Activation of Rap1 Promotes Prostate Cancer Metastasis

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Abstract

Elucidating the mechanisms of prostate cancer (CaP) survival and metastasis are critical to the discovery of novel therapeutic targets. The monomeric G protein Rap1 has been implicated in cancer tumorigenesis. Rap1 signals to pathways involved in cell adhesion, migration, and survival, suggesting Rap1 may promote several processes associated with cancer cell metastasis. Examination of CaP cell lines revealed cells with a high metastatic ability exhibited increased Rap1 activity and reduced expression of the negative regulator Rap1GAP. Rap1 can be further stimulated in these cells by stromal-derived factor (SDF-1), an agonist known to regulate tumor cell metastasis and tropism to bone. Activation of Rap1 increased CaP cell migration and invasion, and inhibition of Rap1A activity via RNAi-mediated knockdown or ectopic expression of Rap1GAP markedly impaired CaP cell migration and invasion. Additional studies implicate integrins α4, β3, and αvβ3 in the mechanism of Rap1-mediated CaP migration and invasion. Extending the effect of Rap1 activity in CaP metastasis in vitro, introduction of activated Rap1 into CaP cells dramatically enhanced the rate and incidence of CaP metastasis in a xenograft mouse model. These studies provide compelling evidence to support a role for aberrant Rap1 activation in CaP progression, and suggest that targeting Rap1 signaling could provide a means to control metastatic progression of this cancer.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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© 2009 American Association for Cancer Research. doi:10.1158/0008-5472.CAN-08-4269

Introduction

In the United States, the second leading cause of cancer deaths in men is carcinoma of the prostate (CaP). Most of these deaths are caused by metastatic spread of the CaP to the bone. CaP metastasis is a complex series of events, which include migration of CaP cells from their primary tumor, survival in circulation, attachment to the metastatic site, and proliferation as micrometastases (1). During several steps in metastasis, cancer cell affinity and avidity for extracellular matrix (ECM) components is altered. Most of these changes are mediated by enhancing the expression or activation of integrins, a family of heterodimeric receptors for cell adhesion molecules crucial for cell behavior and motility (2). In prostate cancer, there are dramatic differences between the distribution and surface expression of integrins in preneoplastic and malignant tumor cells (3). Therefore, an attractive approach to CaP therapy is to antagonize integrin function (4).

Integrin affinity and avidity is regulated by two different signaling methods (a) outside-in signaling from integrin interaction with the extracellular environment, and (b) inside-out signaling from intracellular pathways involving monomeric G proteins such as Rap1 (2). Rap1 is a member of the Ras family of monomeric G proteins that has received recognition for its role in tumorigenesis and cancer progression (5). Studies originating in leukocytes first showed that Rap1 can enhance cell adhesion and migration and activate survival pathways (6, 7). GTPases such as Rap1 are molecular switches regulated positively by guanine nucleotide exchange factors and negatively by GTPase-activating proteins (GAP). Recent in vivo and in vitro data provide evidence that aberrant Rap1 activation contributes to several malignancies (8). In cancer phenotypes attributed to increased Rap1 activity, one common thread seems to be a decrease in expression of a RapGAP by deletion or mutation of the Rap1GAP or Sipa1 gene (9–12). There is a consensus from studies of several cancer types that aberrant Rap1 activation leads to increased cancer cell proliferation and tumorigenesis (9–11). The consequence of Rap1 activation on invasion, however, seems to have a very cell and/or environment-specific context. In osteosarcoma cells, inactivation of DOCK4, a Rap1 activator, allows the cells to become more invasive (13). Additionally, Rap1GAP expression has been correlated with increased in vitro invasion of squamous cell carcinoma (14). On the other hand, Rap1GAP overexpression led to a reduction in pancreatic cancer incidence and local invasion from the primary tumor site (9). Thus, it remains uncertain when and in which cancers Rap1 signaling positively or negatively regulates invasion and metastasis.

