Review Article

A review of similarity of KLF2, MSX1 and SOX9 genes expression in zebrafish and human

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ABSTRACT

In recent studies, the focus on zebrafish, as a suitable laboratory model for studying vertebrate gene function, has increased dramatically. We evaluated the similarity between the three genes KLF2, MSX1 and SOX9 in humans and zebrafish. The KLF2 gene plays a role in heart and immunity of humans and zebrafish. In zebrafish, KLF2 gene is involved in immunogenesis and angiogenesis. This gene can increase the expression of thrombomodulin and endothelial nitric oxide synthase for human angiogenesis and vascular volume modulation. Zebrafish and human MSX genes are involved in the development of the ethmoid plate and are expressed in the neural crest and pre-placodal ectoderm. In zebrafish and humans, members of the SOX family play an important role in embryogenesis and sex determination. Therefore, understanding the relationship between zebrafish and human genes is important for effective modeling of human genetic diseases.

***Keywords*:** Zebrafish, KLF2, MSX1**,** SOX9

INTRODUCTION

KLF2 genes, in both humans and zebrafish, it plays a role in many processes that occur. In human KLF2 genes, play a role in heart, prevent inflammation and. In addition, KLF2 genes also plays a role in hematopoietic system and arteries, and the

kidney of zebrafish which we explained all of these roles in this article.

The MSX family that regulate Transcription, have to paralogs MSX1 and MSX2, and the pseudogene MSX2P, while zebrafish have 5 genes that include MSXa ، MSXb ،MSXc ،MSXd and MSXe [1]. MSX1 in human is homologue of zebrafish MSXe [2]. Targeted Msx1 disruption in mice leads to the phenotype of cleft palate, whereas in zebrafish, simultaneous knockdowns of MSXB, MSXC, and MSXE are required to significantly [3] disrupt ethmoid plate [4]. In the neural crest and preplacodal ectoderm, MSXB, MSXC, and MSXE are expressed in partially overlapping regions [5]. A homeobox gene called MSX1, sometimes referred to as HOX7, controls cellular differentiation during development [6].

The Msx homeobox gene family encodes homeodomain transcription factors that are critical for dorsoventral patterning during early foetal development. Many organs, including the limbs, teeth, and neural crest, require Msx proteins for proper development. Both the MSX1 and MSX2 genes are expressed in ovarian germ cells during foetal life [7]. MSX1 protein acts as a transcriptional repressor during embryogenesis. Given the importance of the cell cycle in endometrial cancer and MSX1's ability to induce G0/G1 arrest, MSX1 can be a good prognostic factor for patients with this kind of tumor [8].

SOX genes are growth regulators that can be divided into several categories based on their evolutionary structure and conservation. SOX9 gene is a type of SRY protein, which is known as an important transcription factor. This gene also plays a role in important processes such as proliferation, apoptosis and angiogenesis and can regulate many growth pathways [9]. Sox8, SOX9, and SOX10 are three members of the SOXE family in mammals that appear to operate as transcriptional regulators [10]. SOX9 is involved in the development of many different cell types and organ systems; for instance, it is necessary for the formation of the mammalian skeleton and the determination of male sex. SOX9 and its closely related family member SOX8 play redundant roles in the control of branching morphogenesis in the urine transport system's arborizing network in the kidney [11]. An essential role in controlling cell fate during embryonic development and adult tissue homeostasis is played by the transcription factor SOX9. The expression of SOX9 in healthy adult kidneys is very low [12].

