Generation of induced pluripotent stem cells (iPSCs) from NY-ESO-I-specific CD8+ T cell isolated from the patient with melanoma

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INTRODUCTION & HYPOTHESIS

Melanoma is well known to be one of the most immunogenic tumor, which may be spontaneously regressed, occasionally. Therefore, immunotherapy for melanoma has been tried to develop and markedly advanced in recent years. Unresectable and distant metastatic melanomas can be treated using immune checkpoint modulators, such as anti-

PD-1 and anti-CTLA-4 antibodies. In addition, adoptive immunotherapy using tumor antigen-specific cytotoxic T cells is also developing. However, treatment efficacy of anti-cancer agents and adoptive immunotherapies are still limited. Moreover, there are several issues for commercial viability of adoptive immunotherapy, especially in the case using T cell receptor (TCR) gene-modified T cells. Recently, it has been reported that induced pluripotent stem cells (iPSCs) can be generated from established CD8+ T cell lines recognizing MART-1, one of cancer-testis antigens expressed in melanoma, and re-differentiated into rejuvenated T cells to provide enough number of functional T cells for adoptive immunotherapy.

HYPOTHESIS

The sequence of rearranged TCR gene in T cells should be maintained even after reprogramming into iPSCs and re-differentiating into T cells again.

However, it is unclear whether tumor antigen-specific T cells can be enough in number and freshly isolated from MHC-unidentified patients with melanoma, and reprogrammed into iPSCs.

METHODS & RESULTS

Isolation of NY-ESO-I-specific CD8+ T cells

We first found a patient with NY-ESO-I-positive melanoma and confirmed the patient had the population of T cell which reacts NY-ESO-I peptide by lymphocyte transformation test. Next, we isolated NY-ESO-I-specific CD8+ T cells from patient's PBMC by the method mentioned below.

MHC-dependent tetramer technology was not used for antigen-specific T cell isolation, since this patient has minor HLA types.

Characteristics of isolated T cells

Rank	TRAV	TRAJ	CDR3	Frame	Reads	%Reads
1	TRAV8-3	TRAJ54	CAVGAVIQGAQKLVF	in-frame	60856	60.62
2	TRAV25	TRAJ4	CAGPFGGYNKLI	in-frame	20869	20.79
3	TRAV13-1	TRAJ49	CAAKRIHYHQVTSS*F	out-of-frame	2568	2.56
4	TRAV21	TRAJ32	CAVPYGGATNKLI	in-frame	581	0.58
5	TRAV25	TRAJ4	CAGPFGGYNKLVF	in-frame	519	0.52

Rank	TRBV	TRBJ	CDR3	Frame	Reads	%Reads
1	TRBV4-3	TRBJ2-1	CASSQEWHSYNEQFF	in-frame	208330	74.21
2	TRBV5-6	TRBJ2-7	CASSQLGMNYYEQYF	in-frame	17631	6.28
3	TRBV4-1	TRBJ2-7	CASSQLAGAATSS*F	out-of-frame	6531	2.33
4	TRBV4-3	TRBJ2-1	CASSQAWHSYNEQFF	in-frame	1423	0.51
5	TRBV4-3	TRBJ2-1	CASSQDWHHSYNEQFF	in-frame	987	0.35

TCR repertoire analysis showed that the group of isolated T cells were almost monoclonal, indicating that we succeeded in isolating NY-ESO-I-specific T cells without tetramer technology.

Establishment of T cell-derived iPSCs

We next generated transgene-free iPSCs from isolated NY-ESO-I-specific T cells using Sendai virus vectors.

The authenticity of generated iPSCs was confirmed by checking the expression of stem cell markers by immunostaining and RT-PCR, and the differentiation capability by teratoma formation (partly data not shown).

RAG2 gene knockout in T cell-derived iPSCs

We knocked out RAG2 gene, a critical gene for TCR gene rearrangement, using hCAS9/CRISPR system to prevent TCR re-rearrangement during re-differentiation process. We provided CAS9 protein/synthesized gRNA in T cell-derived iPSCs by lipofection. We finally obtained iPSC line knocked out RAG2 gene in both two alleles.

Re-differentiation of iPSCs into CD8+ T cells

iPSC-derived T cell TCRα

Rank	TRAV	TRAJ	CDR3	Reads	%Reads
1	TRAV8-3	TRAJ54	CAVGAVIQGAQKLVF	39,535	42.93
2	TRAV12-1	TRAJ53	CVVNSGGSNYKLT	2,967	3.22
3	TRAV8-2	TRAJ53	CVVSDSGGSNYKLT	1,361	1.48
4	TRAV12-1	TRAJ52	CVVNAGGTSYGLTF	1,156	1.26
5	TRAV8-2	TRAJ48	CVVSDNFGNEKLT	1,143	1.24

RAG2-KO iPSC-derived T cell TCRα

Rank	TRAV	TRAJ	CDR3	Reads	%Reads
1	TRAV8-3	TRAJ54	CAVGAVIQGAQKLVF	10,781	92.64
2	TRAV9-2	TRAJ37	CALSDPPNGTGLIF	39	0.34
3	TRAV30	TRAJ40	CGTTTTSGTYKYIF	29	0.25
4	TRAV19	TRAJ42	CALSEAGNYGSGQNLIF	28	0.24
5	TRAV17	TRAJ23	CATHLGNQGGKLI	26	0.22

We are now trying to re-differentiate iPSCs into CD8+ cytotoxic T cells on OP9 co-culture system (Maeda T et al. Cancer Res 2016; 76: 6839-50). Not enough in number, but CD8+ T cells could be re-differentiated from T cell-derived iPSCs. iPSC-derived T cells had oligoclonal TCR by TCR re-rearrangement during differentiation process, however, diversity of TCR in RAG2-KO iPSC-derived iPSCs is limited compared with that in iPSC-derived T cells.

CONCLUSIONS

We succeeded in isolating NY-ESO-I-specific CD8+ T cells from a melanoma patient, and generating transgene-free iPSCs from these T cells. We are now in the process for re-differentiating T cell-derived iPSCs into functional cytotoxic T cells using the OP9 co-culture system. Our study is capable of being a "touchstone" to develop iPSC-based adoptive immunotherapy for the treatment of melanoma for the future clinical use.