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Activating Wnt/β-catenin signaling pathway for disease therapy: Challenges and opportunities

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ABSTRACT

Wnt/β-catenin signaling pathway is essential for embryo development and adult tissue homeostasis and regeneration, abnormal regulation of the pathway is tightly associated with many disease types, suggesting that Wnt/β-catenin signaling pathway is an attractive target for disease therapy. While the Wnt inhibitors have been extensively reviewed, small molecules activating Wnt/β-catenin signaling were rarely addressed. In this article, we firstly reviewed the diseases that were associated with disruption of Wnt/β-catenin signaling pathway, including hair loss, pigmentary disorders, wound healing, bone diseases, neurodegenerative diseases and chronic obstructive pulmonary diseases, *etc.* We also comprehensively summarized small molecules that activated Wnt/β-catenin signaling pathway in various models *in vitro* and *in vivo*. To evaluate the therapeutic potential of Wnt activation, we focused on the discovery strategies, phenotypic characterization, and target identification of the Wnt activators. Finally, we proposed the challenges and opportunities in development of Wnt activators for pharmacological agents in term of targeting safety and selectivity.

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Abbreviations: 5α-DHT, 5-alpha-dihydrotestosterone; AGA, androgenetic alopecia; APC, adenomatous polyposis coli; ARFGAP1, GTPase activating protein of ADP-ribosylation factor 1; BIO, 6-bromoindirubin-3′-oxime; BMSCs, bone marrow-derived mesenchymal stem cells; CK1, casein kinase 1; COPD, chronic obstructive pulmonary disease; DAX, DIX domain of Axin; DKK-1, Dickkopf-1; ESC, embryonic stem cell; GSK-3, glycogen synthase kinase-3; HFSCs, hair follicle stem cells; HLY78, 4-ethyl-5-methyl-5,6-dihydro-[1,3] dioxolo[4,5-j] phenanthridine; HT22 cells, mouse-derived hippocampal HT22 cells; Lrp, low density lipoprotein receptor-related protein; MV, methyl vanillate; P19 cells, mouse embryo teratocarcinoma epithelial cells (P19 line); PP2A, protein phosphatase 2A; QS11, purine derivative; SAHPAs, stapled α-helical peptides targeting the Axin-β-catenin complex; SB-216763, 3-(2,4-dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole −2,5-dione; SB-415286, 3-(3-chloro-4-hydroxyphenylamino)-4-(2-nitrophenyl)-1H- pyrrole-2,5-dione; SFRP-1, secreted frizzled-related protein-1; SH-SY5Y cells, human dopaminergic SH-SY5Y cells; SKL2001, 5-furan-2yl-isoxazole-3-carboxylic acid (3-imidazol-1yl-propyl)-amide; SPR, surface plasmon resonance; ST2 cells, bone marrow derived ST2 stromal cells; TCF, T cell transcriptional factor; TOP-Flash, TCF optimal promoter driven luciferase reporter; TWS119, 4,6-disubstituted pyrrolopyrimidine; U2OS cells, Human osteosarcoma U2OS cells; VPA, valproic acid; WAY-316606, trifluoromethyl analog of diphenylsulfone sulfonamide.

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1. Introduction

The Wnt/β-catenin signaling pathway is one of the most evolutionarily conserved regulators of embryo development and adult tissue homeostasis (Clevers & Nusse, 2012; Logan & Nusse, 2004; Verkaar, Cadigan, & van Amerongen, 2012; Watanabe & Dai, 2011). Historically, Wnt gene was originally discovered as *Int1* (integration 1) in mice (Nusse & Varmus, 1982), later a homolog was identified in *Drosophila melanogaster* with a wingless phenotype, and the *Int* gene was renamed as *Wnt* (Nusse et al., 1991; Rijsewijk et al., 1987). Further studies revealed that Wnt proteins controls a canonical signaling pathway with a key effector β-catenin, therefore the pathway is also known as Wnt/β-catenin signaling pathway (Willert & Nusse, 1998).

In mammals, the Wnt/β-catenin signaling pathway mainly consists of three steps, including Wnt signaling transduction in the membrane, regulation of β-catenin stabilization in the cytoplasm and activation of Wnt target genes in the nucleus (Clevers & Nusse, 2012). Briefly, when Wnt signal is absent, β-catenin is degraded by a protein destruction complex consist of Axin, adenomatous polyposis coli (APC), glycogen synthase kinase-3 (GSK-3) and casein kinase 1 (CK1) (MacDonald, Tamai, & He, 2009; Stamos & Weis, 2013). In the destruction complex, β-catenin will be firstly phosphorylated in the N-terminal by GSK-3 and CK1, the phosphorylated β-catenin is then ubiquitinated by the F box/WD repeat protein β-TrCP and consequently degraded by proteasomes (Aberle, Bauer, Stappert, Kispert, & Kemler, 1997; Rao & Kuhl, 2010; Rubinfeld et al., 1996). When Wnt ligands are present, the binding of Wnts and the receptors leads to the dissociation of the destruction complex and the accumulation of β-catenin in the nucleus, where β-catenin binds to the T-cell factors/lymphoid enhancing factors (TCFs/LEFs) and activates the expression of target genes (Clevers, 2006).

In adults, tissue homeostasis is controlled by the coordination of self-renewal, regeneration, and reprogramming of adult stem cells, in which spatiotemporal gene expression is precisely regulated by intrinsic and extrinsic signals including Wnt/β-catenin signaling (Lian et al., 2013; Wray & Hartmann, 2012). Abnormal activation or disruption of Wnt/β-catenin pathway is tightly associated with many types of diseases, including cancer, hair loss, wound healing and osteoporosis (Kretzschmar & Clevers, 2017; Rabbani et al., 2011; Sato, Meijer, Skaltsounis, Greengard, & Brivanlou, 2004). Although the Wnt inhibitors have been extensively reviewed (Anastas & Moon, 2013), Wnt activators were rarely addressed. Here, we summarized the diseases that were associated with abnormal inactivation of Wnt/β-catenin signaling pathway, and reviewed small molecules activating Wnt/β-catenin signaling pathway. We emphasized on the discovery strategies and models, phenotype, and target proteins of these Wnt activators. We also proposed the challenges and opportunities in development of Wnt activators for disease therapy.

2. Wnt/β-catenin signaling and diseases

As an essential regulator of tissue homeostasis and regeneration, Wnt/β-catenin pathway is tightly controlled in spatiotemporal patterns, and abnormal inactivation of the pathway was found in many types of diseases (Fig. 1 and Table 1).

