Translational Medicine

Research Article | **Open Access**

CD8+ T Cells Are Compromised In Human Pancreatic Cancer

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Summary

Objectives and background: An efficient cellular immune response against cancer requires a robust population of CD8+ T cells with optimal cytotoxic functions. The aim of this study was to define the percentage of CD8+ T cells and the perforin expression level of the CD8+ T cells in the peripheral blood from patients with pancreatic adenocarcinoma.

Methods: Fourty-six pancreatic cancer patients in our center between August 2011 and April 2012 were recruited. Thirty-five healthy persons were included as controls. Blood was drawn before surgery and one and a half months after surgery. Cells were labeled with monoclonal antibodies against perforin and surface antigen CD8 and were analyzed by flow cytometry.

Results: Compared with the healthy controls, the percentage of CD8+ T cells was significantly decreased in pancreatic cancer patients. The level of perforin expression was also decreased in pancreatic cancer patients, whereas the percentage of CD8+ T cells was significantly increased after radical tumor resection.

Conclusions: Both the number and function of CD8+ T cells were compromised in pancreatic cancer patients, which were partially recovered after the surgery.

Keywords: CD8+ T cells; Pancreatic cancer

Abbreviations: APC: Allophycocyanin; FITC: Fluorescein isothiocyanate; PC: Pancreatic Cancer; CTL, Cytotoxic T lymphocyte

Introduction

Pancreatic cancer is the fourth most common cause of adult cancer death in the United States. A total of 43,920 new cases and 37,390 deaths from pancreatic cancer are estimated to occur in the USA in 2012 [1]. Although great progress has been made in the diagnosis and therapy of cancer, the prognosis of pancreatic cancer still remains poor. Due to the lack of early diagnosis and high incidence of metastatic disease at initial diagnosis, only 15%-20% of patients are resectable [2-4]. Even so, the 5-year survival rate amongst radically operated patients is only about 5-20% [2,5,6].

Chemotherapy still remains the most important adjuvant therapy in patients with pancreatic malignancies. Since the introduction of gemcitabine, it has become the first-line treatment for advanced pancreatic cancer patients [7]. However, due to the high degree of inherent or acquired chemoresistance, only modest benefit was recorded in patients with advanced disease, even when combined with other chemotherapeutic agents [8,9]. No consensus regarding the role of radiotherapy in pancreatic cancer has been reached. Given the limited therapeutic options available for pancreatic cancer and high rate of recurrence and metastasis after surgical resection, new therapies, such as immunotherapy, are needed. Immunotherapy acts through a mechanism that is distinct from chemotherapy or radiation therapy, which may serve as a new treatment strategy. In theory, cancer cells are vulnerable to immune effector cells such as tumor-specific CD8+ T cells and natural killer cells (NK). However, most cancers can evade this immune surveillance by several distinct mechanisms, such as production of immunosuppressive factors (e.g. TGF-β), or inactivation of infiltrating lymphocytes by the FAS/FAS ligand system [10].

Tumor-specific CD8+ T cells (also known as cytotoxic T lymphocytes, CTL) play a vital role in cell-mediated immune response. They secrete cytolytic granules containing proteins, such as perforin, granzymes, which are able to destroy cancerous cells or virus-infected cells. Among them, perforin plays a critical role in their cytotoxic

activity by forming pores in the plasma membrane, facilitating delivery of pro-apoptotic granzymes into the target cells [11]. One possible reason for ineffective surveillance in patients with cancer could be the insufficiency of perforin expression and dysfunction of perforin release in and around the tumor [12].

The aim of this study was to investigate the frequency and function of CD8+ T cells in pancreatic cancer patients. The expression status of perforin in circulating CD8+ T cells was also analyzed in pancreatic cancer patients. The presence of CD8+ T cells before and after surgery was compared.

