Biochemical and Biophysical Research Communications 449 (2014) 163–168

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

PFTK1 interacts with cyclin Y to activate non-canonical Wnt signaling in hepatocellular carcinoma

Tingting Sun [a], Ngai Na Co [a], Nathalie Wong [a,b,*]

[a] Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Shatin, Hong Kong, China
[b] State Key Laboratory in Oncology in South China, The Chinese University of Hong Kong, Shatin, Hong Kong, China

ARTICLE INFO

Article history:
Received 30 April 2014
Available online 10 May 2014

Keywords:
Hepatocellular carcinoma
PFTK1
Cyclin Y
Cell motility
Wnt pathway

ABSTRACT

PFTK1 is a Cdc2-related protein kinase that is frequently upregulated in human hepatocellular carcinoma (HCC) where it correlates with metastatic features and motile phenotypes. To understand the modulated pathway underlining the PFTK1 action, here we show a physical interaction between PFTK1 and cyclin Y (CCNY) in promoting noncanonical Wnt signaling. In HCC cells, we found PFTK1 forms a direct complex with CCNY, and together readily upregulate key components of Wnt signaling (Dvl2 and Naked1). Exogenous expression of PFTK1 and CCNY activated Rho GTPases, which are known targets of the noncanonical path. In line with Rho GTPases activation, we also found marked actin polymerizations in cells with PFTK1–CCNY co-expressions. Our findings highlight a PFTK1–CCNY complex in activating noncanonical Wnt signaling in HCC cells.

1. Introduction

Wnt protein is a family of secreted glycoproteins that could trigger intracellular signalings through binding with the receptor Frizzled family and other Wnt ligands on cell membrane. The Wnt pathway functions in multiple cellular biological processes and is involved in various human diseases including cancer [1–4]. The complex Wnt signalings are divided into two branches: the canonical β-catenin-dependent pathway and the noncanonical planar cell polarity (PCP) and Wnt/Ca^{2+} pathways. Canonical Wnt signals activate target genes through stabilization and nuclear translocation of β-catenin, controlling cell proliferation and cell fate determination; while noncanonical Wnt signals involve activation of Rho family of small GTPases and an increase of intracellular Ca^{2+}, thereby regulating cell polarity and motility [5].

PFTK1, also named PFTAIRE1, is a member of Cdc2-related serine/threonine protein kinases which share a highly conserved motif PSTAIRE and are crucial regulators of cyclins and cell cycle [6]. Human PFTK1 is highly expressed in the brain, pancreas, kidney, heart, testis and ovary, but low in placenta, lung and the liver [6]. The function of PFTK1 is recently reported as a cyclin-dependent kinase (CDK) regulating cell cycle progression and cell proliferation by specifically interacting with members of cyclin proteins such as cyclin D3 (CCND3) and cyclin Y (CCNY) [7,8]. Interestingly, the interaction of PFTK1 with CCNY, a cyclin protein with key functions in *Drosophila* embryogenesis [9], is reported to corporately mediate phosphorylation of low-density-lipoprotein receptor-related protein 6 (LRP6) in *Drosophila* [8–10]. It is well known that phosphorylation of transmembrane receptor LRP6 represents an important event and an initial step of the canonical Wnt signaling cascade [11–13], suggesting a potential role of PFTK1–CCNY complex in controlling the Wnt pathway. However whether this PFTK1–CCNY protein interaction also exists in cancer and its effect on the Wnt pathway in tumor cells remain to be explored.

Hepatocellular Carcinoma (HCC) is the fifth most common cancer and ranks third among causes of cancer-related deaths in men worldwide [14]. It is highly fatal since survival patients from diagnosis is often less than six months and only 5–9% of patients survive over five years [14]. High incidence of recurrence and metastasis is the principal causes of poor prognosis after hepatic resection in individuals with HCC [15]. The elucidation of molecular basis and biological mechanisms by which HCC cells disseminate hence hold much importance in understanding HCC metastasis. Previously our group reported regional chr. 7q21-q22 gain is closely associated with advanced metastatic HCC [16]. PFTK1 was further defined as a target proto-oncogene from this aberrant region [17]. We found common overexpression of PFTK1 in HCC tumor tissue compared to adjacent non-tumoral liver, and more interestingly upregulation of PFTK1 was more profound in

* Corresponding author at: Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, N.T., Hong Kong, China. Fax: +852 2637 6274.
E-mail address: natwong@cuhk.edu.hk (N. Wong).

http://dx.doi.org/10.1016/j.bbrc.2014.05.002
0006-291X/

cases with microvascular invasion and associated with poorly differentiated tumors [17,18]. Functionally, PFTK1 could confer cell invasive potentials and cell motilities through actin cytoskeletal polymerization as evident by both ectopic expression and knockdown investigations in HCC cells [17].

Given that HCC is one of the most common cancer type that shows activation of Wnt pathway [19], we thus hypothesis that PFTK1 might control the Wnt signaling pathway in HCC cells via interacting with cyclin Y.

