Clinical and Experimental Health Sciences

Dose-Dependent Antiproliferation and αvβ3 Expression Downregulation by Phenylboronic Acid in SK-OV-3 and MIA-Pa-Ca-2

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ABSTRACT

Objective: Metastasis, a critical process in cancer progression, is mediated predominantly by integrins, including the αvβ3 integrin, which plays a key role in cellular adhesion, migration, and survival. Both cell lines have significant metastatic potential, and αvβ3 integrin plays a role in their interaction with the extracellular matrix and invasiveness. This study investigated the antiproliferative potential of phenylboronic acid in SK-OV-3 ovarian adenocarcinoma and MIA-Pa-Ca-2 pancreatic carcinoma cell lines and its effects on integrin αvβ3 expression.

Methods: SK-OV-3 and MIA-Pa-Ca-2 cells were cultured under standard conditions.

Cells were treated with phenylboronic acid concentrations of 10–500 µM for cell viability analysis (MTT) and 50, 100, 500 µM for immunofluorescence staining analyses. An immunofluorescence assay was performed to assess αvβ3 integrin expression levels, and fluorescence intensity was analyzed as a surrogate marker of protein expression.

Results: MTT analysis showed that the IC_{50} values of PBA after 24 h exposure were 264.8 µM for SK-OV-3 and 294.4 µM for MIA-Pa-Ca-2 cells. Immunofluorescence revealed a dose-dependent decrease in αvβ3 integrin intensity in both cell lines ($p<.0001$). In SK-OV-3 cells, 50 µM PBA reduced fluorescence to ~45% of control, whereas MIA-Pa-Ca-2 required 100–500 µM for similar effects.

Conclusion: This study demonstrated that phenylboronic acid exerts a dose-dependent antiproliferative effect in SK-OV-3 and MIA-Pa-Ca-2 cells and is associated with a concentration-dependent reduction in αvβ3 immunofluorescence signal, particularly at lower doses in SK-OV-3 cells. Given the well-described role of αvβ3 in cell adhesion, migration and metastatic spread, these observations raise the possibility that PBA may influence metastasis-related pathways, a hypothesis that should be tested in future functional studies.

Keywords: Integrin αvβ3, metastasis, MIA-Pa-Ca-2, phenylboronic acid, SK-OV-3

1. INTRODUCTION

More than 90% of cancer-related deaths are caused by metastasis (1). Metastasis is a multi-step process in which normal cells undergo oncogenic transformation, allowing them to evade immune surveillance, proliferate uncontrollably, resist apoptosis, survive in the bloodstream, promote the formation of new blood vessels (angiogenesis), invade surrounding tissues, and ultimately establish secondary tumors in distant organs (2, 3). To prevent metastasis, a comprehensive understanding of the molecular mechanisms underlying each step of the metastatic process is essential. Integrins play a crucial role in this process by mediating cell adhesion, migration, and interactions with the tumor microenvironment (4, 5).

In most solid tumors, the metastatic cascade begins with the breakdown of the underlying basement membrane by cancer cells. Integrins contribute to this process by upregulating the expression of matrix metalloproteinase genes, thereby facilitating protease activation and extracellular matrix degradation. (6). Integrins are transmembrane glycoproteins composed of an α subunit and a β subunit. Among them, β1 and β3 integrins play key roles in the progression of various types of cancer (7, 8). Among β3 integrins, αvβ3 integrin

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exhibits the broadest range of functions. It is implicated in the growth, survival, invasion, and metastasis of various cancer cells. Additionally, αvβ3 serves as a marker of increased lymph node or bone metastasis and is associated with decreased patient survival in certain cancers (9-11). The expression of integrins varies significantly between normal and tumor tissues. For instance, integrins αvβ3, α5β1, and αvβ6 are typically expressed at low or undetectable levels in most adult epithelial tissues but are highly upregulated in certain tumors. Among them, αvβ3 integrin plays a particularly prominent role in promoting tumor progression and metastasis (12).

αvβ3 integrin is a transmembrane receptor that mediates cell adhesion, migration, and signaling by binding to extracellular matrix proteins such as vitronectin and fibronectin. It plays a crucial role in physiological processes, including angiogenesis, wound healing, and bone remodeling, while also contributing to pathological conditions such as cancer progression and metastasis. (13, 14). Extensive evidence supports the clinical and therapeutic significance of αvβ3 integrin in cancer progression, metastasis, and angiogenesis. Studies have shown that targeting αvβ3 integrin with RGD-based nanocarriers enhances drug delivery and therapeutic efficacy, effectively suppressing tumor growth. In melanoma and breast cancer models, αvβ3 integrin is associated with increased cell invasion and adhesion to extracellular matrix components such as vitronectin, highlighting its role in promoting tumor aggressiveness. Additionally, in breast cancer bone metastases, αvβ3 integrin contributes to tumor survival by inducing osteoprotegerin production, a key survival factor within the bone microenvironment (15, 16).

Further investigations into pancreatic cancer have revealed that αvβ3 integrin expression is regulated by ENO1, and its downregulation significantly impairs cancer cell adhesion, invasion, and metastatic potential. In angiogenesis models, αvβ3 integrin blockade suppresses blood vessel formation and tumor growth, further emphasizing its role in tumor vascularization. Collectively, these findings establish αvβ3 integrin as a critical target in cancer diagnostics and treatment, with its involvement in tumor adhesion, invasion, and angiogenesis presenting new opportunities for therapeutic intervention (16, 17).

Current cancer treatments primarily involve the use of chemotherapeutic agents designed to control the rapid proliferation of primary tumor cells (18). This approach often damages normal tissues, leading to severe side effects such as nausea, significant weight loss, fatigue, and immune system suppression (19-21). Additionally, cancer cells often develop mechanisms to evade the effects of treatment, leading to drug resistance (22, 23). These challenges have heightened interest in the discovery of novel chemotherapeutic agents and have popularized the use of naturally derived compounds with anticancer and anti-metastatic properties.

