Akt1 and focal adhesion kinase (FAK) are protein kinases that play key roles in normal cell signaling. Individually, aberrant expression of these kinases has been linked to a variety of cancers. Together, Akt1/FAK interactions facilitate cancer metastasis by increasing cell adhesion under conditions of increased extracellular pressure. Pathological and iatrogenic sources of pressure arise from tumor growth against constraining stroma or direct perioperative manipulation. We previously reported that 15 mmHg increased extracellular pressure causes Akt1 to both directly interact with FAK and to phosphorylate and activate it. We investigated the nature of the Akt1/FAK binding by creating truncations of recombinant FAK, conjugated to glutathione S-transferase (GST), to pull down full-length Akt1. Western blots probing for Akt1 showed that FAK/Akt1 binding persisted in FAK truncations consisting of only amino acids 1-126, FAK(NT1), which contains the F1 subdomain of its band 4.1, ezrin, radixin, and moesin (FERM) domain. Using FAK(NT1) as bait, we then pulled down truncated versions of recombinant Akt1 conjugated to HA (human influenza hemagglutinin). Probes for GST-FAK(NT1) showed Akt1-FAK binding to occur in the absence of the both the Akt1 (N)-terminal pleckstrin homology (PH) domain and its adjacent hinge region. The Akt1 (C)-terminal regulatory domain was equally unnecessary for Akt1/FAK co-immunoprecipitation. Truncations involving the Akt1 catalytic domain showed that the domain by itself was enough to pull down FAK. Additionally, a fragment spanning from the PH domain to half way through the catalytic domain demonstrated increased FAK binding compared to full length Akt1. These results begin to delineate the Akt1/FAK interaction and can be used to manipulate their force-activated signal interactions. Furthermore, the finding that the N-terminal half of the Akt1 catalytic domain binds so strongly to FAK when cleaved from the rest of the protein may suggest a means for developing novel inhibitors that target this specific Akt1/FAK interaction.

Key words: adhesion, Akt, cancer, focal adhesion kinase, metastasis, glutathione S-transferase, pleckstrin homology domain

INTRODUCTION

Cancer metastasis requires adhesion to a new substrate by cancer cells that have traveled from the primary tumor to the metastatic site. Circulating tumor cells increase dramatically during oncologic surgery while tumor cells are also easily recovered from the peritoneal cavity after colon cancer resection (1-8). It is not possible to quantitate the impact of surgical intervention upon tumor dissemination, since new metastases cannot be distinguished from pre-existing metastases that were simply too small to detect. However, tumor dissemination remains a concern for the oncologic surgeon, and designing a drug to block tumor cell adhesion would seem a worthwhile goal.

Forces such as pressure and shear are present in the circulation due to hemodynamic forces and in the surgical environment due to laparoscopic insufflation pressures, tumor manipulation, and irrigation forces. Modest (15 mmHg) increases in extracellular pressure activate a signal cascade within cancer cells that ultimately results in phosphorylation of the cytoplasmic tail of the beta 1 integrin subunit, propagating a conformational change that opens the extracellular matrix binding domain of beta 1 integrin heterodimers and increases cancer cell adhesiveness (9, 10). Other physical forces, such as shear force have similar effects (11). This pathway can be targeted either pharmacologically or by more specific molecular techniques to block this increase in adhesion and substantially increase tumor free survival in animal models (12, 13). However, blocking common intracellular signals leads also to non-specific toxicity, as these signals are likely to regulate other important aspects of the organism's biology. Indeed, the dose of colchicine used in previous pharmacologic blockade studies of pressure stimulated cancer adhesion is too high for human therapy (12). Blocking a less common element of the signal pathway that regulates cancer cell adhesiveness would seem more likely to be tolerated by a patient.

