deficiency of the receptor for interleukin-4 (IL-4, a type 2 cytokine) mitigated the biogenesis of beige fat[3].

The findings from Suárez-Zamorano *et al.*[3] provide an additional link between the microbiota and the gut-adipose axis[8] in obesity. More work is needed to unravel the mechanism(s) through which the microbiota influence fat browning in mice. For example, restoration of the gut microbiota in germ-free mice suppresses the expression of the fasting-induced adipocyte factor[9] (FIAF, an inhibitor of lipoprotein lipase) in adipose tissue, liver and intestines, and dietary saturated fat–induced alterations in the microbiota can both exacerbate pro-inflammatory responses in the WAT and impair insulin sensitivity[10] in mice. These processes may also influence the browning of fat in microbiota-ablated mice.

Determining which gut bacteria and which metabolites inhibit WAT browning in mice will also be important. However, the identification of non-invasive authentic and universal biomarkers of beige fat biogenesis and activation is an essential first step in the process to supplant the expensive and tedious imaging techniques that are currently in use. Once identified, these biomarkers can be integrated with multiple 'omics' approaches such as metagenomics, metatranscriptomics, lipidomics, glycomics and metabolomics to shed more light on the microbial regulation of browning and de-browning cascades in adipose tissue. A reductionist approach starting with the delineation of whether Gram-positive and/or Gram-negative bacteria are involved, followed by the monocolonization of germ-free mice with specific bacterial species that belong to major gut bacterial phyla, would reveal which gut bacteria are involved. Metabolomics approaches could then identify the metabolite(s) that connect these bacteria to beige fat adipogenesis.

Finally, additional work is needed to determine whether this process operates in humans, and whether these findings can be exploited for long-term therapeutic gain. For example, it is unclear how these findings fit into the large body of work suggesting that frequent antibiotic exposure in infancy and childhood promotes weight gain[11,12]. In addition, although microbiota ablation by means of antibiotics treatment may be feasible in mice, such an approach may not be a sustainable treatment option for humans. Mechanistic studies will help to determine whether approaches based on fecal transplantation, probiotics and/or targeting of specific microbiotal metabolite(s) would promote WAT-to-BAT polarization, and whether these therapeutic strategies are viable for weight management and the treatment of metabolic dysfunction, obesity or type 2 diabetes.

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1. Cannon, B. & Nedergaard, J. *Physiol. Rev.* **84**, 277–359 (2004).
2. Lidell, M.E., Betz, M.J. & Enerback, S. *J. Intern. Med.* **276**, 364–377 (2014).
3. Suárez-Zamorano, N. *et al. Nat. Med.* **21**, 1497–1501 (2015).
4. Lee, M.W. *et al. Cell* **160**, 74–87 (2015).
5. Brestoff, J.R. *et al. Nature* **519**, 242–246 (2015).
6. Qiu, Y. *et al. Cell* **157**, 1292–1308 (2014).
7. Herbst, T. *et al. Am. J. Respir. Crit. Care Med.* **184**, 198–205 (2011).
8. Mestdagh, R. *et al. J. Proteome Res.* **11**, 620–630 (2012).
9. Bäckhed, F. *et al. Proc. Natl. Acad. Sci. USA* **101**, 15718–15723 (2004).
10. Caesar, R., Tremaroli, V., Kovatcheva-Datchary, P., Cani, P.D. & Backhed, F. *Cell Metab.* **22**, 658–668 (2015).
11. Trasande, L. *et al. Int. J. Obes.* **37**, 16–23 (2013).
12. Cox, L.M. *et al. Cell* **158**, 705–721 (2014).

A new role for dystrophin in muscle stem cells

Alexandra C Keefe & Gabrielle Kardon

Duchenne muscular dystrophy (DMD) is a devastating X-linked disease that is characterized by progressive muscle degeneration and caused by mutations in dystrophin. Dystrophin is critical for myofiber structural integrity, but a new study reveals an additional important role for this protein in muscle stem cells.

