Curcumin Inhibition of Integrin ($\alpha_6\beta_4$)-Dependent Breast Cancer Cell Motility and Invasion

Hong Im Kim, Huang Huang, Satish Cheepala, Shile Huang and Jun Chung

Abstract Curcumin, a polyphenol natural product isolated from the rhizome of the plant *Curcuma longa*, has emerged as a promising anticancer therapeutic agent. However, the mechanism by which curcumin inhibits cancer cell functions such as cell growth, survival, and cell motility is largely unknown. We explored whether curcumin affects the function of integrin $\alpha_6\beta_4$, a laminin adhesion receptor with an established role in invasion and migration of cancer cells. Here we show that curcumin significantly reduced $\alpha_6\beta_4$-dependent breast cancer cell motility and invasion in a concentration-dependent manner without affecting apoptosis in MDA-MB-435/β4 (β_4-integrin transfectants) and MDA-MB-231 breast cancer cell lines. Further, curcumin selectively reduced the basal phosphorylation of β_4 integrin (Y1494), which has been reported to be essential in mediating $\alpha_6\beta_4$-dependent phosphatidylinositol 3-kinase activation and cell motility. Consistent with this finding, curcumin also blocked $\alpha_6\beta_4$-dependent Akt activation and expression of the cell motility–promoting factor ENPP2 in MDA-MB-435/β4 cell line. A multimodality approach using curcumin in combination with other pharmacologic inhibitors of $\alpha_6\beta_4$ signaling pathways showed an additive effect to block breast cancer cell motility and invasion. Taken together, these findings show that curcumin inhibits breast cancer cell motility and invasion by directly inhibiting the function of $\alpha_6\beta_4$ integrin, and suggest that curcumin can serve as an effective therapeutic agent in tumors that overexpress $\alpha_6\beta_4$.

Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione], also called diferuloylmethane, is a natural yellow-orange–colored compound extracted from the rhizome of *Curcuma longa* (1, 2). For centuries, curcumin has been a vital component of Indian medicine and culinary traditions (1). A number of recent studies have shown that curcumin displays a wide variety of pharmacologic effects, including antioxidant (3), antiviral (4), anti-inflammatory (5), and anticancer (1, 2). In particular, the anticancer effect of curcumin has garnered considerable attention in recent years and has been shown in many human cancer cells including breast (6–8), colon (9, 10), prostate (11, 12), hepatocellular carcinoma (13), T-cell leukemia (14), B-cell lymphoma (15), melanoma (16), and neuroblastoma (17). Currently, curcumin is being tested in early clinical trials as an anticancer agent (1). However, the anticancer mechanism of curcumin remains to be determined. In particular, how curcumin inhibits cancer cell motility is largely unknown.

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In this study, we tested the effect of curcumin treatment on the function of a well-known tumor antigen, $\alpha_6\beta_4$ integrin. Initially, $\alpha_6\beta_4$ has been characterized as a receptor for laminin family members of extracellular matrix. It mediates hemidesmosome formation and tissue integrity in normal epithelia where it serves as an adhesion receptor (18). In aggressive cancer cells, however, the host-tumor microenvironment induces mobilization of $\alpha_6\beta_4$ from hemidesmosome into filamentous actin–based structures such as lamellipodia and filopodia, where this integrin becomes signaling competent by functionally interacting with other growth factor receptors and G-protein–coupled receptors (19). The enhanced signaling function of $\alpha_6\beta_4$ contributes to the malignant behaviors of cancer cells. For example, the level of $\alpha_6\beta_4$ expression in breast carcinoma cells correlates with their ability to invade (20, 21) and to survive under conditions of nutrition deprivation (22, 23). This is associated with its activation of the phosphatidylinositol 3-kinase (PI3K)-Akt (protein kinase B) signaling pathway (20, 22, 24), which is critical for cell proliferation, growth, survival, and migration.

In this study, we tested whether curcumin inhibits $\alpha_6\beta_4$ signaling and functions important for breast cancer cell motility and invasion using MDA-MB-435/β4 and MDA-MB-231 breast cancer cells because curcumin has been shown to inhibit Akt (11, 24) and nuclear factor kB (25, 26), both of which are known downstream effectors of $\alpha_6\beta_4$ (20, 27). We found that curcumin inhibits $\alpha_6\beta_4$-dependent cancer cell motility and invasion by reducing the key tyrosine residue (Y1494) phosphorylation of the β_4-integrin cytoplasmic tail as well

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as phosphorylation of Akt, a downstream target of $\alpha_6\beta_4$. Reduction of cell motility and invasion by curcumin also correlates with the reduction in expression of ENPP2, which is the key cell motility –promoting factor whose expression is selectively enhanced by $\alpha_6\beta_4$ (28). Curcumin also works cooperatively with other pharmacologic inhibitors known to block $\alpha_6\beta_4$ signaling pathways at lower concentrations. All together, our studies present the first evidence of curcumin inhibition of integrin function, which leads to the blockade of integrin-dependent cancer cell motility and invasion.

