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A comparative study of SOX9, HOXA10 and OCT4 gene expression in human and Zebrafish reproduction and embryogenesis

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ABSTRACT

So far, many studies have been conducted on the importance of expressing different genes in humans and zebrafish. In this regard, the expression of OCT4, HOX and SOX genes as influential genes in the embryonic period is very thought-provoking. At different embryonic stages, including morula, middle blastula, gastrula, and segmentation, the expression of these genes is very important in the growth, immunity, tissue repair, and viability of different cells. The aim of this study was to investigate the expression of common genes between humans and zebrafish in the embryonic period and their efficiency in different parts of the body.

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Abbreviations

SRY, Sex-determining region Y; HOX, homeobox; OCT4, Octamer-binding transcription factor 4; ESC, Embryonic Stem Cell; Pou5f1, POU domain-class 5-transcription factor 1; ICM, Inner Cell Mass; CBX2, Chromobox2; AMH, Anti-Müllerian hormone; CBX2, Chromobox2; EMX2, Empty Spiracles Homeobox 2; TDO, tryptophan 2,3-dioxygenase MSCs; iPSC, induced pluripotent stem cell; MSCs, Mesenchymal Stem Cells; HESCs, Human Embryonic Stem Cells; CDK4.6, cyclin-dependent kinase 4.6; miRNA, microRNA; GADD-45, growth is stunted and the susceptible induction protein DNA-45; DSD, disorders of sexual development; PCOS, polycystic ovarian syndrome; IVF, in vitro fertilization; jef, Jellyfish mutation; crx, Cone-rod homeobox; rs1, Retinoschisis 1; calb2, Calbindin 2; col2α-1a, collagen type II α-1a; col11α-2, collagen type XI α-2; CNS, central nervous system; Zspg, Zygotic pou5f1 loss-of-function mutant spiel ohne Grenzen; TCA, tricarboxylic acid; HK2, Hexokinase 2; PKM2, Pyruvate kinase M2.

Introduction

SOX9 belongs to the SOX family and the SOXE subgroup (1). The SOX9 gene provides instructions for making proteins which play a key role in fetal development. The SOX9 protein plays an important role, particularly in skeletal growth and in determining prenatal sex. The SOX9 protein binds to specific regions of DNA and regulates the activity of other genes, especially those that control skeletal growth and sex determination. Based on the function mentioned, the SOX9 protein is called a transcription factor (2). Studies have been performed on SOX9 in terms of growth, especially during chondrogenesis and the onset of male gonads (3). However, a key role in the stem cell biology of mesoderm-, ectoderm-, and endoderm-derived tissues and organs has been illuminated by recent practical and molecular analyzes (3, 4). Significantly, both adult stem and congenital cells with high turnover are maintained by SOX9 as in intestine and hair follicles, and also that is determining for repairing postpartum damage to the endodermic and ectodermic organs (3, 4). In the first stage, if there is a Y chromosome, the Sex-determining region Y (SRY) gene is expressed in somatic cells of undifferentiated gonad (5). The product of this gene, which is a transcription factor, leads to testicular formation and inhibition of a number of genes involved in determining female sex (5). The formation of the testis and the expression of a number of genes, including SOX9, lead to the production of the Anti-Müllerian hormone (AMH), which inhibits the formation of the Müllerian ducts and eventually, by secreting testosterone and other male sex hormones, the internal structures of the male reproductive system are formed (5-7).

Homeobox (HOX) genes encode transcription factors that are needed for development and are evolutionarily conserved (8, 9).In the developing reproductive system, four HOXA genes (HOXA9, HOXA10, HOXA11, and HOXA13) are expressed. The primordia of the fallopian tube and developing uterus express HOXA9 and HOXA10, respectively. The lower uterine segment and cervix express HOXA11, while the developing upper vagina expresses HOXA13 (8, 10). Since HOXA10 regulates a number of downstream genes, including cell adhesion molecules, signal transduction factors, and metabolic mediators, its function is critical for normal uterine embryogenesis and menstrual cycle regulation (11, 12).

Octamer-binding transcription factor 4 (OCT4) is a transcription factor for Embryonic Stem Cell (ESC) pluripotency and reprogramming that is encoded by the POU domain, class 5, transcription factor 1 (Pou5f1) gene (13). OCT4A, OCT4B and OCT4B1 are three isoforms of OCT4 that are generated in humans by the OTF3 gene (14). OCT4 is required for early embryonic development in terms of function (14, 15). Embryos that lack OCT4 die during uterine wall replacement due to a lack of growing Inner Cell Mass (ICM) cells (14, 16). Thus, OCT4 is considered as the main regulator of pluripotent cell start and maintenance during embryonic development. Interestingly, the exact level of OCT4 expression is an important predictor of ESC fates, and their potential can only be sustained if OCT4 expression is kept at a normal level (14, 17-19).

Mechanism of action and role of SOX9 gene in human

SOX9 is involved in sex evolution. In general, sex evolution takes place in two stages: in the first stage, when sex determination occurs, the primary gonad is able to distinguish between two types of testis or ovary, and in the next stage, sexual differentiation is mediated by the secretion of relevant sex hormones and the formation of internal and external sexual organs in the individual (5). At the end of these two stages, the undifferentiated gonad is differentiated into one of two types of sexual gonads. In the first stage, if the Y chromosome is present, the SRY gene is expressed in somatic cells of undifferentiated gonad. The expression of this gene is regulated by some of upstream factors. The product of the gene is a transcription factor that causes testicular formation and inhibits a number of genes involved in determining female sex (5). The Anti-Müllerian hormone (AMH) is produced as a result of the creation of the testis and the expression of some genes, including SOX9, which prevents the growth of the Müllerian ducts and finally, the internal structures of the male reproductive system are established by secreting testosterone and other male sex hormones (5-7). The two most important signal transduction pathways involved in sex determination are the Map-kinase and WNT4-RSPO1 pathways, which are involved in testicular and ovarian formation, respectively (5). In the male sexual development process, due to SRY, RAC1, AXIN1, and MAP3K4 factors, SOX9 is expressed, forming a feed-forward loop that induces FGF9 expression (Fig.1a).

