The Planar Cell Polarity Pathway Drives Pathogenesis of Chronic Lymphocytic Leukemia by the Regulation of B-Lymphocyte Migration

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Abstract

The planar cell polarity (PCP) pathway is a conserved pathway that regulates cell migration and polarity in various contexts. Here we show that key PCP pathway components such as Vang2, Celsr1, Prickle1, FZD3, FZD7, Dvl2, Dvl3, and casein kinase 1 (CK1)-ε are upregulated in B lymphocytes with chronic lymphocytic leukemia (CLL). Elevated levels of PCP proteins accumulate in advanced stages of the disease. Here, we show that PCP pathway is required for the migration and transendothelial invasion of CLL cells and that patients with high expression of PCP genes, FZD3, FZD7, and PRICKLE1, have a less favorable clinical prognosis. Our findings establish that the PCP pathway acts as an important regulator of CLL cell migration and invasion. PCP proteins represent an important class of molecules regulating pathogenic interaction of CLL cells with their microenvironment. Cancer Res; 73(5); 1491–501. ©2012 AACR.

Introduction

Wnt signaling pathways are crucial for cell-to-cell communication, differentiation, and morphogenesis in embryonic development. Dysfunction or deregulation of Wnt signaling accounts for a number of developmental defects, inherited diseases, and many types of cancer (1). The best-known Wnt pathway—canonical or Wnt/β-catenin pathway—induces β-catenin–dependent activation of T-cell factor/lymphoid enhancer factor (TCF/LEF)-mediated transcription. However, Wnt ligands can activate other so-called noncanonical Wnt pathways, which are β-catenin–independent and biochemically distinct from the canonical Wnt pathway (2). Among those, the pathway that regulates planar cell polarity (Wnt/PCP pathway) is the best known (3). The core components of the Wnt/PCP pathway (referred here as PCP or polarity proteins) are conserved in evolution and include Vang-like protein 2 (Vang2), cadherin EGF LAG 7-pass transmembrane G-type receptor (Celsr1), Frizzleds (FZD, in mammals mainly FZD3, FZD6, and FZD7), which act as receptors, and cytoplasmic components such as Disheveled (Dvl1-3 in mammals), Prickle-like proteins (mainly Prickle1), casein kinase 1ε (CK1ε), and the small GTPases Rho/Rac and downstream kinases ROCK/JNK. PCP proteins regulate cell polarity (3), convergent extension movements during gastrulation and neurulation, and polarity of the hair cells in the inner ear (3, 4).

In the present study, we investigated the role of PCP proteins in chronic lymphocytic leukemia (CLL). CLL is characterized by clonal expansion and apoptosis dysregulation of CD5+ B lymphocytes. Neoplastic CLL cells accumulate in blood, bone marrow, and lymphoid tissue. It is recognized that cell migration and recirculation between peripheral blood and lymphoid niches in bone marrow and lymphoid tissue are important factors contributing to CLL biology (5–7) and that alterations in survival and proliferation of CLL are affected by their interaction with the microenvironment. Here, we show for the first time that the core cassette of the PCP pathway composed of Celsr1, Prickle1, Vang2, Dvl2, Dvl3, FZD3, and FZD7 is upregulated in B cells of patients with CLLs. We show that PCP proteins, which are conserved regulators of the cell–cell interaction and cell migration, are important mediators of CLL chemotactic responses and homing.

Materials and Methods

Cell isolation, purification, and cell treatments
Primary B cells from previously untreated patients with CLLs or healthy volunteers were separated using gradient centrifugation followed by non-B-cell depletion (RosetteSep B Cell Enrichment Kit and Human CD3+ Depletion Kit;
StemCell Technologies; or MACS B cell Isolation Kit II; Miltenyi Biotec) according to the manufacturer’s instructions. Flow cytometry was conducted for evaluation of CD5 and CD19 expression on purified cells (tricolor panel: CD45-TRI-COLOR, MHCD45065, Invitrogen, CD5-FTTC, A08932, CD19-PE, A07769, Beckman Coulter). For further analyses, samples with the purity higher than 95% B cells were used. The cell line MEC1 was obtained from DSMZ, cell line was tested by DSMZ using molecular genetic methods; MEC1 cells were cultured in RPMI-1640 supplemented with 10% FBS and antibiotics at 37°C and 5% CO2.

