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ROLES OF FAK FAMILY KINASES IN NERVOUS SYSTEM

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1. ABSTRACT

Focal adhesion kinase (FAK) and its related kinase, proline rich tyrosine kinase 2 (PYK2), are major kinases activated by cell-extracellular matrix adhesion. Although they are highly expressed in the nervous system, the functions of these two kinases in the nervous system remained unclear until recently. FAK and PYK2 appear to play an important role in developing nervous system as well as adult brain. Importantly the two kinases are activated by different extracellular stimuli and execute distinct regulatory effects on various aspects of neural developmental processes and neuronal function. This review summarizes the potential roles of FAK and PYK2 in axon path-finding and synaptic plasticity.

2. INTRODUCTION

FAK, a major tyrosine kinase activated by cell-extracellular matrix (ECM) adhesion, plays an important role in regulating actin cytoskeletal organization and cell migration in multiple types of cells (1, 2). FAK targets at focal adhesions via the FAT domain (3) and at the same time interacts with paxillin and talin (4-6). In addition, FAK recruits signaling proteins into the cell adhesion complex, including Src family kinases (7), $p130^{Cas}$ (Crk associated substrate) (8, 9), Graf (GTPase regulator associated with FAK) (10), PSGAP (a PH and SH3 domain containing RhoGAP protein) (11), and ASAP1 family ArfGAP proteins (12). Expression of FAK in Chinese hamster ovary cells stimulates cell migration (13). On the other hand, expression of dominant negative FAK mutants inhibits cell spreading and cell migration (14, 15). Furthermore, fak-/- mice die at early embryonic development due to the defects on cell migration, and fibroblasts derived from fak-/- mice exhibit increased numbers of focal adhesions, and reduced rates of cell migration (16). These observations indicate a role of FAK in regulating cell motility.

The other member of the FAK-family kinases is PYK2 [also called cell adhesion kinase β (CAKβ), related adhesion focal tyrosine kinase (RAFTK), or calcium dependent protein tyrosine kinase (CADTK)] (17-20). This kinase has similar domains or motifs to FAK, sharing 45% overall sequence identity and 60% identity in the catalytic domain. However, PYK2 interacts with several proteins that do not bind to FAK although the two kinases share binding partners (21, 22). Moreover, PYK2 has different tissue distribution pattern. Last PYK2 kinase activity increases in response to stimuli including TNFα, UV lights, and neuronal depolarization (17, 23-25). Therefore FAK and PYK2 are likely to play distinct roles in regulating cell motility and cell adhesion, some of which are beginning to be elucidated. This review will focus on their potential functions in the neural development and neuronal synaptic plasticity.

3. DISCUSSION

3.1. Role of FAK/PYK2 in Axon Path-finding

3.1.1. Growth cone is a cell-ECM adhesion structure that has both sensory and motor functions

In neural development, neurons differentiate to grow neurites that develop into axons and dendrites. Axons often travel long distance, making stereotypical turning decisions along the paths – a process called “path finding”. During this process, the growth cone, a leading edge of

