The Tortoise, the Hare, and the FoxO

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Maintaining stem cell quiescence is intimately connected to preserving long-term self-renewal potential. In this issue of Cell Stem Cell, Paik et al. (2009) and Renault et al. (2009) demonstrate a role for FoxO transcription factors in regulating neural stem cell proliferation and in maintaining stem and progenitor cell homeostasis.

Most of us remember the childhood tale “The Tortoise and the Hare,” in which the hare begins a race with a burst of energy and enthusiasm to take an early lead, but winds up losing to the tortoise in the end. In the current issue of Cell Stem Cell, two groups show that the lessons of Aesop’s fable may be equally applied to the biology of the neural stem cell (NSC). Together, these two manuscripts demonstrate that the FoxO family of transcription factors exerts an important constraint on NSC proliferation (Paik et al., 2009; Renault et al., 2009). Like the proverbial hare, FoxO-deficient NSCs initially seem robust and invincible. Yet this initial burst of postnatal activity quickly disappears and as the mice age, FoxO-deficient stem cells appear to rapidly decrease in both number and function. In contrast, wild-type mice—although slower out of the gate—appear to better preserve their stem cell function throughout adulthood. As such, it would seem that in both childhood tales and stem cell biology, slow and steady wins the race.

In mammals, the FoxO family of transcription factors consists of four members: FoxO1, FoxO3, FoxO4, and FoxO6. Activation of the PI3K/Akt pathway after insulin or growth factor stimulation results in FoxO phosphorylation and inactivation due to retention in the cytoplasm. In contrast, certain stresses, such as oxidative or nutrient stress, result in FoxO nuclear accumulation. Once in the nucleus, FoxO proteins transactivate a large array of downstream targets including cell-cycle inhibitors, metabolic regulators, and genes involved in stress resistance. The precise targets appear to vary depending on both what kind of stress is involved and the specific tissue being studied (Salih and Brunet, 2008). In contrast to mammals, worms and flies express only one FoxO member. In C. elegans, the single FoxO isoform is denoted as DAF-16, whereas in flies, it is known as dFOXO. Interestingly, the general transcriptional targets of DAF-16 include both metabolic and stress resistance genes that in many cases are similar to those regulated by mammalian FoxO factors. Even more intriguing, increased expression of DAF-16 in worms or dFOXO in flies results in lifespan extension in these model organisms.

Currently, the function of mammalian FoxO factors in lifespan regulation is unknown, although mounting evidence suggests a role for these factors in the maintenance of certain long-lived cell types such as endothelium and thymocytes (Paik et al., 2007). In contrast to the postmitotic worm, most mammalian tissues require the constant contribution of stem and progenitor cells to maintain tissue homeostasis. As such, it would seem possible that genes implicated in regulating organismal lifespan in lower organisms might have evolved to play a role in stem cell homeostasis.

In these two new reports, the Brunet and DePinho lab demonstrate the plausibility of this hypothesis by implicating mammalian FoxO activity in the regulation of NSC proliferation and self-renewal. The two manuscripts use two slightly different models, with one group studying a whole body or conditional knockout of FoxO3a−/−, and the other group employing a brain-specific conditional triple knockout of FoxO1/3/4. Both groups find that reducing FoxO activity leads to an in vivo increase in NSC/progenitor proliferation immediately after birth. Indeed, both animal models resulted in a larger overall brain size presumably due to this enhanced proliferation. However, long-term BrdU labeling or staining with Ki67 reveals that as the mice age, proliferation within the stem and progenitor compartment falls off precipitously. Complementary in vitro experimentation demonstrates an age-dependent decline in primary and secondary neurosphere formation, consistent with a decline in the number and self-renewal capacity of NSCs that lack FoxO activity. Thus, FoxO activity within the NSC compartment appears to be required to maintain stem cell quiescence. In the absence of FoxO activity, the early expansion of progenitors cannot be sustained, and as the animals matures, the number and activity of the NSC pool declines rapidly.

