An Intracellular Signal Pathway That Regulates Cancer Cell Adhesion in Response to Extracellular Forces

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Abstract

Increasing evidence suggests that tumor cells can regulate their own adhesion via intracellular signals that modulate integrin binding affinity. Although the full pathway has not yet been elucidated, the effects of pressure seem likely to require cytoskeletal mechanosensing, Src, phosphatidylinositol 3-kinase, focal adhesion kinase, and Akt-1 activation. Ultimately, activated focal adhesion kinase accumulates at the membrane in association with β1-integrin heterodimers and may modulate integrin binding affinity. This pathway may be a promising target for manipulation to inhibit metastatic cancer cell adhesion. [Cancer Res 2008;68(1):2-4]

Background

The adhesion of metastasizing cancer cells is often viewed as a passive process on the part of the cancer cells themselves. Cells swirling through the lymphatic or venous circulation or around a body cavity or surgical site contact endothelial or other cells or extracellular matrix proteins, adhering if the correct membrane receptors are deployed at the right place to bind to adhesion sites and if the energy of cell movement is not so great that it overcomes binding affinity. However, work from our laboratory (1–3) and others (4, 5) suggests that cancer cells may regulate their own adhesion to matrix proteins, endothelial cells, or surgical wounds by intracellular signals that regulate the binding affinity of matrix receptors including β1-integrin heterodimers. At least one pathway by which this occurs seems to involve focal adhesion kinase (FAK) and Src activation (2) and paxillin (6, 7) and has been shown to be active in breast (8), head and neck (6), and colon (1) cancer cell lines, as well as in primary human colon cancer cells isolated directly from body cavity or surgical site contact endothelial or other cells or extracellular matrix proteins, adhering if the correct membrane receptors are deployed at the right place to bind to adhesion sites and if the energy of cell movement is not so great that it overcomes binding affinity. However, work from our laboratory (1–3) and others (4, 5) suggests that cancer cells may regulate their own adhesion to matrix proteins, endothelial cells, or surgical wounds by intracellular signals that regulate the binding affinity of matrix receptors including β1-integrin heterodimers. At least one pathway by which this occurs seems to involve focal adhesion kinase (FAK) and Src activation (2) and paxillin (6, 7) and has been shown to be active in breast (8), head and neck (6), and colon (1) cancer cell lines, as well as in primary human colon cancer cells isolated directly from surgical specimens (2). These signals mediating increased adhesion are stimulated by increased extracellular pressure (2) as well as by laminar or nonlaminar shear stress (4, 5, 9).

Although the entire pathway has not yet been elucidated, previous work suggested that the signal events involved occurred primarily within the focal adhesion complex (where FAK and Src bind to the adapter protein paxillin), could be activated by the stimulation of pressure or shear acting on the cell, and could then modulate β1-integrin binding affinity. An intact cytoskeleton seems to be required for FAK activation and the ultimate effect on adhesion (10), so the cytoskeleton may act as a tensility style sensor for external forces that activate this pathway. Src activation by extracellular pressure is not affected by cytoskeletal manipulation, however, and what causes Src to be activated when extracellular pressure is increased is as yet unclear.

Key Findings

These additional studies led to several important additional conclusions in our recent study. These are briefly enumerated here, although the interested reader is referred back to the original publication for more details (11). First, PI3K and Akt are also activated by extracellular pressure and required for the stimulation of adhesion by extracellular pressure. The downstream S6 kinase is not involved, however, because rapamycin does not block the effect. This was particularly interesting because rapamycin does block the effect of increased extracellular pressure on macrophage phagocytosis (12). We have previously described other differences between macrophage and cancer cell signaling in response to extracellular pressure.

Second, Src activation seems to be required for PI3K activation, which is in turn required for the activation of FAK and Akt. Indeed, the p85 subunit of PI3K directly associates with FAK in increased proportions in response to increased extracellular pressure, based on coprecipitation studies. This changed our view of the more proximal part of this signal pathway because we had previously postulated that FAK-397 autophosphorylation within focal adhesions was an early event in the pressure-induced pathway, which is required for subsequent FAK-Src interaction and FAK-576/577 phosphorylation by Src (2). In contrast, this observation suggested that FAK-397 phosphorylation in cancer cells responding to extracellular pressure actually requires prior activation of PI3K.

Third, the proportion of cellular phosphorylated (and presumably active) Akt localized within the membrane/cytoskeletal fraction then increases, along with the proportion of cellular phosphorylated (activated) FAK in the same fraction. This is an important finding that may be explained by alternate models. The pressure stimulus may cause one or both of these important kinases to translocate to the membrane either before activation or after activation. Alternatively, the apparent shift in localization of these activated kinases may reflect an inhibition of movement back into the cytosol after activation at the cell membrane. The shifts in Akt and FAK do seem to be linked in some fashion. An Akt inhibitor prevented the shift into the membrane/cytoskeletal fraction of FAK as well as the shift of Akt itself; conversely, FAK seemed to be required for Akt activation because small interfering RNA (siRNA) reduction of FAK blocked Akt activation. A nonphosphorylatable FAK mutant also
seemed to shift into the membrane/cytoskeletal fraction with pressure. Although this does not tell us whether FAK activation occurs at the membrane or within the cytosol, it does suggest that the shift in FAK localization in response to extracellular pressure does not require FAK activity. Distinguishing among these competing possibilities will require further study.

However, regardless of the details of these shifts in FAK and Akt activation and localization, the end result of these signals, and our fourth significant finding, is that increased extracellular pressure results in increased association of FAK with β1-integrin heterodimers in a manner sensitive to blockade of Src or PI3K. Such FAK-integrin association could directly modulate integrin binding affinity and explain the ultimate increase in adhesiveness observed in response to increased extracellular pressure. Previous observations confirm that the expression of integrins on the cell surface is not altered in response to extracellular pressure (2).

