


A Comment on “Muscle Xenografts Reproduce Key Molecular Features of Facioscapulohumeral Muscular Dystrophy”: What Is New and What Has Already been Done and Reported but Was Not Quoted?

Cell Transplantation
 Volume 29: 1–4
 © The Author(s) 2020
 Article reuse guidelines:
sagepub.com/journals-permissions
 DOI: 10.1177/0963689720939120
journals.sagepub.com/home/ccl


Daniel Skuk¹  and Jacques P Tremblay¹

Abstract

A study was recently published that sought to develop an *in vivo* model of facioscapulohumeral muscular dystrophy by transplanting muscle precursor cells from a patient into immunodeficient mice. The study largely applied the methodology used by our team in a study published more than two decades ago with a similar objective, albeit for another muscular dystrophy. However, our study is not cited, leaving the wrong idea that the concept, methodology, and part of the results are original to this recent study. Although the recent study is of interest, the omission of our publication, as well as other relevant references, deprives it of an adequate scientific context. We, therefore, want to point out the importance of a careful bibliographic search in any scientific work.

Keywords

disease model, immunodeficient mice, muscle cell transplantation, xenotransplantation

Recently, Mueller et al.¹ have published a study in which they attempted to develop an *in vivo* model of facioscapulohumeral muscular myopathy (FSHMD). To this end they transplanted muscle precursor cells (MPCs) from a patient with FSHMD into immunodeficient mice. At first, we were enthusiastic to learn that another research group took up an idea that we had more than two decades ago and that we published in 1999². At the time, we sought to develop an *in vivo* model of another muscular dystrophy, namely myotonic dystrophy (MD). In our study, MPCs from five adult MD patients as well as MPCs from a fetus with the congenital form of MD were transplanted into severe combined immunodeficiency (SCID) mice, with MPCs of other origins serving as controls (Table 1). In the following 1 to 4 mo, we observed human-derived myofibers in all mouse muscles grafted with human MPCs. Mouse muscles grafted with human MD cells had the same genetic abnormalities as the donors, and we found electromyographic abnormalities characteristic of MD in some of them².

FSHMD: facioscapulohumeral muscular myopathy; iNMES: intermittent neuromuscular electrical stimulation; MD: myotonic dystrophy; MPC: muscle precursor cell; SCID: severe combined immunodeficiency.

Since the study published by Mueller et al.¹ reproduced much of our previous methodology and had an objective similar to that of our 1999 study² (Table 1), it is understandable that our enthusiasm turned into disappointment as we noted that the recent manuscript did not mention our work as a precedent in terms of concept and methodology.

Referencing is an important part of any scientific work. Quoting an editorial from B. Penders on the rules for responsible referencing³, citations in a scientific article “demonstrate the foundation upon which our studies rest as well as how they are different from previous work”³. As this editorial pointed out, the omission of essential citations “can wrongfully suggest that your own publication is the origin of an idea, a question, a method, or a critique, thereby

¹ Axe Neurosciences, Research Center of the CHU de Quebec - CHUL, Quebec, Canada

Submitted: June 4, 2020. Accepted: June 10, 2020.

Corresponding Author:

Daniel Skuk, MD, Axe Neurosciences, P-09300, CHUL, 2705 boulevard Laurier, Québec (QC), Canada G1V 4G2.
 Email: daniel.skuk@crchudequebec.ulaval.ca



Table 1. Comparison Between the Study of Mueller et al.¹ and Our Previous Study², Noting Similarities and Differences in the Objectives and Methodology.

Article	Skuk et al. (1999)	Mueller et al. (2019)
Purpose of the study	Develop an <i>in vivo</i> model of a muscular dystrophy (myotonic dystrophy) by creating human myofibers in a mouse muscle	Develop an <i>in vivo</i> model of a muscular dystrophy (FSHD) by creating human myofibers in a mouse muscle
Cells transplanted	Human MPCs	Human MPCs
Origin of cells to model pathology	Adult patients with MD and a fetus with congenital MD	Adult patient with FSHMD
Origin of cells used for controls	Normal adults Normal newborn	Normal adult
Receptor	Immunodeficient mice (SCID mice)	Immunodeficient mice (NOD-Rag1 ^{null} IL2rg ^{null} mice)
Muscle for cell grafts	Tibialis anterior	Tibialis anterior
Muscle pretreatment	X-ray irradiation 15 to 20 Gy 1 wk before MPC transplantation Injection of a snake-derived myotoxin (notexin) 1 d before MPC transplantation or during MPC transplantation	X-ray irradiation 25 Gy 1 wk before MPC transplantation Injection of a snake-derived myotoxin (cardiotoxin) 1 d before MPC transplantation
Number of cells grafted	2 to 5 × 10 ⁶	2 × 10 ⁶
Time of sampling	1 to 4 mo	1 mo (4 to 5 wk)
Muscle post-treatment	No	iNMES
Identification of human-derived myofibers in mouse muscle sections	Immunodetection of a sarcolemmal protein of human origin (dystrophin)	Immunodetection of a sarcolemmal protein of human origin (β-spectrin)
Best engraftment reported	An average of 725 human-derived myofibers per mouse muscle section, using control newborn cells	An average of around 400 human-derived myofibers per mouse muscle section, using control cells in muscles treated with iNMES