Promising candidates for pharmacologic targeting are the non-receptor tyrosine kinase focal adhesion kinase (FAK) and the serine/threonine kinase Akt. Both FAK and Akt play important roles in normal cell physiology, and both kinases are activated and/or overexpressed in a variety of cancers (14, 15). Classically, FAK activation is the result of surface integrin engagement, which places FAK near the top of several signaling cascades that work to transmit information from the external environment to actors within the cell (16). FAK associates with Cas and Src to promote the focal adhesion turnover required in cell migration,
and it is also required in the cooperative signaling pathway that exists between integrins and growth factors, which controls cell cycle progression and proliferation through the MEK-ERK axis (16, 17). Indeed, FAK signaling is required for cell survival in many adherent cells, and its absence triggers anoikis (18). However, FAK also participates in a range of physical force-mediated signaling events. For instance, repetitive deformation of adherent intestinal epithelial monolayers flexes the cytoskeleton mediated signaling events. For instance, repetitive deformation of FAK in a manner that varies with matrix substratum and integrin-binding (19-22). Conversely, increases in extracellular pressure can stimulate cancer cell adhesiveness by increasing FAK activation before the cell's integrins have engaged with the matrix (23). Interestingly, similarly increased extracellular pressure stimulates phagocytic cells to increase their phagocytic ability via a decrease in FAK activation (24, 25).

Akt also contributes to a range of cell processes such as cell proliferation and survival. Akt classically has a complex activation scheme that involves PIP3-mediated membrane translocation and phosphorylation by both PDK1 and mTORC2. Upon activation, Akt loses its membrane restrictions and enjoys a high degree of intracellular mobility, which is directed in part by isoform preference (26). While Akt isoforms have a limited capability to compensate for one another, they also exhibit an assortment of unique functions (26). In murine knockout models, Akt1 proved to be responsible for overall growth, Akt2 was required for proper insulin signaling, and the loss of Akt3 manifested in a decrease in brain size (27-29). In the context of cancer, unregulated FAK and Akt activity have been repeatedly implicated in the machinations of tumors. However, as previously mentioned, the non-specific inhibition of such wide-reaching cellular elements may prove to be inextricably tied to unintended toxicities that limit their feasibility as drug targets.

While it may not be prudent to base treatments off of global kinase blockade, a viable option could be the inhibition of unique interactions exhibited by these kinases. FAK and Akt have been tied to numerous processes that are initiated by physical forces. Pressure-mediated macrophage phagocytosis progresses through a mechanism that inhibits FAK but activated Akt2 (24, 30). In Caco-2 colon cancer cells, both cyclic strain-mediated migration and proliferation require the activation of FAK and Akt (31, 32). Similarly, pressure-mediated Caco-2 adhesion also requires FAK and Akt activation (33). We have previously delineated a novel interaction between focal adhesion kinase (FAK) and Akt1 that is required for pressure to stimulate cancer cell adhesion. While FAK is normally considered to act at the focal adhesion complex, there are much larger pools of FAK within the cytosol, and there is a constant equilibrium between the two (34). The signals activated by increases in extracellular pressure in cancer cells in suspension cause Akt-1 to bind to FAK in the cytosol and phosphorylate FAK at three previously uncharacterized serines (33). This interaction is required for subsequent FAK tyrosine 397 autophosphorylation, activation, and translocation to the focal adhesion complex where it further influences integrin binding strength (33). Although it is not known how this specific FAK-Akt1 interaction responds to variations in the magnitude and duration of extracellular pressure, at the cellular level, increases in adhesion have been observed over a range of pressures (10 – 30 mmHg) and can be elicited equally well by exposures of 1 minute as by those lasting 30 minutes (10, 35). The increase in adhesiveness engendered by increased pressure persists for at least 30 minutes after the higher pressure has been returned to baseline (10).

