RESEARCH ARTICLE

Curcumin Enhances Antimigration of Pentagamavunon-1 by Suppressing MMP-2 and MMP-9 Expression in Triple-Negative (4T1) and Luminal A (T47D) Breast Cancer Cells

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Abstract

BACKGROUND: Breast cancer remains the leading cause of cancer death worldwide. Migration and invasion of cancer cells are still crucial stages in the metastasis process, highlighting the urgent need for treatments that target both proliferation and metastatic progression. Curcumin and its synthetic analogue, pentagamavunon (PGV)-1, exhibit antiproliferative effects in breast cancer cells. However, the effects of combining curcumin and PGV-1 on cancer cell migration have not yet been explored. Therefore, this study was conducted to examine the antimigratory effects of curcumin and PGV-1 combination on 4T1 and T47D breast cancer cells.

METHODS: Cytotoxicity effects of curcumin and PGV-1 were examined using an MTT assay to determine their effects on 4T1 and T47D cell viability. The antimigration activity was assessed using a scratch wound healing assay by measuring the closure of artificially created wounds on monolayer cells. Expression of matrix metalloproteinases (MMPs) that play a crucial role in cancer cell migration was analyzed using gelatin zymography to measure their enzymatic activities.

RESULTS: The IC_{50} of PGV-1 and curcumin were 4.88 μM and 37.62 μM in 4T1 cells and 3.16 μM and 23.15 μM in T47D cells, respectively. Furthermore, combination of PGV-1 and curcumin effectively inhibited 4T1 and T47D cell migration. PGV-1 (0.5–2 μM) demonstrated superior antimigratory activity compared to curcumin (5–20 μM) by suppressing MMP-2 and MMP-9 expression in both cell lines. Significantly, curcumin was shown to synergistically enhance the antimigratory effects of PGV-1, leading to a further decrease in MMP-2 and MMP-9 expression.

CONCLUSION: The combination of PGV-1 and curcumin may provide a promising antimigratory agent, potentially leading to enhanced antimetastatic strategies and more efficacious treatments for triple-negative and luminal breast cancer patients.

KEYWORDS: antimigration, curcumin, luminal breast cancer, MMP-2, MMP-9, pentagamavunon-1, triple-negative breast cancer

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Introduction

Breast cancer is the most commonly diagnosed cancer worldwide. In 2020, there were more than 2.3 million new cases and 685,000 deaths due to breast cancer. It is projected that by 2040, the impact of breast cancer will rise to over 3 million new cases and 1 million deaths annually, solely due to population growth and aging.(1) Most women with advanced-stage breast cancer have experienced metastasis of their cancer cells.(1,2) Migration and invasion of cancer cells are crucial stages in the metastasis process, and its

metastasis is a significant cause of treatment failure and poor patient outcomes.(3-5) The metastatic process involves complex molecular mechanisms, such as cell migration, invasion, angiogenesis, and colonization at distant sites.(6-9)

Triple-negative breast cancer (TNBC), which accounts for about 20% of all breast cancer cases, lacks estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2), contributing to its high metastatic potential. Luminal breast cancer, the most common subtype, makes up nearly 70% of cases, and in luminal A breast cancer, ER activation promotes metastasis by increasing matrix metalloproteinase (MMP)-2 expression, a key protein involved in cell invasion.(10) MMPs are enzymes that play a key role in the breakdown of the extracellular matrix (ECM), a key process in cancer development, including breast cancer. Breast cancer cells often increase the expression of MMPs, such as MMP-2 and MMP-9, to facilitate invasion and metastasis by degrading ECM components, such as collagen and gelatin, which support cancer cell migration.(11) Focusing on MMP-2 and MMP-9 is essential to effectively inhibit the migration and metastasis of luminal breast cancer and TNBC cells. These proteases break down ECM, promoting cell invasion and migration.(11,12) Gelatinase, which consists of MMP-2 and MMP-9, is an enzyme from the MMP family that plays a role in collagen degradation. MMP-2 can break down gelatin and type I/IV collagen, and MMP-9 only digests type IV collagen.(11) MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are essential to degrade collagen through gelatin digestion, which is activated after fibrillar collagen denaturation by collagenase (MMP-1).(11) In summary, MMP-2 and MMP-9 play important roles in collagen degradation and growth factor activation, both promoting tissue remodeling and cancer metastasis. Elevated MMP-2 and MMP-9 levels are found in invasive breast cancer types, contributing to increased tumorigenicity in TNBC.(13) These MMPs are also overexpressed in luminal breast cancer, thereby enhancing tumor invasive and metastatic characteristics.