Boron and its derivatives have emerged as promising anticancer agents, with boric acid (BA)—the most abundant physiological form of boron in plasma—demonstrating significant therapeutic potential. Naturally present in fruits, vegetables, and drinking water, boron is a common dietary component, with an estimated human intake of approximately 15 μM. Recent epidemiological, in vitro, and animal studies have highlighted boric acid's potential as a chemotherapeutic agent, owing to its unique biochemical properties.

Boric acid exhibits antioxidant activity by neutralizing reactive oxygen species (ROS), which are often elevated in cancerous environments, and exerts anti-inflammatory effects by modulating cytokine production within the tumor microenvironment. These properties contribute to the inhibition of angiogenesis and tumor cell proliferation, both of which are critical for cancer progression. Additionally, boric acid has shown promise in innovative therapeutic approaches such as boron neutron capture therapy (BNCT), where its selective accumulation in tumor tissues enables precise cancer cell destruction while sparing healthy tissues. As a biologically active compound that is rapidly absorbed and excreted, boric acid's dual role as both a micronutrient and a therapeutic agent underscores its potential in advancing cancer treatment (24-27). These findings highlight the potential of boron and its derivatives as promising candidates for the development of novel anticancer therapies.

Phenylboronic acid (PBA), a derivative of boric acid (BA), binds to cis-diol-containing compounds, including carbohydrates, in aqueous environments through the reversible formation of boronate esters. This property allows PBA to function as a synthetic mimic of lectins. Often referred to as "boronolectins," these compounds are valued for their high stability and selective binding specificity, making them a viable alternative to lectins in chemical applications (28). Structurally, phenylboronic acid consists of a phenyl group attached to a boron atom, which is bonded to two hydroxyl groups (29, 30). In a study on breast and prostate cancer cell lines, phenylboronic acid (PBA) exhibited greater potency than its parent compound, boric acid, in inhibiting cancer cell migration and survival (31).

The SK-OV-3 and MIA-Pa-Ca-2 cell lines represent human ovarian adenocarcinoma and human pancreatic carcinoma, respectively. The SK-OV-3 cell line exhibits significant plasticity in apoptosis pathways, characterized by the downregulation of anti-apoptotic genes such as *BCL2* and *BRAF*. These cells are highly adaptable within the tumor microenvironment, facilitated by the expression of genes involved in DNA repair and survival pathways under stress conditions. Notably, αvβ3 integrin is highly expressed in SK-OV-3 cells and plays a crucial role in mediating adhesion to vitronectin and facilitating extracellular matrix (ECM) degradation through the activation of matrix metalloproteinases (32, 33).

The MIA-Pa-Ca-2 cell line represents one of the most therapeutically challenging models of pancreatic cancer. These cells exhibit high levels of epithelial-mesenchymal transition (EMT) markers and weak cell-cell interactions, both of which contribute to their metastatic potential (34) This cell line exhibits notable methionine dependency, a

hallmark of pancreatic cancer, and shows vulnerabilities in pathways related to oxidative stress and apoptosis (35).

Previous studies have shown that αVβ3 integrin plays a key role in lymph node metastasis in pancreatic carcinoma (9) and that the induction of αvβ3 integrin in SK-OV-3 cells inhibits cell invasion (36). In our study, we evaluated the dose-dependent effects of PBA on αvβ3 integrin immunofluorescence and cell viability in SK-OV-3 and MIA-Pa-Ca-2 cells, with the aim of generating hypotheses about possible links between PBA, αvβ3-related signalling and metastatic pathways.

2. METHODS

2.1. Cell Culture

The SK-OV-3 (HTB-77, ATCC) cell line used in this study is a hypodiploid human ovarian adenocarcinoma line with a modal chromosome number of 43 (range: 42-45) observed in 63.3% of cells and higher ploidy levels in approximately 32%. Characteristic chromosomal abnormalities include del(1)(q21), der(13)t(1;?;13)(q11;?;q34), der(11)t(11;?)(q12), del(10)(q22), and several marker chromosomes. The MIA-Pa-Ca-2 (CRL-1420, ATCC) cell line is a hypotriploid human pancreatic carcinoma line with a modal chromosome number of 61, usually showing 16-20 marker chromosomes, and lacking several normal chromosomes. SK-OV-3 was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with %10 fetal bovine serum (FBS), %2,5 horse serum (HS) and %1 penicilin-streptomycin (PenStrep) solution (37). The human pancreatic carcinoma cell line MIA-Pa-Ca-2 was cultured in RPMI medium supplemented with %10 FBS and %1 PenStrep solution (38). When cultures reached 80–90% confluence, cells were trypsinized using 0.25% trypsin-EDTA (39).

2.2. MTT Cell Viability Assay

The cytotoxic potential of phenylboronic acid (PBA) was evaluated using the MTT colorimetric assay. SK-OV-3 and MIA-Pa-Ca-2 cells were plated in 96-well culture plates at a density of 1×10^4 cells per well and allowed to attach for 24 hours. Once the cells reached approximately 70% confluence, the medium was replaced with fresh medium containing the indicated concentrations (10-50-100-150-200-500 µM) of PBA. PBA stock solutions were prepared in DMSO+PBS and diluted in culture medium; the final vehicle concentration did not exceed 0.1% (v/v) in any well, including the control group. Vehicle-treated cells (medium + 0.1% DMSO) served as the negative control. As a positive control for cytotoxicity, cells were treated with Triton X-100 at a final concentration of 0,01%. Following 24 hours of incubation, the treatment medium was discarded and replaced with MTT reagent (0,5 mg/mL; final dilution 1:20). The cells were then incubated for an additional 3 hours at 37 °C in a humidified 5% CO_2 atmosphere. Subsequently, the MTT solution was removed, and the resulting formazan crystals were solubilized in a 1:1 mixture of DMSO and complete medium. The plates were gently agitated for 1 hour in the dark to ensure complete dissolution. Absorbance was recorded at 570 nm using a microplate spectrophotometer, and half-maximal inhibitory concentration (IC_{50}) values were calculated from the absorbance data using GraphPad Prism version 10.3 (40).

