In Vitro and in Vivo Study of Individualized Cancer Immunotherapy and a Pilot Trail on Patients with Gastric Cancer

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Abstract:

Introduction:

Cancer immunotherapy has been used in clinical trials and shows a powerful life force. But if it is benefit for the treatment of gastric cancer is still not well studied. Present study discussed the antitumor efficiency of individualized immunotherapy induced by dendritic cells transfected with total antigen of autologous gastric cancer cells and reported the initial results of our pilot clinical trail.

Methods:

Autologous DCs were isolated from PBMC and proliferated in supplement with GM-CSF and IL-4. Resulting immature DCs were loaded with autologous tumor antigen (tumor lysate or total RNA) and matured in supplement with TNF-α (tu-DC). Autologous T lymphocytes were isolated from PBMC and cocultured with tu-DC to prepare antigen-specific CTLs. Autologous primary cancer cells were obtained from resected specimens of the patients with gastric cancer and cultured as target cells. Phenotypic characters of DCs were detected and evaluated by FCM. The efficiency of cytotoxicity and cytokine release was measured and estimated by using assays of Cytotoxicity and ELISA.

Humanized immune system was reconstructed on 4-week-old nude mice by intraperitoneal (i.p.) injection of human PBMC with cell number of $3 \times 10^7$ each. Prophylactic assay and therapeutic assay were performed by subcutaneously challenge of primary cancer cells after or before autologous DC vaccination, respectively, and the tumor burden were observed.

Nine patients with gastric cancer were subjected to a pilot clinical trail. Two patients with stage Ib-IIIa underwent a radical D2 resection, 5 patients with stage IIIb-IV underwent palliative resection, and 2 patients with stage IV underwent an open-and-close operation. Autologous tumor lysate were prepared from fresh specimens of the patients. DC vaccines and tumor specific CTLs were prepared by using the methods above mentioned. To suppress Treg and to promote calreticulin (CRT) exposure, CTX 1000 mg and ADM 60 mg were intravenously infused a day before immunotherapy. Tumor-specific CTLs were theraped by intravenous infusing with one day interval, and DC vaccines were inoculated to the axillary lymph nodes with two therapies interval. Thymosin alpha 1 (Ta1) was injected once a day subcutaneously during the therapy. The levels of Treg were measured before and after chemotherapy by FCM, and the Incidence of recurrence, Karnofsky performance scale, tumor burden, and side effects were observed and evaluated.

Results:

1) Modality of target cells: Primary cancer cells were well growing on 15 to 18 days of culturing, and most of the cells showed a typical modality of cancer cells with more than 80% of the cells being PAS positive histologically.

2) Modality and function of DCs: A typical modality of mature DC is presented on day 7 of culturing. HLA-I molecule was persistently high expressed by immature and mature DCs. The expression of HLA-II, CD83, CD80 and CD86
were significantly going higher when immature DCs were matured. T cell proliferation assay indicated that mature DCs and tu-DCs have a stronger capability to induce autologous and allogenic T cell proliferation (CD3$^+$ >95%).

3) Cytokine release: The IL-12 and INF-γ production in the culture supernatant of tu-DCs and CTLs was significantly increased (P<0.05, respectively).

4) Cytotoxicity of CTL to cancer cells: CTLs induced by tu-DC have a much stronger cytotoxicity to autologous tumor cells than the allogenic tumor cells (P<0.05).

5) Prophylactic and therapeutic efficiency: In the prophylactic panel, final tumor volume and tumor dry weight in tu-DC vaccinated mice was significantly less than controls (P<0.05, respectively). Tumor inhibition rate in tu-DC group (53.7%) was significantly higher than controls (25.1%; P<0.05). Production of IL-12 and IFN-γ in tu-DC group was also significantly higher than controls (P<0.05, respectively). In the therapeutic panel, final tumor volume and tumor dry weight in tu-DC vaccinated mice was significantly less than controls (P<0.01, respectively). Tumor inhibition rate in tu-DC group (38.8%) was significantly higher than controls (13.2%; P<0.05). No autoimmune change was found histologicaly in all the tissues harvested from the experimental mice.

6) Results of the pilot clinical trial: The percentage of Treg in peripheral blood was significantly decreased in all the nine patients 4 days after chemotherapy (25.51%) compared with that before chemotherapy (12.56%; P<0.05). Two patients with radical surgery were tumor-free survived with 20-23 months. Five with palliative and two with open-and-close surgery were steady with durable disease stabilization for 5 to 19 months. Karnofsky performance scale was significantly improved in all nine patients. No severe side effects was found except low fever with 37.5°C-38.5°C and fatigue.

**Discussion and Conclusions:**

Our ex vivo study indicated that individualized immunotherapy, by using autologous resources of total antigen of the tumor, DCs and effector cells, can induce a strong antitumor responses. Our pilot clinical trial demonstrated the feasibility of using individualized immunotherapy based on regulation of tumor microenvironment to induce immune responses against gastric cancer clinically and benefit to the overall survival and alive with neoplasm of the patients.