2. Materials and methods

2.1. Cell culture

HCC cell lines, HKCI-3 and HKCI-C3, were established from patients who underwent curative surgery for HCC at Prince of Wales Hospital, Hong Kong [20]. These cell lines were cultured in AIM-V medium supplemented with 10% fetal bovine serum, 1× L-glutamine and 1× NEAA (Life Technologies, Carlsbad, CA).

2.2. Transfection

Full length cDNA of PFTK1 and CCNY were cloned to pEF6/V5-His and pCMV-Flag-3A vectors, respectively. The authenticity of both constructs was verified by DNA sequencing. PFTK1-pEF6/V5-His and CCNY-pCMV-Flag-3A constructs were allowed to form complexes with Lipofectamine 2000 (Life Technologies) according to the manufacturer's recommendations and then incubated with HKCI-3 and HKCI-C3 cells. The overexpression of PFTK1 and CCNY in cells was confirmed by Western blot analysis using anti-V5 and anti-Flag antibodies, respectively.

2.3. Western blot analysis

The cells were washed thrice with ice-cold PBS and lysed in RIPA lysis buffer supplemented with 1× protease inhibitor cocktail and 1× PhosStop (Roche Diagnostics, Indianapolis, IN). The proteins were separated on SDS-polyacrylamide gels and electrophoretically transferred to PVDF membranes (Roche Diagnostics). The membranes were incubated with primary antibodies against Flag (#F3165; Sigma, St. Louis, MO), V5 (#46-0705, Life Technologies), LRP6 (#2560; Cell Signaling, Danvers, MA), phospho-LRP6 (Ser1490) (#2568, Cell Signaling), Axin 1 (#2087, Cell Signaling), β-catenin (#610153; BD, Franklin Lakes, NJ), active β-catenin (#05-665; Millipore, Billerica, MA), GSK-3β (#9315, Cell Signaling), Phospho-GSK-3β (Ser9) (#5558, Cell Signaling), Naked1 (#2201, Cell Signaling), Dvl2 (#3224, Cell Signaling), Dvl3 (#3218, Cell Signaling) or GAPDH (#MAB374, Millipore) for overnight followed by appropriate horseradish peroxidase-conjugated secondary antibodies at 1:10,000 dilution (Santa Cruz Biotechnology, Santa Cruz, CA) for 2 h at room temperature. Signals were developed by using ECL chemiluminescence detection reagents (Thermo Scientific, Franklin, MA) and visualized on X-ray film (Fuji Photo Film, Tokyo, Japan).

2.4. Immunoprecipitation

Cells were seeded in 100 mm plates and each plate was transfected with V5, PFTK1-V5, Flag or CCNY-Flag, respectively. After 48 h, cells were washed twice using ice cold PBS and lysed in RIPA lysis buffer containing 1× protease inhibitor cocktail and 1× PhosStop (Roche Diagnostics). The cell lysate was incubated on ice for 30 min and centrifuged at 14,000 rpm at 4 °C for 10 min. Protein concentrations were measured by the Bradford assay (Bio-Rad Laboratories, Hercules, CA) at an absorbance of 595 nm. Two milligram protein lysates were pre-cleared with 40 μl of rec-Protein G-Sepharose resin slurry (#101242, Life Technologies) at 4 °C for 1 h to eliminate non-specific protein bindings. The precleared supernatants were incubated with 1 μg anti-V5 or anti-Flag antibodies at 4 °C for overnight with agitation. Immunocomplexes were collected using 50 μl of rec-Protein G-Sepharose resin slurry at 4 °C for 4 h with agitation. The resin was washed with lysis buffer four times and the immunocomplexes were recovered by heating at 100 °C for 2 min in 2× SDS Laemmli sample buffer. The recovered immunocomplexes were subjected to Western blot analysis using anti-Flag and digoxigenin-labeled anti-V5 as primary antibodies and anti-mouse IgG HRP (Santa Cruz Biotechnology) and anti-digoxigenin-POD (Roche Diagnostics) as secondary antibodies, respectively. Signals were developed by using ECL chemiluminescence detection reagents (Thermo Scientific) and visualized on X-ray film (Fuji Photo Film).

2.5. GTPase activity assays

HKCI-3 and HKCI-C3 cells were transiently transfected with V5 tagged PFTK1 and/or Flag tagged CCNY for 48 h and subjected to Rho Activation Assay or Rac1/Cdc42 Activation Assay (Millipore). In brief, cells were washed twice with 1× PBS and then lysed in Mg^{2+} lysis buffer supplemented with 10% glycerol, 1× protease inhibitor cocktail and 1× PhosStop (Roche Diagnostics). The lysed cells were collected, incubated on ice for 30 min and centrifuged 14,000*g* at 4 °C for 5 min. The lysates were incubated with 15 μl slurry of Rho Assay Reagent (Rhotekin Rho binding domain) or 10 μl slurry of Rac1/Cdc42 assay reagent (PAK-1 PBD, agarose) at 4 °C for 1 h. The agarose beads were washed thrice with Mg^{2+} lysis buffer supplemented with 10% glycerol and collected by centrifugation at 14,000*g*. The agarose beads were resuspended in 2× Laemmli reducing sample buffer and boiled for 5 min prior to resolution on SDS–PAGE. Western blots were performed using primary antibodies against RhoA (#2117, Cell Signaling), Rac1 (#2467, Cell Signaling) and Cdc42 (#2466, Cell Signaling).

