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Research Article

Holistic Transcriptomic Analysis Identifies Prospective Reprogramming Factors for Induced Pluripotent Stem Cell Manufacturing

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ABSTRACT

Induced pluripotent stem cells (iPSCs) offer a groundbreaking technology, which has transformed translational research and clinical applications in a wide range of fields, such as regenerative medicine, tissue engineering, cell therapy, disease modeling, developmental biology, etc. iPSCs are derived from terminally differentiated somatic cells by reprogramming the genetic and epigenetic program back to the pluripotent stem cell characteristics. iPSCs are very identical to embryonic stem cells in regards to differentiation into many cell types; however, iPSCs are exempt from the legal or ethical issues. These advantages enable iPSCs to advance the cell therapy and transplantation strategies. Nonetheless, low reprogramming efficiency and the risk for tumorigenicity are still limitations in the application of iPSCs in practice because the usage of the same pluripotency factors in all somatic cell types remains incapable of an efficient reprogramming. Here, we accomplished a holistic meta-analysis of the transcriptome datasets in a bidirectional perspective to achieve significant pluripotency-related genes that can commonly be applicable in all origin cells. The current study suggested prospective reprogramming factors, such as *POLR3G*, *TERF1*, and *PHC1*. Meanwhile, integrated drug repurposing also revealed certain small chemical molecules, which can promote transgene-free reprogramming and safer iPSC generation protocols.

1. Introduction

Induced Pluripotent Stem Cells (iPSCs) were first created utilizing retroviral transduction of certain genes into adult somatic cells by Takahashi and Yamanaka [1]. The basic principle is to switch terminally differentiated cells back into the embryonic stem cell characteristics by delivering certain nuclear reprogramming factors that sustain capacity for potency and self-renewal. *Octamer-binding transcription factor 4* (*OCT4*), *Sex-determining region Y-box 2* (*SOX2*), *Kruppel Like Factor 4* (*KLF4*), and the oncogene *c-MYC*, collectively known as OSKM or Yamanaka factors, were able to induce the transdifferentiation (reprogramming) of somatic cells to the embryonic-like pluripotent cells. These genes emerged as the crucial ones in the initial stages of embryonic stem cell (ESC) formation as the output of a comprehensive screening assay [2, 3].

Since then, multiple studies revealed that ectopic expression of Yamanaka factors in the somatic cells is not sufficient alone to re-alter the complex epigenetic program required for the iPSC generation [4]. Hereby, only a small fraction of somatic cells (up to 1%) can successfully be reprogrammed into iPCSs from the terminally differentiated cells [5]. Another disadvantage is that overexpression of oncogenic genes such as *KLF4* and *c-MYC* is associated with a high carcinogenic risk in reprogrammed cells through various mechanisms, including p53 inactivation, development of drug resistance, inhibition of cell cycle suppressors, and epithelial-mesenchymal transition (EMT) [6, 7].

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Over the last 15 years, a wide range of protocols, which are especially capable of overcoming epigenetic barriers, have been developed to generate clinical-grade iPSCs and/or to enhance reprogramming efficiency [2, 8]. To date, a) identification of alternative or additional transcription factors to OSKM, b) addition of small chemical molecules that induce transdifferentiation (chemical induction) into the growth medium, c) use of non-integrative viral and non-viral gene transfer methods (e.g., episomal plasmids, SeVdp, PiggyBac, etc.), d) origination from the alternate cell sources that are more talented for somatic reprogramming, and e) transfection of non-coding RNAs as epigenetic regulators have all been reported to increase the efficiency and in vivo safety of iPSC production [2, 4, 9].

Researchers have mainly focused on the gene delivery methods for improved reprogramming efficacy and biological safety [10-12]. However, exploration of alternate reprogramming factors, which re-establish the pluripotent nature within the somatic cells, has been overshadowed so far. This is because each somatic cell type requires a divergent reprogramming factor cocktail due to having a unique epigenetic profile, transcriptional regulatory networks, and molecular roadblocks [13-15].

Indeed, there are numerous studies that explored the transcriptomic signature and regulation during the differentiation processes (from somatic cell to iPSC and from iPSC to somatic cell) [16-19]. Nevertheless, this research only discussed the transcriptomic changes for a specific somatic cell. In the present study, we aimed to investigate common pluripotency-associated genes that can be used jointly as reprogramming factors independent of the origin somatic cell by evaluating the outcomes of multiple transcriptome analyses together.

Otherwise, chemical reprogramming is another approach for overcoming the challenges in efficient and safer iPSC manufacturing. Chemical reprogramming is based on the administration of small chemical compounds to somatic cells, which enable reprogramming of somatic cells by robustly tuning ESC-related signaling pathways [20]. For instance, Li et al. defined a small-molecule cocktail, namely VC6T, consisting of valproic acid (an HDAC inhibitor), CHIR99021 (a GSK3 inhibitor), 616 452 (a TGF-β inhibitor), and tranylcypromine (an LSD1 inhibitor) that could substitute for *SOX2*, *KLF4*, and *c-MYC* in somatic cell reprogramming [21]. Thus, it offers a transgene-free, cost-effective, and customizable method to generate clinical-grade iPSCs [22]. Even so, the discovery of versatile chemical compounds simultaneously governing a wide range of pathways is still a necessity. Herein, our data also proposed several transcriptional targets to eliminate epigenetic barriers when downregulated and novel agents for the generation of chemically induced iPSCs (ciPSCs). In this regard, we sought differentially expressed genes (DEGs) in the microarray data published on the Gene Expression Omnibus (GEO) database. Here, we settled on a bidirectional approach through the meta-analysis of sorts of datasets: 1) detection of upregulated genes in the course of reprogramming various somatic cells to iPSCs, and 2) extraction of DEGs between the iPSC-derived somatic cells and the parental iPSCs.