2.1. Hair growth disorders

Under normal condition, the adult hair follicle can be activated cyclically at the quiescent phase (telogen), hair growth (anagen) and regression phase (catagen) throughout life (Schneider, Schmidt-Ullrich, & Paus, 2009). The maintenance of hair cycling depends on the activity of the hair follicle stem cells (HFSCs) in the bulge. It has been established that Wnt/β-catenin signaling was activated in HFSCs during the transition from telogen to anagen and reverted to inactivation when the follicle entered catagen and telogen stages (Lien et al., 2014; Tumbar et al., 2004). In addition, Wnt ligands secreted by the hair follicle epithelium

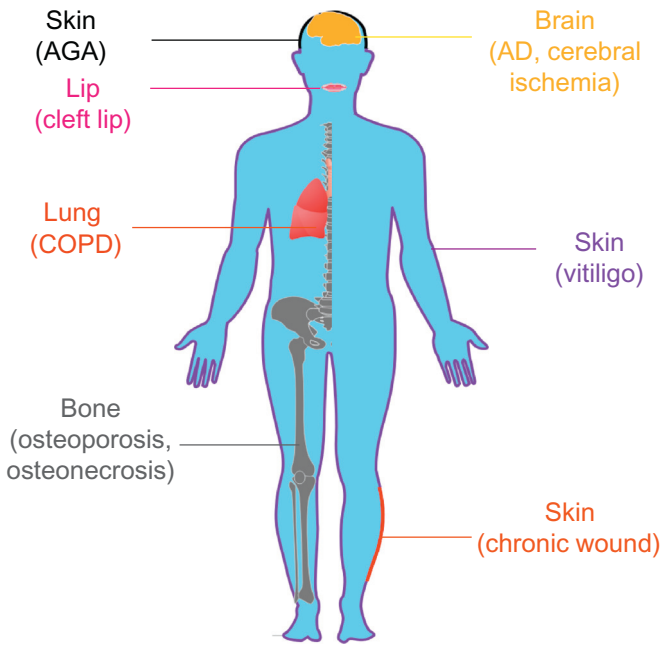


Fig. 1. Clinical features associated with abnormal inactivation of Wnt/β-catenin signaling pathway. Pathological changes are shown by the affected organ system. AGA, androgenetic alopecia; AD, Alzheimer's Disease; COPD, chronic obstructive pulmonary disease.

were required for adult hair follicle regeneration, loss of epithelial Wnt signals resulted in a profound hair cycle arrest (Myung, Takeo, Ito, & Atit, 2013). For the dermal papilla that located at the base of the hair follicle, Wnt signaling from the epidermis is pivotal for its maintenance in an anagen state and growth of the hair shaft (Ito et al., 2007; Kishimoto, Burgeson, & Morgan, 2000; Shimizu & Morgan, 2004). Consistently, depletion of Wnt receptor leucine-rich repeat-containing G-protein-coupled receptor 5 positive (Lgr5+) cells in mice abrogated hair regeneration, and inhibition of Wnt signaling prevented the recovery of Lgr5+ cells and hair germ (Hoeck et al., 2017).

Androgenetic alopecia (AGA) is the most common type of permanent scalp hair loss mediated by androgen's metabolite 5-alpha-dihydrotestosterone (5α-DHT or DHT) (Trueb, 2002). It has been demonstrated that DHT activated the Wnt negative regulators GSK-3 and Dickkopf-1 (DKK-1) and inhibited the Wnt/β-catenin pathway, which subsequently induced anagen-to-catagen transition and inhibited HFSCs differentiation (Leiros, Attorresi, & Balana, 2012). Similarly, in the hypodermis of mice during anagen, injection of recombinant human DKK-1 caused premature onset of catagen, whereas neutralizing DKK-1 antibody delayed anagen-to-catagen transition (Kwack, Kim, Kim, & Sung, 2012). The connection of the Wnt/β-catenin signaling pathway and AGA pathogenesis was also supported by many other studies (Andl, Reddy, Gaddapara, & Millar, 2002; Heilmann et al., 2013; Heilmann-Heimbach, Hochfeld, Paus, & Nothen, 2016; Millar et al., 1999; Reddy et al., 2001). Notably, DKK-1 levels were significantly higher in scalp biopsies with AGA than controls, suggesting that DKK-1 could be a promising therapeutic target for AGA (Fawzi, Mahmoud, Shaker, & Saleh, 2016). Consistently, in female patients with AGA, topical treatment of Wnt activator methyl vanillate (MV) for 6 months significantly increased the hair count and hair mass index (Cha, Shin, Zahoor, Kim, Min Do, & Choi, 2014; Tosti et al., 2016).

2.2. Pigmentary disorders

Vitiligo, the most frequent human pigmentary disorder, is characterized by progressive and declining melanocyte of skin and destruction of

Table 1
Biological processes and pathological changes associated with **abnormal inactivation of Wnt/β-catenin signaling pathway.**

Tissues	Biological processes	Pathological changes	Diseases
Skin	Fate determination of HFSCs	Inhibition of HFSCs differentiation[a]	AGA
	Regeneration of adult hair follicle	Abrogation of hair regeneration[b]	
	Epidermis maintenance	Inhibition of recovery of hair germ[b]	
	Regulation of hair shaft growth	Hair cycle arrest[c]	
	Regeneration of melanocytes	Decrease of epidermal melanocytes[d]	Vitiligo
	Proliferation of epidermal stem cells	Impairment of the wound healing[e]	Chronic wound
	Differentiation and migration of keratinocytes		
	Regeneration hair follicle		
Lips	Fate determination of lip epithelium	Proliferation of epithelial seam cells[f]	Cleft lip
Bone	Differentiation of osteoblasts	Inhibition of osteoblasts[g]	Osteoporosis
	Osteogenesis	Increase of osteoclasts[h]	Osteonecrosis
	Regulation of bone mass, homeostasis, and deposition	Inhibition of bone resorption[h]	
		Induction of osteopenia[i]	
Brain	Formation, stabilization and recycling of synapses	Suppression of amyloid β-induced synapse loss[j]	AD
	Neurogenesis		
	Modulation of adult neurogenesis, vascular integrity and blood-brain barrier permeability	Disruption of blood-brain barrier and microvascular hemorrhage[k]	Cerebral ischemia
Lung	Lung development, homeostasis	Destruction of parenchymal tissue and repair capacity of emphysema[l]	COPD
	Lung epithelial injury and repair processes		

AGA, androgenetic alopecia; AD, Alzheimer's Disease; COPD, chronic obstructive pulmonary disease; HFSCs, hair follicle stem cells.
[a] Leiros et al. (2012)
[b] Hoeck et al. (2017)
[c] Myung et al. (2013)
[d] Sun et al. (2018)
[e] Qi et al. (2015)
[f] Kurosaka, Iulianella, Williams, & Trainor (2014)
[g] Glass II et al. (2005), Uluckan et al. (2016)
[h] Kramer et al. (2010)
[i] Holmen et al. (2005)
[j] Purro et al. (2012)
[k] Chang et al. (2017)
[l] Kneidinger et al. (2011)

mature epidermal melanocytes (Birlea, Costin, Roop, & Norris, 2017). In patients with vitiligo, oxidative stress in melanocytes impaired Wnt/β-catenin signaling, and reactivation of this pathway enhanced melanoblast differentiation, suggesting that activation of the Wnt/β-catenin pathway would be a therapeutic approach to repigmentation in vitiligo patients (Harris, 2015; Regazzetti et al., 2015). The positive role of Wnt/β-catenin signaling in melanocyte regeneration was supported by genetic manipulation of the melanocytes, overexpression of Wnt inhibitor DKK-1 reduced epidermal melanocytes, while activation of Wnt signaling by stabilizing β-catenin increased epidermal melanocytes (Sun et al., 2018). In melanocyte stem cells that are the origin of melanocytes, activation of Wnt signaling dramatically enhanced the periodically regeneration of pigment-producing hair and epidermal melanocytes (Rabbani et al., 2011; Takeo et al., 2016). As a vital regulator of melanocyte regeneration, Wnt/β-catenin pathway is also observed in melanocyte progenitor cell line iMC23, in that activation of the pathway by Wnt3a promoted the differentiation and melanogenesis (Guo et al., 2016).