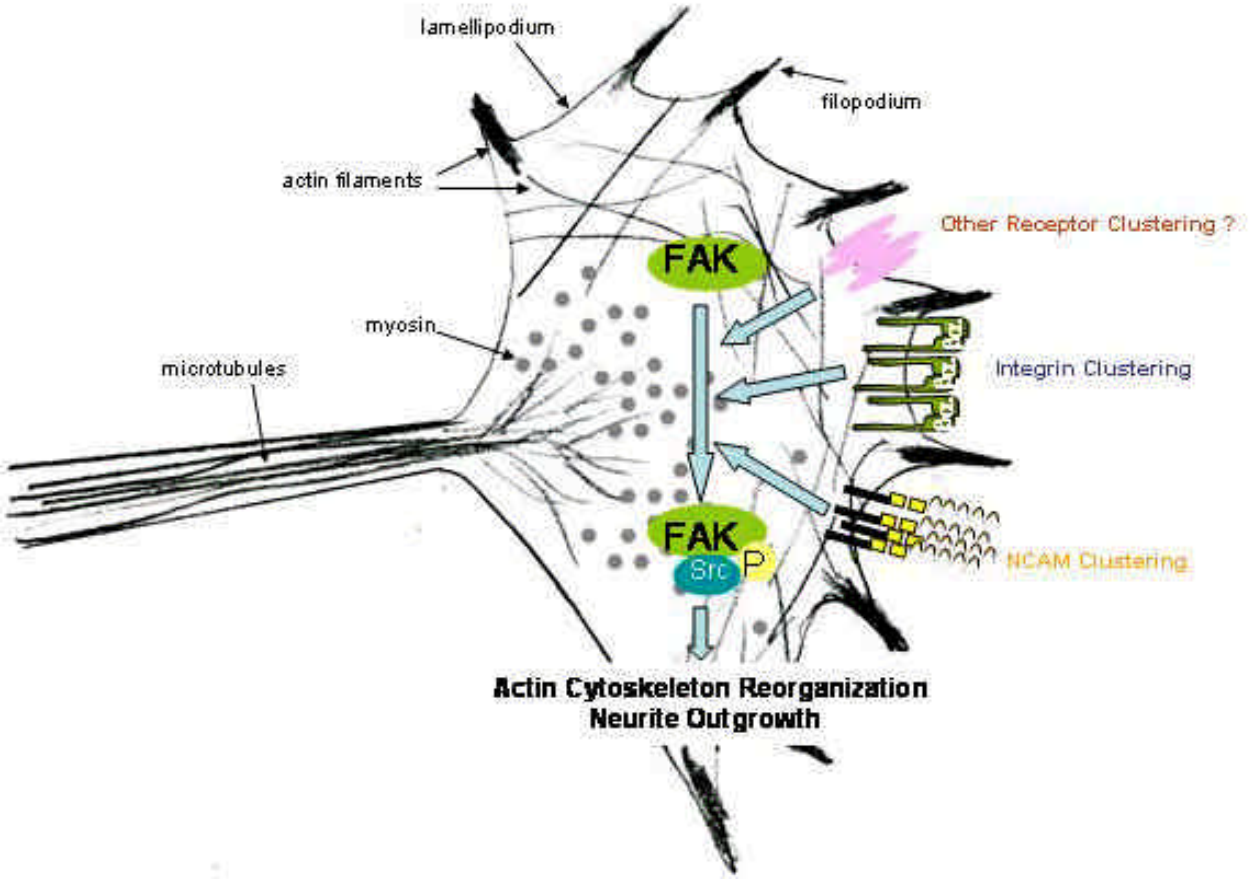

Figure 1. A model to illustrate the potential role of FAK in neuronal growth cones. A growth cone is composed of finger-like filopodia and fan-shaped lamellipodia, as indicated. FAK is enriched at the growth cone. The clustering of integrins receptors or NCAM cell adhesion proteins lead to the activation of FAK, which appears to be important for the outgrowth induced by the extracellular matrix proteins (e.g., laminin) and NCAM.

elongating neurites, plays a crucial role. A growth cone is composed of finger-like filopodia and fan-shaped lamellipodia (Figure 1). It is a cellular structure with two functions: to receive directional cues from the environment and to respond with a change in axon elongation direction. The coupling between the sensory and motor capabilities of the growth cone is critical for axonal guidance (26).

Growth cones possess cell surface receptors for extracellular signals that regulate path-finding. These signals could be permissive such as extracellular matrix (ECM) proteins and cell adhesion molecules or instructive such as attractive and repulsive cues (26, 27). In addition, growth cones contain large amounts of cytoskeleton and signaling proteins potentially important for the motor activity. Although no typical focal adhesion structure is noticeable in growth cones, there are small "point contacts" where growth cones contact the ECM (28, 29). Point contacts exist in highly mobile cells, which are similar to podosomes of transformed cells or osteoclasts (28, 29). Despite morphological difference, the molecular composition of growth cones is similar to that of focal adhesions. Growth cones contain cell adhesion molecules including integrins, cytoskeletal proteins (mena, vinculin, and talin), FAK and PYK2, and adapter proteins ($p130^{Cas}$)(30-33). However, unlike focal adhesions those are arrow-shaped and located at the ends of actin stress fibers; polymerized actin filaments are present in growth cones and are surrounded by cell adhesion proteins (Pasquale, 2000). Thus, actin-binding proteins and motor proteins including gelsolin, cortactin, and myosin are localized at growth cones, but not focal adhesions (34-40). These proteins and regulation of their function appear to be essential for axon path-finding.

3.1.2. FAK and PYK2 expression in the nervous system

The expression and distribution of FAK and PYK2 during development have been studied at the messenger RNA and protein levels (32). Interestingly, FAK and PYK2 appear to be differentially expressed during brain development. The level of PYK2 mRNAs in embryonic brains is low per *In situ* hybridization studies, and its protein is nearly undetectable by western blot analysis (32). In contrast, expression of FAK is relatively high in embryonic stage and decreases afterwards as the development proceeds (32). Such temporal expression patterns of the two kinases suggest that FAK, probably not PYK2, may be important for axon path-finding during brain development.

