Assessing the red blood cell nursery

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Abstract

A healthy human individual makes approximately 2 million red blood cells per second. Red blood cell production (erythropoiesis) occurs in the bone marrow in healthy individuals. Developing red blood cells (erythroblasts) surround a central macrophage, forming multi-cellular cluster known as the erythroblastic island (EBI). EBI, also known as the erythroid niche, serves as a nursery for developing erythroblasts during erythropoiesis. However, our understanding on how EBIs control erythropoiesis remains poorly understood.

As anatomy often dictates physiology, we first assessed the mouse EBIs using the serial-block-face scanning electron microscope (SBF-SEM) at the subcellular level. EBIs observed by the SBF-SEM consist of a central macrophage surrounded by both nucleated erythroid progenitors and anuclear reticulocytes. The central EBI macrophage contains only one nucleus and is the largest cell type in the niche. The macrophage engulfed and destroyed the expelled erythroid nuclei as indicated by the cytoplasmic inclusions in the macrophage. Granulocytes with annular-shaped nucleus and cytoplasmic granules could also be found in the EBIs. Nuclear openings surrounding the nucleus were exclusively found in all erythroblasts but not macrophages or granulocytes. Within these erythroid niches, there are cells without intact nuclear envelopes but exhibit regions of electron dense structures indicative of mitotic cells.

Mitochondrial cristae also varied between the cell types, which were less defined cristae in late-stage erythroblasts and absent in the mitochondria of anuclear reticulocytes. SBF-SEM showed that mitochondrial attached to the erythroid nucleus surface. Three-dimensional rendering of SBF-SEM images revealed that erythroid mitochondria reside in close proximity to erythroid nuclei, forming "grappling-hook" like attachments. Mitochondria in these nucleated progenitors exhibit either a ribbon-like structure or kidney-bean morphology. Erythroblasts with kidney-shaped mitochondria have a tight absolute count of 35-43 mitochondria per cell. Mitochondrial dynamics of erythroid progenitors were also assessed using flow cytometry with conventional MitoTracker dyes and a novel synthetic ratiometric reactive oxidative species probe, FCR-2.

Having observed extracellular exosomes resembling expelled mitochondria in the EBIs, we added mitochondria-labelled erythroid progenitors to isolated single EBI macrophages. Erythroid mitochondria rapidly accumulated in the macrophage. This indicates that mitophagy is not the sole mechanism to remove erythroid mitochondria and the removal of mitochondria does not accompany the erythroid enucleation event. It also indicates that the central macrophage plays an important role in erythropoiesis by erythroid organelle removal. A rare bridge-like connection, known as a tunnelling nanotube (TnT), could also be found connecting two erythroblasts when imaging the EBIs using conventional scanning electron microscopy. These TnTs were observed on one of the clusters imaged on the SBF-SEM, with a cargo present within the TnT. Basic automated thresholding performed on the reconstructed 3D model reveals the cargo to resemble a mitochondrion. This evidence collectively indicates that erythroid mitochondrion trafficking occurs in the niche. We propose that the central macrophage further supports erythropoiesis by shuffling mitochondria among erythroblasts.

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