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The Myostatin Gene

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Summary

Myostatin is an important negative regulator of muscle growth. Natural mutations and knockouts in animals produce a “double-muscled” phenotype the basis of which is a dramatic increase in muscle mass. Therapeutics that target myostatin are currently in development. There are almost no documented side effects of suppression, among which research suggests that myostatin inhibition can restore function in cases of muscular dystrophy. Clinical trials are currently underway, although myostatin research has not taken advantage of some techniques like RNA interference. With the development of myostatin therapies and advances in gene therapy, the social and ethical implications of using these as performance enhancement strategies raise interesting questions about athleticism in general.

History of Discovery

For some time now, scientists have been characterizing the process by which muscle cells are formed by differentiation of myoblasts and subsequent fusion into long fibers with contractile properties. It is clear that muscle growth in humans and other animals is not unlimited; thus, there must be a biochemical signal for myoblasts to stop differentiation and proliferation. McPherron, Lawler, and Lee (1997) first identified the protein they called growth/differentiation factor-8 (GDF-8) using PCR with primers designed from conserved regions of the transforming growth factor-B (TGF-B) superfamily. The same group discovered that knockout of the myostatin gene in mice results in a doubling to tripling in the size of the mouse muscles.

Soon after, Grobet et al. (1997) identified an eleven base pair deletion in the myostatin gene in the Belgian Blue cattle breed (BBCB) expressing the well-known double-muscled phenotype for that species. The double-muscled BBCB was the first observed phenotype resulting from a natural myostatin mutation, and it had been known and intentionally bred into cattle populations for some time. Myostatin overexpression leads to an accumulation of myoblasts in the G0/G1 and G2 phases and thus are not measurable in serum; to a lesser extent in heart muscle. In mice models myostatin and proliferation. McPherron, Lawler, and Lee (1997) first identified the protein they called growth/differentiation factor-8 (GDF-8) using PCR with primers designed from conserved regions of the transforming growth factor-B (TGF-B) superfamily. The same group discovered that knockout of the myostatin gene in mice results in a doubling to tripling in the size of the mouse muscles.

Characterization of the Myostatin Gene and Expression

Gonzalez-Cadavid et al. (1998) first characterized the human myostatin gene by scanning the human EST data bank for orthologs to the well-known mouse myostatin gene. They found that the coding region is approximately 6.2kbp long with a small intron (1.8kbp) and a larger intron (2.4kbp) separating three exons. This sequence resides on the 2q33.2 chromosomal region and is transcribed into a 3.1kbp mRNA, which is then translated into a 375 amino acid myostatin protein precursor of size 26-kDa. The precursor then undergoes further processing to form the mature myostatin protein of size 12-kDa. This mature form of the protein dimerizes to form the active myostatin ligand. Human serum and skeletal muscle were demonstrated to contain only mature myostatin by Western blotting, but other human organs that are composed of smooth muscle tissue such as the colon, intestine, stomach, bladder, and prostate did not show any myostatin precursor or mature protein. It was demonstrated by Sharma et al. (1999) that myostatin is expressed in both fetal and adult hearts and may play a role in the patholgy of heart muscle. In mice models myostatin is also expressed in skeletal muscle tissue and is measurable in serum; to a lesser extent in heart muscle in both developing and adult animals (McPherron et al., 1997). As will become obvious, the role of myostatin in tissues outside of skeletal muscle is poorly understood, but this is changing with the development of drugs that target myostatin.

Biochemical Pathways of Myostatin Action

Because myostatin clearly impacts the formation of muscle, it is necessary to have a basic picture of the process of muscle formation. Muscle fiber formation begins with multipotent cells descended from the mesoderm. In the presence of the transcription factors MyoD or Myf5, which bind to DNA sites that activate muscle-specific genes, these cells commit to becoming mature muscle cells called myoblasts. Myoblasts proliferate until they leave the cell cycle. Then, multiple myoblasts line up together and fuse to form multinucleated myotubes. These myotubes express proteins that allow them to become the contractile units we know as skeletal muscle tissue (Gilbert, 2000).

