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Original Article

Enhanced differentiation capacity of parthenogenetic embryonic stem cells via incorporation of non-growing oocyte genomes in mouse

Hua Shao[1,2], Jie Cao[1], Ronghua Lu[2]

[1]College of Animal Science and Technology, Hunan Agriculture University, Changsha, Hunan, China; [2]Translational Medicine Research Center, Chongqing University Three Gorges Hospital, Chongqing, China

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Abstract: Objectives: To evaluate the in vivo developmental and therapeutic potential of a novel parthenogenetic embryonic stem cell line (NF-pES), which contains genomes from both non-growing and grown oocytes. Methods: NF-pES cells were injected into mouse blastocysts to generate chimeric mice, and their contribution to various tissues was assessed. Skeletal muscle differentiation potential was examined through teratoma assays and analysis of muscle tissue in chimeric mice. For therapeutic assessment, a skeletal muscle injury model was established by cardiotoxin and irradiation treatment of the tibialis anterior muscle. NF-pES-derived precursor cells, obtained through in vitro induction and differentiation, were transplanted into the injured muscle. Results: Notably, NF-pES cells contributed extensively to multiple somatic lineages in chimeric mice, with high levels of chimerism observed in the heart (83.36%) and bone marrow (50.44%). These levels are comparable to those achieved with embryonic stem cells derived from fertilized embryos. Importantly, NF-pES cells demonstrated robust myogenic differentiation capacity, as evidenced by their contribution to skeletal muscle tissues in both teratoma formation assays and in vivo chimeric muscle integration. Following in vitro induction, NF-pES-derived precursors were transplanted into the injured tibialis anterior muscle of recipient mice to assess their regenerative potential in vivo. One month after transplantation, immunohistochemical analysis confirmed the successful engraftment of donor-derived cells within the host muscle tissue. These donor-derived cells expressed markers of terminal myogenic differentiation and were incorporated into mature skeletal muscle fibers. Conclusions: NF-pES cells exhibit strong developmental capacity and therapeutic potential for skeletal muscle regeneration, suggesting their value in future regenerative medicine applications.

Keywords: Parthenogenetic embryonic stem cell (pES), embryonic stem cell (ES), chimeras, non-growing oocyte, grown oocyte, differentiation

Introduction

Over the past two decades, remarkable progress has been achieved in the field of stem cell biology, particularly in the derivation and application of pluripotent stem cell lines. Among these, induced pluripotent stem (iPS) cells [1, 2], embryonic stem (ES) cells [3], and parthenogenetic embryonic stem (pES) cells [4] have emerged as powerful tools for both regenerative medicine and the study of early embryonic development. Each of these pluripotent cell types possesses distinct molecular characteristics and developmental potentials, offering unique advantages while also presenting specific limitations. Accordingly, functional assessments of these cell lines are essential for advancing our understanding of pluripotency and optimizing their clinical utility [5].

In 2006, Takahashi and Yamanaka pioneered the generation of induced pluripotent stem (iPS) cells by reprogramming somatic cells through the ectopic expression of key transcription factors - Oct4, Sox2, Klf4, and c-Myc [1]. iPS cells exhibit molecular and functional characteristics closely resembling those of embryonic stem (ES) cells, including the capacity to differentiate into derivatives of all three germ layers [6]. Subsequent refinements in repro-

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gramming strategies - particularly the development of non-integrating delivery systems such as episomal vectors and synthetic mRNA - have significantly enhanced the safety, efficiency, and clinical feasibility of iPSC production [7, 8]. These advances have laid a robust foundation for the application of iPSCs in disease modeling, drug discovery, and personalized regenerative therapies [9].

Recent progress in directed differentiation protocols has further accelerated the clinical translation of iPSC-based therapies. Notably, the clinical implementation of iPSC-derived myocardial cell sheets in Japan for the treatment of heart failure demonstrated functional cardiac recovery without the requirement for long-term immunosuppression [10]. In parallel, the emergence of chemically induced pluripotent stem cells (CiPSCs), which are generated without the introduction of exogenous genetic material, has addressed key concerns regarding genomic integrity and tumorigenicity [11-13]. A landmark clinical milestone was achieved with the autologous transplantation of CiPSC-derived pancreatic islets into a patient with type 1 diabetes, resulting in sustained insulin independence and improved glycemic control [14]. Together, these developments underscore the transformative potential of iPSC technologies and their rapidly expanding role in regenerative medicine.

