

Review Article

Targeted therapy against hepatocellular carcinoma

Songlin Chen^{1*}, Lei Yang^{1*}, Yanhui Cen^{1,2*}, Jingpeng Feng¹, Ping Li¹, Mingfen Li¹, Xiaoqi Huang¹, Aiping Pan¹, Yinghui Lin¹

¹Department of Clinical Laboratory of First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning, Guangxi, People's Republic of China; ²Guangxi University of Chinese Medicine, Nanning, Guangxi, People's Republic of China. *Equal contributors.

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Abstract: As a frequent solid tumor of the liver, hepatocellular carcinoma (HCC), has a very poor prognosis. It has been the second most common cause of death which led by cancer all of the world. With more and more risk factors, such as fatty liver, hepatitis B and C viral infections, alcohol abuse, and metabolic syndrome, HCC represents an increasing incidence and mortality. It is still a challenge for HCC curative treatment. There are no effective therapeutic strategies to meet the clinical need. Despite increasing research about the novel drugs, most of them ultimately fail in Phase III clinical trials. In this review, we provide key point summaries and present research agenda of targeting therapeutic strategies for HCC regarding biomarkers and targeting delivery vehicles. Then, the CAR-T-cell and Bite targeting strategies efficiency are discussed. Finally, this review provides a critical evaluation of the targets and strategies discussed above for personalized treatment of HCC.

Keywords: HCC, incidence, treatment

Introduction

Hepatocellular carcinoma (HCC), in most cases, occurs as a result of chronic hepatitis, and the biggest risk factor is cirrhosis [1, 2]. Currently, some clinical treatments are available for HCC, surgical approaches, chemotherapeutics, and radiation percutaneous approaches are the main therapeutic strategies. Immunotherapeutics are a promising approach for treating cancer [3]. However, only surgical approaches have proven effective thus far [4, 5]. Surgical approaches are highly limited by the advanced stage HCC. Despite tumor cells and tumor microenvironment are relatively more sensitive than normal tissues to the exposure of chemotherapeutics, non-specific anticancer drugs hold non-selective physiological activity, which contributes to serious systemic cytotoxicity to normal tissues. Radiation percutaneous approaches have a similar situation [6-8]. Some existing chemotherapeutic medicines have been used to treat HCC, such as doxorubicin, sorafenib, cisplatin, and mitomycin, but none of them have shown clinical efficacy without overt toxicity and adverse effects due to their inherent drug resistance and nonspecific bio-distribution. Sorafenib has been approved by the

Food and Drug Administration (FDA) to be used as the first-line clinic treatment for HCC [9, 10]. Although some research has reported that Sorafenib can improve HCC patients' median survival from 7.9 months to 10.7 months, unorderd accumulation and nonspecific distribution in the body lead to much systemic side-effects and also deficiency dosage targeting the tumor cells and tumor microenvironment [11]. Recent discoveries of new cancer biomarkers has been providing novel therapeutic tools for cancer targeting treatments, especially concerning immunotherapeutic approaches [12]. New promising therapeutic strategies, like CAR-T-cell and bispecific antibodies (bsAbs), have paved the ways to anticancer treatments. In this review, biomarkers and the therapeutic tools for HCC will be described as the guide line for clinical treatment [13].

HCC bio-marker

Glypican-3 (GPC3): GPC3 is a heparan sulfate proteoglycan expressed on the HCC cell surface while not in normal hepatocytes or tissue [14, 15]. Previous research has reported that GPC3 can be used as a reliable diagnosis bio-marker of HCC. Furthermore, more and more

