



OPEN ACCESS

EDITED BY

Xiaojun Ren,
University of Colorado Denver, United States

REVIEWED BY

Shilpi Minocha,
Indian Institute of Technology Delhi, India
Domenico Aprile,
University of Campania Luigi Vanvitelli, Italy

*CORRESPONDENCE

Mohamed M. Emara,
✉ memara@qu.edu.qa

[†]These authors have contributed equally to this work and share first authorship

RECEIVED 21 November 2023

ACCEPTED 11 January 2024

PUBLISHED 05 February 2024

CITATION

Swaidan NT, Soliman NH, Aboughalia AT, Darwish T, Almeshal RO, Al-Khulaifi AA, Taha RZ, Alanany R, Hussein AY, Salloum-Asfar S, Abdulla SA, Abdallah AM and Emara MM (2024), *CCN3*, *POSTN*, and *PTHLH* as potential key regulators of genomic integrity and cellular survival in iPSCs. *Front. Mol. Biosci.* 11:1342011. doi: 10.3389/fmolb.2024.1342011

COPYRIGHT

© 2024 Swaidan, Soliman, Aboughalia, Darwish, Almeshal, Al-Khulaifi, Taha, Alanany, Hussein, Salloum-Asfar, Abdulla, Abdallah and Emara. This is an open-access article distributed under the terms of the [Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

CCN3, *POSTN*, and *PTHLH* as potential key regulators of genomic integrity and cellular survival in iPSCs

Nuha T. Swaidan^{1†}, Nada H. Soliman^{1†}, Ahmed T. Aboughalia¹, Toqa Darwish¹, Ruba O. Almeshal¹, Azhar A. Al-Khulaifi¹, Rowaida Z. Taha², Rania Alanany³, Ahmed Y. Hussein⁴, Salam Salloum-Asfar², Sara A. Abdulla², Abdallah M. Abdallah¹ and Mohamed M. Emara^{1*}

¹Basic Medical Sciences Department, College of Medicine, QU Health, Qatar University, Doha, Qatar, ²Neurological Disorders Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Doha, Qatar, ³College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar, ⁴School of Medicine, New Giza University, Giza, Egypt

Reprogramming human somatic cells into a pluripotent state, achieved through the activation of well-defined transcriptional factors known as OSKM factors, offers significant potential for regenerative medicine. While OSKM factors are a robust reprogramming method, efficiency remains a challenge, with only a fraction of cells undergoing successful reprogramming. To address this, we explored genes related to genomic integrity and cellular survival, focusing on iPSCs (A53T-PD1) that displayed enhanced colony stability. Our investigation had revealed three candidate genes *CCN3*, *POSTN*, and *PTHLH* that exhibited differential expression levels and potential roles in iPSC stability. Subsequent analyses identified various protein interactions for these candidate genes. *POSTN*, significantly upregulated in A53T-PD1 iPSC line, showed interactions with extracellular matrix components and potential involvement in Wnt signaling. *CCN3*, also highly upregulated, demonstrated interactions with TP53, CDKN1A, and factors related to apoptosis and proliferation. *PTHLH*, while upregulated, exhibited interactions with CDK2 and genes involved in cell cycle regulation. RT-qPCR validation confirmed elevated *CCN3* and *PTHLH* expression in A53T-PD1 iPSCs, aligning with RNA-seq findings. These genes' roles in preserving pluripotency and cellular stability require further exploration. In conclusion, we identified *CCN3*, *POSTN*, and *PTHLH* as potential contributors to genomic integrity and pluripotency maintenance in iPSCs. Their roles in DNA repair, apoptosis evasion, and signaling pathways could offer valuable insights for enhancing reprogramming efficiency and sustaining pluripotency. Further investigations are essential to unravel the mechanisms underlying their actions.

KEYWORDS

iPSCs, ESCs, transcription factors, genomic integrity, cellular survival

1 Introduction

The reprogramming of human somatic cells into a pluripotent state has been accomplished by the ectopic expression of well-defined transcriptional factors such as *OCT4* (also known as *POU5F1*), *SOX2*, *KLF4*, *MYC* (also known as c-MYC); these factors are collectively referred to as the OSKM factors (Yamanaka, 2012; Swaidan et al., 2020). Human induced pluripotent stem cells (hiPSCs), which possess embryonic stem cell (ESC)-like characteristics, have increased the possibility of generating patient-specific cells (Dimos et al., 2008; Soldner et al., 2009). These hiPSCs are distinguished by their ability to self-renew and differentiate into any cell type of the body. Consequently, these cells can serve purposes such as comprehending disease progression, developing and testing novel drugs, and conducting autologous replacement therapies for degenerated cells (Dimos et al., 2008). This is particularly relevant to degenerative diseases of the central nervous system (CNS) such as Parkinson's disease (PD), in which dopaminergic neurons are lost (Soldner et al., 2009).

PD is the second most common chronic progressive neurodegenerative disease after Alzheimer's disease. It is characterized by the loss of the nigrostriatal dopamine-releasing neurons. Despite the fact that genetic factors are implicated in the pathogenesis of PD, the majority of PD cases are sporadic, not associated with a known genetic mutation (Soldner et al., 2009). The pathological hallmark of PD is the aggregation and deposition of abnormal α -synuclein protein within the neuronal cell bodies (Stefanis, 2012). Numerous mutations have been reported in the gene responsible for encoding this protein. The α -synuclein point mutation (A53T) is believed to be the most frequent and associated with the familial type of PD (Swaidan et al., 2020).

While OSKM factors have demonstrated a robust method for reprogramming somatic cells that has potential for regenerative medicine, the process is still inefficient since only a small fraction of cells undergoes a complete and successful reprogramming (Swaidan et al., 2020). To overcome low reprogramming efficiency, several studies have examined factors that could enhance the activation of the pluripotency pathway and prevent the differentiation of stem cells (M. Wang et al., 2017; Bogliotti et al., 2018; Xu et al., 2016; Theka et al., 2017). In our earlier research, we pinpointed five genes (*GBX2*, *NANONGP8*, *SP8*, *PEG3*, and *ZIC1*) exhibiting differential expression in iPSCs generated from fibroblast cells. These genes maintained their differential expression regardless of the presence or absence of the PD A53T mutation, while also showcasing interactions with OSKM factors (Swaidan et al., 2020). Building upon our prior research, this paper focuses on a distinct sample (named A53T-PD1) that displayed enhanced colony integrity and stability in comparison to other samples (A53T-PD2, ID-PD and HC). This observation holds true despite the fact that a smaller number of colonies were formed during the reprogramming process of this sample.

Hence, with the aim of enhancing the regeneration process's efficiency, our focus lies in investigating genes related to genomic integrity/stability and cellular survival. These genes exhibit differential expression in A53T-PD1 cells and could potentially be genetically modified or suppressed to bolster and sustain iPSC pluripotency.

2 Materials and methods

2.1 Cell lines and cell culture

In this study, four established human iPSC cell lines (HC, ID-PD, A53T-PD1, and A53T-PD2) were employed ($n = 3$ per iPSC cell line, total = 12 samples). These stem cells were grown using a feeder-free system with Matrigel (Corning) for cell adherence and maintained in StemFlex media (Termo Fisher Scientific) under controlled conditions at 37°C and 5% CO₂ incubators. Cells were fed every other day, and passage was performed when cell confluency reached approximately 80%, typically within four to 5 days after the previous passage. The passage process involved pre-coating of 35 mm dishes with Matrigel for 30 min, 2 h, or overnight, washing colonies with 1 mL Dulbecco's Phosphate-Buffered Saline (DPBS), gently dissociating the cells into appropriately sized colonies (~100 μ m) using 500 μ L of non-enzymatic reagent (ReLeSR; StemCell Technologies), collecting these colonies with StemFlex media, centrifuging them for 4 min at 800 RPM and 22°C, resuspending the resulting pellet in 1 mL fresh StemFlex media, and finally evenly distributing 70 μ L of cells onto the freshly prepared dishes containing 2 mL fresh StemFlex media.

2.2 RNA and RT-qPCR

Total RNA was extracted with a Direct-zol RNA MicroPrep Extraction kit (Zymo Research) according to manufacturer's instructions. Then, cDNA was synthesized using Superscript IV, First-Strand Synthesis System kit (Invitrogen). The RT-qPCR technique was performed using TaqMan Fast Advanced Master Mix (Applied Biosystems) and TaqMan probes (20 \times) according to the manufacturer's protocol. The list of genes: Assay name: POSTN, Assay ID: Hs0166750, PTHLH, Assay ID: Hs00174969, NOV (CCN3), Assay ID: Hs00159631 and housekeeping gene GAPDH, Assay ID: Hs02786624. The total reaction volume was 10 μ L per well. RT-qPCR reaction was run in triplicates using Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument under default conditions (95°C for 10 min and then PCR reaction: 40 cycles of 95°C, 15 s and 60°C, 1 min).

2.3 RNA sequencing (RNA-Seq)

The transcriptomic sequencing process encompassed two key steps: the first step involved the preparation of mRNA libraries, followed by RNA sequencing. Initially, the quality of the extracted total RNA samples was assessed using a 2100 Bioanalyzer equipped with the Agilent RNA Nano 6000 chip. Subsequently, we carried out mRNA library preparation using the TruSeq Stranded mRNA Sample Prep LS kit (Illumina). To ensure the quality and quantity of the generated mRNA libraries, a thorough quality control analysis was conducted using the Agilent DNA 1000 chip on a 2100 Bioanalyzer, along with the quantification of DNA library templates using a Qubit assay. For cluster generation, indexed DNA libraries were normalized to 10 nM and placed in a diluted cluster template (DCT) plate, after which they were combined in equal volumes in a pooled DCT plate (PDP). The final step involved RNA

sequencing, which was performed utilizing the HiSeq 4000 system (Illumina) at the QBRI genomic core facility.