DMD was first described in the 1860s by the French neurologist Guillaume Duchenne as a disease of progressive muscle weakness in which muscle fibers are frail and surrounded by abundant fibrotic connective tissue[1]. Mutations in the gene encoding dystrophin, *DMD,* are responsible for causing DMD. Dystrophin is a rod-shaped protein that is expressed primarily by differentiated myofibers[2]. Dystrophin, in combination with a complex of other proteins, connects the intracellular cytoskeleton of a myofiber through the cell membrane to the surrounding extracellular matrix; it thus stabilizes the myofiber membrane during muscle contraction. Mutations in *DMD* lead to muscle cell membrane fragility, contraction-induced damage and muscle degeneration. Chronic, successive rounds of muscle degeneration and regeneration with attendant fibrosis and inflammation lead to a loss of muscle mass and function and, ultimately, death. To date, work on the role of dystrophin in muscles has focused exclusively on its requirement in differentiated muscle fibers. However, Dumont *et al.*[3] now show a surprising new role for dystrophin—one in muscle stem cells.

Myofibers are postmitotic, and they are regenerated by a dedicated population of resident muscle stem cells called satellite cells[4]. Quiescent satellite cells reside in a niche between a muscle fiber's cell membrane and its basement lamina. In response to myofiber damage, satellite cells are activated. They then become committed myoblasts and migrate to sites of injury, where differentiated myocytes fuse either with each other or with existing myofibers to repair muscle damage. In addition, some satellite cells or their progeny return to the niche to maintain the population of quiescent satellite cells. Reflecting these dual fates, satellite cells have been found to be a heterogeneous population; some satellite cells are prone to differentiate into myoblasts, whereas others serve as satellite stem cells (identified by Dumont *et al.*[3] via the absence of the transcription factor myogenic factor 5 (Myf5)) responsible for self-renewal.

In DMD, the constant degeneration of dystrophic myofibers necessitates the continuous regeneration of damaged muscle by satellite cells. The progressive loss of muscle mass in people with DMD suggests that, over time, satellite cells become unable to replenish myofibers, possibly owing to a depletion of satellite cell numbers or a decrease in their regenerative function. Indeed, previous studies have

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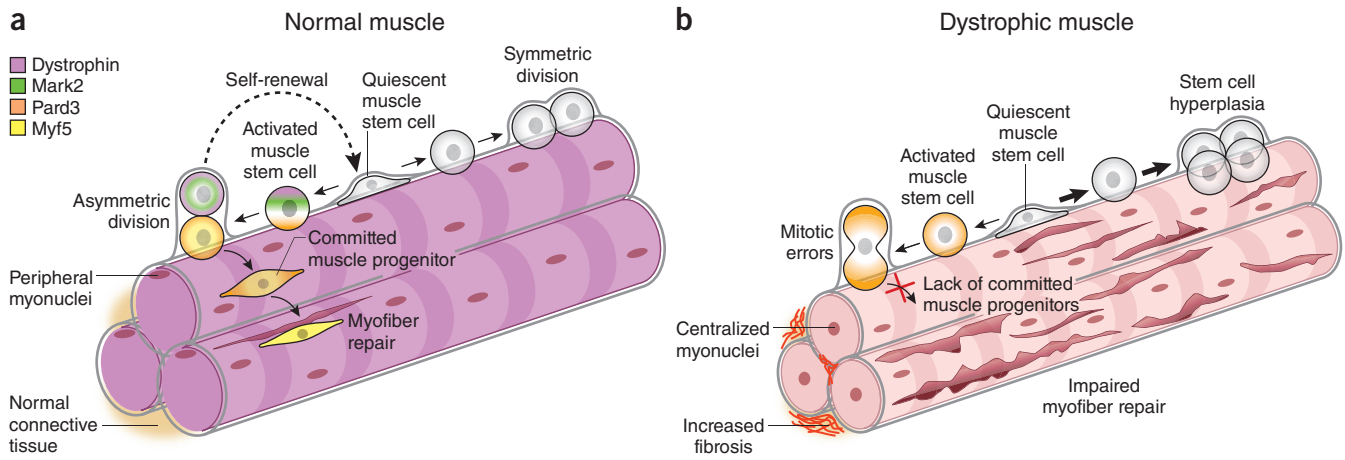