In adult brain, the distribution of FAK and PYK2 has been investigated by immunoperoxidase and *In situ* hybridization studies of cryostat sections. The highest levels of staining of FAK and PYK2 are observed in the hippocampus, cerebral cortex and thalamus (32, 41). In hippocampus, PYK2 is particularly strong in both dentate gyrus and CA3/CA1 regions (32, 41). In addition, PYK2 is primarily concentrated in neurons, where it is detectable in both the soma and dendrites. FAK appears to be in a slightly different pattern as PYK2. FAK is more intense in the CA1/CA3 regions than in the dentate gyrus (32). Although FAK is concentrated in neurons, it is almost exclusively in the somas, but not dendrites (32). These results suggest that PYK2, probably not FAK, may be a component of the postsynaptic density (PSD), a microscopic structure associated with the postsynaptic membrane that contains a variety of signaling proteins. Proteomic analysis demonstrates that PYK2 is abundant in the PSD (42).

Subcellularly, FAK and PYK2 distribution has been examined in primary cultured hippocampal neurons (32). In early differentiating neurons, FAK is distributed as clusters in neuronal cell bodies and growth cones, colocalizing with actin filaments and vinculin, an actin binding focal adhesion protein implicated in control of growth cone motility (32, 33). Interestingly, while FAK is present in the periphery of the growth cone tips, PYK2 appears to be excluded from the lamellipodia of growth cones (32). While the sensory capability of the growth cone depends in large part on its filopodia, lamellipodia of growth cones are critical for growth cone motility and outgrowth. Thus, the distribution of FAK in the periphery of the growth cone tips suggests that FAK may be important for the motility of growth cones and support a role of FAK in regulating axon path-finding.

Several isoforms of FAK and PYK2 are generated by alternative splicing or using different promoters (43-46). Of FAK isoforms, FAK6+, 7+ and FAK+ are abundantly expressed in the brain (44). FAK6+ and FAK7+ contain short inserts located close to the autophosphorylated tyrosine 397 (44). The addition of these inserts appears to increase phosphorylation of tyrosine 397, thus modulate kinase activity (44). FAK+ has a 3 amino acid insert in the carboxyl-terminal FAT domain, and is the predominant form of FAK in the adult CNS (32). PYK2 has three isoforms, The long slice isoform is highly expressed in the brain (41), while the short isoforms are considerably less abundant in the CNS (41).

3.1.3. FAK tyrosine phosphorylation is induced by ECMs and guidance cues in neurons

FAK tyrosine phosphorylation is induced by multiple factors including ECM molecules, growth factors, and guidance cues (1, 2)(Figure 1). Among them, laminins, NCAM, and L1 are particularly interesting since they have been implicated in axon path-finding.

Laminins are a major type of ECM glycoproteins in developing brain (47, 48). They are potent stimulators of neurite outgrowth *in vitro* for a variety of neurons (47, 48). Studies of neurons in culture demonstrate that neurites prefer laminin-coated surface (47, 48). They stay in tracks where active laminins are present and stay away from areas where laminins are inactivated(49, 50). These studies suggest that laminins may have two roles in axon navigation: first, promoting neurite outgrowth and second, serving as a permissive signal. Recent studies of *Xenopus* retinal neurons indicate a novel function of laminins. The growth cones of these neurons turn toward a pipette tip that releases netrin-1, an attractive guidance cue ((51). Interestingly, the netrin-1 attractive response is converted into a repulsive effect when neurons are cultured on a laminin-1 matrix (52). These results suggest that the effect of laminins could be instructive when directional cues are present.

The receptors for laminins include integrins and non-integrin laminin binding proteins. Integrins are a diverse family of integral glycoproteins. They form non-covalent $\alpha\beta$ heterodimers to mediate cell-matrix interactions in multicellular organisms. Integrins bind to proteins in the matrix via the extracellular domains. The cytoplasmic domains of integrins interact with adapter proteins and kinases to activate and/or regulate multiple intracellular signaling pathways that regulate actin cytoskeletal organization. The non-integrin laminin-binding proteins include LBP-110, the 67 kDa laminin-receptor (67LR), α-dystroglycan, and β1, 4 glactosyltransferase (47). FAK is activated by integrin engagement either by cell attachment to the ECM including laminins or antibody cross linking (53). Exact how laminins regulate FAK activity in neurons remain unclear.

