line HepG2.2.15. Inhibition of tumor growth was most significant in the SCID mice model. The above results suggested that a vaccination therapy using DCs co-infected with the two tumor-associated antigen genes is an effective strategy for immunotherapy [48]. This method does not require prior knowledge of relevant T-cell peptide epitope. However, this approach is also limited to the tumors in which TAAs have been identified.

DCs pulsed with tumor lysates of autologous or allogeneic whole tumors or tumor cell lines: Tumor lysates including tumor-derived RNA, cell lysates, and auto phagosome. The major advantages of using whole tumors or tumor cell lines are that antigen presentation is prolonged and that multiple epitopes can be presented on MHC molecules of different haplotypes, allowing the potential to induce both CD4⁺ and CD8⁺ T cell responses to a wide spectrum of antigens [49]. The most common procedures for generating lysates of autologous or allogeneic whole-tumor cells or tumor cell lines include: 1) multiple freeze-thaw cycles [50, 51], which have been demonstrated to release endogenous danger-associated molecular patterns (DAMPs); and 2) UVB irradiation [34], which has been reported to induce the necroptosis and/or apoptosis of tumor cells. Yoshihito et al. examined whether there are important distinctions between TAAs induced by repeated freeze/thaw procedure and UVB light exposure. Their results demonstrated that although some differences exist between the two forms of TAAs in the expression of heat shock proteins, and in the production of interleukin-12 by pulsed DCs, other capacities, such as the capacity to mature DCs phenotypically, and to elicit both effective immune priming and antitumor

therapeutic efficacy *in vivo* when presented by DCs, are equivalent [52]. In another study, DCs were transfected with RNA extracted from HepG2 to induce the expression of specific antigens. Injection of transfected DCs into SCID mice limited the growth of HepG2 tumors. These methods may have a therapeutic application in humans to reduce the recurrence of HCC [53]. Fabian et al. directly compared RNA electroporation and pulsing of DCs with whole tumor cells killed by UVB radiation using a convenient tumor model expressing human papilloma virus (HPV) E6 and E7 oncogenes. They found that electroporation with whole tumor cell RNA and pulsing with UV-irradiated tumor cells are both effective in eliciting antitumor immune response, but RNA electroporation results in more potent tumor vaccination under the examined experimental conditions [54]. A novel therapeutic cancer vaccine based on tumor cell-derived autophagosomes was also developed for cancer immunotherapy. Autophagosome-pulsed DC immunization induced antitumor immunity in a HCC mouse model generated by transplantation of HepG2 into BALB/c-nu mice, resulting in significant inhibition of tumor growth through a T cell specific response [55]. As tumor cells frequently undergo high rates of mutation which could result in the loss of a single or multiple antigens, it would be ideal to choose a source of antigen that can elicit a broad polyclonal tumor-specific response directed against multiple antigenic epitopes. Whole tumor antigen offers this distinct advantage as it allows DCs to process and present numerous tumor antigens to stimulate a strong polyclonal T cell response to prevent tumor escape [56]. Based on these advantages, tumor lysates were widely used to pulse DCs.

Fusion of DCs and tumor cells: Fusion of DC with tumor cells was first described by Gong et al., who used polyethylene glycols (PEGs), a classical fusogenic agent that is widely used in hybridoma technology [37]. The cell fusion method allows DCs to be exposed to a broad array of TAAs originally expressed by whole tumor cells. DCs then process TAAs endogenously and present them through MHC I and II pathways in the context of costimulatory molecules, resulting in simultaneous activation of both CD4⁺ and CD8⁺ T cells [57]. Electrofusion also seems to be an attractive method

for achieving cell-cell fusion. A study comparing therapeutic efficiency of PEG versus electro-fusion showed that electro-fusion was similar to PEG-fusion with regard to vaccine potency in prophylactic anti-tumor immunization assays *in vivo* [58]. This method combined whole antigenic spectrum of the tumor cells with the powerful antigen capabilities. And offers the following advantages that a broad array of known and unidentified TAAs can be simultaneously presented on the surface of DC-tumor fusion cells, which increases the frequency of polyclonal antigen-specific CD4⁺ and CD8⁺ T cells, resulting in long-term efficient antitumor immunity. However, the low fusion efficiency and the limited availability of viable autologous tumor cells as a fusion partner limited its application in research as well as in the clinic [57].

Determining the optimal vaccine administration methods to ensure loaded DCs can migrate to lymph nodes

A variety of routes of vaccine injection, including intradermal (i.d.), subcutaneous (s.c.), intravenous (i.v.), intraperitoneal (i.p.), intranodal (intra-lymphatic) and intratumoral, have been studied [59-68], but the optimal route of administration has yet to be determined. The administration route of antigen-loaded DCs affects the migration of DCs to lymphoid tissues and the magnitude of antigen-specific CTL response. Migration to the LN is critical for inducing immune responses. Footpad injection is a combination of intradermal and subcutaneous injections, with the lymph draining directly up the hind leg to the popliteal LN [69]. The LN is a multifunctional and compartmentalized organ that collectively offers structural guidance for optimal DCs and T cells interaction [70, 71]. After vaccine administration, activated DCs migrate to regional LNs, where they interact with resident T lymphocytes [72].

