Commentary

Stem Cell INVESTIGATION

Heterochromatin compaction is regulated by Suv4-20h1 to maintains skeletal muscle stem cells quiescence

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Abstract: In this report, Boonsanay and colleagues describe a novel mechanism of maintenance of skeletal muscle stem cells [also known as satellite cells (SCs)] quiescence via the di-methyltransferase Suv4-20h1, regulator of heterochromatin formation. Conditional ablation of Suv4-20h1 in SCs leads notably to the loss of the histone modification H4K20me2 on the distal regulatory element of *Myod* combined with a relocation of the *Myod* locus toward a central position in the nucleus. This switch in nuclear compartment is correlated with decreased facultative H3K27me3 associated heterochromatin, and an increase in spontaneously activated MYOD-expressing SCs in homeostatic muscles. Consequently, Suv4-20h1 knock-out SCs demonstrate compromised stem cell potential, as they fail to efficiently self-renew and replenish the SC pool upon muscle injury. Strikingly, restoring MYOD expression alone rescues the levels of facultative chromatin and reverses the loss-of-quiescence phenotype.

Keywords: H3K27me3; MYOD; Suv4-20h1; muscle regeneration; muscle stem cells; quiescence

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The quiescent state of a stem cell is defined by the ability of a cell to remain outside of the cell cycle while maintaining the capacity to proliferate and engage in differentiation and self-renewal programs upon reception of specific signals. Understanding quiescence regulation is critical to translate stem cell knowledge to the medical field where the maintenance and/or restoration of a functional stem cell pool is a promising therapeutic strategy.

The satellite cells (SCs) are the resident stem cells of skeletal muscle tissue. SCs express the paired box factor transcription factor PAX7 and are in a quiescent state in resting adult muscle (1). Upon activation, the SCs induce a sequence of transcription factors defining the various state of the cell, such as activation ($PAX7^+MYOD^+$), differentiation ($MYOG^+MYOD^+PAX7^-$) and renewal ($PAX7^+MYOD^-$). During this sequence the activated SCs proliferate and eventually fuse to form new myofibers while a subpopulation self-renew, return to quiescence to maintain the stem cell pool (2,3).

The recent advancement in sequencing technologies has allowed to define in more detail the quiescent state via transcriptome analysis and profiling of epigenetic marks, including histone modifications. Histones modifications are post-translational modifications of specific histones amino-acids linked with gene expression during cell fate maintenance and/or cellular differentiation (4). Specific histones modifications have been characterized for active enhancers (H3K27ac), active promoters (H3K4me3), silenced genes (H3K27me3) or heterochromatin (H4K20me3). The histone modifications landscape is cell-specific and a determinant of cellular identity and functions. Cell fate changes such as differentiation are accompanied by specific remodeling of the pattern of histones modifications via different epigenetic enzymes such as (de)methylases and (de)acetylases.

Few groups have, however, so far shown how histone

modifications are linked with muscle stem cell function: in 2013, Liu and colleagues (5) described the transcriptome and some epigenetic features (H3K27me3, H3K4me3 and H3K36me3) of quiescent and activated SCs. In a complementary study, Boonsanay and colleagues (6) recently demonstrated the key role for the di-methyltransferase Suv4-20h1 in maintaining quiescence via the histone modification H4K20me2 in SCs. H3K20me2 is a histone modification catalyzed by Suv4-20h1 associated with response to DNA damage and DNA replication and a substrate for H4K20me3 formation (7,8).

Specifically, Boonsanay and colleagues show that disruption of Suv4-20h1 in SCs leads to a striking reduction of H4K20me2 associated with a significant loss of heterochromatin. This loss of H4K20me2 is coupled with an impaired quiescence: immunostaining of muscle sections shows a strong increase of PAX7$^+$/MYOD$^+$ satellite cells in the resting state. The double labeling PAX7$^+$/MYOD$^+$ is the signature of aberrant satellite cells activation (normally PAX7$^+$/MYOD$^-$), a condition rarely observed in resting muscles. Increased activation of satellite cells is also supported by an elevated number of cycling Pax7$^+$ cells in vivo. However, it is surprising that the authors also observed a major reduction in H3K27me3, a marker of facultative heterochromatin (fHC, heterochromatin mostly composed of silent genes that can be regulated by epigenetic enzymes) as this mark is not directly regulated by Suv4-20h1.