illegitimately appropriating them”³. In this sense, the recent report published by Mueller et al.¹ may impart on readers the impression that their study is the origin of an idea and a methodology that are, in effect, over two decades old.

Furthermore, we noted other omissions or citation errors in Mueller et al.¹, which we point out below:

1. Papers published by our group in 2007⁴ and 2012^{5,6} were quoted to say that in earlier studies of human MPC transplantation in the muscles of immunodeficient mice, human-derived myofibers were partially “contaminated” with mouse myonuclei. This mention was used in Mueller et al.¹ to explain that the authors decided to treat the mouse muscles with high doses of X-rays prior to the MPC graft. Expressed in this way and without more appropriate references, this might lead readers to believe that muscle irradiation before MPC transplantation is a novelty of the recent study. However, the suppression of the proliferative capacity of endogenous satellite cells to favor the regeneration of myofibers by the grafted MPCs using high doses of gamma or X-rays is a standard technique whose first use dates back almost three decades^{7,8}. In fact, a careful reading of the 2007 study⁴ would have shown that we did indeed irradiate the host muscles previous to the human MPC

transplantation and there was no mention of mouse myonuclei contamination in the human-derived myofibers⁴. On the other hand, our first studies regarding human MPC transplantation in immunodeficient mice are much earlier, having been published in 1993 and 1994^{9–11}, and one of them¹⁰ used high-dose irradiation (20 Gy) of the muscles prior to the transplantation of human MPCs. In this study, an analysis of whether the human-derived myofibers had mouse myonuclei was made, and we reported that the majority were completely of human origin¹⁰. Therefore, the protocol we used in 1994 already minimized the contamination of human-derived myofibers by mouse myonuclei. This protocol afforded our team a very efficient engraftment, i.e., we reported SCID mouse muscles almost entirely composed by human-derived myofibers (92.8% ± 6.5%) in 1994¹⁰, and up to 725 ± 329 human-derived myofibers per mouse muscle in 1999².

2. MPCs from FSHMD patients have previously been transplanted into immunodeficient mice and compared with MPCs of normal donors in a study published by Vilquin et al.¹². Although the objective of the study was not to develop an *in vivo* model of FSHMD, they reported that the engraftment of FSHMD cells was similar to those from normal

individuals. As this finding was similar to that observed by Mueller et al.¹, proper etiquette dictates that it ought to have been cited³.

3. The fact that some MPCs (from mouse and human origin) give rise to satellite cells once grafted into mice has already been sufficiently established to be “intriguing” (reviewed in reference 13). In fact, one of the experiments proposed by Mueller et al.¹, i.e., treating the muscles that have human-derived myofibers with a myotoxin (which allows to observe if the satellite cells derived from the graft can participate in new muscle regeneration cycles), has already been carried out in studies with murine¹⁴ and human¹³ MPCs. Therefore, these publications should be cited at least in future studies if that methodology is used.

Leaving aside these shortcomings in referencing, we found that the study of Mueller et al.¹ is interesting and surely relevant for the study of FSHMD. From the specific point of view of our expertise with MPC xenografts in immunodeficient mice (which includes not only MPCs of human origin, but also from dogs¹⁵ and monkeys^{4,16}), we found the intermittent neuromuscular electrical stimulation (iNMES) of the peroneal nerve innovative. It is interesting that the authors reported a substantial increase in the number of human-derived myofibers when they used iNMES to induce repeated maximal contractions of the muscle transplanted with human MPCs¹. This good engraftment was achieved, according to what we interpret, with only one longitudinal injection of cells, i.e., without the need to distribute the MPCs throughout the muscle by multiple micro-injections, as we usually do in our studies^{2,10}. Importantly, with this technique Mueller et al.¹ reported an increase in the average diameter of the human-derived myofibers, close to 30 μm according to figure 1c in that article, while in our earlier papers we had reported $22.9 \pm 12.4 \mu\text{m}$ in the best case². They also reported that iNMES enhanced the differentiation of the human-derived myofibers, as shown by a reduction in the levels of embryonic myosin when compared to muscles that were not treated with iNMES¹. Therefore, iNMES could improve the protocols previously developed to create human “mini-muscles” in immunodeficient mice. These updated protocols could more effectively model some human genetic myopathies and help researchers gain more reliable data. Finally, we hope that the references mentioned here will serve to better contextualize future studies in this field.

