mesenchymal progenitors might be the major source of heterotopic bones that develop in FOP.

Recently, PDGFRα has been shown to be a therapeutic target in alveolar rhabdomyosarcoma (40). Alveolar rhabdomyosarcoma is an aggressive skeletal muscle cancer of childhood. PDGFRα is overexpressed in alveolar rhabdomyosarcoma and inhibition of PDGFRα had a dramatic effect on tumor cell growth. Although the relation between PDGFRα$^+$ mesenchymal progenitors and alveolar rhabdomyosarcoma is unclear, the role of PDGFRα$^+$ mesenchymal progenitors in the pathogenesis of this skeletal muscle cancer is of considerable interest.

Importance of the interaction between muscle cells and PDGFRα$^+$ mesenchymal progenitors on muscle homeostasis

To elucidate how in vivo adipogenesis of PDGFRα$^+$ mesenchymal progenitors is regulated, we compared two different muscle injury models; one results in fatty degeneration, the other leads to successful muscle regeneration. Using reciprocal transplantation between regenerating and degenerating muscle, and co-culture experiments, we revealed that muscle fibers themselves have a strong inhibitory effect on the adipogenesis of PDGFRα$^+$ mesenchymal progenitors (under submission). This study highlights the importance of the interaction between muscle cells and PDGFRα$^+$ mesenchymal progenitors on muscle homeostasis. Several studies have shown that macrophages are indispensable for successful muscle regeneration and the inhibition of macrophage activity leads to impaired muscle regeneration with adipocyte accumulation (2, 33, 39). However, we believe that macrophages do not directly play an inhibitory role in adipogenesis of PDGFRα$^+$ mesenchymal progenitors because we could not see any obvious change in adipogenic differentiation of PDGFRα$^+$ cells when PDGFRα$^+$ cells were co-cultured with macrophages. Therefore, adipogenesis observed in this context seems to occur secondary to the effect of macrophage suppression on myogenic cells.

Under the proper interaction with muscle cells, PDGFRα$^+$ mesenchymal progenitors may have supportive functions for muscle tissue, as observed in several developmental contexts where PDGFRα is expressed in the mesenchyme that supports tissue organogenesis (1, 17, 18). Examples of the supportive functions of non-myogenic mesenchymal cells in adult skeletal muscle have been reported. Non-myogenic mesenchymal cells in muscle side population cells (41) stimulate the proliferation and migration of myoblasts (26). CD90$^+$ mesenchymal cells in skeletal muscle produce laminin α2, and may have positive roles in muscle regeneration by producing the basement membrane that acts as a scaffold for efficient myogenesis (13). The interrelationship between PDGFRα$^+$ mesenchymal progenitors and non-myogenic mesenchymal cells remains unclear. However, given the specificity and effectiveness of PDGFRα to identify mesenchymal progenitors, we believe that PDGFRα is the best marker

reported to date.

Conclusions

Here, we have reviewed the potential roles of the newly identified PDGFRα$^+$ mesenchymal progenitors in skeletal muscle. The characteristics of these cells have led to the concept that the cellular balance between muscle cells and mesenchymal progenitors has a considerable impact on tissue homeostasis. The idea of lineage choice in multipotent satellite cells seems to have become less important in an in vivo context. The relevance of PDGFRα$^+$ mesenchymal progenitors in muscle pathology raises the possibility that these cells or PDGFRα itself may be a therapeutic target. Although targeting PDGFRα$^+$ cells or PDGFRα has not been tested in muscle disorders, the potency of this strategy was reported in other pathological conditions, such as cardiac fibrosis (45) and alveolar rhabdomyosarcoma (40). Thus identification of PDGFRα$^+$ mesenchymal progenitors not only provides valuable insight for a better understanding of the pathophysiology of skeletal muscle, but has also opened new opportunities for designing therapeutic strategies for muscle diseases.

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5. 骨格筋

骨格筋は傷害を受けると再生する（図1）．筋傷害シグナルにより骨格筋特異的幹細胞である筋衛星細胞（muscle satellite cells）が活性化され，分裂・増殖し，やがてお互いに融合，あるいは既存の筋線維と融合して筋線維を再生する．デュシェンヌ型筋ジストロフィー（Duchenne muscular dystrophy；DMD）等の重篤な遺伝性筋疾患に対して筋・幹細胞を移植する再生医療が期待されているが，その確立のためには，筋組織の再生がどのように制御されているかを理解することが重要である．

a. 骨格筋衛星細胞

筋衛星細胞は筋基底膜と筋線維の間にある単核の細胞で1961年にAlexander Mauroによって初めてその存在を記載された．通常，細胞周期のG0の状態にあるが，筋傷害時に活性化され，増殖して筋線維を再生する．生直後は骨格筋組織の中の核の30％程度が筋衛星細胞の核であるが，成体になると5％程度とほぼ一定になる．体幹と四肢の骨格筋の発生学的な起源は沿軸中胚葉由来の体節であり，その中に形成されるdermomyotomeに出現するPax3，Pax7陽性の筋前駆細胞（muscle progenitor cells）が増殖し，やがてMyf5，MyoD等の筋分化制御遺伝子を発現して筋芽細胞（myoblast）となり，次に増殖を止め，融合して，筋線維を形成する．筋衛星細胞はその過程で派生してく