This interaction between Akt-1 and FAK does not seem required for most other actions of FAK, suggesting that blocking the Akt-1-FAK interaction might be less toxic than blocking all FAK activity. Indeed, even with regard to physical force effects, the mitogenic effects of pressure in adherent cancer cells are completely independent of the PI-3-kinase-Akt axis while the effects of repetitive deformation on Caco-2 colon cancer cell proliferation require Akt-2, not Akt-1 (32, 36). Previous studies using chimeras made by splicing different domains of Akt-1 and Akt-2 have suggested that the specificity of this interaction for FAK activation might rest in the PH-domain and hinge region of Akt-1 (37). We therefore now sought to further characterize the

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![Fig. 1. The FAK (NT1) region binds Akt1. Recombinant GST-FAK truncations NT (residues 1-415) and NT1 (residues 1-126) (shown in A) were conjugated to Glutathione Sepharose 4B beads and used to pull down recombinant HA-Akt1. All four truncations were able to pull down Akt1 (B, N = 4 similarly). In each lane, the GST blot shows the presence of the FAK truncations (top band), the detached GST tags (bottom band), and protein degradation products (middle bands).](Image 1)
site on Akt which binds to FAK as a preliminary to attempting to
design a drug to block this interaction. Serial truncations of Akt-1 were constructed and tested for their ability to bind to FAK.

MATERIALS AND METHODS

Materials

Caco-2 colon cancer cells were cultured according to American Type Culture Collection (ATCC, Rockville, MD) recommendations. We obtained Lipofectamine 2000 and other transfection supplies from Invitrogen (Carlsbad, CA). Glutathione Sepharose 4B beads from GE Life Sciences (Pittsburg, PA), Akt1 and GST antibodies from Cell Signaling Technology (Beverly, MA), anti-hemagglutinin (HA, clone 12CA5) monoclonal antibodies from Roche Applied Science (Indianapolis, IN). pCMV-HA vector was obtained from Clontech (Mountain View, CA). pcDNA3 myr HA Akt1 was provided by Dr. Paula Herman (Dana Farber Cancer Institute) through Addgene (Cambridge, MA). pcDNA3 HA-FAK was generated as previously described (33). pGEX GST-Akt1 and its truncations were a generous gift from Dr. Chi Bun Chan (Emory University School of Medicine). All primers were purchased from Integrated DNA Technologies (Coralville, IA). QIAquick Gel Extraction, QIAprep spin Miniprep, QIAquick PCR purification and QIAfilter Plasmid Maxi kits were purchased from Qiagen (Valencia, CA).

Generation of constructs

Mammalian expression vectors pCMV-HA-Akt1 and its truncations were constructed via PCR by introducing 5'EcoRI and 3'KpnI cut sites into a pcDNA3 myr HA Akt1 template. Products were then subcloned into the EcoR 1Kpn I double digested pCMV-HA cassette to get pCMV-HA-Akt. A similar protocol was used to generate pGEX-4T-1 GST FAK-NT and pGEX-4T-1 GST FAK-NT1 from pcDNA3 HA-FAK.

Transfections

Caco-2 cells were plated on p100 dishes at 30 – 35% confluence one day prior to transfection. Briefly, the constructed plasmids or empty plasmid were transfected into Caco-2 cells at final concentrations of 2 µg/ml plasmid and 5 µg/ml Lipofectamine 2000. Five hours after transfection, the medium was replaced with 15 ml pre-warmed Caco-2 media without antibiotics. Forty eight hours after DNA transfection, the cells were lysed for pull-down assays.

Glutathione S-transferase pull-down

Glutathione Sepharose 4B beads were blocked with 1% bovine serum albumin for 1 hour at room temperature and washed before being conjugated with recombinant GST-tagged proteins under similar conditions. Conjugated beads were incubated with lysate from transfected cells overnight at 4°C. The beads were washed to remove the unbound proteins. Bound proteins were eluted by addition of loading buffer with sodium dodecyl sulfate (SDS) and heating at 95°C for 5 min in preparation for western analysis.

Western blotting

Western blots were performed as previously described (38). Eluate from the pull-downs were resolved by SDS-polyacrylamide gel electrophoresis and transferred to Hybond ECL nitrocellulose membrane (Amersham Pharmacia Biotech, Sweden).
Piscataway, NJ). Membranes were blotted with specific antibodies directed against either their wild-type structures or recombinant tags with the appropriate secondary antibody coupled to horseradish peroxidase. Bands were detected with enhanced chemiluminescence (Amersham) and analyzed with a Kodak Image Station 440CF (Perkin Elmer, Boston, MA).

