[Cell Cycle 5:5, 478-480, 1 March 2006]; ©2006 Landes Bioscience

Extra View

Focal Adhesion Kinase Signaling and the Aggressive Melanoma Phenotype

Angela R. Hess

Mary J.C. Hendrix*

Children's Memorial Research Center; Feinberg School of Medicine; Northwestern University; Chicago, Illinois USA

*Correspondence to: Mary J.C. Hendrix; Children's Memorial Research Center; Feinberg School of Medicine; Northwestern University; 2300 Children's Plaza, Box 222; Chicago, Illinois 60614-3394 USA; Tel.: 773.755.6528; Fax: 773.755.6534; Email: mjchendrix@childrensmemorial.org

Original manuscript submitted: 01/11/06
Manuscript accepted: 01/17/06

Previously published as a Cell Cycle E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=2518

KEY WORDS

melanoma, vasculogenic mimicry, focal adhesion kinase (FAK), cell invasion, cell migration, Erk1/2, urokinase, proliferation

ABBREVIATIONS

VM	Vasculogenic mimicry
FAK	focal adhesion kinase
FRNK	FAK-related non-kinase

ACKNOWLEDGEMENTS

This work was supported by NIH Grants CA59702, CA80318 and CA88046-02S.

ABSTRACT

Focal adhesion kinase (FAK) mediates myriad cellular functions and has been found to be overexpressed in numerous human cancers. We recently explored the role of FAK in promoting the aggressive phenotype of melanoma cells, characterized by increased invasion, migration, and vasculogenic mimicry (VM) potential. We found FAK to be phosphorylated on its key tyrosine residues (397 and 576) in aggressive melanoma cells cultured on a three-dimensional type 1 collagen matrix in vitro, as well as in radial and vertical growth phase melanomas in situ. Furthermore, expressing FAK-related non-kinase (FRNK) in melanoma cells directly resulted in the inhibition of the aggressive phenotype, as demonstrated by decreased invasion, migration and VM potential, in part by blocking an Erk1/2 mediated signaling pathway. Additional data indicated that increased FAK activity may promote cellular proliferation and anchorage independent growth of aggressive melanoma. Together these observations implicate FAK as a promoter of the aggressive melanoma phenotype, thereby identifying a rational target for therapeutic intervention of malignant melanoma.

Malignant melanoma is becoming a major health threat as the incidence continues to rise. Although cutaneous melanoma is more common, uveal melanoma remains the primary intraocular malignancy.[1] For both cutaneous and uveal melanoma, the major health threat is death from metastasis which involves invasion and migration of tumor cells from the primary tumor to distant sites within the body. Identifying prognostic indicators useful for predicting an aggressive melanoma phenotype and the likelihood of metastasis would offer new avenues for therapeutic intervention for this devastating disease.

As an attempt to understand the signal transduction events which promote melanoma metastasis, our laboratory recently identified focal adhesion kinase (FAK) as a key mediator of the aggressive melanoma phenotype.[2] We screened numerous human melanoma cell lines with varying degrees of aggressiveness, as characterized by increased invasion, migration, and vasculogenic mimicry (VM) potential, for FAK protein phosphorylated on tyrosines 397 and 576. Our results demonstrated that only the most aggressive melanoma cell lines contained phosphorylated FAK whereas the poorly aggressive melanoma cell lines did not. These data suggest a correlation exists between FAK signaling and increased aggressive behavior. In order to provide evidence that FAK signaling may translate to an increased risk for metastatic disease in patients, we screened 14 different patient tumors containing either radial or vertical growth phase melanomas and found a significant amount of phosphorylated FAK on both tyrosines 397 and 576 in situ. In order to test the role of FAK signaling in promoting the aggressive melanoma phenotype we expressed the FAK-related non-kinase (FRNK), which acts to disrupt FAK signaling, in an aggressive human cutaneous melanoma cell line. Expression of FRNK resulted in a significant decrease in the invasive, migratory, and VM potential of these cells. Furthermore, we found a decrease in the level of Erk1/2 phosphorylation which was concomitant with a decrease in urokinase, MMP-2, and MT1-MMP activities. Collectively, these data indicated an important role for FAK signaling in promoting the aggressive melanoma phenotype.

One significant problem with treatment strategies for melanoma is its resistance to anti-tumor agents.[3] This is often thought to be due to the ability of aggressive melanoma cells to resist apoptosis, which may in part be due to aberrantly regulated signal transduction pathways, including those mediated by Ras and NF-κB.[4,5] Recently, proteosome inhibitors have gained momentum as an effective cancer therapy because they selectively trigger apoptosis in tumor cells but not in normal cells. For example, reports testing the effects of proteosome inhibitors for the induction of apoptosis in melanoma have been promising.[4,6] These studies have shown that apoptosis can be induced in melanoma cell lines after

treatment with the Federal Food and Drug Administration-approved bortezomib. Furthermore, bortezomib has been found to induce apoptosis of melanoma cells in vivo, perhaps through an NF-κB mediated pathway.[4]