Materials and Methods

Patients

Between August 2011 and April 2012, fourty-six patients diagnosed with pancreatic adenocarcinoma were enrolled in this study. The diagnosis was confirmed by pathological examinations. The male to female ratio was 1:1. The mean age was 64 years, ranging from 36 to 76 years. In all patients, 1 ml of heparinated blood samples was collected before treatment. Blood samples of 13 patients who received radical pancreatectomy were collected approximately one and a half months after the surgery and tested for immunological parameters. Thirty-five healthy persons, consisting of laboratory personnel and blood volunteer donors, corresponding by age (age 32 to 68) and sexes (17 females and 18 males) to the examined patients groups were also studied.

Informed consent forms were obtained from all patients and

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volunteers enrolled, which was performed according to the guidelines of the Ethics Committee of the Medical Faculty at Shanghai Cancer Center, Fudan University.

Monoclonal Antibodies (MoAbs)

FITC-conjugated anti-CD8, APC-conjugated anti-perforin MoAbs were purchased from eBiosciences (San Diego, CA). Isotype-matched mouse antibodies, directly conjugated with FITC and APC were used as negative controls for each class of antibody used.

Flow cytometric analysis

For cellular surface staining of CD8 antigen, thirty-one pancreatic cancer patients and twenty healthy donors were enrolled. 100 µl of thoroughly mixed blood were prepared and labeled with primary-conjugated antibodies against cell-surface markers (anti-human CD8). They were mixed gently and incubated in tubes for 15 minutes at room temperature (20° to 25°C) in the dark. 2 ml of FCM Lysing solution was added to the tubes, which were then vortexed thoroughly and incubated for 10 minutes at room temperature (20° to 25°C) in the dark. The tubes were spun at 400 × g for 5 min and the supernatant was removed. The cell pellets were resuspended in tubes in 2 ml of PBS, and centrifuged at 400 × g for 5 min. The supernatant was removed and the cell pellets were resuspended in tubes of 1 ml of PBS for immediate analysis.

For intracellular staining of perforin molecules, another fifteen pancreatic cancer patients and fifteen healthy donors were enrolled. Blood samples were first stained with FITC-conjugated anti-CD8 and incubated for 15 minutes at room temperature. 100 µl of reagent A (Fixation Medium, Invitrogen) was added and then incubated for 15 minutes followed by a single wash with PBS. The tubes were centrifuged to remove the supernatant, and we then added 100 µl of reagent B (permeabilization Medium, Invitrogen) and 5 µl of the APC-conjugated anti-perforin antibody. We vortexed and incubated the tubes for 20 minutes, washed and centrifuged for 5 minutes at 400 × g and then removed and aspirated the supernatant. We resuspended the cells in 1 ml of PBS. Sample data was acquired on Beckman FACScan (German) and analyzed with CXP 2.1 software.

Statistical analysis

Student's t test and paired t-test were used to determine statistical significance (SPSS 13.0). A p value of <0.05 was considered significant.

Results

Percentage of CD8+ T cells and perforin levels in pancreatic cancer patients and healthy donors

To investigate the basal immune status of patients, the subpopulation of CD8+ T cells in the peripheral blood were analyzed. Healthy donors were used as controls. The percentage of CD8+ T cells in the pancreatic cancer group was (22.55 ± 5.22)%, which was remarkably lower than that in the healthy control group (26.96 ± 3.72%) (Figure 1A, P=0.00195).

No differences in the percentage of CD8+ perforin+ T cells were observed in patients versus healthy donors (Figure 1B, P>0.05, data not shown). However, patients had significantly lower mean fluorescence intensity (MFI) than the normal controls (Figure 1C, 38.32 ± 10.67 vs. 50.33 ± 8.6, P=0.00208). The value of mean fluorescence intensity (MFI) correlates with the number of total perforin molecules present in the cells.

