Wnt Signaling and the Control of Human Stem Cell Fate

J. K. Van Camp · S. Beckers · D. Zegers · W. Van Hul

Abstract Wnt signaling determines major developmental processes in the embryonic state and regulates maintenance, self-renewal and differentiation of adult mammalian tissue stem cells. Both β-catenin dependent and independent Wnt pathways exist, and both affect stem cell fate in developing and adult tissues. In this review, we debate the response to Wnt signal activation in embryonic stem cells and human, adult stem cells of mesenchymal, hematopoetic, intestinal, gastric, epidermal, mammary and neural lineages, and discuss the need for Wnt signaling in these cell types. Due to the vital actions of Wnt signaling in developmental and maintenance processes, deregulation of the pathway can culminate into a broad spectrum of developmental and genetic diseases, including cancer. The way in which Wnt signals can feed tumors and maintain cancer stem stells is discussed as well. Manipulation of Wnt signals both in vivo and in vitro thus carries potential for therapeutic approaches such as tissue engineering for regenerative medicine and anti-cancer treatment. Although many questions remain regarding the complete Wnt signal cell-type specific response and interplay of Wnt signaling with pathways such as BMP, Hedgehog and Notch, we hereby provide an overview of current knowledge on Wnt signaling and its control over human stem cell fate.

Keywords Wnt · Human · Embryonic stem cells · Adult stem cells · Canonical · Noncanonical

Introduction

Research on the Wnt pathway started about 30 years ago with the discovery of Int-1, a proto-oncogene that, when activated by insertion of the Mouse Mammary Tumor Virus (MMTV),

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leads to malignant transformation of mammary tissues in mouse [1]. Int-1 was shown to be a homolog of *Drosophila wingless* (*wg*), which controls segment polarity during larval development, and the gene was subsequently named *Wnt1* (wingless-type MMTV integration site family member 1).

Currently, the Wnt pathway is known as an important regulatory signaling axis that influences developmental processes in the embryo and regulates maintenance, self-renewal and differentiation of adult mammalian tissue stem cells.

Wnt Signaling Pathways

The involvement of numerous Wnt ligands and (co-)receptors accounts for a myriad of possible interactions, that are distilled intracellularly into a limited number of established responses. In the past, these were divided into canonical and noncanonical Wnt pathways, defined by their requirement or independence of intracellular β-catenin, respectively. In recent years however, we have been encouraged not to view canonical and noncanonical pathways as independent, linear pathways but to view them as part of a complex and dynamic signaling network. When Wnt ligands bind to receptors and co-receptors on the cell surface, both β-catenin dependent and β-catenin independent answers can be set in motion, which can reinforce or even oppose each other. At this point, the cell will respond to the net result of the intracellular signaling activities, and this will depend on factors intrinsic to both the cell and its environment.

In absence of Wnt ligands, intracellular levels of β-catenin are kept low by ubiquitin-dependent proteasomal degradation, set in motion by a multicomponent degradation complex, consisting of Axin, caseine kinase 1α (CK1α), Adenomatous polyposis coli (APC) and glycogen synthase kinase 3β (GSK3β). When Wnt proteins bind to the seven transmembrane (7-Tm) Frizzled (Fz) receptor and low density lipoprotein receptor-related protein co-receptor 5/6 (LRP5/6), the formation of the degradation complex is inhibited, and β-catenin is stabilized [2–4]. β-catenin then accumulates in the

cytoplasm and migrates to the nucleus where it acts as a transcriptional co-activator for transcription factors of the T-cell factor/lymfoid-enhancing factor (TCF/LEF) family, among others [5] (Fig. 1b). Additional co-activators, such as B-cell lymphoma 9 (BCL9), cAMP response element-binding protein (CREB)-Binding Protein (CBP)/p300 and brahma-related gene 1 (BRG1) bind the β-catenin-TCF complex and

ensure cell- and tissue-specific activation or suppression of transcription of numerous Wnt responsive genes [6–10]. When β-catenin is not present in the nucleus, TCF/LEF binds TLE1, which promotes histone deacetylation and chromatin compaction, thereby repressing transcription [11] (Fig. 1a).

Through their efforts to find proteins homologous to Wnt1, a protein shown to signal through the Wnt β-catenin dependent
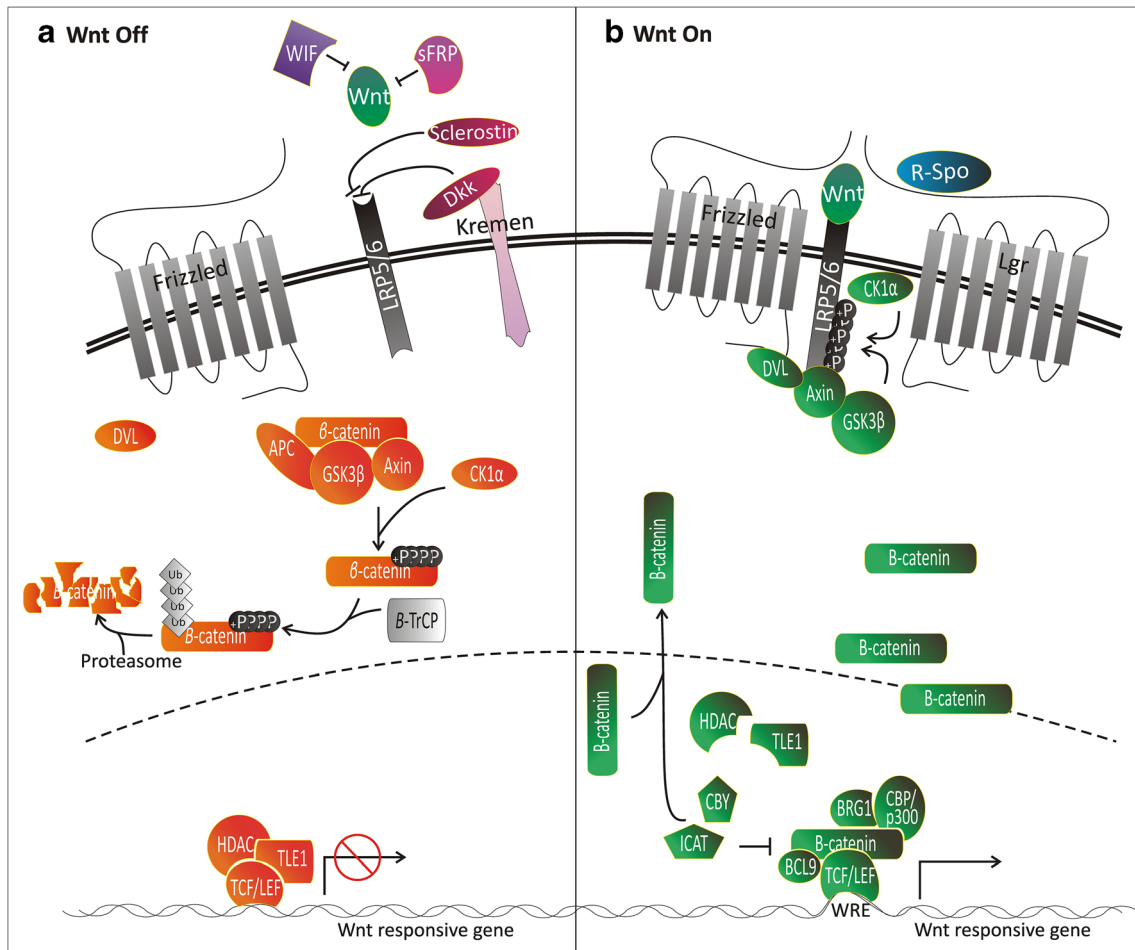

Fig. 1 β-catenin dependent Wnt signal transduction pathway. A graphical representation of the β-catenin dependent Wnt pathway. **a** In the canonical Wnt pathway, the central co-activator β-catenin is kept at low levels by a degradation complex, consisting of Axin, CK1α, GSK3β and APC. Phosphorylation of β-catenin by this complex leads to poly-ubiquitination by the E3 ligase β-TrCP and subsequent degradation by the proteasome. In the inactive state, TCF/LEF transcription factors are bound to TLE1, which recruits HDACs to silence chromatin and thereby repress transcription. The Wnt pathway is regulated by both extracellular and intracellular inhibitors, intruding at every step of the pathway. sFRPs and WIF both work in an autocrine and paracrine manner and bind Wnts, preventing their interaction with Fz. DKK proteins inhibit the canonical pathway by binding the extracellular part of LRP co-receptors and, together with Kremen, mediating the internalisation of LRP. Sclerostin binds LRP co-receptors and impairs the binding to Wnt ligands. **b** When Wnt proteins bind the 7-Tm Frizzled receptor and LRP5/6 on the cell surface, CK1α and GSK3β are diverted to the plasma membrane and phosphorylate LRP5/6. In addition, DVL molecules gather to the plasma membrane to interact with Fz. Interaction of Axin with phosphorylated

