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BRIEF ARTICLE

Increasing the frequency of CIK cells adoptive immunotherapy may decrease risk of death in gastric cancer patients

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Abstract

AIM: To analyze the correlation between cytokine-induced killer (CIK) cells adoptive immunotherapy and cancer-related death in gastric cancer patients.

METHODS: One hundred and fifty-six gastric cancer patients after operation at the Third Affiliated Hospital of Soochow University were enrolled in this study. Their clinical data including demographic characteristics, operation time, tumor size, pathological type and staging, tumor metastasis, outcome of chemotherapy or CIK cells adoptive immunotherapy, survival time or time of death were collected with a standard structured questionnaire. Kaplan-Meier method was used to estimate the median survival time, and the 2- and 5- year survival rates. Hazard risk (HR) and 95% confidence interval (95% CI) of CIK cells adoptive immunotherapy for gastric cancer were calculated using the two-stage time-dependent covariates Cox model.

RESULTS: The survival time of gastric cancer patients was longer after CIK cells adoptive immunotherapy than after chemotherapy (χ^2 = 10.907, P = 0.001). The median survival time of gastric cancer patients was also longer after CIK cells adoptive immunotherapy than after chemotherapy (49 mo *vs* 27 mo, P < 0.05). The 2- and 5-year survival rates of gastric cancer patients were significantly higher after CIK cells adoptive immunotherapy than after chemotherapy (73.5% *vs* 52.6%, 40.4% *vs* 23.9%, P < 0.05). A significant difference was observed in the survival curve for patients who received CIK cells adoptive immunotherapy (0, 1-10, 11-25, and over 25 frequencies) (χ^2 = 14.534, P = 0.002). The frequencies of CIK cells adoptive immunotherapy were significantly related with the decreasing risk of death in gastric cancer patients after adjustment for sex and age of the patients, tumor stage and relapse (HR = 0.54, 95% CI: 0.36-0.80) when the first stage Cox model was used to define the subjects who remained alive beyond 36 mo as survivors. However, no correlation was observed between the frequencies of death in CIK cells adoptive immunotherapy and the risk of gastric cancer patients (HR = 1.09, 95% CI: 0.63-0.89) when the second stage Cox model was used to define the subjects who survived for more than 36 mo as survivors.

CONCLUSION: The survival time of the gastric cancer

patients treated with chemotherapy combined with CIK cells adoptive immunotherapy is significantly longer than that of the patients treated with chemotherapy alone and increasing the frequency of CIK cells adoptive immunotherapy seems to benefit patients more.

Key words: Immunotherapy; Cytokine-induced killer cells; Gastric cancer; Survival analysis; Probability

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INTRODUCTION

Gastric cancer is one of the most common causes of cancer-related death in China[1]. Its incidence in Jiangsu Province of China is particularly high, and its mortality rate is much higher than the national average[2]. The early clinical detection rate of gastric cancer is less than 15%, and about 85% cases of gastric cancer are at the advanced stage when their gastric cancer is diagnosed[3]. Surgery is the standard treatment procedure for localized and resectable gastric cancer[4]. However, surgery alone does not improve the 5-year survival rate of local advanced gastric cancer patients[3]. Although standardized surgical resection and adjuvant therapeutic modalities are available for gastric cancer, the survival rate of advanced gastric cancer patients remains very low after operation[5]. About 60% of gastric cancer patients usually experience local recurrence and metastasis to other organs[6]. It has been demonstrated that local recurrence and distant metastasis constitute a major problem in the failure of cancer therapies[7]. Therefore, considerable efforts are needed to improve the current therapeutic modalities and to explore new therapies. In recent years, immune therapy has become the fourth important treatment modality for malignant tumors following surgery, radiotherapy and chemotherapy[8-10].

