Research Article

An update on Dendritic Cell-Based Cancer Immunotherapy

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Abstract

Although treating advanced cancers that affect organs with distant metastasis remains challenging, the pace of recent advances has accelerated; these advances have particularly focused on the inhibitors of key immune potentiates. Research on therapeutic vaccination involving active dendritic cell (DC)-based immunotherapy is also being performed for the induction of an efficient immune response against cancer-associated antigens by the acquired immune system. Cancer vaccines prepared with autologous monocyte-derived mature DCs have been generated using granulocyte–macrophage colony-stimulating factor and interleukin-4, which are principally attributed to the presence of tumor-associated antigens. Wilms' tumor 1 (WT1) is an attractive target antigen that is widely detected in many cancers. DC-based immunotherapy targeting WT1 may elicit a strong therapeutic response to cancers. DC vaccines primed with HLA class I/II-restricted WT1 peptides (WT1-DC) are a feasible option for patients with advanced cancers. Immune response monitoring using tetramer analysis and/or enzyme-linked immunosorbent spot assay has been applied to determine the efficacy of WT1-DC. The inhibition of immune suppressors and acceleration of anti-cancer immunity with WT1-DC may comprise a promising future therapeutic strategy for treating advanced cancers.

Abbreviations: APC: Antigen-Presenting Cell; DCs: Dendritic Cells; RECIST: Response Evaluation Criteria in Solid Tumors; HLA: Human Leukocyte Antigen; MDSCs: Myeloid-Derived Suppressor Cells; CTLs: Cytotoxic T Cells; GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor; TGF: Transforming Growth Factor; IL: Interleukin ; VEGF: Vascular Endothelial Growth Factor; PGE2: Prostaglandin E2; PD-L1: Programmed Death-Ligand 1; FDA: Food and Drug Administration; WT1: Wilms' Tumor 1; MUC1: Mucin 1 Cell Surface Associated; HER2: Human Epidermal Growth Factor Receptor 2; CEA: Carcinoembryonic Antigen; PSA: Prostate-Specific Antigen; WT1-DC: DC Vaccines Primed with HLA class I/II-Restricted WT1 Peptides; ELISPOT: Enzyme-Linked Immunosorbent Spot; WT1-CTLs: WT1 Antigen-Specific Cytotoxic T Lymphocytes; IFN: Interferon; PBMCs: Peripheral Blood Mononuclear Cells; HSCT: Hematopoietic Stem Cell Transplantation; G-CSF: Granulocyte Colony-Stimulating Factor

Introduction

Immune checkpoint inhibitors have resulted in significant advances in cancer therapeutics however; the treatment of advanced cancers affecting organs and involving distant metastasis remains extremely difficult [1-6]. Clinical benefit of an anti-programmed death 1 (PD1) immune checkpoint inhibitor was shown in patients with progressive metastatic colorectal cancer with mismatch repair-deficiency [7].

Antigen-presenting cell (APC)-based immunotherapy using active dendritic cells (DCs) is under investigation for developing therapeutic vaccination against cancer [8]. Autologous DC-based immunotherapy appears to trigger few adverse reactions with limited clinical effectiveness if conventional evaluation procedures such as response evaluation criteria in solid tumors (RECIST) are employed [9,10]. Long-term acquired immunity following DC vaccination produces a delayed separation of survival curves with an advantage pertaining to prolonging the overall survival [11,12].

