The role of Maged1 in skeletal muscle myogenesis

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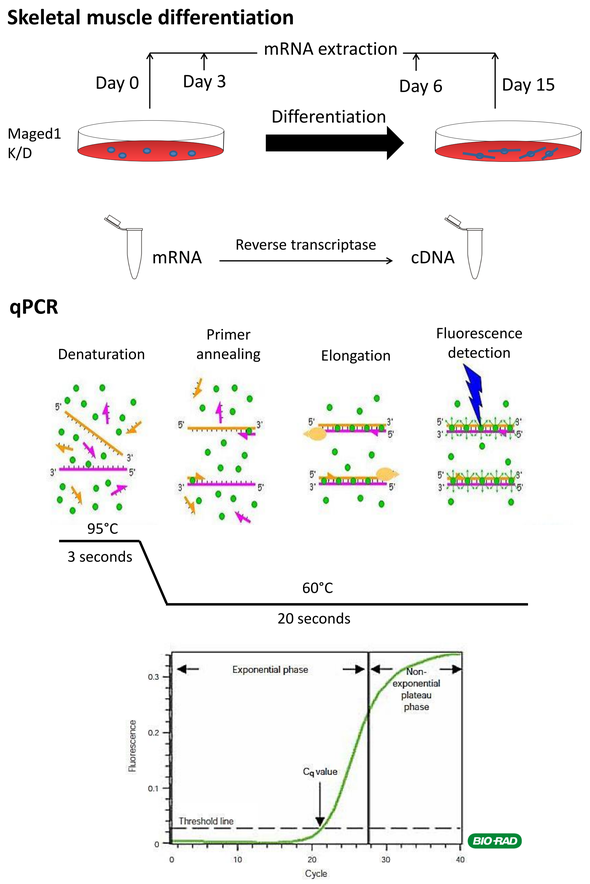
Abstract

The study of molecular mechanisms involving the regulation of skeletal muscle growth may play an important role in the development of future treatments for patients suffering from muscle loss due to aging, disease or injury. Knowledge concerning which factors can activate skeletal myogenesis will facilitate the advancement of stem cell therapies to replace damaged tissue. Melanoma antigen gene-D1 (Maged1) is a member of the MAGE family of proteins that has recently been shown to be important in terminal skeletal myogenesis. It is known to alleviate Msx-dependent repression of myogenic genes during terminal myoblast differentiation (1). Our lab has linked Maged1 to an important early developmental transcription factor Pax3 through Co-Immunoprecipitation (CoIP) and mass spectrometry experiments. Pax3 is expressed in myogenic progenitors and plays a role in the activation of myogenic regulatory factors. To further study the role of Maged1 in skeletal myogenesis, we examined the differentiation of shMaged1 mouse embryonic stem cells (mESc). Quantitative polymerase chain reaction (qPCR) was used to investigate the impact of the knockdown on important myogenic genes. The knockdown resulted in a significant decrease in the expression of myogenic regulatory factors, Myogenin, MyoD and myogenic progenitor marker Pax3. Trends towards reduced expression of mesodermal marker Brachyury T and myogenic regulatory factor Myf5 were also observed. These results show that Maged1 is important for the expression of important myogenic genes required for embryonic skeletal myogenesis.

Methods



Background

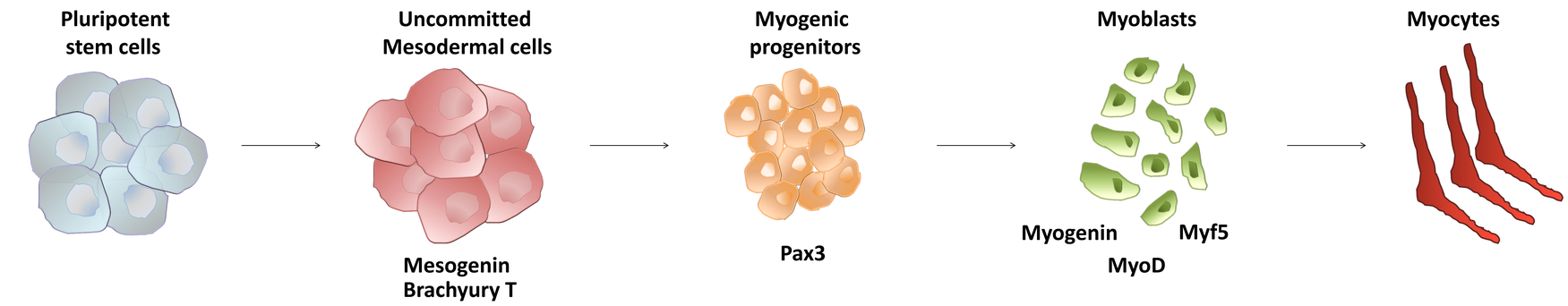


Figure 1. Signalling proteins and transcription factors involved in skeletal myogenesis. Stem cells differentiate into uncommitted mesodermal cells, where the transcription factors BrachyuryT (BraT) and Mesogenin are expressed. This is followed by the formation of myogenic progenitors, where Pax3, an early developmental transcription factor, is expressed. Myogenic progenitors then differentiate into myoblasts, where the expression of myogenic regulatory factors (MRFs), MyoD, Myf5, and Myogenin (MyoG) are observed. In the final step, myoctyes are formed from myoblasts.