Intratumoral administration of DC vaccines showed retention at the injection site with a low number of DCs detected in the draining lymph nodes [59], indicating failure of the vaccines to reach their targets. Another possible route of DC administration, which avoids the need for DC migration, is to inject directly into lymph nodes. It offers the advantage of DCs not needing to migrate, as they are already in close proximity with T cells in the lymph nodes. Vac-

inations of mice and humans were performed by direct injection of vaccine into inguinal lymph nodes. In humans, the procedure is guided by ultrasound. In mice, the procedure is invasive [60]. Laura et al. found that bone marrow-derived, tumor lysate-pulsed DCs administered intranodally generated more potent protective antitumor immunity than s.c. or i.v. DC immunizations [61]. Others report that despite direct delivery of DCs into the lymph nodes, the elicited immunologic responses were comparable to [62] or no better [59] than intradermally administered DC vaccines. The intra-lymphatic method is more invasive than other injectable methods such as i.v. and s.c. injections, and the proper injection of vaccines into lymph nodes is also technically difficult; an improper injection could disrupt the lymph node architecture.

Previous studies examining the capacity of DCs to immunize when given through i.v. and s.c. routes have demonstrated the superiority of s.c. injection over i.v. injection in the induction of CTL, as s.c. injected DCs accumulated in the draining lymph node, while i.v. injected DCs were sequestered in the spleen [63, 64]. Okada et al. investigated the vaccine efficiency of DC2.4 cells, a murine dendritic cell line, pulsed with ovalbumin (OVA) in the murine E.G7-OVA tumour model after immunization via i.d., and s.c., they found that DC2.4 cells accumulated in the regional lymph nodes in the i.d.-and s.c.-injected groups [65]. Song et al. compared the efficacy of DC vaccine immunized via footpad injections, i.v. injections, or intratumoral injections in treating melanoma and priming tumor-specific immune responses using a B16-HBc melanoma murine model. They found that although all vaccination approaches protected mice from developing melanoma, only three intratumoral injections of DCs could induce a significant anti-tumor response [66]. In contrast, a statistically significant increase in survival was seen after i.v. immunization with adenoviral peptide-pulsed, spleen derived DCs compared with s.c. immunization of similar DCs in a study of protective immunity against adenovirus-peptide expressing tumors. However, when bone marrow-derived DCs were used, no statistically significant difference in survival could be attributed to route of immunization [67]. Irvine et al. showed that i.v. and i.m. immunization with recombinant tumor antigen-expressing poxviruses was significantly more ef-

Dendritic cells based immunotherapy

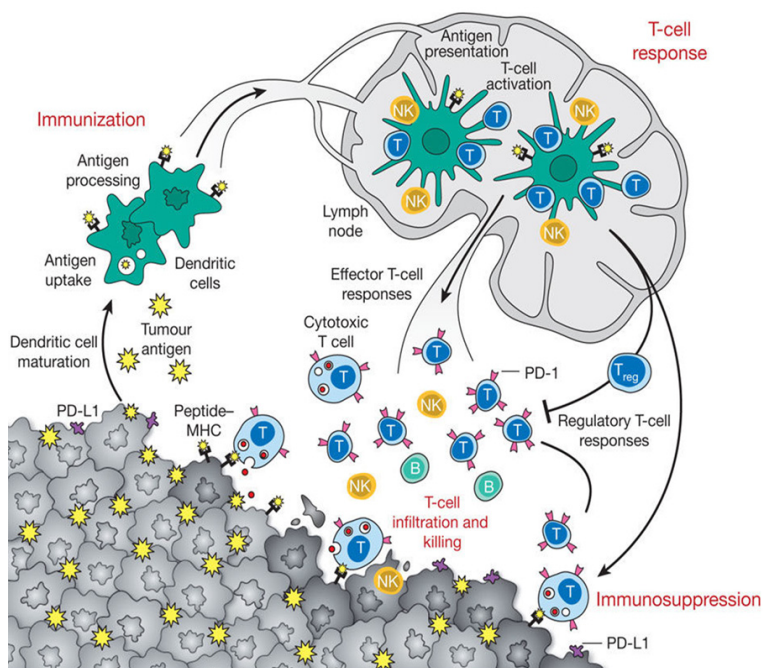


Figure 3. Events regulating the antitumor immunity. The dendritic cells capture and present antigens, and migrate to draining lymph nodes. In response to activation via dendritic cell signaling, T cells will elicit anti-tumor responses. Immune checkpoints are present at several points of the immune response, which contributing to the immunosuppression, making it a potential therapeutic target. Reproduced from http://www.nature.com/nature/journal/v480/n7378/fig_tab/nature10673_F1.html.

fective at inducing antitumor immunity than either s.c. or tail scarification [68]. Thus, there appears to be some controversy in the literature regarding the optimal route of immunization with a DC vaccine, and these routes of immunization have not reached a satisfactory level. IP injection of DC-based vaccine is rarely reported. It may be a potential regimen for gastrointestinal tumors since huge populations of LNs are present throughout the gastrointestinal track, accelerating the delivery of DC-based vaccines and improving the subsequent migration in a viable status.

The current outcomes of DC vaccine in HCC and pancreatic cancer treatments

DC based immunotherapy has been tested in clinical trials in melanoma, prostate cancer, renal cell cancer, and HCC [73]. However, the clinical outcomes were not as exciting as expected. Several mechanisms may account for the limited effectiveness of DC vaccine-induced immune responses to tumors. A major obstacle to the success of cancer vaccines might be the

presence of regulatory T cells (Tregs) and suppressive pathways established by tumors (Figure 3).

