Phosphorylation of Focal Adhesion Kinase at Tyr925: Role in Glia-Dependent and Independent Migration Through Regulating Cofilin and N-Cadherin

Lingzhen Song

Center for Molecular Neurobiology, University Medical Center Hamburg-Eppendorf

<https://orcid.org/0000-0001-8768-1256>

Shanting Zhao

College of Veterinary Medicine, Northwest A&F University

Michael Frotscher

Universitätsklinikum Hamburg-Eppendorf

Xuejun Chai (✉ xchai@xiyi.edu.cn)

College of Basic Medicine, Xi'an Medical University <https://orcid.org/0000-0002-5590-4328>

Research Article

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Abstract

The adult neocortex is a six-layered structure, consisting of nearly continuous layers of neurons that are generated with large temporal diversity. During development, cortical neurons originating from the ventricular zone migrate towards the Reelin-containing marginal zone in an inside-out arrangement. Focal adhesion kinase (FAK), one tyrosine kinase localizing to focal adhesions, has been shown to be activated by Src, an important downstream molecule of Reelin signaling, at tyrosine 925 (Y925). Up to date, the precise molecular mechanisms of FAK and its phosphorylation at Y925 during neuronal migration are still unclear. Combining *in utero* electroporation with immunohistochemistry and live imaging, we examined the function of FAK in regulating neuronal migration. We show that phosphorylated FAK is colocalized with Reelin positive cells in the developing neocortex and hippocampus. Phosphorylation of FAK at Y925 is significantly reduced in reeler mice. Overexpression and dephosphorylation of FAK impair locomotion and translocation, resulting in migration inhibition and dislocation of both late-born and early-born neurons. These migration defects are highly correlated to the function of FAK in regulating cofilin phosphorylation and N-Cadherin expression, both are involved in Reelin signaling pathway. Thus, phosphorylation of focal adhesion kinase at Y925 is crucial for both glia-dependent and independent neuronal migration.

Highlight

1. FAK and phosphorylation of FAK at Y925 are required for the migration of both early-born and late-born neurons.
2. FAK is crucial for regulating migratory speed and soma translocation.
3. The function of FAK in neuronal migration is mediated via cofilin and N-Cadherin, which are related to Reelin signaling pathway.

Introduction

The adult mammalian cerebral cortex consists of six layers that are formed by the precisely coordinated proliferation, migration and lamination of cortical neurons during development, thereby providing a basis for the establishment of functional neuronal connectivity [1]. These neurons originate from radial glia cells in the ventricular zone (VZ) via asymmetric proliferation, migrate towards the pial surface and finally localize their cell bodies in their final destination and form the six aligned layers of the cortical plate (CP) [2–5].

The neurons of layer VI are established first and layers II–V are subsequently formed in an inside-out manner. Two distinct forms of cell movement: locomotion and translocation, are responsible for the migration of cortical neurons [6]. During the early neocortical development, early-born neurons destined to form deep layers migrate in radial glia-independent manner [7], which means they extend leading processes to the superficial marginal zone (MZ) and then shorten their leading processes to lift their cell bodies. This migration model is called somal translocation. Migration of late-born neurons is more

complex, consisting of multipolar migration, morphology transformation, glia-dependent locomotion and terminal translocation [7–9]. When the leading process of locomoting neurons reaches MZ, they switch to terminal translocation mode, in which the somata move rapidly in a radial glia-independent manner [10]. Even though early-born and late-born neurons apply different migrating strategies, precisely regulated adhesion and cytoskeleton dynamics are involved in controlling lamination [11–13]. Impairment in any of these processes has been related to cortical malformation and neurological disorders, which shows life-long effects on daily life and cognitive function of human beings, as well as of mice [14–17]. Therefore, investigation on the molecular mechanisms underlying cortical lamination, although fairly well established, is still the subject of ongoing refinements.

Among all the known signaling cascades, the Reelin signaling pathway is one of the most studied. Reelin, an extracellular matrix protein, is crucial for controlling layer formation of brain structures, such as the neocortex, hippocampus and cerebellum [18–21]. *Reeler* mice lacking Reelin expression, mutants deficient in Reelin signaling molecules as well, show severe defects of neuronal migration and layer lamination, as a consequence of which, dampened learning and memory and cognitive and behavioral abnormalities [22–27]. It has been shown that $\alpha_3\beta_1$ integrins are shown to bind Reelin and control neuronal migration [28]. Moreover, treatment of cortical tissue with Reelin induces phosphorylation of actin depolymerizing protein Cofilin and Src, resulting in stabilization of the actin cytoskeleton [18]. The involvement of Cofilin and Src in Reelin signaling and in neuronal migration is experimental confirmation by our subsequent studies [29, 30]. Focal adhesion kinase (FAK), one tyrosine kinase localizing to focal adhesions, has been shown to be activated at tyrosine 925 (Y925) by Src [30]. FAK expression is related to cell motility, thus elevated FAK activity in human cancers promotes cell invasion through matrix and tissue barriers [31]. By contrast, FAK-deficient cells have more focal adhesions, spread slower than control cells and respond poorly to multiple cues [32]. It was shown that phosphorylation of FAK by Cdk5 at serine 732 is critical for microtubule organization, nuclear movement and neuronal migration [33]. Consistently, conditional FAK knockdown and ablation in migrating cells display abnormal morphology and defective turnover of Connexins 26, suggesting FAK is required for radial glia-dependent neuronal migration [34]. Recently, impairment of neuronal migration by overexpressing FAK and its mutant was reported during corticogenesis [35].

Despite this wealth of studies, it has remained unclear how FAK and its phosphorylation at Y925 are involved in neuronal migration and whether the function of FAK is regulated by Reelin. Here, we address these knowledge gaps and investigate the role of FAK in neuronal migration. The present results show that FAK phosphorylation at Y925 is highly related to Reelin in both the neocortex and hippocampus. The migration of both late-born and early-born cortical neurons is inhibited by overexpressing FAK and its dominant-negative mutant. We found that FAK controls the speed of neuronal locomotion within the CP and the final migrating step: terminal translocation and somal translocation of late-born and early-born neurons, respectively. Furthermore, our findings suggest that regulation of these processes by FAK is mediated at the level of actin and cell-cell adhesion, possibly through regulation of activity of cofilin and expression of N-Cadherin through its phosphorylation by Reelin.

