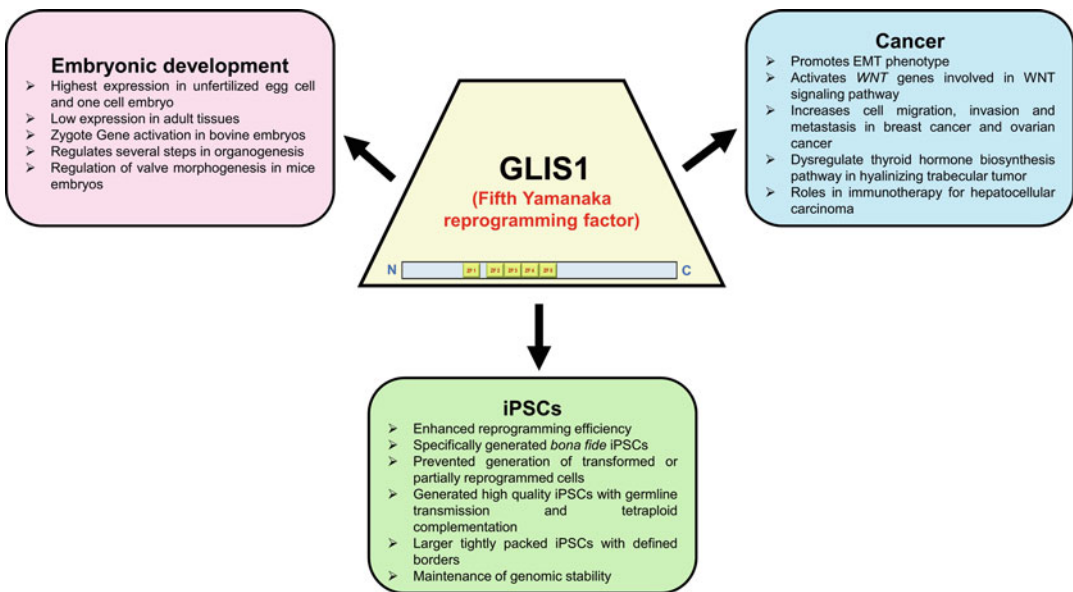




An Insight into the Role of GLIS1 in Embryonic Development, iPSC Generation, and Cancer

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Graphical abstract



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Abstract

The curiosity to discover transcription factors to reprogram somatic cells to induced pluripotent stem cells (iPSCs) resulted in the identification of a reprogramming factor, Gli-similar transcription factor GLIS1. This proline-rich Kruppel-like zinc finger transcription factor has a role in embryonic development, iPSC generation, and cancer. The spatial and temporal expression of GLIS1 during embryonic development implicates that it can control gene expression at specific developmental stages. Moreover, GLIS1 in combination with OCT4, SOX2, and KLF4 reprogramming factors resulted in an increase in reprogramming efficiency, giving rise to primarily bona fide iPSCs. Mutations in the *GLIS1* gene are associated with several types of tumors and cancers, and it shows a tissue-specific function where it acts either as an oncogene or as a tumor suppressor gene. This review gives a comprehensive overview of GLIS1 and its important role in embryonic development, cancer, and the generation of iPSCs.

Keywords

Cancer · Cell reprogramming · Embryonic development · GLIS1 · Induced pluripotent stem cells · Transcription factor

Abbreviations

ALL	Acute lymphoblastic leukemia
CAFs	Cancer-associated fibroblasts
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
EMT	Epithelial–mesenchymal transition
ESCs	Embryonic stem cells
HTT	Hyalinizing trabecular tumor
iPSCs	Induced pluripotent stem cells
MET	Mesenchymal–epithelial transition
OSKMG	OCT4, SOX2, KLF4, c-MYC, and GLIS1

1 Introduction

Stem cells and their role in regenerative medicine are a developing field in biomedical science. Understanding the mechanisms involved in stem cell self-renewal and differentiation holds great importance in unraveling the potential and their subsequent use in various biomedical applications. Fundamentally, they are undifferentiated cells that have the potential to self-renew and differentiate and are classified into different stem cell types such as embryonic stem cells (ESCs), fetal stem cells, and adult stem cells (Roobrouck et al. 2008). ESCs are pluripotent cells that can be differentiated into cells of all three germ layers, namely ectoderm, endoderm, and mesoderm (Poh et al. 2014), thereby having immense utility in disease modeling and other biomedical applications. The ethical limitations associated with the use of ESCs restrict their use in patient-specific applications. To overcome this limitation and generate pluripotent stem cells in a laboratory, Yamanaka and group found that using a combination of OCT4, SOX2, KLF4, and c-MYC (OSKM) transcription factors, mouse and human fibroblasts can be reprogrammed to an early embryonic stem cell-like state, which they termed as induced pluripotent stem cells (iPSCs) (Takahashi et al. 2007; Takahashi and Yamanaka 2006). In the subsequent year, Thomson and group also independently generated iPSCs using the factors OCT4, SOX2, LIN28A, and NANOG, which were capable of reprogramming human somatic cells to iPSCs (Yu et al. 2007). These two findings revolutionized the field of regenerative medicine and since then, multiple transcription factors have been screened for elucidating their roles in enhancing or substituting OSKM reprogramming transcription factors for the generation of mature iPSCs (Dey et al. 2021a, b, c; Raina et al. 2021; Thool et al. 2022a). The caveat of using OSKM for the generation of clinical-grade iPSCs was the formation of a heterogeneous population of iPSC colonies comprising fully reprogrammed cells (bona fide iPSCs; rarely) and incompletely reprogrammed cells (transformed colonies; mostly) (Maekawa et al. 2011). Also, the presence of c-MYC

makes OSKM-based reprogramming less worthy for clinical applications due to its tumorigenic potential (Akifuji et al. 2021). Gli-similar 1 (GLIS1) is one of the factors that was reported to enhance the formation of fully mature iPSC colonies when included with OSK (Maekawa et al. 2011).

GLIS1 gene was identified independently by two groups based on sequence homology to Gli zinc finger domain (Kim et al. 2002; Nakashima et al. 2002). It belongs to the family of Kruppel-like zinc finger transcription factor, which is one of the largest family of transcription factors that work as activators and repressors of gene transcription (Kim et al. 2002). Moreover, *GLIS1* is found to be expressed during early embryonic development and has a critical role in the control of gene expression during specific stages of embryonic development (Kim et al. 2002). These attributes made GLIS1 to be quoted as “the fifth Yamanaka factor” in reprogramming. In addition, high expression of GLIS1 is also found to be associated with different tumors and cancers (Kim et al. 2022; Shimamoto et al. 2020; Vadnais et al. 2014). In this review, a detailed discussion on the importance of GLIS1 in embryonic development, cancer, and iPSC generation is reported.

2 GLIS1 Gene

GLIS1 is a protein-coding gene (Table 1) that acts as both an activator and repressor of transcription (Kim et al. 2002). It is localized on chromosome 1 at 1p32.3 in humans and chromosome 4 at 4C6 position in mice (Kim et al. 2002). The gene is conserved in chimpanzees, rhesus monkeys, dogs, cows, mice, rats, chickens, and zebrafish, with 250 organisms having orthologs with the human *GLIS1* gene (Yasuoka et al. 2020). It is also reported that GLIS1 has paralogs such as GLIS3, GLI2, GLI3, GLIS2, GLI1, ZIC5, ZIC2, ZXDC, ZIC3, ZIC1, ZXDB, ZXDA, AEBP2, and ZIC4.

Table 1 Physiochemical parameters of human GLIS1 sequence

Parameters	Values
<i>Protein-coding DNA sequence</i>	
Number of nucleotides (bp)	1863
Average GC content (%)	66
Nucleotide composition	Adenine (A): 17.77% (331 bases) Thymine (T): 16.21% (302 bases) Guanine (G): 27.69% (516 bases) Cytosine (C): 38.33% (714 bases)
<i>Protein</i>	
Number of amino acids	620
Molecular weight (kDa)	65.976
Theoretical pI	7.56
Total number of negatively charged residues	Asp + Glu: 53
Total number of positively charged residues	Arg + Lys: 54

3 GLIS1 Protein

Human GLIS1 protein is 620 amino acids in length (Fig. 1) having a molecular weight of ~66 kDa, while mouse GLIS1 protein consists of 789 amino acids with a molecular weight of ~84 kDa (Lee et al. 2017). It belongs to the GLIS subfamily of Kruppel-like zinc finger transcription factors, which is the largest family of transcription factors (Kim et al. 2002; Scoville et al. 2017). It is closely related to GLI and ZIC subfamilies with GLIS2 and GLIS3 as other protein members of the same family (Scoville et al. 2017). GLIS1 is a neutral protein with a theoretical pI of 7.56. It is also a proline-rich protein with 12.4% proline content. The sequence identity between human and mouse GLIS1 proteins is 83.2%. Furthermore, on analyzing the protein sequence using the InterPro database, the zinc finger domain was found to have five Cys2-His2-type zinc finger motifs with the consensus Cys-X4-Cys-X12,15-His-X3,4-His belonging to GLI Cys2-His2-type zinc-finger protein family (Fig. 1). InterPro scan and profile scan

