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Hematopoietic Stem Cells Rock Around The Clock: Circadian Fate Control via TNF/ROS Signals

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https://doi.org/10.1016/j.stem.2018.09.002

Hematopoietic stem cell function is closely tied to circadian rhythms. In this issue of *Cell Stem Cell*, Golan et al. (2018) identify crosstalk between circadian hormone signals, the inflammatory cytokine TNF, and bone marrow macrophages as a key regulator of HSC proliferation, differentiation, and self-renewal in the bone marrow.

A wide range of organisms tie their physiology and behavior to highly conserved circadian clocks as a means of regulating daily biological functions (Cuninkova and Brown, 2008). Diverse functions such as tissue repair and maintenance, metabolism, and the length and timing of periods of activity and dormancy are tied to a set of "clock" genes whose expression varies over the length of a day. Circadian control of these genes in most vertebrates is tied to visual light/dark cues transmitted from the eye to the suprachiasmatic nucleus (SCN) in the brain. The SCN in turn functions as a central oscillator that governs circadian activity of the organism based in part on triggering the release of hormonal signals such as norepinephrine (NE) and melatonin by components of the sympathetic nervous system (SNS). Circadian variation in bone marrow (BM) cell proliferation was first described in humans in the 1960s (Mauer, 1965). Subsequently, elegant studies in the mouse have identified direct light-dark control of BM functions including timing of hematopoietic stem cell (HSC) egress, regulated by NE secretion that regulates the balance between expression of the BM chemokine CXCL12 and the Sp1 transcription factor (Méndez-Ferrer et al., 2008). A new publication in *Cell Stem Cell* now provides exciting mechanistic insight into how HSC proliferation and maintenance are regulated in a circadian fashion by an interplay between hormones, inflammatory cytokines, and BM macrophages (Golan et al., 2018).

Tumor necrosis factor (TNF) is a pluripotent cytokine that activates pro-inflammatory signaling cascades including the NF-κB pathway in a wide variety of cell types. The role of TNF in regulating HSC biology has been somewhat controversial, with some studies indicating that TNF suppresses HSC function while others suggest that it activates HSCs (Pietras, 2017). Strikingly, in mice, Golan et al. find that two daily "peaks" of BM hematopoietic stem and progenitor cell (HSPC) numbers at 11 a.m. and 11 p.m. (based on a 12-hour light/dark cycle that transitions at 6 a.m. and 6 p.m.) are preceded by elevated TNF levels in the BM at 7 a.m. and 7 p.m., with higher TNF expression at the 7 a.m. peak. These findings closely correlate with prior work showing diurnal shifts in TNF production in blood plasma from human subjects (Petrovsky and Harrison, 1998). The authors find that these TNF peaks induce a transient increase in HSPC reactive oxygen species (ROS) levels that are responsible for the transient increases in HSPC numbers, as blockade of TNF or treatment with the antioxidant N-acetylcysteine (NAC) at the time of light/dark transition significantly attenuates HSPC expansion. Interestingly, TNF levels can be transiently increased if mice are briefly switched into either a light or dark environment for 1 hr, indicating direct regulation of the TNF oscillations by light/dark cues. Collectively these data implicate a TNF/ROS axis as a regulator of homeostatic HSPC proliferation in the BM, regulated by daily light/dark cues. While TNF and increased ROS levels are often associated with pathogenic phenotypes in which HSC function is dysregulated (Pietras, 2017), TNF and ROS are also important for HSC specification during fetal life (Espín-Palazón et al., 2014). Hence, pro-inflammatory signals like TNF may have evolved to play dual homeostatic and "emergency" roles by virtue of their cell-activating properties, with timing and dosage likely dictating which role the signal plays in a given scenario.

Notably, Golan et al. find that while HSPC numbers peak twice per day, the fate of the cells appears to differ based on the time of day. Exclusively in the morning, HSPC egress is increased, in line with prior work (Méndez-Ferrer et al., 2008), with a corresponding increase in the number of lineage-committed myeloid and lymphoid progenitors present in the BM. Along these lines, transplantation assays reveal that donor BM cells exhibit decreased repopulating activity when harvested in the morning versus the evening, suggesting that TNF/ROS peaks are interpreted as distinct cell fate cues by HSPCs based on time of day. Addressing this question, Golan et al. find a peak of NE in the BM that corresponds to the transient increase in BM TNF/ROS, as well as increased sinusoidal permeability in the BM. NE blockade results in decreased BM and circulating HSPC numbers and attenuation of TNF/ROS, related to lower expression of the TNF activating enzyme TACE in BM cells. Conversely, BM levels of melatonin increase in a TNF-dependent fashion during the evening, and blockade of this hormone has the opposite effect, leading to increased numbers of lineage-committed progenitors, along with HSPC mobilization and decreased engraftment efficiency. Likewise, administration of NE or melatonin to mice at the opposite time of day could induce HSPC differentiation and retention, respectively. Hence, distinct HSPC fate choices are entrained to levels of these two hormones, with TNF itself functioning to induce melatonin. Further investigations can identify the

precise cellular sources of these hormones. Additionally, these data reinforce the point that time of day could have a profound impact on outcomes of phenotypic and functional studies of HSCs; the data show that key HSC markers such as CD150 are hormonally regulated and change throughout the day, whereas the abundance and engraftment efficiency of phenotypic HSCs also shift drastically. Future investigations may need to take these potentially confounding variables into account when analyzing certain biological parameters in the BM including HSPC number, phenotype, metabolic state, and response to inflammatory factors.

Macrophages are well established as critical regulators of tissue remodeling and are important for maintaining HSC homeostasis in the BM (McCabe and MacNamara, 2016). Here, the authors show that COX-2/α-smooth muscle actin (SMA) macrophages are induced to expand by melatonin, thereby reducing ROS levels and preventing aberrant HSC differentiation and egress in response to the evening TNF peak in a COX2-dependent fashion. As HSPCs also express melatonin receptors MT1 and MT2, this suggests that melatonin has a broad impact on different hematopoietic components, all likely geared toward promoting HSC quiescence.

Altogether these data define a complex circuit that links the SNS, inflammatory cytokine pathways, and BM macrophages together in regulating distinctive HSPC functional states throughout the day. Important next steps include addressing human biology and investigating how acute and chronic deregulation of the light/dark cycle due to insomnia, jet lag, or evening working hours impacts long-term hematopoietic function. Along these lines, sleep deprivation is shown to reduce HSC engraftment capacity, and disorders including hematological malignancy have significant interplay with circadian control circuits (Puram et al., 2016; Rolls et al., 2015). Hence, understanding the impact of such deregulations on HSC fate may have critical implications in a clinical setting.

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3D Human Esophageal Epithelium Steps Out from hPSCs

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https://doi.org/10.1016/j.stem.2018.09.010

Human pluripotent stem cell (hPSC)-derived organoids can reveal important principles underlying tissue development. In this issue of *Cell Stem Cell*, Zhang et al. (2018) and Trisno et al. (2018) establish protocols for generating esophageal epithelial cells and 3D stratified epithelium from hPSCs, revealing roles for key signaling pathways and how they are controlled by critical transcription factors.

The generation of epithelial structures from human pluripotent stem cells (hPSCs) provides a foundation for new insights into fundamental mechanisms underlying tissue formation as well as windows for studying disease etiology and potential therapeutics. Studies of the esophageal epithelium would benefit also from such platforms as a basis to integrate insights from developmental biology into normal homeostasis and disease pathophysiology. In this issue of *Cell Stem Cell*, Zhang et al. (2018) and Trisno et al. (2018) take orthogonal approaches for deriving three-dimensional (3D) tissues