

# Wnt/Planar Cell Polarity Signaling: New Opportunities for Cancer Treatment

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1	Wnt/Planar Cell Polarity signaling:
2	new opportunities for cancer treatment
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#### 21 Abstract

22

Cancer cells are addicted to a large spectrum of extracellular cues implicated in the initiation, 23 stem cell renewal, tumor growth, dissemination in the body, and resistance to treatment. 24 25 Among these factors, Wnt ligands and their associated signaling cascades contribute to most of these processes, paving the way for opportunities in therapeutic development. The 26 developmental Wnt/Planar Cell Polarity pathway is the most recently described branch of 27 28 Wnt signaling for having a strong implication in cancer development at early and late stages. We will depict here some of the latest knowledge accumulated on this pathway and the 29 pending questions, present the most convincing findings about its role in cancer and review 30 the most promising strategies currently designed to target its components. 31

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#### 34 Not one but several Wnt signaling pathways

Among the developmental signaling pathways defective in cancer (Hedgehog, Notch, Hippo, 35 Wnt), it recently became clear that Wnt signaling plays a pivotal role in all steps of the 36 disease, from tumor initiation to cancer growth and dissemination. During the past three 37 decades since the discovery of the first Wnt ligand and its implication in cancer (**BOX 1**) [1, 38 2], a large number of genetic, functional and translational studies have greatly improved our 39 knowledge on this pathway. In particular, many important Wnt signaling components have 40 been identified and functionally characterized including a network of 19 Wnt ligands, 10 41 membrane Frizzled receptors (Frizzled) or co-receptors (LRP5/6,...) that trigger intracellular 42 43 cascades with multiple outputs [3-5] (Figure 1A). Part of the challenge lies in the complexity of the pathway in which multiple combinatory interactions are generated by the intervening 44 components at the cell surface and inside the cells. Painting a simplistic picture of the 45 46 pathway, Wnt signaling is classically divided in two branches whose delineation relies on differential requirement for  $\beta$ -catenin, an armadillo-repeat cytoplasmic adaptor protein with 47 membrane and nuclear functions. For many years,  $Wnt/\beta$ -catenin signaling (also referred as 48 canonical Wnt signaling) has been the most extensively characterized pathway at the 49 molecular level and in human diseases. This has led to major discoveries about its mode of 50 action and its prominent implication in cancer (for review, see [6]). The  $\beta$ -catenin-51 independent Wnt pathways (hereafter named non-canonical pathways) utilize a different panel 52 of Wnts and (co)receptors compared to the canonical pathway although some Wnt 53 components such as Dishevelled are shared by both pathways. However, depending on the 54 cellular context and expression of particular co-receptors, a given Wnt can activate either β-55 catenin-dependent or β-catenin-independent Wnt signaling. The non-canonical Wnt pathway 56 is often seen as subdivided in two branches, one using intracellular calcium as a second 57 messenger (Wnt/Ca<sup>2+</sup>) and the other one defined by a conserved genetic pathway (Wnt/Planar 58

Cell Polarity or PCP) (Figure 1). However, as discussed below, one can envision these two branches as a single one on the basis of biochemical arguments. Recent reviews have depicted canonical and Wnt/Ca2+ pathways in great details (see for example [3]). In this review, we will focus on the Wnt/PCP pathway whose contribution in many cancers has been recently uncovered.

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#### 65 The evolutionary conserved Planar Cell Polarity

In 1982, Gubb and Garcia-Bellido studied mutants of the fruit fly (Drosophila melanogaster) 66 harboring defects in the orientation of hairs and sensory bristles on the wings, describing for 67 68 the first time what is now routinely called PCP defects [7]. PCP refers to the organization of the epithelium orthogonal to the apico-basal polarity axis which orients epithelial cells 69 attached to extracellular matrices. Genetic studies in flies further identified a group of genes, 70 71 so-called core PCP genes, responsible for the establishment and maintenance of PCP in wings and ommatidia [8, 9]. The encoded molecules are evolutionary conserved and are members of 72 73 diverse family of receptors (Flamingo, Frizzled, Vang-Gogh-like, Ptk7, Ryk, Ror2, Daschous, Fat) and cytoplasmic adaptors (Dishevelled, Prickle, Diego). Asymmetric distribution of PCP 74 components in polarized cells, - Vangl-like, Prickle and Diego localize at the anterior sides of 75 the plasma membrane and Frizzled, Dishevelled and Dachsous at the posterior sides of the 76 77 plasma membrane -, is inherent to PCP functions, and is achieved through diverse and not completely understood molecular mechanisms [10]. PCP components are highly conserved 78 throughout evolution, from drosophila to humans, albeit one fruit fly gene has usually several 79 paralogs in vertebrates. For example, the drosophila Vang-Gogh and Prickle1 genes have two 80 (Vangl1 and Vangl2) and four (Prickle1-4) mammalian paralogs, respectively. In the mouse, 81 82 PCP is required for orientation of stereocilia in the cochlea, orientation of hairs in the skin and morphogenesis of many organs [11-13]. In the early 2000s, PCP molecules were found 83

implicated in the regulation of convergent extension (C-E), a process characterized by cellular 84 movements controlled by JNK and RHO activities required at early stages of development 85 (gastrulation), and in ciliogenesis in vertebrates [3, 8, 10, 13-15]. The central role of PCP in 86 vertebrate development is further evidenced by the dramatic impact of misexpression of its 87 components in embryos, leading to neural tube defects, - the most obvious and dramatic being 88 craniorachischisis in mice and humans mutant for Vangl-like -, and to abnormal formation of 89 the body axis during gastrulation [16]. Work from many laboratories has assigned PCP to a 90 non-canonical Wnt pathway referred thereafter as Wnt/PCP pathway. 91

