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Dendritic cell targeting vaccine for HPV-associated cancer

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Abstract

Dendritic cells (DCs) are major antigen presenting cells that can efficiently prime and activate cellular immune responses. Delivering antigens to *in vivo* DCs has thus been considered as a promising strategy that could allow us to mount T cell-mediated therapeutic immunity against cancers in patients. Successful development of such types of cancer vaccines that can target *in vivo* DCs, however, requires a series of outstanding questions that need to be addressed. These include the proper selection of which DC surface receptors, specific DC subsets and DC activators that can further enhance the efficacy of vaccines by promoting effector T cell infiltration and retention in tumors and their actions against tumors. Supplementing these areas of research with additional strategies that can counteract tumor immune evasion mechanisms is also expected to enhance the efficacy of such therapeutic vaccines against cancers. After more than a decade of study, we have concluded that antigen targeting to DCs via CD40 to evoke cellular responses is more efficient than targeting antigens to the same types of DCs via eleven other DC surface receptors tested. In recent work, we have further demonstrated that a prototype vaccine (anti-CD40-HPV16.E6/7, a recombinant fusion protein of anti-human CD40 and HPV16.E6/7 protein) for HPV16-associated cancers can efficiently activate HPV16.E6/7-specific T cells, particularly $CD8^+$ T cells, from the blood of $HPV16^+$ head-and-neck cancer patients. Moreover, anti-CD40-HPV16.E6/7 plus poly(I:C) can mount potent therapeutic immunity against TC-1 tumor expressing HPV16.E6/7 protein in human CD40 transgenic mice. In this manuscript, we thus highlight our recent findings for the development of novel CD40 targeting immunotherapeutic vaccines for HPV16-associated malignancies. In addition, we further discuss several of key questions that still remain to be addressed for enhancing therapeutic immunity elicited by our prototype vaccine against HPV16-associated malignancies.

Keywords

Dendritic cell; CD40; Cross-presentation; Lectin; Vaccine; Cancer; Immunotherapy; HPV

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Conflicting interests

The authors declare no conflict of interests except that SO is a named inventor of patents relating to DC targeting that are held by Baylor Research Institute.

Author contributions

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Dendritic cells (DCs) are the major antigen presenting cells (APCs) that can efficiently cross-prime antigen-specific $CD8^+$ T cells [1, 2]. Such functional specialty in turn makes DCs the ideal cellular targets for the rational design of vaccine against cancers. In line with these notions, Bonifaz *et al.* demonstrated that antigen targeting to *in vivo* DCs via DEC-205 using conjugates of anti-DEC-205 and antigen is far more efficient than antigen alone at eliciting antigen-specific cellular immunity [3].

For more than a decade after the initial report on DC targeting vaccines [3], groups of scientists have been trying to optimize DC-targeting vaccines by delivering antigens to different DC surface receptors. These receptors include c-type lectins (e.g., DEC205, DC-SIGN, CD207, LOX-1, DC-ASGPR, Dectin-1, DCIR, DCIR2, CLEC6, CLEC9A, and CLEC12A) [3–22], as well as non-lectin receptors, including CD40 [22–26], mannose receptor [27], and integrins [28]. Antigens delivered to DCs via these receptors have been shown to elicit certain levels of antigen-specific $CD8^+$ CTL responses *in vitro* in humans and/or *in vivo* in mice or non-human primates (NHPs). However, it remains unclear which targeted receptors are the most efficient at priming and boosting antigen-specific $CD8^+$ and $CD4^+$ T cell responses. Finding a specific DC surface receptor through which potent T cell responses, particularly $CD8^+$ T cell responses, can be elicited is fundamental for the rational design and development of effective DC-targeting vaccines against cancers.

In our previous study [29], we tested 11 different human DC surface receptors (CD40, LOX-1, Dectin-1, DEC-205, DC-ASGPR, DC-SIGN, DC-SIGN/L, DCIR, CLEC6, MARCO, and CD1d) for their ability to elicit antigen-specific $CD8^+$ T cell responses. We found that CD40 was the most efficient at both priming and boosting antigen-specific functional $CD8^+$T cell responses in a human *in vitro* system. Interestingly, however, lectin-like receptors (LOX-1 and Dectin-1) were more efficient than CD40 at eliciting antigen-specific $CD4^+$ T cell responses in a human *in vitro* system. *In vivo* data generated in mice also showed that CD40 was more efficient than Langerin at eliciting antigen-specific $CD8^+$ T cell responses; whereas Langerin, another lectin-like receptor, was more efficient than CD40 at eliciting antigen-specific $CD4^+$ T cell responses. Although antigens fused to anti-CD40 and anti-Langerin antibodies may not target the same subsets of DCs in mice, these data further support our conclusion that antigen targeting to DCs via CD40 is an efficient way to elicit antigen-specific $CD8^+$ T cell responses.