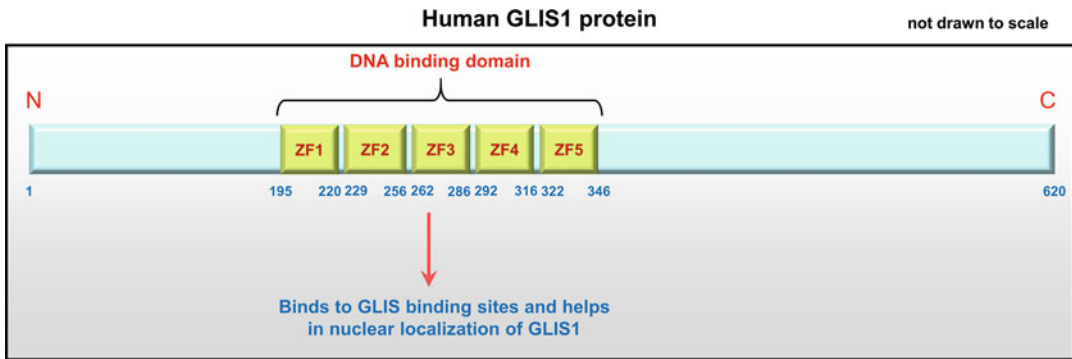


Fig. 1 Pictorial representation of domains in human GLIS1 protein. The highly conserved DNA-binding domain of human GLIS1 with five tandem repeats of

Cys2-His2 zinc finger motifs is present toward the N terminal (ZF – zinc finger)

analysis also identified a potential bipartite nuclear localization signal between Arg511 and Lys527 that overlaps with the fifth zinc finger motif of GLIS1. The region containing the zinc finger domain and bipartite nuclear localization signal was important for the nuclear localization of GLIS1. It has a strong activation domain at the C-terminal end that can mediate its interaction with a co-activator, thus functioning primarily as a transcriptional activator (Jetten et al. 2022; Kim et al. 2002). The main co-activator of GLIS1 is EP300/p300, which is recruited to the transactivation domain present at the C-terminal end (Jetten et al. 2022). It also contains a repressor domain in its N-terminal end that functions as a transcriptional repressor (Jetten et al. 2022). The highly conserved DNA-binding domain (zinc finger domains) has five tandem repeats of Cys2-His2 zinc finger motifs. These motifs bind to the GLIS binding sites, which are oligonucleotides containing G-rich consensus sequence ((A/G) GG (G/A) GG) (Scoville et al. 2017). In general, this protein acts as an activator or repressor of gene transcription as it contains both transactivation and repressor domains (Kim et al. 2002).

The three-dimensional crystal structure of GLIS1 protein is not reported to date. The 3D structure of the protein was obtained from the AlphaFold database (Fig. 2a). The secondary structure of the protein was predicted using the online bioinformatics tools GOR4, SOPMA, and PSIPRED (Fig. 2b). Using these tools, the

predicted structure reveals that GLIS1 mainly consists of random coils (77%) (Fig. 2b). The amount of α -helices is around 17% and β -sheets is around 6% (Fig. 2b). Random coils or intrinsically disordered regions are a characteristic feature of transcription factors, which is necessary for its functionality (Garza et al. 2009). The amino acid composition of GLIS1 protein reveals that it contains more proline (12.4%), serine (11%), and leucine (11%) residues that might contribute to the transactivation activity of GLIS1 (Chin et al. 1997).

The interacting partners of GLIS1 protein were analyzed using the STRINGv11 database as depicted in Fig. 3. The suppressor of fused homolog protein, which is a negative regulator of Hedgehog signaling pathway (Takenaka 2007), regulates developmental processes in multicellular embryos and is found to be a direct interacting partner of GLIS1 and other GLI family proteins GLI1, GLI2, and GLI3. NR2C1, an interacting partner of GLIS1, is also reported to activate OCT4 gene expression and forms the core of direct repeat erythroid-definitive complex that represses the transcription of embryonic and fetal β -globin (Park et al. 2007; Tanabe 2002; Tanabe et al. 2007). GLIS1 also interacts with MYCL, which is a proto-oncogene belonging to the MYC family, thereby having the potential to replace c-MYC in OKSM-based reprogramming (Schwab 2004). Furthermore, CAMK4 is a direct interacting partner of GLIS1, which

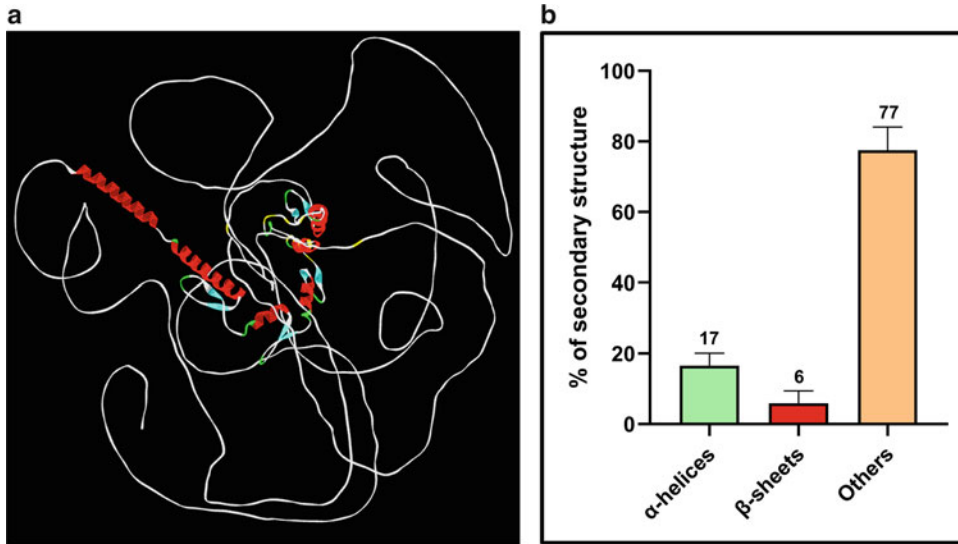
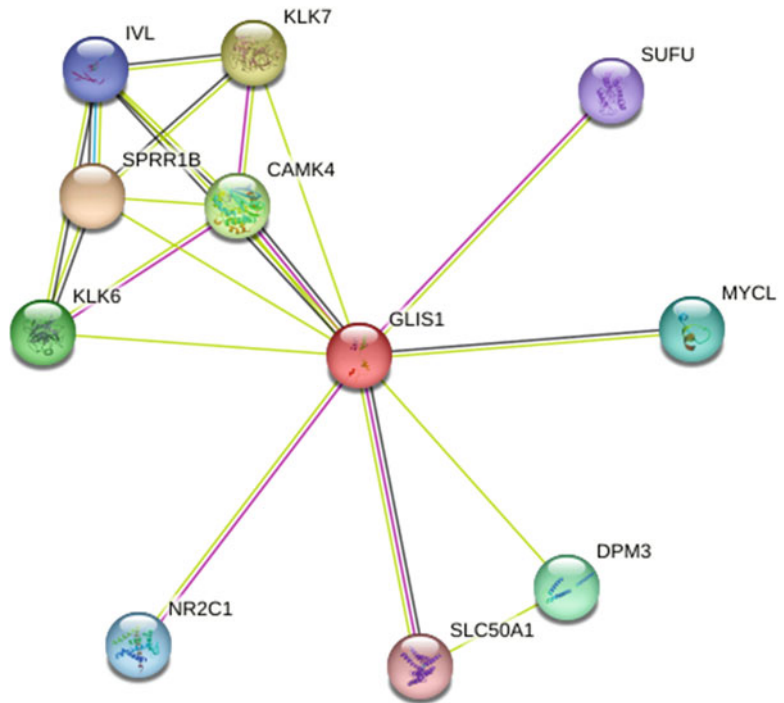


Fig. 2 The three-dimensional and secondary structure of human GLIS1 protein. (a) The three-dimensional structure of the human GLIS1 protein was obtained from the AlphaFold database. It was visualized with BIOVIA

discovery studio visualizer. (b) The amount of different secondary structures in human GLIS1 protein was predicted using different web servers (GOR4, PSIPRED, SOPMA, and AlphaFold) are indicated in the graph

Fig. 3 Protein-interacting partners of human GLIS1. The proteins that interact or are predicted to be interacting with human GLIS1 protein were obtained from the STRING v11 database



regulates the activity of transcriptional activators such as CREB1, MEF2D, JUN, and RORA by phosphorylation. This plays important roles in

immune response, inflammation, and memory consolidation (Naz et al. 2020). Also, GLIS1 was found to have three potential CAMK4

phosphorylation sites at Ser72, Ser187, and Thr458 (Kim et al. 2002). It was reported that CAMK4 can enhance the transcriptional activation mediated by GLIS1 fourfold (Kim et al. 2002). Interestingly, an association of GLIS1 with KLK4 was reported, which might be an indication of the involvement of GLIS1 in the pathogenesis of Parkinson's disease, since KLK4 has roles in degrading α -synuclein and preventing its aggregation (Song et al. 2012; Sotiropoulou et al. 2009). These experimental evidence indicate the association of GLIS1 in various physiological functions.