NCAM and L1 are neural cell adhesion molecules of the immunoglobin super family(54). They promote axon growth, fasciculation, and cell adhesion by homophilic and heterophilic interactions(54). Alternative splicing of a single NCAM gene results in three major NCAM isoforms: two transmembrane isoforms of 140 and 180 kDa, and a 120-kDa glycophosphatidylinositol linked isoform (54). The cytoplasmic domains of transmembrane NCAMs lack catalytic activity but may mediate interactions with intracellular signaling proteins. NCAM140 is present in free, migratory growth cones, whereas NCAM180 is found at sites of cell-cell contact, where it may involved in stabilization of synapses (54). NCAM120 is present mainly in glia (54). All three isoforms can be post-translationally modified by addition of polysialic acid, a carbohydrate moiety that modulates axon guidance (55, 56). Stimulation of NCAM or L1 by homophilic binding or by binding of antibodies that recognize extracellular determinants of NCAM or L1 evokes changes in protein tyrosine phosphorylation and activation of FAK (Figure 1). Interestingly, FAK is found to associate with NCAM140 (57). This interaction may be important for NCAM induced activation of FAK.

3.1.4. FAK plays an important role in regulating axon outgrowth

FAK is activated by factors that promote axon outgrowth. Upon activation, FAK is phosphorylated at multiple tyrosine residues. Tyrosine (Y) 397 is a major auto-

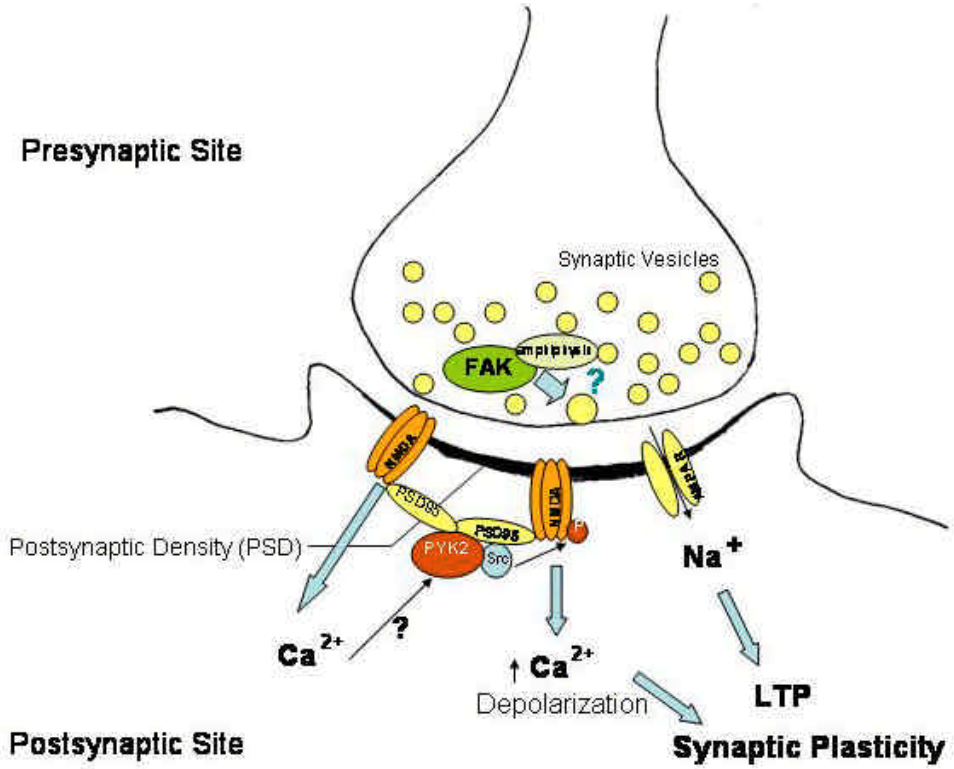

Figure 2. A schematic of the protein complex of NMDA-PSD-95-PYK2 in postsynaptic membrane of a central synapse. A central synapse is a cell-cell adhesion structure that permits signaling between nerve cells. In this diagram, the presynaptic terminal and postsynaptic membrane are indicated. NMDA receptor is one of the glutamate receptors in the PSD that is calcium permeable and opens in response to glutamate only when the postsynaptic membrane is concomitantly depolarized. PSD-95 is a scaffolding protein that interacts with multiple proteins in the PSD including NMDA receptor and PYK2.

hosphorylation site in response to integrin engagement (7, 58). Y397 phosphorylation increases the catalytic activity (58) and creates a binding site for SH2 domain containing proteins including Src family kinases, PI-3 kinase, PLCγ, or Grb7 (7, 59-61). Y577 in the catalytic domain of FAK is phosphorylated by c-Src *in vitro* and v-Src *in vivo* (62). Y577 phosphorylation also leads to increased FAK catalytic activity (62). Y861 is a major site phosphorylated in v-Src transformed cells and phosphorylation of this site may regulate FAK binding to integrins (63, 64). Integrin mediated FAK tyrosine phosphorylation is inhibited by cells expressing FRNK (FAK related non-kinase), an autonomously expressed C-terminal fragment of FAK that is transcribed from an internal promoter of FAK gene (14, 43).

