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Promise of cancer stem cell vaccine

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Dendritic cell (DC)-based vaccines designed to target cancer stem cells (CSC) can induce significant antitumor responses *via* conferring host anti-CSC immunity. Our recent studies have demonstrated that CSC-DC vaccine could inhibit metastasis of primary tumors and induce humoral immune responses against cancer stem cells. This approach highlights the promise of cancer stem cell vaccine in cancer immunotherapy.

Keywords: cancer stem cell (CSC), vaccine, dendritic cells (DC), programmed death-1 (PD-1)

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To date, the available cancer-preventive vaccines function by preventing viral infections rather than cancer per se. For example, there are 2 vaccines that prevent viral infections whose long-term consequence of chronic infection include cancer. Hepatitis B vaccine prevents infection by hepatitis B virus (HBV), where chronic HBV infection results in a high risk of progression to cirrhosis and hepatocellular carcinoma (HCC).[1,2] In this regard, it has been shown that Hepatitis B vaccination can lower the long-term rate of development of HCC in populations with a high level of vaccination.[3,4] Similarly, human papilloma virus (HPV) vaccine prevents infection, where chronic HPV infection is a risk factor for development of cervical cancer.[5,6] HPV vaccine is now being used in 11–14 year-old girls. It is reported that about 90% of invasive cervical cancer, high-grade cervical neoplasia, genitals warts and anal cancer cases could be prevented by a HPV vaccine.[7]

The field of 'cancer vaccines' that are not anti-infection vaccines is directed to immunotherapy of diagnosed tumors. For therapy, dendritic cells (DC) pulsed with tumor antigen have shown efficiency and DC-based vaccine represents a promising immunotherapy for cancers.[8,9] For example, sipuleucel-T immunotherapy for castration-resistant prostate cancer revealed encouraging result. Sipuleucel-T is a cancer vaccine developed from autologous dendritic cells loaded with engineered fusion protein of prostatic acid phosphatase (PAP) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Recent Phase III studies show that sipuleucel-T therapy can prolong median survival in patients with castration-resistant prostate cancer (CRPC). Use of sipuleucel-T vaccine has been approved by the United States Food and Drug Administration (Clinical Trials.gov number, NCT00065442).[10]

Many human malignancies are associated with quantitative and qualitative deficiencies in the immune system. Immunotherapy thus holds the promise for cancer treatment, and has demonstrated the complementary role to traditional cancer treatments, e. g. surgery, chemotherapy and radiotherapy. Immunotherapy has revealed several advantages over the traditional cancer treatment, such as less toxicity.[11] The current immunological strategies for cancer therapy include vaccines, monoclonal antibodies, and cellular therapies. However, despite of the encouraging preclinical studies in cancer immunotherapy, the clinical responses are limited to a confined patient population.

There is accumulating evidence that tumors contain a distinct subpopulation of stem-like cells, or cancer stem cells (CSCs).[12-14] Recent studies have described several biomarkers for cancer stem cells *e.g.* $CD44^+/ CD24^-$ for breast

cancer,[15] $CD133^+$ for brain tumor[16] and colon cancer,[17] and $CD44^+$ for gastric cancer.[18] The isolation and identification of cancer stem cells or CSC-like cells (CSC-LCs) in ovarian cancer have also been reported.[19] Importantly, Du et al found that use of anti-CD44 monoclonal antibodies may be a potential strategy for the treatment of human ovarian cancer after conventional therapy *via* inhibiting tumor growth and promoting apoptosis in sphere-forming cells (SFCs) with stemness.[20] More recently, high aldehyde dehydrogenase (ALDH) activity, measured *via* ALDEFLUOR assay, has been successfully used as a marker to enrich cancer stem cell populations from multiple tumor types.[21-27] Ithimakin et al. reported that higher expression of HER2 in the $ALDEFLUOR^+$/ $ALDH^{high}$ cancer stem cells further enhances tumor initiation.[28] Cancer stem cells are resistant to traditional chemotherapy and radiotherapy,[29,30] and have been found to be responsible for the initiation, relapse as well as metastasis of tumor.[31,32] Immunological targeting of cancer stem cells may therefore represent a novel approach in cancer immunotherapy.

We have reported the protective antitumor immunity induced by $ALDEFLUOR^+$/ $ALDH^{high}$ cancer stem cell-dendritic cell (CSC-DC) vaccine using histologically distinct murine tumor models syngeneic to different immunocompetent hosts. Enriched $ALDEFLUOR^+$/ $ALDH^{high}$ cancer stem cells were immunogenic and more effective as an antigen source than unsorted bulk tumor cells or $ALDEFLUOR^-$/ $ALDH^{low}$ cells in inducing protective antitumor immunity.[27] Mechanistic investigations established that CSC-primed antibodies and T cells were capable of selectively targeting cancer stem cells and therefore conferring antitumor immunity.[27] The inability to target cancer stem cells with the current immunological approaches may be a significant factor contributing to treatment failure. These results provide a rationale for a new type of cancer immunotherapy based on the development of CSC-DC vaccines that can induce host immunity specifically targeting cancer stem cells.

