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Boric Acid Modulates Hippo, TGF-β/SMAD, and Epigenetic Signaling Pathways in MDA-MB-231 Triple-Negative Breast Cancer Cells

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Abstract

Background: The Hippo signaling pathway plays a critical role in the regulation of cell proliferation, apoptosis, and tumor progression, and its dysregulation has been strongly associated with triple-negative breast cancer (TNBC). Boric acid has been reported to exhibit anti-proliferative and pro-apoptotic effects in various cancer models; however, its effects on Hippo signaling and related molecular pathways in TNBC remain poorly understood. This study aimed to investigate the time-dependent effects of boric acid on Hippo pathway-related gene expression and associated protein markers in MDA-MB-231 breast cancer cells.

Methods: MDA-MB-231 cells were treated with boric acid, and cell viability was assessed using the MTT assay. The half-maximal inhibitory concentration (IC_{50}) was determined and used for subsequent analyses. Expression levels of Hippo pathway-related genes (*YAP, TAZ, TEAD1–4*), TGF-β/SMAD pathway components (*SMAD2, SMAD3*), the chromatin remodeling factor CHD2, and extracellular matrix remodeling genes (*COL1A1, MMP2, TIMP1–3*) were evaluated by quantitative real-time PCR after 24, 48, and 72 h of treatment. Immunocytochemical analyses were performed to assess Cleaved Caspase-3, TNF-α, and TGFβR1 expression.

Results: Boric acid reduced cell viability in a concentration-dependent manner, with an IC_{50} value of approximately 240 µg/mL after 48 h of treatment. Gene expression analyses revealed significant downregulation of *YAP* and *TEAD2* at 24 h, *CHD2* at 48 h, and *TAZ, YAP, SMAD2*, and *CHD2* at 72 h. In contrast, *MMP2* expression was significantly increased at 72 h. Immunocytochemical evaluation demonstrated a time-dependent increase in Cleaved Caspase-3 immunoreactivity, moderate alterations in TNF-α expression, and reduced TGFβR1 staining intensity following boric acid treatment.

Conclusions: These findings indicate that boric acid modulates multiple interconnected signaling pathways in TNBC cells, including Hippo, TGF-β/SMAD, apoptotic, inflammatory, and extracellular matrix remodeling pathways. The observed suppression of YAP/TAZ-associated signaling together with *CHD2* downregulation suggests that both transcriptional and epigenetic mechanisms contribute to the anti-proliferative effects of boric acid. Boric acid may therefore represent a promising modulatory agent for targeting key regulatory networks in aggressive breast cancer subtypes.

Keywords: Boric acid; Triple-negative breast cancer; Hippo signaling pathway; TGF-β/SMAD; Apoptosis

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Borik Asit, MDA-MB-231 Üçlü Negatif Meme Kanseri Hücrelerinde Hippo, TGF-β/SMAD ve Epigenetik Sinyal Yollarını Modüle Eder

Öz

Amaç: Hippo sinyal yolakları, hücre proliferasyonu, apoptoz ve tümör ilerlemesinin düzenlenmesinde kritik bir rol oynar ve bu yolakların düzensizliği, üçlü negatif meme kanseri (TNBC) ile güçlü bir şekilde ilişkilendirilmiştir. Borik asidin çeşitli kanser modellerinde anti-proliferatif ve pro-apoptotik etkiler gösterdiği bildirilmiştir; ancak, TNBC'de Hippo sinyalizasyonu ve ilgili moleküler yollar üzerindeki etkileri henüz tam olarak anlaşılamamaktadır. Bu çalışma, borik asidin MDA-MB-231 meme kanseri hücrelerinde Hippo yolu ile ilgili gen ekspresyonu ve ilişkili protein belirteçleri üzerindeki zaman bağımlı etkilerini araştırmayı amaçlamıştır.

Yöntemler: MDA-MB-231 hücreleri borik asit ile tedavi edildi ve hücre canlılığı MTT testi kullanılarak değerlendirildi. Yarı maksimum inhibitör konsantrasyonu (IC50) belirlendi ve sonraki analizler için kullanıldı. Hippo yolu ile ilgili genlerin (*YAP, TAZ, TEAD1–4*), TGF-β/SMAD yolu bileşenlerinin (*SMAD2, SMAD3*), kromatin yeniden şekillendirme faktörü *CHD2*'nin ve ekstraselüler matris yeniden şekillendirme genlerinin (*COL1A1, MMP2, TIMP1–3*) ekspresyon seviyeleri, tedaviden sonra 24, 48 ve 72 saatlik sürelerde kantitatif gerçek zamanlı PCR ile değerlendirildi. İmmünositokimyasal analizler, Cleaved Caspase-3, TNF-α ve TGFβR1 ekspresyonunu değerlendirmek için yapıldı.

Sonuçlar: Borik asit, hücre canlılığını konsantrasyona bağlı olarak azalttı ve 48 saatlik muamele sonrası yaklaşık 240 µg/mL'lik bir IC50 değeri gösterdi. Gen ekspresyon analizleri, 24 saatte YAP ve TEAD2'nin, 48 saatte *CHD2*'nin ve 72 saatte *TAZ, YAP, SMAD2* ve *CHD2*'nin önemli ölçüde aşağı regüle olduğunu ortaya koydu. Buna karşılık, MMP2 ifadesi 72 saatte önemli ölçüde arttı. İmmünositokimyasal değerlendirme, borik asit muamelesinin ardından Cleaved Caspase-3 immünoreaktivitesinde zamanla artan bir artış, TNF-α ifadesinde orta dereceli değişiklikler ve TGFβR1 boyama yoğunluğunda azalma gösterdi.

