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Wnt Signalling in Implantation, Decidualisation and Placental Differentiation – Review

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Abstract

The family of secreted Wingless ligands plays major roles in embryonic development, stem cell maintenance, differentiation and tissue homeostasis. Accumulating evidence suggests that the canonical Wnt pathway involving nuclear recruitment of β-catenin and activation of Wnt-dependent transcription factors is also critically involved in development and differentiation of the diverse reproductive tissues. Here, we summarise our present knowledge about expression, regulation and function of Wnt ligands and their frizzled receptors in murine and human endometrial and placental cell types. In mice, Wnt signalling promotes early trophoblast lineage development, blastocyst activation, implantation and chorion–allantois fusion. Moreover, different Wnt ligands play essential roles in the development of the murine uterine tract, in cycling endometrial cells and during decidualisation. In humans, estrogen-dependent endometrial cell proliferation, decidualisation, trophoblast attachment and invasion were shown to be controlled by the particular signalling pathway. Failures in Wnt signalling are associated with infertility, endometriosis, endometrial cancer and gestational diseases such as complete mole placentae and choriocarcinomas. However, our present knowledge is still scarce due to the complexity of the Wnt network involving numerous ligands, receptors and non-canonical pathways. Hence, much remains to be learned about the role of different Wnt signalling cascades in reproductive cell types and their changes under pathological conditions.

Keywords

Placenta; Trophoblast; Endometrium; Wnt

1. General role of Wnt signalling

A small number of signalling pathways are critically involved in the early development of complex, multi-cellular organisms controlling early axis formation, limb patterning and organogenesis. Such crucial and conserved signalling pathways include Hedgehog, transforming growth factor β(TGF-β)/bone morphogenetic protein (BMP), Notch and Wingless (Wnt) which are active from drosophila to human [1-3]. Wnt ligands are secreted, palmitoylated glycoproteins playing central roles in embryogenesis and tissue homeostasis of adult organisms [3,4]. Maintenance of stem cells and their differentiation processes are regulated by the particular factors [5-8]. Historically, it was shown that the murine proto-oncogene Int-1 shares the same origin with the drosophila segment polarity gene Wingless leading to the creation of the term Wnt (combination of Wg (Wingless) and Int) [9].

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Abnormal Wnt signalling is often associated with severe human diseases, including cancer, osteoporosis and other degenerative disorders [3,4,10].

Up to now, 19 Wnt ligands, 10 transmembrane, G-protein coupled frizzled receptors (FZD) and 2 low density lipoprotein receptor-related protein co-receptors (LRP-5 and -6) have been identified in mammals [11]. In the well studied, canonical Wnt pathway the signal is transduced by FZD–LRP heterodimeric receptors, regulating stability and nuclear recruitment of the transcriptional co-activator β-catenin. However, some Wnts also activate non-canonical, β-catenin-independent cascades such as the Wnt/Ca^{2+} and the Wnt/planar cell polarity (PCP) pathway [12,13]. Moreover, Wnt ligands can bind to receptor tyrosine kinases such as Ror and Ryk the latter playing a role in neuronal development [14]. Hence, Wnt signalling can be regarded as a highly organised network of different ligands, receptors and downstream effectors controlling complex cellular responses [15].