Many studies have used natural agents in monotherapy or in combination with co-chemotherapy to increase anticancer effects (14), such as galangin (15), extract of *Vernonia amygdalina* leaf (16,17), *Citrus sinensis* peel extract (18), and *Chromolaena odorata* (19). As one of the largest turmeric producers, Indonesia provides an abundant supply of curcumin from various species, such as *Curcuma longa*, *Curcuma heyneana*, *Curcuma xanthorrhiza*, and *Curcuma manga*.(20) This abundance offers opportunities to develop curcumin-based products and therapies targeting luminal breast cancer and TNBC. Curcumin, a polyphenolic compound found in turmeric, exhibits antimetastatic properties against various cancer types.(21) It inhibits the activation of signaling pathways that regulate MMP-2 and MMP-9 expression, thereby suppressing their activity. Additionally, curcumin can prevent the migration and metastasis of breast cancer cells by targeting MMPs.(15,22) However, its efficacy is constrained by its low bioavailability.(23-25)

Curcumin analog pentagamavunon (PGV)-1 also has potent antimigration against various cancer cells, including breast cancer cells.(26) It inhibits MMP-9 and suppresses cell migration.(26,27) In addition, PGV-1 induces cell senescence and apoptosis, further intensifying its antimigration activity.(26,28) These findings suggest that similar to curcumin, PGV-1 may help prevent breast cancer cell migration and metastasis by targeting the key signaling pathways involved in these processes.

The combination of PGV-1 and curcumin has a synergistic effect by increasing the efficiency of curcumin in relation to cell cycle arrest, hence inducing mitotic catastrophe in 4T1 (TBNC) and T47D (luminal A) breast cancer cells.(15) Given the critical role of migration in metastasis, understanding the impact of this synergy on breast cancer cell migration is crucial. The relationship between migration and metastasis, especially those involving MMPs, is critical for understanding the mechanisms of cancer progression. MMPs, especially MMP-2 and MMP-9, facilitate cellular matrix degradation and allow cancer cells to migrate (29) and invade surrounding tissues, an essential step in the metastatic cascade. In breast cancer, inhibiting MMP-9 expression and activity significantly reduces cell migration and metastasis. By evaluating the effects of this compound on MMP-2 and MMP-9 expression will provide insights into their potential to inhibit cellular matrix degradation and the migration and metastasis of luminal breast cancer and TNBC cells, thereby offering a promising strategy to suppress the development of metastases in these highly metastatic breast cancer types. Several lines of evidence reported that curcumin and PGV-1 have individual effects on cell migration and matrix degradation (17-19), but their combined impact on these processes, especially in relation to MMP expression, has not been fully explored. It is, therefore, interesting to examine the potential synergistic effects of PGV-1 and curcumin in inhibiting metastasis by influencing migration and the status of MMP-2 and MMP-9 in TNBC and luminal A breast cancer cells.

Methods

Preparation of Curcumin and PGV-1

Curcumin ((1E,6E)-1-(4-hydroxy-3-methoxyphenyl)-7-(3-methoxy-4-methylphenyl) hepta-1,6-diene-3,5-dione) and PGV-1 ((2E,5E)-2-[(4-hydroxy-3,5-dimethylphenyl) methyldene]-5-[(3-methoxy-4,5-dimethylphenyl) methylidene] cyclopentan-1-one) were prepared through a chemical synthesis process that was reported by previous study.(30) Curcumin and PGV-1, with a purity of ≥93% and ≥99.8%, respectively, were obtained from the Cancer Chemoprevention Research Center (CCRC), Faculty of Pharmacy, Universitas Gadjah Mada.(31)