Materials And Methods

Animals

All experiments were performed in compliance with the German laws and the guidelines of the European Community for the use of animals in research and were approved by the local ethical committee (48/13). *Reeler* (B6C3Fe-a/a-ReI^{nl}) were obtained from Dr. Joachim Herz (University of Texas Southwestern, Dallas) and bred in University Medical Center Hamburg-Eppendorf animal facility. Timed-pregnant wild-type C57BL/6J mice from the animal facility and *reeler* mice were housed individually at a 12 h light/12 h dark cycle and were given access to water and food ad libitum. The day of vaginal plug detection was considered embryonic day (E) 0.5, the day of birth was considered postnatal day (P) 0. All experiments were performed on pups of both sexes.

Plasmid construction

Plasmid pCAG-GFP, a mammalian expression green fluorescent protein (GFP) vector driven by the chicken actin promoter (Addgene, plasmid 11150), was used as a control (CON). pCAG-GFP-FAK (FAK-WT) encoding mouse FAK (GenBank: M95408.1) was constructed by reverse transcription PCR (RT-PCR) and In-Fusion (Takara, Japan). The used primers were designed as follows (S, sense; A, anti-sense; underlines are the sites of restriction enzyme): (S) 5'-GCAAAGAATTGAATTCGCCACCATGGCAGCTGCTTATCTTGACCC-3'; (A) 5'-TGCTCGAGGCAAGCTTGCGTGTGGCCGTGTCTGCCCTAGC-3'. A dominant-negative mutant pCAG-FAK(Y925F)-GFP (FAK-DN) mimics the dephosphorylated form by changing tyrosine 925 into phenylalanine was generated by overlap PCR. The mutant primers were designed as follows: (S) 5'-GACAAGGTATTTGAGAATGTGAC-3'; (A) 5'-GTCACATTCTCAAATACCTTGTC-3'. Underlines are the sites of the gene mutant. The constructs were further tested by sequence analysis and enzyme restriction. The resulting plasmids were then purified using an Endo-free maxi prep kit from Qiagen.

Cell culture and transfection

The neuro-2a mouse neuroblastoma cell line (ATCC, CCL-131) were cultured as monolayer cultures in Dulbecco's modified minimal essential medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS, Life Technologies), penicillin (100 units/ml) and streptomycin (100 mg/ml; Life Technologies) in a humidified atmosphere containing 95% air and 5% CO₂ at 37°C. When used for western blotting, cells were seeded in 60-mm cell/tissue culture dishes 36 h before transfection. Cells were transfected with liposome (Merck) according to the manufacturer's instructions transfection. For CON and the expression of FAK and its mutant, cells were transfected at a similar dose and transfection conditions.

Western blot analysis

Total lysates were obtained from 48h transfected neuro-2a cells or the cortex tissue of the brains of P0. Transfected cells were washed with 2 mL of ice-cold phosphate buffered saline (PBS) and harvested with a cell scraper. For the preparation of brain tissues, hemispheres of newborn pups were isolated in ice-cold

PBS and the tissues were chopped and resuspended in ice-cold PBS. The harvested cells or tissues were centrifuged at 2300 rpm for 5 min and lysed in RIPA lysis buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 2 mM NaF, 5 mM EDTA and 1 mM sodium orthovanadate. The protease inhibitor phenylmethylsulfonyl fluoride (PMSF, 1 mM) and phosphatase inhibitor Cocktail-1 (FIVEphoton Biochemicals) were added to the RIPA buffer in advance. After 1-h incubation on ice, the lysates were centrifuged at 10,000 rpm for 10 min at 4°C to remove cell debris. The supernatants were mixed with SDS sample buffer (50 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 100 mM dithiothreitol, or 5% 2-mercaptoethanol and bromophenol blue). Protein concentrations were measured and equal amounts were loaded in each condition. The extracted protein was electrophoresed in 12% SDS-PAGE gels and transferred to 0.45-µm polyvinylidene fluoride membranes (PVDF, Millipore). The PVDF membranes were blocked in TBST buffer (20 mM Tris-HCl (pH 8.0), 150 mM NaCl and 0.05% Tween 20) containing 5% w/v skimmed milk proteins for 3 h at room temperature (RT) and then incubated with primary antibodies (rabbit polyclonal phospho-FAK (Y925) antibody (1:1000, Cell Signaling Technology), rabbit anti-phospho-cofilin Ser3 (1:1000, Santa Cruz), rabbit anti-Actin (1:5000, Millipore), followed by treatment with horseradish peroxidase (HRP)-conjugated secondary antibodies for 2 h at RT. After washing three times with TBST, targeted proteins were detected using Enhanced Chemiluminescence (ECL) Plus Western blotting detection reagents (Pierce) according to the manufacturer's instruction. At least three Western blots were analyzed for each experiment. The stained bands were scanned and the relative optical densities were measured for quantization of the relative expression levels of each protein using Fiji analysis software (National Institutes of Health).

In utero electroporation (IUE)

Timed-pregnant mice (E12.5 or E14.5) were injected subcutaneously with buprenorphine (0.05 mg/kg body weight) 30 min before surgery. Surgery was performed on a heating blanket and toe pinch and breathing were monitored throughout. Under isoflurane anesthesia (induction: 5%, maintenance: 3.5%), the eyes of the dam were covered with eye ointment to prevent damage before dehairing and disinfecting the abdomen with iodine tincture. A ~3 cm midline laparotomy was performed and the uterine horns were exposed and moistened with warm sterile phosphate buffered saline (PBS, 37°C, pH 7.4). Solution containing 1.0 µg/µl pCAG-GFP (CON), pCAG-FAK-GFP (FAK-WT) or dominant-negative pCAG-FAK(Y925F)-GFP (FAK-DN) and 0.1% fast green dye at a volume of 0.75-1.25 µl was injected into the right lateral ventricle of individual embryos using pulled borosilicate glass capillaries (GB100TF-10; Science Products, Hofheim, Germany), with a sharp and long tip. Plasmid DNA was purified with NucleoBond (Macherey-Nagel, Germany). IUE was performed as described previously [29, 30, 36]. Each embryo within the uterus was placed between the electroporation tweezer-type paddles (5 mm diameter, Protech, TX, USA). Electrode pulses (30 V, 50 ms) were applied six times at intervals of 950 ms controlled by an electroporator (ECM 830 BTX; Harvard Apparatus). Uterine horns were placed back into the abdominal cavity after electroporation. The abdominal cavity was filled with warm sterile PBS and abdominal muscles and skin were sutured individually with absorbable and non-absorbable suture thread, respectively. After recovery, pregnant mice were returned to their home cages, which were half placed on a heating blanket for two days after surgery and received on a daily basis additional wet food

supplemented with 2-4 drops Metacam (0.5 mg/ml, Boehringer-Ingelheim, Germany). Dams were sacrificed and the brains of the embryos were used for the preparation of slice cultures (see below). Alternatively, 3, 5, or 7 days after IUE, brains were fixed in 4% paraformaldehyde (PFA) and sectioned coronally into 50- μ m-thick sections.