Figure 1 The role of dystrophin in satellite cells in Duchenne muscular dystrophy (DMD). (**a**) In normal muscle, dystrophin is expressed in activated satellite cells and in differentiated myofibers. In activated satellite cells, localized dystrophin expression promotes the polarization of Mark2, and of Pard3 to opposite sides of the dividing cell. This polarization promotes asymmetric cell divisions, leading to the generation of satellite stem cells that lack Myf5 and committed muscle progenitors. In myofibers, dystrophin maintains muscle membrane integrity. (**b**) Dumont *et al.*[3] show that dystrophin-null satellite cells have a loss of Par-mediated cell polarity, leading to cell division errors and a decrease in asymmetric cell divisions. The resulting decrease in differentiated myocytes leads to impaired regeneration, and the impaired regeneration of dystrophin-null satellite cells combined with degeneration of dystrophin-null myofibers leads to progressive muscle loss.

demonstrated that in mice lacking dystrophin, the DMD phenotype is significantly worsened if satellite cells are dysfunctional[5]. Nevertheless, any effect on satellite cell numbers or function in DMD was presumed to be indirect, because dystrophin is so highly expressed in muscle fibers and was never reported to be expressed in myoblasts.

Dumont and colleagues[3] make the striking observation that dystrophin is expressed by satellite cells. By using a combination of microarrays and immunofluorescence on satellite cells freshly isolated from mice, they find that dystrophin is transiently expressed during the brief window when satellite cells are activated, but not after these cells become committed myoblasts. Similarly to other types of stem cells, satellite cells have been found to undergo both symmetric cell division (allowing for satellite cell expansion) as well as asymmetric cell division, enabling the generation of two distinct daughter cells—one that maintains its stem cell characteristics and one that differentiates. Intriguingly, the authors report[3] that in most activated satellite cells expressing dystrophin, the protein becomes polarized to the membrane of one side of the cell (**Fig. 1**). After asymmetric cell division occurs, dystrophin is preferentially found in the more stem cell–like daughter cell that does not express Myf5.

Recent research has shown that the evolutionarily conserved Par complex regulates asymmetric division of satellite cells[6]. In myofibers, the Par complex protein MAP/microtubule affinity regulating kinase 2 (Mark2, also known as cell polarity–regulating kinase Par1b) binds dystrophin[7]. This prompted Dumont *et al.*[3] to examine the relationships of dystrophin, Mark2 and other Par complex proteins in satellite cells. By using a proximity ligation assay (a technique that uses a combination of antibodies and oligonucleotide tags to reveal protein interactions *in situ*), they elegantly demonstrate that dystrophin is strongly associated with Mark2 in isolated mouse satellite cells. The localization of Mark2 and par-3 family cell polarity regulator (Pard3) to opposite sides of a cell is crucial for the establishment of cell polarity[8]; consistent with this observation, dystrophin never localizes with Pard3 (**Fig. 1**). To determine whether dystrophin regulates the localization of Par proteins or vice versa, Dumont *et al.*[3] analyzed activated satellite cells lacking dystrophin. They find that, in these cells, Mark2 levels are low and Pard3 is mislocalized around the entire periphery of the activated cells. Conversely, in activated satellite cells that lack Mark2, dystrophin remains polarized. The recruitment of dystrophin to localized membrane regions seems therefore to be the initiating event for satellite cell asymmetry. Dystrophin, in turn, may become polarized in response to extracellular cues (although the exact nature of these cues is not yet known), given that dystrophin was also found to interact with integrin α7, a receptor for extracellular laminin.