Materials and Methods

Cell lines and reagents

The MDA-MB-435 and MDA-MB-231 human breast carcinoma cells were obtained from the Lombardi Breast Cancer Depository at Georgetown University (Washington, DC). The generation of MDA-MB-435 subclones (MDA-MB-435/mock (vector only) and MDA-MB-435/β4 (β_4 integrin) was done as previously described (20, 22, 29). MDA-MB-231 cells were stably infected with lentiviruses that expressed shRNA targeted against either green fluorescent protein or the β_4-integrin subunit as previously described (30). These cells were cultured in low-glucose DMEM with L-glutamine, sodium pyruvate, 10% fetal bovine serum, and 100 units/mL of penicillin and streptomycin. For inhibitor pretreatment, cells were preincubated with the indicated doses (see figure legends) of curcumin (Sigma), SU11274 (Calbiochem), and Akt inhibitor (Calbiochem) for either 3 or 12 h before the assays. Integrin β_4 (clone H-101) and actin (clone C-11) antibodies were purchased from Santa Cruz Biotechnology, and Akt and p-Akt (Ser^{473} and Thr^{308}) antibodies were obtained from Cell Signaling Technology. Phospho-β_4-integrin (Y1494) was obtained from ECM Biosciences.

Cell motility and invasion assays

For the cell motility assay, the upper and lower surfaces of the membrane in transwell inserts (Costar) were coated with collagen I at 4°C overnight. To prepare for the invasion assay, Matrigel (Collaborative Research; 0.5 μg) was diluted with cold water and dried onto each filter overnight at room temperature. On the following day, transwell membranes were blocked with DMEM for 1 h at 37°C. Cells were trypsinized and resuspended in serum-free DMEM/bovine serum albumin. A total of 10^5 cells were added to the upper chamber of each well. Lysophosphatidic acid (LPA; 100 ng/mL) was added to the lower chambers as a chemoattractant. Inserts were incubated for 2 to 3 h, and nonmigrating cells were mechanically removed with cotton swabs. The cells attached to the bottom side of the membrane were stained with crystal violet and counted. Assays were done in triplicate and repeated five times. Cell motility and invasion were quantified by counting the cells that migrated to the lower surface of the membrane per square milliliter using bright-field optics. Data represent the mean ± SD and are pooled from five independent experiments.

Three-dimensional matrix culture

Three-dimensional culture matrix obtained from growth factor–reduced basement membrane extract (RGF BME) was prepared as described in the manufacturer's protocols (Trevigen, Inc.). Briefly, three-dimensional culture matrix was thawed and added to the desired culture plates and incubated at 37°C for 1 h to promote gelling of matrix. Assay medium (2% three-dimensional culture matrix RGF BME in tissue culture media) was prepared to dilute cells. A given number of harvested cells were added to the well of the plate containing three-dimensional culture matrix RGF BME and incubated at 37°C in a CO_2 incubator overnight. Each day, cell growth and structure formation were monitored via an inverted microscope. Assay media were replaced every 3 d.

Western blot analysis

For the analysis of protein expression, cells were lysed in radioimmunoprecipitation assay (RIPA)-EDTA buffer [50 mmol/L Tris (pH 7.4), 150 mmol/L NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, and 5 mmol/L EDTA] containing 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L Na_3VO_4, and protease inhibitor. The lysate was clarified by centrifugation at 4°C. Equal amounts of extracts were resolved in SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride membranes (Millipore). The rest of the Western blot analysis was done as described previously (22).

Apoptosis assay

Cells were incubated in 10% fetal bovine serum–containing DMEM for 12 h at 37°C with or without treatment at the indicated dose of curcumin. Subsequently, both adherent and nonadherent cells were harvested and apoptosis was measured using the Annexin V-PE Apoptosis Detection Kit I (BD Biosciences) as described previously (22). The data represent the mean ± SD and are pooled from three independent experiments.

Quantitative real-time PCR

Quantitative analysis of ENPP2 mRNA expression was done with real-time PCR using the ABI Prism 7700 sequence detection system as described by the manufacturer (Applied Biosystems). The of primers and probes are as follows: ENPP2 forward sequences primer, 5′-CTCACCCTGCAGATCATGA-3′; ENPP2 reverse primer, 5′-CTCAGTTCTATCACATGTGAC-3′; ENPP2 probe, 5′-GGTGTGTCAACCGTCATCT-3′; β-actin forward primer, 5′-TCACCATGGATGATGATATCGC-3′; β-actin reverse primer, 5′-AAGCCGGCCTTGCACAT-3′; and β-actin probe, 5′-CGCTCGTCGTCGA-3′. The data represent the mean ratio of ENPP2 to β-actin mRNA (±SD) obtained from triplicate samples.