2. General Methods

2.1. Transcriptome dataset acquisition

All transcriptomic datasets were acquired from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo). GSE55109 [23], GSE62066 [24], and GSE67915 [19] were the GEO datasets comparing the gene expression levels in iPSCs and their somatic cell origins in the course of somatic cell reprogramming (SomC to iPSC). Concurrently, the microarray datasets GSE175179 [25], GSE43143 [26], GSE55536 [27], GSE65423, GSE117086 [28], and GSE147498 [29] were used to evaluate DEGs between iPSCs and iPSC-derived somatic cells during the conversion of iPSCs into terminally differentiated cells (iPSC to SomC).

2.2. Evaluation of differentially expressed genes (DEGs)

Differentially expressed genes (DEGs) were identified using the GEO2R tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/)

provided by the GEO database. Benjamini & Hochberg adjustment was applied to the p-values [30]. The Log2FoldChange (Log2FC) threshold was set as 1 (|log2foldchange| > 1.0) to consider the DEGs with at least a 2-fold change. Other options were kept as default. Common DEGs were displayed, and Venn diagrams were rendered in Jvenn, an open-source interactive tool [31]. After DEGs in each microarray dataset were individually dissected and descendingly ranked according to enrichment scores as Log2FC, mutual DEGs were revealed by using intersection-based Venn diagrams. Therefore, only concordant genes from independent platforms and biological contexts could be considered to identify the most probable candidates without needing batch correction.

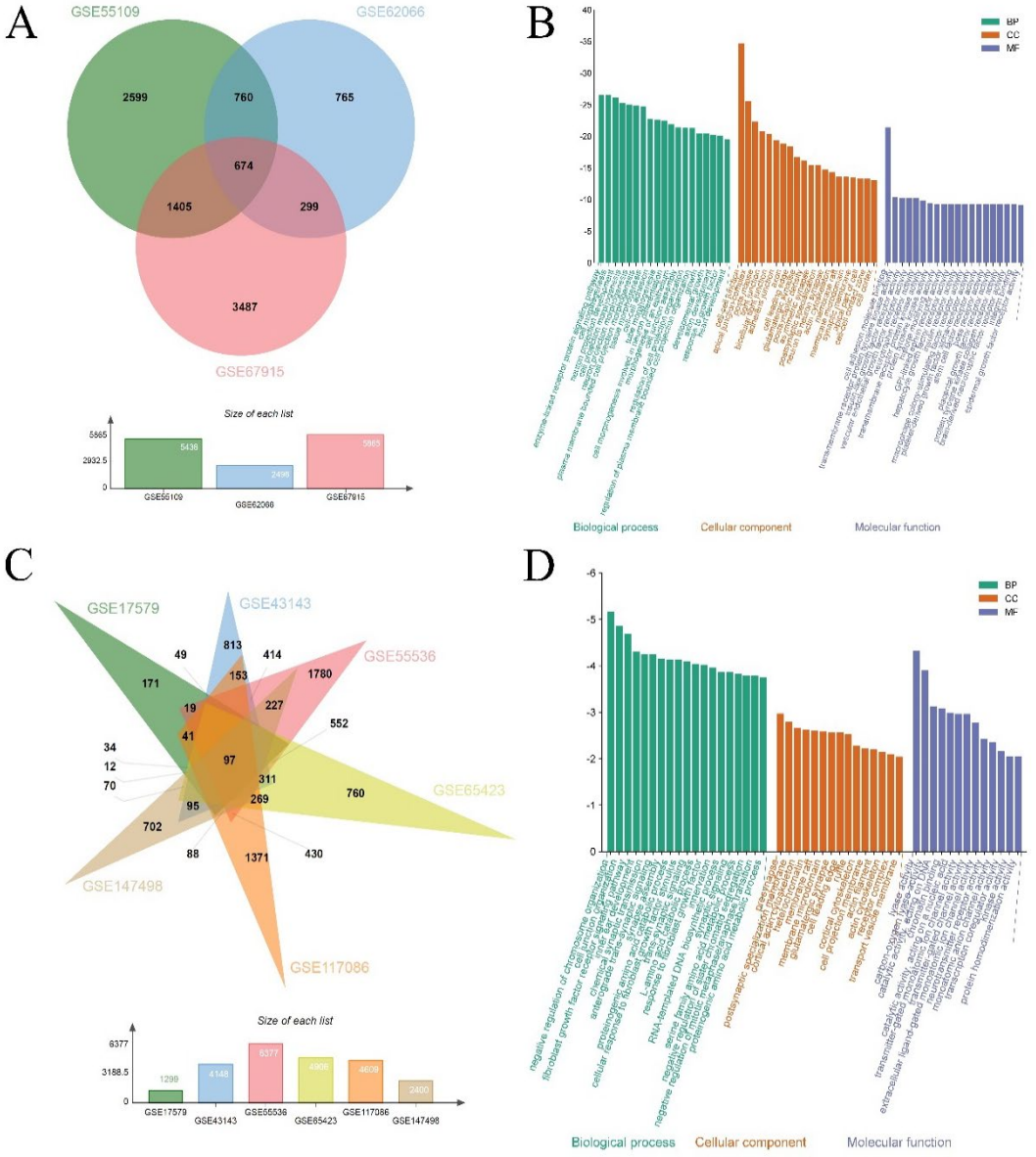

Figure 1. Upregulated genes while somatic cells were reprogrammed into iPSCs (A) and enriched GO terms (B). Downregulated genes during iPSCs were terminally differentiated into somatic cell types (C) and related GO terms (D)

2.3. Gene enrichment analysis

Metascape, a web-based analysis resource, was utilized for gene annotation and interpretation of enriched Gene Onthology (GO) terms, including biological processes, molecular functions, and cellular components, KEGG pathways, etc [32]. For enrichment analysis, minimum overlap, p-value cutoff, and minimum enrichment were selected as 3, 0.01, and 1.5, respectively. Afterwards, charts including the three enriched terms (BP/CC/MF) were plotted using SRplot [33].

