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Scleraxis and MyoD fused with the TAT polypeptide may induce equine adult stem cells towards tenogenic and myogenic fates

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The polypeptide TAT is a small molecule essential for viral replication that possesses the unusual property of entering the cells from the extracellular milieu. Several experiments using recombinant GFP fused, either to TAT or to its basic domain, showed that the internalization process occurs through caveolar endocytosis.

In order to produce tenocytes and myocytes from undifferentiated peripheral blood MSC, we present an innovative methodology based on the use of transcription factors Scleraxis and MyoDfused with the sequence TAT which promotes the cellular internalizationwithout the use of viral vectors. MyoD and Scleraxis are two transcriptional factors that respectively promote myogenesis and tenogenesis. The nucleotide sequence encoding human MyoD was amplified from a human cDNA library while the nucleotide sequence encoding mouse Scleraxis gene was amplified from plasmid RCAS(B) mScx (Addgene). The amplified products were cloned in plasmid pTAT-Ngl to generate the fusion protein TAT-MyoD and TAT-Scleraxis containing the translocation domain of the HIV-1 proteinTAT (underlined)(MRGSHHHHHHGMARGYGRKKGRQRRR). The fusion proteins were then expressed and purified adapting standard recombinant techniques.

In this study we used the undifferentiated MSC collected with a non-invasive withdrawal from peripheral blood (pbMSC) of horse. We have tested different incubation times to detect when the proteins fused with TAT were fully internalized in the nucleus of pbMSC and we have demonstrated that MyoD-TAT and Scleraxis-TAT protein can be found in the cell nucleus after few hours from the inoculation.

Finally, we have evaluated the myogenic and tenogenic differentiation of MSC; for myogenic induction cells were induced by MyoD-TAT and co-cultured with mouse myoblast cell line C2C12 whereas, for tenogenic induction, cells were induced by Scleraxis-TAT with the addition of specific growth factorsas BMP-12 and FGF-2.The differentiation towards myogenic and tenogenic lineages of pbMSC were analysed using immunofluorescent specific antibodies.

Biography

Chiara Gomiero completed her Degree in Industrial Biotechnology at University of Padua, Italy in 2010. Since then, she was a Temporary Research Assistant in Xeptagen SpA in Venice and she worked, for one year, in the diagnosis of new tumor biomarkers for the diagnosis and treatment of the main solid tumors of the adult. Then she won a bursary with Telethon at the Department of Biomedical Sciences of University of Padua where she studied three genetic diseases that affect striated muscles: type 2D Limb Girdle Muscolar Dystropy, Brody's disease and CPVT. Actually is a PhD Student in Dept. Comparative Biomedicine & Food Science at University of Padua and works with stem cells for the regeneration of muscle, tendon and epithelial pathologies. She presented abstracts/posters/talks in some conferences.

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