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Review

Wnt Signaling in Cell Motility and Invasion: Drawing Parallels between Development and Cancer

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Abstract: The importance of canonical and non-canonical Wnt signal transduction cascades in embryonic development and tissue homeostasis is well recognized. The aberrant activation of these pathways in the adult leads to abnormal cellular behaviors, and tumor progression is frequently a consequence. Here we discuss recent findings and analogies between Wnt signaling in developmental processes and tumor progression, with a particular focus on cell motility and matrix invasion and highlight the roles of the ARF (ADP-Ribosylation Factor) and Rho-family small GTP-binding proteins. Wnt-regulated signal transduction from cell surface receptors, signaling endosomes and/or extracellular vesicles has the potential to profoundly influence cell movement, matrix degradation and paracrine signaling in both development and disease.

Keywords: Wnts; cell motility; cell invasion; cancer; development; small GTP-binding proteins

1. Introduction

The loss of normal cell polarity and adhesion, along with the acquisition of motility and invasiveness, are fundamental steps during tumor progression and metastasis. The process of metastasis, wherein cells disseminate from a tumor and grow at distant locations, remains the largest contributor to cancer mortality [1,2]. The dysregulation of many signaling pathways, including Wnt signaling, contribute to this behavior. Wnts are well-characterized for critical functions during normal embryogenesis and tissue homeostasis, regulating processes such as cell motility, adhesion, invasion, tissue patterning, and proliferation [3,4]. However, aberrant Wnt signaling in the adult frequently leads to abnormal cellular behaviors, progressing to the onset of disease. Many aspects of Wnt signaling have been reviewed extensively in the literature, and here we describe known and predicted parallels between Wnt-mediated regulation of normal embryonic and tissue homeostatic behavior, and the abnormal activation of these pathways in cancer progression, with particular focus on tumor cell motility and invasion. Wnt-mediated regulation of the dynamics of signaling endosomes, extracellular vesicles, and invadopodia have the capacity to impact tumor cell invasion and extracellular matrix degradation. We discuss recent findings on the roles of canonical and non-canonical Wnt pathways and small GTPase-mediated signaling in the modulation of these processes, and outline unresolved gaps in the field that merit further study.

2. Canonical and Non-Canonical Wnt Signaling—An Overview

There are currently nineteen Wnt ligands identified for both canonical and non-canonical signaling axes, with some ligands functioning through both pathways [5,6]. Wnt receptors LRP5 and LRP6, alongside the ten members of the frizzled (Fzd) family of G-protein-coupled receptors, mediate canonical signaling pathways [7,8]. ROR1, ROR2 (receptor tyrosine kinases), and RYK (receptor-like tyrosine kinase) function as alternative Wnt receptors in non-canonical signaling pathways, though

this signal transduction may also modulate canonical signal transduction [5,9–11]. The large number of Wnt ligands and receptors potentially allows for great diversity in signaling outcomes [5].

The cellular processes modulated by Wnts range from stem cell self-renewal to cell motility, and are mediated by transcriptional activation as well as through direct effects on cytoplasmic targets [3,12]. β-catenin is a critical component in many Wnt pathways, and functions as both a cell-cell adhesion protein and also an intracellular signaling molecule [13,14]. Cytoplasmic β-catenin is typically degraded in the proteasome following phosphorylation by the destruction complex, which is composed of adenomatous polyposis coli (APC), Axin 1/2, casein kinase I (CKI), and glycogen synthase kinase 3β (GSK3β), and subsequent ubiquitination by β-transducin repeat-containing protein (β-Trcp). Wnts are the best known inhibitors of this degradation. Wnt ligands activate canonical signaling by binding Fzd and LRP5/6 receptors at the cell surface, and LRP phosphorylation mediates the recruitment of Axin and its subsequent inactivation, prompting the dissociation of the destruction complex and freeing β-catenin to translocate to the nucleus [4,15] (Figure 1). Nuclear β-catenin acts as a transcriptional co-activator for a variety of downstream targets of the TCF/LEF family of transcription factors, affecting the transcription of target genes which regulate a large and diverse set of cellular processes including apoptosis, metabolism, proliferation, motility, cell cycle progression, and differentiation [16,17].

