INTRODUCTION: The future success of myogenic-based treatment modalities will require an enhanced understanding of the highly heterogeneous nature of the myogenic progenitor cell pool, which has been previously documented by numerous researchers. Further, for translation of experimental animal results to clinical applications, reliable in vitro selection criteria must be established and must be translatable across species. While research into the utility of surface markers is ongoing, as an alternative we have investigated in vitro cell behavioral characteristics under imposed conditions that challenge the propensity of myogenic progenitors to undergo differentiation. Previous observations in the mouse suggest an enhanced in vivo regenerative capacity of myogenic populations attributable to their in vitro ability to maintain a proliferative and undifferentiated state (1). Based on these observations we hypothesized that such behavior may represent an a priori indicator of regenerative capacity following transplantation. To characterize this proposition, a rat cell isolation and transplantation model was evaluated in an identical manner. In agreement with the results obtained from the mouse, we observed a significant correlation between regeneration capacity and induction of differentiation.

METHODS: Cell Isolation. A modified version of the preplate technique (1,2,3) was utilized to obtain various myogenic cultures from dissociated skeletal muscle. Both hindlimb gastrocnemius muscles from a single female rat (Sprague Dawley, 6-10 weeks old) were combined for enzymatic digestion and cell isolation. Evaluation of the percentage of myogenic cells initially contained within each preplate culture was performed via desmin immunocytochemistry (1,2,3,4). In addition to primary cultures (utilized within 1 week of isolation), a previously prepared rat muscle-derived stem cell (MDSC) population was utilized in the experiments. This cell population was obtained via an identical preplate isolation procedure using neonatal rat hindlimb muscle (6-2 weeks old), and was derived from a PP6 culture using a previously described methodology (1,2,3,4). Evaluation of Myogenic Commitment. A previously described protocol (1,3,4) was used to evaluate each myogenic population for the expression of Myogenic Regulatory Factor family (MRF) proteins Myf5, MyoD, and myogenin in order to gauge their respective progression toward end-stage myogenic differentiation. Evaluation of CD34 expression. Flow cytometric analysis of CD34 expression within each preplate was performed in a manner similar to that previously described (1,3,4). 7-AAD was added for dead cell exclusion, and viable and non-hematopoietic lineage cells (as distinguished by CD45 expression) were analyzed by appropriate gating. Cell Transplantation and Dystrophin Restoration. Myofiber regeneration was assessed via dystrophin restoration. Rat cell populations were transplanted by single direct injection into the gastrocnemius muscles of dystrophic and immune-deficient (mdx/Scid) mice. Dystrophin restoration was evaluated at 7 days post-injection via immunohistochemistry, and a regeneration index defining the number of dystrophin-positive myofibers generated per 1×10^5 injected myogenic cells was determined for each population, as described previously (1,3,4). Proliferation and Differentiation/Fusion Characteristics. An automated microscopic imaging system (Automated Cell Technologies, Inc.) was used to acquire time-lapsed brightfield imaging data pertaining to cell division and temporal fusion characteristics, as previously described (5). Immediately following this 96-hour observation period, dual immunofluorescence staining was performed for evaluation of desmin and myosin heavy chain (1:250; clone MY-32, Sigma). Statistics. Multiple comparisons were made among all four cell populations in terms of regenerative efficiency and proliferation and fusion behavior using the brightfield/immunofluorescence observations and a one-way ANOVA as well as Student-Newman-Keuls post-hoc pairwise comparisons (when a significant F-value was obtained).

RESULTS: Desmin and CD34 Expression. The average percentage of myogenic cells within each preplate population from PP1 to PP6, as determined through desmin expression, was 14±2, 34±13, 58±4, 86±4, 86±2, and 81, respectively. MDSC cultures contained an average percentage of 66±2 desmin-expressing cells. Flow cytometric evaluation of non-hematopoietic cells within the rat cell cultures revealed minimal CD34 expression. Less than 2% of the cells within each preplate (PP1-PP6) expressed CD34, regardless of donor age (7-10 days or 6-10 weeks) or the length of time spent in culture (less than 24 hours or 5-7 days). Similar results were observed in the rat-derived MDSC population. Evaluation of Myogenic Commitment. High percentage myogenic cultures could not be obtained from PP1 and PP2 through replating, and thus were omitted from MRF expression analysis and evaluation of transplantation and fusion characteristics. Overall, the MDSC population demonstrated a comparatively lower myogenic commitment, with a small percentage of cells expressing both early- and late-stage MRF proteins (MyoD and myogenin, respectively).

DISCUSSION: We have sought to distinguish cellular traits that may be used to identify efficient myogenic progenitor cells within culture (i.e., cells that possess a high regenerative capacity following isolation from muscle biopsy and transplantation into skeletal muscle). Using previously described procedures and parameters to define their behavior within culture, we observed that the in vitro reduced fusion behavior of a given rat myogenic cell population correlates positively with its regenerative performance in vivo. These findings closely parallel those obtained earlier using a mouse model. Such results imply that inherent discrepancies in cellular differentiation behavior may have a significant impact upon the ability of a particular population of implanted cells to efficiently regenerate skeletal myofibers.

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