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Tumor immunotherapy by an engineering T cell receptor: a proposed hypothesis and study design

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Abstract

Harnessing immune system to recognize and eliminate tumor cells by engineering T cells has recently emerged as important approach for cancer treatment. Currently, chimeric antigen receptor (CAR) T cell designs targeting antigens expressed by hematological malignancies are becoming more available. However, the most commonly used targets are also shared by normal tissues, resulting in off-tumor toxicity. On the other hand, T cell receptor (TCR) engineered T cells, which recognize antigens presented by human leukocyte antigen (HLA), can target internally processed tumor specific peptides (neoantigens). Targeting such neoantigens can minimize the toxicity effects. Wide application of this approach is limited in solid tumors due to paucity of identified tumor specific neoantigens shared among different cancer types and/or patients. Hence, I hypothesize that identification and targeting of personalized immunogenic neoantigens by highly avid T cell receptor repertoires can result in a durable tumor regression with minimal off-tumor side-effects. To address this hypothesis, I propose two objectives: i) to identify tumor specific immunogenic neoantigen(s) and reactive TCR repertoire(s) in a given solid tumor; and ii) to engineer the neoantigen reactive TCR repertoires for affinity enhancement and validate their functionality. These aims will be elucidated by employing personalized immunogenomic techniques. In general, this project aims to provide innovative immune-based cancer therapy approach against solid tumors to improve survival rate of cancer patients.

Keywords: Immunotherapy; neoantigen; TCR engineered T cells; cancer

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1. Introduction

Cancer immunotherapy has lagged behind for decades1 due to the ability of cancer cells to invade anticancer immune response[2]. However, recent advances in monoclonal antibody (mAb) - and T cell

engineering-based therapies have revolutionized cancer immunotherapy. Despite the difference in mechanism of action of these approaches – mAb therapy unleashes immune checkpoint while genetically engineered T cells target specific antigens – both activate T cell response[3]. In the past few years, stunning results have been observed in clinical trials of refractory or relapsed hematological malignancies using CAR and TCR engineered T cells[4–8].

CAR T cell is combined from antibody's single chain variable fragment that recognizes specific antigen expressed on a cell surface independent of HLA and T cell activation endodomain motifs[2,3,9]. However, the most commonly used CAR targeted antigens are also expressed on the surface of normal cells at lower concentration, implicating potential toxicity to normal tissues. In addition, majority of the internally processed point mutation peptides responsible for T cell activation escape recognition by antibodies – and thus targeting by CAR T cells – due to less efficiency of antibodies to recognize point mutations[10].

On the other hand, engineered TCR T cell recognizes internally processed antigens presented in peptide-HLA complex[10]. Although this approach has shown great promise in hematological malignancies to target antigens like BOB111 and NY-ESO-17, these targets also are not tumor specific. Alternatively, genetic alteration occurring in protein coding region of a gene can generate tumor specific mutated peptides, called neoantigens[12], that can trigger T cell response. But the ability to trigger T cell response depends on concentration the neoantigens, and affinity and expression level of the TCR[10]. Thus, targeting such neoantigens by high avidity TCR engineered T cells can generate high specificity and low potential off-tumor side-effects, which has been a major safety issue[13,14]. Moving to solid tumors, application of the technique is hampered by paucity of identified immune reactive tumor specific neoantigens shared among different cancer types and/or patients[15]. Therefore, further works emphasizing on identification of personalized neoantigen and engineering TCR of T cells against specific immunogenic neoantigen are required to realize engineered T cell therapy in solid tumors.

2. Hypothesis and objectives

Neoantigens derived from mutated genes can provide opportunity to target tumor cells specifically and reduce off-tumor effects. I hypothesize that targeting personalized tumor specific immunogenic neoantigen(s) by affinity enhanced antigen reactive TCR repertoire(s) can result in a durable solid tumor regression with minimal off-tumor effects. To address the hypothesis, I propose the following objectives:

To identify tumor specific immunogenic neoantigen(s) and neoantigen-reactive TCR repertoire(s) in a given solid tumor; and

To engineer the neoantigen reactive TCR repertoires for affinity enhancement and validate their functionality.