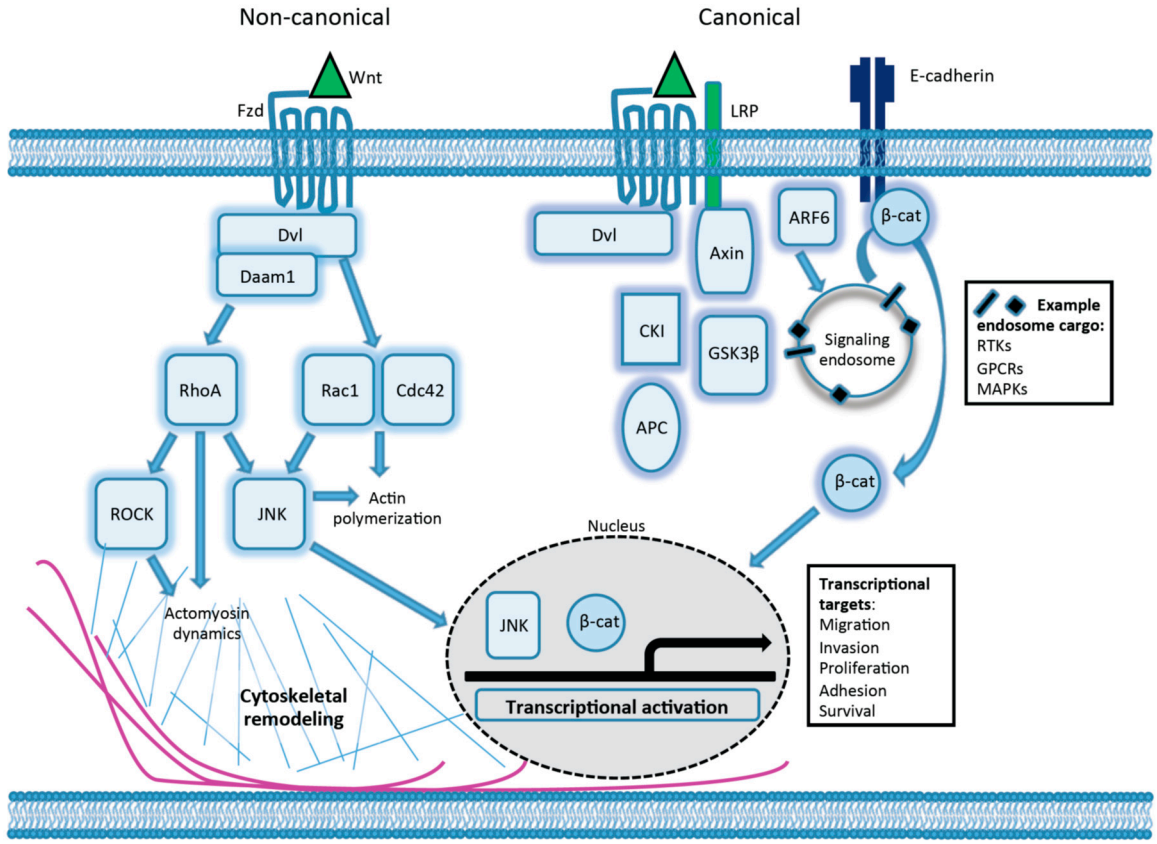

Figure 1. Canonical and non-canonical Wnt pathways influence cell migration and invasion. Wnt activation may prompt the activation of multiple downstream signaling pathways, selected examples of which are shown here. Canonical Wnt signal transduction results in the dissociation of the "destruction complex" and stabilization of cytoplasmic β-catenin, which then translocates to the nucleus to regulate the transcription of downstream targets. Canonical Wnt signaling has also been shown to stimulate ARF6 activity, which in turn promotes the internalization of transcriptionally active β-catenin from sites of cadherin-based adhesion. Non-canonical signaling cascades promote the activation of the small GTPases Rac1, RhoA, and Cdc42 to promote the cytoskeletal remodeling needed for cell invasion and migration, as well as JNK to regulate additional transcriptional targets.

Non-canonical Wnt signaling, which is independent of β-catenin transcriptional activity, encompasses multiple signaling cascades which signal through Fzd; Fzd alternative receptors ROR1, ROR2, or RYK; or possibly through Fzd with ROR or RYK as co-receptors [5,7,9,10,18]. Non-canonical signal transduction engages several downstream effectors, including calmodulin/calcium, protein kinase C (PKC), heterotrimeric G proteins, Src, JNK, and multiple small GTPases [19].