According to studies, three KLF2 genes in zebrafish are identified in the hematopoietic system and arteries, and the kidney, which is the site of hematopoiesis in zebrafish, suggests that these genes are involved in blood production. Other genes termed KLF2a and KLF2b have been discovered to be homologous to the KLF2 gene. The KLF2a and KLF2b genes were discovered in the developing pectoral fins. The activities of the two genes KLF2a and KLF2b can be generalized to the KLF2 gene because we discovered that they are homologous. The KLF2a gene is found in the head, trunk, and tail, as well as blood vessel cells. The KLF2a and KLF2b genes are also expressed in the mesenchymal of the pectoral fins and the mesenchyme beneath the body wall, suggesting that KLF2 gene orthologues are involved in limb muscle differentiation [13]. The KLF2 gene is involved in activities such as immunology, metabolism, and angiogenesis in adult zebrafish fast muscle [14]. In the vascular endothelium, the human KLF2 gene can increase the expression of molecules including Thrombomodulin (THBD) and endothelial Nitric Oxide Synthase (eNOS). Angiogenesis, immune cell mobilization, and vascular volume modulation are all

roles that the human KLF2 gene can play in the endothelium [15]. Additionally, the KLF2a and KLF2b genes are triggered as zygotes in the gastrula and abdominal ectoderm stages, and after activation, the KLF2a gene expresses in blood vessels while the KLF2b gene expresses in the epidermis. The KLF2a gene, of course, is involved in the development of heart valves and, as previously stated, is critical for blood flow in the spleen of zebrafish [16]. The zebrafish heart can heal after being injured. To regenerate the cardiac muscle, the Notch signal must be activated, which can be accomplished through a number of alterations such as hemodynamic shifts. The above-mentioned KLF2 gene homologs are capable of responding to hemodynamic alterations that occur after damage. To activate the Notch signal and regenerate the heart, KLF2a and KLF2b are necessary [17]. The expression of the KLF2 gene is also quite visible in the human heart. The findings suggest that the KLF2 gene can inhibit inflammation, which is known to be one of the main causes of heart failure. The KLF2 gene suppresses inflammation in immune cells, preventing inflammation and heart issues, and so the KLF2 gene is implicated in both the heart and immunity in humans and zebrafish [18]. One of the immunological roles of the

KLF2 gene is to limit the production of inflammatory genes in the endothelium while also regulating genes with anti-thrombotic properties that help to keep the endothelium healthy [19]. We discovered what genes are expressed in the oocytes by studying single-celled zebrafish RNA-seq and human oocytes RNA-seq. At the gene sequence level, researchers discovered a significant degree of orthology between the human and zebrafish genomes in a study. Meanwhile, due to the strong genetic similarity between the two creatures, some genes related to pluripotency, such as the KLF2 gene, which is found in both human and zebrafish eggs, are expressed uniquely in zebrafish eggs [20]. The KLF2 gene is expressed in the human testis, and it functions as an inhibitory factor in the treatment of tumors as shown in Figure 1.

KLF2 gene in other fishes

The KLF2 gene is found as an epidermal growth factor on the metacentric chromosome of the flatfish Solea senegalensis, which belongs to the Pleuronectiformes order [3].

The repairing role of KLF2 in kidney disease and injury

The KLF2 gene is one of the genes involved in kidney damage regulation and

illness treatment. Fibrosis, which is caused by inflammation, is one of the most prevalent kidney ailments. By expressing a range of pharmacological activators such as statins, suberanylhydroxamic acid, tannic acid, and resveratrol, the KLF2 gene can prevent kidney inflammation. As a result, it helps to avoid general fibrosis and is used to treat the condition. In addition, if the KLF2 gene is not expressed, the renal glomeruli can be damaged. KLF-like factors are involved in a number of kidney physiological processes [22].

Expression of the KLF genes during development in zebrafish

KLFs genes are involved in a variety of developmental phases, including cardiovascular development and hematopoiesis. The KLF17 gene is one of the KLF genes that is involved in the development and generation of body parts in the zebrafish. The klf17/biklf gene (blood island-enriched krupple-like factor) is a zygote-activated gene that is identified and expressed during zebrafish development. The KLF17 gene is also expressed during gastrolysis and in cell mass, as well as for the goal of organogenesis [23]. The KLF4 gene, which can stimulate cell proliferation in epidermal stem cells, is another type of KLFs gene. The KLF4 gene has also been found to be

involved in the formation of zebrafish skin in the ventral ectoderm and embryonic stages [24].

