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Focal Adhesion Kinase and p53 signal transduction pathways in cancer

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1. ABSTRACT

Human cancer is characterized by a process of tumor cell motility, invasion, and metastasis. One of the critical tyrosine kinases that is linked to these processes of tumor invasion and survival is the Focal Adhesion Kinase (FAK). Our laboratory was the first to isolate FAK from human tumors, and we had demonstrated that FAK mRNA was up-regulated in invasive and metastatic human breast and colon cancer samples. We have cloned FAK promoter and have found that FAK promoter contains p53 binding sites, and that p53 inhibits FAK transcription and regulates its expression in tumor samples. In addition, we have found a high correlation between FAK overexpression and p53 mutations in 600 population-based series of breast cancer patients. found that N-myc binds FAK promoter and induces FAK transcription in neuroblastoma cells. Thus, this review will be focused on FAK and p53 signal transduction pathways in cancer.

2. INTRODUCTION

Focal Adhesion Kinase was discovered more than 17 years ago, as a protein that plays a critical role in intracellular processes of cell spreading, adhesion, motility, survival and cell cycle progression. One of the critical tyrosine kinases that are linked to the processes of tumor invasion and survival is the Focal Adhesion Kinase (FAK). The FAK gene encodes a non receptor tyrosine kinase that localizes at contact points of cells with extracellular matrix, and is activated by integrin (cell surface receptor) signaling. 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 tumors, and we demonstrated that FAK mRNA was up-regulated in invasive and metastatic human breast and colon cancer samples (2). At the same time, matched samples of normal colon and breast tissue from the same patients had almost no detectable FAK expression. This

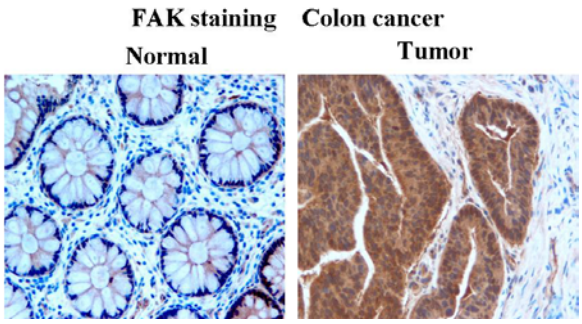

Figure 1. Focal Adhesion Kinase is overexpressed in tumor samples. Immunohistochemical staining is shown for colon cancer sample. Left panel: normal tissue, right panel: matched tumor tissue from the same patient.

was the first evidence that FAK can be regulated at the level of gene transcription, as well as other mechanisms (such as gene amplification). Subsequently, we have demonstrated up-regulation of FAK at the protein level in numerous types of human tumors, including colon, breast, thyroid, ovarian, melanoma, and sarcoma (3),(4-6), (7, 8) (Figure 1). In addition, we have found novel interaction of FAK with several binding partners, such as: RIP (9), linking FAK with the death-receptor pathways and p53 (10), linking FAK with the apoptotic/survival nuclear pathways (11), (12). In addition, we have cloned the regulatory promoter region of the FAK gene, and found its transcriptional up-regulation in cancer cell lines (13). We have found that FAK promoter contains p53 binding sites, and that p53 inhibits FAK transcription both *in vitro* (13) and *in vivo* (14). Thus, this review will be focused on FAK intracellular signaling in cancer, linking signaling from extracellular matrix to the nucleus. We will discuss the role Focal Adhesion Kinase (FAK) expression, localization, activity, protein-protein interaction, and survival signaling in the development of cancer. We will discuss the FAK structure, function, and the novel FAK-p53 cross-talk pathways in the junction of death and growth factor receptors and apoptotic and survival pathways. Then we will pay attention to novel therapeutics approaches to target these interaction and pathways in cancer.

3. FAK GENE STRUCTURE

First, FAK cDNA encoding 125 kDa protein was isolated from chicken embryo cells (1). The human FAK (also known as PTK2,protein tyrosine kinase 2) gene has been mapped to chromosome 8 (15, 16). Human complete FAK mRNA sequence is a 3791 bases long sequence (17). We were the first group to isolate human FAK cDNA from the primary sarcoma tissue and found increased FAKmRNA in tumor samples compared with normal tissue samples (2).

