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# Converting Pathological Cells to Therapeutic Ones: An Odyssey Through Pluripotency

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In a study reported in the 27 June 2012 issue of *Science Translational Medicine*, Tedesco and collaborators[1] put into practice methodologies for generating, differentiating, and transplanting pluripotent cells derived from human patients with limb-girdle muscular dystrophy type 2D (LGMD2D, also called α-sarcoglycanopathy). Starting from patient fibroblasts or myoblasts, the authors first established and validated human induced pluripotent stem (iPS) cell lines, which were then coaxed toward differentiation into mesoangioblast-like cells termed "human iPS-derived mesoangioblast-like stem/progenitor cells" (HIDEMs). These cells were then supplemented with complementary DNAs encoding human α-sarcoglycan (Sgca) and a tamoxifen-inducible myogenic transcription factor (MyoD). These cells participated in muscle regeneration after intramuscular administration into the Sgca-null mouse, a model of the human disease. Moreover, when injected by the arterial route, the cells were able to colonize the downstream muscles and to improve their phenotype and their functional capacities. The findings suggest that cells prepared from simple biopsy specimens from human patients can be committed through a multistep procedure into corrected cells able to cross the endothelial barriers, allowing systemic distribution over large muscle volumes and widespread correction of myopathic features.

Muscular dystrophies are a heterogeneous family of orphan genetic diseases for which no cure is yet available. LGMD2D is a rare disorder (prevalence between 1 and 9 per million worldwide) caused by mutations in the small gene encoding Sgca, a member of the sarcoglycan complex located at the muscle membrane and involved in linking the cytoskeleton to the extracellular matrix. LGMD2D is characterized by a symmetrical involvement of trunk and limb muscles, calf hypertrophy, proximal weakness, high levels of serum creatine kinase, but absence of cardiac dysfunction.[2] Muscular dystrophies are being targeted by innovative therapeutic strategies.[3] Gene supplementation approaches using viral vectors[4] and post-transcriptional "gene surgery" targeting messenger RNAs are being investigated to restore the expression of functional proteins, but these approaches do not supply a pool of regenerative cells to the degenerating muscle tissues of the patients.

Cell therapy approaches take advantage of the natural fusion between syncytial fibers and individual progenitors during muscle regeneration. The transplanted cells not only constitute a myogenic supply but also are able to carry new genetic material into the hybrid muscle fibers.[5] Initial cell transplantation trials for muscular dystrophies involved myoblasts, which are committed myogenic progenitors expanded in culture from satellite cells, and produced mitigated results underlining their limitations. Although localized tissue repair could be observed, the myoblasts were susceptible to high mortality and could not be delivered through systemic routes. Several new myogenic cell types have been identified, and some among the family of perivascular cells might be amenable to systemic delivery.[6–10] Mesoangioblasts (MABs) in particular are a type of pericyte of muscle origin that can cross endothelial barriers and are under clinical testing in an allogeneic context in children affected by Duchenne muscular dystrophy.[1,6]

The initial goal of the authors of the new study was to produce and use MABs extracted from muscles of LGMD2D patients to correct the pathology in Sgca-null mice. Unexpectedly, the choice of this model disease underlined a potential involvement of pericytes in the physiopathological process of LGMD2D. It was indeed impossible to expand MABs from biopsy samples of adult patients or of adult Sgca-null mice, whereas MABs had been prepared from juvenile Sgca-null mice in a previous study.[7] Therefore, the exhaustion of these cells might constitute a hallmark of LGMD2D and appears comparable to the exhaustion of satellite cells associated with the ongoing degeneration in Duchenne muscular dystrophy.[11] To obtain the desired MABs, the authors then decided to convert somatic cells first into pluripotent cells, and then into MAB-like cells.