KLF12, KLF4, and KLFd expression patterns in zebrafish

KLF12: Hatch gland cells, which express this gene between 24 and 36 hours past fertilization (hpf), are one of its expression sites. In pronephric cells, expression of the KLF12 gene is quite high with 36 hpf.

KLF4: At 48 hpf, this gene was shown to be expressed in circulating red blood cells. The KLF4 gene is expressed in the same way as the KLF12 gene in hatch gland cells, following the same instructions. The limb-derived organ has significant levels of KLF4 gene expression at hpf 12.

KLFd: Similar to KLF4, the KLFd gene has been discovered in circulating red blood cells at hpf 48. The expression of these genes has also been demonstrated in hematopoietic cells [13].

Roles of Msx1 gene

The processes that control germ line sex determination and meiosis initiation are unclear. There is small evidence that homeobox Msx transcription factors are involved in the beginning of foetal meiosis in mammalian germ cells. During the beginning of meiosis, the Msx1 and Msx2

genes are strongly expressed in the fetal ovary. Researches shown the majority of undifferentiated germ cells remained in the ovaries of Msx1/Msx2 double mutant embryos, as well as a decrease in the amount of meiotic cells. In human fetal gonads, Msx1 and Msx2 were strongly expressed in the ovary during the beginning of meiosis at 14.5 wpf. The Stra8 gene, required for meiosis, was prevented from fully activating in vivo by the Msx1/Msx2 double-null mutant. Msx1 binds to Stra8 regulatory sequences in F9 cells, and Msx1 overexpression promotes Stra8 transcription. In sum, it has been shown that some homeobox genes are required to initiate meiosis in the female germ line [7].

MSX1 has been demonstrated to induce apoptosis in HeLa cancer cells, which results in a change in cell morphology and a reduction in cell growth. MSX1 overexpression in cisplatin-resistant ovarian cancer cell lines, leads to cisplatin sensitization, enhanced apoptosis, and increased cisplatin-induced p21 expression. MSX1 has been shown to inhibit cell growth and promote apoptosis in HeLa cell lines by stabilizing p53, decreasing degradation, and increasing nuclear localisation in previous research [6]. MSX1 overexpression reduced cell proliferation by lengthening the G1 phase

without causing a reciprocal increase in the S and G2-M phases. Msx1's ability to suppress growth suggested that it might play a function in cell cycle regulation [25].

MSX1 and MSX2 are transcription factors whose role is reported to be to suppress Wnt5a to modulate luminal epithelial cell polarity for blastocyst attachment. MSX1 may interact with the restrictive PRC2 complex in myoblast cells, inhibiting target gene expression by upregulating H3K27Me3 [26].

Mechanism of Msx1 gene in pregnancy

MSX1 and MSX2 reduce stromal-epithelial cross-talk in normal pregnancy by repressing WNT and b catenin signaling and inhibiting FGF production in the uterine stroma. FGFs are elevated in the absence of MSX1 and MSX2, activating the epithelial FGFR ERK1/2 pathway and encouraging epithelial proliferation. Phosphorylation of epithelial ESR1 by activated ERK1/2 follows. This causes ESR1 transcriptional activation and production of its target genes, such as Muc-1, which prevents the luminal epithelium from functionally transforming to a receptive state, preventing embryo implantation as shown in Figure 2 [27].

Msx1 deletion in the uterus inhibits implantation. The luminal epithelium lacks

well-defined crypts for blastocyst homing and attachment, according to histological study of MSX1-/- implantation sites. Furthermore, knocking out both Msx1 and MSX2 in the uterus causes complete implantation failure, as well as altered luminal epithelial cell polarity and impaired stromal epithelial communication, suggesting that MSX2 plays a compensatory role in the establishment of uterine receptivity in the absence of MSX1. Nevertheless, studies show that the MSX1/2 genes are crucial for uterine epithelial integrity and receptivity in mice [28].