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#### 93 Wnt/PCP is a $\beta$ -catenin independent Wnt pathway

Wnts (Wingless/Int-1) are secreted glycoproteins expressed during embryonic development 94 and throughout adult life, acting in diverse processes such as cell proliferation, stem cell 95 96 maintenance, cell migration, survival, and cell fate determination. In vertebrates, Wnts can activate β-catenin-dependent and -independent pathways, however genetic studies conducted 97 in Drosophila concluded that Wingless, the sole fly Wnt, does not control PCP [8]. 98 Organization of Wnt pathway is very complex owing to the important number of Wnt ligands 99 and membrane receptors of the seven transmembrane Frizzled family or single-spanning 100 101 transmembrane (co)receptors able to enter into multiple Wnt/(co)receptor combinations at the plasma membrane [3, 17]. As mentioned above, use of  $\beta$ -catenin defines the canonical and 102 best studied Wnt pathway (**BOX 2**) [3]. Depending on the cellular context, Wnt/PCP pathway 103 can be triggered by various Wnts including some which generally activate the  $\beta$ -catenin 104 105 dependent pathway. There is a large body of evidence that shows how the presence of coreceptors at proximity of Frizzled receptors orients the decision toward either  $\beta$ -catenin 106 dependent or independent pathways. However, among the Wnts, WNT-5A, WNT-7 or WNT-107 11 are known to mostly favor Wnt/PCP pathway. In parallel, it is commonly admitted that 108

FRIZZLED-3, -6 and -7 mostly orient toward Wnt/PCP signaling whereas canonical Wnt 109 pathway usually utilizes FRIZZLED-1 and -4. Wnt co-receptors such as tyrosine kinase 110 receptor family members (PTK7, ROR2, RYK) or membrane proteins (CD146, VANGL2, 111 Syndecan, Glypican) have a demonstrated implication in Wnt/PCP pathway [18, 19]. They 112 can directly bind to Wnts (CD146, ROR2, RYK) or facilitate interaction between Wnts and 113 their cognate Frizzled receptors. For example, formation of a Wnt-Frizzled-ROR2 complex 114 triggers Wnt/PCP signaling during development and in cancer cells. The ability of ROR2 to 115 116 heterodimerize with PTK7, another tyrosine kinase receptor, and to transduce downstream events on its own adds additional layers of complexity to this signaling platform [20-22]. 117 Furthermore, oligomerization of Frizzled receptors is not only necessary for their maturation 118 and membrane localization [23] but also represents, as demonstrated for other seven 119 transmembrane proteins [24, 25], an opportunity for the recruitment of signaling molecules 120 121 implicated in canonical or non-canonical Wnt pathways, increasing again system complexity.

Binding of Wnt ligands leads to recruitment of Dishevelled to Wnt/PCP Frizzled receptors 122 123 and activation of small GTPases of the Rho family (Cdc42, Rac1, RhoA) involved in actin cytoskeleton remodeling and cell contractility, and JNK pathway [14]. In vertebrates,  $\beta$ -124 catenin independent signaling is traditionally divided into Wnt/Ca<sup>2+</sup> and Wnt/PCP JNK-125 dependent signaling which can be both activated by Wnt5a. However, in Xenopus, Randall 126 Moon's team showed that Prickle1 and Dishevelled can trigger JNK (monitored by AP1 127 reporter assays) and calcium (increase of Ca<sup>2+</sup> dynamics and activation of CAMKII and PKC, 128 two  $Ca^{2+}$  dependent kinases) signaling, leaving the possibility open that Wnt/Ca<sup>2+</sup> and 129 Wnt/PCP branches function as one signaling pathway or, at least, that they cross-talk [26, 27]. 130 None of these biochemical assays have been applied to *Drosophila* where PCP signaling was 131 genetically, not biochemically, defined. Together, these data suggest subtle regulation and 132 overlap between these two Wnt pathways. 133

134 Canonical Wnt and non-canonical Wnt/PCP pathways are engaged in intimate relationships 135 by sharing common components such as Dishevelled, PTK7 and RYK which can positively or 136 negatively act on both signaling cascades [14, 15]. For example, Wnt-5a can trigger  $\beta$ -catenin 137 dependent or independent pathway [28, 29] and PTK7 can directly interact with  $\beta$ -catenin, 138 promoting a canonical Wnt signaling during Xenopus development, or activate Wnt/PCP 139 signaling [20, 30-32]. Finally, like most Wnt/PCP components, Prickle behaves as an 140 inhibitor of  $\beta$ -catenin dependent signaling [3, 33].