Cell Culture and Treatment

TBNC cell line, 4T1 (CRL-2539TM; ATCC, Manassas, Virginia) was kept in Roswell Park Memorial Institute 1640 (RPMI) media (Cat. No. 31800-022; Gibco, Waltham, MA, USA), while the luminal A breast cancer cell line, T47D (HTB133TM; ATCC) was kept in Dulbecco's modified Eagle's medium (DMEM) media (Cat. No. 11965092; Gibco). Both media were high-glucose culture media that were previously supplemented with sodium bicarbonate (Cat. No. S5761-500G; Sigma-Aldrich, St. Louis, MO, USA), N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (Cat. No. 7365-45-9; Sigma-Aldrich), 10% (v/v) fetal bovine serum (FBS) (Cat. No. 16000-044; Gibco), and 1% penicillin/streptomycin (Cat. No. 15140-122; Gibco). The cells were kept in an incubator with 5% CO2 in a humid environment at 37°C and sub-cultured every 3–4 days. For the selection of individual dose levels, a wide range of 1–100 µM for curcumin and PGV-1 was initially screened. The final dose range was determined based on at least three independent tests to ensure consistency, resulting in a narrower range of 1–10 µM for PGV-1 and 1–100 µM for curcumin. Combination doses were selected based on the sub-IC_{50} levels, specifically ½, ¼, and ⅛ of the IC_{50} values of each compound.

Cell Viability Test

MTT assay was used to determine the viability of the cells. In brief, 4T1 (4×10^3 cells/well) and T47D (7×10^3 cells/well) were incubated in 96-well plates for 24 h at 37°C until they reached 80% confluence. The cells were then incubated for 24 h with various doses of PGV-1 (0.1–10 µM) and curcumin (1–100 µM). After incubation, the cells were treated with 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution (Merck, Darmstadt, Germany) for 4 h and then diluted in a culture medium until formazan crystals had formed. The samples were incubated in the dark overnight at room temperature by adding a 10% solution of sodium dodecyl sulfate (Merck) in 0.01 N hydrogen chloride. Each well's absorbance was measured at 595 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). Three data sets were collected, and IC_{50} was calculated.(32)

Wound Healing Assay

In brief, 4T1 and T47D cells (8.5×10^4 cells/well in 500 µL of complete medium) were seeded into 24-well plates and incubated for 24 h. Cell starvation was induced by replacing the medium with a culture medium containing 0.5% FBS, and cell proliferation was inhibited with mitomycin C treatment. Both procedures were performed for 24 h. Post-starvation, a sterile yellow tip was used to create a scratch wound in the cell monolayer. The cells were then treated with 2 mL of various concentrations, approximately a quarter and a half of IC_{50} of PGV-1 and curcumin. The 4T1 cells were treated with PGV-1 at 1 and 2 µM and curcumin at 10 and 20 µM, while T47D cells were treated with PGV-1 at 0.5 and 1 µM and curcumin at 5 and 10 µM, both as a single treatment and in combination. The cell gap closure was observed, and images were captured at 0, 12, 24, 36, and 48 h post-treatment using an inverted microscope (Olympus, Tokyo, Japan) and a digital camera (Samsung, Seoul, South Korea) at 100× magnification. The wound closure was quantified using ImageJ 1.51j8 software (National Institute of Health, Bethesda, MD, UDA) with Java 1.8.0_1 12, and the results were statistically analyzed with SPSS Statistics 25.0 (IBM Corporation, Armonk, NY, USA).

Gelatinolytic Zymography

The activity of MMP-2 and MMP-9 proteins in 4T1 and T47D culture media was assessed by gelatin zymography. The 4T1 and T47D cells (2.5×10^5) were grown in six-well plates and treated with approximately half of the IC_{50} of PGV-1 and curcumin. The 4T1 cells were treated with PGV-1 at 2 µM and curcumin at 20 µM, while T47D cells were treated with PGV-1 at 1 µM and curcumin at 10 µM, both as a single treatment and in combination for 24 h in a serum-free medium. The culture medium was collected as protein lysate, and the total protein lysate was normalized with the Bradford method and used for gelatin zymography. The medium was collected, and gel electrophoresis (125 volts, 60 mA, 100 min) on an 8% SDS-PAGE gel (Cat. No. 8220501000; Merck), containing 0.1% gelatin (Cat. No. 1610732; Bio-Rad) was performed to determine the activity of MMP-2 and MMP-9 in the culture medium by using a