Preparation of embryonic cortical slice cultures

Three days after electroporation, pregnant mice at E17.5 were decapitated under hypothermic anesthesia. The embryos at E17.5 (CON, n=6; FAK-WT, n=6; FAK-DN, n=8) were collected and rapidly placed in ice-cold Hank's balanced salt solution (HBSS; Invitrogen). The brains were dissected from the skull and checked under a fluorescence microscope. GFP-positive cerebral cortices were then dissected and sectioned (300 μ m) perpendicular to the longitudinal axis of the cerebral cortex using a McIlwain tissue chopper. The slices were placed onto culture inserts (Millipore) and transferred to 6-well plates with 1 ml/well nutrition medium containing 25% heat-inactivated horse serum, 25% Hank's balanced salt solution, 50% minimal essential medium and 2 mM glutamine (pH 7.2; Invitrogen) and incubated in 5% CO₂ at 37°C for at least 3 h. After recovery, the culture inserts were placed in Petri dishes (30 mm diameter) with a glass-bottom containing fresh medium and then transferred for live imaging.

Live imaging of slice cultures

For live imaging of slice cultures, an Impropvision confocal spinning-disc microscope (Zeiss) and 20 $\times$ air immersion objective was used to acquire z-series of cortical slices at 5 μ m intervals through a tissue depth of about 20 μ m. The z-series were then visualized as single optical scans with concurrent orthogonal views using Volocity6 software (Perkin Elmer). The time interval was 10 min. Duration of imaging was up to 15 h. This microscope was equipped with a chamber for the control of temperature, humidity and CO₂. Results of time-lapse imaging of the slice cultures were analyzed using Volocity6 software and the average speed of neuronal cell migration was measured by using Imaris software. Measurements from 50 GFP-expressing cells were collected from three videos of each different plasmid transfection and stage (3 days after IUE). Only neurons whose migration could be tracked for a substantial distance were included. In these cells, the average speed of movement and the direction of migration (towards the marginal zone or ventricular zone) were measured in the x- and y-axes.

Histology and immunohistochemistry and imaging

Brains were prepared for histological analysis on 3, 5 and 7 days after IUE or from non-treated pregnant dams or newborn pups at the desired date. Pregnant wild-type mice were anesthetized with isoflurane and were decapitated under hypothermic anesthesia and the embryos were dissected in ice-cold HBSS. Five days after IUE, corresponding to the day of birth or seven days after IUE, newborn mice were sacrificed by cervical dislocation under hypothermia and the brains were dissected and rapidly placed in HBSS. Brains after IUE were viewed under a fluorescence microscope to verify transfection. Brains were fixed in 4% PFA overnight at 4°C.

After washing in 0.1 M PBS at RT for several hours, brains were embedded in 5% agar and cut transversally into 50-µm-thick slices on a Leica VT 1000S vibratome (Leica Microsystems) and the sections were placed in a 24-well plate containing 0.1 M PBS. For immunohistochemistry, free floating slices were permeabilized and blocked with PBS containing 0.3% Triton X-100 (Sigma Aldrich), 5% normal bovine serum albumin (BSA, Jackson ImmunoResearch). Subsequently, slices were incubated overnight with mouse monoclonal anti-Reelin G10 (1:1000, Millipore), rabbit polyclonal anti-phospho-FAK (Y925) (1:1000, Cell Signaling Technology), mouse monoclonal anti-NeuN (1:1000, Millipore) or rabbit monoclonal anti-Brn2 (1:1000, Cell Signaling Technology) diluted in PBS containing 0.03% Triton X-100, 0.5% BSA and 1% normal goat serum (NGS, Sigma), followed by 2 h incubation with secondary antibody Alexa Fluor-568 goat anti-mouse (1:500, Invitrogen), Alexa Fluor-488 goat anti-rabbit (1:500, Invitrogen), Alexa Fluor-568 donkey anti-mouse (1:500, Invitrogen), Alexa Fluor-647 donkey anti-rabbit (1:500, Invitrogen). DAPI (1:5000) was added to the second antibody for the nuclear labeling. Finally, slices were transferred to glass slides and covered with Vecta-Shield (Vector Laboratories). Sections were photographed using a confocal laser-scanning microscope (Leica TCS SP5) and 20× air or 63× oil immersion objectives. z-series of brain sections at 0.5 µm intervals through a tissue depth of 9 µm were acquired and visualized as single optical scans with concurrent orthogonal views using Leica LAS software (Leica).

Data Analysis

Of the embryos transfected with the different constructs, at least three brains per different construct and stage were used. Statistical analyses were performed in Graphpad Prism environment. Student's t-tests were used to determine the statistical significance of differences between the two groups. Comparisons among multiple groups were evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni-corrected post hoc analysis. Data are presented as mean ± SEM. Significance levels of $p < 0.05$ (*, #), $p < 0.01$ (**, ##) or $p < 0.001$ (***, ###) were used.

Results

Phosphorylation of FAK at Y925 is related to Reelin expression

FAK is involved in cell motility through regulating by different molecules and plays a critical role in corticogenesis [34, 33, 35]. However, the function of FAK, especially its phosphorylation of at Y925 (p-FAK925) during neuronal migration *in vivo* and the underlying molecular mechanism is still poorly understood.

To close this knowledge gap, we first performed immunostaining in the developing brain (Fig. 1A, B). p-FAK925 was expressed in both neocortex and hippocampal formation at E17.5 (Fig. 1A). p-FAK925 did not show layer preference but was ubiquitously distributed throughout the cortical plate (CP) and subareas of the hippocampus. Exceptions were marginal zone (MZ) of the neocortex, the MZ of the

dentate gyrus (DG), the prospective outer molecular layer (oml) of the DG, where populate Reelin-positive Cajal–Retzius (CR) cells. Strong p-FAK expression was highly overlapped with Reelin. A similar expression profile was noticed in neocortical and hippocampal slices from P0 mice (Fig. 1B). In addition, we performed Western blots of p-FAK925 in cortical lysates prepared from P0 wild-type mice ($rl^{+/+}$) or their *reeler* ($rl^{-/-}$) littermates (Fig. 1C). Densitometric analysis from three independent experiments showed that the protein level of p-FAK925 was significantly downregulated in *reeler* mutant mice (Fig. 1D, $rl^{+/+}$: 1.00 ± 0.0 , $rl^{-/-}$: 0.66 ± 0.038 , $p < 0.0001$). Our observations suggest that the function of FAK is highly related to the Reelin signaling pathway during brain development.