A number of adoptive immunotherapy with killer cells have been reported, including lymphokine-activated killer cells[11], tumor infiltrating lymphocytes[12], or anti-CD3 monoclonal antibody-induced killer cells[13]. However, their therapeutic efficacy is limited due to their low anti-tumor activities[14]. At present, cytokine-induced killer (CIK) cells are a new type of anti-tumor effector cells, which can proliferate rapidly *in vitro*, with a stronger anti-tumor activity and a broader target tumor spectrum than the reported anti-tumor effector cells[10,15]. Moreover, CIK cells can regulate and enhance immune function[16]. Studies have reported the level of tumor markers, change in cellular immune functions, exploration of molecule targets and a short-term efficacy in gastric cancer patients after chemotherapy plus CIK cells immunotherapy despite some side effects[17-19]. However, the relation between the frequencies of CIK cells immunotherapy and its clinical efficacy has not been examined. In the present study, data obtained from 156 gastric cancer patients in fitting multivariate Cox model showed that more frequencies of CIK cells immunotherapy could improve the survival rate of gastric cancer patients.

MATERIALS AND METHODS

Patients

One hundred and fifty-six primary gastric cancer patients after operation at the Third Affiliated Hospital of Soochow University (Jiangsu Province, China) were enrolled in this study. Those who did not meet the inclusion criteria, or had other tumors were excluded.

A standard questionnaire was designed to collect the data from the patients, including demographic characteristics, operation time, tumor size and location, pathological type and staging, tumor metastasis, outcome of chemotherapy or CIK cells immunotherapy. Meanwhile, time of relapse and death of the patients was recorded. Patients received 6 cycles of chemotherapy before CIK cells immunotherapy. Some patients underwent CIK cells immunotherapy due to cancer recurrence during chemotherapy. Recurrence of gastric cancer was defined when local, peritoneal or distant metastasis was detected at any site during chemotherapy[20]. The study was conducted according to the principles of the Declaration of Helsinki. All patients gave their informed consent prior to inclusion in the study. The study was approved by the Ethics Committee of the Third Affiliated Hospital of Soochow University.

Eighty-one patients (62 males, 19 females) at the age of 59.9 ± 10.5 years with a median age of 60.5 years who underwent chemotherapy alone served as chemotherapy group (group Ⅰ), and those (60 males at the age of 62.4 ± 10.8 years with a median age 60.5 years, 15 females at the age of 51.0 ± 10.7 years with a median age of 50 years) who received chemotherapy plus CIK cells immunotherapy served as treatment group (group Ⅱ) (Table 1).

Preparation of CIK cells and treatment

Peripheral blood mononuclear cells (PBMC) were collected with a COBE spectra blood cell separator (Gambro BCT, Inc., Lakewood, USA). Viability of PBMC was assessed by trypan blue exclusion. PBMC (2.0×10^6/mL) were plated onto 6-well dishes (Nunc, Denmark) and cultured with medium I containing RPMI 1640 in the presence of 1.0×10^6 U/L human interferon-γ (IFN-γ, Shanghai Fosun Pharma Co., China), 5.0×10^5 U/L recombinant human interleukin-2 (IL-2, Shangdong Quangang Pharmaceutical Co., China), 10% inactivated human serum, 25 mmol/L HEPES, 2 mmol/L L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin. The cells were incubated in a humidified atmosphere containing 5% CO_2 at 37℃. Monoclonal antibody (MAb) against CD3