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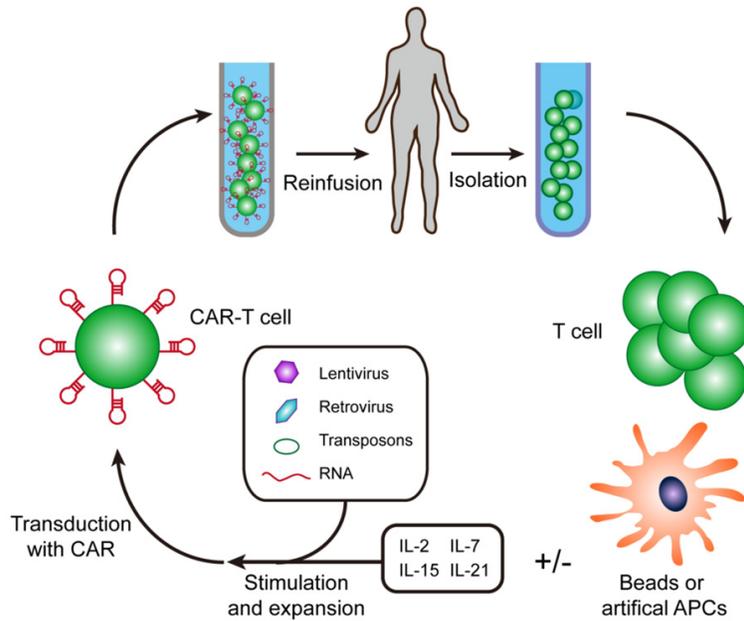


Figure 1. Therapeutic strategy CAR-T-cells.

studies indicated that GPC3 worked as a co-receptor which play a part in promoting neoplastic transformation [16, 17]. In addition, Li et al. has reported that GPC3 could upregulate c-Myc expression which is a typical target of the classical Wnt signaling pathway [18]. Furthermore, reducing expression GPC3 could suppress the volume and survival of HCC cells *in vivo* and *in vitro*. Moreover, further studies indicated that GPC3 is treated as a potential biomarker for HCC diagnosis and treatment [19, 20].

Some anti-GPC3 monoclonal antibodies (mAbs) has been designated according to their structure and bioactivity toward the GPC3 molecule [21]. The earliest therapeutic mAb against GPC3, named GC33 which specifically binds to the C-terminal of GPC3 spatial structure with a high affinity. GC33 showed cytotoxic activity target GPC3-positive hepatoma cells and exhibited potential antitumor activity in xenograft models [22]. Furthermore, antibodies which arm to GPC3 for HCC treatment have been reported, such as humanized mouse YP7 antibodies, human antibodies MDX-1414 and HN3. Further research has been toward preclinical evaluation [23].

Asialoglycoprotein receptor

The asialoglycoprotein receptor (ASGPR) is largely expressed on hepatocytes without dis-

tributed in extra-hepatic tissues [24, 25]. As one of the most studied HCC targets, ASGPR is expressed on the early and advanced HCC patient tumor cells. Researchers have exploited galactose, lactoferrin moieties, lactose, to produce drug delivery systems targeting ASGPR. Their studies reported that drug delivery systems are significant for effective recognition by ASGPR [26-28].

Lactobionic acid (LA) is a specific binding ligand for galactose group [29, 30]. It can be used as hepatoma-targeted bio-mark to improve the effective to target hepatocytes. In these models, the medicines were released in tumor cells by

receptor-mediated endocytosis between ASGPR and galactose residues on the cells. Additionally, some studies have reported that LA-modified target systems could improve the incept drugs as described in the murine models bearing hepatoma. The galactosylated nanoparticles incorporated with Docetaxel and conferred anti-tumor efficacy and enhanced cytotoxicity with better tolerance for treatment *in vivo* [31, 32].

SerpinB3

Recently studies have certified the serpin-protease inhibitor SerpinB3, a novel molecule in hepatocyte malignant transformation [33, 34]. It is expressed on the hepatocyte as soon as it turns to malignant transformation. Hence, SerpinB3 can be exploited for diagnostic and therapeutic purposes targeting in the primary liver cancer. SerpinB3 and SerpinB4 are isoform which highly expressed in hepatocellular tumor and in dysplastic nodules without detectable in normal hepatocytes [35, 36]. They are suggesting a diagnostic in relatively early phase of HCC. Novel cellular localization skills have been used to describe for SerpinB3 and its isoform SerpinB4, while cytosolic localization and surface localization was both initially reported. Moreover, nuclear localization mediated by JNK1 (c-Jun NH2-terminal kinase-1) has been described, like following exposure to ultraviolet

irradiation [37, 38]. Researchers have reported that SerpinB3 promotes epithelial-mesenchymal transition and inhibits apoptosis. It acts as a key process of differentiation and migration of epithelial cells by reducing intercellular adhesion and increasing motility into motile mesenchymal cells. Recently, studies indicate that hypoxia can upregulate SerpinB3 by hypoxia-inducible factor-2 α (HIF-2 α). Besides, the ETS family transcription factor PEA3 and oncogenic Ras via MAPK is also induced the expression of SerpinB3 [39-41].