protein marker ExcelBand™ 3-color Pre-Stained Protein Ladder, High Range (9–245 kDa) (Cat. No. PM5100; SMOBIO, New Taipei City, Taiwan). The gel was then washed and incubated at room temperature with a shaker for 30 min in a denaturing solution with 2.5% Triton mM $CaCl_2$, 0.02% NaN_3 for 30 min, washed, and continued to be incubated with a new buffer for 24 h at 37°C. After removing the process buffer, the gel was stained with 0.5% Coomassie Brilliant Blue R-250 (CBB) (Catalog No. D0770-25G; Sigma-Aldrich) solution for 30 min. The stain was removed with a de-staining solution (20% methanol, 10% acetic acid, and 70% water (1:1:8)) until a clear band appeared on a dark blue background.(33) The gel was visualized using a gel documentation system with a white tray from GelDoc Go Gel Imaging System (Bio-Rad). ImageJ software was employed to calculate band intensities.

Statistical Analysis

Data are expressed as mean±standard error mean (SEM) of three independent experiments. The data were analyzed and visualized using GraphPad Prism 10.3.1 (GraphPad Software, Boston, MA, USA). Statistical analyses were performed using one-way ANOVA followed by Tukey's multiple comparison. Statistical significance was indicated by p-values, with asterisks denoting the levels of significance (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Results

Cytotoxic Effect of Curcumin and PGV-1 on 4T1 and T47D Cells

The findings of single compound cytotoxicity assessment showed that curcumin exhibited more potent cytotoxicity against T47D cells compared with 4T1 cells, with IC_{50} values of 37.62 (Figure 1A) and 23.15 µM (Figure 1C) for 4T1 and T47D cells, respectively. In addition, a single application of PGV-1 to both cell lines showed about seven times stronger cytotoxicity than curcumin, with IC_{50} of 4.88 µM against 4T1 cells (Figure 1B) and 3.16 µM against T47D cells (Figure 1D). These data indicated that PGV-1 has superior cytotoxic activity compared to curcumin in both cell lines. However, these results were limited to short-term exposure (24 h), and further studies with extended incubation times are needed to confirm their cytotoxic effects.

Antimigratory Effects of PGV-1 and Curcumin on 4T1 and T47D Cells

The results of the antimigratory effect of PGV-1 and curcumin on 4T1 and T47D cells were shown in Figure 2A and 2B, respectively. Compared with the untreated control group, PGV-1 significantly inhibited the migration of 4T1 and T47D cells. Curcumin also suppressed the