Table 1 Distribution of demographic and clinical characteristics in two groups

Demographic and clinical features	*n*	Groups, *n* (%)		χ^2	*P*
		Group Ⅰ	Group Ⅱ		
Sex					
Men	122	62 (76.5)	60 (80)	0.273	0.601
Women	34	19 (23.5)	15 (20)		
Age (yr)					
≤ 45	14	7 (8.6)	7 (9.3)		
45 < age ≤ 60	71	36 (44.5)	35 (46.7)	0.047[a]	0.977
> 60	71	38 (46.9)	33 (44.0)		
Tumor site[b]					
Gastric cardia					
Yes	58	30 (37.0)	28 (37.3)	0.002	0.970
No	98	51 (63.0)	47 (62.7)		
Gastric body					
Yes	64	35 (43.2)	29 (38.7)	0.332	0.564
No	92	46 (56.8)	46 (61.3)		
Gastric antrum					
Yes	36	17 (21.0)	19 (25.3)	0.414	0.520
No	120	64 (79.0)	56 (74.7)		
Tumor size[c] (cm)					
< 5	76	43 (59.7)	33 (55.0)	0.299	0.585
≥ 5	56	29 (40.3)	27 (45.0)		
Histological type[c]					
Differentiated	52	28 (35.4)	24 (64.6)	0.002	0.962
Poorly-differentiated	94	51 (38.5)	43 (64.2)		
Invasion[c]					
Yes	112	55 (76.4)	57 (89.1)	3.745	0.053
No	24	17 (23.6)	7 (10.9)		
Lymph node metastasis[c]					
Yes	100	55 (76.4)	45 (70.3)	0.643	0.423
No	36	17 (23.6)	19 (29.7)		
Relapse					
Yes	98	58 (28.4)	40 (46.7)	5.566	0.018
No	58	23 (71.6)	35 (53.3)		
Pathological grade					
1	2	1 (1.3)	1 (1.3)		
2	27	18 (22.2)	9 (12.0)	2.976	0.395
3	92	44 (54.3)	48 (64.0)		
4	35	18 (22.2)	17 (22.7)		
Tumor stage					
Ⅰ	14	9 (11.1)	5 (6.7)		
Ⅱ	22	12 (14.8)	10 (13.3)	2.129[a]	0.546
Ⅲ	102	49 (60.5)	53 (70.7)		
Ⅳ	18	11 (13.6)	7 (9.3)		

[a]cmh2 χ^2-test; [b]Two cases of gastric cancer involving gastric cardia and body; [c]Missed cases.

(100 μg/L, Antibody Diagnostic Inc., USA) and IL-1α (1.0 × 10^5 U/L, Promega) were added after 24 h culture. Supernatant was aspirated and the cells were cultured in Medium Ⅱ in the absence of INF-γ after another 48 h culture. The cells were transferred to Kolle flasks (Nunc, Denmark) and cultured in the same medium after 1 wk. The medium was changed every 3 d. The cytotoxic activity was assayed as previously described[17,21]. Patients in group Ⅰ were treated with oxaliplatin (120 mg/m^2 D1, 5-Fu 400 mg/m^2 CIV 24 h D1-5, CF 200 mg/m^2 D1-5) as previously described[22] with the doses adjusted according to the toxicity. Patients in group Ⅱ received CIK cells immunotherapy as previously described[17] after 6 cycles of chemotherapy, with 1.0 × 10^9 CIK cells transfused into the patients within 1 h.

Statistical analysis

All data were loaded into the Epidata3.0 database with double-check, and analyzed with the SAS software package (version 9.13; SAS Institute, Cary, NC, USA). The data were expressed as mean ± SD. χ^2-test or cmh2 χ^2-test was used to compare the difference in balance between the concerned clinical indexes and to find the confounding factors between the two groups. Survival data were analyzed using Kaplan-Meier method and log-rank test to estimate the median survival time, 2- and 5-year survival rates, and to determine if the survival curves for the two groups were different.

When the frequency of CIK cells immunotherapy and the survival time were introduced into the Cox model, the interaction item was significantly associated with the death of gastric cancer patients (wald-χ^2 = 4.946, P = 0.0261). A two-stage time-dependent Cox model was established to precisely estimate the hazard risk (HR) and 95% confidence interval (95% CI) of the association between the frequency of CIK cells immunotherapy and the death of gastric cancer patients. Because the median survival time of gastric cancer patients was about 36 mo, 36 mo was used as the optimum cutoff point.

The first stage Cox model involved 154 patients with a survival time of over 36 mo who were defined as survivors. Otherwise, the survival status was the same as the original definition.

The second stage Cox model only involved 56 patients with a survival time longer than 36 mo, and their survival status was defined as the original definition.

Pearson correlation test was performed between Schoenfeld residual of the frequencies of CIK cells immunotherapy and the survival time of gastric cancer patients to determine whether the frequency of CIK cells immunotherapy is a time-dependent variable in the two Cox models[23].