Preclinical results of DC vaccine in HCC

HCCs are phenotypically and genetically heterogeneous tumors [74, 75], it possesses characteristics that render it a potential target for immunotherapy. HCC can actively recruit tumor-infiltrating lymphocytes that are capable of lysing autologous tumor cells in *ex vivo* studies [76]. Several rodent models have been used for defining DCs-based immunotherapy. Using an experimental mouse HCC model, Lee et al. showed that DC pulsed with hepatoma cell-lysate can be applied to treat small HCCs effectively *in vivo*. The small hepatocellular tumors, up to 3×3 mm in diameter, were eradicated entirely in more

than half of the experimental mice after two courses of DC treatments. This study showed that efficacy of DCs-based immunotherapy decreases while tumors grow [77]. Shu et al. evaluated the effectiveness of tumor cell derived autophagosomes (DRibbles)-pulsed dendritic cell immunization to induce anti-tumor immunity in BALB/c mouse HCC. They found that DRibbles-pulsed DC immunization induced a specific T cell response against HCC and resulted in significant inhibition of tumor growth [55]. Another study demonstrated that the intratumoral injection of IL-12 encoding plasmid followed by intra-tumoral DC vaccination led to the suppression of HCC and metastases in mice [78]. Wang et al. found that bone marrow-derived DC vaccines loaded with Hepa1-6 cell lysate inhibited tumor progression *in vivo*, as demonstrated by improved overall survival rate and bioluminescence measurement in an orthotopic murine HCC model *in vivo* [79]. However, these results have not reached a satisfactory level until now. Although DCs vaccines are currently used in various stages of clinical trials, no vaccine has been

Dendritic cells based immunotherapy

Table 1. Recent studies of DC vaccine in HCC and PDAC treatment

Year	Tumor model	Cancer type	DC load method	Conclusion	Ref.
2013	Humanized immune reconstituted HepG2 HCC murine model	HCC	DRibbles-pulsed dendritic cell	DRibbles-pulsed DC immunization induced a specific T cell response against HCC and resulted in significant inhibition of tumor growth compared to mice treated with DCs alone.	[55]
2013	Orthotopic pancreatic tumors	PDAC	Non-loaded Bone marrow-derived DC	DC vaccination followed by Gem treatment led to a significant delay in tumor growth and improved survival in pancreatic cancer-bearing mice.	[85]
2014	Subcutaneous or orthotopic pancreatic tumors	PDAC	Bone marrow-derived DC were loaded with soluble OVA protein	Gemcitabine enhances therapeutic efficacy of DC vaccination despite its negative influence on vaccine-induced T-cell proliferation.	[84]
2014	Xenograft model using immunodeficient mice	PDAC	Baculovirus (BV)-infected dendritic cells (DCs)	After treatment with BV-infected bone marrow-derived dendritic cells (BMDCs), human pancreatic tumors caused by AsPC-1 cells in a nude mouse model were significantly reduced in size, and the survival of the mice was improved compared with that of non-immature BMDC (iDC)- and BV-DC-immunized mice.	[87]
2016	MH134-bearing mice model	HCC	DC pulsed with a MH134 cell lysate	DC + CIK vaccination is more effective than DC or CIK alone therapy for the treatment of hepatocarcinoma cancer.	[89]
2016	Orthotopic murine HCC model	HCC	DC pulsed with a Hepa1-6 cell lysate	90 % survival rate by day 60 compared with a survival rate lower than 5 % for untreated mice.	[79]
2016	Nude mice co-injected with MHCC97 cells and Hepa 1-6 induced tumor-bearing C57/BL6 immune competent mice	HCC	SP cell lysate-pulsed DCs	DCs loaded with SP cell lysates could induce a T cell response <i>in vivo</i> and suppress the tumor growth.	[90]

approved so far for HCC. There are only very few therapeutic options for intermediate and advanced HCC; HCC prognosis remains very poor in these stages of disease. Thus, finding novel therapies for HCC remains an urgent need.

Preclinical results of DC vaccine in pancreatic cancer

Pancreatic cancer is generally considered a non-immunogenic malignancy, as tumor-infiltrating effector T lymphocytes do not represent a histopathologic hallmark of this disease [80, 81]. Immunotherapy studies using vaccines for advanced pancreatic cancer have resulted in little clinical success, possibly because of rapid tumor growth, insufficient CTL expansion, and tumor-associated immune suppression. Scientists have been exploring how and why pancreatic cancer evades immune surveillance, and several potential strategies were proposed [82]. A recent clinical report demonstrated that DC vaccine based immunotherapy combined with chemotherapy was somewhat effective in patients with advanced pancreatic cancer refractory to standard treatment [83]. In another study, DC-based vaccination and systemic administration of gemcitabine resulted in longer survival of mice bearing pancreatic cancer

