

LITTLE EVIDENCE FOR CONTINUAL MUSCLE FIBER REPAIR BY PRESUMED CIRCULATING STEM CELLS.

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It has recently been claimed that bone marrow derived blood cells can contribute to the myogenic compartment. Such occurrence would bear considerable therapeutic potential in genetically determined muscle diseases like Duchenne Muscular Dystrophy due to a loss in the structural muscle protein dystrophin.

In order to identify circulating stem cells we transplanted male bone marrow carrying EGFP reporter and intact dystrophin genes into female MDX mice. Throughout life MDX mice suffer muscle fiber damage, thus, if circulating cells do repair damaged muscle fibers (by fusing), the frequency of green fluorescent protein (GFP) and dystrophin positive muscle fibers should continually increase with time.

Upon sacrifice at 16 to 46 weeks after bone marrow transplantation, soleus and e.d.l. muscles typically contained some 0.5 - 2% GFP-positive muscle fibers (n=44), TA muscles around 1 - 5% (n=24). However, no increase with time in the frequency of GFP labelled muscle fibers was detectable. On frozen sections, dystrophin positive muscle fibers were present in all muscles, but there was no increase in frequency with age either and no difference between experimental and untreated MDX mice. Thus, most dystrophin positive fibers are probably revertant fibers, rather than fibers which have acquired the donor gene.

Also, Y-chromosome specific DNA (a separate marker of the male donor cells in female recipients) extracted from whole muscles did not increase with time (n=18). These findings and the lack of increase in both GFP and dystrophin positive fiber numbers speaks against the existence of circulating stem cells which would enter damaged skeletal muscle fibers and acquire the myogenic program.

Similarly, cryodamaging or crushing muscles 8 and 16 weeks after transplantation did not produce more GFP or dystrophin positive muscle fibers. There was, however, a higher yield in Y-chromosome related DNA in the damaged versus undamaged muscles and in untreated MDX versus non-MDX muscles due to accumulation of donor derived cells in the interstitial space. The finding of donor derived nuclei within structurally intact GFP negative or dystrophin negative muscle fibers indicates that donor cells enter (damaged) muscle fibers but may remain there without expressing or acquiring a myogenic program.

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