

Perspective

The axis of mTOR-mitochondria-ROS and stemness of the hematopoietic stem cells

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It has been hypothesized that adult hematopoietic stem cells (HSCs) need to remain quiescent to retain their long-term self-renewal activity and multipotency. However, it is still unclear how lack of quiescence is detrimental to HSC. We identified that the mTOR pathway is the key to HSCs quiescence. mTOR overactivation caused increased mitochondrial biogenesis and accumulation of much higher level of reactive oxygen species (ROS). Removal of ROS rescued HSC defects associated with hyperactivated mTOR. We propose susceptibility to ROS as the underlying cause for HSC's general requirement for quiescence.

The stemness of HSCs is defined by self-renewal and multipotency. Studies from several laboratories showed that adult HSCs are relatively quiescent, loss of which is associated with loss of HSC stemness.¹⁻⁶ However, since proliferation per se is not necessarily incompatible with the stem cell function,⁷ it is still unclear why quiescence is required.⁸ By studying HSCs with targeted mutation of *Tsc1*, a negative regulator of mTOR pathway,^{9,10} we demonstrated that the mitochondrial metabolite ROS as the missing link between quiescence and stemness of HSCs.⁸

The Proliferative Quiescence and Stemness of HSCs

During fetal and neonatal periods, HSCs divides rapidly to expand the HSC pool and set up the hematopoietic system.¹¹ Thereafter, HSCs switches from active to low proliferation. At normal homeostasis conditions, most adult HSCs are in G₀ phase of cell cycle, while a small subset of HSCs are dividing.⁶ By BrdU incorporation and DNA content assays, it was found that less than 5% HSCs were in S/G₂/M phases of cell cycle, 20% were in G₁ phase, while more than 70% HSCs were in G₀ phase.^{2,3} The notion of HSC quiescence is consistent with the staining results using Pyronin Y,^{12,13} a dye widely used to monitor gene expression

in general. Pyronin Y staining showed that more than 70% HSCs are quiescent.^{3,4}

Quiescence is suggested as one of the major mechanisms to maintain HSC function.⁷ Multiple studies showed that when HSCs underwent active proliferation, they also lost their long-term functions. This can be effected by environmental changes.¹⁴⁻¹⁷ Moreover, in multiple mouse genetics models, high proliferation of HSCs is also associated with defects of their functions.^{4,18-21} For example, targeted mutation of *p21^{Cdkn1}*, a cyclin-dependent kinase inhibitor (CDKI) that is known to inhibit cyclin E-CDK2 activity,^{22,23} resulted in increased cellular RNA contents and enhanced replication of DNA. Correspondingly, a much reduced regenerative capacity was revealed by serial transplantation. Nevertheless, it should be noted that the correlation between quiescence and stemness is not universal. Thus, deletion of *p18^{INK4c}* in HSC increased both rates of proliferation and stem cell function,²⁴ while ectopic expression or deletion of *HoxB4* in HSCs both proliferation and stem cell function in parallel.^{25,26}

The Tsc-mTOR Pathway Regulates the Quiescence and Functions of HSCs

Compared with proliferating cells, quiescent cells display reduced overall transcription and translation rates (Fig. 1).²⁷ The mTOR pathway has been shown to be a key regulator for cellular metabolism.²⁸ Active mTOR promotes translation through S6K and 4E-BP1. mTOR signaling also induces transcription of many genes, including those involved in metabolic and biosynthetic pathways.²⁹ By promoting translation and transcription as well as responding to Wnt signaling, mTOR promotes cell growth and proliferation.²⁰

Studies on yeast suggested that the TOR pathway might regulate the entry of quiescence.^{27,30} To study the role of the TSC-mTOR pathway on the quiescence and functions of HSCs, we used conditionally allele to delete *Tsc1* in HSCs.⁸ We found that *Tsc1* deficiency driven HSCs from quiescence into rapid cycling, as demonstrated by pyronin Y staining and BrdU incorporation. Thus, only 10% of *Tsc1*-deficient HSCs exhibited low pyronin Y staining while 70% of wildtype HSCs did. After 24 hour labeling, 12% of wildtype HSCs were BrdU⁺ while more than 50% of *Tsc1*^{-/-} HSCs were. These data indicated that Tsc1 was

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an important regulator for the quiescence of HSCs.

Even though the *Tsc1*-deficiency increased the number of HSCs, the mutant HSCs displayed multiple hematogenesis defects, including progressive bone marrow failure and hypocellularities of leukocytes. Serial bone marrow transplant (BMT) and competitive BMT confirmed the loss of long-term functions is cell-intrinsic for the mutant HSCs. The phenotypes of *Tsc1*-deficient HSCs provide another example that quiescence is linked with the functions of HSCs. A subsequent study by Gan et al. confirmed Tsc-mTOR pathway as a regulator of HSC quiescence and functions.³¹

Mitochondria and ROS Suggest a Link between Quiescence and Stemness of HSCs

Recently, it has been shown that the TSC-mTOR pathway controls the mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex in cultured muscle cells.³² By MitoTracker staining and real-time PCR, we found that the mitochondrial mass and mitochondrial DNA copy numbers were increased by approximately two-fold in *Tsc1*^{-/-} HSCs when compared with the wild-type. The expression levels of the mitochondrial oxidative genes were also significantly enhanced in *Tsc1*-deficient HSCs, which suggests high levels of mitochondrial oxidative activities.