2.3. Immunofluoroscence Assay

SK-OV-3 and MIA-Pa-Ca-2 cells were seeded into 8-well chambers at a density of 2×10^4 cells per well and allowed to attach for 24 h at 37 °C in a humidified 5% CO_2 incubator. After attachment, the medium was replaced with fresh medium containing PBA at 50, 100 or 500 µM to establish the experimental groups. After 24h of PBA incubation, immunofluoroscence assay was conducted as described below.

PBA concentrations were aspirated from the 8-well chambers. For fixation and permeabilization, %100 ice cold methanol was used. After 5 min of ice-cold methanol fixation, the samples were washed with PBS, 3 times. To block non-specific protein binding; %1 BSA, 22 mg/ml glycine and %0,1 Tween 20 containing PBST solution was added to the samples and incubated for 30 min. Subsequently, 1:100 diluted integrin αVβ3 primary antibody (%1 BSA in PBST) was added and incubated in +4 C overnight. The following day, primary antibody solution aspirated and the samples were washed with PBS threetimes. Secondary antibody (1:200) (%1 BSA in PBST) added to the cells and incubated at room temperature for 1h. Then secondary antibody solution aspirated and the cells were washed with PBS three times. For counter-staining, DAPI was added and incubated for 5 min. Then the cells were washed three times with PBS. Following staining procedures, the silicon wells of 8-well chamber were removed using sterileforceps. Aqueous mounting medium added applied tothe slides, which were then mounted using coverslips. Immunofluroscence imaging was conducted with a Zeiss LSM800 confocal microscope using appropriate wavelenghts. For each condition, three images were acquired from non-overlapping fields, and αvβ3 fluorescence was quantified using ImageJ as integrated density (sum of pixel intensities within each region of interest). Regions of interest were drawn over DAPI-positive, morphologically intact cells; integrated density values were background-subtracted and normalised to the number of nuclei in each field, yielding arbitrary units (AU) that minimise the influence of cell number on the readout. These measurements provide a semi-quantitative estimate of αvβ3-associated immunofluorescence signal rather than an absolute expression level (41, 42).

2.4. Statistical Analysis

Experiments were performed in triplicates. Statistical analysis was performed using GraphPad Prism 10.3 software. One-way ANOVA followed by Dunnett's multiple comparisons test versus the vehicle control and the Brown–Forsythe test for homogeneity of variances were carried out. The differences

between PBA concentration groups and the control group were statistically evaluated (43).

3. RESULTS

To define the cytotoxic window of phenylboronic acid (PBA), MIA-Pa-Ca-2 and SK-OV-3 cells were exposed to 10–500 µM PBA for 24 h and viability was quantified by MTT. Four-parameter logistic (4PL) fits to normalized absorbance yielded IC_{50} = 294.4 µM ($logIC_{50}$ = 2.469; R^2 = 0.9801) for MIA-Pa-Ca-2 and IC_{50} = 264.8 µM ($logIC_{50}$ = 2.423; R^2 = 0.9797) for SK-OV-3 (Figure 1A–B). One-way ANOVA indicated a significant effect of dose in both cell lines (Dunnett's multiple comparisons versus vehicle, (****: P <.0001).; Figure 1C–D).

Based on the MTT results, three concentrations of PBA (50, 100 and 500 µM) were selected for αvβ3 immunofluorescence analysis. For each condition, three images were acquired from non-overlapping fields, and fluorescence intensities from 10 regions of interest per image were quantified and compared with the vehicle control. Even at 500 µM, a high-dose and above-IC_{50} condition, a distinct population of DAPI-positive, morphologically intact cells remained and was used for semi-quantitative analysis of αvβ3-associated fluorescence. Although cells were still detectable at 500 µM, this concentration is well above the IC_{50} range (~264–294 µM) and therefore represents a surviving outlier sub-population under highly cytotoxic conditions. These cells may reflect physiologically abnormal or senescent survivors rather than a biologically relevant proliferative response. Quantitative analysis of immunofluorescence intensity showed a significant reduction in αvβ3-associated fluorescence signal in both SK-OV-3 and MIA-Pa-Ca-2 cells compared with the control group. This reduction exhibited a clear dose-dependent relationship, with increasing PBA concentrations corresponding to progressively lower αvβ3 fluorescence intensities (Figures 2 and 3). In the control group, MIA-Pa-Ca-2 cells exhibited higher fluorescence intensity values for integrin αVβ3 expression compared to SK-OV-3 cells. Despite this baseline difference, PBA treatment demonstrated a more pronounced reduction in fluorescence intensity in SK-OV-3 cells than in MIA-Pa-Ca-2 cells. Specifically, a 50 µM PBA concentration reduced fluorescence intensity levels in SK-OV-3 cells to approximately 45% of the control, whereas in MIA-Pa-Ca-2 cells, a comparable reduction was achieved at concentrations between 100 µM and 500 µM. This observation indicates that SK-OV-3 cells exhibit greater sensitivity to PBA, at least in terms of reduction of αvβ3 immunofluorescence signal at sub-IC_{50} concentrations.