LRP5/6 and DVL leads to disintegration of the destruction complex and accumulation of β-catenin in the cytoplasm. β-catenin can then migrate to the nucleus where it binds transcription factors of the TCF/LEF family, among others. When activated, β-catenin converts TCF/LEF into a transcriptional activator and the complex binds Wnt response elements in the DNA. In addition, several co-activators are recruited (BCL9, CBP/p300, BRG1) to ensure efficient transcription of target genes. Chibby and ICAT inhibit β-catenin action in the nucleus by preventing the formation of the β-catenin-TCF complex. In addition, Chibby was shown to promote β-catenin export out of the nucleus. CK1α, caseine kinase 1α; GSK3β, glycogen synthase kinase 3β; APC, Adenomatous polyposis coli; β-TrCP, β- transducin repeat-containing protein; HDAC, histone deacetylase; TCF/LEF, T-cell factor/lymfoid-enhancing factor; sFRP, secreted frizzled-related protein; WIF, Wnt-inhibitory factor; DKK, Dickkopf; LRP5/6, low density lipoprotein receptor-related protein co-receptor 5/6; DVL, dishevelled; BCL9, B-cell lymphoma 9; CBP, cAMP response element-binding protein-binding protein; BRG1, brahma-related gene 1; WRE, Wnt response element

pathway, Christian et al. identified XWnt5a which, as opposed to XWnt1, did not lead to duplication of the embryonic axis when injected in *Xenopus* embryos. Administration of XWnt5a however lead to developmental defects of head and tail and even antagonized the ability of other Wnts to induce an ectopic axis [12, 13]. This implied that some Wnts signal through an alternative pathway, that differs from standard β-catenin signaling [14, 15]. Current studies have however shown that Wnt ligands elicit a canonical or noncanonical response dependent on cell type, environment and receptor milieu.

Depending on the major intracellular mediators used, the noncanonical signaling cascades are subdivided into several Wnt/Ca^{2+} pathways and a Dishevelled (DVL)-c-Jun N-terminal kinase (JNK) pathway (Fig. 2).

In the noncanonical Ca^{2+}-mediated pathways (Fig. 2a), stimulation of heterotrimeric G-proteins and DVL, associated with Frizzled, leads to the activation of phospholipase C (PLC) [14, 16–18]. The resulting IP3 then diffuses through the cytosol and interacts with the Ca^{2+} channels present on the membrane of endoplasmic reticulum (ER) resulting in release of Ca^{2+} ions. Ca^{2+} ions then function as secondary messengers, leading to the activation of protein kinase C (PKC)-Cdc42 [19, 20], Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), TGFβ activated kinase (TAK1), Nemo-like kinase (NLK) [21, 22] and/or calcineurin (CaCN)-nuclear factor of activated T cells (NFAT) [23, 24].

In the DVL-JNK pathway (Fig. 2b), after binding of Wnts to the Frizzled receptor, Dishevelled can aid in the activation of JNK through the downstream action of small GTPases Rho and Rac. This pathway is involved in cellular polarity and cytoskeletal modulation as well as the morphology and migration of cells during gastrulation in Xenopus embryos, and has therefore been termed the 'planar cell polarity' or PCP pathway [25, 26].

Wnt Ligands

Nineteen Wnt ligands have been identified in vertebrates to date (Table 1) but their classification is based on amino acid sequence rather than on functional properties [27]. The common structural features of all Wnt proteins include a signal sequence for secretion, several highly charged amino acid residues, and multiple glycosylation sites [28, 29]. In addition, Wnt ligands display a characteristic distribution of 22–24 cysteine residues [29–31]. On the first conserved cysteine residue, Wnts carry a lipid modification, in the form of a palmitate group [32]. Palmitoylation of Wnts occurs at the level of the endoplasmatic reticulum of the Wnt secreting cell, and is performed by a porcupine acyl-transferase (PRCN). Further transport and secretion of the Wnt protein in secretory vesicles is controlled by Wntless (Wls)/Evenness interrupted (Evi), a multipass transmembrane protein that is present in the Golgi and/or on the plasma membrane [33]. The presence of

palmitate on Wnts was shown to be necessary for Wnt signaling and is proposed to aid in the N-linked glycosylation of Wnt ligands [32, 34]. Glycosylation might support Wnt transport by increasing Wnt interactions with heparin sulfate proteoglycans (HSPGs) present on the surface of Wnt responding cells [35, 36].

As described previously, some Wnts signal through a "noncanonical" pathway, that differs from standard β-catenin signaling [14, 15]. Although the intracellular response to Wnt activation does not depend solely on the Wnt ligand, Wnt4, Wnt5a, Wnt5b and Wnt11 are known as "noncanonical-Wnts", whereas Wnt1, Wnt3 and Wnt10b are typically "canonical-Wnts". This signaling specificity is achieved in part by the preference for a particular type of Fz receptor (Table 1) in addition to the choice of (a) specific co-receptor(s). However, as mentioned previously, specific Wnt ligands are not restricted to activating the canonical or noncanonical pathway and several Wnts have been shown to activate both, depending on the receptor and co-receptor usage (Table 1).

As can be predicted from their important role in embryonic and adult tissue development and maintenance, mutations in Wnt ligands lead to a broad spectrum of developmental, genetic diseases (Table 1). In addition, overexpression of Wnt ligands and repression of Wnt pathway inhibitors was shown to cause human cancer (reviewed in [37]).

Wnt Receptors

Wnts bind to the Frizzled (Fz) family of 7-Tm receptors, comprised of 10 members in humans (Table 1). Fz proteins are characterized by a large extracellular part containing a conserved cysteine-rich domain (CRD) [2]. The cytoplasmic side of Fz interacts directly with the PDZ domain of Dishevelled. It is at the level of DVL that the Wnt signal branches into the three separate pathways i.e. the canonical, Wnt/Ca^{2+} and DVL/JNK pathway.

Unique for the canonical Wnt pathway, is the use of LRP5/6 as co-receptors. LRP5 and LRP6 are single-pass transmembrane proteins with a relatively small intracellular domain and a large extracellular domain, containing several potential regions for protein interaction [38]. Intracellularly, LRP5/6 is phosphorylated by the action of GSK3β and CKγ. This results in the interaction of LRP5/6 with Axin, leading to the release of Axin from the destruction complex, and the subsequent stabilization and nuclear localization of β-catenin (Fig. 1). LRP4 is another Wnt co-receptor that shares structural elements within the extracellular ligand binding domain with LRP5 and LRP6 [39].

