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FAK and p53 Protein Interactions

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Abstract

Focal Adhesion Kinase plays a major role in cell adhesion, motility, survival, proliferation, metastasis, angiogenesis and lymphangiogenesis. In 2004, we have cloned the promoter sequence of FAK and found that p53 inhibits its activity (*BBA*, v. 1678, 2004). In 2005, we were the first group to show that FAK and p53 proteins directly interact in the cells (*JBC*, v. 280, 2005). We have shown that FAK and p53 proteins interact in the cytoplasm and in the nucleus by immunoprecipitation, pull-down and confocal microscopy assays. We have shown that FAK inhibited activity of p53 with the transcriptional targets: p21, Bax and Mdm-2 through protein-protein interactions. We identified the 7 amino-acid site in p53 that is involved in interaction with FAK protein. The present review will discuss the interaction of FAK and p53 proteins and discuss the mechanism of FAK-p53 loop regulation: inhibition of FAK promoter activity by p53 protein and also inhibition of p53 transcriptional activity by FAK protein.

Keywords

Focal Adhesion Kinase; p53; metastasis; tumor; protein interaction

INTRODUCTION

Focal Adhesion Kinase was discovered almost 20 years ago, as a protein that plays a major role in different cellular functions such as adhesion, motility, survival, proliferation and cell cycle. The FAK gene encodes a non receptor tyrosine kinase that localizes at focal adhesions: contact points of cells with extracellular matrix, and is activated by integrin (cell surface receptor) signaling or by growth factor receptor (c-Met, EGFR, PDGFR) or by angiogenesis receptors. The FAK gene was first isolated from chicken embryo fibroblasts transformed by v-src [1]. Our laboratory was the first to isolate FAK gene from human osteosarcoma tumors and to demonstrate that FAK mRNA was up-regulated in invasive and metastatic human tumor samples [1]. This was the first evidence that FAK can be regulated at the level of gene transcription, as well as by other mechanisms (gene amplification), reported by other groups. Subsequently, we have demonstrated up-regulation of FAK protein by immunohistochemical staining in different types of human tumors, including colon, breast, thyroid, ovarian, melanoma, and sarcoma [1,2–4,5,6]. In addition, we have found novel interaction of FAK with several binding partners, such as: RIP [7], and p53 [8], linking FAK with the apoptotic/survival nuclear pathways [9,10]. In addition, we have

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DISCLOSURE OF POTENTIAL CONFLICT OF INTEREST
Dr. Cance and Dr. Golubovskaya are inventors of patents and Co-Founders and shareholders of CureFAKtor Pharmaceuticals.

cloned the promoter region of the FAK gene [11]. We have found that FAK promoter contains p53 binding sites, and that p53 inhibits FAK transcription both *in vitro* [11] and *in vivo* [12]. Thus, this review will be focused on FAK intracellular signaling in cancer, linking signaling from extracellular matrix to the nucleus. We found the p53 and FAK interaction as an example focal adhesion protein functioning in the signaling between extracellular matrix and nucleus. The report of Frame *et al*. discussed that many FERM domain proteins have nuclear export and nuclear localization signals, suggesting of other proteins involvement in the shuttling of proteins from extracellular matrix to the nucleus and exchange of this signaling [13].

FAK has several binding partners in the N-terminal, Central and C-terminal domains. The N-terminal domain of FAK contains one proline-rich domain, and the C-terminal domain of FAK contains another two proline-rich domains that are sites of binding proteins, containing SH3 domains. The C-terminal part of C-terminal domain of FAK (853–1012 a.a) called FAT (Focal adhesion targeting domain) domain that is necessary for targeting of FAK to focal adhesion complexes through binding with different proteins (paxillin, talin, Rho, etc).

The first indirect link of FAK and p53 was provided by [14]. The authors showed that extracellular matrix survival signals mediated by FAK suppressed p53-directed apoptosis [14]. We were the first group to discover the direct binding of FAK and p53 proteins in different cancer cells [8]. The N-terminal domain of p53 (1–92 a.a.) binds the N-terminal domain of FAK [8]. We have shown that p53 can bind FAK promoter and inhibit its luciferase activity [8]. Moreover, FAK can also block p53 transcriptional activity of p21, BAX and Mdm-2. Thus, there is a feedback loop mechanism of regulation of these two proteins [10]. The recent report confirmed direct binding of the N-terminal domain of FAK with p53 and also found interaction of FAK and Mdm-2 providing a novel mechanism of FAK-Mmd-2-mediated ubiquitination of p53 in the nucleus [15]. These data link FAK with the p53 tumor suppressor signaling that we will discuss below. Thus, we will discuss the novel FAK-p53 cross-talk pathways in apoptotic and survival pathways. Then we will pay attention to novel therapeutics approaches to target the FAK-p53 interaction in cancer.

