# Roles of Wnt Signaling Pathway and ROR2 Receptor in Embryonic Development: An Update Review Article

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**ABSTRACT:** The Wnt family is a large class of highly conserved cysteine-rich secretory glycoproteins that play a vital role in various cellular and physiological courses through different signaling pathways during embryogenesis and tissue homeostasis 3. Wnt5a is a secreted glycoprotein that belongs to the noncanonical Wnt family and is involved in a wide range of developmental and tissue homeostasis. A growing body of evidence suggests that Wnt5a affects embryonic development, signaling through various receptors, starting with the activation of β-catenin by Wnt5a. In addition to affecting planar cell polarity and $Ca^{2+}$ pathways, β-catenin also includes multiple signaling cascades that regulate various cell functions. Secondly, Wnt5a can bind to Ror receptors to mediate noncanonical Wnt signaling and a significant ligand for Ror2 in vertebrates. Consistent with the multiple functions of Wnt5A/Ror2 signaling, Wnt5A knockout mice exhibited various phenotypic defects, including an inability to extend the anterior and posterior axes of the embryo. Numerous essential roles of Wnt5a/Ror2 in development have been demonstrated. Therefore, Ror signaling pathway become a necessary target for diagnosing and treating human diseases. The Wnt5a- Ror2 signaling pathway as a critical factor has attracted extensive attention.

**KEYWORDS:** Wnt5a, Ror2, tetralogy of Fallot, β-catenin-mediated and β-catenin-independent

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## Overview

Every year, hundreds of thousands of children are born with congenital disabilities and leading infant mortality derivations. There are surgical interventions and medical treatment protocols for some congenital disabilities. However, many of these children live with congenital disabilities that leave them permanently disabled. The factors which might induce those congenital disabilities can be genetic, such as genetic mutations in specific genes or chromosomal abnormalities,[1] or those provoked by teratogens (environmental factors), such as by drugs, environmental chemicals, and abnormal concentrations of natural metabolites (such as folate deficiency).[2-5] Of all genetic birth anomalies, cardiac defects are the most predominant. Its incidence is up to 1% of live births is one of the leading causes of neonatal disability and death in China. Congenital cardiovascular disease includes various conditions, such as atrial septal defect, ventricular septal defect, patent ductus arteriosus, and tetralogy of Fallot.[6] These diseases seem to have little in common, but the underlying disease mechanisms are similar, and the onset is related to environmental and genetic factors.[2] It is essential to understand the heart's embryonic development to identify the underlying pathology of these defects'. The linear tube forms the mesoderm's anterior lateral plate during embryonic development before undergoing extensive remodeling. Additionally, the second heart field assigned a late differentiating lateral plate of mesoderm expands and initiates the right ventricle and atria's growth in the mammals. Formerly neural crest cell migration has occurred and contributes to heart formation, particularly the outflow tract. With the continuous extending of the research on the pathogenesis of congenital heart disease (CHD), especially the rapid development of molecular biology and genetics, the pathogenic factors of CHD have been profoundly understood. Recently, the discovery of monoclonal genetic defects associated with an isolated illness or non-syndromic CHD has revealed critical molecular pathways in heart morphogenesis. Multiple genes are associated with CHD, such as NKX2.5,[7] GATA4,[8] MYH6,[9] BMPR2,[10] CRELD1 and ALK2, and NOTCH1.[11] However, the genetic mutation of these genes causes severe cardiac defects.

Nonetheless, other associated factors influence cardiac mesoderm alteration. However, most of the patients with congenital heart disease, especially the genetic causes of sporadic patients, are still unclear. The study of Wnt signaling pathways has always been an area of concern. Therefore, this review article will outline Wnt signaling pathways, new cellular and molecular data to investigate the potential mechanisms in vertebrate models associated with congenital heart disease induction. In addition, we will specify brief details on environmental factors related to early heart development and new evidence on folic acid and inositol supplementation.

