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microRNA regulation of Wnt signaling pathways in development and disease

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Abstract

Wnt signaling pathways and microRNAs (miRNAs) are critical regulators of development. Aberrant Wnt signaling pathways and miRNA levels lead to developmental defects and diverse human pathologies including but not limited to cancer. Wnt signaling pathways regulate a plethora of cellular processes during embryonic development and maintain homeostasis of adult tissues. A majority of Wnt signaling components are regulated by miRNAs which are small noncoding RNAs that are expressed in both animals and plants. In animal cells, miRNAs fine tune gene expression by pairing primarily to the 3′untranslated region of protein coding mRNAs to repress target mRNA translation and/or induce target degradation. miRNA-mediated regulation of signaling transduction pathways is important in modulating dose-sensitive response of cells to signaling molecules. This review discusses components of the Wnt signaling pathways that are regulated by miRNAs in the context of development and diseases. A fundamental understanding of miRNA functions in Wnt signaling transduction pathways may yield new insight into crosstalks of regulatory mechanisms essential for development and disease pathophysiology leading to novel therapeutics.

Keywords

Post-transcriptional regulation; Regulatory network; β-catenin; Non-canonical Wnt signaling; Cancer

1. Introduction

The Wnt signaling pathways are highly conserved throughout evolution. Wnt signaling can be classified as (a) canonical β-catenin-dependent Wnt pathway and (b) non-canonical β-catenin-independent Wnt/Planar cell polarity (Wnt/PCP) and Wnt/calcium (Ca^{2+}) pathways (Fig. 1A). The Wnt signaling pathways are employed in a plethora of cellular processes during embryonic development and in adult animals. Wnt signaling pathways are tightly regulated at all levels from transcriptional regulation to post-translational modification [1]. Inappropriate Wnt pathway activity results in developmental disorders and diseases,

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including cancer, skeletal disorders, neuronal diseases, and cardiovascular diseases [1–6]. Recently microRNAs (miRNAs) are found to regulate components of the Wnt signaling pathways (Table 1) [7,8]. In addition, the Wnt signaling cascades have been linked to the production and activity of miRNAs (reviewed in [8]). Thus, both miRNAs and Wnt signaling pathways interact to regulate various biological processes in the developing embryo. This review is divided into four main sections. The first section discusses the current knowledge on Wnt signaling pathways in the context of development. The second section discusses the biogenesis and function of microRNAs. The third and fourth sections of the review summarize miRNAs that are known to target the Wnt pathways in the context of development and disease, respectively.

2. Wnt signaling pathways in development

In 1982, Nusse and Varmus identified the mouse proto-oncogene Wnt1 (Int1) in Wnt signaling [9]. Inappropriate expression of *Wnt1* induces a transformed phenotype in mammary epithelial cells [10] and in transgenic mice [11]. Injection of *Wnt1* mRNA into *Xenopus* embryos led to duplication of the embryonic axis, revealing its role in the canonical Wnt pathway [12]. In efforts to identify homologs of *Wnt1*, Moon and coworkers identified *Wnt5a* in *Xenopus*. However, when *Wnt5a* mRNA was injected into *Xenopus* embryos, it led to developmental defects of the head and tail as a result of cellular movement perturbation which are different than the defects induced by *Wnt1*. Ectopic expression of the *Xenopus Wnt5a* in zebrafish embryos caused increased intracellular calcium concentration and the stimulation of calcium signaling phenocopied that of Wnt5a signaling, indicating that Wnt5a is one of the major ligands responsible for non-canonical Wnt signaling [13]. These seminal publications elucidated the critical roles that Wnt signaling pathways play in development.

2.1. The canonical β-catenin-dependent Wnt pathway

Canonical Wnt signaling has been shown to control diverse biological processes and functions, including cellular proliferation and differentiation [14], survival [15], cell fate decisions [16], stem cell maintenance and somatic cell reprogramming [17]. Furthermore, canonical Wnt signaling has been shown to be critical in important embryological events, including axis specification [18] and gastrulation [19], as well as in organogenesis, including development of the breast [20], limb [21], heart [22], central nervous system [23], and bone [24].

In canonical Wnt pathway, the absence of the Wnt ligand leads to rapid phosphorylation of cytoplasmic β-catenin by glycogen synthase kinase 3β (GSK3β) at Ser33, Ser37 and Thr41 [25] and by casein kinase Ia (CK Ia) at Ser45 [26]. GSK3β is found as a part of the destruction complex, which includes Axin and adenomatous polyposis coli (APC) [4] (Fig. 1). The post-translational phosphorylation of β-catenin targets it for ubiquitinylation and subsequent proteasomal degradation [27], preventing its nuclear accumulation. However, binding of canonical Wnt ligand to its corresponding transmembrane receptor frizzled (FZ/FZD) as well as co-receptor LRP5/6 [28] results in recruitment of cytoplasmic protein disheveled (Dsh/Dvl) to the cell membrane. Dsh/Dvl transduces Wnt ligand activation of both canonical Wnt/β-catenin and the non-canonical Wnt signaling pathways [29]. Dsh/Dvl protein has three highly conserved domains, an N-terminal DIX (disheveled, Axin) domain,

a central PDZ (postsynaptic density 95, discs large, zonula occludens-1) domain, and a C-terminal DEP (Dvl, Egl-10, Pleckstrin) domain [29]. The Dsh/Dvl DIX domain and its proximal region are important for Dsh/Dvl oligomerization which is required for relay of signal and subsequent stabilization of β-catenin [30]. The binding of FZ to the activated Dsh/Dvl recruits Axin and GSK3β to cell membrane, thereby, dismantling the destruction complex and inhibiting phosphorylation of β-catenin [31]. This results in increased stability of β-catenin, facilitating its nuclear accumulation to one pole of the embryo. Asymmetric localization of nuclear β-catenin is conserved in invertebrate sea urchin species [32,33], starfish *Asterina pectinifera* [34], ascidian *Halocynthia roretzi* [35], and vertebrate *Xenopus laevis* [36]. Together with the Tcf/Lef family of transcription factors, β-catenin activates transcription of several genes involved in diverse biological processes, such as cellular proliferation, apoptosis, and differentiation [14,17].

Besides functioning as a transcriptional co-activator, β-catenin is also a component of the adherens junction [37]. β-catenin links the cadherin molecules to the α-catenin, leading to strong cadherin-mediated cell adhesion [38]. The level of β-catenin in the adhesion complex at the plasma membrane affects the availability of β-catenin functioning as a transcription co-activator in the nucleus [38]. This is demonstrated with experiments in which perturbation of cadherin complexes has an effect on Wnt/β-catenin regulated processes. For example, overexpression of cadherins in *Xenopus* embryos results in inhibited dorsal axis formation because binding of cadherin to endogenous β-catenin antagonizes β-catenin's role as a nuclear transcription co-activator [39,40]. Similarly, overexpression of sea urchin cadherin results in depletion of nuclear β-catenin, abrogating endomesodermal cell types [32,41].

Wnt/β-catenin signaling is regulated at many levels, including by secreted proteins that antagonize the Wnt ligand [3]. Among these are secreted frizzled-related proteins (SFRPs) and Wnt inhibitory protein (WIF) that bind to Wnt ligands, preventing interaction between the Wnt ligand with the frizzled receptor [42]. Other Wnt inhibitors include Dikkopf (DKK) and WISE/SOST protein families that bind to LRP5/6 in inhibiting the Wnt signaling pathway [43,44].