The use of embryonic stem (ES) cells for the treatment of myopathies was conceptualized long ago but was hampered by the low efficiency of myogenic commitment. Recently several groups have developed multistep procedures to produce myogenic cells (**Table 1**).[12–23] These are generally based on combinations of mesodermal conversion of ES or iPS cells using specific media; the induction of early progenitors using commitment factors such as Pax3, Pax7, and MyoD; their nesting within embryoid bodies; and, finally, the sorting of differentiated progenitors using flow cytometry or immunomagnetic beads.[24] The originality of the approach by Tedesco and collaborators lies in the commitment toward MAB-like cells (HIDEMs) instead of satellite- or myoblast-like cells, so as to benefit from the ability of MABs to cross endothelial barriers. A few teams have attempted to produce vasculogenic or pericyte-like cells from healthy ES or iPS cells but did not evaluate their capacities in the context of myopathies or through systemic distribution, or both.[19,25] These HIDEMs harbored some characteristics of pericytes such as membrane markers (CD13, CD44, CD49b, and CD146), the expression of molecular markers, the proliferative capacity, and karyotype stability; moreover, they were

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**Table 1 Recent protocols developed to commit pluripotent cells toward myogenic lineages**

| Reference | Line type | Gene introduced | EBs (substrate) | Characterization (FACS) | Selection (FACS) | *In vitro* differentiation | *In vivo* differentiation, route | Formation of teratoma |
|---|---|---|---|---|---|---|---|---|
| Ozasa *et al.*[12] | Mouse ES | *MyoD* (Tet-inducible) | No EBs (none) | CD34, Sca-1, c-kit | No | Markers + | + (Mdx), i.m. | Yes (tumors) |
| Darabi *et al.*[13] | Mouse ES | *Pax3* | EBs (feeder layer) | CD29, CD44, M-Cad, CXCR4 | Flk1$^-$/PDGFαR$^+$ | Markers +, MT + | + (Mdx), i.m., i.a, i.v. | Yes (unselected) |
| Sakurai *et al.*[14] | Mouse ES | None | No EBs (collagen IV) | None | Flk1$^-$/PDGFαR$^+$ | Markers +, MT + | + (IMD), i.m. | Yes (uncommitted) |
| Chang *et al.*[15] | Mouse ES | None | EBs (Matrigel) | M-Cad, Pax7 | SM/C-2.6$^+$ | Markers +, MT + | + (Mdx), i.m. | No |
| Darabi *et al.*[16] | Mouse ES | *Pax3* or *Pax7* | EBs (feeder layer) | CD29, CD34, CD44, CXCR4, M-Cad, syndecan-4, Sca-1 | Flk1$^-$/PDGFαR$^+$ | Markers +, MT + | + (Mdx), i.m., i.a. | No |
| Mizuno *et al.*[17] | Mouse iPS | *Oct4, Sox2, Klf4, c-Myc* | EBs (Matrigel) | CD34, CD56, M-Cad, c-Met, Itg α7 | SM/C-2.6$^+$ | Markers +, MT + | + (Mdx), i.m. | No |
| Darabi *et al.*[18] | Mouse iPS | *Oct4, Sox2, Klf4, Pax7* | EBs (feeder layer) | CD29, CD44, M-Cad, Sca-1 | Flk1$^-$/PDGFαR$^+$ | Markers +, MT + | + (Mdx), i.m. | Yes (unselected) |
| Quattrocelli *et al.*[19] | Mouse iPS from MABs | *Oct4, Sox2, Klf4, c-Myc, Pax3, Pax7* | EBs (collagen) | Initial MABs: AP, Sca-1, CD44, CD140a, CD140b Committed iPS: CD56$^+$ | CD56$^+$ | Markers +, MT+ in cells and in teratomas | + (Sgca-null), i.m. | Yes (unselected) |
| Zheng *et al.*[20] | Human ES | None | EBs (gelatin) | Not done | No | Markers + | + (IMD), i.m. | No |
| Barberi *et al.*[21] | Human ES | None | No EBs (gelatin) | CD44, CD54, CD105, CD106, CD166 | CD73$^+$, then CD56$^+$ | Markers +, MT + | + (IMD), i.m. | No |
| Darabi *et al.*[22] | Human ES, Human iPS | *Pax7* | EBs (Matrigel) | CD13, CD29, CD44, CD56, CD63, CD90, CD105, CD146, Itg α7, M-Cad, MHC 1 | Flk1$^-$/PDGFαR$^+$ | Markers +, MT + | + (IMD-Mdx), i.m. | No |
| Goudenege *et al.*[23] | Human ES, Human iPS | *MyoD1* | No EBs | CD56, CD73 | No | Markers +, MT + | + (IMD-Mdx), i.m. | No |