## Wnt Signaling Pathway

Since the first Wnt gene (Int-1) in 1982, Wnt proteins constitute an important family of signaling molecules that coordinate and influence many cellular biological and developmental processes.[12] A total of 19 Wnt proteins associated with this family and their function and related disease have been

mentioned in Table 1. The Wnt signaling pathway is a multichannel signal transduction pathway activated by binding ligands and proteins during embryonic development and tumorigenesis. Through this pathway, Wnt protein is secreted as cysteine-rich glycolipoproteins that's act as extracellular signals. This further transmits the signals into the cell by activating intracellular segments of receptors on the cell surface, playing an essential role in regulating cell proliferation, differentiation, survival, migration, polarity, and other physiological processes.[3] The Wnt pathway was designated as both the canonical (β-catenin-mediated) and non-canonical (β-catenin-independent) pathways. In addition to the listed in Table 1, there are some other ligand and receptor proteins associated with these pathways, such as Wnt receptor (Frizzled family proteins),[13] LDL receptor-related protein (LRP), tyrosine kinase Ror,[14] Disheveled (DSH/DVL) protein, β-catenin, glycogen synthase kinase C3β (GSK-3β),[15] axin/conductin, and adenomatous polyposis coli (APC).[16] Furthermore, the non-canonical Wnt signals were additionally classified (based on downstream signaling) into the following categories: the non-canonical Planar cell polarity pathway (PCP pathway), the non-classical Wnt/$Ca^{2+}$ pathway (Wnt/$Ca^{2+}$ pathway), and intracellular pathways. The canonical Wnt signaling pathway is tangled in the cell cycle. The non-classical PCP pathway affects the cytoskeleton's formation, and the non-canonical Wnt signaling/calcium pathway involves DNA transcription. Besides, recent studies have pointed to the crucial role of Wnt signaling in maintaining adult homeostasis of stem cell pluripotency. Wnt binds to the Frizzled and LRP5/6 co-receptors to induce stable β-catenin proteins and enter the nucleus affecting the target transcription genes via the canonical Wnt signaling pathway.[13] In addition, it is highly genetically conserved in animals and is very similar in different animal species. Subsequently, overt Wnt signaling of uncontrolled embryonic development is a hallmark of congenital disabilities, cancer, and other degenerative diseases; understanding how this pathway is regulated is crucial,[16,17] as shown in Figure 1.

## Wnt Signaling Pathway and Embryonic Cardiac Development

Genetics is dependent on the intensification of a small number of primitive germ cells (PGCs) in the early embryo. The derivative (stem cells) of the PGCs differentiates into multiple organ development. In mammals, stem cells or progenitor cells reside in a particular microenvironment termed a niche. Its microenvironment influences progenitor cell proliferation in different directions, including neuronal differentiation, liver, myogenic, chondrogenic, osteogenic, adipogenic, and cardiogenic cells.[31] The growth of progenitor cells is regulated by both circulating and local signaling pathways. These multiple regulatory pathways, including receptor tyrosine kinases (RTKs), Notch (N), Hedgehog (Hh), TGFβ, Jak/Stat, nuclear receptors, and Wnt, are involved in cell differentiation. During embryonic growth, the Wnt/b-catenin dependent pathway regulates the early differentiation of mouse embryonic stem cells.[32] Thus, Wnt and membrane protein receptors are essential regulators of heart development in vertebrates.[4] Furthermore, Wnt signaling activity is low in a healthy adult cardiovascular system under normal physiological conditions. Nevertheless, the reactivation of this pathway has been observed in cardiovascular disease.[5] Additionally, many fetal genomes are activated during the heart's pathological remodeling, including Wnt singling pathway. Experimental studies have shown that the application of Wnt signaling activators increases the myogenic differentiation of mesenchymal stem cells from cardiac patients. However, the Wnt pathway is active in developing the pericardial, the adjacent cardiac mesoderm, the endocardial cushions, and the early outflow tract. However, it retains almost no role in the developing ventricular myocardium.[33] Surprisingly, several Wnt ligands have a high expression level in the heart's early development, including Wnt2, Wnt2b, and Wnt8a.[34-36] Furthermore, cardiac genes' expression significantly increases via Wnt3A. On the other hand, inhibition of the Wnt signaling pathway resulted in the embryo's complete disappearance.[17] These findings suggest that the canonical Wnt pathway not only drives the formation of embryonic stem cell cardiomyocytes but is also an essential pathway for development.