In summary, the canonical Wnt/β-catenin signaling pathway is highly conserved for anterior–posterior axis formation, endodermal specification, and organ formation in most animals [39,41,45–48].

2.2. The non-canonical Wnt pathways

The non-canonical Wnt pathways or the β-catenin independent Wnt signaling includes the Wnt/Planar cell polarity (PCP) pathway and the Wnt/calcium (Ca^{2+}) pathway (Fig. 1A). The non-canonical Wnt signaling pathways regulate convergence and extension of the body axis during embryogenesis in *Xenopus* [49,50], zebrafish [51], and mice [52,53]. In addition, the non-canonical Wnt pathways regulate cell motility which is critical for many cell type establishment and organ development, such as kidney ureteric branching [54], neural crest cell migration [55], neural axon guidance [56,57], neuroectoderm formation [23,58], female gonadal development [59], skeletogenesis [60], and osteogenesis [61].

In the Wnt/PCP signaling pathway, the Wnt ligand binds to non-canonical FZ and its co-receptors to activate two parallel pathways stimulating small GTPases RhoA and Rac1 [62,63]. This signaling is transduced through the PDZ and DEP domains of Dsh/Dvl that directly interact with FZ at the cell membrane [31] and indirectly activate RhoA [64]. Activation of RhoA GTPase turns on the Rho-associated kinase (ROCK) cascade, which in turn results in dynamic cytoskeletal rearrangement, leading to formation of lamellopodia and filopodia-like structures associated with the leading edge during cell migration [62]. Further, ROCK phosphorylates Myosin Light Chain (MLC) phosphatase, which associates with acto-myosin filaments [65]. Wnt/PCP signaling has been shown to be important for the initiation of sea urchin primitive gut (archenteron) invagination by activating RhoA [66,67]. Inhibition of RhoA results in failure to initiate invagination movements; whereas, constitutively active RhoA induced precocious invagination of the archenteron [66]. Similarly, inhibition of ROCK also results in blocking sea urchin gut invagination [66].

The second branch of Wnt/PCP signaling activates the Rac GTPase which in turn stimulates c-Jun N-terminal kinase (JNK) activity, resulting in modification of actin cytoskeleton [68]. Moreover, JNK activation which occurs downstream of RhoA, but independent of ROCK, has been shown to be essential during *Xenopus* convergent extension movements [69]. JNK phosphorylates focal adhesion adaptor protein paxillin to promote cellular migration. Paxillin and its interacting proteins, such as focal adhesion kinase, c-Src, vinculin, Erk, p38 MAPK, MEK, have all been implicated in cell migration [70].

In the Wnt/Ca^{2+} pathway, binding of non-canonical Wnt to its cognate receptor FZ/FZD results in activation of heterotrimeric G proteins that regulate phospholipase C (PLC), PDZ, and p38 [71]. This activation of PLC is coupled with short-lived increase in concentration of intracellular second messengers such as inositol 1,4,5-triphosphate (IP_3), 1,2-diacylglycerol (DAG), and Ca^{2+} with the consequences of rapid alteration of the cellular functions [72,73]. Elevated levels of intracellular Ca^{2+} is sufficient for activation of calcium-sensitive enzymes such as calmodulin dependent protein kinase II (CaMKII), protein kinase C (PKC) [74], calcineurin (Cn) [75] and calpain (a non-lysosomal cysteine protease) [76]. PKC activates small GTPase Cdc42 which remodels actin cytoskeleton and shape cell polarity [77–79]. Activated CaMKII, PKC as well as Cn, mediate gene transcription via NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells), CREB (cAMP response element-binding protein) and NFAT (nuclear factor of activated T cells) transcription factors [62]. NFAT has been shown to regulate transcription of heavy chains of several isoforms of motor protein Myosin (Delling et al., 2000), implicating its role in regulation cellular movements by controlling actomyosin contractility [80]. Using *Xenopus* embryos, it was shown that expression of truncated Dsh/Dvl possessing only PDZ and DEP domain relayed Wnt signaling predominantly via Wnt/Ca^{2+} cascade as seen in elevated levels of intracellular Ca^{2+} and activation of Ca^{2+}-dependent enzymes such as PKC and calcineurin [62]. An overexpression of Dsh-DEP domain in sea urchin embryos manifests a dominant negative effect over endogenous Dsh/Dvl and blocks specifically non-canonical signaling pathways with developmental defects, including a lack of endodermal markers and organized skeleton [60].

Co-receptors for the non-canonical Wnt/PCP pathway include the receptor tyrosine kinases of Ryk and Ror families (Ror1 and Ror2), which are orphan receptors that have been shown to mediate polarized cell migration [81,82]. In development, Ryk (receptor tyrosine kinase-related tyrosine kinase) together with Wnt5 mediate growth cone repulsion in *Drosophila* commissural axons [83]. In *Xenopus*, Ryk cooperates with FZ7 and Wnt11 during gastrulation and convergent extension movements [84,85]. As in *Drosophila*, mammalian Ryk is required for axon guidance and neurite outgrowth [86–90]. Further, Ryk-deficient mice exhibit defects similar to Wnt/PCP mutants, such as the misorientation of stereocilia in the inner ear [91]. In glioma-derived cells, Ryk is important for the Wnt5a-dependent induction of matrix metalloproteinase (MMP-2) that degrades extracellular matrix to facilitate cancer cell motility and increase invasive activity [92]. Thus, Ryk is a pharmacological target for human glioma.

Studies have shown that Ror1 and Ror2 interact genetically to regulate skeletal and cardiovascular systems [93]. Ror2 mutants exhibit similar phenotypes as the Wnt5a mutant mice in that they show dwarfism, facial anomalies, short limbs and tails, and respiratory dysfunction that lead to neonatal lethality [94]. In contrast to Ror2 mutant mice, Ror1 mutant mice do not have apparent morphological abnormalities, yet they die neonatally due to respiratory dysfunction and cyanosis, similar to Wnt5a and Ror2 mutants [93]. Ror1 and Ror2 double mutant mice exhibit enhanced Ror2 mutant phenotypes with skeletal defects, as well as complete transposition of great arteries [93]. In humans, mutation of Ror2 co-receptors results in skeletal disorders dominant brachydactyly type B and recessive Robinow Syndrome [95,96]. In addition, overexpression of Ror1 and Ror2 results in various cancers [97–104]. Ror2 expression is observed in squamous cell carcinoma of the oral cavity, osteosarcoma cell lines, melanoma, renal cell carcinoma, leiomyosarcoma, and gastrointestinal stromal tumor. Ror1 overexpression is involved in hematopoietic malignancies of B cell lineage in humans, neuroblastoma, breast cancer, renal cancer, and lung adenocarcinoma. Because of their role in activating Wnt/PCP pathway to promote cell migration, small molecules and antibody-based therapies against Ror1 and Ror2 are potential targets for cancer therapy [105].

In summary, the non-canonical Wnt/PCP pathway regulates cell polarity in morphogenetic processes [60,106–108], and the Wnt/Ca^{2+} pathway regulates the transcription of genes controlling cell fate and cell migration [62]. Both branches of the non-canonical Wnt pathways have the ability to mediate actin polymerization in morphogenetic movements which are critical for proper development.

