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Asia-Pacific Journal of Blood Types and Genes

2017, 1(3):1-6

APJBG

CAR–T cell therapy and its application in clinical cancer treatment

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ABSTRACT

Immunotherapy is regarded as the most significant method for cancer treatment in recent years. Chimeric antigen receptor T cell(CAR–T) technology , one form of target immunotherapy, has made a great breakthrough in hematological malignancies treatment and also a few progress in solid tumor treatment. This article reviews the history and mechanism of CAR–T, as well as the advantages and limitations of CAR–T in clinical cancer treatment. Then the review mainly discussed the clinical trial progress of CAR–T cell therapy in solid tumor treatment. A primary obstacle of CAR–T therapy is the heterogeneity in solid tumors. With an increasing number of solid tumor surface antigens being discovered, different varieties of CARs have been designed to treat solid tumors and have made some progress in clinical trial. In the end, this review puts forward a possible development direction of CAR–T.

Keywords: immunotherapy, CAR–T, solid tumors

The treatment of malignant tumors has long been a disturbing problem for the public. Traditional methods of cancer treatment include surgery, chemotherapy, radiotherapy, while most treatments have no satisfactory therapeutic effect. With the rapid development of immunology, immunotherapy has become a significant treatment method for malignant tumors in recent years. T lymphocytes, the most effective anti–tumor immune cells, attract the most widespread attention. Combined the high specificity of antibody with the cytotoxic activity of T cells, chimeric antigen receptor T cell (CAR–T) technology considered as a pearl of cellular immunotherapy has made great progress in recent years. This review summarizes the progress of CAR–T cell technology and its clinical application in cancer treatment.

OVERVIEW OF CAR–T TECHNOLOGY

The history of CAR–T technology development

Chimeric antigen receptor (CAR) contains extracellular antigen binding domains, transmembrane and intracellular signaling domains. Extracellular antigen binding domains are usually derived from a single chain antibody (single chain variable fragment, scFv). The intracellular signaling domain is immunoreceptor tyrosine–based activation motif (ITAM), generally including CD3 ζ or Fc ε RI γ [1–2], etc. Fc ε RI γ includes 1 ITAM, however, only CD3 ζ including 3 ITAMs can activate T cells effectively. Therefore it is more common that CD3 ζ is taken as the intracellular signaling domain.

The first generation of CAR had only one intracellular signaling domain. The signal mainly transducts through the ITAM of CD3 ζ . Owing to the lack of the second signal of T cell proliferation, T cells are difficult to proliferate further after combining with tumor antigen. Therefore it only has a weak anti–tumor effect.

Based on the first generation of CAR, the second generation of CAR was produced by adding a stimulatory molecule (CM) to the CAR, which is able to transfer the second signal, such as CD28 or 4–1BB molecules connected with CD3 ζ . Thus the second generation of CAR improves the effects of T cells. Af–

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The authors reported no conflict of interests.

ter combining with the tumor antigen *in vivo*, the sec–ond generation CAR–T cells can kill cancer cells and complete their amplification at the same time. Adding the co–stimulatory signal 4–1BB extends the survival time of CAR–T cells *in vivo* and increases the amount of CAR–T cells located in the tumor. Meanwhile it im–proves the anti–tumor ability.

The third generation of CAR intracellular signaling domain contains two CM (such as CD28 and CD137/CD134 chimera connected with CD3 ζ)[3]. These transgenic CAR–T cells can produce enough IL–2 to promote its proliferation with the effect of exogenous co–stimulatory molecules. Compared with the second generation CAR, the third generation CAR has stronger cytotoxicity, amplification and capacity of cytokine production *in vivo*.

The fourth generation of CAR, called T cells redi–rected for universal cytokine killing (TRUCK), intro–duces proinflammatory cytokines and costimulatory ligands which cause greater antitumor effects widely in immune suppression of the tumor microenvironment by releasing proinflammatory cytokines, recruiting and activating more immune cells.

The fifth generation of CAR is also called general CAR. The French Cellectis Company applied TALEN technology to knock down the T cell receptor (TCR) and CD52, which could reduce or avoid the risk of graft–versus–host disease (GVHD) produced by al–logeneic cells[4]. CD52, a target for the anticancer drug alemtuzumab, can protect the CAR–T from killing by drugs after knocked down.

