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Efficacy of cytokine-induced killer cells in the treatment of elderly patients with metastatic pancreatic adenocarcinoma

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Wei Li and Yaomei Wang contributed the same work to this study.

Abstract

Currently, metastatic pancreatic cancer is associated with disappointing survival outcomes. This is largely due to a rapid progression of the disease and a precipitous deterioration in the health of affected individuals, especially elderly patients who are often unable to tolerate chemotherapy. The aim of this study was to evaluate the efficacy and safety of adoptive immunotherapy using cytokine-induced killer cells (CIK) as a first-line treatment for metastatic pancreatic cancer. Between December 2010 and June 2012 eight patients were enrolled in this study. All participants were elderly, suffering from metastatic pancreatic cancer, and unable to tolerate chemotherapy. All patients in this study received R-CIK therapy only as a first-line treatment. In the eight patients, 1 had complete response (CR), 5 had stable disease (SD) and 2 had progression disease (PD). Therefore, the overall response rate (ORR) was 12.5% (1/8) and the disease control rate (DCR) was 75.0% (6/8 patients). The 1-year survival rate was 37.5%, and the median overall survival time (mOS) was 13.04 months (95% CI: 5.9-20.2). The results indicated that no significant positive or negative predictive factors were identified by univariate analysis. The main adverse effect of R-CIK was fever and the side effect rate was 25.0% (2/8). Adoptive immunotherapy using R-CIK cells showed comparable OS to survival data seen in previous trials assessing conventional chemotherapies in elderly patients and the adverse effect is less pronounced.

Key words: *cytokine-induced killer cells, elderly pancreatic adenocarcinoma, immunotherapy.*

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Introduction

Pancreatic adenocarcinoma is one of the most aggressive malignant cancers known, and the associated mortality rates have remained largely unchanged despite active research into therapies that have been successful against other malignant neoplasia [1]. Despite improvements in imaging and surgery, long-term survival remains poor with a median survival of one year for patients with resected disease, 8-10 months for those with locally advanced, unresectable disease and less than 6 months for patients with metastatic disease [2, 3]. This poor prognosis is attributed mainly to the high incidence of metastasis at the time of presentation, the aggressive course of disease and limited efficacy of available systemic treatments [4]. At present, chemotherapy is still the primary therapeutic option for metastatic pancreatic adenocarcinoma. While some studies have yielded improvements in ORR and progression free survival time (PFS), benefits in OS have been limited. Additionally, the mOS of elderly patients that received chemotherapy was only 5.9-7.3 months [5]. Therefore, the need to explore a new treatment to prolong the mOS of elderly patients is of the utmost importance.

Over the past decade, adoptive immunotherapy has gained more and more attention as a treatment option for many kinds of malignant carcinoma, including metastatic pancreatic adenocarcinoma [6-10]. Recently, CIK therapy has become the most widely used immunotherapeutic method, largely due to the rapid proliferation of this cell type in vitro as well as its strong anti-tumor activity against a broad spectrum of solid tumors. It is well known that the effect of CIK therapy depends on the quality and quantity of

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the cultured cells. Recently, studies have reported that use of RetroNectin can improve the conglutination, extension, differentiation and proliferation of cells, resulting in more efficient stimulation of T cells [11-14]. In this study, we investigated the clinical effects and safety of RetroNectin treated CIK cells (called R-CIK) in the treatment of elderly patients with metastatic pancreatic carcinoma in the hopes of establishing a new and much needed treatment option.

Material and methods

Patients

Eight patients with metastatic pancreatic adenocarcinoma were enrolled in this study. Selected participants had a minimum age of 65 years and the median age was 71.3 years (with an age range of 65-79 years). The Eastern Cooperative Oncology Group (ECOG) scores were 2 or 3. All selected patients were unable to tolerate chemotherapy because of advanced age and poor performance. The eight patients' characteristics are detailed in Table 1. The study was approved by the ethics committee at The Affiliated Cancer Hospital of Zhengzhou University, and approved consent forms were signed by all patients. The procedures were in accord with the Helsinki Declaration of 1975.

