Derivation and characterization of induced pluripotent stem cells lines with inactivation of the beta-2-microblogulin gene by CRISPR/Cas9 genome editing

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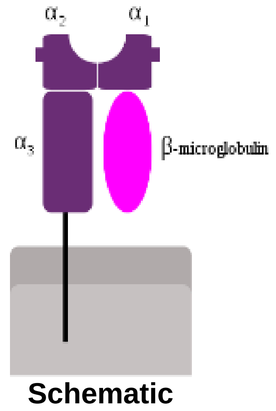
Introduction

The main cause of tissue rejection during transplantation is the mismatch of HLA haplotypes between donor and recipient. The discovery of induced pluripotent stem cells (iPSC) likewise the development of targeted differentiation protocols opens up broad prospects for the progress in regenerative medicine. Reprogramming technology allows establishing autologous iPSC that resolve the issue of immune rejection. However, obtaining patient-specific iPSC is very expensive, and requires the characterization and the quality control of each reprogrammed cell line. One possible solution is a creation of universally compatible characterized iPS cell lines that will be suitable for transplantation to any patient.

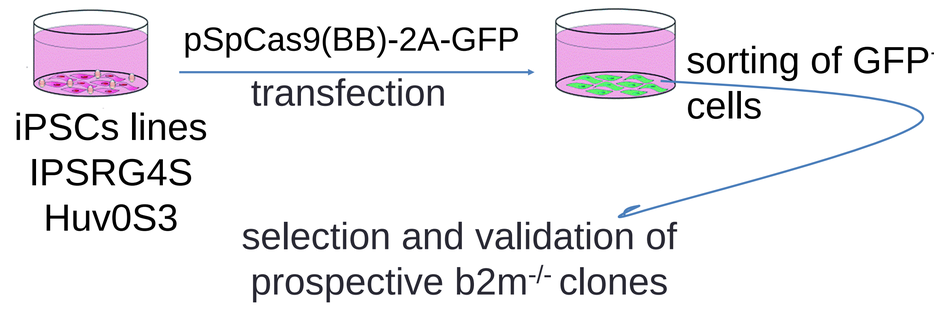


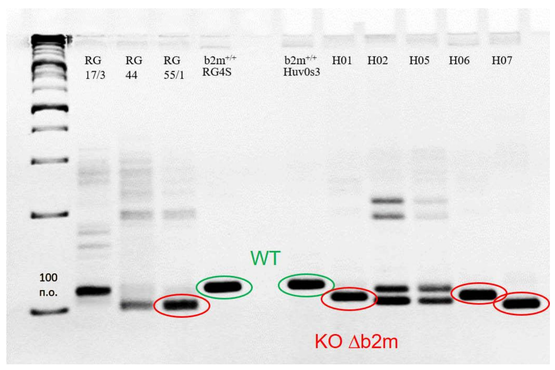
Schematic representation of HLA-I molecule

HLA I proteins form heterodimers that consist of a polymorphic heavy α chain and a light β-2-microglobulin (*b2m*) chain. The inactivation of *b2m* in iPSC leads to shortage of HLA I expression on cell surface, thus, these cells should have reduced immunogenicity to allogeneic CD8⁺ T cells. It should be noted that cells that do not carry the HLA class I molecules on their surface may become targets for NK cells.

Generation of the b2m knockout iPS cell lines

b2m knockout iPS cell lines were established by CRISP/Cas9-mediated genome editing using transfection of pSpCas9(BB)-2A-GFP plasmid containing Cas9 and guide RNA followed by GFP-based cell sorting. Selected clones were analysed by PCR analysis and sequencing.