RESULTS

Distribution of demographic and clinical characteristics in two groups

No statistical difference was found in sex and age of the patients, tumor site, histological type, invasion depth, lymph node metastasis, pathological grade, tumor size and stage between the two groups. However, the number of patients was significantly greater in group Ⅱ with recurrent disease than in group Ⅰ (46.7% *vs* 28.4%, χ^2 = 5.566, P = 0.018) (Table 1), suggesting that more patients with relapse should receive CIK cells immunotherapy.

Demographic and clinical characteristics of patients after CIK cells immunotherapy

No statistical difference was observed in sex and age of the patients, tumor site, histological type, invasion depth, lymph node metastasis, pathological grade or tumor size after CIK cells immunotherapy (0, 1-10, 11-25, and over 25 frequencies). However, a significant difference was found in cancer recurrence and stage after CIK cells immunotherapy (Table 2).

Table 2 Distribution of demographic and clinical characteristics in group Ⅱ

Demographic and clinical features	*n*	Frequency of CIK cells immunotherapy, *n* (%)				χ^2	*P*
		0	1-10	11-25	> 25		
Sex							
Men	122	62 (76.5)	39 (84.8)	13 (86.7)	8 (57.1)	5.573	0.134
Women	34	19 (23.5)	7 (15.2)	2 (13.3)	6 (42.9)		
Age (yr)							
⩽ 45	14	7 (8.6)	5 (10.9)	1 (6.6)	1 (7.1)	1.153	0.979
45 < age ⩽ 60	71	36 (44.4)	20 (43.5)	7 (46.7)	8 (57.1)		
> 60	71	38 (47.0)	21 (45.6)	7 (46.7)	5 (35.8)		
Tumor site[a]							
Gastric cardia							
Yes	58	30 (37.0)	17 (37.0)	5 (33.3)	6 (42.9)	0.290	0.962
No	98	51 (63.0)	29 (63.0)	10 (66.7)	8 (57.1)		
Gastric body							
Yes	64	35 (43.2)	19 (41.3)	5 (33.3)	5 (35.7)	0.691	0.875
No	92	46 (56.8)	27 (58.7)	10 (66.7)	9 (64.3)		
Gastric antrum							
Yes	36	17 (21.0)	10 (21.7)	4 (26.7)	5 (35.7)	1.614	0.656
No	120	64 (79.0)	36 (78.3)	11 (73.3)	9 (64.3)		
Tumor size[c] (cm)							
< 5	76	43 (59.7)	17 (45.9)	7 (58.3)	9 (81.8)	4.834	0.184
⩾ 5	56	29 (40.3)	20 (54.1)	5 (41.7)	2 (18.2)		
Histological type[c]							
Differentiated	52	28 (35.4)	12 (30.0)	6 (40.0)	6 (50.0)	1.760	0.624
Poorly-differentiated	94	51 (64.6)	28 (70.0)	9 (60.0)	6 (50.0)		
Invasion[c]							
Yes	112	55 (76.4)	38 (90.5)	10 (90.9)	9 (81.8)	4.226	0.238
No	24	17 (23.6)	4 (9.5)	1 (9.1)	2 (18.2)		
Lymph node metastasis[c]							
Yes	100	55 (76.4)	31 (75.6)	7 (58.3)	7 (63.6)	2.371	0.499
No	36	17 (23.6)	10 (24.4)	5 (41.7)	4 (36.4)		
Relapse							
Yes	98	58 (71.6)	31 (67.4)	8 (53.3)	1 (7.1)	15.633	0.0004
No	58	23 (28.4)	15 (32.6)	7 (46.7)	13 (92.9)		
Pathological grade							
1	2	1 (1.2)	1 (2.2)	0 (0.0)	0 (0.0)	2.976[b]	0.3953
2	27	18 (22.2)	8 (17.4)	0 (0.0)	1 (7.1)		
3	92	44 (54.3)	25 (54.4)	12 (80.0)	11 (78.6)		
4	35	18 (22.2)	12 (26.0)	3 (20.0)	2 (14.3)		
Tumor stage							
Ⅰ	14	9 (11.1)	3 (6.5)	0 (0.0)	2 (14.2)	13.66[b]	0.0386
Ⅱ	22	23 (28.4)	3 (6.5)	1 (6.7)	6 (42.9)		
Ⅲ	102	38 (46.9)	34 (73.9)	13 (86.7)	6 (42.9)		
Ⅳ	18	11 (13.6)	6 (13.1)	1 (6.6)	0 (0.0)		