2.6. TOP/FOPflash reporter assay

Luciferase activities were measured in HKCI-3 and HKCI-C3 following transiently transfection with PFTK1 and/or CCNY constructs. Five hundred nanograms of pTOPflash or pFOPflash with 20 ng Renilla plasmid were co-transfected. At 24 h and 48 h after transfection, the activities of firefly and Renilla luciferases were detected using Dual-Luciferase® Reporter Assay System (Promega, Madison, WI). Relative luciferase activities were calculated by normalizing the ratios of TOP/FOP with Renilla luciferase activities.

2.7. Immunofluorescence staining

Transfected HKCI-3 and HKCI-C3 cells with PFTK1 and/or CCNY were seeded onto glass coverslips and cultured in complete medium for 24 h. Cells fixed in 4% paraformaldehyde were permeabilized with 0.5% Triton X-100. Filamentous actin was stained with tetramethylrhodamine B isothiocyanate (TRITC)-labeled phalloidin (Sigma). Finally, cells were counterstained with DAPI and examined by confocal microscopy (LSM5 PASCAL; Carl Zeiss, Gottingen, Germany).

2.8. Statistical analysis

All values were presented as the mean ± S.E.M. from at least 3 independent experiments. Student's *t* test was used to compare differences of experimental data obtained between groups. *P*-value less than 0.05 was considered a statistically significant difference.

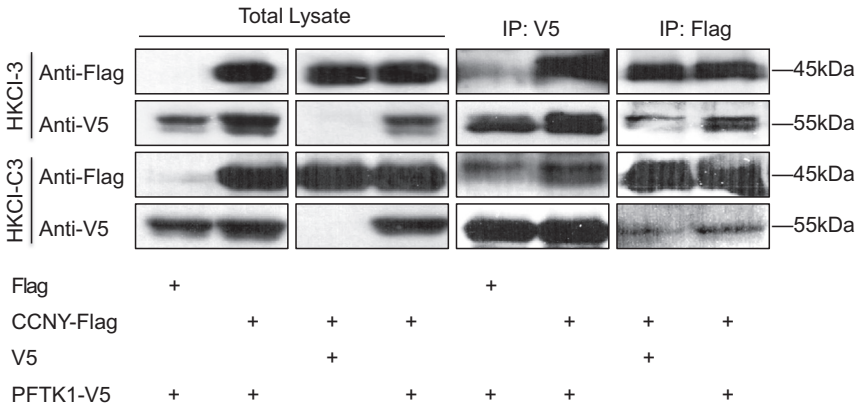

Fig. 1. PFTK1 interacts with cyclin Y (CCNY) in hepatocellular carcinoma (HCC) cells. HCC cells HKCI-3 and HKCI-C3 were transfected with PFTK1-V5 and CCNY-Flag plasmids alone or combined. Transfections of Flag and/or V5 vector plasmids were used as controls. Ectopic expressions of PFTK1 and CCNY in total lysates were confirmed by anti-Flag and anti-V5 antibodies, respectively. After cell lysates were immunoprecipitated with anti-V5 antibody, anti-Flag antibody was used to detect the existence of CCNY in protein complex pulled down by anti-V5 antibody. Co-immunoprecipitation was repeated using anti-Flag antibody reversely.

3. Results

3.1. Protein interaction between PFTK1 and CCNY in HCC cells

We first determined the physical association of *PFTK1* and cyclin Y (CCNY) in two HCC cell lines. Full length PFTK1 and cyclin Y were cloned into V5-pEF6 and Flag-pCMV vectors respectively, and cotransfected into HKCI-3 and HKCI-C3 cells both of which express low levels of PFTK1. Forced expression of PFTK1 and CCNY in total lysates was first verified by Western blot using anti-V5 and anti-Flag antibodies, respectively (Fig. 1). Coimmunoprecipitation was then applied to investigate the protein interaction between ectopic expressed PFTK1 and CCNY in the two cell lines. Western blot showed that in both HKCI-3 and HKCI-C3 cells PFTK1 could be identified in the immunocomplex pull-down by anti-Flag (CCNY) antibody in the co-transfected cells; whereas no PFTK1 was detected in control samples without PFTK1 transfection (Fig. 1). Inverse immunoprecipitation was repeated using anti-V5 (PFTK1) antibody and CCNY was also detectable in co-transfected cells. The result confirmed a direct physical interaction between PFTK1 and cyclin Y in HCC cells.

3.2. Interaction of PFTK1 with CCNY did not alter canonical Wnt pathway

To delineate the effect of PFTK1–CCNY complex on the canonical β-catenin-dependent Wnt signaling, we examined the expression of several key Wnt components by Western blot. In cells coexpressing PFTK1 and CCNY, results showed that neither PFTK1 nor CCNY alone or in combination could affect the phosophorylation of LRP6 at Ser-1490 when compared with total LRP6 levels (Fig. 2A). This suggested that PFTK1 and CCNY might not cooperate to induce the activity of LRP6 in HKCI-3 and HKCI-C3. Furthermore, PFTK1 and/or CCNY overexpression has no effect on the protein levels of Axin1, GSK-3β, phosphorylated GSK-3β at Ser-9, β-catenin and active β-catenin in both HKCI-3 and HKCI-C3 (Fig. 2A), indicating PFTK1 and CCNY might not play a role in canonical Wnt/β-catenin signaling.

