審査の結果の要旨

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The thesis paper consists of 10 chapters: abstract, introduction, purpose/hypothesis, material&methods, results, discussion, conclusion, references, annex and acknowledgement. The abstract states that epidermal growth factor-like domain 7 (Egfl7) context dependent on the cellular expression of beta 3 integrin (Itgb3) altered Notch signaling. Egfl7 blocked Notch signaling when hematopoietic stem cells (HSCs) express Itgb3 leading to progenitor proliferation and myeloid differentiation. In contrast, Egfl7 activated Notch signaling when Itgb3 was either genetically ablated or when a Itgb3 inhibitor was used leading to HSC without cell differentiation. In the introduction, the Egfl7 gene-knockout mouse phenotype is discussed, and knockout studies using miR126 are introduced. miR126 is located in intron 7 of Egfl7. In the second part, the role of cytokines like Kit ligand (KitL) or FMS–like tyrosine kinase 4 (Flt3) and Notch signaling for HSC expansion, and differentiation are introduced. The third part describes the role of Flt3 and Notch signaling for thymic T cell development. The forth part of the introduction gives a background on integrins. The hypothesis of the study is presented, namely that Egfl7 dictates HSC and early progenitor fate i.e. dependent on the cellular Itgb3 expression level. The method part covers: a description of human and mouse samples, and details on adenoviral administration of Egfl7 in vivo. Details on the generation of adenovirus for the overexpression of Egfl7 are presented, and how inhibitors against Itgb3 or Flt3 were administered. Competitive transplantation experiments are described. Lentivirus generation/production for the knockdown (kd) of Egfl7, and cell analysis or isolation strategies are described (FACS, MACS). In vitro assays (ETP-, KSL-, and CD34 delta expansion and colony assay) and culture conditions are mentioned. In the result part data are presented describing Egfl7 expression mainly on the earliest progenitors and HSCs, and its upregulation after myelosuppression in hematopietic cells. This is followed by data demonstrating that overexpression of Egfl7 *in vivo* and *in vitro* expanded murine and human HSCs. Then experiments using shRNA lentivirus strategies Egfl7kd in HSC are presented, demonstrating its importance for cell survival in vitro. Egfl7kd KSL cell transplantation experiments revealed that once Egfl7kd KSL cells were transplanted, the Egfl7kd similar to Egfl7wt cells showed identical three-lineage differentiation potential and competitive repopulation capacity, suggesting exogenous Eglf7 can rescuethe Egfl7kd phenotype in KSL cells. Data are presented showing that Egfl7 upregulated its own receptor Itgb3 on HEL cells. Then both in vitro and in vivo data are given showing that Egfl7 expanded HSC expansion in the absence of Itgb3. This was further confirmed by showing that an Egfl7 mutant, where the RGD domain that conveys Itgb3 binding, showed highest KSL expansion potential. Next, the mechanism how Egfl7 drives HSC differentiation and proliferation was elucidated. HEL cells were used to

show that Egfl7 in a Itgb3 dependent fashion regulated its own expression, the hematopoietic transcription factor GATA-2, c-Kit and Flt3 receptors that lead to phosphorylation of FAK, AKT, ERK1/2 and STAT-3. Furthermore, Egfl7 driven c-Kit upregulation on HSC was shown to be responsible for enhanced HSC survival and differentiation in vivo and in vitro. Impaired survival of Egfl7kd in KSL cells could e.g. be rescued using c-Kitmut(Kit-KitW-v) cells. Egfl7 in the absence of Itgb3 was shown to activates Notch and Flt3 signaling in HSCs, and that Flt3 signaling was critical for HSC expansion as shown using a Flt3 inhibitor. Another set of experiments demonstrate that Egfl7 is upregulated after irradiation in the thymus, where its overexpression led to the expansion of early thymic progenitors through a mechanism involving Flt3 receptor upregulation on ETP and endothelial cells. The conclusion part summarizes the results and discusses the importance of Itgb3 to regulate Notch signaling and thereby regulating stem cell self-renewal fate versus HSC differentiation. The data on upregulation of the stem cell active cytokine receptors c-Kit and Flt3 after Egfl7 are discussed with literature showing the critical importance of both the c-Ki/Kit ligand and and Flt3/Flt3 ligand pathway for the expansion and maintenance of HSCs. The data on HSC expansion after Egfl7 overexpression are compared to studies on the role of miR126. Finally, it is discussed that Egfl7 might be important for stem cell aging, but it is acknowledged that further studies here are required. RT-PCRhuman and murine primer set details are given in the annex.

Yousef is the *main contributor* of this study. Under my supervision he designed, and performed the experiments. He analyzed the data, followed up on the literature and showed good team spirit. He established skills in doing *in vivo* and in vitro experiments, and showed great skills in techniques of molecular biology, including viral vector design. He showed a good working ethics, problem-solving skills, and great enthusiasm for the scientific projects he performed. I hereby guarantee that her work makes her eligible to receive a PhD (Medical Science) from the graduate school of Frontier Sciences (CBMS), Tokyo, Japan. 以上 799 字