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Commentary

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Illuminating regeneration: noninvasive imaging of disease progression in muscular dystrophy

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Muscular dystrophies are characterized by progressive muscle weakness and wasting. Among the key obstacles to the development of therapies is the absence of an assay to monitor disease progression in live animals. In this issue of the *JCI*, Maguire and colleagues use noninvasive bioluminescence imaging to monitor luciferase activity in mice expressing an inducible luciferase reporter gene in satellite cells. These cells proliferate in response to degeneration, therefore increasing the level of luciferase expression in dystrophic muscle.

Introduction

Skeletal muscle has a robust regenerative capacity, with rapid reestablishment of full strength, even after severe damage to the tissue. Regeneration is mediated by muscle stem cells, called satellite cells. In response to muscle damage, satellite cells proliferate, differentiate into myoblasts, and fuse into myotubes, which act to repair damaged muscle. In muscular dystrophies, continuous muscle degeneration is accompanied by regeneration of muscle fibers mediated by satellite cell progeny (1).

Currently, the standard method for evaluating disease progression in muscular dystrophy animal models is muscle histopathology. This approach is labor intensive, as it involves the removal and processing of the tissue of interest, imaging of the slides, and analysis of the images. Furthermore, the invasiveness of this approach does not permit consecutive sampling, hindering the ability to evaluate the course of a disease or success of a therapeutic strategy. Other methods for evaluating muscle disease include behavior testing and force testing of the dissected muscle, although the specificity of the results obtained from these tests can often be difficult to assess. High levels of serum biomarkers, such as serum creatine kinase, can be indicative of muscle damage, but levels depend on muscle mass and can be widely variable over time in individual dystrophic mice (2).

Perhaps the best candidate technology for studying muscle disease in live animals is MRI, which can reveal the permeability of muscle fibers correlating with disease severity (3). While MRI is noninvasive, it is more expensive and less widely available than bioluminescence imaging systems in animal research laboratories.

A "regeneration reporter" mouse strain

The first group to use bioluminescence imaging to reveal satellite cell proliferation was Sacco et al., who transplanted a single luciferase-expressing satellite cell into the tibialis anterior (TA) muscle of NOD/SCID mice that were depleted of endogenous satellite cells by irradiation (4). They observed that a single luciferase-expressing satellite cell is capable of self renewal after transplantation. Further, they found a substantial increase in satellite cell proliferation, as indicated by increased bioluminescence values, in response to muscle tissue damage by notexin.

In this issue, Maguire et al. (5) utilized the *Pax7Cre*^{ER}*/LuSEAP* mouse first generated by Nishijo et al. (6) to develop a mouse model that could be used to monitor muscle regeneration in response to disease and injury. This mouse expresses a Cre-dependent firefly luciferase gene and an estrogen-responsive Cre-recombinase under the control of the Pax7 locus. Because satellite cells are the only muscle cells in the adult that express Pax7, these mice

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Figure 1
A "regeneration reporter" mouse strain. Pax7-positive muscle satellite cells express luciferase (indicated by green shading) following tamoxifen injection. Upon damage to muscle by either cardiotoxin injury or disease, satellite cells differentiate, and the progeny also express luciferase. Satellite cell progeny proliferate and differentiate into myotubes to repair the muscle, increasing the luciferase signal that can be detected by a bioluminescence imager.

express the bioreporter following tamoxifen treatment specifically in satellite cells. This expression is maintained as satellite cell progeny proliferate and differentiate into myotubes to repair the muscle, and expression of luciferase can be monitored by noninvasive, bioluminescence imaging (Figure 1). Nishijo et al. previously utilized the *Pax7Cre*^{ER}*/LuSEAP* mice to characterize satellite cell kinetics in growing postnatal muscle and found a doubling of the signal every 3.93 weeks from adolescence to young adulthood (6). Maguire et al. use the *Pax7Cre*^{ER}*/LuSEAP* mouse to assess satellite cell activation and proliferation as an indicator of regeneration. They first injured the TA muscles with cardiotoxin and compared the luciferase signal between the injured and uninjured limbs. Luciferase activity increased following injury, and immunohistochemical studies confirmed that luciferase-expressing cells were present in the injured TA as nascent myofibers and myotubes (5). This suggests that the noninvasive measurement of luciferase activity in the *Pax7Cre*^{ER}*/LuSEAP* strain is an accurate measure of the proliferative activity of satellite cells and therefore has potential to be used to measure muscle regeneration in muscular dystrophy models as well.