2.4. Identification of small chemical compounds

Upregulated common DEGs (37 genes) and downregulated core DEGs (9 genes) in iPSCs were submitted to L1000 Characteristic Direction Signature Search Engine (L1000CDS²) developed by the Ma'ayan Laboratory at the Icahn School of Medicine at Mount Sinai to extract repurposing small chemical ligands. There configuration was selected as “mimic” to search for the small molecules that may provide the input common gene expression profile.

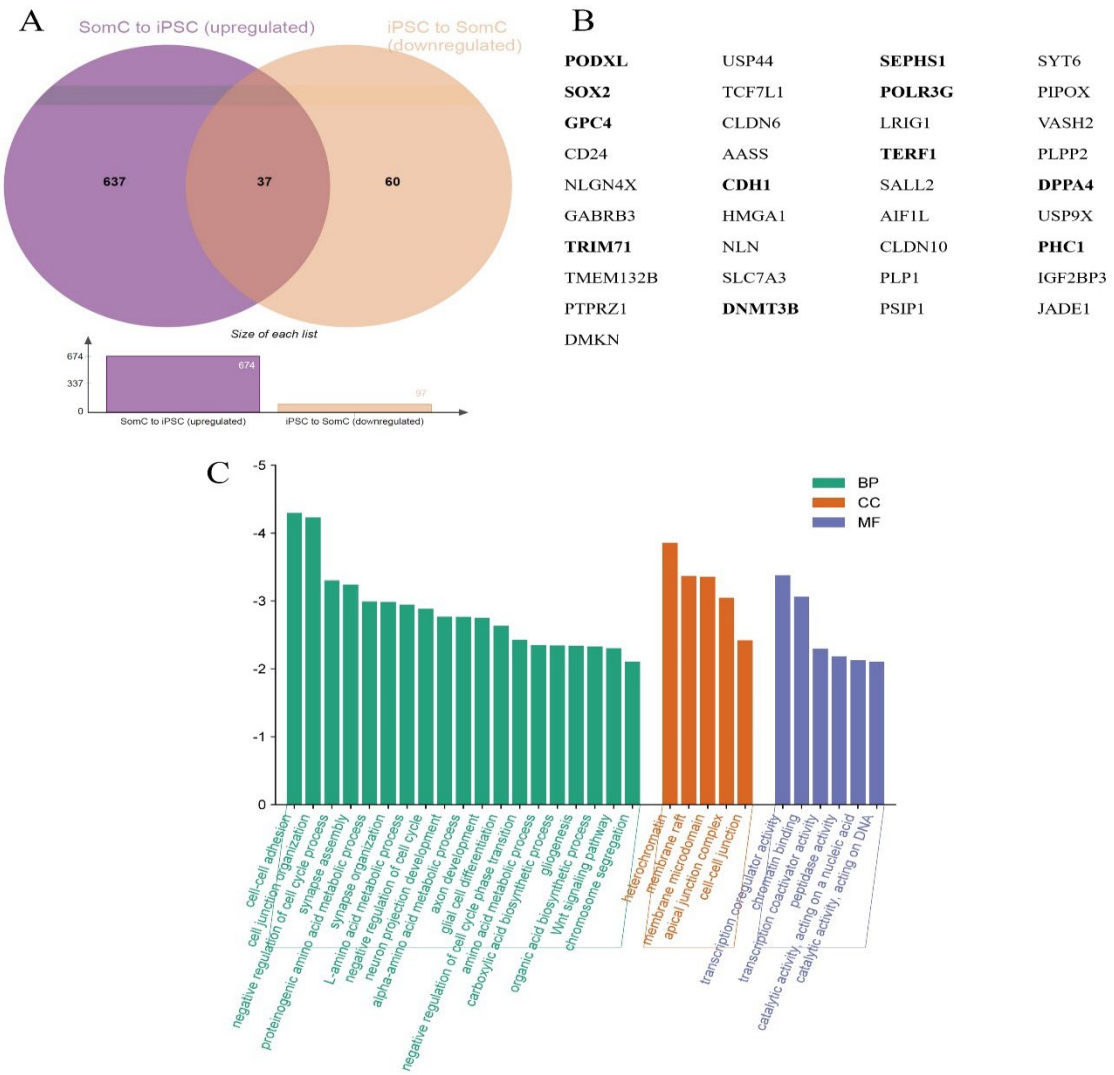

Figure 1. Common genes potentially linked to pluripotency in iPSCs (A, B) and related GO terms (C)

3. Results

3.1. Evaluation of the DEGs related to pluripotent state

Ongoing research has shown that each somatic cell type has a unique epigenetic profile that distinguishes transcriptional circuits [34, 35] and constitutes an epigenetic memory [36]. Thus, different somatic origins exhibit unusual iPSC production efficiency and quality during OSKM-induced reprogramming. In other words, utilization of the OSKM cocktail in all somatic cell types might give rise to nonequivalent impacts on the epigenetic memory-associated barriers [37-39]. Given that discrimination, we decided to collectively reevaluate the microarray data to unravel alternate or supporting pluripotency factors, comparing the transcriptomic profiles during reprogramming of different kinds of somatic cell origins to iPSCs.

First, we aimed to identify the common upregulated genes, whose overexpression in resultant iPSC lines was probably independent from the origin cell. After the increased DEGs in

iPSCs by at least 2-fold were listed for each dataset (GSE55109, GSE62066, and GSE67915), mutual DEGs were represented by creating a Venn scheme. It was found that 674 genes were upregulated in all iPSC lines (Figure 1A) reprogrammed from the human adult dermal fibroblasts and CD34+ cord blood cells from three different studies [19, 23, 24]. GO enrichment analysis showed that these upregulated genes are related to reasonable GO biological process, cellular component, and molecular function terms, including cell-cell interaction, cytoskeleton, adhesion, stem cell factor receptor activation, histone modifying activity, tube morphogenesis, and neuronal development (Figure 1B).