Besides, MAP3K4 with the help of its cofactor called Gadd45y increases SRY expression (5). Also, SOX9, AXIN1, and GSK3B destabilize β-catenin and cause an obstacle in the ovarian development pathway. During the sexual evolution of female, FOXL2 negatively regulates the expression of the SOX9 gene (5). As a result, testicular formation is inhibited and on the other hand, by performing a series of phosphorylation reactions, β-catenin is stabilized and positively regulates the expression of FST and FOXL2 genes (Fig.1b) (5, 20).

In the epigenetic pathway, Chromobox2 (CBX2) is part of the polycomb inhibitor complex, which binds to the H3K27me3 (7), histone sequence upstream of the SRY, then positively regulates it and suppresses the pathways that cause ovarian formation (5, 21). The product of this gene binds to the SRY gene promoter and also the SOX9 gene promoter in connection with NR5A1 (5). As a result, the SRY gene will have a protein product th at has the ability to turn on and activate its downstream genes, such as SOX9, which are involved in the expression of genes involved in testicular formation, and also suppress and inactivate the expression of genes needed for ovarian formation, such as β-catenin and RSPO1 (5, 6).

Mechanism of action of HOXA10 gene in humans

HOXA10 and HOXA11 are generally expressed within the endometrium of adult mice and humans. The expression of these two genes is littered with the estrous/menstrual cycle. Within the proliferative process of the endometrium, HOXA10 and HOXA11 are expressed, and their level increases during the stage (22). During the embryonic stage, HOX genes expressed within the reproductive tract control normal developmental patterning, and they still function in adults. Throughout the menstrual cycle, the endometrial glands and stroma express HOXA10, which is regulated by estradiol and progesterone (12, 23, 24). Empty Spiracles Homeobox 2 (EMX2) is expressed during the embryonic period within the developing female reproductive tract yet as within the adult endometrium, and HOXA10 functions as a transcriptional repressor of Emx2 (23, 25). Endometrial β3-integrin subunit gene expression is directly upregulated by HOXA10 (23, 26). Several target genes were found to have substantially increased expression when HOXA10 was overexpressed.

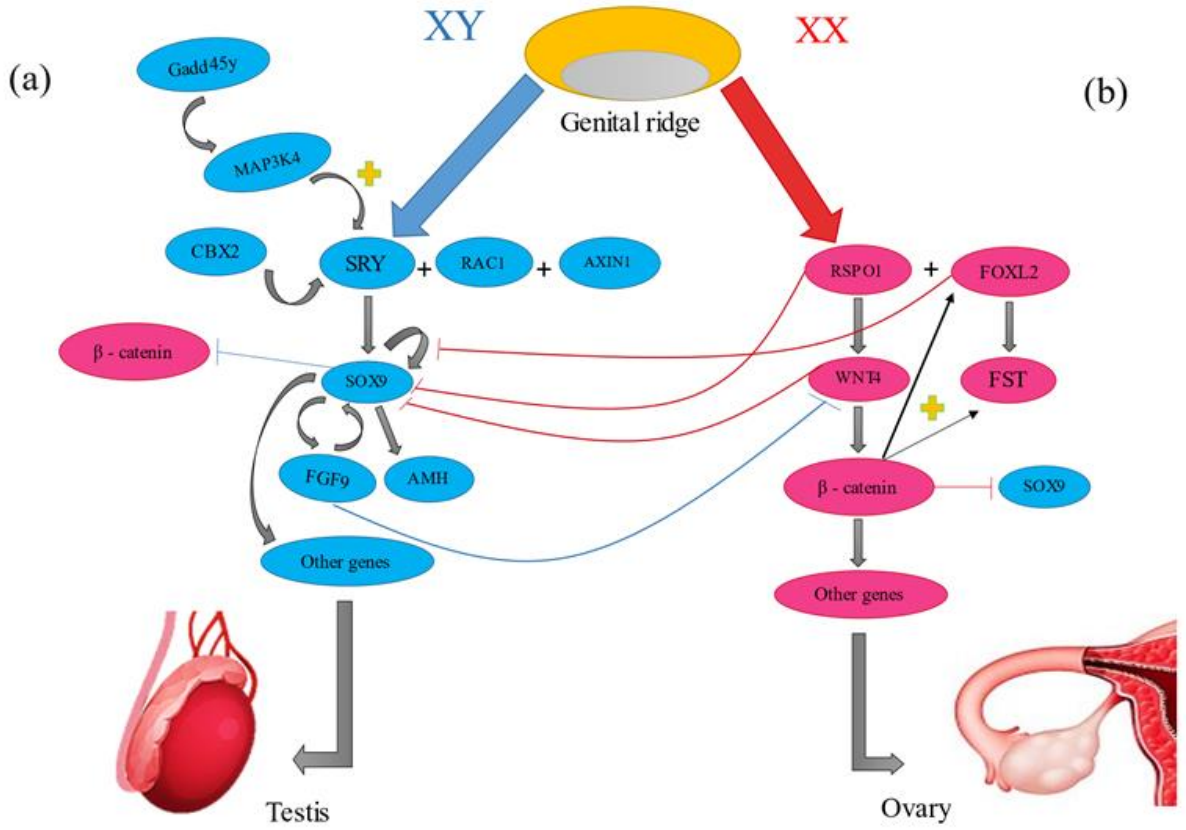

Figure 1. Evolution of sexual gonads and related genes in the embryonic period. a) The path of male sexual development; The map-kinase signaling pathway and the expression of the SRY gene and its SOX9 product, as well as other genetic factors involved, cause testicular formation. b) The path of female sexual evolution; The ovary is formed by the WNT4-RSPO1 signaling pathway and the expression of a number of genes, including β-catenin and inhibition of SOX9.

The tryptophan 2,3-dioxygenase (TDO) gene, which increased eightfold in response to constitutive expression of HOXA10, has the largest increase (23, 27). In the mid-secretory phase, around the time of implantation, HOXA10 and HOXA11 expression increases, and they keep increasing throughout the secretory phase. This increased expression is required for embryonic implantation; in both mice and humans, decreased HOXA10 and HOXA11 expression at this time results in lower implantation rates (Figure 2) (22).

Also, estrogen and progesterone control its expression, in the mid-luteal process. At the time of implantation, endometrial epithelial and stromal HOXA10 expression levels are upregulated (12, 32). In the nonpregnant condition, the normal human fallopian tube displayed very little HOXA10 gene messenger RNA during pregnancy, the normal fallopian tube showed a tendency toward higher HOXA10 gene expression (32).