For a vaccine to be clinically relevant, it needs to be examined in the therapeutic models. In a recent study, we developed a strategy to target the cancer stem cell populations in melanoma and squamous cell carcinoma using cancer stem cell lysate-pulsed dendritic cells in established tumors.[33] The CSC-DC vaccine was administered after localized radiation therapy of the established tumor. Using mouse models we demonstrated that dendritic cells pulsed with cancer stem cells enriched by virtue of their expression of the CSC marker ALDH (CSC-DC) significantly inhibited tumor growth, reduced development of pulmonary metastases of established primary tumor and prolonged survival. In these experiments, B6 or C3H mice were inoculated s.c. with 0.05×10^6 D5 melanoma cells or 0.5×10^6 SCC7 squamous cell carcinoma cells respectively on day 0. The mice were treated with localized radiation therapy (RT) on day 5 and day 6 followed by the 1st DC vaccine on day 7. The combined RT + vaccine treatment was repeated on day 12, 13, 14 and 19, 20, 21 respectively. Thus, the RT was delivered 6 times, which were on days 5, 6, 12, 13, 19 and 20 with a total dose of 51 Gy (8.5 Gy × 6), while vaccines were administrated 3 times, 1 week apart, which were on days 7, 14 and 21 (**Fig. 1**).

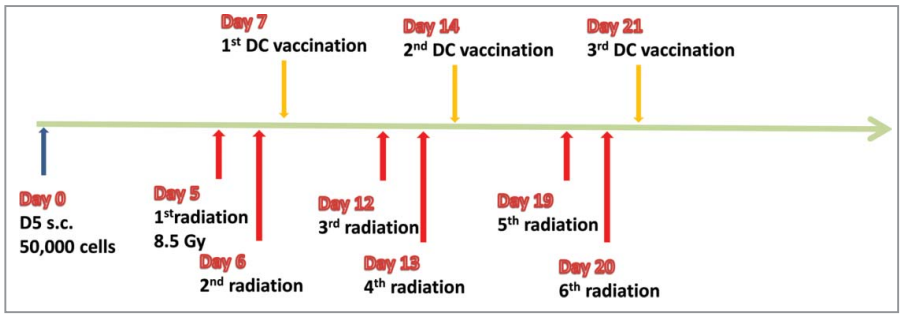

Figure 1. Tumor treatment protocol.

Mice treated with local tumor radiation therapy (RT) plus $ALDH^{high}$ CSC-DC vaccine showed a 20.5-day survival advantage over the RT plus $ALDH^{low}$-DC vaccinated mice ($P = 0.0036$), and a 17-day survival advantage over the RT plus heterogeneous, unsorted tumor cell lysate-pulsed dendritic cell (H-DC) vaccinated mice ($P = 0.0121$). We found that RT followed by $ALDH^{high}$ CSC-DC vaccination significantly inhibited the lung metastasis in the established tumor models (**Fig. 2**). Specifically, in each experiment group ($n = 11$), there were 10, 9 and 8 mice that resulted in distant lung metastasis after treatment with PBS (control), RT only, or RT plus $ALDH^{low}$-DC vaccine respectively. In contrast, RT plus $ALDH^{high}$ CSC-DC vaccine treatment only had 2 mice with lung metastasis. As a result, RT plus $ALDH^{high}$ CSC-DC vaccination significantly prolonged the overall survival of the animals, showing the significant therapeutic efficacy of $ALDH^{high}$ CSC-DC vaccine in combination with radiation therapy in the management of established tumors. CSC-DC vaccine significantly reduced $ALDH^{high}$ CSCs in the primary tumors. Direct targeting of CSCs was demonstrated by specific binding of IgG produced by $ALDH^{high}$ CSC-DC vaccine-primed B cells to $ALDH^{high}$ CSCs, resulting in lysis of these target CSCs in the presence of complement, thus showing the humoral immune responses against cancer stem cells. These data suggest that the CSC-DC vaccine approach may be useful after conventional treatment of cancers, such as radiotherapy, where local and systemic relapse are high.

In order to improve vaccine efficiency, Mitchell et al used tetanus toxoid as an adjuvant. They randomized patients with glioblastoma for pre-conditioning with either mature DCs or TD (tetanus/diphtheria) unilaterally before bilateral vaccination with DCs pulsed with cytomegalovirus phosphoprotein 65 (pp65) RNA. Twelve patients were randomized into this clinical trial.