2.3. Pathological wound healing

Wound healing is usually completed in days through tight orchestration of inflammation, blood clotting, cell proliferation and remodeling (Seifert et al., 2012). As a pathological healing, chronic wound is a barrier defect that has not healed in 3 months and have become a major therapeutic challenge (Martin & Nunan, 2015). In Sprague-Dawley rats with full-thickness skin wounds, activation of Wnt/β-catenin signaling promoted wound closure by regulating the proliferation of epidermal stem cells, the differentiation and migration of keratinocytes, and follicle regeneration (Shi et al., 2015). Consistently, suppression of the Wnt signaling pathway impaired the healing of the wound skin, and activation of the pathway rescued the wound-healing deficiency in diabetic mice (Qi et al., 2015).

Cleft lip is a kind of pathological wound in lips, it is a common craniofacial birth defect containing an opening in the upper lip (Seto-Salvia & Stanier, 2014). It has been demonstrated that disruption of Wnt signaling pathway was associated with the etiology of cleft lip (Brugmann et al., 2007), reactivation of Wnt pathway in lips resulted in increased proliferation and decreased cell death in the lip epithelium (Chiquet et al., 2008; Song et al., 2009).

Cerebral ischemia is a kind of wound in brain, which is generated by insufficient blood flow to meet metabolic demand in the brain (Lee et al., 2018). In rat middle cerebral artery occlusion model, activation of Wnt/β-catenin signaling attenuated early ischemia-reperfusion stroke injury by reducing neurologic deficits, brain edema, infarct volume, and blood-brain barrier permeability (W. Wang et al., 2017). In mouse model of ischemic stroke, the disruption of blood-brain barrier and microvascular hemorrhage was accompanied by decrease of Wnt/β-catenin signaling, and constitutive activation of the pathway fully rescued the defects (Chang et al., 2017), implicating that Wnt/β-catenin signaling is a potential therapeutic target for human cerebral ischemia.

2.4. Bone diseases

Osteoporosis is one of the mostly observed bone diseases that is characterized with low bone mass, ocular defects, and a predisposition to fractures (Alejandro & Constantinescu, 2018). It has been reported that inactivation of Wnt/β-catenin signaling inhibited the activities of osteoblasts, bone resorption and finally led to osteopenia (Glass II et al., 2005; Uluckan et al., 2016). On the other hand, stabilization of β-catenin in differentiated osteoblasts resulted in high bone mass (Glass II et al., 2005), and treatment of Wnt3a restored osteogenic capacity in autograft models, indicating that activation of the Wnt/β-catenin pathway is a new target therapeutic approach for osteonecrosis, especially in aged patients (Salmon et al., 2017). The crucial function of Wnt/β-catenin signaling in controlling bone homeostasis was also supported by genetically modified mouse models, knockout of β-catenin

produced severe osteopenia with striking increases in osteoclasts, whereas constitutive activation of β-catenin resulted in dramatic increase of bone deposition and a disappearance of osteoclasts (Holmen et al., 2005; Kramer et al., 2010).

2.5. Neurodegenerative diseases

In the adult nervous system, activation of Wnt pathway enhanced the formation, stabilization and recycling of synapses and promoted neurogenesis (Inestrosa & Arenas, 2010; Tiwari et al., 2014). Alzheimer's Disease is characterized by loss of neurons and synapses in the cerebral cortex and certain subcortical regions (Burns & Iliffe, 2009). Genome-wide linkage studies revealed the association between inactive mutations of Wnt receptor Lrp6 gene and late-onset Alzheimer's disease (De Ferrari et al., 2007). In addition, induction of DKK-1, a negative modulator of the canonical Wnt signaling pathway, contributed to the beta-amyloid triggered pathological cascade, and targeting DKK-1 suppressed beta-amyloid-induced synapse loss (Caricasole et al., 2004; Purro, Dickins, & Salinas, 2012). In Alzheimer's models, chemical activation of Wnt/β-catenin signaling promoted synaptic growth, reduced spatial memory impairment and neurodegeneration in brains (Jin et al., 2017; Lu, Yamamoto, Ortega, & Baltimore, 2004; Toledo & Inestrosa, 2010).

2.6. Chronic obstructive pulmonary diseases

Chronic obstructive pulmonary disease (COPD) is characterized by long-term breathing problems and loss of functional pulmonary tissue with no available causal therapy (Cantor & Turino, 2018). Earlier studies revealed that Wnt/β-catenin signaling was involved in lung development, homeostasis, lung epithelial injury and repair processes (Konigshoff & Eickelberg, 2010). In lung tissues from patients with COPD, immunofluorescence staining detected significant decrease of nuclear β-catenin staining (Kneidinger et al., 2011). In mouse models, Wnt/β-catenin signaling was down-regulated in COPD tissue that was challenged by cigarette smoke exposure and elastase instillation (Kneidinger et al., 2011). On the other hand, activation of Wnt/β-catenin signaling attenuated COPD symptoms by reducing airspace enlargement and collagen content (Kneidinger et al., 2011), indicating that Wnt/β-catenin activation is a potential therapeutic approach of COPD.

3. Discovery of Wnt activators

Given that abnormal inactivation of Wnt/β-catenin pathway is essential for many types of diseases, small molecules activating the pathway would be potential therapeutic agents for these diseases. During the last decades, a number of small molecules have been identified to activate the pathway and some of them exhibited promising effects in preclinical models (Table 2). To facilitate further development of Wnt agonists, we summarized the cell models, strategies and phenotypes that were used in discovery and evaluation of these small molecules.