The differential roles of Rap1 require that its effects be addressed in different malignancies, and thus far, the importance of Rap1 in CaP metastasis had not been examined. In this study, we found CaP cell lines with high metastatic potential had increased Rap1 activity levels and decreased Rap1GAP expression. Activation of Rap1 in CaP cells enhanced their migration and invasion via a process that involves αvβ3, β3, and α4 integrins. Additionally, activated Rap1 dramatically enhanced the rate and incidence of CaP metastasis in vitro. These findings show a role for Rap1 in CaP metastasis and suggest a novel, targeted therapeutic strategy for prostate cancer.

Materials and Methods

Prostate cancer cell lines, antibodies, plasmids, and reagents. The PC3-M, PC3, DU-145, LNCap, and LNCap-c81 cell lines were obtained from the Duke University Medical Center Cell Culture Facility or American Type Culture Collection. The VCaP cells were from Donald McDonnell (Duke University). The PC3, DU-145, and LNCap lines were maintained as described (15). The VCaP line was maintained in DMEM with 10% fetal bovine serum (FBS), nonessential amino acids, and 1 mmol/L sodium pyruvate. Antibodies to Rap1 were obtained from Santa Cruz Biotechnology, to Rap1GAP from EMD/Merck KGaA, and to actin from Sigma-Aldrich. Ll-COR Odyssey-compatible secondary antibodies were from Rockland and Molecular Probes. Antibodies to integrins α4, β3, and αvβ3 were from Cell Signaling, and that to integrin α2β1 was from Abcam, Inc. Rap1-63E and Rap1GAP constructs were gifts of Lawrence Quilliam (University of Indiana). Growth factor–reduced Matrigel was from BD Biosciences, SDF-1α was from LI-COR Odyssey-compatible secondary antibodies were from Rockland.
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R&D Systems, and D-Luciferin was from Xenogen. The GST-RalGDS-RBD protein was produced in the BL21DE3 strain of Escherichia coli (Novagen/Merck KGaA) as described previously (15). Recombinant retroviruses were constructed by subcloning the Rapi-63E and Rap1GAP plasmids into the pMscv-IRES-GFP vector (Clontech) and then cloning Luciferase into the pLPCX vector (Clontech), as described previously (15).

Immunoblot analysis. Cells, grown to ∼80% to 90% confluency, were washed once with cold PBS, and lysed with cold Lysis Buffer (described in the Rap1 Activity Assay). Lysates were cleared by centrifugation, supernatants assayed for protein concentration, and then equal amounts of total protein from lysates separated by SDS-PAGE and subjected to immunoblot analysis using the Odyssey System (LICOR) according to the manufacturer's instructions.

Rap1 activity assays. The levels of activated Rap1 were determined using pull-down assays with a glutathione S-transferase (GST) fusion of the Ras-binding domain of RalGDS as previously described (16). Briefly, cells that had been starved for 18 h were stimulated by addition of media containing 10% FBS or SDF-1, harvested, lysed, and GST-RalGDS-RBD beads used to precipitate activated Rap1. Pelleted material was resuspended in SDS-PAGE sample buffer, proteins separated by gel electrophoresis, and subjected to immunoblot analysis to detect Rap1.

RNA interference studies. The siRNA oligonucleotides were synthesized by Integrated DNA Technologies, Inc., based on a published Ambion sequences for both targeted and control oligonucleotides (10). siRNAs were introduced into PC3 cells via transient transfection with Oligofectamine reagent (Invitrogen) for 72 h. Immunoblot analysis of total cell lysates was used to confirm the efficacy of knockdown of Rap1 expression in siRNA-treated cells.