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## 142 Deregulation of Wnt/PCP signaling in cancer

Whereas loss of Wnt/PCP signaling, mostly by gene mutations, is linked to human genetic 143 disorders (defects of neural tube closure or palate cleft, kidney diseases, ciliopathies) (for 144 reviews see [8, 9, 14]), up-regulation of Wnt/PCP components is observed in many cancers, 145 146 this event being most of the time, although not exclusively, associated to a poor prognosis (Table 1). Recent studies have highlighted how these deregulations are associated to the 147 classical features of cancer progression. We will describe thereafter how Wnt/PCP proteins 148 are involved in processes such as cell proliferation, stemness, epithelial-mesenchymal 149 transition (EMT), cell migration, tumor invasiveness and resistance to treatment (Figure 1B). 150

151 *Cancer cell proliferation and stemness* 

Involvement of canonical Wnt signaling in the early stages of colon cancer is well established as mutations in up to 90% of colorectal cancers (CRCs) of *APC* (80%),  $\beta$ -catenin (10%) or *AXIN* which constitutively activate the pathway were found [3]. A recent study analyzing a large cohort of non-metastatic CRC patients showed that enhanced expression of noncanonical Wnt-5a confers a better clinical outcome [34]. Accordingly, xenografts of colorectal HCT116 cells overexpressing Wnt-5a are less proliferative and less tumorigenic [34]. This tumor suppressing role of Wnt-5a has also been described in other cancer types (lymphoma,

thyroid cancer) [35-37] and is due to inhibition of canonical Wnt pathway [29]. Paradoxically, 159 in other studies, Wnt-5a is described as a poor prognosis marker for patients suffering from 160 CRC and gastric cancer and behaves as a pro-migratory and pro-invasive ligand [38, 39]. 161 downregulation of the Wnt/PCP receptors 162 Along the same line. PTK7 and VANGL1/VANGL2 impairs cell proliferation of colon and breast cancer cells, respectively 163 [40-43]. A recent report showed that FRIZZLED-7 along with other Wnt/PCP components is 164 upregulated in ovarian cancer cells conferring cell proliferation, cell cycle progression, and 165 166 stemness mediated in part by RhoA activity [44]. The PDZ protein SCRIBBLE plays a prominent role in apico-basal polarity and behaves as a tumor suppressor in *Drosophila* [45]. 167 Its function has somehow diverged along evolution as it participates to Wnt/PCP signaling in 168 vertebrates [46]. However, its tumor suppressing function is conserved in breast and prostate 169 cancer through an increase of cancer cell survival [47, 48], MAPK and Hippo pathways which 170 171 promote polarity defects, cancer cell proliferation and invasiveness, self-renewal and tumorigenic capacities of cancer stem cells [48-51]. In stark contrast with canonical Wnt 172 173 pathway, little is known about the transcriptional events and target genes of Wnt/PCP signaling which makes its study difficult. However, GRHL3, a transcription factor involved 174 in epidermal wound repair and acting as a tumor suppressor in squamous cell carcinoma, 175 represents a notable exception [52, 53]. GHRL3 is the orthologous to Drosophila Grainy head 176 177 (Grh), a protein involved in PCP signaling in wing hair formation and ommatidium orientation in flies [54, 55]. Mechanistically, human GRHL3 controls the expression of 178 RhoGEF19, an activator of the small GTPase family RhoA, leading to actin polymerization, 179 cytoskeletal rearrangement and directed migration in wound healing [52]. Recently, Jarid2, a 180 developmental transcription factor member of the Polycomp Repressor complex 2, has been 181 182 shown to control expression of Wnt/PCP components such as Wnt-9a, Frizzled-2, and Prickle1 which are important for stemness of murine embryonic stem cells [56]. Such a causal 183

link has not been yet established in cancer stem cells; however human JARID2 promotes
EMT in hepatocellular, lung and colon cancer cells [57, 58]. Lastly, PTK7 was recently
described as a hematopoietic and colon cancer stem cell marker whose function has yet to be
determined [59, 60].