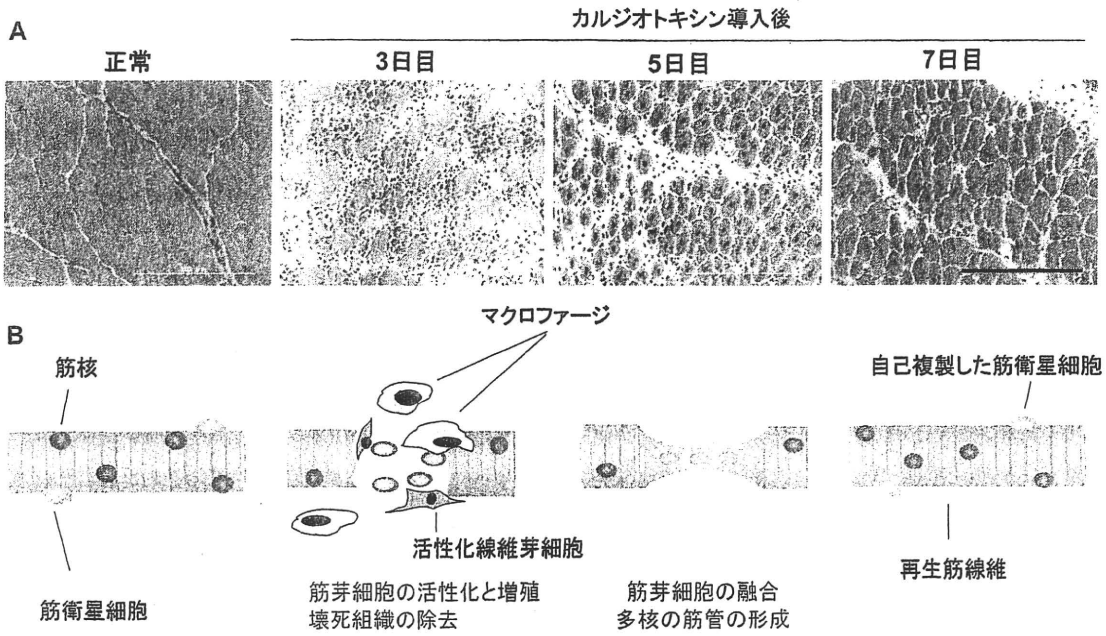

図1 骨格筋の再生

A） C57Bl/6マウス骨格筋に蛇毒であるカルジオトキシンを導入して筋傷害を引き起こした後の組織修復過程を示す．ヘマトキシリン・エオジン染色．スケールバー：200ミクロン．

B） 骨格筋特異的幹細胞である筋衛星細胞は，静止期の状態では筋基底膜と筋線維の間に存在するが，筋傷害時には活性化し，増殖する（筋芽細胞）．やがてお互いに融合し，あるいは既存の筋線維と融合して筋再生が完了する．この過程には好中球やマクロファージ等による壊死組織の貪食機能が重要である．活性化した筋衛星細胞の一部は，元の筋衛星細胞の状態に戻る（自己複製）．

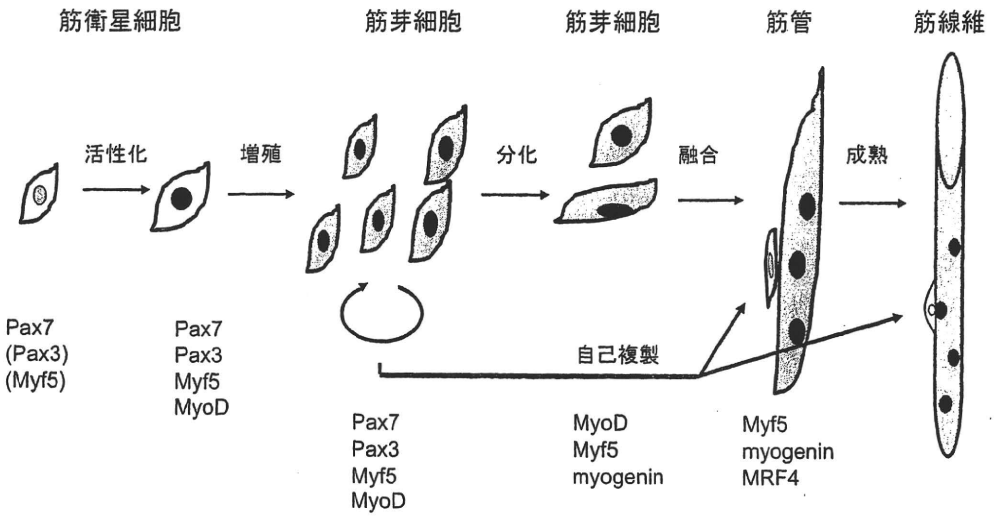

図2　筋衛星細胞の分化過程とその制御因子
筋衛星細胞の筋分化は，発生過程の筋分化と類似しているが，その維持にはPax7が重要な働きを持つ．筋衛星細胞が不均等分裂により自己複製すると考えられているが，そのタイミングや制御分子に関しては不明な点が多い．

る．c-Met，Pax7，Myf5，M-cadherin，CD34等が筋衛星細胞特異的マーカーとして知られているが，昨今の網羅的遺伝子発現研究等の結果，新しいマーカー（カルシトニン・レセプター，odz4等）がリストに加わってきた．筋衛星細胞研究には実験動物の骨格筋から筋衛星細胞を高い純度で分離する方法が有用だが，従来は，線維芽細胞との培養皿への接着性の違いを利用したpreplating法，現在は各種細胞表面マーカーで染色しセル・ソーターで分離する方法が用いられている．筋衛星細胞は自己複製することで，一生涯にわたって筋再生能を維持する（図1，図2）．その機構として不均等分裂が提唱されているが，その分裂様式，制御因子等，不明な点が残されている．

b．筋衛星細胞の活性化，増殖，分化

骨格筋が傷害されるとnitric oxide synthase（NOS）が活性化され，nitric oxide（NO）が産生され，hepatocyte growth factor（HGF；肝細胞増殖因子）を活性化し，c-Metレセプターへ結合する．c-Metを介したシグナルが筋衛星細胞を活性化し，筋衛星細胞は活発に増殖する．筋衛星細胞の増殖能は分裂を繰り返すと徐々に低下する．とくに筋ジストロフィー等の，筋変性・壊死，再生を繰り返す筋疾患では，筋衛星細胞の増殖能は徐々に低下し，筋再生が筋壊死に追いつかなくなり，筋線維が脱落し，筋力が低下していく．筋衛星細胞は筋細胞の他に脂肪細胞，骨細胞にも分化することが報告されているので，筋疾患の進行した段階で認められる脂肪変性や，徐々に筋組織の骨化が進行する進行性骨化性線維異形成症等の遺伝性の疾患の発症に関与する可能性がある．

c．筋・幹細胞と再生医療

1990年代前半，近親者から得た筋衛星細胞を培養後，DMD患者の骨格筋へ移植する筋芽細胞移植が行われたが，その効率は低かった．移植直後に多くの筋芽細胞が死んでしまうこと，移植後筋芽細胞があまり移動しないこと，免疫抑制が不十分であったこと等が原因であったと推察されている．1998年，骨髄細胞が筋線維へ分化し，さらに筋衛星細胞へ分化することが示され[1]，造血幹細胞の可塑性との関連で，DMDへの治療応用が期待されたが，その筋線維再生への寄与率はわずかであり，またその分化機序は依然不明で，大部分は細胞融合に