The killing mechanism of CAR–T cells

Compared with traditional T cell treatment, CAR recognizes the tumor antigen by passing the antigen presenting stage without the restriction of MHC. And CAR also has the recombinant costimulatory signal which enhances the cytotoxicity of T cells. Therefore CAR–T can overcome the adverse effects of tumor im–mune escape caused by down–regulation of MHC of tumor cells and reduced CM expression and so on.

The CAR was transfected into T cells *in vitro* to form CAR–T cells. CAR–T cells can specifically recognize tumor associated antigens (TAA), which increases the effects of CAR–T cell targeting, killing activity and durability compared with traditional immune cells. CAR–T cells combine with the corresponding tumor cell specifically and secrete a variety of enzyme pro–teins including perforin and granzyme and various cytokines to kill tumor cells[5]. CAR–T cells can over–come the local immune suppression microenvironment of the tumor and breaks the state of immune tolerance. Antitumor antibodies usually lose their effect in a few days or weeks, while CAR–T cells are expected to cir–culate in the body and inhibit tumor recurrence for sev–eral years.

ADVANTAGES AND LIMITATIONS OF CAR–T CELL THERAPIES

Advantages of CAR–T cell therapy

Compared with traditional ways of tumor therapy such as radiotherapy and chemotherapy, CAR–T cells therapy has the following advantages: ① The preci–sion killing of tumor cells. CAR–T cell is the geneti–cally modified autologous T cells. Owing to the an–tigen and antibody binding mechanism, CAR–T can overcome tumor immune escape, which is the result of tumor cells lowering the expression of MHC and reducing antigen presentation. ② Multiple target ef–fect. CAR can recognize tumor cells by not only the tumor protein antigen, but also glycolipid non–protein antigens, which expands the range of tumor antigen target. ③ Wider action range. In view of the variety of tumor cells that express the same tumor antigen, once the CAR gene construction targeted a certain tumor antigen has completed, it can be widely used in the treatment of multiple tumors. ④ Persistent anti–tumor effects.

Limitations of CAR–T cell therapy

Target antigen

B lymphocyte tumor cells often express CD19 mol–ecules on the surface of cells, so CD19 CAR–T has a significant effect on B cell tumors. However, hetero–geneity is one of the more prominent features of solid tumors, which means that a tumor does not express the same tumor correlative antigen. Even on the same tumor lesion, none of the target antigens can cover all tumor cells. Therefore, if a single target CAR–T cell is used to treat a solid tumor, the high heterogeneity of the solid tumor is thus that it will inevitably relapse or will not be fully eradicated[6]. In addition, the target antigen of CAR–T cell is also expressed in normal cells or tis–sue in a certain extent. The cross immune killing effect can be controlled through the strict screening of target antigens, but still can´t completely be avoided, which leads to on–target off–tumor effects [7].

Cytokine release storm

Another serious complication of CAR–T therapy is cytokine release storm (CRS), which has a high le–thality. An important pathway for CAR–T cells to kill tumor cells is by secreting a large number of cytokines to promote the proliferation of immune cells and to

enhance their immune response to tumor cells. After CAR–T cells enter the body, T cells, B cells, NK cells, monocytes and macrophages can release a large number of inflammatory cytokines, including TNF–α, IL–1, IL–6, IL–12, IFN, etc. These cytokines cause acute inflammation which leading to adverse reactions such as nausea, headache, tachycardia, hypotension, rash and tachypnea, of which severe cases can lead to multiple organ failure or acute respiratory distress syndrome, known as CRS. The application of an anti–inflammatory glucocorticoid or cytokine antagonist can reduce the adverse reactions caused by CRS. Reducing the initial dose of CAR–T and increasing from low doses to high doses can effectively prevent CRS[8]. In addition, the CAR–T cell with the suicide gene switch system can also effectively control the occurrence of CRS. Pre–setting the suicide gene switch system in the CAR–T cells, which can be activated by small molecule and clear away the input CAR–T cells when necessary, consequently reduces the adverse reactions caused by CRS. There are several suicide gene switch systems, such as the herpes simplex virus–thymidine kinase(HSV–TK) system, which are applied in CRS therapy and show wide application prospects[9].

Homing of CAR–T cells

In the development process of solid tumors, a tumor microenvironment (TME) is formed. There are infiltration of regulatory T cells (Treg), tumor associated macrophages and high expressions of PD–1 and inhibitory cytokines (IL–10, TGF–ß), which protect the tumor very closely. The CAR–T cells transfused through vein can hardly home to the tumor; therefore, the treatment effects are poor.