1.1. Canonical Wnt pathway

The central player in canonical Wnt signalling is β-catenin. In unstimulated cells β-catenin is mainly located to adherens junctions where it is critically involved in maintaining epithelial integrity by binding to E-cadherin and α-catenin. In the absence of Wnt ligands (off-state) excess, cytoplasmic β-catenin is complexed with APC (adenomatous polyposis coli) and Axin both facilitating the phosphorylation of the protein by casein kinase Iα (CKIα) and glycogen synthase kinase 3β (GSK-3β) (Fig. 1). This provokes degradation of β-catenin through the β-TrCP (β-transducin repeat-containing protein) mediated ubiquitin/proteasome pathway resulting in low cytosolic levels [16,17]. Binding of a Wnt ligand (on-state) to the cysteine-rich domain (CRD) of FZD promotes FZD–LRP heterodimerisation triggering a series of events that disrupt the Axin/APC/GSK-3β/CK1α destruction complex [12]. In detail, Wnt stimulation induces recruitment of Dishevelled (Dsh) to the FZD receptor forming a so called signalosome [18]. Moreover Axin, a key negative regulator of β-catenin stability, translocates to the cytoplasmatic tail of LRP catalysed by CK1γ- and GSK-3β-dependent phosphorylation of the FZD co-receptor [19,20]. Sequestration of a critical component (Axin) of the destruction complex and activation of Dsh finally result in impaired degradation and accumulation of β-catenin in the cytosol [21]. Active β-catenin then translocates into the nucleus where it functions as a transcriptional co-regulator [4,12]. It displaces transcriptional inhibitors of the Groucho protein family and histone deacetylases (HDACs) from the T cell-specific factors (TCFs)/lymphoid enhancer-binding factor 1 (LEF-1) and recruits histone acetylases, the Legless family docking proteins (Bcl9) and CBP/p300 thereby converting TFC/LEF-1 into transcriptional activators [12]. Axin, APC and other Wnt components can also enter the nucleus, thereby modulating nuclear trafficking and transcription [22]. For example, APC was suggested to play a critical role in the exchange of co-activator and co-repressor complexes at Wnt target genes [22]. These include genes involved in cell proliferation and migration such as c-myc, c-jun, cyclin D1, CD44, matrilysin, matrix metalloproteinases (MMPs) and urokinase plasminogen activator receptor (uPAR) as well as others summarised at the Wnt homepage (http://www.stanford.edu/~rnusse/wntwindow.html). In addition, TCF/β-catenin dependent expression of Axin-2, FZDs, TCF-1 and other Wnt pathway components was noticed indicating that feedback control is also a feature of Wnt signalling. In addition, the canonical Wnt pathway can be negatively affected by endogenous β-catenin inhibitors such as inhibitor of beta-catenin (ICAT) and Chibby or soluble inhibitors such as secreted frizzled-related protein (sFRPs) or members of the Dickkopf (Dkk) family [12]. The latter bind to LRP and induce receptor internalisation leading to downregulation of Wnt signalling [23].

1.2. Non-canonical Wnt pathways

Non-canonical Wnt pathways have been less well characterized due to the diverse receptors and downstream effectors involved. They do not operate through β-catenin and may even inhibit nuclear β-catenin activity [24]. In the Wnt/planar cell polarity (PCP) and the Wnt/Ca^{2+} pathway, stimulated by the non-canonical Wnt ligands Wnt5a or Wnt11, signalling is transduced upon FZD binding and Dsh activation without requiring LRP as a co-receptor [13]. Signalling through the Wnt/PCP pathway results in activation of the GTPases RhoA and Rac and their downstream targets ROCK (Rho-associated kinase) and JNK, respectively (Fig. 2). Originally identified in Drosophila, PCP was also shown to play a crucial role in developmental processes of higher organisms such as angiogenesis, bone morphogenesis or convergent extension movements of mesenchymal cells during gastrulation [25]. Interestingly, non-canonical Wnt ligands such as Wnt5a may also act in a canonical manner (upon binding to FZD4) suggesting that the effects of Wnt stimulation depend on the cellular receptor context [26].

The Wnt/Ca^{2+} pathway was discovered by the fact that stimulation with particular Wnt ligands leads to intracellular Ca^{2+} release from the ER [27,28]. Wnt-dependent increase in Ca^{2+} levels is achieved either through inhibition of cGMP-dependent protein kinase (PKG) which blocks Ca^{2+} release in unstimulated cells [29] or through activation of PLC (phospholipase C) and elevation of inositol 1,4,5-trisphosphate (IP_3). Intracellular Ca^{2+} in turn activates protein kinase C (PKC), calcium/calmodulin-dependent kinase II (CamKII) and calcineurin. CamKII activates TGF-β-activated kinase (TAK1) and Nemo-like kinase (NEMO) which can antagonize Wnt/β-catenin signalling by phosphorylation and inactivation of TCF [30]. The protein phosphatase calcineurin unmasks nuclear localization sequences of nuclear factor of activated T cells (NF-AT) allowing NF-AT to enter the nucleus and to activate gene expression [31]. Wnt/Ca^{2+}/NF-AT signalling, for example, was shown to control dorsoventral axis formation in Xenopus embryos [32].

Wnts can also signal through the receptor tyrosine kinase Ryk and the receptor tyrosine kinase-like orphan receptor (Ror) upon binding to their extracellular WIF and CRD domains, respectively [14]. Ryk activation provokes canonical, TCF/β-catenin-dependent signalling, activation of Src family kinases and, similar to Notch signalling, cleavage and nuclear recruitment of the Ryk intracellular domain [33]. Ryk was shown to play a critical role in neuronal development controlling axon guidance, neurite outgrowth and synaptogenesis [33]. Wnt5a-dependent Ror activation results in polarised cell movement involving Dsh and JNK [34]. Also, Ror-2-deficient mice display similar abnormalities as Wnt5a mutant mice reflecting changes in non-canonical Wnt pathways such as Wnt/PCP [34].

Moreover, ligands such as Wnt3a or Wnt16b may also activate other β-catenin-independent cascades such as ERK or PI3K/AKT signalling [35-37], again emphasizing the complexity of the Wnt signalling network.