Cell migration and invasion assays. Cell migration assays were performed as described previously (15, 17). Briefly, cells were starved for 12 to 18 h, detached with Cellstripper (Mediatech), and 3 × 10^4 cells in 100 μL were placed into the upper chamber of the transwell. For experiments using SDF-1, the ligand (200 ng/mL) was added to the cells after harvesting. For migration studies the transwell was transferred into a well containing 600 μL of conditioned medium from MG-63 osteosarcoma cells (18). Cells were permitted to migrate for 4 h at 37°C and the cells on the underside of the membrane were counted. Four representative high-powered fields were selected for each membrane. Invasion assays were performed similarly to the migration assays with a few modifications. Cells were added to transwells previously coated with 50 μg of growth factor–reduced Matrigel (BD Biosciences) for 4 h, after which the transwell was transferred into a well containing 600 μL of growth medium containing 10% FBS. Cells were incubated for 24 h at 37°C, after which the transwell membranes were stained and analyzed as described above.

In vivo metastasis. All animal handling and procedures were conducted in accordance with an approved Institutional Animal Care and Use Committee protocol. PC3 cells expressing firefly luciferase (PC3-Luc) were generated by retroviral transduction. Under anesthesia, 4- to 6-wk-old male athymic NCr nu/nu mice (National Cancer Institute-Frederick) were injected with 1 × 10^5 PC3-Luc cells into their left cardiac ventricle using a published procedure (19). Bioluminescence imaging was performed on the mice at 4 to 7 d intervals as described previously (17) using a charge-coupled device camera (IVIS; Xenogen Corp) with a 15-cm field of view. Data were analyzed using IVIS Living Image software (Xenogen Corp).

Statistical analysis. Data were analyzed using GraphPad Prism v5 (GraphPad Software, Inc.). Data are given as mean ± SE and compared by one- or two-way ANOVA as appropriate, followed by the relevant post t test to determine P values. A P value of <0.05 was considered significant.

Results

Rap1 activity is increased in progressively metastatic human prostate cancer cell lines. Reports of anomalous Rap1 signaling in some types of cancers (5) prompted our assessment of Rap1 activation status in prostate cancer. Because analyzing Rap1 activation status requires the use of fresh samples, it was necessary use prostate cancer cells lines. We chose a spectrum of prostate cancer cell lines of varying invasiveness and metastatic potential (20). Two major criteria were used; first, the expression status of the androgen receptor because expression of this receptor is lost in most human metastatic CaP, and second, the capacity for aggressive tumor formation in mouse xenografts if such data existed. The well-characterized RalGDS pulldown assay was used to directly examine levels of activated (GTP-ligated) Rap1, and levels of Rap1GAP, the negative regulator of Rap1, assessed by immunoblot analysis. These analyses showed the general trend that the more invasive, androgen-independent cell lines DU-145, PC3, and PC3-M, had higher levels of Rap1-GTP and lower levels of Rap1GAP when compared with the less metastatic, androgen-sensitive, cell lines VCaP, LNCaP, and LNCaP-c81 cells (Fig. 1). Furthermore, this trend of decreased Rap1GAP expression and increased Rap1-GTP levels was observed in direct comparison of the LNCap and PC3 cell lines with their more invasive derivative lines, LNCap-c81 and PC3-M, respectively (Fig. 1, lanes 2–3 and 5–6). The PC3 cell line was chosen for the subsequent studies, as these cells have been widely used in similar in vitro and in vivo assays. Furthermore, it was hoped their moderate metastatic potential would allow assessment of both increasing and decreasing Rap1 activity on cellular functions related to metastasis.

Modulation of Rap1 activity affects CaP cell migration and invasion. The finding that increased Rap1 activity correlated with more metastatic CaP cells prompted an evaluation of the effect of Rap1 activation on CaP cell metastatic behavior. Given that the classic metastatic site of prostate cancer is bone, we used conditioned media from bone-derived osteosarcoma cells (MG63) as the source of chemotactant in the migration assays (18). We confirmed that this conditioned media contains ECM proteins, specifically vitronectin (integrin αvβ3), and fibronectin (integrin α4β1 ligand; data not shown). Additionally, invasion assays through Matrigel were performed to model a metastatic microenvironment.

