

MODULATING THE NICHE IN MUSCULAR DYSTROPHY INCREASES HOST REGENERATIVE CAPACITY

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BACKGROUND: The hostile microenvironment characteristic of dystrophic skeletal muscle has been shown to severely limit skeletal muscle regenerative capacity and the regenerative potential of corresponding stem cell populations. Cellular therapies have been suggested as a potential method to restore muscle function and healing capacity by reinstating the stem cell reservoir. Recent studies investigating the trophic influence of donor stem cells on surrounding tissues have created an interest in examining this role as it applies to muscular dystrophy and factors dictating stem cell engraftment and myogenic differentiation.

METHODS: Proliferation Assay: Muscle derived stem cells were harvested from 3 week-old *mdx* mice using a modified pre-plate technique (MDSC^{mdx}). MDSC^{mdx} were co-cultured with MDSCs isolated from female wild type mice (MDSC^{WT})(isolated through the same technique), in variable transwell arrays. Paracrine influences were measured under 4 conditions: MDSC^{mdx}/MDSC^{WT}, MDSC^{WT}/MDSC^{mdx}, MDSC^{mdx}/MDSC^{mdx} and MDSC^{WT}/MDSC^{WT}. The population in the bottom well was analyzed for all variables considered. This array allowed for paracrine effects to be observed without direct cell-to-cell contact. Proliferation was measured using Live Automated Cell Imaging of co-cultured cells. Images were analyzed at 12 hour intervals for 60 hours, at which point confluence was achieved. Population doubling time was calculated as $T/[\log_2(N_F/N_0)]$ (T=time in culture; N₀, N_F=cell number, initial and final).

Differentiation Assay: A transwell differentiation assay was created based on the proliferation setup as described above. Cells were plated at a density of 500 cells/cm², adequate to achieve >70% confluence after 72 hours of proliferation. After 72 hours, cell media was removed and replaced with serum starved differentiation-inducing media, and the cells were co-cultured an additional 48 hours. Immunocytochemistry for fast isoform myosin heavy chain (fMHC) and nuclei staining with DAPI was used to calculate the fusion index (total number of nuclei within myotubes relative to the total number of nuclei present in each image). ELISA was performed using the media in the 4 co-culture groups in order to quantify cytokine secretions for Vascular Endothelial Growth Factor (VEGF) and Transforming Growth Factor β 1 (TGF- β 1), a stimulator of VEGF secretion, after 48 hours of co-culture.

RESULTS: There was no difference in MDSC^{mdx} proliferation in the presence or absence of MDSC^{WT}. However, a significant increase in MDSC^{mdx} fusion was observed when MDSC^{mdx} were co-cultured with MDSC^{WT} cells, when compared to MDSC^{mdx}/MDSC^{mdx} controls. TGF- β 1 levels were found to be highest in MDSC^{mdx}/MDSC^{mdx} cultures, with an average relative concentration of 1534 pg/mL, compared to MDSC^{WT}/MDSC^{WT} with an average concentration of 810 pg/mL. Co-culture TGF- β 1 concentrations followed a predictable pattern based on cell ratio in each experimental setup. VEGF levels were found to be higher in MDSC^{WT}/MDSC^{WT}, with an average relative concentration of 410 pg/mL compared to MDSC^{mdx}/MDSC^{mdx} (329 pg/mL). Interestingly, VEGF concentration was the highest when MDSC^{mdx} were co-cultured with MDSC^{WT}, 442 pg/mL. Additionally, fewer nuclei were observed in MDSC^{WT} groups co-cultured with MDSC^{mdx}.

DISCUSSION: These results reveal that, through cytokine exchange, the regenerative capacity of a severely weakened host may be improved by the trophic influence of transplanted cells. Furthermore, early analysis of TGF- β 1 and VEGF levels suggest that even a hostile host environment can stimulate an increased angiogenic response by donor cells, potentially benefitting the regenerative capacity of both host and transplanted stem cells. Results revealed a significant decrease in nuclei present in MDSC^{WT} when co-cultured with MDSC^{mdx}, suggesting a need to further measure the host effect on donor cell proliferation. This promising look at consequential up-regulation of the VEGF pathway and resulting improved fusion of dystrophic cells in the presence of wild type MDSCs indicates that cellular therapies may play a dual role in dystrophic muscle regeneration, modulating the host environment to benefit both host and donor stem cells. Our findings are consistent with previous studies demonstrating that genetic up-regulation of VEGF results in a similar enhanced myogenicity of dystrophic muscle. In conclusion, this study suggests that a therapeutic benefit from cellular transplantation may be observed simply through modulation of the dystrophic niche with donor MDSC populations.