#### 188 Epithelial-Mesenchymal Transition

EMT is a physiological reversible process which describes the transition of epithelial cells 189 from a polarized to a mesenchymal state prone to motility. It occurs during normal embryonic 190 191 development but also in tumors leading to increased invasion and drug resistance, and is controlled by transcriptional programs driven by many signaling pathways [61]. Up-192 regulation of Wnt-5a has been observed in cellular systems undergoing EMT such as 193 squamous carcinoma cells [62] and Madin-Darby Canine Kidney (MDCK) cells treated with 194 TGF<sub>β</sub> [63]. Levels of FRIZZLED-4 and PTK7 receptors are also high in prostate cancer, and 195 196 in MDCK or human embryonic stem cells in which EMT is triggered [63-65]. Cause or consequence? In melanoma cells, Wnt-5a promotes EMT through a PKC-dependent 197 mechanism, related to Wnt/Ca<sup>2+</sup> and possibly to Wnt/PCP, which leads to expression of 198 199 mesenchymal markers such as Snail and Vimentin and decreases E-cadherin expression [66]. Recent results attribute to SCRIBBLE a direct role in EMT in normal [67] and tumoral [68] 200 situations through the modulation of SMAD3/SMAD4 and MAPK-ERK activities, 201 202 respectively. On the other hand, induction of EMT in breast cancer cells delocalizes SCRIBBLE from the plasma membrane inducing a Hippo-dependent pro-metastatic program 203 [50]. Recently, elevated Wnt-5a/Wnt-5b and FRIZZLED-2 levels have been observed in 204 205 several types of metastatic cancers (liver, lung, colon, and breast) expressing EMT markers. In this study, FRIZZLED-2 is a cornerstone and targetable molecule driving EMT and 206 207 metastasis through FYN and STAT3, two novel components of Wnt pathways [69].

208 *Cell migration and invasiveness* 

Extensive data have been accumulated on the role of Wnt/PCP deregulation in tumoral 209 dissemination through in vitro and in vivo studies [40, 41, 43, 49, 66, 70-73]. In skin cancers 210 such as melanoma, overexpression of Wnt-5a correlates with enhanced cell invasion and 211 212 metastasis, and with poor outcome [73]. These effects can be mediated by different Wnt receptors including FRIZZLED-3, -5 or ROR2 [73-76]. At the cellular level, stimulation of 213 melanoma cells by Wnt-5a leads to an asymmetrical accumulation of FRIZZLED-3 in 214 intracellular Wnt-mediated receptor-actin-myosin polarity (W-RAMP) structures under the 215 216 control of two small GTPases, Rab4 and RhoB [77]. Mechanistically, W-RAMPs localize at the trailing edge of migrating cells where they locally activate PKC leading to increased 217 contractility and focal adhesion disassembly rates [78]. Wnt-5a can also control focal 218 adhesion dynamics through FRIZZLED-2, APC and Dishevelled [79]. APC and Dishevelled 219 220 associate with Focal Adhesion Kinase (FAK) and PAXILLIN which are important for Wnt-221 5a-dependent focal adhesion turnover. This particular pathway seems initiated through an association between Wnt-5a/FRIZZLED-2 and integrins at the leading edge of migrating 222 223 cells. In another study, single molecule RNA sequencing of circulating tumor cells (CTCs) 224 from prostate cancer revealed an increase of Wnt-2 driven non-canonical signaling which suppresses anoikis and increases cell motility [80]. 225

226 Genomic analysis led to the stratification of breast cancers into five main classes according to 227 gene expression. Triple negative breast cancers (TNBCs) are the most aggressive entity, lacking expression of HER2 and estrogen/progesterone receptors, with no yet available 228 targeted therapy. Recent data from our lab found that PRICKLE1 and VANGL2 are both 229 overexpressed in TNBCs [43, 70] whereas high expression of VANGL1, a VANGL2 230 homologue, was associated to poor prognosis in estrogen receptor-positive breast cancer 231 patients [81]. PRICKLE1 which is known to interact with VANGL2 [82] is implicated in 232 focal adhesion dynamics [70, 71, 83]. Interestingly, PRICKLE1 downregulation has been 233

reported to strongly impair cell motility via three possible downstream regulators, Akt [70], 234 and Rho-GEF [83] in TNBC cells or LL5ß [71] in other cell systems. Moreover, activation of 235 surviving Akt-dependent mechanism by Wnt/PCP proteins contributes to cancer progression 236 [70, 84]. Since the first observations that elevated amounts of Wnt-5a are found in invasive 237 breast cancers [85] [86], other Wnt/PCP-related molecules have also been proposed as 238 markers of aggressiveness. This is for instance the case of two other Wnt ligands, Wnt-7a and 239 Wnt-11 [72, 87], as well as receptors (FRIZZLED-2, -7, VANGL2) [43, 69] and adaptors 240 (SCRIBBLE, PRICKLE1) [42, 70, 88]. In TNBCs, VANGL2 is thought to act downstream of 241 ROR2 [89] or Frizzled [90] and its overexpression appears to correlate with JNK pathway 242 activation through a direct interaction with the p62/SQSTM1 scaffold [43]. VANGL2 may 243 also promote cell migration through interaction with SCRIBBLE [41]. Altogether these data 244 suggest that VANGL2 act as signaling platform able to mediate downstream signaling events 245 246 through its interaction with different scaffold proteins (SCRIBBLE, p62/SQSTM1 or PRICKLE1). The finding that, in neuroblastoma and fibrosarcoma cells, PRICKLE1 and 247 248 VANGL2 act as tumor suppressor genes [91, 92] indicates that their implication in cancer 249 might vary depending on the cellular context.

