P1405 ARGinine-Dependent Regulation of the Eukaryotic Translation Initiation Factor (eIF5A) Controls Human Hematopoietic Progenitor Fate to the Erythroid Lineage

Topic: 23. Hematopoiesis, stem cells and microenvironment

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Background:

The production of 200x10^9 red cells per day in the human bone marrow is highly dependent on iron, glucose, fatty acid and amino acid metabolism. Amino acids support multiple aspects of cell metabolism, ranging from precursors of nucleic acids, conversion to glucose and/or lipids, stimulation of the mTOR signaling pathway, production of TCA cycle intermediates, and maintenance of intracellular redox, amongst others. Arginine—a semi-essential dibasic, cationic amino acid—is one of the most versatile molecules, promoting the synthesis of urea, nitric oxide, proline, creatine, agmatine, and polyamines. These diverse properties contribute to arginine’s critical role in a myriad of physiological and pathological processes such as immune function, insulin sensitivity, wound healing, hormone secretion, endothelial function, and cancer proliferation. Notably though, our understanding of the function of arginine metabolism in erythropoiesis is limited.

Aims:

This study aimed to investigate the role of SLC7A1/CAT1-mediated uptake of arginine and its intracellular catabolism in the commitment of human hematopoietic stem and progenitor cells (HSPC) to the erythroid lineage as well as in terminal erythroid differentiation. This study further evaluated the mechanisms via which arginine regulates erythropoiesis in both physiological and pathological contexts.

Methods:

EPO-induced differentiation of human CD34+ progenitors was evaluated following alterations in arginine transport and metabolism by different pharmacological inhibitors and shRNA-mediated knockdown of identified genes. Polyamine-mediated regulation of eukaryotic translation factor 5A (eIF5A) activity was evaluated by direct hypusination, metabolic assays were performed as indicated, and proteomics profiling was performed by LC-MS/MS. The role of arginine in disordered erythropoiesis was evaluated in human HSPC with shRNA-mediated downregulation of RPS19 and RPL11, as well as in del(5q)-MDS progenitors and in murine haploinsufficient Rps14+/− progenitors.

Results:

We find that SLC7A1/CAT1-mediated arginine uptake and its catabolism to spermidine control erythroid commitment and differentiation. Spermidine serves as a precursor for hypusine, required for activation of the eIF5A translation factor. Hypusine levels increased during early erythroid differentiation and importantly, attenuated hypusine synthesis skewed the differentiation of EPO-stimulated HSPCs from an erythroid to a myeloid cell fate. LC-MS/MS analyses together with metabolic assays revealed mitochondrial protein translation and function to be...
critical downstream effectors of hypusinated eIF5A. Finally, within the hypusine network, ribosomal proteins (RPs) are highly enriched. As genetic alterations in RPs drive anemia in Diamond-Blackfan anemia and myelodysplastic syndromes with chromosome 5q deletions, we evaluated eIF5A hypusination in different models of these bone marrow failure syndromes. Abnormal hypusination and attenuated mitochondrial metabolism were hallmarks of progenitors with RP haploinsufficiency, highlighting RP-eIF5A interactions in regulating erythroid differentiation.

**Summary/Conclusion:** Our data show that HSPC commitment to the erythroid lineage requires SLC7A1/CAT1-mediated arginine uptake—driving polyamine metabolism and hypusination of the eIF5A translation elongation factor. Furthermore, our study reveals a novel link between hypusination and RPs in the regulation of erythropoiesis, identifying aberrant eIF5A activity in ribosomal protein-linked disorders of ineffective erythropoiesis.