

TYROSINE KINASES OF THE FOCAL ADHESION KINASES FAMILY

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In this presentation I will address two topics. I will first describe the FAK family of tyrosine kinases and their interest as potential targets for kinase inhibitors. In the second part I will present briefly recent data related to another signaling pathway, the ERK pathway, which indicate that behavioral disorders might also be potential targets for kinase inhibitors.

TYROSINE KINASES OF THE FOCAL ADHESION KINASES FAMILY

Bona fide tyrosine kinases are widespread among metazoans in which they are essential for cell-cell interactions mediated by contact or soluble factors. Among non-receptor tyrosine kinases, focal adhesion kinase (FAK) (Hanks et al., 1992; Schaller et al., 1992) and proline-rich tyrosine kinase 2 (PYK2, 45% amino acid identity with FAK) (Lev et al., 1995) form a small and distinct group (here referred to as FAKs) with original domain organization and properties. PYK2 is also known as cell-adhesion kinase- β (Sasaki et al., 1995), "related adhesion focal tyrosine kinase" (Avraham et al., 1995), or Ca²⁺-dependent tyrosine kinase (Yu et al., 1996).

These kinases are conserved in invertebrates, in which a single orthologous gene is found, more closely related to FAK than to PYK2. Gene duplication probably occurred in deuterostomes after the echinoderma embranchment, leading to the evolution of PYK2 with distinct properties. The amino acid sequence of FAK and PYK2 is conserved in their functional domains but not in their linker regions.

FAK and PYK2 possess a central tyrosine kinase domain flanked by an N-terminal FERM (*four-point-one, ezrin, radixin, moesin*) domain (Girault et al., 1999a) and a multifunctional C-terminal region encompassing several proline-rich motifs and a focal adhesion targeting (FAT) domain. FAK is enriched in focal adhesions and activated following integrins engagement or stimulation of a variety of transmembrane receptors, which trigger its autophosphorylation and the formation of multimolecular signaling complexes. FAK is involved in focal adhesions turnover and integrin-dependent cell survival and migration, and plays an important role in signaling pathways in numerous cell types. FAK is critical for processes ranging from embryo development to cancer invasiveness and metastasis (Schlaepfer and Mitra, 2004; Mitra et al., 2005). Although PYK2 shares many properties with FAK, it has the particularity to be activated in response to increases in cytosolic free Ca²⁺ concentration. PYK2 plays a role in immune responses, synaptic plasticity, bone resorption and many other normal or pathological processes, including cardiac hypertrophy and brain ischemia (Girault et al., 1999b; Avraham et al., 2000; Takahashi, 2004). Because of their implications in human diseases, both FAK and PYK2 have been identified as important potential therapeutic targets

The autophosphorylated residue of FAK and PYK2 (Tyr-397 and -402, respectively) is located in a linker peptide between the N-terminal FERM domain and the central kinase domain. Phosphorylation of this residue allows the docking of proteins containing specific Src-homology 2 (SH2) domains, including Src-family kinases Src and Fyn. Thus recruited and activated, Src or Fyn phosphorylates several tyrosines in FAK or PYK2 and in associated proteins, leading to the activation of multiple signaling pathways. Although it is an essential step in the function of FAK and PYK2, the trigger and precise mechanism of their autophosphorylation are not known. Current evidence indicates that these enzymes are in a basal inactive state, promoted, at least in part, by inhibitory interactions. We and others have

shown that autophosphorylation of FAK and PYK2 is an intermolecular reaction, indicating that activation includes, at least transiently, the formation of multimolecular complexes. It is likely that conformational alterations regulated by protein binding and/or phosphorylation-dephosphorylation of serine and threonine residues are critical to trigger autophosphorylation.