Mechanism of MSX1 gene in endometrial cancer

Endometrial cancer is the most common type of uterine cancer and the fourth most common gynecological cancer and has highest rate in developed countries. The majority of endometrial cancers are caused by a combination of familial, genetic, and lifestyle factors [29]. Cell cycle has an important role in endometrium cancer and MSX1 can induce G0/G1 cell-cycle-arrest in cancer cells. The analysis show that endometrioid endometrial carcinomas have the strongest staining intensity of MSX1, and the expression of the protein MSX1 correlates with the length of survival in patients with endometrioid and clear-cell

carcinomas of the endometrium and the ovary [8]. Bioinformatics and laboratory analysis show that probably MSX1 is one of the main molecular markers of progesterone resistance in endometrial cancer [30].

MSX1 protein levels, which are ordinarily high in the secretory phase of the endometrium, were shown to be considerably lower in endometrial biopsies taken from infertile couples. MSX1 transcript increase 5-fold between the late proliferative and early secretory phases, then declined prior to implantation receptivity. MSX1 protein have strong nuclear localization in the luminal epithelium and glands in fertile patients, but is weakly expressed in stroma nuclei. MSX1 protein levels increase in all endometrial cells compartments throughout the secretory phase. Between the mid- and late-secretory stages, MSX1 protein levels in the glands dropped. During the secretory phase, however, infertile individuals show a wide drop in MSX1 accumulation in all cell types, which was particularly prominent in the stroma and glands. During the mid- and late-secretory stages, prolonged co-localization of E-cadherin and b-catenin in epithelial cell junctions was linked to infertility. MSX1 homeobox protein accumulation is connected with the secretory phase in the endometrium of fertile couples, and is commonly disturbed in infertile individuals, according to researches [31].

Mechanism of MSX1 in inhibition of cancer by prolongation of the G1 phase

Overexpression of some homobox genes increases cell growth and tumorigenesis, but overexpression of MSX1 in human ovarian cancer cell line leads to growth inhibition due to prolongation of G1 phase of cell cycle without reciprocal increase in phase S and G2-M, although the exact biological function of MSX1 is still unclear. Growth inhibition by MSX1 suggests a potential role for MSX1 in cell cycle regulation. Overexpression of the MSX1 gene prevents cell proliferation by increasing cell doubling time. During the G1 phase, cyclin D1-associated kinase gradually phosphorylates pRB to activate E2F activity. This induces the expression of cyclin E, which ensures stable phosphorylation and inactivation of pRB during the remaining period of G1 phase through a positive auto regulatory loop between pRB phosphorylation and cyclin E expression.

Overexpression of cyclin D1 is essential for malignancy, and cyclin E may have a direct role in the process of tumor development.

c-jun and jun-B are immediate primary genes associated with cell proliferation and conversion in various cell types, including ovarian cancer cell lines. The expression patterns of c-jun and jun-B in renal cell cancer tissues and cell lines, and normal kidneys suggest that c-jun may play a role in inducing malignant tumor transformation [25].

Cyclin D1 inhibits multiple lineage differentiation through its ability to block exit from the cell cycle and thus represents a downstream effective factor for MSX1 in culture. Cyclin D1 blocks MyoD activity in myogenic ancestors, which is significant because MSX1 inhibits MyoD expression in cell culture as well as in vivo. However, the consequences of incorrect expression of MSX1 are not exactly the same as the results of cyclin D1, because MSX1 does not significantly enhance cell proliferation, resulting in a higher percentage of cells expressing MSX1 in the G1 phase. This distinction probably reflects the fact that MSX1 regulates cyclin D1 but does not regulate cyclin E or G2 cyclins. Cyclin D1 is activated as an early MSX1 response gene, and evidence suggests that it may be an indirect target. In fact, the MSX genes encode transcription suppressors [32].