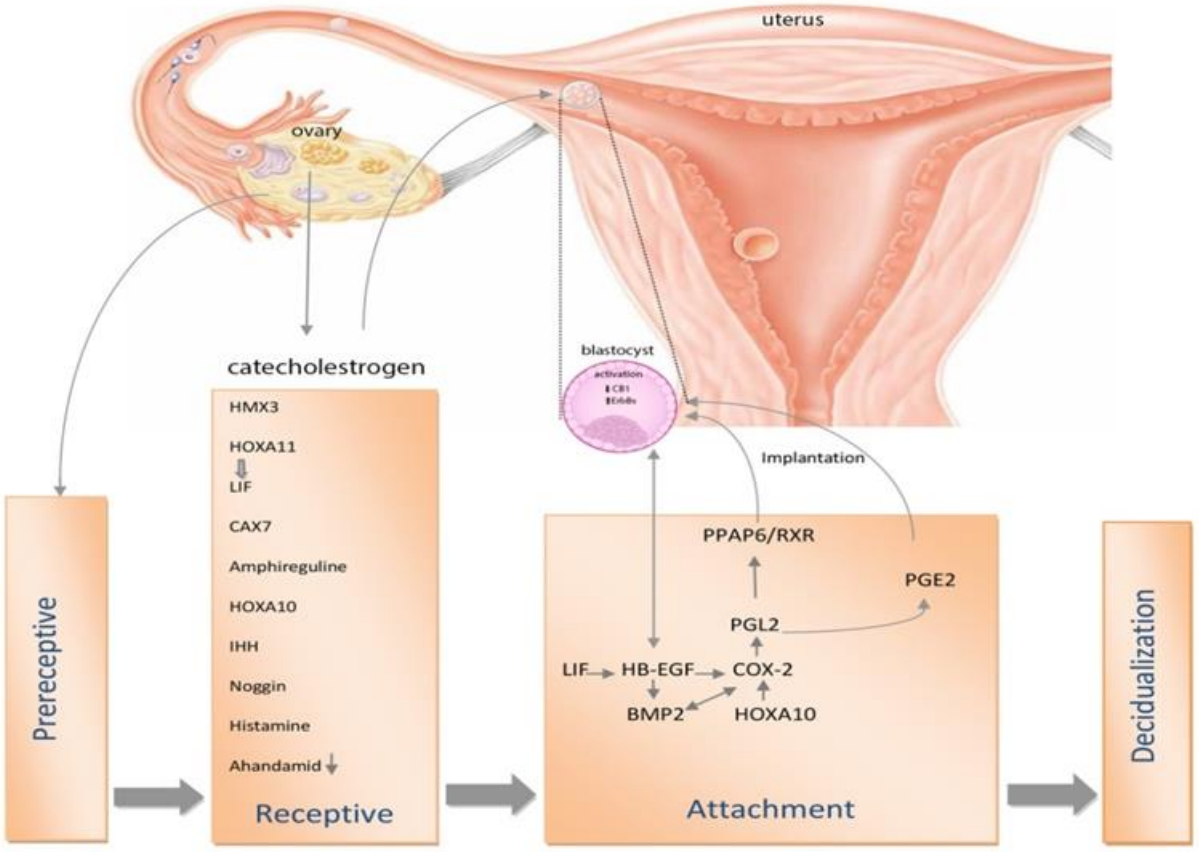

Figure 2. Molecular signaling stages of implantation in rat and human uterus. The pre-receptive uterus for pregnancy does not respond to the blastocyst. Via a variety of uterine stimuli, ovarian estrogen and progesterone turn the pre-receptive uterus into a receptive state. Consecutive signaling events inside the uterus lead to blastocyst implantation during the attachment process. If the expression level of HOXA10 reduced in the attachment stage, the implantation rate in humans will be decreased.

The role of HOXA10 gene in humans

The human HOXA10 gene is a component of the A cluster on chromosome 7 that encodes a DNA-binding transcription factor that regulates morphogenesis, gene expression, and differentiation (28-30). The transcriptional factors involved in embryonic development are encoded by the homeobox genes (31). In humans and mice, the function of these genes in embryogenesis and their expression has been shown that HOXA10/HOXA11 are found in endometrial glands and stroma during the menstrual cycle, with a slightly elevated expression throughout the mid-luteal process, which corresponds to the implantation window, in humans and mice (10, 12, 31).

Endometrial receptivity to blastocyst implantation requires uterine HOXA10 expression, as shown by targeted mutation or HOXA10 antisense therapy (33-35). Although no human females have been identified with the HOXA10 and HOXA11 mutations, in patients with low implantation rates, the expression of HOXA10 and HOXA11 is lower in the secretory stage, indicating that maternal HOX gene expression is preserved and required for endometrial receptivity (22).

Mechanism of action and role of OCT4 gene

OCT4 is highly produced in pluripotent cells, but differentiation causes it to die. Interestingly, OCT4 expression controls the fate of embryonic stem cells. Several regulators, including transcription, mRNA translation, and post-translational modification, all work together to accurately control OCT4 expression. As a result, a number of regulators function at several levels to precisely control OCT4 expression, including transcription, mRNA translation, and post-translational modification. OCT4, in cooperation with SOX2, Nanog, and other components of the nuclear transcription regulation regulatory circuit, enhances differentiation by activating both protein-encoding genes and non-coding RNAs. OCT4 also suppresses another set of targets important in growth processes when used in combination with transcription suppressor sets. Importantly, OCT4 can restore the ability of somatic cells to differentiate, and correct reprogramming of OCT4 expression is required for the extraction of induced pluripotent stem cell (iPSC) lines (14). The dose of OCT4 is very important in determining the fate of Mesenchymal Stem Cells (MSCs). Depending on the level of OCT4 expression, MSCs retain their potency or develop from trophoblasts (low or no OCT4 expression) or endodermal and primary mesoderm lineages (high OCT4) (19). This gene's rheostat activity, which has also been observed in SOX2 (36), shows that these genes have a dose-dependent effect. Furthermore, it has been well established that the overexpression of OCT4 in ESCs enhances the development of ES neurons in serum-free culture conditions (37). OCT4 was also found to play a role in ESC cardiac commitment and mesendoderm differentiation. Also, depending on its degree of expression, OCT4 may form distinct protein complexes with SOX family members: the SOX2/OCT4 complex, which binds to a main binding moiety, and the expression of genes implicated in Maintains induction of multiple power. Increased OCT4 or SOX17 expression. On the other hand, causes gene changes, resulting in SOX2 being replaced by SOX17. This complex subsequently attaches to the promoters of genes involved in primary endoderm and mesenteric differentiation, which plays a distinct role in compressed DNA (36, 38). It's questionable if the OCT4 protein, like SOX2 or SOX17, has complicated activities that are dependent on post-translational modifications (39). OCT4 regulates cell proliferation and protects undifferentiated embryonic stem cells, by genomically identifying the OCT4 target genes and OCT4-axis protein cross-proteins published in recent years. The protein OCT4 is made up of three components: An N-terminal transport domain and a C-terminal domain, which seems to be a particular cell-type domain, make up a central POU domain (Pit-Oct-Unc) for DNA binding (14).

