Redirected T Cells That Target Pancreatic Adenocarcinoma Antigens Eliminate Tumors and Metastases in Mice

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BACKGROUND & AIMS: Pancreatic adenocarcinoma (PAC) is often diagnosed at an advanced and inoperable stage, and standard systemic treatments are generally ineffective. We investigated the effects of adoptive transfer of tumor-specific T cells that express chimeric antibody-based receptors (CAR) to mice with primary and metastatic PAC xenografts. **METHODS:** Human effector T cells were genetically modified to express CAR against Her2/neu or CD24, a putative PAC stem cell antigen. The antitumor reactivity of the engineered T cells (T-bodies) was evaluated in SCID mice with different PAC xenografts. A total of 1×10^7 T-bodies were injected via the tail vein or directly administered to the subcutaneous tumor on 3 or 4 alternating days. Mice were then given twice-daily intraperitoneal injections of interleukin-2 for 10 days. **RESULTS:** Intratumor injection of human CD24 and Her2/neu-specific T-bodies completely eliminated the tumors from most animals. Intravenous injection of T-bodies reduced tumor size and prolonged survival of mice with orthotopically transplanted tumors; more than 50% of animals appeared to be disease-free more than 2 months later. Additional systemic administration of T-bodies 8 weeks after the initial injection eliminated primary tumors, along with liver and draining lymph node metastases. A single administration of the Her2/neu-specific T-bodies prolonged the survival of mice with tumors in which most of the cells expressed the target antigen. In contrast, the CD24-specific T-bodies prolonged survival of mice in which only a subpopulation of the tumor cells expressed the antigen. **CONCLUSIONS: CAR-redirected T cells stop growth and metastasis of PAC xenografts in mice. T-bodies specific to CD24, a putative cancer stem cell antigen, were effective against PAC xenografts that had only a subset of antigen-expressing cells.**

Keywords: Immunotherapy; Pancreatic Cancer; Adoptive Cell Therapy; Chimeric Antigen Receptor.

Pancreatic adenocarcinoma (PAC) is almost always incurable, because the standard therapies are ineffective against the advanced stages of the disease at which this cancer is generally diagnosed. PAC comprises more than 90% of all pancreatic cancers and has the worst prognosis of any major malignancy. Most of the patients already have metastatic disease at the time of diagnosis, and only 10%–15% of the patients present a localized tumor that is suitable for surgical resection; even from this population, only 20% survive 5 years after resection. Systemic chemotherapy and/or radiotherapy of PAC has failed to provide more than a very slight survival benefit for patients with advanced disease.[1] Thus, optimal management of the disease remains a significant therapeutic challenge.

Our laboratory pioneered an immunotherapy approach that uses redirected effector T lymphocytes expressing a chimeric antigen receptor (CAR) with antibody-type specificity (dubbed the "T-body" approach).[2] We and others showed that such T-bodies, expressing tumor-specific CAR, can recognize, kill, and reject, in a non–major histocompatibility complex–restricted manner, a large spectrum of human cancers. Initial clinical trials using T-bodies are ongoing for several types of cancer (see Baxevanis and Papamichail[3] and Sadelain et al[4] for reviews). Most recently, encouraging results were obtained in a pilot clinical trial using T-bodies to treat patients with chronic lymphocytic leukemia.[5] The antitumor effectiveness of the T-body therapy is strongly related to the composition of the CAR and proper selection of the targeted tumor-associated antigen (TAA).[6,7] The curative effect of this form of adoptive cell therapy appears to be directly dependent on the persistence of the modified T cells in the recipient.[8] In recent years, the CAR composition was improved by the addition of intracellular domains derived from various costimulatory receptors such as CD28, CD137 (4-1BB), and CD134 (OX40), which enhance T-body activation and proliferation, independent of costimulatory receptor-ligand interactions.[7]

In this preclinical study, we investigated CARs directed at 2 surface TAAs found in PAC, each having a unique feature. CD24 is a small heavily glycosylated mucin-like cell surface protein anchored to glycosylphosphatidylinositol. It was shown to be expressed in various malignancies, including 72% of PAC tumors, and to correlate

Abbreviations used in this paper: CAR, chimeric antibody-based receptor; CSC, cancer stem cell; FACS, fluorescence-activated cell sorting; MST, mean survival time; PAC, pancreatic adenocarcinoma; TAA, tumor-associated antigen.

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with higher tumor grades and aggression.[9] CD24 is believed to play a role in pancreatic carcinogenesis[10] and was recently suggested to be expressed by the putative PAC cancer stem cells (CSC) along with CD44 and epithelial specific antigen or CD133.[11] Among these antigens, CD24 is the least commonly found on normal tissues, making it a preferable antigen for immunotherapy. Her2/neu is a member of the human epidermal growth factor receptor family and is a potent mediator of normal cell growth and development (reviewed in Baselga and Swain[12]). Her2/neu has been the subject of increased interest due to its oncogenic potential when its signaling function is deregulated. It is overexpressed in approximately 20%–60% of pancreatic cancers,[13,14] and its expression correlates with metastatic disease and with more severe disease and poor outcome.[14] Her2/neu has become an established target for antibody therapy of breast cancer,[15] and several adoptive transfer studies using redirected lymphocytes against Her2/neu were reported (reviewed in Baxevanis et al[16]), although none of these were directed at human PAC.