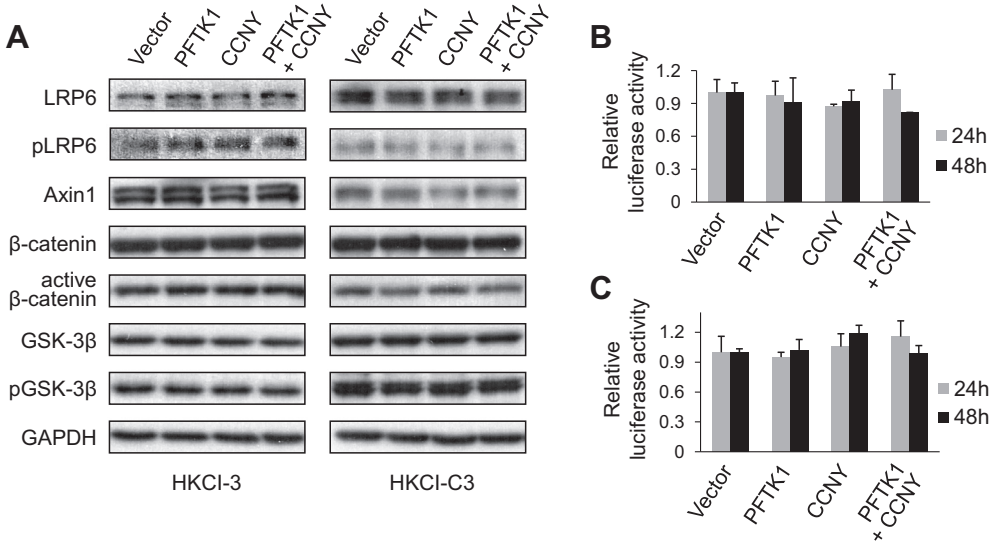

Fig. 2. PFTK1 and cyclin Y (CCNY) do not activate Wnt/β-catenin signaling. (A) HKCI-3 and HKCI-C3 were transfected with PFTK1 and/or CCNY, and key components in Wnt/β-catenin signaling (LRP, pLRP, Axin1, β-catenin, active β-catenin, GSK-3β and pGSK-3β) were analyzed by Western blotting. (B) TOP/FOP Flash luciferase assay showed no significant changes after HKCI-3 and HKCI-C3 cells transfected with PFTK1 and CCNY for 24 h or 48 h. Representative Western blotting images from three independent experiments were shown.