Therapeutic strategies

CAR-T: Adoptive cellular immunotherapy (ACI) has brought new therapeutic strategies for cancer. Since Mitchison found that lymphocyte “adoptive transfer” caused rejection of allograft tumors in the mouse model, adoptive immunotherapy has shown sustainable development. Whether in vitro preparation, cell proliferation and prolong cell survival, or the efficacy of cancer patients and side effects, this therapeutic strategy has advanced considerably [42-44] and CAR-T-cells have undergone four generations of evolution (**Figure 1**).

The principle is that a single-chain fragment variable (scFv) is edited and linked to T-cells *in vitro* to form an artificial T-cell receptor TCR, scFv regulates its targeting function against specific antigens. Compared with the unmodified TCR, CAR can both recognize the targeted biomarker through the extracellular antigen recognition domain scFv and mediate the T-cell activation through the intracellular TCR signal transduction domain. This optimized design allows T-cells recognition to unrestricted from human leukocyte antigen (HLA) and avoid the dependence on antigen presentation [45-48].

A classical CAR structure is composed of an extracellular antigen recognition domain, a transmembrane domain, and an intracellular TCR signaling domain. The extracellular antigen recognition domain is a typical scFv structure. It usually derived from the heavy and light chain variable regions of specific antibodies which target to tumor associated antigen (TAA). In a general way, the TAA includes proteins which specifically expressed in tumor cells, glycoproteins of peptide antigens and surface marker molecules, as well as glycolipids and ganglio-

sides. The transmembrane region of CAR is usually derived from type I membrane proteins such as CD3, CD4, CD8, and CD28. If the transmembrane region is mutated, CAR can reduce the recognition ability of CAR. The intracellular TCR signal transduction domain of CAR is derived from CD3 ζ , as well as co-stimulatory molecules such as CD28 and tumor necrosis factor (TNF) receptor family members. CD3 ζ is involved in the formation of endogenous TCR complexes in the CAR as a key molecule in T-cell active signaling. In contrast to CD3 ζ alone CAR, CAR T-cells with co-stimulatory signal domain have enhanced viability, proliferation ability and cytokine production ability after antigen recognition. At present, the four generations of CAR have been designed and the main differences in the intracellular signal domain (**Figure 2**) [49-53].

The first-generation CAR

The first-generation CAR only has the basic CAR structure consisting of bound scFv epitopes, transmembrane region, and CD3 ζ intracellular signal transduction region. This structure allows the first-generation CAR to activate calcium channels and thus cause transient T-cell activation and proliferation, but does not significantly stimulate T-cells [54, 55].

The second- and third- generation CAR

Compared with the first-generation, the second-generation CARs added a costimulatory domain to the intracellular signal domain. Hence, the second-generation CAR has dual intracellular signal domains: the first is from the CD3 ζ signal chain and the second is from costimulatory molecules. Second-generation CARs are effective at generating repetitive antigenic stimulation, promoting T-cell proliferation and producing IL-2 [52].

In order to improve the clinical efficacy of CAR-T-cell therapeutic strategies, the third-generation of CAR was improved. OX40 and 4-BB (CD137), which are members of the TNF receptor family, can provide co-stimulation signals to activate T-cells to proliferate and produce cytokines. The third-generation CAR utilizes triplex signal domains, including CD3 ζ , co-stimulation signaling domains and OX40/4-1BB (CD137), which perform significantly better effective

