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A complex interplay between PGC-1 co-activators and mTORC1 regulates hematopoietic recovery following 5-fluorouracil treatment

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Abstract In vitro stimulation of HSCs with growth factors generally leads to their depletion. Understanding the molecular mechanisms underlying expansion of HSCs in vivo following myeloablation could lead to successful expansion of HSCs ex vivo for therapeutic purposes. Current findings show that mTORC1 is activated in HSPCs following 5-fluorouracil treatment and that mTORC1 activation is dependent on mitochondrial ETC capacity of HSPCs. Moreover, expression of PGC-1 family members, proteins that regulate mitochondrial biogenesis, in HSPCs following 5-fluorouracil treatment changes; also, these proteins play a stage specific role in hematopoietic recovery. While PRC regulates HSCs' expansion during early recovery phase, PGC-1α regulates progenitor cell proliferation and recovery of hematopoiesis during later phase. During early recovery phase, PRC expression, mitochondrial activity and mTORC1 activation are relatively higher in PGC-1$\alpha^{-/-}$ HSCs compared to WT HSCs, and PGC-1$\alpha^{-/-}$ HSCs show greater expansion. Administration of rapamycin, but not NAC, during early recovery phase improves WT HSC numbers but decreases PGC-1$\alpha^{-/-}$ HSC numbers. The current findings demonstrate that mTOR activation can increase HSC numbers provided that the energy demand created by mTOR activation is successfully met. Thus, critical tuning between mTORC1 activation and mitochondrial ETC capacity is crucial for HSC maintenance/expansion in response to mitogenic stimulation.

Introduction

Expansion of hematopoietic stem cells (HSCs) in vitro using growth factors has been thwarted by findings that there is loss of HSCs during in vitro expansion. In steady-state hematopoiesis, a majority of HSCs remain quiescent; however, in response to 5-fluorouracil (5-FU) treatment, majority of HSCs undergo rapid proliferation and lead to hematopoietic recovery (Harrison and Lerner, 1991). Therefore, a better understanding of molecular pathways that regulate HSC expansion and recovery of hematopoiesis following 5-FU treatment may

Abbreviations: HSC, hematopoietic stem cell; HSPC, hematopoietic stem and progenitor cell; mTORC1, mammalian target of rapamycin complex1; ETC, electron transport chain; PGC-1α, peroxisome proliferator-activated receptor gamma co-activator-1α; PRC, PGC-1 related coactivator; NAC, N-acetyl cysteine.

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allow us to develop protocols that will eventually lead to successful ex vivo expansion of HSCs.

Mammalian target of rapamycin (mTOR) kinase is the catalytic subunit of two complexes, mammalian target of rapamycin complex (mTORC) 1 and 2; mTORC1 and 2 have been respectively characterized as the rapamycin-sensitive and rapamycin-insensitive complexes (Laplante and Sabatini, 2009). Growth factors stimulate the mammalian target of rapamycin complex1 (mTORC1) pathway that controls cell growth and division (Fingar and Blenis, 2004). Activation of mTOR is regulated by adenosine triphosphate (ATP) levels in the cell (Dennis et al., 2001). Oxidative phosphorylation (OXPHOS), an oxygen dependent process that occurs in the mitochondria, is the major source of cellular ATP. Taken together, these findings raise the possibility that the interaction between the molecular pathways, mTOR activation and mitochondrial biogenesis, plays a deterministic role in ability of hematopoietic stem and progenitor cells (HSPCs) to successfully expand following 5-FU treatment. Mitochondria have their own DNA that encodes for 13 essential proteins of inner membrane respiratory apparatus but nuclear genes encode for a majority of respiratory proteins and all other gene products necessary for a variety of mitochondrial functions. The nucleo-mitochondrial interactions depend on regulation of transcription factors and peroxisome proliferator-activated receptor gamma co-activator (PGC-1) family of transcriptional co-activators that includes PGC-1α, PGC-1β and PGC-1 related coactivators (PRC) (Scarpulla, 2008). Although various PGC-1 family members can regulate mitochondrial biogenesis (Scarpulla, 2002; Uldry et al., 2006), PGC-1α is considered the master regulator of mitochondrial biogenesis (Puigserver and Spiegelman, 2003). PGC-1α is expressed in BM hematopoietic stem progenitor cells (HSPCs) (Basu et al., 2013).