Ror2 (orphan receptor tyrosine kinase) is a single-pass transmembrane tyrosine kinase receptor that contains a CRD motif similar to that of the Frizzleds. This receptor is involved in Wnt/Ca^{2+}-signaling and under certain circumstances has
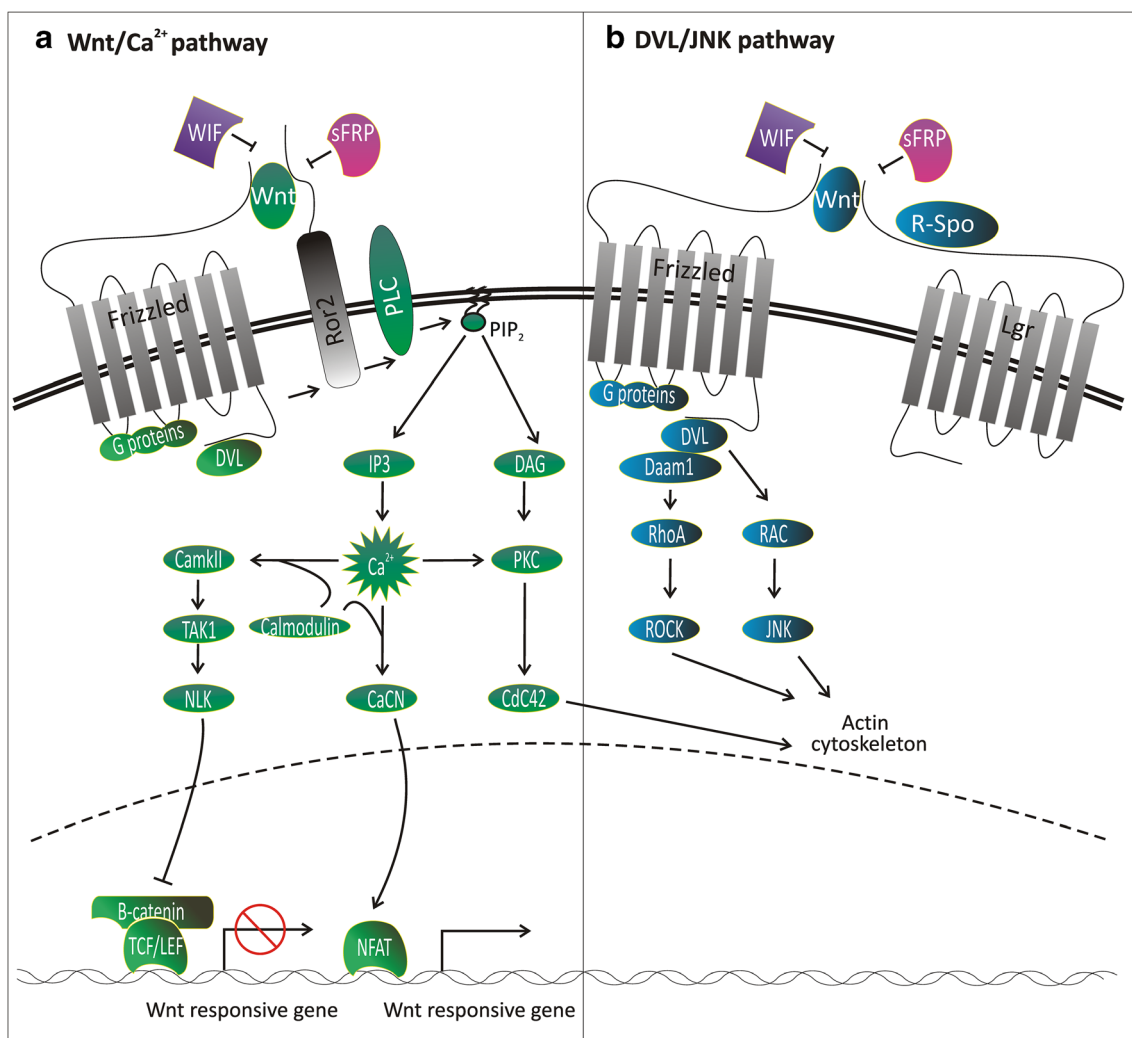
Fig. 2 β-catenin independent Wnt signal transduction pathways. A graphical representation of the different β-catenin independent Wnt pathways. **a** In the Wnt/Ca2+ pathway, Wnt ligand binding to Frizzled and Ror co-receptor leads to stimulation of heterotrimeric G-proteins and DVL. This interaction leads to the activation of PLC. This enzyme converts PIP2 into IP3 and DAG. Subsequently, intracellular Ca^{2+} ions are released from the endoplasmatic reticulum through the action of IP3. These Ca^{2+} ions will now act as secondary messengers, relaying signaling via downstream pathways. The cytosolic rise of Ca2+ ions, together with ubiquitously expressed calmodulin, activates CamKII. CaMKII induces activation of TAK1 and NLK which can eventually lead to NLK mediated inhibition of β-catenin-TCF-dependent transcription through phosphorylation of TCF. Ca^{2+} ions, together with calmodulin, activate the phosphatase CaCN, which in turn leads to the dephosphorylation and activation of NFAT. NFAT will then migrate to the nucleus and alter gene expression. DAG, along with released Ca^{2+} from ER, activates PKC. PKC then regulates small GTPase Cdc42 which is a key regulator of actin cytoskeleton remodeling and cell migration. **b** In the DVL/JNK pathway, Frizzled and Dishevelled function in concert with G proteins and Daam1 to activate small GTPases RhoA and Rac. Activation of Rho GTPase leads to the activation of the ROCK which leads to modification of the actin cytoskeleton. The activation of Rac is independent of Daam1 and stimulates JNK to set up cellular polarity through its effect on the actin cytoskeleton. Ror, orphan receptor tyrosine kinase; DVL, disheveled; PLC, phospholipase C; PIP2, phosphatidyl inositol 4,5-bisphosphate; IP3, inositol 1,4,5-triphosphate; DAG, 1,2 diacylglycerol; CamKII, Ca^{2+}/calmodulin-dependent kinase II; TAK1, TGFβ activated kinase; NLK, nemo-like kinase; CaCN, calcineurin; NFAT, nuclear factor of activated T cells; PKC, protein kinase C; Daam1, Dishevelled associated activator of morphogenesis 1; ROCK, Rho-associated kinase; JNK, c-Jun N-terminal kinase

been shown to bind Wnt5a, leading to the inhibition of Wnt/β-catenin/TCF signaling [40].

Ryk (receptor-like tyrosine kinase) is another single-pass tyrosine kinase receptor, that was shown to activate Wnt signaling by binding Fz, but its function mainly lies within the developing neuron and will therefore be discussed further below (§3.7 Neural stem cells).

The leucine-rich repeat containing, G-protein–coupled receptor (LGR) family of receptors associate with the Frizzled/Lrp Wnt receptor complex and are able to enhance both Wnt β-catenin and PCP signaling [41, 42]. LGRs belong to a 7-Tm evolutionary conserved receptor family, known for their large extracellular ligand binding domain. R-spondins (RSPO1-4) are secreted agonists of the Wnt pathway that interact with

Table 1 Wnt ligands with corresponding effect on stem cells, expression data and link with human disease

	Chrom. position	Receptor	β-catenin	Wnt/Ca²⁺ pathway	DVL/JNK pathway	Proliferation of SC	Differentiation of SC	Expression	KO mouse	Human disease
WNT1	12q13	Fz1 [220], Ryk [183]	Yes[a] [221]	Yes [221]	nd	NSC[a] [222]	MSC: inhibition of adipogenesis and chondrogenesis [94, 141]	Blood, brain [223–225]	Perinatal lethality, lack of cerebellum and part of the midbrain [226], ataxia [227]	Early-onset osteoporosis and osteogenesis imperfecta [228]
WNT2	7q31.2	Fz1 [220]	Yes [229]	nd	nd	NSC[a] [230]	nd	Blood, lung, breast, thalamus and fetal lung, placenta [189, 223–225, 231, 232]	Perinatal lethality, lung hypoplasia [233], placentation defects [234]	nd
WNT2B (WNT13)	1p13	nd	Yes [235]	nd	nd	HSC [147]	nd	Heart, brain, placenta, lung, prostate, testis, ovary, intestine, breast tissue and fetal brain, lung and kidney [236, 237]	No described phenotype [233]	nd
WNT3	17q21	Fz1 [220] Fz7 [238]	Yes [238]	nd	Yes [239]	ISC[a] [163]	nd	Breast, blood, liver [223–225, 232]	No primitive streak, mesoderm or node formation, no anterior-posterior neural patterning [59]	Tetra-amelia [240]
WNT3A	1q42	Fz1 [220], Fz2 [241], Fz5 [242] Ryk [183]	Yes [243]	Yes [244]	Yes [245]	HSC [32] NSC[a] [182, 222] GSC[a] [164]	MSC: bone [243] NSC [246] ESC [247, 248]	Blood, placenta, lung, spleen, liver and prostate [223–225, 249]	Perinatal lethality, lack of caudal somites and tailbud, disrupted notochord in embryos, shortened trunk, kinked neural tube [250], missing or small hippocampus [176]	nd
WNT4	1p36.23-p35.1	nd	Yes [251]	nd	Yes[a] [151]	HSC[a] [151]	MSC: adipocytes [99]	Breast, blood [223–225, 232]	Absence of Müllerian-duct in females [252], defective nephron development [62], defective Sertoli cell differentiation [253], sex reversal [254]	Müllerian-duct regression and Virilization [251] SERKAL syndrome [255] Renal hypodysplasia [256]
WNT5A	3p21-p14	Fz2 [257] Fz3 [258] Fz4 [241], Fz5 [259] Ror2 [40, 260]	Yes [40]	Yes [261]	Yes [262, 263]	HSC [147] MaSC[a] [264] NSC[a] [182]	MSC: bone [263] NSC[a] [222, 262] ESC [265]	Blood, liver, fetal heart and lung [223–225, 266]	perinatal lethality, dwarfism, facial abnormalities, short limbs and tails, dysplasia of lungs and genitals, ventricular septal defects [260, 267]	Robinow Syndrome [268]
WNT5B	12p13.33	Ror2 [269]	nd	Yes[a] [270]	Yes[a] [270]	nd	MSC: adipocytes[a] [271, 272]	Prostate, liver, ovary, small intestine, blood and fetal brain, lung and kidney [223–225, 273]	No described phenotype [274]	nd
WNT6	2q35	nd	Yes[a] [275]	nd	nd	nd	MSC: bone and inhibition of adipogenesis[a] [275]	Spleen, blood, liver and placenta [223–225, 276]	Aberrant stromal cell proliferation [277]	nd
WNT7a	3p25	Fz5 [278, 279], Fz10 [280]	Yes[a] [221]	Yes[a] [221]	Yes[a] [145]	NSC[a] [181, 281] MSC: satellite stem cells[a] [145]	nd	Placenta, kidney, testis, uterus, brain, blood and fetal lung and brain [223–225, 282]	Anterior-Posterior and dorsoventral patterning defects with limb [60] and female reproductive tract [283], failure in Müllerian-duct regression [284], delayed maturation in	Fuhrmann syndrome Al-Awadi/Raas-Rothschild/ Schinzel phocomelia syndrome [287]

Table 1 (continued)

