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Targeting cancer stem cells via dendritic-cell vaccination

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Using syngeneic murine tumor models established in immunocompetent hosts, we showed that cancer stem cells are immunogenic and can be selectively targeted by dendritic cell-based vaccines. This new approach induced both humoral and cellular immune responses and conferred significantly superior antitumor immunity as compared with conventional vaccines.

During the last decade, the identification of cancer stem cells (CSCs) in various human malignancies[1–3] led to substantial research efforts aimed at developing approaches to eliminate this small but critical subpopulation of tumor cells.[4] Once it became evident that CSCs are resistant to major conventional therapeutic strategies,[5] it has been postulated that they play a key role in both local tumor recurrence and systemic relapse. Studies focused on targeting the molecular pathways that are selectively activated in CSCs have recently provided promising results.[6]

Harnessing the potency and specificity of the host immune response to eradicate CSCs represents an attractive approach. However, its feasibility is unknown. Most studies focusing on CSCs are based on the inoculation of human-derived CSCs into severely immunocompromised hosts. These xenograft tumor models offer many advantages, but preclude immunological assessments. Furthermore, most current forms of cancer immunotherapy, such as dendritic cell (DC)-based vaccines or the adoptive transfer of tumor-reactive T cells, are designed to target tumor-associated antigens expressed by the bulk of "differentiated" cancer cells. The ability of these approaches to target CSCs is unclear.

To circumvent these limitations, we used two syngeneic tumor models established in immunocompetent mice: the D5 melanoma growing in C57BL/6 mice and the SCC7 squamous cell carcinoma growing in C3H mice.[7] In both types of cultured cells and freshly harvested tumors, we identified by flow cytometry a 2–5% CSC-enriched population, based on enhanced aldehyde dehydrogenase (ALDH) activity.[8,9] Such ALDH^{bright} cells (as well as their ALDH^{dim} counterparts) could be isolated by flow sorting and bilateral subcutaneous inoculation of naïve syngeneic mice with increasing doses of ALDH^{bright} (right flank) vs. ALDH^{dim} (left flank) cells demonstrated a significant difference in tumorigenicity. Inoculation of as few as 500 D5 or 2,000 SCC7 ALDH^{bright} cells generated tumors, whereas inoculation of 50,000 D5 or 200,000 SCC7 ALDH^{dim} cells did not. These results, as well as in vitro and in vivo self-renewal analyses of ALDH^{bright} cells, validated the use of ALDH^{bright} as a reliable CSC marker in the D5 and the SCC7 murine tumor models.[7]

To test immunogenicity, tumor-derived ALDH^{bright} cells were lysed, and used as a source of antigens to pulse DCs (**Fig. 1**). DCs pulsed with ALDH^{dim} cell- or whole tumor cell-derived lysates were used as controls, the latter representing a conventional form of DC-based cancer vaccine. Thereafter, naïve mice were vaccinated 2–3 times prior to challenge with syngenic tumors. Thus, DCs pulsed with ALDH^{bright} lysates significantly inhibited tumor growth as compared with DCs pulsed with either ALDH^{dim} or whole tumor lysates. These results were obtained in both tumor models mentioned above and were persisted irrespective of the tumor inoculation route (be it either intravenous or subcutaneous). Thus, CSCs appear to be superior to the bulk of tumor cells as a source of antigens to prime DC vaccines.

To examine potential mechanisms underlying these findings, we evaluated the systemic immune responses elicited by DC-based CSC vaccination. The splenocytes of mice receiving ALDH^{bright} lysate-pulsed DCs secreted significantly higher amounts of IgG after in vitro activation as compared with control splenocytes. Further, serum samples collected from these mice were found to contain IgG antibodies that bound ALDH^{bright} cells and mediated their complement-dependent lysis. In contrast, IgG antibodies derived from the sera of control-vaccinated mice had a significantly reduced capacity to bind and lyse ALDH^{bright} cells. Finally, cytotoxicity assays showed that both peripheral blood mononuclear cells and splenocytes from mice receiving ALDH^{bright} lysate-pulsed DCs lysed ALDH^{bright} cells more efficiently than ALDH^{dim} cells. Of note, tumors