Recently, the genomic structure of FAK has been characterized (18). The gene coding sequence contains 34 exons, and genomic sequence spans 230 kb (18). We were the first group to clone and characterize the human FAK promoter, regulating FAK expression (13). The core promoter contains 600 base pairs and includes many transcription binding sites, such as AP-1, AP-2, SP-1, PU.1, GCF, TCF-1, EGR-1, NF-kappa B and p53(13). Interestingly, we found two transcription binding sites for p53 in the FAK promoter, and found that p53 can block FAK promoter activity (13). Recently, mouse promoter has been cloned and it is highly homologous to the human promoter, and contained the same binding sites (18).

4. FAK PROTEIN STRUCTURE

The FAK protein is a 125 kDa tyrosine kinase ($p125^{FAK}$) with a large amino-N-terminal domain, exhibiting homology with a FERM (protein 4.1, ezrin, radixin and moesin) domain with an autophosphorylation site (Y-397), a central catalytic domain, and a large carboxy-C-terminal domain that contains a number of potential protein interacting sites, including two proline-rich domains and FAT domain (19-21) (Figure 2).

4.1. FAK N-terminal domain

The function of the N-terminal, homologous to FERM domain was linked to the binding of integrins, via their β subunits(22). The N-terminal domain (1-415 a.a) of FAK protein contains the major autophosphorylation site Y397-tyrosine, that in phosphorylated form becomes a binding site of SH-2 domain of Src, leading to its conformational changes and activation (19). The crystal structure of the N-terminal domain of avian FAK, containing FERM domain has been recently described (23). Interesting negative regulation of FAK function by FERM domain was revealed by (24) and (25). Tyrosine phosphorylation of FAK causes binding and activation of Src that leads to tyrosine phosphorylation Y407 and Y576,Y577 - major phosphorylation sites in the catalytic domain of FAK; Y861 and Y925 (19, 26), and then to phosphorylation of FAK binding proteins, such as paxillin and Cas (27). This cascade causes subsequent cytoskeletal changes and activation of RAS-MAPK (mitogen-activated protein kinase) signaling pathways (26, 28). Thus, FAK-Src signaling complex activates many other signaling proteins, involved in survival, motility and metastatic, invasive phenotype in cancer cells. Phosphorylated Y397 FAK is able to recruit another important signaling protein, p85 PI3-kinase (phosphoinositide 3-kinase), growth factor receptor bound protein Grb 7, phospholipase Cgamma(PLCgamma) and others. Thus, the N-terminal domain of FAK binds to the extracellular matrix receptors, integrins, growth factor receptors or important signaling cytoplasmic, cytoskeletal and nuclear proteins, mediating signaling from the extracellular matrix to the cytoplasm and nucleus and controlling cytoskeletal changes, survival, motility and invasion.

4.2. FAK Kinase domain

The central kinase (catalytic) domain of FAK (416-676 amino-acids) is the most conserved domain in vertebrate and non-vertebrate organisms (18). The central catalytic domain of FAK contains Y576 and Y577, major phosphorylation sites, and also K454, ATP binding site (Figure 2). Phosphorylation of FAK by Src on Y576 and Y577 is an important step in the formation of active signaling complex and is required for maximal enzymatic