In 1998, Thomson and colleagues successfully derived human embryonic stem (ES) cells from the inner cell mass of blastocyst-stage embryos [15], marking a milestone in developmental biology and regenerative medicine. These pluripotent cells have been instrumental in advancing our understanding of the molecular pathways that govern early human embryogenesis, as well as in developing robust protocols for lineage-specific differentiation in vitro [16]. Despite their scientific utility, the derivation of ES cells involves the destruction of preimplantation embryos, raising profound ethical and societal concerns that have led to regulatory restrictions in many countries. These limitations have, in turn, catalyzed the search for alternative sources of pluripotent stem cells that can bypass the ethical controversies associated with embryo destruction, while retaining comparable developmental potential [17-19].

Parthenogenetic embryonic stem cells (pESCs) are derived from the activation of unfertilized oocytes, thereby offering a pluripotent cell source that circumvents the ethical concerns associated with the destruction of fertilized embryos [20]. Extensive studies have confirmed that pESCs exhibit core features of pluripotency, including sustained self-renewal and the ability to differentiate into cell types representing all three germ layers [21]. A distinct advantage of pESCs lies in their potential to generate human leukocyte antigen (HLA)-matched cell lines from homozygous donors, which may significantly reduce the risk of immune rejection in allogeneic transplantation settings [22]. In addition to their translational potential, pESCs serve as a valuable model for investigating key biological processes such as oocyte activation, epigenetic reprogramming, and genomic imprinting - phenomena that are difficult to interrogate using conventional ES cells derived from fertilized embryos [23, 24]. Recent preclinical studies have demonstrated that parthenogenetic embryonic stem cells (pESCs) possess substantial therapeutic potential across a range of disease models. Notably, pESC-derived cardiomyocytes have been bioengineered into contractile cardiac patches capable of electrically integrating with infarcted myocardium, resulting in improved cardiac function following myocardial infarction [25, 26]. In hepatic injury models, pESC-derived hepatocyte-like cells have been shown to successfully engraft into damaged liver parenchyma and repopulate $Fah^{-/-}$ mouse livers, restoring metabolic function and significantly extending survival [27]. In models of Parkinson's disease, transplantation of pESC-derived neural cells - including dopaminergic neurons and neural stem cell populations - has led to robust engraftment, increased striatal dopamine levels, and marked improvement in motor function in both rodent and non-human primate models, without evidence of tumor formation [28].

Encouraged by these promising outcomes, pESC-derived neural progenitors have recently progressed to clinical evaluation, culminating in the initiation of a first-in-human Phase I/IIa trial for the treatment of Parkinson's disease [29]. Collectively, these findings highlight the broad differentiation potential, regenerative efficacy, and unique immunological compatibility of pESCs. Their capacity to be derived from HLA-

homozygous donors positions them as an attractive, scalable, and potentially immune-matched "off-the-shelf" pluripotent cell source for allogeneic transplantation in cardiac, hepatic, and neurodegenerative disorders.

Parthenogenetic embryos generated through the activation of mature oocytes are inherently limited in their developmental potential, typically undergoing resorption around embryonic day 10.5 (E10.5) due to aberrant genomic imprinting [30-32]. Interestingly, reconstructed parthenogenetic embryos created by combining nuclear material from non-growing (NG) oocytes and mature oocytes have demonstrated extended developmental capacity, progressing to embryonic day 13.5 (E13.5) upon uterine transfer [33]. Transcriptomic analyses of NG oocytes have revealed a partial paternal-like epigenetic profile, characterized by the expression of paternally imprinted genes such as *Peg1/Mest*, *Peg3*, and *Snrpn*, alongside the absence of expression for certain maternally imprinted genes, including *Igf2r* and *p57^Kip2* [34]. These findings suggest that NG oocytes harbor an epigenetic landscape that more closely resembles that of sperm than mature oocytes, thereby partially alleviating the imprinting-related barriers to parthenogenetic development.

Remarkably, full-term development has been achieved in reconstructed parthenogenetic embryos using *H19*-null mature oocytes combined with NG oocytes, resulting in the generation of viable, fertile parthenogenetic mice [35]. These results underscore the critical role of precise imprinting regulation in supporting embryonic viability. Supporting this concept, recent studies have demonstrated that restoring monoallelic expression across 10 key imprinted loci is sufficient to enable the full-term development of mice derived from haploid embryonic stem cells (haESCs), regardless of their maternal or paternal origin [36].