Similarly, the non-canonical Wnt pathways play an endorsement role in the fate of cardiomyocytes. The Wnt11-mediated mesenchymal stem cells have been shown to upregulate GATA-4, conceivably through signaling via the PKC or JNK pathways.[8] Both Wnt5a and Wnt11 promote the fate of embryonic cardiac stem cells. However, there is little literature on the molecular and cellular mechanisms behind the influence of Wnt5a and Wnt11 on cardiac embryonic development. Furthermore, we used human embryonic stem cells (hESC) to systematically analyze the expression of endogenous Wnt signaling elements during the development of Human cardiomyocytes. So, our preliminary results showed the expression of Wnt3 and Wnt8a regulates the short axis and influences mesoderm development through FZD7.[17,28,29] In addition, Wnt5a/5B regulates cardiovascular development through the Rror2 non-canonical signaling pathway. Besides, Wnt2, Wnt5a/5B, and Wnt11 regulate the differentiation of cardiomyocytes via FZD4, FZD6.[24,25,28] The role of classical Wnt signaling in the ordered phase of vertebrate cardiogenesis has been confirmed. The more precise role of single Wnt signaling and Wnt receptor genes in atypical signaling pathways in human cardiomyocyte development has been identified.[37]

## Role of the Ror Receptor in the Cardiac Embryonic Development

Receptor tyrosine kinases (RTK) play a crucial role in cell development by inducing a cascade of homologous ligand binding signals that lead to cell proliferation and differentiation. The Ror-family receptor tyrosine kinases in mammals consist of Ror1 and Ror2, characterized by the extracellular

**Table 1.** Wnt protein associated function, gene location, and related diseases.

| ALIASES | GENE LOCATION (HUMAN) HTTPS://WWW.GENECARDS.ORG | FUNCTION | ASSOCIATED DISEASE |
|---|---|---|---|
| Proto-oncogene Wnt-1 | 12q13.12 | Involve with oncogenesis, cells differentiation in the embryogenesis, formation of the embryonic dorsal neural tube, and initiation of mesencephalon and cerebellum | Joubert syndrome, autism, osteogenesis imperfecta, and type XV[17] |
| WNT2 | 7q31.2 | Involve with oncogenesis, cell differentiation in the embryogenesis, cognitive/linguistic, and midbrain dopaminergic neuron development. | Autism[18,19] |
| Wnt-2b (formerly Wnt-13) | 1p13.2 | Controlling mesoderm specification and some aspects of brain, heart, and lung during gastrulation. | Diarrhea 9 (DIAR9)[17] |
| Wnt3 | 17q21.31-q21.32 | Involve with oncogenesis, cell differentiation in the embryogenesis | Tetraamelia syndrome and tetra-amelia syndrome[17] |
| Wnt3a | 1q42.13 | Involve with oncogenesis, adipogenesis, and cell differentiation in the embryogenesis | Osteoporosis, juvenile, and hypotrichosis[20] |
| Wnt4 | 1p36.12 | Aggravate the testis-determining factor, regulates endometrial stromal cell proliferation, survival, and differentiation | SERKAL syndrome, Mullerian aplasia, and hyperandrogenism[21,22] |
| Wnt5a | 3p14.3 | Cell differentiation in the embryogenesis, posterior development of the female reproductive tract, and cardiac outflow tract | Robinow syndrome, autosomal dominant, congenital heart disease[23-26] |
| Wnt5b | 12p13.33 | Cell differentiation in the embryogenesis, regulation of cardiac development | Type 2 diabetes, fallopian tube serous adenocarcinoma, and colorectal cancer[17] |
| Wnt6 | 2q35 | Key roles in cell differentiation during embryogenesis, carcinogenesis, and inhibits the induction of cardiogenic mesoderm | Mullerian aplasia and hyperandrogenism, and gastric cancer[23] |
| Wnt7a | 3p25.1 | Roles in cell differentiation during embryogenesis, the development of the anterior-posterior axis in the female reproductive tract, and uterine smooth muscle | Fuhrmann syndrome, Schinzel phocomelia syndromes, fibular aplasia, or hypoplasia[23] |
| Wnt7b | 22q13.31 | Regulating of cell fate and patterning during embryogenesis, placental, lung, eye, dendrite, and bone formation along with kidney development | Beckwith-Wiedemann syndrome, microphthalmia, syndromic 9 and colobomatous microphthalmia[27] |
| Wnt8a | 5q31.2 | Development of early embryos and germ cell tumors | Exudative vitreoretinopathy 1 and Norrie disease. |
| Wnt8b | 10q24.31 | Roles in cell differentiation during embryogenesis | Exudative vitreoretinopathy 1, epilepsy, and Alzheimer's disease. |
| Wnt9a (formerly Wnt14) | 1q42.13 | Involves oncogenesis and the regulation of cell differentiation during embryogenesis | Gastric cancer and exudative vitreoretinopathy 1. |
| Wnt9b (formerly Wnt15) | 17q21.32 | Required for craniofacial development, the standard fusion of the palate, embryonic kidney, and urogenital tract development | Cleft lip and Mayer-Rokitansky-Kuster-Hauser syndrome. |
| Wnt10a | 2q35 | Involves oncogenesis and the regulation of cell differentiation during embryogenesis | Odontoonychodermal dysplasia, Schopf-Schulz-Passage syndrome, Tooth agenesis, selective, promyelocytic leukemia, and Burkitt's lymphoma. |
| Wnt10b (formerly Wnt12) | 12q13.12 | Involved in oncogenesis and the regulation of cell differentiation during embryogenesis, breast cancer, and governs adipogenesis | Breast cancer, split-hand/foot malformation, tooth agenesis |
| Wnt11 | 11q13.5 | Development of skeleton, kidney, and regulates cardiac chamber | Fallopian tube serous adenocarcinoma and exudative vitreoretinopathy[28,29] |
| Wnt16 | 7q31.31 | Embryonic regulation of cell growth and differentiation | Basal cell carcinoma and nodular basal cell carcinoma.[30] |