Meanwhile, we sought the common downregulated genes while iPSCs differentiate into somatic cells, suggesting these could also be related to the maintenance of pluripotency and should be silenced for a proper cell fate commitment. Here, we detected 97 genes downregulated (Figure 1C) in iPSC-derived human cardiomyocytes, neurons, macrophages, cerebellar-like neuronal cells, hepatocytes, and primordial germ cell-like cells (hPGCLCs) from 6 different gene expression datasets (GSE175179, GSE43143, GSE55536, GSE65423, GSE117086, and GSE147498). Likewise, downregulated genes were associated with the comparable GO biological process, cellular component, and molecular function terms, such as cell junction organization, synaptic signaling, membrane localization, and so on (Figure 1D).

Actually, genes associated with the pluripotent state are supposed to be upregulated while somatic cells are converted to iPSCs. Besides, similar genes related to pluripotency are likely to be suppressed while iPSCs differentiate into any somatic cell type. Of course, DEGs can differ between iPSCs and terminally differentiated somatic cell states according to the cell types. However, the most critical genes for the iPSC identity are not expected to diverge from cell type to cell type (in both directions; iPSC to somatic cell or somatic cell to iPSC).

Given this, upregulated genes in iPSCs (reprogrammed from somatic cells) and downregulated genes in iPSC-derived somatic cells were compared to unveil the common DEGs, which potentially retain indispensability for the pluripotent state independent from the cell type. Surprisingly, only 37 DEGs were shared between the two conditions explained above (Figure 2A). The genes already associated with stem cell biology, including *SOX2*, *DPPA4*, *DNMT3B*, *USP44*, *HMGA1*, etc., were intriguing among these common DEGs (Figure 2B), demonstrating outcomes of both analyses were consistent with each other. These genes were noticed to be involved in important biological processes and molecular functions, such as cell adhesion, cell junction, heterochromatin, cell cycle regulation, amino acid metabolism, the Wnt pathway, and transcription cofactor activity (Figure 2C). Taken together, these genes in Figure 2B stand out as alternative reprogramming factor candidates that can induce pluripotency alone or improve the efficacy of OSKM-mediated reprogramming protocols.

3.2. Dissecting the DEGs that potentially suppress the pluripotency

From another perspective, downregulated genes during iPSC formation can gain insights into hub proteins whose expression may be redundant in the pluripotent stage. Alternatively, these silenced genes may also hint at candidate targets for new compounds for chemical reprogramming protocols.

While reprogramming the human adult dermal fibroblasts and CD34+ cord blood cells into iPSCs, 215 common genes were downregulated in iPSCs compared to their origin somatic cells in all transcriptomic datasets (Figure 3A). Gene enrichment analysis showed that common DEGs were mainly involved in cellular responses to certain stimuli, cytokine production, immune regulation, secretion, and enzymatic activity (Figure 3B). With a reverse approach, we also assessed upregulated genes in terminally differentiated cells against iPSCs that they were derived from, suggesting that the expression of the pluripotency suppressor genes might be increased along with the lineage-specific genes. Herein, 149 genes were in the intersection of all GEO datasets (Figure 3C) with conceivable GO terms (biological process, molecular function,

and cellular component), such as the development of various tissues, morphogenesis, growth, extracellular matrix structure, transcriptional regulation, and cell adhesion were certain (Figure 3D). Unexpectedly, enriched terms showed a discrepancy between two datasets (Figure 3B and Figure 3D).