Study design and response

In this study, all patients received R-CIK therapy only as a first-line treatment. When disease progression became evident, patients continued to receive R-CIK therapy combined with best supportive care or best supportive care only. During treatment, patients received regular follow-up and were reviewed to evaluate the changes in their condition. The follow-up deadline was August 1, 2013. Response to therapy was evaluated using RECIST 1.1 criteria [15]. According to the criteria, clinical effects were divided into CR, partial response (PR), SD, and PD. When the disease met the criteria for PD, subsequent therapies were applied. OS was calculated from the first R-CIK treatment to death or to the time of re-censoring at the last instance of contact with the patient alive. Evaluation of adverse reactions to the experimental treatment regime was performed using the National Cancer Institute Common Toxicity Criteria 3.0 (NCI-CTC3.0), and adverse reactions were divided into grades 1-4 [16].

Preparation of R-CIK

The method used for R-CIK cell preparation in our study was slightly different from methods described in previous literature [7]. Peripheral blood mononuclear cells (PBMCs) were collected from 50 ml samples of the patient's peripheral blood, coated in with RetroNectin (10 μg/ml) and anti-CD3 antibody (5 μg/ml) for 24 h and then cultured in GT-T551 medium, which contains recombinant human interleukin-2 (rhIL-2, 1000 U/ml), interferon-γ (IFN-γ, 1000 U/ml) and 5% inactive autogeneic plasma. Cells were cultured at 37°C with 5% CO_2 for 4 days. Then, the cells were cultured in the fresh IL-2 and 2% inactive autogeneic plasma-containing medium for 5 days. At day 10, R-CIK were harvested and analyzed for phenotype. All products were free of bacterial, mycoplasma, and fungal contamination. By LAL sensitivity

Table 1. Eight patients' characteristics

Characteristics	
Age (year)	median 71.3 years (65-79 years)
> 70	4
≤ 70	4
Sex	
male	3
female	5
ECOG (scores)	
= 2	6
= 3	2
CA-199 U/ml	
> 1000	2
≤ 1000	6
Cycles of CIK	
≥ 6	3
< 6	5
Once surgery	
yes	3
no	5
ALT (U/l)	
> ULN	3
≤ ULN	5
AST (U/l)	
> ULN	2
≤ ULN	6
γ-GGT (U/l)	
> ULN	2
≤ ULN	6
Bilirubin (μmol/l)	
> ULN	3
≤ ULN	5
Albumin (g/l)	
> LLN	6
≤ LLN	2
PLT (g/l)	
> LLN 6	7
≤ LLN	1
HGB (g/l)	
> LLN 6	4
≤ LLN	4
Calcium (mmol/l)	
> ULN	3
≤ ULN	5

ECOG – Eastern Cooperative Oncology Group; CA-199 – carbohydrate antigen-199; ALT – alanine transaminase; AST – aspartate transaminase; γ-GGT – γ glutamyl transpeptidase; PLT – platelet; HGB – hemoglobin; ULN – upper limit of normal; LLN – lower limit of normal

review, endotoxin levels of the final preparation were less than 5 EU.

Phenotypic assessment of R-CIK

R-CIK (1×10^6) and PBMC (1×10^6) were harvested and double stained with 10 µl fluorescein isothiocyanate (FITC) CD3 and phycoerythrin (PE) CD4, CD8 and CD56. Samples were incubated at 4°C for 30 min, then washed twice with PBS and re-suspended in 500 ml PBS. Fluorescence was detected by FACS Calibur flow cytometer (BD, USA) and data on 10,000 cells were acquired and analyzed. Propidium Iodide and Annexin V-FITC (BD, USA) measured viability and apoptosis.