	Chrom. position	Receptor	β-catenin	Wnt/Ca²⁺ pathway	DVL/JNK pathway	Proliferation of SC	Differentiation of SC	Expression	KO mouse	Human disease
									cerebellum [285], lack of endometrial glands, infertility [286]	
WNT7b	22q13	Fz1 [288], Fz10 [288]	nd	nd	Yes^a [289]	nd	nd	Breast, brain, lung, prostate, blood and fetal brain, lung and kidney [223–225, 232, 290]	Perinatal death due to respiratory failure (lung hypoplasia and pulmonary hemorrhage) [291], deficiencies in formation of the chorio-allantoic placenta [292]	nd
WNT8a	5q31	nd	nd	nd	nd	nd	nd	Blood [223–225]	nd	nd
WNT8b	10q24	nd	nd	nd	nd	nd	nd	Blood, fetal brain [223–225, 293]	No described phenotype [294]	nd
WNT9a (WNT14)	1q42	nd	nd	nd	nd	nd	nd	Blood, liver, muscle, heart, placenta, lungs, pancreas, spleen, ovary and fetal brain and lung [223–225, 249]	Skeletal abnormalities [295]	nd
WNT9b (WNT15)	17q21	nd	Yes^a [296]	nd	Yes^a [297]	nd	nd	Blood [223–225]	Postnatal lethality, vestigial kidneys, absence of reproductive duct [298]	nd
WNT10a	2q35	nd	Yes^a [275]	nd	nd	nd	MSC: bone and inhibition of adipogenesis^a [275]	Spleen, kidney, prostate, ovary, blood and fetal kidney, placenta, lung [223–225, 276]	nd	Odonto-onycho-dermal Dysplasia [299]
WNT10b	12q13	nd	Yes [300]	nd	nd	HSC [147]	MSC: bone and inhibition of adipogenesis [111]	Ubiquitously, highest levels in heart and skeletal muscle [301]	Increased fusion of myoblasts, lipid accumulation in regenerating muscle [97], decreased bone volume, decreased BMD [111]	Obesity [302, 303] Split-Hand/Foot Malformation [304, 305]
WNT11	11q13.5	Fz4 [306] Fz7 [307] Fz8 [306]	Yes^a [308]	Yes^a [309]	Yes^a [310]	ISC^a [309]	MSC: adipocytes [99] ESC [265]	Blood, fetal skeleton, lung mesenchyme, urogenital system and the cortex of the adrenal gland [223–225, 311]	Lethality in utero, kidney hypoplasia [312]	nd
WNT16	7q31	nd	nd	nd	Yes [313]	nd	nd	Adult pancreas, kidney, placenta, brain, heart, blood and spleen [223–225, 314]	Reduced total body BMD [315]	nd

All 19 vertebrate Wnt ligands are described, with human chromosomal position. Different receptors that have been shown to signal via the indicated Wnt ligand are described and the effect of the different Wnt ligands on specific Wnt pathway activation (β-catenin dependent, Ca2+ or DVL/JNK pathway) and on proliferation and/or differentiation of specific stem cell types is described

^{a} Indicates that proof came from studies in mouse cell lines and/or mouse models. Expression data for the different Wnt ligands in human tissues is summarized. Phenotypic observations in homozygous knockout (KO) mice are described and in the last column, human diseases are listed that are caused by (a) mutation(s) in the corresponding Wnt gene. *Nd* not determined; *ESC* Embryonic stem cell; *MSC* mesenchymal stem cell; *ISC* intestinal stem cell; *GSC* gastric stem cell; *HSC* hematopoetic stem cell; *NSC* neural stem cell; *CSC* cancer stem cell; *MaSC* mammary stem cell; *BMD* bone mineral density

LGRs on the cell surface. In addition, R-spondins were shown to bind cell-surface transmembrane E3 ubiquitin ligase zinc and ring finger 3 (ZNRF3) and induce the association between ZNRF3 and LGR4, which results in membrane clearance of ZNRF3 [43]. Because ZNRF3 inhibits Wnt signaling by promoting the turnover of Frizzled and LRP6, this mechanism represents another way in which R-spondins can enhance Wnt signal transduction [43].

Intracellular and Extracellular Regulators of Wnt Pathways

The Wnt pathway can be spatially and temporally regulated by both extracellular and intracellular inhibitors, intruding at every step of the signal transduction (Figs. 1 and 2).

The secreted frizzled-related protein (sFRP) protein family consists of 5 members in humans (sFRP1-5) and all are known modulators of the Wnt pathway that bind Wnt ligands extracellularly [44]. Wnt-inhibitory factor 1 (WIF1) binds Wnt ligands through a unique WIF domain that does not exhibit homology to the cysteine-rich Wnt-binding regions of sFRP or Frizzled proteins, but similarly prevents Wnts from binding their receptors [45]. Another secreted Wnt inhibitor is Dickkopf (Dkk), which works together with Kremen to mediate LRP co-receptor internalization, thereby making it unavailable for Wnt reception [46]. In a similar way, secreted Sclerostin protein can bind to LRP co-receptors and cause the inhibition of the Wnt pathway [47–50].

Chibby (Cby) is an intracellular modulator of the canonical Wnt pathway and has a dual role in inhibiting the pathway. On the one hand, Cby competes with TCF/LEF transcription factors in the nucleus for binding to β-catenin and on the other hand, Cby promotes removal of β-catenin out of the nucleus through interaction with 14-3-3 proteins [51–53]. ICAT (inhibitor of β-catenin and TCF-4) is another intracellular regulator that interferes with the formation of the β-catenin-TCF4 complex by binding to β-catenin [54].

Above mentioned mechanisms are just a fraction of possible ways to dynamically regulate Wnt signaling and it is clear that this pathway contains an enormous potential to control developmental and homeostatic processes.

As mentioned above, Wnt signaling is involved in the upkeep of both embryonic (ESC) and adult (ASC) stem cells. Embryonic stem cells are pluripotent, meaning that they have the ability to differentiate to each of the three germ layers. It is however a transient cell state in the development of all vertebrates. Adult stem cells have a lower ability to differentiate (multi-, oligo- or unipotent) and typically reside within regenerative tissues throughout adult life, playing an important role in replenishment and repairing of these tissues. In the first part of this review, we will discuss the involvement of Wnt signaling in embryonic stem cell proliferation and differentiation. In the second part, we will discuss the majority of human regenerative tissues in which stem cells, that are maintained by Wnt

signaling, reside. Lastly, we will focus on cancer stem cells, a small population of stem cells in tumor tissues, which maintain tumor growth and possess properties of both ESC, ASC and cancer cells.

Role of Wnt Signaling in Stem Cell Proliferation and Differentiation

Embryonic Stem Cells

The decision of an embryonic stem cell to either proliferate, and maintain its pluripotent character, or commit to the differentiation process (to mesoderm, endoderm or ectoderm; Fig. 3) and lose a level of potency depends on an enormous amount of extracellular and intracellular signals, that have to be interpreted by the cell and funneled into a final choice. Whereas in vitro adding of murine leukemia inhibitory factor (LIF) to murine ESCs is sufficient for self-renewal and maintenance of the pluripotent state of these cells, human LIF is not able to maintain a human ESC culture in an undifferentiated state [55]. The pluripotent state of human embryonic stem cells (hESC) is characterized and maintained by the expression of key transcription factors Oct4, Nanog and Sox2 [56]. RNA expression profiling in H7 hESCs showed that these cells express RNA for each of the 19 Wnt genes and in addition, express all 10 Fz receptors and both LRP5 and LRP6 co-receptors [57].

There has been some debate on the role of the canonical and noncanonical Wnt signaling cascades in the decision of the hESC to either proliferate or differentiate. Although it is clear that Wnt signaling is widely utilized during early development to regulate body axis formation [58–60], organogenesis [61, 62] and cell migration [63–65] in vertebrates, its role in the maintenance of pluripotent stem cell character is thus not clear.

While many reports indicated that Wnt pathway activation leads to loss of pluripotency and differentiation toward mesoderm and endoderm lineages [66–69], a number of other reports identified the Wnt pathway as an essential factor for the establishment and maintenance of ESC self-renewal [70–73].

Sato et al. stated that the activation of the Wnt/β-catenin pathway by 6-bromoindirubin-3′-oxime (BIO), a specific pharmacological inhibitor of GSK3β, was sufficient for maintaining the undifferentiated phenotype in both murine and human ESC. Oct3/4, Rex1 and Nanog were expressed in BIO-treated mESC cells, which led the authors to believe that the observed pluripotent state was mediated by Wnt-activation of these transcription factors [70].

