A Novel c-Jun N-terminal Kinase (JNK) Signaling Complex Involved in Neuronal Migration during Brain Development**

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Disturbance of neuronal migration may cause various neurological disorders. Both the transforming growth factor-β (TGF-β) signaling and microcephaly-associated protein WDR62 are important for neuronal migration during brain development; however, the underlying molecular mechanisms involved remain unclear. We show here that knock-out or knockdown of Tak1 (TGFβ-activated kinase 1) and Jnk2 (c-Jun N-terminal kinase 2) perturbs neuronal migration during cortical development and that the migration defects incurred by knock-out and/or knockdown of Tbr2 (type II TGF-β receptor) or Tak1 can be partially rescued by expression of Tak1 and JNK2, respectively. Furthermore, TAK1 forms a protein complex with RAC1 and two scaffold proteins of the JNK pathway, the microcephaly-associated protein WDR62 and the RAC1-interacting protein POSH (plenty of Src homology). Components of the complex coordinate with each other in the regulation of Tak1 as well as JNK activities. We suggest that unique JNK protein complexes are involved in the diversified biological and pathological functions during brain development and pathogenesis of diseases.

Radial migration of neurons plays a critical role in the lamination of the neocortex and functional neuronal connectivity during brain development (1–4). During corticogenesis, newborn neurons derived from the ventricular zone (VZ)2 undergo radial-glia-guided migration to reach their final destination within the cortex. Disturbance of neuronal migration may cause various neurological disorders, such as primary autosomal recessive microcephaly (MCPH) and lissencephaly (2, 5–7).

Transforming growth factor-β (TGF-β) signaling controls developmental processes, including cell proliferation, differentiation, morphogenesis, and tissue homeostasis. Disruption of TGF-β signaling through knock-out of type II TGF-β receptor (Tβr2) has been shown to play important roles in axonal specification and neuronal migration during brain development (8), although the underlying mechanisms involved in TGF-β signaling during neuronal migration are still unclear.

TGF-β signaling is regulated through both Smad-dependent (canonical) and Smad-independent (the non-canonical) pathways (9, 10). One such non-canonical pathway involved in TGF-β signaling is the c-Jun N-terminal kinase (JNK) pathway (9, 10). A JNK pathway protein complex, the POSH-JIP apoptotic complex, which includes two scaffold proteins, plenty of Src homology (POSH) and JNK-interacting protein (JIP), as well as the GTPase family member RAC1, and several members of the mixed-lineage kinases (MLks) and the MAPK kinases (MKks) 4 and 7, has been shown to be involved in neuronal apoptosis (11–13). Interestingly, several POSH-JIP apoptotic complex components, including POSH, RAC1, MKK4, and MKK7, are also indispensable for radial neuronal migration in the developing brain (14–18). However, it remains questionable whether JNK promotes or inhibits neuronal migration during brain development (15, 19).

Mutations of WD40-repeat protein 62 (WDR62) have been identified to cause human MCPH (autosomal-recessive primary microcephaly), a neurodevelopmental disorder characterized by decreased brain size at birth and non-progressive mental retardation (20–22). WDR62 has been shown to control JNK signaling and neurogenesis during brain development, including neuronal migration (23). How WDR62 is involved in the regulation of JNK signaling is still not very clear. Here, we show that TGF-β-activated kinase-1 (TAK1) forms a complex with RAC1 and the scaffolding proteins, WDR62 and POSH, to mediate the TGF-β-JNK signaling and plays an essential role in neuronal migration during cortical development.

Experimental Procedures

Plasmids—TAK1-specific shRNAs (shT-6 and shT-1) and JNK2-specific shRNAs (shJ2-5 and shJ2-1) were cloned into pLL3.7-GFP. The targeted sequences for these shRNAs are shown in Table 1. Mouse Tak1 and Jnk2 were amplified by PCR from E18.5 mouse brain cDNA and cloned into pCMV–tag2b–FLAG and pcDNA3.1–HA plasmids, respectively. Human TAK1 (both wild-type and K63R/K158R mutant) were gifts