HSCs are very sensitive to high levels of ROS.³³⁻³⁵ Mitochondrion generates ROS during its oxidative activities, especially when the mitochondrial potential and the NADH/NAD⁺ ratio are high.³⁶ Consistent with enhanced mitochondrial biogenesis and oxidative activities, we found that the ROS levels were dramatically elevated in *Tsc1*^{-/-} HSCs. In the *Tsc1*^{-/-} HSCs, ROS is the major cause of the loss of stemness because quenching ROS by *N*-acetylcysteine (NAC) significantly rescued the reconstitution capacity of the mutant HSCs.

Taking together, our data reported recently⁸ demonstrated that after the *Tsc1* deletion, high mTOR activity promotes the proliferation of HSCs. These dividing HSCs have enhanced metabolism with increased mitochondrial biogenesis and elevated levels of ROS. Consequently, ROS impairs the functions of HSCs. Therefore, HSCs must remain quiescent to avoid the accumulation of ROS (Fig. 2). The inability to handle toxic metabolites is therefore proposed as the underlying cause for the fundamental requirement for quiescence for the stemness of HSCs.

Concluding Remarks

We hereby present the link between mTOR-mitochondria-ROS axis and HSC stemness as a new concept in stem cell biology. If our hypothesis is correct, it may also find practical application in HSC therapy. One big limitation for current HSC therapy is

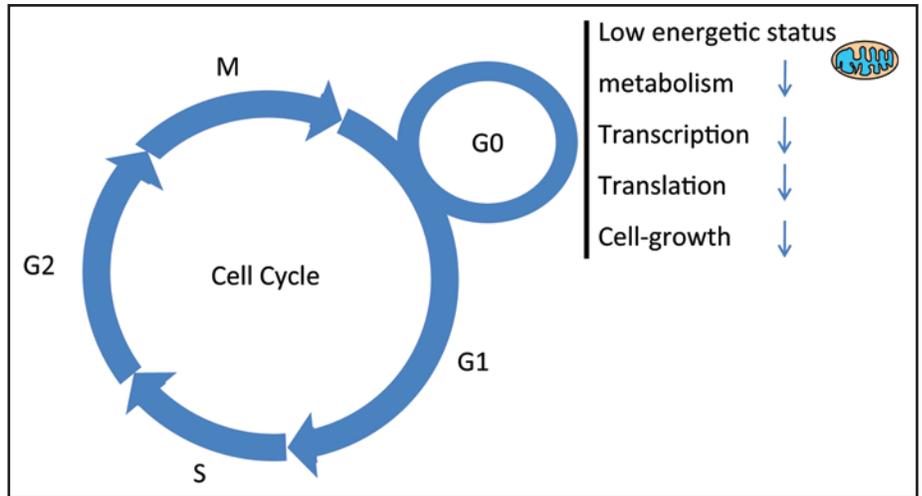


Figure 1. The quiescence and cell cycle in HSCs. Besides not proliferating, quiescent cells display multiple properties, including low energetic status, low level of metabolism, reduced transcription and translation, and thus not increasing cell mass over time.

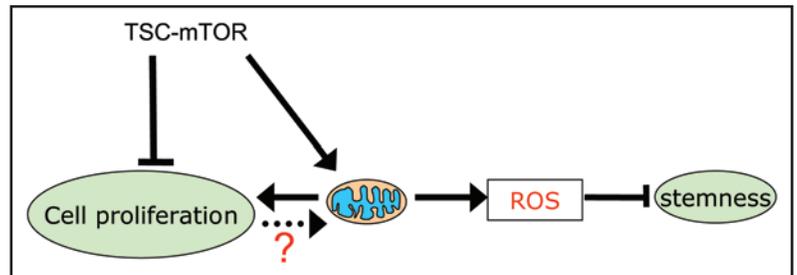


Figure 2. Mitochondria/ROS as a missing link between quiescence and stemness of HSCs. Over-activation of mTOR enhances mitochondrial activities. Enhanced mitochondrial activities in turn lead to the accumulation of high levels of ROS, which cause the loss of stemness of HSCs. We propose that metabolic quiescence in mitochondria rather than the proliferation per se that regulate the functions of HSCs.

that it is very difficult to expand HSCs in vitro while maintaining their functions. According to our model, removal of ROS may disrupt the link between quiescence and stemness. Therefore ROS antagonists may help maintain the stemness while HSCs are proliferating.

In the future, it would be important to test whether the homeostasis of ROS are also disrupted in other models where HSC quiescence is linked with their functions.^{4,18-20} Conversely, it has been noted that fetal HSCs have high proliferating rate while maintaining their stemness. Likewise, a number of genetic models revealed exceptions in which proliferative quiescence are not linked to stemness of adult HSC.^{24,25} It is therefore pressing to test if the levels of ROS in these models are uncoupled to HSC proliferation. These investigations may lead to a fundamental understanding on how proliferation and ROS homeostasis are linked in HSC.

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