Significant clinical response of advanced colorectal cancer to combination therapy involving capecitabine and adoptive cell transfer therapy: a case report

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Abstract: Significant clinical response was obtained in a patient with stage IV colorectal cancer (CRC) following combination therapy involving capecitabine and adoptive cell transfer therapy. She had laparoscopic lower anterior resection and left liver metastatic carcinoma resecting in 20th, February, 2017. Capecitabine was used to further treatment for an unresectable hepatic metastasis. The serum level of carcinoembryonic antigen (CEA) was increased significantly after dropped temporarily. Since then, the patient took the adoptive cell transfer therapy at the same time. αβT cells and NK cells were injected intravenously into the patient. After the first transfusion with αβT cells, the tumor biomarker, CEA, dropped obviously from 14.7 to 6.1 ng/mL. And it came to 1.9 ng/mL after four times treatment, which was back into normal range (<5 ng/mL). Flow cytometry (FCM) was used to reveal the detailed immunological status of this patient before and after adoptive cell transfer therapy. With 19-month follow-up, neither recurrence or complication was founded. Combination therapy involving adoptive immunotherapy and capecitabine may be the potential method for advanced CRC with less complication.

Keywords: Adoptive cell transfer therapy; immunotherapy; colorectal cancer (CRC); metastasis

Introduction

Colorectal cancer (CRC) remains the fourth common cause of death. Because of the low examination rate for colonoscopy, many patients are diagnosed as advanced CRC. Presently, with systematic treatment including surgery, chemotherapy and radiation therapy, more and more patients are cured. While patients with advanced CRC, some with metastasis especially, still don't have effective treatment (1). It is said that Asian and Pacific Islander has the highest overall 5-year survival rates. The 5-year survival rates for patients with a local stage CRC is 92.3%. Survival declines to 74.3% and 15.7% for patients diagnosed as regional and distant stage, respectively (2).

Fluoropyrimidine-based chemotherapy is accepted as the standard treatment for metastasis colorectal cancer (mCRC) (3). Capecitabine is an oral prodrug which is converted to its only active metabolite, 5-fluorouracil (5-FU), by thymidine phosphorylase (4). The fluoropyrimidine chemotherapy to the preoperative regimen reduces local recurrence (5), but does not improve survival (6), and has a negative effect on quality of life (7). Capecitabine combination with oxaliplatin is usually used for better therapeutic effect. While the addition of oxaliplatin doesn't show positive effects in short-time result (8,9), but it has increased complications resulting in dose reductions and treatment interruptions (10,11). For the
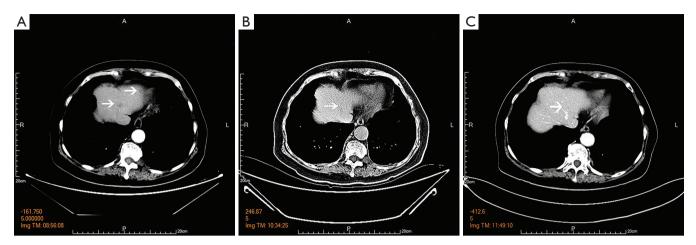
694

Li et al. Act and chemo for mCRC

Figure 1 The CT of the hepatic metastases before (A) and after adoptive immunotherapy [(B) plain CT; (C) enhancement CT]. The metastasis in left lobe was resected. The lesions in liver segment 4a became similar to the density of normal liver and hard to be seen. CT, computed tomography.

mCRC, the treatment is part of a palliative rather than curative treatment and the purpose of that is to prolong overall survival and to maintain quality of life (12). Other monoclonal anti-body (Mab) include cetuximab and panitumumab, both of which are epidermal growth factor receptor (EGFR) targeted Mab. They can regulate signal pathways related with cell survival, angiogenesis, cell migration and cellular invasion (13). Moreover, immunotherapy is a novel strategy for cancer therapy in recent years. Some clinical trials show the potential effectives for CRC patients (1).