Fifth and finally, only Akt-1 seems to participate in this pathway. siRNA reduction of Akt-1 blocks the effect of pressure on cell adhesion, but siRNA reduction of Akt-2 does not. This was true both for SW620 colon cancer cells in which Akt-1 predominates and for Caco-2 colon cancer cells in which Akt-1 and Akt-2 expression levels seem to be similar. One caution about these results was that we were only able to achieve a relatively modest 50% to 60% reduction of each Akt isoform by siRNA transfection. Thus, these studies left unanswered the question of whether a more complete reduction in Akt-2 might have been able to inhibit the pressure effect. More recent unpublished observations have further supported our hypothesis that Akt-2 is not involved, however (13).

Implications
The concept that physical forces such as pressure, shear, and deformation can modulate cell biology without ligand-receptor interactions is not new (Fig. 1). Many cell types exhibit mechanosensitive behavior, including intestinal epithelial cells (14). Cancer cells may experience physical forces in a variety of settings. Cancers growing rapidly against a constraining stroma may exhibit increased interstitial pressures (15, 16), whereas metastasizing tumor cells experience pressure and shear during passage through the lymphatics or vasculature. Perioperatively, cancer cells are commonly shed from tumors into the surgical site or the venous circulation during procedures, and the presence of free tumor cells on the outside of the surgical specimen correlates adversely with prognosis for colon cancers (17). Surgeons apply force to tumors during dissection and insufflate the peritoneal cavity to 15 mm Hg above ambient during laparoscopic surgery.

Figure 1. Physical forces such as shear and pressure stimulate the adhesion of cancer cells in suspension to extracellular matrix proteins (ECM) or nonmalignant host cells in vitro or in vivo. The signal pathway that mediates this effect seems to involve Src and PI3K interaction to activate FAK in a complex manner that also requires an intact cytoskeleton, including paxillin, and Akt-1 activation. These events increase the proportion of phosphorylated FAK (p-FAK) that associates with β1-integrin heterodimers, increasing integrin binding affinity for substrate. This pathway may be one mechanism by which cancer cell adhesiveness is internally regulated.
Irrigation of the surgical site may also generate pressure and shear stress. Finally, patients exhibit similar increases in intra-abdominal pressure for 2 to 3 days after surgery as bowel wall swelling because of third spacing increases the volume of the intra-abdominal viscera, so intra-abdominal cancer cells may experience increased extracellular pressures for days after surgery. Conversely, the adhesiveness of the cancer cells themselves correlates inversely with prognosis (18).

These observations therefore raise the possibility that the adhesiveness of metastasizing epithelial cancer cells may be increased by a force-activated pathway involving Src, PI3K, FAK, and Akt-1. It is important to distinguish here the inside-out events by which intracellular signaling before adhesion regulates integrin binding affinity from the outside-in events that occur after adhesion when integrin binding induces the activation of kinases within the focal adhesion complex and subsequent downstream events. Integrin-induced signaling is well characterized, but the pressure-induced signal events described here occur in suspended cells before adhesion and make adhesion itself more likely.

Although this work has focused on mechanoactivated stimuli to date, growth factors or cytokines that act on various elements of this pathway could also modulate cancer cell adhesiveness. Interestingly, the proliferation of colon cancer cells is also stimulated by increased extracellular pressure over hours, albeit via a different signal mechanism (19). These signals might, in the future, offer new targets for intervention to inhibit metastasis, either in the perioperative period or in the setting of unresectable primary tumors. As the proponents of antiangiogenesis have pointed out, an unresectable primary cancer may not be a life-threatening event in many cases if metastasis can be blocked.

The observation of Akt isoform specificity may be interesting in this regard. Although many of the initial studies of Akt function did not distinguish among Akt isoforms, there are actually three Akt isoforms. Akt-1 and Akt-2 are found in all cells, whereas Akt-3 is chiefly found in the brain, heart, and kidney. There is increasing interest in differentiating the functions of the various Akt isoforms. Although opposing roles have been suggested for Akt-2 and Akt-1 in modulating cell motility (20), the current study suggests that Akt-1 is the sole pressure-responsive element that mediates the effect of pressure on cancer cell adhesion. Akt-1 has also this year been reported to selectively regulate integrin activation in endothelial cells and fibroblasts (21).

It is interesting that the stimulation of macrophage phagocytosis by extracellular pressure seems to depend instead on Akt-2 (12). For some other aspects of cell biology, Akt-2 may have other unique functions or the Akt isoforms may have interchangeable functions. The isoform specificity of the pressure-stimulated adhesion effect suggests that Akt-1 may be an interesting target of opportunity on which to act to inhibit cancer cell adhesion with less toxic inhibition of other effects in which Akt-1 may be replaced by Akt-2.

Like any good study, these observations pose more questions than they answer. These include the nature of the proximal mechanosensor by which the cancer cell perceives the physical force; the mechanism responsible for the Akt isoform specificity; whether FAK and Akt are translocating toward the membrane or inhibited from moving away from it and where within the cell their phosphorylation occurs; and the manner in which integrin binding affinity is altered. Such questions await further research, as does the question of whether growth factors or cytokines modulate cancer cell adhesion by acting on this pathway. However, taken together with previous work from our laboratory and others, this study suggests that internal signaling events within tumor cells modulate tumor cell adhesiveness. These signals may prove important targets for therapeutic intervention to inhibit metastasis in the future.

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