4 Role of GLIS1 in Embryonic Development

The expression of GLIS1 was first observed during early stages of embryonic development in mice (Kim et al. 2002). Predominant expression of GLIS1 was observed in unfertilized oocytes and one-cell stage murine embryo followed by a moderate expression in the two-cell stage and lowest at the four-cell stage (Fig. 4) (Jetten 2018; Nakashima et al. 2002). The expression was absent or observed in trace amounts in the eight-cell stage; however, no expression was

observed in the subsequent stages (16-cell stage, morula and blastocyst) (Fig. 4) (Nakashima et al. 2002). Also, transient expression of GLIS1 was observed in several defined structures of mesodermal lineage, including craniofacial regions, follicles, branchial arches, limb buds, somites and myotomes, genital tubercle, and tailbud during embryonic development (Kim et al. 2002). Its expression was detected in extra-embryonic tissues and lateral mesoderm during gastrulation in mice (Kim et al. 2002). It is expressed both spatially and temporally during embryonic development, implying that it can regulate several steps in organogenesis (Kim et al. 2002). GLIS1 protein showed a low level of expression in mouse embryonic stem cells (ESCs) and its overexpression led to reduced proliferation of ESCs (Maekawa et al. 2011; Wang et al. 2019). Moreover, GLIS1 protein plays a crucial role in controlling gene expression during specific stages of embryonic development (Kim et al. 2002).

GLIS1 plays a regulatory role during heart development and is expressed predominantly in the nuclei of endothelial and interstitial cells of mitral valves in mice during embryonic development (Yu et al. 2019). It was retained in a subset of interstitial and endocardial cells during fetal

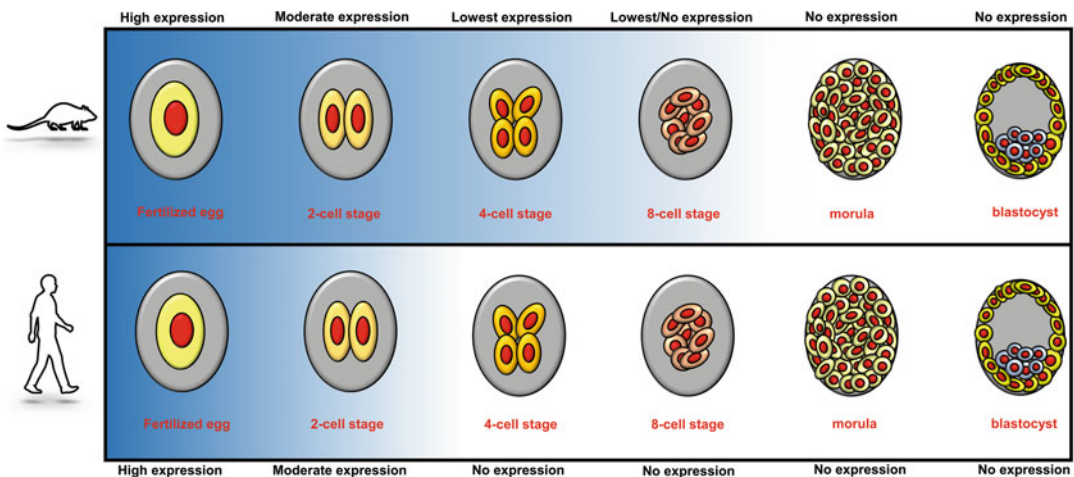


Fig. 4 Expression of GLIS1 during embryonic development. In both mouse and human, high expression of GLIS1 is observed in unfertilized oocyte and one-cell stage. In mouse, a moderate expression is seen in the

two-cell stage, while with the following stages, the expression becomes untraceable. In humans, a moderate expression is seen in the two-cell stage while the expression becomes untraceable in the following stages

gestation. The expression became much weaker during 6 months of age, with weaker expression detected in the myocytes, epicardium, and endocardium of the ventricular myocardium. This suggests that GLIS1 is embryonically regulated and likely to be involved in the regulation of valve morphogenesis during early development (Yu et al. 2019). Furthermore, it was reported that in adult mouse tissues, GLIS1 mRNA is highly expressed in the placenta and kidney, whereas its expression is low in testes, brain, colon, brown fat tissue, and thymus (Nakashima et al. 2002).

Downregulation of GLIS1 in bovine embryos led to failed zygotic genome activation, causing developmental arrest at the 16-cell stage (Takahashi et al. 2015). Studies reported that pyruvate dehydrogenase 1 α and heat shock cognate protein 70 (HSPA8) gene expression was downregulated upon the silencing of *GLIS1* gene (Takahashi et al. 2015). Pyruvate dehydrogenase 1 α helps in murine and bovine embryo development and also in glucose metabolism. HSPA8 codes for heat shock cognate protein 70, which acts as a housekeeping and chaperone protein. This showed the critical importance of GLIS1 in the development of bovine oocytes and embryos (Takahashi et al. 2015).

In humans, GLIS1 is highly expressed in unfertilized eggs and one-cell embryos while it is very low in adult tissues (Fig. 4) (Jetten et al. 2022; Maekawa and Yamanaka 2011; Nakashima et al. 2002). A modest level of expression was seen in two-cell embryos and placenta (Jetten 2018; Nakashima et al. 2002). GLIS1 was most abundantly expressed in the kidney, with moderate expression in various other organs such as brain, thymus, adipose tissue, colon, testis, and placenta (Jetten 2018; Nakashima et al. 2002). The role of GLIS1 in human embryo development remains elusive and requires further investigation.

5 Role of GLIS1 in Cancer

GLIS1 belongs to the Glis subfamily of Kruppel-like transcription factors, which are closely related to the GLI family transcription factors

that are part of the Hedgehog signaling pathway (Kim et al. 2003). These transcription factors are involved in the initiation and regulation of multiple oncogenic signaling pathways (Didiasova et al. 2018). Furthermore, the GLI genes, *GLI2* and *GLI3*, were found to be highly associated with human cancers (Dahmane et al. 1997; Sheng et al. 2002). These transcription factors play a pivotal role in regulating biological processes, which are critical during oncogenesis such as stem cell renewal, differentiation, inflammatory responses, epithelial–mesenchymal transition (EMT), and cell proliferation by regulating gene expression (Dahmane et al. 1997; Sheng et al. 2002). Previous studies reported that GLIS1 protein was associated with several cancers such as breast cancer, colorectal cancer, thyroid tumor, ovarian cancer, hepatocellular carcinoma, and leukemia (Jetten et al. 2022).

GLIS1 was reported to be highly expressed in tumors with elevated Wnt gene expression (Vadnais et al. 2014). Interestingly, GLIS1 was found to act as an oncogene in breast cancer through its cooperation with Cux1 as it activated Wnt genes in human breast cancer cells via autocrine activation of Wnt/ β -catenin pathway (Fig. 5a) (Vadnais et al. 2014). The elevated Wnt expression can be associated with high levels of GLIS1 along with the factor Cux1, which led to EMT (Vadnais et al. 2014). GLIS1 stimulates T cell factor or β -catenin transcriptional activity and helps in increasing cell migration and invasion (Vadnais et al. 2014). Moreover, high expression of GLIS1 in mammary tumor cells led to the activation of several Wnt genes by increasing expression of Wnt ligands such as Wnt3, Wnt4, Wnt5b, Wnt6, Wnt9a, Wnt10a, and Wnt10b in MCF10A cells (Fig. 5a) (Vadnais et al. 2014). It is known that higher expression of Wnt ligands is associated with autocrine activation of Wnt/ β -catenin pathway that is seen in different cancers, especially breast tumors (Vadnais et al. 2014). When both GLIS1 and CUX1 were co-expressed, the highest expression of the Wnt expression was observed, along with an increase in the migration and invasion rate in mammary epithelial cells and enhanced mesenchymal–epithelial transition (MET) (Vadnais et al. 2014). Furthermore, in colorectal cancer, difference in hypermethylation of

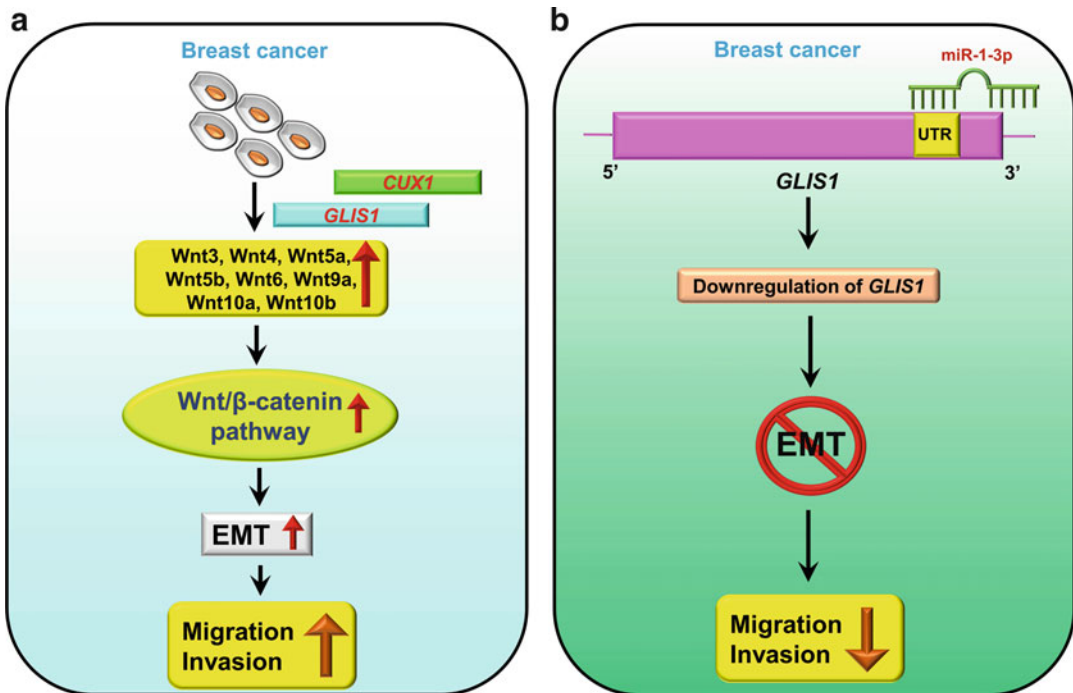


Fig. 5 GLIS1 as an oncogene in breast cancer. (a) GLIS1 cooperates with CUX1 to increase the migration and invasion in breast cancer cells. (b) microRNA 1–3p binds to 3'

untranslated regions of GLIS1 to repress its function, leading to a decrease in migration and invasion in breast cancer cells

