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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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 time-consuming, 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. 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. In the present study, we derived *b2m* knockout iPS cell lines 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. Flow cytometry analyses revealed that both surface b2m and HLA I were not expressed on KO iPSC and their derivatives. The genetic manipulation and the disruption of HLA I expression did not affect pluripotency characteristics. 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. Similar cytotoxic tests will be conducted in co-cultivation with NK cells. This work was supported by the Russian Science Foundation grant #17-75-10206.