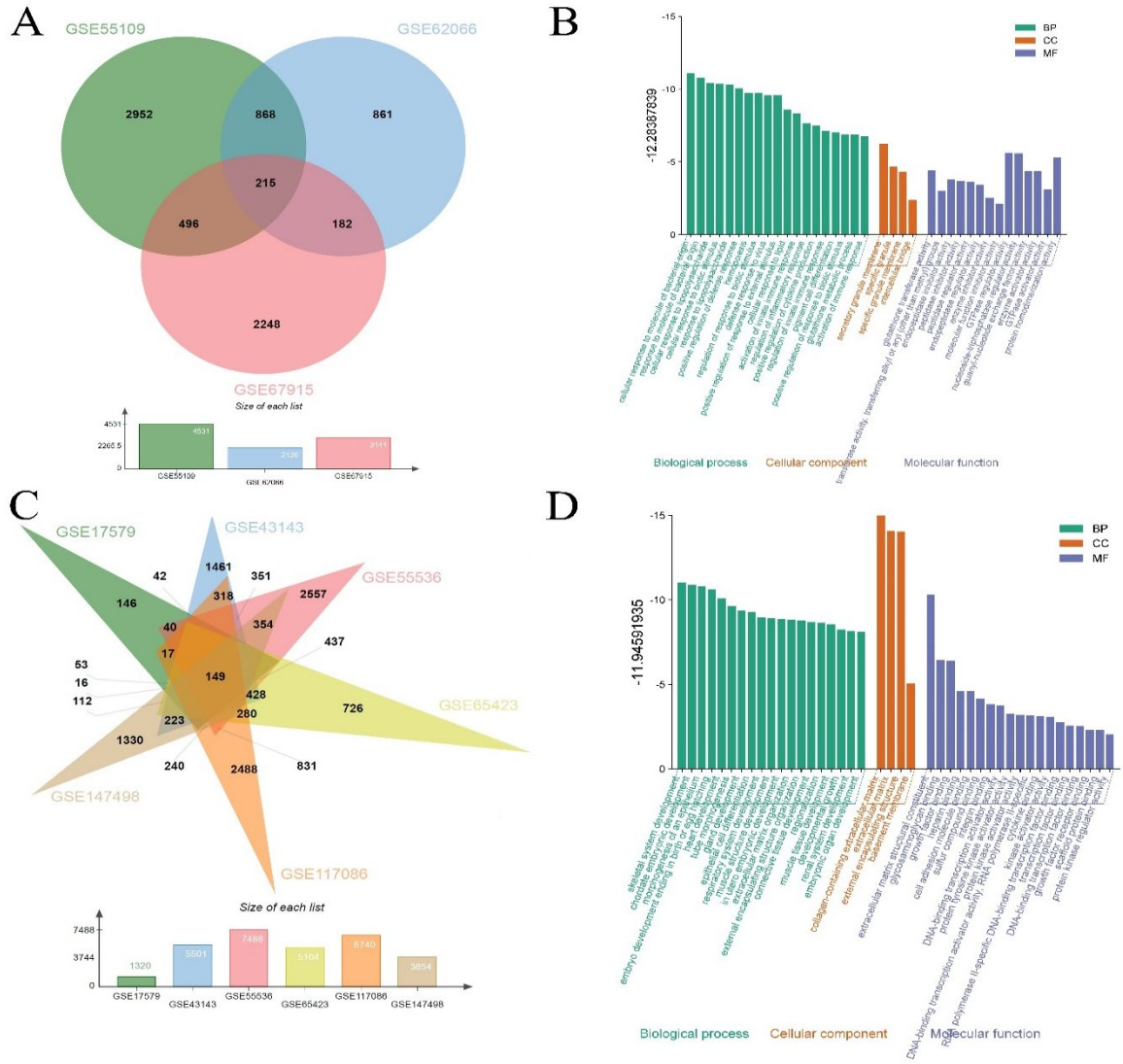

Figure 2. Downregulated genes while somatic cells were reprogrammed into iPSCs (A) and enriched GO terms (B). Upnregulated genes during iPSCs were terminally differentiated into somatic cell types (C) and related GO terms (D)

However, the number of common genes between two datasets was fewer than expected. Only 9 core genes (*RGCC*, *SUSD6*, *YAF2*, *RNF19A*, *ARID5B*, *RIN2*, *GSTM3*, *MTSS1*, *ARMCX3*) were unveiled as the mutual differentially expressed hub genes (Figure 4). Unfortunately, associated GO terms could not be enriched due to the inadequate number of hub genes.

3.3. Exploration of the small chemicals to mimic DEG profile in iPSCs

As figured out, 37 core upregulated genes and 9 core downregulated genes in iPSCs were found in all GEO datasets. This suggests that the differential expression of these genes is probably independent from the type of somatic cell. Nominately, we hypothesized that mimicking the expressional pattern by using small chemical compounds could promote chemical reprogramming of human somatic cells. Repurposed molecules and their potential effects on the core genes were depicted in Figure 5. Treatment of the somatic cells with these promising compounds (alone or in combination) (Table 1) can holistically ensure the DEG signature deduced in our transcriptomic meta-analysis, enhancing OSKM-mediated or transgene-free reprogramming yield.

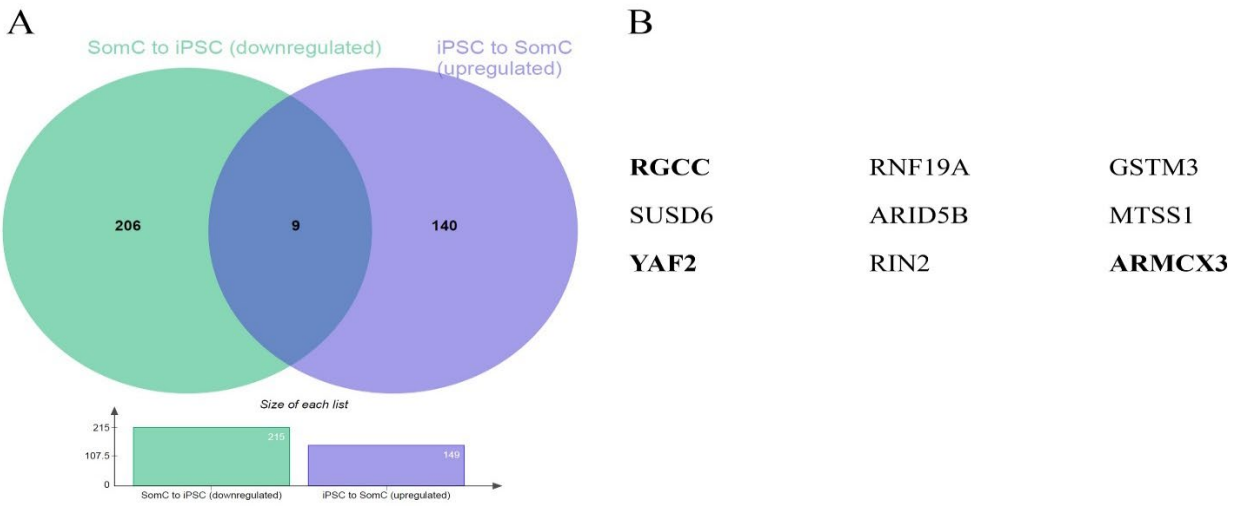

Figure 3. Common genes potentially inhibit pluripotency and promote differentiation in iPSCs (A, B)

