Application Note 001

Qkine's FGF2, TFGβ and Activin A Support Long-Term Maintenance of Dragon Bio Porcine Induced Pluripotent Stem Cells (piPSCs).

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Abstract

Porcine induced pluripotent stem cells (piPSCs) have many desirable characteristics, including self-renewal and the ability to differentiate into multiple cell types. This makes them ideal candidates for cultivated meat applications where the starting cells can divide indefinitely and differentiate into both fat and muscle lineages. Dragon Bio has reprogrammed porcine fibroblasts into piPSCs and fully characterized their self-renewal and differentiation capabilities. Developing the correct medium formulation is critical for maintaining pluripotency during long-term culture. Here, we present data demonstrating the bioactivity of Qkine (Cambridge, UK) FGF2, TGFβ, and Activin A in piPSC medium. These findings confirm that Qkine recombinant growth factors effectively support the long-term maintenance of Dragon Bio piPSCs.

Introduction

Dragon Bio piPSCs have been reprogrammed with non-integrative methods. Our piPSCs have indefinite self-renewal and can differentiate into any cell type. piPSCs, as well as their more studied human counterparts hiPSCs or human embryonic stem cells, require specific growth factors in their culture medium that activate self-renewal intracellular targeting pathways. Fibroblast growth factor 2 (FGF2) mediates the PI3K/AKT/mTOR and MAPK/ERK pathways, whereas TGFβ1 or activin A are required to regulate the TGFβ signalling pathway.

FGF2 regulates both cell proliferation and differentiation in pluripotent stem cells and is included in the growth medium of ESCs and iPSCs to maintain their pluripotency. FGF2 can be included in stem cell growth medium as both a short form (145aa), or a long form (154aa) that contains an extra N terminal 9aa residue. Qkine supplies both isoforms of FGF2 as human proteins (QK025 and QK27) and porcine-specific variants (QK040 and QK056).

Activin A regulates the TGFβ signalling pathway and belongs to the TGFβ family of growth factors. Together with FGF2, activin A is supplemented in many iPSC and ESC medium formulations to maintain pluripotency. Qkine recombinant Activin A (QK001) protein has porcine reactivity.

TGFβ contains 3 isoforms including TGFβ1 and TGFβ3, which together perform important roles in cell proliferation, growth, differentiation and motility. TGFβ1 is used in many commercial hESC and hiPSC mediums such as Essential 8 and mTesR, whereas TGFβ3 is used in B8 medium. Qkine TGFβ1 (QK010) has porcine reactivity whereas TGFB3 (QK054)
is human-specific. QKine growth factors are also free from animal-derived products to support the development of animal component-free media in cultured meat research.

Methods

Cell Culture of piPSCs and Growth Curves

piPSCs were clump passaged using reLEsR on geltrex coated 6-well plates. piPSCs were cultured for a minimum of 5 passages in porcine iPSC medium supplemented with each QKine FGF2 at 100ng/ml (QK025, QK027, QK040 and QK056), Activin A (QK001) at 20ng/ml, TGFβ1 plus (QK010) at 2ng/ml or TGFβ3 (QK054) at 2ng/ml. At passage 5, growth curves were set up for piPSCS cultured with each growth factor. piPSCs were dissociated into single cells using accutase and seeded at a density of 10,000 cells per well of a geltrex-coated 24-well plate, in 500µL of culture medium containing 1x revita cell supplement. The cells were fed daily over 5 days with 500 µL of culture medium containing each QKine growth factor. Triplicate cell counts were performed daily over 5 days, and growth curves were established.

qPCR Gene Expression Analysis of Pluripotency

After 5 passages in medium containing each growth factor (FGF2, Activin A, TGFβ1 or TGFβ3), piPSCs were harvested and RNA extraction was performed using NEB Monarch Total RNA miniprep kit. 2µg of RNA was converted to 2µg CDNA using the Applied Biosystems high-capacity cDNA reverse transcription kit. cDNA samples were diluted to 5ng/µl for qPCR and 10ng of cDNA was used per well of a 96-well PCR plate. Primers against porcine GAPDH (housekeeping), porcine OCT4 (pluripotency) and porcine SOX2 (pluripotency) were ordered from IDT. qPCR was performed using the Quantstudio 1 real-time PCR system. Delta-delta CT analysis was performed to calculate the relative fold change in gene expression of the markers OCT4/SOX2 in QKine supplemented medium vs control piPSC growth medium.