In addition to regulating such cellular functions as invasion and migration, FAK has been found to play a role in mediating cellular proliferation and survival. Constitutive activation of FAK has been linked to increased tumor growth, suggesting that signaling through FAK may be an additional mediator of resistance of tumor cells to apoptosis. In support of this hypothesis, a recent report demonstrated that treatment of aggressive human cutaneous melanoma cell lines, C8161 and BL, with anti-sense oligonucleotides to FAK sensitized them to 5-fluorouracil induced cell death.[7] One possible mechanism by which FAK may promote cellular survival and proliferation is through its ability to activate the MAPK pathways through the activation of Ras.[8] The signal transduction events responsible for the FAK mediated activation of Ras involve the phosphorylation of the adaptor protein $p130^{Cas}$. The C-terminal portion of FAK contains a binding site for $p130^{Cas}$, and upon binding, $p130^{Cas}$ is phosphorylated on tyrosine resulting in the recruitment of Crk, Nck, and SOS leading to the activation of Ras. It is important to note that although FRNK lacks a kinase domain, it does contain the necessary binding sites for $p130^{Cas}$ and may function to sequester this protein from endogenous FAK, thus attenuating downstream activation of the Ras pathway.[9]

Our recent study indicated that expression of FRNK in aggressive melanoma cells decreased the phosphorylation of Erk1/2, demonstrating that FAK can signal through the Ras-MAPK pathway, raising the possiblity that expression of FRNK may affect cellular proliferation of the aggressive melanoma cells. Following this rationale, we tested the effects of FRNK expression on the proliferation rates of the C8161 aggressive melanoma cells and found a significant reduction in the ability of these malignant tumor cells to proliferate (Fig. 1A). These results further support the hypothesis that FAK signaling plays an important role in mediating the proliferation of aggressive melanoma cells, and may be dependent on the Ras/MAPK pathway.

A second method by which FAK signaling promotes cellular survival is by protecting cells from a special form of apoptosis termed "anoikis" which refers to nonadherent cell death.[10] In normal cells, FAK becomes phosphorylated upon focal adhesion formation mediated by integrins and growth factor receptor activation and is commonly believed to help coordinate the signal transduction events activated downstream of both integrins and growth factor receptors, thus promoting cell survival. In nonadherent cells, FAK remains unphosphorylated and survival signals are not propagated thus resulting in anoikis. The role of FAK in rescuing cells from anoikis has been described in both endothelial and epithelial cell types. In these cells, constitutively active FAK was able to promote growth under nonadherent conditions, which also resulted in their transformation.[11] Overcoming anoikis is a key step in the progression to metastasis of a primary tumor. Resistance to anoikis enables tumor cells to survive in the systemic circulation until they find a new site within the body to colonize. Furthermore, dysregulation of anoikis in tumor cells may be caused by an increase in the expression and/or activation of FAK. To simulate nonadherent conditions in our study, we tested the anchorage independent growth properties of the FRNK transfected C8161 aggressive melanoma cells using a soft agar assay. We found a significant decrease in the ability of the C8161 cells expressing FRNK to form colonies in soft agar (Fig. 1B), further demonstrating the importance of the signal transduction pathways mediated by FAK.