FAK disrupts the radial neuronal migration and reduces the migratory speed of late-born neurons

To further gain insight into the function of FAK during neuronal migration, plasmids overexpressing wild-type FAK (FAK-WT) and a dominant-negative mutant (FAK-DN) by changing tyrosine 925 into phenylalanine were constructed (Fig. 2A). These plasmids were transfected into cultured Neuro-2a cells, exogenous p-FAK was detected by immunostaining after 24 h. The elevated phosphorylation of FAK was only seen in the FAK-WT expressing cells but not in GFP (CON) and FAK-DN expressing cells (Fig. 2B). GFP-labelling was found in the whole cell in GFP-transfected control cells, while GFP-labelling was only detected in the cytoplasm and absent in nuclei in cells transfected with plasmids of FAK-WT and FAK-DN. Neuro-2a cells were harvested 48 h after transfection for Western blot. Strong endogenous p-FAK was found in FAK-WT lysate (Fig. 2C). These results indicate FAK and its mutant were expressed and functioned as expected.

To study the effects of FAK on neuronal migration, *in utero* electroporation (Tabata and Nakajima, 2003) was employed to overexpress different plasmids in mouse brains at E14.5. Plasmids were transfected into newly generated late-born neurons in the ventricular zone (VZ). After 3 days at E17.5, transfected brains were sectioned. In CON ($n=10$), numerous GFP-positive cells migrated into both the upper region of the CP (UCP) and the deeper region of the CP (DCP) (Fig. 3A). However, neurons in brains transfected with the plasmid expressing FAK-WT ($n=8$) and FAK-DN ($n=10$) had fewer neurons invaded the CP, but more accumulated in the intermediate zone (IZ) and ventricular zone/subventricular zone (VZ/SVZ) (Fig. 3B, Table 1). In CON group, the transfected cells that were migrating in the CP showed a typical bipolarity morphology *in vivo* (Fig. 3C), which is a long and thick leading process directed toward the pial surface and a short, thin trailing process pointing to the VZ. Similar to Neuro-2a cells *in vitro*, overexpression of FAK-WT and FAK-DN was mainly located in processes and the perinuclear region of migrating neurons. Moreover, compared to the even distribution of GFP signal in CON neurons, aggregates of FAK-WT and FAK-DN were found in the unsmooth-looking membrane of neurons (Fig. 3C), which might be the sites of focal adhesions between neurons or neurons with glial cells (Fig. 3C). Given the polarity of migrating neurons was barely affected, we checked the migratory behavior of neurons transfected with the different constructs using time-lapse imaging. CON cells revealed their characteristic bipolar shape and moved smoothly by nuclear translocation ($n=227$ cells, 18.93 ± 0.646 $\mu\text{m/h}$, Fig. 3D-E, Tabel 1, Movie 1, 2). By

contrast, neurons transfected with FAK-WT showed slower forward movement ($n= 256$ cells, $16.14 \pm 0.515 \mu\text{m/h}$, $p=0.0025$, Fig. 3D-E, Tabel 1, Movie 3, 4), as well as in FAK-DN neurons ($n= 338$ cells, $15.92 \pm 0.510 \mu\text{m/h}$, $p=0.0004$, Fig. 3D-E, Tabel 1, Movie 5, 6). Thus, overexpression and mutation of FAK disrupt the radial neuronal migration of late-born neurons, at least partially by reducing their migratory speed. Of note, since the plasmids we used were fused FAK with GFP, so GFP represented the distribution changing of FAK during locomotion circles. In FAK-WT transfected neurons, the dynamic GFP signal was observed inside the migrating neurons. FAK-WT either spread relatively more 'even' in the cytoplasm and the leading process (Fig. 3D, FAK-WT, 0-40 min) or aggregated in the perinuclear area of the leading process and tail process (Fig. 3D, FAK-WT, 60-100, 180-200 min) or localized on one side of the cell body (possible the interaction side between neuron and radial glial cell, Fig. 3D, FAK-WT, 120-160 min). In contrast, the FAK-DN transfected cells showed a more stable GFP signal (Fig. 3D, FAK-DN 40-200 min), especially in the FAK-DN high-expressing migrating neuron (Movie 7).

Table 1

Summary of the distribution pattern, locomotion speed and soma location of cortical neurons in neocortex after IUE.

Neuronal Distribution		CON	FAK-WT	FAK-DN	F values
E14.5- E17.5	UCP (%)	28.83 ± 3.107	17.41 ± 2.821 *p=0.0179	14.07 ± 1.800 **p=0.0011	F(2,25)=9.203 P=0.001
	DCP (%)	20.58 ± 1.452	15.34 ± 1.108 *p=0.0251	17.86 ± 1.124	F(2,25)=4.113 P=0.0281
	IZ (%)	31.06 ± 3.693	40.81 ± 5.914	33.70 ± 5.811	F(2,25)=1.638 P=0.2146
	VZ/SVZ (%)	19.53 ± 2.135	27.69 ± 4.295	34.37 ± 4.378 *p=0.0205	F(2,25)=4.358 P=0.0228
	Locomotion speed (µm/h)	18.93 ± 0.646	16.14 ± 0.515 **p=0.0025	15.92 ± 0.510 ***p=0.0004	F(2,818)=8.401 P=0.0002
E14.5-P0	II/III (%)	91.02 ± 1.945	59.39 ± 4.156 ***p<0.0001	73.73 ± 1.881 *p=0.0118 #p=0.0273	F(2,16)=23.84 P<0.0001
	IV (%)	4.25 ± 1.246	15.60 ± 1.301 ***p<0.0001	16.08 ± 1.387 ***p<0.0001	F(2,16)=24.33 P<0.0001
	V/VI (%)	3.24 ± 0.829	17.57 ± 2.832 **p=0.0014	8.38 ± 2.398 p=0.0505	F(2,16)=10.10 P=0.0015
	IZ (%)	1.49 ± 0.515	7.44 ± 2.201 *p=0.0414	1.81 ± 0.487	F(2,16)=4.401 P=0.030

Data are shown as mean ± SEM. Significance was assessed using one-way analysis of variance (ANOVA) test followed by Bonferroni-corrected post hoc test and the listed p values correspond to comparisons between CON and FAK-WT CON and FAK-DN (*p< 0.05, **p< 0.01, ***p< 0.001), FAK-WT and FAK-DN (#p< 0.05, ##p< 0.01, ###p< 0.001).