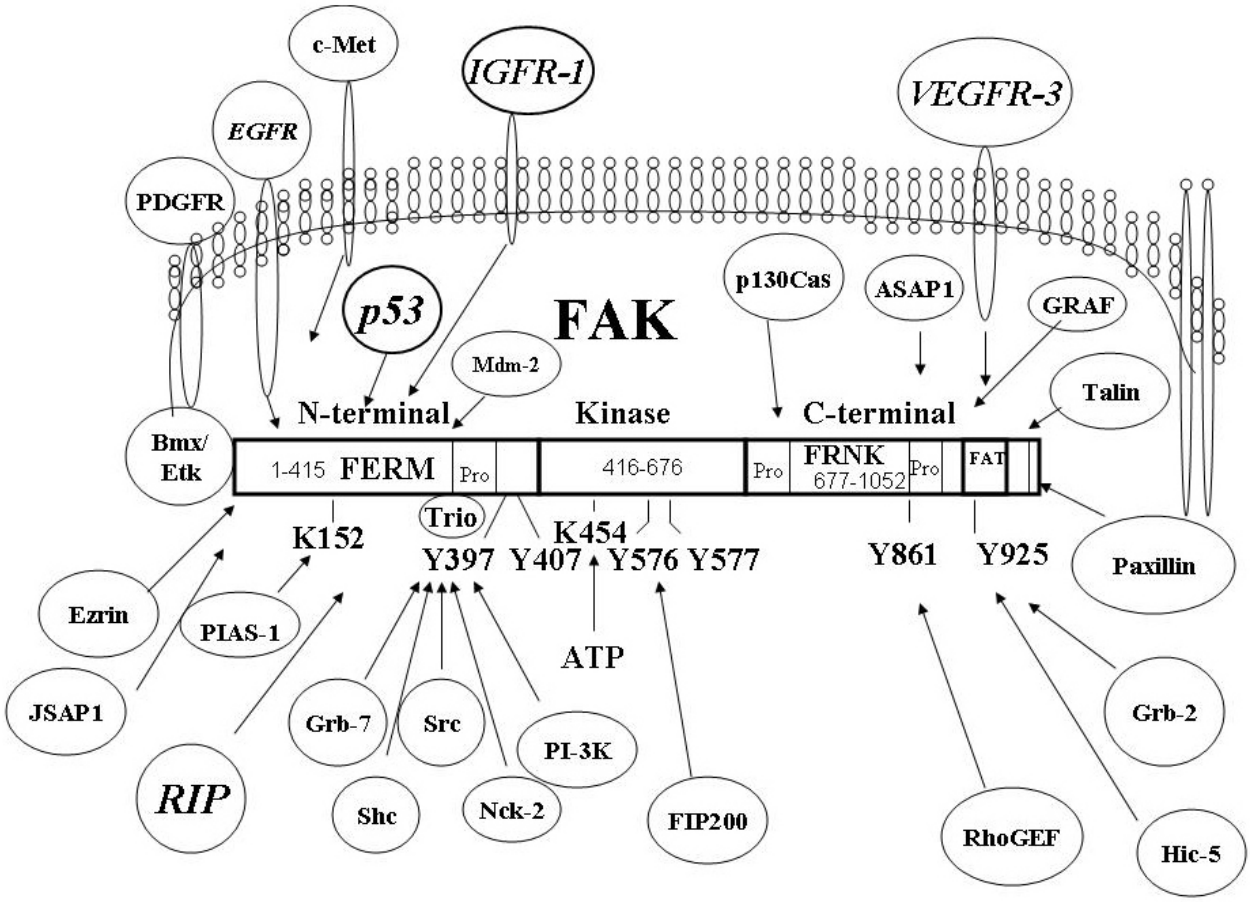

Figure 2. Focal Adhesion Kinase (FAK) structure. FAK has the N-terminal, Kinase domain and the C-terminal domains. The N-terminal domain has Y-397-Y-autophosphorylation site. The Kinase domain has Y576/577 tyrosines important for catalytic activity of FAK. The C-terminal domain of FAK has Y861 and Y925 tyrosines. Different proteins bind to these domains and involved in motility and survival signaling, The N-terminal domain (205-422 a.a.) of FAK is involved in interaction with Src, RIP, p53, PI3Kinase, PIAS-1, PI3Kinase, Grb-7, EGFR/PDGFR, Ezrin, Bmx, Trio and others. Kinase domain is involved in binding with FIP200 protein. ASAP, p130Cas, Grb-2, Paxillin, Talin, RhoGEFp190 and other proteins bind C-terminal domain of FAK. Interactions of FAK and other proteins demonstrated by group are shown in Italics.

activity of FAK (29). The crystal structure of FAK kinase domain revealed open conformation similar to the fibroblast growth factor receptor-1 (FGFR-1) and vascular endothelial growth factor receptor (VEGFR) (30). The FAK kinase domain structure has an unusual has been recently solved (25).

4.3. FAK C-terminal domain

Different proteins can bind to the C-terminal domain of $p125^{FAK}$ (677-1052 amino-acids), including paxillin, p130cas, PI3-kinase, GTP-ase-activating protein Graf, leading to changes in the cytoskeleton and to activation of the Ras-MAP kinase pathway (21, 28, 31, 32). The carboxy-terminal domain of FAK contains sequences responsible for its targeting to focal adhesions, also known as the FAT domain. Alternative splicing of FAK results in autonomous expression of the C-terminal part of FAK, FAK-related non-kinase (FRNK) (33). The crystal structure of the C-terminal domain of FAK, FAT, has been determined recently by several groups (34),(35) that can exist as dimer or monomer, allowing binding of several binding partners. FAT domain mediates signaling through Grb-2 binding to Y925 site of FAK (36).