Case presentation

An 81-year-old woman was diagnosed as CRC in February 2017, and she complained the dark red colored bloody stool without any obviously causative factors. Her previous medical history included hypertension and osteoporosis, which had lasted 20 and 10 years respectively. Colonoscopy showed an irregular lump occupying 2/3 enteric cavity, which the colonoscopy couldn't break through. The patient's serum level of carcinoembryonic antigen (CEA) was 48.13 ng/mL, CA199 was 75.86 U/mL, and CA125 was 64.13 IU/mL. Computed tomography (CT) showed several hypodense lesions in liver, some of which were enhanced in enhancement CT (*Figure 1*). Evaluation of MR images revealed rectosigmoid colon was occupied. The case was suspected CRC (cT4aN1M1). Laparoscopic lower anterior resection and left liver metastatic carcinoma resecting were conducted in 20th, February, 2017 (*Figure 2*). The tumor

was 5 cm-long and 4 cm-wide, cauliflower-like appearance, invading the serosa, and occupying 2/3 enteric cavity. A hepatic metastasis which located at the surface of left lobe was observed in the surgery and was resected at the same time, but some hypodense lesions in the CT images were unresectable (*Figure 1A*). The patient recovered well after the operation. The pathological diagnosis was differentiated adenocarcinoma invading to serosa with lymph node metastasis (4/13), and the resected lesion of left liver was confirmed as metastasis tumor. The surgical margin was free of tumor involvement. The test of immunohistochemistry showed EGFR(–), Ki67(high 80%, average 40%), MLH(+), PMS2(+), MSH2(+), MSH6(+).

After released from the hospital, the patient takes capecitabine (1.5 g po bid, d1–14, 21 d/cycle) for further treatment. The patient's serum level of CEA was dropped after the surgery, which was 19.52 ng/mL, but it increased dramatically 2 weeks later. Because of that, she enrolled immunotherapy six times from April to July 2017. $\alpha\beta$T cells and NK cells were injected intravenously into the patient six courses. Peripheral blood mononuclear cells were harvested using centrifugation. Over 1×10^6 harvested cells were cultured with an immobilized antibody to CD3 and interleukin (IL)-2 for 14 days, and 7.54×10^9 (4.4×10^9–9.2×10^9 on average) lymphocytes were obtained. The cultured lymphocytes included 61%±15% of CD8+ T-cells, 30%±15% of CD4+ T-cells (CD4+: CD8+ ratio, 0.8 on average), and a small percentage of natural killer (NK) cells, indicating that the proliferation of CD8+ T-lymphocytes was much greater than that of CD4+ T-lymphocytes during
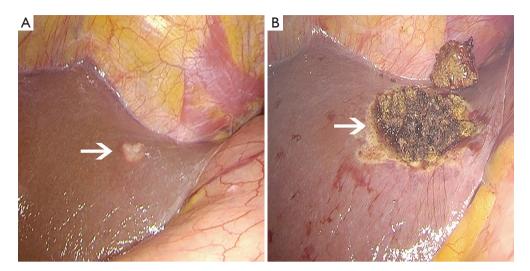
Figure 2 The hepatic metastasis confirmed in surgery. Arrow in (A) represents a metastasis in the surface of liver and it was resected (B).
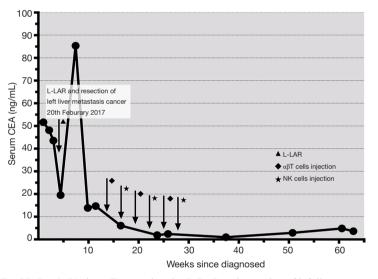

Figure 3 The serum level of CEA (N<5 ng/mL) since diagnosed. ↓▲, L-LAR and resection of left liver metastasis cancer; ↓◆, αβT lymphocytes transfusion; ↓★, NK lymphocytes transfusion. CEA, carcinoembryonic antigen; L-LAR, laparoscopic lower anterior resection; NK, natural killer.

the 2-week culture period. Over 7.54×10^9 αβ T-lymphocytes were injected intravenously into patients once every 3 weeks for 4.5 months (six cycles). After the first transfusion with αβT cells, the tumor biomarker, CEA, dropped obviously from 14.7 to 6.1 ng/mL. And it came to 1.9 ng/mL after four times treatment, which was back into normal range (<5 ng/mL) (*Figure 3*).