Neuronal Distribution		CON	FAK-WT	FAK-DN	F values
PCZ (%)		47.72 ± 1.695	39.34 ± 2.391	30.39 ± 1.920	F(2,9)=18.35 P=0.0007
			*p=0.0403	***p=0.0006 #p=0.0365	
E14.5-P2	II/III (%)	91.71 ± 1.71	80.08 ± 2.352	78.92 ± 2.662	F(2,20)=4.249 P=0.0290
			*p=0.0386	*p=0.0292	
	IV (%)	6.20 ± 1.27	15.78 ± 1.76	12.97 ± 1.46	F(2,20)=5.455 P=0.0129
			*p=0.0111	*p=0.0244	
	V/VI (%)	1.33 ± 0.677	2.13 ± 0.590	6.16 ± 1.27	F(2,20)=4.668 P=0.0217
				*p=0.033	
	IZ (%)	0.76 ± 0.446	2.01 ± 0.637	1.95 ± 0.696	F(2,20)=0.5894 P=0.564
Data are shown as mean ± SEM. Significance was assessed using one-way analysis of variance (ANOVA) test followed by Bonferroni-corrected post hoc test and the listed p values correspond to comparisons between CON and FAK-WT CON and FAK-DN (*p< 0.05, **p< 0.01, ***p< 0.01), FAK-WT and FAK-DN (#p< 0.05, ##p< 0.01, ###p< 0.001).					

The migration defects observed above led to the question of whether the final location of late-born neurons is affected as well. Next, we examined the transfected brains after birth at P0. At this time point, most neurons have terminated their migration and reached their destinations in the CP. More than 90% of all labeled CON cells (n=6, 91.02 ± 1.945%) formed a compact layer subjacent to the MZ, which is layer II/III of the cortex (Fig. 4A, B, Tabel 1). Layer IV, V/VI and IZ contained a few neurons (Fig. 4A, B, Tabel 1). Notably, the distribution pattern of FAK-WT transfected cells was quite different from that in the CON group (Fig. 4A). Significantly fewer cells (n=8, 59.39 ± 4.156%, p<0.0001) were found in the layer II/III of the CP, while significantly more cells in layer IV, V/VI and IZ (Fig. 4B, Tabel 1). The majority of FAK-DN neurons (n=5, 73.73 ± 1.881%, p=0.0118) occupied layer II/III, whose number was significantly lower than in the CON group. Consequently, there were much more neurons stacked in layer IV (Fig. 4B, Tabel 1). Furthermore, the neurons that reached layer II/III were significantly less (p=0.0273) in the FAK-WT group when compared to the FAK-DN group as well. These migration impairments in the FAK-WT and FAK-DN groups lasted till P2 when there were still much more transfected neurons not reaching their final destination in layer II/III (Fig. 4C, D, CON: n=4, 91.71 ± 1.71%; FAK-WT: n=7, 80.08 ± 2.352%, p=0.0386; FAK-DN: n=12, 78.92 ± 2.662%, p=0.0292, Tabel 1), but more neurons stayed in layer IV and V/VI (Fig. 4C, D, Tabel 1). These mislocated neurons gave rise to several branches (Fig. 4C), suggesting their migration stopped and differentiation started.

These data indicate that overexpression of FAK and its mutant induces migration defects of late-born neurons, not only delaying the locomotion but the final destination.

FAK is required for glia-independent terminal translocation in late-born neurons and somal translocation in early-born neurons

The abnormal location of FAK-WT and FAK-DN neurons observed above suggests that their migration disturbance was not solely related to the reduction of locomotion speed, but was contributed by other processes regulated by FAK. One possible process might be the glia-independent terminal translocation. Locomoting neurons directionally migrate toward the pia surface and when they arrive beneath MZ they apply their final migration mode, in which they extend processes to the MZ and shorten their leading processes to move their cell bodies to their final positions [10].

To directly test this hypothesis, we then first monitored the effect of FAK-WT and FAK-DN on terminal translocation in late-born neurons. These neurons transiently pause and accumulate, forming into the primitive cortical zone (PCZ). At P0, PCZ could be identified by dual staining of NeuN and Brn2 (Fig. 5A). Quantification showed that, in the CON group, $47.72 \pm 1.695\%$ (n=4) transfected neurons located their somata in the NeuN⁺Brn2⁺ PCZ region (Fig. 5A, B), whereas there was a much lower number of neurons in FAK-WT (n=4, $39.34 \pm 2.391\%$, p=0.0403, Tabel 1) and FAK-DN group (n=4, $30.39 \pm 1.920\%$, p=0.0006, Tabel 1). Of note, the difference of this number in FAK-WT and FAK-DN was significant as well (p=0.0365). These results suggest that the terminal translocation of late-born neurons was affected by FAK manipulation.

Second, we studied the migration of early-born neurons in the early developmental stages since they undergo somal translocation mode, which is morphologically similar to the final terminal translocation mode of late-born neurons [7]. To target early-born neurons, IUE experiments were performed at E12.5. Transfected brains were sectioned and examined 5 days later at E17.5. Most neurons in all groups migrated into the CP by E17.5 (Fig. 5C, D), while significantly less occupation of FAK-WT (n=4) and FAK-DN (n=4) neurons in the upper region of CP (Bin 2-3) when compared to CON (n=4). More neuronal accumulation was observed around the border between the CP and the IZ (Fig. 5D, Bin 10). These observations suggest that FAK deregulation affected the somal translocation of early-born neurons, similar to the phenotype of FAK-WT and FAK-DN at a later stage (electroporation at E14.5).

Collectively, these series of data suggest that FAK is required for the neuronal migration of both early-born and late-born neurons. However, different mechanisms might be involved in these processes.

FAK regulates the phosphorylation of cofilin and the expression of N-Cadherin.

To investigate the molecular mechanisms of how FAK affects neuronal migration, molecules control cytoskeleton dynamics and cellular adhesion, both processes that are highly involved in regulating neuronal migration, were first examined. Previous results showed that Cofilin and N-Cadherin are critical genes in regulating neuronal migration, whose function was closely related to Reelin signaling cascade

[18, 29, 37, 38]. In the present study, FAK phosphorylation at Y925 was found to be highly related to Reelin expression. Therefore, Neuro-2a cells were transfected with FAK-WT and its mutant FAK-DN and then subjected to immunoblot analysis with antibodies against p-cofilin and N-Cadherin (Fig. 6A).