Results

Curcumin inhibits $\alpha_6\beta_4$-dependent breast cancer cell motility in a dose-dependent manner

Whereas a number of studies have shown that curcumin inhibits the motility of cells derived from hepatocellular carcinoma (31), rhabdomyosarcoma (32), colon (33), and prostate cancer (34), little is known about the mechanism by which curcumin accomplishes this. Based on our previous studies and those of others showing that $\alpha_6\beta_4$ is critical for cell motility, we hypothesized that curcumin targets $\alpha_6\beta_4$ signaling. To test this hypothesis, MDA-MB-231 and MDA-MB-435 breast cancer cell lines were used. MDA-MB-231 cells are highly motile and an invasive breast carcinoma cell line that endogenously expresses high levels of $\alpha_6\beta_4$. The MDA-MB-435 cell line lacks endogenous expression of β_4 integrin. Therefore, the stable clones that express β_4 integrin by transfection ($\alpha_6\beta_4$ positive) and null vector (mock control, $\alpha_6\beta_4$ negative) were used for our studies. The motility of the cells was determined with a transwell assay.

As reported previously (20, 21, 35), the ability of these cells to migrate toward a chemoattractant such as LPA correlates with the level of $\alpha_6\beta_4$ expression (Fig. 1). Briefly, expression of β_4 integrin in MDA-MB-435 cells increased the cell motility by ~7-fold (Fig. 1A), whereas a reduction of β_4-integrin expression in MDA-MB-231 cells by shRNA decreased the cell motility by ~40% (Fig. 1B). In both of the $\alpha_6\beta_4$-positive cell lines (MDA-MB-435 β_4 transfectants and MDA-MB-231 green fluorescent protein shRNA infectants), curcumin effectively blocked $\alpha_6\beta_4$-dependent cell motility in a dose-dependent manner (Fig. 1). Significant inhibition of cell motility was

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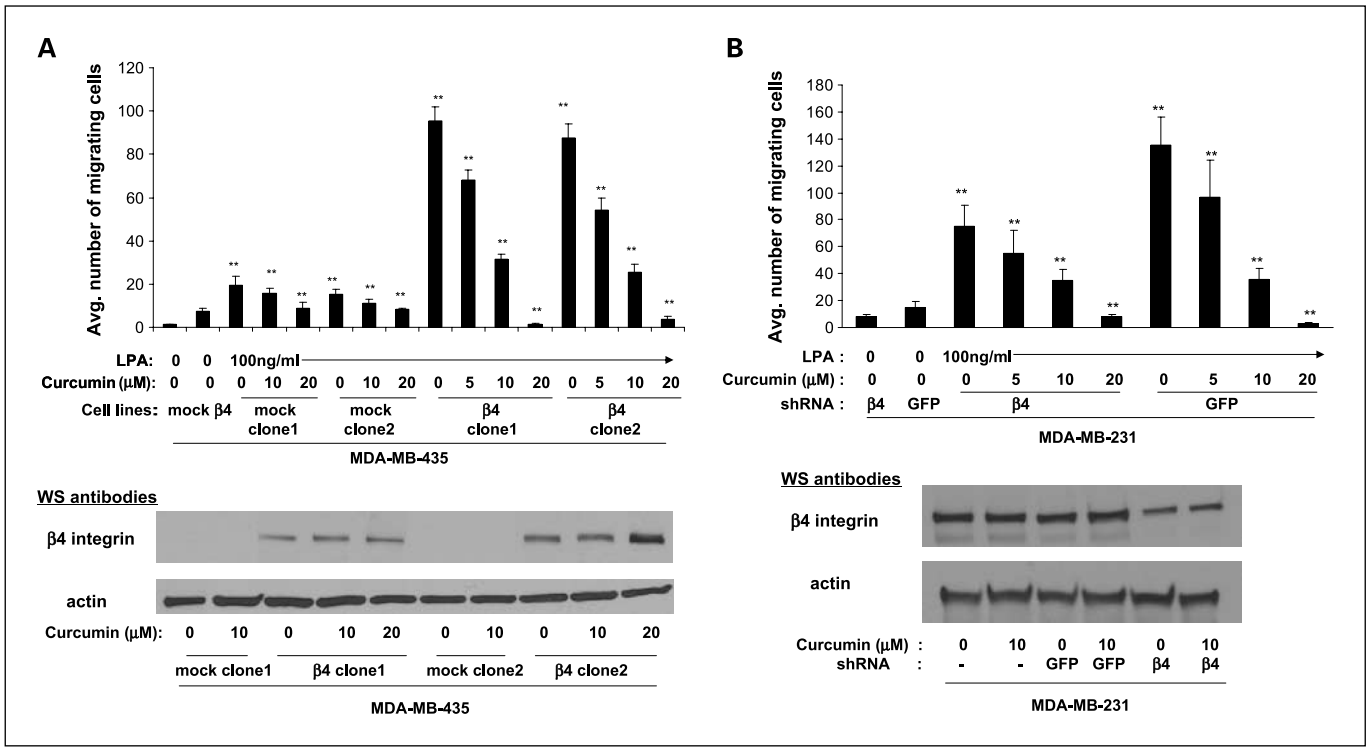