Of the non-canonical pathways, the planar cell polarity (PCP) pathway is the best characterized, with influence over a variety of developmental and disease processes [20–22]. Of the nineteen identified Wnt ligands, seven are characterized to work in non-canonical pathways [23], with Wnts 4, 5a, and 11 as the best characterized to influence PCP signaling. PCP signaling is critical for polarized cell movement and the uniform alignment of cell polarity and patterning during tissue formation [12,21,24–28]. As such, PCP signaling is key during neural crest migration [27], when cells must migrate from the dorsum of the neural tube following an epithelial-to-mesenchymal transition and travel to their final destination where they will differentiate into a wide variety of cells ranging from cartilage to neurons to melanocytes [29]. This migration occurs in highly polarized cell streams which are shaped by the presence of negative signals from the surrounding environment [27]. PCP signaling has also been shown to regulate additional, diverse developmental behaviors, including vertebrate gastrulation [24,28], neural tube closure [25], establishment of normal ciliary function [26], and the planar orientation of cell division [24].

3. Wnt Signaling in Cancer—An Overview

While canonical and non-canonical Wnt regulation of activities such as differentiation, adhesion, cell morphology, and motility are critical for normal development in the embryo [3,13], aberrant signaling through Wnt pathways can promote cancer development and progression [13,19,22,30,31]. A role for Wnt in cancer was initially identified when the tumorigenic mouse mammary tumor virus (MMTV) was frequently found to integrate into a particular region of the genome, then named MMTV int1 [32], and later recognized to encode the first identified Wnt, later to be named as Wnt1 [33]. A strong association of Wnt signaling in human cancer was identified when a correlation was noted between mutations of the β-catenin regulatory protein APC and familial adenomatous polyposis, which confers a greatly increased incidence of colorectal cancer [13,34,35]. Mutations of APC have now been characterized in approximately 80 percent of sporadic colon tumors [36,37], and less frequently in cancer of other tissues such as breast, stomach, and lung [19,38–40]. Mutations in β-catenin itself are also frequently found in tumors of the liver, pituitary gland, endometrium, pancreas, and certain ovarian cancers, with a large number of mutations in regions which prevent its degradation [19,41,42]. Mutation of the destruction complex protein Axin is also common, noted in a variety of cancers including medulloblastoma, melanoma, adenoid cystic carcinoma, and hepatic cancers [19,43].

It is important to note that Wnts do not always promote tumor progression, however, and may have oncogenic or tumor suppressive roles depending on the cellular context. An example of this is Wnt5a, which has been shown to correlate with poor prognosis and promote tumor cell invasion in melanoma, glioma, pancreatic, prostate and gastric cancer [44–49], however in breast cancer, Wnt5a has been better characterized as a tumor suppressor [50–52].

4. Small GTPases in Wnt-Regulated Cell Motility and Invasion: Parallels between Development and Cancer

4.1. Conserved Cell Behavior: Motility and Invasion in Development and Disease

There are many similarities evident between the normal behaviors of cells during embryogenesis, and tumor cells during invasion and metastasis. Genetic mutations that subvert embryonic signaling modules are frequently characterized in cancer development. As an example, many parallels exist between the behavior of migrating neural crest cells in a developing embryo and invasive tumor cells migrating during the course of metastasis to a secondary site. Melanoma, the most frequently fatal

skin cancer, is one such example [53]. One of the characteristics of advanced melanoma is aggressive tissue invasion [54], reminiscent of the activity that neural crest-derived melanocytes display during development, as cells must move through the dermis to colonize the skin and hair follicles [29].

The regulation of such cell motility and invasion is complex and multifaceted, and cells can utilize qualitatively different modes of movement to traverse distances [55,56]. These different motile behaviors are observed during embryogenesis and tissue remodeling, and tumor cells may exploit similar mechanisms. In cells with a rounded or amoeboid morphology, actomyosin at the cell cortex contracts in the direction of the desired flow. On the other hand, cells adopting a flattened or mesenchymal morphology generally form a leading edge that extends actin-rich protrusions such as lamellipodia, alongside adhesive interactions with the substratum, and followed by retraction of the contractile cell rear to achieve cellular movement [56,57]. The cytoskeletal organization required for amoeboid and mesenchymal movement is regulated by the selective activation or suppression of the Rho and ARF families of the Ras superfamily of small GTPases, as described further below. Both canonical and non-canonical Wnt signaling cascades intersect with these regulatory molecules to influence motile behavior [27,31,58,59] (Figure 1).