2.3. Processing of Wnt ligand and its importance in development

While signaling molecules within receiving cells are essential for activation of the various Wnt dependent signaling pathways, Wnt processing within ligand producing cells also significantly influences Wnt signaling. The synthesis and movement of Wnt ligands are strictly regulated in the signal producing cells. Wnts, cysteine rich signaling ligands, are synthesized in the rough endoplasmic reticulum (ER) and simultaneously undergo N-glycosylation by oligosaccharyl transferase complex (OST) and disulfide bond formation facilitated by an ER-resident protein GRP78/BiP [109–113]. Within the ER, Wnts (with the

exception of the fly-specific WntD) are also palmitoylated by the membrane-bound acyltransferase Porcupine (Porc) [109,114–117]. This post-translational lipid modification renders Wnts hydrophobic and prevents them from moving within the cellular secretory pathway as a soluble protein [118,119]. Modified Wnts are escorted through the secretory pathway to the plasma membrane for extracellular release by a dedicated chaperone protein, Wntless (Wls) [120–122]. The post-translational processing of Wnt ligands is essential, as all 19 human Wnts require palmitoylation by Porcupine and interaction with Wls in order to ensure efficient secretion [123]. The exact mechanism of Wnt extracellular release and targeting to receiving cells has not yet been fully elucidated. However, Wnts have been detected in the extracellular space in a variety of different forms that would enable targeting of otherwise hydrophobic Wnts to receiving cells. These include packaging of Wnts into exosomes and lipoprotein particles, delivery of membrane bound Wnts on membrane processes known as cytonemes, and interaction with extracellular proteins that mask Wnt hydrophobic domains. Indeed, the modality of Wnt extracellular travel may also influence specificity of Wnt pathway activation in the receiving cells [124–131].

Changes in the expression of the factors required for Wnt maturation would be expected to have pleiotropic effects, as they will affect all Wnts. Hence, some specific developmental defects and human diseases have been attributed to disrupted expression of Wnt processing factors. Mutations in human *Porcupine* result in focal dermal hypoplasia (FDH) that is characterized by skin deformities and various ocular and dental malformations [132,133]. Knockout mutations in *porcupine* block Wnt signaling, resulting in early embryonic lethality in mice due to gastrulation failure [134,135]. Similarly, the knockdown of *porcupine* or treatment with C59 (specific inhibitor of Porcupine) results in defective sea urchin endodermal gene regulatory network and apical neurogenic domain [136]. Thus, Wnt processing by Porcupine is requisite for proper Wnt signaling.

Wnt–Wls interaction is crucial for proper development. Disruption of mammalian *WLS* (*Gpr 177*) by a *lacZ* insertion exhibits defective body axis formation [137]. While conditional deletion of *WLS* caused pancreatic hypoplasia in pancreatic precursor cells, germline deletion of *WLS* in mice results in failed anterior-posterior axis formation, similar to β-catenin deletion phenotypes [138]. In addition to regulating proper embryonic axis formation, Wnt and Wls levels are also important for maintaining normal tissue homeostasis. Elevated Wnt and Wls protein levels have been observed in patient colorectal cancers, as compared to matched normal colon tissue [139]. Furthermore, knockdown of *WNT* and *WLS* in colon cancer cell lines with activating mutations in *β-catenin* and *APC* show decreased Wnt reporter activity, suggesting the presence of ligand with its chaperone protein further increases Wnt pathway activation [139].

Secreted Wnt ligand can be inactivated by the catalytic activity of extracellular Wnt inhibitors, such as Tiki1 and Notum [140,141]. Tiki1 is a protease that cleaves amino terminal residues of Wnt proteins [141], whereas Notum has recently been shown to cleave the acyl group from Wnts [142,143]. Both changes prevent Wnt binding to the FZ receptor and downstream signaling. Loss of Notum in *Drosophila* leads to increased Wnt signaling activity, resulting in abnormal wing growth [140,144]. The critical role of Notum in head development is conserved from planarians to vertebrate [145,146]. In *Xenopus* embryos,

Notum is critical for neural and head induction by acting within the presumptive neuroectoderm [142]. Inactivation of extracellular WNT inhibitors, Tiki1 and Notum, may lead to the development of therapeutic treatments for degenerative diseases caused by WNT deficiency [147].

2.4. Cross regulation of the Wnt signaling pathways

How Wnt signaling achieves specificity remains unclear. The same Wnt ligands or frizzled receptors can activate either the canonical Wnt/β-catenin or the non-canonical Wnt pathways, such as in the case of Wnt 2 [148–150], 3 [151–153], 4 [154,155], 5 [156,157] and FZ4 [156,158], 7 [156,159–162]. Recent studies have shown that one way Wnt signal transduction achieves specificity is by selective activation of the type of Wnt pathway depending on the concentration of Wnt ligands. For example, in primary human articular chondrocytes, high concentration of Wnt3A activates the Wnt/β-catenin pathway, whereas low concentration of Wnt3A activates the Wnt/Ca^{2+} pathway [163]. Thus, Wnt3 can activate both canonical Wnt/β-catenin and non-canonical signaling in the same cells and turns on different target genes, depending on the level of Wnt3. In the *Xenopus* embryo, Wnt11 ligand is enriched on the dorsal side of the embryo and present in lower concentrations on the ventral side of the embryo [164]. This Wnt11 gradient results in Wnt11 activating CamKII of the non-canonical Wnt/Ca^{2+} pathway on the ventral side of the embryo [165], and Wnt11 activating the Wnt/β-catenin pathway for dorsal axis formation [166]. Further, the canonical and non-canonical Wnt pathways are highly interconnected and they cross-regulate each other. For example, the Wnt/Ca^{2+} pathway has been found to inhibit the Wnt/β-catenin pathway [167–169]. Thus, pattern formation and organogenesis during embryogenesis is achieved by the diversity of Wnt mediated cellular effects, depending on gradients of Wnt ligands and cross regulation of canonical and non-canonical Wnt signaling pathways.

3. microRNAs biogenesis and function in development

miRNAs are non-coding regulatory RNA molecules that are approximately 21–23 nucleotides in length [170]. The first microRNA (miRNA) genes identified were *lin-4* and *let-7* that control developmental timing in nematodes by modulating the expression of *lin-28* and *lin-41*, respectively, at the post-transcriptional level [171,172]. miRNAs are found in viruses, fungi, plants, and animals that are capable of modulating a large fraction of an organism's transcriptome [173–176]. miRNAs are capable of regulating thousands of genes that are involved in diverse biological processes, including cellular differentiation and development [177]. Because a single miRNA regulates numerous gene targets, dysregulation of miRNAs is associated with various pathologies such as cancer [178], autoimmune [179], developmental disorders [180], and many others. The miRNA field has grown tremendously and miRNAs are now recognized as critical regulators of gene expression.

3.1. Synthesis and regulatory mechanism of miRNAs

Most miRNAs in the genome are located in the regions that are distant from previously annotated genes, suggesting that they are in an independent transcription unit with a

promoter of their own [170]. A minority of miRNAs are derived from the intronic regions of the pre-mRNAs transcribed from the protein coding genomic sequences, suggesting that these miRNAs are dependent on the promoter region of the associated gene and the mRNA splicing mechanisms [181]. Furthermore, some miRNA genes are also found clustered in the genome, indicating their transcription as a multi-cistronic primary transcript [182].