Fig. 1. Curcumin inhibits $\alpha_6\beta_4$-dependent breast cancer cell motility in a dose-dependent manner. The ability of MDA-MB-435 (*A*; mock clone1, 6D2; mock clone2, 6D7; β_4-integrin transfectants clone1, 5B3; and β_4-integrin transfectants clone2, 3A7) and MDA-MB-231 breast carcinoma cell lines [*B*; parental, green fluorescent protein (*GFP*), and β_4 shRNA infectants] to migrate toward 100 nmol/L LPA was measured with a transwell cell motility assay. Cells were pretreated with the indicated dose of curcumin for 3 h before the migration assay. Migration was quantified by counting the cells that migrated to the lower surface of the membrane per square milliliter using bright-field optics. *Columns*, mean from five independent experiments; *bars*, SD. **, $P < 0.01$, versus control groups (without LPA and curcumin treatments). Equal amounts of extracts from each sample were used for Western blot analysis with antibodies against β_4 integrin and β-actin.

observed beginning at a dose of 5 μmol/L curcumin, and cell motility was almost completely blocked at 20 μmol/L (Fig. 1). $\alpha_6\beta_4$-null MDA-MB-435 mock transfectants were barely migratory even in the presence of LPA, which means that we did not see a dramatic inhibitory effect of curcumin either (Fig. 1A). On the other hand, we observed that down-regulation of β_4-integrin expression by 70% (via densitometric analysis of Western blot data, Fig. 1B) with shRNA did not completely block their cell motility toward LPA in MDA-MB-231 cells.

Although curcumin effectively blocked cell motility in both MDA-MB-231 green fluorescent protein and β_4-integrin shRNA expressing cells (Fig. 1B), it is interesting to note that its inhibitory effect is less effective in β_4-integrin shRNA expressing cells. For example, treatment with 10 μmol/L curcumin led to a 77% reduction in motility of green fluorescent protein shRNA expressing MDA-MB-231 cells compared with that of control cells (without curcumin treatment), whereas it blocked 56% of motility of β_4-integrin shRNA expressing MDA-MB-231 compared with that of control cells (Fig. 1B). These results suggest that curcumin may target $\alpha_6\beta_4$ signaling to inhibit cell motility, and $\alpha_6\beta_4$ may sensitize breast cancer cells to curcumin treatment.

Curcumin prevents the $\alpha_6\beta_4$-dependent invasive phenotype of breast cancer cells

Cell motility is an essential component of the invasive phenotype of cancer cells. To obtain more conclusive evidence to determine whether curcumin could blunt breast cancer cell invasion, we used three-dimensional culture matrix systems that provide growth factor–reduced Matrigel to mimic the matrix environments breast cancer cells encounter *in vivo* (Fig. 2A). Expression of $\alpha_6\beta_4$ in MDA-MB-435 cells induced a dramatic neomorphic effect, producing protrusive extensions that invaded basement membrane gels (Fig. 2A). Treatment with curcumin efficiently blocked $\alpha_6\beta_4$-dependent protrusive extension as well as the growth of MDA-MB-435 cells under three-dimensional Matrigel culture (Fig. 2A). Transwell-based invasion assays further confirmed that curcumin blocked the invasion of the three $\alpha_6\beta_4$-positive breast cancer cell lines (MDA-MB-435 β_4 transfectants, MDA-MB-231, and SUM-159) in a dose-dependent manner (Fig. 2B). The invasion of these cell lines was previously shown to depend on $\alpha_6\beta_4$ (20, 21, 35).

Curcumin inhibition of $\alpha_6\beta_4$-dependent breast cancer cell motility and invasion is not due to apoptosis

Considering previous reports that curcumin induces apoptosis of cancer cells depending on the cancer cell type and the dosage of curcumin treatment (16, 24, 36), one concern raised in relation to the cell motility and invasion assays is whether the inhibitory effect of curcumin is related to apoptosis. To address this issue, we conducted an apoptosis assay in MDA-MB-435 β_4-integrin transfectants (Fig. 3A) and MDA-MB-231 cells (Fig. 3B) with the Annexin V-PE Apoptosis Detection Kit. As shown in Fig. 3, there was no significant increase in

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apoptosis in either of these cell lines when treated with doses of curcumin from 5 to 20 μmol/L, within which range we saw an inhibitory effect on cell motility and invasion (Fig. 1). We did observe a detectable increase in apoptosis in these cells on curcumin treatment at concentrations ≥30 μmol/L (Fig. 3). Therefore, we concluded that curcumin inhibits cell motility and invasion at relatively low concentrations (5-20 μmol/L) and that this inhibitory effect is not due to its induction of apoptosis.

Curcumin inhibits the phosphorylation of a key tyrosine residue of the β_4 subunit and $\alpha_6\beta_4$ signaling cascades important for cell motility

To address the mechanism by which curcumin inhibits $\alpha_6\beta_4$ functions such as cell motility and invasion, we assessed whether the inhibition of $\alpha_6\beta_4$ occurs directly at the receptor level. A tyrosine residue (Y1494) in the third fibronectin type III domain of the β_4 cytoplasmic tail has been shown to be essential for initiating $\alpha_6\beta_4$-dependent signaling cascades to promote carcinoma invasion and survival (22, 29). The level of Y1494 phosphorylation is also an indication of the signaling competency of $\alpha_6\beta_4$ (29). Therefore, we tested whether curcumin affects the phosphorylation of this key tyrosine residue of β_4 integrin. As shown in Fig. 4A, the basal phosphorylation level of Y1494 was dramatically reduced by even as low as 5 μmol/L curcumin, suggesting that curcumin directly inhibits $\alpha_6\beta_4$.