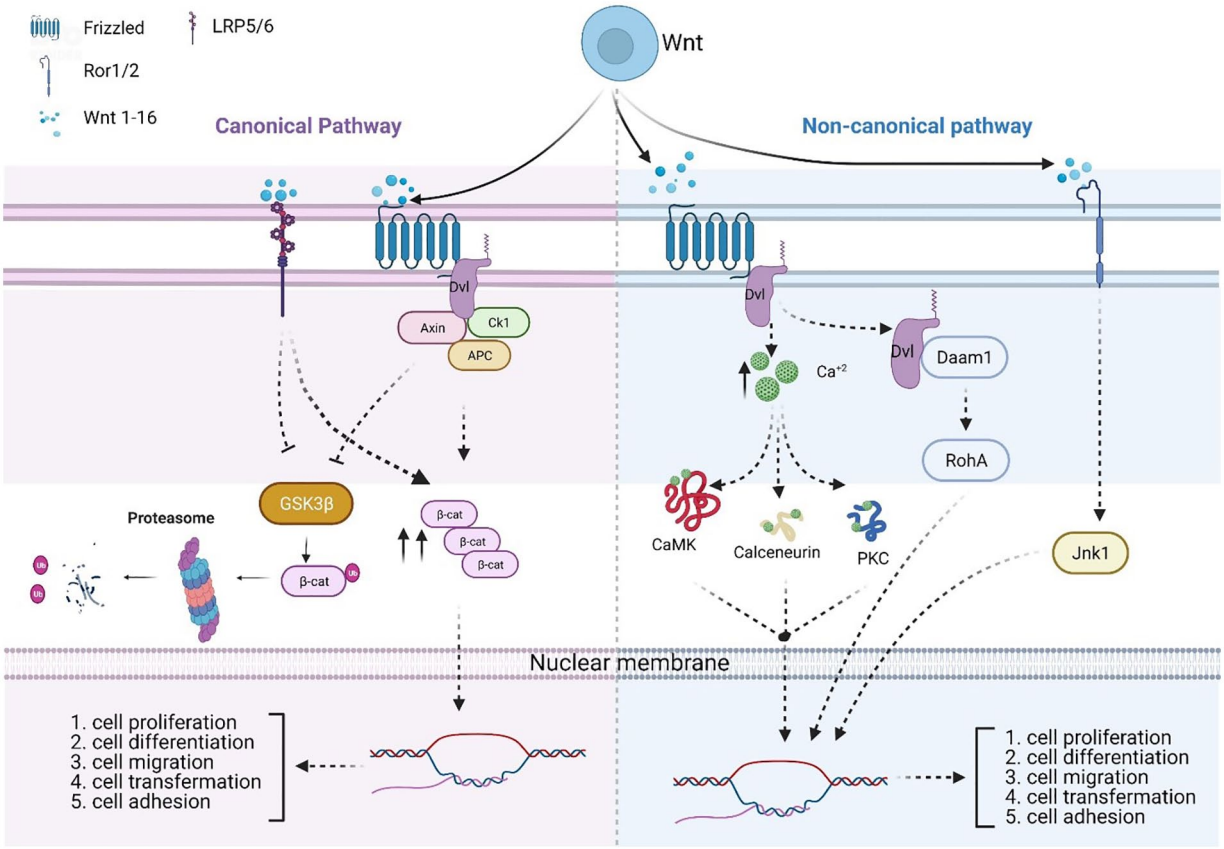


**Figure 1.** Signaling pathways involved in CHD. In normal conditions, the Wnt/β-catenin pathway is kept inactive as the β-catenin is complex with APC/Axin complex. When the Wnt or other specific ligands bind with membrane receptors, including frizzled and Ror, or by situations in which APC/Axin become unstable, cascades become activated and increase the free form of β-catenin and is not being phosphorylated, leading to accumulation in the cytoplasm. Eventually, this free β-catenin is associated with nuclease and promotes the expression of several Wnt target genes. APC helps Axin phosphorylate β-catenin and subsequent degradation of β-catenin through ubiquitination if the cell remains inactive. Wnt/β-catenin non-canonical signaling pathway is transduced independent from β-catenin activation. After ligation, Dvl utilizing Daam 1 activates Rho kinase (RhoA). Dvl also activates CaMK, calcineurin, and PKC, resulting in subsequent activation of JNK. The integrity of these pathways leading to cytoskeletal changes and stimulation of cell polarization and mutilations during gastrulation.

Frizzled-like cysteine-rich domain and membrane-proximal kringle domains. Ror (RTK) and Wnt/β-catenin signals can activate various intracellular signals, including the activation of the planar cell polarity pathway (PCP pathway).[38,39] Another mechanism of ROR2′s action on the PCP signal is activating the classical PTK signaling pathway. By interacting with PTK7 and SFRPs, ROR2 promotes signal transduction to JNK. Therefore, the activation of the PCP pathway and typical RTK signaling pathway by ROR2 may be caused by different signals that are differentially regulated by SFRPs.[13]

The Ror-receptor has previously been multiple signaling functions that regulate several processes during embryonic development, such as developing the heart, nervous system, bone, and kidney. Furthermore, Ror2 knockout mice presented with dwarfism, facial anomalies, and cardiac septal defects. During embryonic development, the expression pattern of the Ror gene is significantly different between invertebrates and vertebrates.[40] In addition, Ror1 and Ror2 were initially being found to regulate the germinal bands of neurogenic ectoderm during Drosophila embryogenesis, suggesting their role in neurodevelopment.