2.4 RNA-seq analysis

We employed the Bcl2fastq Conversion Software to perform two crucial tasks: firstly, to demultiplex the data, and secondly, to convert the BCL files generated by Illumina sequencing systems into standard FASTQ file formats, which are essential for subsequent RNA-Seq analysis. Quality control checks were conducted using FastQC, a specialized tool designed for high-throughput sequence data analysis, accessible at (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>).

The subsequent analysis followed the standard QIAGEN bioinformatics CLC Genomics Workbench 20.0 pipeline (<https://digitalinsights.qiagen.com>). This sequential pipeline comprised four key stages: 1) pre-processing of raw sequencing reads, 2) mapping of reads to a reference genome, 3) quantification of genes and transcripts, and 4) differential expression analysis. Data normalization was performed relative to the healthy control iPS cell line, which served as the baseline for comparison.

2.5 Gene ontology molecular analysis

We utilized the Protein Analysis Through Evolutionary Relationships (PANTHER) 14.1 platform, accessible at (<http://www.pantherdb.org>), as an online classification system. PANTHER is specifically designed for the identification and categorization of proteins and their corresponding genes based on their family/subfamily, molecular function, biological processes, and pathways in which they play integral roles within the cellular context. This software served as a valuable tool for filtering and pinpointing genes relevant to our areas of focus, which encompassed cellular integrity and genomic stability.

2.6 Protein-protein interaction analysis

We employed the Search Tools for the Retrieval of Interacting Genes (STRING) 10.5, an online software dedicated to the exploration of gene and protein interactions, accessible at (<https://string-db.org/>). Our primary objective with STRING was to establish predicted interactions between our selected genes of interest and protein-encoding genes that are involved in cell cycle checkpoints, DNA repair and replication, *TP53* phosphorylation, and apoptosis inhibition. Through the STRING platform, we did not only visualize the nature of interactions between these proteins but also examined the underlying evidence supporting these interactions.

2.7 Databases and literature review

A comprehensive literature review was conducted using medical search engines such as PubMed, Medline, UpToDate, Scopus,

Access Medicine, Genetic Home Reference, and Access Genetics. Specific keywords were employed such as “the name of the chosen candidate gene,” “pluripotency/pluripotent,” “reprogramming,” “genomic stability/integrity in Embryonic stem cells/ESCs,” and “induced pluripotent stem cells/iPSCs”. Relevant articles were screened and their findings documented for confirmation of transcriptional factors’ involvement in genomic integrity and cellular survival.

2.8 Statistical analysis

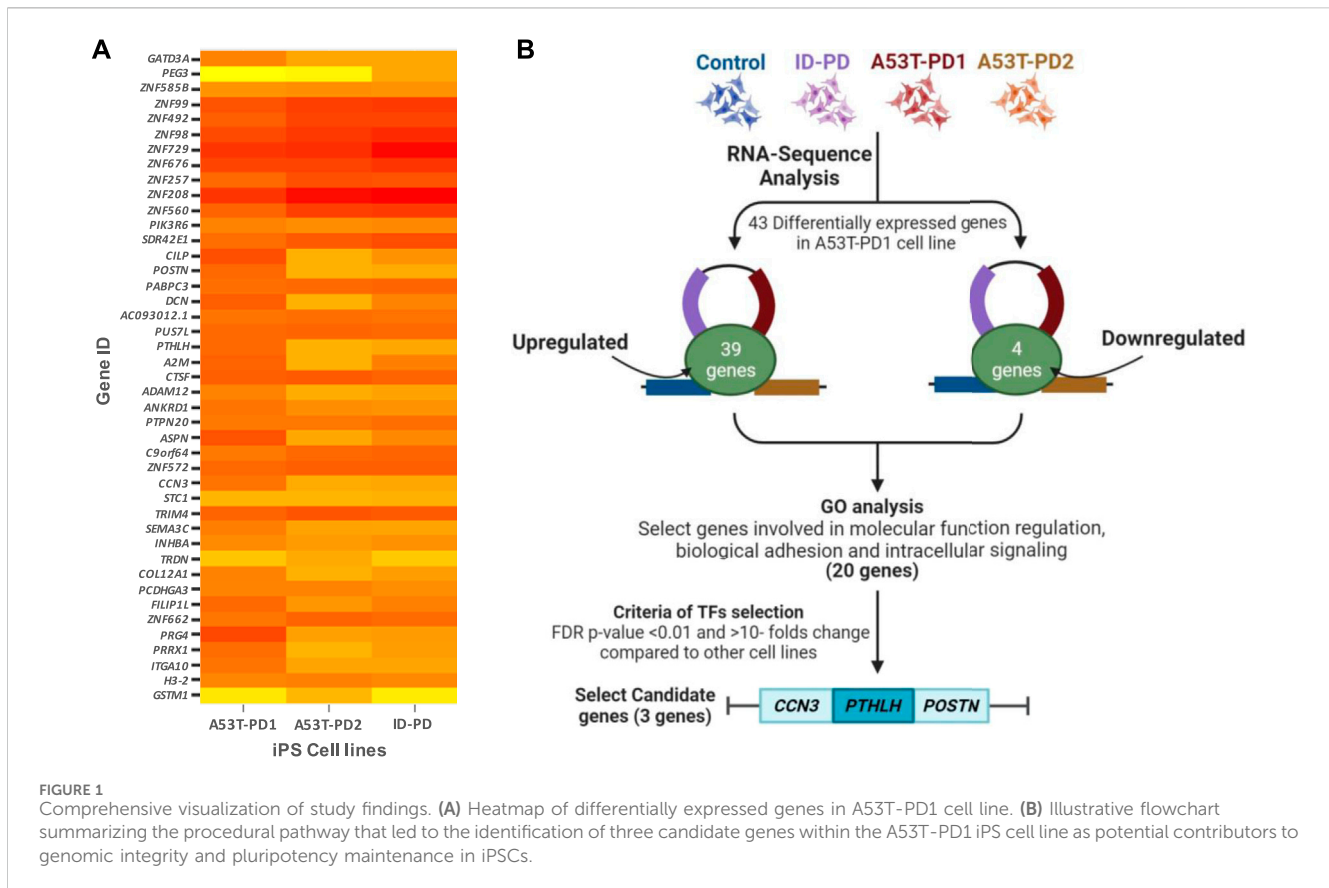
Statistical analyses were conducted using GraphPad Prism 8.4.3 (GraphPad Software, Inc., San Diego, CA). Data were presented as the mean \pm standard deviation (SD) for each group ($N = 3$). Statistical significance was assessed through the Mann–Whitney U test and defined as p -values less than 0.05 ($p < 0.05$) or 0.01 ($p < 0.01$).

3 Results

3.1 A set of genes involved in molecular function regulation, including DNA binding, biological adhesion and intracellular signalling

Due to the superior colony stability observed in A53T-PD1 iPSCs, we turned our focus to the identification of differentially expressed candidate genes across distinct iPS cell lines. Utilizing RNA sequencing data, we conducted a comparative analysis of differentially expressed genes between A53T-PD1 and other iPS cell lines: HC, ID-PD, and A53T-PD2. Our selection criteria involved genes exhibiting a false discovery rate (FDR) of less than 0.05 specifically in A53T-PD1 cell line. This screening resulted in the identification of a total of 43 genes, comprising 39 genes with upregulated expression and 4 genes displaying downregulated expression (Figure 1).

Subsequent to this discovery phase, we proceeded to subject the 43 genes to Gene Ontology analysis (GO), in order to systematically classify them based on their roles and functions within the cellular context (Figure 2). Our selection process was grounded in the prioritization of cellular processes that bear utmost relevance to both cellular integrity and genomic stability. These encompassed processes such as molecular function regulation, DNA binding, biological and cell adhesion, as well as intracellular signaling. Notably, while the statistical significance threshold was not met for DNA binding and cell adhesion molecules (with FDR p -values of 0.42 and 0.25, respectively), the attributes of molecular function regulation, biological adhesion, and intracellular signaling exhibited statistical significance (FDR p -values of 0.003, 0.04, and 0.04, respectively). Guided by these findings, our attention was directed towards refining the gene selection criteria to those involved in molecular functions, biological processes, and protein classes, with a focus on genes that are implicated in molecular function regulation, biological adhesion, and intracellular signaling (as outlined in Figure 2).



3.2 Selection of candidate genes in A53T-PD1 cell line

We successfully identified a total of 20 genes, effectively categorized into two distinct groups. The initial group comprised 11 zinc finger transcription regulator genes, integral to the orchestration of molecular function regulation. The remaining 9 genes, namely, *CCN3*, *INHBA*, *PTHLH*, *PIK3R6*, *PEG3*, *H3.2*, *POSTN*, *PCDHGA3*, and *STC1*, demonstrated their involvement across various functional domains. Further delving into the expression patterns of these 20 genes within each iPS cell line occurred through an in-depth analysis, utilizing the differential expression data generated in relation to control iPSCs.