The results showed that the overexpression of FAK-WT did not affect the phosphorylation of cofilin. However, phosphorylation of cofilin was significantly inhibited in cells transfected with FAK-DN (Fig. 6A, B, CON: 1.00 ± 0.0 , FAK-WT: 0.95 ± 0.081 , FAK-DN: 0.72 ± 0.060 , $F(2,12)=6.451$, $P=0.012$, $*p=0.017$, $^{\#}p=0.049$). In addition, the expression of N-Cadherin was significantly downregulated in the FAK-DN group, while no change was found in cells transfected with FAK-WT (Fig. 6A, B, CON: 1.00 ± 0.0 , FAK-WT: 1.05 ± 0.054 , FAK-DN: 0.63 ± 0.097 , $F(2,6)=12.26$, $P=0.008$, $*p=0.022$, $^{\#}p=0.012$). Given that p-FAK925 was significantly lower in *reeler* mice (Fig. 1D), we next examined whether cofilin and N-Cadherin expression was affected by *reelin* knockout. Western blots of N-Cadherin in cortical lysates prepared from P0 wild-type mice ($rl^{+/+}$) or their *reeler* ($rl^{-/-}$) littermates showed that the protein level of N-Cadherin was downregulated in *reeler* mutant mice (Fig. 6C, D, $rl^{+/+}$: 1.00 ± 0.0 , $rl^{-/-}$: 0.52 ± 0.019 , $p<0.0001$). Phosphorylation of cofilin was lower in *reeler* as well (Fig. 6C, D, $rl^{+/+}$: 1.00 ± 0.0 , $rl^{-/-}$: 0.66 ± 0.036 , $p<0.0001$), which confirm previous investigations that Reelin enhances cofilin activity by regulating its phosphorylation [18].

Thus, our observations suggest that the phosphorylation of FAK at Y925 is crucial for different migrating processes via coordinate cytoskeleton dynamics and adhesion, which are highly related to cofilin and N-Cadherin through the Reelin signaling pathway.

Discussion

Coordinated corticogenesis during early development has been proposed to underlie precise connectivity and higher brain functions such as learning, memory and cognition (Guy and Staiger, 2017; Kast and Levitt, 2019). Neuronal migration, as a core aspect of corticogenesis, is especially being the interest of research in the last decades. However, until recently, the molecular signaling cascades for controlling neuronal migration during development were not fully uncovered. In the present study, we reported that overexpression of FAK in cortical neurons *in vivo* disrupts the normal radial migration. This phenotype is likely to be due to inhibited glia-dependent locomotion speed revealed by time-lapse imaging and glia-independent translocation defects in abundant FAK expression (FAK-WT group) and the absence of FAK Y925 phosphorylation (FAK-DN group). Impaired neuronal migration correlates with actin turnover and focal adhesion site, which appears to be the molecular basis for this phenotype. Indeed, we show that FAK controls actin polymerization and cell adhesion by regulating cofilin phosphorylation and expression of N-Cadherin, respectively. These processes involved phosphorylation of FAK at Y925 and are correlated with Reelin. Our findings reveal a new Reelin regulated mechanism through which FAK controls actin dynamics and cell adhesion, thus neuronal migration during development.

Detailed expression and localization investigation in rat brain revealed ubiquitously presence of FAK in all regions of developing and adult brains, with enrichment in specific neuronal populations of the neocortex,

hippocampus and cerebellum, particularly during development [39]. In contrast to restricted to focal adhesions in astrocytes, FAK was found in cell bodies, axons, dendrites of neurons and showed no enrichment in any specific neuronal compartment [39, 40]. In line with this result, FAK-WT and FAK-DN signal was only found in the cytoplasm of either neuronal cell line *in vitro* or transfected migrating neurons *in vivo*. Tyrosine phosphorylation is a critical regulator of FAK and is mainly regulated by Src family kinases (SFK) [41]. Although FAK hypophosphorylation was only found in *fyn* mutants [40], other members of SFK: *src* and *yes* were proved to activate FAK in various studies [42, 43, 30]. A wealth of studies documented FAK plays a pivotal role in the regulation of cell proliferation, survival and migration in various cell types [44–49]. Given members of the SFKs have been implicated in neuronal migration, as a downstream kinase that can be phosphorylated by SFK, the function of FAK was investigated during neuronal migration. FAK-null mice showed an embryonic lethal phenotype due to defects in the axial mesodermal tissues and cardiovascular system [32], which limited its use for studies on the roles and mechanisms of FAK in embryonic development. Conditional deletion of FAK in migrating pyramidal neurons did not show any obvious migratory alteration [50], which is conflicting with later researches. Alternatively, in the same year, Xie et al. showed Cdk5 dependent FAK phosphorylation at S732 is critical for the organization of microtubules based nuclear translocation and, in turn, neuronal migration [33]. Conditional ablation and knockdown of FAK showed that FAK is required for radial migration of pyramidal cells, but not for tangential migration of cortical interneurons [34]. In addition, FAK-deficiency induced abundant branching and swelling of the leading process of migrating neurons by a defective assembly of Connexin-26 contact points in the adhesion surface between neurons and radial glial cells. Overexpression of FAK *in vivo* impaired neuronal migration and layer formation of the neocortex possibly is related to its relationship with vinculin and F-actin cytoskeleton [35]. The involvement of FAK in brain development was also confirmed in another study of Inositol hexakisphosphate kinase 1 (IP6K1) [51]. IP6K1 physiologically interacts with α -actinin and localizes to focal adhesions, whose deletion abolishes FAK autophosphorylation and α -actinin and leads to brain malformation and abnormalities of neuronal migration. The present results add experimental evidence for the involvement of FAK in neuronal migration, during which actin cytoskeleton dynamic and adhesion are both affected by phosphorylation of FAK at Y925. Collectively, increasing evidence showed FAK participate in neuronal migration in a cell-autonomous manner.