GLIS1 gene was seen along with four other genes involved in Wnt signaling without adenomatous polyposis coli mutation, suggesting a cancer formation mechanism in these tumors (Xicola et al. 2018). In contrary to its weak expression in adult tissues, high expression of *GLIS1* was observed in cancers (Vadnais et al. 2014; Xicola et al. 2018).

GLIS1 is expressed in breast cancer tissues and is an important downstream target gene of microRNA 1-3p, which is a small noncoding RNA that regulates mRNA posttranscriptionally (Tao et al. 2021). Repression of *GLIS1* by microRNA 1-3p by binding to 3' untranslated regions led to impairment in progression and metastasis of breast cancer cells (Fig. 5b) (Tao et al. 2021). This indicates that *GLIS1* downregulation has antitumor and antimetastatic functions. *GLIS1* silencing in breast cancer led to a decline in cell migration and invasion along with the blocking of EMT (Fig. 5b) (Tao et al. 2021).

Previous studies reported *GLIS1* as an important gene in the metastasis process (Kim et al. 2022). In a study to identify cancer-associated fibroblast (CAF)-related genes that regulate progression and metastasis in the tumor microenvironment in ovarian serous carcinoma, it was identified that *GLIS1* expression level increased both mRNA and protein levels in metastatic cancer-associated fibroblasts (CAFs). It was shown that *GLIS1* overexpression facilitated migration, invasion, and wound healing along with peritoneal metastasis in ovarian cancer cells (Fig. 6) (Kim et al. 2022). Moreover, metastasis of ovarian cancer can be inhibited by targeting *GLIS1* in CAFs, thus making this a potent therapeutic target. On silencing *GLIS1*, properties such as motility, adhesion, angiogenesis, and metastasis of ovarian cancer cells were reduced both in vitro and in vivo (Fig. 6) (Kim et al. 2022).

Significantly, *GLIS1* is evident in the regulation of several features that can be associated with

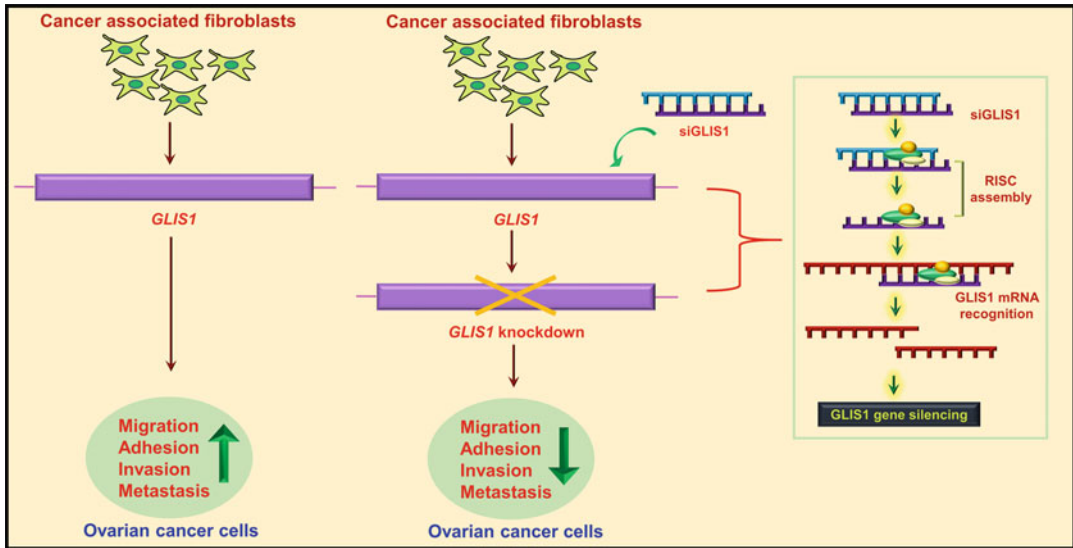


Fig. 6 GLIS1 increased metastasis in ovarian cancer cells. In CAFs, GLIS1 overexpression increased migration, invasion, adhesion, and metastasis of ovarian cancer

cells. Upon the knockdown of GLIS1, the migration, invasion, adhesion, and metastasis were reduced in ovarian cancer cells

malignancy such as EMT and risk of relapse (Chen et al. 2015). In high hyper diploid acute lymphoblastic leukemia (ALL) patients, it was identified that nucleotide variations downstream of GLIS1, which is a cell fate-determining transcription factor, created developmental disturbance. These variations led to increased risk of relapse in patients with high hyper-diploid ALL (Chen et al. 2015). Somatic mutations were also detected in GLIS1 along with other signaling molecules that were involved in B cell differentiation, proliferation, and cell death, and mutation in *GLIS1* gene was deleterious in ALL cases (Chen et al. 2015).

Apart from these, GLIS1 was found to have a significant association with hyalinizing trabecular tumor (HTT), and therefore reclassifying HTT as “GLIS-rearranged hyalinizing trabecular adenoma” (Nikiforova et al. 2019). In this study, it was demonstrated that rearrangements of GLIS and PAX8 genes are a genetic hallmark of HTT. Gene fusions of GLIS1 or GLIS3 to PAX8 were formed by inter-chromosomal rearrangement, leading to overexpression of 3' portion of GLIS genes containing intact DNA-binding zinc finger domain of these transcription factors (Nikiforova

et al. 2019). Moreover, it was shown that GLIS1 fusion was highly specific in HTT, and also the malignant transformation is higher in tumors with GLIS1 fusion than GLIS3 fusion. This specificity and sensitivity of these fusions to HTT led to a remarkable improvement in the clinical management of patients, by detecting GLIS1 fusion in their samples by next-generation sequencing (Nikiforova et al. 2019). To pinpoint, GLIS fusion detection could be used in diagnosing HTT.

Furthermore, GLIS1 has promising roles in immunotherapy for hepatocellular carcinoma (Rong et al. 2023). In hepatocellular carcinoma, GLIS1 regulates the SGK-STAT3-PD1 pathway and promotes CD8⁺ T cell exhaustion. It was reported that the downregulation of GLIS1 led to a change in the infiltration ability of CD8⁺ T cells, blocked cancer development, and raised the anti-PD1 reaction of CD8⁺ T cells (Rong et al. 2023). Thus, GLIS1 can be a potential target for enabling anticancer immunity by the reversal of T cell exhaustion (Rong et al. 2023).

The current treatment strategies for cancer involves chemotherapy, radiotherapy, and surgery. An early diagnosis of cancer can help in a

permanent cure, which is not available till now. Further exploration of the roles of GLIS1 in several cancers, and on using other biomarkers can detect cancer at an early stage, thus paving way to find a permanent cure.

6 Role of GLIS1 in iPSCs

The discovery of iPSCs revolutionized the field of regenerative medicine by unveiling a new research area in stem cell biology. The functional and molecular similarity of iPSCs to ESCs made them potential cell resources for disease modeling, drug discovery, toxicology screening, and autologous cell therapy (Thummer and Kaveeshwar 2023). Moreover, iPSCs can be used as a model to study tumorigenesis and drug resistance and early human development and as a promising tool for regenerative medicine (Ho et al. 2018; Thummer and Kaveeshwar 2023; Shi et al. 2017). Similar gene expression profiles of ESCs and iPSCs opened new frontiers

to identify different pluripotency-associated genes, playing a role in embryonic development, helping overcome the ethical concerns associated with the use of embryos.