For the zinc finger transcription regulator genes, a consistent and statistically significant upregulation was observed across all cell lines (FDR <0.01), compared to control iPSCs (illustrated in Figure 3A). As a result of this uniformity, these genes were subsequently excluded from further gene selection. Conversely, among the remaining genes (Figure 3B), the highest upregulation in A53T-PD1 cell line was notably exhibited by *POSTN*, soaring 70-fold (FDR <0.001), followed closely by *PTHLH* with a 59-fold increase (FDR <0.001). In contrast, both *POSTN* and *PTHLH* showed downregulation in ID-PD and A53T-PD2 cell lines. Additionally, *CCN3*, *INHBA*, and *PIK3R6* displayed substantial upregulation in A53T-PD1 cell line, significantly exceeding other cell lines (FDR <0.001 for all).

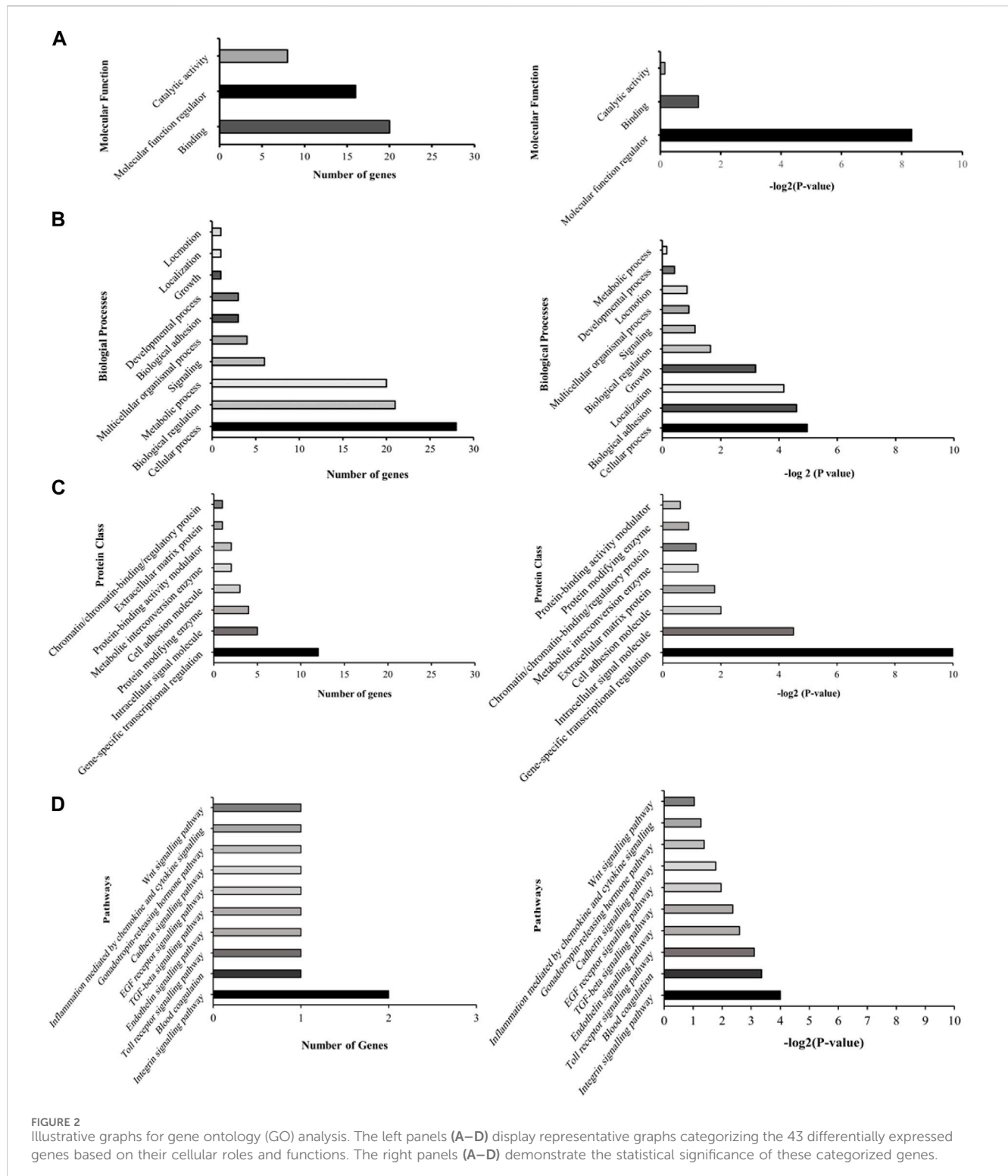
H3.2. displayed an upregulation of 12-fold in A53-PD1 cell line, in contrast to 16-fold in A53T-PD2 and 9-fold in ID-PD cell lines, all

with an FDR <0.001. *PCDHGA3*, on the other hand, exhibited consistent upregulation across all cell lines, demonstrating a similar 15-fold change in both A53T-PD1 and A53T-PD2 cell lines (FDR <0.001). Unlike all the previously mentioned genes, *STC1* and *PEG3* displayed downregulation. While *STC1* demonstrated downregulation across all cell lines without significant variation among them, *PEG3* showcased its most pronounced downregulation in A53T-PD1 cell line at a staggering -990-fold change (FDR <0.001), succeeded by A53T-PD2 cell line at -443-fold change (FDR <0.001) (Figure 3B).

In the quest for a final selection of candidate genes potentially contributing to the generation of the most stable iPSC colonies in A53T-PD1 cell line, we employed two stringent criteria: (a) FDR p -value <0.01 and (b) a significant upregulation or downregulation in A53T-PD1 cell line, displaying a variation of more than 10-fold compared to other cell lines. Consequently, *INHBA*, *PIK3R6*, *H3.2*, *STC1*, and *PCDHGA3* were disregarded. It is important to highlight that *PEG3* was excluded from consideration, as it had already been analyzed in our previous work (Swaidan et al., 2020). Therefore, this rigorous process left us with a final selection of three genes: *CCN3*, *POSTN*, and *PTHLH*.

3.3 Protein-protein interaction analysis

Following gene selection, we utilized STRING software to predict potential protein interactions associated with our chosen



candidate genes. Individually, each gene underwent scrutiny to anticipate its feasible associations with other proteins. Additionally, each gene underwent evaluation in conjunction with a cluster of genes linked to cell cycle regulation and DNA repair/replication.

An illustrative instance of this predictive analysis is evident in *POSTN*, which exhibited a remarkable 70-fold upregulation in

A53T-PD1 cell line. This gene was predicted to stimulate *COL1A1* expression, contributing to the synthesis of a substantial portion of type 1 collagen (as indicated in Figure 4A through the green arrow). Furthermore, *POSTN* demonstrated unknown interactions with other collagen-related genes, including *COL1A2*, *COL5A2*, and *COL3A1*, as well as the *DCN* gene, responsible for encoding the decorin protein. Decorin, in turn, contributes to

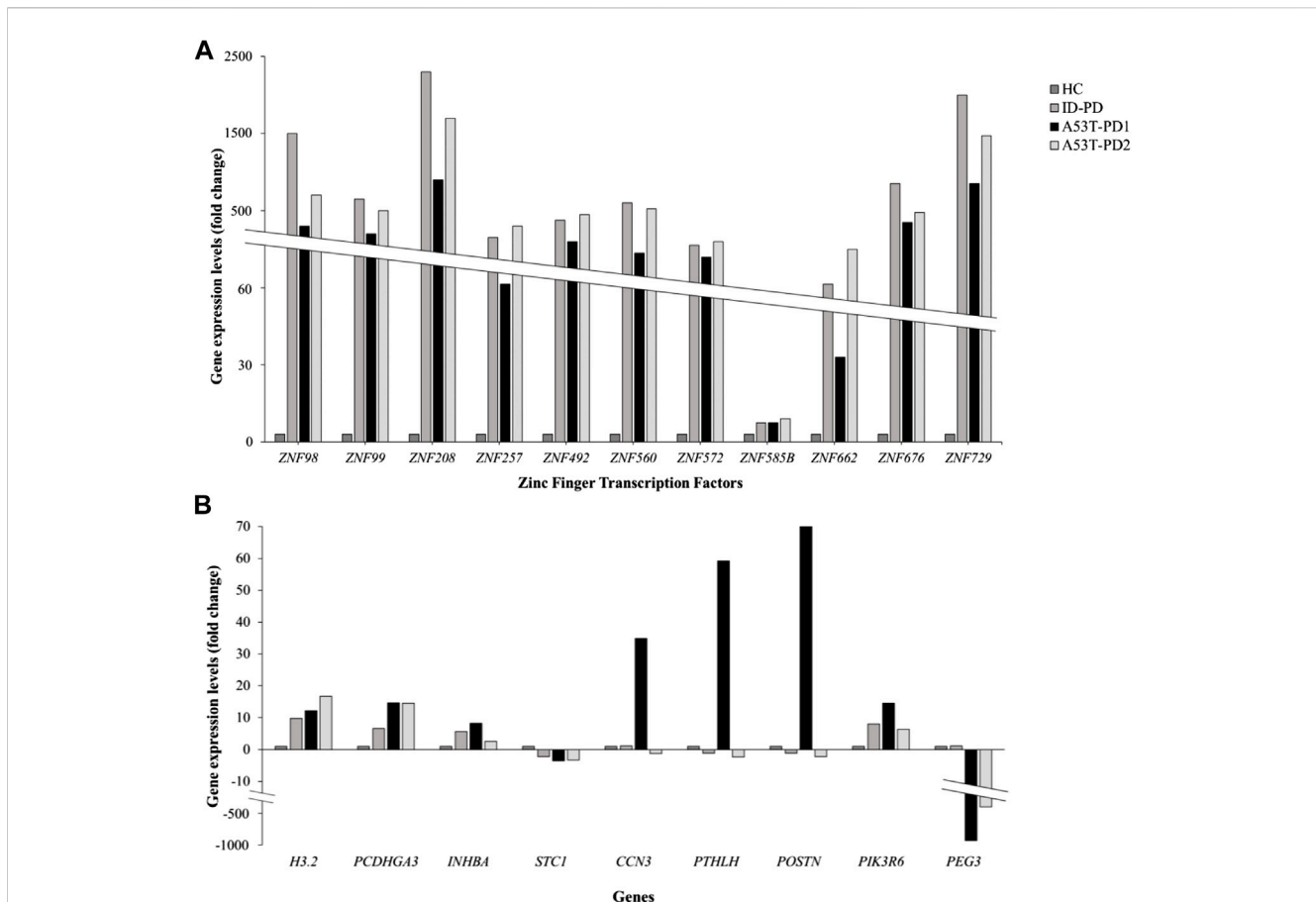


FIGURE 3 Expression profiling of 20 genes involved in genomic integrity and cellular survival. Gene expression levels for a panel of 20 genes associated with genomic integrity/stability and cellular survival were analyzed using RNA-Seq data and represented in two separate graphs. (A) Illustrates the expression levels of 11 zinc finger transcription factors expressed in different iPS cell lines. (B) Illustrates the expression levels of the remaining 9 genes expressed in iPS cell lines.