Is FAK important for neurite outgrowth? Studies using dominant negative FAK proteins (FRNK or FAK tyrosine mutants) point the role of FAK in regulating neurite outgrowth. In PC 12 or neuroblastoma cells, co-stimulation of growth-factor receptors by EGF and integrins promote outgrowth of neurites. Expression of FRNK in these cells inhibits EGF and integrin-induced neurite outgrowth (65, 66). PC12-E2 cells grow neurites when they are co-cultured with fibroblasts expressing NCAM (67). This NCAM induced neurite outgrowth is blocked by the introduction of FRNK in PC12-E2 cells (67). We have recently studied the role of FAK in netrin-1-induced neurite outgrowth using *Xenopus* spinal neurons. These neurons exhibit increased outgrowth and attractive turning response to a gradient of netrin-1 (51). We found that netrin-1-induced neurite outgrowth and growth cone turning are inhibited in neurons expressing FAK-Y397F/Y861F, but not FAK-WT (Ren and Xiong, unpublished results), suggesting the requirement of FAK tyrosine phosphorylation in netrin-1 induced neurite outgrowth and attractive turning. Finally, recent results from FAK conditional knockout mice suggest an important role of FAK in regulating neurite outgrowth and neuronal cell migration during development (Rao, personal communication).

3.2. Role of PYK2 in Synaptic Plasticity

Synaptic plasticity is a long-lasting form of synaptic transmission that includes long-term potentiation (LTP) or long-term depression (LTD)(68). Mechanisms underlying synaptic plasticity have been intensively studied because they may represent ways of encoding "memory" in the brain. LTP or LTD is primarily produced by different patterns of activation of NMDA glutamate receptors, a ligand-gated cation channels that opens in response to glutamate only when the postsynaptic membrane is depolarized (Figure 2). Thus, regulation of NMDA receptor activity is essential for the understanding mechanisms of synaptic plasticity. The complex molecular mechanisms that underlie postsynaptic signaling and plasticity are beginning to emerge.

Several lines of evidence implicate PYK2 in regulating synaptic plasticity. PYK2 tyrosine phosphorylation is increased by stimuli including stimulation that produces LTP. Long electronic stimuli that cause membrane depolarization and LTP increase PYK2 tyrosine phosphorylation (69). Using rat hippocampal slices and cortical synaptosomes, it has been found that not only membrane depolarization, but also glutamate or specific agonists that activate glutamate receptors increase tyrosine phosphorylation of PYK2 (70). Activation of group I metabotropic glutamate receptors (mGluRs) upregulates NMDA receptor function and enhances LTP (68). Selective agonists that activate group I mGluRs can increase PYK2 tyrosine phosphorylation (70). Interestingly, PYK2 can be recruited to NMDA receptors, probably via interacting PSD-95 or SAP102 (71) (Figure 2). It is believed that PYK2 via activating Src kinases causes the increased tyrosine phosphorylation of NMDA receptor subunits 2A and B (NR2A/B) which may increase NMDA receptor activity (69)(Figure 2). Thus, PYK2 could enhance calcium influx in a positive feed-forward manner to promote synaptic potentiation. This notion is supported by the observation that induction of LTP is prevented by blocking PYK2 using catalytically inactive PYK2 (69). These observations demonstrate the importance of PYK2 in the induction of LTP in hippocampal synapses. It remains to be examined whether the induction of LTP is impaired in PYK2 knock-out hippocampus.

FAK has been found to interact with amphiphysin, a protein associated with synaptic vesicles and important for synaptic transmission (72)(Figure 2). The role of FAK in regulating synaptic transmission remains to be examined.

4. SUMMARY

Recent studies of FAK and PYK2 in nervous system have demonstrated the importance of FAK and PYK2 not only in developing nervous system, but also in adult brain. Several lines of evidence that supports a role of FAK in regulating axon guidance, particularly axon outgrowth, include: 1) FAK is highly expressed in developing nervous system and enriched in neuronal growth cones; 2) FAK in neurons is activated by multiple adhesion proteins (e.g., integrins and NCAM) and guidance cues important for axon outgrowth. PYK2, on the other hand, is believed to be involved in regulating synaptic plasticity.

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