Cytotoxicity assessment of R-CIK

The anti-tumor activity of our cultured R-CIK cells was tested in an overnight cytotoxicity assay at the end of cell expansion with K-562 (chronic myeloid leukemia) cell lines used as targets. The cytotoxic activity of cells was investigated in a lactate dehydrogenase (LDH) release assay (CytoTox 96, Promega). This non-radioactive assay is a colorimetric alternative to ^{51}Cr release assay and quantitatively measures LDH that is released upon cell lysis in the same way that ^{51}Cr is released. Every experiment, at each effector cell concentration, was performed in a triplicate set of wells and the mean value was calculated.

Statistical methods

SPSS 17.0 was used to perform the statistical analysis. Data on phenotypic analysis of R-CIK cells and cytotoxicity were analyzed by independent sample *t*-test. For survival time analysis, the Kaplan-Meier method was used and prognostic factors of survival time analysis were used for the log-rank test and multivariable analysis. For all data, $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Phenotypic analysis of R-CIK

Phenotypic analysis of R-CIK in the patients before culture and after 10 days of culture showed that percentages of CD3+, CD3+/CD4+, CD3+/CD8+ and CD3+/CD56+, increased from 41.89 ±3.19%, 27.92 ±2.14%, 17.96 ±1.79% and 4.09 ±0.62% to 91.32 ±4.98%, 63.03 ±4.99%, 51.22 ±2.98% and 17.03 ±2.78% respectively, with *p* values < 0.05. The details are shown in Fig. 1.

Cytotoxicity of R-CIK *in vitro*

In our center, we regularly detected the cytotoxicity of R-CIK *in vitro*. A sample of each R-CIK expansion obtained from a total of eight patients was tested for cytotoxicity against K-562, an NK-sensitive leukemia target cell line. All experiments were performed in triplicate and the mean value for each patient is represented by a single dot. At an effector to target (E/T) cell ratio of 40 : 1, 20 : 1 and 10 : 1, the median levels of cytotoxicity were 35.45 ±5.28%, 27.75 ±4.33% and 21.20 ±4.32%, respectively. The details are shown in Fig. 2.

Viability and apoptosis of R-CIK *in vitro*

At day 10, we regularly detected the viability and apoptosis of R-CIK *in vitro*. The results indicated that the viability rate of R-CIKs was more than 95%, the early apoptotic rate was 7.65 ±1.32% and the late apoptotic rate was 0.69 ±0.27%.