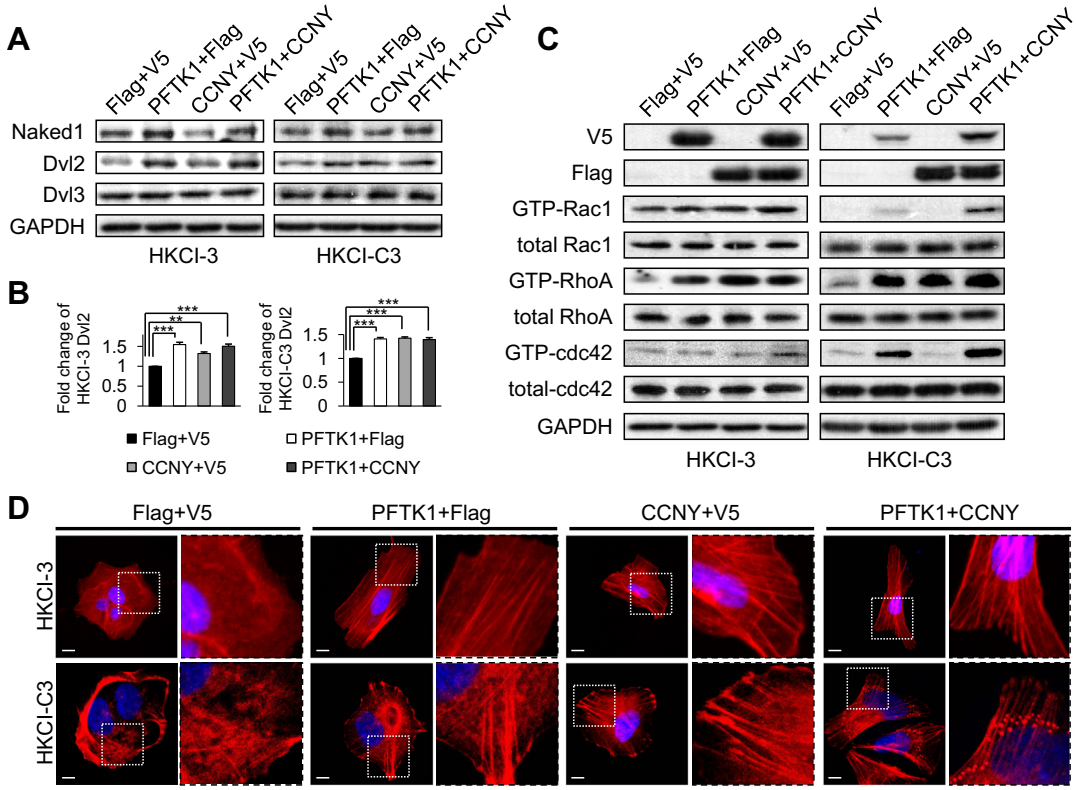

Fig. 3. PFTK1 and cyclin Y (CCNY) activates noncanonical Wnt signaling. (A) Key components in noncanonical Wnt signaling (Naked1, Dvl2 and Dvl3) were analyzed by Western blotting. (B) Densitometry analysis of Dvl2 in HKCI-3 (left) and HKCI-C3 (right) was shown. $^{**}p < 0.01$, $^{***}p < 0.001$, $n = 3$. (C) Cells were transfected with PFTK1 and/or CCNY, and over-expression was confirmed by Western blotting using anti-Flag and anti-V5 antibodies. After GTPase activity assay was used to pull down GTP-bound Rac1, RhoA and Cdc42, activated (GTP-) and total Rac1, RhoA and Cdc42 were analyzed by Western blotting. (D) Immunofluorescence microscopy for phalloidin staining showed the degree of actin polymerization in PFTK1 and/or CCNY transfected cells. Scale bar = 20 μm (HKCI-3). Scale bar = 10 μm (HKCI-C3). Representative Western blotting and immunofluorescence staining images from three independent experiments were shown.

We further affirmed Western analysis by TOP/FOPflash luciferase reporter assay. In concordance with unaffected active β-catenin protein levels by PFTK1 and CCNY, we did not find a significant increase in TOPflash luciferase activities at both 24 h and 48 h after of PFTK1 and CCNY cotransfection in HKCI-3 and HKCI-C3 cells (Fig. 2B and C).

3.3. PFTK1 and CCNY complex activates noncanonical Wnt pathway

Given that the noncanonical Wnt signaling are involved in cytoskeletal movement and cell motility that are closely related to cell migration and cancer metastasis, we continued to elucidate the effect of PFTK1 and CCNY on the expression of key molecules in the β-catenin-independent noncanonical Wnt pathways. In both HKCI-3 and HKCI-C3 cells cotransfected with PFTK1 and CCNY, marked induction in the level of Dvl2 and increase in Naked1 was readily shown from Western blot (Fig. 3A and B). We also noticed that PFTK1 or CCNY alone could induce expression of Dvl2, and Naked1 level was slightly induced by PFTK1 alone.

To further determine whether overexpression of PFTK1 and CCNY could affect the activity of downstream molecules in noncanonical Wnt signaling, the GTP/GDP binding status of RhoA, Rac1 and Cdc42 was investigated. By Rhotekin Rho binding domain (RBD) and PAK p21 binding domain (PBD) pull-down assays, the amounts of GTP-bound RhoA, Rac1 and Cdc42 were found to be significantly increased upon PFTK1 and CCNY cotransfections, while total protein levels of RhoA, Rac1 and Cdc42 remained unchanged (Fig. 3C). In HKCI-C3, PFTK1 alone also increased the active form of Rac1 and Cdc42; and active RhoA levels were induced by PFTK1 or CCNY alone in both cells. Collectively, the results suggested that PFTK1–CCNY complex likely activates the Dvl2 and Naked1 in noncanonical Wnt pathway and the downstream cascade that involves the Rho GTPases.

3.4. PFTK1 and CCNY regulate actin polymerization in HCC cells

Actin stress fibers assembly is a well-known process that is regulated by Rho GTPase family in modulating cell motility [21–23]. We therefore further examined the effect of PFTK1 and CCNY on filamentous actin organization. Immunofluorescence analysis using phalloidin staining revealed marked polymerization of actin stress fibers in PFTK1 and/or CCNY transfected cells (Fig. 3D), suggesting that PFTK1 and CCNY activation of the noncanonical Wnt signaling could result in the active formation of actin stress fibers in HCC cells.

4. Discussion

In this study, we demonstrated the presence of protein interaction between PFTK1 and cyclin Y in HCC cells. The PFTK1–CCNY complex activates the β-catenin-independent noncanonical Wnt pathway but keeps the canonical Wnt/β-catenin intact. The subsequent activation of Rho GTPases (RhoA, Rac1 and Cdc42) in the noncanonical Wnt pathways further leads to actin polymerization and stress fibers formation.