While glycolysis is important for the maintenance of HSCs (Simsek et al., 2010; Takubo et al., 2010), the role of mitochondrial metabolism in hematopoiesis (Nakada et al., 2010; Gurumurthy et al., 2010), particularly in response to stress (Mortensen et al., 2011), is increasingly being realized. Indeed, recovery of peripheral blood cells (PBCs) following 5-FU treatment is significantly impaired in PGC-1α knockout (PGC-1$\alpha^{-/-}$) mice compared to wild type (WT) mice; however, steady-state hematopoiesis is not overtly affected in PGC-1$\alpha^{-/-}$ mice (Basu et al., 2013). Moreover, the impairment of PBC recovery is more profound in older PGC-1$\alpha^{-/-}$ mice (SB unpublished observation) consistent with age related degeneration of mitochondrial function (Pieri et al., 1993). These findings suggest a role of mitochondria in the recovery of hematopoiesis following 5-FU treatment.

To get an insight into mechanisms regulating HSC proliferation in vivo, recovery of hematopoiesis following 5-FU treatment in WT and PGC-1$\alpha^{-/-}$ mice was investigated in this study. The current study demonstrates that mitochondrial biogenesis is critical for HSPCs to optimally respond to mTORC1 activation and proliferate in response to mitogenic signals following 5-FU challenge. Moreover, the level of mTORC1 activation is regulated by the mitochondrial biogenesis potential of HSPCs. Interestingly, different members of the PGC-1 family play stage-specific roles in hematopoietic recovery following 5-FU treatment: while PRC is required for hematopoietic stem cell (HSC) proliferation during early recovery phase, PGC-1α is important for rapid proliferation of progenitors during late phase of hematopoietic recovery following 5-FU treatment.

Material and methods

Animal studies

All animal studies were evaluated and approved by the Institutional Animal Care and Use committee. PGC-1α knockout (PGC-1$\alpha^{-/-}$) mice were a kind gift from Prof. B. Spiegelman (DFCI, Boston, MA) and used after 9 backcrosses to C57/Bl6 mice. PGC-1$\alpha^{+/+}$ litter mate mice were used as a control and are designated as wild type (WT) in this study. C57/Bl6 mice were purchased from Jackson Laboratories; C57/Bl6:BoyJ F1 and Boy/J mice were from the In Vivo Core Therapeutic Facility of the Indiana University School of Medicine.

5-FU and rapamycin treatments

Mice were treated with 150 mg/kg wt of 5-FU intraperitoneally (i.p.) on day 0 and the changes in PBC counts and bone marrow (BM) cellularity was followed thereafter. Rapamycin (Sigma-Aldrich) was made in absolute ethanol at 10 mg/ml and further diluted in 5% Tween-80 (Sigma-Aldrich) and 5% PEG-400 (Hampton Research) (Chen et al., 2008). Rapamycin was delivered i.p. so that the measured dose of rapamycin (adjusted to mouse body weight) could be administered. Mice received 4 mg/kg rapamycin on days 1 and 3 or on day 8 post-5-FU treatment.

Phenotypic and functional analysis of BM cells

BM cells were obtained from the long bones (tibias and femurs) of mice. Lineage positive cells were identified based on staining pattern with fluorescent dye conjugated antibodies against B220, CD3, Gr-1, Mac-1, CD71 and Ter119 (BD Biosciences). The above set of antibodies, except CD71, was used to gate out the lineage positive cells for analysis of HSC/progenitor populations. For analysis of 5-FU treated BM on day 4 Mac-1 was not included in the lineage cocktail antibody for gating out lineage negative cells (Randall and Weissman, 1997). c-Kit$^+$Sca-1$^+$IL-7R$^-$Lin$^-$ cells are designated as KSL and c-kit$^+$IL-7R$^-$Lin$^-$ cells are designated as KL/MP. HSCs are defined as KSL IL-7R$^-$CD34$^-$, and MPPs defined as KSL IL-7R$^-$CD34$^+$. Since the expression of CD34 on HSCs alters following 5-FU treatment (Sato et al., 1999), I used SLAM marker in conjunction with KSL (Yilmaz et al., 2006) to enumerate changes in primitive and progenitor cell populations in the BM for up to 7 days following 5-FU treatment. The combination of SLAM markers used to identify progenitors differs between Yilmaz et al. (2006) and Wilson et al. (2008) and in this study the identification of the progenitors has been done as described by Yilmaz et al. (2006). Fluorochrome conjugated antibodies were purchased from eBioscience, Biolegend and BD Bioscience. Flow cytometry analysis was performed on an LSR II (BD Biosciences), and cells were sorted using FACSAria (BD Biosciences). Cell cycle status was determined by fixing and permeabilizing cells with BD Per/Fix buffer reagents (BD Pharmingen) and staining the cells with 2 μg/ml HOECHST 33342 (Invitrogen)