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Small molecule TCS21311 can replace BMP7 and facilitate cell proliferation in *in vitro* expansion culture of nephron progenitor cells

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ABSTRACT

Several groups have developed *in vitro* expansion cultures for mouse metanephric nephron progenitor cells (NPCs) using cocktails of small molecules and growth factors including BMP7. However, the detailed mechanisms by which BMP7 acts in the NPC expansion remain to be elucidated. Here, by performing chemical screening for BMP substitutes, we identified a small molecule, TCS21311, that can replace BMP7 and revealed a novel inhibitory role of BMP7 in JAK3-STAT3 signaling in NPC expansion culture. Further, we found that TCS21311 facilitates the proliferation of mouse embryonic NPCs and human induced pluripotent stem cell-derived NPCs when added to the expansion culture. These results will contribute to understanding the mechanisms of action of BMP7 in NPC proliferation *in vitro* and *in vivo* and to the stable supply of NPCs for regenerative therapy, disease modeling and drug discovery for kidney diseases.

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1. Introduction

Nephron progenitor cells (NPCs) in the embryonic kidneys give rise to all epithelial components of the nephron, the minimal functional unit of the kidneys [1,2]. Several groups have reported *in vitro* expansion methods for mouse metanephric NPCs (mNPCs), and all use bone morphogenetic protein (BMP)7 [3–5]. In particular, Li et al. developed NPSR medium, which consists of a cocktail of BMP7, fibroblast growth factor (FGF)2, Heparin, Y-27632, CHIR99021, leukemia inhibitory factor (LIF), A83-01 and LDN193189, to expand mNPCs *in vitro* for more than one year [5,6]. Using the same medium, they successfully expanded human fetal NPCs and human induced pluripotent stem cell (hiPSC)-derived NPCs for 7 and 2 months, respectively. Even though none of the growth factors or compounds are dispensable for the culture, the role of BMP7 is not well understood. Attempts to replace BMP7 with other TGF- β superfamily proteins have been made, but at the cost of NPC expansion [7], suggesting that BMP7 is essential for the maintenance of NPCs.

BMP7 regulates many cellular process during development by binding to type I and type II BMP receptors expressed on the cell surface [8]. BMP-mediated signaling mainly activates receptor-

regulated Smads (R-Smads), including Smad1, 2, 3, 5 and 8. Activated R-Smads interact with Smad4 (Co-Smad), and this complex translocates to the nucleus to regulate target gene transcription. Two inhibitory Smads (I-Smads), Smad6 and Smad7, negatively regulate BMP- and TGF- β -mediated signaling by competing with R-Smads for the receptor kinase or by disrupting Co-Smad formation [8]. In mouse kidney development, it was reported that Bmp7 regulates the differentiation and proliferation of NPCs by determining the balance of mitogen-activated protein kinase (MAPK) signaling and R-Smad activation [4].

Signal transducer and activator of transcription (STAT)3 is a member of the STAT protein family [9]. STAT3 signaling can be activated by multiple upstream factors, including cytokines, chemokines and growth factors. The activation of STAT3 is through the phosphorylation of receptor-associated Janus kinases (JAKs), translocating STAT3 to the nucleus where it acts as a transcription activator. Specifically, STAT3 becomes activated after the phosphorylation of tyrosine 705 or serine 727 [9].

In this study, by performing chemical screening for BMP substitutes, we identified a small molecule, TCS21311, which can replace BMP7 in NPSR medium and revealed a novel inhibitory role of BMP7 in JAK3-STAT3 signaling. Further, we found that TCS21311 facilitates the proliferation of embryonic mouse NPCs and hiPSC-derived NPCs when added to NPSR medium.

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2. Materials and methods

2.1. Animal experiments

The Kyoto University Animal Care Committee approved all animal experiments. B6; 129-Six2^{tm3(EGFP/cre/ERT2)Amc/J} mice were acquired from the Jackson Laboratory Inc. [1]. All mice were housed at the laboratory animal facility of CiRA, Kyoto University, under specific pathogen-free (SPF) conditions. Mice were provided *ad libitum* access to food and water.

2.2. Cell culture

The experiments using hiPSCs were approved by the Ethics Committee of the Department of Medicine and Graduated School of Medicine, Kyoto University. hiPSCs were maintained with feeder-free cultures using Stem Fit AK02 N medium (Takara) on cell culture plates coated with Synthemax (Corning). The cells were passaged using 0.5 mM EDTA/PBS (Thermo Fisher Scientific) every five days and routinely tested for mycoplasma contamination. The harvest and maintenance culture of mNPCs were as described previously [5,6]. Details about the differentiation of hiPSCs towards NPCs are described elsewhere [Tsujimoto et al., in revision], 10].

2.3. Immunostaining

Kidney organoids were fixed with 4% PFA (Nacalai tesque)/PBS(–) overnight at 4°C. The fixed organoids were washed with PBS(–) twice, treated with 30% sucrose (Nacalai tesque)/PBS(–) overnight at 4°C and then frozen with OCT compound (Sakura Finetek). The frozen sections were incubated with 2% donkey serum (Millipore)/0.25% Triton X-100 (Nacalai tesque)/PBS(–) solution for 30 min at room temperature. Primary and secondary antibodies were diluted in 2% donkey serum/PBS(–) solution and incubated with the samples overnight and for 2 h at 4°C, respectively. The antibodies and lectins used in this study are listed in Table S1.