Previously Jiang and co-workers identified cyclin Y as a PFTK1 interacting protein by yeast two-hybrid screening [8]. Although the interaction was further confirmed in mouse brain endogenously and 293T cells with exogenous expression of CCNY and PFTK1 [8], whether the two proteins interact with each other in

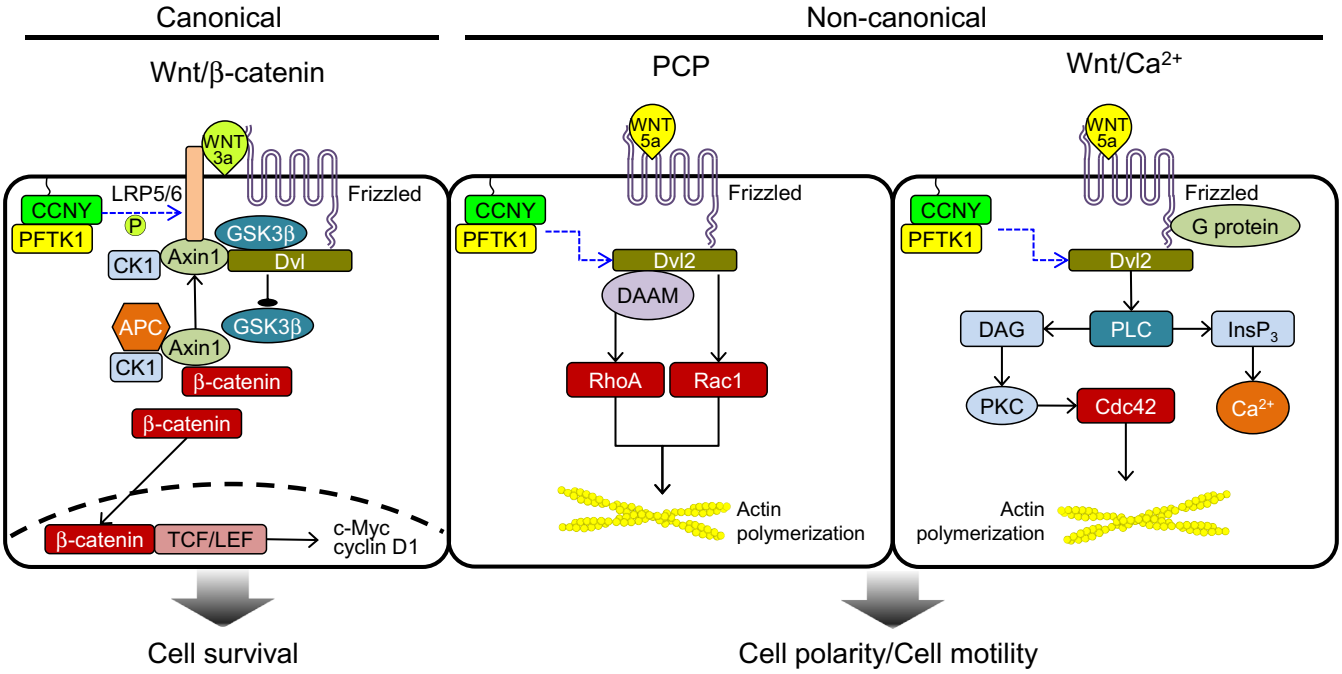

Fig. 4. Schematic diagram of PFTK1/CCNY participating in canonical and noncanonical Wnt pathways. In *Drosophila* cells, PFTK1 interacts with CCNY and cooperates to phosphorylate LRP6 which activates the canonical Wnt/β-catenin cascades. In hepatocellular carcinoma (HCC) cells, interaction of PFTK1 and CCNY activates Dvl2 and the downstream RhoA, Rac1 and Cdc42 in noncanonical planar cell polarity (PCP) pathway and Wnt/Ca^{2+} pathway leading to actin polymerization.

other tissue types and cancer remain unknown. Here, we report in HCC cells there also exists the physical interaction between these two proteins, suggesting that PFTK1 interacting with cyclin Y may be universal and of great significance in cell activities. Nevertheless, different from the findings that PFTK1 and cyclin Y interact and cooperate to phosphorylate LRP6 in *Drosophila* [10], we observe an intact canonical Wnt/β-catenin signaling including unchanged LRP6 phosphorylation in HCC cells. It is thus reasonable to presume that the functions and downstream targets consequential to the activation of PFTK1–CCNY complex vary depending on cell contexts.

It is well-known that canonical Wnt/β-catenin pathway plays an important role in human tumorigenesis [24,25]. Nevertheless, increasing evidence has emerged to indicate components of the noncanonical Wnt pathways are also commonly overexpressed in breast, colon, lung and gastric cancers [26–29]. We observed activation of the noncanonical Wnt pathways in HCC cells with PFTK1 and cyclin Y co-expression, while maintaining a quiescent canonical Wnt pathway. The expression of dishevelled segment polarity protein 2 (Dvl2) was markedly up-regulated in cells co-transfected with PFTK1 and CCNY. Although Dvl2 is able to activate both the noncanonical pathway and the canonical β-catenin pathway [30], we further found the protein expression level of naked cuticle homolog 1 (Naked1) was also up-regulated. Naked1 inhibits the canonical Wnt/β-catenin signaling by directing the protein activity of Dvl towards the noncanonical pathway upon binding with Dvl [31,32].