Using luciferase to monitor disease progression

A key feature of dystrophic muscle is the degeneration of mature muscle fibers and the subsequent fusion of satellite cell–derived myoblasts to form myotubes, which mature into fibers. This degeneration/regeneration is apparent by muscle histopathology, where regenerating fibers can be identified by their centrally located nuclei. Maguire and colleagues hypothesized that the *Pax7Cre*^{ER}*/LuSEAP* strain could be used to develop a noninvasive and quantifiable measure of regeneration activity in dystrophic mice. To test this, they crossed this

reporter strain with a dysferlin-deficient ($Dysf^{-/-}$) mouse model of limb girdle muscular dystrophy 2B (LGMD2B) (7, 8). Dysferlin is a transmembrane protein involved in calcium-mediated plasma membrane repair (9). Like human LGMD2B patients, $Dysf^{-/-}$ mice develop a slowly progressive muscular dystrophy, mainly in proximal limb muscles. Muscles from $Dysf^{-/-}$ mice exhibit centrally nucleated fibers, necrotic fibers, fat deposition, and inflammatory cell infiltrates (10).

The authors injected 2-month-old $Dysf^{-/-}$/*Pax7Cre*^{ER}/*LuSEAP* mice with tamoxifen to induce luciferase expression in satellite cells and found luciferase expression increased over time in the hind limb muscles of dysferlin-deficient, but not wild-type, mice. They found luciferase activity mainly in the proximal limb muscles as early as 3 months of age, with some involvement in the distal muscles starting at 6 months, and increasing luciferase activity in both proximal and distal muscle groups up to 18 months of age. Further examination of these mice showed a correlation between intensity of luciferase signal (as determined by bioluminescent imaging), number of luciferase positive fibers (as determined by immunohistochemistry), and extent of histopathology, including centrally nucleated fibers and fibers that express embryonic myosin heavy chain, a marker for newly regenerated myofibers (5). This validates luciferase imaging as an alternative to conventional measures of disease progression in muscular dystrophy.

There are several immediate advantages to using bioluminescent imaging to determine the extent of disease in muscular dystrophy models. First, the method is less labor intensive and more quantitative than classic histopathology. Second, measurements for individual mice are consistent and show patterns of luciferase activity that parallel the entire cohort. This suggests that a bioluminescence scheme for quantifying regeneration activity could be useful in therapeutic studies, in which each mouse could be used as its own pre- and posttreatment control. A third advantage to this system is the apparent increased sensitivity compared with conventional measures. The $Dysf^{-/-}$ mouse has been previously characterized as having a slowly progressive muscular dystrophy by 6 months of age, followed by rapid disease progression (7, 8). This is similar to other dysferlin-deficient LGMD2B mouse models (the naturally occurring A/J mice (11) and a targeted dysferlin knockout (12) both show active myopathy at approximately 6 to 8 months). Excitingly, the current paper finds a significant increase in luciferase expression in $Dysf^{-/-}$ mice as early as 3 months. This suggests that researchers may now have the opportunity to study the early pathophysiology of dysferlin deficiency and test the effectiveness of early therapeutic interventions.

One major limit to a bioluminescence-based reporter of regeneration activity is that it is light based. Therefore, luciferase signal is influenced by hair, skin pigmentation, thickness of skin and fat, and depth of tissue. This is particularly unfortunate in the case of the diaphragm, which is severely affected in several muscular dystrophies (especially Duchenne, ref. 13). Due to the depth of the diaphragm and the amount of tissue through which light must travel to image it, this method would not be particularly conducive to evaluating regeneration in the diaphragm. For researchers interested in regeneration activity in limb muscles, however, a bioluminescence strategy appears to have potential as a reliable and sensitive technique. Another disadvantage to this technique is the limited potential for an equivalent diagnostic tool for human patients. While therapeutic studies can be designed on animal models using luciferase activity as an indicator of regeneration activity, subsequent testing in humans will need to utilize a different technique, such as MRI imaging, to measure outcome. Finally, regeneration, while closely linked, may not be directly proportional to disease severity. Several mouse models have been generated that have regenerative defects (for examples, see ref. 14). Breeding these mice with the *Pax7Cre*^{ER}/*LuSEAP* reporter line may give an incomplete picture of disease progression. Researchers must keep this caveat in mind when utilizing this technique on mouse models where the satellite cell regenerative capacity has not yet been characterized.

Future applications

Maguire and colleagues have demonstrated that bioluminescence imaging of satellite cells is an accurate representation of regeneration activity in a mouse model of LGMD2B. By crossing the *Pax7Cre*^{ER}/*LuSEAP* mouse to other muscular dystrophy models (the *mdx* model of Duchenne, for example), this strategy can easily be used to study muscle degeneration and regeneration in any number of muscle diseases. Further, the *Pax7Cre*^{ER}/*LuSEAP* mouse can be used to examine satellite cell proliferation in models of atrophy and hypertrophy (hind limb suspension and external loading, respectively), as well as other neuromuscular disorders. In the case of LGMD2B, the authors found that luciferase activity was significantly increased compared with wild-type controls at ages as early as 3 months. Bioluminescence imaging may also reveal early time points at which a difference can be detected in other models of muscle disease, which could provide insights into disease mechanism and alter the timing in which researchers consider applying therapies.