p53 REPRESSES FAK PROMOTER

Our group was first to clone human FAK promoter and to find two p53 binding sites in the FAK promoter [11]. We have shown that p53 can bind FAK promoter and inhibit its transcriptional activity *in vitro* by EMSA [11] and *in vivo* by ChIP (chromatin immunoprecipitation) assay [12]. In addition, several other transcription factors, such as SP-1, AP-2, TCF-1 and NF-kappa B were shown to be present in the FAK promoter. NF-kappa B protein has been shown to be linked to p53 pathway [16]. For example, activation of Cox-2 transcription required co-operation of NF-kappa B and p53 [16]. Thus, regulation of FAK promoter can also include association of these two transcription factors, thus providing additional indirect p53-regulated FAK expression mechanism. Recently, one group demonstrated that bortezomib can down-regulate FAK promoter activity through NF-kappa B-dependent inhibitory, but not through p53-dependent mechanism [17].

The global analysis of p53 transcription factor binding sites demonstrated that induction of HCT116 colon cancer cells with 5-fluorouracil transcriptionally down-regulated FAK [18]. Thus, the authors suggested that p53 can suppress metastasis through down-regulation of metastasis-related genes, such as FAK. We have shown recently that p53 can down-regulate FAK expression in human cancer cells [12]. FAK mRNA and protein was increased in primary colon and breast tumors with mutant p53 versus wild type p53 tumors [12]. We demonstrate that adenoviral p53 directly blocked FAK mRNA and FAK protein levels [12]. In addition, we have demonstrated high correlation between FAK overexpression and p53

mutation in 600 breast cancer tumors [12, 14, 19]. Recently, another group also have shown the p53-dependent repression of FAK in breast cancer in response to estradiol [20]. FAKmRNA and promoter were down-regulated by estradiol in estrogen-dependent breast cancer cell lines with wild type p53, but not with mutant p53 [20]. The data show that p53 is an important regulator of FAK in breast cancer cell lines and that that loss of p53 function in breast cancer may enhance metastasis of estrogen-responsive tumors through upregulated FAK expression upon estrogens stimulation.

DIRECT FAK AND p53 PROTEIN INTERACTION

We were the first group to demonstrate the direct interaction of FAK and p53 proteins by immunoprecipitation, pull-down and confocal microscopy methods [21]. We have demonstrated that the N-terminal domain of p53 (1–92 a.a.) physically directly binds the N-terminal domain of FAK [8]. Three years later another group confirmed interaction of FAK and p53 proteins [15], demonstrating also interaction of FAK and Mdm-2 proteins and providing support for the nuclear function of FAK (Fig. **1**). The authors demonstrate the regions of FAK that bind Mdm-2 and p53. There have been several reports on the localization of the N-terminal part of FAK in the nucleus [22–25]. Furthermore, the N-terminus of FAK was shown to cause apoptosis in breast cancer cell lines [23] and its nuclear localization was regulated by caspase inhibitors in endothelial cells [25]. In addition, p53 has been reported to be localized in the cytoplasm [26]. P53 directly activated Bax and released pro-apoptotic molecules, activating multidomain proteins in the cytoplasm. This mechanism required 62–91 residues in the proline-rich N-terminal domain of p53 [26]. We detected interaction and co-localization of p53 and FAK in tumor colon cancer samples. Moreover, we have shown that 7 amino-acids (65–71 a.a.) from the proline-rich region of p53 were involved in interaction with FAK [27]. Thus, we have shown direct interaction of FAK and p53 proteins [21] and detected exact region of p53 that is involved in interaction with FAK protein [27]. Thus, understanding the detail mechanism and functions of FAK/p53-interaction may ultimately have important implications for targeted cancer therapy.

FEEDBACK MODEL OF FAK-p53 PROTEIN INTERACTION

We have shown that p53 can suppress FAK transcription [11,12]. The global characterization of 65,572 p53 ChIP DNA fragments was done in HCT116 colorectal cancer cell line, treated with 5-fluorouracil to activate p53 [18]. The authors identified novel targets of p53, that are involved in cell adhesion, migration and metastasis, and PTK2 or FAK was one of these kinases [18]. Interestingly, in HCT116 cells, treated with 5-fluorouracil that increases p53 level, the PTK2 (FAK) expression was also inhibited [18].

CONCLUSIONS

We have also shown that FAK can suppress transcriptional activity of p53 through its interaction, as p53-mediated activation of p53-targets: p21, Mdm-2 and Bax was blocked by overexpression of FAK [8]. Thus, p53 can regulate FAK (by inhibiting transcription, and also FAK can regulate p53 by sequestering it from apoptotic signaling and by ubiquitination that decreases p53 transcriptional functions [15] (Fig. **1**). Thus FAK and p53 can be regulated through a feedback mechanism [10]. Mutations of p53 that are frequently found in cancers, can lead to up-regulation and overexpression of FAK. Thus, novel mechanisms of FAK survival function, FAK and wild type or mutant p53 interactions remain to be discovered during carcinogenesis. This novel interaction open avenue for targeting the complex of FAK and p53 and Mdm-2 proteins and developing novel therapeutics.