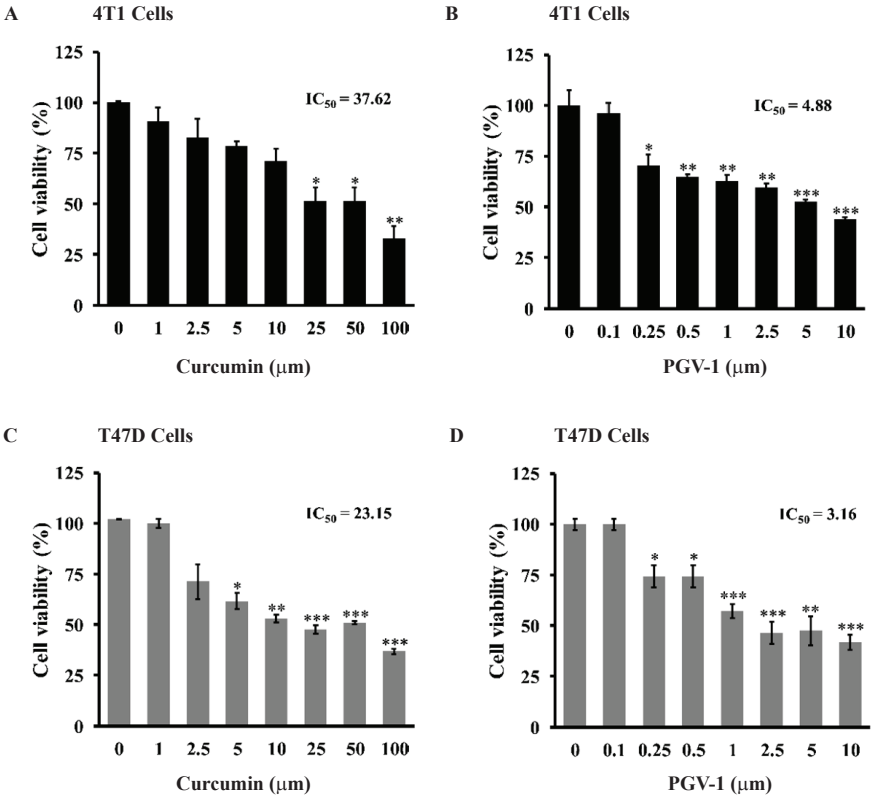

Figure 1. Cytotoxic effects of curcumin and PGV-1 on 4T1 and T47D cells. Cell viability was assessed using the MTT assay after the cells were incubated with the compound for 24 h. A: Viability profile of 4T1 cells after treatment with curcumin. B: Viability profile of 4T1 cells after treatment with PGV-1 C: Viability profile of T47D cells after treatment with curcumin. D: Viability profile of T47D cells after treatment with PGV-1. *p<0.05, **p<0.01, ***p<0.001 were significantly different to untreated cells.