4.2. Roles for Rac1 and RhoA in Wnt-Mediated Cell Motility and Invasion

Intracellular signaling mediated by the small GTPases Rac1 and RhoA is pivotal in mediating Wnt activity in cell motility during development, regulating the formation of cell protrusions and directional migration [12,58]. Rac and Rho can directly influence actin rearrangements, as well as promote JNK activation to further alter cytoskeletal dynamics and gene transcription during several developmental processes [8,18]. In embryonic development, Rac1 activity has been characterized as important for canonical Wnt signaling downstream of Wnt3a and required for the translocation of β-catenin to the nucleus [60]. In this regard, mutation of the JNK2 phosphorylation sites of β-catenin at Ser191 and Ser605 abrogate the activation of Rac1. During neural crest migration, Rac1 is activated at the cell's leading edge, promoting actin polymerization for cell protrusion, with RhoA activated at the retracting rear of the cell, to promote actomyosin contractility [27]. Inhibition of cell-cell contact upon collision of streaming cells is promoted by RhoA activity with a concomitant suppression of Rac1, to promote the retraction of cell protrusions [27].

Upon the binding of Wnt to a Fzd receptor, cytoplasmic Disheveled (Dvl) activates the formin Disheveled Associated Activator of Morphogenesis 1 (Daam1), facilitating the formation of a complex between Daam1, Dvl, and Rho, thereby facilitating Rho activation and subsequent Rho kinase activity [21]. To promote the formation of cell protrusions at the leading edge of the cell, Rac1 and JNK activity may also be enhanced by Dvl, to facilitate actin remodeling [21,61]. In the directional movement of neural crest cells in the Xenopus and zebrafish embryo, Rac1 and RhoA-mediated regulation of cell protrusions is also important. In this context, the proteoglycan Syndecan-4, which is required for directional migration of neural crest cells, downregulates Rac1 activation to control the direction of cell protrusion development [62]. In this process, PCP-mediated RhoA activity inhibits Rac1 in order to regulate directional migration.

During the course of development, embryonic cells adopt distinct morphologies to migrate during tissue organization, and this is also seen in adult tissues during healing and regeneration events [31,63,64]. An antagonistic relationship between Rac1 and RhoA signaling has been well-characterized for regulating mesenchymal versus amoeboid modes of motility [55,65], and an example of this behavior is seen during zebrafish gastrulation, when mesodermal cells must transition from an amoeboid motility to mesenchymal movement and then finally into polarized alignment [66]. This is mediated by RhoA, which promotes the inhibitory phosphorylation of myosin phosphatase to regulate acto-myosin protrusive activity. In the context of skeletal muscle tissue regeneration, the activation of Rho and JNK, believed to be through non-canonical Wnt signaling, is important for the blebbing movement utilized by satellite cells, the resident stem cell population in skeletal muscle [63].

Conversely, in the migration of fibroblasts and epithelial cells, a mesenchymal mode of movement, regulated by Rac1 through Wnt5a, has shown to be important [31].

Amoeboid and mesenchymal modes of motility are also utilized by tumor cells as they navigate the extracellular environment. The alteration of cell morphology and motility is key during the course of cell invasion and metastasis, as cells encounter varied extracellular matrix barriers en route to a secondary site. Rac1 has a number of well-characterized functions in the movement of both normal and tumor cells, traditionally shown to be important for actin polymerization to drive lamellipodia-based movement and the formation of invadopodia to facilitate matrix proteolysis [57,67]. In tumor cells invading a rigid extracellular matrix environment, an upregulation of Rac1 activity and a concomitant downregulation of RhoA has been noted [57], similar to the signaling which has been observed during neural crest migration [62]. Tumor cells invading softer, more compliant extracellular matrix environments adopt rounded, amoeboid morphologies. These cells exhibit RhoA-mediated inactivation of myosin phosphatase, to promote the actomyosin-based contractility required for release of protease-rich invasive microvesicles from the cell surface [57], similar to the regulatory mechanism outlined above for cell motility during zebrafish gastrulation [66]. Both amoeboid and mesenchymal modes of invasion require upstream activation of the ARF6 small GTP-binding protein (described further below), that prompts Rac1 or RhoA activation depending on the compliance of the extracellular matrix [57] (Figure 2).