Akt1 structural analysis

Structures were obtained from the Protein Data Bank (http://www.rcsb.org): 3CQW (active Akt1 with ATP-competitive inhibitor bound kinase) and 3O96 (cytoplasmic PH domain of Akt1) (39, 40). Structures were analyzed using Pymol from DeLano Scientific (San Carlos, CA).

Statistical analysis

Results were compared by Student’s unpaired t-test and considered statistically significant when \( P < 0.05 \). All experiments were done independently at least three times unless indicated otherwise. All data are expressed as mean ± S.E.M.

RESULTS

The FAK (NT1) region is sufficient to bind Akt1

The wild-type full-length FAK molecule was truncated at its band 4.1, ezrin, radixin, and moesin (FERM) domain to discern its necessity for Akt1 binding. These truncations created two shortened FAK molecules. NT (residues 1-415) consisted of only the FERM domain, and NT1 (residues 1-126) contained the F1 subdomain of the FERM domain (Fig. 1A). Each of these truncations was able to bind Akt1 (Fig. 1B, one of four representative blots).

Akt1 binds FAK (NT1) independently of the pleckstrin homology domain and hinge region

Akt1 truncations were generated to determine the role of the pleckstrin homology domain (residues 1-106) and the hinge region (residues 107-147) in binding FAK. These initial serial truncations began with the (N)-terminal half of the PH domain, extended to include the (N)-terminal half of the hinge region, extended to include the (N)-terminal half of the hinge region,

Fig. 3. Akt1 / FAK binding requires the Akt1 kinase domain. (A): The Akt1 pleckstrin homology domain and hinge region span residues 1-147; the kinase domain spans residues 149-408; and the (C)-terminal regulatory domain spans residues 409-480. GST-fused constructs were generated with truncations targeting these domains in their entirety. Construct 1 is a deletion of the PH/hinge domain and the kinase domain. Construct 2 is a deletion of the PH/hinge domain and the (N)-terminal half of the kinase domain. Construct 3 is a deletion of the (C)-terminal half of the kinase domain and the (C)-terminal regulatory domain. Construct 4 is a deletion of the PH/hinge domain and the (C)-terminal regulatory domain. (B): Akt1 constructs were conjugated to Glutathione Sepharose 4B beads and incubated with lysate from transfected Caco-2 cells; and subsequent westerns were blotted for HA-FAK. Akt1 constructs 2 and 4 showed affinities for HA-FAK similar to that of wild-type full-length Akt1 (N = 4) while construct 3 pulled down significantly more FAK than the wild-type Akt1 (N = 4; \( P < 0.05 \)). Compared to the wild-type Akt1, construct 1 demonstrated significantly lower affinity for FAK (N = 6; \( P < 0.05 \)).
and ended with the complete deletion of both domains (Fig. 2A). The endpoints of these truncations were chosen to either disrupt the natural folding of the PH and hinge domains (HA-Akt1Δ1-66, HA-Akt1Δ1-125, respectively) or to remove them outright (HA-Akt1Δ1-147). GST-FAK (NT1), which is a FAK truncation consisting of the first 126 amino acids from its (N)-terminal, was used as bait to bind the Akt1 truncations. Both the most conservative (HA-Akt1Δ1-147) and least conservative (HA-Akt1Δ1-66) Akt1 truncations were pulled down well by the GST-FAK (NT1) coated beads (Fig. 2B, N = 2). However, the intermediate truncation (HA-Akt1Δ1-125) was not detectably bound to the GST-FAK (NT1) coated beads (Fig. 2B, N = 2).