To reveal the detailed immunological status of this patient before and after adoptive cell transfer therapy, we used flow cytometry (FCM) to detect the intracellular or superficial markers of leukocytes. The detail method was described previously (14). We used Flow-Count™ fluorosphere internal standard beads to detect the absolute cell numbers. The OptiLyse C, Flow-Count beads, and monoclonal antibodies (mAbs) against CD3, CD4, CD8, CD14, CD19, CD45, CD56, TCR pan αβ, IFN-γ and IL-4 were purchased from Beckman Coulter (Brea, CA, USA). The result is shown diagrammatically in *Figure 4*. According to the figure, most kinds of lymphocytes were increased after infusion. The number of monocyte (CD14+), Killer T cell (CD3+ CD4- CD8+) and αβT cells (CD3+ TCR αβ+) rose by 259% (from 151 to 392 cells/µL), 159% (from 192 to 305 cells/µL) and

696

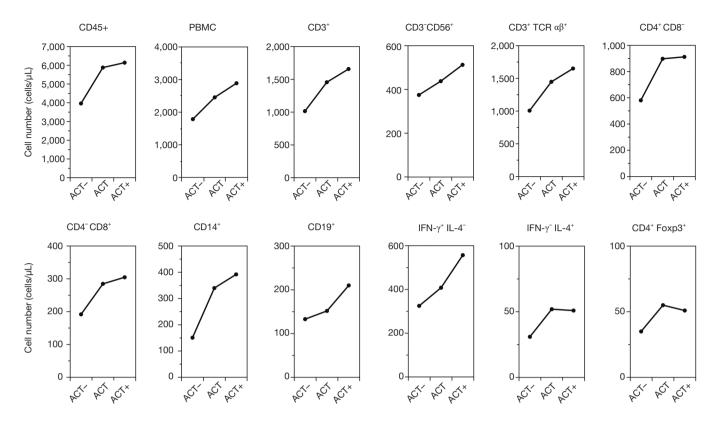Li et al. Act and chemo for mCRC

Figure 4 Changes in immunological cells before and after adoptive cell transfer therapy. CD45$^+$, leukocytes; PBMC, peripheral blood mononuclear cell; CD3$^+$ T cells; CD3$^-$ CD56$^+$, NK cells; CD3$^+$ TCRαβ$^+$, αβT cells; CD4$^+$ CD8$^-$, help T cells; CD4$^-$ CD8$^+$, killer T cells; CD14$^+$, monocytes cells; CD19$^+$, B cells; IFN-γ$^+$ IL-4$^-$, Th1 T cells; IFN-γ$^-$ IL-4$^+$, Th2 T cells; CD4$^+$ Foxp3$^+$, Treg cells; ACT–, before adoptive cell transfer therapy; ACT, six courses adoptive cell transfer therapy finished; ACT$^+$, 1 year after adoptive cell transfer therapy.

165% (from 1,007 to 1,657 cells/μL) respectively.

The CT images revealed the cancer didn't progression or relapse after the immunotherapy. Compared with CT images before the surgery, the metastases are smaller and much harder to see in liver, and are now similar to the density of the normal liver (*Figure 1B,C*). With 19-month follow-up, no relapse was founded, and the serum level of CEA is within normal limits.

The severity of adverse effects was evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 4.0. The patient had slight anemia only, (Hgb 10.5 g/dL), which did not fulfill the definition of adverse effects according to NCI-CTC criteria. There were no other severe treatment-related adverse events and no treatment-related death were founded.