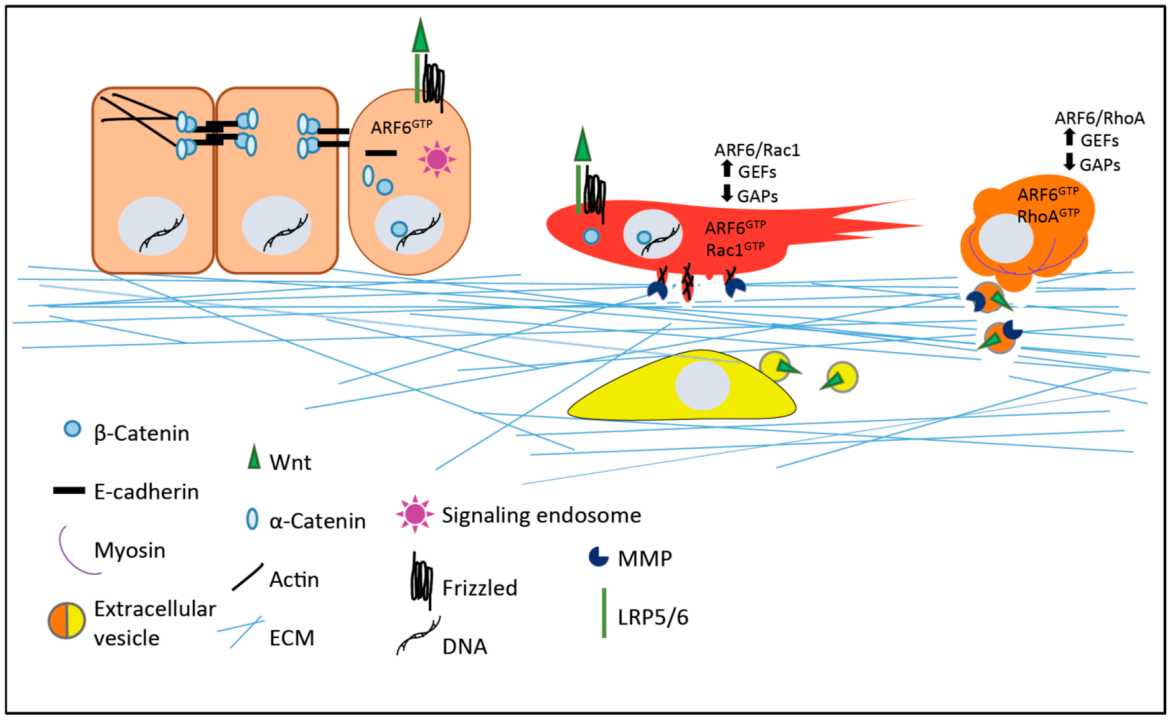

Figure 2. Wnt signaling impacts tumor progression and invasion. Wnt-regulated cellular changes include facilitating the dissociation and internalization of cadherin-based adhesions (cuboidal cells on the left) and the formation of invadopodia in migratory tumor cells (red). Wnt may also be included as cargo in extracellular vesicles released from amoeboid tumor cells (orange) as well as other cells such as fibroblasts in the tumor microenvironment (yellow). Small GTP-binding proteins of the ARF and Rho families, regulated by various GTPase activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs), mediate many of these processes.

The activities of the Rho family proteins downstream of Wnts in cancer are wide-ranging and appear to be dependent on the cellular context. In a model of colorectal cancer, RhoA was found to function as a tumor suppressor, with its inactivation being required for canonical signaling

downstream of Wnt3a to promote metastasis. Further reduced levels of RhoA were observed at metastatic sites when compared to primary lesions [68]. A Chinese hamster ovary cell line model of motility, however, found RhoA to be activated by Wnt3a, promoting actin reorganization and cell movement in concert with Dvl2 [69]. In an oral squamous cell carcinoma model, cells engineered to express β-catenin which lacks the entire GSK3β phosphorylation site showed cytoplasmic and nuclear β-catenin accumulation, and a corresponding increase in Rac1 and Cdc24 activation with the adoption of spindle-like elongated morphologies, loss of cell-cell contacts, and increased invasiveness [70]. During hepatocellular carcinoma progression, Rac1 activity has been shown to promote cell motility, and this can be antagonized by the activation of RhoA and Rho kinase downstream of Wnt activity [71]. In this instance, Wnt11 functions as a tumor suppressor by activating PKC signaling to phosphorylate β-catenin, resulting in reduced TCF-mediated transcriptional activity and decreased cell proliferation.