Majority of miRNAs are transcribed as primary miRNA (pri-miRNA) transcripts by the RNA polymerase II, although some are transcribed by RNA polymerase III as well (Bartel, 2004). Most pri-miRNAs are initially processed by Drosha and its cofactor DGCR8 [183,184] in the nucleus to form the pre-miRNAs which are exported into the cytoplasm. Here, the pre-miRNAs are further processed by Dicer, leading to formation of double-stranded RNA fragments (20–25 bp) [185,186]. One strand of the resulting miRNA:miRNA* duplex, also known as the guide strand, is then loaded on to the RNA-induced silencing (RISC) complex [187], forming mature miRNAs that are able to recognize and bind their target mRNAs. The mechanism of strand selection had been reported to be dependent upon the relative free energies of the duplex ends, as the small RNA whose 5′-end exhibits the less stable end is preferentially maintained in the mature silencing complex [187,188]. The passenger strand denoted with an asterisk (miRNA*) is either degraded or in some cases, may become functional miRNA that can target different mRNA [189]. miRNA* species are bona fide trans-regulatory RNA molecules with demonstrable endogenous regulatory effects in *D. melanogaster* [189]. It was also shown that the miRNA* species are often present at physiologically relevant levels and can associate with Argonaute proteins, similar to their complementary miRNA guide strand. Moreover, using functional assays, the inhibitory activity of miRNA* species in both cultured cells and transgenic animals also validated their functions (Okamura et al., 2008).

A subset of intronic miRNAs, known as mirtrons, is processed independent of Drosha [190–193]. Mirtrons lack the lower hairpin and the flanking single-stranded regions. Instead, they are processed by small nuclear RNAs of the splicing machinery. After debranching of the lariat intron, the spliced introns fold back into a stem-loop structure that obviates the Drosha-mediated processing.

In animals, binding of miRNA to the target mRNA is facilitated by the “seed” or the “core” sequence. The seed sequence is a 6–8 nucleotide stretch present at the 5′ end of the miRNA from positions 2 to 8 and is generally considered necessary and sufficient for miRNA function [194–199]. The miRNA “anchor” sequence (nucleotides 13–16), or 3′-supplementary pairing, plays a modest role in target recognition [173, 200]. Further, a single target mRNA (3′untranslated region in particular) can have more than one different miRNA binding sites, and a single miRNA can also regulate multiple target mRNAs by binding to its corresponding binding site in the 3′UTRs of target mRNAs [195–197,201]. Target seed sites are particularly conserved in the 3′UTR of target mRNAs, suggesting that the predominant regulatory functions of miRNAs are exerted from the 3′UTR [202]. However, recent studies indicate that miRNA binding sites can also occur in the coding sequence [203] as well as in the promoter region of the target gene [204,205].

3.2. miRNAs in developmental processes

Results from various studies have implicated the role of miRNAs in animal development [206–209]. Loss of *dicer1*, a key enzyme in the processing of miRNAs, is lethal to early mouse development and defects become apparent as early as embryonic day 7.5 [210]. Knockdown of maternal and zygotic *Dicer* in zebrafish results in aberrant morphogenesis during gastrulation, brain formation, somitogenesis, and heart development [211]. A global depletion of miRNAs by knockdown of *Dicer* and *Drosha* in the sea urchin embryo results in early developmental defects, including gastrulation failure and embryonic lethality [212].

miRNAs have also been established as key regulators of cell fate and differentiation [213]. miRNAs may function both as a switch and a fine-tuner of the gene regulatory network [214]. Importance of miRNA *lin-4* as a developmental switch during normal temporal regulation of post-embryonic developmental events in *Caenorhabditis elegans* is well established [171,172]. miRNA *lin-4* mediates translational repression of its target mRNA *lin-14* which facilitates switching of *C. elegans* larva from stage L1 to L2 and then to L3.

Studies have also shown that miRNAs play an important role in regulation of cell proliferation and apoptosis during development [215]. *Drosophila melanogaster* miRNA dme-miR-bantam promotes tissue growth by targeting the apoptotic gene *hid*, and the dme-miR-2 family targets the proapoptotic genes *Grim* and *Reaper* [216].

During embryonic development, cells within the embryos are exposed to numerous ligands and morphogens of several signaling pathways (such as TGFβ, Wnt, Hedgehog, Notch, Hippo, EGF, VEGF, PDGF) to allow tissue induction coordination, patterning, growth and morphogenesis [214]. miRNAs may sharpen morphogen gradients in the developing embryo. For example, miR-15/16 targets Nodal receptor activating receptor type 2A to allow selective expression of Nodal in the most dorsal and anterior part of the embryo in forming the Spemann's organizer in *X. laevis* [217,218]. miRNAs can also serve as a positive regulator by amplifying signal strength in duration to allow cell responsiveness to sub-threshold stimuli [7]. This is the case in the RAS-RAF-mitogen activated protein kinase (MAPK) and phosphatidylinositide 3-kinase-AKT cascades where miR-21 enhances in RTK signaling by inhibiting *PTEN* and *Sprouty* [219,220] and miR-126 sustains VEGF signaling by suppressing *SPRED1* and *PIK3R2* mRNAs [221,222].

In summary, during developmental transitions where a tissue-specific miRNA is upregulated and its pool of target genes are downregulated, the effective concentration of the miRNA could greatly increase [214]. The transcription factors of the developmental gene regulatory network, whose protein exerts a switch-like response, may be suppressed in the first place through molecular repression by its regulatory miRNA. In this manner, miRNAs may precisely and promptly fine-tune gene expression thresholds which are an important feature of cell fate decisions [7,214].

4. microRNA regulation of Wnt signaling pathways in the context of development

4.1. Embryonic development

miR-34 has been shown to directly regulate several Wnt/β-catenin components, including *β-catenin*, *Wnt1*, *Wnt3*, *LRP6*, and *LEF1*, affecting *Xenopus* body axis polarity and downstream Wnt-dependent patterning genes [223]. The embryonic axis in early *Xenopus* embryos is controlled by Wnt/β-catenin signaling [39]. Injection of *β-catenin* mRNA lacking its UTR into the *Xenopus* ventral blastomeres resulted in axis duplication, while injection of *β-catenin* constructs with 790 bp of its 3′UTR (containing miR-34 regulatory sites) exhibited significantly less axial defects [223]. This indicates that miRNA repression of β-catenin is critical for establishing embryonic axis.

In the sea urchin, *β-catenin* is regulated by at least two miRNAs [224], one of which has the exact same sequence as the mammalian miR-25 which targets mammalian *β-catenin* [225]. Blocking endogenous miRNA regulation of sea urchin *β-catenin* resulted in increased transcript levels of Wnt responsive endodermal regulatory genes and affected development of the larval gut and the structure of circumesophageal musculature [224]. Thus, regulation of *β-catenin* by miRNAs is a conserved regulatory mechanism utilized by developing vertebrate and invertebrate embryos.