Compared to embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, parthenogenetic embryonic stem cells (pESCs) exhibit notably reduced developmental potential, particularly in their ability to generate mesodermal and endodermal lineages [37-41]. Enhancing the developmental competence of pESCs thus remains a key objective in efforts to advance their applicability in developmental biology and regenerative medicine. The limitations of pESCs have largely been attributed to aberrant epigenetic modifications, most notably the lack of paternally inherited imprints due to their uniparental origin [42]. Emerging evidence suggests that the absence of the paternal genome impairs proper regulation of key imprinted genes critical for early lineage commitment.

Interestingly, previous studies have shown that non-growing (NG) oocytes possess epigenetic characteristics reminiscent of sperm, including the expression of paternally imprinted genes and repression of certain maternally imprinted loci [43]. This paternal-like epigenetic landscape raises the possibility that NG oocyte genomes may partially substitute for the functions of the paternal genome in parthenogenetic development. In our prior work, we established reconstructed parthenogenetic embryos by combining nuclear genomes from NG and fully grown oocytes via germinal vesicle (GV) transplantation [44]. These embryos exhibited more balanced imprinting profiles, resembling those of fertilized embryos, and gave rise to pESC lines derived from their inner cell mass. The resulting pESCs, termed NF-pES cells, expressed core pluripotency markers including alkaline phosphatase (AKP), stage-specific embryonic antigen-1 (SSEA-1), and octamer-binding transcription factor 4 (Oct4) [45]. These lines maintained a stable karyotype (40 chromosomes) and sustained undifferentiated growth in long-term culture. When injected into immunodeficient mice, NF-pES cells formed teratomas containing derivatives of all three germ layers, confirming their pluripotency in vivo. In vitro embryoid body formation further demonstrated their capacity to differentiate into ectoderm (Nestin), mesoderm (Desmin), and endoderm (α-fetoprotein) lineages [44].

Skeletal muscle arises from the paraxial mesoderm through a tightly regulated process of somitogenesis and myogenic lineage specification [46, 47]. Committed myoblasts proliferate and subsequently fuse to form multinucleated myotubes, which further mature into functional myofibers. In the context of injury, muscle regeneration is primarily mediated by muscle satellite cells (MuSCs), a population of tissue-resident stem cells located beneath the basal lamina of muscle fibers [48]. These cells become activated in response to damage, pro-

liferate, and differentiate to facilitate muscle fiber repair and regeneration. Dysfunction or depletion of MuSCs is implicated in a range of myopathies, most notably Duchenne muscular dystrophy (DMD) [49], for which no curative therapy currently exists.

Recent research has highlighted cell transplantation as a promising avenue for skeletal muscle regeneration [50]. However, direct transplantation of MuSCs or cultured myoblasts has yielded limited success in clinical and preclinical settings, largely due to poor cell survival, inadequate engraftment, and insufficient functional integration with host tissue [51, 52]. Alternative myogenic precursors - such as bone marrow-derived progenitor cells and $AC133^+$ adult stem cells - have demonstrated the capacity to contribute to muscle repair and restore dystrophin expression in animal models of DMD [53-55]. Nonetheless, the widespread clinical use of these cell types is hampered by their low abundance and restricted expansion potential.

To address these limitations, we have previously established a novel parthenogenetic embryonic stem cell line (NF-pES) derived from reconstructed oocytes containing genomes from both non-growing and mature oocytes [44]. These NF-pES cells exhibit robust myogenic potential, as demonstrated by their contribution to skeletal muscle in both teratoma assays and chimeric mouse models. To further assess their regenerative capacity in a clinically relevant context, we employed an in vivo model of skeletal muscle injury combined with NF-pES cell-derived precursor transplantation.

Materials and methods

Subjects

Kunming (KM) white mice, C57BL/6J mice, and severe combined immunodeficient (SCID) mice were purchased from the Shanghai Center for Laboratory Animal Sciences, Chinese Academy of Sciences, and were housed in a temperature- and light-controlled environment (14 hours of light and 10 hours of darkness) with free access to food. All animal usage protocols were approved by the Ethics Committees of Inner Mongolia University and Chongqing University Three Gorges Hospital. All mice were euthanized at the end of the study by delivering 100% CO_2, which was maintained for 5 minutes to ensure painless loss of consciousness. After confirmation of respiratory arrest and absence of corneal and pedal reflexes, cervical dislocation was performed to guarantee complete euthanasia.