2.4. Chemical screening for BMP7 substitutes

We established a mouse NPC-3D culture and maintained mNPCs from Six2-GFP reporter mice as reported previously [5,6]. mNPCs passaged 10 to 30 times were used for the screening experiments. mNPCs were dissociated and seeded onto low-attachment u-bottom 384-well plates (Sumitomo Bakelite) at 2.5×10^3 cells/well. A custom-prepared small molecule library was supplied in an arrayed format in the plates with 1 mM stocks in dimethyl sulfoxide (DMSO) per well. Individual compounds were diluted using a Multidrop dispenser (Thermo Fisher Scientific) and transferred into assay plates by using Biomek NX (Beckman Coulter) at a final concentration of 1 μ M. Seventy-two hours after cell seeding, the cells were washed with PBS(–) and analyzed using Array Scan (Thermo Fisher Scientific). The quantification parameters included the size, roundness and average GFP signal of the mNPC aggregates. Each assay plate was designed to include positive and negative control wells containing NPSR medium with (NPSR) and without BMP7 (NPSR-B7), respectively.

2.5. Cell viability assay

For cell viability analysis using ATP quantification, mNPCs were dissociated and seeded onto low-attachment u-bottom 384-well plates at 2.5×10^3 cells/well. A custom made JAK inhibitor library was used. Individual compounds were transferred onto the assay plates by using a Biomek NX (Beckman Coulter) at final concentrations of 0.01, 0.03, 0.1, 0.3, 1 and 3 μ M. Seventy-two hours after

cell seeding, the cells were washed, and the ATP quantity was analyzed using a CellTiter-Glo® 2.0 Cell Viability Assay kit (Promega). Each assay plate was designed to include positive and negative control wells containing NPSR and NPSR-B7, respectively, plus DMSO.

2.6. Imaging

Fluorescent or bright-field images of the organoids were captured by a KEYENCE BZ-X700 or confocal microscope (Zeiss LSM710). Image analysis was performed using Image J version 1.51j8 (National Institutes of Health) [11].

2.7. Flow cytometry and cell sorting

We used OSR1-GFP/SIX2-tdTomato double knock-in hiPSC lines we had previously generated [12] to differentiate NPCs. After dissociation with Accumax (Innovative Cell Technologies, Inc.), the cells were analyzed for SIX2-tdTomato expression using a FACS Arian cell sorter (Becton Dickinson). Dead cells stained with 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI; 0.1 ng/mL; Thermo Fisher Scientific) were excluded from the analysis. The data were analyzed using FACS Diva (BD) software programs.

2.8. RNA sequencing

Previously reported RNA-seq data (GSE78772) was used [5]. The deposited mRNA-seq data of NPC aggregates cultured in NPSR and NPSR-B7 were compared. Transcript quantification was performed using Salmon [13] with GRCm38.p6 cDNA gene annotation (Ensembl). The output from Salmon was then processed using the R/Bioconductor package tximport to get gene expression values. Gene expression levels are represented as log2(RPKM+1). Gene ontology (GO) analysis for the top 500 differentially expressed genes (DEGs) was performed using Metascape [14]. Canonical pathway analysis was performed using Ingenuity Pathway Analysis (IPA; QIAGEN) with a cutoff value of ± 1.5 log2 fold change, and 2647 and 1293 genes upregulated with and without BMP7, respectively, were analyzed.

2.9. Protein assay

mNPC aggregates were dissociated by pipetting in RIPA buffer (Wako) supplemented with an EDTA-free Protease Inhibitor Cocktail Set DMSO Solution (Wako) and Phosphatase Inhibitor Cocktail Solution (Wako) on ice and sonicated, and the cell debris was removed by centrifugation. The protein concentration was analyzed by the XL-Bradford assay (Apro Science) and measured using Power scan (BioTek Instrument Inc.).

Protein measurements for Stat3, Phospho-Stat3 (p-Stat3, Tyr705), p-Stat3 (Ser727), Smad1, p-Smad1/5, Erk1/2, p-Erk1/2, p46 and p54 JNKs, p-p46 and p-p54 JNKs, p38 MAPK and p-p38 MAPK were performed using the cellular lysates from mNPC aggregates in NPSR, NPSR-B7 and NPSR-B7 with 3 μ M TCS21311 (NPSR-B7+TCS). The protein expression was quantified by a capillary western blot assay (Wes Separation Module for 12–230 kDa, ProteinSimple) [15]. Proteins (0.5 μ g/sample) were separated through a size-resolving matrix in the capillaries, immobilized to the inner capillary wall, and incubated with primary antibodies (Table S1) at a concentration of 1:50 in antibody dilution buffer (ProteinSimple) and with anti-rabbit or mouse secondary antibodies before detection using chemiluminescence. Signals reflected as the area under the curve (AUC) of the target proteins were generated automatically at the end of the run. Phosphorylated protein levels were quantified as the ratio of the AUC from each sample that was

normalized by the AUC of the corresponding total target protein. A total protein assay was performed using Total Protein Detection Module (ProteinSimple). Prior to the AUC measurements, the linear dynamic range of the sample concentration was confirmed using linear regression analysis for each target protein.