[34]. Subsequently, they found that gemcitabine enhanced efficacy of the model antigen OVA loaded DC (OVA-DC) vaccine using subcutaneous or orthotopic pancreatic tumors induced by Panc02 cells expressing the model antigen OVA [84]. Ghansah et al. also found that a combination therapy with DC vaccination followed by gemcitabine treatment led to a significant delay in tumor growth and improved survival in pancreatic cancer-bearing mice [85]. Nagaraj et al. challenged Panc02 tumor-bearing mice by intratumoral vaccination with alpha-galactosylceramide (alpha-GalCer)-loaded dendritic cells. They found significant expansion of IFN γ -producing NKT cells which also correlated with decrease in tumor growth *in vivo* [86]. Fujihira et al. found that baculovirus (BV)-infected dendritic cells (BV-DCs) induced antitumor immunity against established tumors in mice. They also examined the antitumor effect of BV-DCs on human pancreatic cancer cells (AsPC-1). After treatment with BV-infected bone marrow-derived dendritic cells (BMDCs), human pancreatic tumors caused by AsPC-1 cells in a nude mouse model were significantly reduced in size, and the survival of the mice was improved compared with that of non-immature BMDC (iDC)- and BV-DC-immunized mice [87]. However, despite all of these reports, the number of pancreatic cancer-related deaths con-

tinues to increase, and pancreatic cancer is expected to represent the second-leading cause of cancer-related death in the United States by the year 2020 [88]. DC vaccine in combination with other treatment options might be a good direction to explore.

A summary of the DC vaccine in HCC and PDAC in recent 5 years is listed in **Table 1**. Based on these encouraging results, DCs vaccination or its combination with other therapeutic treatments appears as a promising treatment option in HCC and pancreatic cancer.

Conclusions

There are currently few therapeutic options for advanced HCC and PDAC. As a result, HCC and PDAC prognosis remains very poor in these stages of disease. DCs is an attractive target for therapeutic manipulation of the immune system to enhance insufficient immune responses in cancer. Immunotherapy has appeared as an attractive option for improving outcome for cancer patients in advanced stage. However, the complexity of the DC system requires rational manipulation of DCs to achieve protective or therapeutic immunity. So, further research is needed to analyze: 1) the immune responses induced by *ex vivo*-generated DC subsets which are activated through different pathways; these *ex vivo* strategies and should help to identify the parameters for *in vivo* targeting of DCs; and 2) the hepatic and pancreatic micro-environment in patients, as understanding the role of the immunological microenvironment in DC maturation is the critical step in the development of DC-based vaccination.

Acknowledgements

This study was supported by grants from U.S. A. National Cancer Institute (R01CA196967 and CA209886).

Disclosure of conflict of interest

None.

Address correspondence to: Zhuoli Zhang, Department of Radiology, Feinberg School of Medicine, Northwestern University, 737 N. Michigan Ave, 16th Floor, Chicago, IL 60611, USA. Tel: (312) 926-3874; Fax: (312) 926-5991; E-mail: zhuoli-zhang@northwestern.edu

References

- [1] Banchereau J and Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998; 392: 245-252.
- [2] Steinman RM. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* 1991; 9: 271-296.
- [3] Wu L and Dakic A. Development of dendritic cell system. *Cell Mol Immunol* 2004; 1: 112-118.
- [4] Steinman RM and Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* 1973; 137: 1142-1162.
- [5] Romani N, Gruner S, Brang D, Kampgen E, Lenz A, Trockenbacher B, Konwalinka G, Fritsch PO, Steinman RM and Schuler G. Proliferating dendritic cell progenitors in human blood. *J Exp Med* 1994; 180: 83-93.
- [6] Osada T, Clay T, Hobeika A, Lyerly HK and Morse MA. NK cell activation by dendritic cell vaccine: a mechanism of action for clinical activity. *Cancer Immunol Immunother* 2006; 55: 1122-1131.
- [7] Sabado RL and Bhardwaj N. Cancer immunotherapy: dendritic-cell vaccines on the move. *Nature* 2015; 519: 300-301.
- [8] Mellman I, Coukos G and Dranoff G. Cancer immunotherapy comes of age. *Nature* 2011; 480: 480-489.
- [9] Hinrichs CS and Rosenberg SA. Exploiting the curative potential of adoptive T-cell therapy for cancer. *Immunol Rev* 2014; 257: 56-71.
- [10] Kazemi T, Younesi V, Jadidi-Niaragh F and Yousefi M. Immunotherapeutic approaches for cancer therapy: an updated review. *Artif Cells Nanomed Biotechnol* 2016; 44: 769-779.
- [11] Palucka K, Ueno H, Roberts L, Fay J and Banchereau J. Dendritic cells: are they clinically relevant? *Cancer J* 2010; 16: 318-324.
- [12] Madan RA, Gulley JL, Fojo T and Dahut WL. Therapeutic cancer vaccines in prostate cancer: the paradox of improved survival without changes in time to progression. *Oncologist* 2010; 15: 969-975.
- [13] Guo C, Manjili MH, Subjeck JR, Sarkar D, Fisher PB and Wang XY. Therapeutic cancer vaccines: past, present, and future. *Adv Cancer Res* 2013; 119: 421-475.
- [14] Nestle FO, Burg G, Fah J, Wrone-Smith T and Nickoloff BJ. Human sunlight-induced basal-cell-carcinoma-associated dendritic cells are deficient in T cell co-stimulatory molecules and are impaired as antigen-presenting cells. *Am J Pathol* 1997; 150: 641-651.
- [15] Enk AH, Jonuleit H, Saloga J and Knop J. Dendritic cells as mediators of tumor-induced tolerance in metastatic melanoma. *Int J Cancer* 1997; 73: 309-316.