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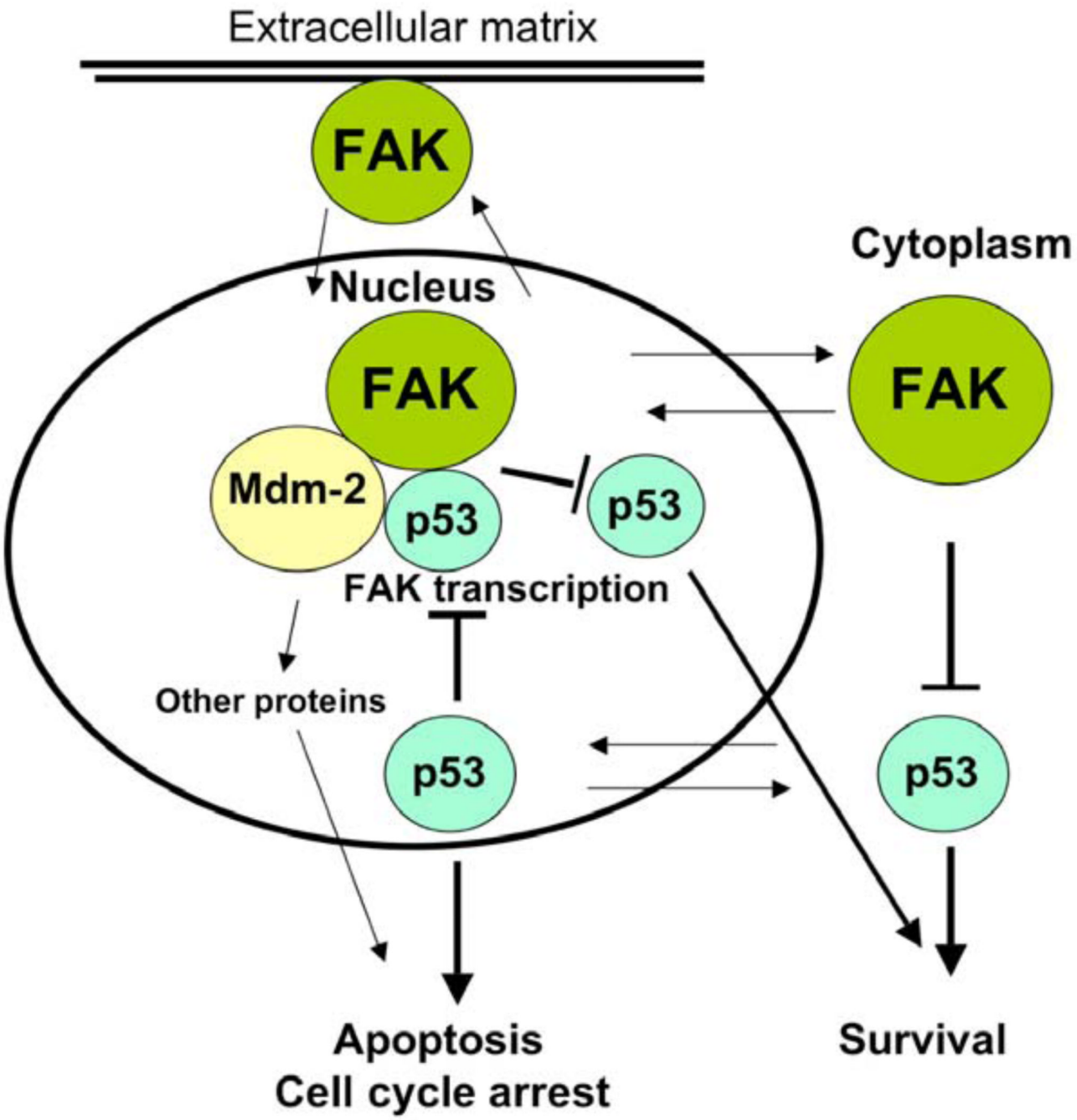

Fig. (1).

A model of FAK and p53 interaction and functions in cells and signal transduction pathways from extracellular matrix to the cytoplasm and to the nucleus. Numerous binding partners of FAK integrate signals from the extracellular matrix through growth factor receptors and integrins to control motility, survival, proliferation, metastasis, lymphangiogenesis and angiogenesis this signaling. In the nucleus, p53 binds FAK promoter and inhibits its transcription and causes cell cycle arrest, apoptosis or other mechanisms of growth inhibition. FAK binds p53 and sequesters p53 from apoptotic signaling and inhibits it growth inhibition function. FAK also binds Mdm-2 to facilitate p53 proteosomal degradation and enhancing cell survival. There is a feedback loop in FAK-p53 regulation.

Thus, FAK and p53 mediate signaling from extracellular matrix to the cytoplasm and nucleus.