2.10. RT-PCR and real-time quantitative RT-PCR (qRT-PCR)

Total RNA was isolated using the RNeasy kit (Qiagen) according to the manufacturer's recommendations followed by cDNA synthesis using standard protocols for ReverTra Ace (TOYOBO). qPCR was performed using the Step One Plus Real-Time PCR System (Thermo Fisher Scientific) and SYBR Green PCR Master Mix (Takara). Denaturation was performed at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and at 60 °C for 34 s. As recommended by the manufacturer, the threshold cycle method was used to analyze the data for the gene expression levels, and the values were normalized to those of the housekeeping gene, β -Actin. The PCR reactions were performed in triplicate for each sample. The primer sequences are listed in Table S2.

2.11. Statistical analysis

Data are expressed as the mean $\pm$ SEM. Student's *t* tests were performed to compare the mean values when the experimental design was composed of two individual groups. One-way ANOVA and the Bonferroni post hoc test were used for multiple group comparisons.

3. Results and discussion

3.1. Chemical screening identified TCS21311 as a BMP7 substitute

In order to examine the roles of BMP7 in NPSR medium, we first cultured mNPCs in NPSR-B7. Consistent with a previous report [5], mNPC aggregates did not grow well compared to mNPC aggregates cultured in NPSR (Fig. 1A). Next, we performed a chemical screen to identify small molecules that increase cell proliferation in the absence of BMP7 by measuring the size and roundness of the aggregate and amount of Six2-GFP expression (Fig. 1B). Of the 4395 chemical compounds tested, 34 were designated as primary hits (data not shown). Each captured image was then manually analyzed based on the size, roundness and GFP signal. We eventually identified a selective JAK3 inhibitor, CP690550, as a candidate compound (Figure S1A).

Next, to confirm whether JAK3 inhibition substitutes BMP7 signaling, we treated mNPCs with multiple JAK inhibitors at final concentrations of 0.01, 0.03, 0.1, 0.3, 1 and 3 μ M and analyzed cell viability using the ATP quantification assay (Fig. 1C). Although cells treated with several JAK3 inhibitors as well as some JAK2/3, JAK1/2 and JAK2 inhibitors showed higher viability than those treated with DMSO, we found that higher concentrations of one JAK3 inhibitor, TCS21311, especially showed potent effects (Fig. 1C). We then maintained mNPC aggregates in NPSR-B7+TCS for 4 days, finding that the aggregates had the potential to differentiate into kidney organoids containing glomeruli and renal tubules (Fig. 1D–F). These results suggest that TCS21311 can replace BMP7 in mNPC maintenance culture.

3.2. BMP7 downregulates JAK3-related signals in *in vitro* NPC expansion culture

We next aimed to elucidate the mechanisms of BMP7 and TCS21311 in NPC expansion. First, to investigate cellular responses to the absence of BMP7, we obtained a previously reported

deposited RNA-seq dataset of mNPCs [5] and performed DEG analysis between mNPCs in NPSR and NPSR-B7 for 4 days. A GO analysis of the DEGs revealed that inflammation response-related signals were upregulated in mNPCs maintained in NPSR-B7 compared to those maintained in NPSR (Table S3). A canonical pathway analysis using IPA identified the predicted upregulation of Stat3-related signals (Fig. 2A). A capillary western blot assay revealed increased phosphorylation of Stat3 (Tyr705 but not Ser727) in NPCs cultured in NPSR-B7, an effect reversed by adding BMP7 or TCS21311 (Fig. 2B and S2A). In mouse embryonic stem cells (ESCs), Smad7 and suppressor of cytokine signaling 3 (Socs3) are reported as target genes of Stat3 signaling [16]. Consistently, qRT-PCR analysis revealed the upregulation of *Smad7*, *Socs3* and endogenous *Bmp7*, but not the other I-Smad, *Smad6*, when cultured in NPSR-B7, which also was reversed by adding BMP7 or TCS21311 (Fig. 2C). A previous report showed that Smad7 interacts with gp130, an upstream activator of Stat3, to prevent dephosphorylation of Stat3 and maintain Stat3 activation in mouse ESCs [16]. Together with the results that multiple JAK3 inhibitors showed positive effects on mNPC viability in NPSR-B7 (Fig. 1C), these data suggest that BMP7 downregulates Jak3-Stat3 signaling in mNPCs in NPSR, possibly through Smad7 downregulation.

