

# FRK Inhibits Migration and Invasion of Human Glioma Cells by Promoting N-cadherin/ $\beta$ -catenin Complex Formation

Qiong Shi · Xu Song · Jun Wang · Jia Gu ·  
Weijian Zhang · Jinxia Hu · Xiuping Zhou · Rutong Yu

Received: 8 May 2014 / Accepted: 11 June 2014 / Published online: 27 June 2014  
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**Abstract** Fyn-related kinase (FRK), a member of Src-related tyrosine kinases, is recently reported to function as a potent tumor suppressor in several cancer types. Our previous study has also shown that FRK over-expression inhibited the migration and invasion of glioma cells. However, the mechanism of FRK effect on glioma cell migration and invasion, a feature of human malignant gliomas, is still not clear. In this study, we found that FRK over-expression increased the protein level of N-cadherin, but not E-cadherin. Meanwhile, FRK over-expression promoted  $\beta$ -catenin translocation to the plasma membrane, where it formed complex with N-cadherin, while decreased  $\beta$ -catenin level in the nuclear fraction. In addition, down-regulation of N-cadherin by siRNA promoted the migration and invasion of glioma U251 and U87 cells and abolished the inhibitory effect of FRK on glioma cell migration and invasion. In summary, these results indicate that FRK inhibits migration and invasion of human glioma cells by promoting N-cadherin/ $\beta$ -catenin complex formation.

**Keywords** Glioma · FRK · Migration · Invasion · N-cadherin ·  $\beta$ -catenin

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Qiong Shi and Xu Song contributed equally to this work

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Q. Shi · J. Gu · J. Hu · X. Zhou · R. Yu (✉)  
Brain Hospital, Affiliated Hospital of Xuzhou Medical College, 99  
West Huai-hai Road, Xuzhou 221002, Jiangsu, People's Republic of  
China  
e-mail: yu.rutong@163.com

Q. Shi · J. Gu · J. Hu · X. Zhou (✉) · R. Yu  
Institute of Nervous System Diseases, Xuzhou Medical College, 84  
West Huai-hai Road, Xuzhou 221002, Jiangsu, People's Republic of  
China  
e-mail: xpzhou@xzmc.edu.cn

X. Song · J. Wang · W. Zhang  
The Graduate School, Xuzhou Medical College, Xuzhou, Jiangsu,  
China

## Introduction

Gliomas are malignant primary brain tumors that, despite advances in multimodal therapies, continue to portend a dismal prognosis (Meyer 2008). This poor prognosis is partly because the diffuse invasiveness of these tumors limits the effectiveness of local therapies such as aggressive resection (Coniglio and Segall 2013).

Tyrosine kinases play important roles in cellular functions including cell migration, differentiation, cell-cell adhesion, exocrine signaling, proliferation, and death (Hunter 2009). The Fyn-related kinase (FRK), originally called RAK, is a 54-kDa tyrosine kinase that belongs to a family of Src kinases. Unlike many other Src-related kinases, FRK acts as a tumor suppressor in cancers that arrests cell growth and suppresses tumorigenesis (Craven et al. 1995; Meyer et al. 2003; Yim et al. 2009b). It is reported that over-expression of FRK suppresses growth through association with phosphoprotein retinoblastoma protein (pRb) during the G1 and S phases (Meyer et al. 2003; Craven et al. 1995). Another study demonstrated that FRK is identified as a phosphatase and tensin homologue (PTEN)-associated protein and ectopic expression of FRK significantly suppressed breast cancer cell proliferation, invasion, and tumor growth (Yim et al. 2009b). Conversely, some studies reported that FRK has oncogenic potential to regulate cell proliferation and invasion. It is reported that FRK contributed directly to leukemogenesis in a patient with acute myelogenous leukemia (Hosoya et al. 2005). Also, Chen et al. found that FRK expression promotes Hep3B but not HepG2 cell growth and transformation as well as enhances invasive ability in both Hep3B and HepG2 cells (Chen et al. 2013). These findings suggested that FRK induces diverse biological responses in different cell types in various micro-environments. Our previous study has shown that FRK over-expression inhibits the migration and invasion of glioma U251 and U87 cells (Zhou et al. 2012), but the mechanism of FRK

involved in glioma migration and invasion has not been well studied.

The process of cell migration and invasion into surrounding tissues requires the coordinated and dynamic assembly and disassembly of cell-cell adhesions (Defamie et al. 2014). Cell-cell adhesion is involved in complex biological processes, including cell growth, proliferation, differentiation, and survival (Halbleib and Nelson 2006). Cell-cell adhesion is mediated by a variety of membrane proteins, including classical cadherins, claudins/occludin, nectin, and desmosomal cadherins (Gumbiner 2005). Cadherins are important for the intercellular contacts in solid tissues, which form the transmembrane part of the adherent junctions. Inactivation of cadherins leads to disruption of the cell-cell adhesion. Among them, epithelial (E)-cadherin, neural (N)-cadherin, and vascular endothelial (VE) cadherin are the better studied (Berx and van Roy 2009; Camand et al. 2012). E-cadherin is mainly expressed in epithelial tissues (Hatta and Takeichi 1986), and loss of E-cadherin is viewed as a triggering event of carcinoma cell detachment from the primary tumor and invasion of the conjunctive tissues (Vleminckx et al. 1991). In contrast to E-cadherin, N-cadherin seems to have a pro-migratory effect, promoting tumor infiltration into the conjunctive tissues (Nieman et al. 1999). However, recently, it is reported that down-regulation of N-cadherin in non-epithelial tumors such as glioma induces an increase in cell migration (Camand et al. 2012).

Cadherins bind to members of the catenin protein family (Grunwald 1993), including  $\beta$ -catenin, a protein with cell-cell adhesion and signaling properties.  $\beta$ -catenin was first found in drosophila as a segment polarity protein in 1987 (Perrimon and Mahowald 1987). Interaction of cadherin and  $\beta$ -catenin promotes actin reorganization and strengthens cell adhesion. The actin cytoskeleton also plays an important role in facilitating the formation of  $\beta$ -catenin and in strengthening cell-cell contacts.  $\beta$ -catenin is localized in different cellular compartments to form different complexes that carry out different cellular functions. In the nucleus,  $\beta$ -catenin regulates the transcription of target genes crucial for cell proliferation and differentiation through the Wnt signaling cascade. However, at the plasma membrane,  $\beta$ -catenin controls cadherin-mediated cell adhesion. Through the integration of these two signaling networks,  $\beta$ -catenin plays a pivotal role in maintaining the integrity of cellular functions (Verras and Sun 2006).

