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Review Article
Dendritic cells based immunotherapy

Na Shang[1], Matteo Figini[1], Junjie Shangguan[1], Bin Wang[1], Chong Sun[1], Liang Pan[1], Quanhong Ma[1], Zhuoli Zhang[1,2]

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

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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

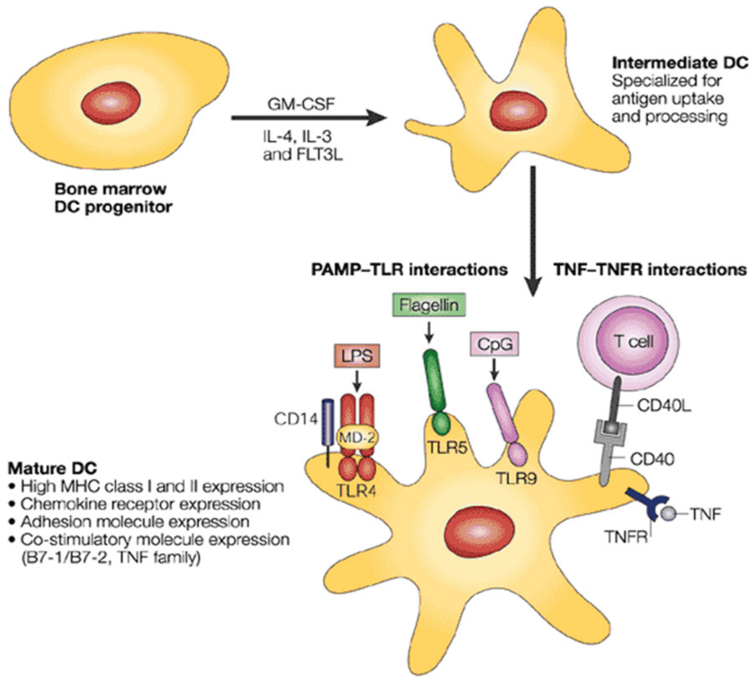

Figure 1. The diagram shows the process of DCs maturation. DC progenitors originate from bone marrow. Certain 'maturation' cytokines including GM-CSF, FLT3, interleukin-3 (IL-3) and/or IL-4 can help DC progenitors different to intermediate DCs. The immature DCs transform to mature DCs in response to 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

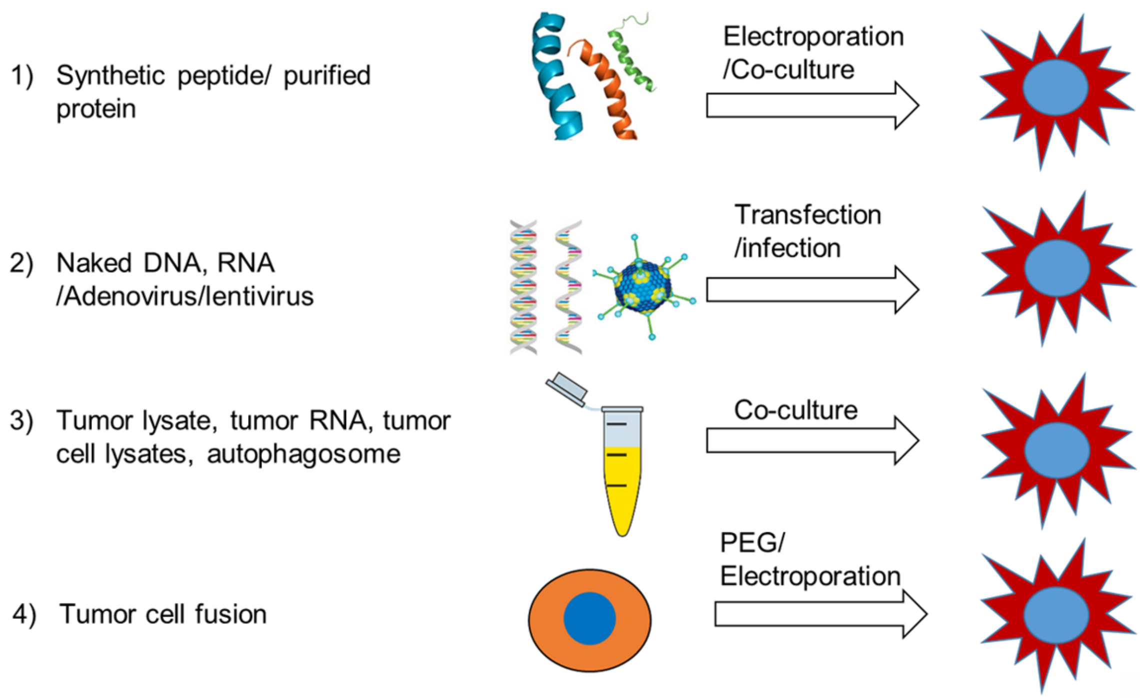

Figure 2. Several strategies have been used to load DC with tumor antigen for antitumor immunity. 1) Synthetic peptide or purified proteins can be pulsed onto the DC surface. 2) DC can be engineered with plasmid DNA, RNA, or viruses to express specific gene products. 3) Tumor lysate, tumor RNA, tumor 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 μg/mL in serum-free RMPI 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 antitumor 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 it's 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 (intralymphatic) 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. Vaccinations 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-

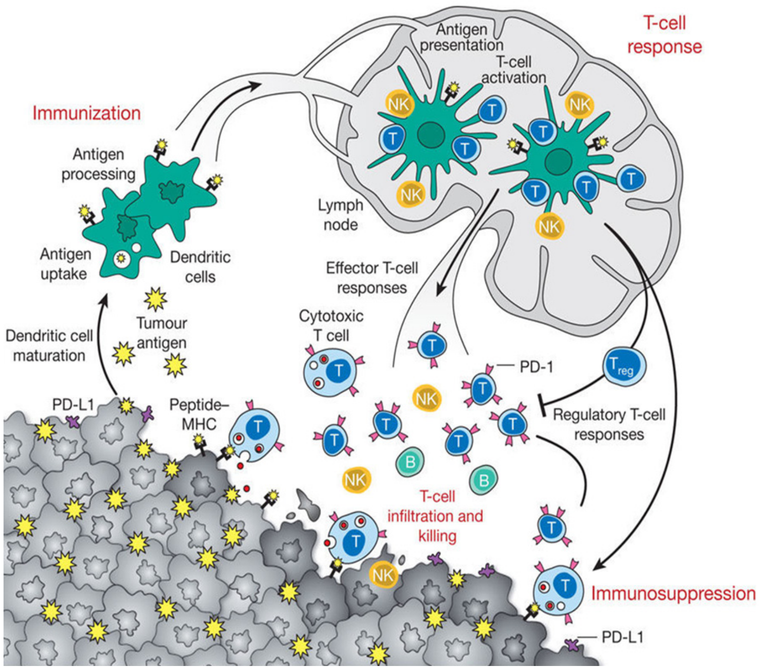

Figure 3. Events regulating the antitumor immunity. The dendritic cells capture and present antigens, and migrate to draining lymph nodes. In response to activation via dendritic cell signaling, T cells will elicit anti-tumor responses. Immune checkpoints are present at several points of the immune response, 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 antitumor 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

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 *in vivo* 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 IFNγ-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, despites all of these reports, the number of pancreatic cancer-related deaths continues 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 it's 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.

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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

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