Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells

Aicha Melouane,Mayumi Yoshioka,Jonny St-Amand

Abstract

Secreted protein, acidic and rich in cysteine (SPARC) is differentially associated with cell proliferation and extracellular matrix (ECM) assembly. We show here the effect of exogenous SPARC inhibition/induction on ECM and mitochondrial proteins expression and on the differentiation of C2C12 cells. The cells were cultured in growth medium (GM) supplemented with different experimental conditions. The differentiation of myoblasts was studied for 5 days, the expressions of ECM and mitochondrial proteins were measured and the formation of the myotubes was quantified after exogenous induction/inhibition of SPARC. The results indicate that the addition of recombinant SPARC protein (rSPARC) in cell culture medium increased the differentiation of C2C12 myoblasts and myogenin expression during the myotube formation. However, the treatment with antibody specific for SPARC (anti-SPARC) prevented the differentiation and decreased myogenin expression. The induction of SPARC in the proliferating and differentiating C2C12 cells increased collagen 1a1 protein expression, whereas the inhibition decreased it. The effects on fibronectin protein expression were opposite. Furthermore, the addition of rSPARC in C2C12 myoblast increased the expression of mitochondrial proteins, ubiquinol-cytochrome c reductase core protein II (UQCRC2) and succinate dehydrogenase iron-sulfur subunit (SDHB), whereas the anti-SPARC decreased them. During the differentiation, only the anti-SPARC had the effects on mitochondrial proteins, NADH dehydrogenase ubiquinone 1 beta subcomplex subunit 8 (NADHB8), SDHB and cytochrome c oxidase 1 (MTCO1). Thus, SPARC plays a crucial role in the proliferation and differentiation of C2C12 and may be involved in the link between the ECM remodeling and mitochondrial function.

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Protocol

Step 1.
Before starting cell culture : General considerations

Step 2.
C2C12 cell line

Step 3.
C2C12 cell culture

Step 4.
Medium and reagents preparation under clean bench

Step 5.
Maintenance of CO2 incubator

Step 6.
Preparation of clean bench before use

Step 7.
Washing glass wares

Step 8.
C2C12 cell culture thaw

Step 9.
C2C12 cell culture passage10. Viability Test

Step 10.
Cleaning of clean bench after use

Step 11.
Storage of cellsII. Effects of SPARC on C2C12 cells differentiation, ECM remodeling andmitochondrial function

Step 12.
Number of cells needed

Step 13.
Differentiation treatment

Step 14.
Differentiation Medium and reagents

Step 15.
General procedure for differentiation

Step 16.
Doubling time of C2C12 and myoblast fusion

Step 17.
Effects of exogenous SPARC inhibition/addition on morphological analysis

Step 18.
Effect of induction / inhibition of SPARC on ECM and mitochondrial proteins

Step 19.

ANNOTATIONS

rimca@live.fr mouloudia87 09 Sep 2017

. Effect of induction / inhibition of SPARC on ECM and mitochondrial function during myoblasts proliferation.