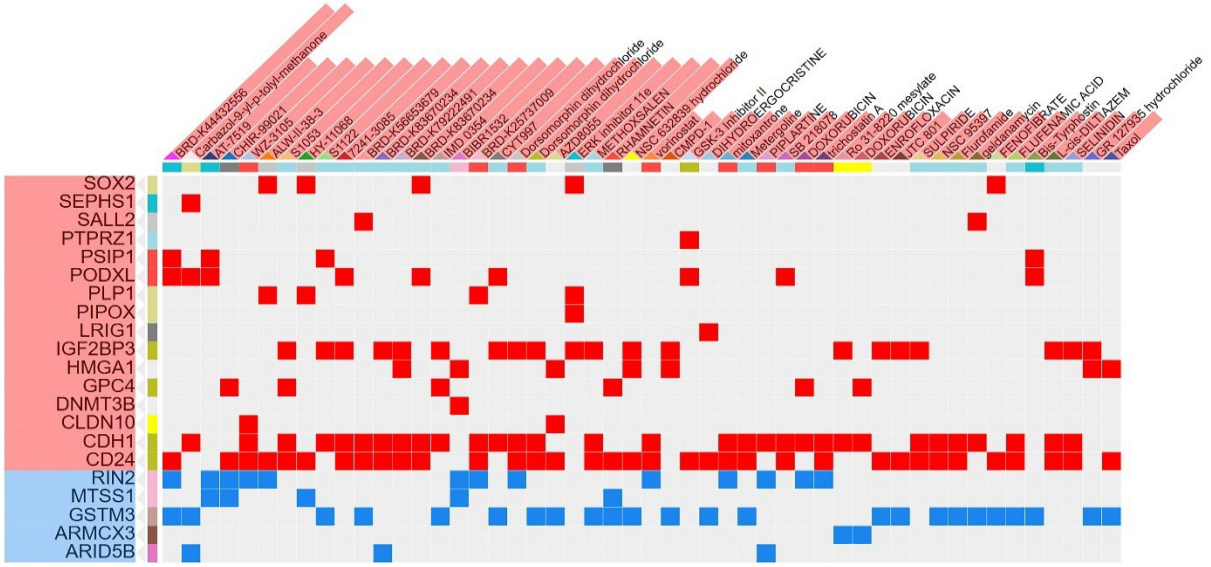

Figure 4. The top ranked repurposed compounds mimicking expressional signature yielded in the transcriptomic meta-analysis. Red and blue squares in the matrix indicate up and down regulation, respectively

4. Discussion

Induced pluripotent stem cells (iPSCs) are marvellous cellular tools offering opportunities in a wide-range of application fields, such as regenerative medicine, developmental biology, disease modeling, tissue and organoid engineering, drug discovery, and cellular therapies. iPSCs are originated from the any terminally differentiated somatic cell by reprogramming them back into an embryonic-like pluripotent state [40]. Even though iPSCs almost represent all embryonic stem cell (ESC) characteristics, their applications are exempt from the legal obstacles and ethical issues in contrast to ESCs since living embryos are not destroyed during ESC isolation. Somatic cell reprogramming have still been accomplished by the delivery of the pluripotency-related transcription factors, such as *OCT4*, *KLF4*, *c-MYC*, *SOX2*, and *LIN28* since these genes were first disscovered by Yamanaka and colleagues [1, 3]. It is challenging to compose a generalizable reprogramming protocol that utilizes the same transgens for each somatic cell type because terminally differentiated cells maintain unique genetic and epigenetic programs [13]. Therefore, utilization of alternative genes related to pluripotent state may enable a more efficient reprogramming for the most of somatic cell types.

Hereby, we sought out the probable genes for the pluripotent state, which jointly showed

significant expression levels in iPSCs during both bidirectional differentiation courses (somatic cell to iPSC and iPSC to somatic cell) by the integrated meta-analysis of transcriptome datasets in the GEO platform. Independent from the somatic cell type, 37 upregulated genes were noticed in iPSCs compared to the microarray data of terminally differentiated cells (Figure 2). Certain genes were quite intriguing among the upregulated transcripts (Figure 2B).

Podocalyxin-like protein (PODXL), a cell-adhesion glycoprotein, has previously been reported to present pluripotency markers, including ALP, SSEA3, SSEA4, SSEA5, TRA-1-60, and TRA-1-81 on the surface of human ESCs [41]. Additionally, *PODXL* gene was activated by KLF4 in human iPSCs at the early stage of reprogramming. Another current study has demonstrated *PODXL* is a crucial factor for primed pluripotency by promoting self-renewal, colony formation, c-Myc expression, and hTERT expression [42]. PODXL also regulated the cholesterol biosynthesis pathway to contribute to pluripotency. Notably, *PODXL* knockdown resulted in the loss of pluripotency, while *PODXL* overexpression augmented the human–mouse chimera formation in the mouse host embryos by regulating cell–cell and cell-extracellular matrix (ECM) interactions.

SOX2 (*SRY-box transcription factor 2*) is expected to appear because it is already an authenticated strong reprogramming factor [43], addressing the reliability of the current meta-analysis. Along with *SOX2*, *OCT4* (*POU5F1* or related isoforms), *LIN28*, *c-MYC*, and *NANOG* were detected in the upregulated transcriptome lists in only one or a few datasets (data not shown) not in the intersection, suggesting expression of other reprogramming factors can differ cell type to cell type.

Glypican 4 (GPC4), a cell surface heparan sulfate proteoglycan, was also reported as required for self-renewal in the mouse ESCs, and its downregulation in human iPSCs led to improvement in neuronal differentiation [44].

Chang et al. showed that expression of the *TRIM71* (T*ripartite motif containing 71*) gene that encodes an E3 ubiquitin-protein ligase was specific to undifferentiated mouse ESCs [45]. As reported, *TRIM71* is associated with the miR-290 and miR-302 families, the best-known ESC-specific miRNA clusters, and the Ago-2 protein to suppress *Cyclin-dependent kinase inhibitor 1A* (*CDKN1A*) expression. Thus, TRIM71 is a significant factor that induces rapid self-renewal of ESCs. Moreover, TRIM71, accompanied by let-7, contributed to human iPSC reprogramming by targeting differentiation genes, particularly the prodifferentiation transcription factor EGR1 [46].