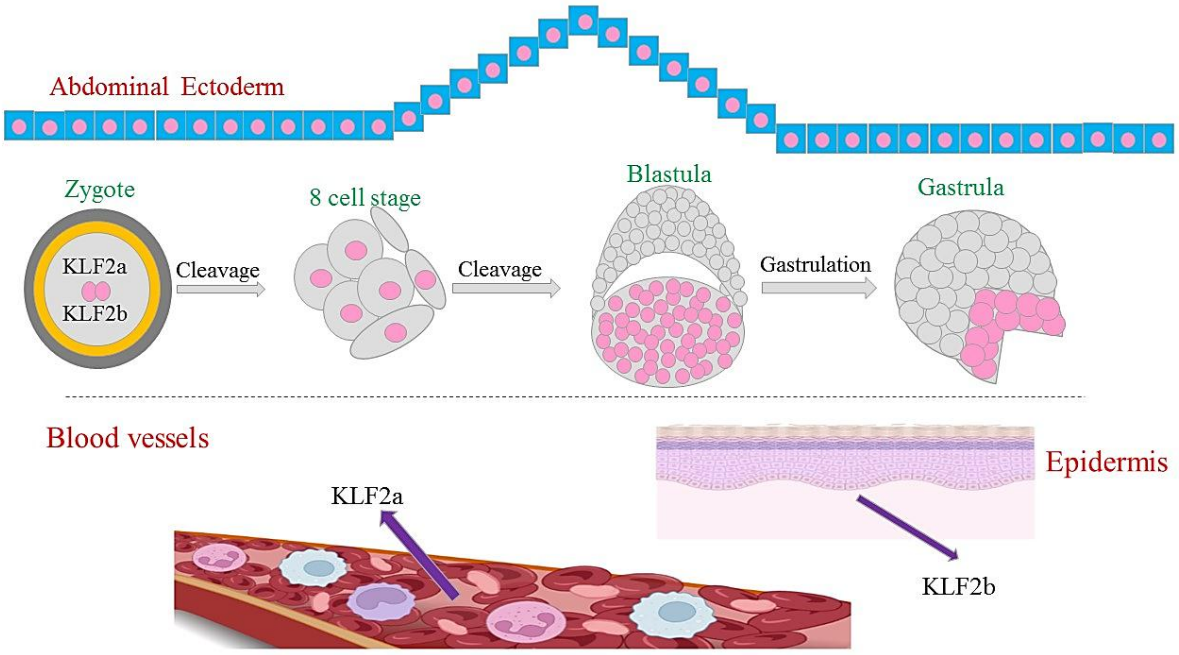

Figure 1. KLF2a and KLF2b genes are expressed in the abdomen ectoderm, blood vessels, and epidermis. Activation of KLF2a and KLF2b in the gastrula stage of the zygote. On the other hand, the KLF2a gene is expressed in blood vessels which is a critical for blood flow. The KLF2b is also expressed in epidermis after activation.