[a]Tumor sites in some cases were repeated; [b]Because the theoretical value is less than 1, χ^2 was performed for patients who received cytokine-induced killer (CIK) cells immunotherapy at the frequencies of 11-25 and > 25, and for those who underwent CIK cells immunotherapy at the frequencies of 1-10, 11-25 and > 25; [c]Missed cases.

Survival time of patients in two groups

The survival time of gastric cancer patients in group Ⅱ was much longer than that of those in group Ⅰ (χ^2 = 10.907, P = 0.001, Figure 1). The median survival time of patients in group Ⅱ was also longer than that of those in group Ⅰ (49 mo *vs* 27 mo).

Two- and 5-year survival rates of patients in two groups

The 2- and 5-year survival rates of patients in group Ⅱ were significantly higher than those of patients in group Ⅰ (73.5% *vs* 52.6%, 40.4% *vs* 23.9%, P <0.05) (Table 3).

Survival time of patients after CIK cells immunotherapy

Because the CIK cells immunotherapy seemed effective against gastric cancer, whether the frequency of CIK cells immunotherapy affects its efficacy was determined. The survival curve was obviously higher for the patients after CIK cells immunotherapy plus chemotherapy than after chemotherapy alone. The survival time of gastric cancer patients was significantly longer after CIK cells immunotherapy than after chemotherapy (χ^2 = 14.534, P = 0.002, Figure 1).

Time-dependent Cox model analysis of CIK cells immunotherapy and prognosis of patients

The frequency of CIK cells immunotherapy was a strong time-dependent variable. A significant difference was observed in the survival time and the frequency of CIK cells immunotherapy between the two models (χ^2 = 27.990, P

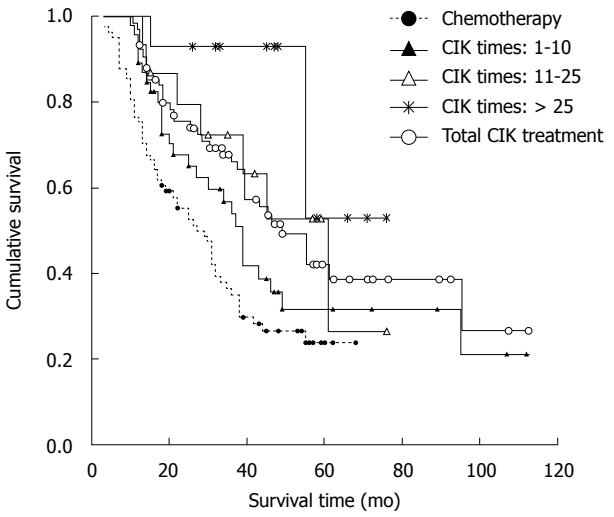

Figure 1 Survival rates of patients after cytokine-induced killer cells immunotherapy plus chemotherapy and chemotherapy alone. CIK: Cytokine-induced killer.

< 0.0001). However, it could not stratify the theoretical hypothesis of proportional hazard model. Hence, we tried to improve the analyzing results of the Cox model by dividing the patients into two stages with a relatively short survival time. Because of the relatively short interval time, the frequency of CIK cells immunotherapy may be a time-independent variable and can satisfy the assumption of proportional hazard model. The median survival time (36 mo) of the patients was used as the optimum cut-point.

In the first stage Cox model, the frequency of CIK cells immunotherapy was not a time-dependent variable, thus refusing the hypothesis of non proportional hazard model by Pearson correlation test between Schoenfeld residual of CIK cells immunotherapy frequency and survival time ($r = 0.04$, $F = 0.10$, $P = 0.751$). After the patients who remained alive beyond 36 mo after treatment were defined as survivors, the frequency of CIK cells immunotherapy was significantly associated with the decreasing risk of death in gastric cancer patients after adjustment for sex and age of the patients, tumor stage and relapse (HR = 0.54, 95% CI: 0.36-0.80) (Table 4).

