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PRECLINICAL RESEARCH

Transplantation of Allogeneic PW1^{pos}/Pax7^{neg} Interstitial Cells Enhance Endogenous Repair of Injured Porcine Skeletal Muscle

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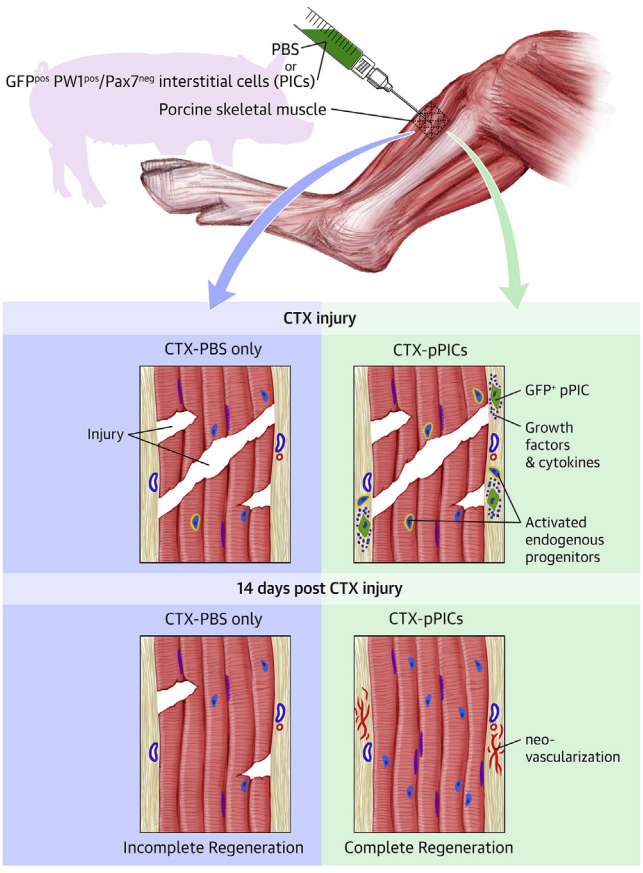
VISUAL ABSTRACT



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HIGHLIGHTS

- **Allogeneic PICs express and secrete an array of pro-regenerative paracrine factors that stimulate a regenerative response in a preclinical muscle injury model applicable to humans.**
- **Paracrine factors secreted by allogeneic PICs stimulate endogenous progenitor cell activation and differentiation, leading to accelerated and improved myofiber regeneration and microvessel formation.**
- **Allogeneic PICs survive long enough to exert their action before being cleared by the host immune system. Therefore, the cells transplanted are allogeneic but the regeneration is completely autologous.**
- **Administration of HGF and IGF-1 improves skeletal muscle regeneration, but not to the same extent as PIC transplantation.**

ABBREVIATIONS AND ACRONYMS

BrdU = 5-bromo-2′-deoxyuridine

CM = pPIC conditioned medium

CSA = cross sectional area

CSC = cardiac stem cell

CTRL = control

CTX = cardiotoxin

FBS = fetal bovine serum

DAPI = 4′,6-diamidino-2-phenylindole

DMEM = Dulbecco's Modified Eagle's medium

GFPpPIC = GFP-positive porcine PW1^{pos}/Pax7^{neg} interstitial cell

GM = growth medium

HUVEC = human umbilical vein endothelial cell

HVG = hematoxylin and van Gieson

ICM = heat-inactivated conditioned medium

IV = intravenous

MHC = myosin heavy chain

MI = myocardial infarction

nMHC = neonatal myosin heavy chain

P = passage

PBMC = peripheral blood mononuclear cell

PBS = phosphate buffered saline

PIC = PW1^{pos}/Pax7^{neg} interstitial cell

pPIC = porcine PW1^{pos}/Pax7^{neg} interstitial cell

qRT-PCR = quantitative reverse transcription polymerase chain reaction

TA = tibialis anterior

UM = unconditioned medium

vWF = Von Willebrand factor

SUMMARY

Skeletal muscle-derived PW1^{pos}/Pax7^{neg} interstitial cells (PICs) express and secrete a multitude of proregenerative growth factors and cytokines. Utilizing a porcine preclinical skeletal muscle injury model, delivery of allogeneic porcine PICs (pPICs) significantly improved and accelerated myofiber regeneration and neocapillarization, compared with saline vehicle control-treated muscles. Allogeneic pPICs did not contribute to new myofibers or capillaries and were eliminated by the host immune system. In conclusion, allogeneic pPIC transplantation stimulated the endogenous stem cell pool to bring about enhanced autologous skeletal muscle repair and regeneration. This allogeneic cell approach is considered a cost-effective, easy to apply, and readily available regenerative therapeutic strategy. (J Am Coll Cardiol Basic Trans Science 2017;2:717–36)

Skeletal muscle is a dynamic, highly plastic tissue that is capable of undergoing repair and regeneration in healthy individuals (1). However, this regenerative capacity can become impaired in muscle diseases, such as muscular dystrophy, and as a result of disuse and ageing (2,3). This leads to decreased proliferation, delayed fusion, and reduced differentiation of muscle progenitors, as well as a gradual replacement of muscle fibers by fat and fibrosis with poor vascularization (4,5). The mechanisms that underpin impaired skeletal muscle regeneration are still unclear; however, recent findings suggest that the microenvironment and/or systemic factors (6,7) affect the response of various endogenous skeletal muscle progenitor cells to repair and regenerate after injury (8). This has led us to focus upon therapeutic strategies that target resident muscle progenitors and stimulate endogenous repair mechanisms.

Autologous stem cell approaches are attractive from a theoretical and biological standpoint; however, for skeletal muscle where the muscle satellite cells cannot be effectively propagated to large numbers in vitro, and like other tissue-specific stem/progenitor cells, such as cardiac, are affected by age and disease, they are impractical. Moreover, administration of cells can induce therapeutic responses by indirect means, such as secretion of growth factors and interaction with endogenous repair processes, represented by the resident stem/progenitor cells (9,10). This has been termed the paracrine effect. Therefore, there seems to be little advantage in the use of autologous cells because a similar, and perhaps enhanced, effect can be obtained by the administration of a cell type isolated from allogeneic sources, which could be made readily available at reduced costs (11). We have previously shown that intracoronary injection of allogeneic porcine cardiac stem cells (CSCs) after myocardial infarction (MI) activates the endogenous, resident CSCs through a paracrine mechanism, resulting in improved myocardial cell survival, function, remodeling, and regeneration (12).

Skeletal muscle satellite cells, although capable of muscle fiber regeneration, have a limited migration capacity with an inability to cross the endothelial wall (13). Moreover, cultured satellite cells ex vivo have decreased stemness, proliferation, and myofiber differentiation, which has subsequently hampered their

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