*Abbreviations*: AP, alkaline phosphatase; Cad, cadherin; c-Met, MNNG HOS transforming gene, or hepatocyte growth factor receptor; CXCR4, chemokine receptor type 4; EBs, embryoid bodies; ES, embryonic stem; FACS, fluorescence-activated cell sorting; Flk1, fetal liver kinase-1; i.a., intra-arterial; i.m., intramuscular; IMD, immunodeficient mouse; iPS, induced pluripotent stem; Itg, integrin; i.v., intravenous; MABs, mesoangioblasts; Markers +, presence of myogenic markers (antibodies, polymerase chain reaction); Mdx, mouse model of Duchenne muscular dystrophy; MHC, myosin heavy chain; MT +, myotube formation *in vitro*; PDGFαR, platelet-derived growth factor receptor α; Sca-1, stem cell antigen-1; Sgca, α-sarcoglycan; SM/C-2.6, a clone of antibody recognizing murine satellite cells.

not tumorigenic *in vivo*. At variance with natural pericytes,[8] HIDEMs required cultivation in the presence of myogenic (i.e., C2C12) cells or transduction with a vector encoding MyoD, a strong and polyvalent muscle transcription factor, to exhibit myogenic differentiation *in vitro*. The HIDEM cells were then engineered to express the therapeutic gene (the complementary DNA encoding Sgca) under control of a muscle-specific promoter so as to avoid ubiquitous expression in other cell types, such as professional immunological presenting cells. To improve their myogenicity, the cells were further transduced with a vector encoding a tamoxifen-inducible *MyoD* transgene, which exerts a potent myogenic commitment in a self-activating manner.[26]

HIDEMs could exit the vascular system upon intra-arterial injection and home into diseased muscle. Some HIDEMs participated in muscle regeneration through fusion with muscle fibers, while others remained within the interstitium in a pericyte-like position, and even replenished this niche in the pathological Sgca-null mouse model. This capacity underlines their angiogenic potential and is reminiscent of the capacity of satellite cells and some myogenic progenitors to home to satellite cell niches when the latter are depleted. In the future, if HIDEMs are to become of widespread use, their efficacy to replenish niches should be assessed in models of muscular dystrophies showing no spontaneous defect in pericyte biology. Their efficacy may also be compared with that of other mesodermal-oriented ES or iPS cell types presenting a homing capacity upon intra-arterial injection.[13,16,18]

Tedesco and collaborators also compared the efficacy of human and murine cells in the Sgca-null model.[1] They observed a fourfold increase in histological integration and improved muscular capacities when murine MAB-like cells (termed MIDEMs) were used in a mouse-to-mouse transplantation context instead of HIDEMs in a human-to-mouse context. This discrepancy might be explained by the difference in size between murine and human cells resulting in divergences in the spreading or clotting into vessels. Also, slight incompatibilities between human and murine molecules or physiological systems may

cause defective recognition of cell surface antigens involved in migration, extravasation, or fusion. Finally, the combination of the exogenous human α-sarcoglycan with the murine sarcoglycans β, γ, δ leads to the formation of a tetrameric but chimeric complex of unknown functionality.

An ultimate goal of cell therapy approaches is to generate biological products amenable to clinical trials, which impose several restrictions regarding production, characterization, quality control, and delivery to prevent the worsening of the pathologies by embolisms, immune reactions, unexpected differentiations, or fibroses. With this in mind the authors have tested the robustness of their protocols using iPS cell lines certified to be free of viral integrations, and the cells proposed by Tedesco and collaborators may constitute the first generation of a new category of progenitors that, despite not being completely equivalent to their natural *in situ* counterparts, may share on demand some angiogenic and/or myogenic capacities. Further work will be necessary to (i) assess their safety and stability, (ii) document the colonization of specific muscles affected in several myopathies (diaphragm, intercostal muscles, heart), (iii) compare HIDEMs derived from various initial cell types, and (iv) assess their potential immunogenicity, even in an autologous context.[27] The production of HIDEMS may benefit from ongoing progress in our understanding of the biology of pluripotent stem cells, which are emerging as important weapons in the armamentarium of cell, gene, and molecular therapy.