The Par complex regulates centrosomes and the alignment of mitotic spindles[8]. The authors therefore analyzed mitotic centrosomes in satellite cells lacking dystrophin. They found a striking increase in the number of abnormal mitotic divisions as well as a decrease in the number of asymmetric cell divisions (**Fig. 1**). As a consequence of the reduced number of asymmetric divisions, significantly fewer differentiating myocytes are generated in mice lacking dystrophin, thus leading to an impaired regenerative response. Cells that lack Mark2 or Pard3 also experience a decrease in satellite cell asymmetric divisions and generate fewer differentiating myocytes, which confirms previous findings[6]. This suggests that an essential function for dystrophin in satellite cells is its regulation of the Par complex. Interestingly, Dumont *et al.*[3] observe that satellite cells lacking dystrophin generate a greater number of Myf5-negative satellite cell stem cells *in vitro* (presumably because of expansion via symmetric cell division). Some (but not all) studies have also found that people with DMD have an increased number of satellite cells[9]. Together, these observations suggest that it is not the depletion of satellite cells, but rather the dysfunction of satellite cells in muscle regeneration that leads to the progressive decline in muscle mass and function in those with DMD.

Dumont and colleagues[3] thus clearly show that DMD is a disease that not only disrupts the structural integrity of differentiated myofibers, but that also affects muscle stem cell function. The extent to which satellite cell dysfunction contributes to the severity and progression of

DMD awaits future research. Experiments in mice lacking dystrophin specifically in satellite cells or myofibers will enable researchers to dissect dystrophin's role in these two cell populations. Another interesting question is whether the devastating connective tissue fibrosis associated with DMD results, in part, from satellite cell dysfunction. Over time, damaged muscle is replaced progressively by fibrotic tissue, and these secondary changes to the muscle are a major contributor to DMD pathology. Changes in satellite cell function could contribute to this fibrosis. Previous studies of muscle regeneration have shown that satellite cells dynamically regulate the number of connective tissue fibroblasts[10]; the observed increase in satellite stem cells may secondarily lead to an expansion of these fibroblasts and the connective tissue that they produce. Alternatively, the increased population of satellite stem cells lacking dystrophin may itself be a source of connective tissue fibrosis, given that satellite cells can convert to a fibrogenic fate under some conditions[11]. Co-culture and lineage-tracing experiments could test these hypotheses.

Finally, this study has important implications for DMD therapeutic interventions. Clinical trials are currently testing direct replacement of the dystrophin gene via viral vectors. However, these therapies are almost exclusively targeted to the differentiated muscle, and given dystrophin's large size and the limits of viral packaging, only reduced versions of the *DMD* gene (containing only the presumed essential regions encoding dystrophin) are currently being tested. These 'mini-dystrophin' genes do not contain the spectrin repeats 8 and 9, which are required for Mark2 binding[12], and so they are unlikely to rescue satellite cell dysfunction in DMD. In future, gene therapy may need to include these regions in order to rescue dystrophin function in both muscle fibers and satellite cells.

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1. Duchenne, G.B. *Arch. Gen. Med.* **11**, 552–588 (1868).
2. Rahimov, F. & Kunkel, L.M. *J. Cell Biol.* **201**, 499–510 (2013).
3. Dumont, N. *et al. Nat. Med.* **21**, 1455–1463 (2015).
4. Relaix, F. & Zammit, P.S. *Development* **139**, 2845–2856 (2012).
5. Sacco, A. *et al. Cell* **143**, 1059–1071 (2010).
6. Troy, A. *et al. Cell Stem Cell* **11**, 541–553 (2012).
7. Yamashita, K. *et al. Biochem. Biophys. Res. Commun.* **391**, 812–817 (2010).
8. Neumüller, R.A. & Knoblich, J.A. *Genes Dev.* **23**, 2675–2699 (2009).
9. Kottlors, M. & Kirschner, J. *Cell Tissue Res.* **340**, 541–548 (2010).
10. Murphy, M.M., Lawson, J.A., Mathew, S.J., Hutcheson, D.A. & Kardon, G. *Development* **138**, 3625–3637 (2011).
11. Brack, A.S. *et al. Science* **317**, 807–810 (2007).
12. Fairclough, R.J., Wood, M.J. & Davies, K.E. *Nat. Rev. Genet.* **14**, 373–378 (2013).