2. Wnt signalling in murine reproduction

Implantation of the blastocyst, i.e. apposition, attachment and subsequent invasion of trophoblast into the uterine luminal epithelium, represents a complex biological process requiring cross-talk between the fetal and maternal tissues. Most important for implantation is synchronisation of blastocyst activation with uterine receptivity, the latter being controlled by ovarian steroid hormones [38]. In mice, estrogen is required for proliferation and differentiation of the uterine luminal and glandular epithelia whereas the coordinated action of estrogen and progesterone promotes stromal cell differentiation. Factors governing blastocyst activation are still poorly understood, however, a range of signalling molecules

preparing the uterus for blastocyst implantation have been identified [39]. Besides the steroid hormones, cytokines such as LIF, the Hedhog morphogens and others were suggested to play critical roles in uterine receptivity of mice. Considering that Wnt signalling plays a pivotal role in embryonic development, it may not be surprising that the particular pathway has also been implicated in uterine growth as well as murine blastocyst activation, implantation and decidualisation [40].

2.1. Uterine development, pre-implantation and decidualisation

In vivo studies using different mouse models support the idea that Wnt signalling is crucially involved in uterine growth and development. Estrogen, for example, induces Wnt4, Wnt5a and FZD2 in the mouse uterus as well as nuclear recruitment of β-catenin in the endometrial epithelium [41]. Inhibition of canonical Wnt signalling trough adenovirus-mediated expression of sFRP2 inhibited estrogen-dependent activation of β-catenin and epithelial cell proliferation suggesting that steroid hormone-dependent uterine cell growth also involves Wnt signalling [41]. Moreover, estrogen was also shown to upregulate the β-catenin-dependent transcription factors TCF-3 and LEF-1 in an estrogen receptor-independent manner [42]. Interestingly, the hormone also provokes physical interaction of ERα with activated LEF-1/TCF-3 and recruits the latter to Wnt/estrogen-dependent target genes suggesting that cross-talk between estrogen and Wnts could be critical for endometrial function [42]. Moreover, progesterone was shown to downregulate GSK-3β in rat uteri, which was suggested as a prerequisite for estrogen-dependent activation of the canonical Wnt pathway [43].

Knock-out studies demonstrated that Wnt4- and Wnt5a-deficient mice display failures in the development of the female reproductive tract [44,45]. Mice lacking Wnt7a do not develop uterine glands and have disorganised uterine smooth muscles [46]. Interestingly, Wnt7a was also identified as a key regulator of female Müllerian duct development which evolves into oviduct, uterus and cervix [47]. In males, Wolffian ducts develop and Sertoli cells of the testes secrete Müllerian-inhibiting substance provoking Müllerian duct regression. In Wnt7a-deficient male mice, however, Müllerian ducts do not regress due to the absence of the receptor for Müllerian-inhibiting substance resulting in generation of pseudohermaphrodites [47]. Wnt7a knock-out mice also lose expression of HoxA10 and HoxA11 in the endometrial stroma, two genes which upon homozygous deletion in mice provoke failures in the decidualisation process resulting in infertility [46].

Canonical Wnt signalling was also shown to be critical for the development of Müllerian duct derivatives. Mice harbouring a conditional deletion of β-catenin in the Müllerian duct mesenchyme display abnormal uteri after birth and largely lack myometrial smooth muscles upon adulthood [48]. Finally, FZD4 knock-out mice were shown to be infertile since formation of the corpus luteum is impaired. Expression of luteal cell-specific genes such as sFRP4 and the LH/hCG receptor was found to be reduced in these mice [49].

With respect to reproduction different FZD receptors (FZD2, FZD4 and FZD6) and Wnt ligands (Wnt4, Wnt5a, Wnt7a, Wnt7b, Wnt11, Wnt16) were shown to be expressed in the mouse uterus before and around the time of implantation as well as during stromal cell differentiation [50]. Expression of most of these genes was highest at implantation (day 5 after fertilisation). Wnt4 and Wnt7b, for example, strongly increase in stromal cells around the implanting blastocyst and in the luminal epithelium, respectively, suggesting specific roles associated with the implantation process [50]. Indeed, Wnt4 has been identified as a critical gene controlling murine as well as human uterine decidualisation since siRNA-mediated silencing of its mRNA impaired the differentiation process [51]. Wnt4 is a target gene of the key regulator BMP2 which is induced upon progesterone treatment of uterine stromal cells [51].

Other Wnts and FZDs, however, also undergo dynamic changes upon implantation and decidualisation and were shown to be regulated by steroid hormones in ovariectomised mice [41,50]. Expression levels of Wnt7a, Wnt7b, Wnt11 and Wnt16 which are significantly higher in uterine implantations sites compared to non-implantation sites decrease upon stromal cell differentiation (days 6 and 7 after fertilisation) [50]. Moreover, non-canonical Wnt receptors such as Ror-2 are expressed in endometrial epithelial and stromal cells of non-pregnant mice [52]. During pregnancy stromal expression of Ror-2 increased and also appeared in uterine NK cells which may suggest a role of the receptor in implantation and regulation of trophoblast invasion, respectively [52].