5. FUNCTIONS OF FAK IN CANCER

FAK has numerous functions in cell survival, motility, metastasis, invasion and angiogenesis.

5.1. Survival function

FAK plays a major role in the survival signaling and has been linked to detachment-induced apoptosis or anoikis (37). It has been shown that constitutively activated forms of FAK rescued epithelial cells from anoikis, suggesting that FAK can regulate this process (32, 37-40). Similarly, both FAK antisense oligonucleotides (41, 42), as well as dominant-negative FAK protein (FAK-CD), caused cell detachment and apoptosis in tumor cells (41, 43-50). The anti-apoptotic role of FAK was also demonstrated in FAK-transfected FAK/HL60 cells that

were highly resistant to apoptosis induced with etoposide and hydrogen peroxide compared with the parental HL-60 cells or the vector-transfected cells (51, 52). HL-60/FAK cells activated the AKT pathway and NF-kappa B survival pathways with the induction of inhibitor-of-apoptosis proteins, IAPs (51). We have demonstrated that EGFR and Src signaling cooperate with FAK survival signaling in colon and breast cancer cells (45),(46, 50, 53). We have also demonstrated that simultaneous inhibition of Src and FAK or EGFR and FAK pathways was able to increase apoptosis in cancer cells (45); (46). Thus, cancer cells use cooperative function of kinases and growth factor receptor signaling to increase survival.

5.2. Motility function

FAK has also been shown to be important for cell motility (28, 54-56). FAK-null embryos exhibit decreased motility *in vitro* (57). Furthermore, enforced expression of FAK stimulated cell migration (58, 59). Cell migration is initiated by protrusion at the leading edge of the cell, by the formation of peripheral adhesions, exertion of force on these adhesions, and then the release of the adhesions at the rear of the cell (60). Focal adhesion kinase (FAK) is involved in the regulation of migration, although the precise mechanism of this FAK-regulated migration is unclear. FAK has been shown to be required for the organization of the leading edge in migrating cells by coordinating integrin signaling in order to direct the correct activation of membrane protrusion (60). SH2 domain of Src, targeting Src to focal adhesions and Y397 activity has been shown to be important for motility (61). PI3 kinase has been also shown to be critical for FAK-mediated motility in Chinese hamster ovary (CHO) cells (62). Tumor suppressor gene PTEN, encoding phosphatase has been shown to interact with FAK, caused its dephosphorylation and blocked motility (63). Moreover, Y397FAK was important for PTEN interaction with FAK (64). Overexpression of FAK reversed the inhibitory effect of PTEN on cell migration (63).

5.3. Metastasis, invasion and angiogenesis functions

Activation of FAK is linked to invasion and metastasis signaling pathways. FAK was important in Erb-2/Erb3-induced oncogenic transformation and invasion (65). Inhibition of FAK in FAK-proficient invasive cancer cells prevented cell invasion and metastasis processes (65). In addition, FAK has been shown to be activated in invading fibrosarcoma and regulated metastasis (66). Inhibition of FAK with dominant-negative FAK-CD disrupted invasion of cancer cells (56). We have also shown that high FAK expression in breast cancers associated with tumor aggressive phenotype (67). Subsequently, we analysed FAK expression in pre-invasive ductal carcinoma *in situ*, DCIS tumors and detected protein overexpression in preivasive tumors (8), suggesting that FAK survival function occurs as an early event in breast tumorigenesis.

Overexpression of focal adhesion kinase in vascular endothelial cells promoted angiogenesis in transgenic mice (68). Overexpression of FAK induced human retinal endothelial cell (HREC) migration and *in vivo* angiogenesis (69). FAK activity and phosphorylation of Y925 site of FAK promoted angiogenic switch during tumor progression (70). FAK-Grb2-MAPK signaling has been shown to be important for promoting angiogenesis. Furthermore, inhibition of FAK resulted in disruption of angiogenesis (70). FAK and Src catalytic activities are important to promote VEGF-dependent angiogenesis (71). Thus, FAK is involved in angiogenesis and plays a major role in tumorigenesis.

