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Editorial

Muscle gets stressed? p53 represses and protects

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Long-living tissues, such as skeletal muscles and neurons, are composed of terminally differentiated cells that irreversibly withdraw the cell cycle and therefore do not have the opportunity to cyclically monitor the integrity of their genome, by means of cell cycle checkpoints, as dividing cells do.[1] Moreover, terminally differentiated cells have an impaired DNA-repair machinery[2] and are typically resistant to apoptosis[3]—an intrinsic trade-off that preserves survival of these tissues throughout the lifespan, at the expense of their nuclear turnover. Thus, the genomic stability of post-mitotic tissues is highly dependent on the accuracy by which progenitor cells protect the integrity of their genome before differentiating.

About a decade ago, it has been proposed a mechanism that safeguards the genomic integrity of terminally differentiated skeletal myofibers, via activation of a myogenic differentiation checkpoint in muscle progenitors exposed to genotoxic stress.[4] Upon DNA damage, myoblasts prioritize DNA repair over the activation of the myogenic program, by transient inhibition of muscle gene expression, via ABL-mediated MyoD tyrosine phosphorylation, to avoid the formation of terminally differentiated myofibers carrying unrepaired DNA. Further studies have shown that the differentiation checkpoint is superimposed to DNA damage-activated checkpoints at the G1 or G2 boundaries of the cell cycle.[5] Interestingly, the differentiation checkpoint seems to be restricted to progenitors of long-living tissues, as tissues with high turnover, such as skin and hematopoietic cells, appear to adopt the opposite strategy—that is, the acute induction of differentiation—to safeguard the integrity of their genome in response to excessive DNA damage.[6,7] Conceivably, in these tissues, confining aberrant DNA structures and aneuploidy into differentiated progeny is tolerated by virtue of their high turnover, while protecting against the oncogenic potential of stem cells with genomic instability.[8]

p53 is a DNA sequence-specific transcription factor that is activated in response to DNA damage and has a central role of in the maintenance of the genome integrity, by inducing cell cycle arrest at specific checkpoints to allow DNA repair.[9] Alternatively, p53 eliminates genetically unstable cells, by inducing apoptosis, or limits their expansion by activating the senescence program. In all these circumstances, DNA damage-activated p53 binds the DNA of target genes that promote cell cycle arrest, apoptosis or senescence.[10]

In this issue, Yang *et al.* propose a role of p53 in the activation of the myogenic differentiation checkpoint that relies on direct repression of myogenin—a MyoD downstream target gene, whose activation is required for myoblast progression toward terminal differentiation into multinucleated myotubes.[11,12] This finding is of particular interest, when considering that in unperturbed myoblasts p53 rather seems to contribute to muscle differentiation,[13,14] as it suggests that in myoblasts the activity of p53 is biased toward inhibiting differentiation by the DNA damage signaling. The authors first discovered the transcriptional repression of myogenin by p53 in the human rhabdomyosarcoma RD cell line. These cells typically express myogenin and are homozygous for p53 Arg248Trp mutation, which impairs DNA binding. When the authors restored wild-type protein by tamoxifen-inducible p53-estrogen receptor fusion, they observed that while the p53-downstream p21 gene was induced and led to cell cycle arrest in G1 phase, myogenin was repressed in a dose-dependent manner by ectopic p53. Interestingly, the authors observed that bypassing p53 by ectopic expression of p21 in RD cells results into G1 arrest, followed by induction of myogenin and enhanced differentiation. This finding prompted an interest toward investigating the mechanism by which p53 could repress myogenin transcription in RD cells. As RMS are tumors bearing high-genomic instability and aneuploidy, which leads to persistent activation of DNA damage response (DDR), the authors surmised that DDR was necessary for p53-mediated repression of myogenin. Therefore, they extended their experiments to mouse embryonic fibroblasts (MEFs) treated with DNA damage-inducing irradiation (IR). These experiments identified a putative p53 response element (p53RE) in the regulatory elements of myogenin locus that mediates p53-dependent repression of myogenin. Interestingly, the p53RE coincided with a distal enhancer of the mouse myogenin gene, recently annotated by genome-wide studies.[15] Using quantitative ChIP analyses, Wang *et al.* found decreased levels of the histone marks H3K27Ac that is typically associated with active enhancers, in myoblasts exposed to IR, suggesting that p53-dependent repression of myogenin could be mediated by preventing the activation of a distal enhancer.

Finally, by using MEF conversion into skeletal muscle up on ectopic expression of MyoD, the authors show that p53 expression prevents the formation of terminally differentiated

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myotubes carrying genomic instability, when derived from MyoD-expressing MEFs exposed to IR. Indeed, bypassing the 'myogenic differentiation checkpoint' in these cells by either p53 deficiency or ectopic expression of myogenin led to the formation of myotubes with abnormal nuclei that presumably reflect genomic instability. The authors concluded that p53 mediates a quality control program in myogenic progenitors by transient repression of the differentiation program, at the stage of myogenin transcription, to temporally coordinate DNA damage repair and muscle gene expression, when the differentiation program intersects with the cell cycle checkpoint control.