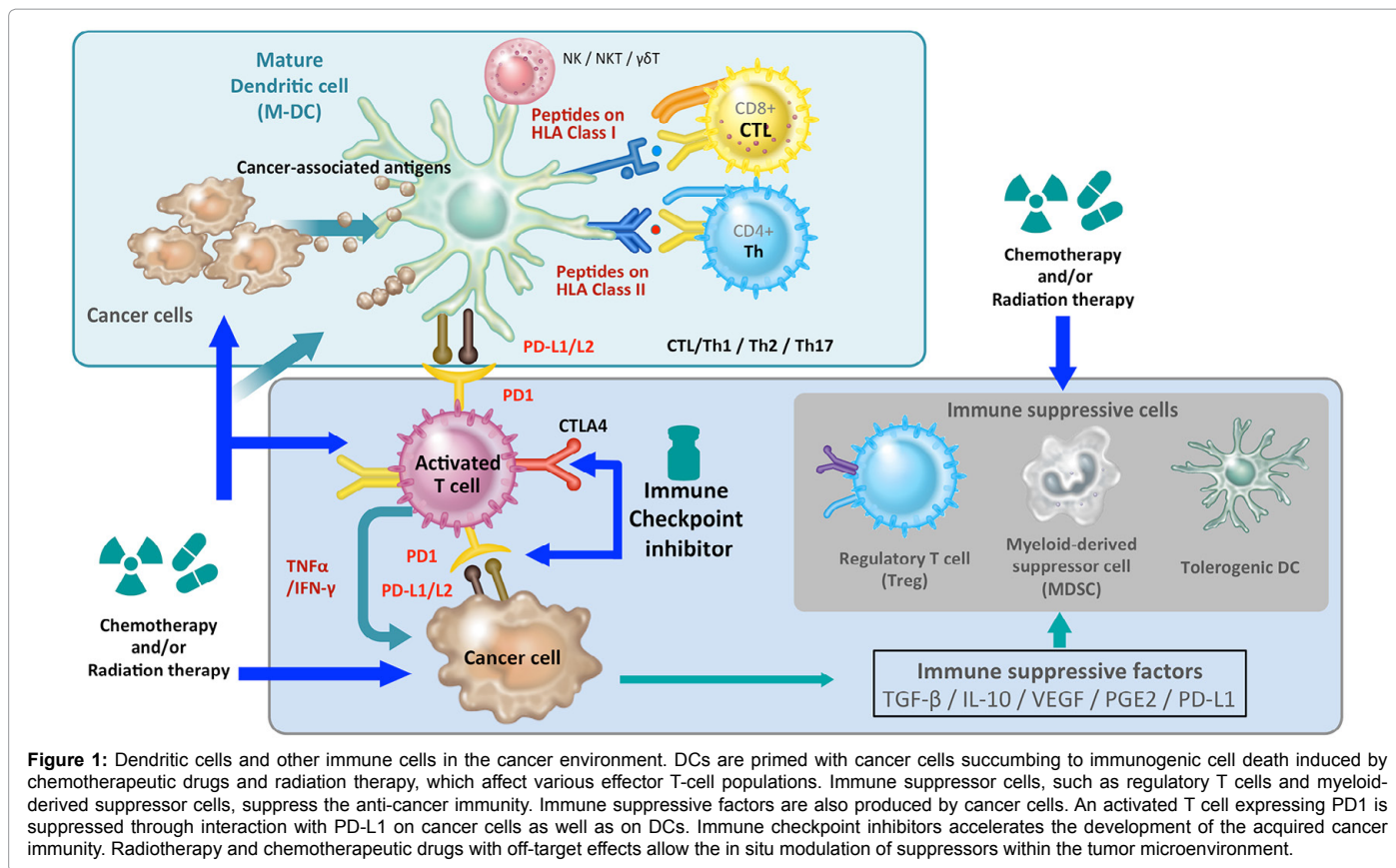Human leukocyte antigen (HLA) molecules containing cancer antigen peptides on DCs bind with T cell receptors on the CD8+ killer and CD4+ helper T cells, producing an immune response against cancers. In contrast, immune suppressor cells such as regulatory T cells, and myeloid-derived suppressor cells (MDSCs) suppress the auto reactive and anti-cancer immunity [13-15]. Moreover, tolerogenic DCs with immunosuppressive cytokines induce antigen-specific anergy and regulatory properties in memory CD4+ T cells [16-18]. Conversely, inflammatory DCs are driven by infection or inflammation release inflammatory cytokines, including tumor necrosis factor and the inducible nitric oxide synthase [19]. Moreover, inflammatory DCs drive Th1- and Th17-mediated immunity and facilitate the induction of regulatory T cells [20,21] (Figure 1). Immune suppressive factors are also produced by cancer cells; these factors include transforming growth factor (TGF)-β, interleukin (IL)-10, vascular endothelial growth factor (VEGF), prostaglandin E2 (PGE2), and programmed death-ligand 1 (PD-L1) [22] (Figure 1). Chemotherapeutic drugs, targeted anti-cancer agents and various other biological and physicochemical therapies such as radiation therapy have been identified as employing immunogenic cell death. DCs are primed with cancer cells succumbing to these immunogenic cell death inducers [23]. Radiation therapy aside from the induction of cancer cell death is useful to shift an immunosuppressive tumor microenvironment to a more beneficial immune stimulation. Radiation therapy can also enhance the expression of HLA class I on the surface of cancer cells, boosting the recognition and killing of irradiated cancer cells through T cells and NK cells [24]. DCs in combination with chemoradiotherapy may accelerate the development of acquired