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FIGURE 1. TAK1 knockdown impairs neuronal migration in the neocortex. A, coronal sections from E18.5 mouse cortex stained with anti-p-TAK1 antibody (red) and anti-Tuj1 antibody (green). Bars, 50 μm. B, high magnification view of the IZ in A. Bars, 10 μm. C, FLAG-tagged-Tak1 (mouse) was co-transfected with control shRNA (shCtrl), shT-6, or shT-1 into HEK293T cells; 24 h later, the FLAG-Tak1 levels were analyzed by immunoblotting (IB) with anti-FLAG antibody, and α-tubulin (α-Tub) served as a loading control and GFP as a transfection efficiency control. D and E, human TAK1 (both wild-type and K63R/K158R mutant (D)) or human TAK1 with synonymous mutation refractory to shRNA, Mut-TAK1 (both wild-type and K63R/K158R mutant (E)) was co-transfected with shCtrl or shT-6 into HEK293 cells; 24 h later, the cell lysates were stained with anti-FLAG, phospho-JNK antibody, GAPDH, and GFP antibodies. F, coronal sections of mouse brains electroporated in utero with shCtrl or shT-6 along with vector, WT-TAK1 (human TAK1 with synonymous mutation refractory to shRNA), Mut-TAK1 (human TAK1 K63R/K158R mutant with synonymous mutation refractory to shRNA), or CA-JNK2 (constitutively active human JNK2) at E14.5 and analyzed at E18.5. Bars, 50 μm. G, quantification of GFP + cell distribution in the cortex in F. Data represent mean ± S.E.; n = 9 (3); shT-6 + Vec, n = 22 (7); shT-6 + WT-TAK1, n = 11 (4); shT-6 + Mut-TAK1, n = 13 (4); shT-6 + CA-JNK2, n = 10 (3); one-way ANOVA). n, number of slices from different brains. Nuclei were stained with DAPI (blue).

### TABLE 1

| Gene cloned | Primers for cloning | Primers for cloning |
|-------------|---------------------|---------------------|
| Mouse Tak1  | 5'-catgtcGAATTCTatgctgacagctccggctc-3' (F) | 5'-acgtcaCTCGAGtcatgaagtggtctgctttc-3' (R) |
| Mouse Jnk2  | 5'-catgtcGAATTCTatgctgacagctccggctc-3' (F) | 5'-acgtcaCTCGAGtcatgaagtggtctgctttc-3' (R) |
| Mutated (shT-6) human TAK1 | 5'-gttgtgctaatcagctctccagcgGccAtcTatggagaaaatggaataaag-3' (F) | 5'-catattttcccacatttctctctatAgaGccGccgtgagagatcttttaggaacaac-3' (R) |

| shRNAs      | Targeted sequences                        |
|-------------|-------------------------------------------|
| shCtrl      | 5'-TCAATGGCTCTCTATCTACGTC-3'              |
| shT-6       | 5'-GCCCTTCAATGGGAGAACTT-3'                |
| shT-1       | 5'-GCCCTTCAATGGGAGAACTT-3'                |
| shJ2-5      | 5'-CCAATCGTCTGTTGGTCAAA-3'                |
| shJ2-1      | 5'-GCCACCCGATATTACTGT-3'                  |

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from Dr. Yang (24). Human TAK1 was synonymously mutated into a form refractory to shT-6 (from 5'-cgccctcaatggag-gaaatt-3' to 5'-cgGccAtcTatggaggaaatt-3'). The pcDNA3.1-FLAG-CA-JNK2 construct was a generous gift from Dr. R. Davis. The gene Cre was cloned and constructed into pCAGIG plasmid, a gift from Connie Cepko (Addgene plasmid 11159) (25). The primers used for cloning are listed in Table 1. POSH, WDR62, and RAC1 were described previously (13, 18, 23).

Cell Culture, Transfection, in Vitro Binding Assay, Western Blotting, and Immunostaining—HEK293 cell culture, transfection, in vitro binding assay, and Western blotting were performed as described previously (12, 13). The antibodies used for Western blotting are as follows: GFP (Abcam, ab290, 1:7000); α-tubulin (Cell Signaling Technology, 3873s, 1:2000); GAPDH (Cell Signaling Technology, 2118s, 1:2000); FLAG (Medical & Biological Laboratories (MBL), M185, 1:5000); HA (MBL, M180, 1:5000); Myc (MBL, M562, 1:2000); POSH (MBL, 1:1000); Wdr62 (Abcam, 1:1000); TAK1 (Cell Signaling Technology, D9407, 1:1000); and p-JNK (Abcam, ab124956, 1:1000). Immunostaining was performed as described previously (18, 23, 26). The antibodies used for immunostaining are as follows: p-TAK1 (Abcam, ab79583, 1:800); TAK1 (Abcam, ab109526, 1:400); GFP (Abcam, ab13970, 1:1000); BrdU (Abcam, ab6326, 1:500); histone H3 (phospho-Ser-28, Abcam, ab10543, 1:1000); Ki67 (Abcam, ab15580, 1:1000); Cre (Novus Biologicals, NB100-56134, 1:400); and β-III tubulin (Abcam, ab7751, 1:1000).