Singh et al. however postulated that different GSK3 complexes are present in the cell, which perform separate biochemical functions. They indicated that low concentrations of
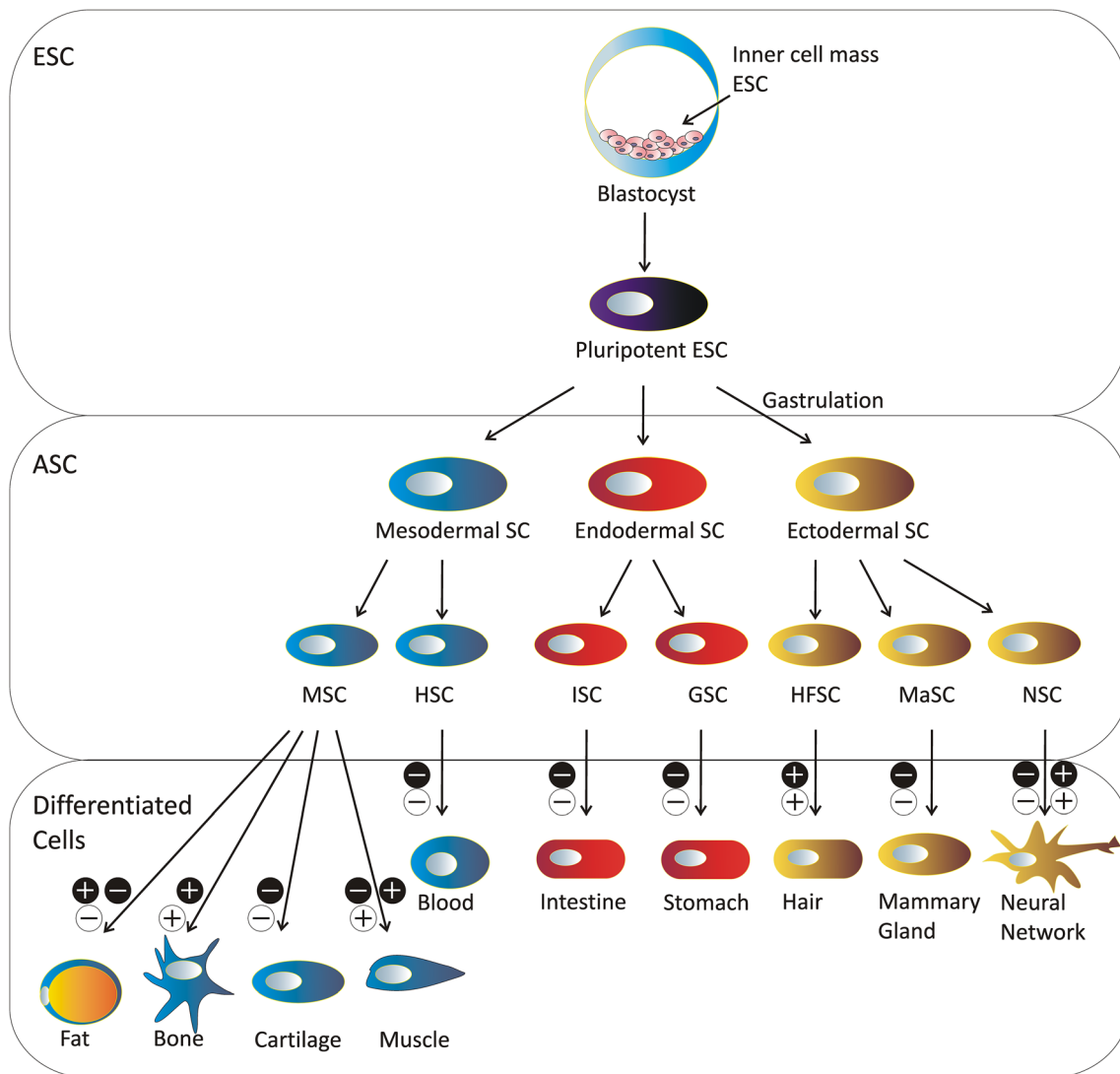
Fig. 3 Stem cell lineages and the effect of Wnt signaling on differentiation. Different stem cell lineages are depicted, starting from the blastocyst and ending with the main differentiated cells of the human body. The effect of canonical (*white circles*) and noncanonical (*black circles*) Wnt signaling on adult stem cells is illustrated, whereby '*plus sign*' indicates stimulation and '*minus sign*' indicates inhibition. In some stem cell types, depending on the Wnt ligand, (co-)receptor usage, activation state of the cell, environmental factors, ect. Wnt signaling can either stimulate or inhibit differentiation. ESC, Embryonic stem cell; ASC, Adult stem cell; MSC, mesenchymal stem cell; HSC, hematopoetic stem cell; ISC, intestinal stem cell; GSC, gastric stem cell; HFSC, hair follicle stem cell; MaSC, mammary stem cell; NSC, neural stem cell

GSK3β inhibitors such as BIO will lead to proliferation of ESC but not by activation of Wnt pathway target genes, as hypothesized by Sato et al., but by stabilization of Myc, acting through PI3K/Akt signaling [74]. Only at high concentrations of GSK3 inhibitor will the Wnt pathway become active, leading to loss of pluripotency markers and increased levels of mesendoderm markers [75].

Another reason for the discrepancy between studies is the applied time frame to study the effect of Wnt ligands on ESC differentiation. While the addition of Wnt3a-conditioned medium to ESC culture for <1 week maintained their undifferentiated cell morphology [70], culturing these cells for longer periods in the presence of Wnt pathway activating conditions did significantly reduce their self-renewing capacity [67, 68].

It appears that only two signaling pathways, dynamically interacting with each other, are required to maintain pluripotency. First, the transforming growth factor β (TGFβ)/Activin A pathway leads to activation of Smad2,3 and its downstream targets, including Nanog [76–78]. However, this pathway switches to a pro-differentiation mode in absence of the PI3K/Akt signaling pathway, which is the second pathway required for maintaining pluripotency. When PI3K/Akt signaling is inactive, Smad2,3 cooperates with β-catenin to up-regulate proteins required for early differentiation, such as the mesendodermal marker MixL1 [75, 79]. Another target of TGFβ signaling is BAMBI (BMP and activin membrane-bound inhibitor), a single-pass transmembrane decoy receptor that was shown to inhibit TGFβ and Activin signaling, but

enhance Wnt signal transduction [80]. In the PI3K/Akt signaling pathway, PI3K and subsequent Akt activation inhibits MAPK, whereby MAPK can no longer prevent GSK3β kinase action in the Wnt pathway, resulting in the downregulation of Wnt pro-differentiation target genes [79]. Taken together, these data indicate that suppression of Wnt signaling stabilizes pluripotent cells [75]. Consistent with this theory, Davidson et al. reported that Wnt/β-catenin signaling is not required for the self-renewal of pluripotent ESC and that Oct4, a key pluripotency factor in hESC, functionally represses Wnt signaling in self-renewing hESC [67].

Teo et al. stated that Wnt signaling can maintain pluripotency of ESC when β-catenin uses cAMP response element-binding protein (CREB)-Binding Protein (CBP) as a cofactor for transcription of target genes (e.g. Oct4, survivin) in the nucleus. However, when switching to the use of p300, a closely related homolog of CBP, differentiation is induced and cellular potency is decreased [9, 81]. Both CBP and p300 are protein acetyltransferases, able to acetylate histones and thereby convert chromatin in an open, activated state, promoting pluripotency [82]. The co-factor switch from CPB to p300 is suggested to be induced by noncanonical Wnt signaling, whereby activation of PKC leads to phosphorylation of p300, thereby increasing the affinity of β-catenin for p300. PKC also activates CDC42, leading to the cytoskeletal rearrangements necessary for differentiation [9].

In a search for cancer therapeutics, ICG-001 was identified as a highly selective inhibitor of the CPB-β-catenin interaction by binding to CPB, but not p300. Confirming the use and success of these type of agents, it has been shown that CBP/catenin antagonism is able to target and eliminate drug-resistant leukemic stem cells both in vitro and in vivo [83, 84].

Adult Stem Cells

One differentiation step further down the line are the multipotent stem cells, no longer able to develop into cells of all three germ lines, but still able to proliferate and maintain the possibility of differentiating into a spectrum of specialized tissues (Fig. 3). ASC are unique in their ability to self-renew while simultaneously generating specialized cells. The balance between self-renewal and differentiation of these cells is determined by factors coming from the stem cell microenvironment, also called the stem cell niche. Below, we will discuss the presence and function of Wnt ligands in the stem cell niches of bone, adipose tissue, muscle, intestine, hair follicles, blood, mammary gland and neurons.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs), otherwise termed as mesenchymal progenitor cells or marrow stromal cells, were first identified within the bone marrow stromal cell compartment,

and researchers at that time observed that different stromal cell lines could be promoted under different culture conditions [85, 86]. Later studies identified synovial tissue, periosteum, blood, adipose tissue, muscle and dermis as alternative tissue sources of MSCs [87, 88]. When supplied with appropriate media and growth factors, MSCs are capable of differentiating into osteoblasts, chondrocytes, adipocytes, and myoblasts [89] (Fig. 3).

Human MSCs (hMSCs) were shown to express a number of Wnt ligands, receptors and regulators, and both β-catenin dependent and independent Wnt pathways are believed to play a crucial role in their capacities of self-renewal, proliferation and differentiation [90, 91]. Below, we will discuss the role of the Wnt pathway in each of the established MSC derived cell types.

a. Adipose tissue

The origination of a new adipocyte, starting from a mesenchymal stem cell, involves a temporally regulated cascade of transcription factor activities and gene expression events. During determination, multipotent MSCs become pre-adipocytes and hereby commit to the adipogenic lineage. A mature fat cell is formed when pre-adipocytes differentiate further. Early in the differentiation process, expression of CCAAT/enhancer binding protein (C/EBP) β and δ is induced and subsequently, these transcription factors bind to the promoters of peroxisome proliferator activated receptor γ (PPARγ) and C/EBPα [92]. When activated, PPARγ and C/EBPα induce expression of genes essential for the adipogenic phenotype [93].

