FAK overexpression and *p53* mutations are highly correlated in human breast cancer

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Abstract

Focal Adhesion Kinase (FAK) is overexpressed in a number of tumors, including breast cancer. Another marker of breast cancer tumorigenesis is the tumor suppressor gene *p53* that is frequently mutated in breast cancer. In the present study, our aim was to find a correlation between FAK overexpression, p53 expression and mutation status in a population-based series of invasive breast cancer tumors from the Carolina Breast Cancer Study. Immunohistochemical analyses of 622 breast cancer tumors revealed that expression of FAK and p53 were highly correlated (P = 0.0002) and FAK positive tumors were 1.8 times more likely to be p53 positive compared to FAK negative tumors [odds ratio (OR) = 1.8; 95% Confidence Interval (CI) 1.2 – 2.8, adjusted for age, race and stage at diagnosis]. Tumors positive for p53 expression showed higher intensity of FAK staining (P<0.0001) and higher percent of FAK positive staining (P<0.0005). From the same study, we evaluated 596 breast tumors for mutations in the *p53* gene, using SSCP (single strand conformational polymorphism) and sequencing. Statistical analyses were performed to determine the correlation between *p53* mutation status and FAK expression in these tumors. We found that FAK expression and *p53* mutation were positively correlated (P<0.0001) and FAK positive tumors were 2.5 times more likely to be *p53* mutation positive compared to FAK negative tumors [adjusted OR = 2.5, 95% CI 1.6–3.9]. This is the first analysis demonstrating a high correlation between FAK expression and *p53* mutations in a population-based series of breast tumors.

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Introduction

Tumor suppressor *p53* is one of the major markers of human tumorigenesis and is mutated in almost 50% of all tumors [1]. Following induction by a variety of cell stresses such as DNA damage, hypoxia, and oncogene activation, p53 up-regulates a number of genes that can promote cell death and growth arrest, such as p21, Bax, cyclin G, GADD45 (reviewed in [2]). Recently, it was shown that p53 can also repress promoter activities of a number of anti-apoptotic genes and cell-cycle genes (survivin, cyclin B1, cdc2, cdc25 c, stathmin, Map4, bcl-2 and FAK) [3].

Focal adhesion kinase (FAK) is a non-receptor cytoplasmic protein tyrosine kinase that controls a number of cellular signaling pathways, including proliferation, cell spreading, motility, angiogenesis, invasion and survival [4]. FAK is overexpressed in many types of tumors, including breast cancer tumors [5]. We have shown that FAK up-regulation occurred in early stages of breast tumorigenesis [5, 6]. In addition, increased FAK mRNA and protein expression has been demonstrated in adenomatous tissues and in matched samples of colorectal carcinoma and liver metastases [7, 8].

The first report on indirect functional link between FAK and tumor suppressor gene *p53* was reported by Ilic *et al.* [9]. The authors demonstrated that p53 controls survival signals from the extracellular matrix transduced by FAK in anchorage-dependent cells [9]. Recently, cloning and characterization of the FAK promoter demonstrated p53 transcription factor binding sites that had potential importance for regulation of FAK transcription [3]. In addition, we and other group demonstrated direct physical interaction of the FAK and p53 proteins [10, 11] providing a novel mechanism for FAK-p53-mediated signaling in tumorigenesis [12, 13].

Recently, global characterization of 65,572 p53 ChIP DNA fragments was done on 5-fluorouracil-treated HCT116 colorectal cancer cells to determine potential targets of activated p53 [14]. The authors identified a number of novel targets, including PTK2 (FAK) that were involved in cell adhesion, migration and metastasis [14]. These 5-fluouracil-treated HCT116 cells inhibited FAK [14], leading the authors to suggest that p53 can suppress metastasis through repression of FAK. We performed ChIP assays on HCT116 colon cancer cells and demonstrated that p53 can bind the FAK promoter both *in vitro* [3] and *in vivo* [15] and repress its promoter activity [3]. Furthermore, recently we demonstrated down-regulation of FAK mRNA and protein levels by adenoviral overexpression of p53 and by endogenous induction of p53 with chemotherapy[15]. The aim of this report was to analyze the p53 status and FAK expression in a population-based series of breast cancers. We clearly show for the first time that high FAK expression and the presence of p53 mutations positively correlated in a population-based series of invasive breast cancer.

Materials and Methods

Study Population

The Carolina Breast Cancer Study (CBCS) is a population-based, case-control study of breast cancer in African American and white women[16, 17]. The CBCS included women 20–74 years of age from 24 contiguous counties of central and eastern North Carolina. This research was approved by the University of North Carolina School of Medicine IRB and University of Florida IRB. Women with a first diagnosis of invasive breast cancer (Phase 1

of the CBCS) were identified by the North Carolina Central Cancer Registry through a rapid case ascertainment system. Increased cases of African American women and women younger than 50 years were included so that these subpopulations would represent approximately 50% of the study population 17.

