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During this fellowship award, I made considerable progress in understanding the contribution of the α6 integrin receptors to breast carcinoma progression. I developed a dominant negative strategy to 'knock-out' the expression of the α6β1 integrin in a highly metastatic breast carcinoma cell line (MDA-MB-435). After depletion of α6β1 surface expression, these cells are deficient in their growth in the mammary fat pad of athymic mice and can no longer survive as metastases in the lungs and liver. These findings suggest that the α6β1 integrin plays an important role in regulating the growth and survival of breast carcinoma. I also established MDA-MB-435 transfectants that express the α6β4 integrin receptor. These cells have a marked increase in their ability to invade through basement membrane matrices *in vitro*. I determined that the mechanism by which this integrin promotes invasion involves a preferential and localized targeting of phosphoinositide-3 OH kinase (PI 3-K) activity. The small GTP-binding protein Rac is downstream of PI 3-K in the cells examined and it is involved in invasion. Collectively, these findings provide a mechanism for the involvement of α6β4 in promoting carcinoma invasion and invoke a novel function for PI 3-K signaling.

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Leslie M Shaw 10/14/97

PI - Signature Date

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## Introduction

A major advance in cancer research made during the last several years has been the realization that integrin adhesion receptors play a major role in regulating tumor behavior. Specific tumor-associated functions that are regulated by integrins include adhesion and migration, differentiation, growth and apoptosis, and angiogenesis (1-5). This array of diverse but important functions reflects the fact that integrins are critical for the function and maintenance of the normal epithelial progenitors of all carcinomas. The importance of integrin-mediated interactions in normal epithelial biology has been particularly well-demonstrated for the mammary epithelium (6). Such studies have identified the laminins, a family of extracellular matrix proteins, as key players in determining the function of both normal and transformed mammary epithelia. For these reasons, integrin laminin receptors are prime candidates for investigating the role of cell adhesion events in breast carcinoma (7).

Although several integrin heterodimers can function as receptors for members of the laminin family, the α6 integrins, α6β1 and α6β4, are the predominant laminin receptors in many cell types (7). The possibility that the α6 integrins play an important role in breast carcinoma progression is supported by a study by Imhof and colleagues (8) which examined the expression of α6 in invasive breast carcinomas of 119 women and correlated levels of expression with patient survival. The results demonstrated that high expression levels of the α6 subunit correlated with reduced patient survival time. In fact, α6 integrin expression was superior in predicting reduced survival than other known factors alone including estrogen receptor status. These data implicate a key role for the α6 integrins in deadly breast carcinomas. Data to support the involvement of the α6 integrins in the progression of other carcimomas has also been reported. Expression of the α6 integrins in prostate carcinoma cell lines correlates directly with the invasive potential of these cells in nude mice (9). In addition, expression of the α6β4 integrin has been reported to correlate with the progression of squamous, gastric, and colon carcinomas (10-12). Taken together, these data, in conjunction with the Imhof data, provide a compelling case for the involvement of α6 integrins in carcinoma progression. However, these studies only emphasize the potential importance of the α6 integrins in breast cancer, but they do not provide mechanistic information. Until this is established, the full potential of α6 for diagnosis, or as a target for therapeutic development, will not be known.

The ability of integrin receptors to influence cellular processes such as growth, differentiation, cell motility, and tumor progression is the result of both their adhesive and signaling capabilities (1,13). Many of the signaling pathways that have been characterized for growth factor receptors can be stimulated by integrin receptor engagement. In fact, these two receptor systems may act in concert to elicit the specific signals that are required for proper cell function (14). There is still much to be learned about how integrin receptors control signaling pathways but it is clear that alterations in integrin expression and function during transformation are likely to have multiple consequences on tumor progression. The purpose of the work supported by this fellowship was to elucidate specific functions of breast carcinoma cells that are influenced by the α6β1 and α6β4 integrins and to elucidate the signaling pathways that control these functions. The work performed under this award has made significant progress toward attaining this goal.

## Body

**The α6β1 integrin is required for the growth and survival of MDA-MB-435 breast carcinoma cells *in vivo*.** To examine the contribution of the α6β1 integrin to breast carcinoma progression, I developed a dominant-negative technique for 'knocking-out' the expression of α6β1 in MDA-MB-435 cells, a human breast carcinoma cell line that is highly metastatic in athymic mice. A mutant β4 subunit that lacked the entire cytoplasmic domain was expressed at high levels in this cell line. β4 dimerizes only with α6, and not other integrin α subunits, and therefore it selectively depleted α6β1 surface expression by forming the α6β4-ΔCYT heterodimer. I found that elimination of α6β1 expression inhibited the ability of these cells to mediate specific *in vitro* functions associated with metastatic spread such as laminin adhesion and migration (Described in previous Annual Report; *Shaw, L.M., C. Chao, U.M. Wewer, and A.M. Mercurio. 1996. Function of the integrin α6β1 in metastatic breast carcinoma cells assessed by expression of a dominant negative receptor. Cancer Res., 56:959-963.*). Subclones of these transfectants were isolated by FACS sorting. Mock (transfected with the vector alone) and β4-ΔCYT transfectant subclones were chosen that expressed similar levels of all integrin subunits. These subclones also adhered to other matrix proteins such as fibronectin, vitronectin, and collagen I to the same extent.