4.3. ARF6 and Wnt Signaling Regulate Tumor Cell Invasion and Signaling Endosome Formation

The small GTP-binding protein ARF6 regulates early endocytic membrane trafficking and rearrangements of the actin cytoskeleton, in part through its effects on phosphoinositide metabolism [72]. Mice homozygous for a null allele display embryonic and perinatal lethality beginning around mid-gestation, with impaired hepatic cord formation during liver development [73]. ARF6 is frequently overexpressed in cancers such as lung, breast, prostate, and colon [74–77], and its activation is associated with increased tissue invasion and increased formation of invadopodia and tumor-derived microvesicles to facilitate movement through the extracellular matrix [75,78–81].

Melanoma provides a prominent example for the role of Wnts and ARF6 in tumor invasion. A role for Wnt5a in melanoma progression through both canonical and non-canonical signal transduction has been demonstrated [46,48], which may be modulated by ARF signaling. Wnt5a, best characterized as a non-canonical signaling ligand, has been shown to promote melanoma invasion in a PKC-dependent manner, signaling through Fzd5 [48]. Additionally, canonical signaling through Wnt5a has been shown to promote ARF6 activation and subsequent melanoma invasion by stimulating β-catenin-mediated transcriptional activity [46]. In this instance, Wnt5a activates ARF6 by stimulating the activity of its exchange factor GEP100, through the receptors Fzd4 and LRP6. Blocking this pathway prevented lung metastasis in mice with melanoma xenograft tumors. In a separate study, the activities of ARF6 and ARF1 were shown to be important regulators of Wnt3a-mediated canonical signaling, through the generation of PIP2, and LRP6 phosphorylation [82]. In this instance, ARF1 and ARF6 activity was mediated by inactivation of the GTPase activating protein ARFGAP [83].

Aside from its role in promoting structural remodeling conducive to the acquisition of invasive phenotypes, ARF6 activation also has a vital role in propagating Wnt signaling through the formation of signaling endosomes [84,85] (Figure 2). Signaling endosomes are unique endosome populations that serve as a platform for robust and long-lived receptor signal propagation, and a host of cargoes including MAP kinases, various G protein-coupled receptors, and receptor tyrosine kinases (RTKs) such as EGFR, have been identified [86]. In three-dimensional basement membrane cell cultures of epithelial ductal units, ARF6 activation has been shown to promote the formation of signaling endosomes containing growth factor receptors, leading to hyperactive ERK signaling and aberrant epithelial glandular phenotypes resembling certain glandular cancers [84,85]. Wnt3a stimulation of epithelial cysts has been observed to promote ARF6 activity and the subsequent formation of signaling endosomes. Resulting hyperactivation of ERK promotes CK2-dependent phosphorylation of α-catenin to promote the disassembly of cadherin-based adhesions and internalization of adhesion molecules, increasing the cytoplasmic pool of transcriptionally active β-catenin [85]. ERK hyperactivation also leads to increased phosphorylation of the Wnt receptor LRP6, which may allow further amplification of canonical signaling by prompting dissociation of the destruction complex and promoting β-catenin stabilization. These cellular events culminate in filled glandular units reminiscent of pathologies observed in ductal carcinoma in situ (DCIS). Together, these studies advocate a potential role for signaling endosomes in Wnt signal transduction and cancer progression.

4.4. Eph and Ephrin Signaling Regulate Small GTPases in Wnt Signaling

Another family of proteins with renewed relevance in cancer, and which regulate Rho GTPases in PCP signaling in development and tissue homeostasis, are ephs and ephrins. Ephrins are membrane-anchored proteins that bind receptor tyrosine kinase eph receptors to stimulate a variety of cell processes mediated by reorganization of the actin cytoskeleton and cell-matrix and cell-cell adhesions, both by forward signaling in the eph-expressing cell and reverse signaling in the ephrin-expressing cell [87,88]. During development, repulsion and attraction cues from ephrin signaling assists in guiding cell movement, attachment, and morphology, mediated by differential activation of Rac, Rho, and Cdc42 [88–92]. Rho exchange factors Ephexin and Vms-RhoGEF preferentially bind to EphA receptors to promote Rho activity, whereas GEFs for Rac1 and Cdc42, Kalirin and Intersectin, associate with EphB receptors [89]. EphrinB1 has been shown to signal through the PCP pathway by interacting with Dvl to activate Rho and JNK, requiring Daam1, to regulate cell migration in Xenopus eye development [93]. An example of ephrin modulation of cell morphology and attachment is seen in melanoma and human embryonic kidney cells, wherein the activation of EphA3 by EphrinA5 activates Rho, causing acto-myosin rearrangement, cell rounding, blebbing, and detachment [94]. Eph-ephrin signaling may function to suppress or promote cancer progression, and many ephrins are overexpressed in cancer [87,95]. An example of the Wnt regulation of differential ephrin signaling in cancer progression is seen in colorectal cancer. In this context, EphB receptors are expressed as Wnt targets, with EphB2 characterized as a tumor suppressor to constrain tumor growth in $Apc^{Min/+}$ mice [96,97], whereas EphB4 promotes cancer progression [98].

