Kicking Up Muscle Cell Engraftment with Notch

September 17, 2012

EM Scherer

Duchenne muscular dystrophy (DMD) is the most common and severe form of muscular dystrophy. It is a lethal X-linked disorder that affects approximately one in 3500 boys worldwide and is caused by mutations in the dystrophin gene.

To restore functional dystrophin levels in individuals suffering from DMD, muscle stem cell transplant therapies are being developed that use healthy donor cells to generate new muscle. Most of these transplant approaches use muscle-derived donor cells, as they contain a minor fraction of satellite cells that act as myogenic progenitor cells (i.e., muscle stem cells). However, one of the current limitations of this approach is that a considerable number of freshly procured donor cells are needed for efficient engraftment, particularly as DMD affects a large mass of tissue. Moreover, the expansion of muscle-derived donor cells *in vitro* actually promotes *ex vivo*differentiation, thus reducing the effective number of myogenic progenitor cells.

Recently, Drs. Colleen Delaney and Irwin Bernstein of the Clinical Research Division showed that culture of human cord blood in the presence of Notch ligand Delta-1^{ext}-IgG increased the number of hematopoietic stem cells for myeloid repopulation in an immunocompromised murine model (NOD/SCID). As activation of Notch signaling is also required for muscle regeneration after injury, Dr. Maura Parker, an associate in Clinical Research, conducted a study with members of the Storb Lab (Clinical Research), members of the Tapscott Lab (Human Biology Division) and outside collaborators to examine whether the culture of canine muscle-derived cells (MDC) with Delta-1^{ext}-IgG improves the yield of myogenic progenitor cells for transplantation.

The authors found that canine MDC cultured with Delta-1^{ext}-IgG for eight days expanded 6.5-20-fold prior to transplantation. Although the number of expanded cells did not statistically differ between MDC cultured with Delta-1^{ext}-IgG and MDC cultured with an irrelevant human IgG, the former did exhibit significantly improved myogenic potential compared to the latter. For example, increased Pax7 expression was observed in Delta-1^{ext}-IgG-expanded MDC by immunohistochemistry, where Pax7 is a transcription factor that specifies the muscle stem cell lineage. Delta-1^{ext}-IgG-expanded MDC also generated significantly more canine dystrophin-positive muscle fibers and more Pax7-positive cells than control MDC following xenotransplantation into the tibialis anterior muscle of NOD/SCID mice hind limbs. Moreover, cells engrafted from Delta-1^{ext}-IgG-expanded MDC gave rise

to more canine dystrophin-positive muscle fibers following a secondary regeneration event induced *in situ* with barium chloride than MDC expanded with an irrelevant human IgG.

Although culture with Delta-1^{ext}-IgG improved the yield of myogenic progenitor cells from *ex vivo* expansion, xenotransplants performed with 10,000 Delta-1^{ext}-IgG-expanded MDC resulted in two-fold fewer canine dystrophin-positive muscle fibers than xenotransplants performed with an equivalent number of fresh MDC. Nevertheless, because Delta-1^{ext}-IgG expands the total number of MDC by up to 20-fold, even if losses in engraftment efficiency compared to fresh MDC are taken into account, the effective yield of Delta-1^{ext}-IgG-based expansion for transplant purposes remains as high as 10-times the starting number of MDC.

These findings demonstrate that expanding MDC on immobilized Delta-1^{ext}-IgG enhances their potential to regenerate dystrophin and maintain myogenic progenitor cell populations in recipient muscle. Consequently, a Notch activation-based expansion approach may help to improve human muscle stem cell transplant therapies in much the same way it has advanced hematopoietic stem cell transplants.

Parker MH, Loretz C, Tyler AE, Duddy WJ, Hall JK, Olwin BB, Bernstein ID, Storb R, Tapscott SJ. 2012. Activation of Notch signaling during ex vivo expansion maintains donor muscle cell engraftment. *Stem Cells*, Epub ahead of print, doi: 10.1002/stem.1181.

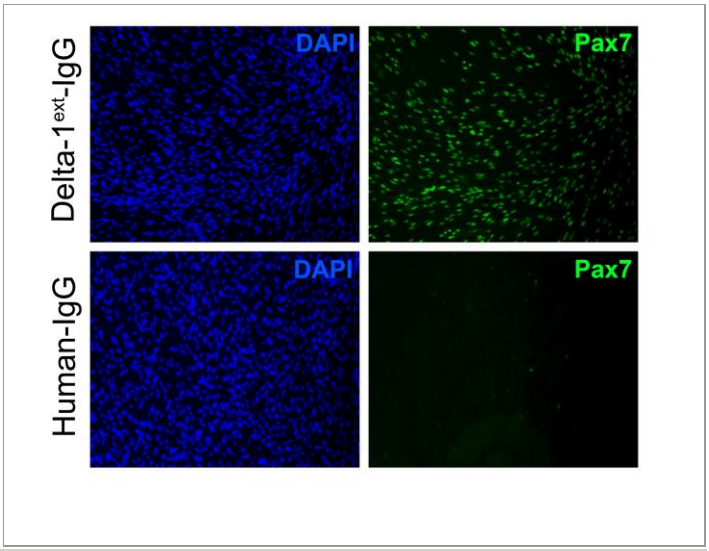

Image provided by Dr. Maura Parker

Prior to transplantation, donor muscle-derived cells (MDC) were expanded for eight days ex vivo with either the Notch ligand Delta-1ext-IgG or an irrelevant human IgG. The number of total expanded cells did not differ between treatment groups; however, Delta-1ext-IgG treatment better maintained the myogenic potential of MDC. As shown above, Delta-1ext-IgG-expanded MDC exhibit increased Pax7 expression (green) by immunohistochemistry compared to human-IgG-expanded MDC, where Pax7 specifies the muscle stem cell lineage. Cell nuclei are stained blue with DAPI.