In this study, we found that FRK over-expression inhibited glioma cell migration and invasion, in line with our previous report. FRK over-expression increased the protein level of N-cadherin and the N-cadherin/ $\beta$ -catenin complex level. Down-regulation of N-cadherin promoted the migration and invasion of glioma U251 and U87 cells. FRK over-expression did not

affect the total protein level of  $\beta$ -catenin, but promoted the membrane translocation of  $\beta$ -catenin. Thus, our findings suggested that FRK inhibits the migration and invasion of glioma cells by promoting N-cadherin/ $\beta$ -catenin complex formation.

## Materials and Methods

### Cell Culture

U251 and U87 glioma cell lines were purchased from Shanghai Cell Bank, Type Culture Collection Committee, Chinese Academy of Sciences. Cells were maintained in DMEM/F12 (Gibco) supplemented with 10 % fetal bovine serum (FBS) (Sijiqing Bio-logical Engineering Materials Co.). All cell lines were cultured at 37 °C in a saturated humidity incubator containing 5 % CO<sub>2</sub>.

### Plasmids, Antibodies, and Reagents

The FRK plasmid was kindly provided by Dr. Shiaw-Yih Lin at MD Anderson Cancer Center, Houston, USA. Antibodies specific for Flag, FRK, N-cadherin, Histone 3, or Na<sup>+</sup>-K<sup>+</sup> ATPase were purchased from Abcam. Antibodies specific for glyceraldehyde phosphate dehydrogenase (GAPDH) or  $\beta$ -catenin were purchased from Goodhere and Novus, respectively. Alexa 488 goat anti-mouse IgG and Alexa 555 goat anti-rabbit IgG were from invitrogen.

### Small Interfering RNA and Plasmids Transfection

For transfection, U251 or U87 cells were transfected with FRK plasmid or N-cadherin siRNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's specifications. To minimize nonspecific effects of interfering RNAs, non-targeting control siRNA (NC) was used as negative control. The sequence of N-cadherin siRNA (purchased from Shanghai GenePharma Co) was: 5'-CUAACAGGGAGUCAUAUGGUGGAGC-TdT-3'. For transfection, 140 pmol N-cadherin siRNA, 3  $\mu$ g FRK plasmid, and 5  $\mu$ l Lipofectamine™ 2000 reagent were used.

### Cell Immunofluorescence Assay

The transfected cells were fixed in 4 % paraformaldehyde for 30 min, washed twice with phosphate-buffered saline (PBS, pH 7.4), blocked with 5 % normal goat serum, and incubated overnight with antibody, then preformed as described previously (Li et al. 2013).

## Wound Healing Assay

A scratch was made in the middle of the well with a plastic pipette tip 24 h after transfection, and the monolayer was washed twice with PBS (pH 7.4) and incubated in serum-free media. At the designated time, five randomly selected fields at the lesion border were acquired under an inverted microscope (Olympus IX71).

## Cell Invasion Assay

Cell invasion ability was assessed by matrigel precoated transwell inserts (8.0  $\mu\text{m}$  pore size with polyethylene tetrathalate membrane) according to the manufacturer's protocol. Filters were precoated with 10  $\mu\text{g}$  of matrigel (BD Biosciences). A pretreated cell suspension ( $1 \times 10^5$ ) in serum-free culture media was added into the inserts, and each insert was placed in the lower chamber filled with culture media containing 10 % FBS as a chemoattractant. And then, the invasion assay was performed as described (Zhou et al. 2012).

## Immunoprecipitation

Proteins were extracted from cells using the NP-40 buffer (2  $\mu\text{g}/\text{ml}$  Aprotinin, 10  $\mu\text{M}$  Leupeptin, 1 % NP-40, 1  $\mu\text{M}$  Pepstatin A, 0.5 mM PMSF, 4 mM Benzamidine, 1 mM DTT) for 30 min at 4 °C. Protein concentration was detected using the BCA Protein Assay Kit (Thermo scientific), and 500  $\mu\text{g}$  lysates were used for immunoprecipitation. Lysates were then incubated with primary antibodies (2  $\mu\text{g}$  anti-N-cadherin or 2  $\mu\text{g}$  anti- $\beta$ -catenin) and 30  $\mu\text{l}$  protein G agarose beads at 4 °C overnight. The beads and antigen banding antibodies complex were boiled and detected by Western blotting (Zhang et al. 2013).

## Western Blot Analysis

U251 and U87 cells ( $5 \times 10^5$ ) were seeded in 6-well plates with complete growth medium and then these cells were transfected with indicated siRNAs or plasmids. Forty-eight hours later, Western blot was performed as described (Zhou et al. 2013).

## Statistical Analysis

The results were representative of experiments repeated at least three times and quantitative data were presented as means  $\pm$  S.E.M. Analysis of variance and Student's *t* test were used to analyze the difference between the means of test samples and controls with  $P < 0.05$  considered statistically significant ( $*P < 0.05$ ). All statistical analyses were performed using Office Excel 2003 (Microsoft Corporation) or SPSS software (SPSS version 16.0).