Culture of NF-pES cells and ES cells

NF-pES cells and embryonic stem (ES) cells were cultured following established protocols [37]. Briefly, the cells were passaged by rinsing adherent cultures with PBS, dissociating them using 0.05% trypsin-EDTA, neutralizing enzymatic activity with serum-containing fresh medium, centrifuging the suspension at 200 × g for 5 minutes, and replating the pelleted cells at a 1:5-1:10 split ratio onto mitotically inactive mouse embryonic fibroblasts in fresh ES cell medium. This medium consisted of DMEM (Gibco, Cat. No: 11965118), supplemented with 15% fetal bovine serum (Hyclone, Cat. No: 10099141C), 1 mM Glutamine (Gibco, Cat. No: 25030081), 0.1 mM β-mercaptoethanol (Sigma, Cat. No: M3148), 1% nonessential amino acids (Gibco, Cat. No: 11130051), and 1000 U of leukemia inhibitory factor (LIF, Gibco, Cat. No: PMC9484) per 1 ml.

Generation of chimeras

NF-pES cells and ES cells were thawed and cultured for 48 hours until the colonies exhibited optimal morphology, characterized by a compact, domed shape, without signs of differentiation. The cells were dissociated into single-cell suspensions using 0.05% trypsin-EDTA, transferred to feeder-free culture dishes, and incubated for 1 hour. Following this incubation, the supernatant was aspirated, and 200 μL of ES cell medium was added to gently resuspend the loosely adherent cells.

For blastocyst microinjection, 15 to 20 embryonic stem (ES) cells were aspirated into a micropipette and injected into the blastocoel cavity at the junction of trophectoderm cells, followed by the collapse of the cavity. The injected blastocysts were incubated at 37°C under 5% CO_2 for 1 to 3 hours until the blastocoel re-expanded. Recovered blastocysts were surgically transferred into both uterine horns of pseudopregnant mice, with 7 to 8 embryos per horn. The foster mothers were housed for

gestation, and the experiment was repeated in eight independent replicates.

Flow cytometry analysis of chimeric tissue cells derived from fertilized embryo ES cells and NF-pES cells

Chimeric mice were euthanized, and the heart, kidney, spleen, and bone marrow were harvested. Bone marrow cells were flushed directly from the marrow cavity. The heart, kidney, and spleen tissues were finely minced using ophthalmic scissors and incubated in 0.05% trypsin at 37°C in a CO_2 incubator for 10 minutes. Mechanical dissociation was performed using a pipette tip with 20 strokes. After allowing the mixture to stand for 2 minutes to facilitate the sedimentation of undigested tissue, the supernatant was transferred to centrifuge tubes containing serum-supplemented medium. The residual tissue fragments underwent two additional rounds of trypsin digestion, each lasting 10 minutes. All tissue suspensions were filtered through a cell strainer, centrifuged at 1500 rpm for 5 minutes, and the resulting pellets were resuspended in a small volume of PBS. Red blood cells were removed via Percoll density gradient centrifugation. The isolated cells were finally resuspended in PBS for flow cytometry analysis.

Distribution of NF-pES cells in the pancreas and skeletal muscle of chimeric mice

Pancreas, tongue, and skeletal muscles from the back and limbs were collected from three chimeric mice derived from NF-pES cells for cryosectioning. Six distinct fields were randomly selected under a fluorescence microscope, and 50 cells were counted in each field. The chimerism rate was defined as the percentage of cells exhibiting green fluorescence relative to the total number of cells analyzed.

Animal model establishment and cell transplantation

Each SCID mouse (8-10 weeks old) received an intramuscular injection of 20 μl of cardiotoxin (1 mg/ml, Sigma, Cat. No: 217503) into the tibialis anterior muscle of the right hind limb. Twenty-four hours later, the same muscle was subjected to 25 Gy of X-ray irradiation. A total of six SCID mice were treated. The left tibialis anterior muscle underwent the same treatment, with the exception of cell injection. NF-pES cells were induced to differentiate in vitro for 14 days, then dissociated into single-cell suspensions and adjusted to a concentration of 5×10^7 cells/ml. Twenty-four hours after irradiation, 20 μl of the cell suspension (1×10^6 cells) was injected into the right tibialis anterior muscle of each mouse.