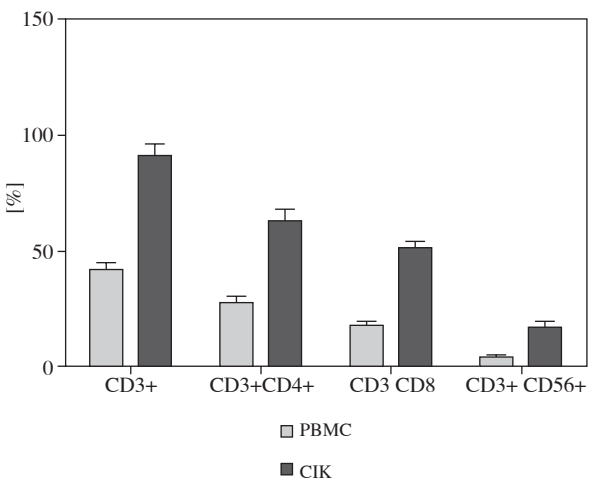

Fig. 1. Phenotype of PBMC and CIK

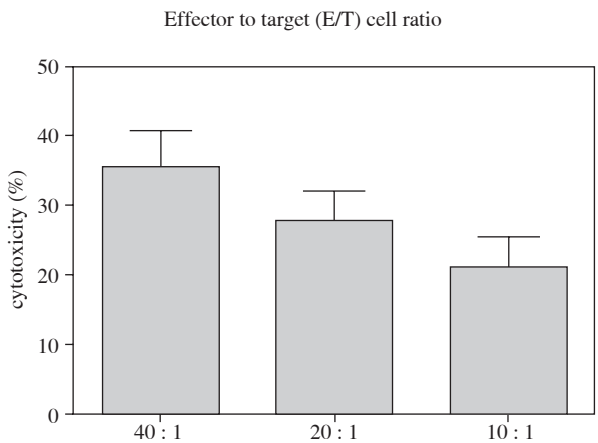

Fig. 2. CIK cell cytotoxicity for K-562 cell line

Table 2. Eight patients' survival time and status

No.	Sex	Age (y)	ECOG	Cycles of CIK	Response	OS (months)
1	Male	66	2	3	SD	5.3
2	Male	65	3	3	PD	5.5
3	Female	67	3	3	PD	5.57
4	Female	76	2	6	SD	7.3
5	Female	75	2	3	SD	9.07
6	Female	79	2	4	SD	23
7	Female	77	2	30	CR	33.53
8	Male	65	2	12	SD	15.07

Treatment outcomes

In the eight patients, 1 had CR, 5 had SD and 2 had PD. Therefore, the ORR was 12.5% (1/8) and the DCR was 75.0% (6/8 patients). The 1-year survival rate was 37.5% and the mOS was 13.04 months (95% CI: 5.9-20.2). All patient survival times and status are shown in Table 2.

Prognosis factors of R-CIK treatment

In this study, in order to explore the prognosis factors of R-CIK treatment, we used univariate analysis, however, the results indicated that no significant positive or negative predictive factors were identified (Table 3).

Adverse events

In this study, eight patients received R-CIK therapy. None of the enrolled patients failed to complete a full course of immunotherapy or were ruled out because of side effects. No severe adverse effects (grade 3 or grade 4) were associated with R-CIK therapy. The primary side effect of immunotherapy (grade 1 or 2) was fever. The side effect rate was 25.0% (2/8). No other side effects emerged.

Discussion

Metastatic pancreatic cancer represents a considerable challenge to the clinician, particularly when working with an elderly patient population. Elderly patients have a higher rate of concomitant disease, which may lead to contraindications for chemotherapy. Additionally, they are more susceptible to the adverse side effects of chemotherapy. For these reasons, both patients and physicians may hesitate to undertake chemotherapy with cytotoxic drugs for elderly patients. Furthermore, the literature reveals that even the most current chemotherapy achieve mOS of only 5.9-10.4 months in elderly pancreatic cancer patients, a modest gain by any standard [5, 17].

In recent years, adoptive immunotherapy with CIK cells has attracted more and more attention from clini-

Table 3. Univariate analyses

	OS	χ^2	*P*-value
Sex			
male	8.623		
female	15.694	1.683	0.195
Cycles of CIK			
≥ 6	18.633		
< 6	9.688	1.458	0.227
Age (years)			
> 70	18.225		
≤ 70	7.860	2.969	0.085
ECOG (scores)			
= 2	15.545		
= 3	5.535	3.174	0.075
CA-199 (U/ml)			
> 1000	6.400		
≤ 1000	15.257	1.745	0.187
Once surgery			
yes	20.610		
no	8.502	1.774	0.183
ALT (U/l)			
> ULN	9.334		
≤ ULN	19.223	2.034	0.154
AST (U/l)			
> ULN	10.918		
≤ ULN	19.415	0.560	0.454
γ-GGT (U/l)			
> ULN	8.803		
≤ ULN	17.283	0.980	0.119
Bilirubin (μmol/l)			
> ULN	8.448		
≤ ULN	20.700	2.427	0.322
Albumin (g/l)			
> LLN	20.415		
≤ LLN	10.585	1.208	0.272
PLT (/l)			
> LLN	15.070		
≤ LLN	12.753	0.050	0.822
HGB (g/l)			
> LLN	13.868		
≤ LLN	12.218	0.268	0.604
Calcium (mmol/l)			
> ULN	11.588		
≤ ULN	15.467	0.380	0.538

