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Biochemical and Biophysical Research Communications 630 (2022) 64–70

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Wnt signaling promotes tooth germ development through YAP1-TGF-β signaling

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ARTICLE INFO

Article history:
Received 5 August 2022
Received in revised form
9 August 2022
Accepted 2 September 2022
Available online 14 September 2022

Keywords:
Wnt/β-catenin
YAP1
TGF-β
Tooth germ development
Odontogenic epithelial cells

ABSTRACT

Tooth germ development involves continuous and sequential steps with reciprocal interactions between odontogenic epithelium and the adjacent mesenchyme. Several growth factors, including Wnt, are essential for tooth germ development. Molecular mechanisms underlying Wnt/β-catenin-regulated tooth germ development are poorly understood. In tooth germ rudiments culture, we recently demonstrated that Semaphorin 3A (Sema3A), an axonal guidance factor, stimulation reversed Wnt/β-catenin signaling-dependent decreased cell proliferation but did not completely rescue the morphological anomalies of tooth germ, suggesting that an uncharacterized signaling pathway may be essential in Wnt/β-catenin signaling-dependent tooth germ development. Herein, an enrichment analysis using DNA microarray data, which was obtained in our previous research, revealed that Wnt/β-catenin signaling negatively regulates YAP1 and/or TGF-β signalings. In odontogenic epithelial cells and tooth germ rudiments, Wnt/β-catenin signaling activation reduced YAP1 expression, thereby suppressing YAP1 and TGF-β signalings sequentially. Additionally, YAP1 signaling induced TGF-β2 expression to promote TGF-β signaling in the cells. Finally, Wnt/β-catenin signaling-dependent disorganized tooth germ development, in which YAP1 signaling was suppressed, was reversed by TGF-β stimulation. These results suggest that Wnt/β-catenin signaling contributes to the tooth germ development through YAP1-TGF-β signaling.

1. Introduction

Mouse tooth germ development involves continuous and sequential steps and is regulated by reciprocal interactions between odontogenic epithelium and the adjacent mesenchyme. Several growth factors, including Wnt, bone morphogenetic protein, fibroblast growth factor and sonic hedgehog, and their signal activation are reported to be involved in tooth germ development [1]. Wnt signaling activates two different pathways: the β-catenin pathway and the β-catenin-independent pathway. In the β-catenin pathway, Adenomatous polyposis coli organizes a multiprotein "destruction complex" that degrades β-catenin. Wnt/β-catenin signaling then regulates gene expression by inhibition the "destruction complex" and β-catenin stabilization [2]. Notably, genetically modified mice with odontogenic epithelium-dependent Wnt/β-catenin pathway activation showed developmental anomalies of tooth germ, resulting in supernumerary teeth resembling odontomas [3]. These reports indicate that the appropriate activation of Wnt/β-catenin signaling is essential for tooth germ development.

We recently demonstrated that Wnt/β-catenin signaling activation, which reduced Semaphorin3A (Sema3A) expression, disrupted tooth germ development with decreased epithelial cellular growth [4]. In this tooth germ rudiments culture, Sema3A stimulation reversed Wnt/β-catenin signaling activation-dependent decreased cell proliferation but did not completely rescue their morphological anomalies. Therefore, we hypothesized that Wnt/β-

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https://doi.org/10.1016/j.bbrc.2022.09.012
0006-291X/

catenin signaling might regulate an uncharacterized signaling pathway, even if the role has been already reported, to regulate tooth germ development.

Inactivation of the Hippo pathway regulates morphogenesis in various organs [5], including tooth development [6,7], by regulating the nuclear localization of major downstream transcription co-activator, yes-associated protein-1 (YAP1)/transcriptional co-activator with PDZ-binding motif (TAZ), resulting in the induction of target gene expression [5].

Transforming growth factor β (TGF-β) family signaling also plays important roles in the development of many organs [8]. TGF-β signaling is activated by the binding of TGF-βs (TGF-β1, TGF-β2 and TGF-β3) to TGF-β type II receptor (TGFBR2), resulting in TGFBR1 activation. The receptor complex phosphorylates receptor-regulated Smads (R-Smads; Smad2, Smad3) and then forms a complex with the common Smad (Co-Smad; Smad4) and translocates into the nucleus to regulate transcription of target genes [9]. Smad-mediated activation of TGF-β signaling has been demonstrated to be essential for proper tooth morphogenesis [10].

However, while these reports indicated the Hippo pathway and TGF-β signaling are involved in tooth germ development, the mechanisms underlying the activation of these signalings and their relationship are unclear. Here, we investigated the regulatory effects of Wnt/β-catenin signaling on YAP1-TGF-β signaling in tooth germ development through gene expression.

2. Materials and methods

2.1. Cells

mDE6 mouse odontogenic epithelial cells and SF2 rat odontogenic epithelial cells were kindly provided from Dr. S. Fukumoto (Kyushu University, Fukuoka, Japan) [4,11]. Lenti-X™ 293T (X293T) cells were purchased from Takara Bio Inc. (Shiga, Japan). mDE6 cells and SF2 cells were cultured in DMEM/F12 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen). X293T cells were grown in DMEM (Invitrogen) supplemented with 10% FBS. See supplementary Materials and methods, and Supplementary Table S2 for further details.

2.2. Mouse tooth germ rudiments culture

Protocols used for all animal experiments in this study were approved by the Animal Research Committee of Kyushu University, Japan (No. A29-277-0, A21-065-0). Embryonic day (E) 15 mouse tooth germ rudiment culture was carried out as described previously [4]. See supplementary Materials and methods for further details.

2.3. Enrichment analysis using DNA microarray data

Previously, microarray analyses were performed using mDE6 cells [4]. Then, the raw data was deposited in NCBI GEO under accession number (GSE116739). Enrichment analysis of the downregulated genes, the expression of which are Z-score ≦ -2.0 and ratio ≦0.66 compared to the normalized signal intensities of each probe, was performed using the DAVID 6.8 (Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway) database (https://david-d.ncifcrf.gov/).