In the second stage Cox model, the frequency of CIK cells immunotherapy was not a time-dependent variable ($r = 0.307$, $F = 1.98$, $P = 0.176$).When the second stage Cox model involving only 56 patients with a survival time of over 36 mo was fitted, no association was observed between the frequency of CIK cells immunotherapy and the survival time of gastric cancer patients (HR = 1.09, 95% CI: 0.63-0.89) (Table 5).

DISCUSSION

In China, gastric cancer patients are usually diagnosed at a relatively advanced stage with metastasis to other organs[24]. A number of treatment modalities are available for gastric cancer, such as surgical resection combined with chemotherapy[25,26], radiotherapy[27,28], thermotherapy[29,30] and/or traditional Chinese medicine[31,32]. However,

Table 3 Two- and five-year survival rates of gastric cancer patients in two groups

Groups	Survival rate (%)	95% CI	u-value	*P*
2-yr				
Chemotherapy	52.6	41.7-63.6	2.721	0.007
CIK treatment	73.5	63.3-83.7		
5-yr				
Chemotherapy	23.9	13.5-34.3	1.913	0.0526
CIK treatment	40.4	27.1-53.7		

CIK: Cytokine-induced killer; 95% CI: 95% confidence interval.

the 5-year survival rate of advanced gastric cancer patients is still very poor[33]. It has been shown that cellular immunotherapy can promote host anti-cancer immunity, thus prolonging the survival time of gastric cancer patients[34]. Treatment of gastric cancer with autologous CIK cells is one of the promising cellular immunotherapies[35].

The results of the present study demonstrate that CIK cells immunotherapy plus chemotherapy for gastric cancer has more potential benefits than chemotherapy alone. Therefore, the effect of adjuvant cellular therapies for gastric cancer has drawn more attention of oncologists. It has been shown that adjuvant radiotherapy and chemotherapy for gastric cancer after curative resection can improve the disease-free and overall survival time of gastric cancer patients[25,36]. Residual tumor cells after chemotherapy can be removed by the host immune system[37]. However, several cycles of chemotherapy can decrease the immune functions of gastric cancer patients[38], and have been suspected to be one of the reasons for the high relapse rate of gastric cancer after postoperative systemic chemotherapy. Immunotherapy can directly kill cancer cells and boost the immune responses against the tumor[39]. Therefore, immunotherapy should be beneficial for gastric cancer patients, and not conducive to the growth of tumor cells.

In the present study, CIK cells were obtained upon culturing PBMC in the presence of IFN-γ, IL-2, anti-CD3MAb, and IL-1α[13]. This method allows us to generate a large number of CIK cells. In addition, the anti-tumor cell activity of CIK cells is stronger than that of anti-tumor effector cells[16]. The effector cells in our culture are believed to be $CD3^+CD56^+$. CIK cells have a higher survival rate, proliferation capacity, and killing activity than their target cells[40,41], and can secrete a variety of cytokines, which further enhance the cytotoxicity of immune effector cells[42] and change the tumor microenvironment to favor cancer eradication. In addition, CIK cells can kill both autologous and allogeneic tumor cells[43,44], as well as multi-drug resistance cells and FasL-positive cells[45,46]. Accordingly, CIK cells immunotherapy combined with chemotherapy may have a synergistic effect.