The Akt1 kinase domain is required to bind FAK

Further Akt1 truncations were generated to determine the role of the kinase domain (residues 149-408) and the (C)-terminal regulatory domain (residues 409-480) in binding FAK. Construct 1 consisted of only the (C)-terminal regulatory domain. Constructs 2 and 3 consisted of the (C) and (N)-terminal halves of the Akt1 molecule, respectively. Construct 4 consisted of only the kinase domain (Fig. 3A). All constructs were fused to GST at the (N)-terminus and were used to pull down endogenous wild type FAK from Caco-2 cell lysate. Construct 3 demonstrated an affinity for FAK that was significantly greater than that of the wild-type full-length Akt1 (Fig. 3B, N = 4; P < 0.05). This is consistent with our data showing the full-length PH and hinge domains to permit Akt1/FAK binding. Construct 4 consisted of only the (C)-terminal regulatory domain, demonstrated a significantly lower affinity for FAK (Fig. 3B, N = 6; P < 0.05).

DISCUSSION

We have previously delineated an intricate intracellular signal pathway activated by extracellular forces such as pressure and shear stress up-regulate integrin binding affinity and metastatic potential in cancer cells (9-11). While many of the elements of this pathway are common signaling elements, one novel aspect of the pathway is the binding of Akt1 to FAK and subsequent serine phosphorylation of FAK by Akt1, which seems required for pressure-stimulated FAK activation in this setting. This unusual interaction seems a promising target for manipulation to inhibit metastasis since FAK activation in response to other stimuli has not been found to require Akt-binding. In this manuscript, we used serial truncations to show that the interaction between Akt1 and FAK is independent of the Akt1 pleckstrin-homology (PH) domain and hinge region. Domain-directed truncations further demonstrate that the Akt1 kinase domain alone is sufficient to pull down FAK. Taken together with our previous studies of Akt1/Akt2 chimeras, these results would be consistent with a model in which the Akt isoforms to bind FAK through their homologous catalytic domain with an affinity which is modulated by their varying PH, hinge, and (C)-terminal regulatory domains.

We have previously described a relationship between Akt1 and FAK wherein pressure-induced membrane translocation of Akt1 and its activation by Ser473 phosphorylation is indirectly dependent upon FAK (FAK itself is a tyrosine kinase and so could not be directly responsible for Akt1 Ser473 phosphorylation (37). This translocation of Akt1 and its phosphorylation at S473 occur in response to a 15 mmHg increase in extracellular pressure and requires the presence of both activatable FAK and the specific PH and hinge regions of the Akt1 isoform (37). While the PH domains of the Akt isoforms share a similar functionality of binding phospholipids, their unique identities contribute to isoform-specific functions ranging from the promotion of cell growth and migration to the inhibition of apoptosis in response to shear stress (41-44). The membrane translocation and activation responses of Akt1 to pressure can be transferred to Akt2-like chimeric molecules that contain the Akt1 PH and hinge regions (37).

In contrast to what might have been expected from those previous studies, we show here that FAK binds to Akt1 even when the Akt1 PH and hinge regions are deleted. Omitting either only the (N)-terminal half of the PH domain or the entire PH and hinge regions did not affect the ability of Akt1 to bind FAK.
fragments (GST-FAK (NT1)). Both the truncation missing the (N)-terminal half of the PH domain (HA-Akt1Δ1-66) and the truncation with completely deleted PH and hinge regions (HA-Akt1Δ1-147) bound FAK strongly. However, the partial hinge region truncation HA-Akt1Δ1-125 was unable to bind FAK (NT1). Akt1 activity can be strongly suppressed by allosteric inhibitors acting through PH domain conformational changes. Crystal structures show these inhibitors locking the PH domain into the kinase domain to prevent both Akt1 activation and membrane translocation, and highlight the inhibitory potential of a disrupted PH domain (40). Thus, one model consistent with these results would postulate that FAK binds to Akt outside the PH and hinge regions but that the PH domain can interfere with access by FAK to the kinase domain depending upon the conformation of the PH domain and hinge region. This would explain why the PH and hinge region confer specificity on Akt1 to permit its interaction with FAK even though they are not directly involved in FAK binding. The deviation from the wild-type structure engendered by the inactivating HA-Akt1Δ1-125 deletion could then disrupt intermolecular interactions more effectively than the full length Akt1 PH and hinge region.