Human iPSCs were first generated by transducing OCT4, SOX2, KLF4, and c-MYC (OSKM) (Takahashi et al. 2007; Yu et al. 2007). The application of using human iPSCs in clinical therapies were restricted due to the presence of oncogenic factors such as c-MYC and KLF4 (Akifuji et al. 2021; Okita et al. 2007). To overcome this limitation and to generate human iPSCs, Maekawa and group evaluated a library of 1437 factors, out of which 18 factors including GLIS1 were found to have the ability to replace c-MYC by reprogramming human skin fibroblasts to iPSCs (Maekawa et al. 2011). In this study, the potential of GLIS1 in cellular reprogramming was highlighted as it markedly increased the number of iPSC colonies when co-introduced with OCT4, SOX2, and KLF4 (Fig. 7) (Maekawa et al. 2011). Furthermore, replacing c-MYC with GLIS1 increased the

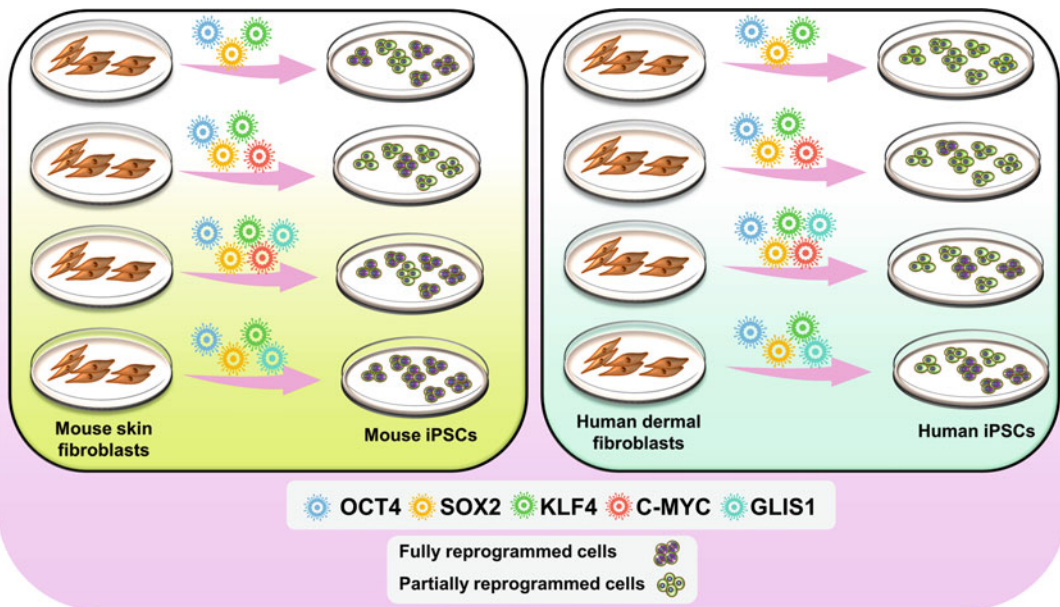


Fig. 7 Effect of GLIS1 on the generation of iPSCs. GLIS1 when used along with the Yamanaka factors increased the efficiency of iPSC generation. Additionally, GLIS1 replaced c-MYC in Yamanaka cocktail and

generated 100% fully reprogrammed iPSCs in the case of mouse and 50% fully reprogrammed iPSCs in the case of human

yield and efficiency of iPSC generation and prevented the generation of transformed colonies. Furthermore, it increased the reprogramming efficiency when synergistically expressed with c-MYC in both mouse and human dermal fibroblasts. Moreover, GLIS1 rescinds the tumorigenic effect by avoiding the potential genomic instability induced by c-MYC when co-expressed (Maekawa et al. 2011). To validate the efficacy of GLIS1 to generate iPSCs from human cancer cells, ovarian adenocarcinoma cells (PEO4) were reprogrammed using OKSG (Bindhya et al. 2021). Twofold increase and larger iPSC colonies were generated on replacing OSKM with OKSG. Notably, GLIS1 successfully replaced c-MYC in reprogramming ovarian cancer cell line and fallopian epithelial cells to iPSCs (Bindhya et al. 2021). A similar study reported the generation of iPSCs from newborn and adult human fibroblasts efficiently using OSKM (Yoshioka and Dowdy 2017). GLIS1 was found to be a successful candidate in the development of cancer model systems, where the elimination of oncogenic factors such as c-MYC is a prerequisite.

Similar reprogramming studies have shown the reprogramming of urine-derived cells to iPSCs using a combination of six factors OCT4, GLIS1, KLF4, SOX2, L-MYC, and miR-302, obviating the use of tumorigenic factors such as c-MYC, SV40-LT, and p53 inhibitor (Wang et al. 2017). Furthermore, the synergistic use of GLIS1 with OSKM cocktail increased the iPSC colonies significantly especially in old human fibroblasts and can be superior to a six-factor combination that includes LIN28A along with OSKM and a four-factor combination using OSKM or OSKG (Yoshioka and Dowdy 2017). Similarly, an increase in iPSC colonies was seen in mouse fibroblasts when OSKM cocktail was used for reprogramming (Yoshioka and Dowdy 2017). Notably, the chimeric mice that were generated from iPSCs induced using OKSG had longer survival times than those from OSKM (Maekawa et al. 2011). In human fibroblasts, similar colonies with better phenotype were obtained using OSKM and OSKM than OSKM cocktail (Yoshioka and Dowdy 2017). In a quest to find other

reprogramming cocktail, it was found that GLIS1 in combination with six factors (JDP2, JHDM1B, MKK6, GLIS1, NANOG, ESSRB, and SALL4) could reprogram mouse embryonic fibroblasts to iPSCs with high quality and efficiency, thus providing an alternative to Yamanaka factors for achieving pluripotency (Wang et al. 2019). These seven-factor colonies were easier to pick and passage to establish iPSC lines. Compared to OKSM factors, these seven-factor iPSC colonies containing GLIS1 are of high-grade ESC quality (Wang et al. 2019). Furthermore, these factors produced high-quality chimeras better than OSKM iPSCs, which support germline transmission and tetraploid complementation. GLIS1 was reported to function as a chromatin remodeler, thereby opening the chromatin structure to assist reprogramming (Wang et al. 2019). Other studies have also reported that iPSC lines can be developed from the fibroblast of cystic fibrosis (CF) patients with mutation in cystic fibrosis transmembrane conductance regulator (*CFTR*) gene by using GLIS1 along with OSKM factors for reprogramming (Kondrateva et al. 2021a, b). Two iPSC cell lines (RCMGi004-A and -B) were derived from human skin fibroblasts of CF patients with heterozygous mutation in *CFTR* gene when GLIS1 along with OKSM factors were used for reprogramming (Kondrateva et al. 2021b). These cell lines showed the potential to differentiate into all three germ layers and expressed all ectodermal, endodermal, and mesodermal markers (Kondrateva et al. 2021b). Another study also showed the derivation of iPSC cell line (RCMGi002-A) from dermal fibroblast of CF patients with a homozygous mutation in the *CFTR* gene. Both these cell lines can be used for disease modeling, drug screening, and genome editing for the personalized treatment of CF (Kondrateva et al. 2021a, b).

Besides iPSCs, the induced tissue-specific stem cells, which does not have the risk of teratoma formation can also be developed from mouse pancreatic tissues using OSKM factors, utilizing single synthetic self-replicative Venezuelan equine encephalitis reprogramming factor RNA replicon (Miyagi-Shiohira et al.

2018). Similarly, induced tissue-specific stem cells were generated from aged mesenchymal cells derived from adipose tissues by expressing OSKG factors. Furthermore, clonal human iPSC colonies were obtained on reprogramming urine-derived cells with OSKMG factors using self-replicative RNA as vector by obtaining a reprogramming efficiency of 0.008–0.17% in a single experiment (Miyagi-Shiohira et al. 2018).

GLIS1 functions as a strong promoter of somatic cell reprogramming by directly interacting with zinc finger domain of KLF4 via its N-terminal domain. It also induced the expression of the forkhead box, FOXA2, and several *Wnt* genes to enhance MET to promote reprogramming (Maekawa et al. 2011). FOXA2 is a transcription factor that antagonizes EMT, which is a prerequisite for iPSC colony formation. GLIS1 increased the expression of several genes such as ESRRB, WNT3, WNT6, WNT8A and WNT10A, LIN281, NANOG, MYCN, MYCL, TSPAN, NRG1, and FOXA2, which can also increase iPSC colony formation (Maekawa et al. 2011). MYCN and MYCL were regulated directly by GLIS1, whereas FOXA2, ESRRB1, and LIN28A transcription was regulated indirectly (Maekawa et al. 2011). GLIS1 also promoted multiple preprogramming pathways including *Wnt* signaling and regulated several genes involved in MET. It also induced multilevel epigenetic and metabolic remodeling in stem cells. During somatic cell reprogramming, GLIS1 indicated a unique multilevel path to pluripotency by initiating an epigenome-metabolome-epigenome cascade without affecting MET (Li et al. 2020). It led to the repression of somatic genes at the chromatin level. Also, it binds to pluripotent and glycolysis genes and opens them, which resulted in the activation of glycolysis and increase in lactate and acetyl-coenzyme A levels (Li et al. 2020). During acquiring pluripotency, glycolysis genes were key targets of GLIS1. Thus, GLIS1 bound to most glycolysis genes, activated their expression, and enhanced reprogramming by upregulating glycolytic activity (Li et al. 2020). It also increased histone acetylation and deacetylation on promoters of pluripotency genes (Li et al.