2.4. Plasmid construction and infection using lentivirus harboring a cDNA

The YAP^{5SA} plasmid, CSII-CMV-MCS-IRES2-Bsd/FLAG-YAP^{5SA}, was used [12]. The vectors were transfected along with the packaging vectors, pCAG-HIV-gp and pCMV-VSV-G-RSV-Rev, into X293T cells using the Lipofectamine LTX reagent (Invitrogen) to generate lentiviruses [13]. See supplementary Materials and methods for further details.

2.5. Knockdown of protein expression by siRNA and quantitative RT-PCR

The effects of siRNA were analyzed as previously described [14]. In brief, siRNAs (final concentration 20 nM) were transfected into SF2 cells using Lipofectamine RNAiMAX (Invitrogen) and used for experiments 48 h post-transfection. Quantitative RT-PCR was performed as described previously [15]. Target sequences and primers are listed in Supplementary Tables S3 and S4, respectively.

2.6. Immunofluorescence staining

Tooth germ rudiments were collected, fixed in 4% (w/v) paraformaldehyde (PFA) buffered by phosphate buffered saline and processed for paraffin embedding. Tissue specimens for examination were sliced into 4-μm-thick sections and stained with H&E [16]. Then, the sections of middle area of mouse tooth germs were selected for immunofluorescence analysis. Immunofluorescence staining was performed as described previously [17,18]. See supplementary Materials and methods for further details.

2.7. Statistical analysis

Statistical analyses were performed using JMP Pro 16 software. Significant differences were determined using Student's *t*-test and one-way ANOVA with *post hoc* Tukey's test. *P* value of <0.05 was considered to indicate statistical significance.

3. Results

3.1. Wnt/β-catenin signaling suppresses YAP1 signaling

To elucidate the underlying molecular mechanisms by which Wnt/β-catenin signaling regulates tooth germ development, we recently performed DNA microarray analysis [4], based on mDE6 cells treated with CHIR99021, an activator of Wnt/β-catenin signaling. In this study, we further carried out an enrichment analysis of the downregulated genes in the previous DNA microarray data using the DAVID (KEGG pathway) database, and then several signals were downregulated by CHIR99021 treatment (Fig. 1A and Supplementary Table S1). Among them, we focused on the Hippo and TGF-β pathways in this study.

Consistent with the KEGG pathway analysis in mDE6 cells (Fig. 1A and Supplementary Table S1), CHIR99021 treatment reduced the expression of connective tissue growth factor (*Ctgf*; a target gene of YAP1/TAZ signaling) [19] and YAP1 but increased *Axin2* (a direct target gene of Wnt/β-catenin signaling) [20] in a dose-dependent manner (Fig. 1B and Supplementary Fig. S1A). The expression of cellular communication network factor 1 (*Ccn1*; also known as *Cyr61*), another target gene of YAP1/TAZ signaling [21], and *Taz* was also suppressed by CHIR99021 treatment (Supplementary Fig. S1A). Similarly, in SF2 cells, CHIR99021 treatment decreased the expression of CTGF, YAP1, TAZ and *Cyr61* but induced the expression of *Axin2* and nuclear translocation of β-catenin (Fig. 1C and Supplementary Figs. S1B and C), suggesting that Wnt/β-catenin signaling negatively regulates YAP1/TAZ signaling. Since Wnt/β-catenin signaling reduced target genes of YAP1/TAZ signaling more efficiently in SF2 cells than in mDE6 cells, we mainly used SF2 cells in the subsequent experiments.