The first study to assess the requirement for Rap1 function in PC3 cell migration was a gain-of-function approach using overexpression of Rap1-63E, a mutationally activated form of Rap1. Comparison with control cells showed that the PC3 cells expressing
Rap1-63E indeed had high levels of activated Rap1 (Fig. 24). Analysis of the migration and invasion properties of the cells revealed that expression of Rap1-63E increased these properties by 2.3 ± 0.52-fold and 2.2 ± 0.08-fold, respectively (Fig. 2B).

Having determined that gain-of-function of Rap1 enhanced CaP cell migration and invasiveness, we asked whether loss of function through expression of the negative regulator Rap1GAP could impair these properties of the PC3 cells. Comparison with vector control cells showed that Rap1GAP-expressing cells indeed had reduced levels of Rap1-GTP (Fig. 2A), and cell migration and invasion were reduced by 47% and 71%, respectively, compared with vector control cells (Fig. 2B). A second loss-of-function study using RNAi-mediated knockdown of Rap1 expression was also used. Similar to Rap1GAP-expressing cells, knockdown of Rap1A expression (Fig. 2C) significantly reduced both migration and invasion of PC3 cells 53% and 67%, respectively, compared with control cells (Fig. 2D). In an attempt to further elucidate which Rap isoform was most important for PC3 cell migration and invasion, as differential effects of Rap isoforms have been described (21, 22), we separately targeted Rap1B and Rap2 using specific RNAi constructs. These results were variable but, in all cases targeting Rap1A expression, was most effective in affecting these cellular processes (data not shown). Together with the finding that expression of Rap1A-63E was sufficient to significantly enhance CaP migration and invasion (Fig. 2B), these data suggest that Rap1A is the primary subtype responsible for these effects. Taken together, these results provide compelling evidence that Rap1A function plays an important role in CaP cell migration and invasion, and that activation of Rap1 enhances CaP cell migration and invasion.

SDF-1 stimulation of CaP cell migration and invasion requires Rap1 function. Having determined that Rap1 was an important player in PC3 cell migration and invasion, we were keen to explore its potential role in an endogenous signaling process known to be involved in cancer metastasis. To this end, we stimulated Rap1 signaling by treatment of PC3 cells with SDF-1. Often present in the bone microenvironment, SDF-1 is a chemokine that has been shown to be up-regulated in and associated with CaP progression (23). Indeed, treatment of PC3 cells with SDF-1 markedly increased the level of activated Rap1 (Fig. 3A). Furthermore, this effect was completely blocked in the PC3 cells overexpressing Rap1GAP (Fig. 3B). Treatment of PC3 cells with SDF-1 stimulated cell migration (Fig. 3B) and invasion (Fig. 3C) by 1.4- and 1.7-fold, respectively, and this stimulation was eliminated in PC3-Rap1GAP cells. Additionally, siRNA-mediated knockdown of Rap1A in both SDF-1 treated and untreated PC3 cells resulted in a ~50% reduction in invasion compared with control cells (Fig. 3D). These results show that SDF-1 stimulates Rap1 activation in CaP cells, and that Rap1 function is required for SDF-1 stimulated CaP cell migration and invasion.

Rap1 promotes CaP migration and invasion through integrins. In lymphocytes and platelets, the downstream signaling event with which Rap1 has been most associated is integrin activation (24). Likewise, activation of several integrins, particularly αvβ3, is thought to be an important component of CaP metastatic progression (25, 26). As an initial screen to determine if the effect of Rap1 activation on PC3 cell migration and invasion involved integrins, we examined whether Rap1 signaling influenced the repertoire of integrins that PC3 cells express. Real-time PCR analysis