Multiple partners of FAK have been identified. Most of them interact with the C-terminal region either with the FAT domain or with the Pro-rich motifs, through SH3-mediated interactions. Other proteins, interact with the N-terminal FERM domain of FAK. Among them interactions with β integrins cytoplasmic tail (Schaller et al., 1995), Etk (Chen et al., 2001) and ezrin (Pouillet et al., 2001) provide direct or indirect links with membrane proteins or cytoskeleton. Such interactions may play a role in FAK activation, possibly by altering its conformation. In addition, FAK also interacts with PIAS-1 (protein interacting with activated STAT-1) (Kadare et al., 2003). PIAS-1 is a SUMO ligase and catalyses the transfer of SUMO to specific lysine residues in targets proteins. PIAS-1 sumoylates Lys-152 in FAK, resulting in a marked activation of autophosphorylation. In transfected cells FAK sumoylation appears to occur within the nucleus. Although in some cells FAK has been reported to be located in the nucleus, the regulation of its translocation and the physiological circumstances in which sumoylation may occur are not known. This, as well as other recent results, indicate that FAK and PYK2 may undergo a regulated cyto-nuclear shuttling.

FAK undergoes multiple alternative splicing in the coding and non-coding regions. As far as the coding sequence is concerned the alternative exons are short, coding for 3 to 28 amino acids. Among them, alternative exons 14 and 16 code for 6 and 7 residues (boxes 6 and 7, respectively, located on either side of the autophosphorylated tyrosine (Tyr-397). The presence of boxes 6 or 7 increases dramatically the autophosphorylation of FAK (Burgaya et al., 1997). This effect results from an alleviation of the inhibitory role exerted by the N-terminal domain of FAK and from the ability of the alternatively spliced isoform to undergo intramolecular autophosphorylation (Toutant et al., 2002). All these alternatively spliced exons are highly conserved among vertebrates, but absent from invertebrate FAK. These splicing events are regulated in a tissue-specific fashion during development (Corsi et al., 2006). For example, the isoform which largely predominates in adult neurons is FAK^{+6,7}, which encompasses exons 14, 16 and 31.

The role of FAK and PYK2 in numerous cell responses is under scrutiny in many laboratories. Their role in cancer as well as in other pathological situations including immune and bone disorders make them primary targets for pharmacological inhibitors. Several molecules with affinities in the nanomolar range for FAK family kinases have been reported (e.g. (Choi et al., 2006)). In addition to designing ATP binding site inhibitors, other strategies aiming to disrupt various aspects of FAK activation, especially its targeting, may provide interesting alternatives. It is likely that this area of research will be very active in the next future.

NEW PROSPECTS FOR PROTEIN KINASES INHIBITORS IN THE NERVOUS SYSTEM

Protein kinase inhibitors are powerful drugs in cancer therapy. They also hold promise in other areas including inflammatory diseases and neurodegenerative disorders. In addition, in the nervous system the identification of signaling mechanisms involved in the action of neurotransmitters provide new targets. On the other hand it should be taken as a note of caution that drugs used for treating non-lethal chronic diseases must be extremely safe. Recent results suggest that short treatments with kinase inhibitors in appropriate conditions might have a potential for improving behavioral disorders. As an example, the role of ERK in the long term effects of drugs of abuse has been well documented. This results from the ability of drugs to activate the ERK pathway through stimulation of dopamine D1 and

glutamate NMDA receptors in the neurons of the striatum (Valjent et al., 2000; Valjent et al., 2005). The ERK pathway triggers the activation of transcription factors and long term alterations in striatal neurons. Such responses can be blocked by MEK inhibitors. It is known that re-exposure of animals to conditioning conditions can reactivate memories in a way which make them sensitive to interferences. Interestingly, re-exposing mice to drugs in the presence of a MEK inhibitor injected i.p. is capable to erase a previously acquired drug-conditioned place preference (Valjent et al., 2006). Although these results are far from any kind of application in humans, they suggest that manipulating signaling pathways with kinase inhibitors has an interesting potential to explore in the context of behavioral or psychiatric disorders.

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