4.2. Bone development: osteoblast differentiation

Recently several studies have reported the role of miRNAs in mediating differentiation of mesenchymal stem cells (MSCs) into osteoblasts. MSCs have the ability to self-renewal and differentiate into multiple cell lineages including osteoblasts, chondrocytes, adipocytes, cartilage, tendons, muscle and skin. miRNAs have the potential to drive differentiation from one to the other lineage by targeting TGFβ, BMP, Wnt signaling pathways that are involved in osteoblast differentiation [226–230]. Several miRNAs have been identified to activate the Wnt signaling pathway which promotes osteoblast differentiation. miR-27, miR-142-3p, and miR-135a-5p target *APC* which leads to accumulation of β-catenin and activation of Wnt signaling to promote osteoblast differentiation in human fetal osteoblastic 1.19 (hFOB1.19) and 3T3-L1 cells [231–233]. miR-29 participates in the fine-tuning of Wnt signaling such that the canonical Wnt signaling induces miR-29a transcription which down regulates inhibitors of canonical Wnt signaling such as *DKK1*, *Kremen2*, and *SFRP2* to promote osteoblast differentiation in hFOB1.19 cells [234]. *DKK1* is also down regulated by miR-335-5p in murine osteoblast-like cells (MC3T3-E1 cells) [235]. miR-218 is another miRNA that directly suppresses *SFRP2* and *DKK2* which result in enhanced Wnt/β-catenin signaling activity that promote osteogenic differentiation in human adipose-derived stem cells [236]. Further, miR-218 is a part of a feed-forward regulatory circuit such that Wnt/β-catenin signaling activity increases the expression of miR-218 [236]. Previous studies using 3T3-L1 preadipocyte cells and human mesenchymal stem cells demonstrated that miR-709, miR-344, and miR-346 directly inhibit 3′UTR *GSK3β*, leading to increased β-catenin which represses adipogenesis [237–239].

In contrast, miR-30e inhibits Wnt signaling by inhibiting *LPR6* co-receptor of Wnt ligands, promoting adipocyte differentiation in mouse bone marrow stromal cell, mesenchymal cell line, C3H10T1/2, and mouse preadipocyte 3T3-L1 cells [240]. A large scale microarray experiment identified miR-210 to target *TCF712* in HEK-293FT cells to promote adipogenesis by repressing Wnt signaling [241]. Similarly, the *Drosophila* miR-8 (homologous to the mammalian miR-200c/141 or miR200b/a429 cluster) directly inhibits *TCF* and *Wls*, resulting in antagonizing Wnt and promotes bone marrow stromal cells to differentiate into adipocytes [242]. An accumulating literature indicates that miRNAs maintain the balance between osteoblast versus adipocyte differentiation as well as osteoblast versus osteoclast differentiation [243]. Thus, miRNAs can potentially be used as a therapeutic treatment of metabolic bone diseases such as osteoporosis.

4.3. Cardiac development

Heart formation and function require precise cardiac gene expression for myogenesis, morphogenesis, and contractility that are controlled by complex regulatory networks at the transcriptional and post-transcriptional levels [244]. miRNAs are important regulators of cardiovascular development, and dysregulation of their expression has been linked to cardiac diseases [245–247]. miR-19b overexpression study revealed its regulation of the left-right symmetry and cardiac development in zebrafish embryo by directly suppressing *β-catenin* (*CTNNB1*) of the Wnt signaling pathway [248,249]. The deformed cardiac phenotype was partially rescued by lithium chloride treatment which inhibits GSK3β, leading to an accumulation of β-catenin [248]. miR-499 in rat bone marrow-derived mesenchymal stem cells was able to drive cardiac differentiation as shown by increased expression of cardiac-specific genes (Nkx2.5, GATA4, MEF2C, and cTnl) through Wnt/β-catenin signaling pathway. miR-499's activation of the Wnt/β-catenin signaling pathway was demonstrated by decreased ratio of phosphorylated/dephosphorylated β-catenin, although its targets within the Wnt pathway have not been directly identified [250]. miR-1 is a key muscle-specific miRNA expressed in cardiac and smooth muscle cells [251,252]. miR-1 promotes differentiation of cardiomyocytes by suppressing the activities of Wnt and FGF signaling pathways [253]. Specifically, miR-1 directly inhibits *FZ7* to repress Wnt signaling [253]. Because miRNAs are able to regulate all stages of cardiac development, certain miRNA expression signatures are used for clinical diagnosis of human heart disease [245]. miRNA-based therapeutics for cardiac disease are also in the near future.

4.4. Other organ development

4.4.1. Brain—Increasing evidence indicates the essential role of miRNAs in brain development [254–256]. A conditional knockout using Wnt-1 Cre-mediated loss of *Dicer* resulted in malformation of midbrain and cerebellum and a failure of neural crest and dopaminergic differentiation [257]. miR-296, -124, and -101 were identified to modulate the *GSK3β* levels which inhibit the Wnt signaling pathway, using luciferase assays, mutagenesis of miRNA binding sites, and β-catenin activity assays in 293 T cells [257]. Further, the size of the midbrain dopaminergic progenitor cells is regulated by miR-135a2, inhibiting the *Wnt1* morphogen in the embryonic midbrain [258]. miR-135a2 suppresses *Lmx1b*, *Ccnd1*, *GSK3β*, and *TCF7l2* in an autoregulatory negative feedback loop to control the size of the dopamine progenitor region [258]. In addition to playing a critical role in various aspects of

normal brain development, miRNAs are also important for neuronal repair. Previous study indicates that the intact Dicer-dependent miRNA pathway is necessary for functional recovery after peripheral nerve injury in adult mouse [259]. Subsequently, miR-431 was identified to stimulate regenerative axon growth by silencing *Kremen1* (*DKK1*), which is a negative regulator of the Wnt/β-catenin signaling pathway [260].

4.4.2. Testis—Wnt signaling plays an important role in sex determination during gonadal development [261,262]. Perturbation of the Wnt pathway with *Drosophila* miR-310 to miR-313 cluster deletion antagonizes Wnt signaling pathway activity, resulting in aberrant germ and somatic cell differentiation in the testis [263]. miR-310/313 is the human ortholog hsa-miR-25 that suppresses the Wnt/β-catenin pathway by directly targeting *Drosophila β-catenin* and *TCF* in vivo [263].

4.4.3. Lung—Alveolar epithelial cell (AEC) trans-differentiation is a process where AECII trans-differentiates into AECI during lung recovery after injuries. miR-375 overexpression inhibited alveolar epithelial trans-differentiation by targeting the *FZ8* of the Wnt/β-catenin pathway [264].

5. microRNA regulation of Wnt signaling pathways in the context of disease

5.1. Heart disease

miRNAs that regulate Wnt/β-catenin signaling pathway have been implicated in contributing to not only normal heart development, but also cardiac dysfunction in disease development. During Coxsackie viral infection, virally induced ERK1/2 activation leads to phosphorylation of ETS-1/2 transcription factors, which induce miR-126 expression [265]. miR-126 inhibits *SPRED1*, *LRP6*, *WRCH1*, to mediate crosstalks between ERK1/2 and Wnt/β-catenin pathways. During Coxsackie viral infection, an upregulation of miR-126 promotes cyto-pathogenicity by stimulating GSK3β and suppressing Wnt/β-catenin signaling, sensitizing the cells to virus-induced cell death and viral progeny release [265]. Also, in patients with Duchenne muscular dystrophy with dystrophin deficiency, miR-199a-5p is elevated in the dystrophic muscle [266]. miR-199a-5p suppresses *FZ4*, *JAG1*, and *Wnt2* that leads to inhibition of Wnt signaling factors that regulate myogenesis [266].