[41] In vertebrates, Ror1 and Ror2 signals are widely expressed in chickens and mice during embryonic development, most notably in the central nervous system, heart, lung, kidney, early limb bud, and cartilage growth plate.[26,42] Furthermore, the PCP pathway controls the direction of the static cilia in the skin, hair, and inner ear and cell polarity and coordination in developing the stomach, nerves, intestine, and limbs.[43] Besides, in humans, mutations in the Ror2 gene cause 2 distinct developmental syndromes, including recessive Robinow syndrome (RRS) and autosomal dominant type B1 brachydactyly.[44,45] In addition, some patients have congenital heart defects, including atrial or ventricular septal defects and cardiac outflow tract defects.[26,46] Another study has shown that Ror2 gene mutations in mice with RRS exhibit multiple bone defects, mainly severe shortening of limbs and tails.[44] Besides, mice with homozygous Ror2 mutations died shortly after birth and had cardiac septal defects, primarily ventricular septal defects. This indicates that Ror2 is essential in forming the heart, especially in developing the cardiac septum.[47]

## Role of Wnt in the Cardiovascular System

Vasculogenesis and angiogenesis control the formation of blood vessels. Vasculogenesis is the process of developing blood vessels from the de novo production of endothelial cells (ECs) in embryos. Later, angiogenesis takes place, forming new blood vessels from pre-existing.[48] Various Wnt ligands appear to affect EC functions (WNT2, -2B, -3, -4, -5A, -7A, -8A, -9A, -9B, -10B, and -11) as mention in Table 1. Vessel maturation and stabilization of blood vessels are also controlled by parietal cells. Pericytes are important pericytes supporting capillaries.[49] Reduced pericyte coverage in the endodermis can lead to vascular loss. In pulmonary arterial hypertension (PAH), the expression of FZD genes required for WNT/PCP activation in the pericyte is reduced, which is an essential mechanism for EC motility and polarity.[5,50] In vitro transfection of the mutant WNT5A reporter gene explains the functional significance of the corresponding mutation that influences amino acid substitution in the epidermal growth factor-like domain (Cys-ARG).[51]