Data analysis

Statistical analyses were conducted using GraphPad Prism software (version 9.5.0; GraphPad Software, San Diego, CA, USA). For intra-group comparisons involving more than two time points, repeated measures analysis of variance (RM ANOVA) was employed following verification of normal distribution and sphericity assumptions. When only two time points were compared within the same group, paired two-tailed t-tests were performed. Where applicable, multiple comparisons were adjusted using the Bonferroni correction method to control for type I error. A *p*-value of less than 0.05 was considered statistically significant.

Results

Chimeric contribution analysis in heart, spleen, and bone marrow by FACS

A previously cryopreserved NF-pES cell line was thawed and cultured under standard conditions. After three days in culture, the cells exhibited the typical morphological features characteristic of murine embryonic stem cells (**Figure 1A**). Due to the presence of an enhanced green fluorescent protein (EGFP) transgene within the NF-pES cell genome, the colonies emitted green fluorescence when exposed to ultraviolet (UV) light (**Figure 1B**). To evaluate the in vivo differentiation capacity of NF-pES cells, they were microinjected into blastocysts derived from KM white mice, which lack the EGFP transgene. The injected blastocysts were subsequently transferred into pseudopregnant recipients to generate chimeric mice. Based on the extent of coat color contribution from the donor cells, the resulting chimeras were classified as exhibiting either high chimerism (**Figure 1C**) or moderate chimerism (**Figure 1D**), with high-chimerism mice displaying a coat color predominantly derived from NF-pES cells.

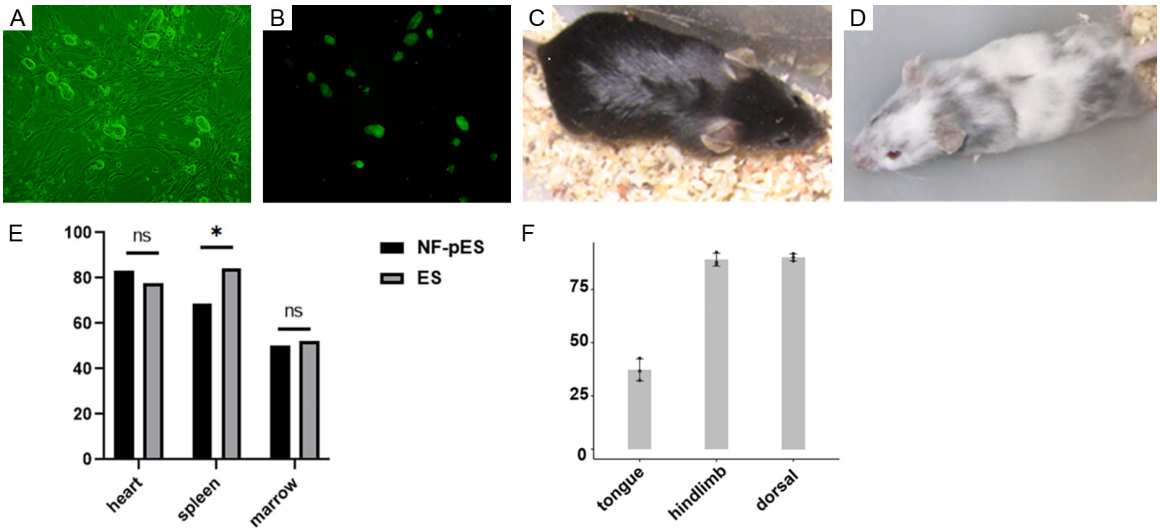

Figure 1. Morphological characteristics of NF-pES cell colonies, chimeric mice, and comparative analysis of chimerism rates in various tissues. A. Morphology of NF-pES cell colonies under bright-field microscopy. B. Fluorescence image of NF-pES cell colonies under UV illumination, highlighting GFP expression. C. High-degree coat color chimeric mouse derived from NF-pES cells. D. Moderate-degree coat color chimeric mouse derived from NF-pES cells. E. Comparative analysis of chimerism rates in various tissues between chimeric mice derived from NF-pES cells and those from conventional ES cells. F. Comparison of chimerism rates of NF-pES cell-derived skeletal muscle cells in dorsal, hindlimb, and tongue muscles.