Tumor specimens for FAK staining

Formalin-fixed, paraffin-embedded tumor blocks were obtained from participating hospital's Pathology Departments and tumors were sectioned by the Specialized Programs of Research Excellence (SPORE) Immunohistochemiostry Core facility. Total 629 breast cancer formalin-fixed, paraffin-embedded tissue cases were used for FAK immunohistochemical analysis.

Immunohistochemistry

FAK immuniohistochemistry was performed using the FAK monoclonal antibody 4.47 (Upstate Biotechnology, USA), as described in 16. In brief, slides were heated at 60°C for one hour prior to deparafinization, rehydration and quenching of endogenous peroxidase activity (3% hydrogen peroxide in methanol). The hydration process was completed by rinsing in DAKO TBS buffer (DAKO Corporation, Carpinteria, CA), containing 0.05% Tween 20 (DAKO Corporation, Carpinteria, CA). During heat-induced epitope recovery, sections were steam-heated while submerged in antigen retrieval Citra buffer (BioGenex, San Ramon, CA) for 30 minutes. Sections were blocked in normal horse serum (Vectastatin Elite Kit, Vector Laboratories, Burlingame, CA) for 15 minutes and then incubated with anti-FAK 4.47 monoclonal antibody (1:250 dilution) overnight at 4°C in a humidity chamber. Sections were washed in DAKO TBS buffer containing 0.05% Tween 20 and then loaded into the DAKO Autostainer for application and incubation of the biotinylated horse antimouse IgG (Vectastatin Elite Kit, Vector Laboratories, Burlingame, CA) for 30 minutes at room temperature. The Avidin-biotin complex (Vectastatin Elite Kit, Vector Laboratories, Burlingame, CA) was applied to the slides for 30 minutes at room temperature. The chromagenic reaction was performed with SG (Vector Laboratories, Burlingame, CA) for 10 minutes at room temperature. Slides were removed from the autostainer and counterstained with Nuclear Fast Red (Vector Laboratories, Burlingame, CA) for 10 minutes and coverslipped with PerMount (Fisher Scientific). Positive and negative controls were included with each run. P53 monoclonal antibody (clone DO-7; Dako, CA, USA) was used for p53 immunohistochemical staining, as described in 18. In brief, the slides were dried and p53 antibody was applied 1:100 dilution to the slides for 30 minutes at 37C. Then slides were processed the same as above. Control sections were incubated with a comparable concentration of isotype-matched IgG_1 monoclonal antibody, MOPC-21 (Sigma, St Louis, Mo).

Immunohistochemistry Scoring

A single experienced board certified pathologist scored blindly each tissue section for FAK expression based on a scoring system that measured intensity (0, none; 1, borderline; 2, weak; 3, moderate; 4, strong) and percentage of positive cells (1–100), as described in 16. FAK was considered high with intensity equal 3 or 4 and with percent (%) of positive cells greater than or equal to 90% and FAK was considered low (0,1 or 2 intensity and/or < 90% positive cells16,18. Positive p53 was considered as at least 2+ nuclear intensity in > 10% of cancer cells.

P53 Mutation Screening

Mutations in exons 4–8 of the *p53* gene were evaluated by SSCP analysis and DNA sequencing, as described before 17. P53 positive tumor contained any p53 mutation,

excluding silent non-clinical-relevant mutations and *p53*-negative included tumors without mutation and tumors with silent mutations, as defined in 17.

Statistical analysis

Statistical analyses were performed with SAS statistical software, Version 9.2, SAS Institute Inc., Cary, NC, USA. Fisher's exact test was used to determine the correlation between FAK expression and *p53* mutation. Chi-square test was used to determine correlation between FAK and *p53* expression. Unconditional logistic regression was implemented to estimate odds ratios for the association of high FAK expression and *p53* mutation. For OR, odds ratio and CI confidence interval calculations unadjusted or adjusted for age (11-ordinal variable value), race (African American, non African American) and stage at diagnosis (1, 2, 3+4) models were used.

Results and Discussion

In the present report we analyzed *p53* status and FAK expression in a population-based series of breast cancer tumors. We screened 596 breast tumors from Phase I of Carolina Breast Cancer Study 17 for mutation status and found *p53* mutations in 169 patients (28%) of the 596 tumors. This group we called *p53* mutation positive (Table 1). The group did not include negative silent mutation of p53, as described in 17. Another group 427 (72%) out of 596 included no *p53* mutations and silent *p53* mutation, called *p53* mutation-negative group (Table 1). Immunohistochemical analysis of FAK expression was performed as described in 16. High FAK expression was considered high with intensity 3+ or 4+ and ≥90% positive cells and not high (0,1 or 2 intensity and/or <90% positive cells 16. Statistical analysis by Fisher's exact test shows that among *p53* mutation-positive tumors FAK expression was high in 38%, while in *p53*-mutation negative cases FAK expression was high in 18% of cases and low in 82%. Thus, high FAK expression positively correlated with *p53* mutation (P<0.0001). An example of a breast tumor with hot-spot mutation and FAK expression is shown on Fig 1. We used logistic regression analysis to calculate odds ratio (OR) and found that tumors with *p53* mutation were significantly more likely to have FAK overexpression than tumors without *p53* mutation (unadjusted OR=2.8; 95% confidence interval CI, 1.8–4.2); adjusted OR = 2.5, 95% CI 1.6–3.9; (adjusted for age (11- level ordinal variable), race (African American, non African American) and stage at diagnosis (1, 2, 3+4). Thus, analysis of 596 breast cancer patients showed that *p53* mutation was correlated with high FAK expression in the breast cancers.