Unlike mice, humans have two OCT3/4 isoforms that are generated via Pou5f1 mRNA replacement splicing. OCT4IA and OCT4IB (360 and 265 amino acids, respectively) are isoforms with 225 amino acids in common in the C-terminal and distinct sequences in the N-terminal. Critical levels of human OCT4IA are necessary for stem cell self-renewal, and OCT4IB has been found to be unrelated to stemness (39). OCT4 mRNA is found at all phases of human development, from infertile eggs to uncompressed and dense morula. These stages, similar to the cytoplasmic localization of OCT4 proteins, indicate a diverse expression pattern of OCT4 mRNA among individual blastomers at the cellular stage. During these stages, there is no OCT4 protein in the nucleus. OCT4 protein expression increases prevalent in the nucleus of all Morula blastomers during compaction. OCT4 transcripts and proteins are found in ICM in blastocysts. OCT4 is found in human embryonic stem cells (HESCs), human embryonic cancer cells, and human embryonic germ cells, as present in mice (39).

OCT4 and its mechanism of action in maintaining G1/S power output and transmission

In somatic cells, the expression of cyclin-dependent kinase 4.6 (CDK4.6) and cyclin D increases during the early G1 phase. Although rat ESCs did not express cyclin D (40), CDK4 and Cyclin D2 mRNA levels were elevated in human ESCs (13, 41). In human ESCs, cyclin D expression rose late in the G1 and G1/S phases, according to further research. Knocking down cyclin D differentiates the endoderm by blocking maternal decapentaplegic nuclear transmission, while overexpression expresses neuroectodermal neurotransmitters (13, 42). There is evidence that appropriate amounts of cyclin D are required for sustaining ESC pluripotency, whereas overexpression induces epidermal cells to convert into stem cells with increased OCT4 and NANOG expression levels (13, 43).

On the other hand, in adult stem cells or cancer cells, OCT4 may bind directly to the promoter area of cyclin D1, controlling G1/S transmission and regulating its transcription (13, 44, 45). Also, OCT4 can bind to the protected microRNA (miRNA) promoter 302 (46), and raise p16 (Ink4a)/p19 (Ink4d) levels while blocking CDK4/6 and Cyclin D interactions (13, 47). Furthermore, OCT4 can interact with SMAD2/3 to regulate the amplitude of ESCs (48, 49). These findings suggest that OCT4 is involved in the control of cyclin D transcription as well as other target genes when taken together (Figure 3) (13).

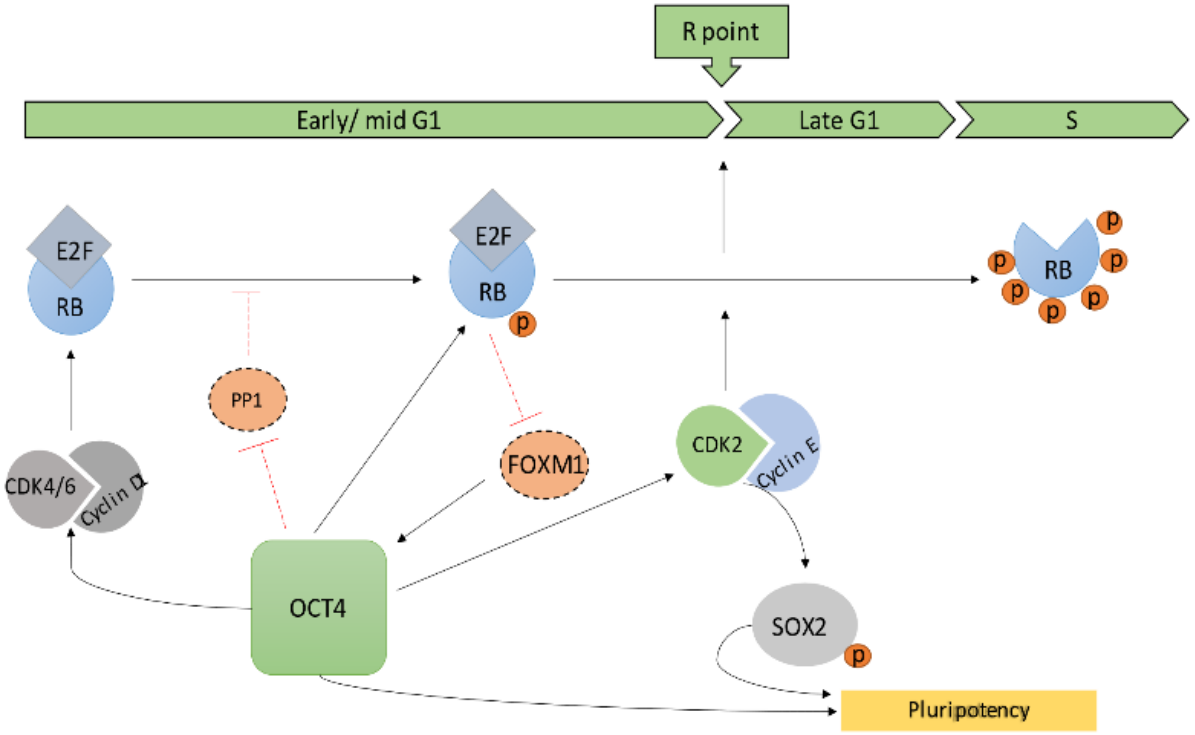

Figure 3. An overview of OCT4's responsibilities in managing the G1/S transition and pluripotency maintenance. In the early and mid G1 phases, OCT4 increases the phosphorylation of hypophosphorylated RB (a need for the R point transition) by downregulating PP1 and upregulating CDK4/6Cyclin D. Phosphorylated RB still binds to E2Fs and inhibits their transcription activating domains, causing the expression of numerous cell cycle promoter genes, including OCT4, to be inhibited. OCT4 can also induce RB hyperphosphorylation by upregulating the CDK2Cyclin E complex, which leads to E2F release, the R point transition, and S phase entrance. CDK2 can also phosphorylate SOX2 to improve the efficacy of reprogramming. Positive regulation is shown by black arrows, whereas negative regulation is shown by red bareheaded lines. PP1 stands for protein phosphatase 1; CDK stands for cyclin dependent kinase; FOXM1 stands for forkhead box protein M1; RB stands for retinoblastoma; E2F stands for E2F transcription factor 1; OCT4 stands for octamer binding transcription factor 4; p stands for phosphorylated.