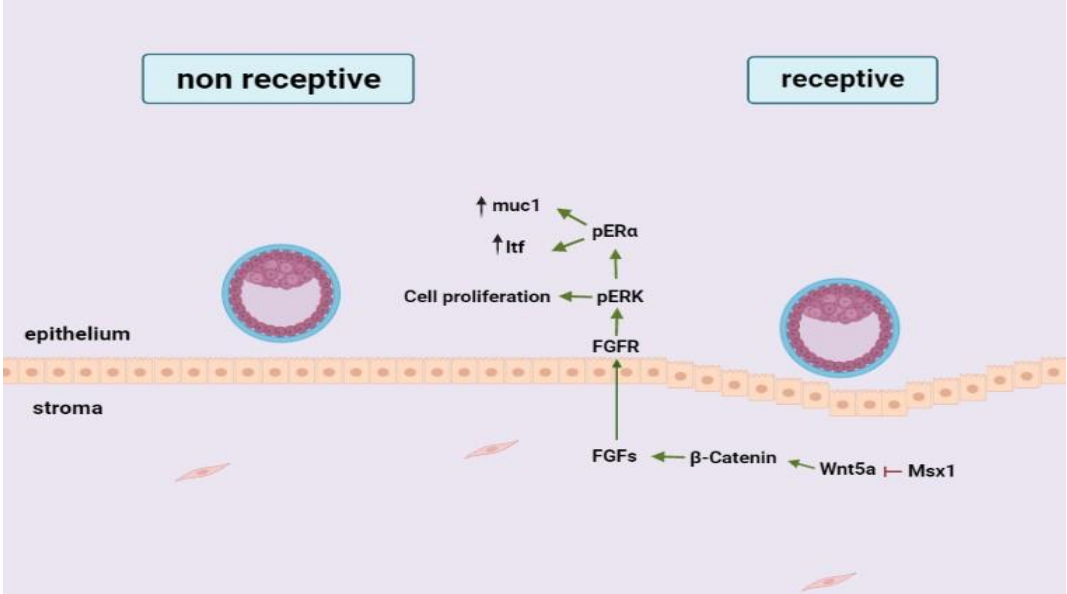

Figure 2. In the absence of MSX1, normal Wnt signaling in stromal cells activates b-catenin and stimulates the production of a subset of Fibroblast Growth Factors (FGFs) in these cells. Paracrine secreted FGFs act through FGF receptors in the epithelium to enhance epithelial proliferation, thus preventing tissue differentiation and creating an non receptive uterus that is resistant to implantation. By increasing MSX1, this pathway is inhibited and the uterus is receptive and ready for pregnancy.

SOX genes in zebrafish

Members of the SOX family also play a significant role in zebrafish embryogenesis and later phases. For example, SOX9a and SOX9b, which are zebrafish orthologs of the SOX9 gene, are expressed in the reproductive sector and are involved in sex determination [33]. The expression of SOX9a and SOX9b is in testis and ovary of zebrafish respectively. Other SOX genes are also expressed in other parts of the zebrafish body and play key roles. For example, SOX7 and SOX18 have a special role during vasculogenesis [34]. SOX18 and SOX7 homologs are expressed in angioblasts and the endothelial component of developing blood vessels in zebrafish embryos [35] and as a result of their simultaneous loss of function, the assumed aorta's vascular identity is severely compromised [33] and according to a study, multiple fusions between the major axial vessels resulted from knockdown of both genes at the same time [35]. The SOX32 gene is expressed in the head kidney (similar to the adrenal

gland in mammals) of the zebrafish and controls the fate of the endoderm. To achieve glomerular filtration and definitive hematopoiesis, the zebrafish head kidney interacts with the Dorsal Aorta (DA) and the Posterior Cardinal Vein (PCV). It was also found that the head kidney was related with PCV but not with DA in endodermless SOX32-deficient embryos [36].

SOX9 gene in other fish

This gene is expressed in most fish in the reproductive sector [33]. However, in some fish, it can be seen that SOX9 is also expressed in other organs [37]. In Madaka, SOX9 was detected using northern blot and in situ hybridization in the mature ovary and by Reverse Transcriptase Polymerase Chain Reaction (RT- PCR) in the testis. It was also expressed in cranial cartilage and pectoral fin endoskeleton during embryogenesis [38]. In Cyprinus carpio, the expression of SOX9b is in the brain, liver, heart, testis and quite low in the kidneys and ovaries, with the highest expression in the brain and testis [39]. In Siberian sturgeons (A. baerii), A. schrenckii and stellate sturgeons (A. stellatus), SOX9 is expressed in gonads, muscles, brains, livers, kidneys, eyes, spleens, fins and gills [37].