First identified in colon cancer cells [93, 94], PTK7 has since been shown to behave as a poor 250 prognosis marker in colon cancer patients and to contribute to the metastatic program [40, 251 252 95]. This inactive tyrosine kinase receptor is a Wnt-5a co-receptor which forms heterodimers with ROR2 and mediates JNK activation [20, 21]. In other contexts, PTK7 can modulate β-253 catenin dependent Wnt pathway [30, 31], induce cell migration [40, 96] and associate with 254 diverse cancer-related membrane receptors (VEGFR1, Plexins) [95]. Moreover, upregulation 255 of PTK7 has been reported in Acute Myeloid Leukemia (AML) and T cell acute 256 257 lymphoblastic leukemia (T-ALL) [96, 97]. High PTK7 levels in AML cells are associated with low circulating blast cell counts most probably because of retention of PTK7 positive 258

cells in the bone marrow [59]. This may well explain the resistance of AML-PTK7 positive 259 patients to chemotherapy as this treatment aims at eliminating circulating cycling blast cells 260 [96]. Other Wnt/PCP components (Wnt-5a, PRICKLE1, VANGL2, CELSR1, FRIZZLED-3, 261 -7) have been also reported to be poor prognosis markers in chronic lymphocytic leukemia 262 (CLL) when overexpressed [98, 99]. Autocrine release of Wnt-5a by CLL cells stimulates 263 ROR1, a ROR2 homologue, and controls the chemotactic response and proliferation by 264 inducing ROR1/ROR2 heterodimerization which activates RhoA and Rac1 [98-100]. This 265 proliferative pathway can be targeted with specific antibodies inducing apoptosis [101]. 266

267

#### 268 Wnt/PCP pathway in host-tumor interaction

Tumor growth and dissemination is not only governed by genetic alterations of cancer cells 269 but also by an altered deregulated communication with adjacent tissues. In Hodgkin 270 271 lymphoma, canonical Wnts were shown to be secreted by the endothelial vasculature, promoting chemotaxis and adhesion of lymphoma cells [102]. In prostate cancer, genotoxic 272 273 treatment leads to WNT16B secretion by the tumor microenvironment which initiates a paracrine activation of canonical Wnt signaling in cancer cells and drug resistance [103]. 274 Wnts are secreted, lipid-modified glycoproteins whose maturation in the endoplasmic 275 reticulum relies on Porcupine, an eight transmembrane spanning acyl transferase [28]. 276 277 Inhibition of Porcupine was thus proposed as a strategy to repress Wnt production and treat squamous cell carcinoma and CRCs (Table 2). Several routes involving protein carriers, 278 filipodia or cytomenes and extracellular vesicles such as exosomes are involved in the 279 extracellular transport of Wnts in the hydrophilic extracellular space to generate signaling 280 gradients in tissues [104]. Exosome are 30-150nm vesicles that allow the transfer of proteins, 281 lipids and genetic materials and play a key role in intercellular communication between 282 cancer cells and their microenvironment [105]. In melanoma, Wnt-5a has been shown to 283

induce a calcium dependent release of exosomes that contain IL-6, VEGF and MMP2. This 284 285 process stimulates the adjacent endothelial cells and favor invasiveness of cancer cells to distant tissues [106]. Recent reports have highlighted the role of Wnt/PCP pathway in cancer-286 287 associated fibroblasts (CAFs) which promote breast cancer metastasis. Indeed, Luga et al. have shown that CAFs generate exosomes which are internalized by cancer cells, loaded with 288 Wnt-11, and released by an autocrine loop promoting metastasis through VANGL1, 289 Dishevelled and PRICKLE1 [72]. Conversely, breast cancer cells can secrete Wnt-7a which 290 291 activates CAFs and promotes invasion through a TGFβ-dependent signaling [87]. Wnt/PCP pathway thus plays an important role in tumor-CAFs communication for the regulation of cell 292 293 motility, invasion and niche formation.

294

#### 295 Wnt/PCP pathway in resistance to treatment

Wnt/PCP can intervene by different mechanisms on drug resistance either indirectly (for 296 297 example through its participation to EMT) or directly. In melanoma, hypoxia induces Wnt-5a 298 expression and contributes to resistance to B-RAF inhibitors [84, 107]. The latter mechanism 299 occurs through ROR2 or FRIZZLED-7, RYK and the pro-survival Akt pathway which blocks apoptosis. Elevation of PRICKLE1 expression in poor prognosis breast cancer is correlated 300 with increased Akt activation; however its contribution to drug resistance has not been 301 evaluated [70]. Overexpression of PTK7 is associated with drug resistance through retention 302 of blasts in the bone marrow of AML patients and to resistance anthracycline-based 303 chemotherapy by an unknown mechanism in breast cancer patients [96, 108]. Recently, 304 305 Wnt/PCP signaling has been shown to be elevated in prostate circulating tumour cells resistant to treatment with enzalutamide, an androgen receptor (AR) inhibitor. Ectopic 306 expression of Wnt-4, -5a, -7b or -11 in LnCAP, an AR-positive cell line, improves cell 307 survival. Interestingly, enzalutamide increases Wnt-5a expression in this cell line suggesting 308

309 the existence of a positive feedback loop whose knockdown decreases overall cell 310 proliferation [109]. Undoubtedly, examining whether other Wnt/PCP proteins also contribute 311 to drug resistance will deserve further investigation.