Figure 1. A normal whippet on the left and a whippet exhibiting the double-muscled phenotype on the right.

A step past proliferation of myoblasts on the way to muscle fibers is their differentiation, which appears to be affected by myostatin. Joulia et al. (2003) demonstrated that the production of myotubes, which are the multinucleated fused form of maturing myoblasts, was inhibited in myostatin-overexpressing cells. Similarly, Langley et al. (2002) showed that adding myostatin to growth medium with bovine myoblasts inhibited the formation of myotubes, and that this inhibition increased as the dose of myostatin increased. Northern blots of the culture medium detected decreased expression of MyoD and myogenin, important determining factors in myogenesis. Rios, Carneiro, Arce, and Devesa (2002) came to the same conclusion by examining the overexpression of endogenous myostatin.

Myostatin seems to have some other peripheral effects. It negatively regulates apoptosis in muscle cells (Rios et al. 2001), while expression of myostatin antisense RNA has the opposite effect. This is an important result because it shows that while myostatin overexpression does not lead to excess muscle production, it does not achieve this by causing the death of muscle cells. This data, along with those showing that myostatin inhibits myoblast proliferation and differentiation, demonstrates how at the biochemical level myostatin mutations can lead to the observed double muscular phenotypes.

Physiological Influences of Myostatin

Myostatin is well characterized in terms of its physiological effects on muscle mass through both hyperplasia and hypertrophy. Muscle hyperplasia refers to an increase in the number of muscle fibers present while muscle hypertrophy refers to an increase in the volume of muscle fibers already present. Hyperplasia is usually regulated at the level of myoblast differentiation and proliferation while hypertrophy is regulated at the biochemical level. Myostatin mutations can lead to the development of both skeletal muscle fibers.

The original study by McPherron et al. (1997) produced a lot of information regarding gross anatomical changes in homozygous myostatin knockout mice. These mice were almost 30% larger than controls and the mass was distributed throughout the body in increased skeletal muscle. The amount of weight increase in individual muscles correlated well with levels of myostatin expression in those muscles. Histological analysis revealed that the increase in muscle mass was due to both hyperplasia and hypertrophy, with the number of fibers increased by 86% and fiber size increased by 49%. Every study done since this original has documented similar increases in muscle size, such as that done by Mosher et al. (2007) examining whippets heterozygous for a myostatin mutation. Amthor, Otto, Macharia, McKinnell, and Patel (2006) noted increased muscle mass as well as some histological changes in muscle fiber composition in a myostatin deficient mouse line. Zebrafish also have a myostatin gene that results in increased muscle mass when knocked out (Xu, Wu, Zohar, and Du, 2003). Although the original studies in mice suggested an increase in muscle mass through both hyperplasia and hypertrophy, Yang et al. (2001) noted only an increase in hypertrophy with no significant hyperplasia. Mccroskey, Thomas, Maxwell, Sharma, and Kambadur (2003) showed that myostatin decreased muscle stem cell activation, consistent with the hypothesis that myostatin deficiency leads to hyperplasia. A novel drug-inducible knockout was used to study the effects of post-developmental myostatin knockout by Welle, Bhatt, Pinkert, Tawil, and Du (2006). They showed that mice with a drug-inducible gene that expresses Cre recombinase and a form of the myostatin gene with loxP sites flanking one of the exons. After the mice reached adulthood, they were given the drug, which knocked out the myostatin gene. The result was mice with 25% greater muscle mass at 3 months of age. Welle et al. (2005) also found that in mice with a drug-inducible gene that expresses Cre recombinase and a myostatin-deficient myostatin mutation, the production of myotubes, which are the multinucleated fused form of maturing myoblasts, was inhibited in myostatin-overexpressing cells. Similarly, Langley et al. (2002) showed that adding myostatin to growth medium with bovine myoblasts inhibited the formation of myotubes, and that this inhibition increased as the dose of myostatin increased. Northern blots of the culture medium detected decreased expression of MyoD and myogenin, important determining factors in myogenesis. Rios, Carneiro, Arce, and Devesa (2002) came to the same conclusion by examining the overexpression of endogenous myostatin.