β-Catenin siRNA rescued the CHIR99021-dependent reduction

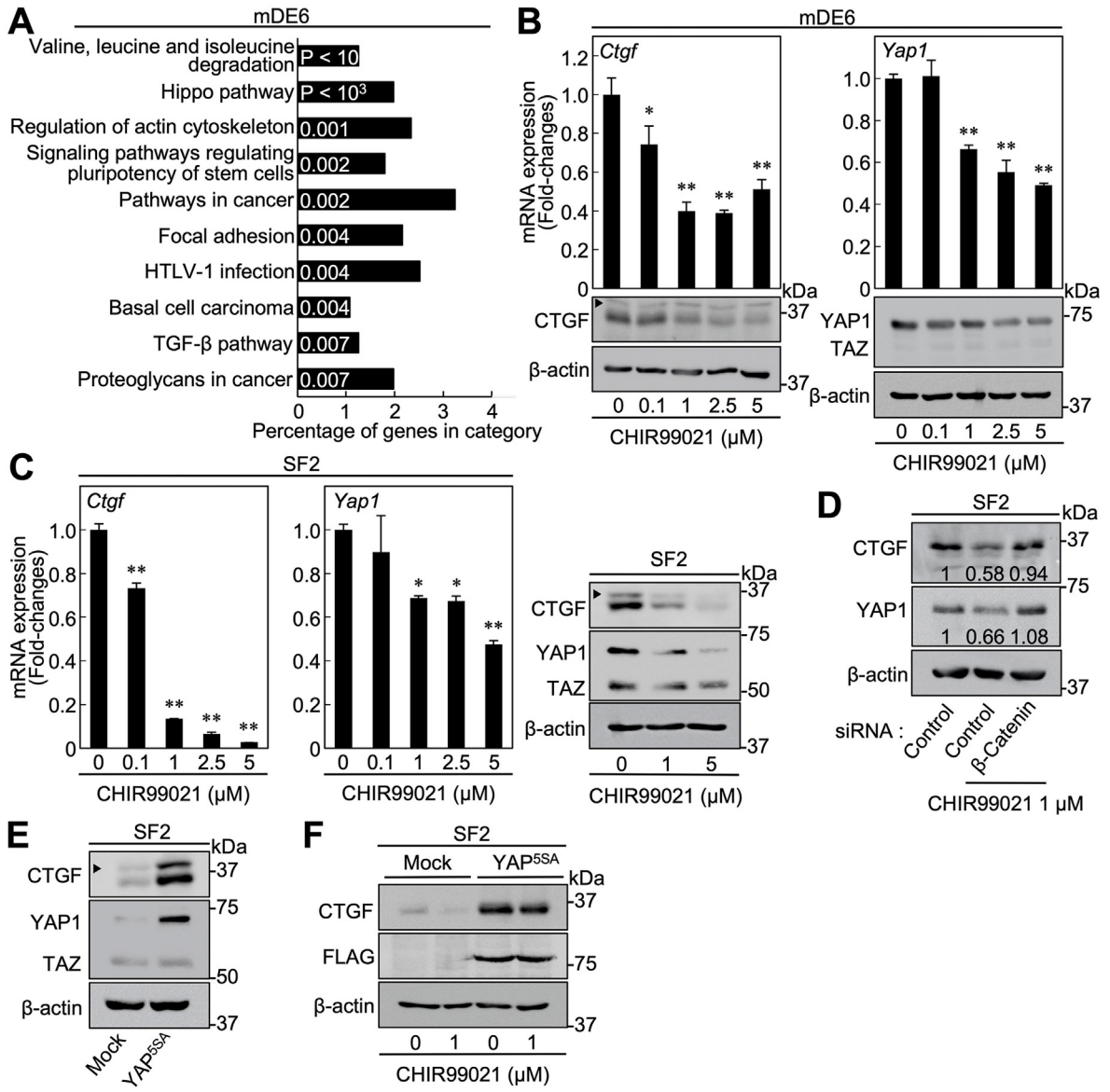

Fig. 1. Activation of Wnt/β-catenin signaling suppresses YAP1 signaling by reducing YAP1 expression.
(**A**) Top 10 significant enriched pathways by KEEG pathway analysis are listed. (**B, C**) mDE6 cells (**B**) and SF2 cells (**C**) were cultured without or with CHIR99021 for 24 h. RT-qPCR for Ctgf and Yap1 mRNA in mDE6 cells (**B**) and SF2 cells (**C**) was performed. Relative mRNA levels were normalized by GAPDH and expressed as fold-changes compared with levels in control cells. Cell lysates were probed with indicated antibodies. (**D**) SF2 cells were transfected with control or β-catenin siRNA for 72 h, and then were cultured with CHIR99021 for last 24 h. Cell lysates were probed with indicated antibodies. Band intensities were quantified using NIH image software and the ratio of each target/β-actin was presented as fold-change compared with control cells. (**E**) SF2 cells expressing mock or YAP^{5SA} were cultured for 24 h, and then cell lysates were probed with indicated antibodies. (**F**) SF2 cells expressing mock or YAP^{5SA} were cultured for 48 h, and then were treated without or with CHIR99021 for last 24 h. Cell lysates were probed with indicated antibodies. Arrowhead indicates non-specific bands. Results are shown as means ± s.d. of three independent experiments. **P < 0.01. *P < 0.05.

of CTGF and YAP1 protein expression, suggesting that endogenous β-catenin was necessary for the inhibition of YAP1 signaling by CHIR99021 treatment in SF2 cells (Fig. 1D and Supplementary Fig. S1D). Next, SF2 cells expressing mock or an active YAP1 mutant, YAP^{5SA}, in which five possible phosphorylation serine residues were changed to alanine, were generated by lentiviral transduction (Fig. 1E). In YAP^{5SA}-expressing cells, CTGF and *Cyr61* expression were elevated compared with mock-expressing cells (Fig. 1E and Supplementary Fig. S1E), and exogenous YAP^{5SA} expression rescued the CHIR99021-dependent reduction in CTGF and *Cyr61* expression (Fig. 1F and Supplementary Fig. S1F). These results suggest that Wnt/β-catenin signaling suppresses YAP1 signaling through a reduction of YAP1 expression in odontogenic epithelial cells.

3.2. Wnt/β-catenin signaling suppresses TGF-β signaling through YAP1 signaling

Consistent with the KEGG pathway analysis (Supplementary Table S1), *Smad3* and inhibitor of DNA binding 2 (*Id2*) expression were indeed reduced by CHIR99021 treatment in SF2 and mDE6 cells (Supplementary Figs. S2A and B). Furthermore, both Smad2 phosphorylation and the expression of downstream transcriptional targets of TGF-β signaling (Vimentin and alpha-smooth muscle actin (SMA; also known as *Acta2*)) [22], were reduced by CHIR99021 treatment (Fig. 2A and Supplementary Fig. S2B). These data suggest that TGF-β signaling is suppressed by Wnt/β-catenin signaling in odontogenic epithelial cells.