6. FAK AND P53 SIGNALING

FAK has numerous 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 (72). The authors showed that extracellular matrix survival signals mediated by FAK suppressed p53-directed apoptosis (72). We were the first to find direct binding of FAK and p53 proteins in different cancer cells (10). The N-terminal domain of p53 (1-92 a.a.) interacts with the N-terminal domain of FAK (10). We have shown previously that p53 can bind FAK promoter and inhibit its luciferase activity (10). Moreover, FAK can block p53 transcriptional activity of p21, BAX and Mdm-2. Thus, there is a feedback loop mechanism of regulation of these two proteins (12). 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 (73). These data link FAK with the p53 tumor suppressor signaling that we will discuss below.

6.1. Structure and function of p53 Protein

p53 is a tumor suppressor gene, which is located at chromosome 17p13 region, spans 20 kb and contains 11 exons (74). The p53 protein is a phosphoprotein transcription factor that binds to 5'Pu-Pu-Pu-C-A/T-T/A-G-Py-Py-Py3' (Pu-purine; Py-pyrimidine) consensus DNA sequence in the promoters of the genes and activates their transcription (75). The p53 gene encodes 393 amino-acid protein. The promoter of p53 lacks TATA box and contains various binding sites for known transcription factors, such as NF-kappa B, Sp1 or c-Jun (76).

p53 protein contains three domains: an acidic N-terminal, transcriptional activating domain (1- 92 aa.), Central, DNA-binding domain (102-292 amino-acids) and C-terminal (102-292 amino-acids), tetramerization domain (325-393 amino-acids). The p53 protein contains many sites for phosphorylation by different kinases: ATM, Chk2, ATR, JNK, MAPK, CKI, CKII).

6.2. Mutations of p53 in cancer

It is known that the p53 tumor suppressor is the most frequent target for genetic alterations in human cancers and

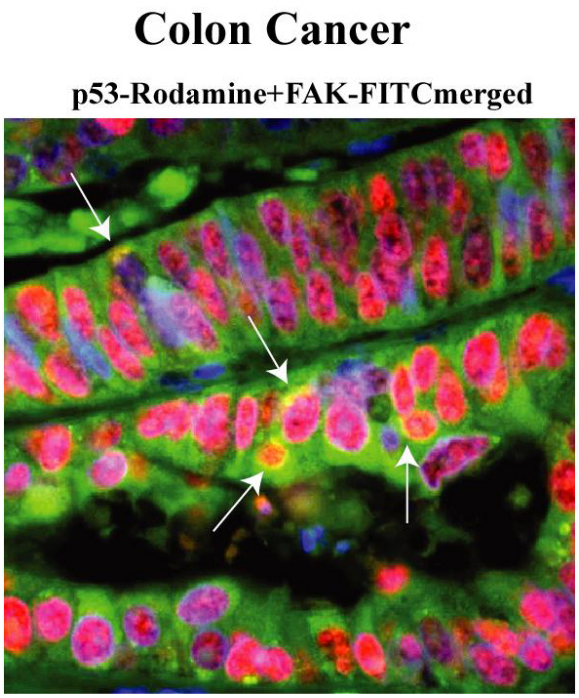

Figure 3. Immunohistochemical staining of FAK and p53 in colon tumor. FAK is stained with FITC, p53 is stained with Rhodamin and nuclei with Hoechst. Arrow indicate co-localization of FAK and p53 (yellow color).

is mutated in almost 50% of all tumors (77-81). Inactivation of p53 gene is a critical step in tumorigenesis (82). Following induction by variety of cell stresses such as DNA damage, hypoxia, presence of activated oncogenes, p53 up-regulates a set of genes that can promote cell death and growth arrest, such as p21, GADD45, cyclin G, Bax, reviewed in (83). Recently, it was shown that p53 can repress promoter activities of a number of anti-apoptotic genes and cell-cycle genes (survivin (84), cyclin B1, cdc2, (85, 86), cdc25 c (87), stathmin (88), Map4(89) , bcl-2 (85).