3. Study design

3.1. Identification of immunogenic neoantigens and reactive TCR repertoires

Neoantigens produced by cancer cells are rarely shared among different cancer types and/ or patients[9,15]. Moreover, only small subset of the generated neonatigens are immune reactive to elicit clonal expansion of cytotoxic T cells[15,16]. Identifying these immune reactive neoantigens and responding TCR repertoires can serve as a milestone to design potent engineered T cell based therapy. This, in turn, requires knowledge of mutational status of genes (Fig. 1). To identify neoantigen generating mutated gene, all protein encoding regions of genes will be sequenced using whole exome sequencing technique. In brief, genomic DNA will be purified from tissue sample and fragmented. Exons will be captured for library construction and sequenced using NextSeq 500/550 (Illumina platform). The generated data will be mapped to reference human genome and analyzed to identify mutation using bioinformatics.

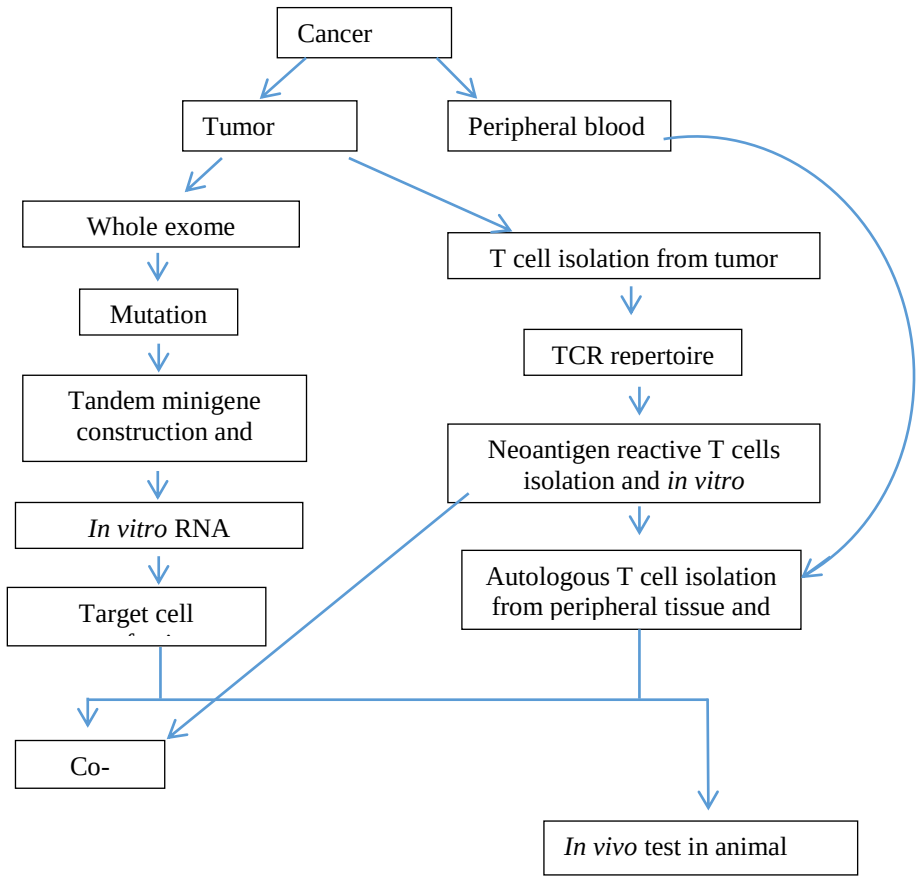

Fig. 1. Work flow of TCR engineering to develop neoantigen based immunotherapy for treatment solid malignancies.

To assess immunogenicity of each of the identified mutations, a tandem minigene (TMG) – which encodes mutated gene product – will be constructed as described previously[17]. Briefly, each minigene will be designed to contain the mutated amino acid flanked by 12 wild-type amino acids sequence on both N- and C- termini of the mutation. Multiple minigenes (up to 12) will be fused together as a DNA string to form TMG. In addition, GFP expressing TMG will also be constructed to verify transfection efficiency. The TMGs will be cloned into plasmid vector. The TMG encoding plasmids will be linearized by restriction enzymes. Agarose gel electrophoresis will be used to ensure complete digestion of the plasmids. Then the linearized plasmid will be used to generate in vitro transcribed (IVT) RNA. In vitro expanded autologous antigen presenting cells (APCs) like dendritic cells will be transfected with the IVT RNA using electroporation.