The results of western blot after FAK overexpression provide first insights into the mechanisms of how FAK is involved in different stages of radial migration. Canonical Reelin signaling involves the very low density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2) [52, 27]. The cellular adaptor protein Disabled 1 (Dab1) is phosphorylated by SFK upon binding of Reelin to its receptors, which further activates SFK and the subsequent downstream molecules [53, 23]. Our observations of colocalization between Reelin and p-FAK and downregulated FAK phosphorylation in *reeler* mutant experimentally proved the involvement of FAK in the “classical” Reelin signaling pathway to control neuronal migration. Reelin induces cofilin phosphorylation at Serine3, thus stabilizing actin dynamics and controlling the orientation and speed of migrating neurons [18, 29]. Consistent with these results, cofilin phosphorylation was also significantly reduced in *reeler* mutants in our observation. Meanwhile,

dephosphorylation of FAK at Y925 strongly inhibited cofilin phosphorylation, suggesting that FAK regulates neuronal migration through influencing actin cytoskeleton dynamics. This mechanism not only underlies the reduced migrating speed, as well as the final translocation—both processes were disturbed by inhibiting FAK. In contrast to that FAK promotes cancer cell migration thus enhancing invasion [31, 54], we showed overexpressing FAK strongly inhibited neuronal migration *in vivo*. Although striking similarities in neuronal and cancer cells, there are more contrasts between cancer metastasis and neuronal motility, e.g., migrating substrates, microenvironment, intracellular forces and cytoskeleton arrangement [55]. Neuronal migration is a more intricate process including synergistic cooperation between neurons, glial cells and the extracellular cues. Compared to other phosphorylation sites of FAK, e.g., Y397, Y576/577; S732; Y861 [56], Y925 is of particular especial. Phosphorylated FAK at Y925 creates a specific SH2-binding site for the GRB2 adaptor protein [57]. It has been shown that only FAK Y925 is required for Strain-stimulated AKT phosphorylation for controlling cell migration [58]. Src-dependent phosphorylation of FAK at Y925 specifically promotes detachment of focal adhesions at the trailing edge of migrating neurons [42]. FAK Y925 phosphorylation is critical for focal adhesions disassembly rate at the cell rear and cell protrusion [47].

In addition, N-Cadherin expression was significantly reduced in *reeler* mice, as well as after FAK dephosphorylation. N-Cadherin is a single-pass transmembrane adhesion receptor required for cell-cell adhesion [59, 60]. The complex migration mode of late-born neurons involves the glia-neuron adhesion during radial locomotion, indicating the regulatory function of N-Cadherin in neuronal migration. Indeed, N-Cadherin controls neuronal migration and lamination in the neocortex under the control of Reelin transduction [37, 38, 12, 61]. Moreover, knockdown of Myosin X abolishes neuronal N-Cadherin expression and disturbs the adherence of migrating neurons to radial glial cells, resulting in neuronal migration defects [62]. N-Cadherin regulates the migration of late-born neurons by regulating their radial orientation of multipolar stage and asymmetric bridge complex that is required for glial-guided migration [12, 61]. In our results, dynamics of FAK distribution in different neuronal subregions were observed by time-lapse imaging, suggesting adhesion sites between neurons and other cells are not stabilized during the one locomotion circle, which is consistent with the function of FAK in regulating focal adhesion turnover [47]. Apparently, N-Cadherin stabilizes the leading process in the MZ and facilitates the somal translocation of early-born cortical neurons [37]. In line with these results, glia-independent terminal translocation of late-born neurons was inhibited after FAK overexpression and dephosphorylation. FAK dephosphorylation at Y925 seems strongly inhibit the dynamic transporting of FAK. Reelin transiently enhances the adhesiveness of neurons to N-Cadherin and promotes neuronal aggregation, which is important for correct stopping and positioning of neurons during migration [38]. Unlike this observation, there was no neuronal aggregation observed after manipulating FAK function, suggesting Reelin-FAK-mediated N-Cadherin expression might not regulate neuron-neuron adhesion but glia-neuron interaction. It seems that function of N-Cadherin is not only due to its role in mediating cell-cell adhesion, but also cytoskeleton dynamics. Above mentioned function of N-Cadherin in controlling both the radial orientation and translocation involved Rap1 GTPases [37, 61], which provide a key nexus for activating other small

GTPases, in turn, regulate the actin cytoskeleton [63]. Cadherin can anchor to the actin cytoskeleton, which is a Rac1-regulated process regulating cell polarity, morphology and motility [64, 65].

Noncanonical signaling can refer to the involvement of transmembrane proteins other than VLDLR and ApoER2, e.g., integrins. Reelin binds directly to the extracellular domain of integrins and interacts with Dab1 through its NPXY motif in the cytoplasmic tail [28, 13, 66], thus facilitating cell-cell and cell-extracellular matrix (ECM) adhesion [67]. Reelin- $\alpha_3\beta_1$ integrin interactions inhibit cortical neuronal migration [28] and are required for the formation of the radial glial scaffold necessary for neuronal migration in the hippocampus [20]. As a non-receptor protein tyrosine kinase, FAK is activated by interactions with integrins and functions as a 'scaffold' for the recruitment signaling proteins needed for proliferation [68], extracellular stimulated cell migration [44], neurites outgrowth and axon pathfinding [69, 70]. Moreover, the SH2 binding site for GRB2 at FAK Y925 partially overlaps with binding sites for paxillin [71], which is an important integrin-binding protein that recruits FAK and vinculin to focal contacts [72–74]. Overexpression of a Y925F mutant of FAK resulted in strong focal contact distribution and blocks the turnover of focal contacts [42, 75]. A point mutation in the Y925 of FAK selectively disrupts GRB2 but promote paxillin binding, then the subsequent association of FAK with β_1 -integrin and prevents the dissociation of FAK from focal contacts and leads to inefficient focal turnover [73], which is consistent with our observation of the relatively stable distribution of FAK-DN signal in the migrating neurons. Therefore, although the direct relation between FAK and integrin was not examined in the present study, it strongly suggests that Reelin-integrin-FAK mediated cell-ECM adhesion might be one underlying mechanism for glial-independent translocation. Moreover, the function of FAK in regulating microtubule organization cannot be neglected during the glial-independent translocation process, during which microtubules generate mechanical forces and drive nuclear translocation [76].

In the present study, we show that FAK-mediated Reelin signaling regulates cortical lamination by controlling two key events during embryonic development: glia-dependent locomotion and glia-independent translocation. Both processes involve dynamic regulating of the actin cytoskeleton and focal adhesions, which might be related to the activity of cofilin and Cadherin. Overall, these data expand the known Reelin signal pathway and provide new insights in understanding neuronal migration mechanisms.

Declarations

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Author contributions

L.S. M.F. and X.C. designed the experiments, L.S. performed experiments and analyzed the data, X.C. helped with IUE and living imaging, S.Z. discussed the data and contributed to unpublished analytic tools, L.S. interpreted the data and wrote the original draft of the paper. L.S. S.Z. and X.C. discussed and commented on the manuscript.

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Availability of data and materials

The data and material supporting the findings of this study are available with the article or is available from the corresponding author upon request.

Code availability

Not applicable.

Ethics approval

The animal study was reviewed and approved by the local ethical committee of the University of Hamburg.