The CDK2 Cyclin E complex is largely expressed and has a role in the establishment of G1/S transmission (50). Inhibition of CDK2 stops the G1 phase human ESCs, which is linked with apoptosis or differentiation. Inhibition of CDK2 can result in persistent genomic damage stimulation of the DNA response, leading to ESC death (13, 51). OCT4 expression may be decreased by lowering CDK2 regulation (52, 53), whereas CDK2 may be reprogrammed by SOX2 phosphorylation at Ser 39 and Ser 253 locations, as reported in earlier investigations, increase reprogramming efficiency (13, 54). Although OCT4 has not been shown to regulate CDK2 Cyclin A/E in the ESC, it has been shown that OCT4 can drive tumor spread by activating cyclin E (55). As a result, it's probable that OCT4 can control the expression of CDK2 Cyclin A/E in ESC (13).

OCT4 mechanism of action in G2/M transmission

CDK1-Cyclin A/B is a crucial cell cycle regulator in somatic cells that can improve G2/M transmission. CDK1 cyclins have been found to play a significant role in the self-renewal and development of ESCs in various investigations. The expression level of cyclin A, the first cloned cyclin protein, is higher in ESCs in the G2 phase than in fibroblasts (56), and rearranging its expression level in induced pluripotent stem cells can improve potency and tumorigenesis in a short period of time (13). In addition, in comparison to somatic cells, Cyclin B1 levels in ESCs are rearranged in the G2 phase. Increased expression of cyclin B1 in the G2 phase can postpone the loss of potency in human ESCs, while degradation of cyclin B1 significantly reduces the expression of potent markers in human ESCs (13, 57). CDK1 is in the same boat. A reduction in CDK1 regulation causes potency loss, increased differentiation markers, the buildup of bifurcated clefts, and the failure to stop at the G2 phase and commit to apoptosis in human ESCs (13, 58, 59).

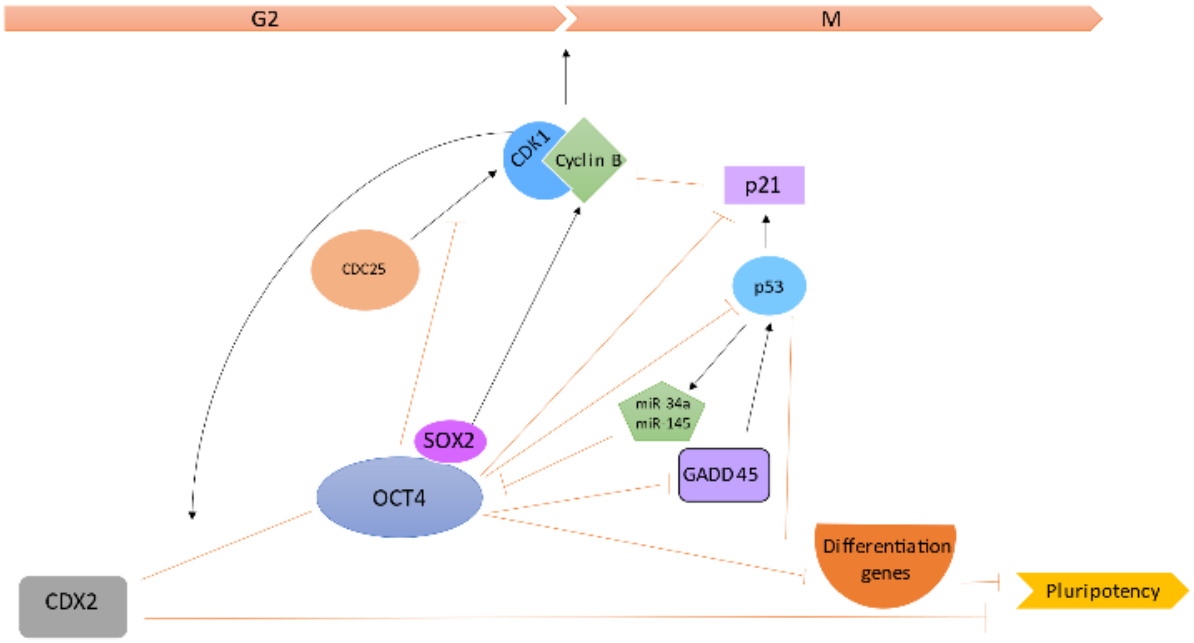

Figure 4. An overview of OCT4's responsibilities in managing the G2/M transition and pluripotency maintenance. OCT4 can suppress CDK1 activation and lead to a longer G2 phase during the G2/M phase via a non-transcriptional mechanism, allowing for future genome integrity checks and minimizing chromosomal mis-segregation. CDK1 can boost OCT4 binding to the CDX2 promoter and repress its transcription in a reciprocal manner, assisting in the maintenance of ESC pluripotency. Positive regulation is shown by black arrows, whereas negative regulation is shown by red bareheaded lines.

CDK1 can enhance the binding of the OCT4 promoter while suppressing the transcription of the CDX2 homeobox protein, which is a marker of normal differentiation (60). Furthermore, rather than ectodermal differentiation, multiple G2/M markers are produced throughout meso- and endodermal differentiation (e.g., the WEE1 G2 kinase checkpoint blocks entrance into mitosis through CDK1 phosphorylation at Y15) (61). By phosphorylating cell division cycle 25, OCT4 can activate CDK1, which is independent of its transcriptional activity (Figure 4) (13). To avoid the inhibitory impact of OCT4, ESCs must produce more CDK1. The inhibition of CDK1 by OCT4 causes the G2 phase to be prolonged, allowing further exploration of genome integration and a reduction in chromosomal segregation (13, 62). In fact, inhibiting CDK1 can stimulate the DNA damage response, leading to nuclear displacement and p53 activation, allowing ESCs to survive (63). Although no studies have found a relation between OCT4 and Cyclin A/B, but there is evidence that SOX2, a critical TF nucleus commonly linked with OCT4, may promote Cyclin A/B expression in cancer cells (64, 65). OCT4's direct control of cyclin CDK1 requires further research (13).