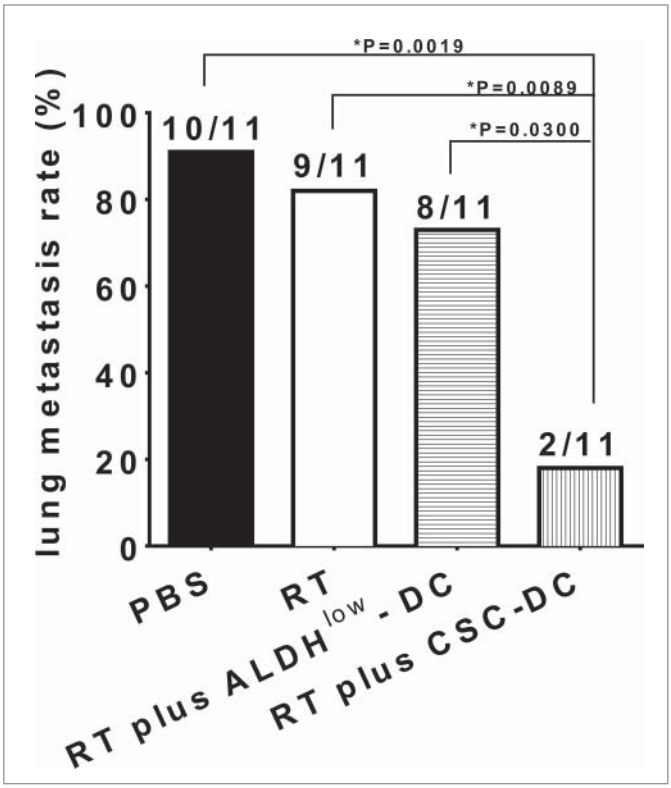

Figure 2. Local tumor radiation therapy (RT) followed by CSC-DC vaccination significantly inhibited the lung metastasis. Bar graph shows the percentage of lung metastasis.

Compared to DC alone-treated patients, TD-pre-conditioned patients showed significantly more draining lymph nodes (DLNs) and increased median progression-free and overall survivals.[34] Bacille Calmette-Guérin (BCG) is well known as another adjuvant used in cancer immunotherapy, particularly for bladder tumor. Rossi et al reported that the number of peripheral blood plasmacytoid DCs (pDCs) was partially affected by BCG administration.[35] Investigation and application of novel adjuvant may enhance the therapeutic efficacy of CSC-DC vaccine.

Programmed death ligand 1 (PD-L1, also known as B7 homolog 1, B7-H1 or CD274) makes tumor-reactive T cells tolerate to tumor cells by binding to programmed death-1 (PD-1 or CD279).[36,37] It is a major barrier to antitumor immunity.[38] Durgan et al found that PD-L1$^{-/-}$ mice given dendritic cells pulsed with antigen and α-GalCer showed decreased tumor size and this was associated with increased trafficking of antigen-presenting cells and CD8^{+} T cells to the tumor. These data demonstrated that interrupting PDL1/PD-1 interaction amplifies an anti-tumor response when coupled with DC vaccination.[39] In a clinical trial, Gettinger et al reported that administration of anti–PD-1 antibody Nivolumab produced durable responses and enhanced survival rates in patients with heavily pretreated non-small cell lung cancer (NSCLC).[40] In addition, Eggermont AM et al conducted a double-blind phase III trial in patients with stage III cutaneous melanoma. Patients received intravenous infusions of 10 mg/kg ipilimumab, a first-in-class immunological checkpoint blockade agent and monoclonal antibody against cytotoxic T-lymphocyte antigen 4 (CTLA-4) or placebo as a control every 3 weeks for 4 doses, then every 3 months for up to 3 y Median recurrence-free survival was 26.1m in the ipilimumab group versus 17.1m in the placebo group; 3-year recurrence-free survival was 46.5% in the ipilimumab group vs. 34.8% in the placebo group.[41] While cancer vaccine and other forms of immunotherapy has been found playing an important role in the treatment of lung cancer,[42] renal carcinoma,[43] and colorectal cancer,[44] immunological targeting of cancer stem cells utilizing cancer stem cell-based vaccine may represent a more potent strategy to prevent tumor metastasis and relapse. Particularly, immunologically targeting cancer stem cells while simultaneously blocking PD-1/PD-L1 and/or CTLA-4-mendiated immune suppression may significantly enhance the outcome of current immunotherapies of cancer.

Disclosure of Potential Conflicts of Interest

There were no conflicts of interest.

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