In collaboration with Ulla Wewer and Reidar Albrechtsen in Copenhagen, Denmark, I examined the *in vivo* behavior of MDA-MB-435 breast carcinoma cells that lack α6β1 expression. Mock and α6β4-ΔCYT transfectant subclones were injected into the mammary fat pad of female athymic mice. After several weeks, the mice were sacrificed and the extent of tumor growth, invasion, and metastasis was analyzed. The tumors formed by the α6β4-ΔCYT transfectant subclones were significantly smaller than those formed by the mock transfectants and metastatic foci were not present in the lungs of the β4-ΔCYT transfectant mice. However, small foci of apoptotic cells were observed in the lungs of the mice injected with the α6β4-ΔCYT transfectant subclones and a high level of apoptosis was observed in the primary tumors (Described in previous Annual Report; *Wewer, U.M, L.M. Shaw, R. Albrechtsen, and A.M. Mercurio. 1997. The integrin α6β1 promotes the survival of metastatic human breast carcinoma cells in mice. Amer. J. Path. (in press)* ). These data suggest that the β4-ΔCYT transfectants were able to metastasize to the lungs but were not capable of surviving in this environment. Taken together, these data indicate that the α6β1 integrin functions in the growth and survival of breast carcinomas.

**The α6β1 integrin can activate MAPK** To begin to understand how the α6β1 integrin functions in the growth and survival of breast carcinomas, I examined the ability of this integrin to activate signaling pathways that regulate these functions. The Mitogen activated protein kinases (MAPK) have been shown to be important for growth control because they regulate the expression of immediate early genes required for cell cycle progression (15). It was recently reported that only a subset of integrin receptors can activate MAPK and that the α6β1 integrin was not included in this group (16). To determine if the ability of α6β1 to activate MAPK was cell type dependent, I assayed MAPK activity after ligation of α6β1 in the MDA-MB-435 cells. As shown in Fig. 1, ligation of α6β1 by clustering with antibodies and attachment to laminin induced MAPK activity in these cells. Interestingly, ligation of the α6β4-ΔCYT receptor did not induce activation of MAPK confirming that this receptor is non-functional for signaling. This inability of the α6β4-ΔCYT receptor to activate MAPK could explain the decrease in the growth of these tumors.

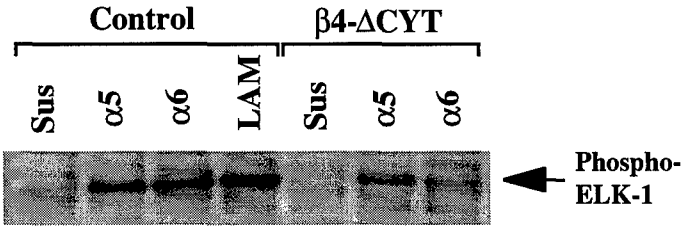


**Figure 1: Activation of MAPK by the α6β1 integrin.** MDA-MB-435 transfectants were maintained in suspension or incubated with α6- or α5-specific antibodies and allowed to adhere to anti-mouse IgG coated plates or laminin-1 coated plates for 30 minutes. Aliquots of cell extracts that contained equivalent amounts of protein were incubated with an anti-phosphoMAPK antibody and then Protein A sepharose. After washing, the beads were resuspended in kinase buffer containing the Elk-1 substrate. The reactions were resolved by 10% SDS-PAGE under reducing conditions, transferred to nitrocellulose, and immunoblotted using an antibody that recognizes the phosphorylated Elk-1.

**The α6β1 integrin can activate S6 kinase and Akt kinase** It has recently been determined that the activity of the Akt serine/threonine kinase is required for survival of several different cell types (17,18). Given the increased apoptosis that was observed in the primary tumors formed by the MDA-MB-435 cells lacking α6β1, we chose to examine if α6β1 could activate Akt. Initially, we examined the activation of the p70 S6 kinase because this kinase is known to be downstream of Akt activity (19). As shown in Fig. 2A, ligation of both the α6β1 and α5β1 integrins activated S6 kinase. We next assayed for Akt kinase activity after ligation of α6β1. As shown in Fig. 2B, Akt was activated after 20 minutes of clustering the receptor with α6 specific antibodies. Importantly, neither kinase was activated upon clustering the α6β4-ΔCYT receptor, as was observed for activation of MAPK.