5.2. Bladder pain syndrome

Human bladder pain syndrome (BPS) is characterized with defects in the proteoglycan core proteins and the tight junction proteins that cause increased urothelial permeability [267,268]. miR-199-5p is highly expressed in the bladder smooth muscle that has previously been found to regulate components of the intercellular junctions [269]. Recently miR-199a-5p was found to directly inhibit *Wnt2* or Wnt-dependent cell proliferation and simultaneously activate myocardin and MRTF-A-dependent smooth muscle differentiation [270]. Attenuation of miR-199a-5p causes a significant reduction of the bladder smooth muscle cell size and increased cell proliferation. Thus, the ability of miR199a-5p to inhibit cell proliferation and activate muscle differentiation makes it important for smooth muscle hypertrophy and fibrosis [270].

5.3. Diabetes

Central to the pathophysiology of diabetic nephropathy is mesangial cells (MCs) that undergo phenotypic transition into myofibroblasts [271,272]. Profibrotic growth factor TGF-β1 responds to high glucose levels in glomerular mesangial cells and regulates MC phenotypic changes during diabetic nephropathy progression [273]. miR-215 directly regulates *Catenin-β Interacting Protein* (*CTNNBIP1*), which suppresses Wnt/β-catenin signaling that leads to activation of α-SMA and fibronectin expression in TGF-β1 treated mouse mesangial cells (MMCs) [274]. Thus, miR-215 suppresses *CTNNBIP1* that promotes the TGF-β1 induced pathogenesis of diabetic nephropathy.

5.4. Various cancers

Both Wnt signaling pathways and miRNAs play essential roles in embryonic development as well as in tissue homeostasis maintenance in adults. Recently microRNAs have been found to regulate tumor-related pathways (reviewed by [275]). As crucial biological regulators, miRNAs suppress targets of the signaling pathway that can act to promote or suppress cancer progression in a context-dependent or target-dependent way. We will discuss examples of miRNA regulation of Wnt signaling pathways that lead to specific types of cancers. Additional miRNAs that regulate Wnt pathways components in other cancers not mentioned in the text are summarized in Table 2.

5.4.1. Brain cancer—miRNAs regulate several types of brain cancer. In meningiomas, one of the most common human brain tumors, miR-200a was found to directly target *β-catenin* to inhibit cell proliferation [276]. miR-200a also acts as a multifunctional tumor suppressor miRNA that promotes cell adhesion and differentiation by directly inhibiting *ZEB* and *SIP1*, leading to increased E-cadherin and cell adhesion [276].

Glioma is a tumor that originates from glial cells in the brain or the spine. It makes up approximately 30% of all brain and central nervous system and 80% of all malignant brain tumors [277]. The majority of known miRNAs that regulate the Wnt pathway in glioma are oncogenic. From miRNA expression profiling of tumor biopsy specimens of human astrocytic gliomas, miR-328 was found to be upregulated in invading glioma cells in vivo and directly suppress *SFRP1* [278]. Oncogenic miR-603 directly inhibits *WIF1* and *CTNNBIP1* and promotes glioma growth [279]. miR-96 was identified to indirectly regulate the Wnt/β-catenin pathway by suppressing *HMG-box transcription factor protein 1* (*HBP-1*) that leads to increased glioma cell proliferation. Importantly, the tumorigenicity of U-87 MG cells was reduced by miR-96 silencing [280]. Oncogenic miR-24 inhibits *ST7L* tumor suppressor gene that negatively regulates the Wnt/β-catenin pathway [281]. miR-222 and miR-92b target *DKK-2* and *DKK-3*, respectively, that lead to constitutive activation of β-catenin in glioma cells [282,283]. Inhibition of miR-222 leads to decreased tumorigenesis in vivo and serves as a potential target in glioma therapy. In addition, miR-30a-5p directly inhibits *PRDM1*, a transcriptional regulator that suppresses the proliferation and invasion by glioma cells by inhibiting Wnt/β-catenin signaling [284].

In medulloblastoma cells, overexpression of miR-193a and miR-224 induced highest Wnt pathway specific upregulation, although their specific targets of the Wnt signaling pathway have not been identified [285].

5.4.2. Colorectal cancer—Colorectal cancer is the second leading cause of cancer-related mortality in Western countries [286]. p53 function and activation of canonical Wnt signaling cascades are often dysregulated in cancer. p53 was found to transactivate miR-34 which suppresses the transcriptional activity of β-catenin and TCF/LEF complexes [223]. Loss of p53 or miR-34 contributes to neoplastic progression in colorectal cancer cells. miR-34-mediated suppression of *Axin2* results in increased nuclear GSK3β and decreased Snail in colorectal cancer [287]. Leading to a similar effect, tumor suppressor miR-23b directly inhibits *FZ7* to halt tumor progression [288]. Tumor suppressor miR-29b down regulates coactivators of β-catenin, including *TCF7L2*, *Snail*, and *BCL9L*, in SW480 cells [289]. Further, ectopic expression of miR-29b inhibits anchorage independent cell growth and promoted EMT in vitro. Targeting the non-canonical Wnt/Ca^{2+} pathway, miR-224 inhibits *Cdc42*, resulting in suppression of cell migration [290]. Dependent on tumor grade, miR-101 is differentially expressed such that its expression suppresses cell growth, hypoxic survival and invasive potential [291]. miR-101 was found to repress activation of Wnt/β-catenin signaling pathway and EMT to hamper the aggressive behavior of colorectal cancer in vivo. miR-93 directly targets *Smad7* which is important for the nuclear accumulation of β-catenin. Indirectly, miR-93 induces downregulation of β-catenin, Axin, c-Myc, and CyclinD [292]. Furthermore, forced expression of miR-93 resulted in reduced colon cancer invasion, migration, and proliferation. A novel regulatory mechanism was discovered in which tumor suppressor miR-145 directly inhibits catenin δ-1 that results in aberrant translocation of β-catenin through defective nuclear shuttling with p21-activated kinase 4 (PAK4) in colon cancer cells [293]. The inability of β-catenin translocation into the nucleus leads to downregulation of key β-catenin activated genes that promote cell proliferation, such as *c-Myc* and *CyclinD1*.

miR135a/b is an oncogenic miRNA that suppresses *APC* directly to promote β-catenin pathway activation in colorectal cancer [294]. Other oncogenic miRs indirectly target various negative regulators of the Wnt pathways. For example, miR-574-5p suppresses Qki6/7, a RNA family protein, leading to increased expression of β-catenin and p27 [295]. This results in increased proliferation, migration and invasion, and decreased differentiation and cell cycle exit of mouse and human colorectal cancer cells. Importantly, inhibition of miR-574-5p suppresses the growth of tumors in the nude mice [295]. miR-21 enhances colon cancer by directly inhibiting a negative regulator of the Wnt/β-catenin pathway, *TGFβ-R2*, which indirectly causes decreased levels of β-catenin, c-Myc, and CyclinD1 [296]. Another example is oncogenic miR-7 that inhibits *Ying Yang 1* transcription factor, which is a negative regulator of Wnt/β-catenin signaling that down regulates *SFRP-1* and *APC* [297].