Acknowledgments

The authors wish to express their gratitude to Gabriel Lamothe for correcting the text as well as for his helpful suggestions.


Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Daniel Skuk  <https://orcid.org/0000-0002-8303-0082>

References

1. Mueller AL, O'Neill A, Jones TI, Llach A, Rojas LA, Sakellariou P, Stadler G, Wright WE, Eyerman D, Jones PL, Bloch RJ. Muscle xenografts reproduce key molecular features of facioscapulohumeral muscular dystrophy. *Exp Neurol*. 2019;320:113011.
2. Skuk D, Furling D, Bouchard JP, Goulet M, Roy B, Lacroix Y, Vilquin JT, Tremblay JP, Puymirat J. Transplantation of human myoblasts in SCID mice as a potential muscular model for myotonic dystrophy. *J Neuropathol Exp Neurol*. 1999; 58(9):921–931.
3. Penders B. Ten simple rules for responsible referencing. *PLoS Comput Biol*. 2018;14(4):e1006036.
4. Quenneville SP, Chapdelaine P, Skuk D, Paradis M, Goulet M, Rousseau J, Xiao X, Garcia L, Tremblay JP. Autologous transplantation of muscle precursor cells modified with a lentivirus for muscular dystrophy: human cells and primate models. *Mol Ther*. 2007;15(2):431–438.
5. Fakhfakh R, Lamarre Y, Skuk D, Tremblay JP. Losartan enhances the success of myoblast transplantation. *Cell Transplant*. 2012;21(1):139–152.
6. Fakhfakh R, Lee SJ, Tremblay JP. Administration of a soluble activin type IIB receptor promotes the transplantation of human myoblasts in dystrophic mice. *Cell Transplant*. 2012; 21(7):1419–1430.
7. Morgan JE, Hoffman EP, Partridge TA. Normal myogenic cells from newborn mice restore normal histology to degenerating muscles of the mdx mouse. *J Cell Biol*. 1990;111(6): 2437–2449.
8. Morgan JE, Pagel CN, Sherratt T, Partridge TA. Long-term persistence and migration of myogenic cells injected into pre-irradiated muscles of mdx mice. *J Neurol Sci*. 1993; 115(2):191–200.
9. Huard J, Roy R, Guerette B, Verreault S, Tremblay G, Tremblay JP. Human myoblast transplantation in immunodeficient and immunosuppressed mice: evidence of rejection. *Muscle Nerve*. 1994;17(2):224–234.
10. Huard J, Verreault S, Roy R, Tremblay M, Tremblay JP. High efficiency of muscle regeneration after human myoblast clone transplantation in SCID mice. *J Clin Invest*. 1994;93(2): 586–599.
11. Huard J, Tremblay G, Verreault S, Labrecque C, Tremblay JP. Utilization of an antibody specific for human dystrophin to follow myoblast transplantation in nude mice. *Cell Transplant*. 1993;2(2):113–118.
12. Vilquin JT, Marolleau JP, Sacconi S, Garcin I, Lacassagne MN, Robert I, Ternaux B, Bouazza B, Larghero J, Desnuelle C. Normal growth and regenerating ability of myoblasts from

- unaffected muscles of facioscapulohumeral muscular dystrophy patients. *Gene Ther.* 2005;12(22):1651–1662.
13. Skuk D, Paradis M, Goulet M, Chapdelaine P, Rothstein DM, Tremblay JP. Intramuscular transplantation of human postnatal myoblasts generates functional donor-derived satellite cells. *Mol Ther.* 2010;18(9):1689–1697.
 14. Gross JG, Morgan JE. Muscle precursor cells injected into irradiated mdx mouse muscle persist after serial injury. *Muscle Nerve.* 1999;22(2):174–185.
 15. Ito H, Vilquin JT, Skuk D, Roy B, Goulet M, Lille S, Dugre FJ, Asselin I, Roy R, Fardeau M, Tremblay JP. Myoblast transplantation in non-dystrophic dog. *Neuromuscul Disord.* 1998; 8(2):95–110.
 16. Skuk D, Tremblay JP. CD56+ muscle derived cells but not retinal NG2+ perivascular cells of nonhuman primates are myogenic after intramuscular transplantation in immunodeficient mice. *J Stem Cell Res Ther.* 2017;7:377. doi: 10.4172/2157-7633.1000377.