Targeting Cancer Associated Fibroblasts for Cancer Immunotherapy

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Introduction

While conventional therapies including surgery, chemotherapy, and radiotherapy for several malignancies have been significantly improved in the last decades, the cure rates for patients with advanced stage tumors are still rather low. Thus, it is desirable to develop new therapies for patients who are resistant to current therapies. Tumor vaccines have the potential to be added to the current treatment armamentarium of cancers due to its potential to eradicate recurrent/metastatic disease after conventional therapies. In addition, vaccines offer the potential of selectively killing malignant cells, thus reducing short and long-term side effects. In this review, we describe a DC vaccine strategy by targeting both cancer cells and cancer associated fibroblasts.

Current limitations of cancer vaccines

While clinical studies have shown that vaccines can induce cancer-specific T cells responses, the antitumor efficacy of the vaccines has been disappointing. The observed limitation could be explained by infiltration of suppressive immune cells within the tumor tissue, such as regulatory T cells (Tregs). Cancer associated fibroblasts (CAFs) are the major component of the tumor supporting stroma. CAFs and Tregs are able to express immunosuppressive factors such as TGF-alpha and/or IL-10, and nutrient depleting enzymes like IDO and arginase that are able to inhibit the function of effector T-cell. In addition, CAFs are the major cells that produce extracellular matrix within tumor tissue, providing a physical barrier for the attack by...
immunotherapies. While vaccine strategies targeting Tregs or CAFs in addition to tumor cells have delayed tumor growth and produced complete responses in animal models, tumors inevitably recur, as effector T-cell functions are eroded and/or subverted. Thus a DC vaccine that 1) targets the cancer cells, 2) counteracts Tregs, and 3) eradicates the tumor stroma has been postulated to be able to overcome the current limitations of DC vaccines (Figure 1). Such a strategy could induce T-cell responses against cancer cells and their supporting stroma, while concomitantly overcoming Treg-mediated immunosuppression in a single vaccine formulation.

Current limitations of inhibiting Tregs

CD4⁺CD25⁺Foxp3⁺ regulatory T cells (Treg) are the major immunosuppressive cells. The number of Treg increases in the peripheral blood and the tumor tissue of patients with various cancers and play an essential role in suppressing antitumor immune responses [4-8]. Therefore, in order to induce effective antitumor immune responses, immune suppression mediated by Treg must be overcome. For example, systemic depletion of Tregs at the time of DC vaccination enhances the induction of anticancer T-cell immunity [11-14]. However most depletion strategies have unwanted side effects since so far no unique Treg surface marker has been identified [15,16]. For example, CD25 antibodies deplete Tregs, but they also deplete activated effector T cells (Teff), which express high levels of CD25. CTLA4 blockade or activating GITR (glucocorticoid-induced TNFR) can overcome Treg-mediated suppression by overactivating cytotoxic T lymphocytes (CTL) and T-helpers (Th) [17-21]. However, these strategies carry the inherent risk of also activating self-reactive T cells, thus restricting their usefulness [22-24]. Thus, a DC vaccination strategy with the ability of overcoming immune suppression mediated by tumor environment will be desirable.

Silencing A20 to overcome Treg mediated immune-suppression

The magnitude and duration of adaptive immunity induced by DC vaccine are tightly controlled by the strength and duration of NF-κB signaling and their regulatory mechanisms [25-29]. A20 is a unique negative regulator that suppresses both TNFR and TLR signaling pathways in DCs [30-34]. Recent studies show that the suppressive function of Treg is critically contingent upon the maturation and activation state of DCs and silencing A20 in DCs by shRNA technology overcomes Treg-mediated immune suppression [35]. Song et al reported that ovalbumin (OVA) expressing DC vaccines, which are genetically enhanced by silencing the NF-kB inhibitor A20, increase T effector cells by inhibiting Tregs and produce tumor regression in a B16-OVA model. A20-silenced DCs were more potent in comparison to DCs lacking the negative regulator SOCS-1, most likely reflecting the broader role of A20 in regulating proinflammtory signaling pathways in DCs [35]. Thus A20-silenced DCs hyperactivated CTL and Th in vivo, and inhibited Tregs in an antigen-specific manner, obviating the need of
unspecific Treg depletion/inhibition. While A20-silenced OVA-DCs target tumor cells and Tregs, the tumor stroma remains and can both inhibit effector T-cell function and sustain residual tumor cells. As a result, the majority of tumors still recur in animals.