Figure 1: Dendritic cells and other immune cells in the cancer environment. DCs are primed with cancer cells succumbing to immunogenic cell death induced by chemotherapeutic drugs and radiation therapy, which affect various effector T-cell populations. Immune suppressor cells, such as regulatory T cells and myeloid-derived suppressor cells, suppress the anti-cancer immunity. Immune suppressive factors are also produced by cancer cells. An activated T cell expressing PD1 is suppressed through interaction with PD-L1 on cancer cells as well as on DCs. Immune checkpoint inhibitors accelerates the development of the acquired cancer immunity. Radiotherapy and chemotherapeutic drugs with off-target effects allow the in situ modulation of suppressors within the tumor microenvironment.

cancer immunity to induce antigen-specific cytotoxic T lymphocytes (CTLs) [25,26]. Chemoradiotherapy induce immunogenic cell death, which could trigger T-cell immunity mediated by high-mobility group box 1 protein in patients with oesophageal squamous cell cancer [27]. Radiotherapy and chemotherapeutic drugs with off-target effects may allow the in situ modulation of regulatory T cells and other suppressors within the tumor microenvironment (Figure 1). DCs and regulatory T cells are more resistant to radiation than other lymphocytes in mouse model [28,29]. Human monocyte-derived DCs as well as macrophages are more radio resistant than monocytes [30]. Tumor necrosis factor α and interferon (IFN)-γ secreted by activated T cells react with cancer cells, which causes the formation of complex of PD-L1 on cancer cells with PD1 on activated T cells. PD1-PDL1 complex suppresses the interactions between HLA molecule and T cell receptor, resulting in regression of anti-cancer immunity [31].

DC Vaccine and Vaccination

A manufacturing technique is being developed to promote the strong induction of T cells against tumor antigens. Oil adjuvants for peptide vaccines act by locally accelerating the activation of lymphocytes [32]. However, DCs have potential bioactivity that can be used as a suitable adjuvant [33-34]. DCs expressing tumor-specific antigens used in active cancer immunotherapies [35,36] have been conventionally generated using peripheral monocytes with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4). Antigenic peptides, protein, tumor lysate, and RNA have been used to deliver cancer-associated antigens into DCs [37-42]. Sipuleucel-T (Provenge®, Dendreon Corporation, Seattle, WA), which has been approved by the US Food and Drug Administration (FDA), is an autologous, DC-based immunotherapy for patients with metastatic hormone-refractory

prostate cancer. Sipuleucel-T is manufactured using patient's blood cells exposed to a recombinant fusion protein comprising a prostatic acid phosphatase with GM-CSF, which enhances its activity against prostate cancer. The autologous DC product is administered as per a 3-dose schedule with approximately 2-week intervals between each dose. This regimen yields a survival benefit of 4.1 months when given to patients with hormone-resistant prostate cancer [43].

Among potential cancer antigens such as Wilms' tumor 1 (WT1); mucin 1, cell surface associated (MUC1); human epidermal growth factor receptor 2 (HER2); carcinoembryonic antigen (CEA); survivin; and prostate-specific antigen (PSA), WT1 was identified to be the most potent cancer-associated antigen fulfilling immunological and clinical effectiveness criteria with respect to therapeutic functions, immunogenicity, specificity, and oncogenicity [44]. WT1 has recently been shown to regulate the expression of VEGF, a major mediator of angiogenesis, suggesting it to be another target within the cancer microenvironment [45,46]. Furthermore, the WT1 peptide was restricted to HLA-A*24:02 and modified WT1235–243 peptide (CYTWNQMNL) and the second amino acid (methionine: M) was replaced with tyrosine (Y), which can more effectively induce cytotoxic T cells than wild-type peptides [47-50]. The WT1-332–347–class II peptide is compatible with HLA-DRB1*04:05, HLA-DRB1*08:03, DRB1*15:01, DRB1*15:02, DPB1*05:01, or DPB1*09:01. Phase I clinical trials of this peptide have been conducted for various types of cancers and hematological malignancies [51-53].