On the other hand, the finding that higher concentrations of TCS21311 exerts much higher activity on mNPC viability than other JAK inhibitors (Fig. 1C) suggests that TCS21311 has other target molecules or pathways than JAK3-STAT3 signaling. Consistently, while the phosphorylation of Smad1/5, Erk1/2 and p46 JNK was decreased when cells were cultured in NPSR-B7, only the phosphorylation of Smad1/5 was reversible by the addition of TCS21311, suggesting that possible downstream targets of TCS21311 include the Smad1/5 pathway (Fig. 2D, S2B–D). Although a previous study showed that Stat3 is an upstream regulator of *Six2* [17], NPSR-B7+TCS did not significantly change the expression level of *Six2* or *Cited1*, markers for the undifferentiated state of NPCs (Figure S1B) [3], or the NPC differentiation markers *Pax8* and *Lhx1* (Figure S1C) [5], compared to NPSR, indicating that TCS21311 maintains the undifferentiated state of NPCs in the absence of BMP7 in NPSR media.

3.3. Addition of TCS 21311 to NPSR media facilitates mNPC proliferation

The finding that higher concentrations of TCS21311 exerts much higher mNPC viability than other JAK inhibitors (Fig. 1C) led us to hypothesize that its addition to NPSR media may produce additive effects on mNPC expansion. We thus tested the addition of several JAK3 inhibitors, including TCS21311, at final concentrations of 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 μ M to mNPCs in NPSR media (Fig. 3A). We found that 0.3 μ M TCS21311 resulted in the highest cell viability among tested JAK3 inhibitors. The total cell number of each mNPC aggregate treated with NPSR media and 0.3 μ M TCS21311 for 4 days was significantly higher than mNPC aggregates treated with NPSR medium alone (Fig. 3B).

We then tested whether the expanded mNPCs in NPSR medium with 0.3 μ M TCS21311 have the potential to differentiate into kidney organoids after 12 days of culture with two passages. Indeed, we confirmed successful differentiation into kidney organoids containing glomeruli and renal tubules (Fig. 3C). These results suggest that TCS21311 has proliferative effects on mNPCs in maintenance culture.

3.4. Addition of TCS 21311 to NPSR media facilitates the proliferation of hiPSC-derived NPCs

Next, to investigate whether the cell proliferative effect of TCS21311 is also applicable to human NPCs, we induced OSR1-GFP/

