



NIH PUBLIC ACCESS

## Author Manuscript

*Int J Cancer*. Author manuscript; available in PMC 2010 October 1.

Published in final edited form as:

*Int J Cancer*. 2009 October 1; 125(7): 1735–1738. doi:10.1002/ijc.24486.

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

Vita M. Golubovskaya<sup>\*,1,2,3</sup>, Kathleen Conway<sup>4,5</sup>, Sharon N. Edmiston<sup>4,5</sup>, Chiu-Kit Tse<sup>4,5</sup>, Amy L. Lark<sup>6</sup>, Chad A. Livasy<sup>7</sup>, Dominic Moore<sup>5,8</sup>, Robert C. Millikan<sup>4,5</sup>, and William G. Cance<sup>\*,3</sup>

<sup>1</sup>Department of Surgery, UF, Gainesville, FL, 32610, USA

<sup>2</sup>UF Shands Cancer Center, Gainesville FL, 32610, USA

<sup>3</sup>Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY, 14263 USA

<sup>4</sup>Department of Epidemiology, School of Public Health, UNC, Chapel Hill, NC, 27599 USA

<sup>5</sup>Lineberger Comprehensive Cancer Center, UNC, Chapel Hill, NC, 27599, USA

<sup>6</sup>Department of Pathology, Duke University Medical Center, Durham, NC, 27710, USA

<sup>7</sup>Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC, 27599

<sup>8</sup>Department of Statistics, University of North Carolina, Chapel Hill, NC, 27599, USA

### 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.

\*Correspondence to: William. Cance, Roswell Park Cancer Institute, Elm@Carlton Streets, Buffalo, NY, 14263; William.Cance@RoswellPark.org; FAX : 716-845-8204. Vita Golubovskaya, Roswell Park Cancer Institute, Elm@Carlton Streets, Buffalo, NY, 14263; Vita.Golubovskaya@Roswellpark.org; FAX : 716-845-3944.

**Novelty and Impact:** This is the first analysis demonstrating a high correlation between FAK expression and *p53* mutations in a population-based series of breast tumors. Since FAK overexpression highly correlate with *p53* mutation, the combined abrogation of these pathways may be important for breast cancer therapeutics.

## Keywords

p53; Focal Adhesion Kinase; breast cancer; tumor

---

## 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-fluorouracil-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) Immunohistochemistry Core facility. Total 629 breast cancer formalin-fixed, paraffin-embedded tissue cases were used for FAK immunohistochemical analysis.

### **Immunohistochemistry**

FAK immunohistochemistry 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 deparaffinization, 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 37°C. Then slides were processed the same as above. Control sections were incubated with a comparable concentration of isotype-matched IgG<sub>1</sub> 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 cells) 16–18. 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  $\geq 90\%$  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 patient 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

<b>FAK</b>	Focal Adhesion Kinase
<b>ChIP</b>	chromatin immunoprecipitation

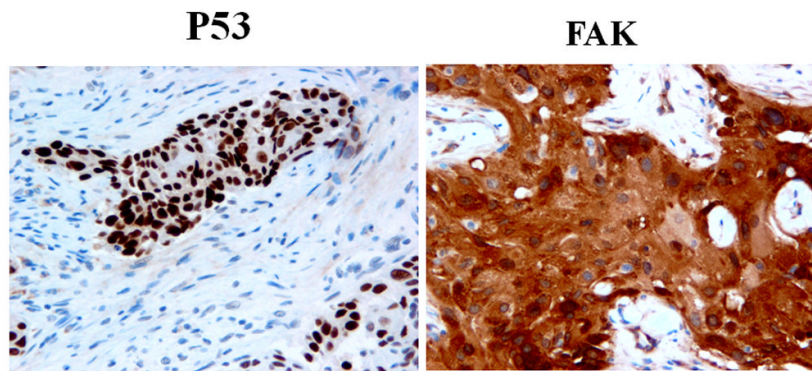
## Acknowledgments

We would like to thank Nicole Massoll for providing an image of immunohistochemical staining of breast cancer tumor. Research is supported by NIH grant CA65910 (W. G. Cance) and Susan G. Komen for the Cure grant BCTR0707148 (V.M.Golubovskaya).