PARP inhibitors: a treatment option for AML?

Lan Wang, Pierre-Jacques Hamard & Stephen D Nimer

A new study provides a rationale for the use of poly (ADP-ribose) polymerase (PARP) inhibitors to trigger irreparable DNA damage as a therapeutic approach in acute myeloid leukemia (AML). It also provides support for combining PARP inhibitors with agents that reduce HOXA9 protein levels.

We continue to struggle to make any clinical headway in the treatment of AML, despite a massive expansion in our knowledge of the underlying genetic abnormalities that drive its growth. AML remains one of the most difficult diseases to treat, and there has been no improvement in the survival of people with AML since the relevant chemotherapy was first initiated in the 1970s. The sole exception is those with acute promyelocytic leukemia (APL), who now have a greater than 90% chance of long-term survival with all-*trans* retinoic acid and arsenic trioxide–based therapies[1].

The first individual ever to have his or her cancer genome sequenced had AML[2]. This effort, followed by the sequencing of genomes from hundreds of other AML patients by The Cancer Genome Atlas project, has shown the tremendous heterogeneity of AML. Nonetheless, certain chromosomal translocations are recurrent in AML genomes: for example, t(15;17), which generates the *PML-RARA* fusion gene, is found in over 90% of APL cases. t(8;21), usually seen in M2 AML, generates the fusion oncogene *RUNX1-RUNX1T1* (encoding the transcription factor AML1-ETO). t(9;11), which generates the *MLL-AF9* fusion oncogene, occurs in various subtypes of AML, with particular prevalence in monocytoid AML genomes.

A study by Esposito *et al.*[3], published in this issue of *Nature Medicine*, is the first to report that the oncogenic products of these fusion genes determine the responsiveness of human AML cells to PARP inhibition. The poly (ADP-ribosyl)ation of nuclear proteins by PARP enzymes has an important role in DNA repair, especially in the base excision repair process. PARP inhibitors (PARPis) can target this critical function; they have been used both alone and with chemotherapy to trigger cell death in a variety of solid tumors, especially those with limited DNA damage repair capacity resulting from genetic or epigenetic mechanisms. PARPis can trigger synthetic lethality in such cells, which may have germline or acquired mutations in *BRCA1* and *2* (*BRCA1/2*), or in other components of the homologous recombination (HR) DNA repair process, such as *RAD51*, *PALB2*, *CHK2*, *ATM* or *PTEN*. Clinical evaluation of PARPis has shown them to have efficacy in people with germline *BRCA* mutant ovarian cancer (which led to US Food and Drug Administration (FDA) approval); in select individuals with breast cancer; and most recently, in individuals with prostate cancer who acquired mutations in *BRCA1/2* or ataxia telangiectasia–mutated (*ATM*)[4].

Esposito *et al.*[3] demonstrate that mouse hematopoietic cells transformed by the AML1-ETO and PML-RARα fusion proteins display a higher level of DNA damage than do those transformed by MLL-AF9. They also show that AML1-ETO and PML-RARα confer sensitivity to the PARPis veliparib and olaparib, whereas MLL-AF9 confers resistance. They find that sensitivity to PARPis is correlated with changes in the expression of genes that control the DNA damage response (DDR); thus, AML cells with lower expression of key members of the DDR pathway, including *Rad51*, *Atm*, *Brca1* and *Brca2*, display increased sensitivity to PARPis. They also show that PARP inhibition triggers the senescence and differentiation of sensitive

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