［課程―2］

審査の結果の要旨

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Epidermal growth factor‐like domain 7 (Egfl7) is a factor expressed in the endothelium and during embryogenesis. Egfl7 is a secreted protein that has been implicated in cell migration and blood vessel formation. Herein, Ismael Gritli provided evidences for a novel role of Egfl7 in hematopoiesis. During his defense, he pinpointed the following results:

1. In wild-type adult mice, *Egfl7* was expressed in hematopoietic cells within the bone marrow (BM), where it was mainly expressed in the most immature (c-Kit^+/$Sca\text{-}1^+$/Lin^-/$CD34^+$) KSL^-/$CD34^+$ and KSL^-/$CD34^-$ fractions after flow cytometric sorting.

2. Using adenovectors, forced expression of Egfl7 (AdEgfl7) in mice resulted in elevated leukocyte levels, corresponding with an increase in granulocytes. Using flow cytometric analysis, Gritli confirmed a rapid increase in the frequencies of neutrophils but monocytes in both peripheral blood and BM after Egfl7 treatment.

3. AdEgfl7 also resulted in thrombocytosis. Gritli investigated the effect of Egfl7 on megakaryocytic (MK) development. He showed an increase in the number of MKs in BM sections of AdEgfl7-treated mice. AdEgfl7 resulted in an increase in the number of platelets in blood circulation. Additionally, Egfl7 increased number of immature CFU-MK colonies.

4. To examine whether Egfl7 acts merely on committed granulocyte progenitors or acts on earlier multipotent levels, Gritli investigated the frequencies HSC-enriched KSL^-/$CD34^-$ cells and found that Egfl7 resulted in an increased frequency of these cells in BM. Associated with this increase, there was an increase in the frequency of GMP (Granulocyte and Monocyte Progenitors) and a decrease in the frequency of CMP (Common-Myeloid Progenitors) within BMs.

5. Gritli has functionally tested the multipotency of Egfl7 stimulated cells by performing colony assays. BM-derived colony forming unit (CFU) cells increased in AdEgfl7-treated mice. Most notably, predominantly immature CFU-granulocyte, erythroid, macrophage and megakaryocyte (CFU-GEMM) increased.

6. AdEgfl7 induced the mobilization of hematopoietic progenitor cells into circulation most likely through the elevation of the chemokine SDF-1α. Plasma SDF-1α levels rose rapidly in AdEgfl7-treated animals, which is known to cause hematopoietic stem and progenitor cells (HSPCs) mobilization.

7. On the protein level, there was an increase of total MMP-9 and KitL in BM culture supernatants and plasma as well as TPO and VEGF-A.

8. Finally, Gritli showed that AdEgfl7 accelerated hematopoietic cell recovery, especially of myeloid cells and platelets after Fluorouracil (5-FU) treatment. 5-FU-treated mice coinjected with AdEgfl7 showed an enhanced recovery of white

blood cells, platelets, and circulating neutrophils, but not monocytes. The percentage of polyploid megakaryocytes increased in AdEgfl7-treated mice as well as platelet counts compared to controls indicating stimulation of the megakaryocytic recovery.

Dissertation result:

Ismael Gritli’s work included promising results that raise the possibility of clinical use of Egfl7 to improve recovery after BM injuries. Gritli’s scientific approach to answer his hypothesis is carefully structured. Experimentally, he used both *in vitro* and *in vivo* models in order to investigate the molecular mechanisms, while at the same time understand the bigger picture. Combined with his excellent presentation performance and being fully prepared to answer the questions asked by the committee, Ismael Gritli is well suited to receive a doctoral degree. Therefore, committee has decided to accept that Ismael Gritli will pass his doctoral dissertation.