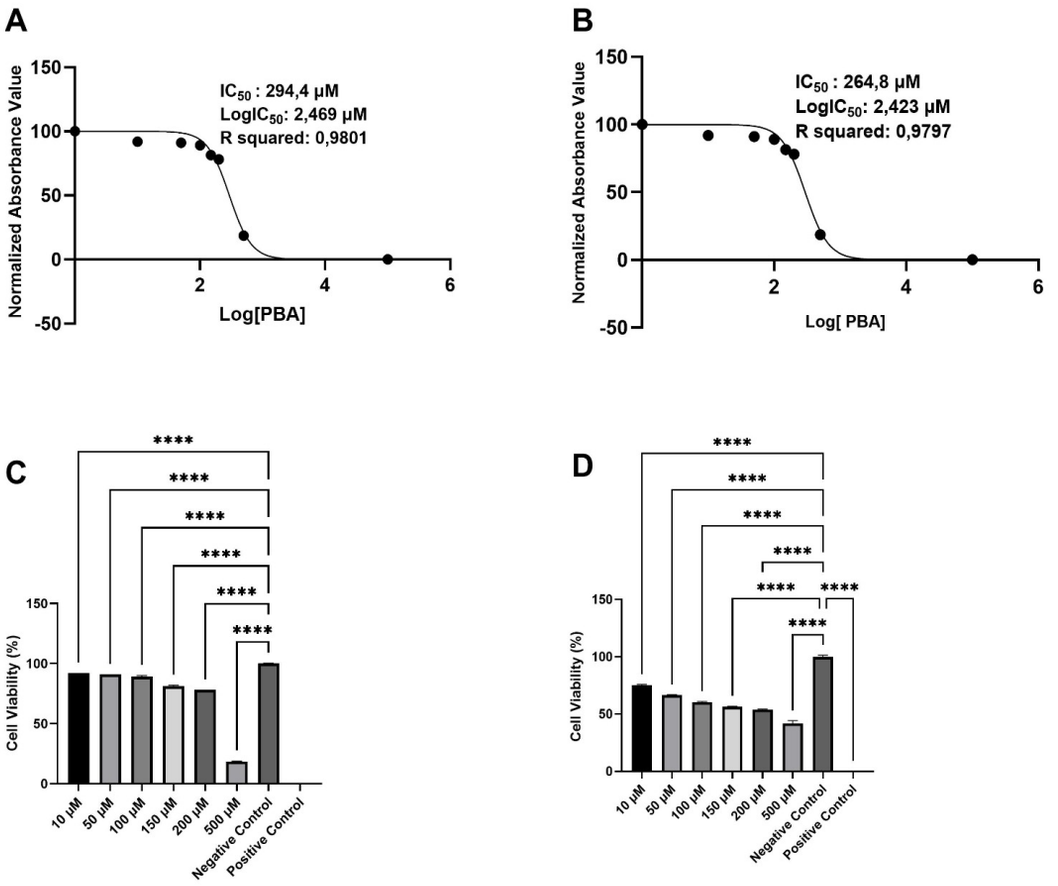

Figure 1. *Effect of PBA on cell viability. (A) MIA-Pa-Ca-2 concentration–response curve (IC_{50} = 294.4 µM; $logIC_{50}$ = 2.469; R^2 = 0.9801). (B) SK-OV-3 concentration–response curve (IC_{50} = 264.8 µM; $logIC_{50}$ = 2.423; R^2 = 0.9797). (C–D) Grouped bar plots for MIA-Pa-Ca-2 (C) and SK-OV-3 (D) showing one-way ANOVA with Dunnett's multiple comparisons versus vehicle (****: P <.0001).*

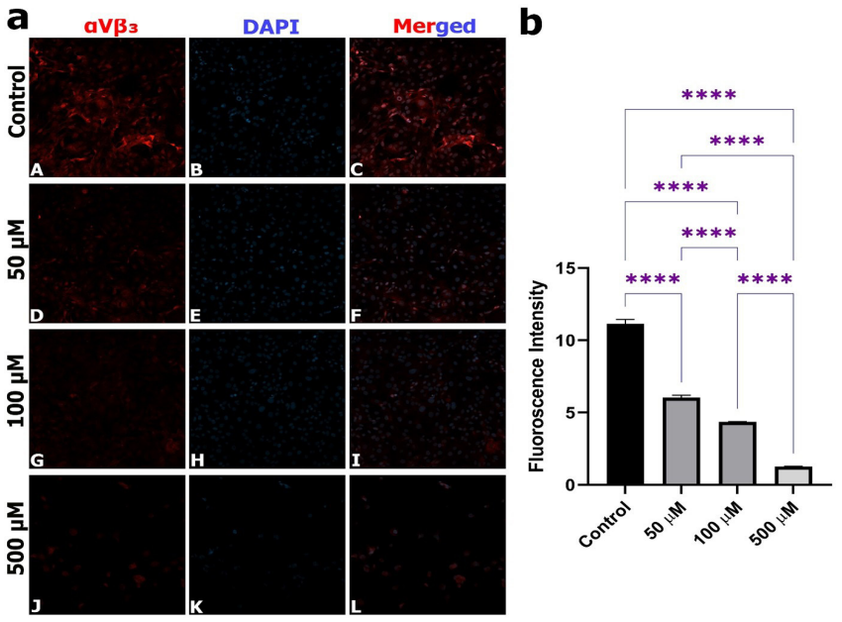

Figure 2. *Effect of the different concentrations of PBA on Integrin αVβ3 levels in SK-OV-3 cells. a) Immunofluoroscence images of SK-OV-3 cells. b) Fluoroscence intensity analysis graphics of SK-OV-3 cells. (****: P <.0001).*

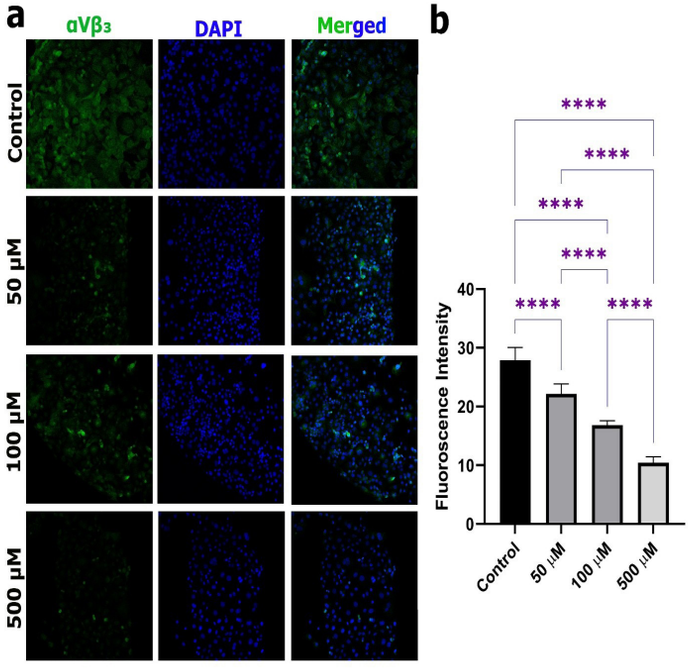

Figure 3. *Effect of the different concentrations of PBA on Integrin αVβ3 levels in MIA-Pa-Ca-2 cells. a) Immunofluoroscence images of MIA-Pa-Ca-2 cells. b) Fluoroscence intensity analysis graphics of MIA-Pa-Ca-2 cells. (****: P <.0001).*