Results

Developing an optimal growth medium for porcine iPSCs is essential to maintain their pluripotency during long-term cell culture. Similar to human iPSCs, porcine iPSCs require both FGF2, TGFβ1 and Activin A supplementation in their growth medium in order to maintain stemness. The following data highlights the bioactivity of porcine and human QKine FGF2 (both 145aa and 154aa forms), TGFβ1 and Activin A to support the successful culture of porcine iPSCs. indicates that species-specific cell lines are a valuable tool to test the efficacy of growth factors and other supply chain reagents in cultivated meat R&D and manufacture.

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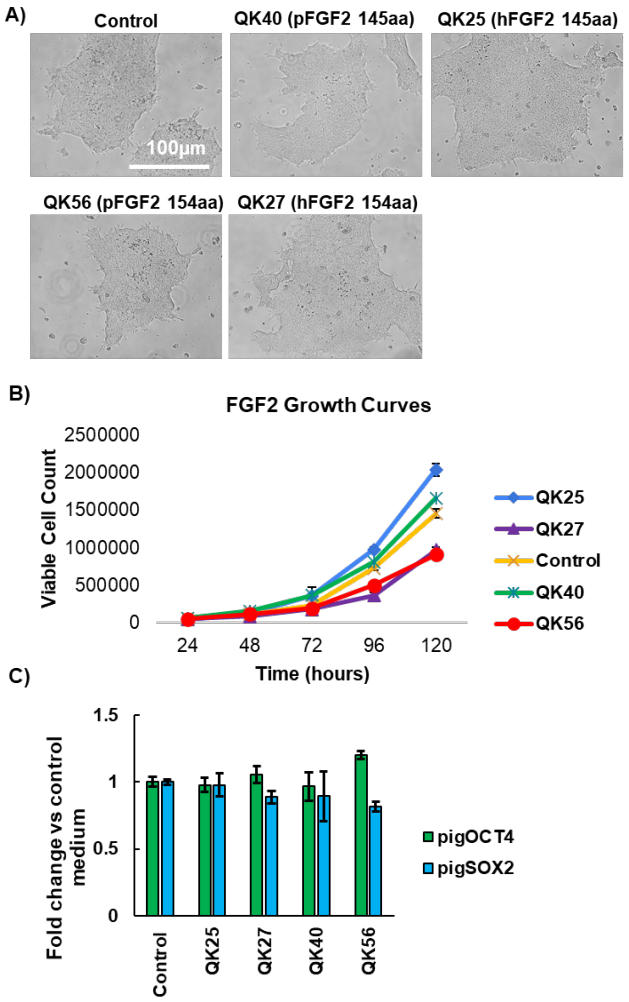

Figure 1: Schematic of cardiomyocyte differentiation from hiPSCs. Overview of the process of hiPSC differentiation into cardiomyocytes using inhibitors for Gsk3 and Wnt. The scale bar represents 500 µm.

QKine FGF2 Supplementation Maintains Pluripotency in piPSCs

Porcine iPSCs cultured with both human and porcine FGF2 isoforms display normal colony morphology (Figure 1A). We observed that piPSCs have increased growth rates compared to control piPSC medium when supplemented with both human and porcine 145aa FGF2 variants. Porcine iPSCs display slower growth rates in medium

containing human and porcine 154aa longer FGF2 isoforms. Expression of the pluripotency markers OCT4 and SOX2 are maintained in piPSCs cultured with all QKine FGF2 growth factors. We observed that the longer 154aa porcine FGF2 variant (QKine 56) had the highest OCT4 to SOX2 ratio, which supports the use of porcine-specific growth factors in piPSC culture medium.