## Results

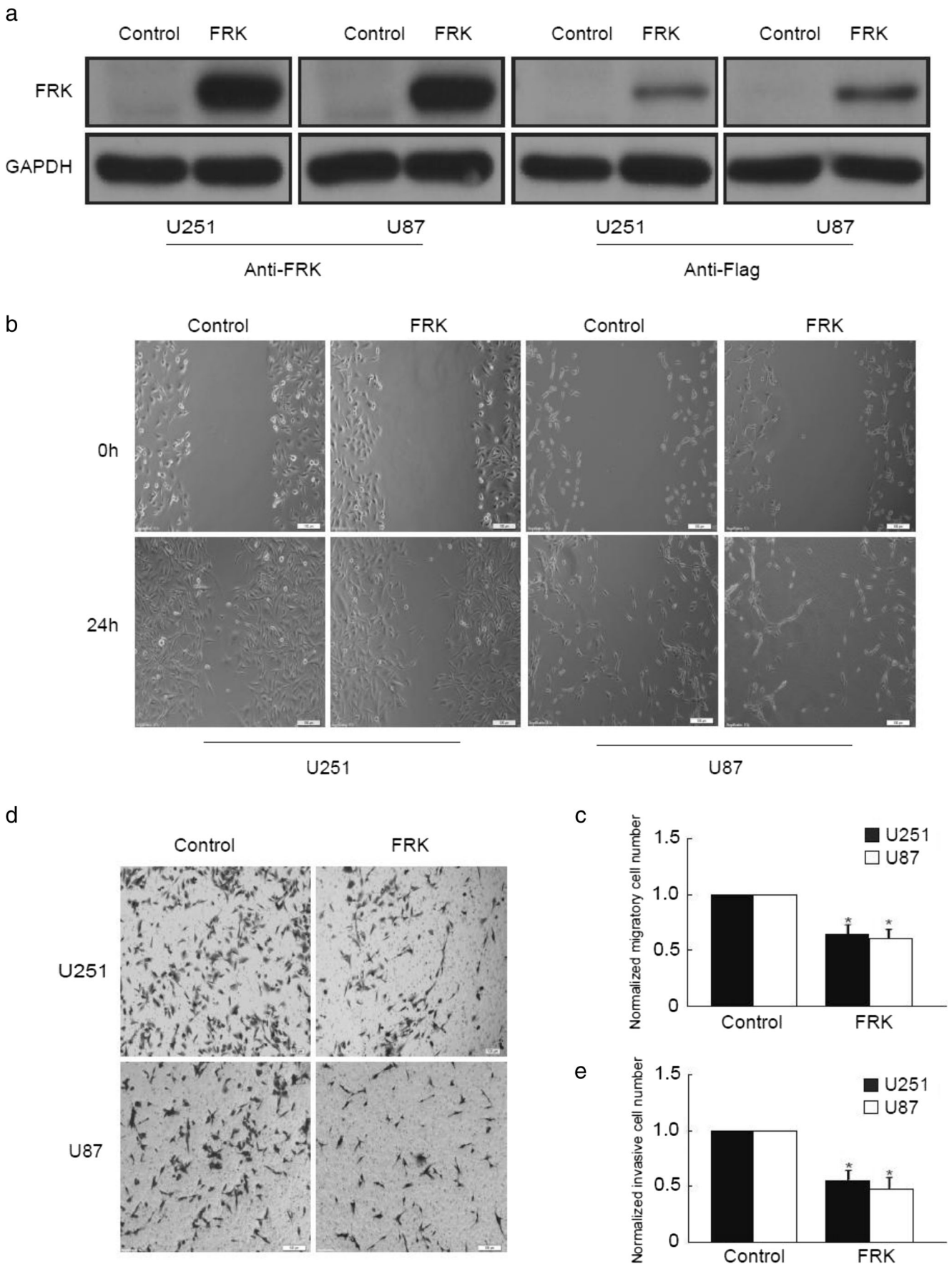
### FRK Over-Expression Inhibits the Migration and Invasion of Glioma U251 and U87 Cells

First, we observed the effect of FRK on cell migration and invasion ability in U251 and U87 cells by using wound healing assay and matrigel precoated transwell chambers, respectively. As Fig. 1a showed, the exogenous FRK (Flag-FRK) expressed very well in glioma U251 and U87 cells examined either by anti-Flag or by anti-FRK antibodies. By using wound healing assay, we found that the wound of control group healed obviously, but FRK over-expressing group had no signs of healing 24 h after being scratched (Fig. 1b). Compared with the control group, the migratory cell numbers of FRK over-expressing group decreased to  $62.23 \pm 2.92$  % ( $P < 0.05$ ) in glioma U251 cells and  $58.62 \pm 2.36$  % ( $P < 0.05$ ) in glioma U87 cells, respectively (Fig. 1c). The matrigel precoated transwell results showed that, compared with the control group, the invasive cell number of FRK over-expressing group was reduced by  $46.52 \pm 2.98$  % and  $52.89 \pm 3.02$  % in U251 and U87 cells, respectively (Fig. 1d, e). These results suggested that over-expression of FRK inhibits the glioma cell migration and invasion, in line with our previous report (Zhou et al. 2012).

### FRK Over-Expression Promotes N-cadherin/ $\beta$ -catenin Complex Formation and Discreases $\beta$ -catenin Nuclear Translocation

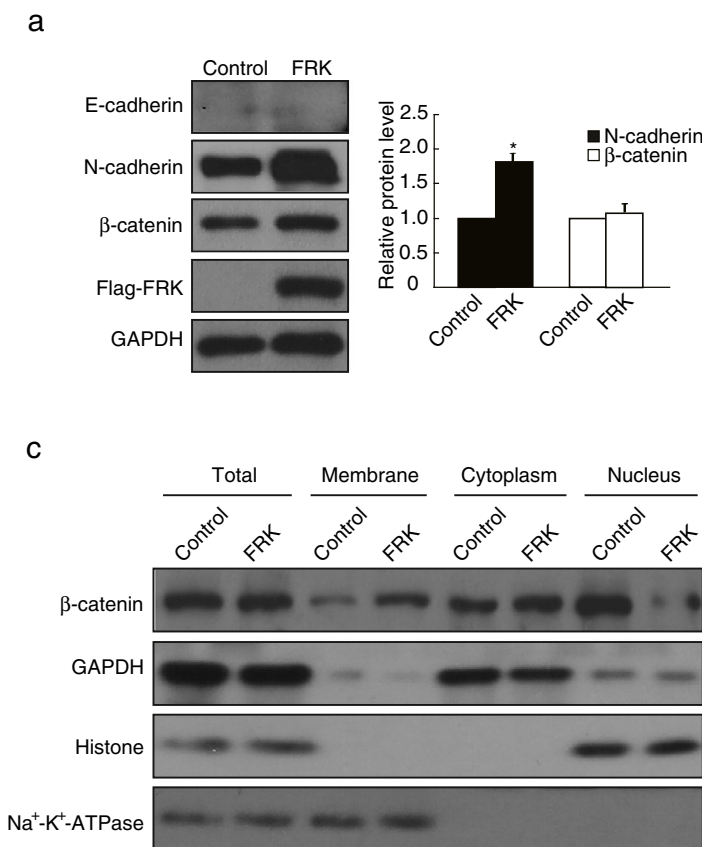
Given cadherin/ $\beta$ -catenin complex plays important roles in cancer migration and invasion (Huang et al. 2012; Kim et al. 2013), we firstly examined the effect of FRK on N-cadherin, E-cadherin, and  $\beta$ -catenin protein levels. As shown in Fig. 2a, both of N-cadherin and E-cadherin expressed in glioma cells and the level of E-cadherin was very low. FRK over-expression significantly increased N-cadherin, but not the E-cadherin protein levels. In addition, the total  $\beta$ -catenin protein level was not altered after over-expression of FRK. Thereafter, we checked whether FRK over-expression could promote N-cadherin/ $\beta$ -catenin complex formation using co-immunoprecipitation assay. The results showed that in glioma U251 cells, N-cadherin could co-precipitate with  $\beta$ -catenin. By contrast, control antibody (i.e., IgG) caused no positive bands (Fig. 2b), suggesting the specificity of this effect. Interestingly, FRK over-expression promoted the interaction of N-cadherin with  $\beta$ -catenin (Fig. 2b).