4. DISCUSSION

In this study, we investigated the antiproliferative effects of PBA and its association with αvβ3 integrin immunofluorescence in two cancer types: pancreatic cancer, represented by the MIA-Pa-Ca-2 cell line, and ovarian cancer, represented by the SK-OV-3 cell line. These cancers were selected due to their high prevalence and aggressive nature, making them critical targets for novel therapeutic strategies. Previous studies conducted by Plopper et al. (31) and Marasovic et al. (44) reported that PBA exhibited limited anticancer activity in prostate and mammary gland cancers. Motivated by these findings, we aimed to broaden the scope of investigation by evaluating the effects of PBA on cancers originating from

different organ systems. This approach enabled us to explore whether PBA's antiproliferative activity and its impact on αvβ3-related fluorescence vary depending on cancer type and biological context.

The SK-OV-3 and MIA-Pa-Ca-2 cell lines were selected because both exhibit high basal αvβ3 integrin expression and are widely used models for studying integrin-mediated metastatic signaling. Moreover, as they originate from distinct organ systems—ovarian and pancreatic carcinomas—this design allows evaluation of whether the effects of phenylboronic acid are tumor-type specific or more broadly applicable across different cancer systems.

Before discussing the integrin-related findings, it is important to note that PBA produced a clear, dose-dependent antiproliferative effect in both models, with SK-OV-3 showing slightly greater sensitivity (lower IC_{50}) than MIA-Pa-Ca-2. This pattern aligns with earlier observations for simple phenylboronic acids in epithelial cancers (31, 32, 43) and primarily served in our study to define a sub-cytotoxic working window. Because αvβ3 is most closely linked to migration and invasion rather than growth control (7, 9), our current data should be viewed as descriptive; we did not directly assess migration or invasion. Future work should therefore combine low, sub-cytotoxic PBA concentrations (e.g. within an IC_{10}–IC_{30} window) with dedicated migration and invasion assays, together with orthogonal viability/apoptosis measurements and αvβ3 perturbation (e.g. cilengitide or siRNA), to disentangle survival effects from any potential influence on metastatic behaviour (45).

Our semi-quantitative immunofluorescence results indicated that PBA treatment was associated with a significant, dose-dependent reduction in αvβ3 fluorescence signal in both cancer cell lines (9, 46-49). Notably, the most pronounced inhibitory effect was observed in SK-OV-3 cells treated with 50 µM PBA. Conversely, in MIA-Pa-Ca-2 cells, the fluorescence intensity of integrin αvβ3 gradually decreased with increasing PBA concentrations, suggesting a concentration-dependent effect. These findings highlight the distinct biological responses of the two cell lines to PBA treatment, emphasizing the complexity of cancer biology and the importance of optimizing therapeutic strategies for specific cancer types. These cell-line specific differences may be due to higher basal αvβ3 expression and integrin-dependent survival signaling in SK-OV-3, versus enhanced EMT/metabolic plasticity and possible ENO1-mediated regulation of αvβ3 in pancreatic MIA-Pa-Ca-2 cells (17, 32, 50). A limitation of the current work is the absence of a non-tumorigenic control cell line; future studies should include normal epithelial control lines (e.g., IOSE for ovarian or hTERT-HPNE/HPDE for pancreatic systems) to evaluate the tumor specificity and safety profile of PBA. In addition, although a 24-hour treatment was sufficient to observe dose-dependent changes in αvβ3 integrin expression and cell viability, further studies assessing shorter and longer exposure times (e.g., 12, 48, and 72 hours) are warranted to provide a more comprehensive understanding of the time-dependent effects of PBA. Previous studies have not examined the impact of PBA on the metastatic potential of SK-OV-3 ovarian cancer cells. However, PBA has been shown to exhibit potent antiproliferative and proapoptotic effects in the ovarian cancer cell line A2780 (51). The most recent research on the relationship between SK-OV-3 cells and PBA focuses on the use of PBA-modified conjugated agents in chemo-photothermal therapy (52). To date, there has been no direct investigation into the relationship between PBA and the metastatic potential of MIA-Pa-Ca-2 pancreatic cancer cells. However, PBA-modified nanoparticles have been shown to inhibit metastasis and reduce immune suppression by targeting the PAN02 pancreatic cancer cell line (53).

Over the past century, boronic acid and its derivatives have been extensively studied, with research primarily focusing on their antifungal, antiviral, and antiprotozoal properties. However, their potential in experimental oncology has been largely overlooked. Previously, only a limited number of studies explored the anticancer and antiproliferative effects of phenylboronic acid (PBA), a simple boronic acid derivative. However, interest in this area has significantly increased since the early 2020s. Notably, research suggests that PBA has not been primarily investigated for its direct effects on cancer cell survival or metastasis but has instead gained prominence as a conjugate in drug delivery systems (54-56).

Taken together, our imaging data suggest that PBA is associated with a reduction in αvβ3-related fluorescence in surviving SK-OV-3 and MIA-Pa-Ca-2 cells, consistent with a possible modulation of αvβ3-mediated pathways. However, in the absence of direct migration or invasion assays, these observations remain descriptive and hypothesis-generating rather than proof of anti-metastatic activity and dedicated functional studies will be required to clarify this aspect.