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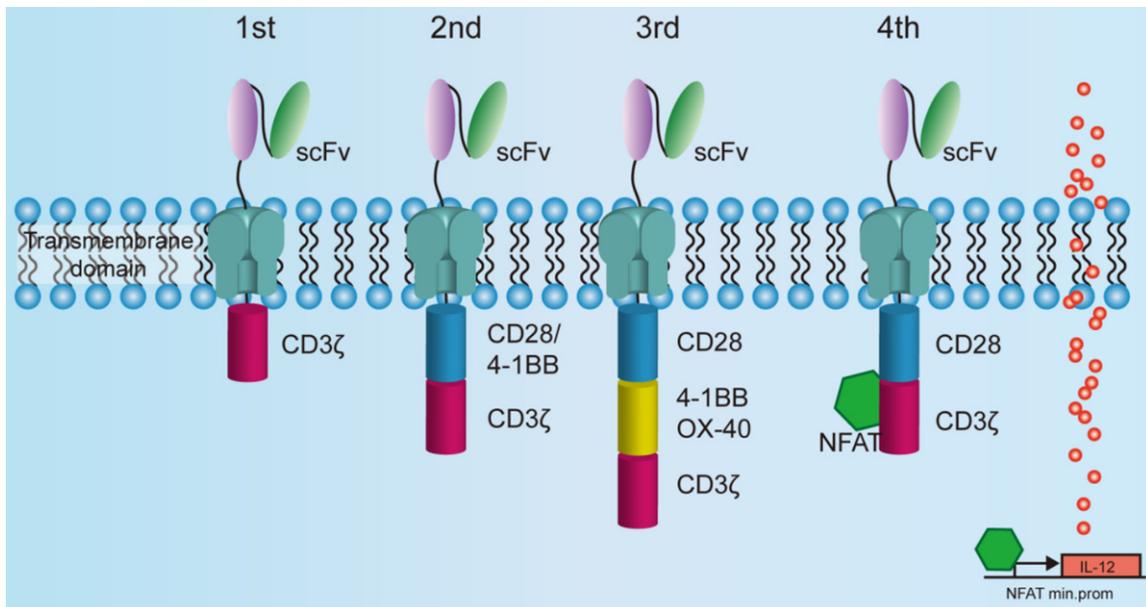


Figure 2. Four generations of CAR-T.

than the second-generation. OX40 signaling not only promotes T-cell production of IL-2 and TNF- α , while maintaining clonal expansion of T-cells, but also induces lysis of target cells. Hombach et al., reported that CD28-CD3 ζ -OX40 signal can reduce IL-10 production, to prevent its inhibition of T-cell function, the study from another side to prove that multiple signal domains on the CAR T-cell function as promotion role. However, the third generation of CARs still lacks clinical support. Currently, the second generation of CARs are still the most widely used generation in clinical practice [56-58].

The factors which influence the effect of CAR T-cells in various clinical stages in vivo can be divided into the following three categories. Such as the combination strategies of infusion products (T-cells and their subtypes), the distribution of tumor as well as its microenvironment and the patient's physical situation. In second-generation of CAR-T-cell applications, the therapeutic effectiveness on hematologic tumors are much better than solid tumors. One of the reasons is that the solid tumor microenvironment is disadvantageous to CAR-T-cell proliferation and tumor targeting. In view of the above problems in practice, the fourth generation of CARs, called T-cells redirected for universal

cytokine killing (TRUCKs), came into being [59, 60].

TRUCKs

The basic design strategy of the fourth-generation of CAR-T-cell is combining with a NFAT (nuclear factor activated T-cell) to provide cytokine. As soon as scFv identified the targeting mark and activated CAR-CD3 ζ signal, the activated NFAT could release transgenes (such as IL-12). As a pro-inflammatory cytokine, IL-12 can recruit NK cells, macrophages, and other non-specific immune responses to kill CAR T-cell unrecognized tumor cells. Additionally, it can continue to stimulate CAR T-cell proliferation and activation, release of IL-2, act as a synergistic cytokine of IL-12 [61-63].

The value of IL-12 in TRUCKs is demonstrated in many pre-clinical model trials. The higher cellular activity, stronger recognition ability, and more sustained release of interferon- γ (IFN- γ) in targeting assays to melanoma models and IL-12 showed by NFAT-driven T-cells are continuously produced. A large number of experiments have shown that: maintaining a certain concentration of IL-12 in local tissues will be more conducive to anti-tumor immune response. Furthermore, IL-2 can be continuous release by T-cells does to maintain the thera-

peutic dose without persistent drug supporting [64-66].