Figure 2. Modulation of Rap1 activity affects CaP cell migration and invasion. A and B, PC3 cells were transduced with retrovirus to establish stable expression of the indicated constructs, vector, Rap1-63E, or Rap1GAP. A, PC3 cells stably expressing the indicated constructs were lysed and levels of Rap1-GTP, total Rap1 and Rap1GAP determined by immunoblot analysis (as described in legend to Fig. 1). B, migration (left) and invasion (right) of PC3 cells stably expressing the indicated constructs. For the migration assays, cells migrated toward a lower chamber containing conditioned media from osteosarcoma cells and were assessed after 4 h. For the invasion assays, cells invaded through a Matrigel layer toward a lower chamber containing 10% serum and were assessed after 24 h. C and D, siRNAs targeting Rap1A or control siRNA, were introduced into PC3 cells and parameters assessed 72 h later. C, levels of Rap1 and actin (loading control) were determined by immunoblot analysis. D, migration (left) and invasion (right) assays of siRNA-treated PC3 cells. The data represent results pooled from three (B) or two (D) independent experiments with at least two replicates each, and are plotted as the ratio of the number of migrated or invaded cells versus that of control cells (B, vector; D, scrambled siRNA) that were determined in parallel. **, P < 0.001; ***, P < 0.005.
of the expression levels of a broad array of integrins in PC3 cells expressing the dominant-active Rap1-63E revealed that these cells exhibited 2.1- and 2.4-fold up-regulated expression of integrins α4 and β3 when compared with vector control cells (Supplementary Table S1). Function-blocking antibodies were then used to inhibit integrins α4 and β3, as well as αvβ3 since this integrin pair has been extensively linked to cancer progression.

In control cells expressing vector alone, neither migration (Fig. 4A) nor invasion (Fig. 4C) was significantly decreased by treatment with 20 μg/mL of integrin blocking antibodies. Importantly, Rap1-63E-mediated migration (Fig. 4B) and invasion (Fig. 4D) of PC3 cells was markedly (40–60%) abrogated by treatment with the same concentration of antibodies targeting integrins α4, β3, and αvβ3. No effect was observed by addition of isotype-matched control IgG (data not shown) or blocking antibody to integrin α2β1 (Fig. 4), an integrin that is not activated by Rap1. These data indicate that the migratory and invasive properties of these three integrins are stimulated by activated Rap1A. These integrins are likely involved in the migration and invasion of the parental cells as well, as both processes were affected at higher concentrations of blocking antibody (data not shown). Additionally, because invasion through Matrigel requires breakdown of the matrix, we also examined a potential role of matrix metalloproteinases (MMP) in Rap1-mediated invasion of these CaP cells. Treatment with a broad-spectrum MMP inhibitor (GM6001 compound) revealed that Rap1-mediated invasion was much more sensitive to blockade of MMP activity than control cells (Supplementary Fig. S1). To further confirm this data, blocking antibodies targeting αvβ3 were used to inhibit invasion through Matrigel. The results showed that αvβ3 antibody abrogated the invasion of PC3 cells expressing vector alone (data not shown). These data indicate that the migratory and invasive properties of these three integrins are stimulated by activated Rap1A. These integrins are likely involved in the migration and invasion of the parental cells as well, as both processes were affected at higher concentrations of blocking antibody (data not shown). Additionally, because invasion through Matrigel requires breakdown of the matrix, we also examined a potential role of matrix metalloproteinases (MMP) in Rap1-mediated invasion of these CaP cells. Treatment with a broad-spectrum MMP inhibitor (GM6001 compound) revealed that Rap1-mediated invasion was much more sensitive to blockade of MMP activity than control cells (Supplementary Fig. S1).

Activation of Rap1 promotes CaP cell metastasis in vivo. The in vitro data supported a role for Rap1 in metastasis and these cell assays were valuable tools to examine the consequence of activated Rap1 in CaP cell migration and invasion. However, it was important to show whether activation of Rap1 could effect CaP metastasis in vivo. To this end, we chose mice to model the later steps in CaP metastasis and to examine bone metastasis (27). Hence, we elected to perform intracardiac injections based on the ability of this technique to permit metastasis to the bone, rather than injections into the tail vein, which usually result in lung metastasis (19, 27). We generated PC3 cells stably expressing luciferase (PC3-Luc) to allow analysis of cancer progression in live animals using bioluminescent imaging technology. The PC3-Luc cells were then engineered to stably express either vector control or Rap1-63E, injected into athymic mice, and their metastatic spread followed. The mice were imaged immediately to verify the cells had been correctly injected into the left cardiac ventricle, instead of in the right ventricle, which pumps blood directly into the lungs. Bioluminescent signal detected in the entire mouse indicated an accurate injection, whereas signal detected only the lungs indicated an incorrect injection; such mice were not included in further analysis.