The *Pax7Cre*^{ER}/*LuSEAP* mouse expresses luciferase in differentiated satellite cells, regardless of fate (myogenic, adipogenic, or fibrogenic). In the future, it would be interesting to utilize key transcription factors in order to design a reporter that could differentiate between self-renewed satellite cells and differentiated satellite cell progeny.

Compared to classical histology, which requires substantial effort in the dissection, processing, imaging, and analysis of each tissue, bioluminescence imaging is high throughput and quantitative. In addition, it is noninvasive and therefore it is ideal for studies in which each animal, and even each muscle, can be used as its own control. This technology is certainly an avenue investigators should consider when designing future studies of regeneration activity and therapeutic intervention in muscular disease.

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Where hypertension happens

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Essential hypertension, which accounts for 90%–95% of all cases of hypertension seen in the clinic, is also referred to as idiopathic hypertension, because we simply don't understand the cause(s). Although many theories have been advanced, in the current issue of the *JCI*, Gonzalez-Villalobos et al. present further evidence implicating the intrarenal renin-angiotensin system and take us one step further by proposing a mechanism underlying this pathology.

We know a lot about the environmental triggers for essential hypertension; there's no doubt that aging, obesity associated with increased insulin resistance, excessive alcohol intake, ethnicity, stress, and (in certain individuals) excessive dietary salt intake or inadequate dietary potassium or calcium can contribute to high blood pressure (1). Nevertheless, we don't have a clear picture of what happens in the body to translate most of these stimuli into a pathological condition. Any attendee of a recent hypertension research meeting will know that there is no shortage of hypotheses, including increased production of reactive oxygen species, enhanced reactivity of resistance blood vessels to vasoconstriction, vascular inflammation involving immune cells, and malfunctioning baroreceptors or triggers in the central nervous system. Although animal models exist in which each of these mechanisms can be shown to alter blood pressure and each may indeed contribute to a greater or lesser degree to elevating blood pressure in hypertensive patients, many lines of evidence suggest that it's in the kidney that it all comes together.

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The kidney as a critical hypertension locus

Several researchers have demonstrated that transplanting the kidneys of hypertensive rats into normotensive control rats rendered the recipients hypertensive (2–4). A molecular-era refinement of such transplant experiments suggests that the hypertension that travels with the kidney might be due in part to the renin-angiotensin system (RAS). In the classical RAS, angiotensinogen from the liver is cleaved by kidney-derived renin to form angiotensin I, which is subsequently cleaved by angiotensin-converting enzyme (ACE) present on the surface of endothelial cells throughout the body to generate the potent vasopeptide angiotensin II (Figure 1A). There are two angiotensin II receptors, and it is the angiotensin II receptor type 1 ($AT1_R$) subtype that increases blood pressure and fluid volume through vasoconstriction and stimulation of aldosterone secretion from the adrenal gland and salt retention by the kidneys. Coffman and colleagues transplanted the kidneys of $AT1_R$-deficient mice into control animals and vice versa (5). Subsequent infusion of these mice for two weeks with a high dose of angiotensin II revealed that the mice with $AT1_R$ expression in the kidney, but a complete lack of this receptor elsewhere in the body, developed hypertension and cardiac hypertrophy. Surprisingly, the mice that lacked $AT1_R$ in the kidney developed neither hypertension nor cardiac hypertrophy, despite expressing $AT1_R$ everywhere else in the body. The overall implication of these studies is that angiotensin II sensing within the kidney is a critical mediator of hypertension.

However, a model in which circulating angiotensin II causes hypertension by a direct action on the kidney fails to explain a long-standing clinical conundrum: how does ACE inhibition remain effective in lowering blood pressure in individuals whose circulating angiotensin II gradually returns to pretreatment levels (so-called angiotensin escape) (6)? In this issue of the *JCI*, Gonzalez-Villalobos et al. address this question by investigating the role of the intrarenal RAS in hypertension by inactivating the *Ace* gene in mouse kidney (7). The way that they achieved this was somewhat unconventional. They began with mice that had a whole-body inactivation of *Ace*. However, because the complete inactivation of any of the RAS genes, including *Ace*, results in severe renal defects and high neonatal lethality in both humans and mice (8), Gonzalez-Villalobos et al. generated *Ace* knockout mice that ectopically expressed ACE in either hepatocytes (ACE 3/3 mice)