筋細胞分化促進ペプチドを導入した新規足場タンパク質の構築

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Construction of new scaffold protein with the peptide that promotes muscle cell differentiation
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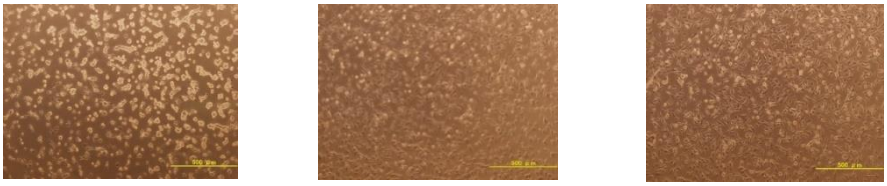
In recent years, skeletal muscle progenitor cells (SMPC) and mature skeletal muscle cells have been attracting an attention as important resources in the fields of skeletal muscle regenerative medicine and drug discovery. Cellular differentiation is regulated by various factors. Interaction between cells and extracellular matrix (ECM) is one of the factors for cellular differentiation. It has been reported that the expression of α6β1 integrin, which is the molecules for the adhesion between cells and ECM, significantly promotes muscle tissue formation during induction of skeletal muscle differentiation. In previously, our laboratory has constructed a scaffold protein that fuses a repeating sequence of elastin-like polypeptide (ELP) with temperature-responsive self-aggregation ability and peptide with cell adhesive ability derived from natural ECM. In this study, we constructed a novel scaffold protein in which a peptide that binds to α6β1 integrin (NPWHSIYITRFG, TWYKIAFQRNRK, SIKVAV) and promotes muscle cell differentiation is fused to the repeated sequence of ELP, and its function was evaluated. Protein was expressed in *E.coli*. After purification, cell adhesive activity was evaluated using mouse myoblast line C2C12 cells. As a result, it was observed that cells attached on the constructed protein.

Keywords : Elastin-like polypeptide; α6β1integrin; muscle cell differentiation

近年、骨格筋再生医療並びに創薬の分野において、骨格筋前駆細胞（SMPC）および成熟骨格筋細胞が重要なリソースとして注目されている。細胞分化を促進する制御機構の 1 つに細胞と細胞外マトリックス（ECM）間の接着がある。筋組織においては、細胞と ECM 間の接着を担う α6β1 インテグリンの発現が、筋組織形成を促進することが報告されている。これまで当研究室では、温度応答性の自己凝集能を有するエラスチン様ポリペプチド(ELP)の繰り返し配列と細胞接着能を持つ ECM 由来ペプチドを融合した足場タンパク質の構築を行ってきた。そこで本研究では、α6β1 インテグリン結合ペプチド（NPWHSIYITRFG、TWYKIAFQRNRK、SIKVAV）を ELP の繰り返し配列に融合した新規足場タンパク質を構築し、その機能評価を行った。マウス筋芽細胞株 C2C12 細胞に対する足場タンパク質の接着能を評価した結果、細胞は足場タンパク質に接着することが示された。



NPWHSIYITRFG　TWYKIAFQRNRK　SIKVAV

図 1 C2C12 細胞への接着能評価