Co-immunoprecipitation (Co-IP)—Co-IP was performed as described previously with some minor modifications (13). For co-IP of endogenous proteins, E14.5 cortical proteins were prepared with cell lysis buffer (20 mM Tris, pH 7.4, 0.5% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, phosphatase inhibitor mixture (Roche Applied Science, 04906845001), protease inhibitor mixture (Roche Applied Science, 04693116001), 0.2 mM PMSF) and sonicated. For each co-IP, 15 μl of protein A-Sepharose with 15 μl of protein G-Sepharose (GE Health-
Academy of Sciences, and the procedures of experiments performed on the mice were approved by the Institutional Animal Care and Use Committee at the Institute of Genetics and Developmental Biology. In utero electroporation experiments were performed as described previously (26).

Imaging and Statistical Analysis—Confocal images of sectioned brain slices were obtained through Zeiss LSM700 and analyzed with Photoshop, ImageJ, or Imaris software. The nucleus density shown by DAPI staining was used to define the boundaries between ventricular zone/subventricular zone (VZ/SVZ, high nucleus density) and intermediate zone (IZ, low nucleus density) or intermediate zone and cortical plate (CP, high nucleus density). The data were analyzed with t test or one-way ANOVA followed by Tukey’ multiple comparison test (without correction of the significance) by using GraphPad Prism software; *, p < 0.05; **, p < 0.01; and ***, p < 0.001 indicated the significant difference. “n = a(b)“ indicated the number of brain slices analyzed, where “a” means the number of brain slices analyzed, and “b” means the number of different mouse brains.

Results

TAK1 Is Essential for Neuronal Migration during Cerebral Cortex Development—TGF-β signaling is essential for neuronal migration and axonal specification (8), and we reported recently that TAK1 is required for TGF-β-induced JNK activation in neuronal cells and axonal outgrowth during neocortical development (27). We therefore postulated that TAK1 may also play a role in neuronal migration. To test this possibility, we examined the distribution of the activated form of TAK1 (Thr-187-phosphorylated TAK1, p-TAK1) in embryonic day 18.5 (E18.5) mouse cortex. Similar to that of TβR2 (8), p-TAK1 labeling was mainly located in the IZ and CP (Fig. 1, A and B). In addition, p-TAK1 co-localized with Tuji, a marker for immature migrating neurons (Fig. 1, A and B). We next screened bicistronic constructs encoding both enhanced GFP and shRNA, which were able to knock down overexpressed mouse wild-type (WT) TAK1 but not human TAK1 with synonymous mutation refractory to shRNA (hTAK1) (Fig. 1, C–E). We employed IUE to deliver control and TAK1 shRNAs into neural progenitor cells (NPCs) of the VZ of E14.5 mouse brain. The most striking difference between TAK1 shRNA and control shRNA-transfected brains at E18.5 was the distribution pattern of neurons (Fig. 1F). In control shRNA-transfected brains, ~52% of transfected cells were in the CP, whereas ~26% were in the IZ (Fig. 1G). In contrast, only ~12% of TAK1 shRNA-transfected cells reached the CP, whereas the majority of cells (~58%) remained in the IZ (Fig. 1G), indicating that TAK1 knockdown perturbs neuronal migration.