Among reported mutations, 75% are missense mutations, with 80% of them located in the DNA-binding domain of p53 (76), and 30% of the mutations are in 5 hot-spot codons: 175, 245, 248, 273 and 282). Arginine residues (248 and 273) involved in interaction of p53 with DNA, and arginines (175 and 282) stabilize DNA-binding sequence (76). Wild type p53 binds to promoters differently, for example, p53 activates p21 promoter with higher affinity than Bax promoter (76). Some p53 mutants are able to trans-activate different genes, such as EGFR, MDR1, c-Myc, PCNA, IGF-2 or VEGF, providing growth-promoting phenotypes and drug-resistance (76).

6.3. p53 binds and represses FAK promoter

Our group was first to clone human FAK promoter and to find two p53 binding sites in the FAK promoter (13). We have shown that p53 can bind FAK promoter and inhibit its transcriptional activity *in vitro* by EMSA (13) and *in vivo* by ChIP (chromatin immunoprecipitation) assay (14) (Figure 3). 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 (90). For example, activation of Cox-2 transcription required co-operation of NF-kappa B and p53 (90). Thus, regulation of FAK promoter can also include association of these two transcription factors, thus providing additional indirect p53-regulated FAK expression mechanism.

Moreover, while wild type inhibited FAK promoter activity, mutant p53 did not inhibit FAK promoter activity, as wild type did. The recent global analysis of p53 transcription factor binding sites demonstrated that induction of HCT116 colon cancer cells with 5-fluorouracil transcriptionally down-regulated FAK (91). Thus, the authors suggested that p53 can suppress metastasis through down-regulation of metastasis-related genes, as FAK. We have shown recently that p53 can regulate FAK expression in human cancer cells (14). FAK mRNA and protein was increased in primary colon and breast tumors with mutant p53 versus wild type p53 tumors (14). In addition population-based study of 600 breast cancer tumors demonstrated high correlation between FAK overexpression and p53 mutation (12,14).

6.4. Direct FAK and p53 protein binding

We have demonstrated that the N-terminal transactivation domain of (1-92 a.a.) of p53 physically directly binds the N-terminal domain of FAK (10) . There have been several reports on the localization of the N-terminal part of FAK in the nucleus (92), (47), (93), (92, 94). Furthermore, the N-terminus of FAK was shown to cause apoptosis in breast cancer cell lines (47) and its nuclear localization was regulated by caspase inhibitors in endothelial cells (94). In addition, p53 has been reported to be localized in the cytoplasm (95). 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 (95). We detected interaction and co-localization of p53 and FAK in tumor colon cancer samples (Figure 3, marked by arrows). Consistent with these findings, recently we have shown that 7 amino-acids (65-71 a.a.) from the proline-rich region of p53 were involved in interaction with FAK (96).

6.5. Direct FAK and Mdm-2 protein binding

The recent report demonstrated that FAK binds also Mdm-2 and causes p53 proteosomal degradation in the nucleus (73) (Figure 4). Thus, understanding the detail mechanism and functions of FAK/p53-interaction may ultimately have important implications for targeted cancer therapy.

6.6. Feedback model of FAK-p53 protein interaction

We have shown that p53 can suppress FAK transcription.(13, 14). Recently, global characterization of 65,572 p53 ChIP DNA fragments was done in HCT116 colorectal cancer cell line, treated with 5-fluorouracil for 6 hours that activated p53 (91). The authors identified novel