**Transwell assay**

The chemotaxis assay was conducted in HTS Transwell-96 well plates (Corning Incorporated) with 5.0-μm pore size polycarbonate membranes following the manufacturer’s instructions. A total of 0.5 × 10⁶ cells (primary CLL or MEC1 cells) were seeded in the Transwell upper insert, which was either nontreated or coated with a human umbilical vein endothelial cells monolayer (HUVEC). Cells in the insert were treated with antibodies or inhibitors as described in Supplementary Material. Cells were incubated overnight in full medium (including 10% fetal calf serum) at 37°C and 5% CO2, and after 18 hours the migration toward the chemokine was analyzed by a Coulter Counter (model Fö, Coulter Electronics; Figs. 2A–C, 3A–D, and 4A–C; Supplementary Figs. S2B and S4E–S4H) or by C6 flow cytometer (Accuri; Figs. 2E–G, 3E–G, and 4D; Supplementary Fig. S2E). The migration index was calculated as the number of cells (treated or untreated) migrating in response to the chemokine divided by the number of cells migrating toward the control medium only. In case of very variable migration indexes (in primary CLL cells), cell numbers were normalized to the chemokine-only condition and expressed as "relative migration."

**In vivo experiments**

Nonirradiated mice (8- to 16-week-old) nonobese diabetic/severe combined immunodeficient (NOD/SCID) IL2Rγ-null (NSG) mice were used for transplantation experiments. Freshly isolated human CLL lymphocytes (25 × 10⁶ per condition) were pretreated as indicated for 20 hours and stained with Calcein AM. Mice underwent transplantation by intraperitoneal injection of 20 × 10⁶ of pretreated and calcein AM–stained human CLL lymphocytes in 120 μL of sterile PBS. Recipient mice were analyzed 24 hours after the injection by fluorescence-activated cell-sorting (FACS) analysis (for details, see Supplementary Material).

Further methods including information on patient samples, quantitative real-time (qRT)-PCR, Western blotting, flow cytometry, nucleofection, and statistics are provided in the Supplementary Material.

**Results**

**Core PCP pathway components are upregulated in CLL patients**

Our earlier microarray expression analysis (8) suggested that components of Wnt/PCP signaling are frequently upregulated in CLLs and expressed differently in prognostically distinct subsets of patients with CLLs. To confirm that the levels of PCP components are indeed altered in CLLs, we collected RNA samples from purified peripheral blood B cells (CD19+ cell population purity ≥ 95%) of control individuals (n = 6) and patients with CLL (n = 93). qRT-PCR analysis showed statistically increased mRNA levels of 3 PCP genes, CELSR1, PRICKLE1, and FZD7, in CLL samples and identified that 2 other genes VANGL2 and FZD3 have high levels in a subset of patients with CLLs [Fig. 1A—normalized to the expression of β2-microglobulin; Supplementary Fig. S1A—normalized to β-actin].

Subsequent analysis of the protein level by Western blotting confirmed increased protein expression of the PCP proteins Vangl2, Dvl2, Dvl3, Prickle1, and CK1ε (Fig. 1B, quantified in Fig. 1C) in patients with CLLs. The level of β-catenin did not differ between CLLs and healthy cells (Fig. 1B), and we failed to detect activated (dephosphorylated) β-catenin (not shown), which suggest that the Wnt/β-catenin pathway is not active in CLL cells. All CLL samples tested had high expression of LEF1 and Ror1 (Fig. 1B), which were previously identified as abundantly and consistently expressed in CLL cells (9, 10). Flow cytometry detection (Fig. 1D) further confirmed increased cell surface levels of Ror1, FZD3, Celsr1, and Vangl2 in comparison to healthy B cells.

Next, we asked whether the levels of PCP proteins are stable or they change during the disease course. When we compared samples of 6 patients with CLLs collected at 2 different time points (t1 and t2; Fig. 1E), we were able to detect changes in the levels of PCP proteins in CLL cells from individual time points. Specifically, we observed a clear increase in Dvl2, Dvl3, and Prickle1 levels in patients followed in time, which is quantified in Supplementary Fig. S1B. The most prominent changes were observed mainly in patients, who progressed from Rai stages (11) 0/1/I to the stage III or IV (see patients C, H, P). The levels of LEF1 and β-catenin do not change (Fig. 1E).