2.2. Blastocyst development and implantation

Despite the facts that various Wnts, such as Wnt5a and Wnt11 [53] and FZDs are detectable in the pre-implanting embryo, different approaches demonstrated that canonical Wnt signalling is dispensable for blastocyst formation. Gene knock-out studies showed that mutant embryos lacking β-catenin develop into blastocysts [54]. However, maternal delivery of β-catenin might have compensated for lack of the zygotic protein during the pre-implantation period. Hence, mice harbouring a conditional deletion of β-catenin in their oocytes were also utilised demonstrating that lack of both maternal and zygotic β-catenin does not impair blastocyst development [55]. In addition, inhibition of the TCF/β-catenin complex through small molecular inhibitors or overexpression of Dkk1 did not negatively affect blastocyst formation [56]. Hence, it is likely that the diverse zygotic Wnts and FZDs expressed operate through non-canonical signalling such as the Ca^{2+}-dependent pathway which was shown to be necessary for pre-implantation development [40].

Whereas β-catenin might be dispensable during the pre-implantation period, blastocyst activation critically involves the canonical Wnt signalling pathway. Upon mating of mice with conditional deletion of β-catenin in oocytes with wildtype male fewer embryos develop as compared to matings with normal mice [55]. In agreement to that, blocking of canonical Wnt signalling through Dkk1 or small molecular inhibitors impairs blastocyst's competency to implant [56]. Along those lines, a shift from non-canonical signalling in the pre-implantation blastocyst towards canonical signalling in the trophectoderm of activated blastocyst could be demonstrated [56].

However, canonical Wnt signalling is not only required for blastocyst activation but also for successful implantation. Indeed, blastocyst attachment was shown to induce TCF/β-catenin-dependent signalling in circular smooth muscle cells of the myometrium and subsequently in the uterine epithelium at the site of implantation [57]. This might involve the blastocyst-derived Wnt7a which upon uterine intraluminal delivery also activates the pathway in the absence of a zygote [57]. Inhibition of the pathway upon administration of sFRP2 decreased the frequency of implantation [57].

2.3. Placental development and trophoblast lineage determination

Although activation of canonical Wnt signalling is not required for blastocyst development, different Wnt ligands could play a role in early trophoblast lineage determination through non-canonical pathways. Wnt3a, for example, was shown to promote trophectoderm formation in embryonic stem cells by inducing Cdx2, one of the critical transcription factors of early trophoblast development [58,59], in an LEF-1-dependent manner [60].

Several in vivo studies demonstrated that Wnt signalling plays a crucial role in extraembryonic development, particularly in placental vascularisation, chorion–allantois fusion and labyrinth function. Mice harbouring a homozygous deletion of R-spondin3, a soluble activator of the canonical Wnt pathway, die around E10 due to defects in the

labyrinth caused by failures in the interaction between chorion and allantois [61]. Similarly, mice with deletions of both TCF-1 and LEF-1 display severe defects in placenta formation due to absence of chorionic–allantois fusion [62]. FZD5 knock-out mice did not survive beyond E10 since their placentae were less vascularised [63]. Also, defects in yolk sac angiogenesis and reduced endothelial cell proliferation were noticed in these mice. Wnt2-deficient mice exhibit reduced birthweight and half of the pups die perinatally [64]. Again, labyrinths of Wnt2 mutant mice show different defects such as oedema, decreased numbers of capillaries and fibrinoid deposition. Deletion of Wnt7b results in embryonic death around midgestation due to placental abnormalities [65]. Chorionic trophoblasts lacking Wnt7b do not express α4 integrin a critical factor required for chorion–allantois fusion suggesting that the ligand is important for organisation and function of the chorionic cell layer.

Whereas Dkk1 was shown to inhibit implantation, its role in murine trophoblast cell invasion might be different. In co-cultivation experiments of ectoplacental cones with decidual cells recombinant Dkk1 promoted whereas Dkk1 antibodies and antisense oligonucleotides reduced invasiveness [66]. Since Dkk1 inhibited canonical Wnt signalling in the in vitro system, the pathway may act antagonistically in murine trophoblast cell invasion.

3. Wnt signalling in human reproductive tissues

Despite the fact that basic research with human material is limited to in vitro studies, accumulating evidence suggests that Wnt signalling could also play a role in endometrial and placental function and undergoes changes in endometrial and gestational diseases. Clearly, Wnt signalling has also been implicated in development and function of ovaries and their diverse cell types. Since this is out of scope if this particular review presented here, we would like to refer to other recent summaries on that issue [67,68].