It is reported that  $\beta$ -catenin is localized in different cellular compartments, such as plasma membrane, cytosol, and nucleus, to form different complexes that carry out differential cellular functions (Verras and Sun 2006). We found that FRK over-expression did not change the total  $\beta$ -catenin protein level but promote N-cadherin/ $\beta$ -catenin complex



**Fig. 1** Over-expression of FRK decreases the migration and invasion ability of glioma cells. **a** Western blot analysis of exogenous FRK expression in U251 and U87 cells. Forty-eight hours after transfection, cells were lysed and protein extraction was assessed by Western blot using FRK antibody (*left*) or Flag antibody (*right*). GAPDH was used as the loading control. **b** Confluent monolayer of cells were wounded by scraping with a micropipette tip and incubated for 24 h. Representative microphotographs are shown. **c** Quantitative migratory cell numbers. **d** Cells were cultured into the top surface of matrigel invasion chamber. Twenty-four hours later, cells that migrated to the opposite side of the insert were stained with crystal violet. Representative microphotographs are shown. **e** Quantitative cell numbers invaded through the filter. Values shown represent the mean $\pm$ SEM from three independent experiments. Scale bar, 100  $\mu$ m.\*;  $P < 0.05$

formation at the plasma membrane (Fig. 2a, b). Thus, we wonder whether FRK over-expression could affect  $\beta$ -catenin subcellular location. To examine this possibility, we separated the membranous, nuclear, and cytosolic fractions of the cells transfected with FRK for 48 h and conducted the Western blot assay to examine the  $\beta$ -catenin levels in each fraction.

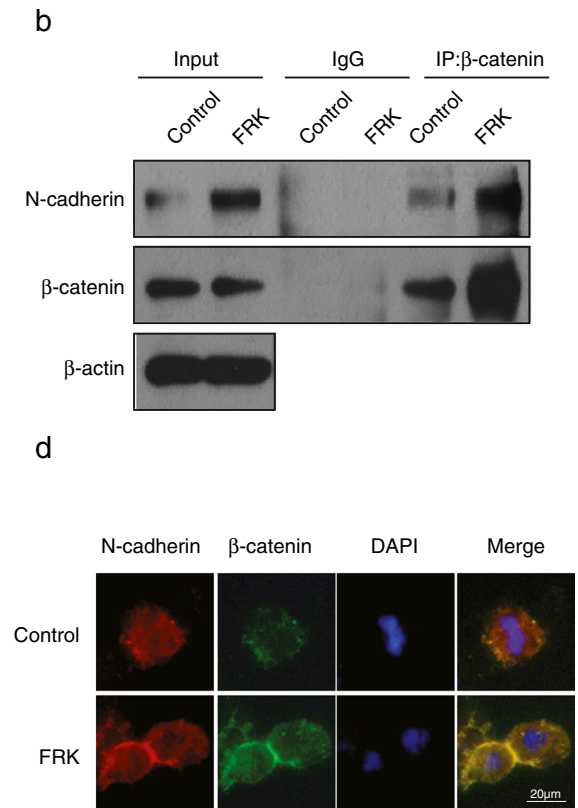


**Fig. 2** FRK over-expression promotes N-cadherin/ $\beta$ -catenin complex formation and decreases  $\beta$ -catenin nuclear translocation. **a** The protein level of E-cadherin, N-cadherin, and  $\beta$ -catenin were detected by Western blot after FRK over-expression. GAPDH was used as the loading control. **b** FRK over-expression promotes the interaction of N-cadherin with  $\beta$ -catenin. Glioma U251 cells were transfected with FRK and lysates were immunoprecipitated with anti- $\beta$ -catenin antibody or anti-IgG to precipitate N-cadherin and immunoblotted with anti-N-cadherin antibody. **c** The cytoplasm, membrane, and nucleus components of U251 cells were

GAPDH, Histone 3, and  $\text{Na}^+\text{-K}^+\text{-ATPase}$  were used as internal loading control for the cytosolic, nuclear, and membranous fraction, respectively. As shown in Fig. 2c, the membrane recruitment of  $\beta$ -catenin increased, while those in the nuclear fraction decreased after FRK over-expression. Furthermore, we also tested the distribution of N-cadherin and  $\beta$ -catenin using immunofluorescence assay and found that FRK over-expression increased the membrane levels of N-cadherin and  $\beta$ -catenin (Fig. 2d). These data suggested that FRK over-expression promotes N-cadherin/ $\beta$ -catenin complex formation by increasing the level of N-cadherin and recruiting  $\beta$ -catenin to plasma membrane.

#### Down-Regulation of N-cadherin Increases Migration and Invasion of Glioma U251 and U87 Cells

Thereafter, we investigated the function of N-cadherin on the migration and invasion of glioma U251 and U87 cells. Firstly,



separated 48 h after FRK transfection. GAPDH, Histone 3, and  $\text{Na}^+\text{-K}^+\text{-ATPase}$  were used as internal loading control for the cytosolic, nuclear, and membranous fraction, respectively. **d** Immunofluorescence assay was used to test the distribution of N-cadherin and  $\beta$ -catenin after FRK over-expression. Immunofluorescent staining was performed with N-cadherin and  $\beta$ -catenin. Cells were also stained with DAPI to identify nuclei. Results are presented as mean $\pm$ SEM from three independent experiments. Scale bar, 100  $\mu$ m.\*;  $P < 0.05$

we observed that the down-regulation efficiency of N-cadherin siRNA reached about 62 % in glioma U251 cells and 76 % in glioma U87 cells, compared with the NC group (negative control siRNA group, Fig. 3a, b). Then, the migration ability of glioma U251 and U87 cells were assessed by wound healing assay after negative control siRNA or N-cadherin siRNA transfection. As shown in Fig. 3c, 48 h after being scratched, compared with NC group, the wound of N-cadherin siRNA group healed obviously, and the migratory cell numbers increased by  $32.16 \pm 2.96$  % and  $39.27 \pm 2.38$  % in glioma U251 and U87 cells, respectively ( $P < 0.05$ , Fig. 3d). Next, we examined the invasion ability of glioma cells using matrigel precoated transwell chambers. As Fig. 3e showed, down-regulation of N-cadherin made a significant increase in the number of invasive cells. Compared with the NC group, the number of invasive cells was increased by  $47.92 \pm 1.87$  % and  $49.53 \pm 2.75$  % in U251 and U87 cells, respectively after N-cadherin siRNA transfection (Fig. 3f), similar to the effect of FRK (Zhou et al. 2012) and previous studies (Camand et al. 2012). These results demonstrated that N-cadherin is involved in glioma cell migration and invasion.