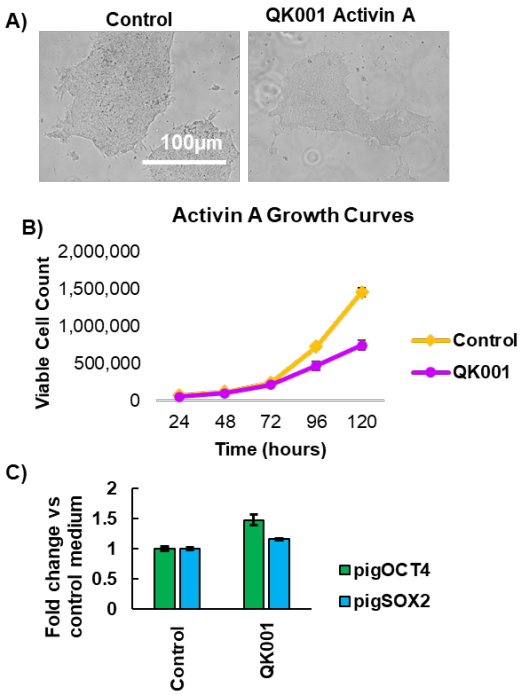

Figure 2: piPSCs cultured with Qkine Activin A. piPSCs were cultured in medium supplemented with 20ng/ml QKine Activin A. A) Brightfield microscopy images. B) Growth curves. C) Gene expression analysis of pluripotency markers (OCT4/SOX2).

Qkine Activin A Enhances Pluripotency in piPSCs

Porcine iPSCs cultured with porcine Activin A (QK001) show normal colony morphology and display slower growth rates than the human Activin A control (Figure 2A-B). However, gene expression analysis of the pluripotency marker OCT4 showed a 1.5-fold increase over 5 passages (Figure 2C). Thus, supplementation with porcine Activin A supports enhanced pluripotency of piPSCs.

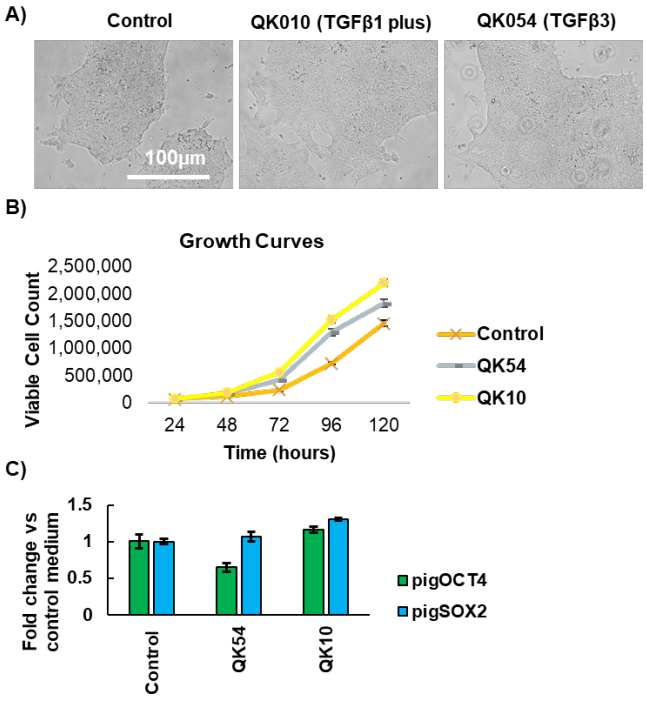

Figure 3: piPSCs cultured with Qkine TGFβ growth factors. piPSCs were cultured in medium supplemented with 2ng/ml QKine TGFβ1 or TGFβ3. A) Brightfield microscopy images. B) Growth curves. C) Gene expression analysis of pluripotency markers (OCT4/SOX2).

Qkine TGFβ1 maintains pluripotency in piPSCs

Porcine iPSCs cultured with either porcine TGFβ1 or human TGFβ3 show normal colony morphology and display higher growth rates than piPSC medium control (Figure 3A-B). We show that the pluripotency markers OCT4 and SOX2 are maintained with TGFβ1 supplementation (Figure 3C). However, the addition of human-specific TGFβ3 led to a decrease in OCT4 suggesting TGFβ3 on its own is not sufficient to maintain pluripotency. Moreover, this data supports that TGFβ1 plus with porcine reactivity is optimal for maintaining piPSC pluripotency.

Discussion

Qkine FGF2, Activin A, and TGFβ1 demonstrated consistent bioactivity in Dragon Bio's piPSCs, maintaining pluripotency markers over multiple passages. Both human and porcine FGF2 isoforms supported normal morphology and growth, with no significant performance differences observed. These findings confirm that Qkine recombinant growth factors are suitable for porcine iPSC maintenance and underscore the value of species-specific cell models for testing reagents in cultivated meat research and manufacturing.