5.4.3. Liver cancer—Dysregulation of the Wnt/β-catenin signaling pathway is observed in hepatocellular carcinoma (HCC). miR-214 directly targets *β-catenin* and the *enhancer of zeste homologue2* (*EZH2*), which also regulates *β-catenin* [298,299]. miR-214 is a tumor

suppressor miRNA that directly and indirectly targets *β-catenin* to inhibit cell growth by downregulating *c-Myc*, *Cyclin D1*, *TCF-1*, and *LEF1* in HCC [299]. Upregulation of miR-139 was observed in HCC cells and demonstrated to directly inhibit *TCF4* via luciferase assay [300]. In addition, overexpression of miR-139 suppressed β-catenin signaling and inhibits cell proliferation and invasion, suggesting it as a potential therapeutic for treating HCC patients. miR-122 and miR-148b directly inhibit *Wnt1*, resulting in reduced β-catenin and c-Myc and prevent HCC cell growth [301,302]. miR-142-3p is found to directly target *Rac1*, a downstream effector of the non-canonical Wnt/PCP pathway and to inhibit migration and invasion of HCC [303]. Tumor suppressor miR-612 directly inhibits *AKT2* accompanied with inhibited epithelial-mesenchymal transition and upregulation of E-cadherin that sequesters β-catenin in the cytoplasm [304,305]. Further, miR-612 level is negatively correlated with TCF/LEF transcriptional activity and transcript levels *CyclinD* and *c-Myc* (downstream genes of β-catenin signaling), indicating that miR-612 indirectly inhibits the Wnt/β-catenin pathway [304]. Another tumor suppressor miR-145 targets *IRS-1* and *IRS-2* in the insulin-like growth factor pathway [306], which is known to cross regulate the Wnt/β-catenin pathway [307,308]. Increased miR-145 expression leads to the reduction of β-catenin protein levels, thus exhibiting its tumor suppressor function in HCC.

miRNAs can also enhance HCC. For example, oncogenic miR-106b and miR-155 were upregulated in hepatoma cells and both directly inhibit *APC*, resulting in an accumulation of β-catenin that promotes the proliferation of human hepatoma cells [309,310]. Although not directly targeting the Wnt pathway, upregulated oncogenic miR-130a directly inhibits tumor suppressor Runx3 expression which results in activation of Wnt/β-catenin signaling and HCC resistance to cisplatin, a commonly used chemotherapeutic drug used for treatment [311]. Recently, oncogenic miR-153 was identified to regulate a tumor suppressor WWOX, inhibitor of the Wnt/β-catenin signaling pathway [312]. Overexpression of miR-153 leads to increased proliferation in HCC in a murine liver cancer model.

5.4.4. Breast cancer—In addition to regulating HCC, miR-145 targets *Mucin*, *Fascin-1*, *c-Myc*, *SMAD2/3*, and *IGF-1R* to inhibit breast cancer tumor cell invasion and indirectly causes downregulation of β-catenin and cadherin11 [313, 314]. miR-31 acts as a tumor suppressor by targeting *RhoA* to inhibit cell motility [315,316]; however, it also acts as an oncogenic miR by targeting Wnt antagonists *DKK1* and indirectly decreases SFRP1, SFRP4, and WIF1 levels [317]. Moreover, tumor suppressor miR-24 inhibits *Wnt4*, resulting in inhibition of cell motility and EMT [318].

Oncogenic miR-374a is overexpressed in breast cancer cell lines and it promotes epithelial to mesenchymal transition (EMT) and metastasis [319]. miR-374a directly inhibits multiple negative regulators of the Wnt/β-catenin signaling cascade, including *Wnt5A* (ligand for non-canonical Wnt), *PTEN*, and *WIF1* [319]. Previously oncogenic miR-142 and miR-150 were increased in human breast cancer stem cells (BCSCs) [320]. miR-142 suppresses *APC* directly and transactivates miR-150 which induces cell growth. Further, knockdown of miR-142 reduced the clonogenicity of BCSCs in vitro and tumor growth in vivo. Interestingly, metastatic breast cancer cells express many osteoblast related genes (osteomimicry) that promote its metastasis to the bone [321]. miRNAs play an important role in regulating osteoblast differentiation during normal bone development [322–324]. For

example, miR-218 is significantly upregulated during osteoblast differentiation and targets multiple inhibitors of Wnt signaling [325]. Oncogenic miR-218 found in metastatic breast cancer cells is a potent activator of Wnt signaling that promotes the osteomimicry to facilitate breast cancer cells that metastasize to the bone.

6. Concluding remarks

Increasing evidence indicates the importance of miRNAs in modulating signal transduction pathways (reviewed in [7]). The highly dose-sensitive nature of developmental signaling pathways is under intense miRNA regulation. Current studies indicate that miRNAs participate in signaling networks, both as additional layer of transcriptional control and as feed-forward or feedback devices that result in robustness to the output of cell signaling. Deciphering miRNA function in the context of a developing embryo where crosstalks of miRNAs, signaling pathways and regulatory complexities are taken into account will be critical in revealing the physiological functions of miRNAs.

Many human diseases result from disruption of developmental signaling pathways where miRNAs play a key role in promoting or inhibiting disease progression. Thus, a thorough understanding of miRNA function and miRNA interaction and crosstalk with various signaling pathways will be critical in understanding normal regulation of development and maintenance of tissue homeostasis. A fundamental understanding of miRNA regulation of signal transduction pathways may lead to discovery of novel anti-miRNA-based diagnostics and therapeutics for cancer and other human diseases.

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Abbreviations

Wls	wntless
Porc	porcupine
HSPG	heparin sulfate proteoglycans
ARF	ADP-Ribosylation Factor
Dsh/Dvl	disheveled
DIX	disheveled Axin domain
PDZ	postsynaptic density 95, discslarge, zonulaoccludens-1
DEP	Dvl, Egl-10, Pleckstrin
LRP5/6	low-density lipoproteinco-receptor 5 and 6
FZ/FZD	frizzled receptor
APC	adenomatous polyposis coli

LEF1	lymphoid enhancer binding factor 1
GSK3β	glycogen synthase kinase3B
SFRPs	secreted frizzled-related proteins
WIF	Wnt inhibitory protein
DKK	dikkopf
ROR	receptorty rosine kinase-like orphan receptor
Ryk	receptorty rosine kinase-related tyrosine kinase
MMP-2	matrix metalloproteinase
Wnt/PCP	Wnt Planar cell polarity
WGEF	weak similarity GEF
PLC	phospho-lipase C
IP3	inositol 1,4,5-triphosphate
DAG	1,2-diacylglycerol
PKC	protein kinase C
CamKII	calmodulin dependent protein kinase II
Cn	calcineurin
NFκβ	nuclear factor kappa-light-chain-enhancer of activated B cells
CREB	cAMP response element-binding protein
NFAT	nuclear factor of activated T cells
EMT	epithelial to mesenchymal transition
DGCR8	DiGeorge syndrome critical region 8
RISC	RNA induced silencing complex
miRNA	microRNA
3′UTR	3′untranslated region

