A new function for odorant receptors
MOR23 is necessary for normal tissue repair in skeletal muscle

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Myofibers with an abnormal branching cytoarchitecture are commonly found in various neuromuscular diseases as well as after severe muscle injury. These aberrant myofibers are fragile and muscles containing a high percentage of these myofibers are weaker and more prone to injury. To date the mechanisms and molecules regulating myofiber branching have been obscure. Recent work analyzing the role of mouse odorant receptor 23 (MOR23) in muscle regeneration revealed that MOR23 is necessary for proper skeletal muscle regeneration in mice as loss of MOR23 leads to increased myofiber branching. Further studies demonstrated that MOR23 expression is induced when muscle cells were extensively fusing and plays an important role in controlling cell migration and adhesion. These data demonstrate a novel role for an odorant receptor in tissue repair and identify the first molecule with a functional role in myofiber branching.

Skeletal muscle is characterized by an extensive ability to regenerate after injury due to trauma or disease. Muscle regeneration results from a finely orchestrated series of steps that are spatially and temporally regulated, many of which are not understood. Elucidation of the mechanisms that regulate muscle regeneration may be beneficial for enhancing the rate or extent of muscle regeneration in injury, disease or age.

Skeletal muscle is composed of myofibers, which are long cylindrical cells containing hundreds of myonuclei in a common cytoplasm (Fig. 1). Each myofiber is surrounded by a basal lamina sheath; between the myofiber and the basal lamina lie myogenic stem cells called satellite cells. When muscle is injured, myofibers degenerate and satellite cells proliferate to give rise to progeny myoblasts. Myoblasts differentiate and undergo migration, adhesion and fusion to form regenerated myofibers and normal tissue architecture is restored. In many neuromuscular diseases muscle regeneration is aberrant and various abnormalities, such as variation in myofiber size, decreased myofiber number, fibrosis and branched myofibers, are observed. In the clinical literature, branched myofibers are more commonly referred to as “split myofibers.”

Branched myofibers are malformed cells which, instead of having a normal cylindrical shape, contain one or more offshoots of small daughter myotubes contiguous with the parent myofiber (Fig. 2). Branched myofibers can be simple with only one branch (Fig. 2A) or complex with many anastomosing branches resembling a gnarled tree root (Fig. 2B). In myofibers with complex cytoarchitecture, individual branches can persist up to hundreds of microns and then eventually recombine with the parent myofiber. Each daughter branch is enclosed in its own basal lamina, which is contiguous with the basal lamina of the parent myofiber. The frequency of branched myofibers in rodent muscle under normal conditions is low, on the order of 0.003%. However, the frequency in both rodent and human muscle is increased in response to hypertrophy as well as regeneration due to induced injury or muscle transplantation.
is unknown and no causative molecules have been identified. The aberrant cytoarchitecture of branched myofibers likely arises from incomplete fusion of small myotubes during muscle regeneration though direct proof is lacking. Evidence in favor of these malformed myofibers arising from abortive regenerative processes includes the expression of neonatal myosin, a marker of early muscle regeneration, in mdx muscles containing a high percentage of branched myofibers are more vulnerable to contraction-induced injury. Thus, decreasing the number of branched myofibers would likely be beneficial to muscle function. Although branched myofibers have been reported in literature for over 100 years, the mechanism by which they arise is unknown and no causative molecules have been identified. The aberrant cytoarchitecture of branched myofibers likely arises from incomplete fusion of small myotubes during muscle regeneration though direct proof is lacking. Evidence in favor of these malformed myofibers arising from abortive regenerative processes includes the expression of neonatal myosin, a marker of early muscle regeneration, in

Figure 1. Myofiber growth during normal muscle regeneration. (A) Myofibers contain many myonuclei within a common cytoplasm and are surrounded by a basal lamina sheath. Underneath the basal lamina lie satellite cells, myogenic stem cells responsible for muscle regeneration. (B) Myofiber degeneration leads to activation of quiescent satellite cells and their reentry into the cell cycle. Their progeny myoblasts proliferate to yield a pool of progenitor cells. (C) Myoblasts differentiate and undergo migration, adhesion and fusion to form nascent myofibers within the original basal lamina sheath. Additional myoblasts fuse with these newly formed myofibers and the myofiber will continue to grow in size. (D) At later time points regenerated myofibers are similar in size to undamaged myofibers but contain centrally located myonuclei, a hallmark of a regenerated myofiber.
the small branches. The presence of centrally located nuclei, a hallmark of regenerated myofibers. The observation that during muscle regeneration multiple small myotubes can form within the old basal lamina sheath leads credence to the idea that aberrant fusion of such small myotubes underlies generation of branched myofibers. Indeed, electron microscopic studies support the ability of myotubes to fuse with one another in vivo; myotubes readily fuse with one another in vitro. Recent studies of odorant receptor function during muscle regeneration in mice have identified the first molecule with a functional role in controlling myofiber branching and suggest that defects in muscle cell migration and/or adhesion may underlie the genesis of these branches.