Although this work clearly assigns to p53 an essential function in the 'myogenic differentiation checkpoint', it also raises the question of what is the relationship between p53-mediated repression myogenin and the previously described ABL-dependent inhibition of MyoD-mediated transcription in myoblasts exposed to genotoxic agents. In both cases, repression is transient and occurs during the G1 cell cycle checkpoint. However, these two mechanisms could be separated and/or interconnected. For instance, they can act redundantly, with one compensating for the failure of the other. Alternatively, ABL-MyoD signaling and p53-mediated repression could work sequentially. In this regard, it is interesting to note that ABL-MyoD signaling also contributes to DNA repair, with ABL-mediated phosphorylation of MyoD promoting DNA repair while repressing its transcriptional activity.[16] Thus, DNA damage-activated p53 might restrict the activation of ABL-MyoD signaling to a specific cell cycle window, by arresting the cell proliferation, via p21 induction. Future genome-wide analysis of MyoD- and p53-chromatin binding, as well as histone modifications, in myoblasts exposed to genotoxic stress should clarify this issue. Likewise, elucidating the kinetics of ABL-MyoD and p53 activation will help understanding the relationship between these two components of the myogenic differentiation checkpoint.

Nonetheless, a direct connection might exist between ABL-mediated repression of MyoD activity and p53-mediated repression of myogenin expression. Functional interactions between nuclear ABL and p53 have been described in various cell types exposed to DNA damage.[17] A common partner of p53 and MyoD is p300/CBP[18]—a transcriptional co-activator that promotes transcription by virtue of its intrinsic acetyltranferase activity. In particular, p300/CBP-mediated H3K27 acetylation has been associated to enhancer activation.[19] As the authors showed that p53-mediated repression of myogenin correlates with reduced H3K27 acetylation at the p53-bound enhancer, it is possible that reversible modulation of the enzymatic activity of p300/CBP by acute DNA damage is implicated in the myogenic differentiation checkpoint. This hypothesis is consistent with the previous observation that transient MyoD inactivation by ABL occurs by tyrosine phosphorylation within the N-terminal domain that mediates interactions with p300/CBP.[4,20] Still, it is puzzling that in response to DNA damage, p53 both activates p21 transcription and represses myogenin transcription. How can p53 simultaneously activate and repress different subset of genes in the same cell? Answering this outstanding question will further elucidate the molecular mechanism underlying the myogenic differentiation checkpoint.

Overall, the differentiation checkpoint appears to rely on a fascinating interplay between DNA transcriptional activators and coactivators in response to DDR (Figure 1).

As the myogenic differentiation checkpoint safeguards the integrity of the genome of skeletal muscles, which

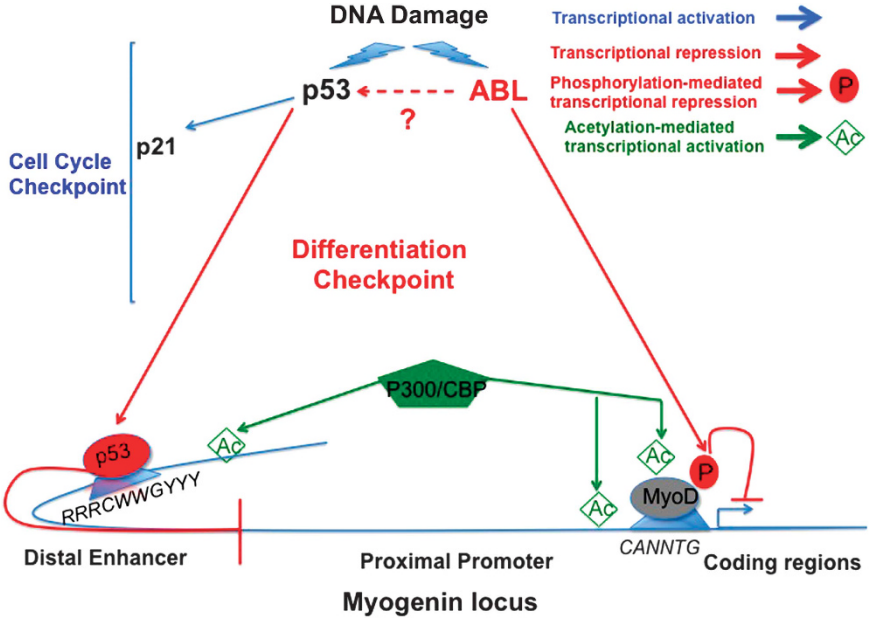

Figure 1 Schematic representation of the DNA damage-activated networks contributing to the myogenic differentiation checkpoint. p53-mediated activation of the cyclin-dependent kinase inhibitor p21 promotes cell cycle arrest at specific checkpoints, to execute the DNA repair in myoblasts exposed to genotoxic agents. Simultaneous binding of p53 to the sequence RRRCWWGYYY (R = purine, W = adenine or thymine and Y = pyrimidine) located in one distal enhancer of myogenin gene represses myogenin transcription. In a parallel signaling, DNA damage-activated ABL tyrosine kinase inactivates MyoD transcriptional activity by phosphorylation of tyrosine 30 within the N-terminal domain, thereby inhibiting myogenin transcription. These two mechanisms of myogenin repression might relies on a common effector—the acetyltransferase p300/CBP—as p53 binding to myogenin enhancer decreases H3K27 acetylation (H3k27Ac) and ABL phosphorylates MyoD on a tyrosine residue previously implicated in the interaction with p300/CBP[20]

account for the most abundant and widely distributed tissue in mammals, future studies should also establish the relationship between proper activation of the differentiation checkpoint and age-associated decline of muscle performance and other pathogenic conditions. Among them, several myopathies and atrophic conditions have been associated with deficient or aberrant activation of p53. Likewise, it will be interesting to elucidate the relationship between age-associated sources of DNA, such as cellular senescence and chronic exposure to stress or inflammation, and the persistent activation of the myogenic differentiation checkpoint as potential mechanism of muscle atrophy.

Conflict of Interest

The authors declare no conflict of interest.

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