We also performed immunohistochemical analysis of FAK and p53 expression in 622 tumors from the Carolina Breast Cancer Study (Table 2). We found that tumors with high FAK expression were more likely to be p53-positive (unadjusted OR=2.0, 95% CI 1.4–3.0; adjusted OR = 1.8, 95% CI 1.2 – 2.8). The correlation was statistically significant (Chi square test, P=0.0002) (Table 2). P53-positive tumors showed higher intensity of FAK staining and higher percent of positive cells (Table 2). These data are consistent with data on endometrial cancers that demonstrated correlation with FAK and p53 overexpression 18. There were also supporting our data reports that showed a positive correlation between p53 mutation and p53 overexpression in breast cancers 19 20. In addition, p53 mutations and overexpression has been demonstrated to be a marker of poor patent prognosis and breast cancer aggressiveness. One of the mechanisms of positive correlation between *p53* mutation and FAK overexpression is based on the repressive function of wild type of p53 on the FAK promoter 3. Once p53 has either a DNA-binding domain mutation or a frameshift mutation, it is not able to bind the FAK promoter, so the repression of p53 is abrogated, and FAK transcription increases due to the non-function of p53.

In summary, this is the first analysis on population-based series of 596 breast cancer tumors demonstrating the high positive correlation between p53 expression and mutations with FAK overexpression and provides an additional basis for FAK-p53 signaling during tumorigenesis. Since FAK overexpression and p53 mutation are associated with worse prognosis, the combined abrogation of these pathways will be important for breast cancer therapeutics.

Abbreviations

FAK   Focal Adhesion Kinase

**ChIP**   chromatin immunoprecipitation

Acknowledgments

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Breast Tumor with mutant p53 and overexpressed FAK
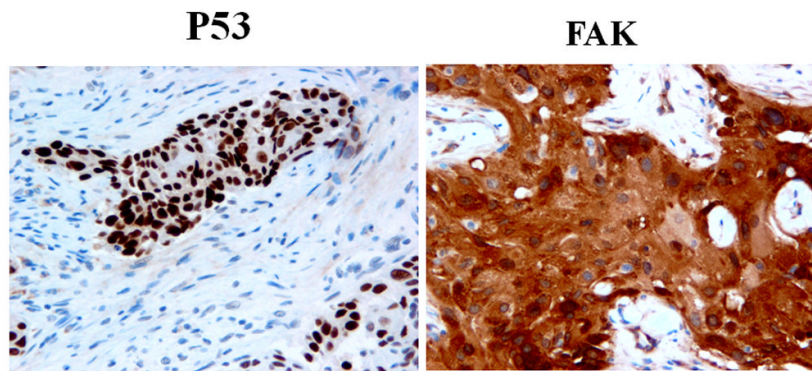
Figure 1. Immunostaining of FAK and p53 expression in breast cancer tumor with p53 mutation
Example of high FAK and p53 staining in breast cancer tumor in patients positive for p53 mutation. Immunostaining of FAK and p53 was performed on different sections of tumors as described in Materials and Methods. FAK 4.47 antibody was used at dilution 1:250; p53 antibody was used (1:500). High FAK expression and p53 are shown. P53 was overexpressed in tumor with p53 mutation.

Table 1

Correlation between FAK and p53 mutations in breast cancer tumors.

FAK expression	*p53* mutation status	
	p53 positive (p53+)	*p53* negative (p53−)
	N = 169	N = 427
	N (%)	N (%)
High FAK	65 (38%)	78 (18%)
Low FAK	104 (62%)	349 (82%)

P<0.0001

OR=2.8 (95% CI, 1.8–4.2)   unadjusted

OR=2.5 (95% CI, 1.6–3.9)   adjusted for age, race and stage

P [a]<0.0001

[a]Fisher's exact test

Table 2

Correlation between FAK and p53 expression in breast cancer tumors.

FAK expression	p53 expression	
	p53 positive (p53+)	p53 negative (p53−)
	N = 292	N = 330
	N (%)	N (%)
High FAK	91 (31%)	60 (18%)
Low FAK	201 (69%)	270 (82%)
OR = 2.0 (95% CI, 1.4–3.0) unadjusted		
OR = 1.8 (95% CI, 1.2–2.8) adjusted for age, race and stage		
P [b] =0.0002		

	FAK intensity [b]	Mean	Median
p53 expression positive (p53+)	2.5	2.0	0–4
p53 expression negative (p53−)	1.9	2.0	0–4
P[c]<0.0001			

	FAK staining		
	% positive [c]	Mean	Median
p53-positive	71.8%	80	0–99
p53-negative	62.9%	80	0–99
P[c]=0.0005			

[b] Chi-square test

[c] Student's t-test