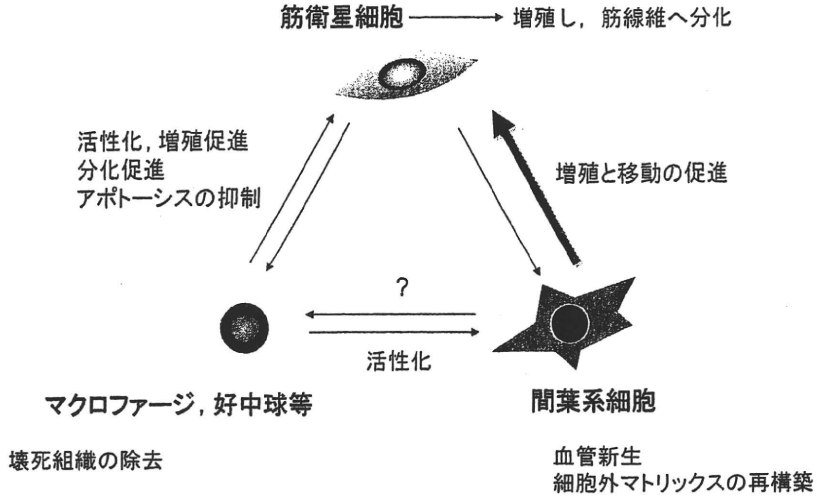

図3　筋再生を制御する細胞とそのネットワーク
筋再生過程では，筋前駆細胞である筋衛星細胞が中心的な役割を果たすが，その他に，壊死組織の除去を担うマクロファージ，好中球等が重要な細胞である．さらに間葉系細胞が間質に存在し，筋傷害時に活性化し，細胞外マトリックスの分解と再構築を促進し，血管新生を制御することで筋再生を制御している．これらの細胞は，直接相互作用する他に，サイトカイン等を介してお互いの活性化，増殖や移動，生存，分化を制御している．

よると思われた．しかし血中の AC133 陽性細胞は移植すると効率よく筋線維に分化するという報告もあり，循環している細胞の中に筋分化能を持つ特別な細胞が存在する可能性は否定できない．一方，骨格筋組織の間質や血管周囲にも，多能性を持ち，筋細胞へも分化する細胞が数多く報告されているが，これらの細胞の相互関係ははっきりしない．それらは，ヘキスト色素を排出する能力に富む side population 細胞（SP cells），血管周囲に存在するペリサイト（pericyte），同じく血管組織に由来するメソアンギオブラスト（mesoangioblast），muscle-derived stem cells，myo-endothelial cells 等である．数量的には筋衛星細胞が筋線維再生に最も寄与していることは広く認められているが，筋変性疾患に対する移植治療という観点では，移植後の生存率が低く，局所にしか生着しない筋衛星細胞に対して，経動脈的，あるいは経静脈的に移植可能なこれらの多能性幹細胞の利用が期待されている[2]．

d．筋再生におけるマクロファージや線維芽細胞の役割

筋再生はさまざまな細胞間の相互作用によって完了する．なかでもとくに重要な細胞はマクロファージと間質の線維芽細胞様の間葉系細胞であろう（図3）．マクロファージは壊死組織の除去の他に，筋衛星細胞の活性化やアポトーシスの抑制，筋分化の促進等の機能があると考えられており[3]，その機能不全で筋再生は障害される．間葉系細胞も筋再生時に活性化され，増殖し，MMPs 等のプロテアーゼを分泌し，細胞移動の促進，細胞外マトリックスの分解・再構築，血管新生，各種成長因子の活性化に関わっている．また，各種ケモカインを分泌しており，炎症細胞，免疫担当細胞の制御にも関与していると思われる．間葉系細胞は in vitro でも脂肪細胞へ分化しやすい傾向を持ち，この細胞の機能低下や異常な活性化が，筋再生の遅延，筋組織の線維化，脂肪変性に関わっていると考えられるので，再生医療の良き標的である．

［鈴木友子，武田伸一］

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疾　患　編

17. 筋ジストロフィー，多発筋炎

国立精神・神経センター神経研究所 遺伝子疾患治療研究部　青木吉嗣・武田伸一

Key words　Duchenne型筋ジストロフィー，多発筋炎，皮膚筋炎

要　点

①Duchenne型筋ジストロフィー（DMD）の確定診断は，遺伝学的検査あるいは筋生検により行う。
②DMDの骨格筋および呼吸機能の改善を目的にステロイド投与を行う。
③DMDでは，心不全と呼吸障害を中心とした全身管理が重要である。
④多発筋炎・皮膚筋炎は，ステロイド，免疫抑制剤，免疫グロブリンにより加療を行う。
⑤多発筋炎・皮膚筋炎は，悪性腫瘍や間質性肺炎の合併に注意する。

重要ポイント

①筋ジストロフィーは，骨格筋の壊死・変性を主病変とし，臨床的には進行性の筋力低下をみる遺伝性の疾患である。筋ジストロフィーのうち，もっとも頻度が高く重症の経過をとるDuchenne型筋ジストロフィーを中心に，呼吸および循環管理に加えて，ステロイド，アンジオテンシン変換酵素阻害薬，交感神経β受容体遮断薬などにより筋障害の改善が試みられている。
②後天性筋疾患の代表である特発性炎症性ミオパチーは，多発筋炎，皮膚筋炎，封入体筋炎に分類される。特に多発筋炎は横紋筋のびまん性炎症性筋疾患であり，特徴的な皮疹を呈するものは皮膚筋炎という。成人に比較的多くみられ，ステロイド，免疫抑制剤，免疫グロブリンが筋力低下に奏効する場合が多いが，悪性腫瘍や間質性肺炎の合併例は予後不良である。

A Duchenne型筋ジストロフィー（Duchenne muscular dystrophy：DMD）

筋ジストロフィーは「骨格筋の壊死・変性を主病変とし，臨床的には進行性の筋力低下をみる遺伝性の疾患である」と定義される。筋ジストロフィーのうち，もっとも頻度が高く重症の経過をとるDuchenne型筋ジストロフィー（DMD）は，ジストロフィン遺伝子（Xp21.2）の変異により，骨格筋膜の安定性に重要なジストロフィンが欠損す

ることで発症する。ジストロフィンの欠損が不完全な場合はベッカー型筋ジストロフィー（Becker muscular dystrophy：BMD）の表現型をとる。DMDは，X染色体連鎖遺伝形式をとり，新生男児3,500人に1人の割合で発症する。