Several studies[17,47-49] showed that CIK cells immunotherapy can significantly improve the immune functions of cancer patients, such as an increase in $CD3^+CD56^+$ level. However, the clinical data are not enough to demonstrate the effectiveness of CIK cells immunotherapy. The results of this retrospective study, based on the follow-up

Table 4 First stage time-dependent multivariate Cox model analysis of cytokine-induced killer cells immunotherapy at different frequencies and prognosis of the patients[1,2] (*n* = 154[3])

Variables[4]	β	$s_β$	Wald-χ^2	*P*-value	HR	95% CI
No. of CIK infusion	-0.620	0.200	9.592	0.002	0.54	0.36-0.80
Sex	0.294	0.298	0.969	0.325	1.34	0.75-2.41
Age	0.508	0.200	6.492	0.011	1.66	1.12-2.46
Tumor stages	0.739	0.202	13.377	0.0003	2.10	1.40-3.11
Relapse (yes or no)	3.363	0.719	21.848	< 0.0001	28.87	7.05-118.25

[1]All patients with survival time of longer than 36 mo (median value for total patients) were defined as the survivors; [2]Pearson correlation test between Schoenfeld residual of cytokine-induced killer (CIK) cells immunotherapy frequency and survival time (r = 0.01, F = 0.01, P = 0.936); [3]Missed patients; [4]Variable value definition in the Cox model (frequency of CIK cells immunotherapy: 0 = 0 time, 1 = 1-10 times, 2 = 11-25 times, 3 = more than 25 times; Sex: 1 = man, 2 = woman), age (0 = ≤ 45 yr, 1 = about 60 yr, 2 = over 60 yr), tumor stage (0 = stage Ⅰ, 1 = stage Ⅱ, 2 = stage Ⅲ, 3 = stage Ⅳ), relapse (1 = yes, 0 = no). HR: Hazard risk; 95% CI: 95% confidence interval.

Table 5 Second stage time-dependent multivariate Cox model analysis of cytokine-induced killer cells immunotherapy at different frequencies and prognosis of patients[1,2] (*n* = 56)

Variables[3]	β	$s_β$	Wald-χ^2	*P*-value	HR	95% CI
Number of CIK infusion	0.089	0.280	0.102	0.750	1.09	0.63-1.89
Sex	0.676	0.619	1.191	0.275	1.97	0.58-6.62
Age	-0.318	0.442	0.518	0.472	0.73	0.31-1.73
Tumor stages	-0.471	0.341	1.909	0.167	0.62	0.32-1.22
Relapse (yes or no)	2.203	0.558	15.582	< 0.0001	9.05	3.03-27.02

[1]This model only involving the patients with a survival time longer than 36 mo. The survival rate of each patient was similar; [2]Pearson correlation test between Schoenfeld residual of cytokine-induced killer (CIK) cells immunotherapy frequency and survival time (r = 0.307, F = 1.98, P = 0.176); [3]Variable value definition in the Cox model (frequency of CIK cells immunotherapy: 0 = 0 time, 1 = 1-10 times, 2 = 11-25 times, 3 = more than 25 times; Sex: 1 = man, 2 = woman), age (0 = ≤ 45 yr, 1 = about 60 yr, 2 = over 60 yr), tumor stage (0 = stage Ⅰ, 1 = stage Ⅱ, 2 = stage Ⅲ, 3 = stage Ⅳ), relapse (1 = yes, 0 = no). HR: Hazard risk; 95% CI: 95% confidence interval.

of 156 gastric cancer patients, show that the frequency of CIK cells immunotherapy can significantly prolong the survival time of gastric cancer patients.

However, the frequency of CIK cells immunotherapy may significantly decrease the risk of cancer-related death in gastric cancer patients. The common Cox model was not preferred in our analysis because the balance test showed that tumor relapse and stage were different in patients of the two groups. There are two reasons that support our conclusion. First, except for tumor relapse and stage, the balance test showed the following factors, including age and sex of the patients, tumor size, site and invasiveness, lymph node metastasis and pathological grade did not affect us to assign the gastric cancer patients into groups Ⅰ and Ⅱ. Second, due to the fact that the frequency of CIK cells immunotherapy was a time-dependent variable in the Cox model, a two-stage time-dependent Cox model adjustment was made for some confounding factors (tumor relapse and stage), thus the false results were avoided when the common Cox model was used to make our analysis more reliable.