312

#### 313 Concluding Remarks

Despite the progress in understanding how Wnt/PCP deregulation contributes to 314 315 tumorigenesis, many questions remain to be answered. In particular, the functions of many components that constitute this pathway are still poorly understood both at the molecular and 316 cellular levels. Wnt-5a is by far the most studied Wnt/PCP ligand in cancer. However, other 317 318 less characterized Wnts such as Wnt-2, -7 or -11 are also playing a crucial role in many steps of tumorigenesis and deserve further attention [72, 80, 87]. Moreover, whereas upregulation 319 320 of Wnt/PCP components is frequently associated to poor prognosis and drug resistance, an 321 inverse correlation has also been reported in the literature leading to conflicting conclusions as to whether Wnt-5a act as a tumor suppressor or pro-metastatic factor, very much like 322 323 TGF<sub>β</sub> [115]. Further contributing to the confusion, Wnt-5a or receptors such as PTK7 have the dual capacity to activate both non-canonical and canonical Wnt pathways. Finally, the 324 frequent repression of canonical Wnt pathway by Wnt/PCP signaling adds an additional layer 325 326 of complexity. More work is thus needed to further dissect the molecular basis of Wnt/PCP regulation which likely varies with (co)receptors composition at the cell surface (Figure 3) 327 328 [29].

Given the large body of evidence demonstrating the importance of Wnt/PCP signaling in cancer, this pathway is now considered as an attractive target for novel therapeutics. Different strategies are currently explored using chemical- or antibody-based compounds able to inhibit or activate the pathway and the list of ongoing clinical trials is growing (Table 2). However, development of these therapeutics will certainly highly benefit from the identification of

reliable biomarkers (assessment of Wnt/PCP component expression levels and/or activation of 334 their associated pathways) able to monitor activation (or inhibition) of the pathway in cells 335 and tissues. Mutations in *PRICKLE1* and *VANGL1* have been recently described in leukemia 336 [116] and squamous cell carcinoma [117]. Interestingly, these mutations lie in regions 337 important for protein-protein interactions. Yet the relevance of these findings will have to be 338 addressed at the functional and clinical levels. During cancer progression, Wnt/PCP 339 molecules can activate signaling pathways that are not classically associated to Wnt signaling 340 such as Akt [70, 84] or Fyn/Stat [69]. Hijacking of these signaling molecules by Wnt/PCP 341 pathway is likely to render cancer cells more efficient at invading tissues or resisting to 342 chemotherapies. Inhibition of Akt or Fyn with available drugs could thus represent a potential 343 strategy to combat Wnt/PCP deregulation in certain cancers. In conclusion, we believe that 344 therapeutics against Wnt/PCP pathway represent an interesting avenue in cancer treatment. 345

347

348 Figures

- 349 **<u>Figure 1</u>** Wnt signaling pathway and cancer progression
- 350 **Figure 2** Protein composition at the cell surface orients Wnt signaling

351

352 Table

353 <u>**Table 1**</u> Deregulation of non-canonical Wnt/PCP signaling components in cancer

354 **<u>Table2</u>**: Clinical trials of compounds targeting Wnt/PCP proteins

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356 Text boxes

BOX 1: In 1982, Roel Nusse and Harold Varnus published a Cell paper using Mouse 357 Mammary Tumour Virus (MMTV) to identify genes involved in tumorigenesis. MMTV is a 358 359 weak oncogenic milk-transmitted retrovirus causing mammary tumours. The authors found that incorporation of MMTV into the mouse genome led to overexpression of a gene 360 361 christened Int-1 (Integration site-1) correlated to high occurrence of cancer. A few years later, Int-1 was recognized as a homologue of the Drosophila Wingless ligand and renamed Wnt-1. 362 WNT1 was later on defined as an oncogenic protein in humans and found implicated in the 363 364 Wnt/ $\beta$ -catenin pathway.

365

**BOX 2:** In the absence of Wnts,  $\beta$ -catenin is associated to a destruction complex comprising adenomatous polyposis coli (APC), Glycogen Synthase Kinase 3 $\beta$  (GSK3 $\beta$ ) and Axin.  $\beta$ catenin is phosphorylated by GSK3 $\beta$  leading to its ubiquitylation by SCF $\beta$ -TrCP and to its degradation by the 26S proteasome. In the absence of  $\beta$ -catenin, the well-defined transcription factors LEF/TCF bind to a repressor called Groucho which recruits histone deacetylases (HDACs) to repress expression of Wnt-target genes. Wnts such as WNT-3A bind to their cognate (co)receptors and form a Frizzled-LRP5/6-Wnt ternary complex. Dishevelled is then associated to Frizzled at the plasma membrane resulting in the recruitment of GSK3β and CK1γ. Both kinases phosphorylate LRP5/6 at the C-terminus resulting in the formation of high affinity binding sites for Axin. Axin sequestration by LRP5/6 competes with the βcatenin destruction complex and leads to accumulation of cytosolic β-catenin and translocation into the nucleus where it displaces Groucho, binds to LEF/TCF and promotes the expression of Wnt-targeted genes.