Neurotoxicity

The use of CAR–T in the treatment of leukemia is known to cause neurological symptoms such as delirium, language impairment, motor disturbance and seizure, etc. The reasons are not clear, although the occurrence time is associated with CRS of the whole body. These symptoms are various and most of them can be cured by themselves. The application of tocilizumab is not effective for neurotoxicity.

APPLICATION OF CAR–T CELL HERAPY IN TREATMENT OF CLINICAL TUMOR

Hematological malignancy

Currently, the clinical application of CAR–T cell therapy focuses on hematopoietic tumors. One example of the clinical application of CAR–T is the case of 6–year–old Emily Whitehead. In April 2012, Emily was highly at risk after experiencing two recurrences of acute lymphoblastic leukemia. The Children´s Hospital of Philadelphia implemented the Novartis CTL–019 phase Ⅰ clinical program for Emily. With this, Emily became the first leukemia child cured by CAR–T cell immunotherapy. During the treatment, she had severe CRS, and fortunately, the CAR–T cell played the therapeutic effect and Emily regained her life. According to the published data by Maude SL *et al*. in 2014, out of 30 cases of different types of B lymphocyte leukemia treated by CAR–T cell therapy, 27 patients (90%) obtained a complete response(CR) and 20 patients (67%) were still clear of cancer cells after half a year[10]. Marco *et al*. applied CAR–T cells targeted CD19 to treat recrudescent and intractable B cell acute lymphoblastic leukemia (B–ALL) patients, and the CR rate reached 88% in 16 cases of patients[11]. On July 12, 2017, the FDA Oncologic Drugs Advisory Committee (ODAC) discussed the biologics license application (BLA) of Novartis´s CAR–T product Tisagenlecleucel (CTL–019) and supported the application of Novartis CAR–T drug CTL–019 to treat children and young adults with advanced leukemia with the vote of 10:0.

Solid tumors

Owing to solid tumors´ atypical, less tumor specific antigen (TSA) and tumor immune editor, nowadays CAR–T therapy has only made small progress on the solid tumors which show obvious TSA, such as lymphoma, melanoma, neuroblastoma, etc.

Lymphoma

In 2010, Kochenderfer *et al*. first reported on the application of CD19 CAR–T cells to treat lymphoma. An advanced lymphoma patient was in a state of lymphoma remission for a long time after administering CD19 CAR–T cells. The normal $CD19^+$ B cells also have long been eradicated, which indicated success in the treatment by CD19 CAR–T[12]. Mishra *et al*.[13] observed the curative effect of CD19 CAR–T and CD38 CAR–T joint Rituxan to treat non–Hodgkin´s lymphoma and found that the CAR–T cells had a strong cytotoxic effect on non–Hodgkin´s lymphoma, with the treatment effect persisting for more than 2 months. Ramos *et al*.[14] reported a treatment concerning relapsed/refractory Hodgkin lymphoma (HL) and anaplastic large cell lymphoma (ALCL). They conducted a phase Ⅰ dose escalation study in which 9 patients with relapsed/refractory HL and ALCL were infused with CD30 CAR–T cells. Of 7 patients with relapsed HL, 1 entered CR lasting more than 2.5 years after the second infusion, 1 remained in continued CR for almost 2 years, and 3 had transient stable disease. Of 2 patients with ALCL, 1 had

a CR that persisted 9 months after the fourth infusion of CD30 CAR−T cells.

Melanoma

In 2011, Rosenberg *et al*.[15] reported that they applied autologous tumor infiltrating lymphocytes (TIL) to treat metastatic melanoma and symptoms were improved. Twenty cases got complete remission among 93 patients and 3−year and 5−year survival rates were 36% and 29% respectively, which proved the efficacy of TIL cell treatment. In Morgan´s[16] study, 17 metastatic melanoma patients received CAR−T treatment (targeted α−MART−1), 2 cases completely subsided, while the other 15 cases continued with a high percentage of CAR−T cells in peripheral blood (9%−56%) and these patients were in stable condition. In addition, Garget *et al*.[17] used GD2 CAR−T and kinase inhibitors in combination in the melanoma experiments *in vitro*. The experimental results showed that the combined application has the function of the synergistic killing of melanoma cells.