2020). Furthermore, the metabolites of glycolysis pathway and tricarboxylic acid cycle increased in the presence of GLIS1, and upon its overexpression, there was an increase in the levels of nucleotide-related molecules except adenosine triphosphate, increase in acetyl-coenzyme A levels, glucose uptake, and secretion of lactate. This study suggested that GLIS1 modulated histone acetylation and lactylation during somatic cell reprogramming by increasing their levels on promoters of pluripotency genes and activating their expression (Li et al. 2020). GLIS1 facilitates a complex sequential closed to open chromatin shift of glycolysis genes, leading to similar shift of pluripotent genes (Li et al. 2020). This study shed light to understand the function of GLIS1 during embryonic development and cancer with respect to the epigenome-metabolome-epigenome cascade mediated by it, as the level of glucose and pentose phosphate pathway metabolized are low in oocytes, and it increases on sperm entry, and cancer also relies on glycolysis to supply energy (Li et al. 2020). In total, 19,494 binding sites were identified for GLIS1 that displayed enrichment close to transcription start site and upstream regions of genes (Li et al. 2020). The genes involved in the glycolytic process such as *Hk2*, *Pgk1*, *Pfk1*, *Pkm*, *Eno1*, and *Ldha* were upregulated, and some of the genes involved in maintaining somatic state such as *Setbp1*, *Thy1*, *Illrn*, *Prss23*, *Col6a2*, *Col6a3*, and *Coll1a1* were downregulated when GLIS1 was used for reprogramming (Li et al. 2020). There was a dual binding mechanism of GLIS1 to upregulated and downregulated gene targets that activated glycolytic process by increasing the expression level of associated genes and suppressing somatic state by inhibiting the gene expression (Li et al. 2020). Studies also identified that GLIS1 enables reprogramming of senescent cells (senescent mouse embryonic fibroblasts) into pluripotent cells and improves the genomic stability, suggesting that it can be used in regenerative medicine for elderly subjects (Li et al. 2020). Furthermore, it reduced p16 expression and alleviated senescent phenotype.

GLIS1 can be a better tool for iPSC technology as it generates completely reprogrammed cells by

suppressing partially reprogrammed cells that still expresses GLIS1. Also, reprogramming by GLIS1 is independent of the cell type and method used (Li et al. 2020). Additionally, it specifically promoted iPSCs and not partially reprogrammed or transformed cells (Maekawa et al. 2011), primarily because it suppressed proliferation when expressed in cells that do not express it. GLIS1 had similar effects in reprogramming in both mice and humans. Moreover, on expressing GLIS1 with OSK factors in mouse fibroblasts, completely reprogrammed cells were produced, and iPSC colonies increased from 10% to 50% in human fibroblasts (Maekawa et al. 2011; Maekawa and Yamanaka 2011). More iPSCs were formed when OSK factors were used, which were larger in size and tightly packed with defined borders compared to those derived from the OSKM combination (Maekawa et al. 2011). GLIS1 increased reprogramming twofold as compared to OSKM and 30-fold as compared to OKS factors, and therefore, it is recognized as an enhancer of reprogramming. It is a powerful reprogramming factor and is called the “fifth Yamanaka reprogramming factor,” thus having great clinical potential. (Li et al. 2020).

7 Conclusion and Future Perspectives

GLIS1 is a nuclear localized protein, which acts as an activator and repressor of gene transcription. It is expressed in unfertilized oocytes and one-cell embryo of both mice and humans. GLIS1 regulates different functions related to cell adhesion, proliferation, differentiation, extracellular matrix, and chemotaxis. Even though not much is known about the physiological functions of GLIS1 in adult tissues, it was found to be associated with different types of cancers and is still elusive in the cancer paradigm. The suppression of GLIS1 was reported to be associated with suppressed carcinoma, metastatic developments, and CD8⁺ T cell exhaustion (Tao et al. 2021; Shimamoto et al. 2020; Kim et al. 2022; Rong et al. 2023). Association of GLIS1 with Wnt and cell cycle pathways (Shimamoto et al. 2020) and its link with SGK1-STAT3-PD1 pathways in

eliciting immune suppressive microenvironments and activation of immune cells, such as natural killer cells, dendritic cells, monocytes, and macrophages upon its downregulation (Rong et al. 2023), opens a plethora of avenues for potential research. Elucidating the role of GLIS1 in the regulation of extracellular vesicles and modulation of immune microenvironment and their role in tumor progression can help generate effective anticancer immunotherapies in near future. Besides cancer, a significant association between a GLIS1 variant and glaucoma was identified in humans (Nair et al. 2021). GLIS1 has an important role in regulating functions of trabecular meshwork whose dysfunction leads to chronically elevated intraocular pressure (Nair et al. 2021). This is a risk factor in glaucoma, which results in blindness. GLIS1 helps in maintaining the structure of trabecular meshwork, aqueous humor dynamics, and normal intraocular pressure, and therefore it was identified as a glaucoma-risk gene (Nair et al. 2021).

The role of GLIS1 in reprogramming fibroblasts and other somatic cells to iPSCs have been established, where several methods can be developed using the potential of GLIS1 for producing clinically safe iPSCs for disease modeling and transplantation applications (Borghain et al. 2019; Dey et al. 2021a, 2022; Haridhasapavalan et al. 2019; Saha et al. 2018). Cell-permeant version of GLIS1 (Dey et al. 2021b) along with OCT4 (Dey et al. 2021c), SOX2 (Thool et al. 2021), NANOG (Thool et al. 2022b), and other reprogramming factors (Raina et al. 2021; Thool et al. 2022a) can be used to derive clinical grade iPSCs for cell therapeutic applications. It is found to replace the proto oncogenes c-MYC and KLF4 in Yamanaka cocktail, thus making it an important factor in reprogramming. The ability of GLIS1 in iPSC production also sheds light about its endogenous role in multipotent stem cells other than pluripotent ESCs (Gérard et al. 2019). GLIS1 is a mesenchymal key transcription factor that is controlled by dynamic cell type-specific super-enhancers that become repressed in both lineages (Gérard et al. 2019). GLIS1 can control differentiation-induced genes; moreover, its overexpression can inhibit the lineage commitment of specific multipotent cells (Gérard et al.

2019). GLIS1 was identified as a central regulator of mesenchymal potency, and it was found to have an important role in adipogenesis (Gérard et al. 2019). Therefore, it is suggested that GLIS1 might contribute to the maintenance of the multipotent state in human development.

The exact mechanisms by which GLIS1 helps in increasing the reprogramming efficiency have not been reported. Online bioinformatics tools can be used for predicting the gene network and structure, which can help researchers identify interacting partners of GLIS1. Although GLIS1 is closely related to GLI-like protein subfamily, an insight into the role of its interacting proteins might give more information about the mechanisms and other roles of GLIS1. The identification of a single nucleotide polymorphism of *GLIS1* gene is linked to Parkinson's disease and that *GLIS1* gene is located in PARK10 loci suggests the association of GLIS1 with Parkinson's disease even though the gene involvement is unclear (Song et al. 2012). Also, studies can be conducted to find the association of *GLIS1* gene in diseases other than cancer. Other members of the GLIS family are found to be associated with primary ciliogenesis, while the connection between GLIS1 and primary cilium has to be established. Targeting GLIS1 in tissues where it is overexpressed and lead to tumor formation can be a potential strategy to help in improving the efficacy of treating cancer. In this review, the multifaceted role of GLIS1 in embryonic development, cancer, and iPSCs is highlighted. The availability of secondary antibodies and unveiling the physiological mechanism would make GLIS1 to be utilized in the fields of stem cell biology, reprogramming, cancer, and other diseases and understanding the exact role in these areas of research.

Competing Interests The authors declare that they have no potential conflict of interest.

Author Contribution RKR and CD wrote the original draft of the manuscript under the guidance of RPT. RPT reviewed and edited the draft. All authors read and approved the final draft of the manuscript and gave consent to submit it for publication.

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References

- Akifuji C, Iwasaki M, Kawahara Y, Sakurai C, Cheng YS, Imai T, Nakagawa M (2021) MYCL promotes iPSC-like colony formation via MYC Box 0 and 2 domains. *Sci Rep* 11:1–30. <https://doi.org/10.1038/s41598-021-03260-5>
- Bindhya S, Sidhanth C, Krishnapriya S, Garg M, Ganesan TS (2021) Development and in vitro characterisation of an induced pluripotent stem cell model of ovarian cancer. *Int J Biochem Cell Biol* 138:106051. <https://doi.org/10.1016/j.biocel.2021.106051>
- Borghain MP, Haridhasapavalan KK, Dey C, Adhikari P, Thummer RP (2019) An insight into DNA-free reprogramming approaches to generate integration-free induced pluripotent stem cells for prospective biomedical applications. *Stem Cell Rev Rep* 15:286–313. <https://doi.org/10.1007/s12015-018-9861-6>
- Chen C, Bartenhagen C, Gombert M, Okpanyi V, Binder V, Röttgers S, Bradtke J, Teigler-Schlegel A, Harbott J, Ginzel S, Thiele R, Husemann P, Krell PFI, Borkhardt A, Dugas M, Hu J, Fischer U (2015) Next-generation-sequencing of recurrent childhood high hyperdiploid acute lymphoblastic leukemia reveals mutations typically associated with high risk patients. *Leuk Res* 39:990–1001. <https://doi.org/10.1016/j.leukres.2015.06.005>
- Chin KC, Li GGX, Ting JPY (1997) Importance of acidic, proline/serine/threonine-rich, and GTP-binding regions in the major histocompatibility complex class II transactivator: generation of transdominant-negative mutants. *Proc Natl Acad Sci U S A* 94:2501–2506. <https://doi.org/10.1073/pnas.94.6.2501>
- Dahmane N, Lee J, Robins P, Heller P, Ruiz i Altaba A (1997) Activation of the transcription factor Gli1 and