3.1. Endometrium, decidualisation and endometrial diseases

Various investigators identified ligands as well as different Wnt signalling components in the endometrium suggesting that the pathway could be involved in the diverse biological roles of human uterine cell types. Wnt2, 3, 4, 5a, 7a, 8b and FZD1, 4, 6 and 10 mRNAs were detected in endometrial samples and endometrial epithelial/stromal cells using different molecular techniques [69,70]. Hormonal control of Wnt2, 3, 4, 5a upon estrogen or progesterone treatment in vitro could not be detected, however, lack of expression was noticed in different endometrial carcinoma cell lines [69]. Wnt7a, the regulator of HoxA10 and HoxA11 [71], was found to be exclusively expressed on luminal epithelial cells, whereas sFRP4, Dkk1 and FZD were detectable in uterine glands and/or stroma [70].

Microarray analyses aiming to analyse global gene expression during the menstrual cycle also revealed expression of mRNAs of the Wnt pathway as well as their potential regulation through steroid hormones. Dkk1 and Wnt10b mRNAs, for example, were shown to strongly increase between early and mid-luteal phases [72]. In contrast, sFRP1 and sFRP4 were found to be downregulated in endometrial samples taken at the time of the LH surge [73,74], suggesting that the decline of inhibitors of Wnt signalling could be important for human implantation and/or decidual differentiation. Indeed, sFRP4 could be involved in endometrial cell proliferation since elevated levels of the inhibitor were noticed in estrogen-dependent endometrial and breast cancer [75]. Similarly, sFRP1 expression was found to be associated with endometrial cell growth and elevated mRNA levels were detected in endometriotic tissues [76]. Since sFRP1 is known to induce angiogenesis, increased expression could be a critical factor in endometriotic cell proliferation [76]. Others, however, found a decrease of sFRP1 [77] and sFRP4 [78] in cultivated endometriotic stromal cells and endometrial carcinoma cells, respectively, and overexpression of sFRP4

decreased cancer cell proliferation in vitro [78]. Finally, treatment of women with the antiprogestin RU486 and subsequent DNA chip analyses revealed elevated expression of sFRP1, Wnt5a, FZD4, 6, 9, 10, β-catenin and Axin-2 [79]. Since RU486 rapidly provokes endometrial breakdown a role of the Wnt pathway during menstruation and/or endometrial repair has been suggested [79].

Whereas Wnt3 was found to be elevated in the proliferative endometrium, Dkk1 increased in the mid-secretory phase and upon in vitro decidualisation of endometrial stromal cells suggesting a possible role in endometrial differentiation and/or implantation [70,73]. Indeed, Dkk1 was shown to be specifically upregulated by progesterone in human endometrial stromal cells, whereas estrogen or cAMP treatment was not effective [80], and progesterone receptor knock-down decreased expression of the inhibitor [81]. Since progesterone-dependent induction of Dkk1 also inhibited Wnt signalling [82], it is likely that repression of the pathway plays a role in decidualisation. Along those lines, TGF-β1, which could play a role in menstruation, was shown to impair Dkk1 expression in decidualising endometrial stromal cells [83]. In addition, Dkk1 mRNA expression is lower in endometriotic fibroblasts compared to normal endometrial fibroblasts which might be associated with a persistent proliferative potential and impaired decidualisation capacity in endometriosis [77]. Moreover, elevated expression of Wnt7a, Wnt2 and FZD1 was observed in endometriotic tissues [84,85]. Abnormal activation of the Wnt pathway in the mid-secretory endometrium, such as persistent expression of activated β-catenin, may also occur in infertile patients with endometriosis [86].

Recent evidence suggests that estrogen-dependent proliferation may, at least partly, depend on activated Wnt signalling since the hormone was shown to induce Wnt pathway components in endometria of estrogen-treated women [82]. This would be in agreement with the fact that up to 30% of estrogen-associated cancers exhibit nuclear β-catenin expression, the hallmark of canonical Wnt signalling [81,87]. Accordingly, reduced levels of Dkk1 were noticed in endometrial carcinomas and treatment of the cells with recombinant Dkk1 inhibited invasiveness in vitro [88].

3.2. Placental function, trophoblast differentiation and gestational disorders

As mentioned above Wnt signalling seems to be required for blastocyst activation and implantation in mice. Similarly, several studies using different trophoblast cell models suggest that the pathway could also be critically involved in human trophoblast implantation and adhesion to maternal uterine tissues. Dkk1, for example, may negatively affect implantation/adhesion of trophoblast to the endometrium. Treatment of JAR spheroids with Dkk1 was shown to impair attachment to endometrial-like Ishikawa cells [89]. Incubation of primary decidualised endometrial stromal cells with trophoblast supernatants provoked downregulation of Wnt4 and FZD2 [90] suggesting a role of Wnt signalling in trophoblast-dependent modulation of the decidualisation process.