CDH1 (C*adherin 1* or *E-cadherin*) is another common upregulated gene in iPSCs in our analysis. *CDH1* was demonstrated to be involved in self-renewal and mesenchymal to epithelial transition (MET) in pluripotent stem cells [47-49]. Notably, An and coworkers exhibited that elevated *CDH1* expression enhanced the reprogramming of human spermatogonial stem cells (SSCs) and male germline stem cells (GSCs) into iPSCs [50], promoting a putative role in the maintenance of pluripotent status.

Otherwise, Qiao and colleagues unraveled that *SEPHS1* (*Selenophosphate synthetase 1*) was dispensable for pluripotency maintenance but critical for three germ layer differentiation of mouse ESCs [51]. Contradictorily, *SEPHS1* upregulation in all iPSCs datasets compared to somatic cells led us to conclude that it may be required at the initial stages of pluripotency and can not induce reprogramming alone.

RNA polymerase III subunit G (*POLR3G*) is another sensible gene yielded in our transcriptomic analysis. *POLR3G* was previously indicated that its level was higher in undifferentiated human ESCs and iPSCs, maintaining the pluripotent state of human ESCs [52]. Then, Lund et al. elaborated that POLR3G was essential to regulate the transcription or splicing of a specific subset of small RNAs (miRNAs, snoRNAs, lincRNAs, etc.) and the pluripotency-linked protein-coding genes, including *DNMT1*, *APC*, *SMARCA4*, *NIPBL*, *RIF1*, *RTF1*, *TET1*, and *LIN28A* [53].

Furthermore, POLR3G could recruit key transcription factors, NANOG, SOX2, and KLF4 on the promoters of POLR3G-regulated

transcripts. Taken together, *POLR3G* stands out as an alternative or supportive pluripotency factor candidate to be utilized in the reprogramming of various somatic cells.

TERF1 (*Telomeric repeat binding factor 1*) encodes a component of the shelterin complex, which is involved in the protection of chromosome ends. Schneider and coworkers pointed out TERF1 as a stem cell marker, which was crucial for both maintenance and induction of pluripotency in the mouse ESCs and iPSCs, respectively [54]. Endogenous *TERF1* expression in mouse iPSCs was enriched because it was a direct target of *OCT4* and *NANOG*. Further evidence about the contribution of *TERT1* to the maintenance of the naive state of mouse ESCs was also manifested by several researchers.

Interestingly, Marión et al. designated that *TERF1* depletion in mouse pluripotent stem cells led to downregulation of a series of genes, including *MYC*, *SOX2*, *NANOG*, *KLF4*, and *BMP*, indicating it might control key pluripotency transcription factors [55]. Liu and colleagues also elucidated the miR-590/Acvr2a/Terf1 axis was substantial for telomere elongation to modulate the pluripotency in the pluripotent cells [56]. Although these findings together reflect the critical involvement of TERT1 in the pluripotency control in ESCs or already reprogrammed iPSCs, there is no evidence about the consequences of ectopic overexpression of *TERT1* in the differentiated cells to induce reprogramming. Given its significance, *TERT1* also emerges as a candidate with high potential for use as a reprogramming factor.

DPPA4 (developmental pluripotency associated 4), a marker of pluripotent stem cells, shows overexpression in ESCs and certain cancers. DPPA4 acts as a transcriptional regulator of endogenous targets in pluripotent cells and tumor cells, promoting cell proliferation [57]. However, *DPPA4* knockout only affected cell fate commitment at later developmental stages but not pluripotency in mice [58]. Nevertheless, its ectopic overexpression in somatic cells came out with oncogenic impacts [57-59]. Therefore, we concluded that *DPPA4* gene is not promising to facilitate clinical-grade iPSC production.

PHC1 (polyhomeotic homolog 1) is a subunit of canonical polycomb repressive complex 1 (PRC1), a crucial regulator of pluripotency and early embryonic development [60]. A recent study revealed that *Phc1* had the highest-ranking score in the mouse integrated stemness signatures (ISSs) composed of a gene regulatory network for self-renewal and differentiation [61]. On the other hand, Chen and colleagues also reported *PHC1* maintained the pluripotency of human ESCs by regulating genome-wide chromatin architecture and particularly activating *NANOG* transcription [62].

Conversely, analysis of transcriptomic datasets revealed a restricted number of genes that were commonly downregulated in iPSCs compared to the related somatic cells (Figure 4). This limitation could have arisen from the biological variability among different somatic cell types and differentiation protocols, as well as technical constraints of the given transcriptomic datasets. For instance, YAF2, a polycomb group (PcG) protein, was shown as a transcriptional repressor and an important regulator for self-renewal of mouse ESCs [63]. Surprisingly, YAF1 also has a function in the differentiation programs in mESCs and the formation of the embryoid body, highlighting that targeting YAF1 by small molecules can indeed gain an advantage in reprogramming protocols. Likewise, there are several studies exhibiting that ARMCX3 (Armadillo repeat containing X-linked 3) and RGCC (regulator of cell cycle) balance self-renewal and neural differentiation of certain progenitors and stem cells [64-67].

Although identified chemical compounds are speculative, they may indirectly support pluripotency *in vitro*. For instance, BRD-K44432556, introduced as a potent HIF1α (hypoxia inducible factor 1α) activator before [68], was quite intriguing. Several studies have reported that HIF1 could contribute to pluripotency during somatic cell reprogramming by promoting the metabolic shift in this process [69]. In this regard, small molecules that can modulate key metabolic players, such as HIFs, are thought to be encouraging in iPSC production [70]. Besides, the studies lasting in recent years have highlighted the importance of managing the ubiquitin–proteasome system (UPS) either in the

induction or maintenance of pluripotency [71, 72]. Thereby, NSC 632839 hydrochloride seems to be plausible, hypothetically controlling UPS because Aleo and coworkers characterized it as the nonselective isopeptidase inhibitor [73]. Additionally, it has been indicated that ERK downregulates *NANOG*, *OCT4*, *KLF2*, and KLF4, and its pharmacological inhibition via VX-11e stimulates self-renewal [74].