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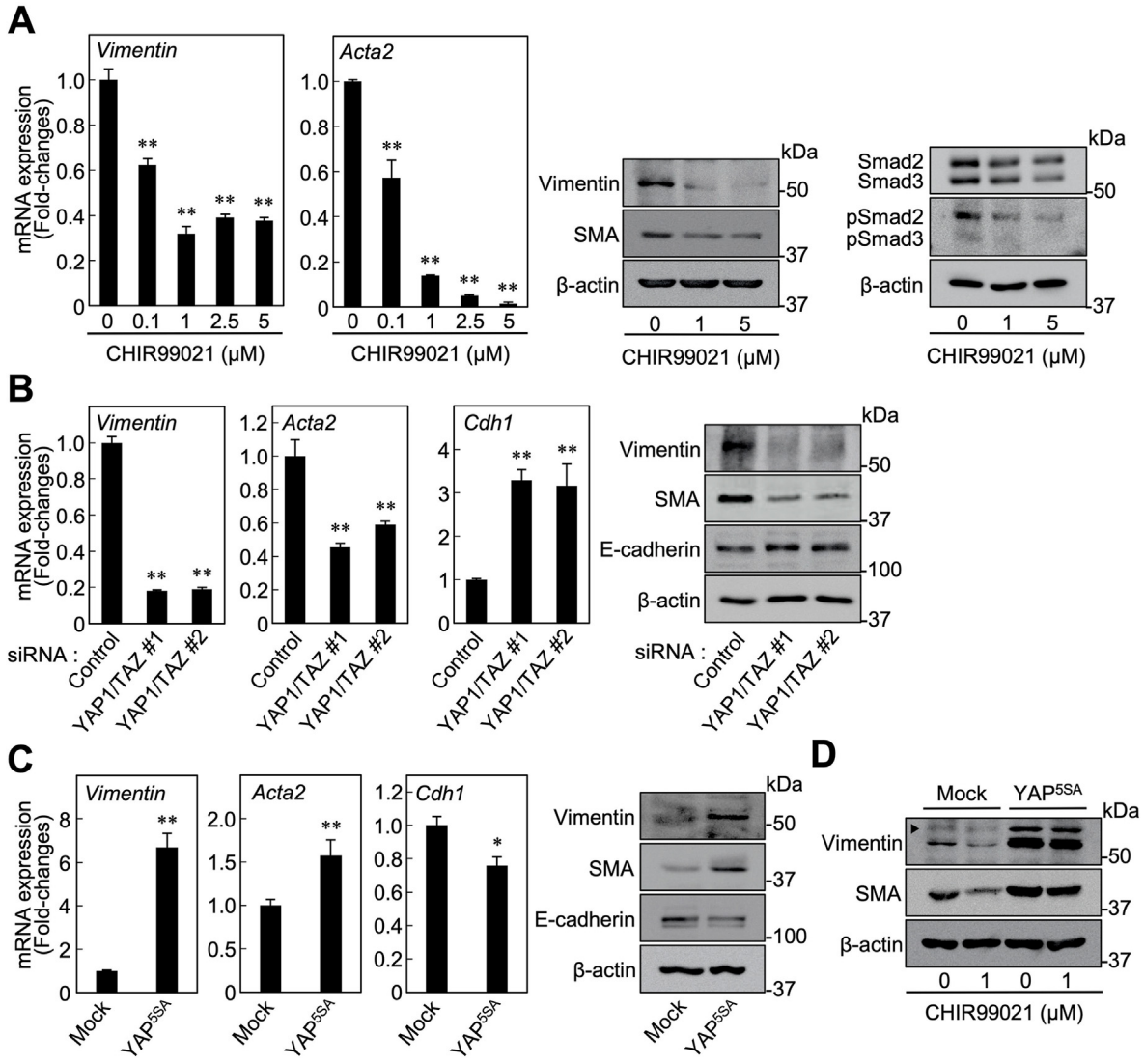

Fig. 2. Activation of Wnt/β-catenin signaling suppresses TGF-β signaling through YAP1 signaling.
(**A**) SF2 cells were cultured without or with CHIR99021 for 24 h. RT-qPCR for Vimentin and Acta2 mRNA was performed. Relative mRNA levels were normalized by GAPDH and expressed as fold-changes compared with levels in control cells. Cell lysates were probed with indicated antibodies. (**B**) SF2 cells were transfected with control or two different YAP1/TAZ siRNAs for 48 h and RT-qPCR for Vimentin, Acta2 and Cdh1 mRNA was performed. Relative mRNA levels were normalized by GAPDH and expressed as fold-changes compared with levels in control cells. Cell lysates were probed with indicated antibodies. (**C**) SF2 cells expressing mock or YAP^{5SA} were cultured for 24 h, and RT-qPCR for Vimentin, Acta2 and Cdh1 mRNA was performed. Relative mRNA levels were normalized by GAPDH and expressed as fold-changes compared with levels in control cells. Cell lysates were probed with indicated antibodies. (**D**) SF2 cells expressing mock or YAP^{5SA} were cultured for 48 h, and then were treated without or with CHIR99021 for last 24 h. Cell lysates were probed with indicated antibodies. Arrowhead indicates non-specific bands. Results are shown as means ± s.d. of three independent experiments. **P < 0.01. *P < 0.05.

Activator (MOB) 1A/1B-deficient mice develop liver cancer through YAP1/TAZ signaling-dependent TGF-β signaling activation [23]. Therefore, to elucidate the effects of YAP1/TAZ signaling on TGF-β signaling in odontogenic epithelial cells, YAP1/TAZ was knocked down by two different siRNAs. YAP1/TAZ siRNAs reduced Vimentin, SMA, CTGF and *Cyr61* expression but promoted E-cadherin (*Cdh1*) expression in SF2 cells (Fig. 2B and Supplementary Fig. S2C). Loss-of-function experiments using YAP1 inhibitors, such as Dasatinib and Verteporfin [24,25], demonstrated that YAP1 signaling is involved in the expression of *Vimentin* and *Acta2* as well as *Ctgf* and *Cyr61* (Supplementary Fig. S2D). Similarly, in YAP^{5SA}-expressing cells, the expression of Vimentin and SMA was elevated, while that of E-cadherin was decreased compared with mock-expressing cells (Fig. 2C). Furthermore, exogenous YAP^{5SA} expression rescued the CHIR99021-dependent reduction of Vimentin and SMA expression (Fig. 2D and Supplementary Fig. S2E). These results suggested that Wnt/β-catenin signaling suppresses TGF-β signaling through YAP1 signaling in odontogenic epithelial cells.

3.3. Activation of Wnt/β-catenin signaling causes abnormal tooth germ development by downregulating YAP1-TGF-β signaling

We evaluated the involvement of Wnt/β-catenin signaling-dependent YAP1-TGF-β signaling in tooth germ development by using organ culture of E15 mouse tooth germ rudiments. Because E15 tooth germ rudiments culture seems to be closer to the *in vivo* scenarios than cell lines, and these cultured rudiments facilitate targeting specific genes/signaling pathways of interests. Consistent with the findings for odontogenic epithelial cells, the expression of *Yap1*, *Ctgf*, *Cyr61*, *Vimentin* and *Acta2* was reduced, while the expression of *Axin2* was elevated in a dose-dependent manner in the E15 tooth germ rudiments treated with CHIR99021 for three

days (Fig. 3A). In addition, YAP1 inhibitors and the ALK5 inhibitor A83-01 reduced the expression of the above genes (Fig. 3B and C). These results suggested that YAP1 signaling and TGF-β signaling are activated in E15 tooth germ rudiments culture, and their signalings may be regulated by Wnt/β-catenin signaling, similar to odontogenic epithelial cells.