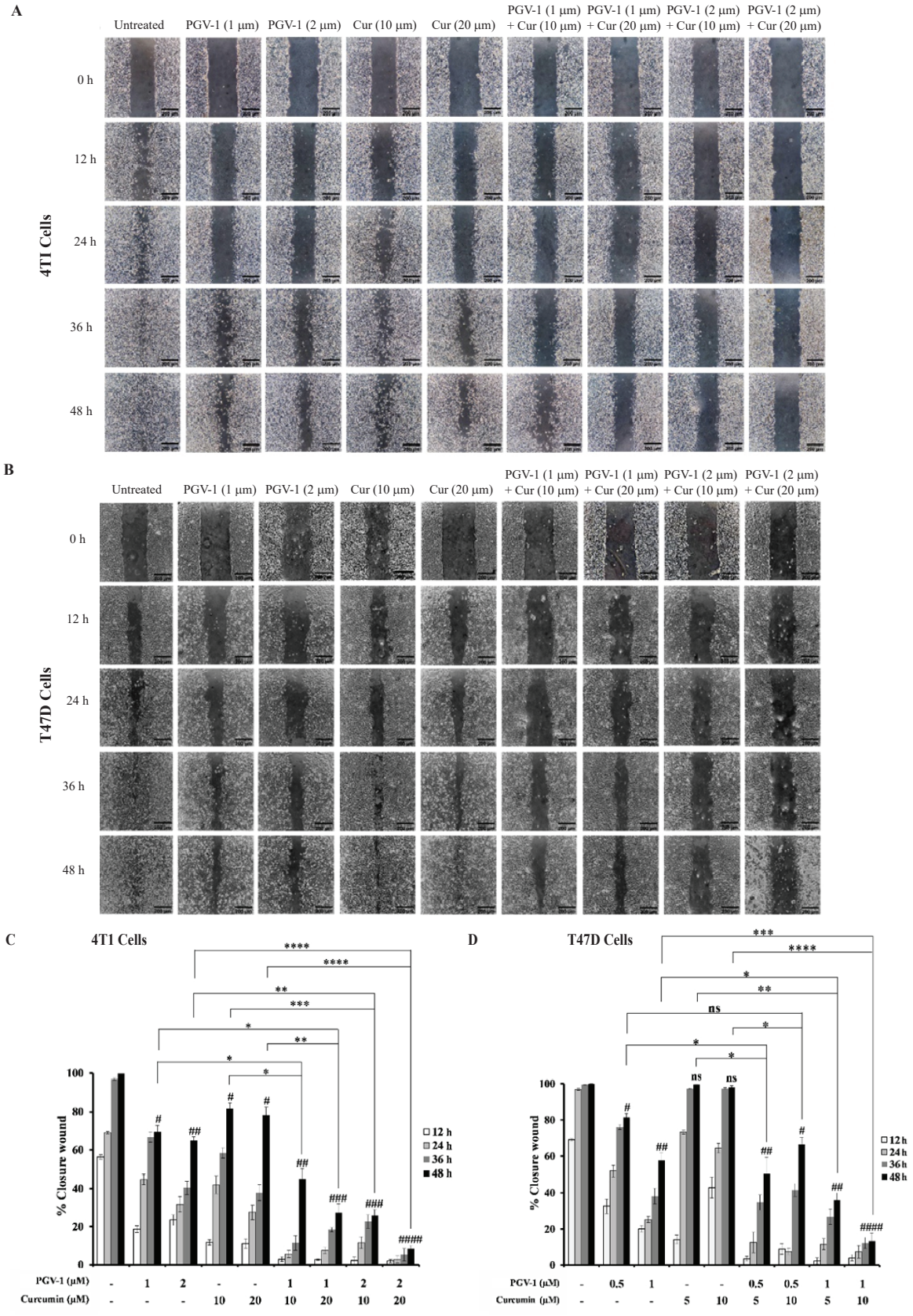

Figure 2. Effect of PGV-1, curcumin, and their combination on cell migration. Both cancerous breast cancer cells, 4T1 and T47D, were scratched and then treated with PGV-1 and curcumin at indicated concentrations, alone and in combination. A: The morphology of the 4T1 cells was observed at 0, 12, 24, 36, and 48 h. B: The morphology of the T47D cells was observed at 0, 12, 24, 36, and 48 h. C: The area of closure of 4T1 cells. D: The area of closure of T47D cells. ($^{\#}$): the significance value of each treatment compared to untreated ($^{\#}p< 0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$; $^{\#\#\#\#}p< 0.0001$; ns: not significant). (*): the significance value of the combination treatment compared to the single treatment (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$; ns: not significant).

migration of 4T1 cells but was less effective on T47D cells. The combination of PGV-1 and curcumin demonstrated a significant migration inhibitory effect on 4T1 cells until around 65% (Figure 2C). Moreover, PGV-1 had a greater antimigratory ability than curcumin on both cell lines within an observation period of 24 h or even up to 48 h. The combination of PGV-1 and curcumin demonstrated potent inhibition in both cell lines compared to when PGV-1 or curcumin were used alone. Among the tested combinations, 2 μM PGV-1 with 20 μM curcumin was the most effective in 4T1 cells (Figure 2C), whereas 1 μM PGV-1 combined with 10 μM curcumi showed optimal results in T47D cells (Figure 2D). These results suggested that the combination of PGV-1 and curcumin exerts a stronger antimigration effect on both TBNC and luminal breast cancer cells than either compound alone. However, the optimal combination was cell line-dependent. Unfortunately, the long-term effects of the compounds on cell migration was not observed in this study.

Effect of PGV-1 and Curcumin on MMP-9 Expression in 4T1 and T47D Cells

This study also investigated the effects of PGV-1 and curcumin on MMP-2 and MMP-9, enzymes critical for the degradation of ECM. MMP-2 and MMP-9 are gelatinases that facilitate ECM breakdown and invasion, thereby enabling cancer cell migration to other tissues. Gelatin zymography was employed to assess the activity of MMPs in cell migration by measuring the expression of MMP-2 (pro: 72 kDa; active: 62 kDa) and MMP-9 (pro: 92 kDa; active: 82 kDa) in degrading gelatin as a substrate, as evidenced by the formation of bands on the gel (Figure 3A–3B). MMP-2 and MMP-9 assays were conducted to evaluate the potential of combined PGV-1 and curcumin treatment in suppressing MMP-2 and MMP-9 expression. We analyzed the expression levels of MMP-2 and MMP-9 in cancer cells treated approximately with half of IC_{50} of PGV-1 and curcumin, both individually and in combination. The results demonstrated that PGV-1, both alone and in combination, significantly affected MMP-9 expression. It exhibited the potential to inhibit the migratory capacity of both cancer cell types. In particular, a single treatment of PGV-1 at a dose of 2 μM in 4T1 cells effectively inhibited MMP-2 and MMP-9 expression by around 70% and 75%, respectively (p<0.01), while curcumin treatment was less effective. Interestingly, the combination of PGV-1 and curcumin has a synergistic effect on suppressing MMP-2 or MMP-9 expression (Figure 3A–3B). On the other hand, in T47D cells, PGV-1 reduced MMP-2 expression by roughly 35% (p<0.01), whereas curcumin alone did not affect MMP-2 or MMP-9 expression. However, when combined with curcumin, there was a marked increase in the inhibition of MMP-2 and MMP-9 expression by approximately 70% and 65%, respectively (p<0.05) (Figure 3C–3D). The combination of PGV-1 and curcumin demonstrated a synergistic effect in reducing MMP-2 and MMP-9 expression across both cell lines, with the inhibitory effect being more pronounced in 4T1 cells than in T47D cells.