After the postoperative adjuvant chemotherapy, most residual tumor cells sensitive to chemotherapy were removed. Chemotherapy may suppress the immune function and therefore immunotherapy is necessary for boosting immunity. It was reported that the number of $CD3^+$ cells and the $CD4^+/CD8^+$ ratio are significantly lower in most gastric cancer patients than in healthy controls after chemotherapy[17]. In the present study, the number of $CD3^+$ and $CD4^+$ cells was significantly increased, while the number of $CD8^+$ cells was declined and the $CD4^+/CD8^+$ ratio was increased after CIK cells immunotherapy, suggesting that CIK cells also have an immune modulating function in addition to their anti-tumor function[50]. Single CIK cells immunotherapy has an *in vivo* effect against gastric cancer for about one month[51]. In contrast, the number of $CD3^+$ cells and the $CD4^+/CD8^+$ ratio maintain at a high level after three cycles of CIK cells immunotherapy[17].

Our study has a few limitations. First, it was a retrospective cohort/observational study rather than a strictly-designed randomized trial. Since the patients were not assigned into CIK cells immunotherapy group and chemotherapy group, imbalance of certain clinical factors between the two groups could not be avoided. Second, there were some unknown potential reasons for choice of treatment regimens, which might affect our conclusion, despite the fact that the balance test was performed and some confounding factors were adjusted in the Cox model. Therefore, a randomized clinical trial is necessary to justify the benefit of CIK cells immunotherapy for gastric cancer. The benefit of radiochemotherapy and S1 chemotherapy for gastric cancers, established in a recent clinical trial[52], is important to determine whether CIK cells immunotherapy provides additional benefit when it is used in combination with radiotherapy and chemotherapy.

In conclusion, more frequencies of CIK cells are necessary for gastric cancer. The survival time of gastric cancer patients is significantly longer after chemotherapy plus CIK cells immunotherapy than after chemotherapy alone.

COMMENTS

Background

Gastric cancer is one of the most common causes of cancer-related death in China. Although standardized surgical resection and numerous adjuvant therapeutic modalities are available for gastric cancer, the postoperative survival rate of advanced stage cancer patients remains very low. In recent years, immune therapy for malignant tumors has become the fourth important tumor treatment modality following surgery, radiotherapy and chemotherapy. Cellular immunotherapy can promote host anti-cancer immunity, thus prolonging the survival time of gastric cancer patients. Treatment with autologous cytokine-induced killer (CIK) cells is one of the promising cellular immunotherapies.

Research frontiers

A number of adoptive cells immunotherapy have been reported, including using lymphokine activated killer cells, tumor infiltrating lymphocytes, and anti-CD3 monoclonal antibody-induced killer cells. However, their therapeutic efficacy is limited due to their low anti-tumor activities. CIK cells, a new type of anti-tumor effector cells, can proliferate rapidly *in vitro*, with a stronger anti-tumor activity and a broader spectrum of tumor targets than the reported anti-tumor effector cells. Moreover, CIK cells can regulate and enhance immune function.

Innovations and breakthroughs

CIK cells immunotherapy can decrease levels of tumor markers, change immune functions, and achieve a short-term efficacy against gastric cancer. However, the relation between the frequency of CIK cells immunotherapy and its clinical efficacy has not been examined. In the present study, data obtained from 156 gastric cancer patients were used in fitting multivariate Cox model, showing that more frequencies of CIK cells immunotherapy improve the survival rate of gastric cancer patients.

Applications

The survival time of gastric cancer patients was significantly longer after chemotherapy plus CIK cells immunotherapy than after chemotherapy alone, and more frequencies of CIK cells immunotherapy benefited gastric cancer patients more. This strategy can be applied in treatment of gastric cancer.

Terminology

CIK cells are cytokine-induced killer cells and a new type of anti-tumor effector cells, which can proliferate rapidly *in vitro* with a stronger anti-tumor activity and a broader spectrum of tumor targets than the reported anti-tumor effector cells. Moreover, CIK cells can regulate and enhance immune function.

Peer review

This study showed beneficial effect of CIK cells immunotherapy on gastric cancer, thus improving the 2- and 5-year survival rates of gastric cancer patients. The study is well designed and the data are believable.

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