b2m locus – 17 bp deletion

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H07 GAGATGTCTCGCTCCGTGGC----------CTCTCTCTTTCTGGCCTGGAG
    ||||||||||||||||||||          ||||||||||||||||||||
WT  GAGATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCTGGAG
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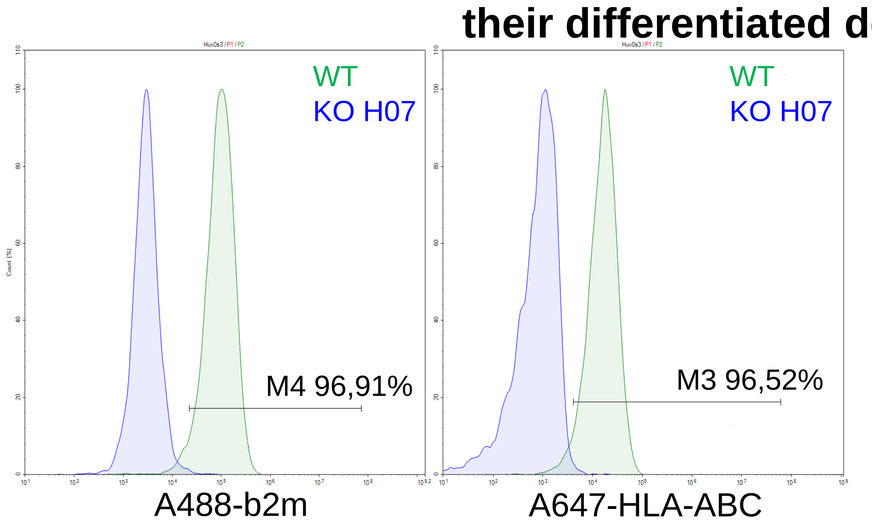
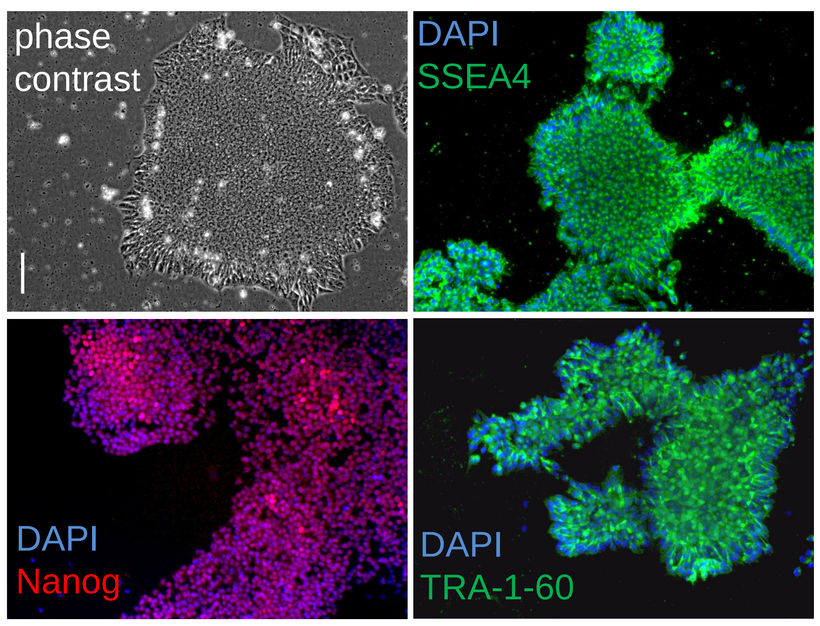
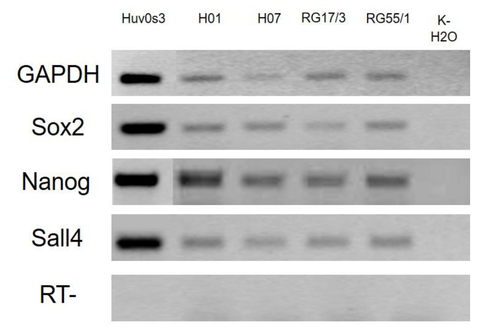
HLA-ABC and b2m are absent on the cell surface of the b2m$^{-/-}$ iPSCs and their differentiated derivatives



Flow cytometry analyses revealed that surface b2m and HLA I were not expressed on KO iPSC and their derivatives.
We analyzed five iPS clones (the figure shows data for the line Huv0SΔb2m cl7).