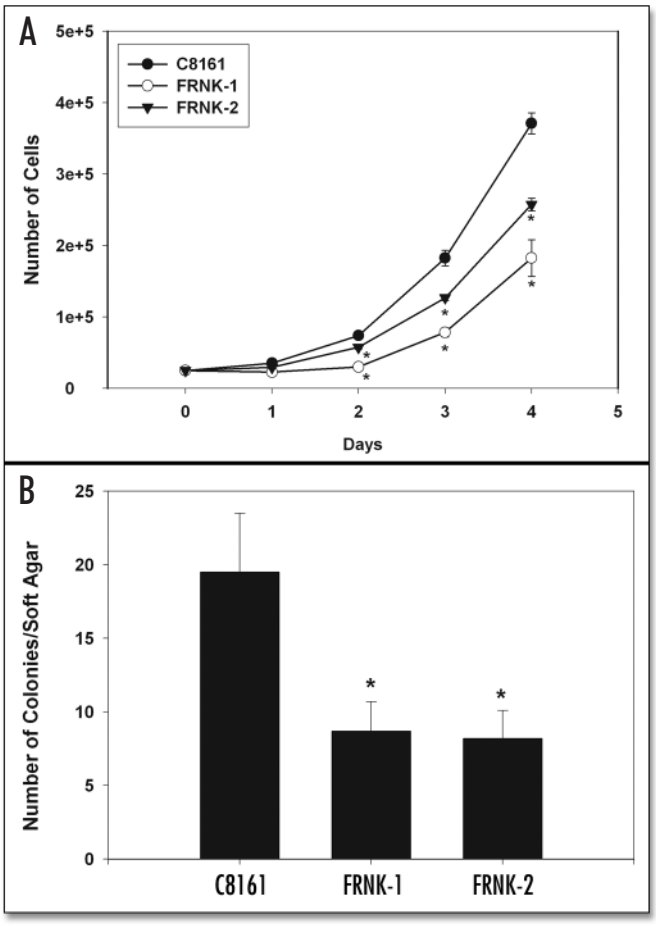

Figure 1. (A) Effect of FRNK overexpression on melanoma cell proliferation. C8161 cells were stably transfected to express the FRNK protein, as previously described.[2] To assess proliferation, 2.5×10^4 C8161, C8161-FRNK-1 (FRNK-1), or C8161-FRNK-2 (FRNK-2) cells were plated into 24-well tissue culture grade dishes in RPMI containing 10% fetal bovine serum. Cells were harvested with trypsin/EDTA and counted every 24 hours for a period of four days. Statistical significance was determined using the student's t-test. ($p < 0.01$; for each time point C8161 n = 6, FRNK-1 n = 6, and FRNK-2 n = 6). (B) Soft agar colony forming assays as a means to test for anchorage independent growth of C8161 cells overexpressing FRNK. To prepare soft agar assays, RPMI containing 20% FBS was mixed in a 1:1 ratio with 1% melted agar, and 1.5 ml were added to each well of a 6-well tissue culture grade dish. Once solidified, 5,000 cells/well were mixed with RPMI containing 20% FBS in a 1:1 ratio with 0.7% melted agarose and 1.5 ml added to each well. Cultures were incubated in a humidified 37°C, 5% CO_2 incubator for 16 days. Subsequently, cultures were stained overnight with 0.005% Crystal Violet/PBS solution, and all visible colonies were counted. Statistical significance was determined using the Student's t-test. ($p < 0.05$; C8161 n = 6, FRNK-1 n = 6, FRNK-2 n = 18).

It has become clear that FAK signaling regulates a multitude of cellular events that collectively promote an aggressive melanoma phenotype. Although we are just beginning to appreciate the complexity of the FAK mediated promotion of the metastatic phenotype in melanoma, there is still much work that needs to be done. Ongoing studies will address the role of FAK signaling in promoting metastasis in an in vivo animal model, and will determine if disruption of FAK signaling in established tumors results in tumor regression. As we continue to acquire a better understanding of the importance of FAK mediated signal transduction events in promoting the aggressive melanoma phenotype, we hope to indentify new avenues for therapeutic intervention.

Reference

1. Cancer Facts and Figures 2005. Altanta, GA: American Cancer Society, 2005, [(Available from http://www.cancer.org) (American Cancer Society, Inc)].
2. Hess AR, Postovit LM, Margaryan NV, Seftor EA, Schneider GB, Seftor RE, Nickoloff BJ, Hendrix MJ. Focal adhesion kinase promotes the aggressive melanoma phenotype. Cancer Res 2005; 65:9851-60.
3. Grossman D, Altieri DC. Drug resistance in melanoma: Mechanisms, apoptosis, and new potential therapeutic targets. Cancer Metastasis Rev 2001; 20:3-11.
4. Amiri KI, Horton LW, LaFleur BJ, Sosman JA, Richmond A. Augmenting chemosensitivity of malignant melanoma tumors via proteasome inhibition: Implication for bortezomib (*VELCADE, PS-341*) as a therapeutic agent for malignant melanoma. Cancer Res 2004; 64:4912-8.
5. Jansen B, Schlagbauer-Wadl H, Eichler HG, Wolff K, van Elsas A, Schrier PI, Pehamberger H. Activated N-ras contributes to the chemoresistance of human melanoma in severe combined immunodeficiency (*SCID*) mice by blocking apoptosis. Cancer Res 1997; 57:362-5.
6. Qin JZ, Ziffra J, Stennett L, Bodner B, Bonish BK, Chaturvedi V, Bennett F, Pollock PM, Trent JM, Hendrix MJ, Rizzo P, Miele L, Nickoloff BJ. Proteasome inhibitors trigger NOXA-mediated apoptosis in melanoma and myeloma cells. Cancer Res 2005; 65:6282-93.
7. Smith CS, Golubovskaya VM, Peck E, Xu LH, Monia BP, Yang X, Cance WG. Effect of focal adhesion kinase (FAK) downregulation with FAK antisense oligonucleotides and 5-fluorouracil on the viability of melanoma cell lines. Melanoma Res 2005; 15:357-62.
8. Schaller MD. Biochemical signals and biological responses elicited by the focal adhesion kinase. Biochim Biophys Acta 2001; 1540:1-21.
9. Almeida EA, Ilic D, Han Q, Hauck CR, Jin F, Kawakatsu H, Schlaepfer DD, Damsky CH. Matrix survival signaling: From fibronectin via focal adhesion kinase to c-Jun NH(2)-terminal kinase. J Cell Biol 2000; 149:741-54.
10. Frisch SM, Screaton RA. Anoikis mechanisms. Curr Opin Cell Biol 2001; 13:555-62.
11. Frisch SM, Vuori K, Ruoslahti E, Chan-Hui PY. Control of adhesion-dependent cell survival by focal adhesion kinase. J Cell Biol 1996; 134:793-9.