The observation that in vitro induction of Wnt1 expression in pre-adipocytes led to inhibition of adipogenesis in the presence of lipogenic medium was the first link between the Wnt pathway and MSC fate [94]. Inhibition of the Wnt pathway, on the other hand, led to adipogenesis and the ligand responsible was shown to be Wnt10b [95]. A FABP4-*Wnt10b* mouse model was later developed, in which *Wnt10b* is expressed after the fatty acid-binding protein-4 (FABP4) promoter, and presented with a reduced amount of both white and brown adipose tissue [96]. In mouse myoblasts, overexpression of *Wnt10b* similarly resulted in inhibition of adipogenic gene expression [97].

In humans, we previously reported a genetic association between *WNT10B* polymorphisms and BMI and weight, further indicating the importance of WNT10B in the formation of fat tissue [98].

It has been shown that in hMSCs, transferred to a lipogenic medium, expression of WNT2, WNT10B, WNT13 and WNT14 decreases, whereas expression of WNT/β-catenin independent WNT4 and WNT11 increases [99]. On the contrary, when activating the WNT/

β-catenin pathway by addition of SB-216763, a highly selective small molecule inhibitor of GSK-3β, induction of adipocyte signature genes LPL, adipsin and PPARγ were blocked and expression of *WNT4* and *WNT11* decreased. This led the authors to conclude that the canonical WNT/β-catenin pathway, activated by *WNT2, WNT10B, WNT13* and *WNT14* inhibits adipogenesis, whereas WNT/β-catenin independent *WNT4* and *WNT11* promote adipogenesis [99].

Not only Wnt ligands, but also secreted regulators of the Wnt pathway, such as DKK and sFRP5, have been linked to human obesity.

DKK gene expression is transiently induced during differentiation of human adipocytes. In vitro studies showed that activating LRP5 mutations, resulting in a decreased affinity for DKK, inhibit adipogenic differentiation of hMSCs [100, 101]. This indicates that DKK promotes human adipogenesis by inhibiting LRP5/6 mediated Wnt signaling [102].

sFRP5 is another extracellular regulator of the Wnt pathway, that has been shown to be expressed in the cytosol of mature adipocytes and to promote preadipocyte differentiation [103]. Plasma sFRP5 levels shown to be differentially expressed in obese compared to lean subjects [104, 105]. sFRP4, a related secreted Wnt inhibitor, was up-regulated with adipogenesis of hMSCs [106]

As discussed previously, in the canonical Wnt pathway cytosolic β-catenin accumulation leads to activation of a TCF/LEF transcription complex. It has been shown that this β-catenin-TCF/LEF complex acts as a direct regulator of chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII). COUP-TFII then recruits the silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT) corepressor complex, which is able to maintain the PPARγ-containing chromatin in an hypoacetylated and repressed state, thereby inhibiting differentiation of pre-adipocytes to mature fat cells [94, 95, 107]. However, activation of the β-catenin independent CaMKII-TAK1-NLK pathway through Wnt5a action similarly results in repression of PPARγ and therefore impediment of adipogenesis. Wnt5a$^{+/-}$ mice present with a significant increase of adipocyte numbers in the bone marrow [108]. This shows that noncanonical Wnt signaling can also cooperate with β-catenin-TCF/LEF to block adipogenesis.

Brown adipose tissue (BAT) is present in very small quantities in humans compared to the lipid-storing white adipose tissue (WAT). Although brown adipocytes express most of the same genes as white adipocytes, they differ in the expression of uncoupling protein 1 (UCP1), which is exclusive for BAT. UCP1 allows dissipation of the electrochemical proton gradient generated by respiration in the form of heat [109]. In a therapeutic view, increasing the ratio of BAT vs. WAT could potentially increase fatty acid oxidation at the expense of the amount of fat stored.

In the past, it was presumed that BAT is of the same adipogenic lineage as WAT. However, recently it was discovered that BAT develops from a myogenic lineage [110]. Still, the main adipogenic transcription factors PPARγ and C/EBPα are induced during brown adipogenesis in an analogous fashion to white adipogenesis. As shown by the FAB4-*Wnt10b* mouse model, in which the development of BAT is completely blocked, Wnt signaling also represses the differentiation to brown adipocytes [93, 96].

b. Bone

Following the observation that Wnt10b is able to block the differentiation of MSCs to adipose tissue, researchers asked what would be the effect of this Wnt ligand on the differentiation to other mesenchymal lineages. To address this question, Bennett et al. analysed the bone characteristics of the FABP4-*Wnt10b* mouse model. Overexpression of Wnt10b in these mice results in skeletons with a higher bone mass, bone strength and bone mineral density (BMD) [111]. However, this was not the first indication that Wnt signaling affects bone formation. In 2001, inactivating mutations in the gene coding for the Wnt coreceptor LRP5 were shown to be causative for osteoporosis-pseudoglioma in human patients [112]. Correspondingly, gain-of-function mutations in *LRP5* impair the action of normal antagonists of the Wnt pathway such as Dkk1 and increase Wnt signaling, which results in a high bone mass phenotype [113–115]. Similarly, loss of function mutations in the *SOST* gene, coding for Sclerostin, lead to sclerosteosis characterized by high bone mass [116–118]. *SOST* is expressed almost exclusively in osteocytes, and the resulting Sclerostin protein is a circulating inhibitor of the Wnt pathway which acts to inhibit LRP co-receptor function [48]. A SOST neutralizing antibody is currently being tested in phase 3 clinical trials and shows great promise as a therapeutic agent for conditions such as osteoporosis, by enhancing bone formation [119]. Finally, loss of Wnt antagonists such as sfrp1, Dkk, Apc or Axin2 results in increased bone mass [120–123].

WNTs are expressed in marrow and appear to be endogeneous regulators of bone formation [90, 124]. In a previously performed genetic association study, we confirmed the importance of WNT10B in bone formation in humans by showing an association between WNT10B polymorphisms and BMD in a population of Danish males [125]. The mechanisms whereby WNT10B induces osteoblastogenesis include C/EBPα and PPARγ suppression in addition to induction of the expression of osteoblastogenic transcription factors such as Runx2, Dlx5 and Osterix

[111, 126–128]. Runt-related transcription factor 2 (Runx2) is the major transcription factor regulating the differentiation of MSCs to osteoblasts, as indicated by mouse studies [129]. Expression of Runx2, Dlx5 and Osterix may in fact be regulated through enhancer regions consisting of adjacent Smad and TCF/LEF1 DNA-binding sites, indicating a cooperative action of both the canonical Wnt and Bone Morphogenic Protein (BMP) pathways [130].

In addition, noncanonical Wnt signaling appears to enhance bone formation of hMSCs. Wnt5a binding to the cell surface leads to activation and homodimerization of Ror2 receptors, which leads to osteogenic differentiation by stimulation of Runx and Osterix expression [131]. Furthermore, CaMKII-TAK1-NLK signaling was shown to repress PPARγ transactivation and induce Runx expression in bone marrow MSCs [132].

In contrast, other studies indicated that high Wnt pathway activation in Wnt1-expressing hMSCs led to reduction of Runx2, Dlx5 and Osterix expression thereby inhibiting osteogenic differentiation. These two seemingly opposite roles of Wnt signaling in osteogenesis can however be combined in a 'Wnt gradient'-theory, as postulated by Liu and coworkers [91]. This theory suggests the presence of a Wnt activity gradient, resulting from the asymmetrical localization of Wnts and antagonists, between hMSC stem/progenitor and osteoblasts/osteocyt compartments. A high level of Wnt activity would permit the expansion of hMSCs, whereas lower Wnt signaling results in the differentiation to bone forming osteoblasts [91].

In contrast, results refuting proliferative and differentiation effects of Wnts in hMSCs may be attributed to the specific Wnt ligands, mode of overexpression, method to measure bone density and cell types used in different studies. In addition, increasing evidence suggests that Wnt signaling effects depend on the stage of differentiation of the cells used. For example, proper osteoblast terminal differentiation appears to require canonical Wnt pathway inhibition by Dkk2, sfrp2 and/or Wif1 [133, 134], instead of Wnt pathway activation.

As shown in human as well as murine in vitro osteoblast cell models, R-spondin1 synergizes with Wnt3a to promote osteoblast differentiation [135, 136]. All four members (RSpo1-4) of the R-spondin family antagonize Dkk and thereby amplify Wnt signaling. R-spondins require Wnt ligands and LRP6 for activity and interfere with DKK1 mediated LRP6 internalisation [137, 138]. In human osteoblasts, isolated from osteoarthritis patients, Rspo2 can counteract the high Dkk activity (and therefore low Wnt signaling) that is seen in osteoarthritic cells. Rspo2 was shown to increase Wnt signaling in the presence, but not in the absence of Wnt3a [139]. R-spondins

therefore provide another link between Wnt signaling and bone formation.

c. Cartilage

Chondrogenesis of human MSCs proceeds in different stages. The first stage is characterized by cell condensation (i.e., increased cell density due to cell aggregation), the second stage by proliferation followed by chondrogenic differentiation (i.e., synthesis of cartilage-specific extracellular matrix proteins, such as type II collagen), and the final stage by hypertrophy and acquisition of a chondrocyte morphology. SOX9 is a high-mobility-group (HMG) domain transcription factor that is expressed in chondrocytes and is required at sequential steps in this pathway [140].