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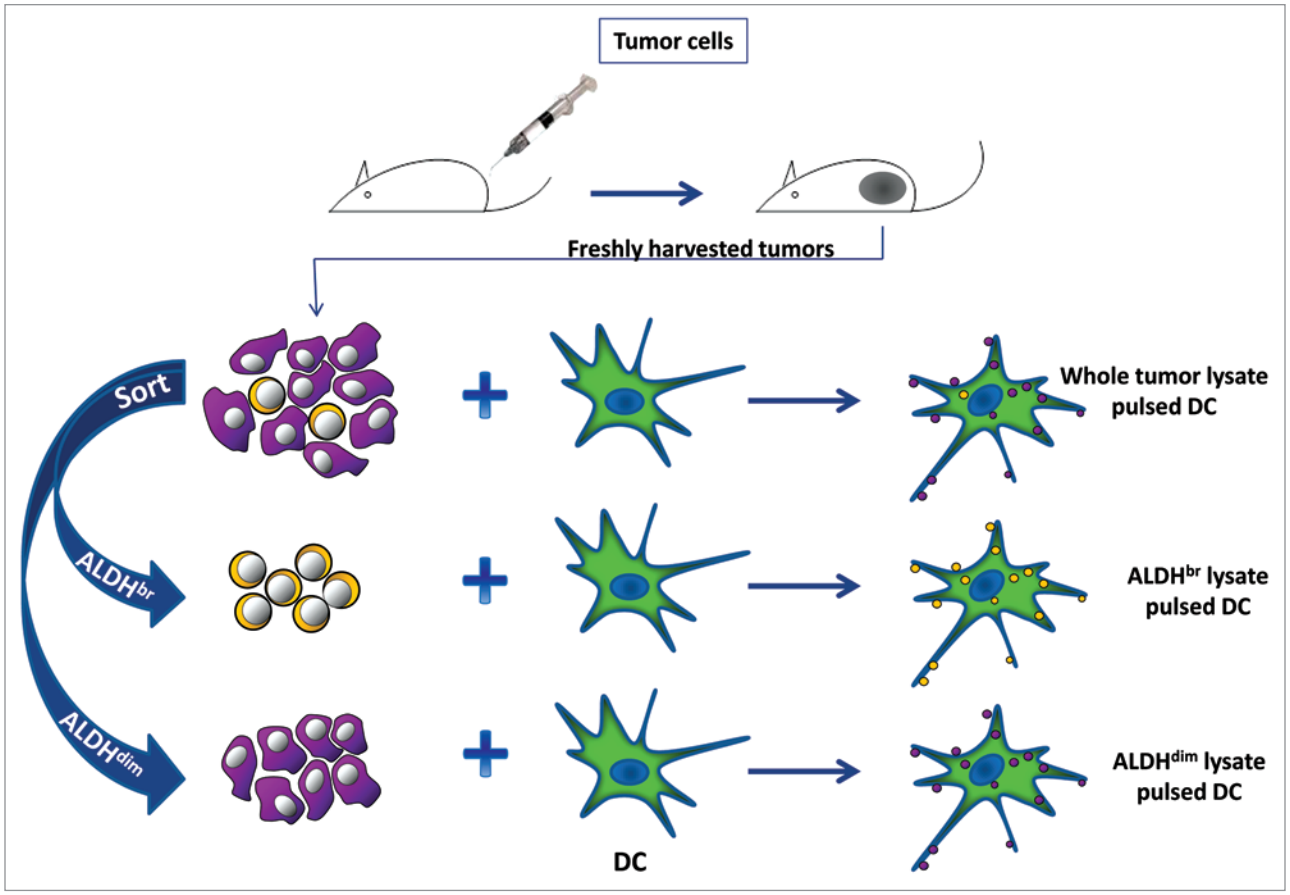

Figure 1. Generation of dendritic cell-based cancer stem cell vaccines. Syngeneic mice were inoculated subcutaneously with D5 melanoma or SCC7 squamous cell carcinoma cells. Established tumors were harvested, dissociated into single cell suspensions, and stained using the ALDEFLUOR kit (StemCell Technologies). ALDH^{bright} and ALDH^{dim} cells were isolated by flow sorting. Bone marrow-derived dendritic cells (DCs) generated from naïve syngeneic mice were pulsed with whole tumor, ALDH^{bright} or ALDH^{dim} cell lysates. ALDH^{bright} cell lysate-pulsed DCs were used as cancer stem cell vaccines, whereas ALDH^{dim} and whole tumor cell lysate-pulsed DCs were used as control and conventional cancer vaccines, respectively.

harvested from these mice contained lower percentages of ALDH^{bright} cells as compared with control tumors. Together, these findings support the notion that DC-based CSC vaccination confers superior protective antitumor immunity by selectively targeting CSCs.

Together, we found that DC-based CSC vaccines can elicit anti-CSC humoral and cellular immune responses, which were associated with the induction of efficient protective antitumor immunity. These studies provide the proof of concept that CSCs can be recognized and eradicated by the immune system and a rationale for designing new immunotherapeutic approaches aimed at targeting CSCs.

Visus et al.[10] have very recently reported that in vitro generated ALDH1A1-specific CD8$^+$ T cells can target ALDH^{bright} cells derived from human head and neck, breast and pancreas cancers in vitro as well as in xenograft models. Adoptive transfer of these cells into xenograft-bearing immunodeficient mice inhibited tumor growth and metastases. Contrarily to the adoptive transfer of in vitro generated effector cells for cancer immunotherapy, the efficacy of tumor vaccines relies on the induction of host antitumor immunity. Further studies are needed in order to evaluate the therapeutic efficacy of DC-based CSC vaccines. The murine tumor models that we have used in this study will allow us to examine whether vaccination with ALDH^{bright} lysate-pulsed DCs can mediate regression of established tumors. Since CSCs constitute a very small percentage of the tumor cell population, selective elimination of these cells may not result in substantial tumor regression. However, combining CSC vaccination with other interventions including chemotherapy, radiotherapy or bulk tumor-targeted immunotherapy, is likely to result in significant antitumor effects. Translation of these combined approaches to the clinical setting holds great promise as it may induce durable responses and reduce the rate of local progression and systemic recurrence.

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