The authors also performed three subsequent muscle injuries to explore the regenerative and self-renewal capacities of satellite cells deleted for *Suv4-20h1*. As predicted they observed a significant decrease in muscle mass and a massive loss of PAX7$^+$ cells. These findings are in agreement with the loss-of-quiescence observed in resting muscles.

Using chromatin immuno-precipitation coupled with qPCR (ChIP-qPCR), the authors then showed a loss of Suv4-20h1 induced H4K20me2 at the DRR regulatory region of *Myod*. Strikingly this epigenetic change was associated with a re-positioning of the *Myod* locus from the periphery of the nucleus toward a central position using fluorescence *in situ* hybridization (FISH). Previous studies have correlated such changes in nuclear localization with increased transcriptional activity. The researchers then demonstrated that crossing the satellite cell-specific conditional *Suv4-20h1* mutant with *Myod*$^{+/-}$ mice, hence adjusting the MYOD levels to normal, was sufficient to partially rescue the skeletal muscle phenotype. Even though the expression of MYOD is not precisely quantified, the authors show a robust amelioration of the mutant phenotype: the SCs showed significantly fewer PAX7$^+$/MYOD$^+$ co-staining, suggesting a partial rescue of quiescence and electron microscopy demonstrated that heterochromatin content was partially restored. Injury experiments indicate that regeneration is functionally rescued, since the number of Pax7$^+$ cells and the muscle mass are similar to control following the three muscle injuries. Of interest, the authors also observed that the levels of H3K27me3 returns to normal values, suggesting that loss of fHC is downstream of MYOD.

The authors propose a model whereby Suv4-20h1 is required to actively maintain the quiescent state through H4K20me2 formation at the DRR region of *Myod*. Loss of this epigenetic mark is correlated with a delocalization of the *Myod* locus toward the center of the nucleus and its transcriptional activation. This leads to SCs activation and to a reduction of H3K27me3 associated facultative heterochromatin (by a mechanism that still requires elucidation).

The work presented in this study provides robust evidences that the quiescent state is actively maintained. Nevertheless the observed pattern of H3K27me3 (a mark of silenced genes) conflict previous work: a precedent report found H3K27me3 levels being low during quiescence and high during activation of SCs (3) while Boonsanay and colleagues found H3K27me3 levels to be high in both quiescent and activated SCs. Divergent methodologies (immunofluorescence based quantification on isolated fibers versus ChIP-sequencing on sorted cells) and differences in the age of the animal models or different antibodies could underlie the observed discrepancies.

While the work from Boonsanay and colleagues is focused on a key epigenetic modifier, additional studies will be required to precisely understand the epigenetic landscape of quiescent muscle stem cells. Many questions remain regarding the molecular events underlying the maintenance of quiescence, early activation and renewal of satellite cells, including the potential involvement of *Suv4-20h1* in these processes.

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Footnote

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References

1. Buckingham M, Relaix F. PAX3 and PAX7 as upstream regulators of myogenesis. Semin Cell Dev Biol 2015;44:115-25.
2. Zammit PS, Golding JP, Nagata Y, et al.Muscle satellite cells adopt divergent fates: a mechanism for self-renewal? J Cell Biol 2004;166:347-57.
3. Zammit PS, Relaix F, Nagata Y, et al. Pax7 and myogenic progression in skeletal muscle satellite cells. J Cell Sci 2006;119:1824-32.
4. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res 2011;21:381-95.
5. Liu L, Cheung TH, Charville GW, et al. Chromatin modifications as determinants of muscle stem cell quiescence and chronological aging. Cell Rep 2013;4:189-204.
6. Boonsanay V, Zhang T, Georgieva A, et al. Regulation of skeletal muscle stem cell quiescence by suv4-20h1-dependent facultative heterochromatin formation. Cell Stem Cell 2016;18:229-42.
7. Schotta G, Sengupta R, Kubicek S, et al. A chromatin-wide transition to H4K20 monomethylation impairs genome integrity and programmed DNA rearrangements in the mouse. Genes Dev 2008;22:2048-61.
8. Kuo AJ, Song J, Cheung P, et al. The BAH domain of ORC1 links H4K20me2 to DNA replication licensing and Meier-Gorlin syndrome. Nature 2012;484:115-9.

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