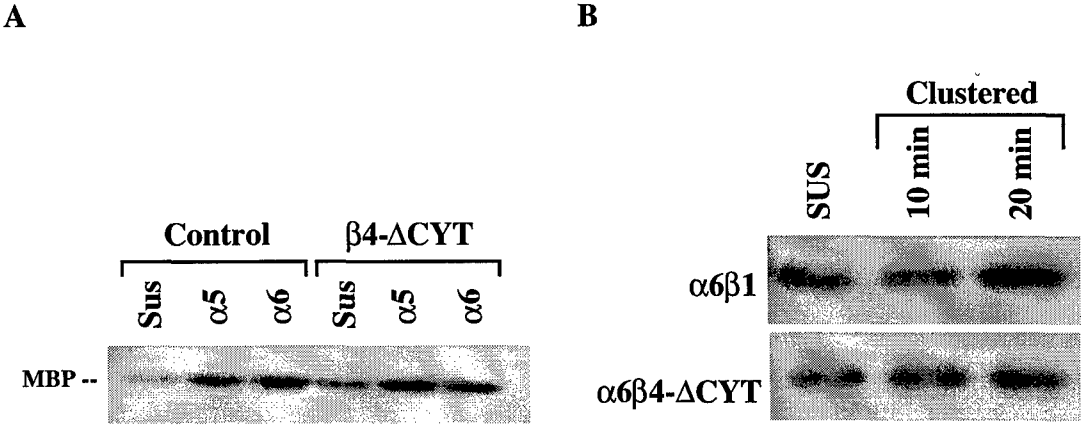


**Figure 2: The α6β1 integrin activates p70 S6 kinase and Akt kinase.** MDA-MB-435 transfectants were maintained in suspension or incubated with α6- or α5-specific antibodies and allowed to adhere to anti-mouse IgG coated plates for 30 minutes. Aliquots of cell extracts that contained equivalent amounts of protein were incubated with an anti-p70 S6 kinase (A) or anti-Akt (B) antibody and then Protein A sepharose. After washing, the beads

were resuspended in kinase buffer containing MBP (A) or histone H2B (B). The reactions were resolved by SDS-PAGE under reducing conditions and transferred to nitrocellulose.

**The α6β1 integrin cooperates with growth factors for activation of signaling pathways** The ability of the α6β1 integrin to activate MAPK, p70-S6 Kinase, and Akt support the hypothesis that this integrin can contribute to the growth and survival of breast carcinoma cells. However, other integrins, such as α5β1, were also capable of activating these important pathways. Therefore, the question arises as to why α6β1 is necessary for breast carcinoma growth and survival *in vivo* when other integrin receptors can also activate these pathways. It has been demonstrated previously that integrins can cooperate with growth factor receptors to enhance the activation of downstream signaling pathways (14). Therefore, we examined the possibility that α6β1 cooperates preferentially with a growth factor signaling pathway to enhance the signals required for growth and survival. As shown in Fig. 3, Insulin-like growth factor 1 (IGF-1) stimulates very little Akt activity when added to cells that are maintained in suspension. However, a marked increase in activity is observed if integrin receptors are ligated when the IGF-1 is added. Importantly, Akt activity was consistently higher when α6β1 was ligated in the presence of IGF-1 than when other integrin receptors were ligated (Fig. 3). This cooperative effect was also observed for p70 S6 kinase. These data support the possibility that α6β1 is important for breast carcinoma survival because this integrin cooperates better with growth factors to activate the signaling pathways that prevent apoptosis, such as those that regulate Akt. Future experiments will continue to examine this cooperation between α6β1 and growth factors and their role in promoting breast carcinoma growth and survival.

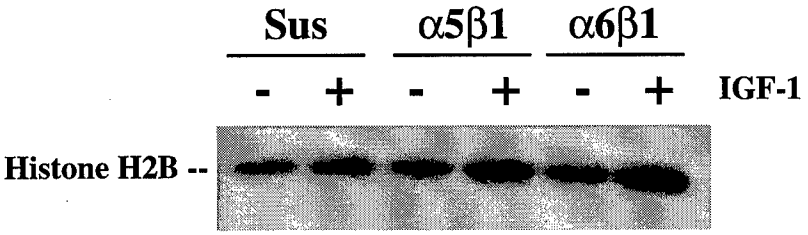


**Figure 3:** MDA-MB-435 transfectants were maintained in suspension or incubated with α6- or α5-specific antibodies and allowed to adhere to anti-mouse IgG coated plates for 30 minutes. IGF-1 was added to some of the plates for the final 10 minutes. Aliquots of cell extracts that contained equivalent amounts of protein were incubated with an anti-Akt antibody and then Protein A sepharose. After washing, the beads were resuspended in kinase buffer containing histone H2B. The reactions were resolved by SDS-PAGE under reducing conditions and transferred to nitrocellulose.