However, there is also evidence that Wnt signalling could be critically involved in differentiation of trophoblasts and contribute to malignant transformation of these cells. Transient loss of components of adherens junctions, i.e. membrane-bound β-catenin and E-cadherin [91], was noticed in the proximal invasion zone of anchoring villi. Recent studies performed in our laboratory revealed nuclear β-catenin expression in a considerable number of invasive trophoblasts in vivo as well as after in vitro differentiation from chorionic villous explant cultures [92]. Elevated numbers of β-catenin-positive nuclei were detectable in invasive trophoblasts of complete hydatidiform mole (CHM) placentae suggesting that abnormal activation of canonical Wnt signalling could play a role in the gestational disease [92].

In normal placentae the Wnt-dependent transcription factors TCF-3 and TCF-4 were predominantly expressed in extravillous trophoblasts (EVT), the latter being almost exclusively expressed in non-proliferating, p57/KIP2-positive trophoblasts [92]. Hence, TCF-4 could be an important transcription factor committing and differentiating the EVT phenotype. Upon stimulation with the recombinant Wnt ligand Wnt3a elevated migration and invasion of primary trophoblasts and the extravillous trophoblast cell line SGHPL-5 was noticed [92]. Moreover, the ligand was shown to increase trophoblast outgrowth from villous explant cultures and activated the canonical pathway as well as AKT in primary EVT and SGHPL-5 cells [93]. Dkk1 failed to inhibit AKT phosphorylation suggesting that the canonical LRP-5/6-FZD receptor is not involved in the activation of the particular kinase. In other cells AKT may induce canonical Wnt signalling by phosphorylation/inactivation of GSK-3β and subsequent accumulation of β-catenin [94]. However, this cross-talk may not exist in trophoblasts since chemical inhibition of AKT did neither affect nuclear abundance of β-catenin nor luciferase activity of a canonical Wnt reporter [93] which would be in agreement with a very recent report [95]. However, stimulation of both canonical Wnt signalling and AKT provoked Wnt-dependent secretion of MMP-2 which could be one of the critical Wnt targets promoting trophoblast invasion [93]. In addition, Dkk1 treatment of primary trophoblasts and SGHPL-5 cells not only abolished Wnt-induced cell motility but also reduced basal migration and invasion suggesting expression of endogenous Wnt ligands [92,93]. Previous descriptive analyses indeed suggested that 14 out of 19 Wnt ligands and 8 out of 10 FZD receptors are expressed in human placenta [96]. Of interest, gestation-dependent expression of Wnts and FZDs was noticed. Wnt1, Wnt7b, Wnt10a and Wnt10b were strongly expressed in first trimester trophoblasts but largely absent in term trophoblasts suggesting that these ligands could play a role in early placental function and differentiation [96]. Moreover, cell-specific distribution of Wnt ligands could also be observed. Wnt10 and Wnt10b were highly expressed in first trimester villous cytotrophoblasts but absent from differentiated EVT or the syncytium suggesting a role in trophoblast proliferation [96]. Primary EVT express canonical as well as non-canonical Wnts indicating that different Wnt signal transduction cascades may influence trophoblast invasion in an autocrine manner. Along those lines, promoters of genes encoding Wnt inhibitors such as sFRP2 were shown to be methylated in first trimester trophoblasts potentially leading to their reduced expression and activation of Wnt signalling [97]. APC and sFRP2 were found to be hypermethylated in choriocarcinomas suggesting that inactivation of negative regulators of Wnt signalling may contribute to trophoblast cancer cell progression [97,98]. JEG-3 and JAR choriocarcinoma cells also lack Dkk1 suggesting that downregulation of the inhibitor could also be involved in tumour formation [99]. Overexpression of Dkk1 in these cells induced apoptosis and growth arrest involving induction of JNK [99].

Wnts may also modulate other trophoblast processes such as phospholipid uptake and transport. StarD7, a member of the StAR1 lipid transfer proteins, was identified as direct target gene of TCF/β-catenin in trophoblasts [100]. Moreover, in addition to Wnts other ligands and receptors may contribute to β-catenin/TCF-dependent signalling in trophoblasts. Gene silencing of protease activated receptor-1, PAR1, provoked β-catenin destabilisation and reduced trophoblast motility [101].