3.1. Screening and discovery

A widely used model for screening of Wnt agonists is HEK293 cells that were originally derived from human embryonic kidney cells (Graham, Smiley, Russell, & Nairn, 1977). HEK293 cells have been extensively used in reporter-based screening due to its high efficiency of transfection and easy reproduction. In Wnt agonist screening, the most commonly used reporter is the T cell transcriptional factor (TCF) optimal promoter driven luciferase (TOP-Flash), in which the promoter contains tandem TCF binding sites and the luciferase level indicates the activity Wnt/β-catenin pathway (Korinek et al., 1997). Luciferase activity is easily detected and quantified by a micro-plate reader, which allows screening of Wnt agonists in a large scale (Gwak et al., 2012; S. Wang et al., 2013). In HEK293 cell harboring TOP-Flash reporter, the luciferase level in control cells is very low and not detectable, it increases significantly upon treatment of Wnt agonists (Sato et al., 2004). To evaluate the activation of Wnt signaling pathway by small molecules, cells are usually treated with a known Wnt agonist as a positive control. For example, in a screening of 100,000 heterocycles in HEK293-TOP-Flash cells, the luciferase activity was increased about fifty times higher by a small molecule QS11 than Wnt3a protein (Q. Zhang et al., 2007). To evaluate the selectivity of Wnt agonists, HEK293 cells are often used for reporter assays of other signaling pathways, such as Gli-luc reporter assay for Hedgehog signaling pathway and NFκB-luc reporter assay for NFκB signaling pathway (Q. Zhang et al., 2007).

For therapeutic purpose, it will be advantageous to discover Wnt agonists using cells that were derived from the tissue relevant to certain diseases. For bone diseases, human osteosarcoma U2OS cells highly responded to Wnt signaling in a β-catenin-dependent fashion (Modder, Oursler, Khosla, & Monroe, 2011). Upon treatment of Wnt3a, murine bone marrow-derived ST2 stromal cells underwent robust osteoblastogenesis with strong increase of alkaline phosphatase activity (Tu et al., 2007). For nerve diseases, mouse-derived hippocampal HT22 cells were used for screening of Wnt agonists that enhanced Wnt3a-stimulated Wnt signaling (Biechele et al., 2010). Wnt/β-catenin signaling was also involved in retinoic acid-induced differentiation of human dopaminergic SH-SY5Y cells, in which the triangular phase-bright bodies with short neurites were transited to typical features of neuron (Uemura et al., 2003). For skin diseases, Wnt agonists were often screened in human dermal papilla cells, in that Wnt/β-catenin signaling was sufficient to maintain the hair-inducing activity and anagen-phase characteristics (Kishimoto et al., 2000; Shimizu & Morgan, 2004).

Multipotent cell lines were also used to discover Wnt agonists because the differentiation potential is dependent on Wnt/β-catenin signaling. For example, bone marrow-derived mesenchymal stem cells (BMSCs) were used to screen therapeutic agents for bone diseases due to the ability to differentiate into a variety of cell types, including osteoblasts, adipoblasts and chondrocytes. It has been reported that Wnt/β-catenin signaling was involved in restore of osteoblast differentiation in fibroblast growth factor 2 deficient BMSC cultures (Colombres et al., 2008; Fei, Xiao, Doetschman, Coffin, & Hurley, 2011; Simann et al., 2015). Mouse P19 cells are a kind of embryonic carcinoma cells that were isolated from a murine embryo-derived teratocarcinoma (Ding et al., 2003). Under normal culture conditions, P19 cells retained a normal karyotype and low frequency of differentiation, and a TOP-Flash luciferase assay revealed that the differentiation of P19 cells to nerve cells was associated with the activation of Wnt/β-catenin signaling (Ding et al., 2003; Lyu, Costantini, Jho, & Joo, 2003). In murine embryonic stem cells (MESCs) that were derived from the inner cell mass of embryos, Wnt/β-catenin signaling was required to maintain the pluripotency and prevent the spontaneous differentiation (Miyabayashi et al., 2007). In murine embryo bodies (EB), activation of Wnt signaling was involved to the skeletal formation out of EB formation (Lee, Haller, et al., 2016).

3.2. Phenotypic characterization

During the past decades, a number of small molecules have been reported to activate Wnt/β-catenin signaling, and various phenotypes were observed in evaluation of the Wnt activators in different models.

In cell models, a small molecule 6-bromoindirubin-3′-oxime (BIO) highly activated Wnt/β-catenin signaling and maintained the pluripotency of human and mouse ESCs, which solved a previous problem in keeping the undifferentiation during ESC expansion *in vitro* (Sato et al., 2004). In ST2 cells, a small molecule 5-furan-2yl-isoxazole-3-carboxylic acid (3-imidazol-1yl-propyl)-amide (SKL2001) activated Wnt/β-catenin signaling and promoted osteoblast differentiation as marked by alkaline phosphatase activity (Gwak et al., 2012). In

Table 2
Phenotypic characterization of Wnt agonists.

Compounds	Cell assays	Animal assays	Relevant diseases
Arylpyrimidines[a]	Increase the TOP-Flash activity in U2OS cells	Increase the β-catenin level and anabolic bone activity in the osteoblastic cells lining the periosteal surface of C57BL/6 mice	Osteoporosis
AMBMP[b]	Increase the TOP-Flash activity in HEK293 cells	Increase the β-catenin level and promote the regeneration and proliferation of hepatocyte in rat I/R model	Liver injury after hepatic I/R
Cyclosporine A[c]	Promote the Wnt signaling activity in human dermal papilla	N.D.	Hair growth disorders
HDT extract[d]	Increase the TOP-Flash activity in HEK293 cells Induce the differentiation of primary calvarial osteoblasts	Increase the β-catenin level in femoral trabecular and cortical bones Increase of bone mass in ICR mice	Osteoporosis
Heparin[e]	Inhibit GSK-3β and increase the β-catenin level Induce the differentiation of murine N2a cells and rat E18 primary hippocampal neurons	N.D.	Neuronal diseases
I3O[f]	Increase the TOP-Flash activity in HEK293 cells Increase the β-catenin level and inhibit the differentiation of 3 T3-L1 cells	Increase the β-catenin level in the adipocytes in high-fat diet male C57BL/6 N mice	Obesity
Icariin[g]	Increase the expression of Wnt1, Wnt3a, β-catenin and TOP-GAL activity in primary bone marrow stromal cells Promote the differentiation of primary bone marrow stromal cells	Increase the expression of Wnt target genes (Axin2, TCF1, and LEF1) in OPG KO mice	Osteoporosis
Ilexonin A[h]	N.D.	Increase the β-catenin level, decrease the GSK-3β and Axin levels in rats Promote neuronal proliferation and regeneration in rat I/R model	ICVD
Lithium chloride[i,j]	Increase the expression of Wnt target genes and TOP-Flash activity in osteoblasts Increase the β-catenin level in *Xenopus laevis* oocytes and Lrp5 mutant calvaria cells	Inhibit GSK-3 activity and increase bone formation and bone mass in Lrp5 KO mice Promote β-catenin accumulation in Xenopus embryos	COPD Osteoporosis
Malva verticillata seed extracts[k]	Increase the β-catenin level, TOP-Flash activity, and proliferation in human dermal papilla cells	N.D.	Hair loss
MV[l]	Increase the TOP-Flash activity in HEK293 cells Increase the β-catenin level and differentiation in primary calvarial osteoblasts	Increase the β-catenin level in femoral trabecular and cortical bones Increase bone loss in OVX mice	Osteoporosis
Riluzole[m]	Increase the Wnt reporter activity in HT22 cells and adult hippocampal progenitor cells Increase the stabilization and nuclear localization of β-catenin in U2OS cells	N.D.	
SKL2001[n]	Increase the β-catenin level and TOP-Flash activity in HEK293 and ST2 cells Increase osteogenesis in ST2 cells Increase the β-catenin level and decrease lipid droplet in 3 T3-L1 preadipocytes	N.D.	Osteoporosis Obesity
SM04554[o]	N.D.	Increase the nuclear β-catenin level in hair follicles Induce hair growth in CD1 mice, depilated C57Bl/6 mice, NIH-III nude mice and mini-pigs	AGA
SMIs[p]	Upregulate the expression of Wnt target genes in embryonic stem cells	N.D.	Muscle disease
Sodium selenate[q]	Decrease the GSK3 activity and increase the β-catenin level, and alleviates amyloidgenesis in primarily cultured neurons of mouse hippocampus	N.D.	AD
WAY-316606[r]	Increase the TOP-Flash activity in U2OS cells Suppress the apoptosis of human pre-osteocytic cells Increase the β-catenin level in human hair pre-cortex keratinocytes and dermal papilla fibroblasts	Increase the total bone area in a murine calvarial organ	Osteoporosis Hair loss