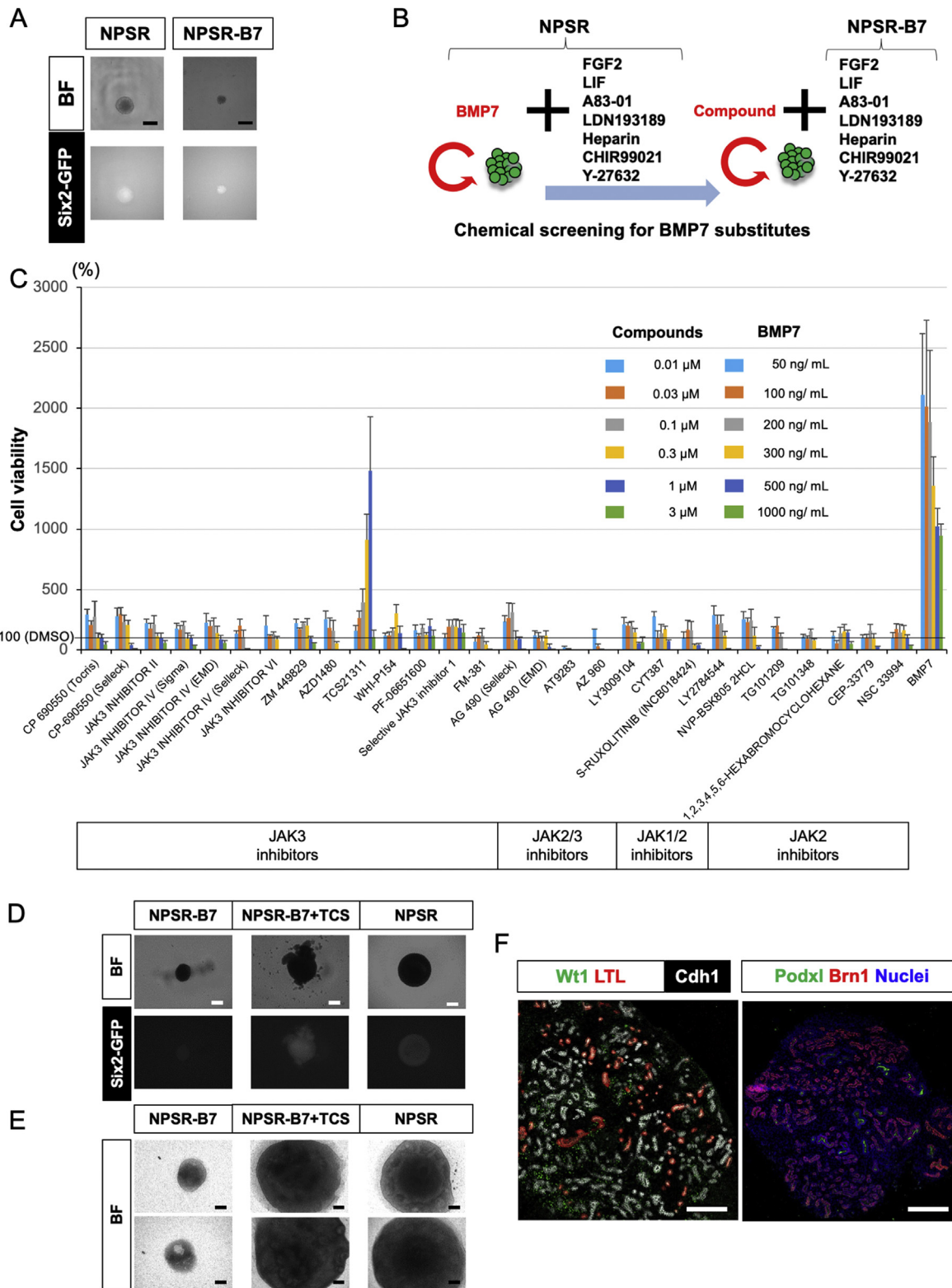


Fig. 1. Chemical screening identified TCS21311 as a BMP7 substitute in NPSR media.

(A) Bright-field (upper panels) and fluorescent images (lower panels) of mNPCs cultured for 3 days in NPSR (left) and NPSR-B7 (right).

(B) Schematic drawing of the screening strategy.

(C) Relative cell viability measured by an ATP assay of mNPCs cultured in NPSR or NPSR-B7 and with various JAK inhibitors. Results are normalized to DMSO control and presented as the mean $\pm$ SEM ($n = 6$ for TCS21311 and $n = 3$ for the others).

(D) Representative bright-field (BF, upper panels) and fluorescent images (lower panels) of mNPC aggregates maintained for 4 days in NPSR-B7 (left), NPSR-B7+TCS (middle) and NPSR (right).

(E) BF images of kidney organoids on culture days 3 (upper panels) and 7 (lower panels) derived from mNPC aggregates maintained in NPSR-B7 (left), NPSR-B7+TCS (middle) and NPSR (right).

(F) Lectin staining and immunostaining analyses for Wt1, *Lotus tetragonolobus* lection (LTL), Cdh1, Podxl and Brn1 in kidney organoids derived from mNPCs maintained in NPSR-B7+TCS. Scale bars: 200 μ m in (A, F) and 300 μ m in (D, E).