We further investigated the functional differences between CD40 and lectins in antigen presentation to $CD8^+$ and $CD4^+$ T cells by examining the subcellular and intracellular trafficking of the three different receptor-bound mAbs in DCs. Anti-CD40 mAb was present mainly on the cell membrane and in early endosomal compartments, which likely contributed to the enhanced antigen cross-presentation to $CD8^+$ T cells [23, 24]. On the other hand, anti-LOX-1 and anti-Dectin-1 localized to both the early and late endosomal compartments. These late endosomal compartments are less efficient for antigen cross-presentation due to a higher concentration of lysosomal enzymes that degrade antigens before they can escape into the cytosol. They are still able to contribute to antigen cross-presentation, especially when their proteolysis is inhibited [24]. We also found that a large fraction of anti-CD40 mAb remained at the plasma membrane, whereas the majority of both

anti-LOX-1 and anti-Dectin-1 mAbs were rapidly internalized into endosomal vesicles. Slow internalization to early endosomes or rapid antigen recycling, as described previously [23, 24], could result in increased antigen stability, followed by prolonged antigen presentation and enhanced $CD8^+$ T cell responses. In line with this, we found that CD40 targeting leads to greater as well as prolonged antigen cross-presentation to $CD8^+$ T cells compared to LOX-1 or Dectin-1 targeting.

To move forward our efforts for the development of CD40-targeting vaccines against cancer, we generated multiple recombinant proteins of humanized anti-CD40 antibody carrying different tumor-associated antigens (TAAs), including prostate-specific antigens and HPV16.E6/7 protein, as prototype vaccines. In our recent studies [29, 30], we have further demonstrated the proof of concept that strongly support the clinical development of CD40-targeting therapeutic vaccines for HPV16-associated cancers using our prototype vaccine, anti-CD40-HPV16.E6/7 protein.

Studies have shown that 79 million people are infected with HPV, with 14 million new cases of infections each year in the United States [31, 32]. Of the more than 150 different types of HPV [33], high-risk HPV strains (HPV16 and 18) are strongly associated with many cancers of the cervix, vagina, vulva, penis, and anus [34, 35]. Up to 22% of adults are HPV16-seropositive, but most primary infections are cleared without sequelae [36–38]. However, in a small but significant proportion of individuals, their immune systems fail to eradicate the virus and it becomes latent. Such persistent infection can lead to cancers. A recent U.S. population-based study conducted by the Centers for Disease Control and Prevention shows that 66% of cervical cancers, 55% of vaginal cancers, 79% of anal cancers, and 62% of oropharyngeal cancers are attributable to HPV16 or 18. Among these cancers, HPV-associated head-and-neck cancers, including oropharyngeal squamous cell carcinomas, have recently risen dramatically in men under 50 years old; whereas the incidence of HPV-negative oropharyngeal cancers has decreased [39]. Although many of the HPV-positive tumors can be cured with modern multidisciplinary treatment approaches, development of new and effective therapeutic vaccines against HPV-associated malignancies is of importance to bring better clinical benefit to cancer patients. Currently available prophylactic vaccines with viral capsid proteins are not effective for the treatment of HPV-associated cancer [40].

Malignant transformation of HPV-infected cells is driven by the products of HPV oncoproteins *E6* and *E7* (E6/7), which inactivate p53 and the retinoblastoma tumor suppressor genes [41]. All HPV-infected cells constitutively express TAAs of viral origin, such as HPV E6/7. As a result, E6/7 are fitting target TAAs for evoking HPV-specific cellular immune responses, particularly $CD8^+$ cytotoxic T lymphocytes (CTLs), which are one of the major tumoricidal effector cell types. Multiple candidate vaccines targeting E6/7 are currently under development, including E6- and/or E7-derived peptide [42–45], protein [46, 47], plasmid [48, 49] and live-vectored vaccines [50–53]. Nevertheless, potential safety concerns for some of these vaccine models, especially for immunocompromised individuals, and the overall weak $CD8^+$ CTL-mediated immunity-remain major challenges in developing a safe and effective vaccine against HPV-associated malignancies [54–56].