#### Down-Regulation of N-cadherin Abolishes the Effect of FRK on Migration and Invasion of Glioma Cells

From the above results, we can see N-cadherin down-regulation inhibited glioma cell migration and invasion, similar to the role of FRK. Also, FRK over-expression promoted N-cadherin/ $\beta$ -catenin complex formation. Thus, we examined the possibility that the N-cadherin/ $\beta$ -catenin complex plays some roles in FRK's effect on glioma cell migration and invasion. To address this question, we co-transfected the FRK and N-cadherin siRNA into glioma cells and examined whether down-regulation of N-cadherin could abolish FRK's effect. As shown in Fig. 4a, b, the co-transfection of FRK and N-cadherin siRNA was shown successfully in glioma U251 and U87 cells. The wound healing assay and transwell assay results showed that FRK over-expression inhibited the migration and invasion of U251 and U87 cells, while down-regulation of N-cadherin promoted the migration and invasion. Interestingly, down-regulation of N-cadherin abolished the inhibitory effect of FRK over-expression on the migration and invasion of glioma U251 and U87 cells (Fig. 4c–f).

Next, we investigated whether down-regulation of N-cadherin could abolish the effect of FRK on the subcellular distribution of  $\beta$ -catenin using immunofluorescence assay. As Fig. 4g showed, FRK over-expression promoted the membrane co-localization of N-cadherin and  $\beta$ -catenin, as well as inhibited  $\beta$ -catenin nuclear translocation. Furthermore, down-regulation of N-cadherin partly inhibited the effect of FRK over-expression on the subcellular distribution of  $\beta$ -catenin. These results suggested FRK inhibits the glioma migration

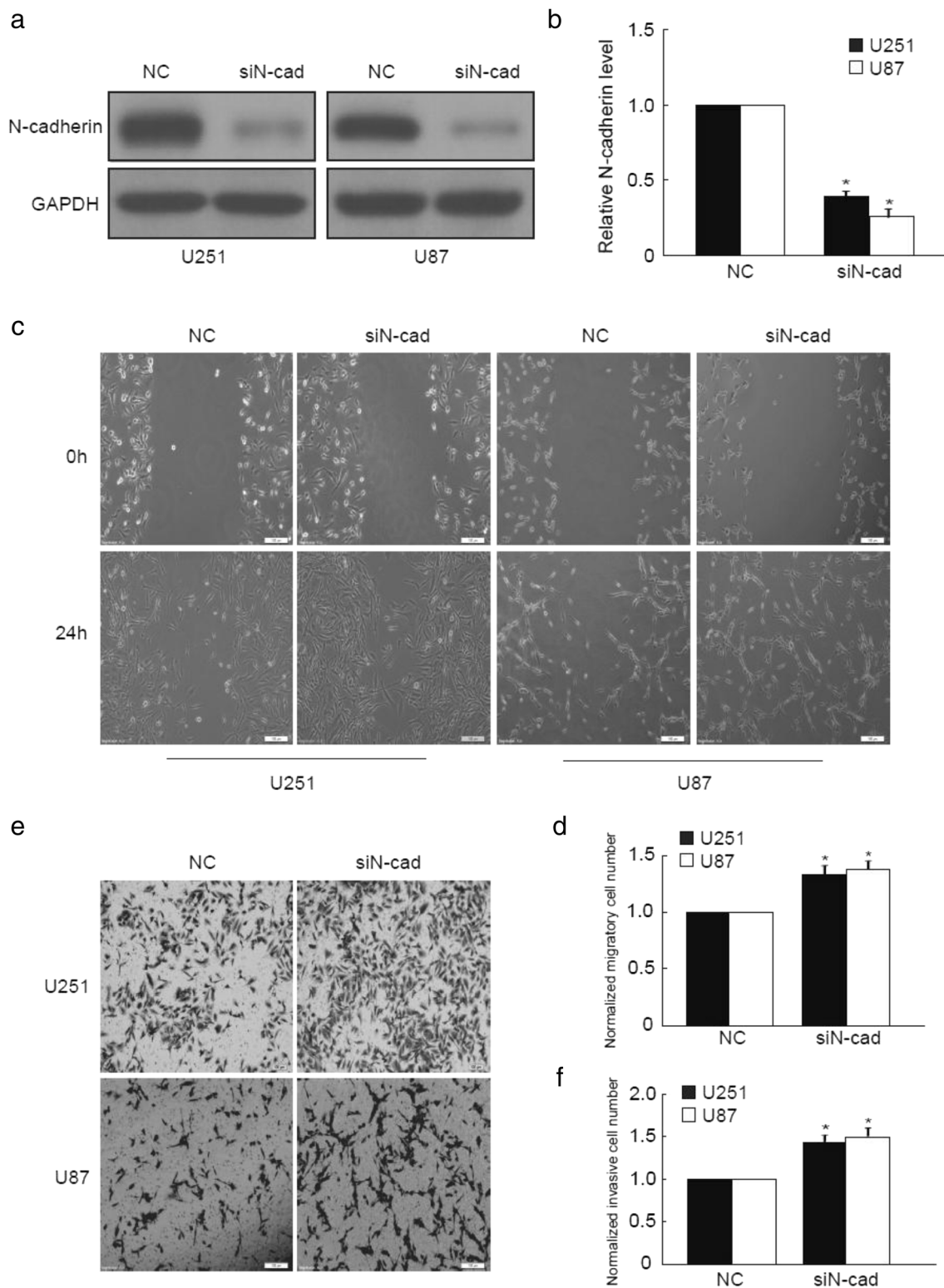
and invasion by regulating N-cadherin/ $\beta$ -catenin complex formation.

#### Discussion

Gliomas account for more than 50 % of all brain tumors and its malignant forms are associated with one of the poorest prognosis for cancer because of their ability to infiltrate diffusely into the normal cerebral parenchyma (Meyer 2008). The causes of glioma invasion remain poorly understood. Our previous study has shown that FRK acts as a potential tumor suppressor in glioma and its over-expression inhibited cell migration and invasion (Zhou et al. 2012). In this study, we found that FRK over-expression inhibited the migration and invasion of glioma cells by regulating N-cadherin/ $\beta$ -catenin complex formation.