Neuroblastoma

In clinical trials of neuroblastoma treatment, Louis *et al*.[18] found that after applying GD2 CAR−T cells to treat patients with neuroblastoma at the active stage, patients attained complete remission, showing a significant treatment effect. In the study of the application of GD2 CAR−T cells to treat 11 children with neuroblastoma, 4 out of 8 cases who could be evaluated with curative effect displayed tumor necrosis and extinction, of which 1 patient got a complete response. These 11 patients were followed up for 2 years with no obvious adverse reactions [19]. A study of 19 high−risk neuroblastoma patients who accepted GD2 CAR−T cell treatment with a long−term follow−up found that GD2 CAR−T cells have a function in the treatment of neuroblastoma. These GD2 CAR−T cells can be amplified in the body and can continue to exist for a long time, showing the persistence of GD2 CAR−T cells and its association with long−term survival [20].

Glioblastoma

In 2016, the City of Hope Research Center reported 1 case of multiple glioblastoma who accepted CAR−T therapy targeted interleukin−13 receptor α2 (IL−13Rα2) and received a treatment breakthrough. After the treatment, which lasting 133 days, they observed that all intracranial and spinal cord tumor volume had narrowed considerably. After the fifth CAR−T cell transfusion (190 days), part of the tumor disappeared and the rest of the tumor had decreased by 77%. After five more sessions of CAR−T cell transfusion, the researchers found that the tumors in the patient had completely disappeared, showing CAR−T cell therapy´s efficacy in treating glioblastoma[21]. In Jun 23, 2017, Dr. Huang and her colleagues[22] published the paper about the treatment of gliomas with CD70−specific CAR−T cells in *Neuro−Oncology*.

Metastatic hepatic cancer

Katz *et al*.[23] reported clinical trials using CAR−T cells targeted carcinoma−embryonic antigen (CEA) via percutaneous hepatic artery injection for the treatment of 6 patients of metastatic hepatic cancer, including 4 cases who have more than 10 liver metastatic carcinoma lesions. One case remained alive after more than 23 months of CAR−T treatment, and 5 died from disease progression. CEA levels fell by an average of 37% (19%−48%). Four cases of biopsy showed necrotic or fibrotic metastatic carcinoma and the serum examination showed higher levels of IFN associated with the decreased CEA. The results suggest that CEA CAR−T therapy is effective for treating metastatic hepatic cancer.

Nasopharyngeal carcinoma

Nasopharyngeal cancer is usually associated with the Epstein−Barr virus (EBV) infection. Tang *et al*.[24] used EBV latent membrane protein 1 (LMP1) as the target to make LMP1 CAR−T cells. *In vitro* experiments showed that LMP1 CAR−T cells had a significant effect on LMP1 positive nasopharyngeal carcinoma cells. Animal experiments show that LMP1 CAR−T cells can obviously inhibit the growth of nasopharyngeal carcinoma, and the results suggest that LMP1 CAR−T has therapeutic effect on the treatment of nasopharyngeal carcinoma expressing LMP1.

Other solid tumors

There have been reports about CAR−T clinical trial treatment for lung cancer, pancreatic cancer, prostate cancer, osteosarcoma, breast cancer, ovarian cancer and so on[25−29], and the therapeutic effects need to be further evaluated. ***Table 1*** shows the clinical trials of CAR−T therapy carried out for solid tumors [30].

FUTURE OF CAR−T THERAPY

At present, researchers are working on the development of a new generation of CAR−T technology. For example, a specific CAR−T cell technology, which includes the second CAR structure, can increase the ability of CAR−T cells to distinguish cancer cells from normal cells. The two CARs can be designed and the second can be reverse. The first CAR identifies the target protein on the surface of cancer cells and causes