The role of SOX9 gene in human kidney development

The GDNF/RET signaling system regulates kidney growth, but the molecular mechanisms that allow RET downstream targets to be activated are still unknown. It is shown that SOX9, a human gene linked to Campomelic Dysplasia (CD), and its near homologue SOX8, perform critical roles in RET signaling. SOX9 expression can be seen in the ureteric tip, the ureter mesenchyme, and in a segment-specific way during nephrogenesis from the earliest stages of renal development. It is discovered that SOX8 and SOX9 are essential for ureter branching in the ureteric tip using a tissue-specific knockout technique, and double knockout mutants have severe kidney abnormalities ranging from hypoplastic kidneys to renal agenesis. SOX8/9 are necessary downstream of GDNF signaling for the activation of RET effector genes like Sprouty1 and Etv5, according to further genetic study. According to the study, unlike SOX9, SOX8 expression is out in growing nephrons and is confined to the growing ureteral epithelial tip. SOX9 is necessary for ureteric tip identification in later stages of development, and SOX9 ablation leads to the creation of ectopic nephrons. Studies indicate that SOX9 is

involved in several stages of kidney organogenesis, and that SOX8 and SOX9 are important components of the RET signaling system. As a result, SOX9 has been identified as a key regulator of kidney growth and as a GDNF/RET signaling controller. Thus, suppresses ectopic nephrogenesis in the kidney's outer cortex [10].

The repairing role of SOX9 in acute kidney injury

SOX9 is also involved in the reconstruction and repair after Acute Kidney Injury (AKI). AKI is a common clinical syndrome that has both short-term and long-term consequences. Renal Tubular Epithelial Cell (RTEC) disorder and cell death are two of AKI most prominent pathological characteristics. RTEC dysfunction can be caused by a variety of systemic and isolated stress situations, including sepsis, rhabdomyolysis, heart surgery, and nephrotoxic medications. Cdkl5, also known as serine/threonine kinase-9, has been identified as an important regulator of nephrotoxic-associated RTEC and ischemia-associated AKI. Evidence suggests that Cdkl5 activation suppresses SOX9 function under renal stress, which has contribution in the development of AKI. SOX9 transcription is increased during AKI. SOX9 functions as a cytoprotector during the early stages of AKI and as a facilitator of repair throughout the recovery phase. SOX9 gene deletion worsens rhabdomyolysis-associated AKI, according to kidney damage analyses. Through functional inhibition of the SOX9 transcription factor, Cdkl5 causes RTEC malfunction and cell death in the Cdkl5-SOX9 signaling pathway (Figure 3) [12].