5. CONCLUSION

This study demonstrated that phenylboronic acid treatment was associated with a dose-dependent reduction in αvβ3 integrin immunofluorescence signal and antiproliferative effects in SK-OV-3 ovarian adenocarcinoma and MIA-Pa-Ca-2 pancreatic carcinoma cells, with greater sensitivity observed in SK-OV-3 at lower concentrations. The concomitant decrease in αvβ3-related fluorescence and cell viability suggests that PBA can influence both proliferative and adhesion-related cellular processes, but the present data do not establish direct anti-metastatic activity. Future work incorporating dedicated migration and invasion assays, as well as in vivo models, will be required to determine whether these descriptive changes in αvβ3 translate into meaningful modulation of metastatic behaviour.

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Availability of Data and Materials

Data available on request from the authors.

Artificial Intelligence Statement

Artificial intelligence (ChatGPT 4o via https://chatgpt.com) was utilized to enhance the clarity and fluency of the language during the writing process of this manuscript.

Author Contributions:

Research idea: BKE, MVB.
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Acquisition of data for the study: TYA, AP, KS, MVB.
Analysis of data for the study: TYA, MVB, BKE, AP, KS.
Interpretation of data for the study: TYA, MVB, BKE.
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REFERENCES

[1] Mittal V. Epithelial mesenchymal transition in tumor metastasis. Annual Review of Pathology 2018;13(1):395–412. https://doi.org/10.1146/annurev-pathol-020.117.043854

[2] Welch DR, Hurst DR. Defining the hallmarks of metastasis. Cancer Research. 2019;79(12):3011–3027. https://doi.org/10.1158/0008-5472.CAN-19-0458

[3] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–674. https://doi.org/10.1016/j.cell.2011.02.013

[4] Castaneda M, den Hollander P, Kuburich NA, Rosen JM, Mani SA. Mechanisms of cancer metastasis. Seminars in Cancer Biology. 2022;87:17–31. https://doi.org/10.1016/j.semcancer.2022.10.006

[5] Rørth P. Collective cell migration. Annual Review of Cell and Developmental Biology. 2009;25:407–429. https://doi.org/10.1146/annurev.cellbio.042.308.113231

[6] Munshi HG, Stack MS. Reciprocal interactions between adhesion receptor signaling and MMP regulation. Cancer Metastasis Reviews. 2006;25(1):45–56. https://doi.org/10.1007/s10555.006.7888-7

[7] Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. Nature Reviews Cancer. 2010;10(1):9–22. https://doi.org/10.1038/nrc2748

[8] Howe GA, Addison CL. β1 integrin: an emerging player in the modulation of tumorigenesis and response to therapy. Cell Adhesion & Migration. 2012;6(2):71–77. https://doi.org/10.4161/cam.20077

[9] Hosotani R, Kawaguchi M, Masui T, Koshiba T, Ida J, Fujimoto K, Wada M, Doi R, Imamura M. Expression of integrin alphaVbeta3 in pancreatic carcinoma: relation to MMP-2 activation and lymph node metastasis. Pancreas. 2002;25(2):e30–e35. https://doi.org/10.1097/00006.676.200208000-00021

[10] McCabe NP, De S, Vasanji A, Brainard J, Byzova TV. Prostate cancer specific integrin alphavbeta3 modulates bone metastatic growth and tissue remodeling. Oncogene. 2007;26(42):6238–6243. https://doi.org/10.1038/sj.onc.1210429

[11] Sloan EK, Pouliot N, Stanley KL, Chia J, Moseley JM, Hards DK, Anderson RL. Tumor-specific expression of alphavbeta3 integrin promotes spontaneous metastasis of breast cancer to bone. Breast Cancer Research. 2006;8(2):R20. https://doi.org/10.1186/bcr1398

[12] Zutter MM, Santoro SA, Staatz WD, Tsung YL. Re-expression of the alpha 2 beta 1 integrin abrogates the malignant phenotype of breast carcinoma cells. Proceedings of the National Academy of Sciences of the United States of America. 1995;92(16):7411–7415. https://doi.org/10.1073/pnas.92.16.7411

[13] Danhier F, Le Breton A, Préat V. RGD-based strategies to target alpha(v) beta(3) ıntegrin in cancer therapy and diagnosis. Molecular Pharmaceutics. 2012;9(11):2961–2973. https://doi.org/10.1021/mp3002733

[14] Seftor RE, Seftor EA, Gehlsen KR, Stetler-Stevenson WG, Brown PD, Ruoslahti E, Hendrix MJC. Role of the alpha v beta 3 integrin in human melanoma cell invasion. Proceedings of the National Academy of Sciences of the United States of America. 1992;89(5):1557–1561. https://doi.org/10.1073/pnas.89.5.1557

[15] Liapis H, Flath A, Kitazawa S. Integrin αvβ3 expression by bone-residing breast cancer metastases. Diagnostic Molecular Pathology. 1996;5(2):127–135. https://doi.org/10.1097/00019.606.199606000-00008

[16] Brooks PC, Strömblad S, Klemke R, Visscher D, Sarkar FH, Cheresh DA. Antiintegrin alpha v beta 3 blocks human breast cancer growth and angiogenesis in human skin. Journal of Clinical Investigation. 1995;96(4):1815–1822. https://doi.org/10.1172/JCI118227

[17] Principe M, Borgoni S, Cascione M, Chattaragada MS, Ferri-Borgogno S, Capello M, Bulfamante S, Chapelle J, Di Modugno F, Defilippi P, Nisticò P, Cappello P, Riganti C, Leporatti S, Novelli F. Alpha-enolase (ENO1) controls alpha v/beta 3 integrin expression and regulates pancreatic cancer adhesion, invasion, and metastasis. Journal of Hematology & Oncology. 2017;10(1):16. https://doi.org/10.1186/s13045.016.0385-8