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Myostatin-based Therapeutics

Myostatin seems to be the ideal candidate for a therapeutic target in diseases that involve muscle wasting. Whittemore et al. (2003) demonstrated that myostatin can determine the fate of multipotent stem cells, but not preadipocytes, and that this effect leads to adipocytes with increased insulin sensitivity. Molecularly, myostatin has favorable effects on the development of both skeletal muscle and adipose tissue.

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developing effective inhibition strategies. The most common methods for knocking out myostatin have been either genetically engineered animals with myostatin mutations, animals expressing a myostatin antisense RNA, or anti-myostatin antibodies. In fact, a phase I/II clinical trial with a myostatin-targeting antibody administered to muscular dystrophy patients was recently completed and although not yet published, preliminary reports verify the safety of the drug (Wagner et al. 2008).

In my opinion, exploiting RNA interference (RNAi) should be the future goal of myostatin therapeutics, although this approach has only been used in a few myostatin-related studies to date. Acosta, Carpio, Borroto, Gonzalez, and Estrada (2005) used double stranded RNA (dsRNA) to suppress myostatin in zebrafish, leading to the double muscled phenotype for that species. Magee et al (2006) published the most impressive report of small interfering RNA (siRNA) being able to suppress myostatin mRNA by 27% leading to a 10% increase in skeletal muscle mass. This is modest compared to other forms of knockout, thus more research needs to be done. Because the RNAi system offers the possibility of tightly controlled and efficient silencing as demonstrated in other studies, this system should be explored as a method of myostatin silencing (Novina & Sharp, 2004).

Social and Ethical Considerations with Myostatin-blocking Treatments

Clearly treatments that target myostatin have the potential to alleviate the suffering of many patients with malunary of diseases. This type of therapy, however, may also attract the interest of people on the other end of the health spectrum: athletes. A recent study by Amthor et al. (2007) shows that myostatin deficient mice display impaired force production. This study stands in contrast to the findings by Mosher et al. (2007) mentioned earlier, that states that heterozygote whippets not only show increased muscle mass but also enhanced racing performance. Clearly racing performance is dependent on the ability to produce force rapidly. The difference may be that the first study examined animals homozygous for myostatin mutations and the second examined heterozygotes, or it may be that there is an interspecies difference. The fact that lack of myostatin to some degree can enhance performance might make myostatin-based therapeutics an attractive option for elite athletes looking to gain an edge in competition. Traditionally, athletes have turned to other performance enhancing drugs like steroids and growth hormone for strength sports, or EPO for endurance sports. Myostatin treatment, because it may prove to have significantly less side effects than other performance enhancers, will certainly be considered by some as a viable and safer method. Even if there are side effects, athletes have proven their willingness to gamble with their health for the sake of greater performance.

This leads directly into a discussion of what we as a society consider athleticism. We watch sporting events and are thrilled to see world records continuously being broken, but we shun professional athletes who achieve these heights by what we consider cheating. Surely taking myostatin inhibitors would be cheating just as much as taking steroids is. But what about boys such as the one described above that have a natural myostatin mutation? Surely he should not be barred from Olympic competitions, but then why should we persecute people who simply try to keep their playing field with genetic wonders like this by taking performance enhancers? These are issues that are important to society and also to me. Having been a competitive athlete for much of my life, I find discussions like this compelling. With the first myostatin inhibitors having already completed phase II clinical trials, the day when we have to deal with this issue is upon us. It is no surprise that here, like in other cases, biomedical technology has advanced far beyond what biomedical ethics has considered.

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