In summary, using several methods, we provide evidence that a host of PCP proteins are overexpressed in B cells of patients with CLLs, with some accumulating to a greater extent in advanced stages of the disease.

**Wnt5a, a PCP ligand, increases migration of primary CLl cells in a chemokine gradient via a Wnt/PCP pathway**

It is becoming increasingly evident that the role of microenvironment in the pathogenesis of CLLs is of a crucial importance (7, 12). Chemokines and their receptors, mainly CXCL12-CXCR4, CXCL9/10/11-CXCR3, and CCL19/21-CCR7, have been shown to mediate invasiveness and transendothelial migration of CLL cells (13–16). In that line, it has been recently shown that Wnt5a, which is the major ligand for Ror1/2 receptors and a crucial component of the Wnt/PCP pathway (17–19), promotes cell polarization and directional movement of melanoma cells and T cells in a chemokine (CXCL12) gradient (20, 21).

We thus hypothesized that Wnt5a, which is also expressed in CLL cells (Supplementary Fig. S2A) together with receptor PCP protein complexes, may contribute to the pathogenesis of CLLs by the effects on the polarized migration of CLL cells toward...
Figure 1. PCP genes and proteins are upregulated in patients with CLL. A, expression of the indicated genes from CD19+ cells of healthy individuals (control; n = 6) and patients with CLLs (n = 93) was analyzed by qRT-PCR. Data represent the fold difference relative to the reference gene 2ΔΔCt. B, the amounts of the indicated PCP proteins were analyzed by Western blotting in B cells of healthy controls and patients with CLLs. One lane represents one individual. C, densitometric quantification of data in B. D, the cell surface expression of the PCP proteins Ror1, FZD3, Celsr1, and VanGll2 in CD19+ B cells of healthy individual and patients with CLLs were analyzed by flow cytometry. E, Western blotting of Dvl2, Dvl3, Prickle1, LEF1, Ror1, and β-catenin in B cells from 6 patients, where each patient has been taken and analyzed at 2 different time points (t1 and t2). β-Actin served as the loading control. The Rai clinical stages of each patient at t1 and t2, and the time between t1 and t2 are indicated. Mean timespan between t1 and t2 is 1,308 days. *** P < 0.001; * P < 0.05. n.s., not significant (Student t test).
chemokines. To test this prediction, we studied primary cells from patients with CLLs for their ability to respond to CXCL12 in the presence and absence of recombinant Wnt5a (200 ng/mL) using Transwell migration chambers (16, 22). As we show in Fig. 2A, CXCL12 alone increased migration of CLL cells, whereas Wnt5a alone had no effect. In combination with CXCL12, Wnt5a significantly increased migration of CLL cells compared with all other experimental conditions. A similar effect was seen for CXCL10, CXCL11, and CCL21 but not for CXCL9 and CCL19 (Supplementary Fig. S2B). Importantly, the Wnt5a-induced synergism was dependent on the activity of CK1, which phosphorylates a key PCP protein Dvl and regulates its activity in the PCP pathway (19, 23–25). Treatment with the CK1 inhibitor D4476 (CK1 inh. I; Fig. 2B) significantly reduced the positive effects of Wnt5a on the chemotaxis toward CXCL12. To exclude that these effects are due to the activation of the Wnt/β-catenin pathway, we tested in primary CLL cells the ability of Wnts to activate sensitive TCF/LEF (TopFlash) luciferase reporter (27) and analyzed the expression of bona fide Wnt/β-catenin target genes AXIN2 and DKK1 by quantitative PCR. As we show in Supplementary Fig. S2C and S2D, no experimental treatments, including treatment with Wnt3a, a typical ligand of the Wnt/β-catenin pathway, trigger the expression from TCF/LEF-responsive promoters.