**Odorant Receptors and Regulation of Myogenesis**

My laboratory has a great interest in understanding the processes that regulate the formation of multinucleated muscle cells, with an emphasis on identifying novel molecules that control myoblast migration, adhesion and fusion. Microarray experiments of mouse muscle cells undergoing extensive migration, adhesion and fusion to form multinucleated muscle cells revealed an induction in expression of mouse odorant receptor 23 (MOR23). Odorant receptors (ORs) are G-protein coupled receptors expressed in the olfactory epithelium necessary for the perception of smell. Canonical OR signaling in olfactory neurons entails Gαolf, a G protein specific to odorant receptors, and activation of adenyl cyclase III and cAMP activated calcium channels. Great diversity in OR genes exists: ORs represent 3–5% of all genes in mammals with approximately 913 OR genes in the mouse genome and 390 in the human genome. Although initially discovered and heavily studied in the olfactory system, ORs appear to be expressed in many tissues. In mouse and human sperm, distinct ORs, MOR23, in mice and OR1D2, in humans, regulate chemotaxis in response to synthetic ligands of these particular ORs. In contrast, in human prostate cancer cells, activation of OR51E2 by the synthetic ligand beta-ionone leads to inhibition of cell proliferation.

To examine OR expression during myogenesis in more depth, we performed a real time PCR screen of 18 ORs in mouse muscle cells in vitro and in vivo. Of these 18 ORs, 13 ORs were expressed and displayed distinct mRNA expression patterns. The majority of ORs in vitro were most highly expressed in proliferating myoblasts, whereas a smaller number of ORs, such as MOR23 were most highly expressed in fusing muscle cells. During muscle regeneration in vivo, the majority of ORs were upregulated when muscle cells are proliferating, differentiating and fusing to form nascent myofibers at early times after injury. The distinct expression patterns observed for these various ORs suggests that individual ORs may have non-redundant functions during myogenesis.

We focused on the specific role of MOR23 in myogenesis of mouse muscle cells as its expression pattern suggested that it may play a role in regulating the processes associated with myoblast fusion to form multinucleated myotubes. Immunoblots confirmed upregulation of MOR23 expression during muscle differentiation in vitro and in vivo and also revealed expression of the canonical signaling OR signaling molecules, Gαolf and adenylyl cyclase III. MOR23 was required in muscle cells in vitro as knockdown of MOR23 by siRNA resulted in defects in directed migration towards a gradient of lyral, a synthetic MOR23 ligand. Interestingly, knockdown of MOR23 also decreased migration of muscle cells to conditioned media collected from fusing muscle cells as well as crushed muscle extract of mouse gastrocnemius muscles. These experiments suggest that an endogenous MOR23 ligand is secreted by both fusing muscle cells as well as injured muscle tissue. Additional defects in MOR23 knockout cells were noted; both cell-cell adhesion and formation of multinucleated myotubes were...
significantly decreased with MOR23 siRNA. Inhibition of adenylate cyclase III by the pharmacologic inhibitor SQ 22536 similarly inhibited cell-cell adhesion in vitro suggesting that MOR23 signaling affects cell-cell adhesion through a downstream adhesion molecule. The defects noted with loss of MOR23 were specific, as siRNAs against two other ORs expressed by muscle cells, did not affect either migration to lyral and conditioned media, or cell adhesion.

Further studies in vivo demonstrated an essential role for MOR23 during muscle repair after injury in mice. Decreasing MOR23 levels during muscle regeneration through electroporation of MOR23 siRNA resulted in aberrant regeneration. Regenerated muscle fibers were smaller in cross-sectional area and increased in number. Closer examination of myofiber morphology revealed that loss of MOR23 led to a 60% increase in regenerating myofibers with branches and 90% more branches per myofiber. Importantly, overexpression of MOR23 during muscle regeneration significantly decreased the percentage of these malformed myofibers. Together these experiments demonstrated a novel role for an olfactory receptor in tissue repair and revealed the first molecule with a functional role in myofiber branching. Furthermore, these results suggested that muscle cell migration and adhesion are critical for proper muscle regeneration in vivo.

**Future Prospects**

A number of interesting questions about MOR23 and odorant receptors are raised by this work. Further studies on MOR23 and other ORs should answer these questions and lead to potential therapies to enhance proper muscle growth during muscle regeneration.

(1) **MOR23 pathway: activators and downstream effectors.** As discussed earlier, dystrophic muscles containing a high percentage of branched myofibers are weaker and more prone to injury.\(^\text{12,14}\)

As overexpression of MOR23 by genetic methods was beneficial in reducing the incidence of branched myofibers during muscle regeneration, potentially the MOR23 pathway may be amenable to pharmacologic manipulation leading to therapeutic applications in various neuromuscular disorders to eliminate branched myofibers. Promoter analyses of the MOR23 gene may help to reveal the signal transduction pathways in skeletal muscle that regulate MOR23 expression during muscle fusion in vitro and in vivo. Although synthetic ligands for various ORs exist, endogenous ligands have not been identified. We show evidence for an endogenous MOR23 ligand expressed by muscle cells and tissue; such a readily available source should facilitate identification of this endogenous ligand. Potentially, modulating the levels of expression of this ligand may prove beneficial in eliminating branched myofibers also. Finally, genes regulated by MOR23 signaling are candidates for a novel class of regulators of muscle growth. In olfactory neurons, odorant receptor signaling leads to changes in the expression of adhesion and migratory molecules.\(^\text{26,31}\) Further work on adenylate cyclase III signaling in muscle cells is likely to identify additional molecules that control myofiber branching.

(2) **Role of other ORs in skeletal muscle.** Our studies reveal multiple ORs are expressed during myogenesis in distinct patterns. ORs are likely to regulate other processes than just cell migration and adhesion shown for MOR23. Given the expression patterns of some ORs in muscle, they may also help to regulate satellite cell quiescence, myoblast proliferation or differentiation. Global analyses of OR expression during both mouse and human myogenesis in vitro and in vivo coupled with functional studies are likely to help reveal a broader role for ORs in skeletal muscle.

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