**Expression of the α6β4 integrin increases the invasiveness of MDA-MB-435 cells** Many studies have implicated the α6β4 integrin in carcinoma progression, however, the mechanism is unknown (9-12)). The MDA-MB-435 cells used in this study do not express the α6β4 integrin although they express the α6β1 integrin. To examine the contribution of α6β4 to breast carcinoma progression, MDA-MB-435 cells were transfected with the full length β4 subunit and stable subclones of these cells that express the α6β4 integrin were isolated (Described in previous Annual Report). These transfectants were examined for their ability to invade Matrigel in a standard chemoinvasion assay. The rate of invasion of the β4 transfectants was approximately 3-4 fold greater than that of the mock transfectants in a 4 hour assay. The β4-ΔCYT transfectants

invaded at a slightly slower rate than that of the mock transfectants (Fig. 4A) indicating that the β4 cytoplasmic domain is essential for stimulating invasion. To examine the contribution of integrin receptors to the invasion of MDA-MB-435 cells, Matrigel chemoinvasion assays were performed in the presence of integrin subunit specific antibodies. A β1-specific antibody (mAb 13) inhibited invasion of the mock and β4-transfectants (Fig. 4B). An α6-specific mAb (2B7) inhibited invasion of the mock transfectants by approximately 60% (Fig. 4B), in agreement with our previous result that these cells use α6β1 as a major laminin receptor. However, the same antibody increased the rate of invasion of the β4 transfectants by approximately 30% (Fig. 4B). The stimulation of invasion observed for the α6 antibody in the MDA-MB-435/β4 transfectants suggests that α6β4 is not required for the adhesive functions involved in invasion but rather acts as a signaling receptor whose function can be enhanced by antibody binding.

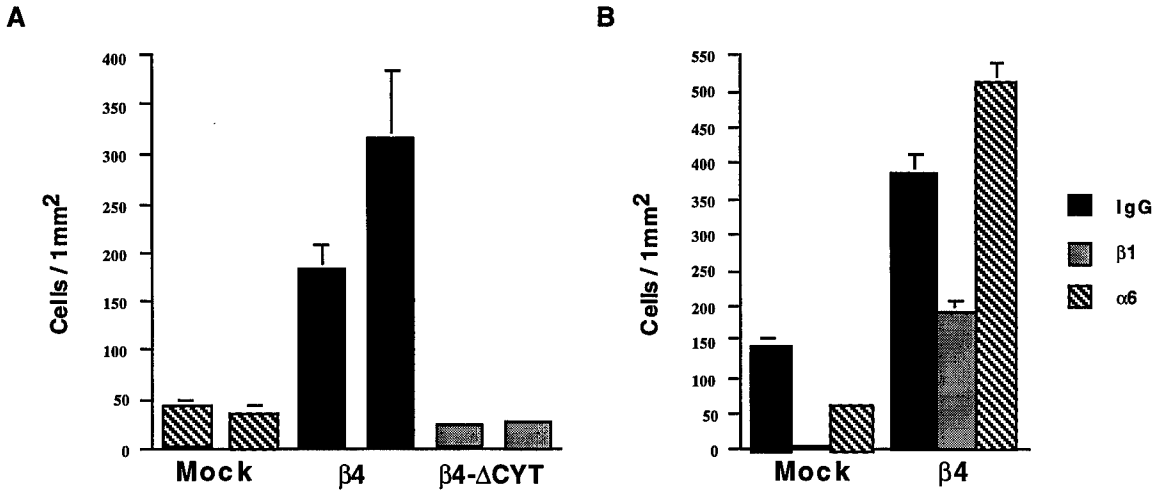


**Figure 4: Invasion of the MDA-MB-435 transfectants.** A) MDA-MB-435 transfectants were assayed for their ability to invade Matrigel. Matrigel was diluted in cold distilled water, added to the upper well of Transwell chambers, and dried in a sterile hood. The Matrigel was reconstituted with medium and the transfectants were added to each well. Conditioned NIH-3T3 medium was added to the bottom wells of the chambers. B) MDA-MB-435 transfectants were assayed for their ability to invade Matrigel in the presence of monoclonal antibodies specific for the indicated integrin subunits. Cells were preincubated for 30 minutes in the presence of antibodies before addition to the Matrigel-coated wells. After 4 hrs, the cells that had not invaded were removed and the cells that had invaded to the lower surface of the filters were fixed, stained, and quantitated. Mock, MDA-MB-435 cells transfected with vector alone; β4, MDA-MB-435 cells transfected with the full length β4 subunit; β4- ΔCYT, MDA-MB-435 cells transfected with the β4 subunit lacking the cytoplasmic domain; IgG; non-specific antibody; β1, mAb 13; α6, 2B7.

**Invasion of MDA-MB-435 cells is dependent on PI 3-K.** As a prelude to identifying the signaling mechanism by which the α6β4 integrin stimulates invasion, we first assessed the effects of the MAPK kinase inhibitor PD98059 on MDA-MB-435 invasion (20). We selected this pathway because it has been reported that MAPK contributes to cell motility by phosphorylating myosin light chain kinase (). As shown in Fig. 5, pre-treatment of these cells with PD98059 (25 uM) resulted in only a modest inhibition (20%) of invasion. We next targeted PI 3-K because of its central involvement in multiple signaling pathways (21). The PI 3-K inhibitor wortmannin

inhibited invasion of both the mock and β4 transfectants by 60-80% (22; Fig. 5). These results suggest that PI 3-K, but not MAPK, is necessary for the invasion of the MDA-MB-435 cells.