- the Sonic hedgehog signalling pathway in skin tumours. *Nature* 389:876–881. <https://doi.org/10.1038/39918>
- Dey C, Raina K, Haridhasapavalan KK, Thool M, Sundaravadivelu PK, Adhikari P, Gogoi R, Thummer RP (2021a) An overview of reprogramming approaches to derive integration-free induced pluripotent stem cells for prospective biomedical applications. In: *Recent advances in iPSC technology*. Elsevier, pp 231–287. <https://doi.org/10.1016/B978-0-12-822231-7.00011-4>
- Dey C, Raina K, Thool M, Adhikari P, Haridhasapavalan KK, Sundaravadivelu PK, Venkatesan V, Gogoi R, Sudhagar S, Thummer RP (2021b) Auxiliary pluripotency-associated genes and their contributions in the generation of induced pluripotent stem cells. In: *Molecular players in iPSC technology*. Elsevier, pp 29–94. <https://doi.org/10.1016/B978-0-323-90059-1.00007-5>
- Dey C, Thool M, Bhattacharyya S, Sudhagar S, Thummer RP (2021c) Generation of biologically active recombinant human OCT4 protein from *E. coli*. *3 Biotech* 11: 1–16. <https://doi.org/10.1007/s12033-021-02758-z>
- Dey C, Venkatesan V, Thummer RP (2022) Identification of optimal expression parameters and purification of a codon—optimized human GLIS1 transcription factor from *Escherichia coli*. *Mol Biotechnol* 64:42–56. <https://doi.org/10.1007/s12033-021-00390-z>
- Didiasova M, Schaefer L, Wygrecka M (2018) Targeting GLI transcription factors in cancer. *Molecules* 23: 1003. <https://doi.org/10.3390/molecules23051003>
- Garza AS, Ahmad N, Kumar R (2009) Role of intrinsically disordered protein regions/domains in transcriptional regulation. *Life Sci* 84:189–193. <https://doi.org/10.1016/j.lfs.2008.12.002>
- Gérard D, Schmidt F, Ginolhac A, Schmitz M, Halder R, Ebert P, Schulz MH, Sauter T, Sinkkonen L (2019) Temporal enhancer profiling of parallel lineages identifies AHR and GLIS1 as regulators of mesenchymal multipotency. *Nucleic Acids Res* 47:1141–1163. <https://doi.org/10.1093/nar/gky1240>
- Haridhasapavalan KK, Borgohain MP, Dey C, Saha B, Narayan G, Kumar S, Thummer RP (2019) An insight into non-integrative gene delivery approaches to generate transgene-free induced pluripotent stem cells. *Gene* 686: 146–159. <https://doi.org/10.1016/j.gene.2018.11.069>
- Ho BX, Min N, Pek Q, Soh B (2018) Disease modeling using 3D organoids derived from human induced pluripotent stem cells. *Int J Mol Sci*. <https://doi.org/10.3390/ijms19040936>
- Jetten AM (2018) GLIS1–3 transcription factors: critical roles in the regulation of multiple physiological processes and diseases. *Cell Mol Life Sci* 75:3473–3494. <https://doi.org/10.1007/s00018-018-2841-9>
- Jetten AM, Scoville DW, Kang HS (2022) GLIS1-3: links to primary cilium, reprogramming, stem cell renewal, and disease. *Cell* 111:1–16. <https://doi.org/10.3390/cells11111833>
- Kim YS, Lewandoski M, Perantoni AO, Kurebayashi S, Nakanishi G, Jetten AM (2002) Identification of Glis1, a novel Gli-related, Krüppel-like zinc finger protein containing transactivation and repressor functions. *J Biol Chem* 277:30901–30913. <https://doi.org/10.1074/jbc.M203563200>
- Kim YS, Nakanishi G, Lewandoski M, Jetten AM (2003) GLIS3, a novel member of the GLIS subfamily of Krüppel-like zinc finger proteins with repressor and activation functions. *Nucleic Acids Res* 31:5513–5525. <https://doi.org/10.1093/nar/gkg776>
- Kim MJ, Jung D, Park JY, Lee SM, An HJ (2022) GLIS1 in cancer-associated fibroblasts regulates the migration and invasion of ovarian cancer cells. *Int J Mol Sci* 23: 2218. <https://doi.org/10.3390/ijms23042218>
- Kondrateva E, Demchenko A, Slesarenko Y, Pozhitnova V, Yasinovsky M, Amelina E, Tabakov V, Voronina E, Lavrov A, Smirnikhina S (2021a) Generation of two induced pluripotent stem cell lines (RCMGi004-A and -B) from human skin fibroblasts of a cystic fibrosis patient with compound heterozygous F508del/W1282X mutations in CFTR gene. *Stem Cell Res* 52:102232. <https://doi.org/10.1016/j.scr.2021.102232>
- Kondrateva E, Demchenko A, Slesarenko Y, Yasinovsky M, Amelina E, Tabakov V, Voronina E, Lavrov A, Smirnikhina S (2021b) Derivation of iPSC line (RCMGi002-A) from dermal fibroblasts of a cystic fibrosis female patient with homozygous F508del mutation. *Stem Cell Res* 53:102251. <https://doi.org/10.1016/j.scr.2021.102251>
- Lee SY, Noh HB, Kim HT, Lee KI, Hwang DY (2017) Glis family proteins are differentially implicated in the cellular reprogramming of human somatic cells. *Oncotarget* 8:77041–77049. <https://doi.org/10.18632/oncotarget.20334>
- Li L, Chen K, Wang T, Wu Y, Xing G, Chen M, Hao Z, Zhang C, Zhang J, Ma B, Liu Z, Yuan H, Liu Z, Long Q, Zhou Y, Qi J, Zhao D, Gao M, Pei D, Nie J, Ye D, Pan G, Liu X (2020) Glis1 facilitates induction of pluripotency via an epigenome–metabolome–epigenome signalling cascade. *Nat Metab* 2:882–892. <https://doi.org/10.1038/s42255-020-0267-9>
- Maekawa M, Yamanaka S (2011) Glis1, a unique pro-reprogramming factor, may facilitate clinical applications of iPSC technology. *Cell Cycle* 10: 3613–3614. <https://doi.org/10.4161/cc.10.21.17834>
- Maekawa M, Yamaguchi K, Nakamura T, Shibukawa R, Kodanaka I, Ichisaka T, Kawamura Y, Mochizuki H, Goshima N, Yamanaka S (2011) Direct reprogramming of somatic cells is promoted by maternal transcription factor Glis1. *Nature* 474:225–229. <https://doi.org/10.1038/nature10106>
- Miyagi-Shiohira C, Nakashima Y, Kobayashi N, Saitoh I, Watanabe M, Noguchi H (2018) Characterization of induced tissue-specific stem cells from pancreas by a synthetic self-replicative RNA. *Sci Rep* 8:1–11. <https://doi.org/10.1038/s41598-018-30784-0>

