Transcriptional regulation of the TCF7L2/TCF4 gene in response to Wnt/β-catenin signaling activation.

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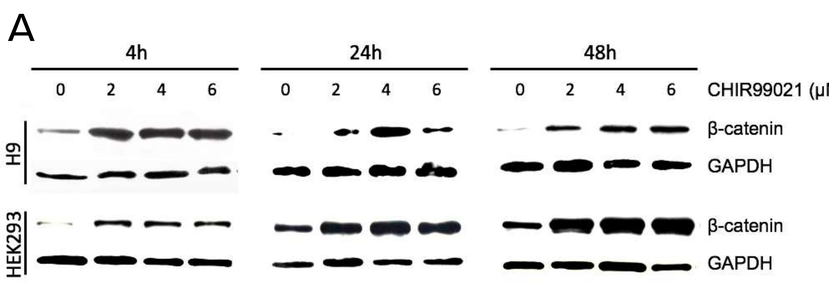
INTRODUCTION

The Wnt/β-catenin signaling pathway plays essential roles in cell differentiation and proliferation, tissue regeneration and cell death, among many other biological processes. Alterations in the levels of the signaling cascade contributes to the development of a wide range of diseases, including cancer, neurodevelopmental disorders and neurodegenerative diseases. The T-cell transcription factor 4 (TCF7L2/TCF4) is considered a key component of the Wnt/β-catenin signaling pathway and acts as the main transcriptional mediator in the nucleus. Although it has been postulated the TCF7L2 could have an important role during neurodevelopment, to date there are no indication of mechanisms regulating its expression. Here we studied the effect of the activation of Wnt/β-catenin and the cellular levels of TCF7L2, using the pharmacological agonist CHIR99021 and a constitutive β-catenin construct in human H9 neuronal progenitor cells and kidney embryonic cells (HEK293).

RESULTS



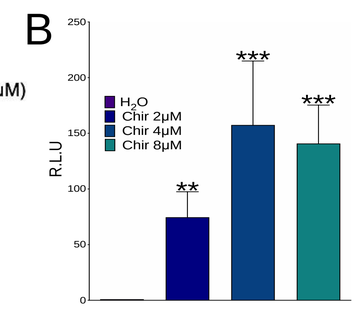


Figure 1. CHIR99021 effects on Wnt/β-catenin signaling activity. (A) Representative Western blot of β-catenin and GAPDH in H9 and HEK293 cells treated with 2, 4 and 6 μM of CHIR99021 for 4, 24 and 48 h. **(B)** Luciferase assays in HEK293 cells transfected with the SuperTOPFlash reporter for 24 h (STF). (n=7).

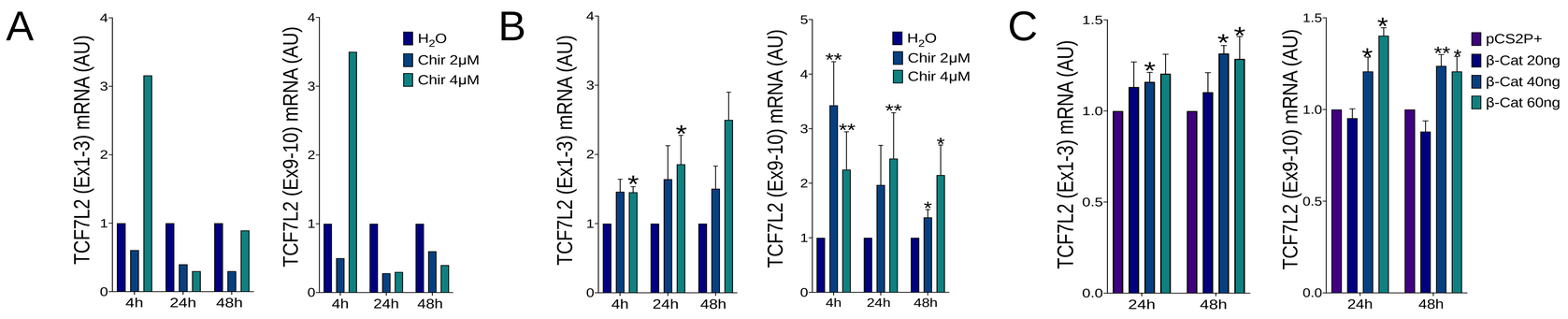


Figure 2. Activation of Wnt/β-catenin signaling induces transcripcion of the TCF7L2 gene. **A)** Quantitative determination (qPCR) of TCF7L2 in H9 cells stimulated with 2 μM and 4 μM of CHIR99021 for 4, 24 and 48 h. Sets of primers for TCF7L2 exons: Ex1-3 detects β-catenin binding site isoforms that participate in the transcriptional activation of Wnt/β-catenin target genes; and Ex9-10 recognizes all isoforms including dominant-negatives ones (n=1). **B)** Quantitative determination (qPCR) of TCF7L2 in HEK293 cells stimulated with 2 μM and 4 μM of CHIR99021 for 4, 24 and 48h (n=4). **C)** Quantitative determination (qPCR) of TCF7L2 in HEK293 cells transfected with a constitutively active β-catenin for 24h and 48h (n=4). qPCR results are shown as relative expression to the control without treatment at each time.

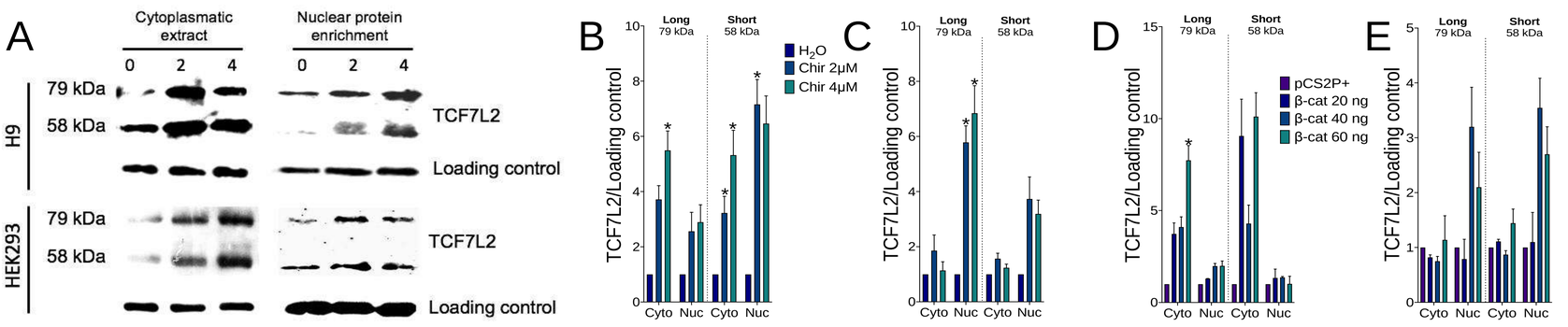


Figure 3. Activation of Wnt/β-catenin signaling pathway increases protein levels of TCF7L2 isoforms. **A)** Representative Western blots of TCF7L2 protein levels in H9 cells and HEK293 cells treated with 2, 4 and 6 μM of CHIR99021 for 24 h. GAPDH was used as control for cytoplasmatic extracts and Histone H3 levels for nuclear protein enrichment. **B and C)** Densitometric analysis of protein levels of TCF7L2 isoforms (long 79 kDa; short: 58 kDa) in HEK293 cells treated with 2, 4 and 6 μM of CHIR99021 for either 24 h (n = 3) **(B)** or 48 h (n = 3) **(C)**. The construct PCS2P+ was used as a transfection control. **D and E)** Densitometric analysis of TCF7L2 protein levels in HEK293 cells transfected with a constitutively active β-catenin for 24 h (n = 3) **(D)** or 48 h (n = 3) **(E)**.

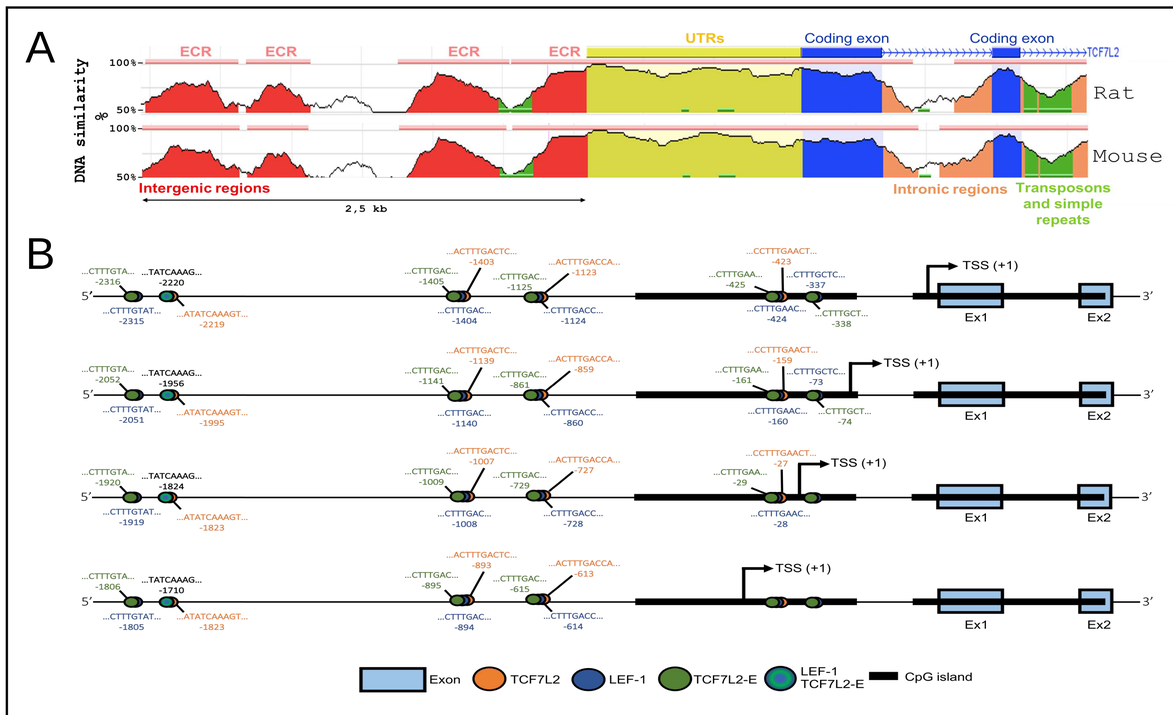


Figure 4. *In silico* characterization of the TCF7L2 promoter region. (A) Genome Browser alignment of Rat and Mouse TCF7L2 gene sequences reveals conserved regions relative to the human genome. Peaks represent regions with high conservation. Pink lines above the peaks denotes Evolutionary Conserved Regions (ECR) that have 80 % or more DNA similarity compared with the human sequence. The promoter region is shown in red, untranslated region (UTR) in yellow, coding region in blue, intronic sequences in peach, transposons and simple repeats are shown in green. **(B)** Schematic representation of putative TCF/LEF binding elements in a window of −2500 bp from TCF7L2 transcriptional start sites (TSS). Putative TCF/LEF binding sites within the promoter region of are shown in orange (TCF7L2), blue (LEF1) or green (TCF7L2-E).

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METHODS

Cells and treatment. Human Neuronal Stem Cells (H9-Derived) were maintained in StemPro NSC SFM complete medium consisting of KnockOut™ D-MEM/F-12 with StemPro Neural Supplement, EGF, bFGF, and GlutaMAX-I. HEK293 (human embryonic kidney) cells were maintained in DMEM supplemented with 10% fetal bovine serum, HEPES, penicillin and streptomycin. Cells were kept at 37 °C in 5% CO_2 incubator and saturated humidity. The cells were stimulated with 2 and 4 μM of CHIR99021, a Wnt/β-catenin signaling activator (Naujok *et al*., 2014), for 4, 24 and 48 h. Hek293 cells were also transfected with a constitutively active β-catenin^{S33Y} construct using Lipofectamine 3000 for 24-48 h (Kolligs *et al*., 1999).