The results of the previous clinical trial showed that IL-2 has some special advantages in the treatment of cancer due to its pleiotropic function. IL-12 is enriched in tumor tissue and thus it can act on tumor stroma and local immune cells particularly by inducing the Fas pathway to promote tumor matrix disintegration. In addition, IL-12 recruits and activates naïve immune cells (such as NK cells, NKT-cells and macrophages), regulates related immunosuppressive cells, induces tumor vessel injury, and necrosis during the target tissue immune response. At the same time, IL-12 is also capable of sustaining T-cell expansion in target tumor tissues and avoiding T-cell depletion by inhibiting BIM proapoptotic molecules which in combination with PD-1 block the enhancement of T-cell function Apoptosis [67-69].

It is worth mentioning that TRUCK releases of IL-12 can motivate and activate non-specific immune cells, such as NK cells, tumor macrophages, and to recruit a variety of other immune cells. They have the ability to recognize antigen-targeted negative tumor cells which common CAR T-cells cannot. As soon as tumor macrophages are activated, the tumor immune response is modulated by the release of TNF- α . Under such a mechanism, mixed-type cancer cell tumors can be effectively removed. In the absence of IL-12, CAR-T-cells without IL-12 structure were able to clear only antigen-targeted tumor cells, and antigen-targeted negative tumor cells would relapse after a reduction in initial T-cells [70-72].

Although TRUCK's therapeutics potential is large, it comes with potential risks. A large number of clinical reports have confirmed that the second generation of CAR-T-cells that have entered clinical practice have achieved gratifying results in the treatment of hematological malignancies. However, irreversible side effects still deserve attention. The same problem appeared in pre-TRUCK clinical trials as well. Among them, one of the most serious side effects is "off-target effects" which produce tissue damage and can lead to the rapid death of CAR T-cells after infusion therapy for severe patients. During targeted clearance of tumor tissue, CAR-T-cells target to recognize normal

tissue which has the same or similar epitope with the tumor tissue and cause cytotoxicity which results in normal tissue damage. On the other hand, normal tissues repeatedly provide CAR T-cell stimulation, thereby amplifying the CAR T-cell effect and prolonging its anti-tumor response, known as cytokine storm. Serum C-reactive protein can be used as a reliable indicator of the cytokine storm [73-75].

Bispecific antibody (BsAb)

The essence of tumor immunotherapy is following the immune response mechanism of the human immune system to treat cancer. Tumor targeting immunotherapy is a big part of tumor immunotherapy which depend on the activation between antibody and targeting bio-mark. Monoclonal antibodies (or other novel functionality peptide and micro-molecule) engage the innate immune system. In the past thirty years, antibody-dependent complement-dependent cytotoxicity and cell-mediated cytotoxicity have been the chief mechanisms of anti-tumor drugs. Researchers have tried to exploit the potential of the immune system. Bispecific antibody (BsAb) drugs have been treated as similar platforms through preclinical and clinical trials. Antibodies are extraordinary molecules has been used over millions of years of evolution. Each antibody molecule has the similar structure. They are two uniform antigen binding sites at the N-terminal variable region which are regulation for antigen specificity and the affinity of these maker molecules, and a steady fragment crystallizable (Fc) region at the C-terminus which triggers multiple effector mechanisms [76-78].

Based on the specific antigen/antibody combine, binding alone just physically block the antigen (targeting marker) or initiate/inhibit signaling via the antigen (targeting marker) result in apoptosis of target cells. As the majority of cancer therapeutic, like IgG antibodies, they work for their immune functions via recruiting natural killer cells and myeloid cells/macrophages by the Fc region. Moreover, as soon as the Fc region initiate the classical complement cascade, they can deposit membrane attack on the surface membrane of tumor cells. Researchers have studied and exploited these Fc dependent targeting lysis mechanisms in human medicine (**Figure 3**) [79-82].