Figure 2. PCP proteins regulate migration of primary CLL cells in a chemokine gradient. A, migration of primary CLL cells (expressed as migration index) in the presence of CXCL12, Wnt5a, and CXCL12/Wnt5a was analyzed using Transwell migration chambers (n = 6). The observed increase is dependent on the activity of CK1 (n = 3; B) and on presence of the Ror1 cell surface receptor (aRor1, Ror1-blocking antibody; n = 4; C). Both CK1 inhibition and Ror1 blocking antibody clearly interfere with Wnt/PCP pathway as shown by their ability to reduce the Wnt-induced mobility shift of Dvl3 (full arrowhead, open arrowhead—non-shifted Dvl3; D). E–G, the effects of the inhibitors of the Rho-associated kinase (Y27632; E), Rac1 (F), and porcupine (IWP-2; G) on the chemotaxis driven by Wnt5a/CXCL12 were tested by Transwell assays. Values represent mean ± SEM. Statistical differences were tested by one-way ANOVA and Tukey post hoc test (*, P < 0.05; **, P < 0.01; ***, P < 0.001). n.s., not significant.
This strongly suggests that Wnt/β-catenin pathway is not involved in the observed phenotypes.

The effects similar to the inhibition of CK1 have been observed, when we blocked the putative Wnt5a receptor in the PCP complex, Ror1, with the goat Ror1 blocking antibody (aRor1) but not with the control goat IgG (Fig. 2C). The efficiency of this antibody, which binds Ror1 and promotes its internalization, has been shown earlier (28). Both D4476 (CK1 inh. I) and aRor1 antibody clearly inhibited activation of PCP pathway in primary CLL cells as shown by their ability to block phosphorylation-dependent shift of endogenous Dvl3 (PS-Dvl3), which is a hallmark of Dvl3 activation in the non-canonical Wnt pathway (Fig. 2D; ref. 19).

In the Wnt/PCP pathway, Dvl mediates the activation of small GTPases RhoA and Rac1, which trigger subsequent cytoskeletal rearrangements and cell migration. As we show in Fig. 2E, the inhibition of RhoA-driven signaling by Y27632, an inhibitor of Rho-associated ROCK protein kinase (29) but not the inhibition of Rac1 by a Rac1 inhibitor NSC23766 (Fig. 2F; ref. 30), blocked the migration of primary CLL cells. No inhibitors used in this section affect the viability of primary CLL cells, as assessed by WST1 test, and the effects on chemotaxis thus do not reflect differences in the condition of CLL cells (Supplementary Fig. S3).

The experiments above suggest that PCP pathway is capable to promote and is required for the efficient chemotactic migration of primary CLL cells. To further elaborate on the mechanism of the interaction between Wnt/PCP and the chemokine signaling, we tested functionally the role of the CXCR4 (the receptor for CXCL12) and the role of...
endogenous Wnts in CLL chemotaxis. Interestingly, CXCR4-blocking antibody was unable to inhibit CLL migration (Supplementary Fig. S2E), whereas the disruption of the endogenous Wnt production by the porcupine inhibitor IWP-2 (31) reduced CLL migration (Fig. 2G). Given the fact that CXCL12-CXCR4 signaling does not regulate Wnt-5a expression and Wnts do not regulate expression of CXCR4 in CLL cells (see Supplementary Fig. S2F), we conclude that (i) the effects do not take place at the level of transcription, (ii) that the autocrine stimulation by Wnts contributes to the CLL chemotaxis, and (iii) that the chemotactic response is not critically mediated by CXCR4.

PCP proteins Dvl and its kinase CK1ε are required for the migration in the chemokine gradient

To study the role of Wnt/PCP pathway in CLL migration in more detail and to overcome the natural variability among patients and limitations in the experimental manipulation with primary CLL cells, we carried out further experiments in MEC1 cells (32). MEC1 is a well-defined cell line derived from a patient with CLLs, which recapitulates many aspects of the CLL biology and is used as the transplantation model of CLL (33). Quantitative analysis of mRNA (WNT5A, CELSR1, PRICKLE1, FZD3, FZD7; Supplementary Fig. S4A) and protein levels (Ror1, Vangl2, Dvl2, Dvl3, CK1ε; Supplementary Fig. S4B) showed that MEC1 cells express most PCP genes in high levels, although the levels of Ror1 were relatively low (Supplementary Fig. S4B).