Adding Wnt1 to hMSCs in vitro culture led to sustained Wnt/β-catenin signaling and suppressed expression of SOX9. Not only does Wnt/β-catenin signaling favor osteoblastogenesis of MSCs, it appears that inactivation of the pathway is necessary for chondrogenesis and the maturation and maintenance of chondroid cells [141].

d. Muscle

In addition to regulating the bone-fat interplay, Wnt signaling was also shown to be important for muscle development and maintenance of skeletal muscle homeostasis in the adult. Also, a role for the Wnt signaling pathway in muscle regeneration after injury by enhancing satellite cell proliferation and differentiation, was found.

Both canonical and noncanonical pathways perform a role, whereby canonical signaling appears to induce differentiation of both satellite stem cells and muscle cells, and noncanonical signaling serves to prevent depletion of the satellite stem cell pool (reviewed in [142]). β-catenin induced Wnt signaling in rat MSCs resulted in the induction of several pro-myogenic factors like Pax 7, MyoD and myogenin, and the simultaneous inhibition of C/EBPα and PPARγ expression [143]. Exogenous induction of canonical Wnt signaling through Wnt3a during the early phase of regeneration resulted in premature differentiation of progenitor cells, thereby leading to depletion of the satellite cell pool [144]. In mouse myoblasts, overexpression of canonical Wnt10b suppressed C/EBPα and PPARγ expression. Consistent with these results, *Wnt10b*$^{-/-}$ mice show lipid accumulation in regenerating muscle but in addition, muscle and isolated myoblasts from these mice undergo myogenic differentiation more efficiently. The authors suggested that Wnt7b, a more myogenic Wnt ligand, may be compensating for Wnt10b deficiency [97].

As for the β-catenin independent pathways, Wnt7a was shown to bind Fz7 and promote expansion of satellite stem cells, by activating the Wnt PCP pathway [145]. Additionally, in differentiated muscle cells, Wnt7a can activate PI3K and subsequently the Akt/mTor growth

pathway. This way, Wnt7a appears to couple the expansion of the satellite cell pool to the mass of the muscle tissue [146].

Hematopoetic Stem Cells

Hematopoetic stem cell (HSC) give rise to all lineages of blood cells, including red blood cells, platelets, lymphocytes, monocytes and macrophages. Wnt signaling has emerged as an important facilitator of HSC fate decisions during differentiation in the bone marrow. HSCs themselves as well as the bone marrow environment can provide Wnt ligands. Conditioned medium containing WNTs promotes the multiplication of human HSCs and inhibits their differentiation, implying that Wnt ligands function as hematopoietic growth factors [147]. In addition, overexpression of activated β-catenin was shown to expand the HSCs in long-term cultures and triggered increased self-renewal [148].

Later studies however indicated that both canonical and noncanonical Wnt signaling in the HSC niche can limit proliferation and are vital for HSC quiescence, which is a critical feature of their reconstituting function [149, 150]. Also, noncanonical Wnt4 ligand regulates HSCs and was shown to activate genes required for cell maintenance in mice [151].

Intestinal Stem Cells

The observation that the deletion of β-catenin or ectopic expression of Dkk in mice resulted in the complete ablation of intestinal crypts, indicated that Wnt signaling was also important for the maintenance of adult intestine [152, 153]. Furthermore, it is known that deregulation of the Wnt pathway, by mutations in APC, Axin or β-catenin, occurs in over 90 % of human colorectal cancers [154].

The epithelium of the small intestine is folded into large numbers of villi and crypts, thus maximizing the surface to allow nutrient uptake. The colon epithelium is also folded into crypts, but the surface does not carry villi. Intestinal stem cells (ISCs) are located at the crypt base and produce progenitor cells that are capable of differentiating towards all epithelial lineages. The proliferating crypt precursors and differentiated villus cells form an adjoining sheet that is in permanent upward motion. These cells keep migrating to the tops of the villi, where they eventually undergo apoptosis and are shed into the lumen [155]. Evidence indicates that the Wnt cascade is the dominant force in controlling cell fate along the crypt-villus axis [154]. A Wnt gradient is established along this axis, whereby β-catenin signaling is highest in stem cells at the base of the crypt and lowest at the villi [156, 157]. In the crypts of the colon, loss of transcription factor TCF4 leads to depletion of stem cells [158]. The Wnt gradient is inversely proportional with a BMP gradient, whereby high Wnt and low BMP signaling results in proliferation of progenitor cells and low Wnt and high BMP signaling allows cells to differentiate [157].

Marker expression studies revealed that ISCs can be identified through their expression of leucine-rich repeat containing, G-protein–coupled receptor 5 (Lgr5) [159]. However, the ligands for Lgr receptors 4, 5 and 6, which represent a subfamily of Lgr receptors, were unknown for a long time. Conditional deletion of Lgr4 and 5 in mice led to the disappearance of intestinal crypts and microarray analysis indicated differential expression of Wnt pathway target genes [41]. Lgr 4, 5 and 6 appeared to be the receptors for R-spondins, molecules that had previously been identified as Wnt pathway agonists [138, 160]. Lgr5 receptors thus are ISC-markers and interact with R-spondins to amplify Wnt β-catenin signaling, allowing proliferation and preservation of stem cell properties.

Paneth cells are located at the base of the crypt, intermingled with intestinal stem cells and thus reside in a high Wnt signal-zone. Rather than proliferating, these post mitotic cells are the only ones in the crypt that use Wnt signals for their terminal differentiation [161, 162]. In vitro culture experiments showed that maintenance of the intestinal stem cell phenotype is dependent on Paneth cells presence. In fact, Paneth cells constitute part of the ISC niche and produce essential stem cell growth factors EGF and Wnt3. ICSs that lose contact with Paneth cells lose multipotency and start differentiation and migration towards the villi [163].

Gastric Stem Cells

Lineage tracing in neonatal mice revealed that cells from all parts of the stomach epithelium originate from Lgr5-expressing cells, as do epithelium cells in the small intestine [164]. However, in adults, Lgr5 cells only occur in the pylorus, the most distal segment of the stomach. Similar to the crypt in the small intestine, the regenerative unit of the stomach resides in an invagination, which is divided in pit, isthmus and gland from top to bottom. It was postulated that each of these gastric units contains more than one multipotent adult stem cell [165]. Gastric stem cells (GSCs) seem to reside at the gland bottom, and push their differentiating progeny up toward the gland. GSCs likewise depend on the presence of a Wnt ligand, and this ligand appears to be Wnt3a [164].

Hair Follicle Stem Cells

Canonical Wnt signals do not appear to regulate epidermal differentiation, but are essential in the normal skin to instruct bulge stem cells toward the hair cell fate [82, 166]. Living human bulge stem cells (or hair follicle stem cells, HFSC) have been isolated in the past, starting from hair follicle

suspensions [167]. These stem cells were shown to reside in a niche, were they respond to a variety of signaling ligands that regulate hair follicle development, maintenance, and the regeneration of hair follicles after wounding. In the mature skin, hair follicles undergo repeated cycles of growth (anagen), death/regression (catagen), and rest (telogen), and in each of these phases, Wnt/β-catenin was shown to play a role. Wnt signaling is up-regulated at the end of telogen and hereby promotes transition to anagen. Indeed, Wnt signaling provides one of the mitogenic stimuli that are necessary for hair growth [168]. During the telogen phase, Wnt upregulation, and subsequent anagen, is repressed by BMP signaling [166]. In addition, telogen follicular bulge cells express secreted Wnt inhibitors such as Sfrp1, Wif1 and Dkk3, both in mice and humans [166, 167, 169].

At different stages of the hair cycle, Lgr5 expression was observed in several distinct locations in the hair follicle. Here, Lgr5+ progeny repopulate stem cell compartments, supporting the existence of a stem or progenitor cell hierarchy [170]. Like the intestine and the stomach, the hair follicle contains rapidly proliferating and unidirectionally migrating epithelial cells, and it was therefore no surprise that hair follicle stem cells could be identified by the presence of Lgr5 on their membrane as well.

Melanocyte stem cells reside adjacent to the bulge stem cells and are activated to differentiate to melanocytes by Wnt10b, expressed by nearby hair germ keratinocytes [166]. Again, as in other adult tissues containing stem cell niches, multiple signaling pathways work together to regulate differentiation, proliferation and normal maintenance of tissue stem cells and in this dynamic network, the Wnt pathway plays a prominent role.