Thereby, ERK inhibitor 11e (VX-11e) in Table 1 would already be anticipated. Kim and colleagues demonstrated that suppression of the mTOR signaling pathway by shRNA constructs augmented pluripotency and ESC-like properties both in humans and mice [75]. Hence, selective mTOR kinase inhibitor AZD8055 was compatible with this data. Numerous studies have already evidenced CHIR-99021's outstanding roles in maintaining self-renewal and pluripotency of the mouse and human pluripotent stem cells [76-78]. This was considered as a corroborative indicator for the relevance of the outputs in Table 1. These findings altogether indicate that repurposed small chemical molecules may be applicable in the maintenance of the pluripotent state since some of them are in conformity with the published research.

Table 1. Top 20 candidate small molecules (alone and in combination) to facilitate somatic cell reprogramming

	Solo Administration		**Administration in Combination**
No	***Compound***	***Actual Target or Cellular Function***	***Compounds***
1	Carbazol-9-yl-p-tolyl-methanone	N/A	1. Carbazol-9-yl-p-tolyl-methanone 6. ERK inhibitor 11e
2	BRD-K44432556	Hypoxia inducible factor activator	2. BRD-K44432556 6. ERK inhibitor 11e
3	NSC 632839 hydrochloride	Ubiquitin isopeptidase inhibitor	1. Carbazol-9-yl-p-tolyl-methanone 11. BIBR1532
4	RHAMNETIN	Plant o-methylated flavonoid (antioxidant)	1. Carbazol-9-yl-p-tolyl-methanone 14. HY-11068
5	METHOXSALEN	Treatment of psoriasis, eczema, vitiligo, and some cutaneous lymphomas	1. Carbazol-9-yl-p-tolyl-methanone 16. ALW-II-38-3
6	ERK inhibitor 11e	Extracellular signal-related kinase 2 (ERK2) inhibitor	1. Carbazol-9-yl-p-tolyl-methanone 18. CHIR-99021
7	AZD8055	ATP-competitive inhibitor of mTOR kinase	1. Carbazol-9-yl-p-tolyl-methanone 2. BRD-K44432556
8	Dorsomorphin dihydrochloride	ATP-competitive AMPK inhibitor	1. Carbazol-9-yl-p-tolyl-methanone 3. NSC 632839 hydrochloride
9	CYT997	Microtubule polymerization inhibitor	1. Carbazol-9-yl-p-tolyl-methanone 4. RHAMNETIN
10	BRD-K25737009	Retinoic Acid Receptor Gamma (predicted)	4. RHAMNETIN 6. ERK inhibitor 11e
11	BIBR1532	Non-competitive human telomerase inhibitor	1. Carbazol-9-yl-p-tolyl-methanone 7. AZD8055
12	IMD 0354	IKKβ inhibitor	6. ERK inhibitor 11e 7. AZD8055
13	SB 239063	p38 MAPK inhibitor	1. Carbazol-9-yl-p-tolyl-methanone 9. Dorsomorphin dihydrochloride
14	ALW-II-38-3	A potent ephrin-A receptor (EphA3) kinase inhibitor	1. Carbazol-9-yl-p-tolyl-methanone 10. BRD-K25737009
15	WZ-3105	Potential targets SRC, ROCK2, NTRK2, FLT3, IRAK1	2. BRD-K44432556 11. BIBR1532
16	CHIR-99021	Selective inhibitor of glycogen synthase kinase 3 (GSK-3)	5. METHOXSALEN 11. BIBR1532
17	AT-7519	Selective inhibitor of certain Cyclin Dependent Kinases (CDKs)	6. ERK inhibitor 11e 11. BIBR1532
18	Taxol	Antimitotic chemotherapy agent	8. Dorsomorphin dihydrochloride 11. BIBR1532
19	GR 127935 hydrochloride	Selective 5-HT1B/1D receptor antagonist.	9. CYT997 11. BIBR1532
20	L-cis-DILTIAZEM	cGMP-activated K+ channel blocker	2. BRD-K44432556 12. IMD 0354

Albeit the current study provides a basis to uncover alternative reprogramming factor candidates, experimental validation for the transcriptomic analyses regarding these factors and small chemical compounds is crucial to substantiate their *in vitro* efficacy in somatic cell reprogramming. For instance, the impacts of the proposed genes (e.g., *POLR3G*, *TERF1*, *PHC1*) on the characteristics of different somatic cell types or the substitution potential of these candidate genes for the Yamanaka factors should be investigated through gain- and loss-of-function strategies. Furthermore, integration of single-cell transcriptomics and epigenomics may also yield deeper insights into cell-type-specific reprogramming dynamics. Meanwhile, bioavailability, cytotoxicity, and potential off-target effects of the small chemical molecules from the in silico repurposing remained unexplored here. Functional assays may also gain original knowledge about how these small molecule candidates could alter cellular identities.

5. Conclusion

Consequently, the current study holistically evaluated transcriptomic data in a bidirectional manner (differentiation from somatic cell to iPSC state, or vice versa) to reveal upregulated genes potentially associated with pluripotency. Otherwise, downregulated genes during iPSC differentiation led us to screen potent chemical molecules to facilitate somatic cell reprogramming. Overall, transcriptomic analysis has released novel findings that are worth taking into account to design more generalizable procedures for iPSC manufacturing.

Article Information Form

The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the author.

Artificial Intelligence Statement

No artificial intelligence tools were used while writing this article.

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