MEC1 cells were unable to respond to CXCL12 but responded clearly to CCL19 and CCL21 in a dose-dependent manner (Fig. 3A; Supplementary Fig. S4C). Interestingly, treatment with Wnt5a did not further promote the promigratory effect of chemokines (Fig. 3A) and Wnt5a-blocking antibody did not block the migration (Supplementary Fig. S4E) despite the high endogenous expression of Wnt5a (see Supplementary Fig. S4A and S4D) Inhibition of CK1ε, a kinase, which is required for PCP signaling due to its role in the phosphorylation of Dvl, with 2 unrelated inhibitors D4476 (CK1 inh. I) and PF670462 (CK1 inh. II; 50 μmol/L; ref. 34) blocked the chemotactic response of MEC1 cells toward CCL21 and CCL19 (Fig. 3B and C). It can be expected that CK1 blocks CLL migration via phosphorylation of the key PCP protein Dvl. Indeed, even partial Dvl2 knockdown efficiently reduced the motility of MEC1 cells (Fig. 3D).

PCP proteins regulate chemokine-driven transendothelial invasion of CLL cells

In the human body, cells behave in a complex environment and have to pass via extracellular matrix or endothelial barriers, which is a process known as cell invasion. We have tested the role of PCP proteins in the invasion of CLL cells by the analysis of their invasion through a layer of HUVEC. In this experimental system, Wnt5a significantly promoted transendothelial migration of primary patient CLL cells in a gradient of
CCL19 (Fig. 4A), to a lesser extent in the gradient of CXCL12 (Supplementary Fig. S4F) but showed no effect in the gradient of CCL21 (Supplementary Fig. S4G). This suggests that cooperation between Wnt/PCP and chemokine signaling is not limited to chemotaxis but takes place also in the process of transendothelial invasion.

To check whether invasion is controlled by PCP signaling, specifically via CK1ε-Dvl2-Rho pathway (as shown in Fig. 3 for chemotaxis), we have carried out another set of experiments in MEC1 cells. Of the 3 tested chemokines, only CCL19 was able to induce transendothelial migration of MEC1 cells (Supplementary Fig. S4H). This response has not been promoted by Wnt5a but has been almost completely abolished by CK1 inhibition (Fig. 4B). Similar to the basal chemotaxis, the downregulation of the key PCP protein Dvl2 by siRNA (Fig. 4C) as well as inhibition of ROCK kinase (by Y27632) and porcupine (by IWP-2; Fig. 4D) efficiently reduced also transendothelial invasion of MEC1 cells. In summary, these results show that PCP proteins mediate both migration/chemotaxis and chemokine-driven invasion of CLL cells.

**PCP proteins regulate CLL homing in vivo**

To test the role of PCP proteins in CLL cells in vivo, we transplanted primary CLL cells into NSG mice (Fig. 5A), which lack mature T cells, B cells, and functional natural killer cells (35). Primary CLL cells efficiently home in NSG mice and can be detected mainly in spleen, liver, and bone marrow. Interestingly, pretreatment with the casine kinase 1 inhibitor D4476 decreased homing to spleen, liver, and bone marrow without any effect on the apoptosis of the cells (Fig. 5B and C; for typical dot plots for apoptosis assay, see Supplementary Fig. S5A). This experiment suggests that inhibition of CK1 activity diminishes the ability of CLL cells to migrate and colonize murine tissues. Interference with the Ror1- and Fzd7-driven signaling by treatment with antibodies directed against Ror1 and Fzd7 (for validation of anti-Fzd7 antibody, see Supplementary Fig. S5B) did not affect the survival of CLL cells either (Fig. 5D). Importantly, treatment with the blocking anti-Ror1 antibody (28) efficiently eliminated homing of CLL cells from most patients to the spleen (Fig. 5E). The homing to bone marrow could not be determined because treatment with any antibody (even control IgG) for unknown reasons completely abolished migration to bone marrow in vivo (data not shown). Liver infiltration was diminished only for some patients (Fig. 5F). Treatment with the anti-Fzd7 antibody did not show any effect on the CLL cells in liver (Fig. 5F); however, decreased spleen infiltration by CLL cells occurred in a subset of patients (Fig. 5E). Interestingly, the patient subset responding to anti-Fzd7 antibody had mutated p53, a strong marker of aggressive disease, whereas the patients not responding to anti-Fzd7 antibody treatment had wild-type (wt) p53.

In summary, these results show that CK1 activity and the cell surface receptors Ror1 and Fzd7 regulate biologic properties of CLL cells in vivo. These observations are consistent with the in vitro results and further strengthen the conclusion that PCP proteins act as regulators of CLL biology and pathogenesis.