Based on a previous report that $\alpha_6\beta_4$ enhances cell motility and invasion through activation of the PI-3K/Akt pathway (20, 24, 35) and up-regulation of cell motility –promoting factors such as ENPP2 (28), we assessed the effect of curcumin on these downstream signaling events of $\alpha_6\beta_4$. Our results indicate that curcumin effectively blocked $\alpha_6\beta_4$-dependent phosphorylation of Akt at Ser^{473} and Thr^{308} (indication of Akt activity) and up-regulation of ENPP2 as assessed by Western blot analysis (Fig. 4B). The data suggest that curcumin directly inhibits $\alpha_6\beta_4$ function and subsequently blocks downstream targets of $\alpha_6\beta_4$. Using quantitative real-time PCR, we further confirmed that curcumin inhibited $\alpha_6\beta_4$-dependent ENPP2 expression, suggesting that ENPP2 expression is regulated by $\alpha_6\beta_4$ and curcumin at the mRNA level (Fig. 4C).

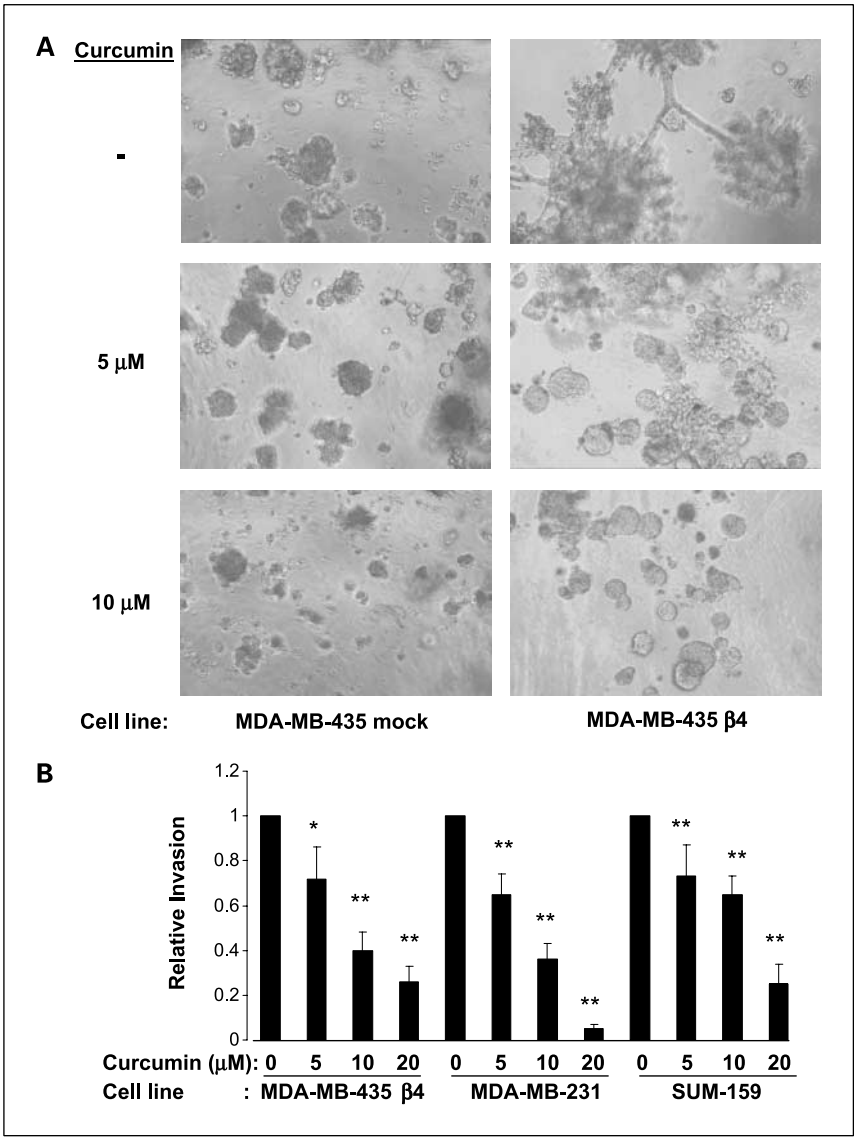

Fig. 2. Curcumin prevents the invasive phenotype of breast cancer cells. *A*, MDA-MB-435 mock and MDA-MB-435 β_4 transfectants were incubated under three-dimensional matrix RGF BME (reduced growth factor basal membrane extracts) with or without curcumin treatment at the indicated concentrations for 7 d. Bright-field images were captured at ×10 magnification. *B*, the ability of MDA-MB-435 β_4 transfectants, MDA-MB-231, and SUM-159 cells to invade Matrigel toward LPA was investigated in a 4-h transwell assay. Before the assay, cells were treated with or without the indicated dose of curcumin for 3 h. *Columns*, mean from five independent experiments; *bars*, SD. *, $P < 0.05$; **, $P < 0.01$, versus control groups (no curcumin treatment).