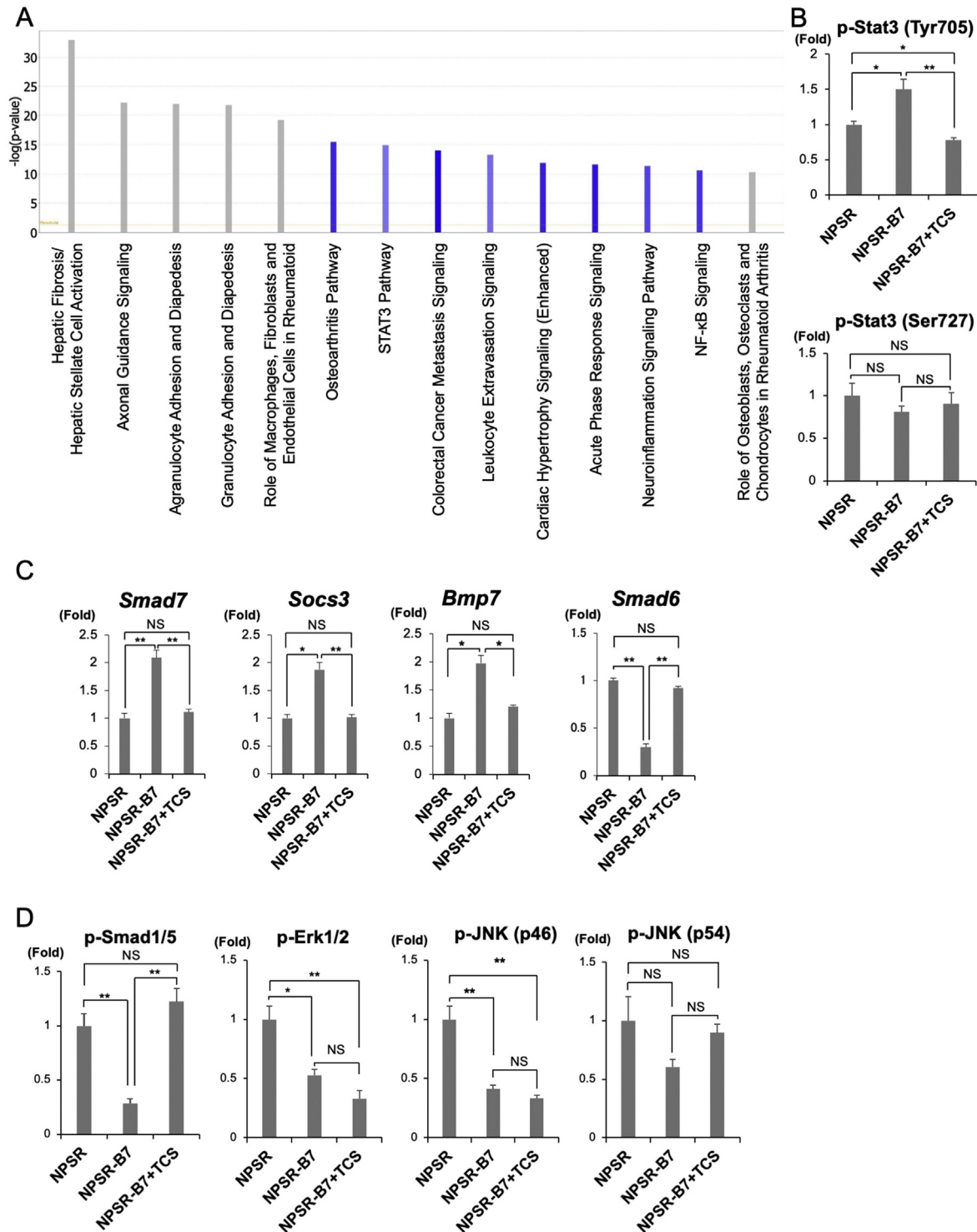


Fig. 2. BMP7 inhibits Jak3-Stat3 signaling in the *in vitro* expansion culture of mNPCs.

(A) Canonical pathway analysis for the differentially expressed genes in mNPCs cultured in NPSR and NPSR-B7. $-\log(p\text{-value})$ scores greater than 10 are shown. The color intensity (from strong blue to gray) represents the predicted overall decrease in activity, with gray indicating pathways that are not eligible for prediction.

(B) The protein levels of Stat3 and phosphorylated Stat3 (p-Stat3) assessed by a capillary western blot assay for mNPCs in NPSR, NPSR-B7 and NPSR-B7+TCS. Values in the bar graph represent means $\pm$ SEM ($n = 4$) of the area under the curve of p-Stat3 signals normalized to those of the corresponding total Stat3 protein. Each value was then normalized to that of control.

(C) qRT-PCR analysis of the *Smad7*, *Socs3*, *Bmp7* and *Smad6* expressions in mNPCs in NPSR, NPSR-B7 and NPSR-B7+TCS. The data from three independent experiments are presented as the mean $\pm$ SEM ($n = 3$). Each value was normalized to that of control.

(D) The protein levels of Smad1, phosphorylated Smad1/5 (p-Smad1/5), Erk1/2, phosphorylated Erk1/2 (p-Erk1/2), JNKs (p46 and p54) and phosphorylated JNKs (p-JNKs (p46 and p54)) were assessed by a capillary western blot assay for mNPCs in NPSR ($n = 6$ for p-Erk1/2 and $n = 4$ for p-Smad1/5 and p-JNKs), NPSR-B7 ($n = 4$ for p-Erk1/2 and p-Smad1/5 and $n = 5$ for p-JNKs) and NPSR-B7+TCS ($n = 6$ for p-Erk1/2, $n = 4$ for p-Smad1/5 and $n = 5$ for p-JNKs). Values in the bar graphs represent means $\pm$ SEM of the area under the curve of p-Smad1/5, p-Erk1/2 and p-JNKs (p46 and p54) signals normalized to that of the corresponding total Smad1, Erk1/2 and JNKs (p46 and p54) proteins, respectively. Each value was then normalized to that of control.

* $p < 0.05$, ** $p < 0.01$ in (B–D). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