Critical findings in our recent study [30] with a prototype vaccine for HPV16-associated malignancies, anti-CD40-HPV16.E6/7, include that it can evoke HPV16.E6/7-specific $CD8^+$ and $CD4^+$ T cell responses in head-and-neck cancer patients *in vitro* and in human CD40 transgenic (hCD40tg) mice *in vivo*. The combination of anti-CD40-HPV16.E6/7 and poly(I:C) efficiently primed HPV16.E6/7-specific T cells, particularly $CD8^+$ T cells, in hCD40tg mice and could thus mount therapeutic immunity against challenged tumors. The observed therapeutic immunity evoked with the prototype vaccine was associated with the frequency of HPV16.E6/7-specific $CD8^+$ T cells in the tumors but not in the blood. Taken together, these data suggest that CD40-targeting vaccines for HPV-associated malignancies can provide a highly immunogenic platform with a strong likelihood of clinical benefit. Data from this study offer strong support for the development of CD40-targeting vaccines for other cancers in the future.

To achieve our ultimate goals with this prototype therapeutic vaccine for HPV16-associated malignancies, however, we still need to consider several factors that could also determine the success or failure of clinical development of this prototype vaccine. These factors include 1) selection of proper adjuvant, 2) route of immunization, 3) finding strategies to promote effector $CD8^+$ T cell migration into mucosal tissues, and 4) finding strategies to efficiently counteract tumor immune evasion mechanisms. Throughout our studies [30], we have used poly(I:C) as an adjuvant, and this decision was based on our human *in vitro* data [30] and mouse *in vivo* data from previous studies [57, 58]. Recent studies in NHPs and humans have also shown that poly(I:C) and it's derivative poly-ICLC are safe [59] and can thus be used as an adjuvant for our prototype vaccine for HPV16-associated cancers. In our study [30], we also found that poly(I:C) in montanide resulted in significantly enhanced HPV16-E6/7-specific $CD4^+$ T cell responses, although there was no significant change in the magnitude of $CD8^+$ T cell responses. If this is the case in humans, montanide might improve the efficacy of this vaccine model in patients because of the critical roles of $CD4^+$ T cells in the maintenance of $CD8^+$ CTLs [60–62]. Other DC activators, including toll-like receptor (TLR) 7/8 ligands, particularly in the form of conjugates to the prototype vaccine, might also promote $CD8^+$ T cell-mediated immunity, as described [63, 64].

Also, the route of immunization could be important in patients, although s.c., i.p., and i.m. delivery of anti-CD40-HPV16.E6/7 plus poly(I:C) resulted in similar outcomes in mice. Alternatively, an experimental protocol using NHPs may provide us with better insights for the selection of an optimal immunization route for evoking strong $CD8^+$ T cell responses.

It will also be important to test whether this vaccine model is capable of evoking mucosal $CD8^+$ CTL-mediated immunity. This might be one of the most important and relevant tasks for the successful development of vaccines against HPV-associated cancers in the mucosa. This question may need to be addressed in the context of selecting the optimal immunization route as well as choosing appropriate adjuvants.

Lastly, the efficacy of this vaccine model might be improved by harnessing tumor immune evasion mechanisms. Although HPV-induced tumors in different mucosal tissues might possess distinct inhibitory mechanisms in patients (and these need to be further studied), recent data suggest that antibodies specific for immune checkpoint inhibitors, including

CTLA-4, PD-1, and PD-L1 [65–68], might also improve the efficacy of this vaccine in patients with HPV-associated malignancies.

In summary, data from our recent studies [29, 30] strongly support the continued clinical development of a CD40-targeting therapeutic vaccine for HPV16-associated malignancies. However, it is also important to note that the chance of successful development of this vaccine will also be largely dependent on multiple factors that have been discussed in this manuscript. Nonetheless, data from our and other recent studies [22–24, 29] support the development of CD40-targeting vaccines against other cancers.

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Abbreviations

HPV	human papillomavirus
TAA	tumor-associated antigen
CTL	cytotoxic T lymphocyte
DC	dendritic cell
APC	antigen-presenting cell
NHP	non-human primate
mAb	monoclonal antibody
MHC	major histocompatibility complex
Poly(I:C)	polyinosinic:polycytidylic acid
PBMC	peripheral blood mononuclear cell
hCD40tg	human CD40 transgenic
TLR	toll-like receptor

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