1．臨床像

2～5歳前後で転びやすい，歩行が遅いなどの症状で気づかれることが多いが，高CK血症を偶発的に指摘され診断に至ることもある。特徴的な登はん性起立（Gowers徴候）を呈する。病初期には明らかな筋萎縮は認めず，腓腹部や舌などの筋肥大を示す場合が多いが，徐々に近位筋優位の筋力低下が進行して歩行は動揺性となり，12歳までに歩行不能となり車椅子生活に移行する。前後して脊柱側彎や関節拘縮の出現をみる。13歳前後で座位の保持も困難となる。呼吸筋の筋力低下のため10歳後半に呼吸不全が生じ，次第に心機能の低下も出現する。主として呼吸管理の進歩により，平均死亡年齢は過去20年で10年程度延長し，30歳前後になった。現在の死因は主に心不全および呼吸不全である。

2．検査所見

1）血液生化学検査

乳児期より著明な高CK血症（20,000～40,000 U/L），アルドラーゼなどの筋原性酵素の上昇をみるが，筋萎縮の進行とともに低下する。AST，ALT，LDHも上昇し，肝機能障害と誤る場合がある。

2）筋電図

随意収縮時に，低振幅・短持続時間の運動単位，運動単位の早期動員（early recruitment）がみられる。

3）画像検査

骨格筋CT，MRIでは5歳頃から大殿筋の脂肪置換を認める。10歳以降では大腿四頭筋（特に大腿直筋），大内転筋と大腿二頭筋，傍脊柱筋を中心に近位筋優位に筋容積の減少や脂肪置換が顕著となるが，薄筋と縫工筋は比較的保たれる（selectivity pattern）。

4）遺伝学的検査

他の検査所見からDMD/BMDの可能性が疑われ，臨床的および遺伝医学的に有用と考えられる場合に実施を検討する。遺伝学的検査は，生涯変化しない個人の重要な遺伝学的情報を扱うため，担当医師から被験者（保護者）に対して，検査を行う意義，利点と限界，その結果が家族や親族に及ぼす影響について十分説明し，書面による同意を得た上で，遺伝子異常が診断されたときの支援まで準備して実施されるべきである。検査実施前後に遺伝カウンセラーが遺伝カウンセリングを実施することが望ましい。

最近，遺伝子変異が確立した男性のDMD/BMD患者を対象に，臨床試験/治験の実施を目的とした筋ジストロフィー患者登録サイトの運用が開始された（Remudy：registry of muscular dystrophy. http://www.remudy.jp/index.html）。登録に際しては，全例にmultiplex ligation-dependent probe amplification（MLPA）法によるスクリーニング検査を行い（保険診療），必要に応じてシークエンス解析，筋生検を実施する。

5）筋生検

筋病理では，筋の壊死・変性，再生線維の混在，筋線維の大小不同，結合織の増生

がみられる。免疫組織化学染色では，DMDの筋細胞膜はジストロフィンをほぼ完全に欠損するが，BMDの細胞膜はまだら状（faint and patchy）に染色される。

3．治療方針

DMDに対するステロイド投与の有効性に関して，筋力の増強あるいは維持と呼吸機能の改善がランダム化比較対照試験により証明されている。5～15歳の症例ではプレドニゾロン（プレドニン®）0.75 mg/kg/日の連続投与が治療の第一選択である。体重増加などの副作用の面から投与量の減量が望ましい場合には，0.5 mg/kg/日に減量し，3～4カ月でさらに0.3 mg/kg/日へと減量する。

【心不全】

定期的に脳性ナトリウム利尿ペプチド（brain natriuretic peptide：BNP）の測定や心エコーを施行し，左室駆出率40％以下（BNP 20～60 pg/mL）で，アンジオテンシン変換酵素阻害薬（レニベース®，2.5 mg/日から開始し漸増），交感神経β受容体遮断薬（アーチスト®，1.25 mg/日から開始し漸増。上限は10 mg/日）を開始する。心筋障害が進行した際は拡張型心筋症の心不全に準じ，強心薬，利尿薬も加える。

【呼吸障害】

定期的なSpO_2，％VC，ピークカフフロー，終末呼気炭酸ガス濃度の測定が重要である。開始時期は，低酸素に基づく症状がある場合，睡眠時にSpO_2低下がある場合，VCが1L（あるいは％VCが20％）を下回る時期の前後，$PaCO_2$が55 Torr以上であれば夜間に非侵襲的陽圧換気療法（Noninvasive Positive Pressure Ventilation：NPPV）を開始する。病状，病態に応じて昼間にもNPPVを追加する。排痰障害にはカフレーター（Mechanical In-Exsufflator：MI-E）や肺内パーカッション換気療法（IPV）も有効である。

4．患者指導とリハビリテーション

早期より側彎と関節拘縮の予防に努め，必要に応じて装具，コルセットを作製する。最大強制吸気量維持のため呼吸訓練を行い，舌咽頭呼吸の習得を試みる。側彎は外科的治療も含め積極的に治療する。過度の痩せは消化管機能を低下させるため栄養指導が大切である。

5．根本的治療開発の動向

現在DMDに対して，PTC124によるリード・スルー療法，ES/iPS細胞や筋芽細胞の移植治療，ウイルスベクターによる遺伝子治療などの開発が進められている。当研究部では，これまでアンチセンス・モルフォリノを用いたエクソン・スキッピング療法の前臨床試験を行ってきた。この成果を踏まえて，DMDを対象にしたエクソン51スキッピングの臨床治験を実施する準備を進めている。

B 多発筋炎（polymyositis：PM），皮膚筋炎（dermatomyositis：DM）

後天性筋疾患の代表である特発性炎症性ミオパチーは，多発筋炎，皮膚筋炎，封入体筋炎に分類される。特に多発筋炎は横紋筋のびまん性炎症性筋疾患であり，特徴的な皮疹を呈するものは皮膚筋炎という。多発筋炎・皮膚筋炎の有病率は人口10万あた

り約6人と推定される。男女比は女性が約2倍と多い。発症年齢の約半数は40～60歳である。

1. 病因

発症には自己免疫機序が関与する。多発筋炎では，筋線維・間質・血管周囲にマクロファージ，$CD8^+$T細胞が浸潤し，筋線維内のカルパインなどのタンパク分解酵素を活性化する結果，筋線維は壊死する（細胞性免疫）。皮膚筋炎では，主に筋周膜の血管周囲や間質にB細胞，$CD4^+$T細胞（ヘルパーT細胞）が浸潤する（液性免疫）。筋内微小血管の内皮細胞が傷害される結果，循環障害による筋束周囲萎縮が生じる。