Figure 2. NF-pES-derived chimeric mice exhibit donor cell integration in dorsal and hindlimb muscles. (A) Control group (KM white mice): Absence of GFP signal in muscle tissue. (B, C) Experimental group: GFP+ donor cells (green) localized in dorsal (B) and hindlimb (C) muscles.

Chimerism levels of embryonic stem (ES) cells from various sources were evaluated in the heart, spleen, and bone marrow of chimeric mice using fluorescence-activated cell sorting (FACS). In chimeras generated from NF-pES cells (n = 3), the mean chimerism rates were 83.36% in the heart, 68.74% in the spleen, and 50.44% in the bone marrow (**Figure 1E**). In contrast, chimeric mice derived from fertilized blastocyst-derived ES cells (n = 3) exhibited mean chimerism rates of 77.96% in the heart, 84.06% in the spleen, and 52.02% in the bone marrow (**Figure 1E**). One-way analysis of variance (ANOVA) revealed no statistically significant differences between the two groups regarding heart and bone marrow chimerism ($P > 0.05$). However, spleen chimerism was significantly higher in the fertilized blastocyst-derived ES cell group compared to the NF-pES group ($P < 0.05$).

Chimeric contribution in the pancreas and skeletal muscles by immunofluorescence

Macroscopic examination of NF-pES cell-derived chimeric mice revealed pronounced green fluorescence in the exposed dorsal and hindlimb skeletal muscles (**Figure 2B**, **2C**). In contrast, control Kunming mice displayed normal tissue coloration without any detectable fluorescence (**Figure 2A**).

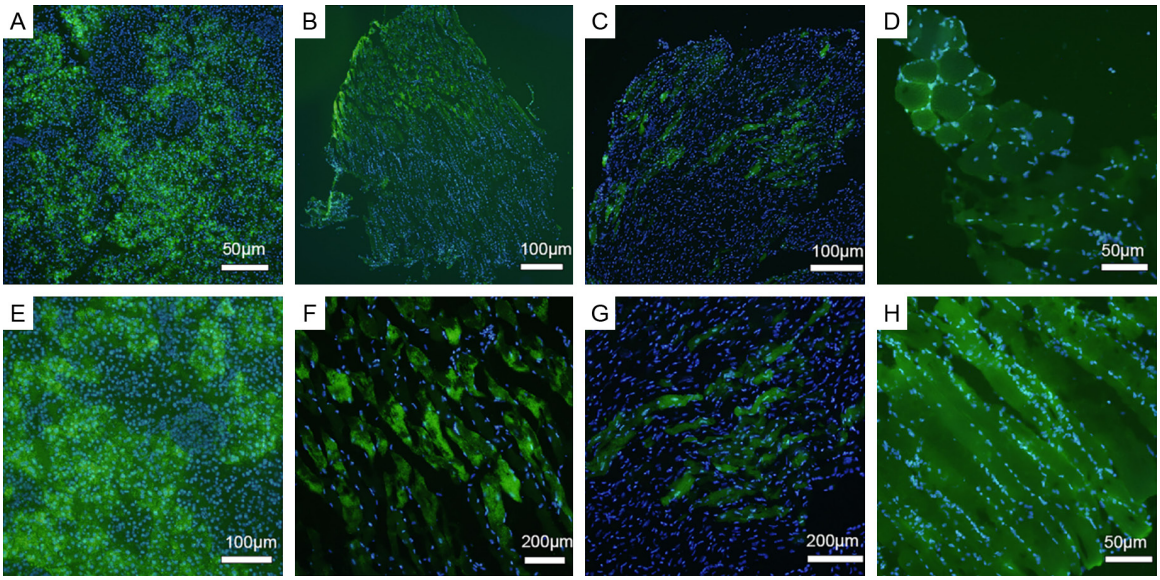

Figure 3. Distribution of NF-pES cell-derived skeletal muscle cells in chimeric tissues. (A) Pancreatic tissue; (B) Dorsal muscle; (C) Tongue muscle; (D) Hindlimb muscle; (E) Enlarged view of (A); (F) Enlarged view of (B); (G) Enlarged view of (C); (H) Longitudinal section of Hindlimb muscle.

