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論文題目	Mitsugumin 56 (hedgehog acyltransferase-like) is a sarcoplasmic reticulum-resident protein essential for postnatal muscle maturation (ミツグミン56は小胞体タンパク質であり、生後筋成熟に必須である)		
(論文内容の要旨) The sarcoplasmic reticulum (SR) is the powerful Ca ²⁺ -handling organelle of muscle cells and evolutionally represents a highly specialized form of the endoplasmic reticulum (ER). During contraction in striated muscle, the activation of dihydropyridine-sensitive Ca ²⁺ channels in the transverse (T-) tubule opens ryanodine receptor (RyR) channels to trigger SR Ca ²⁺ release. Such functional coupling between the T-tubular and SR Ca ²⁺ channels takes place in junctional membrane complexes constructed by junctophilin subtypes, the triad in skeletal muscle and the diad in cardiac muscle. The SR region closely associated with the T-tubule is called the junctional SR or the terminal cisternae, and contains abundant RyRs that control Ca ²⁺ release. The rest of the SR portion, called the longitudinal SR, is responsible for Ca ²⁺ uptake mediated by enriched Ca ²⁺ -pump proteins. The major SR Ca ²⁺ -handling proteins, including RyRs, Ca ²⁺ pumps and luminal Ca ²⁺ -binding proteins, have been extensively characterized, and such studies have deepened and improved our understanding of the structure and function of intracellular Ca ²⁺ stores. However, there are still many SR components with no functional annotation, and therefore, it is important to examine such as-yet-unknown proteins in muscle physiology. MG56 was identified as a new SR protein from rabbit skeletal muscle. Purified rabbit MG56 was analyzed with an automated Edman sequencer to yield the N-terminal sequence of 17 residues (GVKTALPAAELGLYSLV in one-letter code). The determined sequence is almost identical to those of the hypothetical HHATL(hedgehog acyltransferase-like) proteins deduced from mouse and human databases (16/17 identity). Therefore, MG56 corresponds to the rabbit HHATL counterpart. MG56/HHATL contains an MBOAT (membrane-bound O-acyltransferase) motif and multiple transmembrane segments. Among the MBOAT family members, MG56 has high sequence similarity with vertebrate HHAT, and these proteins form a sub-cluster together with the invertebrate HHAT related proteins and yeast Gup proteins, all of which bear no functional annotations in databases. Northern blot analysis in mouse tissues indicated that MG56 is predominantly expressed in skeletal and cardiac muscle. In longitudinal sections of mature skeletal muscle, MG56-immunoreactivity formed a clear striation-staining pattern at the A-I junction, suggesting its specific localization in the triad junction. By means of sucrose density gradient centrifugation, muscle microsomes can be separated into several fractions; low, intermediate and high-density fractions are enriched in the T-tubule, longitudinal SR and junctional SR, respectively. In this separation process, MG56 was highly enriched in the junctional SR fraction. Moreover, the MG56 and RyR/JP			

signals were approximately merged in the immunohistochemical staining and co-enriched in the membrane preparation. Therefore, MG56 is specifically localized in the junctional SR in mature skeletal muscle.

Mg56-knockout mice showed regular locomotion and grew normally during the postnatal lactation period, but stopped growing approximately on postnatal day 7 (P7) and gradually lost body weight thereafter. All of the knockout mice were severely debilitated and died within two weeks after birth, even though mother mice engaged in pup-rearing irrespective of the genotypes. The weight reduction was most likely due to suckling failure in Mg56-knockout mice, because their gastric milk contents were clearly insufficient after P7. Based on these observations, together with the gene expression profile, Mg56 deficiency may result in skeletal muscle dysfunction leading to suckling failure. Morphological analysis of skeletal muscle in Mg56-knockout mice shows that in thigh muscle from P5 Mg56-knockout mice, the sectioned profiles of SR elements appeared to be dilated in considerable portions.

Light microscopy also detected the formation of the SR vacuoles in Mg56-knockout muscle fibers. Muscle contractility during P7-9 was remarkably enhanced in wild-type mice, but marginally reduced in the knockout mice. Therefore, in the P9 knockout EDL bundles, the weakened tension seems to reflect disrupted development as well as dysfunctioning SR Ca^{2+} handling in the vacuole-containing fibers. To roughly survey altered gene expression in Mg56-knockout muscle, total RNA preparations from lower limb muscle were subjected to gene microarray analysis. Data comparison between the genotypes indicated that an extensive set of transcripts with the “ER stress” annotation were upregulated in the knockout muscle.

In short, *Mg56*-knockout mice grew normally for a week after birth, however, they gradually developed suckling failure and died within two weeks under starvation conditions. In the skeletal muscle of *Mg56*-knockout mice, the SR elements began to swell near the Z-line prior to physical debilitation, and further developed enormous vacuoles spreading over the sarcomeres. However, in tension measurements, regular contractile features were largely preserved in *Mg56*-knockout muscle that contained swelling SR elements. Meanwhile, biochemical analysis demonstrated that unfolded protein response was highly activated in *Mg56*-knockout muscle, suggesting that the suckling failure was caused by disrupted muscle maturation under ER stress conditions. Therefore, MG56 exerts anti-ER stress activity in the developing SR and is essential for postnatal muscle maturation.

(続紙 2)

(論文審査の結果の要旨)

心筋・骨格筋の筋小胞体の新規な構成成分として膜タンパク質MG56を分子同定し、その生理機能の究明のためにMG56欠損マウスを作製・解析した。そのノックアウトマウスは骨格筋にて小胞体ストレスを示し、哺乳障害により生後3週までに死亡する。従って、MG56は骨格筋の生後成熟に不可欠な分子であることが示された。一方、MBOATファミリーに所属するMG56は新規の膜内臓型の脂肪酸転移酵素であることが示唆されるが、その基質の同定に至らず、本研究ではMG56の触媒活性の有無が未解決課題として残されたことが残念な点である。しかしながら、本論文は独自性の高いデータを多分に含んでおり、その研究内容は国際学会において発表され、学術雑誌にも既に掲載され、筋生理学分野に評価されている。

従って、本論文は博士(薬科学)の学位論文として価値あるものと認める。また、平成28年10月24日、論文内容とそれに関連した事項について試問を行った結果、合格と認めた。