Discussion

Nowadays the main immunotherapy for CRC include tumor

vaccines, cytokine treatment, mAbs, checkpoint therapies and adoptive cell transfer therapy (15). Previous study for metastasis CRC patients revealed chemoimmunotherapy can enhance proliferative response to colon carcinoma antigen and a significant reduction in suppressive regulatory T lymphocytes (Treg cells), resulting in enhanced T cells activities (16). It gives a prospect of combination of chemotherapy and immunotherapy. The conception of adoptive cell transfer therapy is that specific effector cells are directly infused within the cancer patient. Because most tumor cells express MHC class I-peptide, which can be recognized by antigen-specific CD8$^+$ cytotoxic T lymphocytes (CTLs). Therefore, adoptive cell transfer of activated CTLs successfully used in patients with advanced cancer (17).

The treatment of this patient includes immunotherapy and chemotherapy. αβT cells and NK cells were injected intravenously into the patient. Capecitabine was taken as well. Tumors were measured after adoptive cell transfer therapy, and responses were evaluated according to the

response evaluation criteria for solid tumors (RECIST), version 1.1 (18). The evaluation of responses was based on computed tomography measurements. Complete response (CR) was observed in this patient. The biggest lesion was 13.44 mm × 10.81 mm before the adoptive cell transfer therapy, while, it was unable to measure after the therapy. The CEA was back to the normal levels as well. It is confirmed combination therapy involving adoptive immunotherapy and chemotherapy for stage IV CRC is safe and feasible (19). In the research, therapy in each 21-day treatment cycle involved XELOX (130 mg/m^2 of oxaliplatin on day 1 plus 1,000 mg/m^2 of capecitabine twice daily on days 1–14), bevacizumab (7.5 mg/kg on day 1), and αβ T-lymphocytes (over 5×10^9 on day 18) cultured *ex vivo* with an immobilized antibody to CD3 and IL-2. The overall response rate was 83.3%. The median progression-free and overall survival durations were 567 and 966 days, respectively. The result provides a prospect for adoptive immunotherapy.

Immunotherapy has become a novel choice for solid cancer, though its effect still needs clinical trials to confirmed. PD-1, a check point inhibitor target, is one of the most popular strategies in immunotherapy. PD-1 inhibits T cells by down-regulating IL-2 expression through the PI3K/AKT pathway (20). While it is necessary to find reliable parameters for non-specific immunotherapy, such as adoptive cell transfer therapy. In previous works, we performed a detailed evaluation of the immunological status of 47 patients with advanced solid cancer, compared with 32 healthy subjects by FCM. And peripheral blood from 26 of 47 cancer patients was analyzed again after adoptive cell transfer therapy (14). Absolute numbers of T cells, several T cell subsets, B cells, and NK cells were significantly decreased in patients compared with healthy subjects. And adoptive cell transfer therapy increased the number of T cell subsets. This case provides an example for the conclusions. Before the adoptive cell transfer therapy, the absolute number of T cells is low, αβT cells especially, which are the majority of human T cells. After infusion cultured cells, the number of T cells increased, and it was much higher in 1 year assessment. This indicated adoptive cell transfer therapy can restore impaired T cells status. The patient was regarded as CR.

Adoptive immunotherapy still has some drawbacks (21), including a potential lack of immunologic memory, poor persistence of activated effector cells in patients and the time required to expand the cells. But, compared with chemotherapy, seldom serious complications of adoptive immunotherapy were recorded, except Parkhurst *et al*. reported three patients with mCRC were treated with autologous T lymphocytes genetically engineered to express a murine T cell receptor (TCR), which is against human CEA. All patients experienced profound decreases in serum CEA, however, a severe transient inflammatory colitis that represented a dose limiting toxicity was induced in all three patients (22).

The application of adoptive cell transfer therapy combined with capecitabine chemotherapy in patients with mCRC can ensure clinical efficacy with less complications. For some advanced CRC after primary site resection, the result of systematic treatment is still poor. Combination therapy involving adoptive immunotherapy and chemotherapy may be a feasible method to prolong the survival.

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.02.06). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this manuscript and any accompanying images.

698

Li et al. Act and chemo for mCRC

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