The noncanonical Wnt pathways consist of the planar cell polarity (PCP) pathway and the calcium signaling pathway [1,33], both of which lead to the activation of Rho family of small GTPases (RhoA, Rac1 and Cdc42) and actin polymerization (Fig. 4). Our data verified the activation of the noncanonical Wnt pathway by demonstrating the increased activated form of RhoA, Rac1 and Cdc42 and marked polymerization of filamentous actin in PFTK1 and CCNY co-transfected cells. Because Rho GTPases regulate the processes of cytoskeletal dynamics and formation of lamellipodia, filopodia and actin stress fibers, it is conceivable these features are important in the dissemination of primary cancer cells [34–36]. Our findings may be interpreted as PFTK1 promotes HCC metastasis through interacting with cyclin Y and activating the noncanonical Wnt pathway. Interestingly, we found a portion of the noncanonical Wnt pathway molecules was activated following transfection of PFTK1 only (Fig. 3A Naked1&Dvl2, Fig. 3C Cdc42 in HKCI-C3). It is probable that ectopic expressed PFTK1 may interact with endogenous CCNY in specific cell line. Given that the effect of PFTK1 alone on GTP-Cdc42 is less strong than that of PFTK1 and CCNY co-transfection (Fig. 3C HKCI-C3), another possibility is PFTK1 itself may be able to induce Rho GTPases through an alternate mechanism, which requires further investigation.

To date, HCC is still a high malignant human tumor that is associated with high mortality rate due to frequent metastasis. In this study, we continue to delineate the role of PFTK1 in HCC. Our work outlined a molecule pathway in HCC cells that PFTK1 binds with cyclin Y in activating the Rho GTPases in the noncanonical Wnt pathways which result in the actin polymerization that underlies cell motility and migration. The identification of signaling cascade might provide understandings on HCC metastasis and novel information for development of new drugs and regimen.

Acknowledgment

This work was supported by a General Research Fund from the Hong Kong Research Grants Council (Ref. No.: CUHK 476711).

References

[1] T.P. Rao, M. Kuhl, An updated overview on wnt signaling pathways: a prelude for more, Circ. Res. 106 (2010) 1798–1806.
[2] C.Y. Logan, R. Nusse, The wnt signaling pathway in development and disease, Annu. Rev. Cell Dev. Biol. 20 (2004) 781–810.
[3] H. Clevers, Wnt/beta-catenin signaling in development and disease, Cell 127 (2006) 469–480.
[4] K. Schlessinger, A. Hall, N. Tolwinski, Wnt signaling pathways meet rho GTPases, Genes Dev. 23 (2009) 265–277.