extracellular matrix strength and cell stability. Notably, *POSTN* also exhibited a binding interaction with *TNC*, which encodes Tenascin C, a crucial extracellular matrix protein involved in guiding migrating neurons and axons during development, synaptic plasticity, and neuronal regeneration (as indicated in Figure 4A by the blue line).

In our subsequent exploration, we aimed to determine whether *POSTN* had any potential interactions with gene sets pivotal to fundamental cellular processes such as cellular stability and genomic integrity in ESCs. These encompassed genes that are responsible for DNA repair and replication, phosphorylation of p53, and other molecules like Rb and ATM. For this analysis, we included genes related to cell cycle checkpoints (*CDK1/2*), the MRN complex intrinsic to the homologous recombination pathway of DNA repair (*MRE11*, *RAD50*, and *NBS1*), alongside *BRCA1/2*, *RAD51*, and *SALL4*. However, it's important to highlight that *POSTN* did not display any predicted interactions with the aforementioned genes.

Similarly, *PTHLH* which experienced a significant upregulation in A53T-PD1 cell line (an impressive 59-fold increase) was predicted to exert an inhibitory influence on the *IHH* gene (as indicated in Figure 4B by the red line). This

particular gene plays a pivotal role in governing the differentiation of progenitor cells into osteoblasts, thus exerting a significant influence over cellular differentiation dynamics. Additionally, *PTHLH* exhibited interactions with parathyroid hormone-related genes including *PTH1R*, *PTH2*, *PTH2R*, as well as calcitonin-related genes such as *CALCR* and *CALCA*. Upon closer examination of *PTHLH*'s interactions with the aforementioned gene set that is vital to substantial cellular processes, an intriguing and as yet unidentified interaction emerged with cyclin-dependent kinase 2 (*CDK2*) (Figure 4C). *CDK2*, a pivotal player in cell cycle control, undertakes the phosphorylation of *TP53*, thereby impeding apoptosis. Furthermore, *CDK2*'s involvement extends to the activation of *E2F1* gene expression, an essential precursor for the initiation of DNA synthesis. The orchestrated actions of *CDK2* and *E2F1* contribute to the hyperphosphorylation of the Rb protein-encoding gene, ultimately facilitating ESCs' efficient transition into the S phase of the cell cycle and thereby shortening the duration of the G1 phase.

Interestingly, further exploration of *CCN3* (which exhibited a significant upregulation, 34.9-fold increase in A53T-PD1 cell line) revealed that *CCN3*'s transcriptional regulation, as an extracellular

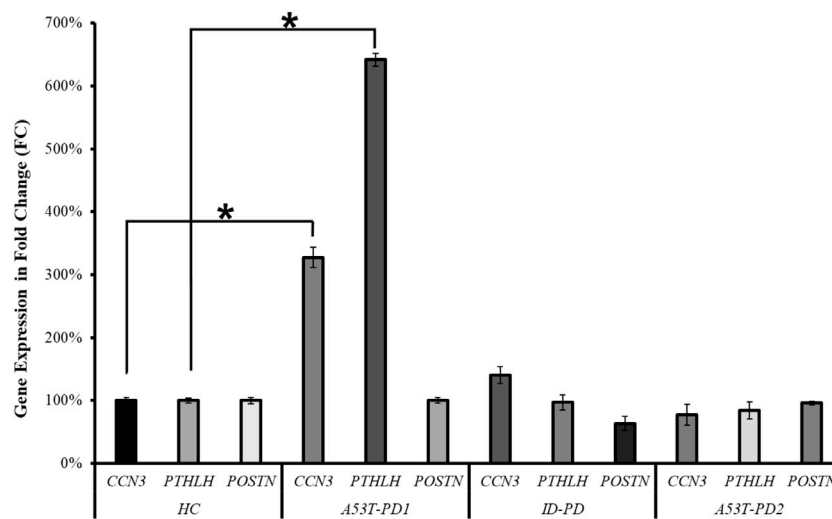


FIGURE 5

RT-qPCR validation of three candidate genes in various iPS cell lines. RT-qPCR analysis was conducted to validate the expression of the three candidate genes (*CCN3*, *PTHLH*, *POSTN*) across different iPS cell lines. The statistical significance was determined using the Mann–Whitney *U* test, with $*p < 0.0001$ indicating significance.

It is imperative to note that differences, whether insignificant or significant, may exist between RNA-seq and RT-qPCR analyses of gene expression levels. Such variations can be attributed to several factors, including methodological disparities. RNA-seq provides a comprehensive, unbiased assessment of the entire transcriptome, detecting both known and novel transcripts, whereas RT-qPCR focuses on specific transcripts, offering a more targeted analysis. Additionally, differences in sensitivity and dynamic ranges are evident, with RNA-seq possessing a broader dynamic range capable of detecting low-abundance transcripts and capturing alternative splicing events, while qPCR, though highly sensitive, may have a more limited dynamic range compared to RNA-seq. Furthermore, normalization methods diverge, with RNA-seq utilizing techniques like TPM (transcripts per million) or FPKM (fragments per kilobase of transcript per million mapped reads) to account for library size and transcript length, while qPCR relies on reference genes for normalization, and the choice of reference genes can influence results. Lastly, considerations of gene isoforms and alternative splicing reveal that RNA-seq detects multiple isoforms and alternative splicing events, whereas primers designed for qPCR may target specific isoforms, potentially missing alternative splicing events (Marioni et al., 2008; Mortazavi et al., 2008; Bustin et al., 2009; Wang et al., 2009; Derveaux et al., 2010).

3.5 CCN3 involvement in cell apoptosis and FGFR2 signaling pathways

To further explore *CCN3*'s role in the regulation of cellular apoptosis and proliferation, we conducted a comprehensive investigation using the Reactome Pathway Database. The data obtained from this database has revealed its participation in cellular apoptosis through an interaction with the POU4F2 domain, known to bind to TP53, thereby modulating the transcription of pro-apoptotic

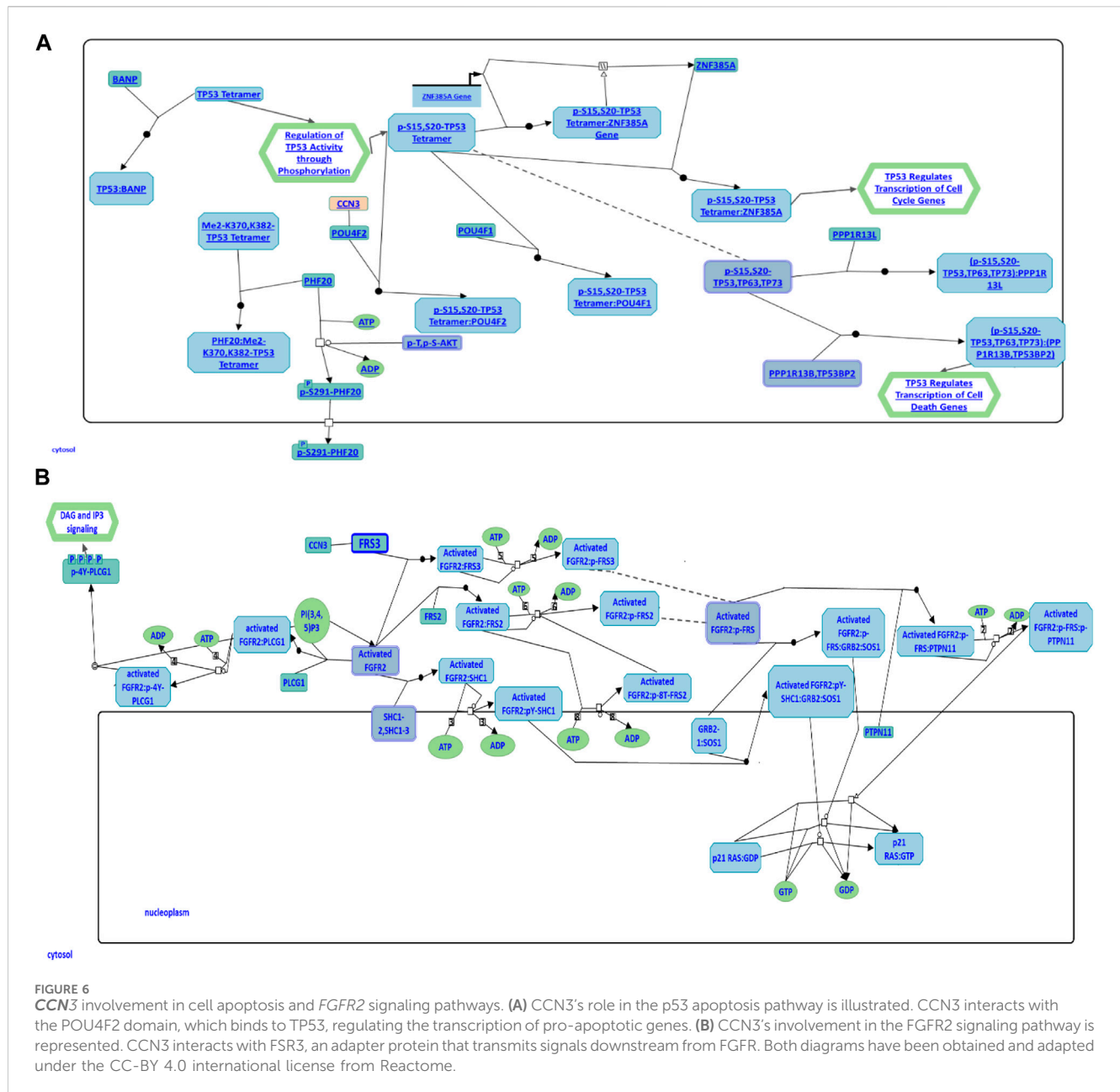
genes (Figure 6A). Inhibition of this pathway has been observed to augment iPS reprogramming efficiency by fostering cell proliferation. Furthermore, *CCN3* has also been found to be a participant in the fibroblast growth factor receptor 2 (FGFR2) signaling pathway, where it interacts with fibroblast growth factor receptor substrate 3 (FSR3), an adapter protein responsible for transmitting signals downstream from FGFR (Figure 6B). Activation of this pathway has been demonstrated to play a crucial role in both the initiation and stabilization phases of reprogramming by promoting cellular proliferation.