In reciprocal binding experiments using FAK as bait, the FAK (NT1) fragment proved to be sufficient to pull down Akt1. The FERM domain is responsible for FAK's ability to bind a variety of proteins and seemed a suitable candidate for mediating FAK-Akt1 interaction (45). The FERM domain consists of three subdomains F1, F2, and F3. The F2 and F3 subdomain resemble acyl-CoA binding proteins and PH domains, respectively (46). No region of Akt1 proposes to be a good binding target for an acyl-CoA binding protein, and while PH-PH interactions do exist, they are highly specific as the PH domains from even related isoforms of Fak fail to bind (47). The F1 subdomain, however, displays structural similarities to ubiquitin (46). Not only is this ubiquitin-like fold found in several unrelated proteins, which makes it a good contender in the context of protein-protein interactions, Akt1 also plays a role in several ubiquitylation pathways (48, 49).

Indeed, our second series of Akt1 truncations instead demonstrated the Akt1 kinase domain alone to be sufficient to bind wild-type FAK. Domain-directed truncations of Akt1 consistently showed strong FAK binding to be dependent on the presence of some portion of the kinase domain. Truncations lacking either the (N) or (C)-terminal half of the Akt1 kinase domain (constructs 2, 3) were able to pull down wild type FAK equally, if not more strongly, than the full Akt1 kinase domain by itself (construct 4). The only truncation in this series that lost FAK-binding affinity was construct 1, which consisted of only the (C)-terminal regulatory domain. Taken along with the PH and hinge domain truncation data, these results point to the kinase domain as the key region needed to allow for FAK binding, and suggests that there may be at least two separate sites in the Akt1 kinase domain, one in the C terminal segment and one in the N terminal segment, that each interact with and bind FAK. A model of the Akt1 surface structure shows the area where the PH domain and (N) and (C)-terminal halves of the kinase domain meet (Fig. 4). In light of the ability of either half of the kinase domain to bind FAK, and our previous data showing the PH domain to confer FAK specificity amongst Akt isoforms, this region of Akt1 presents a promising target for disrupting FAK-specific binding.

There are several global FAK inhibitors undergoing trials, but they share the distinct disadvantage of targeting FAK catalytic activity indiscriminately. In doing so they either also inhibit other kinases with some structural similarity or have profound downstream consequences because of global FAK inhibition. Inhibitors such as TAE226 also inhibit IGF-R1, MAPK, and Akt activity while PF-228 inhibits only motility and not cell growth or viability (50, 51). The promising FAK inhibitor Y15 decreased FAK Y397 autophosphorylation, cancer cell viability, and colony formation, and in mice xenograft in vivo models, intraperitoneal Y15 (30 mg/kg) blocked tumor growth of colon, pancreatic, and breast cancer (52-54). However, toxicity studies conducted in mice showed intraperitoneal Y15 at 45 mg/kg to be lethal with mortality associated with peritonitis (55). In addition to exhibiting a narrow range of safe effectiveness, intraperitoneal Y15 at 30 mg/kg only reaches plasma concentrations of 110 nM whereas in vitro inhibition of cell viability of colon, pancreatic, and breast cancer requires concentrations of at 1, 10, and 50 µM, respectively (52-54). The off-target effects of current FAK inhibitors and the general toxicity that arises from such nonspecific highlight the potential desirability of a therapeutic intervention that not only specifically targets FAK but even more specifically inhibits only certain deleterious aspects of FAK signaling in cancer cells.