The b2m$^{-/-}$ iPS cell lines express pluripotency markers



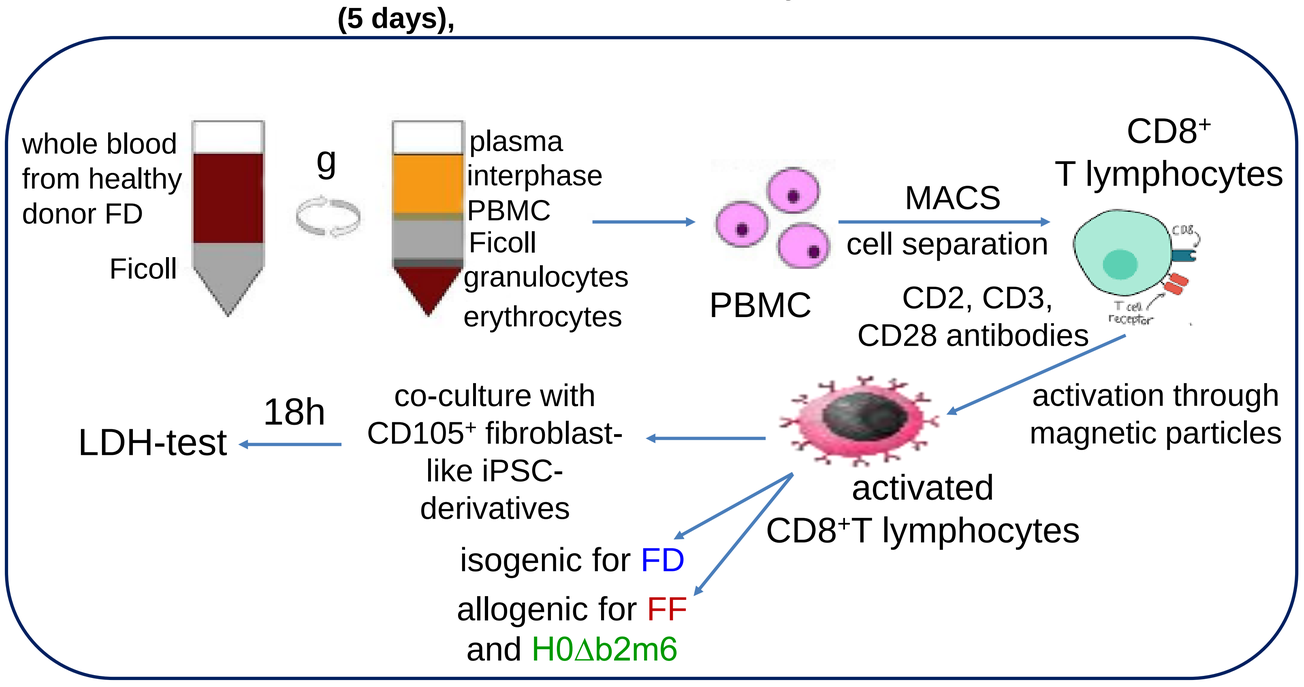


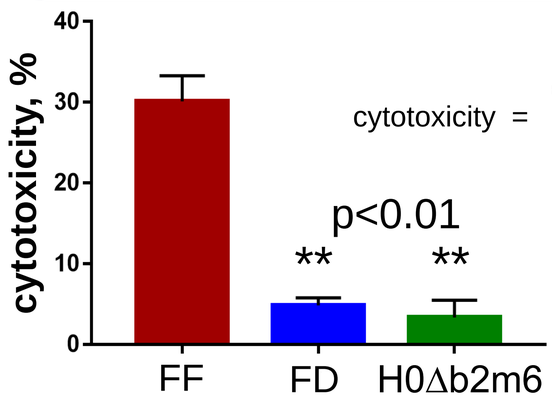
The genetic manipulation and the disruption of HLA I expression did not affect pluripotency characteristics.
All clones exhibit a normal caryotype, and retains their self-renewal capacity, genomic stability and pluripotency.

Resistance of the the b2m$^{-/-}$ iPSC-derivatives to alloreactive CD8⁺ T cell-mediated killing *in vitro*

Immunogenicity of KO cell lines was tested according to standard immunological protocols. Differentiated iPSC derivatives were resistant to allogenic CD8⁺ T cell-mediated killing *in vitro* in comparison with allogeneic fibroblasts of a healthy donor. Similar cytotoxic tests will be conducted in co-cultivation with NK cells.

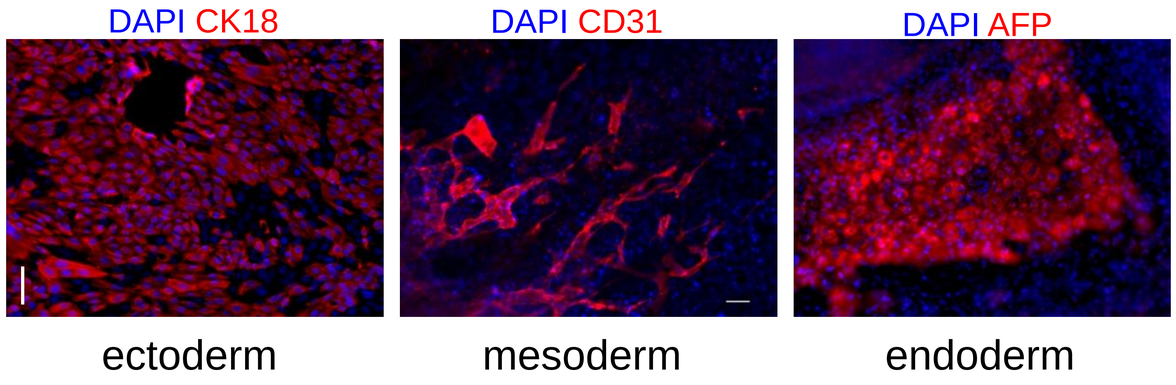
iPSCs —AggreWell™→ **EB** —**Activin A (2 days) / BMP4 (5 days),**→ **Adherent culture** —**bFGF 14 days**→ **CD105⁺ fibroblast-like cells**





$$\text{cytotoxicity} = \frac{\text{OD cell mix} - \text{OD T cells} - \text{OD low control}}{(\text{OD high control} - \text{OD low control})} * 100\%$$

b2m KO iPS cells are able to form embryoid bodies and to differentiate into all three germ layers derivatives



ectoderm | mesoderm | endoderm

Conclusions

Novel integration-free iPSCs lines with biallelic knockout of beta-2-microglobulin gene were generated by CRISPR/Cas9 genome editing technology.
These lines and their differentiated fibroblast-like derivatives do not express cell surface b2m and HLA-I molecules.
The genetic manipulations and the disruption of HLA-I expression did not affect the main pluripotency characteristics of obtained cell lines.
CD105⁺ fibroblast-like derivatives of the iPSCs with b2m gene knockout demonstrated increased resistance to allogeneic CD8⁺ T lymphocytes *in vitro*.
Further genetic modifications are necessary to avoid NK cell recognition and lysis. Such modified iPS cell lines can serve as prototypes of “universal” cell line with reduced immunogenicity.

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