**PCP-high patient cohorts show worse clinical characteristics**

The expression as well as functional data suggested that PCP proteins are strongly associated with CLL cell behavior. In the next step, we analyze the clinical significance of PCP protein expression by analysis of samples from 93 previously untreated patients (identical to patients analyzed in Fig. 1A). The normalized expression of Fzd3, Fzd7, Celsr1, Vangl2, and Prickle1 assessed by qRT-PCR (see also Fig. 1A) was correlated to the clinical parameters.

Importantly, the increased expression of Fzd3, Fzd7, Vangl2, and Prickle1 correlated positively with unmutated immunoglobulin heavy chain (IGHV; Table 1), which is strongly associated with worse prognosis. In contrast, patients with unfavorable chromosomal aberrations del11q22-23 (ATM) or del17p13 (TP53) did not show significantly higher expression of PCP genes. However, it should be noted that the number of patients with del11q22-23 (n = 17; 18%) or del17p13 (n = 7; 7.5%) was rather low and insufficient for robust statistical analysis.

Next, we analyzed the association of PCP gene expression with the treatment-free survival (TFS) using Kaplan–Meier survival curves. Patients were sorted into 2 groups (high vs. low) based on the mRNA levels (as determined by qRT-PCR) of individual PCP genes (Fzd3, Fzd7, Celsr1, Vangl2, and Prickle1). The cutoff for each gene was set up as the value corresponding to 75% percentile for the expression levels in normal B cells from the periphery (see Fig. 1A and below). TFS analysis was conducted in both groups of patients. Importantly, Fzd3-, Fzd7-, and Prickle1-high patient cohorts had significantly (Breslow test, P < 0.05) worse prognosis than the group of patients with low expression of these genes (Fig. 6). Specifically, the median TFS of Fzd3-low (cutoff 1.2) patients was 40 months compared with 17 months in Fzd3-high group, 54 versus 25 months for Fzd7 low versus high (cutoff 2.0), and 54 versus 31 months for Prickle1-low versus Prickle1-high (cutoff 1.0) patient cohorts. Very similar data were obtained when we conducted the analysis with the data normalized to β-actin (Supplementary Fig. S6).

These findings suggest that mainly more aggressive CLL cells with unmutated IGHV use PCP pathway to regulate their chemotaxis toward chemokines. Indeed, when we looked at unmutated IGHV patient subset only, we have not observed any statistically significant difference in survival curves between PCP-high and -low patient cohorts (data not shown). In summary, the expression analyses in patients with CLls showed that (i) the expression of key PCP genes correlates with clinically important mutational status of IGHV and (ii) on the clinical sample show the relevance of processes controlled by PCP pathway for disease pathogenesis.

**Discussion**

In the present study we, for the first time, show that (i) core PCP components such as Vangl2, Prickle1, CK1ε, Dvl, and Celrs1 are upregulated in CLls, (ii) PCP pathway components help regulate chemotaxis and transendothelial migration in the chemokine gradient and in vivo homing of CLL cells, and
(iii) PCP-high patient cohorts show worse clinical parameters such as TFS. Our findings suggest that PCP proteins affect CLL pathogenesis via regulation of chemokine-driven migration and as such influence disease progression.

Our data suggest that PCP pathway contributes to the CLL pathogenesis mainly via regulation of chemotactic responses to chemokines, which are the leading mediators of the interaction between CLL cells and their microenvi-

Figure 5. PCP proteins affect engraftment of CLL cells in vivo. A, scheme of the experiment. Typical FACS dot blot is shown (see gate G1 for transplanted cells). B, the level of apoptosis in primary CLL cells after dimethyl sulfoxide (DMSO)/D4476 (CK1 inh. I) was determined by flow cytometric analysis of the mitochondrial membrane potential using tetramethylrhodamine ethyl ester dye in combination with the green calcine staining. C, proportion of CLL cells (number of positive cells/number of total cells in the organ) treated with DMSO or D4476 (CK1 inh. I) that were recovered from the spleen (C1), liver (C2), and bone marrow (C3) of transplanted mice. D, treatment with the αRor1 and αFZD7 antibodies does not induce apoptosis as determined by tetramethylrhodamine ethyl ester analysis. E and F, proportion of CLL cells, when treated with a nonspecific antibody (IgG) or antibodies directed against Ror1 and FZD7, were recovered from the mouse spleen (E) or liver (F). Please note that samples responding to αFZD7 come from patients with mutated p53 (p53 MUT). B and D, graphs show mean ± SEM. C and E, one symbol on the graph represents one patient. * P < 0.05, Wilcoxon paired t test (C) or one-way ANOVA and Tukey post hoc test (E).
ronment (for review, see ref. 36). Components of the PCP machinery thus become an interesting target for the novel therapies, which interfere with the communication between CLL cells and their environment, similarly to the inhibitors of chemokine receptor or B-cell receptor (BCR) signaling pathways (37–39). In agreement with these data, our observations show that PCP-controlled migration is mainly relevant for the CLL cells with unmutated IGHV, which were shown to be more dependent on chemokine and BCR signaling.