Table 1 **Current CAR–T clinical trials for solid tumors**

Targets	Tumors	CAR functional domains	Clinical trial stage	Country
CEA	adenocarcinoma	NA	Ⅰ	USA
CEA	colorectal carcinoma	CD3ζ/CD28	Ⅰ	USA
CEA	breast cancer	CD3ζ/CD28	Ⅰ	USA
CEA	solid tumors	NA	Ⅰ	UK
CEA	hepatic metastases	CD3ζ/CD28	Ⅰ	USA
CEA	adenocarcinoma	CD3ζ/CD28	Ⅱ	USA
EGFR	advanced EGFR+ tumor	CD3ζ/CD4–1BB	Ⅰ/Ⅱ	USA
EGFR	head and neck cancer	CD3ζ/CD28	Ⅰ	USA
EGFR Ⅷ	glioblastoma	CD3ζ/CD28/4–1BB	Ⅰ/Ⅱ	USA
FAP	MPM	NA	Ⅰ	Switzerland
FBP	ovarian cancer	NA	Ⅰ	USA
GD2	neuroblastoma	CD3ζ	Ⅰ	USA
GD2	neuroblastoma	NA	Ⅰ	USA
GD2	neuroblastoma	CD3ζ/CD28/OX–40	Ⅰ	USA
GD2	sarcoma	CD3ζ/CD28/OX–40	Ⅰ	USA
GD2	non–neuroblastoma	CD3ζ/CD28/OX–40	Ⅰ	USA
Her2	Her2+ tumor	CD3ζ/CD28	Ⅰ	USA
Her2	advanced osteosarcoma	CD3ζ/CD28	Ⅰ	USA
Her2	metastatic carcinoma	CD3ζ/CD28	Ⅰ/Ⅱ	USA
Her2	glioblastoma multiforme	CD3ζ/CD28	Ⅰ	USA
Her2	solid tumor	CD3ζ/CD4–1BB	Ⅰ/Ⅱ	China
IL–13Rα2	malignant glioma	CD3ζ	Ⅰ	USA
PSMA	prostatic cancer	CD3ζ/CD28	Ⅰ	USA

T cell activation, and the second one recognizes the specific protein of normal cell surface and can inhibit T cell activation, which is beneficial to reduce the on–target off–tumor effects. In addition, CAR can also be designed into two positive identification CAR structures. When two target proteins exist on the cell surface, the T cell activation is maximized.

Although the application of CAR–T cells in the treatment of solid tumors is in its infancy, it has shown great promise in clinical curative effects. CAR–T therapy is an attractive prospect; that is, not only that it will be an academic research hot spot, but will also become a hot destination for biological medicine enterprise pursuit. As the progress of CAR–T cell technology and improvement of related clinical research, CAR–T cell therapy will certainly play a more and more important role in tumor immunotherapy, which will absolutely be of benefit to human life and health.

Acknowledgments and funding

This work is supported by the grant from the Natural Science Foundation of China (81773268 to Qi Tang).

References

[1] Cai L, Zhang L, Yang Z, et al. Chimeric antigen receptor T–cell immunotherapy: A new approach to cancer therapy. *Chinese Journal of Nature*(*in Chinese*), 2016, 38(3):228–31

[2] Zhai Y, Li T, Liang J, et al. Introduction and research progress of chimeric antigen receptor T cells. *International Journal of Immunology(in Chinese)*, 2016, 39(6): 612–9.

[3] Shi Y, Liao X. Chimeric antigen receptor–engineered T cells and its clinical application. *Journal of Leukemia & Lymphoma(in Chinese)*, 2016, 25(10): 629–32.

[4] Zhang X, Song Y. Progress of chimeric antigen receptor T cells in the treatment of hematologic malignancies: reports from the 57th American Society of Hematology annual meeting. *Journal of Leukemia & Lymphoma(in Chinese)*, 2016, 25(2):65–8.

[5] Grada Z, Hegde M, Byrd T, et al. TanCAR: A novel bispecific chimeric antigen receptor for cancer immunotherapy. *Mol Ther Nucleic Acids(in Chinese)*, 2013, 2: e105.

[6] Liu Y, Zhu H, Hang W. Chimeric antigen receptor T cell based therapeutic modality in solid tumors. *Chinese Journal of Clinical Oncology(in Chinese),* 2017, 44(9):415–7.

[7] Wu C, Jiang J. Toxic adverse reactions of CAR–T cellular immunotherapy for the patients with carcinoma and its clinical strategies. *Chinese Journal of Cancer Biotherapy(in Chinese)*, 2016, 23(6):745–50.

[8] Ertl HC, Zaia J, Rosenberg SA, et al. Considerations for the clinical application of chimeric antigen receptor T cells: observations from a recombinant DNA Advisory Committee Symposium held June 15, 2010. *Cancer Res*, 2011, 71(9): 3175–81.

[9] Oliveira G, Greco R, Lupo–Stanghellini MT, et al. Use of TK–cells in haploidentical hematopoietic stem cell transplantation. *Curr Opin Hematol,* 2012, 19(6):427–33.

[10] Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*, 2014, 371(16):1507–17.

[11] Davila ML, Riviere I, Wang X, et al. Efficacy and toxic–

ity management of 19–28z CAR–T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med*, 2014, 6(224):224ra25.