Bulk tumors consist of various types of cells, including tumor cells and surrounding stromal cells. Stromal cells within tumor tissues have the ability to promote the tumor cell growth by secreting tumor favorable cytokines, chemokines, and growth factors [9,10]. Targeting stromal cells has been proposed as a tumor immunotherapy strategy. Several studies have highlighted that both cancer and stroma cells have to be killed in order to eliminate bulky tumors. Elimination of tumor stroma allows the killing of antigen loss variant by an antigen-independent mechanism [36,37].

The tumor stroma consists of a variety of nonmalignant cells including CAFs, endothelial cells, and tumor-associated macrophages. CAFs play a central role in shaping the tumor microenvironment. CAFs secrete 1) cytokines like TGF-β, which inhibit immune responses and promote tumor progression, and 2) chemokines like CXCL12 or CXCL14, which are involved in recruiting bone-marrow-derived cells (BMC) and immune suppressive cells, such as tumor-associated macrophages, to the tumor site [10,38-42]. In addition, the tumor stroma may act as a physical barrier for tumor-specific CTL and Th. Expressing profiling of CAFs has shown more than 170 genes that are overexpressed or less expressed in comparison to normal fibroblasts [35]. Among these, FAP has emerged as a promising immunotherapeutic target. FAP is serine protease overexpressed on the cell surface. FAP is highly overexpressed by tumor associated fibroblasts compared to normal fibroblasts. FAP was overexpressed by most epithelial cancers [38-45]. A Phase I clinical studies using a humanized FAP 131I monoclonal antibody (sibrotuzumab) showed no uptake in normal organs but good tumor uptake within 24 to 48 hours post infusion [46].

FAP has been used as a target for cancer immunotherapies. FAP-specific T cells can be activated in humans ex vivo [47] and two groups of investigators have shown in preclinical animal models that DNA or DC FAP vaccines had antitumor effects in several murine models, including melanoma, thymoma, and metastatic breast cancer [48-51]. There is evidence that FAP vaccines enhance CD8+ T-cell infiltration in tumors, likely via the destruction of the tumor. However, most tumors recurred, indicating that the vaccine-induced FAP-specific immune responses were suboptimal, likely due to the tumor-derived immune suppression.

DC vaccine targeting both cancer and CAFs

To enhance the antitumor efficacy of current DC vaccines, an improved vaccine strategy with the ability to overcome tumor-derived immune suppression, such as A20 silencing in DC, is also required to induce robust FAP-specific immune response. In addition, one impediment in the development of effective cancer vaccine is immune escape by tumor through the emergence of treatment-resistant tumor variant. Targeting the tumor stroma instead of tumor cells might overcome this limitation, since tumor stroma are genetically more stable compared to tumor cells. It has been suggested that destruction of tumor stroma is essential for induction of bystander CTLs and eradication of established solid tumor. Importantly, recent studies have demonstrated that DC vaccine targeting FAP as well as an melanoma antigen TRP2 more efficiently eradicate established B16 tumors by inducing bystander CTLs against other B16-associated tumor antigens such as gp100, MART-1, and/or Tyrosinase [51].

While targeting the FAP-tumor stroma is beneficial for inducing potent antitumor responses, two recent studies indicate that FAP is expressed in stromal ‘stem cells’, and that elimination of these cells causes bone marrow failure and/or cachexia [52,53]. The results of Tran and Roberts et al raised a safety concern for FAP-targeted immunotherapies. To address this issue, local targeting of the FAP-positive stroma within tumors would be advisable. For example, FAP-expressing oncolytic viruses (OVs) that selectively express antigen within tumor tissue should allow for inducing immune responses to FAP-positive stroma within the tumors, avoiding systemic induction of FAP-specific T-cell responses.

Conclusions

This review described an new DC vaccine strategy that combines three powerful components: i) cells (DCs) that can be readily obtained from human donors, ii) shRNA to silence the antigen attenuator A20 in DCs, and (iii) antigens to target tumor cells and their associated tumor stroma (FAP). Co-targeting tumor and tumor stroma should gain substantial benefit, because the destruction of the tumor stroma should i) reverse the immune-suppressive microenvironment, and ii) induce bystander T-cell responses (epitope spreading), leading to elimination of tumor antigen loss variants.

Conflicting interests

The authors have declared that no competing interests exist.

References