In addition, the thickening of the pulmonary arterial wall is observed in PAH due to the proliferation of vascular smooth muscle cells. This was associated with increased levels of β-catenin activity in pulmonary artery smooth muscle cells (PASMCs).[52] In addition, β-catenin is located at the adhesion junction of the Catenin /cadherin complex and plays a crucial role in cell adhesion and intercellular communication.[53] mXinα, a protein of the intercalated disk (a critical point for cardiac hypertrophy), contains a β-catenin-binding domain that plays a crucial role in bundling actin filaments.[54] Remarkably, accumulation of β-catenin was found in the intercalated disk of hypertrophic cardiomyopathy hamsters that possibly leads to stiffening of the myocardial wall and harmful structural.[55] However, in vitro, the exact roles of β-catenin are still not fully understood.[56] Further studies are needed to clarify the precise mechanisms by which β-catenin(and WNT signaling) reveal the effects in hypertrophic reactions.

## The Wnt5a–Ror

Wnt5a is a member of the Wnt family and consists of 1172 adenines, 884 cytosines, 946 guanines, and 1172 thymine. In recent years, Wnt5a has attracted extensive attention due to its regulatory effects on both the canonical Wnt Wnt/β-catenin pathway and the non-canonical Wnt/$Ca^{2+}$ pathway. Previous studies have shown that Wnt5a is involved in intercellular signal transduction during embryogenesis. Again, Wnt5a produces a series of intracellular reactions by binding to Frizzled receptor (a 7-fold transmembrane receptor protein), which transmits signals into the cell.[21] Furthermore, Ror2 is a single transmembrane receptor protein containing a tyrosine kinase that acts by forming a complex with Wnt5a that transmits signals into cells. In addition, Ror2 and Vangl2 procedure a Wnt-induced receptor complex, and Wnt5a signaling controls cell polarity by regulating Vangl2 phosphorylation.[57]

As previously established, the Wnt5a-Ror signaling pathway is an important regulatory pathway for tissue regeneration, embryonic tissue formation, and reproduction. The Wnt5a facilitates tissue homeostasis by activating transforming growth factor-B (TGF- B). The core of the downstream TGF-B signaling pathway involves ROR1/2 and Smad proteins.[58] Meanwhile, TGF- B enhanced the transcriptional activity of the Smad protein complex through ROR1/2 signaling. Besides, kinesin superfamily protein KIF26b acts as a downstream target of the Wnt5a-Ror pathway. Wnt5a-Ror induces degradation of KIF26B through a ubiquitin-proteasome system and modulates its cell stability through the canonical Wnt/β-catenin-dependent pathways. Wnt5a regulates epithelial-mesenchymal cell migration through this mechanism. Genetic distress of KiF26b function in vivo leads to axial malformation in embryos and loss of primitive germ cells, 2 phenotypic characteristics Wnt5a-RORs signal disruption. Wnt5a binds to Ror2 (as a ligand in PGC) to promote the polarization, elongation, and redirection of PGC. Furthermore, Wnt5a enhances the chemotaxis of PGC by redistributing intracellular Ror2.[59] Besides, Wnt5a inhibits the Wnt signaling pathway through Ror2. It activates the expression of nuclear target genes, which is beneficial to cells' early and efficient migration and has advantages in cloning germ cells.[60] However, mutations in Ror2 and Wnt5a signaling leads to the formation of developmental defects. In Ror2 mutant mice, the number of PGCs surviving is reduced due to dysregulated migration of PGC in the embryo. In conclusion, Wnt signals affect cell proliferation and differentiation, while Ror mainly controls the process of cell polarization migration.