2. 臨床像

急性ないし亜急性（数週～数ヵ月）に進行する。初発症状は，四肢近位筋・頸筋・体幹の筋力低下，筋痛，関節痛がみられることが多い。遠位筋力の低下は遅れて生ずる。進行例では筋萎縮を認めることがある。嚥下障害が生じることがあるが，構音障害を伴うことは少ない。びまん性間質性肺炎，肺線維症，心筋炎をしばしば合併する。自然寛解や増悪を繰り返しつつ徐々に進行し，5年生存率は約75％である。皮膚筋炎は多発筋炎と類似した臨床像を呈するが，ゴットロン徴候（指関節伸側で肥厚した紅斑），ヘリオトロープ疹（上眼瞼の紫紅色の浮腫性紅斑）を伴うことを特徴とする。皮膚筋炎の悪性腫瘍合併頻度は約20％であり，多発筋炎と比べて2.1～6.5倍高い。女性では，乳癌・卵巣腫瘍，男性では肺癌・消化器癌・前立腺癌が多い。女性の悪性腫瘍合併率は男性の約2倍で，50歳以上は高い。

3. 検査所見

1) 血液生化学検査

活動期には血清CK値は正常値の約10倍に上昇し，ミオグロビン値も上昇する。アルドラーゼ，AST，ALT，LDH，％クレアチン尿（尿中クレアチン/尿中クレアチン＋尿中クレアチニン）が上昇し，活動性の指標判定に有用である。

2) 筋電図

随意収縮時には，低振幅・短持続時間の運動単位，運動単位の早期動員（early recruitment）がみられる。刺入電位は亢進していることが多い。安静時には線維性収縮電位，陽性鋭波を認める。

3) 画像検査

急性期の骨格筋MRIは，STIR（Short TI Inversion Recovery）法および脂肪抑制T2強調画像では，病変は多巣性あるいはびまん性の高信号を示す。進行例は，筋萎縮およびT1強調画像で高信号を示す。

4) 筋生検

筋束内の周辺・筋線維の内部・血管周囲に$CD8^+$T細胞やマクロファージの浸潤像，筋線維の変性と再生，結合織の増生を認める。特に皮膚筋炎では血管周囲の細胞浸潤が主体であり，筋束周囲萎縮が認められることが多い。

5) 自己抗体

抗Jo-1抗体は肺線維症の合併のある多発筋炎の50％，皮膚筋炎の20％に認められる。抗シグナル認識粒子（SRP）抗体は筋炎と心障害を伴う急性発症の重症皮膚筋炎および多発筋炎の5％に検出される。皮膚筋炎に特異的な抗Mi-2抗体は35％で検出され，抗

p155抗体は悪性腫瘍合併例で高率とされる。その他の膠原病を合併するオーバーラップ（重複）症候群では，抗PM-1抗体（強皮症），抗Ku抗体（強皮症・全身性エリテマトーデス），抗nRNP抗体（混合性結合組織病）が陽性となることがある。

4．診断

BohanとPeterの診断基準や皮膚筋炎・多発筋炎の改訂診断基準（厚生省特定疾患自己免疫疾患調査研究班平成4年度研究報告，pp25-28，1993）が汎用される。

5．治療方針

多発筋炎あるいは皮膚筋炎の確定診断後は，プレドニン®1～1.5 mg/kg/日を1～2ヵ月間連日投与する。筋力の改善，血清CK値の減少がみられれば2週間に10％の割合でプレドニン®を減量し，2～3年程度は維持療法を行う。筋症状は早期治療例ほど回復がよい。改善がないときは同量を1～2ヵ月間投与するか，ステロイドパルス療法を2～3クール行う（メチルプレドニゾロン1 g/回の3日間連続投与）。効果がない場合はメトトレキサート（メソトレキセート®，5～25 mg/週，経口あるいは筋肉内投与），アザチオプリン（イムラン®，50～100 mg/日，経口投与）などを併用する。ステロイド，免疫抑制薬の無効例では，追加療法として免疫グロブリン療法を併用する。

6．治療のポイント

筋症状増悪時には筋炎の再燃か，ステロイドミオパチーの合併かの鑑別が重要である。血清CK値上昇，筋電図で線維性収縮電位や陽性鋭波の出現頻度が上昇した場合には再燃を疑う。ステロイドミオパチーは，プレドニン内服を4週間以上続けた場合に発症し，下肢近位筋優位の筋力低下・筋萎縮を呈するが，顔面筋および頸部伸展筋は保たれ，血清CK値は低下する。

7．患者指導とリハビリテーション

急性期は等尺性収縮以外の運動は避ける。安定後は誤嚥性肺炎，廃用性筋萎縮，関節拘縮予防のための理学療法を早期に開始する。

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Caveolin-3 regulates myostatin signaling. Mini-review

Y. Ohsawa[1], T. Okada[1], A. Kuga[1], S. Hayashi[1], T. Murakami[1], K. Tsuchida[2], S. Noji[3], Y. Sunada[1]

[1] Division of Neurology, Department of Internal Medicine, Kawasaki Medical School, 577 Matsushima, Kurashiki-City, Okayama 701-0192, Japan; [2] Division for Therapies against Intractable Diseases, Institute for Comprehensive Medical Science, Fujita Health University, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake-City, Aichi 470-1192, Japan; [3] Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, 2-1 Minami-Jyosanjima-cho, Tokushima-City, Tokushima 770-8506, Japan

Caveolins, components of the uncoated invaginations of plasma membrane, regulate signal transduction and vesicular trafficking. Loss of caveolin-3, resulting from dominant negative mutations of caveolin-3 causes autosomal dominant limb-girdle muscular dystrophy (LGMD) 1C and autosomal dominant rippling muscle disease (AD-RMD). Myostatin, a member of the muscle-specific transforming growth factor (TGF)-β superfamily, negatively regulates skeletal muscle volume. Herein we review caveolin-3 suppressing of activation of type I myostatin receptor, thereby inhibiting subsequent intracellular signaling. In addition, a mouse model of LGMD1C has shown atrophic myopathy with enhanced myostatin signaling. Myostatin inhibition ameliorates muscular phenotype in the model mouse, accompanied by normalized myostatin signaling. Enhanced myostatin signaling by caveolin-3 mutation in human may contribute to the pathogenesis of LGMD1C. Therefore, myostatin inhibition therapy may be a promising treatment for patients with LGMD1C. More recent studies concerning regulation of TGF-β superfamily signaling by caveolins have provided new insights into the pathogenesis of several human diseases.