By slowing the cell cycle and repairing DNA, growth is stunted, and the susceptible induction protein DNA-45 (GADD-45), which has multiple isoforms, is crucial for safeguarding the genome's integrity during G2/M translocation. By generating numerous cell cycle-related inhibitors, such as p53, p21, and Cyclin G1, knocking down GADD45ag morpholino in Xenopus can stimulate differentiation of neural embryonic cells. Furthermore, GADD45ag morphine increases the expression of the Xenopus OCT4 homologue, indicating the necessity of GADD45ag existence to escape multiplicative power and enter differentiation for early embryonic cells (13). GADD45a may also attach to the OCT4 promoter in xenopus oocytes and alter its methylation, which is linked to DNA repair (66). Furthermore, human cell experiments have revealed that GADD45 G is an OCT4 downstream target that is greatly elevated in the OCT4 knockdown system (13, 67).

Knockout, mutations and SOX9 related diseases in humans

Many congenital disorders are caused by mutations in the SOX9 gene or variations in its expression levels (68). In the human SOX9 gene, losing mutations in heterozygous function cause a semi-lethal skeletal malformation syndrome known as campomelic dysplasia (68).

In general, sex-determining genes are involved in diseases and disorders of sexual development (DSD). These genes include SRY and SOX9. Various mutations, including deletion, translocation, duplication, and misunderstanding of the gene, can lead to these disorders (5). Mutations such as deletion, misexpression, or non-expression of the SRY gene in men lead to disorders whose DSD phenotypes include testicular dysgenesis or ovotestis, the formation of Mullerian ducts, feminize, or ambiguous external genitalia (5). The deletion mutation of the SOX9 gene also causes these phenotypes and, in addition, causes compulsive dysplasia. In females, SRY-related translocation mutation leads to testicular formation (testicular DSD) or ovotestis, lack of Mullerian ducts formation, masculinity or ambiguous external genitalia (5). Also, SOX9-related mutations such as doubling, balanced translocations in the forms of t(12;17)(q14.3;q24.3) and t(11;17)(p13;q24.3) lead to the mentioned phenotypes and also facial deformities (5). Experimental and clinical data have shown that SOX9 plays an important role in tumorigenesis because it is overexpressed in a wide range of human cancers and is associated with tumor progression and clinical evidence (69). In addition, SOX9 interacted with various transcription factors and displayed many pro-oncogenic properties in combination with other oncogenes, including proliferation promotion, senescence inhibition, and neoplastic transformation (69). According to COSMIC research, 572 cancer samples out of 46,601 have mutations in SOX9. The most common mutation form is missense substitution (38.81%), with 113(33.63%) being C>transitions. Researches have shown that the SOX9 gene has been studied in a number of human cancers, including breast cancer, bladder cancer, gastric cancer, pancreatic cancer, prostate cancer, ovarian cancer, and colon cancer (69).

Knockout in HOXA10 gene

Patients with endometriosis, who lack rotational expression and up-regulation of HOXA10/HOXA11 during the implantation window, may explain some of the infertilities associated with the disease (31, 70). Further research into the HOXA10/HOXA11 gene expression pattern has indicated DNA methylation as a potential mechanism for altered gene expression (31).

The role of HOXA10 gene in congenital diseases

Endometrial receptivity requires controlled HOXA10 and HOXA11 expression; reduced HOXA10 or HOXA11 expression results in lower implantation rates. Endometriosis, polycystic ovarian syndrome (PCOS), leiomyoma, polyps, adenomyosis, and hydrosalpinx have all been linked to changes in HOXA10 and HOXA11 expression. Changes in HOX gene expression result in uterine developmental defects as well as impaired adult endometrial development, preventing implantation and resulting in female infertility (22). The homeotic transformation of the anterior part of the uterus into an oviduct-like structure is caused by HOXA10 deficiency (22, 71, 72). Rare DNA sequence variations in the HOXA10 gene can play a role in female internal genitalia mis-development (70). Polycystic ovarian syndrome is a prevalent endocrine disease that affects 5% of women of reproductive age. It's marked by anovulation and increased androgen activity. Chronic anovulation is the cause of infertility in people with PCOS. Despite the fact that ovulation defects may be corrected, fertility rates are still poor, and accidental pregnancy failure is common (22, 73). Increased HOXA10 expression in the endometrium is needed for embryo implantation receptivity. However, during the secretory process, endometrial biopsies from women with PCOS in ovulatory cycles revealed that HOXA10 expression is lower than in regular fertile women. Testosterone suppresses HOXA10 expression in vitro (22, 74). Estradiol and progesterone also improve the expression of HOXA10. Flutamide blocked the testosterone effect, while dihydrotestosterone had a similar effect to testosterone. Women with PCOS may have lower reproduction capacity due to decreased uterine HOXA10 expression, indicating that the disease has a major impact on receptivity. Elevated androgen levels can cause PCOS-related infertility by altering HOX gene expression (22).

Knockout, mutations and disease (disorders) related to OCT4 in humans

For the first time, scientists have used gene editing techniques on human embryos to study their development. In 2015, Kathy Niakan, a growth biologist at London's Francis Creek Institute, utilized the CRISPR editing technology on human embryos to learn more about genes active throughout the early stages of development. The researchers intended to start with OCT4, which is a stem cell potency marker that may be converted to any tissue in the body.

A protein called OCT4 is active earlier in human embryos than in mouse embryos, according to this study (75). In 37 human single-cell embryos provided by couples and acquired via therapy and in vitro fertilization (IVF), the Niakan team employed the CRISPR technology to "delete" or "deactivate" the gene encoded for OCT4. Placental cells failed to develop in human embryonic gene deletions, showing that OCT4 is more important in human embryos than in mouse embryos. In fact, knocking down this gene prevents the formation of both embryonic and embryonic generating cells, as well as placental cells, indicating that OCT4 is important in the creation of both cell types in humans (75).

