Generation of CTLs from iPSCs transduced with TCR genes: development of “TCR cassette” method

Seiji Nagano[1], Koji Terada[2], Yasutoshi Agata[2], Hiroshi Kawamoto[1]

1. *Laboratory of Immunology, Institute for Life and Medical Sciences, Kyoto University*
2. *Department of Biochemistry and Molecular Biology, Shiga University of Medical Science*

Abstract

We have proposed a strategy to use the iPSC technology for expansion of tumor antigen specific T cells; iPSCs produced from T cells (T-iPSCs) inherit rearranged TCR genes, and thus all regenerated T cells from T-iPSCs express the same TCR. To apply this approach in allogeneic setting, we thought of a method in which non-T cell-derived iPSCs are lentivirally transduced with exogenous TCR genes (TCR-iPSCs). Furthermore, we decided to improve this TCR-iPS method, since this method still has some concerns: i) risk of damaging genome by lentiviral transduction and ii) some difficulty in controlling expression level of TCR.

It is therefore preferable that an exogenous TCR gene is integrated into TCR gene locus so that it is expressed under the control of endogenous promoter/enhancer. Concurrently, we adopted RMCE (recombinase-mediated cassette exchange) in our “TCR cassette method”, in which we can insert a TCR gene just like “a cassette tape”. First, we largely deleted the region from Vβ20-1 to Cβ2 of TCRβ gene of non-T cell-derived iPSCs and inserted a "cassette deck" structure in this region so that the promoter located upstream and the enhancer located downstream of the cassette deck to which a desired TCR gene can be subsequently inserted.

Based on such ideas, we have succeeded in producing antigen specific CTLs expressing the exogenous TCR, which exerted antigen specific cytotoxic activity, confirming that this new method is applicable in producing T cells for cell therapy against cancer and viral infection.