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#### **FIGURE 1: Wnt signaling pathway and cancer progression**

665 **A.** Wnts bind either to Frizzled receptors or to co-receptors leading to activation of diverse 666 intracellular signaling pathways including Wnt/Ca<sup>2+</sup> or Wnt/β-catenin and Wnt/PCP which 667 acts through the activation of small G-proteins such as Rho, Rac and Cdc42. Signaling 668 molecules with unknown function in Wnt signaling are in light green.

#### 669 **B.** <u>Top panel</u>. **Development and progression of epithelium-derived cancer.**

After transformation of the cell of origin (green) (a.), cancer stem cells (red) start to proliferate (b.). Cells escape from the site of the primary tumour after gaining cell motility and invasive abilities (c.). Circulating stem cells (red) found in the bloodstream and the lymphatic system invade distal organs and prime sites of metastasis by activating fibroblasts (e.). Some cells can resist to chemotherapy and contribute to patient relapse (f.).

675 <u>Bottom panel.</u> Contribution of Wnt/β-catenin and Wnt/PCP signaling in the progression

of cancer disease. Wnt/ $\beta$ -catenin signaling occurs at the early stage of cancer progression providing proliferative cues to cells. Wnt/ $\beta$ -catenin signaling can be inhibited by components of Wnt/PCP signaling. During cancer progression, a shift of Wnt signaling occurs: Wnt/PCP signaling takes over and contributes to cancer progression by increasing cell motility, invasion, priming metastasis niche and resistance to therapy.

681

#### 682 FIGURE 2: Protein composition at cell surface orients Wnt signaling

Depending on a particular cellular context (A or B), a given Wnt binds to Frizzled and its coreceptors and activates downstream signaling pathways leading to A or B signaling.

685

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# Table 1: Deregulation of non-canonical Wnt/PCP signaling

components in cancer

Type of cancer	Name of the genes	Features	References		
Breast					
	WNT-5A	Increased tumorigenesis	Lejeune S et al., 1995; MacMillan, C.D., et		
			al.,2014		
	WNT-11	Increased tumorigenesis	Avgustinova et al., 2016		
	WNT-7A	Increased tumorigenesis	Luga V et al., 2012		
	FRIZZLED-7	Increased tumorigenesis	Yang et al., 2011		
	VANGL-1	Increased tumorigenesis	Anastas et al., 2012		
	VANGL-2	Increased tumorigenesis	Purivajessinghe et al., 2016		
	SCRIBBLE	Increased tumorigenesis	Anastas et al., 2012		
	PRICKLE-1	Increased tumorigenesis	Daulat et al., 2016		
	FRIZZLED-2	Increased tumorigenesis	Gujral et al., 2014		
	PTK-7	Increased tumorigenesis	Gärtner et al., 2014		
	ROR2	Increased tumorigenesis	Henry et al., 2015		
	PTK7	Increased resistance to therapy	Ataseven et al., 2013		
Ovarian	FRIZZLED-7	Increased tumorigenesis	Asad et al., 2014		
Squamous carcinoma	WNT-5A	Increased tumorigenesis	Taki et al., 2003		
	VANGL-1	Increased tumorigenesis	Qin et al., 2016		
Neuroblastoma	VANGL-2	Decreased tumorigenesis	Dyberg et al., 2016		
	PRICKLE-1	Decreased tumorigenesis	Dyberg et al., 2016		
Melanoma					
	WNT-5A	Increased tumorigenesis	Dissanyake et al., 2007		
	WNT-5A	Increased tumorigenesis	Weeraratna et al., 2002		
	WNT-5A	Increased tumorigenesis	Da Forno et al., 2008		
	WNT-5A	Increased resistance to therapy	Anastas et al., 2014		
	FRIZZLED-7	Increased tumorigenesis	Tiwary et al., Plos One 2016		
	FRIZZLED-7	Increased resistance to therapy	Anastas et al., JCI 2014		
	SFRP3	Methylation, loss of expression.	Ekström et al., Plos One 2011		