Consent for publication

Not applicable.

Consent to Participate

Not applicable.

Competing interests

The authors declare no competing interests.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest

Not applicable.

Research involving animals

The animal study was reviewed and approved by the local ethical committee of the University of Hamburg. Welfare of animals were considered during experiments.

Informed consent

Not applicable.

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Figures

Figure 1 -Song et al.

Figure 1

p-FAK925 was highly expressed in Reelin-positive Cajal-Retzius cells in the marginal zone of developing neocortex and hippocampus. A, Representative fluorescent images of phosphorylated focal adhesion kinase (p-FAK925, green) in neocortex (up row) and hippocampus (bottom row) slices from E17.5 mice when co-stained with Reelin (red) and DAPI (blue). Insets, colocalization of p-FAK and Reelin (arrows) shown at higher magnification. Dotted lines mark area borders. Scale bar, 100 μ m. MZ: marginal zone, CP: cortical plate, IZ: intermediate zone, VZ: ventricular zone, CA1: Cornu Ammonis 1, DG: dentate gyrus, H: hilus, ML: molecular layer. B, Same as A, for P0 mice. Scale bar, 50 μ m. C, Western blotting for p-FAK925 expression in cortical lysates from P0 wild-type mice (*rl*^{+/+}) or their reeler (*rl*^{-/-}) littermates. Actin

was used as a loading control. D, Densitometric analysis of relative p-FAK925 expression level from three independent experiments. Data are presented as mean $\pm$ SEM. Student's t-test, ***p < 0.001.

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Figure 2

Construction and verification of wild-type and dominant-negative FAK plasmids. A, Schematic diagrams of FAK function motifs and the mutated amino acid (Phe925). All constructs express a fusion protein of FAK or its mutants and GFP. B, Representative images of neuro-2a cells after 24h transfection with different constructs. GFP (green) expression was detected in the whole cells transfected with control plasmid expressing GFP (up row), while only in the cytoplasm of cells transfected with plasmids expressing FAK-WT (middle row) and FAK-DN (bottom row). Cells were co-stained with p-FAK (red) and DAPI (blue). Scale bar, 20 μ m. C, Western blotting p-FAK925 expression from neuro-2a cells after 48h transfection with different constructs. Actin (upper band) was used as a loading control.

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Figure 3

Overexpression of FAK-WT and FAK-DN disrupted the migration of late-born neurons in the neocortex. A, Representative images showing the distribution of late-born cortical neurons (green) in DAPI (blue) stained coronal slices from E17.5 mice electroporated with CON (left), FAK-WT (middle), FAK-DN (right) at E14.5. Scale bar, 100 μ m. B, Quantitative analysis of the distribution pattern of electroporated cortical neurons across four different cortical zones at E17.5. C, Higher magnifications of migrating neurons in

CP of E17.5 cortical sections electroporated at E14.5. Sections were counter stained with DAPI (red). Scale bar, 10 μ m. D, Migratory behavior of neurons transfected with the different constructs 3 days after IUE. Individual neurons were monitored over a period of 200 min (selected neurons from Movies 1-7). White arrowheads indicate the somata. Scale bar, 10 μ m. E, Quantitative assessment (left) and cumulative distribution (right) of locomotion speed of migrating neurons 3 days after IUE. Data are presented as mean $\pm$ SEM. One-way ANOVA test followed by Bonferroni-corrected post hoc test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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Figure 4

Late-born neurons transfected with FAK-WT and FAK-DN were unable to reach their final destination. Retarded and inhibited neuronal migration of late-born neurons after transfection of FAK-WT and FAK-DN. A, Representative images showing the distribution of the transfected late-born cortical neurons (green) from P0 mice electroporated with CON (left), FAK-WT (middle), FAK-DN (right) at E14.5. The sections were counterstained with DAPI (blue). Scale bar, 100 μ m. B, Quantitative analysis of the distribution pattern of electroporated cortical neurons across four different cortical zones at P0. C-D, Same as A-B, for P2 mice. White arrowheads in C indicate neurons with multiple branches. Scale bar, 100 μ m. Data are presented as mean $\pm$ SEM. One-way ANOVA test followed by Bonferroni-corrected post hoc test, *, #p < 0.05, **p < 0.01, ***p < 0.001.

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Figure 5

Overexpression of FAK-WT and FAK-DN disrupted the translocation of late-born and early-born neurons in the neocortex. A, Representative images showing the soma location of transfected late-born cortical neurons (green) in upper layers of coronal slices from P0 mice electroporated with CON (left), FAK-WT (middle), FAK-DN (right) at E14.5. Brn2 (cyan) and NeuN (Magenta) were used to identify the primitive cortical zone (PCZ) (Brn2+NeuN-). Scale bar, 100 μ m. B, Quantitative analysis of the percentage of

electroporated cortical neurons in PCZ at P0. C, Representative images showing the distribution of the transfected early-born cortical neurons (green) in neocortex from E17.5 mice electroporated with CON (left), FAK-WT (middle), FAK-DN (right) at E12.5. The sections were counterstained with DAPI (blue). Scale bar, 100 μ m. D, Bin analysis of the distribution pattern of electroporated cortical neurons across cortex at E17.5. The area from the outer margin of the CP to the inner margin of the VZ was divided into 10 equally spaced bins and the transfected neurons in each bin were counted. Data are presented as mean $\pm$ SEM. One-way ANOVA test followed by Bonferroni-corrected post hoc test, *, # $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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Figure 6

Reelin and FAK regulate cofilin phosphorylation and N-Cadherin expression. A, Western blotting for p-cofilin and N-Cadherin expression from neuro-2a cells after 48h transfection with different constructs. Actin was used as a loading control. B, Densitometric analysis of relative p-cofilin and N-Cadherin expression level from three independent experiments (t-tests, *** $p < 0.001$). C, Western blotting for p-cofilin and N-Cadherin expression in cortical lysates from P0 wild-type mice ($rl^{+/+}$) or their reeler ($rl^{-/-}$) littermates. Actin was used as a loading control. D, Densitometric analysis of relative p-cofilin and N-Cadherin expression level from three independent experiments. Data are presented as mean $\pm$ SEM. Student's t-test, *** $p < 0.001$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Movie1CON.avi](#)
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- [Movie3FAKWT.avi](#)
- [Movie4FAKWTcotransfectedwithRFP.avi](#)
- [Movie5FAKDN.avi](#)
- [Movie6FAKDNcontransfectedwithRFP.avi](#)
- [Movie7FAKDNhigherexpression.avi](#)