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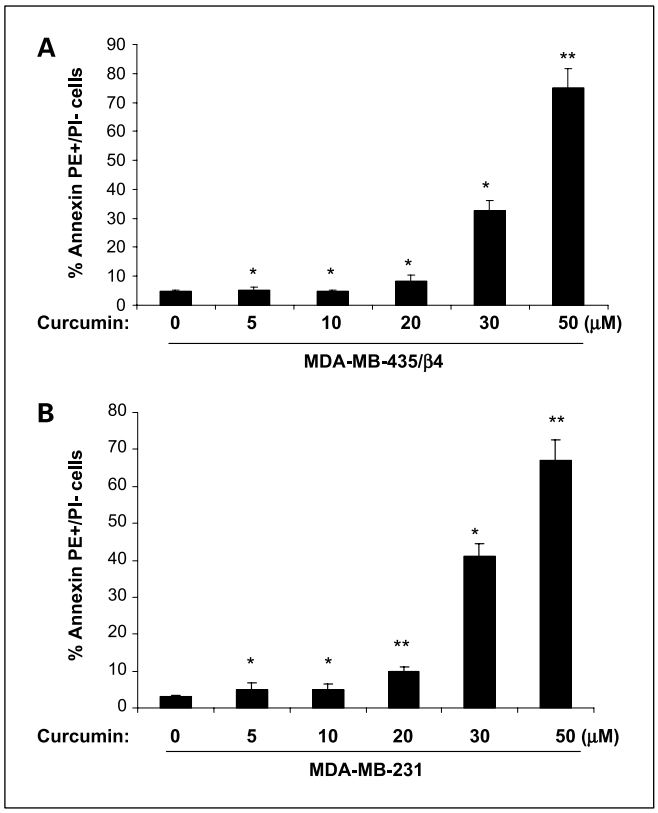

Fig. 3. Curcumin inhibition of $\alpha_6\beta_4$-dependent cancer cell motility is not due to apoptosis. MDA-MB-435 β_4 transfectants (*A*) and MDA-MB-231 cells (*B*) were maintained under regular growth media with or without the indicated dose of curcumin for 12 h. Apoptosis was measured by staining with Annexin V and propidium iodide, followed by fluorescence-activated cell sorting assay. *Columns*, mean from three independent experiments; *bars*, SD. *, $P < 0.05$; **, $P < 0.01$, versus the control group (no curcumin treatment).

The multimodality approach using curcumin with pharmacologic inhibitors of c-Met and Akt enhances inhibition of $\alpha_6\beta_4$-dependent breast cancer cell motility and invasion

Because the combination of chemotherapeutic agents generally results in greater tumor suppressive responses and fewer side effects in cancer patients, we investigated multimodality approaches by combining curcumin with other pharmacologic inhibitors to see whether additive effects occurred in the inhibition of $\alpha_6\beta_4$-dependent cancer cell motility and invasion. We chose a pharmacologic inhibitor against c-Met, the HGF receptor tyrosine kinase, because $\alpha_6\beta_4$ has been shown to functionally interact with c-Met (35, 37). Another target that we chose is Akt, a well-known downstream effector of $\alpha_6\beta_4$ (20, 29). Inhibition of c-Met activity by the c-Met specific inihibitor SU11274 at 5 μmol/L reduced $\alpha_6\beta_4$-dependent cell motility by ~45%, which is similar to its inhibition by 5 μmol/L curcumin in MDA-MB-435 β_4 transfectants (Fig. 5A). However, when these two compounds were used in combination at the above concentration, the inhibitory effect increased to 75% (Fig. 5A). When curcumin was combined with Akt inhibitor, the inhibitory effect increased to 95% (Fig. 5A). The additive effect of curcumin with either SU11274 or Akt inhibitor was also observed to prevent the invasive phenotype of MDA-MB-453 cells in three-dimensional matrix culture (Fig. 5B). Whereas combining curcumin with SU11274 or Akt inhibitor at the indicated dose (Fig. 5) dramatically increased the inhibitory effect on cell motility and invasion, there was no significant increase in apoptosis (data not shown), suggesting that