After intracardiac injection of the engineered PC3 cells, mice were imaged every 4 to 7 days for 30 days or until humane end points had been reached. Bioluminescent imaging showed a significantly increased formation of metastatic lesions, mostly in the bone, in the mice that received Rap1-63E cells compared with the mice injected with vector control PC3-Luc cells (Fig. 5A). As later confirmed by necropsy, these metastatic lesions were mostly observed in the mandible, tibia, femur, and thoracic cavity (data not shown). A summary of the data obtained from this study is...
shown in Fig. 5B to D. Metastasis-free survival (Fig. 5B) was dramatically reduced in the mice injected with Rap1-63E–expressing PC3-Luc cells. All of these mice had detectable metastases within 15 days of injection, whereas only 2 of 5 of the vector control mice had detectable metastasis even after 30 days. Furthermore, all six mice bearing the Rap1-63E cells developed metastatic lesions in multiple (3–10) regions, whereas the two of five vector control cell–bearing mice that exhibited metastatic lesions had only 1 and 3 visible metastases (Fig. 5C). Lastly, assessment of the individual number of metastatic lesions in each mouse at the termination of the study (Fig. 5D) again showed a marked difference in lesion formation between the two groups. Taken together, this in vivo data revealed a dramatic increase in the rate and incidence of metastatic lesion formation of PC3 cells expressing activated Rap1. Combined with the in vitro and in vivo data, this study provides a compelling demonstration of the potent effect of Rap1 activation on CaP migration, invasion, and metastasis.

**Discussion**

Elucidating the precise contributions of Rap1 signaling to any particular cancer is complicated by cancer cell– and/or environment-specific differences that may alter the consequences of Rap1 activation. In the current study, we show that activation of Rap1 promotes CaP metastasis. Our findings revealed that CaP cells with moderate to high metastatic potential have increased Rap1 activity and reduced expression of the negative Rap1 regulator, Rap1GAP (Fig. 1). These findings in CaP are similar to those reported in several cancers where a reduction or loss of Rap1GAP expression correlated with enhanced tumorigenesis or cancer progression (9, 28, 29).

In dissecting the role for Rap1 in CaP metastasis in vitro, activation of Rap1 was shown to enhance CaP cell migration and invasion, and furthermore, these processes were abrogated by Rap1 inhibition (Figs. 2 and 3). These findings corroborate with others that showed inhibition of Rap1 reduced local invasion of pancreatic cancer (9), and inhibited thyroid cancer cell migration and invasion (29). On the other hand, reduction in Rap1 activation reduced invasion of osteosarcoma cells, a process ascribed to less adherens junction formation (13). Also differing from our results, inhibition of Rap1 has been correlated with increased in vitro invasion of squamous cell carcinoma (14). These opposing findings highlight the need to assess the effect of Rap1 activation in individual cancer types, as clearly some aspect of the cells or their environment is important in dictating the response of the cell to Rap1 activation.

Recently, a "tumor microenvironment invasion model" proposed that sequential stable genetic changes during metastasis may give
rise to a tumor microenvironment that induces transient gene expression patterns that contribute to transient cell migration and invasion (30). These microenvironment alterations could generate the transient expression of different integrins and adhesion molecules (some Rap1 regulated) to affect the invasive and metastatic ability of the tumor cells. For example, alterations in CaP cell expression of laminin integrin receptors (α6/β4, α6/β1) was attributed to CaP escape from the laminin-rich primary site (31). The effect of Rap1 activation likely depends on the particular stage in metastatic progression of that cancer because different integrins and ECM are involved and expressed (3, 30). Our results implicate Rap1 in the later stages of CaP metastasis, particularly by demonstrating its involvement with αvβ3 integrin, an integrin important for CaP cell navigation of the circulatory system and metastasis to the bone (31). This conclusion is supported by studies in other cell types showing that Rap1 signaling affects activation of integrins (10, 24, 32). Our results provide the first direct evidence that activation of Rap1 affects integrins involved in CaP migration, invasion, and metastasis to the bone.