DC vaccines primed with HLA class I/II-restricted WT1 peptides (WT1-DC) have been shown to be safe and feasible with few adverse reactions reported for patients with advanced cancers, including lung, breast, stomach, biliary tract, pancreatic, colorectal, ovarian,

and high-grade gliomas [54-62]. Clinical studies have indicated the efficacy of DC vaccination as an add-on to chemotherapy [54-59] and chemoradiotherapy [62,63] and even suggested a survival benefit in some patients. Combinations of adjuvant chemotherapy and/or radiotherapy as well as the periods required for adaptation to these therapies have been investigated. The development of combination therapies that potentially include immune checkpoint inhibitors may help improve the outcomes of personalized therapy for cancer patients.

Immune Monitoring

Immunological monitoring of DC vaccination using tetramer analysis and/or enzyme-linked immunosorbent spot (ELISPOT) assays in clinical studies and trials requires both reproducibility and validation. The presence of WT1 antigen-specific cytotoxic T cells (WT1-CTLs) is defined according to the following criteria: presence of greater than 0.02% WT1-positive CD8$^+$ T cells among the 50,000–10,000 lymphocytes analyzed with no evidence of false positive cells and WT1-positive cell population being clustered but not diffused with slight modification, as previously described [64]. ELISPOT assays were performed to measure WT1-specific IFN-γ production by peripheral blood mononuclear cells (PBMCs). The presence of WT-CTLs was defined according to the following criteria: presence of at least 15 WT1-specific spots per 1×10^6 PBMCs and at least 50% more WT1-specific spots than negative peptide (HIV peptide) spots [64].

We determined WT1-CTLs by both WT1-peptide/HLA-A*24:02 tetramer analysis and ELISPOT assay after one course of DC vaccination during maintenance chemotherapy in a patient with gastric cancer. After one course of DC vaccination, the immune monitoring assay demonstrated that WT1-CTLs comprised 1.10% of the CD8$^+$ T cell population (Figure 2A), with over 100 WT1-specific spots observed in ELISPOT assays (Figure 2B, upper panel). ELISPOT assays performed using WT1-332 (HLA-class II peptide) also demonstrated a specific number of IFN-γ-spots (Figure 2B, lower panel). Specific WT-CTLs were persisted for more than 1 year after DC vaccination.
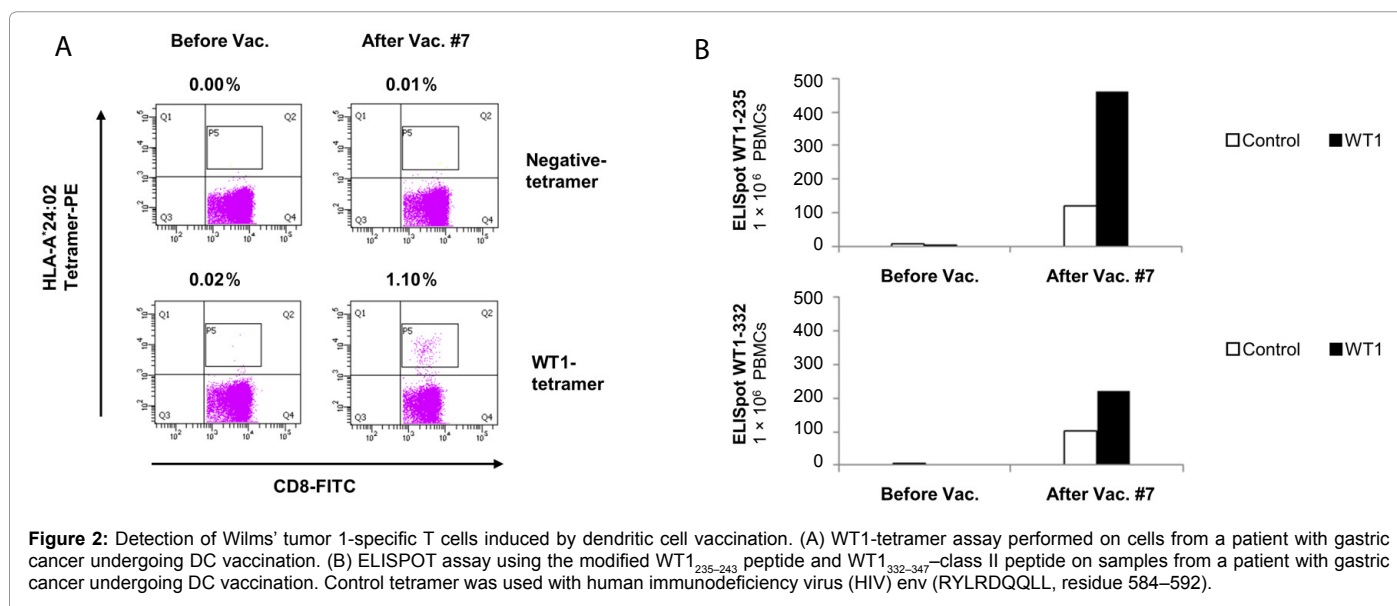
DC Vaccination Technology