To validate that the migratory defects were caused by TAK1 knockdown, we co-electroporated TAK1 shRNA and hTAK1 into the E14.5 brain and found that the migration defect could be partially rescued by co-expression of WT hTAK1, with 32% cells reaching in the CP. However, the impaired neuronal migration caused by TAK1 knockdown could not be rescued by the hTAK1 K63R/K158R mutant, which fails to promote TGF-β-induced JNK activation (24), and only 13% of cells reached in CP, which was similar to that of TAK1 knockdown group (12%
These results indicate that the shRNA-incurred phenotype is not the result of off-target effects and that TAK1 activity is required during neuronal migration. To further verify the role of TAK1 in neuronal migration during cortical development, we deleted Tak1 by expression of Cre in Tak1flox/flox mouse brain via IUE at E14.5 and analyzed 3 days later (E17.5). An obvious migration defect was detected in TAK1 knock-out cells (with 23% of cells in the CP compared with 41% in control group), similar to the phenotype in TAK1 knockdown cells, and this defect could be rescued by co-expression of hTAK1 (37% in the CP, Fig. 2, C and D). We also inspected brains at postnatal day 7 and found that the vast majority of control cells reached the CP, although many of the Tak1 knock-out cells remained in the white matter region (Fig. 2E). The knock-out of Tak1 expression was confirmed in Tak1 Nestin-Cre conditional knock-out (Tak1 cKO) brains (Fig. 3A), as well as Cre-expressing cells in the Tak1flox/flox brains (Fig. 2, A and B).

To further validate the role of TAK1 during cortical development, we performed BrdU birth date analysis. Control or Tak1 cKO mice were labeled with BrdU at E14.5 and examined at E17.5. As shown in Fig. 3, B and C, there were significantly more BrdU+/Ki67− cells in the IZ instead of in the CP in cKO brains than there were in control brains. We went on to analyze the effect of Tak1 cKO on the cell cycle of NPCs and observed no apparent difference between control and Tak1 cKO in mitotic activity based on phospho-histone H3 staining and cell cycle exit index illustrated by the percentage of Ki67+/BrdU+ cells among BrdU+ cells (Fig. 4). Taken together, our results show that TAK1 plays a critical role in neuronal migration but not in NPC proliferation during cortical development.

Because TAK1 is essential for TGF-β-induced JNK activation in neuronal cells during neocortex development (27), we investigated whether the expression of hTAK1 can rescue impaired neuronal migration caused by knock-out of Tβr2 (8). As shown in Fig. 5, deletion of Tβr2 by transfection of Cre in Tβr2flox/flox mice via IUE at E14.5 leads to significantly less cell migration into the CP at E17.5 (with 17% of cells staying in the CP compared with 30% in the control group). The migration defect could be partially rescued by co-expression of WT TGF-β-TAK1-JNK Signaling Regulates Neuronal Migration

FIGURE 4. Knock-out of Tak1 has no significant effect on neuronal proliferation and cell cycle exit. A, coronal sections of E15.5 wild-type or Tak1 cKO mouse brain were stained with anti-phospho-histone H3 (phospho-Ser-28, P-H3) antibody (green). Bars, 50 μm. Right panels, quantification analysis of p-H3 positive cells along the 100 μm border of lateral ventricle. Data represent mean ± S.E. (WT, n = 6(3); cKO, n = 6(3); t test). ns, not significant. B, coronal sections of E15.5 wild-type or Tak1 cKO mouse brain labeled with BrdU (100 μg/kg) at E14.5 were immunostained with anti-BrdU (green) and Ki67 (red) antibody. Bars, 50 μm. C, quantification of BrdU+ cell distribution in the cortex in B. D, quantification analysis of the fractions of BrdU+/Ki67− cells in B. C and D, data represent mean ± S.E. (WT, n = 11(3); cKO, n = 12(3); t test). ns, not significant. n, number of slices from different brains. Nuclei were labeled with DAPI (blue).
hTAK1 (28% in the CP), but not very significantly by hTAK1 K63R/K158R mutant, indicating an important role for TAK1 activity in TGF signaling controlled neuronal migration.

JNK2 Is Required for Cortical Neuron Migration during Development—Considering the role of TAK1 in TGF-β-induced JNK activation, it is reasonable to postulate that TAK1 participates in neuronal migration through JNK. In fact, a dominant negative JNK and a JNK inhibitor, SP600125, have been reported to suppress cortical neuron migration (15). However, both dominant negative JNK and SP600125 may not be specific for the inhibition of JNK activity (12). In addition, there are three different isoforms of JNKs (JNK1, -2, and -3) expressed in the brain. Whether these JNK isoforms play a role in neuronal migration remains to be determined. As the effects of JNK1 knockdown have been reported (23), we decided to focus on shRNAs targeting different regions in Jnk2 and -3. Embryos were electroporated with control, JNK2, or JNK3 shRNAs at E14.5. Four days later, most of the cells transfected with different JNK2 shRNAs remained in the IZ or subventricular zone, very similar to that of shTAK1 (Fig. 6, A–C). However, the distribution of JNK3 shRNA-transfected cells was largely normal (data not shown). The possibility of off-target effects of JNK2 shRNAs was excluded as the migratory defects could be partially rescued by co-expression of constitutively active human JNK2 (CA-JNK2, Fig. 6, A–C). These results indicate that JNK2 but not JNK3 is essential for the migration of cortical neurons. In addition, expression of CA-JNK2 could partially rescue the impaired neuronal migration incurred by knockdown of TAK1 (Fig. 1, F and G), whereas overexpression of WT-TAK1 could not rescue the migration defects caused by JNK2 knockdown (Fig. 6, A and B), confirming the involvement of TAK1 as an upstream kinase in the regulation of JNK-mediated neuronal migration. Similar to knock-out of Tak1, JNK2 knockdown did not have an apparent effect on the cell cycle exit index of NPCs based on the percentage of Ki67−/BrdU+/GFP+ cells among BrdU+/GFP+ cells (Fig. 7).