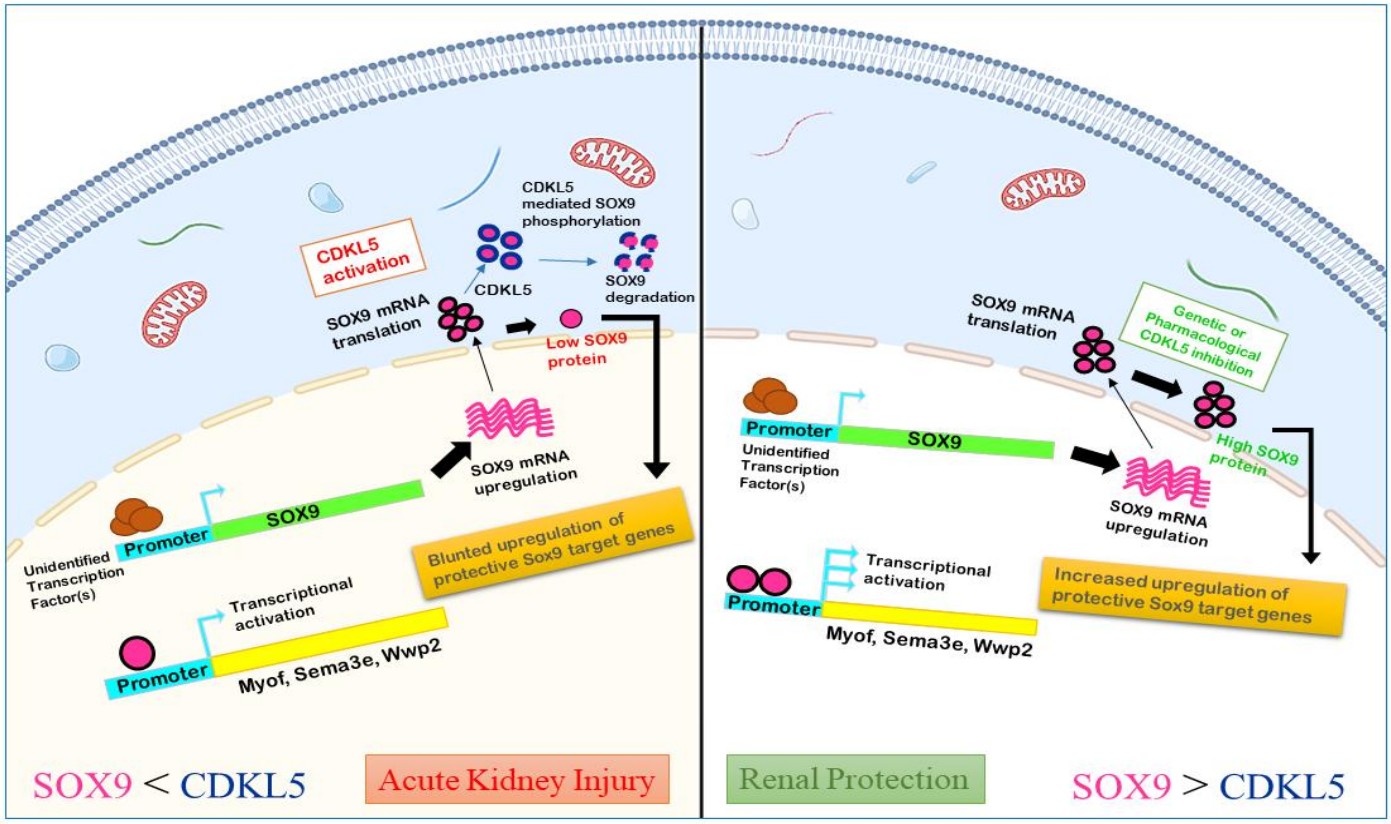

Figure 3. Cdlk5-SOX9 signaling pathway through AKI. Activation of Cdlk5 suppresses SOX9 factor and reduces transcription of protective genes. On the other hand, inhibition of Cdlk5 causes more expression of SOX protein and consequently more transcription of protective genes.

CONCLUSION

Researchers discovered a significant degree of orthology between the human and zebrafish KLF2 genes. As a result, different types of KLF2 genes have been found in zebrafish. Other genes termed KLF2a and KLF2b have been discovered to be homologous to the KLF2 gene. The activities of the two genes KLF2a and KLF2b can be generalized to the KLF2 gene because we discovered that they are homologous. These genes are expressed in different places such as developing pectoral

fins and so on. These genes have very high immune effects.

In other hand, we discuss about MSX family genes in human which has two paralogs, MSX1 and MSX2. However, zebrafish has five genes include: MSXa, MSXb, MSXc, MSXd, MSXe. Zebrafish MSX genes involved in development of ethmoid plate and expression in overlapping reigns of neural crest and preplacodal ectoderm.in human, MSX genes have various roles. MSX genes repress transcription during embryogenesis. MSX genes affect cancer's cell cycle and suppress their growth. In addition, these genes have positive effect on pregnancy by repressing WNT and b catenin signaling and inhibiting FGF production in the uterine stroma.

SOX9 is another gene that was discussed. SOX9 is a transcription factor that plays a key role in determining cell fate during embryonic development and adult tissue homeostasis. In normal adult kidneys, SOX9 has a quite low expression. In zebrafish, members of the SOX family also play important roles during embryogenesis as well as its later stages. This gene is expressed in most fish in the reproductive sector. However, in some fish, it can be seen that SOX9 is also expressed in other organs. As investigated, the SOX9 gene plays a role in the ret signaling pathway in the human kidney, and mutations in the SOX9 and SOX8 genes cause severe renal abnormalities. It was also found that SOX are involved in regeneration and repair after AKI.

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