In CaP metastasis to bone, a critical regulator is SDF-1 (23), a chemokine recognized for its role in metastatic progression and bone tropism (33, 34). The current study shows that SDF-1 stimulates Rap1 in CaP cells, and enhances CaP migration and invasion via a process that depends on Rap1 function (Fig. 3). Although we do not suggest that all SDF-1/CXCR4 mediated events go through Rap1, or that Rap1-independent SDF-1 stimulation of Rap1 in the later stages of CaP metastasis, particularly by demonstrating its involvement with αvβ3 integrin, an integrin important for CaP cell navigation of the circulatory system and metastasis to the bone (31). This conclusion is supported by studies in other cell types showing that Rap1 signaling affects activation of integrins (10, 24, 32). Our results provide the first direct evidence that activation of Rap1 affects integrins involved in CaP migration, invasion, and metastasis to the bone.

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In addition to in vitro findings, the current study also showed that Rap1 activation dramatically enhances CaP metastasis in vivo. Specially, in our in vivo model of PC3 cell metastasis, activation of Rap1 had a potent ability to promote the rate and incidence of CaP metastatic lesion formation (Fig. 5). This model takes into account several steps involved in the metastatic process: survival in circulation, adhesion to and transmigration through the endothelial cell lining, migration to and invasion of the metastatic site, colonization, and growth (35). Inferring from the literature and our study, we would propose that Rap1-mediated integrin activation may explain the dramatic rate and occurrence of metastatic lesion formation in vivo. Collectively, these findings indicate Rap1 may play roles at several points during CaP metastasis.

Previous studies have examined Rap proteins in other aspects of prostate cancer cell biology. In LNCaP cells, vasoactive intestinal peptide promoted androgen-independent transactivation of the androgen receptor through a PKA/Rap1/extracellular signal-regulated kinase pathway to enhance growth (36), and Rap2A has been linked to androgen-stimulated growth of LNCaP cells (37). In CaP and other epithelial cancers, Rap1 function has been linked to cancer cell growth and proliferation through MAP kinase signaling (5, 38). Two recent studies also highlight other potential roles for Rap1 signaling in metastasis, these being the regulation of secretion of metalloproteinases and an involvement in angiogenesis (14, 39). Hence, it is important to note that
Rap1 activation may be promoting several aspects involved in CaP progression.

In conclusion, the current study provides compelling evidence of a role for Rap1 in the metastatic progression of CaP. The increased Rap1 activity detected in the more metastatic human CaP cells was shown to contribute to processes fundamental to CaP metastasis, i.e., cell migration and invasion mediated through specific integrins. Extending these findings to an in vivo model, activation of Rap1 was shown to dramatically enhance the rate and incidence of CaP metastatic lesion formation. Taken together, these data indicate that regulation of the Rap1 signaling pathway may constitute an important therapeutic target in CaP metastasis.

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Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
Received 11/10/08; revised 3/9/09; accepted 3/29/09; published OnlineFirst 5/26/09.  
Grant support: NIH grants CA100869 (P.J. Casey), Department of Defense Grant DAMD17-0510560 (C.L. Bailey), and Duke University Medical School Alumni Scholarship (P. Kelly).  
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.  
We thank Drs. Michelle Kimple, Ian Cushman, and Juhn Jumelia for careful review of this manuscript; and Drs. Timothy Fields, Leslie Parsie, and Gerard Blobe for their expertise, suggestions, and unpublished materials related to this work.

Cancer Research
Vol. 69, No. 12
June 15, 2009
4968
www.aacrjournals.org
Published OnlineFirst May 26, 2009; DOI: 10.1158/0008-5472.CAN-08-4269

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