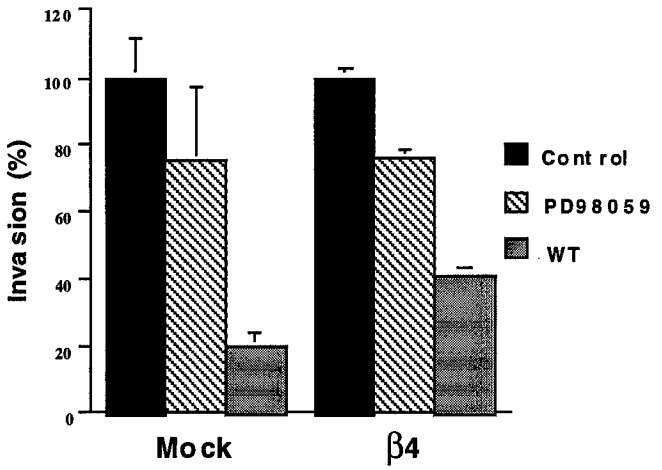


**Figure 5: Analysis of MAPK and PI 3-K involvement in MDA-MB-435 invasion.** MDA-MB-435 transfectants were assayed for their ability to invade Matrigel in the presence of the MEK inhibitor PD98059 (25uM) or the PI 3-K inhibitor wortmannin (WT;100uM). Cells were preincubated for 10 minutes in the presence of the inhibitors before addition to the Matrigel-coated wells. After 4 hrs, the cells that had not invaded were removed and the cells that had invaded to the lower surface of the filters were fixed, stained, and quantitated. The data shown are the mean values (±SD) of one experiment done in duplicate. Mock, MDA-MB-435 cells transfected with vector alone; β4, MDA-MB-435 cells transfected with the full length β4 subunit.

**Activation of PI 3-K by the α6β4 integrin** To determine if the α6β4 integrin can stimulate PI 3-K activity, *in vitro* kinase assays were performed using the mock, β4, and β4-ΔCYT transfectants of MDA-MB-435 cells. After ligation of the α6 integrins with 2B7, extracts were immunoprecipitated with a phosphotyrosine-specific antibody to capture the activated population of PI 3-K and these immunoprecipitates were assayed for their ability to phosphorylate crude brain phosphoinositides. As shown in Fig. 6A, an increase in PI 3-K activity, indicated by the appearance of PtdIns-3,4,5-$P_3$, was observed upon clustering the α6β1 integrin in the mock transfectants and the α6β4 integrin in the β4 transfectants. More importantly, PI 3-K activity stimulated by clustering the α6β4 integrin was markedly greater than that observed after clustering the α6β1 receptor. This enhanced stimulation of PI 3-K was also seen using a β4-specific mAb to ligate the α6β4 integrin in the β4 transfectants (Fig. 6A). PI 3-K activity was also higher in the β4 transfectants than in the mock transfectants after adhesion to laminin-1 (Fig. 6A). This observation suggests that even though α6β4 is not used as the major adhesion receptor in these cells, interactions with laminin through this receptor can stimulate PI 3-K activity. PI 3-K activity was not increased upon ligation of the α6β4-ΔCYT receptor and little activity was evident when the transfectants were maintained in suspension (Fig. 6).

Our data suggested that the ability of the α6β4 integrin to activate PI 3-K may be quantitatively greater than that of β1 integrins in MDA-MB-435 cells. This possibility was examined by comparing PI 3-K activation in the β4 transfectants in response to antibody-ligation of either β1 integrins or the α6β4-integrin. As shown in Fig. 6B, ligation of the α6β4 integrin with β4-specific antibodies stimulated PI 3-K activity approximately two fold greater than β1 integrin ligation demonstrating that PI 3-K is activated preferentially by the α6β4 integrin. The differences between the abilities of the α6β4 and β1 integrins to activate PI 3-K are most likely even greater

than what was observed given the two-three fold higher level of expression of β1 than β4 on the cell surface.