AMBMP, 2-amino-4-[3,4-(methylenedioxy)benzyl-amino]-6-(3-methoxyphenyl)pyrimidine; I/R, ischemia/reperfusion; I3O, indirubin-3′-oxime; ICR, institute of cancer research; ICVD, ischemic cerebrovascular disease; KO, knockout; N.D., None detection; OPG, osteoprotegerin; OVX, ovariectomized; HDT, *Hovenia dulcis* thunb; TOP-GAL, TCF optimal promoter driven luciferase reporter.

[a] Gilbert et al. (2010)
[b] Liu et al. (2005).
[c] Hawkshaw et al. (2018)
[d] Cha et al. (2014)
[e] Colombres et al. (2008)
[f] Choi et al. (2014)
[g] Li et al. (2013)
[h] Zhang et al. (2016)
[i] Clement-Lacroix et al. (2005), Kneidinger et al. (2011)
[j] Klein & Melton (1996)
[k] Lee, Choi, et al. (2016)
[l] Cha et al. (2014)
[m] Biechele et al. (2010)
[n] Gwak et al. (2012)
[o] Deshmukh et al. (2017)
[p] Lee, Haller, et al. (2016)
[q] Jin et al. (2017)
[r] Bodine et al. (2009)

preadipocytes 3 T3-L1 cells, exposure of SKL2001 induced lipid droplet accumulation in response to dexamethasone and insulin as visualized by Oil Red O staining (Gwak et al., 2012). In murine P19 cells, a small molecule 4, 6-disubstituted pyrrolopyrimidine (TWS119) activated Wnt/β-catenin signaling and induced neurogenesis based on counting of TuJ1 positive cells with correct neuronal morphology (Ding et al., 2003). Promotion of hair growth was often observed upon treatment of Wnt agonists (Andl et al., 2002; Gat, DasGupta, Degenstein, & Fuchs, 1998; Kitagawa et al., 2009; Leiros et al., 2012). In dermal papilla cells, *Malva verticillata* seed extracts significantly activated Wnt signaling and promoted the proliferation of human dermal papilla (Lee, Choi, et al., 2016). In human hair follicle organ culture, small molecules Cyclosporine A and WAY-316606 effectively enhanced Wnt/β-catenin signaling activity in hair pre-cortex keratinocytes and dermal papilla fibroblasts (Hawkshaw et al., 2018).

In animal models, Wnt agonists induced various phenotypes in different tissues. In low density lipoprotein receptor-related protein 5 (Lrp5) knockout mice, lithium chloride increased bone formation and mass *via* activating Wnt/β-catenin signaling (Clement-Lacroix et al., 2005). In murine COPD models that were challenged by cigarette smoke exposure and elastase instillation, treatment of lithium chloride activated Wnt/β-catenin signaling and induced an attenuation of COPD features, including constant airspace enlargement and altered lung function (Kneidinger et al., 2011). In Xenopus embryo, a purine derivative QS11 together with Wnt3a synergistically activated Wnt signaling and induced significantly more full and partial double axis formation (Q. Zhang et al., 2007). In C57BL/6 mice, (hetero) arylpyrimidines elevated active β-catenin in the surface of the calvaria, increased mineral apposition in calvaria and promoted osteogenesis (Gilbert et al., 2010). On the other hand, in osteoprotegerin knockout mice, local injection of Wnt agonist Icariin over the surface of calvaria stimulated new bone formation, and significantly reversed osteoprotegerin-deficient-induced bone loss and bone strength reduction (Li et al., 2013). Consistently, in high-fat diet (HFD)-induced obesity mouse model, a Wnt agonist indirubin-3′-oxime (I3O) attenuated HFD-induced body weight gain and visceral fat accumulation without any significant toxicity (Choi et al., 2014). In Sprague-Dawley male rats, Ilexonin A (IA) significantly diminished neurological deficits associated with cerebral ischemia reperfusion as a result of increased neuronal survival *via* activation of the canonical Wnt pathway (B. Q. Zhang, Zheng, Han, Chen, & Jiang, 2016). For hair growth, a small molecule SM04554 increased nuclear β-catenin in hair follicles and induced hair growth with increased hair-follicle counts in CD1 mice, depilated C57Bl/6 mice, NIH-III nude mice and mini-pigs (Deshmukh, Pedraza, Barroga, Seykora, & Yazici, 2017).

4. Target proteins of Wnt activators

In molecular level, the effects of therapeutic agents on cells or bodies were mediated by the engagement with cellular components especially proteins. Identification of target proteins of small molecules is essential to understand the precise mechanism of the action of drugs. Although not all the Wnt agonist targets have been clarified, some studies have attempted to identify the proteins that were required or associated to Wnt activation by small molecules (Fig. 2).

4.1. GSK-3

Among the components of Wnt/β-catenin signaling pathway, GSK-3 is the major target protein of the Wnt agonists described above. GSK-3 is a serine-threonine kinase and was initially discovered as a regulatory kinase in rabbit skeletal muscle (Hemmings & Cohen, 1983). GSK-3 phosphorylates a serine or threonine of the substrate within an active site containing residues 181, 200, 97, and 85 (Dajani et al., 2001). In Wnt/β-catenin signaling pathway, phosphorylation of the key effector β-catenin at the N-terminal by GSK-3 is required for following unbiquitination and proteasome degradation, which eventually leads to inactivation of the Wnt signaling pathway (Dajani et al., 2001). As shown in Table 3, a number of small molecules have been reported to activate Wnt signaling by inhibiting the phosphorylation activity of GSK-3, including SB216763 (Coghlan et al., 2000), L807mts (Licht-Murava et al., 2016), Valproic acid (VPA) (G. Chen, Huang, Jiang, & Manji, 1999), BIO (Sato et al., 2004), lithum chloride (Clement-Lacroix et al., 2005) and TWS119 (Ding et al., 2003).