Dendritic cells based immunotherapy

- [16] Thurnher M, Radmayr C, Ramoner R, Ebner S, Bock G, Klocker H, Romani N and Bartsch G. Human renal-cell carcinoma tissue contains dendritic cells. *Int J Cancer* 1996; 68: 1-7.
- [17] Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, Kavanaugh D and Carbone DP. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nat Med* 1996; 2: 1096-1103.
- [18] Merad M, Sugie T, Engleman EG and Fong L. In vivo manipulation of dendritic cells to induce therapeutic immunity. *Blood* 2002; 99: 1676-1682.
- [19] Pizzurro GA and Barrio MM. Dendritic cell-based vaccine efficacy: aiming for hot spots. *Front Immunol* 2015; 6: 91.
- [20] Lutz MB, Kukulski N, Ogilvie AL, Rossner S, Koch F, Romani N and Schuler G. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J Immunol Methods* 1999; 223: 77-92.
- [21] Antonios JP, Soto H, Everson RG, Orpilla J, Moughon D, Shin N, Sedighim S, Yong WH, Li G, Cloughesy TF, Liau LM and Prins RM. PD-1 blockade enhances the vaccination-induced immune response in glioma. *JCI Insight* 2016; 1.
- [22] Cohn L and Delamarre L. Dendritic cell-targeted vaccines. *Front Immunol* 2014; 5: 255.
- [23] Pardoll DM. Spinning molecular immunology into successful immunotherapy. *Nat Rev Immunol* 2002; 2: 227-238.
- [24] Gurunathan S, Klinman DM and Seder RA. DNA vaccines: immunology, application, and optimization*. *Annu Rev Immunol* 2000; 18: 927-974.
- [25] Stober D, Trobonjaca Z, Reimann J and Schirmbeck R. Dendritic cells pulsed with exogenous hepatitis B surface antigen particles efficiently present epitopes to MHC class I-restricted cytotoxic T cells. *Eur J Immunol* 2002; 32: 1099-1108.
- [26] Butterfield LH, Ribas A, Potter DM and Economou JS. Spontaneous and vaccine induced AFP-specific T cell phenotypes in subjects with AFP-positive hepatocellular cancer. *Cancer Immunol Immunother* 2007; 56: 1931-1943.
- [27] Daftarian P, Kaifer AE, Li W, Blomberg BB, Frasca D, Roth F, Chowdhury R, Berg EA, Fishman JB, Al Sayegh HA, Blackwelder P, Inverardi L, Perez VL, Lemmon V and Serafini P. Peptide-conjugated PAMAM dendrimer as a universal DNA vaccine platform to target antigen-presenting cells. *Cancer Res* 2011; 71: 7452-7462.
- [28] Koido S, Kashiwaba M, Chen D, Gendler S, Kufe D and Gong J. Induction of antitumor immunity by vaccination of dendritic cells transfected with MUC1 RNA. *J Immunol* 2000; 165: 5713-5719.
- [29] Sun QF, Zhao XN, Peng CL, Hao YT, Zhao YP, Jiang N, Xue H, Guo JZ, Yun CH, Cong B and Zhao XG. Immunotherapy for Lewis lung carcinoma utilizing dendritic cells infected with CK19 gene recombinant adenoviral vectors. *Oncol Rep* 2015; 34: 2289-2295.
- [30] Hangalapura BN, Oosterhoff D, de Groot J, Boon L, Tuting T, van den Eertwegh AJ, Gerritsen WR, van Beusechem VW, Pereboev A, Curriel DT, Scheper RJ and de Gruijl TD. Potent antitumor immunity generated by a CD40-targeted adenoviral vaccine. *Cancer Res* 2011; 71: 5827-5837.
- [31] Zhou J, Ma P, Li J and Song W. Comparative analysis of cytotoxic T lymphocyte response induced by dendritic cells pulsed with recombinant adeno-associated virus carrying alpha-fetoprotein gene or cancer cell lysate. *Mol Med Rep* 2015; 11: 3174-3180.
- [32] Palucka AK, Ueno H, Connolly J, Kerneis-Norvell F, Blanck JP, Johnston DA, Fay J and Banchereau J. Dendritic cells loaded with killed allogeneic melanoma cells can induce objective clinical responses and MART-1 specific CD8+ T-cell immunity. *J Immunother* 2006; 29: 545-557.
- [33] Mahdian R, Kokhaei P, Najari HM, Derkow K, Choudhury A and Mellstedt H. Dendritic cells, pulsed with lysate of allogeneic tumor cells, are capable of stimulating MHC-restricted antigen-specific antitumor T cells. *Med Oncol* 2006; 23: 273-282.
- [34] Bauer C, Bauernfeind F, Sterzik A, Orban M, Schnurr M, Lehr HA, Endres S, Eigler A and Dauer M. Dendritic cell-based vaccination combined with gemcitabine increases survival in a murine pancreatic carcinoma model. *Gut* 2007; 56: 1275-1282.
- [35] Dashti A, Ebrahimi M, Hadjati J, Memarnejadian A and Moazzeni SM. Dendritic cell based immunotherapy using tumor stem cells mediates potent antitumor immune responses. *Cancer Lett* 2016; 374: 175-185.
- [36] Zhou L, Lu L, Wicha MS, Chang AE, Xia JC, Ren X and Li Q. Promise of cancer stem cell vaccine. *Hum Vaccin Immunother* 2015; 11: 2796-2799.
- [37] Gong J, Chen D, Kashiwaba M and Kufe D. Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. *Nat Med* 1997; 3: 558-561.
- [38] Inaba K, Metlay JP, Crowley MT and Steinman RM. Dendritic cells pulsed with protein antigens in vitro can prime antigen-specific, MHC-restricted T cells in situ. *J Exp Med* 1990; 172: 631-640.