Discussion

Metastatic cancer is characterized by cell migration, which is closely associated with the increased expression of MMPs, especially MMP-2 and MMP-9.(29) In breast cancer tissues, MMP-2 and MMP-9 are overexpressed to facilitate cell migration, invasion, and metastasis compared to normal tissues.(34,35) TNBC, which lacks HER2, PR, and ER, has a higher metastatic potential than other breast cancer subtypes.(36) Meanwhile, ER activation in luminal A breast cancer promotes metastasis by increasing invasiveness-associated MMP-2 expression.(37,38) This current study explored the synergistic effects of PGV-1 and curcumin in inhibiting migration of TNBC (4T1) and luminal A (T47D) cells. The selected two cell lines also show high migratory activity and MMP-2 and MMP-9 expression. (37,39) Cytotoxicity assays revealed that PGV-1 exhibited 8–10 times stronger potency than curcumin against both cell lines, with higher sensitivity in T47D cells. These findings align with the previous studies showing cytotoxic effects of PGV-1 and curcumin on TNBC (MDA-MB-231, 4T1) and luminal (MCF-7, T47D) cells.(40,41) Additionally, PGV-1 combined with curcumin synergistically inhibited proliferation, cell cycle progression, and induced apoptosis in breast cancer cells.(15)

PGV-1 effectively inhibits migration in highly metastatic TNBC cells (26), and its combination with hesperidin suppresses T47D migration. (41) In this study, PGV-1 significantly inhibited migration and reduced MMP-2 and MMP-9 expression in both cell lines. In contrast, curcumin selectively inhibited migration and MMP expression in 4T1 cells but not in T47D cells. This difference may be attributed to intrinsic cellular properties, including aggressiveness and receptor profiles. For instance, 4T1 cells are highly aggressive and chemotherapy-resistant, whereas T47D cells exhibit lower migratory activity. Curcumin's limited effect on T47D cells may be due to low MMP expression or receptor-related mechanisms.(42)