- Nair KS, Srivastava C, Brown RV, Koli S, Choquet H, Kang HS, Kuo YM, Grimm SA, Sutherland C, Badea A, Johnson GA, Zhao Y, Yin J, Okamoto K, Clark G, Borrás J, Zode G, Kizhatil K, Chakrabarti S, John SWM, Jorgenson E, Jetten AM (2021) GLIS1 regulates trabecular meshwork function and intraocular pressure and is associated with glaucoma in humans. *Nat Commun* 12:1–15. <https://doi.org/10.1038/s41467-021-25181-7>
- Nakashima M, Tanese N, Ito M, Auerbach W, Bai C, Furukawa T, Toyono T, Akamine A, Joyner AL (2002) A novel gene, GliH1, with homology to the Gli zinc finger domain not required for mouse development. *Mech Dev* 119:21–34. [https://doi.org/10.1016/S0925-4773\(02\)00291-5](https://doi.org/10.1016/S0925-4773(02)00291-5)
- Naz H, Tarique M, Suhail M, Shankar H, Muhammad N, Usmani D, Ashraf M, Zughaibi TA (2020) Calcium-/calmodulin-dependent protein kinase IV (CAMKIV): a multifunctional enzyme and its role in various cancer: an update. *Curr Mol Biol Rep* 6:139–147. <https://doi.org/10.1007/s40610-020-00138-9>
- Nikiforova MN, Nikiforov YE, Ohori NP (2019) GLIS rearrangements in thyroid nodules: a key to preoperative diagnosis of hyalinizing trabecular tumor. *Cancer Cytopathol* 127:560–566. <https://doi.org/10.1002/cncy.22163>
- Okita K, Ichisaka T, Yamanaka S (2007) Generation of germline-competent induced pluripotent stem cells. *Nature* 448:313–318. <https://doi.org/10.1038/nature05934>
- Park SW, Hu X, Gupta P, Lin Y-P, Ha SG, Wei L-N (2007) SUMOylation of Tr2 orphan receptor involves Pml and fine-tunes Oct4 expression in stem cells. *Nat Struct Mol Biol* 14:68–75. <https://doi.org/10.1038/nsmb1185>
- Poh YC, Chen J, Hong Y, Yi H, Zhang S, Chen J, Wu DC, Wang L, Jia Q, Singh R, Yao W, Tan Y, Tajik A, Tanaka TS, Wang N (2014) Generation of organized germ layers from a single mouse embryonic stem cell. *Nat Commun* 5(1):1–12. <https://doi.org/10.1038/ncomms5000>
- Raina K, Dey C, Thool M, Sudhagar S, Thummer RP (2021) An insight into the role of UTF1 in development, stem cells, and cancer. *Stem Cell Rev Rep* 17:1280–1293. <https://doi.org/10.1007/s12015-021-10127-9>
- Rong D, Wang Y, Liu L, Cao H, Huang T, Liu H, Hao X, Sun G, Sun G, Zheng Z, Kang J, Xia Y, Chen Z, Tang W, Wang X (2023) GLIS1 intervention enhances anti-therapy for hepatocellular carcinoma by targeting SGK1-STAT3-PD1 pathway. *BMJ* 3:1–15. <https://doi.org/10.1136/jitc-2022-005126>
- Roobrouck VD, Ulloa-Montoya F, Verfaillie CM (2008) Self-renewal and differentiation capacity of young and aged stem cells. *Exp Cell Res* 314:1937–1944. <https://doi.org/10.1016/j.yexcr.2008.03.006>
- Saha B, Krishna Kumar H, Borgohain MP, Thummer RP (2018) Prospective applications of induced pluripotent stem cells in military medicine. *Med J Armed Forces India* 74:313–320. <https://doi.org/10.1016/j.mjafi.2018.03.005>
- Schwab M (2004) MYCN in neuronal tumours. *Cancer Lett* 204:179–187. [https://doi.org/10.1016/S0304-3835\(03\)00454-3](https://doi.org/10.1016/S0304-3835(03)00454-3)
- Scoville DW, Kang HS, Jetten AM (2017) GLIS1-3: emerging roles in reprogramming, stem and progenitor cell differentiation and maintenance. *Stem Cell Investig* 4:1–11. <https://doi.org/10.21037/sci.2017.09.01>
- Sheng H, Goich S, Wang A, Grachtchouk M, Lowe L, Mo R, Lin K, De Sauvage FJ, Sasaki H, Hui CC, Dlugosz AA (2002) Dissecting the oncogenic potential of Gli2: deletion of an NH2-terminal fragment alters skin tumor phenotype. *Cancer Res* 62:5308–5316
- Shi ZD, Lee K, Yang D, Amin S, Verma N, Li QV, Zhu Z, Soh CL, Kumar R, Evans T, Chen S, Huangfu D (2017) Genome editing in hPSCs reveals GATA6 haploinsufficiency and a genetic interaction with GATA4 in human pancreatic development. *Cell Stem Cell* 20:675–688.e6. <https://doi.org/10.1016/j.stem.2017.01.001>
- Shimamoto K, Tanimoto K, Fukazawa T, Nakamura H, Kanai A, Bono H, Ono H, Eguchi H, Hirohashi N (2020) GLIS1, a novel hypoxia-inducible transcription factor, promotes breast cancer cell motility via activation of WNT5A. *Carcinogenesis* 41:1184–1194. <https://doi.org/10.1093/carcin/bgaa010>
- Song W, Chen YP, Huang R, Chen K, Pan PL, Li J, Yang Y, Shang HF (2012) GLIS1 rs797906: an increased risk factor for late-onset Parkinson's disease in the Han Chinese population. *Eur Neurol* 68:89–92. <https://doi.org/10.1159/000337955>
- Sotiropoulou G, Pampalakis G, Diamandis EP (2009) Functional roles of human. *J Biol Chem* 284:32989–32994. <https://doi.org/10.1074/jbc.R109.027946>
- Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663–676. <https://doi.org/10.1016/j.cell.2006.07.024>
- Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131:861–872. <https://doi.org/10.1016/j.cell.2007.11.019>
- Takahashi K, Sakurai N, Emura N, Hashizume T, Sawai K (2015) Effects of downregulating GLIS1 transcript on preimplantation development and gene expression of bovine embryos. *J Reprod Dev* 61:369–374. <https://doi.org/10.1262/jrd.2015-029>
- Takenaka K (2007) GSK3 b positively regulates Hedgehog signaling through Sufu in mammalian cells. *Biochem Biophys Res Commun* 353:501–508. <https://doi.org/10.1016/j.bbrc.2006.12.058>
- Tanabe O (2002) An embryonic/fetal beta-type globin gene repressor contains a nuclear receptor TR2/TR4 heterodimer. *EMBO J* 21:3434–3442. <https://doi.org/10.1093/emboj/cdf340>
- Tanabe O, McPhee D, Shen Y, Jiang X, Campbell AD, Chen Y, Chang C, Yamamoto M, Tanimoto K, Engel JD (2007) Embryonic and fetal beta-globin gene repression by the orphan nuclear receptors, TR2 and TR4. *EMBO J* 26:2295–2306. <https://doi.org/10.1038/sj.emboj.7601676>

- Tao S, Li H, Ma X, Ma Y, He J, Gao Y, Li J (2021) Elevating microRNA-1-3p shuttled by cancer-associated fibroblasts-derived extracellular vesicles suppresses breast cancer progression and metastasis by inhibiting GLIS1. *Cancer Gene Ther* 28:634–648. <https://doi.org/10.1038/s41417-020-00244-x>
- Thool M, Dey C, Bhattacharyya S, Sudhagar S, Thummer RP (2021) Generation of a recombinant stem cell-specific human SOX2 protein from *Escherichia coli* under native conditions. *Mol Biotechnol* 63:327–338. <https://doi.org/10.1007/s12033-021-00305-y>
- Thool M, Sundaravivelu PK, Sudhagar S, Thummer RP (2022a) A comprehensive review on the role of ZSCAN4 in embryonic development, stem cells, and cancer. *Stem Cell Rev Rep* 18:2740–2756. <https://doi.org/10.1007/s12015-022-10412-1>
- Thool M, Sudhagar S, Thummer RP (2022b) Soluble expression and purification of biologically active human NANOG from *Escherichia coli*. In: North-East research conclave. Springer Nature, Singapore, pp 99–118. https://doi.org/10.1007/978-981-99-4056-1_6
- Thummer RP, Kaveeshwar VNU (2023) Induced pluripotent stem cell biobanking: a future perspective for regenerative and personalized medicine. *Bio Bank Afford Healthc Pract Res*. ISBN: 978-8195573028
- Vadnais C, Shooshtarizadeh P, Rajadurai CV, Lesurf R, Hulea L, Davoudi S, Cadieux C, Hallett M, Park M, Nepveu A (2014) Autocrine activation of the Wnt/b-catenin pathway by CUX1 and GLIS1 in breast cancers. *Biol Open* 3:937–946. <https://doi.org/10.1242/bio.20148193>
- Wang L, Chen Y, Guan C, Zhao Z, Li Q, Yang J, Mo J, Wang B, Wu W, Yang X, Song L, Li J (2017) Using low-risk factors to generate non-integrated human induced pluripotent stem cells from urine-derived cells. *Stem Cell Res Ther* 8:1–13. <https://doi.org/10.1186/s13287-017-0698-8>
- Wang B, Wu L, Li D, Liu Y, Guo J, Li C, Yao Y, Wang Y, Zhao G, Wang X, Fu M, Liu H, Cao S, Wu C, Yu S, Zhou C, Qin Y, Kuang J, Ming J, Chu S, Yang X, Zhu P, Pan G, Chen J, Liu J, Pei D (2019) Induction of pluripotent stem cells from mouse embryonic fibroblasts by Jdp2-Jhdm1b-Mkk6-Glis1-Nanog-Essrb-Sall4. *Cell Rep* 27:3473–3485.e5. <https://doi.org/10.1016/j.celrep.2019.05.068>
- Xicola RM, Manojlovic Z, Augustus GJ, Kupfer SS, Emmadi R, Alagiozian-Angelova V, Triche T, Salhia B, Carpten J, Llor X, Ellis NA (2018) Lack of APC somatic mutation is associated with early-onset colorectal cancer in African Americans. *Carcinogenesis* 39:1331–1341. <https://doi.org/10.1093/carcin/bgy122>
- Yasuoka Y, Matsumoto M, Yagi K, Okazaki Y (2020) Evolutionary history of GLIS genes illuminates their roles in cell reprogramming and ciliogenesis. *Mol Biol Evol* 37:100–109. <https://doi.org/10.1093/molbev/msz205>
- Yoshioka N, Dowdy SF (2017) Enhanced generation of iPSCs from older adult human cells by a synthetic five-factor self-replicative RNA. *PLoS One* 12:1–17. <https://doi.org/10.1371/journal.pone.0182018>
- Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318(80):1917–1920. <https://doi.org/10.1126/science.1151526>
- Yu M, Georges A, Tucker NR, Kyryachenko S, Toomer K, Schott J-J, Delling FN, Fernandez-Friera L, Solis J, Ellinor PT, Levine RA, Slauchhaupt SA, Hagège AA, Dina C, Jeunemaitre X, Milan DJ, Norris RA, Bouatia-Naji N (2019) Genome-wide association study-driven gene-set analyses, genetic, and functional follow-up suggest GLIS1 as a susceptibility gene for mitral valve prolapse. *Circ Genomic Precis Med* 12:139500836. <https://doi.org/10.1161/CIRCGEN.119.002497>