NF-pES cells exhibited distinct distribution patterns in the pancreas and skeletal muscles of chimeric mice. In all three analyzed chimeric mice, NF-pES cells were widely distributed within the pancreatic acinar tissue, with no GFP-positive cells detected in the islets (**Figure 3A**, **3E**). Furthermore, dissection revealed that both the dorsal and hindlimb muscles exhibited a pronounced green coloration under visible light, contrasting sharply with control tissues (**Figure 3B**, **3D**, **3F**, **3H**). Cryosections from the dorsal musculature, hindlimb muscle, and tongue were prepared for quantitative chimerism analysis. Fluorescence microscopy revealed chimerism rates of 90.00% in the dorsal muscle, 88.89% in the hindlimb muscle, and 37.22% in the tongue muscle (**Figures 1F**, **3C**, **3G**).

Skeletal muscle injury repair following transplantation of NF-pES cell-derived precursor cells

One month post-transplantation, progenitor cells derived from NF-pES cell differentiation induced teratoma formation at the injection site in two out of six mice. The remaining four mice were sacrificed for histopathological examination of the tibialis anterior muscle. In one recipient, no GFP-positive tissue was detected, while robust GFP signals were observed in three recipients. Laminin immunostaining and morphological analysis confirmed that the GFP-positive regions corresponded to differentiated skeletal muscle fibers (**Figure 4C**, **4D**), in contrast to the control group that did not receive NF-pES precursor cell transplantation (**Figure 4A**, **4B**). These findings indicate that NF-pES cell-derived precursor cells successfully engraft in host skeletal muscle tissue and differentiate into mature muscle fibers, thereby contributing to the repair of injured tissue.

Discussion

The derivation of embryonic stem (ES) cells from parthenogenetic embryos is generally regarded as posing fewer ethical concerns and holds promise for generating autologous donor cells, particularly for female patients [4, 56]. However, compared to ES cells derived from fertilized embryos, parthenogenetic ES (pES) cells - typically generated through the artificial activation of metaphase II (MII) oocytes - exhibit markedly reduced developmental potential, significantly limiting their therapeutic applications. This discrepancy may arise from fundamental genomic differences: parthenogenetic blastocysts contain two identical sets of maternally derived chromosomes, whereas fertilized blastocysts comprise both maternal

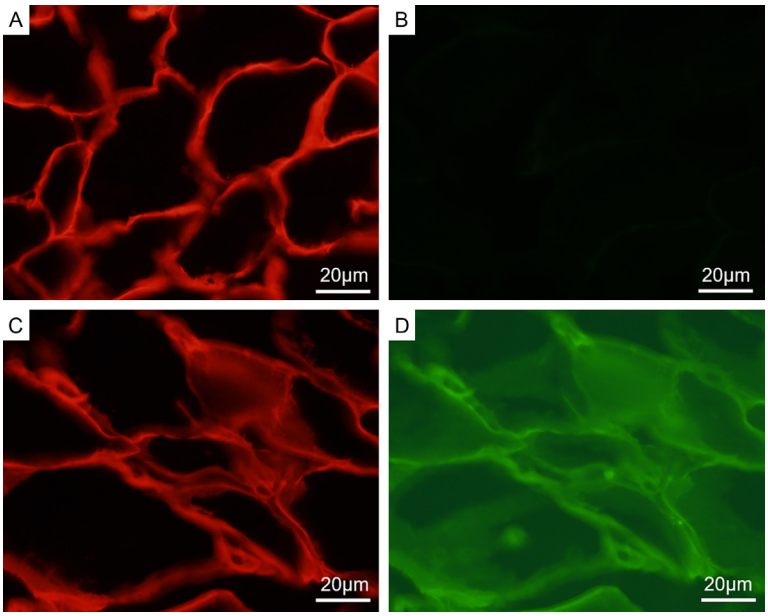

Figure 4. Histological analysis of tibialis anterior muscle regeneration following transplantation of in vitro-differentiated NF-pES precursor cells. (A, B) Depict control group samples, while (C, D) represent the experimental group. (A, C) Immunofluorescence staining for laminin (red) highlights skeletal muscle fibers. (B, D) Fluorescence microscopy images showing the absence (B) or presence (D) of green fluorescent protein (GFP) expression, indicating the presence of transplanted cells.

and paternal genomes. The absence of paternal genetic contribution in parthenogenetic embryos represents the most critical distinction; however, it remains unclear whether this factor alone accounts for the observed differences in the developmental competence of pES cells compared to their fertilized counterparts.