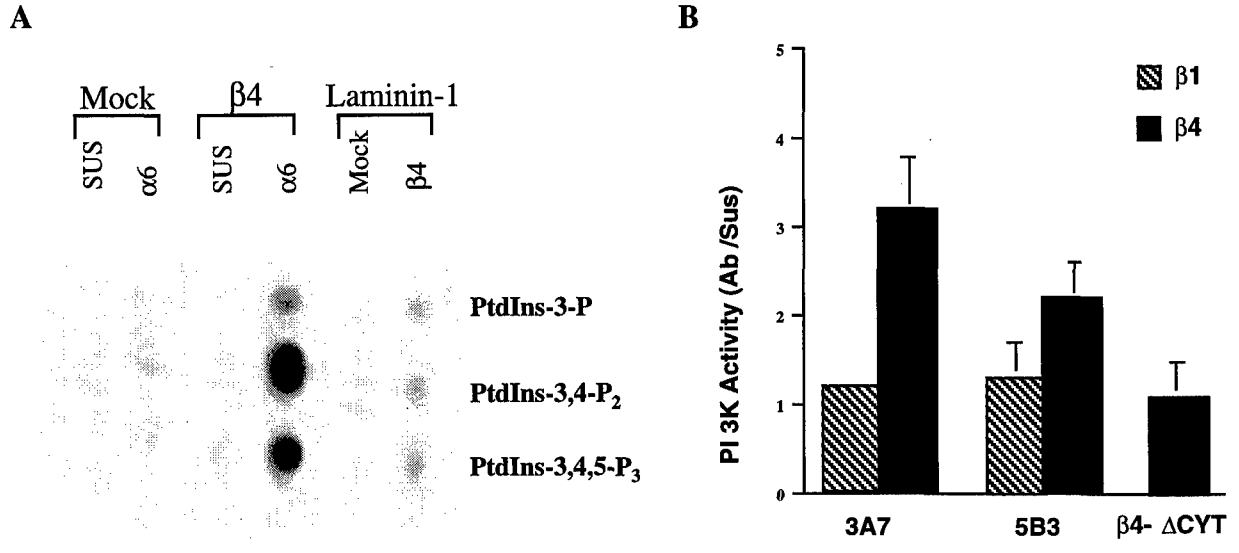


**Figure 6: Analysis of PI 3-K activity in the MDA-MB-435 transfectants.** MDA-MB-435 transfectants were maintained in suspension or incubated with α6-, β1-, or β4-specific antibodies and allowed to adhere to anti-mouse IgG coated plates or laminin-1 coated plates for 30 minutes. Aliquots of cell extracts that contained equivalent amounts of protein were incubated with the anti-phosphotyrosine mAb 4G10 and Protein A sepharose for 3hrs. After washing, the beads were resuspended in kinase buffer and incubated for 10 minutes at room temperature. The phosphorylated lipids were resolved by thin layer chromatography. A) The D3-phosphoinositides are indicated by arrows. B) The amount of radiolabeled PtdIns-3,4,5-$P_3$ was determined for each condition by densitometry. The integrin activated levels of PtdIns-3,4,5-$P_3$ were compared to the level observed for the cells that were maintained in suspension. The value from this ratio was determined to be the relative PI 3-K activity stimulated by each integrin subunit. Mock, MDA-MB-435 cells transfected with vector alone; β4, MDA-MB-435 cells transfected with the full length β4 subunit; SUS, cells maintained in suspension; α6, cells clustered with the α6-specific antibody.

**Constitutively active PI-3K stimulates invasion of MDA-MB-435 cells** The hypothesis that the α6β4 integrin promotes invasion of MDA-MB-435 cells by enhancing the activity of PI 3-K implies that: 1) expression of a constitutively active form of PI 3-K in the parental cells should increase their invasion in the absence of α6β4 expression and 2) expression of a dominant negative PI 3-K subunit in the β4 transfectants should decrease their invasion. To validate these predictions, a constitutively active, membrane-targeted PI 3-K (Myr-p110-Myc) was expressed transiently in MDA-MB-435 cells and the ability of these cells to invade Matrigel was compared to cells transfected with an empty vector. As shown in Fig. 7A, constitutively active PI 3-K increased invasion two-fold and this invasion was inhibited by wortmannin. Overexpression of the wild type p85 regulatory subunit has been demonstrated to function in a dominant negative manner and inhibit endogenous PI 3-K activation (23). Transient expression of a myc-tagged p85

subunit inhibited the invasion of the MDA-MB-435/β4 transfectants by 50% (Fig. 7B). The data obtained with the wild-type p85 subunit substantiate the wortmannin data shown in Fig.5.