Effect of surgical treatment on the percentage of CD8+ T cells

Thirteen pancreatic cancer patients underwent radical pancreatectomy were analyzed. Post-operative analyses were performed approximately one and a half months after surgery, when no patients had yet received any further anti-cancer treatment. There was significant change in CD8+ T cells one and a half month after radical surgery. The percentage of CD8+ T cells increased significantly in patients after radical surgery (Figure 2B, 1 vs. 1.20 ± 0.18, P=0.00186).
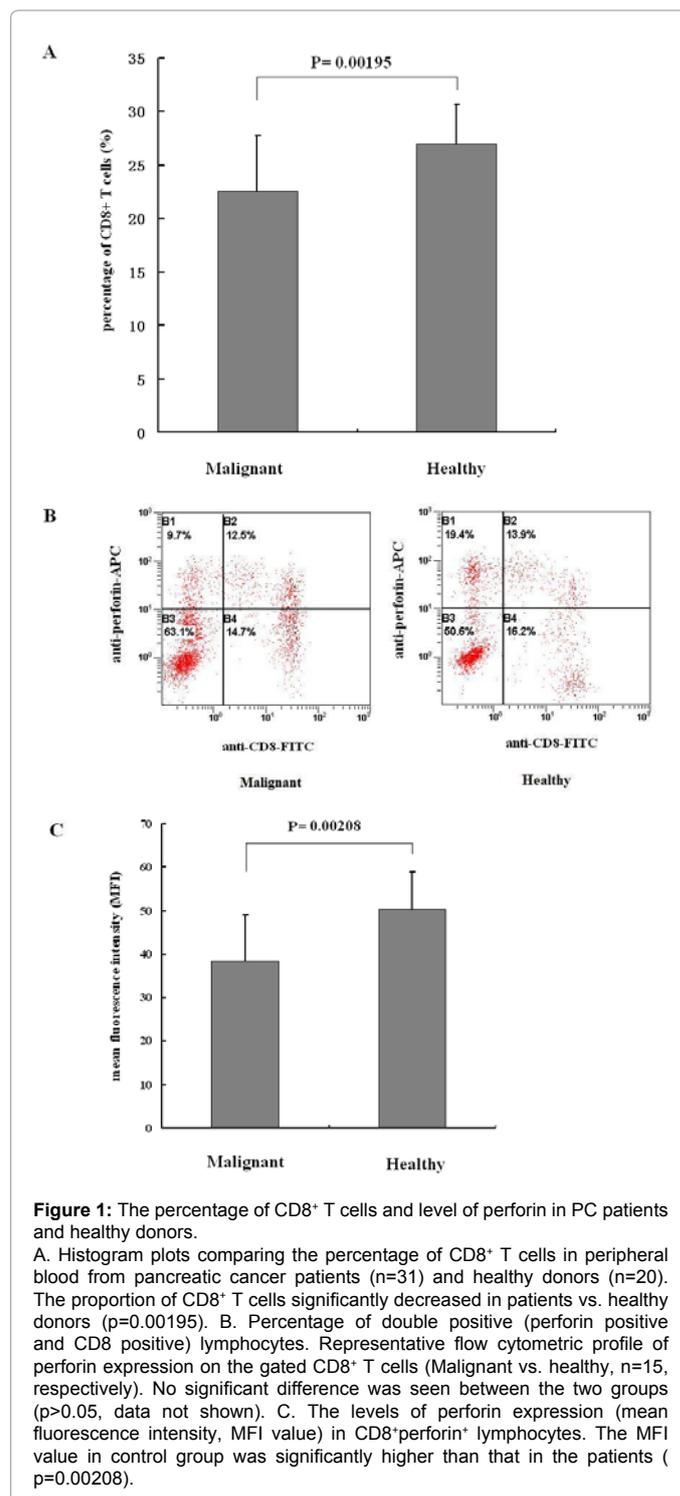
Figure 1: The percentage of CD8+ T cells and level of perforin in PC patients and healthy donors.
A. Histogram plots comparing the percentage of CD8+ T cells in peripheral blood from pancreatic cancer patients (n=31) and healthy donors (n=20). The proportion of CD8+ T cells significantly decreased in patients vs. healthy donors (p=0.00195). B. Percentage of double positive (perforin positive and CD8 positive) lymphocytes. Representative flow cytometric profile of perforin expression on the gated CD8+ T cells (Malignant vs. healthy, n=15, respectively). No significant difference was seen between the two groups (p>0.05, data not shown). C. The levels of perforin expression (mean fluorescence intensity, MFI value) in CD8+perforin+ lymphocytes. The MFI value in control group was significantly higher than that in the patients (p=0.00208).
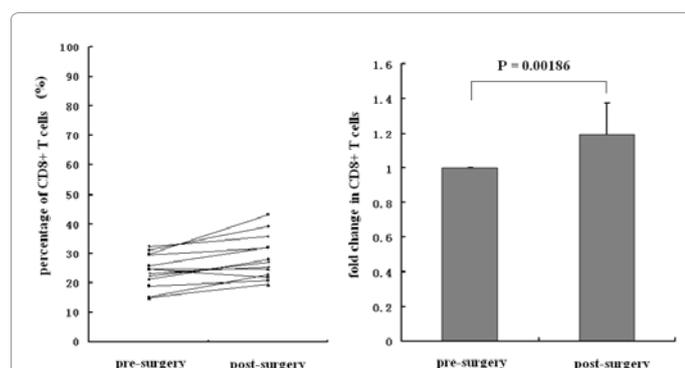
Figure 2: The changes of percentage of CD8+ T cells after radical pancreatectomy.
A. The percentage of CD8+ T cells for each patient (pre-surgery vs. post-surgery, n=13) B. The percentage of CD8+ T significantly increased after radical pancreatectomy (p=0.00186). Data are expressed as fold change (post-surgery vs. pre-surgery). Statistical significance was determined using the paired t-test.