Among these GSK3 inhibitors, BIO is the best-characterized and widely used to active Wnt signaling. BIO is a synthetic derivative of bromo-substituted indirubins from the Mediterranean mollusk Hexaplex trunculus, it selectively inhibited the phosphorylation of GSK-3 at Tyr216/276 (Sato et al., 2004). According to the cocrystal structure of GSK-3 and BIO with 2.8 Å resolution, BIO bound to GSK-3 in a narrow hydrophobic binding pocket of ATP, this planar conformation pocket was defined by two sides, one side was Ile62, Val70, Ala83, Leu132 and Tyr134, another side was Thr138, Arg141, Leu188 and Cys199 (Meijer et al., 2003). In human and mouse ESCs, BIO highly activated the Wnt signaling pathway to maintain the pluripotency of cells (Meijer et al., 2003).

TWS119 (4,6- disubstituted pyrrolopyrimidine) is a small molecule that also directly bound to GSK-3 (Ding et al., 2003). To identify the target protein, TWS119 was linked to an agarose affinity matrix, sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the pull-down samples revealed protein bands at 47- and 49-kDa, and GSK3 was identified by liquid chromatograph-mass spectrometer (Ding et al., 2003). In surface plasmon resonance (SPR) assay, TWS119 exhibited high binding affinity ($K_D = 126$ nM) and strong kinase inhibition ($IC_{50} = 30$ nM) to GSK-3 (Ding et al., 2003).

There are some Wnt agonists that have been proved to target GSK-3 but the exact binding sites were not clear. For example, VPA inhibited the activity of GSK-3 in the absence of magnesium ion (G. Chen et al., 1999). In a peptide-based protein kinase assay, small molecules SB-216763 and SB-415286 inhibited GSK-3 with an inhibition constant (KI) of 9 nM and 31 nM, respectively (Coghlan et al., 2000). Notably, in human liver cells and HEK293 cells, SB-216763 and SB-415286 strongly inhibited GSK-3 activity with little inhibition to other 24 kinds of kinases (Coghlan et al., 2000).

4.2. Axin

In β-catenin degradation complex, Axin is the central phosphorylation scaffold that contains CK1, GSK-3, and β-catenin-binding sites, the simultaneous binding of Axin to a kinase and β-catenin enforces close proximity and thereby increases the effective concentration of enzyme and substrate (Stamos & Weis, 2013). In the target identification of a Wnt agonist HLY78, a label-free quantitative proteomic analysis of the affinity-capture cellular proteins revealed that Axin was a potential target, and the binding of HLY78 and Axin was confirmed by a pulldown assay with HEK293T cell lysates (S. Wang et al., 2013). Subsequent *in vitro* pulldown analysis using a purified recombinant DIX domain of Axin (DAX) confirmed that the DAX was a direct target of HLY78. Further molecular docking and mutagenesis assays identified critical residues (R765, E776 and V810) of DAX for binding HLY78 (S. Wang et al., 2013). The authors also performed SPR spectroscopy and detected high binding affinity ($Kd = 8.83$ μM) of a wild-type DAX and HLY78 (S. Wang et al., 2013).

4.3. β-Catenin

Beta-catenin is the key effector of the canonical Wnt signaling pathway. The interaction between β-catenin and Axin is required for β-catenin phosphorylation and subsequent ubiqutination and degradation (Kimelman & Xu, 2006), therefore, disruption of the protein complex could be an attractive strategy to activate Wnt signaling. For this purpose, two stapled α-helical peptides targeting the Axin-β-catenin

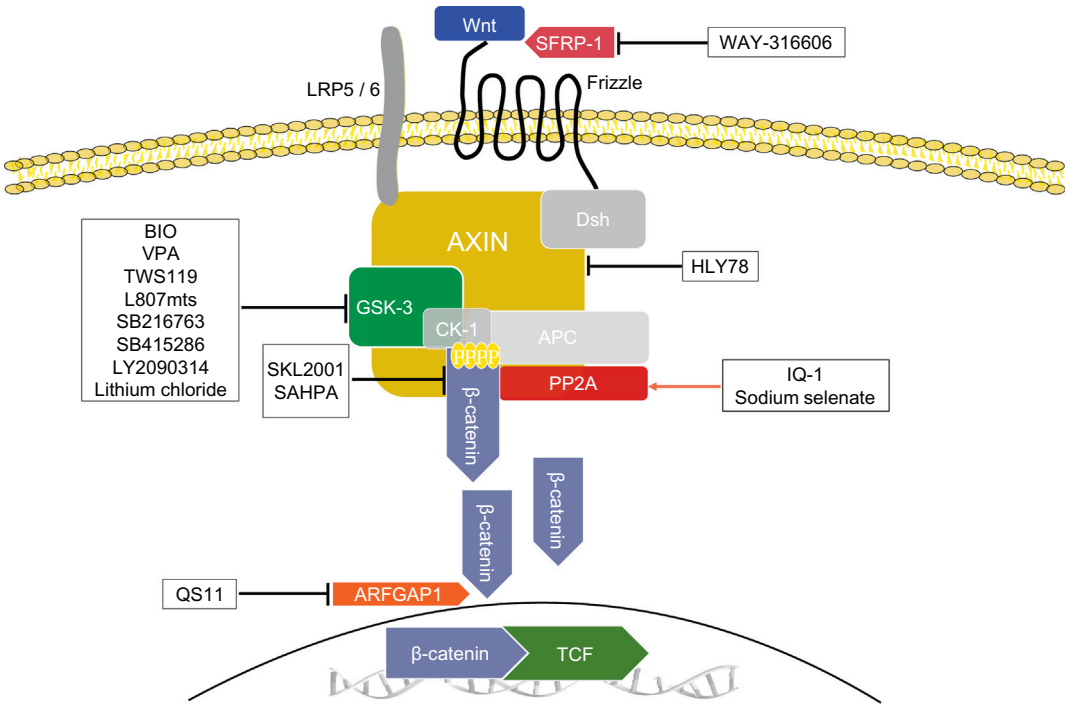


Fig. 2. Wnt agonists and the target proteins. As shown in the schematic diagram, SFRP-1, GSK-3, Axin, and ARFGAP1 negatively regulate the pathway at different steps, while PP2A positively regulates the pathway by directly interacting multiple components of β-catenin destruction complex. Inhibitors of SFRP-1, GSK-3, Axin, or ARFGAP1 were indicated in black and activators of PP2A were indicated by red.

complex (SAHPAs) were generated, the binding of SAHPAs to β-catenin was confirmed by an pull-down assay, and the directing binding was further quantified by isothermal titration calorimetry with a dissociation constant (Kd) of 30.1 μM (H. K. Cui et al., 2013). Consistently, SAHPAs treatment greatly enhanced the TOP-Flash reporter activity in the presence of Wnt3a (H. K. Cui et al., 2013). For the Wnt agonist SKL2001, a pulldown assay using purified fragment of Axin (residue 362–500) and β-catenin revealed that SKL2001 disrupted the Axin/β-catenin interaction, which was further confirmed by immunoprecipitation assay in HEK293 cells (Gwak et al., 2012).