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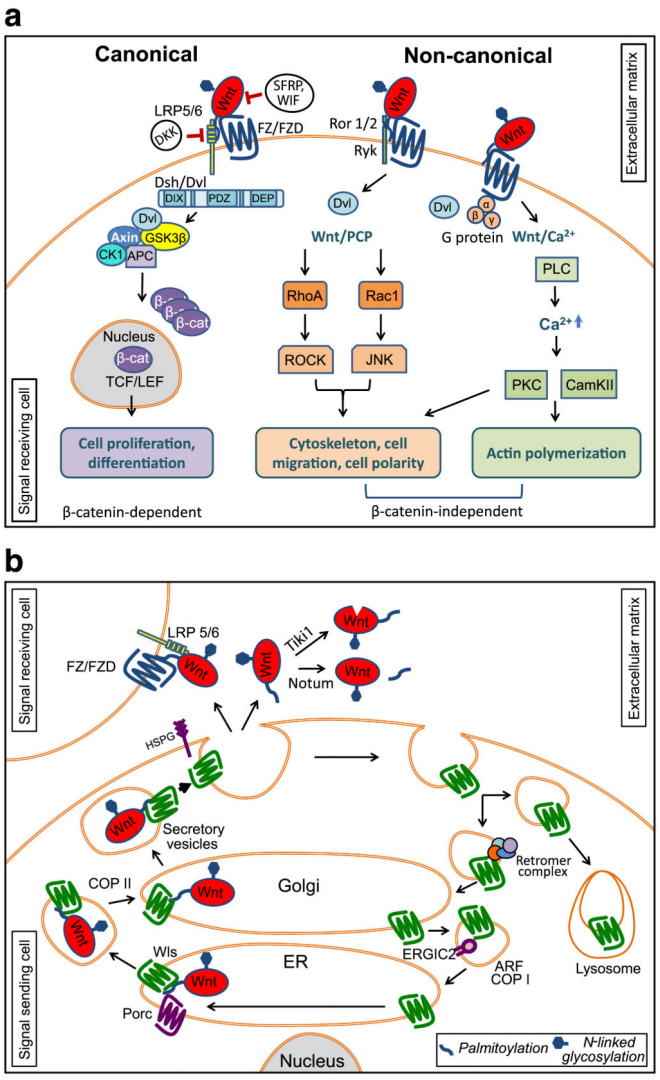

Fig. 1.

Wnt signaling pathways. a) In canonical Wnt pathway, β-catenin is normally degraded by the destruction complex composed of Axin, APC, CK1, and GSK3β. Upon Wnt ligand binding to the frizzled (FZ/FDZ) and LRP5/6 co-receptor, disheveled (Dsh/Dvl) disassembles the β-catenin destruction complex, resulting in an accumulation of β-catenin that can enter the nucleus. In conjunction with TCF/LEF, β-catenin transcriptionally activates target genes. In non-canonical Wnt/Planar cell polarity pathway (Wnt/PCP), its activation is mediated by Wnt ligand binding to the FZ/FDZ and co-receptors Ror1, Ror2, or Ryk. Wnt/PCP is mediated by small GTPases RhoA and Rac1 to activate JNK and ROCK pathways, respectively, resulting in actin remodeling events. In Wnt/Ca^{2+} pathway, the activation of FZ/FDZ is mediated by disheveled and heterotrimetic G-proteins. Calcium release activates PKC, calcineurin, and CamKII that activates cell migration. b) Wnt proteins are N-glycosylated and lipid modified by the oligosacchary transferase complex and acyltransferase Porcupine within the endoplasmic reticulum (ER), respectively. Wnts are then engaged by Wntless (Wls) for secretory pathway transit and extracellular secretion.

Wnt-Wls complex is transferred from ER to Golgi in COP II regulated vesicles, and from the Golgi to extracellular matrix in secretory vesicles [351,352]. Secretion mechanisms of Wnts are not completely understood; however, it has been reported that Heparan Sulfate Proteoglycans (HSPGs) are involved in Wnt stabilization in extracellular matrix [353]. After secretion, the Wnt ligand may be enzymatically inactivated by Tiki1 or Notum. Tiki1 is a protease that cleaves the amino terminal residues of Wnt [141], and Notum removes an essential palmitoleate moiety from Wnt [143]. Functional Wnts are then targeted to their receptors (FZ/FZD) via one of the proposed models mentioned in the text. Following extracellular release of Wnts, Wls is routed to multicellular vesicles for lysosomal degradation or shuttled back to the Golgi by retromer mediated targeting [354–356]. Golgi targeted Wls is transported to the ER by ADP-Ribosylation Factor family proteins (ARF) regulated COP I vesicles and Endoplasmic Reticulum-Golgi Intermediate Compartment protein 2 (ERGIC2) for subsequent rounds of loading ([357]; EMS and SST, unpublished).

Table 1

miRNAs regulate components of Wnt signaling pathway in various cancers.

microRNA	Target gene	Type of cancer	Effect	Reference
miR-499	Wnt1, Inducible Signaling Pathway Protein 2 (WISP-2)	Adrenocortical tumors	Tumor suppressor	[326]
miR-21	TGFβR2	Bladder cancer	Oncogene	[296]
miR-144	EZH2	Bladder cancer	Oncogene Inhibits SFRP-1, APC	[327]
miR-182-5p	Smad4	Bladder cancer	Oncogene	[328]
miR-493	FZ4, RhoC	Bladder cancer cell lines	Tumor suppressor Inhibits cell motility	[329]
miR-1862	CTNNB1, MEK	Bladder cancer	Tumor suppressor	[330,331]
miR-200b	PKCα	Bronchial epithelial cell cancer	Tumor suppressor Inhibits Rac1 mediated motility	[332]
miR-26a	GSK3β	Cholangiocarcinoma	Oncogene	[333]
miR-148a	Wnt10B	Endometrial cancer	Tumor suppressor	[334]
miR-141	Sox-17	Esophageal cancer	Oncogene	[335]
miR-27	APC	Gastric cancer	Oncogene	[336]
miR-218	LEF1	Glioblastoma	Tumor suppressor	[337]
miR-577	LRP6, β-catenin	Glioblastoma	Tumor suppressor	[338]
miR-155	CK1α	Liposarcoma	Oncogene	[339]
miR-31	SFRP-4	Lung cancer	Oncogene	[317]
miR-708	TMEM88	Lung adenocarcinoma	Oncogene	[340]
miR-200a	β-catenin	Meningiomas, nasopharyngeal carcinoma, gastric adenocarcinoma	Tumor suppressor	[276,341,342]
miR-200a	ZEB1/SIP1	Meningiomas	Tumor suppressor	[276]
miR-155	APC	Papillary thyroid carcinoma tumor	Oncogene	[343]
miR-15a	Wnt3a	Prostate cancer	Tumor suppressor	[344]
miR-16-1	Wnt3a	Prostate cancer	Tumor suppressor	[344]
miR-34a	LEF1	Prostate cancer	Tumor suppressor	[345]
miR-34b/c	β-catenin	Prostate cancer	Tumor suppressor	[346]
miR-221	DVL2	Prostate cancer	Tumor suppressor Inhibits migration	[347]
miR-320	β-catenin	Prostate cancer	Tumor suppressor	[348]
miR-490 (5p and 3p)	DAAM1	Thrombocythemia platelets	Tumor suppressor Interacts with DVL that activates Wnt/PCP pathway	[349]
miR-483-3p	β-catenin	Urological cancer	Tumor suppressor	[350]
miR-1862	β-catenin	Urological cancer	Tumor suppressor	[350]