Dendritic cells based immunotherapy

- [39] Yu Z and Restifo NP. Cancer vaccines: progress reveals new complexities. *J Clin Invest* 2002; 110: 289-294.
- [40] Thumann P, Moc I, Humrich J, Berger TG, Schultz ES, Schuler G and Jenne L. Antigen loading of dendritic cells with whole tumor cell preparations. *J Immunol Methods* 2003; 277: 1-16.
- [41] O'Neill D and Bhardwaj N. Generation of autologous peptide- and protein-pulsed dendritic cells for patient-specific immunotherapy. *Methods Mol Med* 2005; 109: 97-112.
- [42] Vollmer CM Jr, Eilber FC, Butterfield LH, Ribas A, Dissette VB, Koh A, Montejo LD, Lee MC, Andrews KJ, McBride WH, Glaspy JA and Economou JS. Alpha-fetoprotein-specific genetic immunotherapy for hepatocellular carcinoma. *Cancer Res* 1999; 59: 3064-3067.
- [43] Liu Y, Butterfield LH, Fu X, Song Z, Zhang X, Lu C, Ding G and Wu M. Lentivirally engineered dendritic cells activate AFP-specific T cells which inhibit hepatocellular carcinoma growth in vitro and in vivo. *Int J Oncol* 2011; 39: 245-253.
- [44] O'Beirne J, Farzaneh F and Harrison PM. Generation of functional CD8+ T cells by human dendritic cells expressing glypican-3 epitopes. *J Exp Clin Cancer Res* 2010; 29: 48.
- [45] Sadanaga N, Nagashima H, Mashino K, Tahara K, Yamaguchi H, Ohta M, Fujie T, Tanaka F, Inoue H, Takesako K, Akiyoshi T and Mori M. Dendritic cell vaccination with MAGE peptide is a novel therapeutic approach for gastrointestinal carcinomas. *Clin Cancer Res* 2001; 7: 2277-2284.
- [46] Simon GG, Hu Y, Khan AM, Zhou J, Salmon J, Chikhlikar PR, Jung KO, Marques ET and August JT. Dendritic cell mediated delivery of plasmid DNA encoding LAMP/HIV-1 Gag fusion immunogen enhances T cell epitope responses in HLA DR4 transgenic mice. *PLoS One* 2010; 5: e8574.
- [47] Wang Y, Wang Y, Mu H, Liu T, Chen X and Shen Z. Enhanced specific antitumor immunity of dendritic cells transduced with the glypican 3 gene and co-cultured with cytokine-induced killer cells against hepatocellular carcinoma cells. *Mol Med Rep* 2015; 11: 3361-3367.
- [48] Yang JY, Cao DY, Xue Y, Yu ZC and Liu WC. Improvement of dendritic-based vaccine efficacy against hepatitis B virus-related hepatocellular carcinoma by two tumor-associated antigen gene-infected dendritic cells. *Hum Immunol* 2010; 71: 255-262.
- [49] Schnurr M, Chen Q, Shin A, Chen W, Toy T, Jenderek C, Green S, Miloradovic L, Drane D, Davis ID, Villadangos J, Shortman K, Maraskovsky E and Cebon J. Tumor antigen processing and presentation depend critically on dendritic cell type and the mode of antigen delivery. *Blood* 2005; 105: 2465-2472.
- [50] Hatfield P, Merrick AE, West E, O'Donnell D, Selby P, Vile R and Melcher AA. Optimization of dendritic cell loading with tumor cell lysates for cancer immunotherapy. *J Immunother* 2008; 31: 620-632.
- [51] Chiang CL, Kandalaf LE, Tanyi J, Hagemann AR, Motz GT, Svoronos N, Montone K, Mantia-Smaldone GM, Smith L, Nisenbaum HL, Levine BL, Kalos M, Czerniecki BJ, Torigian DA, Powell DJ, Jr., Mick R and Coukos G. A dendritic cell vaccine pulsed with autologous hypochlorous acid-oxidized ovarian cancer lysate primes effective broad antitumor immunity: from bench to bedside. *Clin Cancer Res* 2013; 19: 4801-4815.
- [52] Kotera Y, Shimizu K and Mule JJ. Comparative analysis of necrotic and apoptotic tumor cells as a source of antigen(s) in dendritic cell-based immunization. *Cancer Res* 2001; 61: 8105-8109.
- [53] Xie BH, Yang JY, Li HP, Zhang B, Chen W, Zhou B, Peng BG, Liang LJ and He Q. Dendritic cells transfected with hepatocellular carcinoma (HCC) total RNA induce specific immune responses against HCC in vitro and in vivo. *Clin Transl Oncol* 2014; 16: 753-760.
- [54] Benencia F, Courreges MC and Coukos G. Whole tumor antigen vaccination using dendritic cells: comparison of RNA electroporation and pulsing with UV-irradiated tumor cells. *J Transl Med* 2008; 6: 21.
- [55] Su S, Zhou H, Xue M, Liu JY, Ding L, Cao M, Zhou ZX, Hu HM and Wang LX. Anti-tumor efficacy of a hepatocellular carcinoma vaccine based on dendritic cells combined with tumor-derived autophagosomes in murine models. *Asian Pac J Cancer Prev* 2013; 14: 3109-3116.
- [56] Chiang CL, Coukos G and Kandalaf LE. Whole tumor antigen vaccines: where are we? *Vaccines (Basel)* 2015; 3: 344-372.
- [57] Koido S. Dendritic-tumor fusion cell-based cancer vaccines. *Int J Mol Sci* 2016; 17.
- [58] Lindner M and Schirrmacher V. Tumour cell-dendritic cell fusion for cancer immunotherapy: comparison of therapeutic efficiency of polyethylen-glycol versus electro-fusion protocols. *Eur J Clin Invest* 2002; 32: 207-217.
- [59] Lesterhuis WJ, de Vries IJ, Schreiber G, Lambeck AJ, Aarntzen EH, Jacobs JF, Scharenborg NM, van de Rakt MW, de Boer AJ, Croockewit S, van Rossum MM, Mus R, Oyen WJ, Boerman OC, Lucas S, Adema GJ, Punt CJ and Figdor CG. Route of administration modulates the induction of dendritic cell vaccine-induced antigen-specific T cells in advanced melanoma patients. *Clin Cancer Res* 2011; 17: 5725-5735.
- [60] Johansen P and Kundig TM. Intralymphatic immunotherapy and vaccination in mice. *J Vis Exp* 2014; e51031.