4. Conclusions

In conclusion, Wnt signalling has been identified as an essential signalling pathway promoting murine uterine development, blastocyst activation, implantation, chorion–allantois fusion and early trophoblast development. In humans, Wnts promote decidualisation, endometrial function and trophoblast differentiation and changes in Wnt signalling components were noticed in cancers of reproductive tissues, in endometriosis and in gestational diseases (summarised in Fig. 3). Despite the fact that activity of the canonical

Wnt pathway has been identified in human reproductive cell types many open questions remain. With few exceptions individual functions of different Wnts and FZDs in endometrial and placental differentiation have not been elucidated. Specific Wnt–FZD interactions and their downstream targets are largely unknown. Also, the properties of cell-restricted Wnts and of Wnt-dependent transcription factors, such as TCF-4, during placental differentiation remain elusive. Moreover, non-canonical Wnt signalling pathways and their roles in the cycling endometrium, in decidualisation and in trophoblast differentiation have not been investigated so far. Hence, given the importance of the pathway in development and disease Wnt signalling in reproductive tissues remains an interesting research area in the future.

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Abbreviations

APC	adenomatosis polyposis coli protein
Bcl9	similar to B-cell lymphoma 9 protein
BMP	bone morphogenetic protein
β-TrCP	β-transducin repeat-containing protein
CamKII	calcium/calmodulin-dependent protein kinase II
CBP	CREB-binding protein
CDC42	cell division cycle 42
Cer	cerberus
CKI	casein kinase I
CRD	cysteine-rich domain
Daam1	dishevelled associated activator of morphogenesis 1
Dkk	Dickkopf
Dsh	Dishevelled
ER	estrogen receptor
EVT	extravillous trophoblast
FZD	frizzled
ERK	extracellular regulated kinase
GSK-3β	glycogen synthase kinase 3 beta
hCG	human chorionic gonadotrophin
HDAC	histone deacetylases
IP_3	inositol 1,4,5-trisphosphate
Jnk	c-Jun N-terminal kinase
LIF	leukemia inhibitory factor
LRP	low density lipoprotein receptor-related protein

LH	luteinizing hormone
MMP	matrix metalloproteinase
NF-AT	nuclear factor of activated T cells
NLK	Nemo-like kinase
PCP	planar cell polarity
PKC	protein kinase C
PDE	phosphodiesterase
PI3K	phosphoinositid-3-kinase
PKG	cGMP-dependent protein kinase
PLC	phospholipase C
Rac	Ras-related C3 botulinum toxin substrate
RhoA	Ras homolog gene family member A
Rock	Rho-associated, coiled-coil containing protein kinase
sFRP	secreted frizzled-related protein
TAK1	TGF-β-activated kinase-1
TCF/LEF	T cell-specific factor/lymphoid enhancer-binding factor
TGF-β	transforming growth factor β
uPAR	urokinase plasminogen activator receptor
WIF	Wnt inhibitory factor
Wnt	wingless

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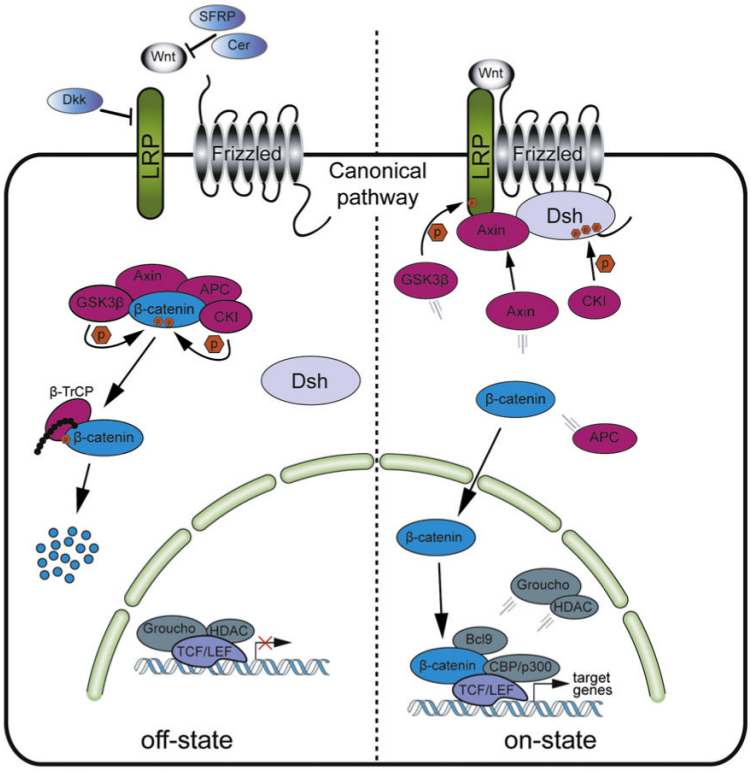

Fig. 1.