[12] Kochenderfer JN, Rosenberg SA. Treating B–cell cancer with T cells expressing anti–CD19 chimeric antigen receptors. *Nat Rev Clin Oncol*, 2013, 10(5):267–76.

[13] Mihara K, Yanagihara K, Takigahira M, et al. Synergistic and persistent effect of T–cell immunotherapy with anti–CD19 or anti–CD38 chimeric receptor in conjunction with rituximab on B–cell non–Hodgkin lymphoma. *Br J Haematol*, 2010, 151(1):37–46.

[14] Ramos CA, Ballard B, Zhang H, et al. Clinical and immunological responses after CD30–specific chimeric antigen receptor–redirected lymphocytes. *J Clin Invest*, 2017, pii: 94306. doi: 10.1172/JCI94306.

[15] Rosenberg SA, Yang JC, Sherry RM, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T–cell transfer immunotherapy. *Clin Cancer Res*, 2011, 17(13): 4550–7.

[16] Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science*, 2006, 314(5796): 126–9.

[17] Gargett T, Fraser CK, Dotti G, et al. BRAF and MEK inhibition variably affect GD2–specific chimeric antigen receptor (CAR) T–cell function *in vitro*. *J Immunother*, 2015, 38(1): 12–23.

[18] Louis CU, Savoldo B, Dotti G, et al. Antitumor activity and long–term fate of chimeric antigen receptor–positive T cells in patients with neuroblastoma. *Blood*, 2011, 118(23): 6050–6.

[19] Shen CJ, Yang YX, Han EQ, et al. Chimeric antigen receptor containing ICOS signaling domain mediates specific and efficient antitumor effect of T cells against EGFRvIII expressing glioma. *J Hematol Oncol*, 2013, 6: 33.

[20] Song DG, Ye Q, Carpenito C, et al. *In vivo* persistence, tumor localization, and antitumor activity of CAR–engineered T cells is enhanced by costimulatory signaling through CD137 (4–1BB). *Cancer Res*, 2011, 71(13): 4617–27.

[21] Brown CE, Alizadeh D, Starr R, et al. Regression of glioblastoma after Chimeric Antigen Receptor T–cell therapy. *N Engl J Med*, 2016, 375(26): 2561–9.

[22] Jin L, Ge H, Long Y, et al. CD70, a novel target of CAR–T–cell therapy for gliomas. *Neuro Oncol*, 2016, vi91–2. doi: 10.1093/neuonc/nox116.

[23] Katz SC, Burga RA, McCormack E, et al. Phase Ⅰ hepatic immunotherapy for metastases study of intra–arterial chimeric antigen receptor–modified T–cell therapy for CEA+ liver metastases. *Clin Cancer Res*, 2015, 21(14): 3149–59.

[24] Tang X, Zhou Y, Li W, et al. T cells expressing a lmp1–specific chimeric antigen receptor mediateantitumor effects against lmp1–positive nasopharyngeal carcinoma cells *in vitro* and *in vivo*. *J Biomed Res*, 2014, 28(6):468–75.

[25] Koneru M, Purdon TJ, Spriggs D, et al. Il–12 secreting tumor–targeted Chimeric Antigen Receptor T cells eradicate ovarian tumors *in vivo*. *Oncoimmunology*, 2015, 4(3): e994446.

[26] Deng Z, Wu Y, Ma W, et al. Adoptive T–cell therapy of prostatecancer targeting the cancer stem cell antigen EpCAM. *BMC Immunol*, 2015, 16: 1.

[27] Criscitiello C, Curigliano G. Immunotherapy of breast cancer. *Prog Tumor Res,* 2015, 42: 30–43.

[28] Beatty GL, Haas AR, Maus MV, et al. Mesothelin–specific chimeric antigenreceptor mRNA–engineered T cells induce anti–tumor activity in solidmalignancies. *Cancer Immunol Res*, 2014, 2(2): 112–20.

[29] Zuccolotto G, Fracasso G, Merlo A, et al. PSMA–specificCAR–engineered T cells eradicate disseminated prostate cancer in preclinicalmodels. *PLoS One*. 2014, 9(10): e109427.

[30] Chang Z, Chen X, Wang L, et al. Research progress of chimeric antigen receptor modified T cells in malignant tumor. *Journal of Pharmaceutical Research*, 2015, 14(9):534–6.

(Received 19 July 2017, Revised 06 August 2017, Accepted 09 August 2017)