Dendritic cells based immunotherapy

- [61] Lambert LA, Gibson GR, Maloney M, Durell B, Noelle RJ and Barth RJ Jr. Intranodal immunization with tumor lysate-pulsed dendritic cells enhances protective antitumor immunity. *Cancer Res* 2001; 61: 641-646.
- [62] Verdijk P, Aarntzen EH, Lesterhuis WJ, Boullart AC, Kok E, van Rossum MM, Strijk S, Eijckeler F, Bonenkamp JJ, Jacobs JF, Blokk W, Vankrieken JH, Joosten I, Boerman OC, Oyen WJ, Adema G, Punt CJ, Figdor CG and de Vries IJ. Limited amounts of dendritic cells migrate into the T-cell area of lymph nodes but have high immune activating potential in melanoma patients. *Clin Cancer Res* 2009; 15: 2531-2540.
- [63] Lappin MB, Weiss JM, Delattre V, Mai B, Dittmar H, Maier C, Manke K, Grabbe S, Martin S and Simon JC. Analysis of mouse dendritic cell migration in vivo upon subcutaneous and intravenous injection. *Immunology* 1999; 98: 181-188.
- [64] Eggert AA, Schreurs MW, Boerman OC, Oyen WJ, de Boer AJ, Punt CJ, Figdor CG and Adema GJ. Biodistribution and vaccine efficiency of murine dendritic cells are dependent on the route of administration. *Cancer Res* 1999; 59: 3340-3345.
- [65] Okada N, Tsujino M, Hagiwara Y, Tada A, Tamura Y, Mori K, Saito T, Nakagawa S, Mayumi T, Fujita T and Yamamoto A. Administration route-dependent vaccine efficiency of murine dendritic cells pulsed with antigens. *Br J Cancer* 2001; 84: 1564-1570.
- [66] Song S, Zhang K, You H, Wang J, Wang Z, Yan C and Liu F. Significant anti-tumour activity of adoptively transferred T cells elicited by intratumoral dendritic cell vaccine injection through enhancing the ratio of CD8(+) T cell/regulatory T cells in tumour. *Clin Exp Immunol* 2010; 162: 75-83.
- [67] Toes RE, van der Voort EI, Schoenberger SP, Drijfhout JW, van Bloois L, Storm G, Kast WM, Offringa R and Melief CJ. Enhancement of tumor outgrowth through CTL tolerization after peptide vaccination is avoided by peptide presentation on dendritic cells. *J Immunol* 1998; 160: 4449-4456.
- [68] Irvine KR, Parkhurst MR, Shulman EP, Tupesis JP, Custer M, Touloukian CE, Robbins PF, Yafal AG, Greenhalgh P, Suttmuller RP, Offringa R, Rosenberg SA and Restifo NP. Recombinant virus vaccination against "self" antigens using anchor-fixed immunogens. *Cancer Res* 1999; 59: 2536-2540.
- [69] Van den Broeck W, Derore A and Simoens P. Anatomy and nomenclature of murine lymph nodes: descriptive study and nomenclatory standardization in BALB/cAnNCrI mice. *J Immunol Methods* 2006; 312: 12-19.
- [70] Castellino F, Huang AY, Altan-Bonnet G, Stoll S, Scheinecker C and Germain RN. Chemokines enhance immunity by guiding naive CD8+ T cells to sites of CD4+ T cell-dendritic cell interaction. *Nature* 2006; 440: 890-895.
- [71] Munoz MA, Biro M and Weninger W. T cell migration in intact lymph nodes in vivo. *Curr Opin Cell Biol* 2014; 30: 17-24.
- [72] Sixt M, Kanazawa N, Selg M, Samson T, Roos G, Reinhardt DP, Pabst R, Lutz MB and Sorokin L. The conduit system transports soluble antigens from the afferent lymph to resident dendritic cells in the T cell area of the lymph node. *Immunity* 2005; 22: 19-29.
- [73] Palmer DH, Midgley RS, Mirza N, Torr EE, Ahmed F, Steele JC, Steven NM, Kerr DJ, Young LS and Adams DH. A phase II study of adoptive immunotherapy using dendritic cells pulsed with tumor lysate in patients with hepatocellular carcinoma. *Hepatology* 2009; 49: 124-132.
- [74] Bertino G, Demma S, Ardiri A, Proiti M, Gruttadauria S, Toro A, Malaguarnera G, Bertino N, Malaguarnera M, Malaguarnera M and Di Carlo I. Hepatocellular carcinoma: novel molecular targets in carcinogenesis for future therapies. *Biomed Res Int* 2014; 2014: 203693.
- [75] Zheng C, Zheng L, Yoo JK, Guo H, Zhang Y, Guo X, Kang B, Hu R, Huang JY, Zhang Q, Liu Z, Dong M, Hu X, Ouyang W, Peng J and Zhang Z. Landscape of infiltrating T cells in liver cancer revealed by single-cell sequencing. *Cell* 2017; 169: 1342-1356, e16.
- [76] Qiu Y, Xu MB, Yun MM, Wang YZ, Zhang RM, Meng XK, Ou-Yang XH and Yun S. Hepatocellular carcinoma-specific immunotherapy with synthesized alpha1,3-galactosyl epitope-pulsed dendritic cells and cytokine-induced killer cells. *World J Gastroenterol* 2011; 17: 5260-5266.
- [77] Lee WC, Wang HC, Jeng LB, Chiang YJ, Lia CR, Huang PF, Chen MF, Qian S and Lu L. Effective treatment of small murine hepatocellular carcinoma by dendritic cells. *Hepatology* 2001; 34: 896-905.
- [78] Kayashima H, Toshima T, Okano S, Taketomi A, Harada N, Yamashita Y, Tomita Y, Shirabe K and Maehara Y. Intratumoral neoadjuvant immunotherapy using IL-12 and dendritic cells is an effective strategy to control recurrence of murine hepatocellular carcinoma in immunosuppressed mice. *J Immunol* 2010; 185: 698-708.
- [79] Wang Q, Luan W, Warren L, Kadri H, Kim KW, Goz V, Blank S, Isabel Fiel M and Hiottis SP. Autologous tumor cell lysate-loaded dendritic cell vaccine inhibited tumor progression in an orthotopic murine model for hepatocellular carcinoma. *Ann Surg Oncol* 2016; 23: 574-582.
- [80] von Bernstorff W, Voss M, Freichel S, Schmid A, Vogel I, Johnk C, Henne-Bruns D, Kremer B and Kalthoff H. Systemic and local immuno-

