［課程－２］

審査の結果の要旨

氏名ベッカー　ハンス　次郎

This thesis examines the potential of *ex vivo* expansion of hematopoietic stem cells (HSCs) in conjunction with CRISPR/Cas9 gene editing to generate large quantities of *bona fide*, genetically modified HSCs for stem cell transplantation. In murine transplantation models, it is shown that:

1. Using CRISPR/Cas9 gene editing, the *Prkdc*^{SCID} mutation in the CB17/SCID murine immunodeficiency model can be corrected in a relevant fraction of HSCs. The edited bulk population can be expanded *ex vivo* using a serum-free, synthetic polymer-based medium, producing a large population of HSCs harboring the corrected *Prkdc* gene. Upon transplantation, edited HSCs give rise to B and T lymphocytes which demonstrate a functional restoration of immunity *in vivo*.

2. The pan-leukocyte antigen CD45.2, expressed on HSCs from C57BL/6 mice, can efficiently be converted to the CD45.1 allele with CRISPR/Cas9. Using a novel synthetic polymer, Soluplus, single-cell cloned CD45.1$^+$ HSCs can be expanded while preserving the expression of phenotypic stemness markers. After transplantation, clonally expanded, edited HSCs display self-renewal and differentiation characteristics. Peripheral blood chimerism correlated with the fraction of CD201(EPCR)$^+$ and CD150$^+$ double positive cells in the expanded population.

3. Following these results, the single cell expansion approach was applied to the SCID model. Bulk edited SCID HSCs were cloned and colonies were screened for high expression of CD201 and CD150 as well as for the presence of a corrected *Prkdc* allele by means of direct sequencing. Selected clones were pooled and transplanted into CB17/SCID recipients, which subsequently showed reconstitution of B and T cell compartments.

This work demonstrates that gene editing of HSCs can restore immunity in SCID mice on a molecular and functional level. Furthermore, a polymer-based culture system permits single cell expansion of HSCs and direct sequencing, enabling marker-free selection of clones with the desired modifications. Engraftment of these clones can be estimated by quantifying the expression of HSC stemness markers. These results significantly contribute to the field of genome editing and has major potential for adoption in hematopoietic gene therapy.

よって本論文は博士（ 医学 ）の学位請求論文として合格と認められる。