The canonical Wnt/β-catenin pathway. **Off-state**; in the absence of a Wnt ligand, β-catenin is bound in a multiprotein degradation complex containing the scaffold protein Axin, the tumour suppressor gene product APC, as well as the kinases CKI and GSK-3β, among others. Upon phosphorylation, β-catenin is ubiquitinated by the β-TrCP–E3-ligase complex and subsequently degraded by the proteasomes. **On-state**; Wnt ligand associates with FZD and LRP-5/6 co-receptors. This leads to the translocation of Axin to the plasma membrane through direct interaction with LRP-5/6 and Dsh/FZD. β-catenin is released from the multiprotein complex, accumulates in the cytoplasm in a non-phosphorylated form, and subsequently translocates into the nucleus where it promotes transcription of Wnt target genes upon binding to TCF/LEF.

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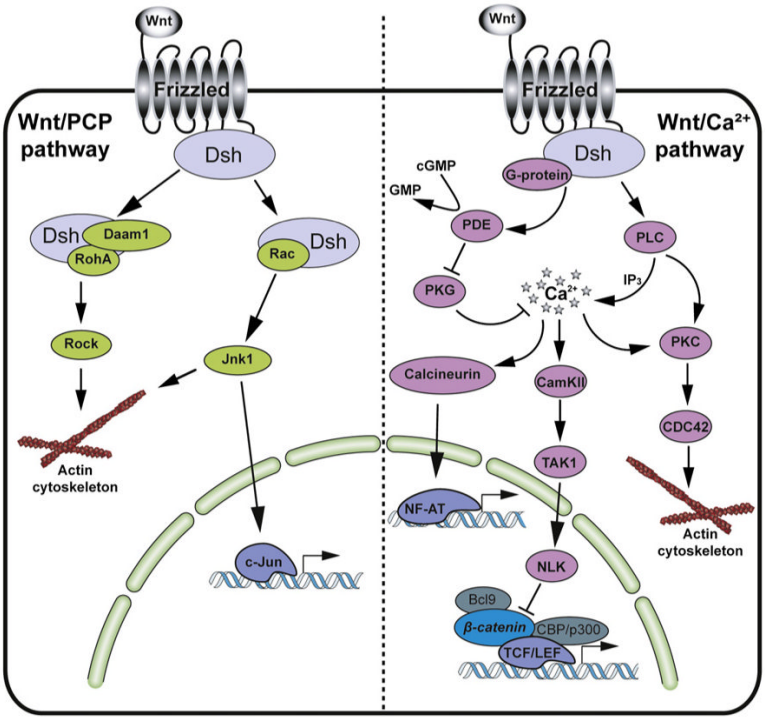

Fig. 2.

Non-canonical Wnt pathways. **Wnt/PCP pathway**; the Wnt/PCP pathway is characterized by asymmetric distribution of FZD receptors resulting in cell polarity and the activation of RhoA/Rock GTPases and JNK through Dsh and DAAM1. **Wnt/Ca^{2+} pathway**; activation of the Wnt-calcium pathway by interaction of Wnts with Frizzled receptors increases the intracellular Ca^{2+} level which subsequently activates calcineurin, CAMKII and PKC.

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Placenta	References
Descriptive data	
- Transient loss of membranous E-cadherin/β-catenin during EVT differentiation	[91]
- Cell specific expression and gestational changes of different Wnt ligands and FZD receptors in trophoblasts	[96]
- Specific expression of TCF-4 and nuclear β-catenin in EVTs	[92]
Functional data	
- Canonical and non-canonical Wnt-dependent regulation of trophoblast migration and invasion	[92, 93, 101]
Pathologies	
- Elevated nuclear β-catenin expression in CHM	[92]
- Hypermethylation and downregulation of Wnt antagonists in choriocarcinoma cells	[97-99]
Endometrium	
Descriptive data	
- Menstrual cycle-dependent and cell type-specific expression of Wnt ligands and FZD receptors in the endometrium	[69-74, 76-79, 84, 85]
Functional data	
- Estrogen-dependent regulation of Wnt signaling in proliferative endometrial cells	[75]
- Progesterone/TGFβ-dependent Dkk1 expression in endometrial stromal cells	[80, 83]
- Antagonistic function of progesterone-dependent Dkk1 expression in decidualisation	[82]
Pathologies	
- Abnormal production of Wnt ligands and antagonists in endometriosis	[76-78, 84-86]
- Frequent accumulation of nuclear β-catenin and decreased Dkk1 in endometrial cancer	[81, 87]
- Aberrant expression of Wnt-ligands in endometrial carcinoma cells	[75, 69, 81, 87]

Fig. 3.

Wnt signalling in human endometrium and placenta under normal and pathological conditions. A summary on descriptive analyses and functional studies performed in endometrial and trophoblast cells is shown.