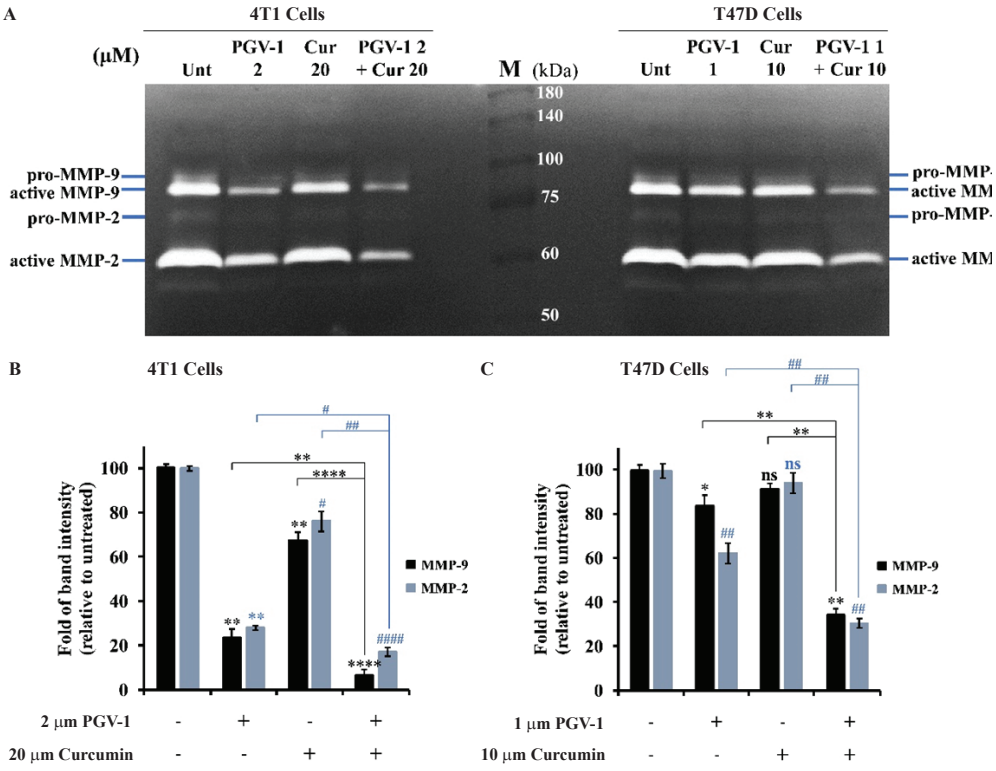

Figure 3. Effect of PGV-1, curcumin, and their combination on MMP-2 and MMP-9 expression in 4T1 and T47D cells. A: MMP-2 and MMP-9 expression were observed by gelatin zymography. B: 4T1 cells (2×10^5 cells/mL) treated with PGV-1 and curcumin alone and in combination. C: T47D cells (2×10^5 cells/mL) treated with PGV-1 and curcumin alone and in combination. The band intensities were quantified using ImageJ (n = 3). (*): the significance value of MMP-9 (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; ns: not significant). ($^{\#}$): the significance value of MMP-2 ($^{\#}p$< 0.05; $^{\#\#}p$<0.01; $^{\#\#\#}p$<0.001; $^{\#\#\#\#}p$< 0.0001; ns: not significant).

The combination of PGV-1 and curcumin demonstrated promising antimigration effects in TNBC and luminal A cells. Specifically, the combination inhibited proliferation, induced apoptosis, reduced migration, and suppressed MMP-2 and MMP-9 expression, with optimal effects observed at concentrations below IC_{50}. MMPs play a crucial role in ECM degradation and migration (11), and their inhibition reduces invasion capacity.(12,27) MMP-9 can be activated by local growth hormones and inflammatory cytokines, such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α, and regulates nuclear factor (NF)-κB, a well-known inducer of MMP formation.(25,37) PGV-1 inhibits NF-κB, sensitizes cancer cells, and reduces Cyclooxygenase-2 (COX-2) and Vascular endothelial growth factor (VEGF) production.(43) Interestingly, the combination treatment showed greater effectiveness in suppressing migration and MMP expression in 4T1 cells compared to T47D cells. This may be attributed to the higher aggressiveness and metastatic potential of TNBC cells, which are more reliant on MMP pathways for their invasive properties. In contrast, luminal A T47D cells exhibit lower metastatic behavior and may be less sensitive to this combination. These findings highlight the higher potential of PGV-1 and curcumin as a therapeutic strategy to limit metastasis, particularly in aggressive TNBC subtypes. Therefore, targeting MMP pathways with PGV-1 and curcumin presents a promising strategy to limit metastasis in aggressive breast cancer.

Despite the promising findings, this study has limitations, including the use of *in vitro* models that do not fully mimic *in vivo* tumor complexity. Additionally, receptor heterogeneity and other metastasis-related pathways, such as epithelial-mesenchymal transition (EMT) and angiogenesis, warrant further exploration. Future studies should validate these results in 3D spheroid and *in vivo* models, address curcumin's bioavailability issues, and investigate the precise molecular mechanisms underlying the synergistic effects of PGV-1 and curcumin. These efforts are essential to advance their development as novel antimetastatic therapies for breast cancer.

Conclusion

This study concluded that curcumin enhances the antimigration effect of PGV-1 by suppressing the expression of MMP-2 and MMP-9 in triple-negative (4T1) and luminal

A (T47D) breast cancer cells. Inhibition of MMP-2 and MMP-9 effectively reduced matrix degradation and cell migration, key processes in metastasis. The combination of PGV-1 and curcumin was most effective at 2 and 20 µM concentrations for 4T1 cells and 1 and 10 µM for T47D cells. Notably, the combination treatment exhibited greater effectiveness in inhibiting migration in the highly aggressive 4T1 cells compared to T47D cells, suggesting its higher potential as an antimetastatic strategy for TNBC therapy. The combination of the two showed synergistic antimigration effects, making it a potential strategy for antimetastatic therapy in highly metastatic breast cancer subtypes.

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Authors Contribution

EM and AN were involved in the concepting and planning, funding acquisition, validation, supervision, also writing-review and editing of the manuscript. DRR conducted the experimental data, calculated and data analysis, as well as drafted and wrote the original manuscript. RIJ performed supervision, validation, review and editing the manuscript. All authors contributed to the critical editing of the manuscript.

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