Muscle: A Nontraditional 1,25-Dihydroxyvitamin D Target Tissue Exhibiting Classic Hormone-Dependent Vitamin D Receptor Actions

Investigations in both cell and animal models have demonstrated important effects of 1,25-dihydroxyvitamin D in several target tissues. These 1,25-dihydroxyvitamin D-responsive target tissues can be divided into two categories, the traditional target tissues, which contribute to the regulation of mineral ion homeostasis, and the nontraditional target tissues, which do not. Before the generation of vitamin D receptor (VDR) knockout mice, it remained uncertain which effects of 1,25-dihydroxyvitamin D observed in vitro were important in vivo and which of the effects observed in vitro were a result of classic hormone-dependent actions of the VDR. Studies in vitamin D-deficient animals were unable to satisfactorily resolve this issue because of two problems. First, these animals have profound abnormalities in mineral ion homeostasis, and it was unclear to what degree this was responsible for the phenotype observed vs. what was a direct consequence of vitamin D deficiency. Secondly, difficulties have been encountered rendering animals truly vitamin D deficient, because breeding must be done in UV-free conditions without vitamin D supplementation for several generations to obtain mice with absence of detectable circulating vitamin D metabolites. Furthermore, the generation of truly vitamin D-deficient animals has been complicated by the finding that they have decreased fertility and lifespan (1). Once again, whether this phenotype was a consequence of vitamin D deficiency or abnormalities in mineral ion homeostasis was uncertain.

The generation of VDR knockout mice and the ability to maintain normal mineral ion homeostasis in these mice using a diet enriched in calcium and lactose (2) has permitted investigations directed at identifying target tissues in which the actions of the VDR are critical for normal development, maturation, and homeostasis. These studies have demonstrated a redundant role for the VDR in two traditional target tissues and an important function in several nontraditional target tissues, including muscle.

Profound vitamin D deficiency, with its accompanying abnormalities in mineral ion homeostasis, can lead to a severe myopathy. Although vitamin D repletion leads to rapid resolution of the myopathy, it is uncertain what role abnormal mineral ion homeostasis plays in its etiology. More recently, modest vitamin D deficiency has also been shown to be associated with decreased stability and an increased number of falls in the elderly population (3).

In vitro studies of vitamin D action on muscle have demonstrated that 1,25-dihydroxyvitamin D exerts rapid effects, including activation of second messengers and phosphorylation. Activation of some of these pathways is blocked by antisense oligonucleotides directed against the VDR (4).

In a study reported in this issue, Endo et al. (5) examined whether 1,25-dihydroxyvitamin D and its receptor have a physiological role in skeletal muscle development. To exclude abnormalities secondary to impaired mineral ion homeostasis, they performed studies in VDR-null mice at 3 wk of age, a time when the mice still have normal levels of calcium, phosphorus, and vitamin D metabolites. They found that the muscle fibers of the VDR-null mice were smaller and had persistently elevated expression of early markers of myogenic differentiation (Fig. 1), including embryonic and neonatal forms of myosin heavy chain, as well as Myf5 and myogenin. There were no changes in the expression of MyoD or MRF4. To determine whether down-regulation of the early myogenic genes by the VDR was ligand dependent, studies were performed in C2C12 myoblasts. These analyses demonstrated that 1,25-dihydroxyvitamin D caused a decrease in the expression of myogenein, Myf5, and neonatal myosin heavy chain.

Myf5, which is genetically redundant with MyoD, plays a regulatory role in the specification and differentiation of early muscle progenitors. Although mice lacking either MyoD or Myf5 are viable and fertile, mice lacking both of these genes have complete absence of skeletal muscle, demonstrating a critical role for these factors in myogenesis (6). Interestingly, in birds, Myf5 is expressed in proliferating progenitors, whereas MyoD is expressed in differentiated muscle, suggesting that Myf5 may be required for proliferation and maintenance of cells of the myogenic lineage, and MyoD may act downstream of Myf5 to induce differentiation (7). In support of this hypothesis is the observation that myoblasts from MyoD-null mice that express high levels of Myf5 proliferate rapidly and differentiate poorly, whereas myoblasts from Myf5-null mice exhibit decreased proliferation (8). Once myogenic cells are committed, they must differentiate and fuse into multinucleated myotubes. Like, MRF4, myogenin is expressed at a later stage in myogenic differentiation than Myf5 and MyoD, playing a role in terminal differentiation and fusion. Myogenin and MRF4 double knockout embryos have Myf5/MyoD-expressing muscle progenitors, but are deficient in differentiated muscles (9).

Thus, there is persistent expression of some early markers of myogenic differentiation in the VDR-null mice. What is presently known about the role of these factors and the regulation of their expression does not clarify why
MyoD is elevated and why MyoD is not, nor does present knowledge clarify why myogenin levels are increased and those of MRF4 are not. It is, however, possible that the expression of Myf 5 and myogenin, or their upstream regulators, are direct transcriptional targets of the VDR, their expression being repressed by classic ligand-dependent actions of the VDR.

Other nontraditional targets in which VDR ablation interferes with classical hormone-dependent receptor activity include the renin-angiotensin system (10) and the mammary gland (11). Hypertension in VDR-null mice is thought to be a consequence of impaired VDR-mediated transcriptional repression of the renin gene by 1,25-dihydroxyvitamin D (10). Like the effects of the VDR on muscle reported in this issue, the effects of the VDR on the mammary gland involve hormone-dependent effects on maturation (11). However, the hormone-dependent effects of the VDR serve to attenuate mammary development, whereas they are thought to promote myogenic differentiation.

The skin is another nontraditional target tissue in which the VDR is required. However, the actions of the VDR in the skin are thought to involve a nonclassical hormone-independent mechanism (12). Analogous to some human kindreds with VDR mutations, VDR knockout mice develop alopecia (13, 14). Hair follicle morphogenesis appears to be unaffected; however, the VDR-null mice exhibit a dramatic defect in postmorphogenic hair cycling due to absence of VDR expression in the epithelial component of the hair follicle, the keratinocyte (15, 16). Alopecia in the VDR-null mice develops regardless of mineral ion status or circulating hormone levels. Studies performed to address whether the alopecia was a consequence of impaired hormone-dependent VDR actions demonstrated that wild-type mice, with undetectable circulating levels of 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D, do not develop alopecia (12). Thus, the actions of the VDR in this nontraditional target tissue are nonclassical, in that they appear to be hormone independent.

The consequences of VDR ablation in other nontraditional target tissues, including the reproductive (17) and immune systems (18), are largely reversed by correction of mineral ion levels. Similarly, the effects of VDR ablation on two traditional targets, the parathyroid gland and the skeleton, are a consequence of impaired intestinal calcium absorption and the resultant abnormalities in mineral ion homeostasis rather than of the absence of a functional VDR in these two tissues (2, 19). Thus, the only traditional target tissue that appears to require classical hormone-dependent receptor actions is the intestine.

Studies in VDR-null mice have revealed important roles for this receptor in the development or regeneration of three nontraditional target tissues: skeletal muscle, the mammary gland, and the skin. Characterization of the pathways interrupted by VDR ablation in these tissues will reveal novel functions of this steroid receptor and extend our understanding of the molecular mechanisms that regulate development or regeneration of these tissues.

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