In this study, we developed adoptive cellular immunotherapy for metastatic PAC using effector lymphocytes expressing CARs specific to either the PAC stem cell antigen CD24 or the Her2/neu (ErbB2) growth factor receptor. Interestingly, the CSC-specific T-bodies prolonged the life of mice bearing xenografts, even those in which only a minority of the cancer cells expressed the target antigen, whereas the anti-Her2/neu T-bodies eradicated only tumors in which the majority of the cells expressed the target antigen. We show here that effector lymphocytes, redirected against these surface antigens, are powerful enough to offer curative treatment for a currently intractable tumor such as PAC.

Materials and Methods

Establishment and Characterization of PAC Xenografts

Fresh PAC specimens were taken from patients diagnosed with PAC who underwent tumor excision surgery (Whipple procedure) at Assaf Harofeh Medical Center. The study received institutional ethics committee approval, and informed consent was obtained from all patients. Pieces of tumors were mixed with Matrigel (Becton Dickenson, Bedford, MA) and transplanted subcutaneously in SCID-beige mice (see Supplementary Materials and Methods). The xenografts that developed after several months (denoted Wapac 1–5) were serially transferred in SCID mice. Samples were cryopreserved and fixed for histologic examination.

Two xenografts were further characterized and used in this study. Wapac-4 was taken from a 68-year-old black man with ductal adenocarcinoma of the pancreas, and Wapac-5 was taken from a 63-year-old white man with moderately differentiated adenocarcinoma of the pancreas. Unlike the original tumor, in mice this xenograft exhibited histopathologic characteristics of mucinous adenocarcinoma.

T-Body Treatment of Tumor-Bearing Mice

Mice bearing either orthotopic or subcutaneous Capan-1 tumors or Wapac xenografts were divided into groups with similar tumor burden 3–4 weeks after tumor transplantation. Mice were either total body sublethally irradiated (200 rad) or injected with cyclophosphamide (200 mg/kg) 1 day before initiation of T-body treatment. For treatment, 1×10^7 T-bodies were injected on alternate days for 3 or 4 treatments, intravenously to the tail vein or directly into the subcutaneous tumor. Mice were injected intraperitoneally twice daily for 10 days with 1000 U interleukin-2 in 200 μL Hank's balanced salt solution. Drinking water was supplemented for 4 weeks with 340 μg/mL enrofloxacin (Baytril 10%; Bayer Healthcare AG, Leverkusen, Germany).

Statistical Analysis

Due to the 3-dimensional nature of the tumors, statistical evaluation was performed based on the calculated cubic root of the volume[17,18] or on bioluminescence measurement. The differences between treatment groups and the control group and those between the different measurement times were analyzed by using 2-way analysis of variance with Bonferroni posttests. Survival statistics were calculated using the log-rank (Mantel–Cox) test. All calculations were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). $P < .05$ was considered significant.

Please see Supplementary Materials and Methods for additional information.

Results

In Vitro Antitumor Activity Against PAC Cell Lines

To study the therapeutic potential of T-bodies toward human PAC, we focused on 2 TAAs: HER2/neu, a growth factor receptor that may have a role in the oncogenic process, and CD24, which was suggested to be a putative tumor stem cell antigen of PAC. As a target cell, we used the human Capan-1 PAC cell line and 2 newly established PAC xenografts: Wapac-4 and Wapac-5. Figure 1 depicts the schematic composition of the CAR (Figure 1*A*), surface expression of the selected target antigens on Capan-1 cells (Figure 1*B*), cytotoxicity (Figure 1*C*), and stimulation-induced cytokine production by T-bodies (Figure 1*D*). For additional information, see Supplementary Results.

T-Body Reactivity Toward Subcutaneous Capan-1 Tumor

In the first experimental model, SCID mice bearing a subcutaneous, established, encapsulated Capan-1 tumor (grown for 4 weeks to a palpable tumor of an average volume of 35 mm^3) were preconditioned by sublethal irradiation (200 rad). After 1 day, T–bodies were injected intratumorally in a split dose (1×10^7 cells/day) on 3 alternate days. This protocol mimics a clinical setting of direct injection to a pancreatic tumor, which is clinically feasible in humans under endoscopic ultrasonography or computed tomography guidance. As shown in Figure 2, the tumors in the groups treated with the control T–bodies grew continuously at a constant rate. In contrast, treatment with specific T-bodies reduced the tumor volume in all mice. After 70 days, 3 mice from the αHER2–