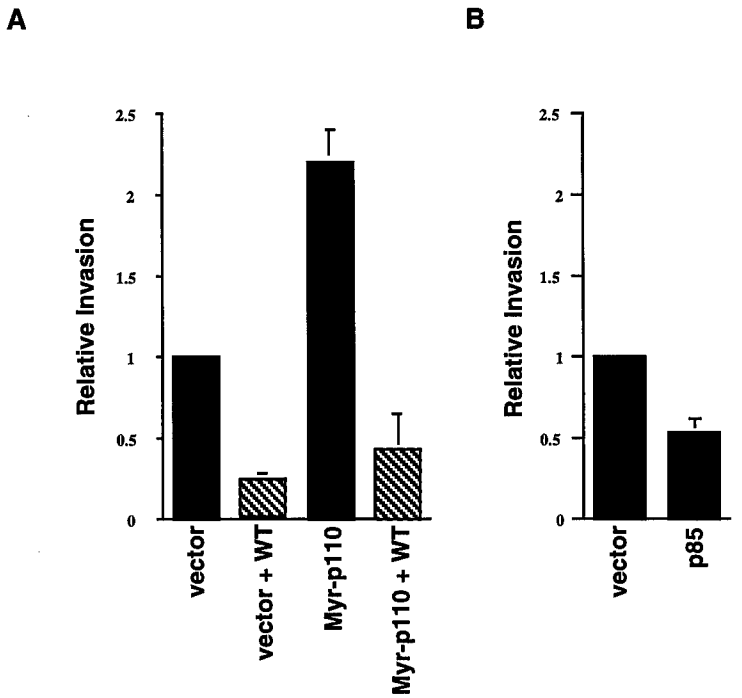


**Figure 7: Analysis of PI 3-K involvement in invasion of MDA-MB-435 cells by transient transfections.** A) MDA-MB-435 cells were transiently transfected with a Myc-tagged constitutively active form of the PI 3-K p110 catalytic subunit, Myr-p110, and assayed for their ability to invade Matrigel in the absence or presence of wortmannin (WT; 100nM). B) MDA-MB-435/b4 transfectants were transiently transfected with a wild-type PI 3-K p85 regulatory subunit, p85, and assayed for their ability to invade Matrigel. After 5 hrs, the cells that had not invaded were removed and the cells that had invaded to the lower surface of the filters were fixed and stained. Invasion was quantitated by counting the cells that stained positively for β-galactosidase expression. Relative invasion was determined by comparing the amount of invasion obtained for the experimental transfections to that observed for the cells transfected with the vector alone, which was given the value of 1.

**The Akt/PKB kinase, a downstream effector of PI-3K, is not required for invasion** The Akt/PKB serine/threonine kinase (Akt) is activated downstream of PI 3-K and, for this reason, could play an important role in invasion (24). This possibility was supported by our finding that ligation of the α6β4 integrin in the MDA-MB-435 transfectants can activate Akt. Based on these observations, we examined the ability of MDA-MB-435 cells that expressed a constitutively active form of Akt (Myr-Akt) to invade Matrigel. Surprisingly, this constitutively active form of Akt actually decreased the rate of invasion in comparison to the control cells even though it was expressed at relatively high levels (Fig. 8A). Most likely, the exogenously expressed Akt sequestered a significant fraction of D3 phosphoinositides and precluded the use of these lipids in those signaling pathways downstream of PI 3-K that are required for invasion. Based on these results, we conclude that Akt is not required for MDA-MB-435 invasion.

**The small G-protein Rac is required for MDA-MB-435 invasion** The small G-protein Rac is a downstream effector of PI 3-K that is involved in the actin rearrangements that result in the formation of membrane ruffles and lamellae (25). The ability of cells to form lamellae is strongly correlated with their motility and therefore influences their invasive potential. To examine the role of Rac in PI 3-K dependent invasion, a dominant negative mutant of Rac, N17Rac, was transiently expressed along with the Myr-p110-Myc construct in the MDA-MB-435 cells. As shown in Fig. 8A, co-expression of the N17Rac inhibited the increased invasion that was observed when the constitutively active p110 subunit of PI 3-K was expressed alone. In addition to this experiment, the N17Rac construct was expressed transiently in the MDA-MB-435/β4 transfectants and a 50% reduction in invasion was observed (Fig. 8B). Taken together, these results demonstrate that Rac is an essential downstream mediator of the α6β4/PI 3-K signaling pathway involved in invasion. Expression of a constitutively active mutant of Rac, V12Rac, did not significantly increase the invasion of the MDA-MB-435 cells suggesting that other PI-3K downstream effectors, in addition to Rac, are important for invasion in these cells. (data not shown).