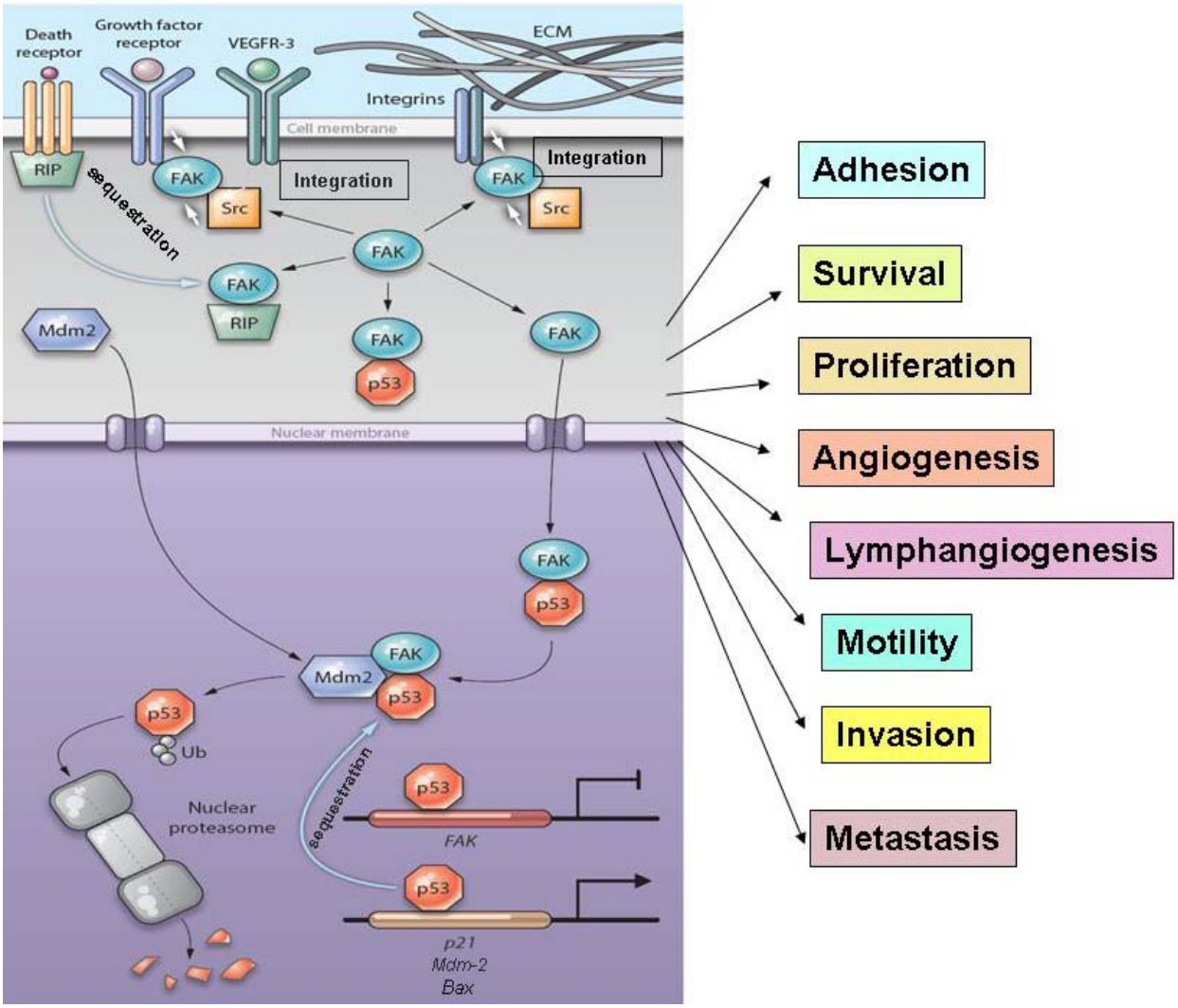

Figure 4. Sequestration and Integration Model of FAK functions in cells and signal transduction pathways from extracellular matrix to the cytoplasm and nucleus. The central part of the scheme is modified from (12). Focal Adhesion Kinase integrates signals from growth factor receptors (EGFR, IGFR), vascular endothelial growth factor receptors (VEGFR-3), Src and integrins to control motility, survival, proliferation, metastasis, lymphangiogenesis and angiogenesis. Numerous binding partners of FAK mediate this signaling. FAK sequesters pro-apoptotic proteins, such as p53 from apoptotic signaling. P53 binds FAK promoter and inhibits its transcription. There is a feedback loop in FAK-p53 regulation. Thus, FAK mediate signaling from extracellular matrix to the cytoplasm and nucleus.

targets of p53, that are involved in cell adhesion, migration and metastasis, and PTK2 or FAK was one of these kinases (91). Interestingly, in HCT116 cells, treated with 5-fluorouracil that increases p53 level, PTK2 (FAK) expression was also inhibited (91).

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 (10). (Figure 4). Thus, p53 can regulate FAK (by inhibiting transcription, and in turn, FAK can regulate p53 by sequestering it from apoptotic signaling and then ubiquitination that decreases p53 transcriptional functions (73). Thus FAK and p53 can be regulated through a comprising a feedback mechanism (12) (Figure 4). 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.