4.4. SFRP1

Secreted frizzled-related protein-1 (SFRP-1) is a negative regulator of Wnt/β-catenin signaling, it is essential for human osteoblast differentiation and in response to treatment with bone forming agents (Bodine et al., 2005). In U2OS cells, a trifluoromethyl analog of diphenylsulfone sulfonamide (WAY-316606) inhibited SFRP-1 activity and activated Wnt signaling at nanomole concentration (Bodine et al., 2009). In a tryptophan fluorescence quenching binding assay, WAY-316606 bound stoichiometrically to purified human SFRP-1 protein with a Kd about 0.08 μM (Bodine et al., 2009). In a fluorescence polarization binding assay that employed WAY-316606 and purified human SFRP-1 protein in a competitive-binding format, the authors detected a half maximal inhibitory concentration at 0.5 μM (Bodine et al., 2009). To identify the binding region, the authors compared the inhibition of WAY-316606 to other SFRP family members and SFRP-1 in other species, the results indicated that WAY-316606 inhibited SFRP-1 *via* the netrin domain (Bodine et al., 2009).

4.5. PP2A

Protein phosphatase-2A (PP2A) is a multi-subunit serine/threonine phosphatase and positively regulates the Wnt pathway at multiple levels by directly interacting with Axin, APC, and β-catenin (Ikeda, Kishida, Matsuura, Usui, & Kikuchi, 2000; Ratcliffe, Itoh, & Sokol, 2000; W. Zhang et al., 2009). Recently, PP2A was identified as a target of Wnt agonists IQ-1 and sodium selenate (Jin et al., 2017). In a pulldown assay with biotinylated IQ-1, coomassie blue staining and mass spectrometry detected that two of the three selectively bound proteins were the alternatively spliced regulatory subunits (PR72/130) of PP2A, co-immunoprecipitation assay revealed that interaction of IQ-1 and PR72/130 subunits disrupted the PP2A/Nkd complex (Miyabayashi et al., 2007).

4.6. ARFGAP1

The GTPase activating protein of ADP-ribosylation factor 1 (ARFGAP1) is a regulator of Wnt signaling by inhibiting the nuclear translocation of β-catenin (Q. Zhang et al., 2007). ARFGAP1 has been identified as a selective target of a Wnt agonist QS11 in pulldown and Western blot assays, the direct binding of QS11 and ARFGAP1 was confirmed by the SPR analysis with a Kd of 620 nM (Q. Zhang et al., 2007). Consistently, treatment of QS11 enhanced the nuclear translocation of β-catenin (Q. Zhang et al., 2007). The direct inhibition of enzymatic activity of ARFGAP1 by QS11 was further confirmed by structure-activity relationship studies (Singh et al., 2015). In addition, a serial of QS11 analogs were developed to directly inhibit the enzymatic activity of purified ARFGAP1 protein and activate the Wnt/β-catenin pathway in cells (Singh et al., 2015).

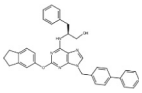
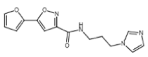
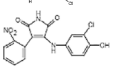
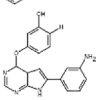
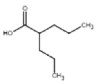
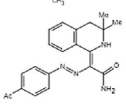
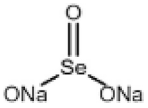
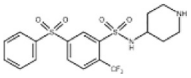
5. Challenges and opportunities

Although activation of Wnt/β-catenin signaling is an attractive therapeutic approach, it remains challenges in developing Wnt activators to pharmacological agents regarding the safety and selectivity.

5.1. Safety

Given that Wnt/β-catenin signaling is essential for many biological processes, the safety of targeting the pathway is an important issue in developing Wnt inhibitor for therapeutic purpose (Kahn, 2014). For

Table 3
The target proteins of Wnt agonists.

Target protein	Compound	Structure	Binding site/region	Binding assay	Mechanism
ARFGAP1	QS11[a]		ARF binding domain	Pulldown and Western blot assays SPR assay (KD =620 nM)	Inhibit ARFGAP1 and promote β-catenin translocation
Axin	HLY78[b]		DIX domain (residues R765, E776 and V810)	Pulldown and MS assay SPR assay (KD = 8.83 μM) Mutagenesis assay	Enhance the association of Axin and LRP6 Enhance LRP6 phosphorylation
	SAHPA[c]	Peptide	β-catenin-binding domain (residues 469 to 482)	Pulldown assay ITC assay (KD = 30.1 μM)	Disrupt the Axin/β-catenin interaction Stabilize the active β-catenin
Axin/β-catenin complex	SKL2001[d]		N.D.	Pulldown assay Immunoprecipitation assay	Disrupt the Axin/β-catenin interaction Inhibit β-catenin phosphorylation
GSK-3	BIO[e]		ATP binding site	Cocrystal	Inhibit GSK3-mediated β-catenin phosphorylation
	L807mts[f]	Peptide	N.D.	Kinase activity assay	Inhibit GSK3-mediated β-catenin phosphorylation
	Lithium chloride[g]		N.D.	Kinase activity assay (KI = 2 mM)	Inhibit GSK3-mediated β-catenin phosphorylation
	SB216763[h]		N.D.	Kinase activity assay (KI =9 nM)	Inhibit GSK3-mediated β-catenin phosphorylation
	SB415286[h]		N.D.	Kinase activity assay (KI =31 nM)	Inhibit GSK3-mediated β-catenin phosphorylation
	TWS119[i]		N.D.	Pulldown and MS assay SPR assay (KD = 126 nM)	Inhibit GSK3-mediated β-catenin phosphorylation
	VPA[j]		N.D.	Kinase activity assay	Inhibit GSK3-mediated β-catenin phosphorylation
PP2A	IQ-1[k]		PR72/130	Pulldown and MS assay	Activate PP2A activity Inhibit the GSK3 activity Increase active β-catenin
	Sodium selenate[l]		N.D.	N.D.	Activate PP2A activity Dephosphorylate β-catenin on S33/S37/T41 Inhibit GSK3 on Y216
SFRP-1	WAY-316606[m]		Netrin domain	Fluorescence binding assay (KD ≈ 0.08 μM) FP assay (KD = 20–30 μM)	Inhibit the interaction between SFRP-1 and wingless

ARFs, ADP-ribosylation factors; FP, Fluorescence polarization binding assay; ITC, Isothermal titration calorimetry; KD, Dissociation constant; KI, Inhibitor constant; MS, mass spectrometry; N.D., None detection; SPR, Surface plasmon resonance.