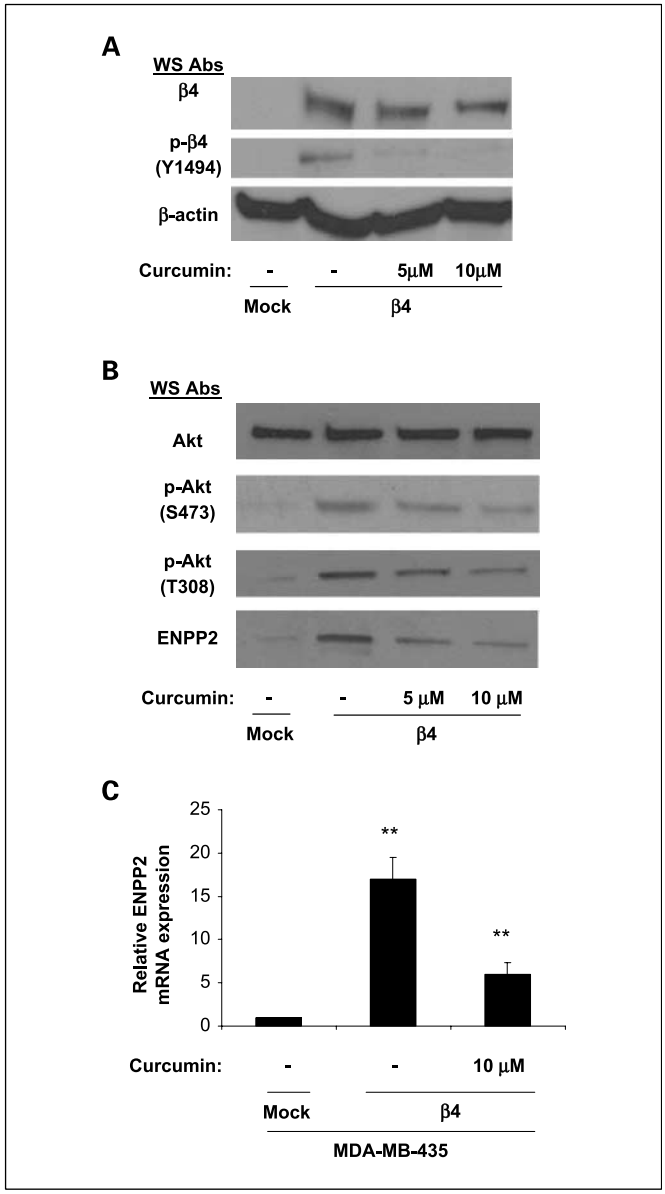

Fig. 4. Curcumin inhibits the phosphorylation of a key tyrosine residue of the β_4 subunit and $\alpha_6\beta_4$ signaling cascades important for cell motility. *A*, MDA-MB-435 β_4 transfectants were pretreated with or without the indicated doses of curcumin for 3 h before lysis with RIPA buffer. Equal amounts of extracts from each sample were used for Western blot analysis with antibodies against β_4 integrin, phospho-β_4-integrin (Y1494), and β-actin. *B*, MDA-MB-435 mock and β_4 transfectants were pretreated with or without the indicated doses of curcumin for 3 h before lysis with RIPA buffer. Extracts from these cells were analyzed for Western blot analysis with antibodies against Akt, phospho-Akt (S473 and T308), and ENPP2. *C*, RNAs were isolated from MDA-MB-435 mock and β_4 transfectants, which were incubated with or without 10 μmol/L curcumin for 12 h. Quantitative real-time PCR reactions to assess ENPP2 were done with 100 ng of RNA for ENPP2 and β-actin (endogenous control). The amount of ENPP2 message was normalized to β-actin levels and reported as a relative value. Representative data of three independent experiments. **, $P < 0.01$, versus the control group (mock control cell line without curcumin treatment).

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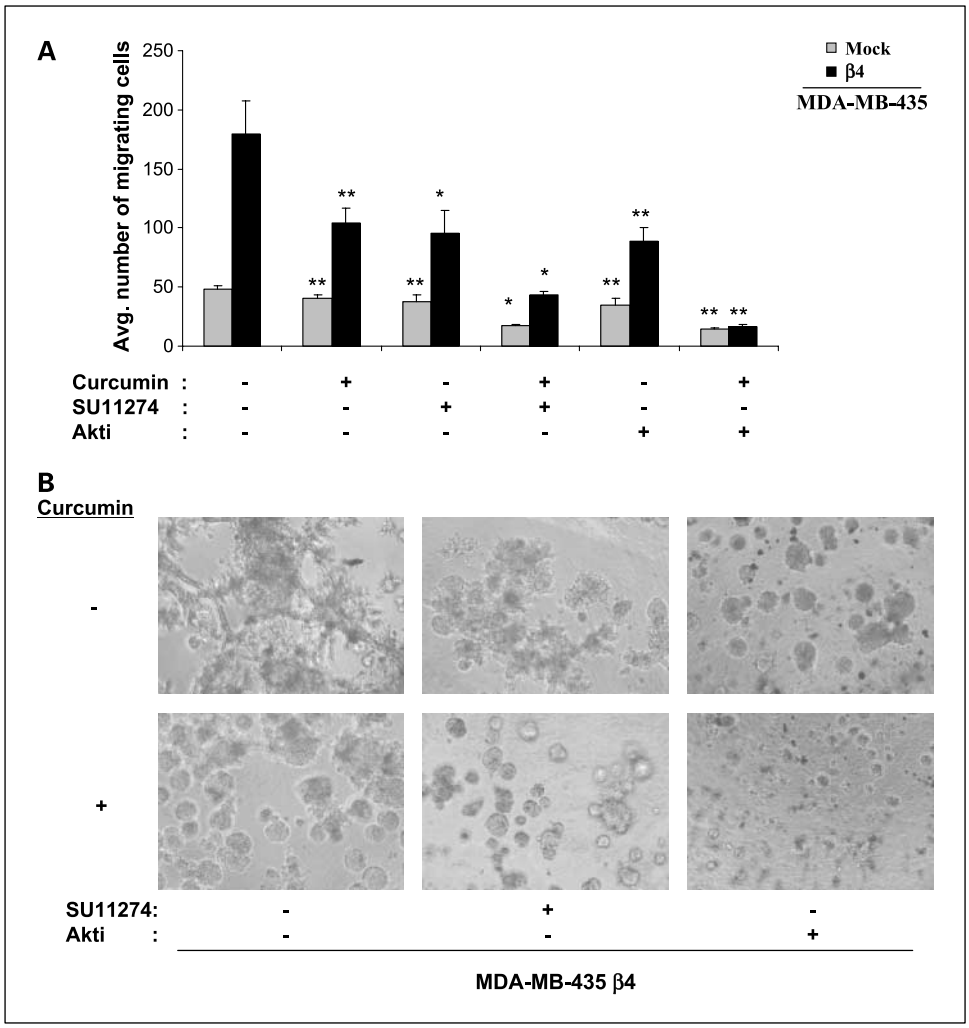