In parallel, autologous tumor infiltrating T lymphocytes (TIL) will be generated from tumor sections. After in vitro expansion, the TILs or tumor tissue will be sequenced using Anchored Multiplex PCR-based targeted RNA sequencing method[18] to profile TCR repertoires and/or single cell sequencing to identify productive TCRα and TCRβ pairs. Clonally expanded TCR repertoires will be sorted by flow cytometry and expanded in vitro. Then these TILs will be co-cultured with IVT RNA transfected APCs. Immunogenic TMG will be identified by assessing cytokine production level (like IFNγ and GM-CSF) and expression of T cell activation markers like OX40, 4-1BB and CD137 at 18-24 hrs by enzyme-linked immunosorbent spot (ELISPOT) assay

and flow cytometry, respectively. Mock and anti-CD3 (OKT3) will be used as negative and positive controls, respectively.

To identify the immunogenic mini-gene(s), APCs will be pulsed with each minigene of the immunogenic TMG and its wild type peptides and co-cultured with reactive TILs. At 18-24 hrs IFNγ and expression of T cell activation level will be measured. Then HLA binding peptide sequence will be computationally predicted. Top hits will be functionally validated by constructing truncated minigenes of different sizes (7-25-mer amino acids) and their wild-type reversion. The reactivity of identified T cells will be further tested against various stimulator cell lines pulsed with the identified peptide or cell lines transduced with IVT RNA to endogenously express the peptide and tumor cell lines those endogenously process and present the peptide.

3.2. Engineering TCR and functional validation

Tumor specific immune reactive neoantigens elicit clonal expansion of T cells. But in vitro expansion of small number of TILs isolated from tumor section can cause loss of a replicative ability and state of terminal differentiation[19]. The affinity of TCR also determines the extent of T cell response. Thus, engineering TCR of T cells from peripheral blood on the bases personalized neoantigens to enhance their affinity would help to realize the personalized T cells based therapy in solid tumors (Fig. 1).

To modify the neoantigen reactive TCR, autologous T cells will be isolated from the patient peripheral blood. Then, isolated T cells will be engineered to express the clonally expanded peptide reactive TCR repertoire(s) as described previously[16]. Briefly, mutant reactive TCR will be constructed by fusing productive TCRα and TCRβ to mouse TCRα and TCRβ constant chain, respectively and separated by a linker. The construct will be cloned into a plasmid vector and retrovirally transduced into the isolated T cells. To enhance TCR affinity, amino acid substitution will be made in binding sites of the TCR and tested as described previously[20].

The engineered T cells will be co-cultured with APCs and target cell lines pulsed with different concentrations of the mutant peptide or transfected with IVT RNA, mock (negative control), or OKT3 (positive control). At the end of the incubation, cytokines production level and lytic capability of the engineered T cells will be determined. In addition, MCH class restriction will also be identified by co-culturing the engineered T cells with target cell lines expressing different HLA alleles. Off-tumor effects will be assessed by co-incubating the transduced TCR cells with different normal and tumor cell lines followed by cytokine production measurement and cytotoxicity assay.

In vivo study: Appropriate humanized mice model will be intravenously inoculated with required HLA restricted tumor cell line expressing luciferase. Then, the mice will be inoculated with TCR- or mock-transduced (control) T cells and monitored for tumor growth by bioluminescence for a fixed period of time. Finally, the mice will be sacrificed and analyzed as needed.

4. Expected result and limitation:

At the end of this study, immunogenic neoantigen and reacting avid TCR engineered T cell will be expected to be elucidated. Successful tumor control in in vitro and in vivo models will be used as a baseline to plan for a clinical trial. On the other hand, the proposed approach is dependent on presentation of endogenously processed antigens by HLA molecules to the engineered TCR. Hence, fraction of cancer patients with down-regulated antigen processing and presenting genes may not benefit.

Conflicts of interest

The authors declare no conflicts of interest.

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