Function and role of sox9 gene and related disease (disorders) in Zebrafish

Since the SOX9 protein controls the expression of target genes, identifying SOX9 targets should help researchers better understand how SOX9 works. Researchers compared wild-type embryos to mutant embryos missing activity for both sox9a and sox9b, the zebrafish co-orthologs of sox9, using microarray expression profiling to help distinguish sox9 targets (76). Sox9a and sox9b are the two copies of sox9 found in zebrafish (76, 77). These mammalian SOX9 co-orthologs originated from a whole genome replication event that occurred after the teleost lineage split from the tetrapod lineage and before teleost fish diversification (76). Following the genome replication case, duplicate gene copies often evolved in a paralog-specific fashion, involving subfunction partitioning at least in part (76, 78). Sox9a and sox9b expression patterns overlap in some regions and are gene-specific in others, and the number of their expression patterns is close to Sox9 patterns in mouse (77). Jellyfish (jef), a zebrafish mutation that disrupts sox9a, results in skeletal defects due to irregular cartilage formation (76). The zebrafish sox9b deletion mutant has less cartilage, and the sox9a sox9b double mutant has more serious defects, indicating that the two zebrafish sox9 co-orthologs have synergistic, additive, and redundant features (76, 79). Microarray analysis is used to compare the expression profiles of homozygous mutant and wild-type embryos in zebrafish sox9a and sox9b mutants, whose heterozygotes are viable and fertile. Microarray is a powerful technique that allows us to test for changes in gene expression around the genome in various biological classes (76).

However, any changes in gene expression observed may be due to secondary effects, such as indirect Noncell-autonomous effects. The expression patterns of putative sox9 targets were compared to the expression patterns of sox9a and sox9b to assess possible sox9 targets found by microarray (76). Previous identified targets such as collagen type II alpha-1a (col2α-1a) and collagen type XI alpha-2 (col11α-2), as well as novel targets such as Cone-rod homeobox (crx), Retinoschisis 1 (rs1), Calbindin 2 (calb2) genes and other genes expressed in developing retina, were among the validated downstream candidate targets of sox9 (76). The presence of eye defects in sox9b mutant embryos supports the function of sox9 candidate targets. Sox9 is expressed in the developing retina of mouse embryos, but its role in the vertebrate retina is still unknown (80). Therefore, microarray analysis, using zebrafish mutations with mutant phenotypes, showed the previously unknown feature of sox9 (76). Also, extended studies of eye defects seen in sox9b mutants is presented, demonstrating the importance of sox9 in retinal differentiation. Genes expressed in cartilage (col2α-1a and col11α-2), retina (calb2a, calb2b, crx, neurod, rs1, sox4a, and vsx1), and pectoral fin bud (klf2b and EST AI722369) were known as potential sox9 targets (76). Cartilage is a well-studied sox9 target, indicating that this strategy is suitable, while retina is a novel sox9 feature. Sox9 regulates the number of Müller glia and photoreceptor cells, according to mutant phenotypes, and also helps coordinate the neural retina. These findings indicate that sox9b plays a significant role in the ganglion cell layer, the inner nuclear layer, and the outer nuclear layer of retinal cells (76). In the preotic region of zebrafish, FGF-dependent sox9a is expressed (81). In fact, in the otic vesicles, both sox9a and sox9b are expressed, although they overlap in separate domains (76). Both otic sensory lineages are lost in embryos bearing a homozygous deletion of dlx3b, dlx4b, and sox9a (Dfb380), but expression of otic neuroblast markers persists in residual otic cells (81). As a result, sox9, especially sox9a (which is upstream of sox9b), has been linked to otic neurogenesis (81). Homozygous zebrafish embryos for jellyfish mutations, similar to humans with compulsive dysplasia, have craniofacial defects and lack cartilage elements of the neurocranium, pharyngeal arches, and pectoral girdle (82).

The role of hoxa10 gene in zebrafish

The central nervous system (CNS) and the sclerotomal component of the somites are two major sites of hox gene expression. Within the mesoderm, hox gene products indicate a specific axial identity (83, 84). Various genes are also expressed in specific regions of the developing endoderm, may indicating a role in gut structure patterning (22). During the early stages of pectoral fin differentiation, members of the hoxa10 complex were enabled. Before any fin outgrowth, larvae accumulated hoxa9 transcripts in the presumptive fin primordia, while only a small percentage of embryos (less than 10%) expressed hoxa10 in the same site (85).

The role and function of oct4 and its related disorders in zebrafish

Only one Pou5f1/Pou2 gene was found in all five sequenced fish species, suggesting that Pou5f1/Pou2 gene amplification happened later in evolution, presumably in the common ancestor of tetrapods. As a result, the Pou5f1/Pou2 gene in zebrafish is an ortholog of the Pou5f1/oct4 gene in mice and other Pou5 class genes in vertebrates (86). The Pou5f1 gene is broadly expressed in vertebrates through pregastrulation and gastrulation, indicating that its function is retained. Pou5f1 is also expressed on the neural plate in fish and mice until mid-mitogenesis, which is less well recognized. On the other hand, expression in primary germ cells is seen only in mice and chickens, but not in zebrafish (86). In zebrafish, the oct4 gene is known as Pou5f1. This gene is one of the so-called potency indicators. According to previous studies, the oct4 gene is the most essential element in the plural network in the examined animals, and its expression has been shown from early embryonic stages to the end of the gastrula stage. This gene is one of the most important predictors of plurality in embryonic stem cell lines (87). On the other hand, the expression of this gene has been found in other pluripotent classes. For example, in mice, medaka, and chickens, it is substantially expressed in primordial germ cells, but not in frogs or zebrafish (87). In a zebrafish research, it was discovered that the expression of this gene was substantially higher in blastomers than in differentiated cells, and that the maximum expression of oct4 was likewise associated to the middle blastola stage (87). It also shows that oct4 expression is directly related to the plurality phenomena in this species.

The requirement of this gene for early development in the embryonic stage was demonstrated by the deletion of its function in model animals. The endodermal germ layer was formed in zebrafish when the gene was knocked out or deleted (87).The zygotic pou5f1 loss-of-function mutant spiel ohne Grenzen (Zspg) is deadly in zebrafish owing to abnormalities in neural plate patterning (86). Pou5f1 mRNA rescue of Zspg embryos permits the creation of homozygous mutant fish that can yield embryos devoid of maternal Pou5f1, Mspg (abbreviated ‘M'), in which the zygotes are rescued by paternal allele expression, and MZspg embryos (abbreviated ‘MZ'), which are entirely devoid of maternal and zygotic Pou5f1 activity. MZ embryos feature defective gastrulation, dorsoventral patterning errors, and no endoderm development. Sox17 during endoderm specification is the sole direct Pou5f1 transcriptional target identified in zebrafish so far (86).