Similar toxicities may develop from complete inhibition of the Akt1 kinase. Like FAK blockade, global Akt1 blockade also affects other signaling pathways. Not only are the general mechanics of cell-survival affected by Akt1 blockade, which may be compensated for by parallel signaling cascades, but more specific responses processes are also compromised. As an anabolic kinase, Akt is well-known for its ability to rescue cells from detachment-induced apoptosis (26). In the context of mechanical forces, Akt has also been shown to be an upstream regulator of a process that combats disuse-atrophy of skeletal muscles, which is a central issue in the microgravitational environment of space medicine but may also be relevant for the challenges seen in rehabilitation medicine (63), and thus may be important for debilitated cancer patients. Current Akt inhibitors undergoing clinical trials have demonstrated deleterious effects when used in combination with well-established anti-neoplastic agents (64). Both novel anti-neoplastic drugs (such as the DNA intercalating agents tosypquin and tosind) and conventional drugs (such as the topoisomerase inhibitors camptothecin and etoposide) depend on DNA synthesis to achieve efficacy. Akt inhibition, on the other hand, fundamentally antagonizes these therapies by causing a decrease in cell proliferation and thus active DNA replication (65, 64). The inability to use general Akt inhibitors in chemotherapeutic cocktails limits its usefulness and can be avoided by designing drugs that target more specific actions such as FAK-AKT interactions rather than the overall catalytic activity of the kinase.

Our previous studies suggest that Akt-FAK interaction may therefore be a desirable target for the inhibition of this prometastatic-pathway, since Akt-FAK interaction could conceivably be blocked, and the force-activated signals that stimulate cancer cell adhesiveness inhibited, without altering many other activities of either FAK or Akt. This would require a precise understanding of the mechanism by which FAK and Akt interact. Coupling this knowledge with protein-model based drug discovery techniques, one could conceivably generate treatments aimed at blocking, competing with, or destabilizing the Akt-FAK interaction. To maximize the efficacy of these drugs, more studies are needed to delineate how Akt and FAK behave after force activation. For instance, the pressure-mediated adhesion return to baseline by 60 minutes after extracellular pressure has returned to ambient, whereas pressure-mediated β1-integrin T88/9 phosphorylation is only measurably increased for 30 minutes, perhaps because the kinetics of the adhesion assay are slower and thus more sensitive to the adhesion that has occurred before the phosphorylation events receded (23). Our present results represent an initial step in this direction, focusing attention on the Akt kinase domain. Further studies to delineate more precisely the two specific FAK binding sites we now postulate within this domain may
ultimately lay the groundwork for the design of a specific therapeutic to block this pathway and inhibit metastasis.

In a broader context, 90% of cancer deaths are attributed not to the original tumor but to cancer metastases (56). Central to metastatic development are processes of intravasation, extravasation, and implantation all of which require cell adhesion. Such adhesion could be modulated by pressurization from within the initial tumor environment itself (which range from 15 – 38 mmHg across a variety of tumors), pressures within the circulation (5 – 10 mmHg in venous and 90 – 120 mmHg in arterial circulation) or shear stresses within the same environment, or by iatrogenic manipulation such as the peritoneal insufflation in laparoscopic-assisted surgeries that generates 15 mmHg pressures, shear stress from peritoneal irrigation, or even direct surgical tumor manipulation (11, 57-59). Force-activated increased adhesiveness appears common to a variety of malignant cell types, including colon cancers, squamous head and neck cancers, breast cancers, and even sarcomas (35, 60-62). By targeting pressure-mediated adhesion through Akt-FAK interaction we target an element shared across cancer pathology that seems less likely to be important in other biology since this Akt-FAK interaction has not previously been described. The tendrils of such an interaction may not be limited to adhesion and may in fact be far reaching. Indeed, FAK and Akt2 have been implicated in pressure-induced phagocytosis, and we have previously described in LPS-stimulated monocytes an inhibitory effect of pressure on the generation of IL-6 and other inflammatory markers (24, 30, 66, 67). In the setting of colorectal cancer, serum levels of IL-6 are also significantly elevated (68). Connections like these not only broaden our view of the effects of mechanical forces on cell pathology and physiology, but also provide us with leads.

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