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Stem-ing mTOR: p53 maintains the male germline

Comment on: Xiong M, et al. Quantitative analysis of male germline stem cell differentiation reveals a role for the p53-mTORC1 pathway in spermatogonial maintenance. Cell Cycle 2015; 14(18):2905-13; PMID: 26177380; http://dx.doi.org/10.1080/15384101.2015.1069928.

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The life-long maintenance of spermatogenesis depends upon the precise balance of self-renewal and differentiation in male germline stem cells. In rodents, the predominant model for this system consists of a putative stem cell referred to as an A_{single} (A_s) spermatogonium, which divides to generate either 2 additional A_s spermatogonia or interconnected A_{paired} (A_{pr}) daughter cells that do not complete cytokinesis.[1] While both A_s and A_{pr} spermatogonia are referred to as 'undifferentiated' cells, A_s are considered to be 'actual' stem cells; A_{pr} are considered to be progenitor cells. Additional divisions of the A_{pr} cells generate chains of 4, 8, 16, and 32 interconnected, undifferentiated Type A spermatogonia ($A_{aligned}$, or A_{al}). These chains of A_{al} then become A_1 differentiating spermatogonia, which further divide to generate A_2, A_3, A_4, Intermediate (In), and Type B differentiating spermatogonia before forming pre-leptotene spermatocytes that initiate entry into meiosis. Despite many years of research on male germline stem cell differentiation, this process has not been fully deciphered at the molecular level.

Spermatogonial differentiation is promoted by the activity of mammalian target of rapamycin complex 1 (mTORC1), which is inhibited in undifferentiated spermatogonia by downstream targets of the Promyelocytic leukemia zinc finger (PLZF; ZBTB16) transcription factor.[2] Loss of functional *Plzf* in male mice results in germ cell depletion due to insufficient stem cell self-renewal.[3,4] mTORC1 is negatively regulated by p53 following DNA damage in multiple cell types to allow for repair in a quiescent, low metabolic environment, but whether this mechanism regulates the balance of germline stem cell self-renewal and differentiation was not known until now.

In this issue of Cell Cycle, Mulin Xiong and colleagues disseminate exciting new findings from an elegant fluorescence-activated cell sorting (FACS)-based approach that separates mouse spermatogonia at consecutive differentiation stages for examination.[5] They use a transgenic reporter mouse line expressing GFP from a promoter fragment of the stimulated by retinoic acid 8 gene (p*Stra8*-GFP), combined with antibodies against 2 cell surface markers: α6 integrin, which is enriched in undifferentiated spermatogonia (A_s, A_{pr}, A_{al}), and c-Kit, which is enriched in differentiating spermatogonia (A_1-A_4, In, B). This *Stra8* promoter fragment was previously shown to become active within chains of undifferentiated spermatogonia (A_{al}), reflecting a progenitor cell population.[6]

For this current study,[5] testicular cells were initially sorted for the c-Kit ^{negative} α6 integrin ^{high} (undifferentiated) fraction. Upon collection, this cell population was then sorted for GFP− and GFP+ fractions that represent the most primitive undifferentiated spermatogonia [p*Stra8*-inactive; denoted population 1 (P1)] and the spermatogonial cell population destined for subsequent differentiation (p*Stra8*-active; P2), respectively. Testicular cells were also sorted for the c-Kit^{positive} α6 integrin^{low} (differentiating) fraction, which was secondarily sorted by GFP expression into cell populations P3 (GFP^{high}) through P6 (GFP^{low}). By crossing the p*Stra8*-GFP mouse line into a p53 knockout mouse line, the authors found that the loss of p53 resulted in premature phosphorylation of ribosomal protein S6 Kinase 1 (pRPS6), a readout of mTORC1 activity, in the P2 (undifferentiated) cell population when compared to control conditions in which pRPS6 was not prominent until the P3 (differentiating) cell population. Notably, pRPS6 was detected in PLZF+ spermatogonia in p53 knockout testes, an occurrence not observed in control testes.

Further analysis of the P1-P5 cell populations in p53 knockout testes revealed that the P1 population decreases when p53 is absent, while populations P2-P5 increase in the absence of p53. These findings demonstrated that while the pool of differentiating spermatogonia (P3-P5) and undifferentiated spermatogonia destined for subsequent differentiation (P2) expanded when p53 was lost and mTORC1 was prematurely activated, the pool of the most primitive undifferentiated spermatogonia (P1) contracted under these conditions.

The results from this study have important implications. Maintenance of male germline stem cells relies upon the presence of p53. Moreover, spermatogonial differentiation is promoted by mTORC1 activity, a mechanism that can be completely disrupted by the chronic exposure of testes to rapamycin.[2,7] The balance of stem cell self-renewal and differentiation therefore appears to be regulated by the relative activities of p53 and mTORC1, which are subjected to alteration during aging, cancer and other biological phenomena. The research findings and novel FACS-based approach from Xiong et al.[5] should open up new lines of inquiry into the molecular mechanisms of male germline stem cell differentiation.

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