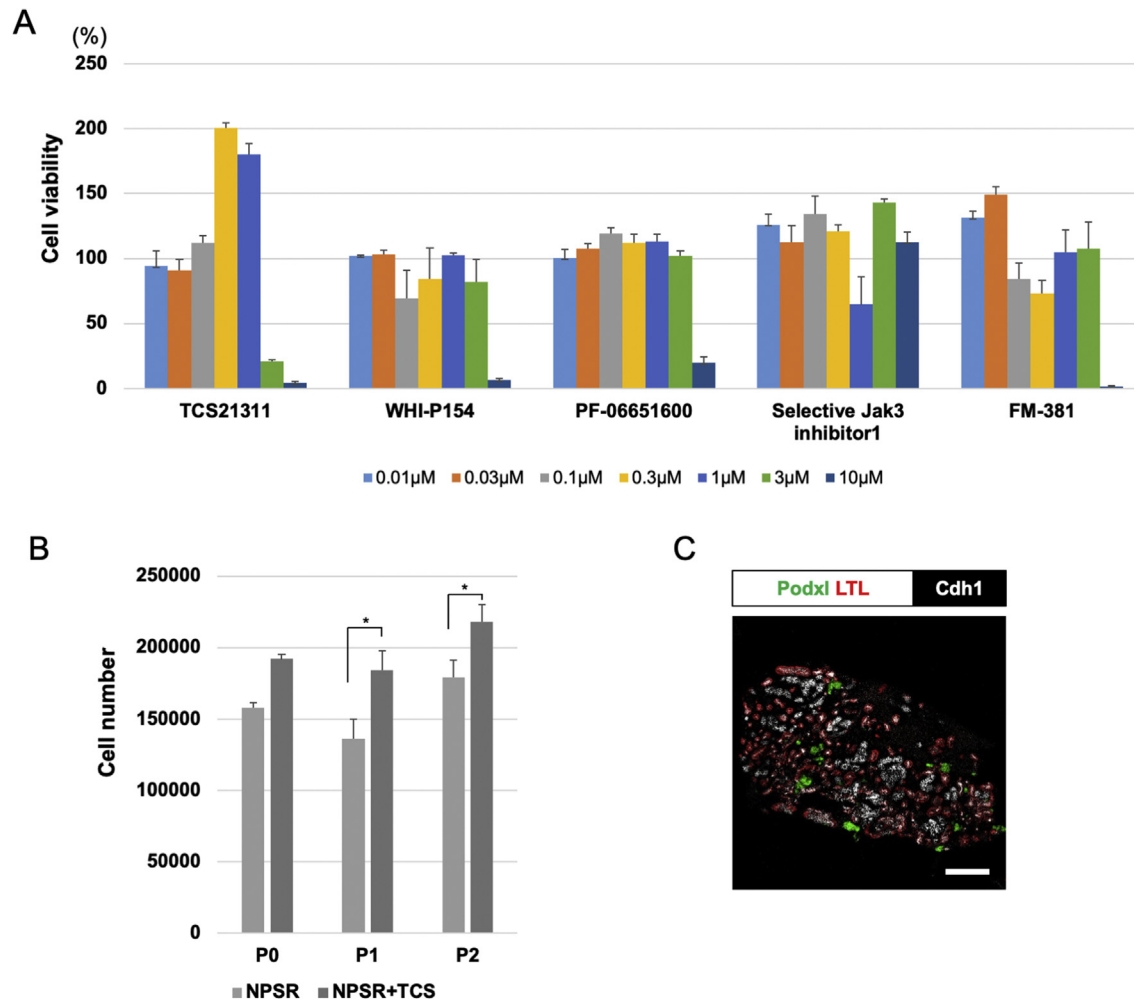


Fig. 3. TCS21311 facilitates the proliferation of mNPCs in NPSR media.

(A) Cell viability measured by an ATP assay of mNPCs cultured in NPSR media with various JAK3 inhibitors compared to controls in NPSR media plus DMSO (100%). Data are presented as the mean $\pm$ SEM (n = 3).

(B) The number of cells in day 4 mNPC aggregates in NPSR (light gray) and NPSR medium with TCS21311 (NPSR + TCS; dark gray) are presented as the mean $\pm$ SEM (n = 3). P: passage.

(C) Lectin staining and immunostaining analyses for Podxl, LTL and Cdh1 of kidney organoids derived from mNPCs passaged twice (day 12) with NPSR + TCS.

*p < 0.05 in (B). Scale bar: 200 μ m.

SIX2-tdTomato knockin hiPSCs [12] to differentiate into NPCs using a previously reported induction method [10] or our original method (Tsujimoto et al. in revision). OSR1(GFP)(+)SIX2(tdTomato)(+) NPCs were purified by flow cytometry sorting and used for the *in vitro* expansion culture experiments. The hiPSC-derived OSR1(+)SIX2(+) NPCs generated by our method were then maintained in NPSR medium or NPSR medium with 0.3 μ M TCS21311 (Fig. 4A), which confirmed the proliferative effect of TCS21311 on hiPSC-derived NPCs. Furthermore, we successfully maintained hiPSC-derived OSR1(+)SIX2(+) NPCs generated by either of the two methods in NPSR medium with TCS21311 for 10 days. The number of hiPSC-derived NPCs was increased around ten times (Fig. 4B), and over 90% of cells were SIX2(+) after 10 days of culture (Fig. 4C and D). Considering the above, these results suggest that TCS21311 exerts proliferative effects on both mNPCs and hiPSC-derived NPCs.

In the current study, by screening a chemical library consisting of approximately 4400 small molecule compounds or natural products for BMP7 substitutes in NPSR conditions [5], we identified JAK3 inhibitors as primary hits and revealed a novel inhibitory role of BMP7 in JAK3-STAT3 signaling, possibly through Smad7

downregulation. We then unexpectedly observed that higher concentrations of TCS21311 facilitate the proliferation of mouse embryonic NPCs and hiPSC-derived NPCs, but the mechanisms of action remain unknown, since other selective JAK3 inhibitors showed weaker effects. These results suggest that there might be biological targets of TCS21311 besides JAK3 or distinctive regulatory roles on the signaling axis in the balance of NPC proliferation and differentiation. Our data indicate that the possible targets of TCS21311 include the Smad1/5 pathway, but not the MAPK pathways. Further mechanistic elucidation of the signaling pathways will provide novel insights into NPC proliferation.

While mNPCs expanded *in vitro* with NPSR medium plus TCS21311 maintained the developmental capacity to differentiate into kidney organoids (Fig. 3C), it was difficult to stably generate kidney organoids from hiPSC-derived NPCs expanded with the same conditions (data not shown). Previous reports also found difficulty in the long-term *in vitro* expansion of hiPSC-derived NPCs while maintaining the developmental capacity of NPCs [5,7]. Future studies should establish expansion methods for hiPSC-derived NPCs.