TAK1 Forms a Complex with POSH, WDR62, and RAC1 to Regulate JNK Activity—We have recently shown that two scaffold proteins involved in the JNK pathway, POSH and the microcephaly-associated protein WDR62, are essential for neuronal migration during rodent cortical development (18, 23). We hypothesized that TAK1 might form a complex with POSH and WDR62 to regulate JNK activity. To test this hypothesis, TAK1, POSH, and WDR62 were transfected into HEK293T cells individually or in combination, and reciprocal co-IP experiments were performed to determine possible interactions. Results revealed that TAK1 interacted with POSH and WDR62 (Fig. 8, A–D). In addition, we found that POSH could interact with WDR62 (Fig. 8, E and F), as has been previously shown for JIPs (11, 12). Furthermore, TAK1 antiserum was able to co-IP with endogenous WDR62, and POSH antiserum was able to co-IP with endogenous TAK1 and WDR62 from E14.5 mouse neocortex (Fig. 8, G and H). These results indicate that TAK1 can form a complex with POSH and WDR62 during neocortical development.

Because RAC1 interacts with POSH and regulates neuronal migration (14, 18, 28, 29), and it also interacts with MLK family members to activate the JNK pathway (30), we therefore investigated the relationship between RAC1, TAK1, and POSH. As shown in Fig. 8, I and J, TAK1 interacted with RAC1 as illustrated by co-IP and GST pulldown analysis. Co-expression of TAK1 with either RAC1(V12) (the activated form of RAC1) or POSH was able to significantly enhance TAK1-induced JNK activation (Fig. 8, K and L). Although TAK1 could interact with
**Figure 6.** JNK2 knockdown impairs neuronal migration in the neocortex. A, coronal sections of mouse brains electroporated in utero with shCtrl, shJ2-5 (shRNA JNK2-5), or shJ2-1 along with vector or CA-JNK2 or WT-TAK1 at E14.5 and examined at E18.5. Nuclei were stained with DAPI. Bars, 50 μm. B, quantification of GFP^+^ cell distribution in the cortex in A. Data represent mean ± S.E. *, p < 0.05; ***, p < 0.001, ns, not significant. (shCtrl + Vec, n = 9(3); shJ2-5 + Vec, n = 18(6); shJ2-5 + CA-JNK2, n = 16(5); shJ2-5 + WT-TAK1, n = 12(4); shJ2-1 + Vec, n = 14(5); shJ2-1 + CA-JNK2, n = 10(3); one-way ANOVA). n, number of slices from different brains. C and D, mouse Jnk2, but not human CA-JNK2, was efficiently knocked down by JNK2 shRNAs. Mouse Jnk2 (C) or human CA-JNK2 (D) was co-transfected with shCtrl, shJ2-5, or shJ2-1 into HEK293 cells; 24 h later, exogenous JNK2 levels were analyzed by immunoblotting (IB) with anti-HA (C) and anti-FLAG (D) antibodies, with α-tubulin (α-tub) as a loading control and GFP as a control for transfection efficiency.

