INTRODUCTION: Muscle-derived stem cells have significant potential in several biomedical applications, including cell-based therapies and tissue engineering. Their demonstrated multipotentiality is valuable for both bone healing and muscle regeneration (1). However, in vitro expansion is necessary, as the cells represent a small percentage of the adult muscle biopsy.

MATERIALS AND METHODS: A primary cell culture, MDSC, was obtained from newborn C5710J newborn mice using the preplate technique (2). The clone of muscle-derived stem cells, MC13 cells, at passage 25, was derived from newborn mdc mice. Cells were cultured DMEM with 5% horse serum, 5% fetal calf serum, 1.25% chick embryo extract, and 1% penicillin/streptomycin. Cytokines examined were human recombinant EGF (100 ng/mL), human recombinant FGF-2 (100 ng/mL), murine natural IGF-1 (100 ng/mL), and mouse recombinant SCF (25 ng/mL).

RESULTS: Colony Growth: The MDSC and MC13 were plated at a density of 250 cells/cm² in 10% serum medium. Growth factors were added at the time of plating and again with fresh medium at t=60 hours. Using a microscopic imaging system, time-lapsed visible imaging was obtained for individual cells growing colonies (4). Cell population growth was monitored by counting the total number of cells, N. Continual viewing of the time-lapsed video allowed us to count the number of dead cells, M. Consequently, cell death was < 5% and did not contribute significantly to the growth rate. Cell Cycle Duration: From the video images, 100 cells were then selected and tracked. The division time, DT, of each cell was determined by direct observation of the time lapsed between cytokinesis. Mitotic Fraction: To determine the mitotic fraction, α, the best fit of the population growth data to the model equation was determined by nonlinear regression using the SigmaStat package (Marquardt-Levenberg algorithm). Six curves for each treatment were fitted and the best fit of the population growth data to the model equation was estimated as the average of the fitted curves. Statistical Testing: Comparisons of cell numbers at each time point were made using the Student’s t-test. Division times were analyzed using the Mann-Whitney rank sum test, p<0.05. The division fraction was determined using nonlinear regression with correlation coefficients R² > 0.90. A Student’s t-test (p=0.05) was used to determine differences.

RESULTS: Colony Growth: The MDSC population size, N, was increased significantly with EGF (72 hrs, 96 hrs, p<0.05), FGF-2 (96 hrs, p<0.05), SCF (72 hrs, 96 hrs, p<0.05), and IGF-1 (96 hrs, p<0.05) (Fig. 1). The cultured MC13 cells also responded to FGF-2 (72 hrs, 96 hrs, p<0.05), EGF (72 hrs, 96 hrs, p<0.05), IGF-1 (72 hrs, 96 hrs, p<0.05) and SCF (96 hrs, p<0.05) (Fig. 2).

Cell Cycle Duration: To examine the contribution of division time on proliferation kinetics, the mean cell division time for uninstantiated MDSC was determined to be 15.8±3.8 hrs; and 16.0±5.3 hrs for MC13 (Table 1). Of the four growth factors (EGF, FGF-2, IGF-1, and SCF) that expanded the primary MDSC population size, only (FGF-2) reduced the division time (13.8±2.5, p<0.01), suggesting that other mechanisms were responsible for the MDSC expansion. For the cultured MC13 cells, the division time was significantly reduced with the presence of factors which led to an increase of total cell numbers (EGF, FGF-2, IGF-1, and SCF) (Table 1). Mitotic Fraction: The contribution of the division fraction, α, on proliferation kinetics revealed that addition of EGF, IGF-1, or SCF increased the number of mitotically active MDSC cells to 86% (p<0.01), 84% (p<0.04), and 92% (p<0.001), respectively. This likely accounts for the increase in population size, which could not be attributed to division time shortening (Table 1). The cultured MC13 cell clones had no significant increases in the mitotic fraction, α, with any of the growth factors.

DISCUSSION: The results showed that freshly-isolated myogenic stem cells (MDSC) and cultured stem cells (MC13) responded differently to cytokines. Expansion of the freshly-isolated, primary culture, MDSC, occurred with SCF, EGF, and IGF-1 by recruiting cells into the cell division cycle (Figure 3). However, expansion of MC13 is attributed to reduced cell cycle length with FGF-2, EGF, SCF and IGF-1. The study identifies several cytokines to expand stem cells and illustrates that culturing affects the response mechanism. Moreover, the study demonstrates the importance of monitoring nonexponential growth parameters which control cell expansion.

REFERENCES:

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