4.5. Regulation of Small GTPases in Wnt Signal Transduction—The Roles of GAPs and GEFs

Important for regulating the activation of small GTP-binding proteins are their guanine nucleotide exchange factors (GEFs), which facilitate activation by exchanging GTP for GDP, and GTPase activating proteins (GAPs), which promote GTP hydrolysis, rendering the proteins to their inactive, GDP-bound state [99]. Many Wnt signal transduction pathways aided by small GTP-binding proteins, as mentioned in sections above, involve direct targeting of GEFs and GAPs, and a few examples of this regulation are provided.

As mentioned above, there are multiple GAPs and GEFs which regulate ARF activation in Wnt signaling cascades. The activity of the GEF GEP100, stimulated downstream of Wnt5a binding to Fzd4 and LRP6, promoted ARF6 activity and subsequent metastasis in a melanoma xenograft model [46]. ARFGAP, a protein which inactivates ARF1 and ARF6, has been shown to be an important modulator of ARF activity downstream of Wnt3a, regulating LRP6 phosphorylation and the generation of PIP2 [82]. In the case of Rho GTPases, APC has been shown to interact with both APC-stimulated GEF (ASEF), a GEF for Rac1, and IQGAP1, a GAP for both Rac1 and Cdc42. Both ASEF and IQGAP1 are important for regulating directional migration [100]. The activation of Rac1 upon Wnt3a stimulation requires the interaction of the Rac1 GEF Vav2 with p120-catenin, upon the release of catenin from E-cadherin [59]. Additionally, the activation of the planar cell polarity proteins PTK7 (protein tyrosine kinase 7), CELSR (Cadherin EGF LAG seven-pass G-type receptor), and VANG (Van Gogh-like) by Wnts or Fzd receptors may support PCP GTPase signaling by recruiting the Rac1 and Cdc42 GEF PAK, the Rac1 GEF ARHGEF7, and the ARF GAP GIT1 [19,20].

4.6. Extracellular Vesicles and Wnt Signaling

In recent years, the study of extracellular vesicles such as exosomes and microvesicles has grown exponentially, largely owing to their importance for the transfer of cargo during normal cellular processes and in disease, and their potential utility as biomarkers [101–104]. The formation of these vesicles is regulated by a variety of small GTPases [57,78,104,105], and they are enriched with a multitude of cargoes which may facilitate cancer progression, such as nucleic acids and oncogenic receptors which can be transferred to recipient cells to modulate the metastatic niche, and matrix

proteases to facilitate cell invasion [101–104]. The aforementioned ephrin family of signaling molecules has also been characterized as microvesicle cargo [106,107]. Recent work has begun to identify a role for extracellular vesicles in the transfer of Wnts.