**Figure 7.** Knockdown of JNK2 does not have significant effect on NPC cell cycle exit. A, mouse brains were electroporated in utero with shCtrl or shJ2-5 at E14.5. 48 h later, mouse were labeled with BrdU (100 μg/kg) and sacrificed at E17.5. Coronal sections were immunostained with anti-GFP (green), BrdU (red), and Ki67 (blue) antibody. Arrow indicates GFP^+/BrdU^+/Ki67^+^ cells and arrowhead points to GFP^+/BrdU^-/Ki67^- cells. Bars, 50 μm. B, quantification analysis of the fractions of GFP^+/BrdU^+/Ki67^- cells among GFP^+/BrdU^- cells in A. Data represent mean ± S.E. (shCtrl, n = 7(3); shJ2-5, n = 8(4); t test). ns, not significant. n, number of slices from different brains.
RAC1(N17) (the dominant negative form of RAC1), RAC1 N17 could not increase TAK1-induced JNK activation (Fig. 8 and data not shown).

Taken together, our results indicate that TAK1 forms a complex with RAC1, WDR62, and POSH to regulate JNK activity, and it plays an important role in TGF-β signaling and neuronal migration.

**Discussion**

Different JNK pathway components, including WDR62, POSH, RAC1, MKK4, and MKK7, are important for radial migration in the developing neocortex (14–17, 31). In this study, we have characterized two new protein kinases involved in neuronal migration, TAK1 and JNK2. Our results support a model in which RAC1, TAK1, MKK4/7, and JNK2 are orga-
nized by WDR62 and POSH to form a protein complex (PACTM: POSH-associated complex involved in TGF-β signaling and migration) (Fig. 8M). TGF-β signaling is likely to control neuronal migration through PACTM-mediated JNK2 activation. Our study may also provide an insight into the molecular mechanisms underlying WDR62-associated MCPH pathogenesis.

TAK1 activity is prominent in migrating neurons in the developing neocortex, similar to that of TβR2 and phosphorylated JNK (8, 27). Through knockdown and knock-out of Tak1 expression and rescue experiments, we provide evidence that TAK1 activity is essential for neuronal migration. Interestingly, TAK1 can rescue the impaired neuronal migration caused by deletion of TβR2, although JNK2 can rescue the migration defect caused by Tak1 knockdown. This indicates that TAK1 is downstream of TGF-β signaling in the regulation of JNK activity and neuronal migration. In addition to TAK1, two other MAPK kinase kinases, dual leucine zipper kinase and MEKK4, were reported to be important for neuronal migration (32, 33). This is in support of the notion that neuronal migration requires a sophisticated interplay of complex molecular machinery and involves multiple steps (2, 5–7, 28, 34). TAK1, dual leucine zipper kinase, and MEKK4 may be involved in different steps of neuronal migration, and further investigation is needed to define these steps.

There is discrepancy on whether JNK inhibits or is required for neuronal migration (15, 19). Through knockdown and rescue experiments, we found that JNK2, but not JNK3, plays an essential role in neuronal migration. Our studies with Jnk1 and Jnk1 knockdown and Jnk1 knock-out mice indicate that, although JNK1 is indispensable for neuronal migration, it is more important for the self-renewal of NPC (23).3 The enhanced neuronal migration in Jnk1 knockout cortices reported by Westerlund et al. (19) is more likely due to premature differentiation of NPC and the earlier migration of newborn neurons from the VZ. Even though JNK1 and JNK2 have been indicated to compensate each other during development (35), JNK1 and JNK2 may have different biological functions during neurogenesis.

Although TAK1 was identified 20 years ago, how it is regulated is still not very clear. Here, we show that endogenous TAK1, WDR62, and POSH interact with each other in the developing brain. This is in support of the perception that scaffold proteins are important for the correct assembly of the stimulus- and compartment-specific JNK signalosomes. Interestingly, co-expression of TAK1 with either RAC1 or POSH induces the activation of JNK more significantly than TAK1 alone, indicating that RAC1 may activate TAK1 and MLK family members in a similar way (30, 36). Together with the evidence that TAK1 plays an essential role in TGF-β-induced JNK activation in neuronal cells (27), we propose a model in which TGF-β activates JNK2 through a novel protein complex consisting of RAC1, TAK1, and MKK4/7, which is organized by WDR62 and POSH and plays an essential role in neuronal migration during brain development.

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Author Contributions—F. Z. conducted most of the experiments and analyzed the results. J. Y. conducted most of co-IP and pulldown experiments. T. Y. designed some of the in utero electroporation experiments. D. X. constructed plasmids expressing Wdr62. Z. C. constructed plasmids expressing different forms of Tak1. Y. X. bred the TβR2-flox/flox mice and conducted some of the in utero electroporation experiments. Z. X. designed the project, analyzed the results, and wrote the paper.

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