The neural crest is a unique cell group of vertebrates originating from the neural plate. The craniofacial skeleton and cardiac outflow tract are all derivatives of the neural crest cells. Cranial neural crest cells migrate ventrally to the heart and form the cardiac outflow tract with the detached aorta and pulmonary trunk.[61] Ror2 expression is critical during mouse embryonic development in various tissues, including the skeletal system and internal organs. Furthermore, Ror2-deficient and Wnt5a-deficient mice were born with craniofacial abnormalities and significantly shortened limbs and tails. However, the craniofacial and cardiac defects of Wnt5a-deficient mice were more severe than those of Ror2-deficient mice. This is because the Wnt5A mutation induces the downregulation of cardiac neural rest through plexus protein 2 (PlxNA2), and the animals exhibit outflow tract defects similar to those of Ror2-deficient mice.[62]

Furthermore, cardiac phenotypic defects in Wnt5a-deficient mice are attributed to defective Wnt/$Ca^{2+}$ signaling in CaMK2,[25] closely related to Wnt/PCP signaling.[63] Regarding the cardiac neural crest, Wnt/PCP signals are required to form other cardiac regions, contributing to the cardiac outflow tract formation.[64] Therefore, simultaneous craniofacial and cardiac outflow tract malformations in patients with RRS and corresponding mouse models suggest that the Wnt5a/Ror2

signaling pathway plays a crucial role in heart development. Mice deficient in both Wnt5a and Ror genes show abnormal bone and heart elevation.[46] This is because Wnt5a-Ror signals affect tissue pattern, cell polarity, and cell migration during organ development. Besides, Wnt5a-Ror signaling is active in adult uteri and crucial for early pregnancy. The Wnt5a-Ror signaling pathway promotes embryo implantation, and the disorder of this pathway leads to reduced fertility. An abnormal Wnt5a-Ror signal can lead to abnormal decidua growth. One of the main functions of the decidua is to regulate the entry of the trophoblast into the stroma and guide placenta formation. Implantation defects and decidual growth restriction were found in uteruses with varying Wnt5a levels.[65] However, the molecular mechanism of the Wnt5a-Ror pathway mediating these processes remains unclear.[38]

## Myoinositol and Folic Acid Role in Embryogenesis

Many studies have confirmed that folic acid (FA) has been generally accepted as a preventive effect on neurodevelopmental abnormalities. Folic acid deficiency and elevated folic acid levels impair the Wnt pathway function in developmental signaling pathways. Depending on the individual genotype, FA supplementation may be detrimental to neurodevelopment and embryogenesis. A recent review article suggested that multivitamins may also reduce the risk for certain heart defects but at higher concentrations than those used for neural tube defects.[66] Furthermore, a cohort study in Denmark and Norway has shown that FA is not associated with the risk of heart defects (including severe defects and septal defects) in offspring.[67] However, another study has confirmed that FA is related to decreased risk of congenital heart defects.[68] It is suggested that women of reproductive age take FA supplements before pregnancy to prevent neural tube defects, including spina bifida and anencephaly. Although the link between the Wnt pathway and FA is not well established, further studies are needed to elucidate it. Inositol is essential in the inositol-lipid cycle and provides metabolic substrates for the signal transduction process, including the Wnt pathway.[69] Furthermore, the depletion of inositol in the Wnt pathway resulted in the cell's inhibition of GSK3β.[70] Wnt3A triggers the phosphatidylinositol signal attached to the G protein to generate inositol polyphosphates instantaneously, including inositol pentaphosphate (IP5). Further, IP5 inhibits GSK-3β activity. By blocking IP5 formation inhibits the buildup of β-catenin.[71] The association between the inositol phosphatidyl pathway and its intermediates with the canonical Wnt signaling pathway may explain the severity of heart defects and different phenotypes. Besides, several studies have found that once mothers increase their folic acid intake, their risk of other congenital disabilities, such as cleft palate, appears to be decline.[72] The addition of myoinositol combined with folate resulted in even better protection of the chick embryo from the adverse effects of the environmental factors.[62,63]

There are some natural compounds other than folic acid and myoinositol, phytochemicals, especially polyphenols, are among the most studied groups of the flavonoids and non-flavonoids subgroups. These drugs overcome the need to introduce foreign compounds with multiple complications into individuals.[73] They are generally non-toxic, more readily available, and less costly than synthetic drugs, preventing several diseases in healthy individuals. Recent studies have also identified several potent inhibitors of the Wnt/β-catenin and hedgehog pathways, including curcumin,[74] resveratrol, epigallocatechin-3-gallate (EGCG), lycopene, and retinoids.[74-76] Analysis of these plant components revealed several unique inhibitors of the Wnt/β-catenin and hedgehog signaling pathways.[77]