Discussion

To date, many studies have been performed to expand the number of tumor-specific immune effector cells with tumor vaccines [13]. However, despite intensive effort, the anti-tumor efficacy remains limited. It has become increasingly recognized that the function of the immune effector cells is impaired due to the tumor microenvironment [14,15]. To improve the efficacy of immunotherapy for pancreatic cancer, it is critical to have a better understanding of the function of the immune effector cells in pancreatic cancer patients.

In the present study, we detected the percentage of CD8+ T cells in pancreatic cancer patients and investigated the effect of surgery on the number of CD8+ T cells. Decreased CD8+ T cell percentage was observed in pancreatic cancer patients. The level of perforin expression was also decreased in pancreatic cancer patients. The percentage of CD8+ T cells was significantly increased after radical tumor resection.

Effective CD8+ T cell mediated cytotoxic killing may play a crucial role in the control of cancer development. However, as has been reported in lung cancer, colorectal cancer, and melanoma patients, the number and especially the function of CD8+ T cells were limited due to the existence of tumor milieu [16-19]. In pancreatic cancer patients, we also found that both the number and function of CD8+ T cells were decreased when compared with healthy donors. After tumor resection, CD8+ T cell numbers were elevated significantly.

So far, many studies have found that the tumor continued to grow despite the presence of high numbers of tumor-specific CD8+ T cells [18,19]. Thus, established tumors must have developed strategies which could inhibit or evade the immune system. Among the multiple suppressive mechanisms, regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) were the most commonly reported mechanisms that might inhibit the function of T effector cells [20-22]. Impaired CD8+ T cell function at the final stage of degranulation can also lead to disease progression [23]. However, the exact cause of functional impairment is still unknown. Pattu et al. [24] found that syntaxin7, a member of SNARE proteins, was required for lytic granule release from CD8+ T cells. In our previous study, we found that profilin-1, the most common isoforms of actin-binding proteins, promoted the stabilization and polymerization of actin [25], which might also facilitated granule release from CD8+ T cells [26,27].

In conclusion, we found that both the number and function of CD8+ T cells in pancreatic cancer patients were compromised. The reason for impaired CD8+ T cells in pancreatic cancer needs further investigation. Since the presence of many suppressive factors in the tumor microenvironment, multiple approaches should be incorporated to recover the function of CD8+ T cells and improve the anti-tumor efficacy of the current immunotherapy in pancreatic cancer.

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