Allogeneic vaccines induce T cell infiltration and aggregate formation in pancreatic cancer, resulting in the induction of immunosuppressive regulatory mechanisms [65]. However, allogeneic DC vaccination targeting WT1 also represents a potential strategy for treating patients with relapsed leukemia following hematopoietic stem cell transplantation (HSCT). Subsequent evidence of immune monitoring would provide proof of the validity of this concept. This strategy may be safe, tolerable, and even feasible for pediatric donors and patients with relapsed leukemia following HSCT, as previously described [66]. In this report, a 15-year-old girl with acute lymphoblastic leukemia received allogeneic DC vaccination pulsed with WT1 peptide after her third HSCT. The vaccines were generated from the third HSCT donor, the patient's younger 12-year-old sister, who was a HLA-A*24:02 match. The patient received 14 vaccine doses with no occurrence of graft-versus-host disease or any systemic adverse effects, except for a grade 2 local skin reaction at the injection site. WT1-specific immune responses were detected post-vaccination by both WT1-tetramer analysis and ELISPOT assay. The patient experienced 44 months of remission after the third HSCT with DC vaccination, whereas she had been in remission for less than 14 months between her second and third HSCT. This suggests that WT1-specific DC vaccination contributed to extended remission after the patient's third HSCT. These findings form the basis for developing individualized therapy for future prospective HSCT clinical trials.

One potential way to overcome the phenomenon of tumor cells escaping immune detection is the generation of IFN-DCs from monocytes using GM-CSF and IFN-α previously described in a clinical study on metastasized medullary thyroid carcinoma [67] under Good Gene, Cell and Tissue Manufacturing Practice conditions. Mature IFN-DCs would induce CTLs together with their adaptive antitumor effects as well adoptive immunotherapy with natural killer cell activity independent of HLA antigen expression [67,68]. Another approach is the administration of granulocyte colony-stimulating factor (G-CSF), resulting in the upregulation of adhesion molecules, to evaluate the hypothesis of increased acquired immunity using G-CSF-primed DC vaccines.

Immune checkpoint inhibitors are rapidly being developed as cancer therapeutic agents [69]. In combination with DC vaccination and immune checkpoint inhibition, further studies are required to evaluate whether the number of effector memory T cell present

Figure 2: Detection of Wilms' tumor 1-specific T cells induced by dendritic cell vaccination. (A) WT1-tetramer assay performed on cells from a patient with gastric cancer undergoing DC vaccination. (B) ELISPOT assay using the modified WT1$_{235-243}$ peptide and WT1$_{332-347}$–class II peptide on samples from a patient with gastric cancer undergoing DC vaccination. Control tetramer was used with human immunodeficiency virus (HIV) env (RYLRDQQLL, residue 584–592).

prior to vaccination and the exhausted marker of PD1-positive CTLs after DC vaccination influence the efficacy of DC vaccination and/or immune check point inhibitors. Such future clinical trials could reveal the effectiveness of DC vaccine in combination with immune checkpoint inhibitors in treating cancers, sarcomas, and hematopoietic malignancies in the near future. Biomarkers that predict the potential efficacy of DC vaccination targeting WT1 are highly relevant to current standards of personalized cancer therapy.

Conflict of Interest

All authors declare no conflict of interest that could be perceived as prejudicing the impartiality of the reported research.

Acknowledgments

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