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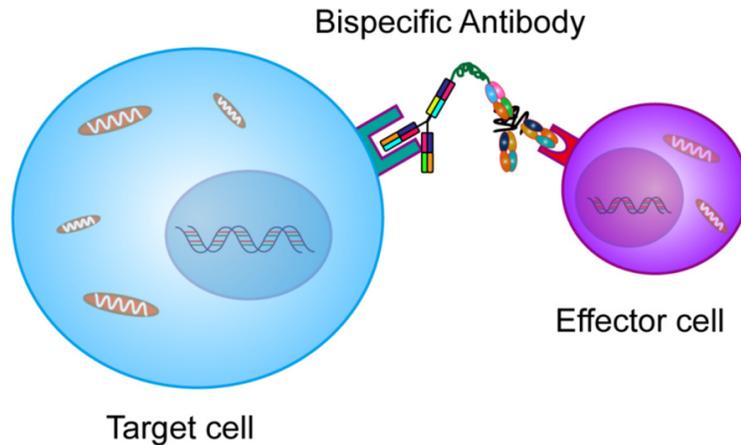


Figure 3. Bispecific antibodies recruit effector cells to the proximity target cells.

Since 1960s, when Nisonoff predicted the potential value of combining one of the two uniform antigen binding arms with a different antigen binding specificity, the concept of bispecific antibodies has captured attention. Researchers have developed this concept further in the 1980s for a second specificity against T-cell determinants. Over the past three decades, bispecific antibodies have been widely developed. The molecular details differ considerably, while they are all similar as the basic design of targeting tumor antigen binding specificity and effective cell binding specificity within one molecule. Currently, only two BsAbs, catumaxomab and blinatumomab, have been authorized for clinical use in humans. They are used as the cooperation to the other IgG based antibody drugs. The delay is great attributed to the difficulties in antibody engineering. Little is known about potential clinical toxicities with these new constructs. Furthermore, in the past 30 years, union molecular constructs have been invented. Some of them have entered clinical stages even in preclinical testing [83-85].

The classical anti-EpCAM-anti-CD3 Triomab named Catumaxomab has been the first Triomab drug in clinical. In the phase-I trial, the dose of catumaxomab single intravenous in patients who have non-small cell lung cancer (NSCLC) has established a maximum tolerated dose as a stand. Additionally, this trial has reported favorable survival in several patients who are in advanced-stage disease surviving past 26 months. More and more researchers

have evaluated lumbar injection administration of catumaxomab in patients with malignant ascites caused by various EpCAM+ tumors. In a clinical phase II/III study, researchers increased the dose of heavily pre-treated patients with symptomatic malignant ascites. Compared with both non-treated control patients and baseline levels, analyses of ascites indicated the reduced level of vascular endothelial growth factor (VEGF), and raising levels of activated CD8+ and CD4+ T-cells together with the distortion

level of CD133+/EpCAM+ cancer stem cells (CSC). Particularly, compared with control patients, patients treated by catumaxomab have had significantly prolonged survival. With data described above, European Medicine Agency (EMA) approved catumaxomab for patients with EpCAM-positive carcinomas who cannot be treated with standard therapy. Another classical anti-Her2-anti-CD3 Triomab named Ertumaxomab has been demonstrated to initiate effective immune cytotoxicity against tumor cell lines which expressed Her2 in vitro. Furthermore, it can efficiently lyse low Her2 expressing tumor cells which cannot be treated by trastuzumab (a mAb targeting Her2) [77, 79, 86-88].

Conclusion

Up to now, much hard work has been paid for the development of novel therapeutic approaches to treat HCC [89]. However, only a few products have been put into clinical trials much less the market. Nonetheless, the progress in the HCC targeting treatment research which supported by the advancements in the nanotechnology. Notably, the discovery of novel cell surface bio-marks may lead the way to the successful design of HCC targeting treatment. Difficulties in designing biomarkers and genetic targets treatment therapies result from insufficient understanding of the special biology characters of HCC. Successful treatment cases need strong biological targets and well design of the mode of action of targeting skills. Fur-

thermore, the field needs careful trial design and more robust [90, 91].

As the main therapeutic strategy for the HCC targeting treatment, CAR-T and bispecific antibody limited by the discovery of novel bio-mark. Effective therapeutic strategy will pay back to the research of novel biomarker. Furthermore, requirements of nanoparticle match with the development of bio-nanotechnology and deeper pathological understanding is required. The novel targeting of markers on HCC cells, as well as the correlation of advanced surface ligand chemistry and targeted treatment research will continue to enhance HCC treatment efficiency in the future.

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Disclosure of conflict of interest

None.

Address correspondence to: Yinghui Lin, Department of Clinical Laboratory of First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning 530023, Guangxi, People's Republic of China. E-mail: 313323960@qq.com

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