## Epigenetic Regulation of Ror Receptor Pathway

Epigenetic regulations such as DNA methylation and histone modification closely coordinate the transcriptional activity of genes, and specific epigenetic structures are necessary to maintain normal cell function.[78] DNA methylation occurs in the covalent modification of cytosine 5-C, mainly in the context of cytosine guanine dinucleotide.[79] The mechanism for establishing and maintaining DNA methylation is well established and involves 3 mammalian methyltransferases (Dnmts): Dnmt1, 3A, and 3B.[80] Germ-line deletion studies in mice have identified an essential role for Dnmts in embryonic heart development.[81] Recent genome-wide DNA methylation analysis studies have identified extensive changes in DNA methylation patterns during early embryonic heart development and revealed potentially essential associations with changes in cardiogenic gene transcription.[82] Furthermore, dynamic changes in DNA methylation patterns are associated with changes in gene expression in human heart failure and dilated cardiomyopathy, and inhibition of DNA methylation may have a cardioprotective effect in norepinephrine-induced hypertrophy ischemic heart disease.[83] Inadequate epigenetic modification control activates oncogenes and inhibits gene inactivation, leading to the development of various cancers.[84] It has recently been reported that colon cancer occurs through the accumulation of abnormal DNA methylation and the destruction of histone coding. Abnormal DNA methylation has been detected in normal mucosa in early colon cancer.[85] Overall hypomethylation leads to chromosomal instability and oncogene activation. The promoter -CpG island is a CpG dinucleotide-rich region, and changes in its methylation intensity alter essential tumor suppressor genes.[86]

In addition to DNA methylation, histone modification is considered to be a key regulator of gene activity. Acetylation, methylation, and phosphorylation of histone-specific residues, such as Lys 4, Lys 9, and Ser 10, provide chemical driving forces for chromatin configuration that prevent or allow the initiation of gene transcription.[87] Increased histone deacetylase activity such as HDAC3 in the nucleus is associated with decreased histone acetylation.[88] In addition, the cross-talk between DNA methylation and histone modification is a hot topic in recent years. On the other hand, DNA methylation

also affects histone methylation. It has been shown that nucleosomes composed of acetylated histones assemble unmethylated DNA.[89]

## Summary and Prospect

In the past decade, few studies have been published on the characteristics of Wnt signaling in general and its role in cardiovascular disease in particular. In this review, we focused on the part of Wnt signaling in congenital heart diseases, cardiovascular diseases, including atherosclerosis and cardiac hypertrophy. However, underlying standard features in the pathological mechanisms of these diseases seem to process such as cell proliferation and differentiation, which the role of Wnt signaling has been demonstrated. This explains the regulatory role of Wnt signaling pathways in these diverse cardiovascular conditions.

The Wnt family is a large class of highly conserved cysteine-rich secretory glycoproteins that play a vital role in various cellular and physiological courses through different signaling pathways during embryogenesis and tissue homeostasis.[3] Wnt5a is a secreted glycoprotein that belongs to the non-canonical Wnt family and is involved in a wide range of developmental and tissue homeostasis. A growing body of evidence suggests that Wnt5a affects embryonic development, signaling through various receptors, starting with the activation of β-catenin by Wnt5a. In addition to affecting planar cell polarity and $Ca^{2+}$ pathways, β-catenin also includes multiple signaling cascades that regulate various cell functions. Secondly, Wnt5a can bind to Ror receptors to mediate non-canonical Wnt signaling and is a significant ligand for Ror2 in vertebrates. Consistent with the multiple functions of Wnt5A/Ror2 signaling, Wnt5A knockout mice exhibited various phenotypic defects, including an inability to extend the anterior and posterior axes of the embryo. Numerous essential roles of Wnt5a/Ror2 in development have been demonstrated. The Wnt5a-Ror2 signaling pathway as a critical factor has attracted extensive attention. Although the pathogenicity of the Ror2 receptor in congenital heart disease has not been thoroughly studied, the study of Wnt5a signaling and the Ror2 receptor still has a long way to go.

Overall, this review provides new insights into the role of DNA methylation in the regulation of neonatal cardiac maturation. However, further research is needed to clarify the mechanisms that induce specific methylation changes in embryonic heart development. Further analysis of DNA methylation during embryonic heart development may better understand the mechanisms that drive cardiac cell cycle arrest and binucleate formation.

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