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POSTER ABSTRACTS

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

Circadian Rhythms Metabolically Regulate Bone Marrow Retained Primitive Hematopoietic Stem Cell Size and Function

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Most primitive hematopoietic stem cells (pHSC; LSK SLAM CD34 $^{-}$ EPCR $^{+}$) are bone marrow (BM) retained in a quiescent non motile state, and are the only chemotherapy resistant HSC which requires their rapid exit from homeostatic quiescence on demand to prevent lethal hematology failure. Circadian rhythms induce daily release of progenitor cells and low levels of rarely cycling pHSCs to replenish the blood and lymphatics with new maturing cells. Metabolically, pHSCs are characterized by their dependence on glycolysis, with low mitochondrial activity and membrane potential (MMP), associated with higher competitive repopulation potential. However, if their metabolism is influenced by circadian cues is currently unknown. We report here that pHSCs at night are more glycolytic with higher surface expression of the glucose transporter Glut1, and higher glucose uptake. Repeated administration of the glucose analog, 2-deoxyglucose (2-DG), during daylight mimicked the night phenotype and enhanced HSC engraftment potential. However, nocturnal administration of 2-DG did not further improve glucose uptake nor HSC engraftment potential, suggesting glycolytic saturation.

To further understand these metabolic oscillations of glucose uptake, we investigated the circadian, transcriptional signatures by single pHSC RNA-seq analysis. We found higher expression of mitophagy-related genes at night. Genes regulating mitochondrial function, were also significantly down regulated at night leading to low MMP in pHSC. While mitochondrial ROS levels were higher, cytoplasmic ROS levels were significantly reduced in pHSC at night. Importantly, we further found at night, low-functioning pHSC mitochondria to also undergo higher levels of fission as assessed by increased expression of the active mitochondrial fission marker, phospho-Drp1 (S616).

To better understand the molecular pathways mediating daily circadian metabolic reprogramming between glycolysis and mitochondrial activity, we monitored our gene set enrichment analysis. We found the Wnt/b-catennin and HIF-1a signaling pathways to be significantly increased at night in pHSC. Active Wnt signaling, leads to enhanced b-catenin localization to the nucleus which is enhanced by downstream effector of Wnt activation, FOXM1. We observed higher total protein expression of FOXM1 at night. While its nuclear and cytoplasmic localization were reduced, we observed significantly higher FOXM1 levels in the mitochondria. FOXM1 localization to the energy powerhouse has been shown to inhibit mitochondrial activity. Blocking mitochondrial recruitment of FOXM1 with a specific canonical Wnt inhibitor at night led to upregulation of MMP, reversing mitochondrial metabolic responses in pHSC.

Finally, daily genetic changes between night Wnt, HIF-1a and glycolysis, versus daylight GSK3b, m-TOR signaling and enhanced mitochondrial activity in BM retained pHSC, also led to cell size changes. We documented reduced quiescent pHSC size at night associated with their increased BM maintenance and higher repopulation potential. While larger sized pHSCs in the morning during the time of blood replenishment, increased their migration and development potential, for their rapid exit from quiescence on demand.

Our study reveals daily circadian cues dynamically determine Wnt-mediated FOXM1 bidirectional shuttling between the nucleus and the mitochondria, leading to metabolic switching of pHSCs to maintain their cellular fitness. Our data define key signaling pathways controlling pHSC quiescence and diverse circadian metabolic activities for accumulated ROS and old mitochondria clearance and turnover, keeping them ready for rapid activation in alarm situations.

Disclosures Choudhuri: *Fulcrum Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Dick:** *Graphite Bio:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Celgene/BMS.:* Research Funding; *Trillium Therapeutics Inc/Pfizer:* Patents & Royalties: Trillium Therapeutics.

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