7. FAK INHIBITORS

Recently, Focal Adhesion Kinase has been proposed to be a new therapeutic target (26). Several *in vitro* approaches used to down-regulate FAK-adenoviral

FAK-CD (dominant-negative FAK) (44), anti-sense oligonucleotides (42) and siRNA for FAK (97). The melanoma cells treated with antisense oligonucleotides lost their attachment and underwent apoptosis (41) (42). The same effect was observed with Ad-FAK-CD in different cancer cells. While breast cancer cells underwent apoptosis by down-regulation of FAK with FAK-CD, normal MCF-10A and HMEM cells did not undergo apoptosis (44). These inhibitors applications are limited due to the cell toxicity *in vivo*. Thus, developing small-molecule drugs is critical for future FAK-targeting therapy, involving kinase inhibitors and drugs, targeting FAK-protein interactions.

There were no pharmacological inhibitors, reducing FAK kinase activity. Recently Novartis Inc. developed novel FAK inhibitors down-regulation its kinase activity (98). The novel Novartis FAK inhibitor, TAE-226 recently was employed in brain cancer and effectively inhibited FAK signaling and caused apoptosis in these cells (99). We also used TAE-226 inhibitor in breast (100), neuroblastoma (101) and pancreatic cancer cells (102) and found that this inhibitor can effectively cause apoptosis in these different types of cancer. Although, it can inhibit also other signaling pathways in addition to FAK, such as IGFR-1. Another, ATP-targeting site inhibitor of FAK, Pfizer-PF-573,228 has been recently described (103). Another Pfizer inhibitor PF-562,271 with high specificity in inhibiting FAK activity has been shown to be effective in tumor xenograft models *in vivo* (104) and on bone tumors(105). The future detail studies will be needed to address specificity of these drugs.

8. TARGETING FAK-PROTEIN INTERACTIONS

One of the approaches to inhibit FAK function can be targeting its protein-protein interaction with its binding partners, such p53. Small-molecule drugs can be found either through high-throughput screening or through database searches using protein crystal structures.

Small molecule drug inhibitors are effectively used to target p53 protein-protein interactions, particularly with Mdm-2 protein (106). The first potent inhibitors targeting p53-Mdm-2 interaction have been identified by high-throughput screening followed by structure-based optimization (106). The screening identified nutlins that represent a class of cis-imidazole analogues that bind to the p53 pocket interacting with Mdm-2. The same strategy can be used to target interaction with FAK and p53, FAK and Mdm-2 and combination therapy approaches can be applied.

Recently we used the crystal structure of the N-terminus of FAK, and screened 200,000 small molecules from the NCI bank for their ability to target this binding site and for their oral bioavailability using Lipinski rules. We identified several potential lead compounds and tested them on human breast, colon, and melanoma cell lines for their ability to disrupt p53-FAK or VEGFR-3-FAK binding and to induce cancer cell death. We found several potential compounds that were able to decrease FAK phosphorylation, decrease cell viability and activate PARP, suggesting its potential role in future therapy.

9. PERSPECTIVE

Thus, understanding of FAK biology during tumorigenesis, mechanisms of its up-regulation in different tumors, role in stem cell biology, angiogenesis, motility, and especially mechanisms of its direct physical interaction with protein binding partners and their down-stream signaling pathways will be critical in developing targeted therapeutics.

Studies with peptide inhibitors already have indicated that blockade of specific protein–protein interactions has therapeutic promise for treating a variety of diseases, including cancer (107-112). Small molecule drugs are particularly attractive as inhibitors of intracellular protein–protein interactions due to the ability to modify their structures to achieve optimal target binding. Recently, the N-terminal domain of Pyk-2 (protein highly homologous to FAK) has been shown to interact with p53 (113). Although, we did not find direct interaction of the full length Pyk-2 protein and p53 in pull-down assay (10), the mechanism of this N-terminal domain and p53 interaction is intriguing. Thus, targeting of FAK-53 signaling pathways can be important in cancer treatment programs. The computer modeling approach that we developed recently, targeting FAK autophosphorylation site showed that this inhibitor can decrease breast and pancreatic tumorigenesis (114, 115). Thus, as we further define the mechanisms of FAK and p53 signaling in cancer cells, we will identify the optimal sites for targeting these protein and disrupting its signaling to cause apoptosis in human tumors. The correlation of p53 mutation and FAK overexpression in 600 breast tumors (116) can be a basis for future therapies in tumors with mutant p53 and overexpressed FAK.

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Abbreviations: FAK, Focal Adhesion Kinase; Mdm-2, murine double minute 2

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