Key words: caveolin-3, limb-girdle muscular dystrophy 1C (LGMD1C), autosomal dominant rippling muscle disease (AD-RMD), myostatin, transforming growth factor-β (TGF-β)

Caveolins are primary components of caveolae

Caveolae, uncoated invaginations of the plasma membrane, are an abundant feature of many terminally differentiated cells, such as adipocytes, endothelial cells, and muscle cells. Caveolin family proteins, 21-24 kDa integral membrane proteins, are the principle components of caveolae, designated as caveolin-1, -2, and -3 (1, 2). Caveolin-1 and caveolin-2 are coexpressed and form heterooligomers in nonmuscle cells, whereas caveolin-3 is muscle specific and forms homooligomers in muscle cells (3, 4). De novo synthesized caveolins assemble to about 350 kDa oligomers in the endoplasmic reticulum, subsequently target to the plasma membrane via the trans-Golgi network, and play a crucial role in the formation of caveolae. These caveolin family proteins have been implicated in numerous cellular events including vesicular trafficking, lipid metabolism, and signal transduction (1-6). They directly bind to and regulate specific lipid and lipid-modified proteins including cholesterol, G-protein, G-protein coupled receptors, Src family kinase, Ha-Ras, and nitric oxide synthases (5-7). The interaction between caveolins and these molecules is mediated by a caveolin-binding motif on the target protein and a scaffolding domain in caveolin (7). The number of *in vitro* studies linking caveolins to signal transduction pathways has grown exponentially. To date, however, only a few studies have been concluded the exact roles of caveolins to signal transduction *in vivo* (3).

Dominant-negative mutations of caveolin-3 gene causes LGMD1C/ AR-RMD

Many mutations in caveolin-3 gene have been detected in autosomal dominant limb-girdle muscular dystrophy (LGMD) 1C and autosomal dominant rippling muscle disease (AD-RMD) (8, 9). Mutations of the caveolin-3 gene cause a significant reduction in the cell surface level of caveolin-3 protein in a dominant-negative fashion and, to a lesser extent, mistargeting of the mutant caveolin-3 protein to the Golgi complex (8-10).

The loss of caveolin-3 by mutations of the caveolin-3 gene in LGMD1C/AD-RMD patients has resulted in subsequent abnormalities of caveolin-3-binding molecules. The

Address for correspondence: Yoshihide Sunada, MD, PhD, Division of Neurology, Department of Internal Medicine, Kawasaki Medical School, 577 Matsushima, Kurashiki-City, Okayama 701-0192, Japan. Fax +81 86 4641027. E-mail: ysunada@med.kawasaki-m.ac.jp

enzymatic activity of neuronal nitric oxide synthase, which is strongly suppressed by caveolin-3, increases in the skeletal muscles from a transgenic mouse model of LGMD1C and LGMD1C/AD-RMD patients (11, 12). Consistently, cytokine-induced NO production increases in C2C12 myoblast cells transfected with LGMD1C/AD-RMD-type mutant caveolin-3 compared to ones transfected with wild-type caveolin-3 (9). Src tyrosine kinase, a membrane tyrosine kinase whose activation regulates the balance between cell survival and cell death, is extremely activated and accumulates not in the plasma membrane but in the perinuclear region in cells transfected in LGMD1C/AD-RMD mutant caveolin-3 (13). Muscle-specific phosphofruktokinase, an enzyme of central importance in the regulation of glycolytic metabolism is also significantly reduced in cells transfected with LDMD1C/AD-RMD mutant caveolin-3 probably through ubiquitin-proteasomal degradation (14). Noteworthy also is the finding that dysferlin, a membrane-repair molecule deficient in LGMD2B/Miyoshi myopathy, mistargets to the cytoplasm from sarcolemma in skeletal muscle from LGMD1C/AD-RMD patients, probably due to the caveolin-3's delivery function to the correct targeting of plasma membrane (15-18).

Despite these findings, the underlying molecular mechanism leading to LGMD1C/AD-RMD in caveolin-3-deficient muscle remains to be elucidated.

Myostatin, a muscle-specific TGF-β superfamily member, is a therapeutic target of muscular dystrophy

Myostatin is a muscle-specific transforming growth factor (TGF)-β superfamily member and negatively regulates skeletal muscle growth and skeletal muscle volume (19). Overexpression of myostatin causes severe muscle atrophy, whereas targeted disruption of myostatin increases skeletal muscle mass in mice (19, 20). Like most members of the TGF-β superfamily, myostatin is synthesized as a precursor protein and undergoes proteolytic processing to generate an N-terminal prodomain and a biologically active, C-terminal disulfide-linked dimer (21). In the inactive state, the prodomain strongly inhibits the biological activity of the C-terminal dimer (22, 23), as do follistatin, and the follistatin-related gene (FLRG); which are collectively called natural inhibitors for myostatin (24). The circulating active form of myostatin directly binds to and phosphorylates the type II serine/threonine kinase receptor, namely activin receptor IIB (ActRIIB) (Fig. 1) (25). This, in turn, phosphorylates the type I serine/threonine kinase receptors, namely activin receptor-like kinase 4/5 (ALK4/5) at the plasma membrane (25-27). The activation of a heteromeric receptor complex consisting of phosphorylated type II and type I serine/threonine kinase receptors induces the phosphorylation of intracellular effectors, receptor-regulated Smads (R-Smads), namely Smad2/3 (26, 27). Phosphorylated R-Smads translocate to the nucleus from the cytoplasm, where it regulate the transcription of specific target genes inducing skeletal muscle atrophy (26-28).

Notably, administration of a blocking antibody against myostatin, myostatin vaccine, and myostatin prodomain, or genetic introduction of a follistatin-derivative ameliorates the pathophysiology of dystrophin-deficient *mdx* mice (29-32). In addition, a blocking antibody against myostatin improves the condition of young model mice with δ-sarcoglycan-deficient LGMD2F (33). An adeno-associated virus (AAV)-mediated myostatin prodomain has ameliorates the pathology of calpain-3-deficient LGMD2A model mice (34). Therefore, myostatin inhibition through different strategies has recently come to be considered for a therapeutic option for muscular dystrophies. However, the precise molecular mechanism by which myostatin inhibition improves the above dystrophic skeletal muscle is not fully understood; i.e. the molecular interaction of myostatin and the dystrophin-glycoprotein complex is unknown.