[5] M. Katoh, M. Katoh, WNT signaling pathway and stem cell signaling network, Clin. Cancer Res. 13 (2007) 4042–4045.
[6] T. Yang, J.Y. Chen, Identification and cellular localization of human PFTAIRE1, Gene 267 (2001) 165–172.
[7] F. Shu, S. Lv, Y. Qin, X. Ma, X. Wang, X. Peng, Y. Luo, B.E. Xu, X. Sun, J. Wu, Functional characterization of human PFTK1 as a cyclin-dependent kinase, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 9248–9253.
[8] M. Jiang, Y. Gao, T. Yang, X. Zhu, J. Chen, Cyclin Y, a novel membrane-associated cyclin, interacts with PFTK1, FEBS Lett. 583 (2009) 2171–2178.
[9] D. Liu, R.L. Finley Jr., Cyclin Y is a novel conserved cyclin essential for development in drosophila, Genetics 184 (2010) 1025–1035.
[10] G. Davidson, J. Shen, Y.L. Huang, Y. Su, E. Karaulanov, K. Bartscherer, C. Hassler, P. Stannek, M. Boutros, C. Niehrs, Cell cycle control of wnt receptor activation, Dev. Cell. 17 (2009) 788–799.
[11] X. He, M. Semenov, K. Tamai, X. Zeng, LDL receptor-related proteins 5 and 6 in wnt/beta-catenin signaling: arrows point the way, Development 131 (2004) 1663–1677.
[12] K. Tamai, M. Semenov, Y. Kato, R. Spokony, C. Liu, Y. Katsuyama, F. Hess, J.P. Saint-Jeannet, X. He, LDL-receptor-related proteins in wnt signal transduction, Nature 407 (2000) 530–535.
[13] X. Zeng, H. Huang, K. Tamai, X. Zhang, Y. Harada, C. Yokota, K. Almeida, J. Wang, B. Doble, J. Woodgett, A. Wynshaw-Boris, J.C. Hsieh, X. He, Initiation of wnt signaling: control of wnt coreceptor Lrp6 phosphorylation/activation via frizzled, dishevelled and axin functions, Development 135 (2008) 367–375.
[14] P. Boyle, B. Levin (Eds.), World Health Organization World Cancer Report, IARC Press, Lyon, 2008.
[15] R. Tung-Ping Poon, S.T. Fan, J. Wong, Risk factors, prevention, and management of postoperative recurrence after resection of hepatocellular carcinoma, Ann. Surg. 232 (2000) 10–24.
[16] S.M. Sy, N. Wong, P.B. Lai, K.F. To, P.J. Johnson, Regional over-representations on chromosomes 1q, 3q and 7q in the progression of hepatitis B virus-related hepatocellular carcinoma, Mod. Pathol. 18 (2005) 686–692.
[17] E.Y. Pang, A.H. Bai, K.F. To, S.M. Sy, N.L. Wong, P.B. Lai, J.A. Squire, N. Wong, Identification of PFTAIRE protein kinase 1, a novel cell division cycle-2 related gene, in the motile phenotype of hepatocellular carcinoma cells, Hepatology 46 (2007) 436–445.
[18] W.K. Leung, A.K. Ching, A.W. Chan, T.C. Poon, H. Mian, A.S. Wong, K.F. To, N. Wong, A novel interplay between oncogenic PFTK1 protein kinase and tumor suppressor TAGLN2 in the control of liver cancer cell motility, Oncogene 30 (2011) 4464–4475.
[19] P. Polakis, Wnt signaling in cancer, Cold Spring Harb. Perspect. Biol. 4 (2012), http://dx.doi.org/10.1101/cshperspect.a008052.
[20] N. Wong, K.Y. Chan, P.F. Macgregor, P.B. Lai, J.A. Squire, B. Beheshti, M. Albert, T.W. Leung, Transcriptional profiling identifies gene expression changes associated with IFN-alpha tolerance in hepatitis C-related hepatocellular carcinoma cells, Clin. Cancer Res. 11 (2005) 1319–1326.
[21] A.J. Ridley, A. Hall, The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors, Cell 70 (1992) 389–399.
[22] R. Kaunas, P. Nguyen, S. Usami, S. Chien, Cooperative effects of rho and mechanical stretch on stress fiber organization, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 15895–15900.
[23] P. Hotulainen, P. Lappalainen, Stress fibers are generated by two distinct actin assembly mechanisms in motile cells, J. Cell Biol. 173 (2006) 383–394.
[24] J. Behrens, Control of beta-catenin signaling in tumor development, Ann. N. Y. Acad. Sci. 910 (2000) 21–33 (discussion 33–5).
[25] H.H. Luu, R. Zhang, R.C. Haydon, E. Rayburn, Q. Kang, W. Si, J.K. Park, H. Wang, Y. Peng, W. Jiang, T.C. He, Wnt/beta-catenin signaling pathway as a novel cancer drug target, Curr. Cancer Drug Targets 4 (2004) 653–671.
[26] T. Pukrop, F. Klemm, T. Hagemann, D. Gradl, M. Schulz, S. Siemes, L. Trumper, C. Binder, Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 5454–5459.
[27] J. Dejmek, A. Dejmek, A. Safholm, A. Sjolander, T. Andersson, Wnt-5a protein expression in primary dukes B colon cancers identifies a subgroup of patients with good prognosis, Cancer Res. 65 (2005) 9142–9146.
[28] C.L. Huang, D. Liu, J. Nakano, S. Ishikawa, K. Kontani, H. Yokomise, M. Ueno, Wnt5a expression is associated with the tumor proliferation and the stromal vascular endothelial growth factor–an expression in non-small-cell lung cancer, J. Clin. Oncol. 23 (2005) 8765–8773.
[29] M. Kurayoshi, N. Oue, H. Yamamoto, M. Kishida, A. Inoue, T. Asahara, W. Yasui, A. Kikuchi, Expression of wnt-5a is correlated with aggressiveness of gastric cancer by stimulating cell migration and invasion, Cancer Res. 66 (2006) 10439–10448.
[30] M.J. Smalley, N. Signoret, D. Robertson, A. Tilley, A. Hann, K. Ewan, Y. Ding, H. Paterson, T.C. Dale, Dishevelled (dvl-2) activates canonical wnt signalling in the absence of cytoplasmic puncta, J. Cell. Sci. 118 (2005) 5279–5289.
[31] K.A. Wharton Jr., G. Zimmermann, R. Rousset, M.P. Scott, Vertebrate proteins related to drosophila naked cuticle bind dishevelled and antagonize wnt signaling, Dev. Biol. 234 (2001) 93–106.
[32] D. Yan, J.B. Wallingford, T.Q. Sun, A.M. Nelson, C. Sakanaka, C. Reinhard, R.M. Harland, W.J. Fantl, L.T. Williams, Cell autonomous regulation of multiple dishevelled-dependent pathways by mammalian nkd, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 3802–3807.
[33] M. Tada, M.L. Concha, C.P. Heisenberg, Non-canonical wnt signalling and regulation of gastrulation movements, Semin. Cell Dev. Biol. 13 (2002) 251–260.
[34] K. Asanuma, E. Yanagida-Asanuma, C. Faul, Y. Tomino, K. Kim, P. Mundel, Synaptopodin orchestrates actin organization and cell motility via regulation of RhoA signalling, Nat. Cell Biol. 8 (2006) 485–491.
[35] C.H. Chan, S.W. Lee, C.F. Li, J. Wang, W.L. Yang, C.Y. Wu, J. Wu, K.I. Nakayama, H.Y. Kang, H.Y. Huang, M.C. Hung, P.P. Pandolfi, H.K. Lin, Deciphering the transcriptional complex critical for RhoA gene expression and cancer metastasis, Nat. Cell Biol. 12 (2010) 457–467.
[36] P. Gulhati, K.A. Bowen, J. Liu, P.D. Stevens, P.G. Rychahou, M. Chen, E.Y. Lee, H.L. Weiss, K.L. O'Connor, T. Gao, B.M. Evers, MTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways, Cancer Res. 71 (2011) 3246–3256.