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The role of the adipogenic stimuli in the regulation of myoblast fusion in the process of development and regeneration of skeletal muscles

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BACKGROUND: The key stage of muscle fiber formation is the fusion of myoblasts [1, 2]. Violations of this process due to genetic, metabolic or other factors, lead to the degradation of skeletal muscles, its replacement by fatty or fibrous tissue. It is known that the pathological interaction between muscle and adipose tissue can adversely affect the development and regeneration of muscles [3]. In this study, we used an *in vitro* muscle differentiation model based on the C2C12 myoblast line to investigate the effect of the adipogenic signal on myoblast fusion.

METHODS: Three types of stimulation of C2C12 myoblast differentiation were used: myogenic DM1, adipogenic DM2 and mixed - DM3. RNA was isolated on days zero to seven (d0 - d7) from the start of differentiation. Myotubes were fixed and stained using antibodies to MYHs, the size of the tubes was determined using Zeiss Zen software. RNA expression was determined using qPCR. RNA sequencing was performed on a MiSeq instrument in order to reveal a global picture of the coordinated regulation of myogenic and adipogenic stimuli. Analysis of sequencing data and visualization was performed using the Phantasus web application.
RESULTS: Myotubules were formed in all types of differentiation. Immunocytochemistry confirmed their maturity (MYH +). The myotube morphology was different: in DM1/DM3 predominantly long and thick tubes were observed, in DM2, short and thin tubes (the difference was $p < 0.001$). Interestingly, that DM2 culture showed the highest fusion coefficient. However, 47% of the tubes in DM2 were formed by fusion of 3 or less myoblasts, while in DM1/DM3 ~70% of myotubes were 4-15-nuclear. The morphology of the fibers correlates with the RNA expression of the main regulators of myoblast fusion *Myomaker* and *Myomixer*: it is reduced in DM2 differentiation. Analysis of RNA sequencing showed that the signaling pathways responsible for the myoblast fusion stage during the development/regeneration of myotubes are inhibited by the stimulation of adipogenesis in DM2 culture.

CONCLUSION: The start of adipogenic differentiation of C2C12 myoblasts leads to impaired maturation of myotubes at the stage of myoblasts fusion.

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