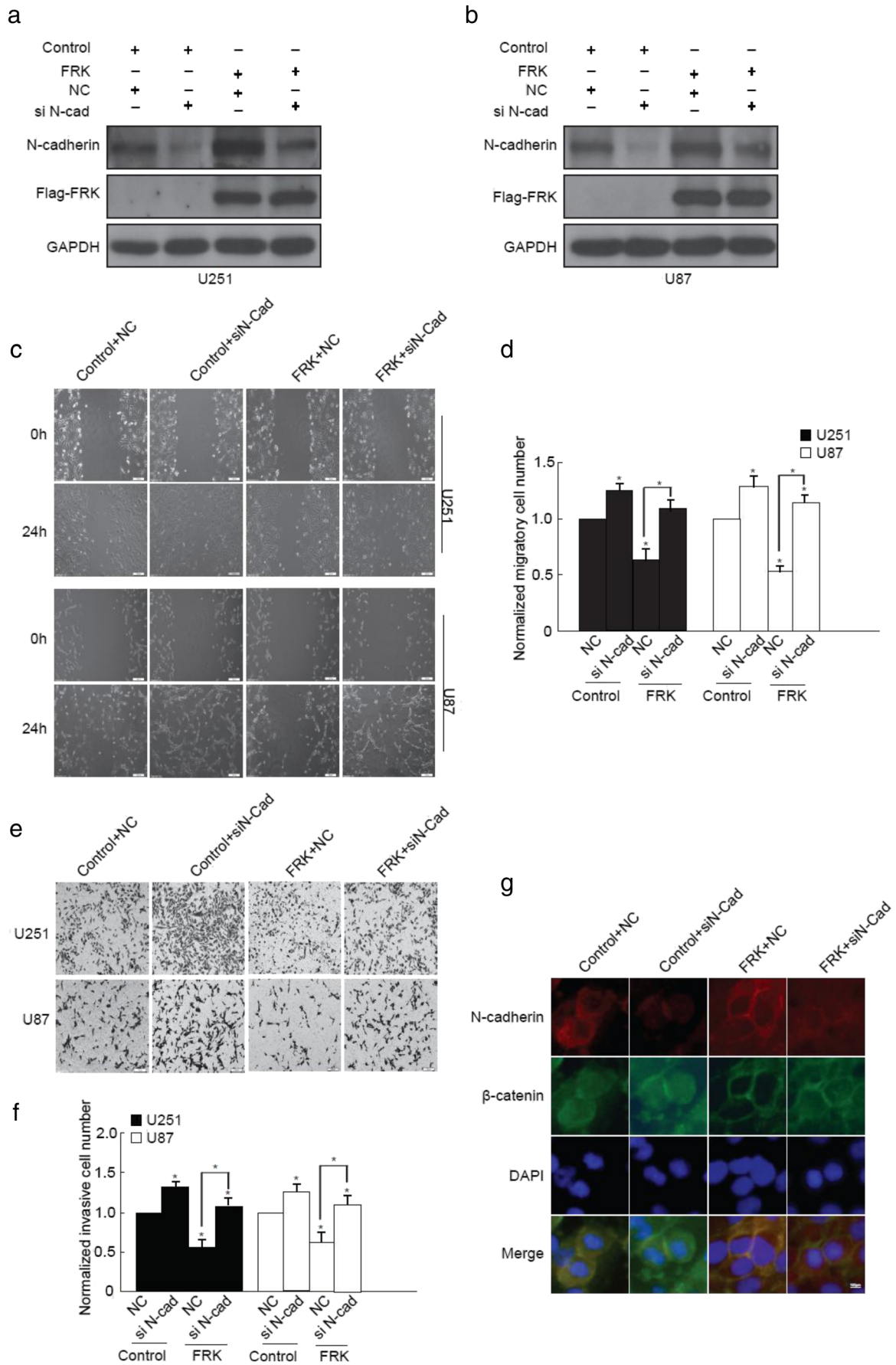
FRK belongs to the Src non-receptor tyrosine kinase family. It is reported that over-expression of FRK suppresses cell growth through association with pRb during the G1 and S phases (Craven et al. 1995; Meyer et al. 2003). Recently, FRK is reported to stabilize PTEN and activates FRK-pRb-induced growth inhibition (Craven et al. 1995; Yim et al. 2009b). However, some studies reported that FRK has oncogenic potential to regulate cell proliferation and invasion. It is reported that high levels of FRK is frequently found in myeloblastosis-associated virus (MAV)-2-induced lung sarcomas in chickens (Pajer et al. 2009). Chen et al. found that FRK was over-expressed in 52 % of hepatocellular carcinoma (HCC) samples (Chen et al. 2013). Furthermore, they also showed that FRK expression promotes Hep3B but not HepG2 cell growth and transformation as well as enhances invasive ability in both Hep3B and HepG2 cells (Chen et al. 2013). In addition, a study reported that FRK contributed directly to leukemogenesis in a patient with acute myelogenous leukemia (Hosoya et al. 2005). All these results showed that FRK induces diverse biological responses in different cell types under various conditions and tissue-specific microenvironments. Our present and previous study (Zhou et al. 2012) showed that FRK over-expression reduced the migration and invasion of glioma cells. Our findings indicated that FRK is a negative regulator of cell proliferation, migration, and invasion, at least in glioma cells.

Cadherins are a class of glycoproteins expressed on the surface of cells involved in calcium-dependent cell-cell adhesion. Although the changes in cadherin levels during epithelial carcinoma progression are now well documented, the possibility that such changes occur in non-epithelial tumors has only recently begun to be explored (Peglion and Etienne-Manneville 2012). The decrease of E-cadherin expression is frequently associated with a cadherin switch resulting in the concomitant increase in N-cadherin expression (Wheelock



**Fig. 3** Down-regulation of N-cadherin increases migration and invasion of glioma U251 and U87 cells. **a, b** Immunoblot was applied to detect the efficiency of N-cadherin siRNA in glioma U251 and U87 cells. GAPDH was used as the loading control. **c** Twenty-four hours after being transfected with N-cadherin siRNA, cells were subjected to wounding assay. **d** Quantitative migratory cell numbers. **e** Representative digital

pictures of three dimensional invasive assay. After 24 h of transfection, cell suspension was added into the matrigel precoated transwell chambers and the cells invaded through the matrigel were stained and photoed. **f** Quantitative cell numbers invaded through the filter. Results are expressed as the mean $\pm$ SEM from three independent experiments. Scale bar, 100  $\mu$ m.\*;  $P < 0.05$