## REFERENCES


1. Tedesco, FS, Gerli, MF, Perani, L, Benedetti, S, Ungaro, F, Cassano, M *et al.* (2012). Transplantation of genetically corrected Human iPSC-derived progenitors in mice with limb-girdle muscular dystrophy. *Sci Transl Med* **4**: 140ra89.
2. Eymard, B, Romero, NB, Leturcq, F, Piccolo, F, Carrié, A, Jeanpierre, M *et al.* (1997). Primary adhalinopathy (alpha-sarcoglycanopathy): clinical, pathologic, and genetic correlation in 20 patients with autosomal recessive muscular dystrophy. *Neurology* **48**: 1227–1234.
3. Pichavant, C, Aartsma-Rus, A, Clemens, PR, Davies, KE, Dickson, G, Takeda, S *et al.* (2011). Current status of pharmaceutical and genetic therapeutic approaches to treat DMD. *Mol Ther* **19**: 830–840.
4. Mendell, JR, Rodino-Klapac, LR, Rosales, XQ, Coley, BD, Galloway, G, Lewis, S *et al.* (2010). Sustained alpha-sarcoglycan gene expression after gene transfer in limb-girdle muscular dystrophy, type 2D. *Ann Neurol* **68**: 629–638.
5. Skuk, D and Tremblay, JP (2011). Intramuscular cell transplantation as a potential treatment of myopathies: clinical and preclinical relevant data. *Expert Opin Biol Ther* **11**: 359–374.
6. Tedesco, FS, Dellavalle, A, Diaz-Manera, J, Messina, G and Cossu, G (2010). Repairing skeletal muscle: regenerative potential of skeletal muscle stem cells. *J Clin Invest* **120**: 11–19.
7. Sampaolesi, M, Torrente, Y, Innocenzi, A, Tonlorenzi, R, D'Antona, G, Pellegrino, MA *et al.* (2003). Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. *Science* **301**: 487–492.
8. Dellavalle, A, Sampaolesi, M, Tonlorenzi, R, Tagliafico, E, Sacchetti, B, Perani, L *et al.* (2007). Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. *Nat Cell Biol* **9**: 255–267.
9. Dellavalle, A, Maroli, G, Covarello, D, Azzoni, E, Innocenzi, A, Perani, L *et al.* (2011). Pericytes resident in postnatal skeletal muscle differentiate into muscle fibres and generate satellite cells. *Nat Commun* **2**: 499.
10. Crisan, M, Yap, S, Casteilla, L, Chen, CW, Corselli, M, Park, TS *et al.* (2008). A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* **3**: 301–313.
11. Morgan, JE and Zammit, PS (2010). Direct effects of the pathogenic mutation on satellite cell function in muscular dystrophy. *Exp Cell Res* **316**: 3100–3108.
12. Ozasa, S, Kimura, S, Ito, K, Ueno, H, Ikezawa, M, Matsukura, M *et al.* (2007). Efficient conversion of ES cells into myogenic lineage using the gene-inducible system. *Biochem Biophys Res Commun* **357**: 957–963.
13. Darabi, R, Gehlbach, K, Bachoo, RM, Kamath, S, Osawa, M, Kamm, KE *et al.* (2008). Functional skeletal muscle regeneration from differentiating embryonic stem cells. *Nat Med* **14**: 134–143.
14. Sakurai, H, Okawa, Y, Inami, Y, Nishio, N and Isobe, K (2008). Paraxial mesodermal progenitors derived from mouse embryonic stem cells contribute to muscle regeneration via differentiation into muscle satellite cells. *Stem Cells* **26**: 1865–1873.
15. Chang, H, Yoshimoto, M, Umeda, K, Iwasa, T, Mizuno, Y, Fukada, S *et al.* (2009). Generation of transplantable, functional satellite-like cells from mouse embryonic stem cells. *FASEB J* **23**: 1907–1919.
16. Darabi, R, Santos, FN, Filareto, A, Pan, W, Koene, R, Rudnicki, MA *et al.* (2011). Assessment of the myogenic stem cell compartment following transplantation of Pax3/Pax7-induced embryonic stem cell–derived progenitors. *Stem Cells* **29**: 777–790.