Our data provide to our best knowledge (i) the first evidence for the increased levels of the conserved proteins unique for the PCP pathway—Prickle1, Vangl2, Celsr1—in leukemia and (ii) the first implication for the role of Celsr1 in human cancer. PCP signaling has been, however, functionally implicated earlier in the metastatic process of various solid tumors with the best defined role in melanoma, breast, and gastric cancer (40–42). It has been convincingly shown, mainly in melanoma, that PCP pathway increases the capacity of tumor cells to migrate and invade into surrounding tissue (43). Our data support the possibility that PCP pathway mediates physical aspects of cell migration via its effects on cytoskeleton and that chemokine signaling can serve as the navigator of invading cells into their final destination.

Interesting prediction of this hypothesis is that PCP pathway is dynamically and temporarily used by migrating immune

Table 1. The expression of PCP genes determined by qRT-PCR in the patient cohorts with mutated (IGHV MUT) and unmutated (IGHV UNMUT) immunoglobulin heavy chain

<table>
<thead>
<tr>
<th>Gene name</th>
<th>IGHV MUT (n = 39)</th>
<th>IGHV UNMUT (n = 54)</th>
<th>P (Mann-Whitney test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FZD7</td>
<td>2.697 (±0.9081)</td>
<td>3.674 (±0.6834)</td>
<td>0.0075</td>
</tr>
<tr>
<td>FZD3</td>
<td>0.7684 (±0.07373)</td>
<td>1.217 (±0.1130)</td>
<td>0.0054</td>
</tr>
<tr>
<td>VANGL2</td>
<td>4.937 (±2.137)</td>
<td>11.12 (± 6.119)</td>
<td>0.007</td>
</tr>
<tr>
<td>PRICKLE1</td>
<td>1.942 (±0.2322)</td>
<td>2.978 (±0.2950)</td>
<td>0.0113</td>
</tr>
</tbody>
</table>

NOTE: Numbers indicate the mean, ±SEM; n, number of patients; P, statistical significance of the difference between both cohorts determined by Mann-Whitney test.

Figure 6. Expression of PCP genes defines CLL progression. Patients were sorted into 2 groups (high vs. low expression; cutoff indicated by the line in the upper graph) based on the mRNA levels of individual PCP genes (FZD3, FZD7, and PRICKLE1), and Kaplan-Meier survival curves (TFS) were plotted and survival analysis was conducted in both groups of patients. PRICKLE1-(A), FZD3- (B), and FZD7-high (C) patient cohorts had a significantly (Breslow test, P < 0.05) worse prognosis than the group of patients with low expression of these genes.
cells during the immune response. This interesting topic clearly exceeds the current study and has to be tested in future.

In summary, our findings provide strong evidence that core components of the Wnt/PCP pathway play an important role in CLL pathogenesis via regulation of CLL migration. We for the first time show the biologic importance of the crosstalk between chemokine and PCP signaling, which might have implication for understanding of the biology of invasiveness in other tumors. Given the fact that Wnt/PCP pathway is an evolutionary conserved regulator of cell polarity and migration, our data further emphasize the role of cell migration in the development of CLLs.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: M. Kaucká, P. Krejčí, Š. Pospíšilová, V. Bryja Development of methodology: M. Kaucká, P. Krejčí Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Kaucká, K. Plevová, S. Pavlova, P. Janovská, A. Mishra, J. Verner, J. Procházková, J. Kotalová, B. Tichý, Y. Brychtová, M. Doubek, J. Mayer
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References
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