3.6 PTHLH implication in G protein activation pathway

Similarly, we conducted a comprehensive investigation utilizing the Reactome Pathway Database analysis to elucidate the cellular processes governed by *PTHLH*, leading to the discovery of its central role in G protein activation (Figure 7). *PTHLH*'s interaction with a G-protein-coupled receptor (GPCR) forms a liganded-GPCR complex, initiating the exchange of GDP for GTP within the G-protein alpha subunit (GNAS), ultimately resulting in G-protein activation. Once activated, the GNAS directly interacts with the proto-oncogene tyrosine kinase Src (SRC), leading to SRC autophosphorylation and subsequent activation. Functionally, the SRC kinase family plays a pivotal role in cellular growth and cancer. Additionally, the G-protein family, particularly the GNAS, is involved in the regulation of cellular apoptosis and the maintenance of pluripotency in ESCs.

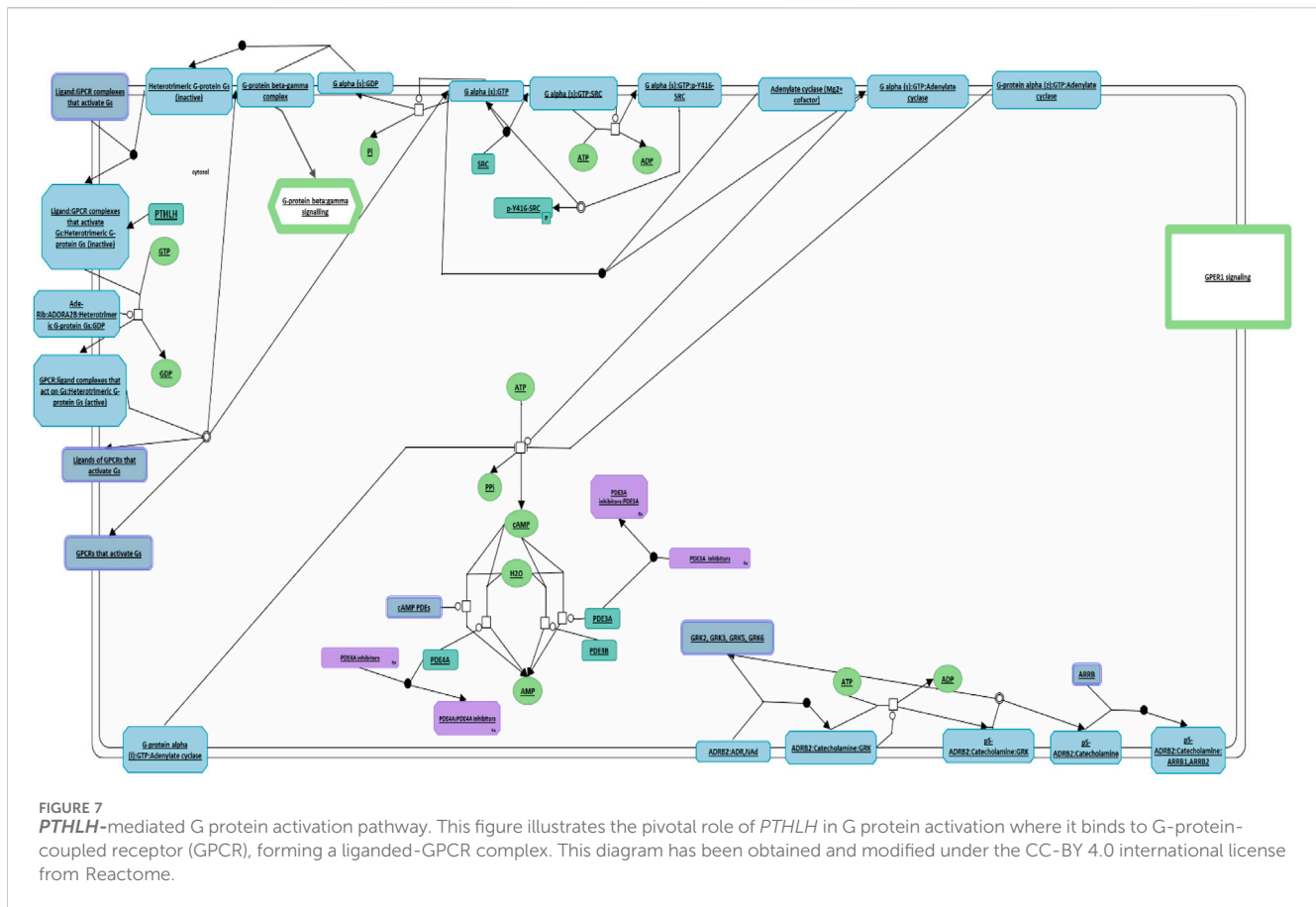
4 Discussion

ESCs and iPSCs offer immense potential, not only in the field of regenerative medicine but also as valuable tools for investigating



developmental processes, pathologies, and the assessment of novel drug interventions. However, the maintenance of genomic integrity and cellular stability presents a formidable challenge to sustaining the self-renewal and pluripotency of PSCs. Genomic instability can impair self-renewal pathways, activate the p53 apoptotic pathway, and promote PSC differentiation (Su et al., 2019). It is therefore imperative to safeguard the genomic integrity of PSCs and explore the genes that may be involved in this process, all while promoting robust growth to preserve their vital functions and self-renewal capability. Several factors, including culture conditions, can contribute to genomic instability in ESCs, and exposure to endogenous or exogenous genotoxic stresses can lead to cell apoptosis due to DNA damage (Martins-Taylor and Xu, 2012). It has been noted that ESCs have shown distinct mechanisms to shield themselves from DNA double-strand breaks, setting them apart from somatic cells (Nagaraja et al., 2013).

In our previous study (Swaidan et al., 2020), iPS cell lines were successfully generated from a PD patient carrying the A53T mutation (A53T-PD1). Although the generated colonies number was limited, these colonies exhibited remarkable genomic resilience and prolonged viability compared to other iPS cell lines that were generated in parallel. This intriguing finding prompted us to consider additional factors influencing genomic integrity beyond age and disease mutation. Our hypothesis regarding the potential roles of certain molecules specific to A53T-PD1 cell lines, at both nuclear and cellular levels, in maintaining genomic and cellular stability were substantiated by transcriptomic analysis. This analysis revealed three differentially expressed genes in the A53T-PD1 cell line (*CCN3*, *POSTN*, and *PTHLH*), all of which are implicated in cellular stability, DNA repair mechanisms, and apoptosis inhibition.



Among these genes, *CCN3*, also known as *NOV*, exhibited significant upregulation in the A5T-PD1 iPS cell line compared to other cell lines (34.9-fold, FDR <0.001). *CCN3* belongs to the CCN family of matricellular proteins, known for its pivotal role in extracellular signaling and the maintenance of cellular viability (Kubota and Takigawa, 2013). Previous studies have highlighted the importance of *CCN3*, particularly its role in cellular adhesion and the regulation of integrin expression (Lafont et al., 2005; Lin et al., 2005; Vallacchi et al., 2008). These findings support our hypothesis that *CCN3* plays a crucial role in pluripotency circuits and their maintenance, particularly in cell adhesion receptor systems and integrin expression (Vitulo and Kimber, 2017; Yu et al., 2018). Moreover, the involvement of *CCN3* in regulating integrin expression not only underscores its significance in initiating and sustaining pluripotency but also implies its role in maintaining the compact morphology of pluripotent colonies, dependent on integrin activity (Yu et al., 2018). In addition, increased cellular proliferation, a hallmark of cells undergoing reprogramming, can be achieved by inhibiting *p53*, which is responsible for programmed cell death, and promoting *LIN28* expression, which regulates cell cycle genes like *CDK* genes. This mechanism contributes to the efficiency of reprogramming processes and the stability of hiPSCs (Hong et al., 2009; Kawamura et al., 2009; Hawkins et al., 2014).

Through STRING analysis, we observed that *CCN3* is involved in promoting cell survival. Notably, it directly interacts with key players in the *p53* pathway, including *TP53* and *CDKN1A*, which regulate cell apoptosis and survival. Reactome analysis further elucidated these interactions, revealing *CCN3*'s direct interaction

with POU domain transcription factors that modulate *p53* and regulate the transcription of pro-apoptotic genes (Figure 6A). When the *P53* pathway is activated in stem cells, it initiates a cascade of events leading to cell cycle arrest, commitment to a differentiation pathway, and the formation of progenitor cells. *P53* serves as a guardian of genomic stability, and its activation is pivotal for maintaining cellular integrity (Levine et al., 2016). Furthermore, *CCN3*'s interaction with *FGF2*, a key factor in the *FGF2* pathway governing reprogramming initiation and stabilization, provides additional evidence of its role in improving reprogramming efficiency. *FGF2* accelerates cell proliferation during the initial phases of reprogramming, and it has been noted that hiPSCs generated in the presence of Activin/Nodal and *FGF2* ligands tend to stabilize in the primed state (Jiao et al., 2013; Hawkins et al., 2014). In addition, *FGF2* is known to influence the self-renewal and clonogenic capacity of pluripotent stem cells (Aprile et al., 2023). These findings highlight *CCN3*'s significance in orchestrating key signaling pathways that govern cellular fate and function. Its active participation in both the *P53* and *FGF2* pathways positions *CCN3* as a central player in the intricate network of molecular events that determine cell behavior, stability, and responsiveness to environmental cues. Our results were further substantiated by pluripotency pathways extracted from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, which highlighted the significance of the *FGF2* pathway in governing reprogramming process in primed stem cells (Figure 8). Interestingly, the *CCN3* gene has been documented to collaborate with *FGF2* as a substrate, leading to enhanced cell proliferation and improved cell survival

osteogenic differentiation in MEFs with higher *CCN3* expression, primarily attributed to the suppression of BMP signaling and the reciprocal inhibition with *DLL1* (Su et al., 2018). Mechanistic analyses further uncovered *CCN3*'s suppressive effects on BMP/Smad and BMP/MAPK signaling pathways, along with its mutual inhibition with *DLL1*, a prominent membrane protein ligand associated with the Notch signaling pathway. Significantly, *Hey1*, a target gene shared by both BMP and Notch signaling pathways, emerged as a pivotal factor, partially counteracting the inhibitory impact of *CCN3* on osteoblastic differentiation (Su et al., 2018). These regulatory roles of *CCN3* in both bone formation and hematopoiesis underscores its vitality in maintaining cellular stability within distinct cellular contexts.

Another gene exclusively upregulated in the A53T-PD1 iPSC cell line compared to other cell lines was *PTHLH* (59-fold, FDR <0.001). STRING analysis unveiled an unknown interaction between *PTHLH* and *CDK2*, which plays a role in the cell cycle and *TP53* inhibition. Previous research has highlighted *PTHLH* as a crucial molecular regulator of cellular development and survival (Guo et al., 2012). Additionally, in *Pthlh*-depleted embryos at the morula stage, the expression of pluripotency-related genes *NANOG* and *POU5F1* was markedly diminished (Min et al., 2022). While there is no direct evidence suggesting *PTHLH*'s involvement in histone acetylation during early embryonic development before implantation, previous research has indicated its direct influence on histone deacetylase 4 (*HDAC4*) (Kozhemyakina et al., 2009; Correa et al., 2010). Moreover, *PTHLH* exerts its influence through a complex network of signaling pathways involving ERK/MAPK pathways (Datta et al., 2005). ERK depletion has been associated with dysregulated pluripotency gene expression, decreased proliferation rates, G1 cell cycle arrest, increased apoptosis, faster telomere shortening, and impaired genomic stability (Chen et al., 2015). This data correlates well our data that *PTHLH* may play a role in increasing the cell survival and stability.

Reactome analysis confirmed *PTHLH*'s role in intracellular signaling, through its binding to GPCR leading to the activation of *GNAS* (Figure 7). G-proteins are essential regulators of cellular apoptotic cascades, with *GNAS* being linked to both proapoptotic and anti-apoptotic pathways (Melien, 2007; Yanamadala et al., 2009). *GNAS* overexpression has been shown to inhibit hydrogen peroxide-induced apoptosis by downregulating *Bcl-xl* (Zhao et al., 2006). Furthermore, endogenous *GNAS* activation through agonists has inhibited apoptosis mediated by cAMP production and PKA activation, leading to the inhibition of pro-apoptotic transcription factors such as AP-1, NF- κ B, and NFAT (Choi et al., 2009). Interestingly, the Gs-alpha pathway has been proposed to regulate pluripotency in ESCs. Activation of Gs-alpha and amplification of cAMP levels have been shown to promote Oct4 expression, stimulating ESC self-renewal and pluripotency (Faherty et al., 2007; Layden et al., 2010). Therefore, we hypothesize that *PTHLH* may exert indirect influences on the expression of pluripotency-related genes, leading to more cellular stability, although the precise underlying mechanism warrants further investigation.

PTHLH has shown to be an upstream activator of *GNAS* which directly interacts with SRC, leading to SRC autophosphorylation and subsequent activation. SRC plays a diverse role in iPSCs, influencing key cellular processes such as survival, proliferation, and cell fate decisions. Its signaling pathways support iPSC self-renewal, prevent apoptosis, maintain stability, and impact commitment to specific lineages (Lu et al., 2007; Shoni et al., 2014; Zhang et al., 2014;

Chaudhari et al., 2019). SRC also modulates pluripotency-associated pathways, including Wnt/ β -catenin (Min et al., 2022), which is crucial for maintaining stem cell identity. During cellular reprogramming, SRC activation facilitates the transition to a pluripotent state, contributing to the erasure of cell identity (Ma et al., 2013; González and Huangfu, 2016). Additionally, SRC is associated with cellular senescence, potentially regulating senescence during reprogramming (Anerillas et al., 2022). The context-dependent nature of SRC in iPSCs, influenced by cellular context, reprogramming stage, and interactions with other pathways, underscores the dynamic complexity of its functions. Altogether, emphasize the important role of *PTHLH* in maintaining iPSC stability and integrity. Indeed, the downregulation of *PTHLH* emerged as a critical factor influencing cellular stability and integrity, particularly evident in a *MYCN*-amplified, *TP53*-mutated neuroblastoma cell line. The reduction of *PTHLH* levels led to a consequential decrease in *MYCN* expression. This event triggered a cascade of effects, including cell cycle arrest, induction of senescence, and impairment of migration and invasion capabilities within the neuroblastoma cell line (García et al., 2019). These findings highlight the intricate role of *PTHLH* in orchestrating key cellular processes and maintaining the equilibrium necessary for stability and integrity.

POSTN exhibited the highest upregulation, increasing by 70-fold (FDR <0.001), in A53T-PD1 iPSC cell line compared to other iPSC cell lines (Figure 3B). STRING analysis predicted that *POSTN* interacts with extracellular matrix components, including collagen-related proteins, Decorin and Tenascin C. Our findings align with previous research indicating that *POSTN* is highly expressed in a mouse ES cell line treated with Mek inhibitor PD0325901, in contrast to other untreated cell lines (Chen et al., 2015). PD is a pharmacological agent that inhibits the Mek/Erk signaling pathway, promoting self-renewal and pluripotency in mESCs (Chen et al., 2015). Additionally, *POSTN* has been associated with the augmentation of the Wnt signaling pathway in mouse breast cancer stem cells (Wang et al., 2013). This suggests a potential role for *POSTN* in maintaining self-renewal and pluripotency through the activation of the Wnt signaling pathway, which is essential for preserving naïve pluripotency and epigenetic stability in mESCs (de Jaime-Soguero et al., 2018; Theka et al., 2019).

Previous studies elucidate the intricate and context-dependent role of *POSTN* in cellular stability and integrity across various biological systems. In renal cell carcinoma (RCC), *POSTN* emerges as a key regulator, where its knockdown significantly suppresses epithelial-mesenchymal transition (EMT) through the IKL/AKT/mTOR pathway. Conversely, overexpression of *POSTN* facilitates EMT (Jia et al., 2021), indicating the crucial role of *POSTN* in cell stability and integrity. Moreover, overexpression of *POSTN* in the context of osteoblasts acts as a protective factor by impeding melatonin-induced apoptosis; hence, increase cell stability and integrity. This protective effect is achieved by inhibiting the eIF2 α -ATF4 pathway through the suppression of the protein kinase R-like endoplasmic reticulum kinase (PERK) pathway (Zhu et al., 2021). These roles of *POSTN* highlights its versatile functions in maintaining cellular homeostasis. Furthermore, using mesenchymal stem cells (MSCs), *POSTN* plays a crucial role in the mineralization of the extracellular matrix (ECM) and supports tendon formation when overexpressed. This emphasizes *POSTN*'s involvement in ECM dynamics, which is essential for tissue development and integrity (Zhu et al., 2021).

In conclusion, our study sheds light on the potential roles of *CCN3*, *PTHLH*, and *POSTN* in maintaining genomic and cellular stability while preserving pluripotency in iPSCs. These findings warrant further investigations to unravel the precise mechanisms through which these genes contribute to these processes.

5 Conclusion

In summary, we identified three genes that were highly expressed in A53T-PD1 iPS cell line, from which robust and stable colonies were generated. The implications of these identified genes extend to potential selective interactions, which are vital for upholding the genomic integrity and maintaining pluripotency in both ESCs and iPSCs. Their functions may encompass activating DNA repair mechanisms, evading apoptosis through TP53 phosphorylation, and triggering signaling pathways that are crucial for preserving the pluripotent state of ESCs. To substantiate this conceptual framework and gain a deeper understanding of the underlying mechanisms, it becomes imperative to conduct thorough functional investigations. These endeavors hold the promise of unraveling crucial insights into the precise roles of these genes in ensuring genomic stability while upholding the self-renewal and pluripotency of the generated iPSCs.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the studies involving humans because these are iPS cell lines established in our lab, hence no need for ethical approval. In addition, ethical approval was obtained for the original research work (Swaidan et al., 2020; Scientific Reports) where we established these cell lines. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which ethical approval was obtained. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

References

- Anerillas, C., Herman, A. B., Rossi, M., Munk, R., Lehrmann, E., Martindale, J. L., et al. (2022). Early SRC activation skews cell fate from apoptosis to senescence. *Sci. Adv.* 8 (14), eabm0756. doi:10.1126/sciadv.abm0756
- Aprile, D., Alessio, N., Squillaro, T., Di Bernardo, G., Peluso, G., and Galderisi, U. (2023). Role of glycosphingolipid SSEA-3 and FGF2 in the stemness and lineage commitment of multilineage differentiating stress enduring (MUSE) cells. *Cell Prolif.* 56 (1), e13345. doi:10.1111/cpr.13345
- Bogliotti, Y. S., Wu, J., Vilarino, M., Okamura, D., Alba Soto, D., Zhong, C., et al. (2018). Efficient derivation of stable primed pluripotent embryonic stem cells from bovine blastocysts. *Proc. Natl. Acad. Sci.* 115 (9), 2090–2095. doi:10.1073/pnas.1716161115
- Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., et al. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* 55 (4), 611–622. doi:10.1373/clinchem.2008.112797
- Chaudhari, P., Tian, L., Kim, A., Zhu, Q., Anders, R., Schwarz, K. B., et al. (2019). Transient c-src suppression during endodermal commitment of human induced pluripotent stem cells results in abnormal profibrotic cholangiocyte-like cells. *Stem cells Dayt. Ohio* 37 (3), 306–317. doi:10.1002/stem.2950

Author contributions

NTS: Conceptualization, Data curation, Formal Analysis, Methodology, Validation, Visualization, Writing–original draft, Writing–review and editing. NHS: Conceptualization, Data curation, Formal Analysis, Methodology, Validation, Visualization, Writing–original draft, Writing–review and editing. ATA: Formal Analysis, Methodology, Validation, Writing–original draft. TD: Formal Analysis, Writing–original draft. RTA: Formal Analysis, Validation, Writing–original draft. AAA: Formal Analysis, Validation, Writing–original draft. RT: Validation, Writing–original draft. RA: Visualization, Writing–original draft. AH: Visualization, Writing–original draft. SS-A: Validation, Writing–original draft. SA: Writing–review and editing, Resources. AMA: Supervision, Writing–review and editing. ME: Project administration, Supervision, Writing–review and editing, Conceptualization, Data curation.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Qatar University funded the publication of this article. This research work was supported by QRDI [grant number: UREP27-156-3-044].

Acknowledgments

We extend our gratitude to Qatar University which funded the publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