## References

1. Vogelstein B, Kinzler KW. p53 function and dysfunction. *Cell*. 1992; 70:523–526. [PubMed: 1505019]
2. Giaccia AJ, Kastan MB. The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev*. 1998; 12:2973–2983. [PubMed: 9765199]
3. Golubovskaya V, Kaur A, Cance W. Cloning and characterization of the promoter region of human focal adhesion kinase gene: nuclear factor kappa B and p53 binding sites. *Biochim et Biophys Acta (BBA) - Gene Structure and Expression*. 2004; 1678:111–125.
4. Hanks SK, Ryzhova L, Shin NY, Brabek J. Focal adhesion kinase signaling activities and their implications in the control of cell survival and motility. *Front Biosci*. 2003; 8:d982–d996. [PubMed: 12700132]
5. Lightfoot HM, Lark A, Livasy CA, Moore DT, Cowan D, Dressler L, Craven RJ, Cance WG. Upregulation of focal adhesion kinase (FAK) expression in ductal carcinoma in situ (DCIS) is an early event in breast tumorigenesis. *Breast Cancer Res Treat*. 2004; 88:109–116. [PubMed: 15564794]
6. Cance WG, Harris JE, Iacocca MV, Roche E, Yang X, Chang J, Simkins S, Xu L. Immunohistochemical analyses of focal adhesion kinase expression in benign and malignant human breast and colon tissues: correlation with preinvasive and invasive phenotypes. *Clin Cancer Res*. 2000; 6:2417–2423. [PubMed: 10873094]
7. Weiner TM, Liu ET, Craven RJ, Cance WG. Expression of focal adhesion kinase gene and invasive cancer. *Lancet*. 1993; 342:1024–1025. [PubMed: 8105266]
8. Lark AL, Livasy CA, Calvo B, Caskey L, Moore DT, Yang X, Cance W. Overexpression of focal adhesion kinase in primary colorectal carcinomas and colorectal liver metastases: immunohistochemistry and real-time PCR analyses. *Clin Cancer Res*. 2003; 9:215–222. [PubMed: 12538472]
9. Ilic D, Almeida EA, Schlaepfer DD, Dazin P, Aizawa S, Damsky CH. Extracellular matrix survival signals transduced by focal adhesion kinase suppress p53-mediated apoptosis. *J Cell Biol*. 1998; 143:547–560. [PubMed: 9786962]
10. Golubovskaya VM, Finch R, Cance WG. Direct interaction of the N-terminal domain of focal adhesion kinase with the N-terminal transactivation domain of p53. *J Biol Chem*. 2005; 280:25008–25021. [PubMed: 15855171]
11. Lim ST, Chen XL, Lim Y, Hanson DA, Vo TT, Howerton K, Larocque N, Fisher SJ, Schlaepfer DD, Ilic D. Nuclear FAK promotes cell proliferation and survival through FERM-enhanced p53 degradation. *Mol Cell*. 2008; 29:9–22. [PubMed: 18206965]

12. Golubovskaya VM, Cance WG. Focal adhesion kinase and p53 signaling in cancer cells. *Int Rev Cytol.* 2007; 263:103–153. [PubMed: 17725966]
13. Cance WG, Golubovskaya VM. Focal Adhesion Kinase Versus p53: Apoptosis or Survival? *Sci Signal.* 2008; 1:pe22. [PubMed: 18493017]
14. Wei CL, Wu Q, Vega VB, Chiu KP, Ng P, Zhang T, Shahab A, Yong HC, Fu Y, Weng Z, Liu J, Zhao XD, Chew JL, Lee YL, Kuznetsov VA, Sung WK, Miller LD, Lim B, Liu ET, Yu Q, Ng HH, Ruan Y. A global map of p53 transcription-factor binding sites in the human genome. *Cell.* 2006; 124:207–219. [PubMed: 16413492]
15. Golubovskaya VM, Finch R, Kweh F, Massoll NA, Campbell-Thompson M, Wallace MR, Cance WG. p53 regulates FAK expression in human tumor cells. *Mol Carcinog.* 2008; 47:373–382. [PubMed: 17999388]
16. Lark AL, Livasy CA, Dressler L, Moore DT, Millikan RC, Geradts J, Iacocca M, Cowan D, Little D, Craven RJ, Cance W. High focal adhesion kinase expression in invasive breast carcinomas is associated with an aggressive phenotype. *Mod Pathol.* 2005; 18:1289–1294. [PubMed: 15861214]
17. Conway K, Edmiston SN, Cui L, Drouin SS, Pang J, He M, Tse CK, Geradts J, Dressler L, Liu ET, Millikan R, Newman B. Prevalence and spectrum of p53 mutations associated with smoking in breast cancer. *Cancer Res.* 2002; 62:1987–1995. [PubMed: 11929815]
18. Livasy CA, Moore D, Cance WG, Lininger RA. Focal adhesion kinase overexpression in endometrial neoplasia. *Appl Immunohistochem Mol Morphol.* 2004; 12:342–345. [PubMed: 15536334]
19. Fukushima T, Onda M, Abe R, Otake T, Kimijima I, Tsuchiya A. p53 mutations and overexpressions in Japanese breast cancer. *Eur J Surg Oncol.* 1995; 21:595–600. [PubMed: 8631401]
20. Lien HC, Lin CW, Mao TL, Kuo SH, Hsiao CH, Huang CS. p53 overexpression and mutation in metaplastic carcinoma of the breast: genetic evidence for a monoclonal origin of both the carcinomatous and the heterogeneous sarcomatous components. *J Pathol.* 2004; 204:131–139. [PubMed: 15376261]

**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&lt;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<sup>a</sup><0.0001<sup>a</sup>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 <sup>b</sup> = 0.0002		

	FAK intensity <sup>b</sup>	Mean	Median
p53 expression positive (p53+)	2.5	2.0	0–4
p53 expression negative (p53-)	1.9	2.0	0–4
P <sup>c</sup> < 0.0001			

FAK staining			
	% positive <sup>c</sup>	Mean	Median
p53-positive	71.8%	80	0–99
p53-negative	62.9%	80	0–99
P <sup>c</sup> = 0.0005			

<sup>b</sup> Chi-square test

<sup>c</sup> Student's t-test