To gain further mechanistic insight into the details concerning the association of these signals, we investigated TGF-βs (TGF-β1, TGF-β2 and TGF-β3) expression in SF2 cells. Among TGF-βs, the expression of *TGF-β2* was reduced by CHIR99021 treatment or YAP1/TAZ siRNAs, while its expression was induced in YAP^{5SA}-expressing cells compared with mock-expressing cells (Fig. 3D–F and Supplementary Figs. S3A–C). Furthermore, YAP^{5SA}-dependent upregulated *Acta2* and *Ctgf* expression were decreased by A83-01 stimulation (Supplementary Fig. S3D). These data suggested that YAP1 signaling induces TGF-β2 expression, resulting in the promoting of TGF-β signaling. In contrast, A83-01 stimulation did not affect the expression of YAP^{5SA}-dependent upregulated *Vimentin* expression, indicating that *Vimentin* was regulated not by TGF-β signaling but by YAP1 signaling. In addition, stimulation with TGF-β2 partially reversed the CHIR99021-dependent reduction of *Acta2* and *Ctgf* as well as TGF-β1 (Fig. 3G and Supplementary Fig. S3E). However, TGF-β1 stimulation partially reversed this reduction more efficiently, so TGF-β1 was used in the subsequent experiments. These findings suggest that Wnt/β-catenin signaling may suppress TGF-β signaling through YAP1 singling-dependent TGF-β2 expression.

Finally, we evaluated the involvement of Wnt/β-catenin-TGF-β signaling in tooth germ morphogenesis. In the E15 tooth germ rudiments treated with CHIR99021 for seven days, tooth germ

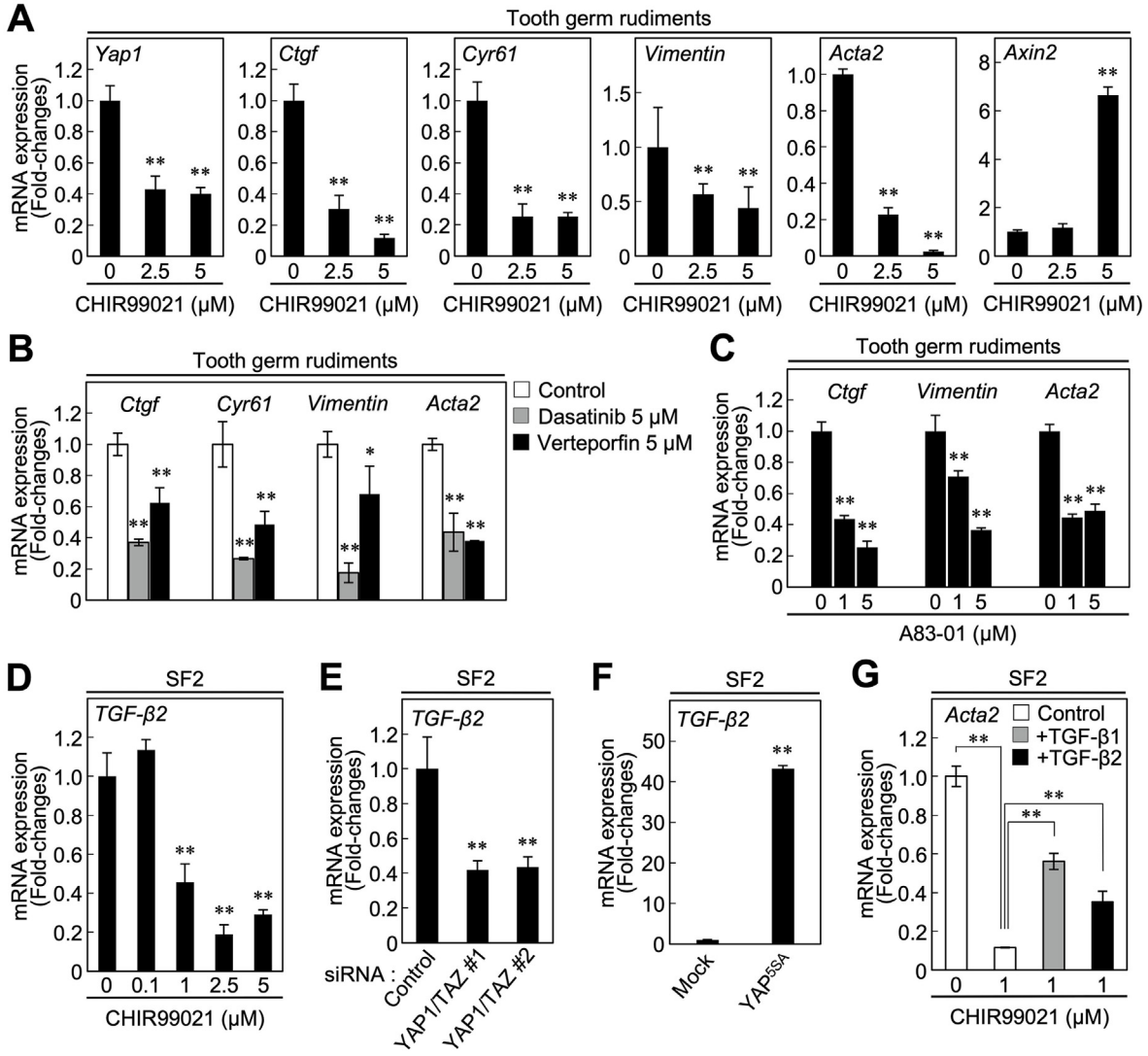

Fig. 3. The effects of Wnt/β-catenin on YAP1 signaling and TGF-β signaling.
(**A-C**) E15 tooth germ rudiments were cultured without or with CHIR99021 (**A**), Dasatinib or Verteporfin (**B**) or A83-01 (**C**) for 3 days, respectively. RT-qPCR for Yap1, Ctgf, Cyr61, Vimentin, Acta2 and Axin2 (**A**), Ctgf, Cyr61, Vimentin and Acta2 (**B**) or Ctgf, Vimentin and Acta2 (**C**) mRNA was performed, respectively. (**D**) SF2 cells were cultured without or with CHIR99021 for 24 h, and RT-qPCR for TGF-β2 mRNA was performed. Relative mRNA levels were normalized by GAPDH and expressed as fold-changes compared with levels in control cells. (**E**) SF2 cells were transfected with control or two different YAP1/TAZ siRNAs for 48 h, and RT-qPCR for TGF-β2 mRNA was performed. Relative mRNA levels were normalized by GAPDH and expressed as fold-changes compared with levels in control cells. (**F**) SF2 cells expressing mock or YAP^{5SA} were cultured for 24 h, and RT-qPCR for TGF-β2 mRNA was performed. Relative mRNA levels were normalized by GAPDH and expressed as fold-changes compared with levels in control cells. (**G**) SF2 cells were cultured for 48 h, and then were treated without or with CHIR99021, 10 ng/ml TGF-β1 and/or TGF-β2 for last 24 h. Acta2 mRNA levels were measured in the cells by RT-qPCR. Relative mRNA levels were normalized by GAPDH and expressed as fold-changes compared with levels in control cells. Relative levels of indicated mRNA expression were normalized to GAPDH and expressed as fold-changes compared with expression in control cells. Results are shown as means ± s.d. of three independent experiments. **P < 0.01. *P < 0.05.