◀ **Fig. 4** Down-regulation of N-cadherin abolishes the effect of FRK on migration and invasion of glioma cells. **a, b** The protein level of N-cadherin and exogenous FRK were detected by Western blot after FRK and N-cadherin siRNA co-transfected into glioma U251 (**a**) and U87 (**b**) cells. **c** After 24 h of FRK and N-cadherin siRNA co-transfection, cells were subjected to wounding assay. **d** Quantitative migratory cell numbers. **e** After 24 h of FRK and N-cadherin siRNA co-transfection, cells were subjected to transwell invasion assay. **f** Quantitative cell numbers invaded through the filter. **g** Down-regulation of N-cadherin abolished FRK over-expression-induced subcellular distribution of N-cadherin and  $\beta$ -catenin, detected by immunofluorescence assay. Immunofluorescent staining was performed with N-cadherin (*red*) and  $\beta$ -catenin (*green*). Cells were also stained with DAPI to identify nuclei. Results are expressed as the mean $\pm$ SEM from three independent experiments. Scale bar, 100  $\mu$ m.\*;  $P < 0.05$

et al. 2008). Recent study has shown that in non-epithelial tumors, such as glioma, there was no obvious staining reaction for E-cadherin in non-epithelial tumors-glioma (Wu et al. 2013b). Our result also showed that E-cadherin was very low in glioma cells, and FRK over-expression has no effect on the protein level of E-cadherin, but increased the protein level of N-cadherin (Fig. 2a).

It is reported that the level of E-cadherin is inversely correlated with the migratory ability of cancer cells (Bex and van Roy 2009; Vleminckx et al. 1991). In contrast to E-cadherin, some studies reported that N-cadherin promotes tumor infiltration in the conjunctive tissues (Agiostatidou et al. 2007; Nieman et al. 1999). However, other studies about the effect of N-cadherin on cancer migration were contrary (Camand et al. 2012; Peglion and Etienne-Manneville 2012; Rappl et al. 2008). For example, some studies show an inverse correlation between N-cadherin level and glioma invasiveness. Using a wound healing assay, Camand et al. reported that down-regulation of N-cadherin in primary astrocytes and glioma cells leads to a faster and less-directed migration (Camand et al. 2012). Rappl et al. reported that down-regulation of N-cadherin in LN18 cells induces an increase in cell migration (Rappl et al. 2008). Our present study also showed that down-regulation of N-cadherin promoted the migration and invasion of glioma U251 and U87 cells (Fig. 3). This apparent contradiction may result from the use of different animal models or from the fact that, in some studies, the level of N-cadherin mRNA is analyzed, while other studies are based on the level of N-cadherin protein (Camand et al. 2012; Utsuki et al. 2002).

The intracellular regions of the cadherins interact with cytoplasmic proteins,  $\beta$ -catenin, which is also a core factor in Wnt/ $\beta$ -catenin signaling. As an essential activator downstream of Wnt signaling,  $\beta$ -catenin moves from the cytoplasm into the nucleus and forms stabilized complexes with TCF4/LEF to activate Wnt target genes (Brantjes et al. 2002). Thus, the activation of  $\beta$ -catenin has been tested in a range of cancers, including gliomas and breast cancer (Uchino et al. 2010; Wu et al. 2013a). In glioma tissues,  $\beta$ -catenin total

level and its nuclear accumulation were significantly higher than in normal tissues. The level of  $\beta$ -catenin also was positively correlated with World Health Organization (WHO) grades of patients with gliomas (Wu et al. 2013a). Moreover, the high level of  $\beta$ -catenin has a poor prognostic impact on glioblastoma patients (Rossi et al. 2011). Additionally, reduction of  $\beta$ -catenin/TCF4 activity using aspirin leads to glioma cell cycle G0/G1 phase arrest, invasion decrease, and subcutaneous tumor growth inhibition (Lan et al. 2011). In the present study, we showed that FRK over-expression did not affect the total level of  $\beta$ -catenin, but promoted the membrane translocation of  $\beta$ -catenin while inhibited its nuclear-accumulation (Fig. 2).

As the process of cell migration and invasion into surrounding tissue probably requires the coordinated and dynamic assembly and disassembly of cell-cell adhesions, the acquisition of invasive properties by tumor cells may depend on changes in the stability and organization of cell-cell contacts, rather than on modifications to the total expression of the molecular components of cell-cell adhesion. Perego et al. investigated the role of N-cadherin and  $\beta$ -catenin-mediated adhesion in primary cultured astrocytes and glioblastoma cell lines (Perego et al. 2002). Their study revealed that, rather than altered expression of cadherin-catenin system components, it is the instability and disorganization of cadherin-mediated junctions that is required to promote migration and invasiveness in glioblastoma cell lines (Perego et al. 2002). N-cadherin/ $\beta$ -catenin complex plays an important role in cancer cell migration and invasion (Huang et al. 2012). Our study showed that FRK over-expression promoted the formation of N-cadherin/ $\beta$ -catenin complex, as well as increased the co-localization of N-cadherin and  $\beta$ -catenin on the membrane. In addition, we showed that down-regulation of N-cadherin eliminated FRK over-expression-induced migration and invasion inhibition and  $\beta$ -catenin distribution.

In summary, we reported that FRK plays an important role in regulating glioma cell migration and invasion through promoting N-cadherin/ $\beta$ -catenin complex formation. FRK over-expression increased the N-cadherin protein level, recruited  $\beta$ -catenin to membrane, promoted N-cadherin/ $\beta$ -catenin complex formation, and inhibited  $\beta$ -catenin's translocation to nuclear at the same time. Because FRK is a non-receptor tyrosine kinase and distributed in the cell cytosol and nucleus (Cance et al. 1994; Yim et al. 2009a; Zhou et al. 2012), how FRK increases the N-cadherin level will be studied in the future.

**Acknowledgments** The project was supported by National Natural Science Foundation of China (No. 81272777; No. 81372699; No. 81302175), Natural Science Foundation of Higher Education Institutions of Jiangsu province (No. 11KJB320019), Natural Science Foundation of Jiangsu Province (No. BK2011195), and the guidance problem of Science and Technology Bureau of Xuzhou (No. XZZD1155).