[18] Fallowfield LJ. Evolution of breast cancer treatments: current options and quality-of-life considerations. European Journal of Oncology Nursing. 2004;8(Suppl 2):S75–S82. https://doi.org/10.1016/j.ejon.2004.09.005

[19] Theobald DE. Cancer pain, fatigue, distress, and insomnia in cancer patients. Clinical Cornerstone. 2004;6(Suppl 1D):S15–S21. https://doi.org/10.1016/S1098-3597(05)80003-1

[20] Amado F, Lourenço MTC, Deheinzelin D. Metastatic breast cancer: do current treatments improve quality of life? A prospective study. Sao Paulo Medical Journal. 2006;124(4):203–207. https://doi.org/10.1590/S1516.318.0200600.040.0006

[21] Mattox TW. Treatment of unintentional weight loss in patients with cancer. Nutrition in Clinical Practice. 2005;20(4):400–410. https://doi.org/10.1177/011.542.6505020004400

[22] Perez-Tomas R. Multidrug resistance: retrospect and prospects in anti-cancer drug treatment. Current Medicinal Chemistry. 2006;13(16):1859–1876. https://doi.org/10.2174/092.986.706777585077

[23] Lehnert M. Multidrug resistance in human cancer. Journal of Neuro-Oncology. 1994;22(3):239–243. https://doi.org/10.1007/BF01052927

[24] Rainey CJ, Nyquist LA, Christensen RE, Strong PL, Culver BD, Coughlin JR. Daily boron intake from the American diet. Journal of the American Dietetic Association. 1999;99(3):335–340. https://doi.org/10.1016/S0002-8223(99)00085-1

[25] Kar E, Kar F, Can B, Çakır Gündoğdu A, Özbayer C, Koçak FE, Şentürk H. Prophylactic and Therapeutic Efficacy of Boric Acid on Lipopolysaccharide-Induced Liver and Kidney Inflammation in Rats. Biological Trace Element Research. 2024;202(8):3701–3713. https://doi.org/10.1007/s12011.023.03941-4

[26] Zumreoglu-Karan B, Kose DA. Boric acid: a simple molecule of physiologic, therapeutic and prebiotic significance. Pure

and Applied Chemistry. 2015;87(2):155–162. https://doi.org/10.1515/pac-2014-0909

[27] Khaliq H, Juming Z, Ke-Mei P. The Physiological Role of Boron on Health. Biological Trace Element Research. 2018;186(1):31–51. https://doi.org/10.1007/s12011.018.1284-3

[28] Sanjoh M, Miyahara Y, Kataoka K, Matsumoto A. Phenylboronic acids-based diagnostic and therapeutic applications. Analytical Sciences. 2014;30(1):111–117. https://doi.org/10.2116/analsci.30.111

[29] Nielsen FH. The emergence of boron as nutritionally important throughout the life cycle. Nutrition. 2000;16(7-8):512–514. https://doi.org/10.1016/S0899-9007(00)00324-5

[30] Woods WG. An introduction to boron: history, sources, uses, and chemistry. Environmental Health Perspectives. 1994;102(Suppl 7):5–11. https://doi.org/10.1289/ehp.94102s75

[31] Bradke TM, Hall C, Carper SW, Plopper GE. Phenylboronic acid selectively inhibits human prostate and breast cancer cell migration and decreases viability. Cell Adhesion & Migration. 2008;2(3):153–160. https://doi.org/10.4161/cam.2.3.6484

[32] Reuning U. Integrin αvβ3 promotes vitronectin gene expression in human ovarian cancer cells by implicating Rel transcription factors. Journal of Cellular Biochemistry. 2011;112(7):1909–1919. https://doi.org/10.1002/jcb.23111

[33] Zoń A, Bednarek IA. Hyperthermia potentiates the effectiveness of anticancer drugs—cisplatin and tamoxifen on ovarian cancer cells in vitro. International Journal of Molecular Sciences. 2024;25(24):13187. https://doi.org/10.3390/ijms252413664

[34] Sasaki N, Gomi F, Hasegawa F, Hirano K, Fujiwara M, Toyoda M, Okabe H, Nishida N, Nakashima O, Maehara Y. Characterization of the metastatic potential of the floating cell component of MIA PaCa-2, a human pancreatic cancer cell line. Biochemical and Biophysical Research Communications. 2020;522(4):881–888. https://doi.org/10.1016/j.bbrc.2019.11.120

[35] Morinaga SEI, Han Q, Mizuta K, Kang BM, Bouvet M, Yamamoto N, Hoffman RM. Ivermectin combined with recombinant methioninase (rMETase) synergistically eradicates MiaPaCa-2 pancreatic cancer cells. Anticancer Research. 2025;45(1):97–104. https://doi.org/10.21873/anticanres.17396

[36] Zoppi N, Ritelli M, Salvi A, Colombi M, Barlati S. The FN13 peptide inhibits human tumor cell invasion through modulation of αvβ3 integrin organization and inactivation of the ILK pathway. Biochimica et Biophysica Acta – Molecular Cell Research. 2007;1773(6):747–763. https://doi.org/10.1016/j.bbamcr.2007.02.007

[37] Jabir M, Sahib UI, Taqi Z, Taha A, Sulaiman G, Albukhaty S, Al-Shammari AM, Al-Jubori A. Linalool-loaded glutathione-modified gold nanoparticles conjugated with CALNN peptide as apoptosis inducer and NF-κB translocation inhibitor in SKOV-3 cell line. International Journal of Nanomedicine. 2020;15:9025–9047. https://doi.org/10.2147/IJN.S276714

[38] Khandakar GI, Satoh R, Takasaki T, Fujitani K, Tanabe G, Sakai K, Nakagawa H. ACAGT-007a, an ERK MAPK signaling modulator, in combination with AKT signaling inhibition induces apoptosis in KRAS mutant pancreatic cancer T3M4 and MIA-Pa-Ca-2 cells. Cells. 2022;11(4):627. https://doi.org/10.3390/cells11040702