development was severely disrupted compared with controls (Fig. 4A and Supplementary Fig. S4A). In detail, treatment with CHIR99021 did not affect the areas of total enamel organ, inner enamel epithelium (IEE) or outer enamel epithelium (OEE) but did increase the areas of stellate reticulum (SR) and E-cadherin-positive intensity of SR (Fig. 4B and Supplementary Figs. S4B and C). Importantly, CHIR99021-dependent disrupted tooth germ morphogenesis involving the areas and E-cadherin-positive intensity of SR was rescued by TGF-β1 stimulation (Figs. 4A and 4B and Supplementary Figs. S4A, B and C). Furthermore, the cell number was counted, and then the cell numbers in the total enamel organ and IEE did not significantly differ among rudiments (Fig. 4C). Treatment with CHIR99021 decreased the cell number in the OEE and increased the cell number in the SR, whereas TGF-β1 stimulation reversed the CHIR99021-dependent phenotype of SR (Fig. 4C). These data suggest that Wnt/β-catenin signaling regulates tooth germ development through TGF-β signaling.

4. Discussion

In studies with genetically modified mice, Wnt/β-catenin signaling regulates tooth germ development [3,26], but the molecular mechanism remains unclear. In this study, we demonstrated that activation of Wnt/β-catenin signaling decreased the expression of YAP1, leading to inactivation of YAP1 signaling and TGF-β signaling sequentially, thereby resulting in abnormal tooth germ development.

Recent reports demonstrated that Wnt/β-catenin signaling promotes YAP1 signaling through several mechanisms [27]. In our study, Wnt/β-catenin signaling downregulated YAP1 signaling involving the expression of downstream molecules in two kinds of odontogenic epithelial cells and tooth germ rudiments, and exogenous YAP^{5SA} expression rescued their expression in the odontogenic epithelial cells. While the effects of Wnt/β-catenin signaling on YAP1 signaling may be cell-context-dependent, the current results confirm that Wnt/β-catenin signaling suppresses YAP1 signaling through a reduction of YAP1 itself in odontogenic epithelial cells and tooth germ rudiments. Further studies are needed to clarify the precise mechanism by which Wnt/β-catenin signaling negatively regulates YAP1 expression.

Reportedly, YAP1/TAZ signaling regulates TGF-β signaling at the transcriptional level, such as via the expression of TGF-βs [23], or at the protein level, such as via nuclear translocation of Smads through association with YAP1/TAZ [28,29]. In the present study, TGF-β2 could be a transcriptional target of YAP1/TAZ signaling in odontogenic epithelial cells. Furthermore, CHIR99021-dependent disorganized tooth morphogenesis, in which YAP1 signaling was suppressed, was reversed by activation of TGF-β signaling. Accumulating evidence suggests the importance of activated intracellular signaling at the individual cell levels, but the precise function at the tissue or organ levels as a complex of cells remains unclear. It is intriguing to speculate that the tooth germ rudiments culture may be a more convenient model for examining multicellular behaviors, such as the association of intracellular signaling or cell-cell interaction, than using genetically modified mice.

Treatment with CHIR99021 affected both the SR and OEE but not the IEE in the rudiments culture, suggesting that the CHIR99021-dependent cellular behavior of the SR and OEE may differ from that of the IEE. While we know that the IEE differentiates into ameloblasts to produce enamel matrix [1], information on the roles of the SR and OEE in tooth germ development is limited. Furthermore, CHIR99021 stimulation decreased the cell number in the OEE and increased it in the SR, whereas TGF-β1 stimulation reversed the phenotype of the SR (see Fig. 4C). TGF-β signaling usually inhibits cell proliferation in a cell-type specific manner and decreased E-cadherin expression [9], so it is possible that CHIR99021 stimulation reduced TGF-β signaling, resulting in the stimulation of cell proliferation and induction of expansion of areas of the SR. Consistently, CHIR99021-dependent increased E-cadherin-positive intensity was decreased by TGF-β signaling. In contrast, in the current CHIR99021-treated tooth germ rudiments without or with TGF-β1, Ki-67-positive cells were mainly observed in the cervical