Due to their lipid modification, Wnt ligands are highly hydrophobic and traditionally thought to act as short-range signaling molecules [3], constraining their activity to adjacent cells to regulate their potent effects. Wnts have a strong affinity for membranes and the extracellular matrix, with a particular affinity for heparin sulfate proteoglycans [108–111]. These short range signaling effects of Wnts have been well-described in the literature, identifying important mechanisms that restrict signaling to adjacent cells during certain cellular processes [3,112,113]. There is accumulating evidence that Wnts or downstream Wnt targets may also be modified or packaged into extracellular vesicles to facilitate further distribution, alter gradient formation, or otherwise modulate signaling [13,114–124]. For example, evidence has arisen that modifications which shield the hydrophobic moieties may function as carrier proteins to facilitate long range signaling. In Drosophila, the lipoprotein modification of the Wnt homolog Wingless (Wg) and its packaging into "argosomes" has been shown to be required for its long-range signaling [118]. Additionally, the Wg-interacting lipoprotein Secreted Wingless-Interacting Molecule (SWIM) has been shown to promote solubility of Wg in flies, important for normal wing development [123]. Similarly, mammalian Wnt3a has been shown to be associated with lipoprotein particles [122]. Wnts have been characterized as cargo in extracellular vesicles including exosomes and microvesicles [114–117,119,120], which may regulate their availability as a ligand. In certain contexts, the packaging of Wnts into vesicles may still mediate short range transfer, such as that which has been demonstrated in synaptic transmission in the Drosophila nervous system [115]. In the context of stem cell renewal, the loading of Wnt3 into embryonic stem cell-derived microvesicles, alongside a complement of other proteins and nucleic acids, has been shown to promote the survival and proliferation of hematopoietic progenitor cells [119]. It has been demonstrated that exosomes may function to remove β-catenin from cells, acting as a mechanism to suppress signaling downstream of Wnt activation [121], and β-catenin has been characterized in exosomes derived from colon cancer cells [106]. Yet another study has shown that carcinoma-associated fibroblasts release extracellular vesicles that stimulate breast cancer cell (BCC) motility and metastasis by mobilizing the non-canonical Wnt/PCP pathway in BCCs [116]. Fibroblast-derived vesicles functioned as a vehicle to tether BCC-produced Wnt11 and facilitate the autocrine signaling of Wnt11 in BCCs. Thus, as an emerging signaling platform, extracellular vesicles could play an important role in facilitating Wnt secretion, transport, and distal signaling.

5. Concluding Remarks and Perspectives

Given that Wnt signal transduction is highly influential in regulating GTPase signaling in a variety of normal developmental behaviors and homeostatic tissue maintenance [27,31,58,59], and Wnt signaling is frequently associated with promoting tumor progression [13,22,30,31], the possibility that canonical and non-canonical Wnt signaling cascades may be modulating the GTPase-driven plasticity that cells use to invade through varied environments merits future exploration. Wnt stimulation may provide an additional means for guiding the processes of tissue invasion by specifically targeting key GTPases and/or their effectors.

Though largely unexplored to date, it is possible that the long-range transmission of Wnts, particularly in extracellular vesicles, may play roles in development and disease progression. While exosomes and microvesicles are formed by normal cells, their formation is frequently upregulated in disease states, including cancer [101,102,104]. As has been seen in the case of cancer-associated fibroblasts promoting breast cancer progression through the release of exosomes which modulate Wnt-PCP signaling [116], it is possible that extracellular vesicles may serve as platforms for the dissemination of Wnt proteins and activate canonical or non-canonical pathways in target cells. In development, the highly regulated structure of Wnt gradients is important to constrain Wnt stimulation to isolated cells or cell groups [13], and in some cases, the distribution of Wnt

ligands throughout the developing tissue may not be required for the entirety of tissue patterning and development [125]. In the case of cancer, however, the dysregulation of Wnt release which allows for the broadcast of ligand to larger areas may be utilized to promote cell proliferation and motility in tumors or motile cell groups, as well as potentially condition the microenvironment ahead of invasion and colonization. Wnt3a has been shown to function as a chemotactic agent, recruiting cells in a transwell assay, an effect that was distinct from simply upregulating motility [69]. Examples such as this raise the possibility that the deposition of Wnt by extracellular vesicles may promote the directional movement of cells.

Protease deposition from invasive cells is an important mediator of cell invasion which may be accomplished by the release of protease-loaded microvesicles [57,101,102], and matrix metalloproteinase (MMP) upregulation. MMPs are upregulated in response to Wnt stimulation during development and tissue homeostasis [125–128], required for tissue remodeling, cell migration, and the cleavage of developmental regulatory molecules to mediate their activation [129]. Increased MMP expression is also frequently noted upon Wnt stimulation in tumor cell invasion, including MMP-2 [49], MMP-7 [130], MMP-9 [131], MMP14 [132], and MMP16 [133]. Though currently not investigated, the possibility for an increase in protease expression and its release in extracellular vesicles from invasive tumor cells in response to Wnt stimulation is an area that merits future investigation. Finally, the biomedical community recognizes that extracellular vesicles represent potentially novel avenues for the targeted delivery of customized cargo during disease. These types of research efforts will not only promote the understanding of vesicle-mediated Wnt signaling, but will also advance the exploration of their roles in transmitting other morphogens and signaling molecules, thereby rendering important insights into the biology of Wnt signaling, and ultimately into strategies for intervention in the treatment of Wnt-related diseases.

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