Satellite cell addition is not obligatory for skeletal muscle hypertrophy

POINT: SATELLITE CELL ADDITION IS OBLIGATORY FOR SKELETAL MUSCLE HYPERTROPHY

Myofiber size is dynamically regulated, increasing and decreasing depending on muscle use. Hypertrophy is defined by increases in myofiber cross-sectional area and mass as well as myofibrillar protein content. Myofibers contain many hundreds of nuclei, each of which has a nuclear domain. A nuclear domain is the volume of cytoplasm within the myonuclei regulated by the gene products of a single myonucleus. Skeletal muscle hypertrophy is accompanied by proportional increases in both myofiber volume and myonuclei, such that the ratio of myonuclei/cytoplasm, or nuclear domain, remains relatively constant (23). As myonuclei are terminally differentiated, myonuclear addition during skeletal muscle hypertrophy is dependent on activation of a local pool of quiescent muscle precursor cells. Satellite cells are located between the basal lamina and cell membrane of each myofiber. Satellite cells are also responsible for myonuclear addition during postnatal muscle growth and new myofiber formation during regeneration. Below we discuss the evidence that supports hypertrophy of myofibers is dependent on satellite cell proliferation and fusion with myofibers.

Central to our viewpoint is that muscle hypertrophy is a continuum characterized by different molecular phases. Early responses to hypertrophy include enhanced transcriptional and translational activities. This is followed by myonuclear addition in a satellite cell-dependent manner. As discussed below, the recruitment of myonuclei to growing myofibers occurs at later times after application of a hypertrophic stimulus and contributes to muscle growth at later phases. The temporal regulation of muscle hypertrophy is integral to our viewpoint on the role of satellite cells in hypertrophy.

Hypertrophic stimuli enhance satellite cell proliferation. Satellite cells reenter the cell cycle in response to various hypertrophic stimuli including synergist ablation (3, 24, 26), testosterone (25), clenbuterol (27), stretch overload (16, 30), and exercise (13, 15). This satellite cell activation occurs in both slow and fast muscles as well as in rodents, birds, and humans. Enhanced satellite cell proliferation is observed as early as 1–2 days after application of a hypertrophic stimulus (24, 30) and continues up to 7 days at least (26). Thus satellite cells begin to expand in number prior to significant increases in myofiber growth.

Inhibition of myonuclear addition prevents hypertrophy. To ablate satellite cells, local gamma irradiation of rodent hindlimbs is performed prior to application of a hypertrophic stimulus. Subsequently, either muscle mass or myofiber cross-sectional area is measured. Satellite cell activity can be assessed in multiple ways, including enumeration of satellite cell numbers, bromodeoxyuridine labeling, myoD expression, and increases in myonuclear number. Although irradiated cell populations may undergo one to three additional cell divisions (29), irradiation may also inhibit satellite cell differentiation and fusion (8, 28). The DNA lesions induced by gamma irradiation may occur either in satellite cells themselves or in non-muscle cell types that may prevent these cells from secreting factors that regulate satellite cell activity. Key to the interpretation of any satellite cell ablation study is the use of an effective local method to prevent increases in myonuclear number. The drawback to using pharmacologic DNA synthesis inhibitors rather than irradiation is that these drugs are administered systemically with potential indirect effects on muscle growth and, in addition, may incompletely inhibit cell proliferation (7).

Ablation of satellite cells and inhibition of myonuclear addition using gamma irradiation totally prevented hypertrophy in adult rodent EDL, soleus, and plantaris muscles following synergist ablation (1, 20, 21) and in adult plantaris muscles subjected to voluntary running (15). In contrast, gamma irradiation decreased only 50% of the muscle hypertrophy in response to either IGF-1 gene delivery (2) or reloading after hindlimb suspension-induced atrophy (18). Thus satellite cells are required for hypertrophy in response to various stimuli. Differing degrees of growth inhibition following irradiation indicate the cellular mechanisms regulating increases in myofiber size may differ depending on the hypertrophic stimulus. The relative importance of satellite cell activity vs. increased protein synthesis within myofibers may vary depending on the stimulus used to induce muscle growth.

Is gamma irradiation merely exerting a non-specific toxic effect on muscle growth? Attenuation of muscle growth is not likely due to a general toxic effect of gamma irradiation on skeletal muscle with the 1,800–3,000 rads used in the studies cited above. No adverse effects on myofiber permeability as measured by release of creatine kinase were observed with 1,800 rads (9), indicating that the inhibition of muscle growth is not merely due to gross myofiber defects. Indeed, doses between 12,000 and 18,000 rads are required for myofiber damage and necrosis (14). Importantly, transcriptional and translational activities are sustained in irradiated muscle subjected to hypertrophic stimuli (1, 15, 20, 21). For example, adaptive changes in myosin heavy chain expression that occur in response to synergist ablation (1, 20, 21) or endurance exercise (15) occurred normally following irradiation. In addition, irradiation had no significant impact on the increase in CD31+/endothelial cells with endurance exercise, suggesting that inhibition of angiogenesis did not contribute to the inhibition of muscle growth. Furthermore, myofiber cross-sectional area increased 50% at early stages of reloading in both control and irradiated soleus muscles after hindlimb suspension-induced atrophy (18). Thus low doses of irradiation do not elicit widespread toxic effects on muscle tissue that would nonspecifically inhibit myofiber growth.

Different molecular phases of myofiber growth are temporally regulated. The initial phase of myofiber hypertrophy is characterized by enhanced transcription and translation (4), leading to enhanced protein accretion and a small expansion of the myonuclear domain. In the later phase, fusion of satellite cells also occurs with growing myofibers to reestablish the ratio of DNA to cytoplasmic volume (22, 23). Thus small increments in myofiber cross-sectional area and cytoplasmic volume can be supported by increased transcriptional and translational activity of myonuclei but a threshold or “myo-
nuclear domain ceiling” exists such that additional increases in myofiber cross-sectional area require myonuclear addition. Accordingly, satellite cell-independent and -dependent phases of myofiber growth occur as shown by analyzing irradiated soleus muscles at multiple time points after a growth stimulus (17). As distinct phases of muscle growth exist, multiple time points are necessary to conclusively determine the role of satellite cells in any hypertrophic model. Although some studies concluded that satellite cells are not required for hypertrophy (6, 7, 16), their analyses were limited only to early times points after the application of a growth stimulus.

What about human muscle hypertrophy? Mechanistic studies can only be performed in animal models where anti-proliferative treatments can be administered or in transgenic models displaying defects in satellite number or function. Thus human studies are limited to demonstrating correlative changes in myonuclear number with increases in myofiber size (5, 11, 19, 25). Myofiber hypertrophy in humans independent of myonuclear addition (10, 12, 19) may be due to not having reached a set myonuclear domain ceiling. A theoretical myonuclear domain ceiling of ~2,000 μm² has been recently proposed beyond which myonuclear addition occurs (19).

In summary, we believe satellite cell myogenesis is necessary for muscle hypertrophy at later phases of growth to maintain a constant myonuclear domain.

REFERENCES