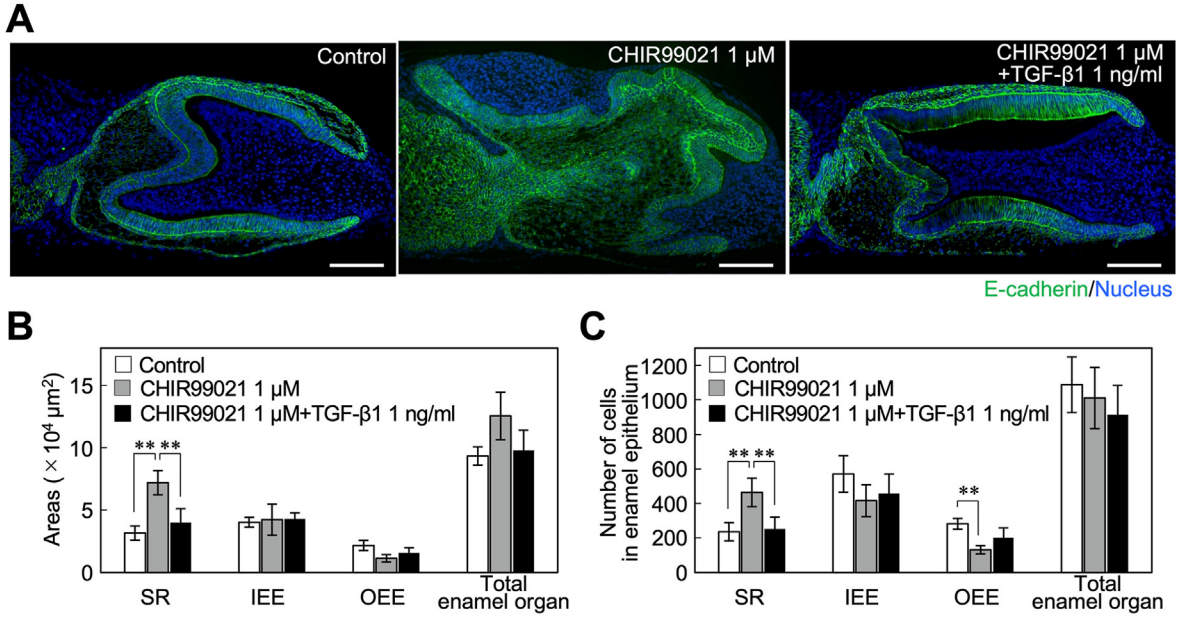

Fig. 4. Wnt/β-catenin signaling regulates tooth germ development through TGF-β signaling.
(**A**) E15 tooth germ rudiments were cultured for 7 days. They were treated without or with CHIR99021 and/or TGF-β1 for initial 3 days, and then the culture medium was changed every second or third day. The rudiment sections were stained with anti-E-cadherin antibody and Hoechst 33342. (**B**) The areas of the stellate reticulum (SR), inner enamel epithelium (IEE), outer enamel epithelium (OEE) and total enamel organ in the rudiments were measured (see Supplementary Fig. S4B). Scale bars: 100 μm. Results are shown as means ± s.d. of three independent experiments. **P < 0.01.

loops of the tooth germ (data not shown). Considering with previous report [4], it is possible that Wnt/β-catenin signaling regulates cell proliferation through at least two kinds of signaling, such as Sema3A signaling on the cervical loops and YAP1-TGF-β signaling on the SR. As treatment did not change the cell number of the total enamel organ, it is possible that Wnt/β-catenin signaling may regulate a specific epithelial cell fate, such as the OEE or SR. But the precise mechanisms are unknown. TGF-β signaling induces Smad4 activity [30], which is involved in tooth germ development and cytodifferentiation in odontogenic epithelial cells [11]. In this study, TGF-β stimulation did not affect differentiation markers, such as *Amelogenin*, *Ameloblastin*, *Enamerin*, *Matrix metalloeptidase 20* (*Mmp20*), *T-box transcription factor 1 (Tbx1)*, *Secreted frizzled related protein 5 (Sfrp5)*, *notch receptor 1* (*Notch1*), *Notch2*, *Cadherin 2*, *Msh homeobox 2 (Msx2)* and *Nerve growth factor receptor (p75)* (data not shown).

In summary, Wnt/β-catenin signaling reduced the expression of YAP1, thereby suppressing YAP1 signaling and TGF-β signaling sequentially. These results suggest that the Wnt-YAP1-TGF-β axis contributes to tooth germ development.

Funding

This work was supported by JSPS KAKENHI Grant to S.F. (2020–2022; JP20K09906), K.H. (2021–2023; JP21K09843) and T.K. (2020–2022; JP20K10096), Takeda Science Foundation to S.F. and K.H., and The Shin-Nihon Foundation of Advanced Medical Research to S.F.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank Drs. H. Wada, M. Kokura and T. Fujimoto and appreciate the technical assistance from The Joint Use Laboratories, Faculty of Dental Science, Kyushu University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2022.09.012.

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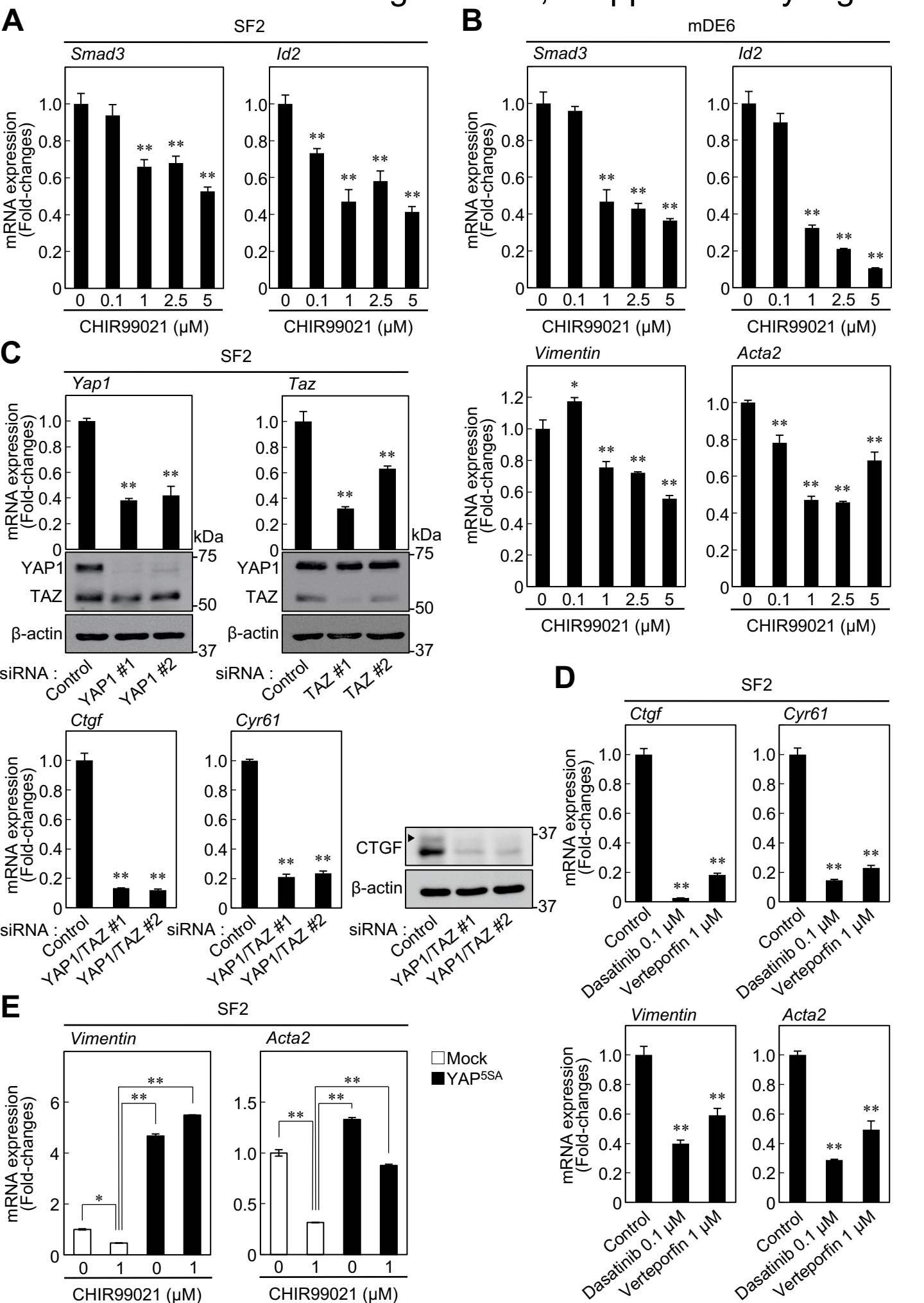
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SF2
Smad3
Id2
mRNA expression (Fold-changes)
CHIR99021 (μM)
B
mDE6
Smad3
Id2
Vimentin
Acta2
C
SF2
Yap1
Taz
kDa
YAP1
TAZ
β-actin
siRNA : Control YAP1 #1 YAP1 #2
siRNA : Control TAZ #1 TAZ #2
Ctgf
Cyr61
CTGF
siRNA : Control YAP1/TAZ #1 YAP1/TAZ #2
D
SF2
Control
Dasatinib 0.1 μM
Verteporfin 1 μM
E
SF2
Vimentin
Acta2
Mock
YAP5SA