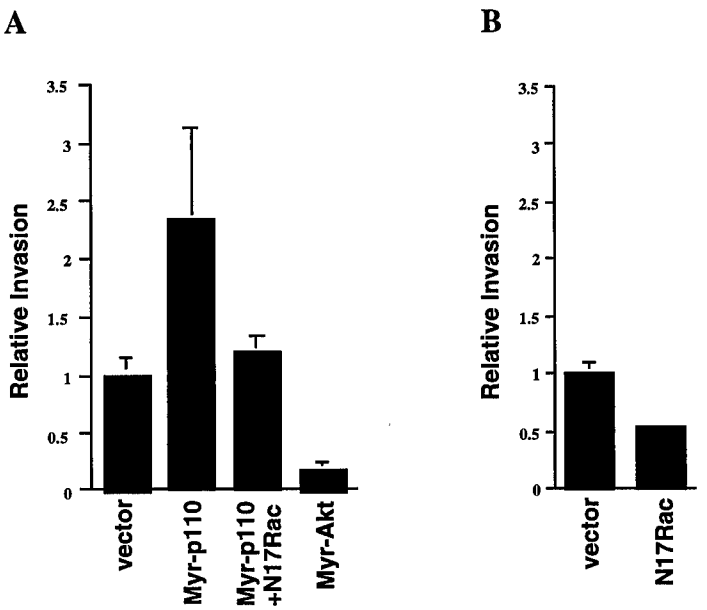


**Figure 8: Analysis of Rac and Akt involvement in PI 3-K-dependent invasion of MDA-MB-435 cells.** A) MDA-MB-435 cells were transiently transfected with either the vector alone, Myr-p110, Myr-p110 and a dominant negative mutant of Rac, GST-N17Rac, or a constitutively active form of Akt, Myr-Akt, and assayed for their ability to invade Matrigel. B) MDA-MB-435/β4 transfectants were transiently transfected with vector alone or N17Rac and assayed for their ability to invade Matrigel. After 5 hrs, the cells that had not invaded were removed and the cells that had invaded to the lower surface of the filters were fixed and stained. Invasion was quantitated by counting the cells that stained positive for β-galactosidase expression. Relative invasion was determined by comparing the amount of invasion obtained for the experimental transfections to that observed for the cells transfected with the vector alone, which was given the value of 1.

## Conclusions

In order to take full advantage of the α6 subunit as a marker for predicting the prognosis of breast cancer, it is necessary to understand mechanistically how the α6β1 and α6β4 integrins promote aggressive tumor behavior. Until this is established, the full potential of α6 for diagnosis, or as a target for therapeutic development, will not be known. The goal of this postdoctoral fellowship was to examine the role of the α6 integrins in breast carcinoma progression. Extensive progress has been made toward developing an understanding of the importance of these receptors in breast carcinoma biology. Although the original aims of this proposal were to compare the cytoplasmic domain variants of the α6 subunit, early studies directed the research in different, exciting directions. The work from this award has demonstrated that the α6 integrins have specific roles in breast carcinoma progression that involve distinct signaling functions of α6β1 and α6β4.

Elimination of α6β1 expression from a highly metastatic breast carcinoma cell line inhibited the ability of these cells to metastasize in athymic mice. However, this was not due to an inability to invade as was predicted from *in vitro* experiments that showed a decreased ability to adhere and migrate on laminin substrates. Rather, the decreased metastasis appears to be due to the inability of these cells to survive in distant organs. These cells are also inhibited in their primary tumor growth because of a decreased proliferative rate and an increased apoptotic rate. Therefore, the conclusion can be drawn that the α6β1 integrin contributes to breast carcinoma progression by controling signaling pathways that are involved in cell cycle progression and/or apoptotic regulation. The work in the final year of this fellowship has focused on identifying specific signaling pathways that are activated by the α6β1 integrin that would contribute to these functions. The data indicate that the α6β1 integrin may cooperate with growth factor receptors to enhance growth and survival signals such as the activation of Akt and p70 S6 kinase.

The α6β4 integrin regulates signaling pathways that influence carcinoma invasion. Expression of the β4 integrin subunit in a highly metastatic breast carcinoma cell line markedly increased the *in vitro* invasive potential of these cells. The mechanism by which this integrin promotes invasion involves a preferential and localized targeting of phosphoinositide-3 OH kinase (PI 3-K) activity. Exogenous expression of α6β4 increased carcinoma invasion in a PI 3-K-dependent manner and transient expression of a constitutively active PI 3-K increased invasion in the absence of α6β4. Ligation of α6β4 stimulated significantly more PI 3-K activity than ligation of either α6β1 or other β1 integrins establishing specificity among integrins for PI 3-K activation. An essential role for PI 3-K in invasion constitutes a novel function for this kinase and implies that downstream effectors of PI 3-K are critical for the invasive process. We provide evidence in fact that the small GTP-binding protein Rac is downstream of PI 3-K in the cells examined and that it is involved in invasion. In contrast, the serine/threonine kinase Akt does not contribute to the invasive process even though it is regulated by PI 3-K and is activated by the α6β4 integrin. Collectively, our findings provide a mechanism for the involvement of α6β4 in promoting carcinoma invasion and invoke a novel function for PI 3-K signaling. These data support the previous findings that the expression of α6β4 is associated with invasive carcinoma. A fascinating problem is raised by these observations because carcinoma progression involves both tumor cell invasion and survival. The recent demonstration that Akt is required for the survival of several cell types (17,18) coupled with our finding that PI-3K is required for invasion suggests that two essential functions of progression may require the products of PI 3-K and that the balance between the use of these pathways may impact tumor cell invasion or survival. These findings are particularly important because they suggest that this α6β4/PI 3-K signaling pathway is a potential target for inhibiting tumor spread.

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Shaw, L.M., C. Chao, U.M. Wewer, and A.M. Mercurio. 1996. Function of the integrin α6β1 in metastatic breast carcinoma cells assessed by expression of a dominant negative receptor. Cancer Res., *56*:959-963.

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Shaw, L.M., U.M. Wewer, and A.M. Mercurio. 1996. Function of the integrin α6β1 in metastatic breast carcinoma cells. Amer. Assoc. Cancer Res. Annual Meeting

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