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POSTER ABSTRACTS

501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Roles for Heme Synthesis in the Maintenance of Hematopoietic Stem and Progenitor Cells

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Heme is required not only in red blood cells for oxygen transport but also in non-erythroid cells for the proper function of hemoproteins. Cytochrome c is one of the hemoproteins and inevitable for mitochondrial oxidative phosphorylation, suggesting that heme possesses important roles for ATP synthesis and mitochondria maintenance. Heme also can skew the differentiation of hematopoietic stem and progenitor cells (HSPCs) by altering the functions of transcription factors. Recent CRISPR Cas9 dropout screens repeatedly showed that some of the heme synthases, such as CPOX, might be inevitable for the survival and/or proliferation of HSPCs. However, still little is known about the roles for heme synthesis in the maintenance (survival, proliferation and differentiation) of HSPCs.

To reveal the roles for heme synthesis in HSPCs *in vivo*, we developed polyI:C inducible hematopoietic cell specific Cpox knock-out mice (*Cpox* $^{f/f}$; Mx1-Cre). PolyI:C treated *Cpox* $^{f/f}$; Mx1-Cre mice (KO mice) showed significant increase in the numbers of hematopoietic stem cells (HSCs) and multipotent stem and progenitor cells (MPP2s and MPP3s) but decrease in the numbers of common myeloid progenitors (CMPs) and some of their progenies (i.e., Gr1 positive cells and platelets). In contrast, its effects on erythroid and lymphoid lineages were limited. This result suggests that heme synthesis inhibition in HSPCs (HSCs, MPP2 and MPP3s) can cause their differentiation arrest. On the other hand, competitive transplantation analysis of LSKs (lineage $^{-}$sca1 $^{+}$c-kit $^{+}$) or HSCs revealed that myelopoiesis were markedly decreased compared to control, suggesting that heme synthesis in HSPCs is important for the proper reconstitution of hematopoiesis. These results indicate that cell intrinsic heme synthesis is inevitable for the maintenance of HSPCs and their lineage specific output *in vivo*. In depth, we revealed that mitochondrial respiratory activity of Cpox KO HSCs were increased compared to control HSCs, suggesting that alterations of mitochondrial status and/or functions by the inhibition of heme synthesis might contribute to the aberrance in Cpox KO HSPCs. Comprehensive gene expression analysis is now ongoing to support this notion.

To further reveal the roles for heme synthesis in human HSPCs, we treated human leukemia cell lines (MV4-11, KG1a and U937) with heme synthesis inhibitors (SA or NMPP). Concentration-dependent cell growth inhibitory effect and cell death were observed with increased apoptosis factors (cleaved caspase 3 and cleaved PARP), suggesting that heme synthesis inhibition triggered apoptosis. We also revealed that CPOX inhibition under doxycycline inducible CRISPR Cas9 system caused cell growth inhibitory effect and cell death in these cells. Second mitochondria-derived activator of caspases (Smac) and cytochrome c were released to cytoplasm, and the inhibitor of apoptosis protein (XIAP) was decreased by heme synthesis inhibitions. Therefore, caspase-3 activation by accumulation of cytoplasmic cytochrome c and Smac might be the inducer of this apoptosis pathway. Comprehensive transcriptomic analysis (RNA-seq) revealed that heme synthesis inhibition induced the release of cytochrome c and Smac from mitochondria into cytoplasm by the transcriptomic upregulation of Oxidative Stress Induced Growth Inhibitor 1 (Osgin1) that is the known target of NRF2.

Collectively, this study demonstrated the significance of heme synthesis in the maintenance of HSPCs especially *in vivo* (mouse) and *in vitro* (human). Dysfunctions of heme synthesis in HSPCs can cause their aberrance by the alterations in their mitochondrial status which can cause apoptosis and/or differentiation arrest. Considering the metabolic differences (i.e., demands for mitochondrial oxidative phosphorylation and subsequent ATP synthesis) between normal HSPCs and leukemia or unperturbed hematopoiesis and perturbed hematopoiesis (i.e., infections and hematopoietic stem cell transplantations), further analysis to reveal the roles for heme synthesis in the maintenance of HSPCs will pave the way to establish optical approaches in these clinical settings.

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