**Conflict of Interest Statement** The authors have no conflict of interest.

## References

- Agiostatidou G, Hult J, Phillips GR, Hazan RB (2007) Differential cadherin expression: potential markers for epithelial to mesenchymal transformation during tumor progression. *J Mammary Gland Biol Neoplasia* 12:127–133
- Berx G, van Roy F (2009) Involvement of members of the cadherin superfamily in cancer. *Cold Spring Harb Perspect Biol* 1:a003129
- Brantjes H, Barker N, van Es J, Clevers H (2002) TCF: Lady Justice casting the final verdict on the outcome of Wnt signalling. *Biol Chem* 383:255–261
- Camand E, Peglion F, Osmani N, Sanson M, Etienne-Manneville S (2012) N-cadherin expression level modulates integrin-mediated polarity and strongly impacts on the speed and directionality of glial cell migration. *J Cell Sci* 125:844–857
- Cance WG, Craven RJ, Bergman M, Xu L, Alitalo K, Liu ET (1994) Rak, a novel nuclear tyrosine kinase expressed in epithelial cells. *Cell Growth Differ* 5:1347–1355
- Chen JS, Hung WS, Chan HH, Tsai SJ, Sun HS (2013) In silico identification of oncogenic potential of fyn-related kinase in hepatocellular carcinoma. *Bioinformatics* 29:420–427
- Coniglio SJ, Segall JE (2013) Review: molecular mechanism of microglia stimulated glioblastoma invasion. *Matrix Biol* 32:372–380
- Craven RJ, Cance WG, Liu ET (1995) The nuclear tyrosine kinase Rak associates with the retinoblastoma protein pRb. *Cancer Res* 55:3969–3972
- Defamie N, Chepied A, Mesnil M (2014) Connexins, gap junctions and tissue invasion. *FEBS Lett* 588:1331–1338
- Grunwald GB (1993) The structural and functional analysis of cadherin calcium-dependent cell adhesion molecules. *Curr Opin Cell Biol* 5:797–805
- Gumbiner BM (2005) Regulation of cadherin-mediated adhesion in morphogenesis. *Nat Rev Mol Cell Biol* 6:622–634
- Halbleib JM, Nelson WJ (2006) Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. *Genes Dev* 20:3199–3214
- Hatta K, Takeichi M (1986) Expression of N-cadherin adhesion molecules associated with early morphogenetic events in chick development. *Nature* 320:447–449
- Hosoya N, Qiao Y, Hangaishi A et al (2005) Identification of a SRC-like tyrosine kinase gene, FRK, fused with ETV6 in a patient with acute myelogenous leukemia carrying a t(6;12)(q21;p13) translocation. *Genes Chromosomes Cancer* 42:269–279
- Huang RY, Wen CC, Liao CK, Wang SH, Chou LY, Wu JC (2012) Lysophosphatidic acid modulates the association of PTP1B with N-cadherin/catenin complex in SKOV3 ovarian cancer cells. *Cell Biol Int* 36:833–841
- Hunter T (2009) Tyrosine phosphorylation: thirty years and counting. *Curr Opin Cell Biol* 21:140–146
- Kim H, Yoo SB, Sun P et al (2013) Alteration of the E-cadherin/beta-catenin complex is an independent poor prognostic factor in lung adenocarcinoma. *Korean J Pathol* 47:44–51
- Lan F, Yue X, Han L et al (2011) Antitumor effect of aspirin in glioblastoma cells by modulation of beta-catenin/T-cell factor-mediated transcriptional activity. *J Neurosurg* 115:780–788
- Li Z, Sun C, Zhang T et al (2013) Geranylgeranyltransferase I mediates BDNF-induced synaptogenesis. *J Neurochem* 125:698–712
- Meyer MA (2008) Malignant gliomas in adults. *N Engl J Med* 359:1850, Author reply 1850
- Meyer T, Xu L, Chang J, Liu ET, Craven RJ, Cance WG (2003) Breast cancer cell line proliferation blocked by the Src-related Rak tyrosine kinase. *Int J Cancer* 104:139–146
- Nieman MT, Prudoff RS, Johnson KR, Wheelock MJ (1999) N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression. *J Cell Biol* 147:631–644
- Pajer P, Karafiat V, Pecenka V et al (2009) Industasis, a promotion of tumor formation by nontumorigenic stray cells. *Cancer Res* 69:4605–4612
- Peglion F, Etienne-Manneville S (2012) N-cadherin expression level as a critical indicator of invasion in non-epithelial tumors. *Cell Adh Migr* 6:327–332
- Perego C, Vanoni C, Massari S et al (2002) Invasive behaviour of glioblastoma cell lines is associated with altered organisation of the cadherin-catenin adhesion system. *J Cell Sci* 115:3331–3340
- Perrimon N, Mahowald AP (1987) Multiple functions of segment polarity genes in *Drosophila*. *Dev Biol* 119:587–600
- Rappl A, Piontek G, Schlegel J (2008) EGFR-dependent migration of glial cells is mediated by reorganisation of N-cadherin. *J Cell Sci* 121:4089–4097
- Rossi M, Magnoni L, Miracco C et al (2011) Beta-catenin and Gli1 are prognostic markers in glioblastoma. *Cancer Biol Ther* 11:753–761
- Uchino M, Kojima H, Wada K et al (2010) Nuclear beta-catenin and CD44 upregulation characterize invasive cell populations in non-aggressive MCF-7 breast cancer cells. *BMC Cancer* 10:414
- Utsuki S, Sato Y, Oka H, Tsuchiya B, Suzuki S, Fujii K (2002) Relationship between the expression of E-, N-cadherins and beta-catenin and tumor grade in astrocytomas. *J Neurooncol* 57:187–192
- Verras M, Sun Z (2006) Roles and regulation of Wnt signaling and beta-catenin in prostate cancer. *Cancer Lett* 237:22–32
- Vlemminckx K, Vakaet L Jr, Mareel M, Fiers W, van Roy F (1991) Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell* 66:107–119
- Wheelock MJ, Shintani Y, Maeda M, Fukumoto Y, Johnson KR (2008) Cadherin switching. *J Cell Sci* 121:727–735
- Wu W, Tian Y, Wan H, Song Y, Li J, Zhang L (2013a) The expressions of Wnt/beta-catenin pathway-related components in brainstem gliomas. *Can J Neurol Sci* 40:355–360
- Wu W, Tian Y, Wan H et al (2013b) Expression of beta-catenin and E- and N-cadherin in human brainstem gliomas and clinicopathological correlations. *Int J Neurosci* 123:318–323
- Yim EK, Siwko S, Lin SY (2009a) Exploring Rak tyrosine kinase function in breast cancer. *Cell Cycle* 8:2360–2364
- Yim EK, Peng G, Dai H et al (2009b) Rak functions as a tumor suppressor by regulating PTEN protein stability and function. *Cancer Cell* 15:304–314
- Zhang H, Nie W, Zhang X et al (2013) NEDD4-1 regulates migration and invasion of glioma cells through CNrasGEF ubiquitination in vitro. *PLoS One* 8:e82789
- Zhou X, Hua L, Zhang W et al (2012) FRK controls migration and invasion of human glioma cells by regulating JNK/c-Jun signaling. *J Neurooncol* 110:9–19
- Zhou X, Liu Z, Shi Q et al (2013) Geranylgeranyltransferase I regulates HIF-1alpha promoting glioblastoma cell migration and invasion. *J Neurooncol* 112:365–374