ECOG – Eastern Cooperative Oncology Group; CA-199 – carbohydrate antigen-199; ALT – alanine transaminase; AST – aspartate transaminase; γ-GGT – γ glutamyl transpeptidase; PLT – platelet; HGB – hemoglobin; ULN – upper limit of normal; LLN – lower limit of normal

cians. CIK therapy is a realistic new option in the field of cancer immunotherapy, showing strong anti-tumor activity and improving the overall survival time of cancer patients when used alone or combined with other conventional therapies. For example, Wang *et al*. reported that a regimen of S-1 plus CIK therapy was well tolerated in a second-line setting in patients with gemcitabine-refractory and advanced pancreatic cancer [18]. Chung *et al.* reported that adoptive immunotherapy using CIK cells showed comparable PFS and OS to survival data of previous trials that assessed conventional chemotherapies while maintaining tolerability and showing encouraging results in terms of patient quality of life (QOL) in gemcitabine-refractory advanced pancreatic cancer [7]. These reports indicate that CIK immunotherapy has potential benefits in advanced pancreatic cancer patients.

As we all know, the efficacy of CIK therapy depends on the quality and quantity of CIK. In our CIK preparation, our method was slightly different from previous literatures [7]. We added RetroNectin (RN) in the culture of CIK preparation (called R-CIK), which could improve the conglutination, extension, differentiation and proliferation of cells [19]. Therefore, through this method we could obtain R-CIK cells with stronger anti-tumor activity and transfer them to patients. In our previous work, one enrolled patient, a 77-year-old female, underwent resection of the pancreatic body and tail as well as the spleen. Analysis of the pancreatic mass revealed poorly differentiated adenocarcinoma, including squamous cell carcinoma differentiation, with positive bilateral margins. One month later, imaging examination revealed that a low-density nodule had emerged at the resection margin, and carbohydrate antigen-199 (CA-199) level had risen to 1,000 U/ml (reference range, 0-35 U/ml). This patient had been reported in our previous work, and was notable for a PFS of more than 19 months [6]. When the patient's disease resumed progression, we continued to apply R-CIK therapy combined with best supportive care. The patient then experienced slow progression of disease which ultimately resulted in an OS of 33.53 months. In our current study, we have continued to apply R-CIK therapy to treat elderly patients with metastatic pancreatic cancer. Encouragingly, in the eight patients, 1 had CR, 5 had SD and 2 had PD. Therefore, the ORR was 12.5% (1/8) and the DCR was 75.0% (6/8 patients). The 1-year survival rate was 37.5% and the mOS was 13.04 months (95% CI: 5.9-20.2). In predictive factor analyses, no significant positive or negative factors were found, indicating that the effects of R-CIK treatment cannot be predicted based on preliminary laboratory tests or imaging reports. And our results indicated that no severe side effects emerged, all the patients could tolerate the side effect. Compared with previous trials in elderly patients, the mOS was obviously prolonged. Although, this study only enrolled eight patients, the patients clearly benefited from R-CIK therapy. This is certainly a cause to conduct further research in the treatment of elderly patients with metastatic pancreatic cancer using R-CIK therapy or R-CIK therapy combined with mono-chemotherapy.

In conclusion, adoptive immunotherapy using R-CIK showed comparable OS to data from previous trials assessing the use of conventional chemotherapy in elderly patients with similar diseases. Because of the poorly tolerated side effects of these conventional therapies, it appears that R-CIK immunotherapy has the potential to fulfill a much needed role in the clinical treatment of this deadly and difficult to treat illness.

Conclusions

Adoptive immunotherapy using R-CIK cells showed comparable OS to survival data seen in previous trials assessing conventional chemotherapies in elderly patients and the adverse effect is less pronounced.

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The authors declare no conflict of interest.

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