Caveolin-1 regulates TGF-β superfamily signaling *in vitro*

Recently, caveolin-1 has drawn attention as a regulator of TGF-β superfamily signaling. Caveolin-1 binds to and suppresses activation of the type I receptor of TGF-β1, which induces growth arrest in nonmuscle cells (35). Consistently, the binding affinity of caveolin-1 with type I TGF-β1 receptor decreases after stimulation with TGF-β1. In addition, caveolin-1 associates with the type II receptor of TGF-β1 (36-38). Caveolin-1 also facilitates ligand–bound TGF-β1 receptors internalization and degradation via the formation of endocytic vesicles with ubiquitin-ligase (39, 40). In addition, caveolin-1 interacts with type II and type I receptors of bone morphogenic proteins (BMPs) *in vivo* (41). These findings indicate that caveolin-1 regulates TGF-β superfamily signaling, including TGF-β1 and BMPs, at its receptor level.

Caveolin-3 suppresses myostatin signaling through its type I receptor *in vitro*

Upon consideration of molecular analogy and tissue distribution, we hypothesized that caveolin-3 inhibits myostatin signaling in a similar manner to that of inhibition

of caveolin-1 to multiple TGF-β superfamily signaling in nonmuscle cells. We found several caveolin-3 binding motifs (7); ϕXϕXXXXϕXXϕX, where ϕ indicates aromatic or aromatic-like amino acids in the cytoplasmic kinase domain of type I serine/threonine myostatin receptors, ALK4/5 (42). Therefore, we cotransfected caveolin-3 and these type I myostatin receptors in COS-7 monkey kidney cells and found that caveolin-3 colocalized with type I myostatin receptor. Immunoprecipitation and subsequent immunoblot analysis revealed that caveolin-3 associates with the type I myostatin receptor. In addition, phosphorylation level of the type I myostatin receptor decreased with the addition of caveolin-3 in cells cotransfected with constitutively active type I receptor and caveolin-3. Moreover, caveolin-3 eventually suppressed subsequent intracellular myostatin signaling; the phosphorylation level of an R-Smad of myostatin, Smad2 as well as the transcription level of the Smad-sensitive $(CAGA)_{12}$-reporter gene. Therefore, caveolin-3 suppresses the myostatin signal at its type I receptor level, in a similar manner to caveolin-1 for TGF-β1 signaling *in vitro*.

Caveolin-3 deficient muscles exhibit enhanced intracellular myostatin signaling

We previously generated transgenic (Tg) mice overexpressing mutant caveolin-3 (CAV-3^{P104L}) to develop a mouse model of LGMD1C/AD-RMD (11). The skeletal muscle phenotype of the transgenic mice showed severe myopathy with loss of caveolin-3. To determine whether caveolin-3 regulates myostatin signaling *in vivo*, we generated and characterized the double-transgenic mice showing myostatin deficiency and myostatin inhibition. Heterozygous mating of mutant caveolin-3 Tg mice with other Tg mice overexpressing myostatin prodomain ($MSTN^{Pro}$) (43), a potent inhibitor of myostatin signaling, gave rise to mice with four distinct phenotypes: wild-type, mutant caveolin-3 Tg, mutant MSTN Tg, and double-mutant Tg (CAV-3^{P104L}/$MSTN^{Pro}$). Growth curves revealed that the double-mutant Tg mice were significantly larger than the mutant caveolin-3 Tg mice and similar in size to the wild-type mice beginning at 6 weeks until 16 weeks of age (42). The muscle atrophy seen in the mutant caveolin-3 Tg was reversed in the double-mutant Tg with increased myofiber size and myofiber number. Thus, myostatin inhibition reverses caveolin-3-deficient muscular atrophy *in vivo*.

Caveolin-3-deficient muscle from mutant caveolin-3 Tg mice showed hyperphosphorylation of an R-Smad of myostatin, Smad2 and significant upregulation of a myostatin target gene, p21. These *in vivo* findings were consistent with our *in vitro* study in which caveolin-3 suppresses myostatin signaling. In the double-mutant Tg mouse, the levels of phospho-Smad2 and p21 gene expression were significantly reduced compared to those in the mutant caveolin-3 Tg mice and were similar to those in the wild-type mice. Thus, myostatin inhibition by genetic introduction of myostatin inhibitor normalized enhanced myostatin signaling and also reversed muscular phenotype in the caveolin-3 deficient mouse.

Myostatin inhibition therapy reversed muscular atrophy in caveolin-3 deficiency

We injected a soluble form of the extracellular domain of type II myostatin receptor, ActRIIB, which can inhibit myostatin-its type II receptor binding (25, 44), into the mutant caveolin-3 Tg mice to develop myostatin inhibition through its type II receptor as a therapeutic strategy for patients with LGMD1C. Intraperitoneal injection of soluble ActRIIB four times significantly increased skeletal muscle mass and reversed myofiber hypotrophy accompanied with suppression of Smad2 phosphorylation and downregulation of p21. This finding, therefore, suggests that myostatin inhibition therapy may be a reasonable and promising therapy for caveolin-3-deficient muscular dystrophy associated with enhanced myostatin signaling.

Conclusions and prospective for future research

Caveolin-3 has been considered to regulate numerous signal pathways for maintaining the normal integrity of skeletal muscles, but the *in vivo* significance of signal alterations by loss of caveolin-3 in the pathogenesis of LGMD1C/AD-RMD has not been well delineated. As reviewed herein, caveolin-3 regulates myostatin signaling *in vitro*, and thus disrupted interaction between caveolin-3 and myostatin could contribute to the pathogenesis of caveolin-3-deficient muscular dystrophy (Fig. 1).

We could not conclude that activated intracellular signaling molecules, hyperphosphorylation of an R-Smad, Smad2, and upregulation of p21 in the caeveolin-3 deficient skeletal muscle result simply from enhanced myostatin signaling by loss of caveolin-3, because the myostatin prodomain or the soluble myostatin receptor suppresses not only myostatin, but also other TGF-β ligands including growth and differentiation factor 11 (GDF11) (22, 25, 44, 45). In fact, evidence of an unknown TGF-β ligand exists in the form of a similar negative regulator of muscle mass like myostatin (45, 46). Thus TGF-β ligands other than myostatin also could be

involved in the pathogenesis of caveolin-3 deficieny via the Smad2-p21-mediated pathway. Crossing of mutant caveolin-3 mice with myostatin-null mice is a prospective project for obtaining straightforward evidence that hyperphosphorylation of Smad2 and upregulation of p21 in caveolin-3-deficient muscles is the simple result of enhanced myostatin signaling.