[a] Zhang et al. (2007)
[b] Wang et al. (2013)
[c] Cui et al. (2013)
[d] Gwak et al. (2012)
[e] Sato et al. (2004)
[f] Licht-Murava et al. (2016)
[g] Kneidinger et al. (2011)
[h] Coghlan et al. (2000)
[i] Ding et al. (2003)
[j] Chen et al. (1999)
[k] Miyabayashi et al. (2007)
[l] Jin et al. (2017)
[m] Bodine et al. (2009)

Wnt activators, one would concern if there is a risk of activation of Wnt/β-catenin signaling in leading diseases especially tumor. Notably, in mice with germ-line loss-of-function mutations of the APC, abnormal activation of Wnt/β-catenin signaling induced multiple intestinal neoplasia that were similar to familial adenomatous polyposis (FAP) in human (Moser, Pitot, & Dove, 1990; Su et al., 1992), but very few tumors were detected in other organs or tissues in human and mice with *APC* mutations (Moser et al., 1990; Su et al., 1992). The fact that Wnt signaling-initiated tumors were detected only in intestine suggests that topical treatment of Wnt activators in organs or tissues other than intestine should be safe therapeutic approach.

The safety of Wnt activator as therapeutic agents was supported by a recent clinical study. A small molecule MV, an ingredient of an extract from *Hovenia dulcis* Thunb (HDT), has been demonstrated to significantly increase the nuclear accumulation of β-catenin and activate TOP-Flash assay (Cha et al., 2014). In an uncontrolled and open-label clinical study including 20 Caucasian women with AGA (Sinclair grade 1–2), topical treatment of MV for 6 months significantly increased the hair count and hair mass index without participant discontinued treatment due to adverse effects (Tosti et al., 2016). The association of Wnt/β-catenin signaling activation and the therapeutic effects of MV was supported by the increase of Wnt10b mRNA expression in the scalp biopsies of MV treated AGA patients (Tosti et al., 2016). The pilot clinical study suggests that topical application of MV potentially serves as a novel therapeutic approach for female AGA patients.

5.2. Selectivity

Selectivity is an important issue of all pharmacological agents. For Wnt activators, the desired selectivity would be that only Wnt/β-catenin signaling is activated without change of other signaling pathways. However, as described above, many small molecules activated Wnt/β-catenin signaling by targeting regulators that are essential for many other biological processes. For instances, GSK-3 is a kinase with over 100 known substrates and is involved in many prevalent disorders, including psychiatric and neurological diseases, inflammatory diseases, and cancer (Beurel, Grieco, & Jope, 2015). PP2A regulates multiple signaling pathways and is involved to many cellular events including viral and cellular transformation, centromere localization and chromosome segregation (Arroyo & Hahn, 2005; Tang et al., 2006; Westermarck & Hahn, 2008). ARFGAP1 is involved in ***s***orting of Golgi resident proteins, formation of components of coat protein vesicles, adaptor protein 2-dependent endocytosis, cytoskeleton remodeling and cell movement (Bai et al., 2011; Lanoix et al., 2001; Luo et al., 2016). It is hard to expect selective activation of Wnt/β-catenin signaling by targeting these proteins. On the other hand, Axin, SFRP1 and β-catenin are proteins that are majorly involved in Wnt signaling (B. Chen et al., 2009; Huang et al., 2009; Valenta, Hausmann, & Basler, 2012; Warrier et al., 2016), therefore, small molecules targeting these proteins are more likely to reach selective activation of the Wnt signaling for intended purpose.

5.3. Target identification

The selectivity of pharmacological agents is dependent on their engaged proteins *in vivo*, identification of the target protein is required to dissect the mechanism of action of the Wnt activators and further optimization for drug development. Beside the Wnt activators with clarified target proteins described above, there are some small molecules that have been demonstrated to activate Wnt/β-catenin signaling, but their target proteins are to be identified. For examples, (hetero) arylpyrimidines strongly activated TOP-Flash activity in U2OS cells and activated non-phosphorylated β-catenin formation in bone, its exact target protein is not clear although the minimal GSK-3 inhibition indicated that it was most likely involved to the interaction with Wnt3a/DKK-1 (Gilbert et al., 2010). Similarly, for 2-amino-4-[3,4-(methylenedioxy) benzyl-amino]-6-(3-methoxyphenyl)pyrimidine that activated Wnt/β-catenin signaling independently of GSK-3 but in a TCF-dependent manner, the molecular targets of the active compound is still under investigation (Liu et al., 2005).

There are also small molecules that exhibited effects of Wnt activation without any clue of target proteins. For examples, Cyclosporine A effectively enhanced Wnt/β-catenin signaling activity by down-regulating the expression of SFRP1 in human dermal papilla (Hawkshaw et al., 2018), small molecule MV significantly activated TOP-Flash activity and increased the nuclear accumulation of β-catenin (Cha et al., 2014), small molecules SMIs induced skeletal muscle differentiation in embryonic stem cells *via* activation of the Wnt signaling (Lee, Haller, et al., 2016). Therefore, identification of the target proteins is required to further development of these Wnt activators.

Drug target identification is very challenging due to technical issues (*C.cui*, Zhou, Zhang, Qu, & Ke, 2018). Generally the first step of target identification is to find the direct binding proteins, and the most commonly used technique is affinity pulldown assay, such as the target identification of Wnt agonists HLY78 (S. Wang et al., 2013), SKL2001 (Gwak et al., 2012), IQ-1 (Creyghton et al., 2006), and QS11 (Q. Zhang et al., 2007). However, small molecules need to be labeled in pulldown assay, which is potential to interfere the binding between the small molecule and target proteins. Furthermore, the pulldown assay cannot exclude proteins that bind to small molecules indirectly, additional biochemical or biophysical assay is need to confirm the direct binding. For these reasons, researchers have developed label-free biochemical methods for target identification and validation. For examples, DARTS (target identification using drug affinity responsive target stability) coupled to mass spectrometry allows global identification of drug direct binding proteins (Lomenick et al., 2009; Y. Qu et al., 2016). Microscale thermophoresis is also a label-free biochemical assay for quantification of the binding affinity between small molecule and target protein (Y Qu et al., 2018; Wienken, Baaske, Rothbauer, Braun, & Duhr, 2010). Notably, both assays can be performed with cell lysates, which is particularly advantageous for small molecules that the purified protein of candidate target is not available.

6. Conclusions

As an essential regulator in embryonic development and adult tissue homeostasis, Wnt/β-catenin signaling is tightly associated with many types of diseases and serves as an attractive target for pharmacological therapy. During the past decades, a number of small molecules have been reported to activate Wnt/β-catenin signaling in various *in vitro* and *in vivo* models, some of them showed promising therapeutic effects for several types of diseases in preclinical models. For therapeutic purpose, there are some challenges for Wnt activators regarding the safety and selectivity, which could be addressed by choosing topical treatment and target identification, respectively.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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