As described earlier, the genome of non-growing oocytes exhibits characteristics that are analogous to those of the sperm genome. We generated diploid parthenogenetic embryos containing genomes from both non-growing and fully grown oocytes, subsequently deriving embryonic stem cells from these embryos, which we term NF-pES cells [44]. The developmental potential of these ES cells was evaluated by analyzing their chimerism rates across various tissues in chimeric mice. Our results demonstrated that the differentiation capabilities of NF-pES cells in both the heart and bone marrow were comparable to those of embryonic stem cells derived from fertilized embryos.

Typically, the chimerism rate of parthenogenetic cells declines with the aging of chimeric mice [37]. However, in our study, NF-pES-derived cells exhibited significantly higher chimerism rates in the heart, spleen, and bone marrow of 6-month-old chimeric mice compared to previously reported rates for conventional pES-derived chimeras of the same age [37]. Prior research has demonstrated that the nuclear transfer of parthenogenetic embryonic stem (ES) cell nuclei into enucleated oocytes, followed by activation and blastocyst formation, can generate nuclear transfer embryonic stem (NT-ES) cells with enhanced developmental potential, particularly toward neural lineages [42], albeit still lower than that of fertilized embryo-derived ES cells. Notably, NF-p blastocysts have been shown to develop to embryonic day 13.5 (E13.5) following transfer into pseudopregnant recipients [32], extending their in vivo developmental window by approximately 3.5 days compared to conventional parthenogenetic blastocysts. Furthermore, H19 gene knockout in NF-p blastocysts enables full-term development and the birth of viable offspring [35]. These findings suggest that NF-p blastocysts may exhibit a gene expression profile more closely aligned with that of fertilized embryos, which could underlie the enhanced differentiation capacity observed in NF-pES cells. Consequently, the improved developmental potential of NF-pES cells is likely attributable, at least in part, to the increased embryonic competency of their source blastocysts.

Although the epigenetic state of mouse embryonic stem (ES) cells is known to undergo changes during in vitro culture, these modifications do not compromise their developmental totipotency [57]. Similarly, parthenogenetic ES (pES) cells also experience epigenetic alterations during culture, which may facilitate the ectopic expression of certain paternally imprinted genes [37]. However, these culture-induced changes appear insufficient to overcome the inherently restricted developmental potential

of pES cells. Therefore, we speculate that the epigenetic characteristics inherited from the embryos continue to influence the future differentiation potential of ES cells.

Previous studies have demonstrated that parthenogenetic embryonic stem (pES) cells contribute poorly to skeletal muscle in chimeric mice, with chimerism rates declining further as the animals age [37]. In contrast, our findings reveal that NF-pES cells exhibit sustained and robust contributions to skeletal muscle tissue, even at 6 months of age. Specifically, chimerism rates in the dorsal and hindlimb muscles reached 90.00% and 88.89%, respectively. Notably, macroscopic evaluation showed strong green fluorescence in these muscle groups, indicating a high degree of tissue integration derived from NF-pES cells. These results suggest that NF-pES cells possess significant developmental potential toward the skeletal muscle lineage.

However, pES cells derived from activated mature oocytes have consistently exhibited limited developmental potential, particularly concerning skeletal muscle differentiation [37-42]. To further evaluate the regenerative capacity of NF-pES cells, we employed a skeletal muscle injury model, demonstrating that NF-pES-derived progenitor cells could differentiate into skeletal muscle fibers at the injury site. This finding suggests that, unlike conventional pES cells, NF-pES cells possess greater myogenic potential and may represent a promising cell source for regenerative therapies targeting skeletal muscle injuries and disorders characterized by muscle degeneration or functional loss.

Conclusions

In summary, NF-pES cells demonstrate a developmental potential that is comparable to that of embryonic stem (ES) cells across various lineages, including cardiac, bone marrow, and skeletal muscle. Furthermore, they possess the capability to repair damaged skeletal muscle tissue. These findings establish NF-pES cells as a clinically valuable source of stem cells, alongside induced pluripotent stem (iPS) cells, ES cells, and parthenogenetic embryonic stem (pES) cells, highlighting their significant therapeutic potential.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hua Shao, Translational Medicine Research Center, Chongqing University Three Gorges Hospital, Wanzhou, Chongqing, China. Tel: +86-13262566308; E-mail: hshao@cqu.edu.cn

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