More recent studies have shown to be caveolins as an exact negative regulator of TGF-β superfamily signaling because the loss of caveolins has play important roles in the pathogenesis of human disorders. Mutations of the caveolin-1 gene or downregulation of caveolin-1 protein have been detected in some sporadic breast cancers (47) and epithelial cells derived from caveolin-1 null mice have shown hyperphosphorylation of Smad2 and epithelial mesenchymal transition, corresponding to premalignant status (48). In addition, loss of caveolin-1 has been strongly associates with idiopathic pulmonary fibrosis (49, 50). Caveolin-1 protein has been found to be reduced in the lung tissue from patients with idiopathic pulmonary fibrosis. TGF-β1-induced extarcellular matrix production, which is indicative of fibrosis, significantly increases in

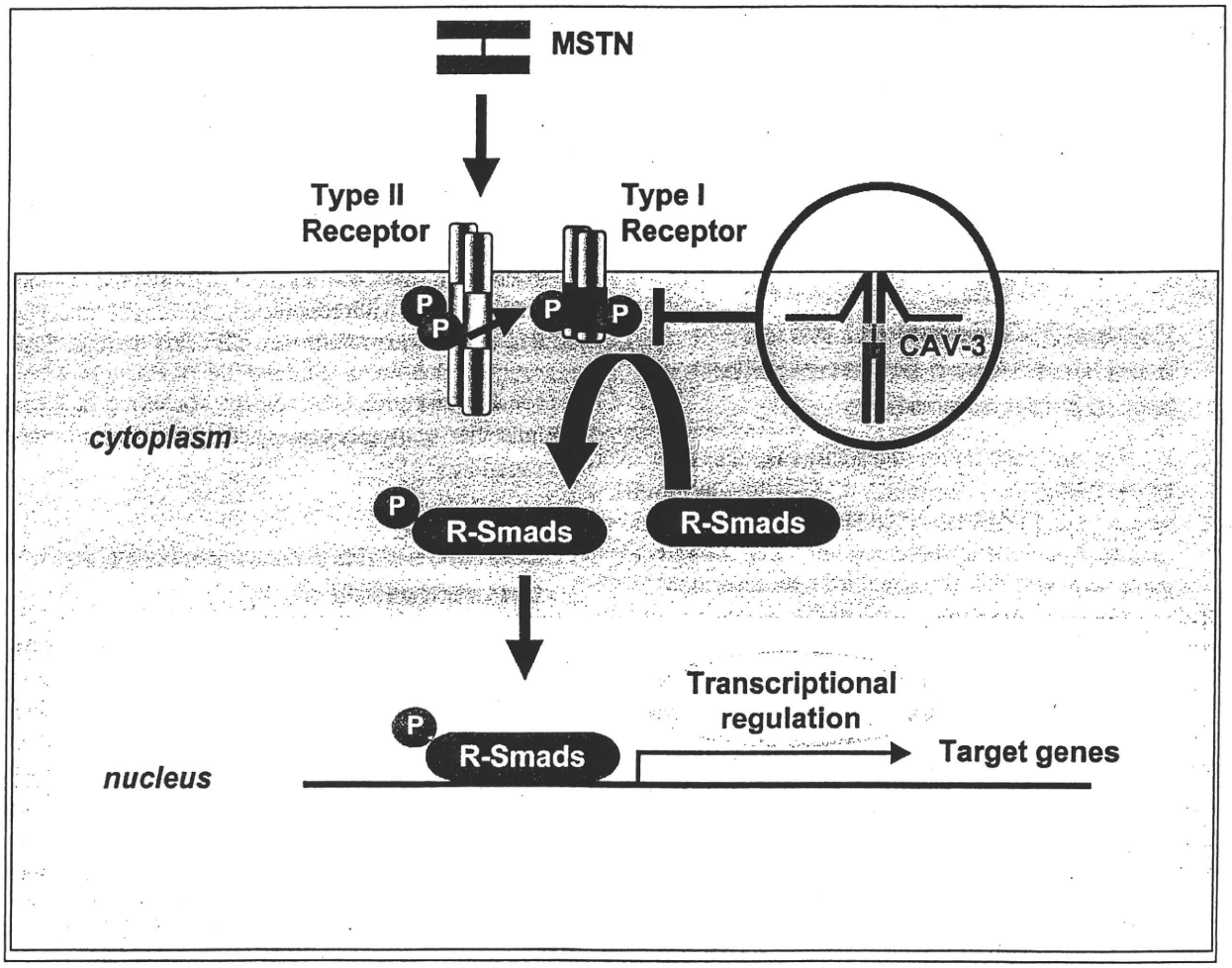

Figure 1. Putative scheme of the regulation of myostatin signaling by caveolin-3. Myostatin (MSTN) signaling is propagated through the myostatin receptor, a heteromeric complex consisting with transmembrane receptor serine/threonine kinases. Myostatin binds to and phosphorylates its type II serine/threonine kinase receptor (Type II Receptor). Subsequently, its type I serine/threonine kinase receptor (Type I Receptor) is phosphorylated by Type II Receptor and is recruited into the heteromeric complex, which in turn phosphorylates receptor-regulated Smads (R-Smads), a family of transcription factor controlling the expression of specific target genes. Caveolin-3 (CAV-3) binds to and suppresses activation of the Type I Receptor of MSTN at the plasma membrane and suppresses intracellular myostatin signaling, including phosphorylation of R-Smads and transcription of specific target genes. Loss of caveolin-3 resulting from dominant negative mutations of the caveolin-3 genes in patients with LGMD1C could enhance intracellular myostatin signaling, and thereby result in muscle mass reduction. Type II Receptor, ActRIIB; Type I Receptor, ALK4/5; R-Smads, Smad2/3. P indicates phosphorylation.

primary fibroblasts isolated from patients with idiopathic pulmonary fibrosis. Moreover, retroviral introduction of caveolin-1 ameliorates bleomycin-induced lung fibrosis in mice. Together with this review, it may be concluded that aberrant TGF-β superfamily signaling by loss of caveolins participate in the pathogenesis of some human diseases, including LGMD1C/AD-RMD, breast cancer, and idiopathic pulmonary fibrosis.

Myostatin inhibition therapy is effective, to some extent, with mouse models of several types of muscular dystrophies (29-34). Further investigation is needed to determine which types of myostatin inhibition therapy could be applied and to clarify the molecular mechanism by which myostatin-inhibition improves muscular dystrophy for prospective treatment of patients with muscular dystrophy. As reviewed herein, myostatin inhibition may be a potent therapy for caveolin-3-deficient muscular dystrophy with enhanced myostatin signaling.

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