- Chen, H., Guo, R., Zhang, Q., Guo, H., Yang, M., Wu, Z., et al. (2015). Erk signaling is indispensable for genomic stability and self-renewal of mouse embryonic stem cells. *Proc. Natl. Acad. Sci.* 112 (44), E5936–E5943. doi:10.1073/pnas.1516319112
- Choi, Y. J., Kim, S. Y., Oh, J. M., and Juhn, Y. S. (2009). Stimulatory heterotrimeric G protein augments gamma ray-induced apoptosis by up-regulation of Bak expression via CREB and AP-1 in H1299 human lung cancer cells. *Exp. Mol. Med.* 41 (8), 592–600. doi:10.3858/emmm.2009.41.8.065
- Correa, D., Hesse, E., Seriwatanachai, D., Kiviranta, R., Saito, H., Yamana, K., et al. (2010). Zfp521 is a target gene and key effector of parathyroid hormone-related peptide signaling in growth plate chondrocytes. *Dev. Cell* 19 (4), 533–546. doi:10.1016/j.devcel.2010.09.008
- Datta, N. S., Chen, C., Berry, J. E., and McCauley, L. K. (2005). PTHrP signaling targets cyclin D1 and induces osteoblastic cell growth arrest. *J. Bone Min. Res.* 20 (6), 1051–1064. doi:10.1359/jbmr.050106
- de Jaime-Soguero, A., Abreu de Oliveira, W. A., and Llus, F. (2018). The pleiotropic effects of the canonical Wnt pathway in early development and pluripotency. *Genes (Basel)* 9 (2), 93. doi:10.3390/genes9020093
- Derveaux, S., Vandesompele, J., and Hellemans, J. (2010). How to do successful gene expression analysis using real-time PCR. *Methods (San Diego, Calif.)* 50 (4), 227–230. doi:10.1016/j.ymeth.2009.11.001
- Dimos, J. T., Rodolfa, K. T., Niakan, K. K., Weisenthal, L. M., Mitsumoto, H., Chung, W., et al. (2008). Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 321 (5893), 1218–1221. doi:10.1126/science.1158799
- Faherty, S., Fitzgerald, A., Keohan, M., and Quinlan, L. R. (2007). Self-renewal and differentiation of mouse embryonic stem cells as measured by Oct4 expression: the role of the cAMP/PKA pathway. *Vitro Cell Dev. Biol. Anim.* 43 (1), 37–47. doi:10.1007/s11626-006-9001-5
- García, M., Rodríguez-Hernández, C. J., Mateo-Lozano, S., Pérez-Jaume, S., Gonçalves-Alves, E., Lavarino, C., et al. (2019). Parathyroid hormone-like hormone plays a dual role in neuroblastoma depending on PTH1R expression. *Mol. Oncol.* 13 (9), 1959–1975. doi:10.1002/1878-0261.12542
- González, F., and Huangfu, D. (2016). Mechanisms underlying the formation of induced pluripotent stem cells. *Wiley Interdiscip. Rev. Dev. Biol.* 5 (1), 39–65. doi:10.1002/wdev.206
- Guo, L., Qi, S. T., Miao, D. Q., Liang, X. W., Li, H., Ou, X. H., et al. (2012). The roles of parathyroid hormone-like hormone during mouse preimplantation embryonic development. *PLoS One* 7 (7), e40528. doi:10.1371/journal.pone.0040528
- Hawkins, K., Joy, S., and McKay, T. (2014). Cell signalling pathways underlying induced pluripotent stem cell reprogramming. *World J. Stem Cells* 6 (5), 620–628. doi:10.4252/wjsc.v6.i5.620
- Hong, H., Takahashi, K., Ichisaka, T., Aoi, T., Kanagawa, O., Nakagawa, M., et al. (2009). Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. *Nature* 460 (7259), 1132–1135. doi:10.1038/nature08235
- Jia, Y. Y., Yu, Y., and Li, H. J. (2021). POSTN promotes proliferation and epithelial-mesenchymal transition in renal cell carcinoma through ILK/AKT/mTOR pathway. *J. Cancer* 12 (14), 4183–4195. doi:10.7150/jca.51253
- Jiao, J., Dang, Y., Yang, Y., Gao, R., Zhang, Y., Kou, Z., et al. (2013). Promoting reprogramming by FGF2 reveals that the extracellular matrix is a barrier for reprogramming fibroblasts to pluripotency. *Stem Cells* 31 (4), 729–740. doi:10.1002/stem.1318
- Kawamura, T., Suzuki, J., Wang, Y. V., Menendez, S., Morera, L. B., Raya, A., et al. (2009). Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature* 460 (7259), 1140–1144. doi:10.1038/nature08311
- Kozhemyakina, E., Cohen, T., Yao, T. P., and Lassar, A. B. (2009). Parathyroid hormone-related peptide represses chondrocyte hypertrophy through a protein phosphatase 2A/histone deacetylase 4/MEF2 pathway. *Mol. Cell Biol.* 29 (21), 5751–5762. doi:10.1128/mcb.00415-09
- Kubota, S., and Takigawa, M. (2013). The CCN family acting throughout the body: recent research developments. *Biomol. Concepts* 4 (5), 477–494. doi:10.1515/bmc-2013-0018
- Lafont, J., Thibout, H., Dubois, C., Laurent, M., and Martinier, C. (2005). NOV/CCN3 induces adhesion of muscle skeletal cells and cooperates with FGF2 and IGF-1 to promote proliferation and survival. *Cell Commun. Adhesion* 12 (1–2), 41–57. doi:10.1080/15419060500383069
- Layden, B. T., Newman, M., Chen, F., Fisher, A., and Lowe, W. L., Jr. (2010). G protein coupled receptors in embryonic stem cells: a role for Gs-alpha signaling. *PLoS One* 5 (2), e9105. doi:10.1371/journal.pone.0009105
- Levine, A. J., Puzio-Kuter, A. M., Chan, C. S., and Hainaut, P. (2016). The role of the p53 protein in stem-cell biology and epigenetic regulation. *Cold Spring Harb. Perspect. Med.* 6 (9), a026153. doi:10.1101/cshperspect.a026153
- Lin, C. G., Chen, C. C., Leu, S. J., Grzeszkiewicz, T. M., and Lau, L. F. (2005). Integrin-dependent functions of the angiogenic inducer NOV (CCN3): implication in wound healing. *J. Biol. Chem.* 280 (9), 8229–8237. doi:10.1074/jbc.M404903200
- Lu, M., Glover, C. H., Tien, A. H., Humphries, R. K., Piret, J. M., and Helgason, C. D. (2007). Involvement of tyrosine kinase signaling in maintaining murine embryonic stem cell functionality. *Exp. Hematol.* 35 (8), 1293–1302. doi:10.1016/j.exphem.2007.04.010
- Ma, T., Xie, M., Laurent, T., and Ding, S. (2013). Progress in the reprogramming of somatic cells. *Circulation Res.* 112 (3), 562–574. doi:10.1161/CIRCRESAHA.111.249235
- Marioni, J. C., Mason, C. E., Mane, S. M., Stephens, M., and Gilad, Y. (2008). RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.* 18 (9), 1509–1517. doi:10.1101/gr.079558.108
- Martins-Taylor, K., and Xu, R. H. (2012). Concise review: genomic stability of human induced pluripotent stem cells. *Stem Cells* 30 (1), 22–27. doi:10.1002/stem.705
- Melien, O. (2007). Heterotrimeric G proteins and disease. *Methods Mol. Biol.* 361, 119–144. doi:10.1385/1-59745-208-4:119
- Min, J. K., Park, H. S., Lee, Y. B., Kim, J. G., Kim, J. I., and Park, J. B. (2022). Cross-talk between Wnt signaling and Src tyrosine kinase. *Biomedicines* 10 (5), 1112. doi:10.3390/biomedicines10051112
- Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L., and Wold, B. (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat. methods* 5 (7), 621–628. doi:10.1038/nmeth.1226
- Nagaraja, P., Robert, C., and Rassoul, F. V. (2013). DNA double-strand break response in stem cells: mechanisms to maintain genomic integrity. *Biochim. Biophys. Acta* 1830 (2), 2345–2353. doi:10.1016/j.bbagen.2012.09.001
- Qi, X., Li, T. G., Hao, J., Hu, J., Wang, J., Simmons, H., et al. (2004). BMP4 supports self-renewal of embryonic stem cells by inhibiting mitogen-activated protein kinase pathways. *Proc. Natl. Acad. Sci. U. S. A.* 101 (16), 6027–6032. doi:10.1073/pnas.0401367101
- Shoni, M., Lui, K. O., Vavvas, D. G., Muto, M. G., Berkowitz, R. S., Vlahos, N., et al. (2014). Protein kinases and associated pathways in pluripotent state and lineage differentiation. *Curr. stem Cell Res. Ther.* 9 (5), 366–387. doi:10.2174/1574888x09666140616130217
- Soldner, F., Hockemeyer, D., Beard, C., Gao, Q., Bell, G. W., Cook, E. G., et al. (2009). Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell* 136 (5), 964–977. doi:10.1016/j.cell.2009.02.013
- Stefanis, L. (2012). α -Synuclein in Parkinson's disease. *Cold Spring Harb. Perspect. Med.* 2 (2), a009399. doi:10.1101/cshperspect.a009399
- Su, J., Zhu, D., Huo, Z., Gingold, J. A., Ang, Y. S., Tu, J., et al. (2019). Genomic integrity safeguards self-renewal in embryonic stem cells. *Cell Rep.* 28 (6), 1400–1409. doi:10.1016/j.celrep.2019.07.011
- Su, X., Wei, Y., Cao, J., Wu, X., Mou, D., Luo, J., et al. (2018). CCN3 and DLL1 co-regulate osteogenic differentiation of mouse embryonic fibroblasts in a Hey1-dependent manner. *Cell Death Dis.* 9 (12), 1188. doi:10.1038/s41419-018-1234-1
- Swaidan, N. T., Salloum-Asfar, S., Palangi, F., Errafi, K., Soliman, N. H., Aboughalia, A. T., et al. (2020). Identification of potential transcription factors that enhance human iPSC generation. *Sci. Rep.* 10 (1), 21950. doi:10.1038/s41598-020-78932-9
- Tan, T. W., Huang, Y. L., Chang, J. T., Lin, J. J., Fong, Y. C., Kuo, C. C., et al. (2012). CCN3 increases BMP-4 expression and bone mineralization in osteoblasts. *J. Cell Physiol.* 227 (6), 2531–2541. doi:10.1002/jcp.22991
- Theka, I., Sottile, F., Aulicino, F., Garcia, A. C., and Cosma, M. P. (2017). Reduced expression of Paternally Expressed Gene-3 enhances somatic cell reprogramming through mitochondrial activity perturbation. *Sci. Rep.* 7 (1), 9705. doi:10.1038/s41598-017-10016-7
- Theka, I., Sottile, F., Cammisà, M., Bonnin, S., Sanchez-Delgado, M., Di Vicino, U., et al. (2019). Wnt/ β -catenin signaling pathway safeguards epigenetic stability and homeostasis of mouse embryonic stem cells. *Sci. Rep.* 9 (1), 948. doi:10.1038/s41598-018-37442-5
- Vallacchi, V., Daniotti, M., Ratti, F., Di Stasi, D., Deho, P., De Filippo, A., et al. (2008). CCN3/Nephroblastoma overexpressed matricellular protein regulates integrin expression, adhesion, and dissemination in melanoma. *Cancer Res.* 68 (3), 715–723. doi:10.1158/0008-5472.can-07-2103
- van Roeyen, C. R. C., Eitner, F., Scholl, T., Boor, P., Kunter, U., Planque, N., et al. (2008). CCN3 is a novel endogenous PDGF-regulated inhibitor of glomerular cell proliferation. *Kidney Int.* 73 (1), 86–94. doi:10.1038/sj.ki.5002584
- Vitillo, L., and Kimber, S. J. (2017). Integrin and FAK regulation of human pluripotent stem cells. *Curr. Stem Cell Rep.* 3 (4), 358–365. doi:10.1007/s40778-017-0100-x
- Wang, M., Tang, L., Liu, D., Ying, Q. L., and Ye, S. (2017). The transcription factor Gbx2 induces expression of Kruppel-like factor 4 to maintain and induce naive pluripotency of embryonic stem cells. *J. Biol. Chem.* 292 (41), 17121–17128. doi:10.1074/jbc.M117.803254
- Wang, X., Jia, L., Wang, Z., Huang, Y., Liu, W., Zhu, X., et al. (2013). Periostin contributes to the acquisition of multipotent stem cell-like properties in human mammary epithelial cells and breast cancer cells. *PLOS ONE* 8 (8), e72962. doi:10.1371/journal.pone.0072962
- Wang, Z., Gerstein, M., and Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.* 10 (1), 57–63. doi:10.1038/nrg2484

- Xu, Z., Robitaille, A. M., Berndt, J. D., Davidson, K. C., Fischer, K. A., Mathieu, J., et al. (2016). Wnt/ β -catenin signaling promotes self-renewal and inhibits the primed state transition in naïve human embryonic stem cells. *Proc. Natl. Acad. Sci. U. S. A.* 113 (42), E6382–e6390. doi:10.1073/pnas.1613849113
- Yamanaka, S. (2012). Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell* 10 (6), 678–684. doi:10.1016/j.stem.2012.05.005
- Yanamadala, V., Negoro, H., and Denker, B. M. (2009). Heterotrimeric G proteins and apoptosis: intersecting signaling pathways leading to context dependent phenotypes. *Curr. Mol. Med.* 9 (5), 527–545. doi:10.2174/156652409788488784
- Yu, L., Li, J., Hong, J., Takashima, Y., Fujimoto, N., Nakajima, M., et al. (2018). Low cell-matrix adhesion reveals two subtypes of human pluripotent stem cells. *Stem Cell Rep.* 11 (1), 142–156. doi:10.1016/j.stemcr.2018.06.003
- Yu, S., Zhou, C., Cao, S., He, J., Cai, B., Wu, K., et al. (2020). BMP4 resets mouse epiblast stem cells to naïve pluripotency through ZBTB7A/B-mediated chromatin remodelling. *Nat. Cell Biol.* 22 (6), 651–662. doi:10.1038/s41556-020-0516-x
- Zhang, X., Simerly, C., Hartnett, C., Schatten, G., and Smithgall, T. E. (2014). Src-family tyrosine kinase activities are essential for differentiation of human embryonic stem cells. *Stem Cell Res.* 13 (3), 379–389. doi:10.1016/j.scr.2014.09.007
- Zhao, C., Lai, J. S., Warsh, J. J., and Li, P. P. (2006). Galpha(s) sensitizes human SH-SY5Y cells to apoptosis independently of the protein kinase A pathway. *J. Neurosci. Res.* 84 (2), 389–397. doi:10.1002/jnr.20875
- Zhu, D., Zhou, W., Wang, Z., Wang, Y., Liu, M., Zhang, G., et al. (2021). Periostin: an emerging molecule with a potential role in spinal degenerative diseases. *Front. Med.* 8, 694800. doi:10.3389/fmed.2021.694800
- Zuo, G. W., Kohls, C. D., He, B. C., Chen, L., Zhang, W., Shi, Q., et al. (2010). The CCN proteins: important signaling mediators in stem cell differentiation and tumorigenesis. *Histology Histopathol.* 25 (6), 795–806. doi:10.14670/HH-25.795