Quantitative-PCR analysis. Total RNA was extracted using Trizol reagent and 2 μg of RNA was reverse transcribed with SuperScript II. q-PCR was performed in the StepOne Plus thermal cycler using Brilliant II SYBR Green qPCR Master Mix and 200 nM of primers targeting known TCF7L2 isoforms. Thermal cycling conditions included an initial activation step at 95 °C for 10 min and 40 cycles of denaturing at 95 °C, annealing at 60 °C, and amplification at 72 °C for 20 s. The expression levels of TCF7L2 were normalized to Rpl13a expression in H9 cells and to GAPDH in HEK293 cells using the delta-delta Ct method (2-ΔΔCt) as described (Alarcón *et al.,* 2013).

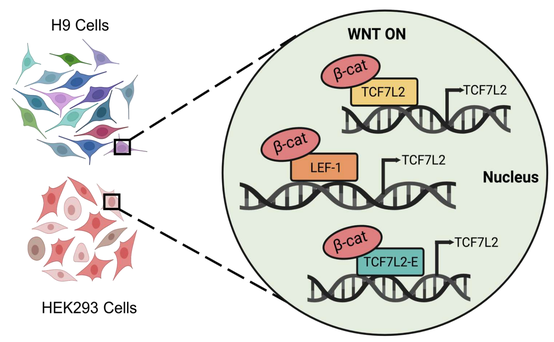
Western blot. Protein extracts were obtained from H9 and HEK293 cells using a lysis buffer (20 mM Tris pH=7,5, 100 mM NaCl, EDTA 0,1 mM pH=8 and 0,5% NP-40) and 20 μg were resolved by SDS-PAGE and transferred onto a Nitrocellulose Membrane. Western blots experiments were performed using anti-β-catenin antibody (E-5; Santa Cruz Biotechnology Cat#sc- 7963, 1:1000), anti- GAPDH antibody (6C5; Santa Cruz Biotechnology Cat#sc-32233, 1:1000), Anti-Histone H3 antibody (ab1791; Abcam, 1:3000) and anti-TCF7L2/TCF4 antibody (C48H11; Cell Signaling, 1:3000). Secondary antibodies with horseradish peroxidase-conjugated (Jackson) were used at 1:5000.

***In silico* characterization of the promoter region of TCF7L2.** Evolutionary Conserved Regions were identified in ECR browser (https://ecrbrowser.dcode.org/) with an 80% or more DNA similarity with the human sequence. TCF/LEF-binding elements (TBE: CTTTG) known to mediate β-catenin transcriptional activation of Wnt/β-catenin target genes (Cadigan & Waterman, 2012) were predicted using software PROMO v3.0.2 (which utilizes TRANSFAC 8.3) with a 15% Maximum matrix dissimilarity rate (Messeguer *et al*., 2002; Farre *et al*., 2018).

Statistical analysis. Statistical significance was determined through a one-way ANOVA with multiple comparisons test Dunnett (*p<0.05; ***p<0.001; ****p<0.0001). Data represent mean ± s.e.m.

CONCLUSIONS

In summary, here we show that sustained activation of Wnt/β-catenin signaling pathway in H9 and HEK293 cells induces a rapid increase in the transcripcion and expression of TCF7L2 isoforms and that the promoter region of TCF7L2 contains potential TCF/LEF-binding elements suggesting that they would be direct targets of the canonical pathway. Our results indicate that TCF7L2 is an interesting biomarker for several pathologies with high levels of Wnt/β-catenin signaling activity.



A model of the regulation of TCF7L2 in response to Wnt/β-catenin signaling activation

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