17. Mizuno, Y, Chang, H, Umeda, K, Niwa, A, Iwasa, T, Awaya, T *et al.* (2010). Generation of skeletal muscle stem/progenitor cells from murine induced pluripotent stem cells. *FASEB J* **24**: 2245–2253.
18. Darabi, R, Pan, W, Bosnakovski, D, Baik, J, Kyba, M and Perlingeiro, RC (2011). Functional myogenic engraftment from mouse iPS cells. *Stem Cell Rev* **7**: 948–957.
19. Quattrocelli, M, Palazzolo, G, Floris, G, Schöffski, P, Anastasia, L, Orlacchio, A *et al.* (2011). Intrinsic cell memory reinforces myogenic commitment of pericyte-derived iPSCs. *J Pathol* **225**: 593–603.
20. Zheng, JK, Wang, Y, Karandikar, A, Wang, Q, Gai, H, Liu, AL *et al.* (2006). Skeletal myogenesis by human embryonic stem cells. *Cell Res* **16**: 713–722.
21. Barberi, T, Bradbury, M, Dincer, Z, Panagiotakos, G, Socci, ND and Studer, L (2007). Derivation of engraftable skeletal myoblasts from human embryonic stem cells. *Nat Med* **13**: 642–648.
22. Darabi, R, Arpke, RW, Irion, S, Dimos, JT, Grskovic, M, Kyba, M *et al.* (2012). Human ES- and iPS-derived myogenic progenitors restore dystrophin and improve contractility upon transplantation in dystrophic mice. *Cell Stem Cell* **10**: 610–619.
23. Goudenege, S, Lebel, C, Huot, NB, Dufour, C, Fujii, I, Gekas, J *et al.* (2012). Myoblasts derived from normal hESCs and dystrophic hiPSCs efficiently fuse with existing muscle fibers following transplantation. *Mol Ther* **20**: 2153–2167.
24. Salani, S, Donadoni, C, Rizzo, F, Bresolin, N, Comi, GP and Corti, S (2012). Generation of skeletal muscle cells from embryonic and induced pluripotent stem cells as an *in vitro* model and for therapy of muscular dystrophies. *J Cell Mol Med* **16**: 1353–1364.
25. Dar, A, Domev, H, Ben-Yosef, O, Tzukerman, M, Zeevi-Levin, N, Novak, A *et al.* (2012). Multipotent vasculogenic pericytes from human pluripotent stem cells promote recovery of murine ischemic limb. *Circulation* **125**: 87–99.
26. Shani, M, Faerman, A, Emerson, CP, Pearson-White, S, Dekel, I and Magal, Y (1992). The consequences of a constitutive expression of MyoD1 in ES cells and mouse embryos. *Symp Soc Exp Biol* **46**: 19–36.
27. Zhao, T, Zhang, ZN, Rong, Z and Xu, Y (2011). Immunogenicity of induced pluripotent stem cells. *Nature* **474**: 212–215.


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# rAAV-Mediated Tumorigenesis: Still Unresolved After an AAV Assault

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The risk of oncogenesis mediated by vector-induced insertional mutagenesis during therapeutic gene transfer has received much attention in recent years. Any nucleic acid, regardless of how it is delivered, can cause insertional mutagenesis if it integrates into the genome. The two parameters that define this risk are integration site preference and frequency of integration. Recombinant adeno-associated viral (rAAV) vectors have been shown to be safe and efficacious in early gene therapy clinical trials,[1–4] although such vectors do integrate into the genome at a low but measureable rate (0.1 to 1% of transduction events) in animal models.[5,6] In this issue of *Molecular Therapy*, Rosas and colleagues test various conditions that are hypothesized to facilitate rAAV integration so as to determine whether these events can lead to an increased rate of oncogenesis.[7]

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