OCT4 and its role in ESC metabolism

Preliminary data suggest that OCT4, as it enhances tricarboxylic acid (TCA) cycle activity while decreasing glycolytic flow, may play a role in regulating metabolism (13). Further research revealed that OCT4 may directly transcribe the glycolytic enzymes Hexokinase 2 (HK2) and Pyruvate kinase M2 (PKM2), the two most important glycolytic enzymes. Overexpression of HK2 and PKM2 contributes to the maintenance of elevated glycolysis levels and ESC potency (88). PKM2 can bind to OCT4 directly and promote OCT4-mediated transcription (13).

Results

What stands out of researches is the fact that SOX9 gene in human plays a significant role in determining fetal sex. It was also observed that two important signaling pathways have an important role in sex determination:

First, WNT4-RSPO1 pathway in the direction of ovarian formation and second, Map-kinase pathway in the direction of testicular formation. If there is a Y chromosome in the embryo, the SRY gene is expressed and then its product, which is SOX9 and is itself a transcription factor, with the help of other genes and interactions between them, inhibits the genes involved in female sex determination, and it causes testicular formation. However, in the absence of the Y chromosome, the WNT4-RSPO1 pathway is activated, leading to the production of B-catenin. Then, SOX9, with the help of a number of other factors, is inhibited and the ovary is formed.

In line with research on gene mutations and knockouts, the results showed that changes in the expression level and various mutations in the SRY and SOX9 genes could lead to congenital diseases. For example, mutations like the loss of heterozygous function in SOX9 cause compomelic dysplasia, a semi-lethal skeletal abnormality syndrome. Also, other mutations, such as misexpression, doubling, and other SOX9 and SRY disorders, cause DSD (Difference in sex development) phenotypes, such as ambiguous external genitalia in males and females. According to clinical research on the expression level of the SOX9 gene, researchers have found that this gene can also play a role in various human cancers. However, this issue is still under investigation. Regarding the function of sox9 gene in Zebrafish, researchers have used common orthologs of this gene, sox9a and sox9b, and it has been observed that the target tissues of it are cartilage, pectoral fin and retina. The jef mutation or zebrafish mutation jellyfish disrupts sox9a and causes skeletal defect through the formation of abnormal cartilage. It has also been found that the sox9b deletion mutation leads to cartilage reduction. Given the role of sox9b in the retina, the results show that embryos with mutations in this gene have ocular defects. On the other hand, it was observed that sox9a is involved in otic neurogenesis. Finally, it can be said that in the jef mutation, zebrafish get a disease similar to human campomelic dysplasia.

Homeobox genes generally encode transcription factors related to embryo growth. The HOXA10 gene is expressed in the endometrial glands and stroma throughout the human menstrual cycle and its expression increases during the intermediate secretion phase during implantation, which is essential for embryo implantation and can be regulated by estradiol, progesterone and testosterone. In the fallopian tube, we see the lowest expression of the HOXA10 gene during the period when the person is not pregnant. HOX genes are involved in regulating the normal growth pattern in the embryonic period and maintain their function in adults. Decreased expression of the HOXA10 gene causes diseases such as endometriosis, polycystic ovary syndrome, leiomyoma, polyps, adenomyosis, and hydrosalpinx. In endometriosis, the expression of the HOXA10 and HOXA11 genes are not performed during the implantation window and can lead to infertility.

In polycystic ovary disease, the HOXA10 gene is reduced in the ovulatory cycle during the secretion phase. In this disease, gene expression may be altered by testosterone or androgen, causing infertility associated with polycystic ovary disease. hox family genes are expressed in various tissues in the zebrafish body and have different roles, including modeling of intestinal structures and axial identity transfer in the zebrafish and in the early stages of pectoral fin differentiation.

According to studies, OCT4 is one of the most important factors in maintaining the strength of stem cells in the human fetus. OCT4 can also be mentioned as an important factor in the differentiation of differentiated somatic cells. As a result, cells acquire the condition of stem cells, because these stem cells can induce. It should also be noted that OCT4 is also involved in the metabolism of ESCs. This factor increases the activity of the TCA cycle and reduces the glycolytic flux and also has a direct effect on the transcription of hexokinase 2 and pyruvate kinase M2, which are two important enzymes in the glycolytic pathways.

On the other hand, based on studies, we found that OCT4 plays a very important role in placenta formation in human embryos, and if the gene encoding OCT4 is deleted or inactivated, placental cells will not form. Thus, not only are embryonic and embryonic cells not formed by the knockout of OCT4, but also placental cells are not formed; This means that OCT4 is involved in the growth of both cell types. Also, the oct4 gene, known as pou5f1 in its zebrafish, is one of the potency factors in this fish. In the zebrafish, if the pou5f1 is removed or damaged, the germinal layer of the endoderm will not form.

Conclusion

The SOX9 gene is one of the most important genes in sex determination in the embryonic period and related diseases. Studies on the level of expression of this gene indicate that this gene is also involved in various human cancers. According to researches, scientists have reached a new function of this gene in zebrafish, which explains its role in retinal function. Also, in zebrafish, the disease caused by mutations and knockouts of this gene is similar to human compomelic dysplasia, which is related to cartilage tissue.

HOXA10 gene expression, which is important for successful implantation, is reduced in women with endometriosis. HOXA10 gene expression is essential for embryo implantation. As investigated, the OCT4 gene is a transcription factor that controls the expression of a number of genes involved in embryonic development and is important for early embryogenesis and the pluripotency of embryonic stem cells. By knocking out the OCT4 gene, embryo-producing cells as well as placental cells are not formed. This gene is also mentioned in (Zebrafish) as one of the plurality markers. In Zebrafish, this gene is known as pou5f1. In this animal, the maximum expression of this gene is related to the middle blastola stage, which indicates that oct4 in this species is directly related to the phenomenon of cell proliferation, and deletion or knockout of this gene prevents the formation of a layer.

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M.A. conceived and designed the format of the manuscript. MM.G. and S.R., drafted and edited the manuscript. Z.J. designed the figures. All authors contributed to the critical reading and discussion of the manuscript. All authors have read and agreed to the published version of the manuscript.

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