These results are not the first to demonstrate a role for FoxO proteins in maintaining stem cell homeostasis. A previous analysis of the FoxO3−/− mouse as well as examination of a conditional triple FoxO1/3/4 knockout within hematopoietic stem cells (HSCs) revealed that the HSC compartment is also regulated by FoxO activity (Miyamoto et al., 2007; Tothova et al., 2007). In both of these models, reduced FoxO activity was associated with a rise in reactive oxygen species (ROS) levels within HSCs and a subsequent defect in quiescence, a decrease in self-renewal, and a depletion of stem cell number. The rise in ROS levels within HSCs appears to relate to the known role of FoxO proteins in regulating the transcription of various antioxidant gene products such as superoxide dismutase and catalase (Kops et al., 2002; Nemoto and Finkel, 2002). These results suggest that alterations in various critical tissue-specific transcriptional
metabolism to ultimately regulate the
relationship between preserving quiescence and the age-dependent maintenance of overall stem and progenitor cell function. Previous studies analyzing mice deficient for the cell-cycle regulator p21 have also demonstrated an increase in early proliferation of NSCs followed by an accelerated age-dependent loss of NSC self-renewal capacity (Kippin et al., 2005). In contrast, NSCs lacking the phosphatase PTEN appear to demonstrate augmented proliferation without an apparent decrease in stem cell maintenance (Gregorian et al., 2009). The basis for such differences remains obscure. Perhaps within the NSC, PTEN can simultaneously regulate the activity of multiple pathways including both FoxO and mTOR that are linked to maintaining "stemness." Perhaps this capacity to induce an increase in proliferation without an apparent corresponding decrease in self-renewal is also why mutations in PTEN are so commonly found in deadly brain tumors such as glioblastomas. Further understanding of the connection between quiescence and stem cell maintenance promises to provide important insights into the intersection of stem cell biology with both aging and cancer. Indeed, it is likely that the molecular regulators of quiescence and self-renewal will continue to be heavily enriched for genes previously implicated in either tumor formation or lifespan regulation. Significant gaps remain, including the development of an integrative picture that incorporates how within the context of the niche, extracellular cues from molecules such as Wnt are potentially coupled to intracellular parameters such as glucose metabolism to ultimately regulate the balance between stem cell growth and quiescence. As Aesop would say, let the race begin!

Figure 1. A Conserved Role for FoxO Family Members
In C. elegans, a single FoxO family member DAF-16 regulates lifespan by altering the expression of numerous transcriptional targets, many of which are involved in cellular metabolism and augmenting stress resistance. In contrast to the postmitotic worm, FoxO transcription factors in mammals have four family members and their role appears to have expanded to include regulation of neural and hematopoietic stem cell proliferation and self-renewal.

Although these studies definitively implicate the FoxO family of transcription factors in maintaining neural stem and progenitor cell homeostasis, caveats and questions remain. First, the caveat: in contrast to HSCs for which self-renewal assays can involve the transplantation of single immunopurified cells to an irradiated host, followed by large-scale cellular expansion, the assay for NSC self-renewal relies instead on a tissue-culture-based assay known as secondary neurosphere formation. Questions persist regarding the physiological relevance of this in vitro assay and whether it truly measures the self-renewal capacity of a stem cell as opposed to the activity of an immature progenitor (Reynolds and Rietze, 2005). Assay aside, these new studies do seemingly highlight the tight relationship between preserving quiescence and the age-dependent maintenance of overall stem and progenitor cell function. Previous studies analyzing mice deficient for the cell-cycle regulator p21 have also demonstrated an increase in early proliferation of NSCs followed by an accelerated age-dependent loss of NSC self-renewal capacity (Kippin et al., 2005). In contrast, NSCs lacking the phosphatase PTEN appear to demonstrate augmented proliferation without an apparent decrease in stem cell maintenance (Gregorian et al., 2009). The basis for such differences remains obscure. Perhaps within the NSC, PTEN can simultaneously regulate the activity of multiple pathways including both FoxO and mTOR that are linked to maintaining "stemness." Perhaps this capacity to induce an increase in proliferation without an apparent corresponding decrease in self-renewal is also why mutations in PTEN are so commonly found in deadly brain tumors such as glioblastomas. Further understanding of the connection between quiescence and stem cell maintenance promises to provide important insights into the intersection of stem cell biology with both aging and cancer. Indeed, it is likely that the molecular regulators of quiescence and self-renewal will continue to be heavily enriched for genes previously implicated in either tumor formation or lifespan regulation. Significant gaps remain, including the development of an integrative picture that incorporates how within the context of the niche, extracellular cues from molecules such as Wnt are potentially coupled to intracellular parameters such as glucose metabolism to ultimately regulate the balance between stem cell growth and quiescence. As Aesop would say, let the race begin!

REFERENCES