Fig. 5. Multimodality approaches using curcumin to inhibit $\alpha_6\beta_4$-dependent breast cancer cell motility and invasion. *A*, MDA-MB-435 mock and β_4 transfectants were incubated with or without 5 μmol/L curcumin, 5 μmol/L SU11274 (c-Met inhibitor), or 25 μmol/L Akt inhibitor (*Akti*), or the combination of curcumin and SU11274 or curcumin and Akt inhibitor for 3 h. The migration assay was done as described in Fig. 1. *Columns*, mean from five independent experiments; *bars*, SD. *, $P < 0.05$; **, $P < 0.01$, versus control groups (no inhibitors added). *B*, MDA-MB-435 mock and β_4 transfectants were treated as described above and were incubated under three-dimensional matrix RGF BME for 7 d. Bright-field images were captured at ×10 magnification.

the multimodality approach using curcumin may lead to the development of an efficient antimetastatic agent without increased toxicity.

Discussion

Our study establishes a novel mechanism by which curcumin regulates integrin function. Specifically, we have shown that curcumin effectively inhibits cancer cell mobility and invasion toward a chemoattractant in a concentration-dependent manner by suppressing $\alpha_6\beta_4$-dependent Akt activation and expression of the cell motility –promoting factor ENPP2. Even at low doses, curcumin also acts cooperatively with other pharmacologic inhibitors against key signaling effectors of $\alpha_6\beta_4$, such as c-Met and Akt. Considering the fact that $\alpha_6\beta_4$ enhances migration and invasion of aggressive cancer cells, our data suggest a potential role of curcumin as an antimetastatic agent.

High doses (8 g/d) of curcumin can be delivered to patients with virtually no deleterious side effects and were found to generate a 1.77 ± 1.87 μmol/L average peak serum concentration in a phase I clinical trial (38). We found that even at the lower doses (2-5 μmol/L), curcumin effectively reduced cancer cell motility and invasion. Therefore, our finding is encouraging because the low concentrations are well within a range physiologically achievable in cancer patients. The multimodality approach using curcumin (Fig. 5) also suggests the possibility that combination of curcumin with other pharmacologic inhibitors could potentially create effective anticancer therapeutic cocktails in which the overall dose of each component could be reduced enough to lower the risk of side effects.

Whereas our data show that curcumin reduces the phosphorylation of the key tyrosine residue (Y1494) of the β_4-integrin cytoplasmic tail, which is important for its function (28), the mechanism by which curcumin regulates $\alpha_6\beta_4$ function remains to be determined. If curcumin inhibition of $\alpha_6\beta_4$ is direct, it is likely that curcumin inhibits the kinase that phosphorylates Y1494 (identity currently unknown) or activates a phosphatase that dephosphorylates this residue. If the inhibition is indirect, curcumin may regulate the expression of some genes involved in the regulation of $\alpha_6\beta_4$ activity. These possibilities are currently under investigation. Regardless of whether curcumin inhibition of $\alpha_6\beta_4$ function is direct or indirect, its inhibitory effect on cancer cell motility seems to be more pronounced in the $\alpha_6\beta_4$-positive cancer cell lines (Fig. 1). $\alpha_6\beta_4$-negative cancer cell lines such as the MDA-MB-435 mock clone are far less motile and invasive compared with $\alpha_6\beta_4$-positive cancer cell lines (Fig. 1), and the effect of curcumin on their growth or motility was minimal. These

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results suggest the novel possibility that the presence of $\alpha_6\beta_4$ may sensitize cancer cells to curcumin treatment. In other words, frequent up-regulation of $\alpha_6\beta_4$ in cancer cells may explain why curcumin selectively targets cancer cells over normal cells. This possibility will be tested in our future studies.

Based on our findings, we conclude that curcumin may prove to be a potent antimigratory agent that potentially prevents the spread of the breast cancer from its primary origin to distant organs. This activity is associated with the successful inhibition of Akt, the downstream target of $\alpha_6\beta_4$ integrin. Therefore, our findings suggest that curcumin could be used in novel anticancer therapeutics for breast cancer patients whose primary tumors overexpress $\alpha_6\beta_4$ integrin.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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