		Decreased tumorigenesis		
	FRIZZLED-5	Increased tumorigenesis	Weeraratna et al., 2002 O'Connell et al., Oncogene 2010	
	ROR2	Increased tumorigenesis		
	ROR1	Increased tumorigenesis	O'Connell et al., Cancer Discovery 2013	
Colorectal	PTK7	Increased tumorigenesis	Lhoumeau et al., Plos One 2015	
	ROR2	Increased tumorigenesis	Mei et al., BBRC 2014	
	WNT-5A	Tumor suppressor	Cheng et al., J Cell Physiology 2014	
	WNT-5A	Increased tumorigenesis	Bakker et al., 2013	
Gastric cancer	WNT-5A	Increased tumorigenesis	Kurayoshi et al., 2006	
Pancreas	FRIZZLED-5	Increased tumorigenesis	Steinhart et al., Biorxiv.org	
	FRIZZLED-4	Increased tumorigenesis	Gupta et al., 2010	
	WNT-2	Increased tumorigenesis	Yu et al. 2012	
	WNT-5A	Increased resistance to therapy	Miyamoto et al., 2015	
Thyroid	WNT-5A	Tumour suppressor	Kremenevskaja et al., 2005	
Leukemia				
CLL				
	PRICKLE1	Increased tumorigenesis	Kaucka et al., 2013	
	VANGL2	Increased tumorigenesis	Kaucka et al., 2013	
	CELSR1	Increased tumorigenesis	Kaucka et al., 2013	
	Frizzled-3	Increased tumorigenesis	Kaucka et al., 2013	
	Frizzled-7	Increased tumorigenesis	Kaucka et al., 2013	
	Dishevelled 2	Increased tumorigenesis	Kaucka et al., 2013	
	Dishevelled 3	Increased tumorigenesis	Kaucka et al., 2013	
	Casein kinase 1	Increased tumorigenesis	Kaucka et al., 2013	
	ROR1	Increased tumorigenesis	Janovska et al., 2016	
	WNT-5A	Increased tumorigenesis	Kaucka et al., 2013	
		Increased tumorigenesis	Yu et al., 2016	
	WNT-5A	Tumor suppressor	Liang, H et al., 2003	
AML	PTK7	Increased tumorigenesis	Prebet et al., Blood 2010	

ALL

PTK7

## 695

# Table 2: Clinical trials of compounds targeting Wnt/PCP proteins

Target genes	Compound	Cancer	Trial	Company	Description	Trial phase
			number			
WNT-5A	Foxy-5	Breast, Colon and Prostate	NCT02655	WntResearch AB	Establishment of the	Phase-I
		Cancer	952		recommended dose for	completed
					Phase-II clinical trial	
Inhibitor of	WNT974	Colorectal Cancer with	NCT02278	Array BioPharma /	In combination with	Phase-I
Porcupine		BRAF mutation	133	Novartis	LGX818 (RAF inhibitor)	(WNT974);
					and Cetuximib	Phase-II
						(LGX818)
Inhibitor of	WNT974	Squamous Cell Carcinoma,	NCT02649	Novartis		Phase-II
Porcupine		Head And Neck	530			
PTK7	PF-	Advanced solid tumors	NCT02222	Pfizer	In combination with	Phase-I
	06647020		922		fluconazole. The aim of	
					the study is to assess the	
					maximum tolerated dose.	
FRIZZLED-	SYNFRIZZ	Advanced synovial sarcoma	NCT01469	OncoTherapy Science,	Assesment of	Phase-I
10			975	Inc.	biodistribution and	completed
					tumour uptake	
FRIZZLED-7	OMP18R5	Non-small cell lung cancer	NCT01957	OncoMed	In combination with	Phase-I
	(Vantictuma		007	Pharmaceuticals, Inc.	Docetaxel	
	b)					
FRIZZLED-7	OMP18R5	Metastatic Breast cancer	NCT01973	OncoMed	In combination with	Phase-I
	(Vantictuma		309	Pharmaceuticals, Inc.	Paclitaxel	
	b)					
FRIZZLED-7	OMP18R5	Pancreatic cancer	NCT02005	OncoMed	In combnation with Nab-	Phase-I
	(Vantictuma		315	Pharmaceuticals, Inc.	Paclitaxel and	
	b)				Gemcitabine	
FRIZZLED-8	OMP54F28	Ovarian cancer	NCT02092	OncoMed	In combination with	Phase-I
			363	Pharmaceuticals, Inc.	Paclitaxel and	
					Carboplatin	
FRIZZLED-8	OMP54F28	Pancreatic cancer	NCT02050	OncoMed	In combnation with Nab-	Phase-I
-						

			178	Pharmaceuticals, Inc.	Paclitaxel and	
					Gemcitabine	
FRIZZLED-8	OMP54F28	Hepatocellular Cancer	NCT02069	OncoMed	In combination with	Phase-I
			145	Pharmaceuticals, Inc.	Sorafenib	

## **Outstanding questions box**

- How does the Wnt/PCP pathway function in normal and cancer cells?
- What are the mechanisms that localize and regulate Wnt/PCP molecules at the plasma membrane?
- What is the best readout to monitor Wnt/PCP activation in normal and cancer cells?
- What are the mechanisms that up-regulate expression of Wnt/PCP molecules in cancer cells?
- Are Wnt ligands and receptors mandatory to trigger abnormal activation of Wnt/PCP signaling in cancer cells?
- Which Wnt/PCP components should be targeted in priority in tumors and how?
- Is potential upregulation of canonical Wnt pathway activity upon inhibition of the Wnt/PCP pathway a problem?
- Is it possible to anticipate the side effects of therapeutic inhibition of Wnt/PCP pathway?





# FIGURE 2:

