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Focal Adhesion Kinase: An Old Protein with New Roles

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Abstract: Focal adhesion kinase, FAK is a tyrosine kinase which is prominently localized to focal adhesions and therefore its name, is an indispensable protein in integrin signaling. Since the discovery of FAK, this tyrosine kinase had been shown to regulate a variety of cell behavior like cell migration, proliferation, apoptosis and metastasis which makes this molecule at a centre stage of cell and developmental biology research. We want to take this opportunity to briefly review the current state of knowledge about FAK and why FAK was so important in cell migration, apoptosis, cardiovascular and metastatic research. Since the FAK signalling was very crucial for normal cellular development and cell physiology and since its deregulation prompts the oneset of a variety of diseases like cancer and cardiovascular diseases, an updated knowledge about its signalling mechanism and how FAK interacts with other signalling molecules can not only offer newer understanding in this field but also attract new methods and pathways to explore and investigate its biology, which can further open new avenues in anticancer research targetting FAK.

Key words: FAK, cell migration, angiogenesis and cancer

INTRODUCTION

Focal Adhesion Kinase (FAK) is a cytoplasmic protein tyrosine kinase that plays an important role during embryonic development and in different diseases including cancer and cardiovascular disorders (Golubovskaya *et al*., 2009). Basically, FAK is a 125-kDa protein tyrosine kinase which is made up of an N-terminal FERM domain, a central kinase domain, two proline-rich motifs and a C-terminal focal adhesion targeting, FAT domain, as shown in Fig. 1 (Girault *et al*., 1999; Sun *et al*., 2002).

The most important event in FAK signaling is the phosphorylation of Tyr397 (Schaller *et al*., 1994; Lai *et al*., 2010), which eventually promotes the recruitment and activation of Src to cell-extracellular matrix adhesion, sites (Yeo *et al*., 2006). This Tyr397 phosphorylation can then trigger the phosphorylation and activation of a variety of other kinases including its own tyrosine kinase residue FAK Tyr576/Tyr577 which promotes maximal FAK catalytic activity (Caron-Lormier and Berry, 2005). The phosphorylation of FAK Tyr925 located in the FAT domain is probably a late event but recognized as one of the several mechanisms through which adhesion can promote the activation of Ras/mitogen-activated protein kinase pathway (Mitra *et al*., 2005). FAK also has a paxillin binding site, which is considered as a mediator of FAK binding to integrins in the process of formation of adhesion complexes. Largely, the FAK signaling is explained in terms of its interaction with integrins, however, several growth factors like EGFRs, chemokines and G-protein coupled receptors also interacts and activate the FAK signaling, therefore it seems like FAK activation is a secondary event and the result from other pathways affecting integrin function. The complete mechanism of FAK signaling and its downstream signaling has been reviewed in several recent articles, therefore, we want to briefly emphasize the various role of FAK and will focus on how FAK could be an attractive target in anticancer research and beyond.

FAK and cell migration: Cell migration is a coordinated and complex process and evidence indicates the involvement of FAK in cell migration (Erickson, 1990). FAK signaling enhances cell motility, whereas inhibition of FAK signaling impairs cell migration (Mitra *et al*., 2005).

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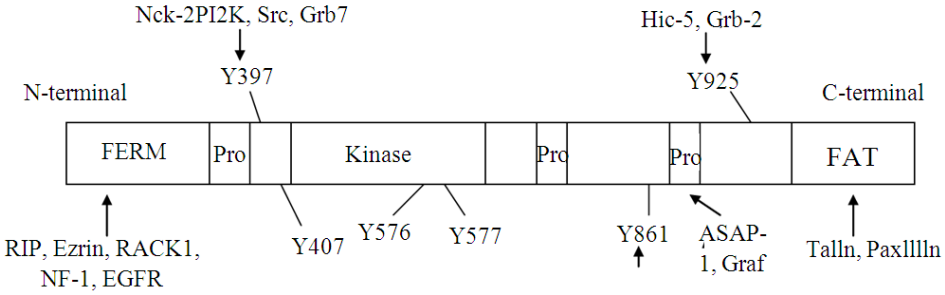

Fig. 1:Structure of Focal Adhesion Kinase and interaction proteins. FAK has N-terminal, central kinase and C-terminal domains. There are several known phosphorylation sites and interacting partners, indicated in the figure

FAK regulates the motility in randomly migrating cells and in response to a broad range of stimuli, including chemotactic, haptotactic and durotactic signals. Embryonic cells from FAK-deficient mice exhibited a decreased migration in culture, which was suggested to be responsible for a defect in mesodermal migration resulting in an embryonic lethal phenotype of the FAK-deficient ice, providing direct evidence for a role of FAK in promoting migration (Ilic *et al*., 1995). *In vivo,* microinjection of the FAK C-terminal recombinant protein reduced FAK activation and reduced migration of fibroblasts (Gilmore and Romer, 1996). There are reports showing FAK promote cell migration trough its interactions with PI3K and an adaptor molecule Grb7 (Han and Guan , 1999; Han *et al*., 2000). Similarly, expression of FAT, FRNK, or mutant FAK (397F) constructs, which typically act as dominant interfering forms of FAK, in various cell types usually results in partial inhibition of FAK activation and partial inhibition of cell migration (Hauck *et al*., 2001; Ding *et al*., 2005). Conversely, the overexpression of wild-type FAK in various different cell types enhances cell migration (Wang *et al*., 2000).

FAK and cell survival: Cell growth and survival is a very tightly regulated process and the cell fate is always a pre-determined event. The mechanism of apoptosis insures that the senescent and dead cells are cleared and this process is highly regulated. However this normal mechanism of apoptosis is also the target of tumor promoting factors, proto-oncogenes and several kinases that are up regulated in tumor cells. In fact this normal mechanism of apoptosis is actually hijacked to promote cell growth without inhibition. FAK knockout in mouse endothelial cells have shown to cause vascular defects and an increase in cell apoptosis and aberrant cell migration (Braren *et al*., 2006).

Several pathways downstream of FAK could be involved in cell survival. A phosphorylated FAK at Tyr397 can also bind to the p85 subunit of phosphatidylinositol 3-kinase, PI-3K and the production of phospholipids by phosphatidylinositol 3-kinase can lead to the activation of Akt kinase that inhibits apoptosis by regulating various components of the cell death machinery. The role of FAK have been investigated to promote cell proliferation by suppressing the process of apoptosis and also by some other mechanisms like activation of c-Jun NH2-terminal kinase downstream of CAS (Almeida *et al*., 2000). FAK therefore acts like a double edge sword where it's knocking down cause's severe defects in cell motility and embryonic lethality and on the other side this molecule is involved in suppressing apoptosis and therefore acts like a tumor promoter kinase.

FAK in cardiovascular development: Recent studies using animal models have shown an important role for FAK in the cardiovascular physiology. In particular, FAK is essential for angiogenesis in the embryo, functions in heart development and modulates the response of cardiomyocytes to pressure overload in adult mice. FAK inactivated mice were dead within hours of birth, mainly due to defective heart function and incomplete formation of the septum (Hakim *et al*., 2007). FAK gene knockout in mouse model have shown early embryonic lethality and severe cardiovascular defects (Ilic *et al*., 1995). In FAK conditional knockout mice there are defects in cardiac developmental and eccentric right ventricular hypertrophy in cardiomyocyte (Peng *et al*., 2008). FAK also play a modifying role in cardiac hypertrophy. *In vivo,* loss of FAK expression resulted in increased dilation of the ventricle lumen upon AngII induced hypertrophy and aortic banding. This study suggests that Inactivation of focal adhesion kinase in cardiomyocytes promotes eccentric cardiac hypertrophy and fibrosis (Peng *et al*., 2006).

FAK in angiogenesis: Angiogenesis, the formation of new blood vessels from pre-existing ones, is essential for tumor development. Several angiogenic growth factor receptors regulate FAK expression. A number of studies in recent days have shown direct evidence about the role of FAK in angiogenesis utilizing transgenic and knockout mouse model system. Global mutant *FAK*-knockin mouse model reporting, specific roles for the kinase activity of FAK in embryonic vessel development. Lim *et al*. (2010) generated a knockin mouse model in which a FAK utation abolished the catalytic activity of the kinase domain. They reported that the inhibition of FAK kinase activity led to a lethal embryonic phenotype at E9.5, associated with hemorrhage and damage of blood vessels (Lim *et al*., 2010). FAK has been shown as an important modulator of angiogenesis, as transgenic mouse models have indicated that endothelial FAK expression and activity are essential for the formation of new blood vessel networks during embryonic development (Braren *et al*., 2006). Recently, using a tissue-restricted knockout mouse model, it was demonstrated that endothelial FAK was essential for tumor growth and tumor-associated angiogenesis, as mice lacking endothelial-specific FAK expression exhibited reduced tumor angiogenesis and hence reduced tumor growth in vivo (Tavora *et al*., 2010). In addition to its putative role in angiogenesis, altered FAK activity and expression have been directly linked to tumor genesis and metastasis since interference with FAK signaling led to decreased metastasis in a variety of tumor models, including breast and lung cancer (Golubovskaya *et al*., 2009; Zhao and Guan, 2009).

Targeting FAK in cancer: future direction in FAK research: FAK is an important regulator of cell migration and angiogenesis that is why FAK may be a best target in different diseases those that are very dependent on these biological process. A good example will be cancer metastasis where both of these processes involved. Pharmacological inhibition of FAK is one of the several exciting area in anticancer research as the FAK activity have been found up regulated in a variety of cancers such as ovarian cancer, neck, colon, prostate, thyroid and many other cancer types (McLean *et al*., 2005; Brunton and Frame, 2008). Therefore there is a big surge in research targeting FAK as a possible means to suppress the tumor cells. Several small molecule inhibitors are under way at various stages and few are undergoing clinical trials for cancer treatment (McLean *et al*., 2012). Several lines of evidence in cultured cells and conditional knock-out mouse models have suggested a critical role of FAK in angiogenesis during embryonic development and tumorigenesis (Lechertier and Hodivala-Dilke, 2012). Pfizer has developed a FAK specific inhibitor, PF-573,228 and another dual inhibitor PF-562,271, targeting FAK and a related molecule Pyk2, to check phosphorylation of FAK Tyr397. However the strategy is not as simple as it sounds because most of the inhibitors so far have targeted only the catalytic activity of FAK thereby targeting Tyr397 and suppressing the FAK-Src complex formation. But the kinase-independent functions of FAK observed in endothelial cells and mouse embryonic fibroblasts may also be true in cancer cells, therefore the small molecule inhibitors specifically designed to target both kinase dependent and kinase-independent functions of FAK along with targeting FAk's interaction with other molecules and pathways may prove much more promising. Another line of research could be two separate investigations, each targeting for kinase dependent and independent functions of FAK and then trying both inhibitors in combination. Much more reproducible results in different cancer cell types and mouse models would be needed before we reach to any conclusion and therefore we observe at this stage that both kinase dependent and independent inhibition of FAK will remain an active area of investigation in search of better anticancer drugs for the future.

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