[39] Wang J, Chen L, Zheng Q, Chen S, Hou Z, Liu P. Indirubin induces apoptosis in ovarian cancer cells via the mitochondrial pathway. American Journal of Translational Research. 2024;16(10):6119–6129. https://doi.org/10.62347/IOFY5604

[40] Acar TY, Bulbul MV, Vatandaslar E, Kurt A, Kalender SM, Keskin I. Linarin inhibits survival and metastasis in triple-negative breast cancer cell line MDA-MB-231 by downregulating NF-κB p-p65/MMP-9, upregulating Bax, and enhancing Caspase 3/7 expression. Natural Product Research. 2025;39(2):1–10. https://doi.org/10.1080/14786.419.2025.2507160

[41] Bulbul MV, Mermer A, Kolbasi B, Kocabas F, Kalender SM, Kirectepe Aydin KA. mTOR pathway inhibition, anticancer activity and in silico calculations of novel hydrazone derivatives in two – and three-dimensional cultured type 1 endometrial cancer cells. Molecules. 2024;17(12):1562. https://doi.org/10.3390/ph17121562

[42] Kalender M, Bulbul MV, Kolbasi B, Keskin I. In 2D and 3D cell culture models, effects of endothelial cells on E-cadherin / β-catenin expression levels and spheroid sizes in Ishikawa cells. Asian Pacific Journal of Cancer Prevention. 2022;23(1):39–51. https://doi.org/10.31557/APJCP.2022.23.1.39

[43] Lončarić M, Stojanović N, Rac-Justament A, Coopmans K, Majhen D, Humphries JD. Talin2 and KANK2 functionally interact to regulate microtubule dynamics, paclitaxel sensitivity and cell migration in the MDA-MB-435S melanoma cell line. Cell & Molecular Biology Letters. 2023;28(1):56. https://doi.org/10.1186/s11658.023.00473-6

[44] Marasovic M, Ivankovic S, Stojkovic R, Djermic D, Galic B, Milos M. In vitro and in vivo antitumour effects of phenylboronic acid against mouse mammary adenocarcinoma 4T1 and squamous carcinoma SCCVII cells. Journal of Enzyme Inhibition and Medicinal Chemistry. 2017;32(1):1299–1304. https://doi.org/10.1080/14756.366.2017.1384823

[45] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983;65(1-2):55–63. https://doi.org/10.1016/0022-1759(83)90303-4

[46] Heyman L, Kellouche S, Fernandes J, Dutoit S, Poulain L, Carreiras F. Vitronectin and its receptors partly mediate adhesion of ovarian cancer cells to peritoneal mesothelium in vitro. Tumour Biology. 2008;29(4):231–244. https://doi.org/10.1159/000152941

[47] Wang H, Xu YH, Guo Y. Novel prognostic marker TGFBI affects the migration and invasion function of ovarian cancer cells and activates the integrin αvβ3-PI3K-Akt signaling pathway. Journal of Ovarian Research. 2024;17(1):50. https://doi.org/10.1186/s13048.024.01377-5

[48] Walsh N, Clynes M, Crown J, O'Donovan N. Alterations in integrin expression modulates invasion of pancreatic cancer cells. Journal of Experimental & Clinical Cancer Research. 2009;28(1):140. https://doi.org/10.1186/1756-9966-28-140

[49] Vannini A, Leoni V, Barboni C, Sanapo M, Zaghini A, Malatesta P. αvβ3-integrin regulates PD-L1 expression and is involved in cancer immune evasion. Proceedings of the National Academy of Sciences of the United States of America. 2019;116(40):20141–20150. https://doi.org/10.1073/pnas.190.193.1116

[50] Hamidi H, Ivaska J. Every step of the way: integrins in cancer progression and metastasis. Nature Reviews Cancer. 2018;18(9):533–548. https://doi.org/10.1038/s41568.018.0038-z

[51] Psurski M, Łupicka-Słowik A, Adamczyk-Woźniak A, Wietrzyk J, Sporzyński A. Discovering simple phenylboronic acid and benzoxaborole derivatives for experimental oncology – phase cycle-specific inducers of apoptosis in A2780 ovarian cancer

cells. Investigational New Drugs. 2019;37(1):35–46. https://doi.org/10.1007/s10637.018.0611-z

[52] Sun P, Hu D, Chen P, Wang X, Shen Q, Chen S. Anti-quenching NIR-II excitation phenylboronic acid modified conjugated polyelectrolyte for intracellular peroxynitrite-enhanced chemo-photothermal therapy. Advanced Science. 2024;11(30):e2309446. https://doi.org/10.1002/advs.202309446

[53] Lu Z, Long Y, Wang Y, Wang X, Xia C, Li M. Phenylboronic acid modified nanoparticles simultaneously target pancreatic cancer and its metastasis and alleviate immunosuppression. European Journal of Pharmaceutics and Biopharmaceutics. 2021;165:164–173.https://doi.org/10.1016/j.ejpb.2021.05.014

[54] angid AK, Kim K. Phenylboronic acid-functionalized biomaterials for improved cancer immunotherapy via sialic acid targeting. Advances in Colloid and Interface Science. 2024;333:103301. https://doi.org/10.1016/j.cis.2024.103301

[55] Solanki R, Parmar B, Jadav M, Pooja D, Kulhari H, Patel S. Berberine encapsulated phenylboronic acid-conjugated pullulan nanoparticles: synthesis, characterization and anticancer activity validated in A431 skin cancer cells and 3D spheroids. International Journal of Biological Macromolecules. 2024;273(Pt 1):132737. https://doi.org/10.1016/j.ijbiomac.2024.132737

[56] Kawish M, Siddiqui NN, Jahan H, Elhissi A, Zahid H, Khatoon B. Targeted pH-responsive delivery of rosmarinic acid via phenylboronic acid functionalized mesoporous silica nanoparticles for liver and lung cancer therapy. Pharmaceutical Development and Technology. 2024;29(6):541–550. https://doi.org/10.1080/10837.450.2024.2356210