Dendritic cells based immunotherapy

- suppression in pancreatic cancer patients. *Clin Cancer Res* 2001; 7: 925s-932s.
- [81] Clark CE, Beatty GL and Vonderheide RH. Immunosurveillance of pancreatic adenocarcinoma: insights from genetically engineered mouse models of cancer. *Cancer Lett* 2009; 279: 1-7.
- [82] Ko AH. Progress in the treatment of metastatic pancreatic cancer and the search for next opportunities. *J Clin Oncol* 2015; 33: 1779-1786.
- [83] Kimura Y, Tsukada J, Tomoda T, Takahashi H, Imai K, Shimamura K, Sunamura M, Yonemitsu Y, Shimodaira S, Koido S, Homma S and Okamoto M. Clinical and immunologic evaluation of dendritic cell-based immunotherapy in combination with gemcitabine and/or S-1 in patients with advanced pancreatic carcinoma. *Pancreas* 2012; 41: 195-205.
- [84] Bauer C, Sterzik A, Bauernfeind F, Duedell P, Conrad C, Kiehl R, Endres S, Eigler A, Schnurr M and Dauer M. Concomitant gemcitabine therapy negatively affects DC vaccine-induced CD8(+) T-cell and B-cell responses but improves clinical efficacy in a murine pancreatic carcinoma model. *Cancer Immunol Immunother* 2014; 63: 321-333.
- [85] Ghansah T, Vohra N, Kinney K, Weber A, Kodumudi K, Springett G, Sarnaik AA and Pilon-Thomas S. Dendritic cell immunotherapy combined with gemcitabine chemotherapy enhances survival in a murine model of pancreatic carcinoma. *Cancer Immunol Immunother* 2013; 62: 1083-1091.
- [86] Nagaraj S, Ziske C, Strehl J, Messmer D, Sauerbruch T and Schmidt-Wolf IG. Dendritic cells pulsed with alpha-galactosylceramide induce anti-tumor immunity against pancreatic cancer in vivo. *Int Immunol* 2006; 18: 1279-1283.
- [87] Fujihira A, Suzuki T, Chang MO, Moriyama T, Kitajima M and Takaku H. Antitumor effects of baculovirus-infected dendritic cells against human pancreatic carcinoma. *Gene Ther* 2014; 21: 849-854.
- [88] Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM and Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* 2014; 74: 2913-2921.
- [89] Jung NC, Lee JH, Choi HJ, Hwang SU, Song JY, Seo HG, Choi J, Jung SY, Han SG and Lim DS. Dendritic cell immunotherapy combined with cytokine-induced killer cells effectively suppresses established hepatocellular carcinomas in mice. *Immunol Invest* 2016; 45: 553-565.
- [90] Li X, Zhang Z, Lin G, Gao Y, Yan Z, Yin H, Sun B, Wang F, Zhang H, Chen H and Cao D. Antigen-specific T cell response from dendritic cell vaccination using side population cell-associated antigens targets hepatocellular carcinoma. *Tumour Biol* 2016; 37: 11267-11278.