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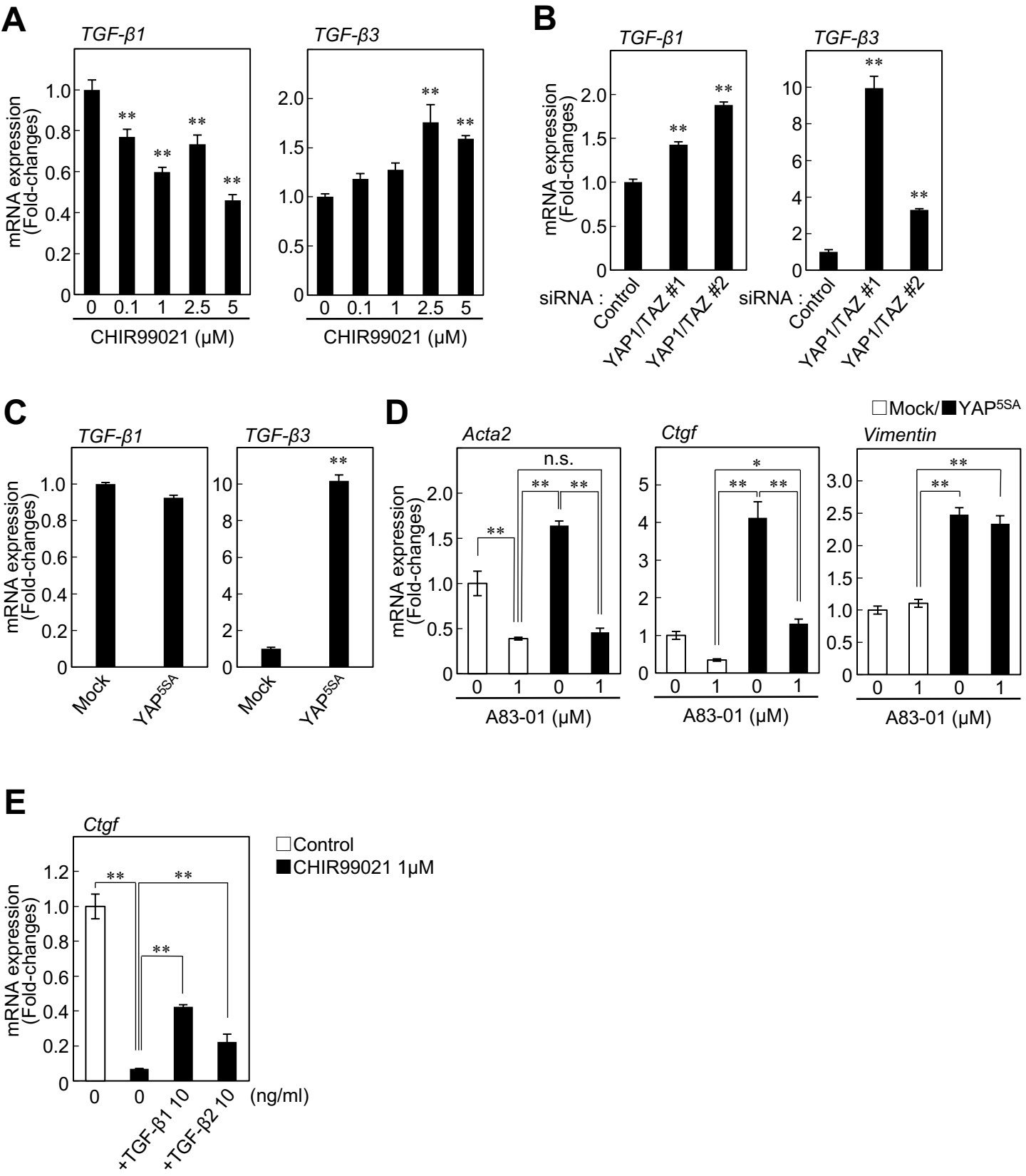

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