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MYOGENIC REGULATORY FACTOR EXPRESSION IN SATELLITE CELLS CULTURED FROM ADULT AND OLD RAT SKELETAL MUSCLES

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Introduction: The family of myogenic regulatory factors (MRFs) includes four known transcription factors, MyoD, Myf-5, myogenin, and MRF4/herculin, which are able to induce muscle-specific gene expression. We developed a method of satellite cell culture that produces a three-dimensional skeletal muscle organoid, a myooid. Our hypothesis is that the temporal expression of MRFs during the proliferation and differentiation of myooids cultured from adult and old rat skeletal muscle is the same.

Methods: Satellite cells were cultured from soleus muscle of adult and old rats. Total RNA was extracted during proliferation and differentiation of the cultures. Total cellular RNA was hybridized with P32-labeled RNA probes for myogenic regulatory factors. RNase T2 was used to digest the single-stranded RNA. RNase-resistant fragments were separated by electrophoresis on acrylamide/urea gels. After exposure to x-ray film, gels were analyzed by scanning densitometry.

Results: In the adult and old rat satellite cell cultures, the first MRF transcripts to be expressed were MyoD and Myf-5. Both factors were expressed early and at the time of cell confluence. Myogenin expression correlated with myoblast fusion and myotube formation at 72 hours in adult rat cultures and after 144 hours in old rat cultures. The last factor, MRF4/herculin, was expressed by 96 hours in the adult rat cultures, and by 360 hours in the old rat cultures.

Conclusions: These preliminary data support our hypothesis. MyoD and Myf-5, which function as commitment factors for the myogenic phenotype, were present early in the course of satellite cell proliferation in both adult and old rat cultures. Myogenin and MRF4/herculin, which are necessary for terminal differentiation of myogenic cells, were expressed later at the time of myotube fusion in both adult and old rat cultures. The pattern of MRF gene expression during the proliferation and differentiation of skeletal muscle satellite cells in our organoid model was consistent with the roles of the MRFs in muscle development. Thus, although the time course was prolonged in old rat satellite cell cultures compared to adult rats, the pattern of expression of the MRFs during the growth and differentiation of the satellite cells cultures was the same.

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