Mammary Stem Cells

Transplantation assays in mice have demonstrated the existence of a rare population of mammary stem cells (MaSCs) that are able to re-form a functional mammary gland [171, 172]. As shown by in vitro and in vivo experiments, Wnt signals serve as important self-renewing signals for MaSCs. In the mammary stem cell niche, Wnt signaling supposedly acts to inhibit differentiation by suppression of Gata-3, a transcription factor that promotes differentiation of mammary stem cells into luminal cells [173]. For a normal mammary gland to develop, both canonical Wnt signaling co-receptors Lrp5 and Lrp6 are required [174, 175]. In addition, loss of Lrp5 in MMTV-Wnt1 transgenic mice reduces both the early proliferation of the progenitor cell population and the subsequent formation of mammary tumors [174]. Together, these results demonstrate that Wnt signaling (through Lrp5/6) is an important component of normal mammary stem cell function.

Neural Stem Cells

When looking at mouse models in which Wnt ligands (Wnt1, Wnt3a) are mutated, it is clear that Wnt signaling has an important role to play in the proper development of telencephalon, metencephalon and hippocampus [61, 176–178]. Also in the adult neuronal tissue, it appears that sustained Wnt signaling is necessary to regulate neural stem cell (NSC) activity and maintenance [177, 179].

Several components of the canonical Wnt pathway (Wnt ligands, Fz receptors and Wnt pathway inhibitors) have been described in the adult mice neurogenic niche, consisting of the subventricular zone (SVZ) of the lateral ventricle in the cortex and subgranular zone (SGZ) in the hippocampus [177, 180].

When Wnt/β-catenin signaling is inhibited in adult neural progenitor cells (aNPCs) co-cultured with hippocampal astrocytes, by the WNT inhibitor sFRP2/3 or ectopic expression of dominant negative Lef1, the percentage of neuronal lineage cells decreases, indicating that β-catenin-dependent Wnt signaling is a principal positive regulator of neurogenesis in the adult hippocampus [177]. Investigations on the role of β-catenin signaling in SVZ neurogenesis demonstrated a mitogenic effect of Wnt signaling in mice aNPCs. Activation of β-catenin by Wnt3a, Wnt5a, or Wnt7a [181, 182] or the ectopic expression of a stable β-catenin form increased the proliferation of neural progenitors in vitro, whereas blockade of the canonical Wnt pathway by a Wnt antagonist or ectopic expression of Axin had an opposite effect [181].

It has been shown that Wnt3a signals through the Ryk receptor-like tyrosine kinase in developing neurons. The Ryk receptor is another single-pass Wnt receptor, characterized by an unusually short intracellular region and unique substitutions in its catalytic tyrosine kinase domain. However, Ryk is still able to activate downstream pathways such as the Wnt pathway and has been shown to form a ternary complex with Fz and Wnt1 [183]. Extracellularly, Ryk contains a binding domain similar to the Wnt-inhibitory factor (WIF) proteins, a group of secreted Wnt binding factors [184]. Transgenic mice expressing anti-Ryk siRNA have defects in axon guidance and in addition, Ryk allows Wnt3a to induce neurite outgrowth in dorsal root ganglia explants, suggesting that Ryk is a functional and key receptor for Wnt in the nervous system [183].

Although most studies indicate that Wnt/β-catenin signaling promotes the expansion of the neural progenitor pool [181, 182, 185] and determination of neuronal fate [177, 182], conflicting results have been obtained as well [186]. This may be due to the context-dependent signaling ability of the Wnt pathway, whereby β-catenin can activate different and sometimes opposing genetic programs depending on cellular signaling history, availability of signaling partners, ect. [187].

In humans, polymorphisms in several genes from the pathway (WNT2 [188, 189], FZ3 [190, 191], TCF7L2 [192, 193], DKK4 [194] and APC [195]) have been associated with psychiatric disorders such as schizophrenia and autism confirming the important role of Wnt signaling in adult neuron activity and maintenance. In addition, recent studies indicate a role of Wnt signaling in synapse maintenance and plasticity [196], and link the increased expression of the Wnt antagonist Dkk1 in brains of Alzheimer patients and in Alzheimer transgenic mouse models, to the pathology of Alzheimer's disease [197, 198]. It is not clear whether loss-of-function of Wnt genes would affect proper neuronal maintenance and development in the human brain, and whether this could lead to a neuronal disorder, but it is definitely an interesting topic to investigate further.

Cancer Stem Cells

Emerging evidence has indicated a subpopulation of multipotent cells, termed cancer stem cells (CSC) within cancer tissue. CSCs behave as both stem cells and cancer cells, being able to self-renew and differentiate to cell types present in the tumor [199, 200]. Indeed, there is an overlap between the expression patterns of transcriptional regulators in human embryonic and cancer SCs. The shared transcriptome, including genes regulating stemness and pluripotency such as NANOG and OCT3/4, determines in part the differentiation status of a tumor and, in addition, tumor aggressiveness. Among the genes expressed both in ESCs and CSCs is the Fz7 receptor, an important Wnt receptor, known to activate both canonical and noncanonical Wnt signaling [201, 202].

Several of the signaling pathways that are active in normal adult stem cells (such as Wnt, Hedgehog, Notch) appear to be involved in regulation of CSC as well, and aberrant regulation of these same pathways leads to neoplastic proliferation and tumor formation [202, 203]. Many of the genes activated by the β-catenin/TCF transcription complex, including cyclin D1 [204] and c-myc [205], which play critical roles in cell growth, proliferation, and differentiation, are inappropriately activated in colon cancer. In addition, up-regulation and overexpression of DVL proteins has been reported in many cancers, including those of prostate, mesothelioma and lung (non-small cell) [206–208].

LaBarge and co-authors present a model whereby a malignant cell overtakes a normal adult stem cell niche, becoming the initial CSC that nurtures a tumor. The tumor microenvironment may then evolve, and impose CSC-like functions onto other cells. As in normal tissue, this pool of undifferentiated stem cells gives rise to less potent progenitors, which then produce the most specialized cells of a given tissue [203]. In this hierarchical model, only the small, self-renewing population of CSCs in the tumor is responsible for tumor initiation and growth maintenance.

CSC present a challenge for cancer therapeutic treatment, seeing that eradication of bulk tumor usually does not include the population of CSCs, leaving them at liberty to re-establish the complete heterogeneity of cancer tissue. This may be due in part by the fact that CSCs, like normal adult stem cells, express multidrug resistance genes such as MDR-1 and BRCP1, making them resistant to chemotherapy [209]. In addition, CSCs are often highly resistant to radiation therapy, owing to their ability to rapidly induce DNA repair mechanisms [209, 210]. Furthermore, CSCs appear to be particularly adept in stimulating angiogenesis, nurturing tumor development [209]. To circumvent these problems, alternative therapeutic methods of selectively targeting CSCs are currently in development, including antagonists of the Wnt/β-catenin pathway.

Future Perspectives

It is clear that Wnt signaling is a conserved, ubiquitously functioning pathway that acts in both embryonal and adult stages. In this review we discussed the specifics of the pathway in stem cells residing in several main tissues and we tried to focus on the knowledge in humans. Because of the fact that this pathway exerts great control over stem cell behavior, it is inevitable that deregulation results in grave cell and tissue malfunctioning such as cancer. Further knowledge on the pathway and on control points herein have definite therapeutic potential, not in the least in the field of regenerative medicine where the ex vivo fabrication of tissues, containing multiple cell types, or even complete organs, will possibly revolutionize medicine.

Autologous pluripotent stem cells themselves can be used for the ex vivo engineering of tissues, which can then be applied for regenerative purposes [88]. There is a clear immunological advantage of using autologous stem cells, and in addition, these cells are not burdened with ethical considerations.

A historically important source of adult stem cells is the bone marrow, but pluripotent bone marrow cells may be as rare as 1 in 10^7 to 10^8 [211]. A second possible source of multipotent cells is processed lipoaspirate cells from adipose tissue [212]. However, a common restriction for tissue engineering is the necessity to grow and expand stem cells in a culture. Protocols have been developed to allow long-term culture of human adult stem cells, and these include coculturing with other human or mammalian cell lines [213, 214], coating culture dishes with matrix components [215], reducing oxygen tension [216] and the use of exogenous scaffolds to guide the 3D organization of cells [217, 218], among others. However, because stem cells reside within their own, carefully organized niche in vivo, they will self-renew and proliferate most efficiently when grown in a similar

environment. Recently, Sato et al., developed self-organizing 3D intestinal epithelial organoids or "mini-guts" from a single Lgr5$^+$ ISC. The minimal, essential stem cell maintenance factor cocktail for these mini-guts consisted out of R-Spondin, epidermal growth factor (EGF) and Noggin [219]. Colon crypt culture requires an additional Wnt ligand for the maintenance of cells, once again showing the importance of this pathway in stem cell preservation. Epithelial colon-derived mini-guts were reintroduced into the colons of mice with chemical-induced mucosal lesions and regenerated epithelial patches indiscernible from surrounding recipient epithelium. These patches lasted for several months without changing histological appearance [219].

For the ex vivo development of such single or multiple cell-derived tissues, it is crucial to have knowledge about signaling molecules that are involved within the stem cell niche in vivo. As discussed in this review, Wnt ligands have an important role to play in the stem cell environment of multiple tissues and an increased understanding of the Wnt pathways will possibly lead to the development of therapeutic options for a range of diseases manifesting in both embryonic and adult stages.

Conflict of Interest The authors declare no conflict of interest.

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