Results

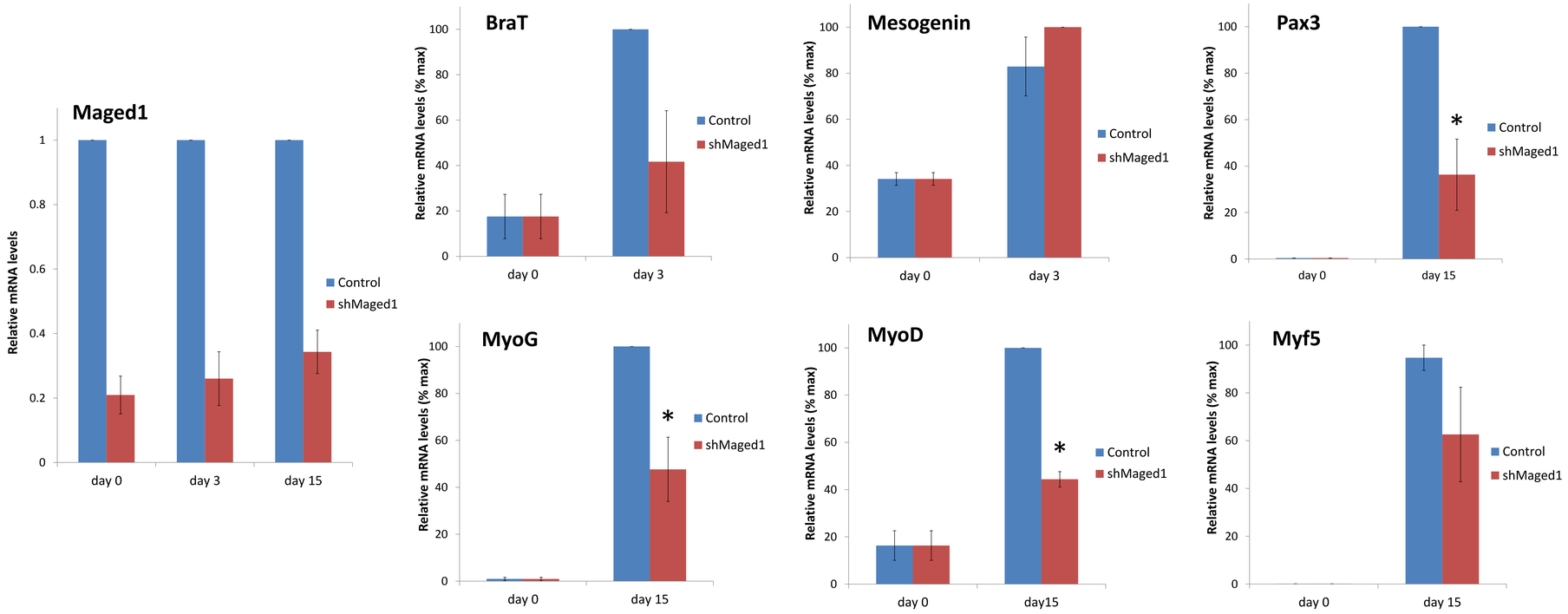


Figure 2. qPCR profiling of important myogenic genes in Maged1 knock down and control mouse embryonic stem cells during *in vitro* differentiation. Total mRNA was collected at the indicated time points of myogenic differentiation. cDNA was synthesized using a reverse transcriptase kit and qPCR was run on the samples using the primers for the indicated genes. Results are normalized against β-actin and expressed as a fold change over day 0 for each respective cell line. Two-tailed Student's t test was performed to assess the significance of the results, where $p<0.05$ was considered significant (*). Error bars represent +/- standard error, n=3.

Conclusion

Maged1 is important for the expression of the late important myogenic genes, Myf5, MyoD, Pax3 and Myogenin, in embryonic skeletal myogenesis.

Maged1 does not seem to play a role in the expression of mesodermal genes, BraT and Mesogenin, during the early stages of myogenesis.

Further analysis may include profiling of other myogenic markers and protein analysis through Western blotting and immunofluorescence.

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References

Nguyen, T. H. N., Bertrand, M. J. M., Sterpin, C., Achouri, Y., & De Backer, O. R. Y. (2010). Maged1, a new regulator of skeletal myogenic differentiation and muscle regeneration. *BMC cell biology*, *11*, 57.

Kuwajima, T., Taniura, H., Nishimura, I., & Yoshikawa, K. (2004). Necdin interacts with the Msx2 homeodomain protein via MAGE-D1 to promote myogenic differentiation of C2C12 cells. *The Journal of biological chemistry*, *279*(39), 40484–93.