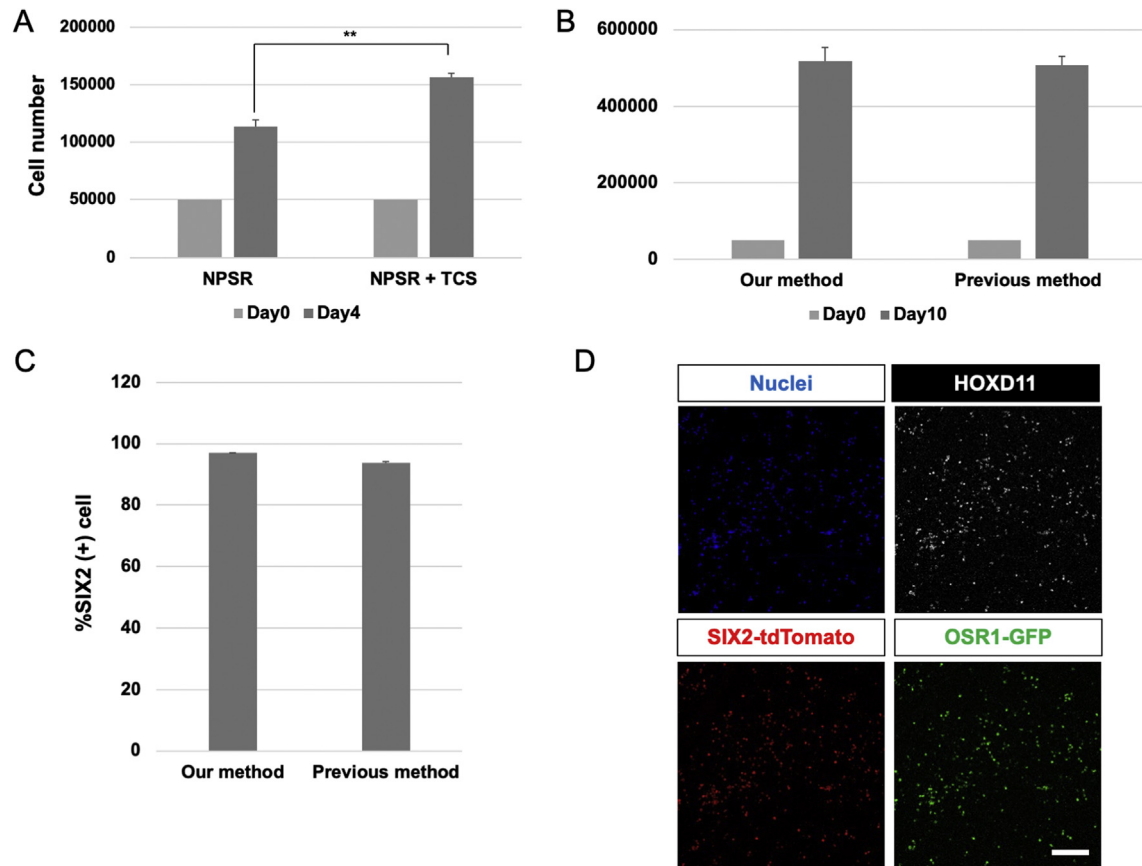


Fig. 4. TCS21311 facilitates the proliferation of hiPSC-derived NPCs in NPSR media.

(A) The number of cells in day 4 hiPSC-derived NPC aggregates in NPSR (light gray) and NPSR medium with TCS21311 (NPSR + TCS; dark gray) are presented as the mean $\pm$ SEM (n = 4).

(B) The number of cells in day 0 (light gray) and day 10 (dark gray) hiPSC-derived NPC aggregates in NPSR + TCS are presented as the mean $\pm$ SEM (n = 3). (C) The percentage of SIX2 (tdTomato) (+) cells in DAPI (–) living cells from hiPSC-derived NPC aggregates maintained in NPSR + TCS for 10 days.

(D) Immunostaining analysis for SIX2 (tdTomato), OSR1 (GFP) and HOXD11 in hiPSC-derived NPCs maintained in NPSR + TCS for 10 days.

**p < 0.01 in (A). Scale bar: 200 μ m in (D).

In conclusion, our findings will contribute to understanding the mechanism of action by which BMP7 proliferates NPCs *in vitro* and *in vivo*. Novel NPC expansion methods using TCS21311 will reduce the workload in cell preparation for iPSC/ESC technology-based regenerative therapy, disease modeling and drug discovery for kidney diseases.

Author contributions

H.T., T.A., Y.N., A.O., T.N. and K.O. designed the study. H.T. performed the experiments. H.T. and K.O. analyzed the data. K.O. supervised the study. H.T. and K.O. drafted the manuscript. All authors discussed the results and commented on the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: K.O. is a founder and member without the salary of the scientific advisory boards of iPS Portal, Inc., and a founder and chief scientific advisor of RegeNephron Co., Ltd. T.A. is a founder and scientific advisor of RegeNephron Co., Ltd.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrc.2020.02.130>.

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