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Dendritic/pancreatic carcinoma fusions for clinical use: Comparative functional analysis of healthy- versus patient-derived fusions

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Abstract Fetal calf serum (FCS)-independent pancreatic cancer cells were established in plasma protein fraction (PPF)-supplemented medium that is an agent of good manufacturing practice (GMP) grade. Dendritic cells (DCs) were activated with the Toll-like receptor agonist, penicillin-inactivated *Streptococcus pyogenes* (OK-432) that is also a GMP grade agent. Therefore, sufficient amounts of FCS-independent fusions were successfully generated with decreased potential hazards of FCS. The FCS-independent fusions expressed tumor-associated antigens, HLA-DR, costimulatory molecules, IL-12, and IL-10. Stimulation of T cells with fusions from healthy donors resulted in proliferation of T cells with high expression levels of perforin/granzyme B and IFN-γ and efficient induction of antigen-specific cytotoxic T lymphocytes (CTLs). Selection and expansion of T-cell clones were confirmed by TCR Vβ analysis. However, fusions from patients with metastatic pancreatic cancer induced increased expression levels of TGF-β1 in CD4+ CD25high T cells and low levels of CTLs with decreased IFN-γ production.

Abbreviations: DC, dendritic cell; Ab, antibody; TAA, tumor-associated antigen.

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Introduction

Pancreatic cancer is the fifth most common cancer worldwide. Despite aggressive surgical and non-surgical treatment, the mean life expectancy is approximately 15–18 months for patients with local and regional tumors and 3–6 months for patients with metastatic tumors [1]. Because only 10–15% of patients diagnosed are eligible for surgical treatment, the 5-year overall survival rate is less than 4% [2,3]. For patients with advanced pancreatic cancer, the treatment options are limited. Therefore, the development of new treatment modalities, including specific immunotherapy, is of great importance in the treatment of pancreatic cancer. In support of the immunotherapy approach are the findings that pancreatic cancer cells express tumor-associated antigens (TAAs) such as MUC1 [4], WT1 [5], carcinoembryonic antigen (CEA) [6], HER-2/neu [7], mutated KRAS [8], and p53 [9] as potential targets for immunotherapy. Immunotherapy for pancreatic cancer is one approach that is at a crucial crossroads, as therapeutics that are designed to target TAAs and regulatory signaling molecules are entering clinical trials.

Antigen-specific cytotoxic T lymphocytes (CTLs) can be activated by vaccination with dendritic cells (DCs). DCs are specialized antigen-presenting cells (APCs) with the unique capacity to elicit primary immune responses [10]. DCs derive their potency from the prominent expression of major histocompatibility complex (MHC) class I and II, costimulatory, and adhesion molecules that provide secondary signals for the activation of naive CD4+ and CD8+ T cells [11]. Various strategies to deliver TAAs into DCs have been developed to generate a potent CTL response against autologous tumor cells. DCs have been pulsed with synthetic peptides derived from the known TAAs, tumor cell lysates, apoptotic tumor cells, and tumor RNA [12–15]. Although the production of cancer vaccines for individual patients with pancreatic cancer has currently been addressed in clinical trials, a major drawback of this strategy comes from the limited number of known tumor peptides available in many HLA contexts. Moreover, DCs pulsed with antigen-specific peptides have been used in clinical trials for patients with cancer, but the results show that clinical responses have been found in a small number of patients [16,17]. An alternative approach to induce efficient antitumor immunity is the use of fusions of DCs with tumor cells [18–20]. Because few other pancreatic tumor antigens have been identified, the whole tumor cells may be postulated to serve as the best source of antigens. The strategy for DCs/tumor fusion cell vaccine is based on the fact that DCs are the most potent APCs, whereas tumor cells express abundant TAAs. In this approach, TAAs, including both known and unidentified antigens are delivered to DCs, processed, and presented through both MHC class I and II pathways in the context of costimulatory molecules [19,20]. We have previously reported that autologous DCs fused with autologous tumor cells induce antigen-specific polyclonal CTLs able to kill autologous tumor cells in vitro [21–31]. This fusion cell technology eliminates the need to isolate specific TAAs, as the original tumor is utilized in the fusions. However, in the clinical setting of the patients with advanced pancreatic cancer, a major problem of the DCs/tumor fusion cell vaccine is the preparation of sufficient amounts of autologous tumor cells. Only 10–15% of pancreatic cancer patients diagnosed are eligible for surgical treatment. Therefore, autologous tumor cells may not be provided in almost of the patients. Moreover, even if the patients are treated by surgical resection, it is difficult to prepare sufficient numbers of viable tumor cells due to the length of culture time and potential contamination of bacteria and fungus [27]. To circumvent this problem, allogeneic tumor cells may be used instead of autologous tumor cells as a fusion partner to deliver shared TAAs into autologous DCs. We have reported that fusion cells generated with autologous DCs and allogeneic tumor cells can induce antigen-specific CTLs able to kill autologous tumor cells through the cross-priming against shared tumor antigens in vitro [27,29]. These reports suggest that CTLs induced by fusions generated with autologous DCs and allogeneic pancreatic cancer cells kill autologous targets in TAAs-specific and HLA restriction manners. Indeed, a phase III clinical trial of GM-CSF secreting allogeneic cancer cell based-vaccine approach has been tested in patients with prostate cancer [32].

Although fetal calf serum (FCS) has usually been used for culture of various types of tumor cells as a component of the culture medium, the use of FCS in clinical trials has a possible risk of infection with pathogens of prion diseases such as bovine spongiform encephalopathy (BSE) that is regulatory issue. Therefore, a major challenge to develop a DCs/allogeneic tumor fusions vaccine strategy is to overcome the potential hazards of FCS that limit safety in clinical trials. One alternative approach for culture of cancer cells is the use of autologous serum from patients. However this approach can present a problem for clinical application such as the significant amounts of autologous serum necessary to expand the sufficient amounts of cancer cells in vitro. The clinical grade preparation necessitates adhering to good manufacturing practices (GMP) to insure the delivery of a "cell drug" that is safe, reproducible, and efficient. Here, we have successfully established FCS-independent pancreatic cancer cells able to grow in human plasma protein fraction (PPF)-supplemented (tumor/PPF) or protein-free medium (tumor/free). Human PPF is mainly composed of albumin and a GMP grade agent. Moreover, human PPF has been widely used in patients. In this study, DCs maturation was performed with the Toll-like receptor (TLR) agonist, penicillin-inactivated *Streptococcus pyogenes* (OK-432) that is also a GMP grade agent. Therefore, an important aspect of our work is its potential clinical relevance. Fusions of the OK-432-stimulated DCs either from healthy donors or patients with metastatic pancreatic cancer and FCS-independent tumor cells (FCS-independent fusions) elicited T cells able to kill tumor targets. This alternative approach may provide the opportunity to continue clinical trials for treating pancreatic cancer patients who do not respond to conventional therapy. Moreover, well-established treatment strategies such as surgical resection, chemotherapy, or radiation may successfully combine with immunotherapeutic approaches. The present studies demonstrate that FCS-independent fusions are effective in the generation of CTL responses with decreased potential hazards.