Sonuçlar: Bu bulgular, borik asidin TNBC hücrelerinde Hippo, TGF-β/SMAD, apoptoz, inflamatuar ve ekstraselüler matris yeniden şekillendirme yolları da dahil olmak üzere çok sayıda birbirine bağlı sinyal yollarını modüle ettiğini göstermektedir. Gözlemlenen YAP/TAZ ile ilişkili sinyal yolaklarının baskılanması ve *CHD2*'nin aşağı regülasyonu, hem transkripsiyonel hem de epigenetik mekanizmaların borik asidin anti-proliferatif etkilerine katkıda bulunduğunu düşündürmektedir. Bor asidi bu nedenle, agresif meme kanseri alt tiplerinde anahtar düzenleyici ağları hedeflemek için umut verici bir modülatör ajanı temsil edebilir.

Anahtar kelimeler: Borik asit; Üçlü-negatif meme kanseri; Hippo sinyal yolakları; TGF-β/SMAD; Apoptoz.

INTRODUCTION

Breast cancer remains one of the leading causes of cancer-related mortality among women worldwide owing to its molecular heterogeneity and metastatic potential[1,2]. Among its subtypes, triple-negative breast cancer (TNBC) is particularly aggressive and is characterized by poor prognosis, early metastasis, and limited treatment options[2,3]. Therefore, identifying the molecular mechanisms that drive TNBC progression and discovering novel therapeutic targets remain major research priorities.

The MDA-MB-231 cell line is one of the most widely used in vitro models of TNBC. Derived from metastatic breast adenocarcinoma, these cells lack estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression and exhibit highly invasive and mesenchymal-like characteristics associated with enhanced metastatic potential[3–5].

The Hippo signaling pathway is a highly conserved regulatory network that controls cell proliferation, apoptosis, and tissue homeostasis[6,7]. Its principal downstream effectors, Yes-associated protein (*YAP*) and transcriptional co-activator with PDZ-binding motif (*TAZ*), interact with TEA domain (*TEAD1–4*) transcription factors to regulate genes involved in cell growth, survival, invasion, and tumor progression[7,8]. Aberrant activation of

YAP/TAZ signaling has been implicated in several cancers, including breast cancer, where it is associated with tumor aggressiveness and poor prognosis[8,9].

Transforming growth factor-β (TGF-β) signaling also plays a pivotal role in breast cancer progression. While it may function as a tumor suppressor during early stages, it promotes epithelial–mesenchymal transition (EMT), invasion, and metastasis in advanced disease [10,11]. Activation of TGF-β receptors initiates downstream SMAD-dependent and SMAD-independent signaling pathways that contribute to tumor progression[12]. Importantly, accumulating evidence demonstrates functional crosstalk between Hippo and TGF-β signaling, as *YAP/TAZ* cooperate with *SMAD2/3* to regulate genes involved in EMT and metastasis[13,14]. In addition, inflammatory mediators such as tumor necrosis factor-α (*TNF-α*) and apoptotic regulators including caspase-3 (*Cas-3*) contribute to tumor progression and therapeutic responses through interactions with these signaling networks[15].

Boric acid is a biologically active boron compound reported to modulate cell proliferation, oxidative stress responses, and cancer-related signaling pathways[16]. Previous studies have demonstrated dose-dependent anti-proliferative and pro-apoptotic effects of boron-containing compounds in various cancer models[16,17]. However, their effects on Hippo and TGF-β signaling remain poorly understood. Emerging evidence further suggests that epigenetic regulators may participate in these processes. Chromodomain helicase DNA-binding protein 2 (CHD2), a chromatin-remodeling factor involved in transcriptional regulation and genome stability, has recently attracted attention because of its potential role in cancer biology[18].

Therefore, the present study aimed to investigate the time-dependent effects of boric acid on Hippo pathway-related gene expression in MDA-MB-231 breast cancer cells. In addition, the effects of boric acid on TGF-β signaling, apoptosis, inflammation, and the epigenetic regulator CHD2 were evaluated through gene expression and immunocytochemical analyses.

METHODS

Cell Culture

MDA-MB-231 human breast cancer cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 and subcultured at 70–80% confluence using trypsinization.

Boric Acid Preparation

Boric acid (Eti Maden, Türkiye) was sterilized before use and prepared as stock solutions, which were diluted to the required concentrations in culture medium immediately before treatment.

Cell Viability Assay (MTT)

Cell viability was evaluated using the MTT assay. MDA-MB-231 cells were seeded in 96-well plates at a density of 5×10^3 cells/well and incubated for 24 h. Cells were then exposed to serial concentrations of boric acid for 48 h. Subsequently, 15 µL of MTT solution (5 mg/mL; Sigma-Aldrich, USA) was added to each well and incubated for 4 h at 37°C. The medium was removed and formazan crystals were dissolved in 100 µL dimethyl sulfoxide (DMSO; Merck, Germany). Optical density was measured at 570 nm using a microplate reader. Cell viability was expressed relative to untreated controls. Experiments were performed in five independent replicates, and the IC_{50} value was determined by nonlinear regression analysis.

Experimental Design

Following determination of the IC_{50} concentration, MDA-MB-231 cells were treated

with boric acid for 24, 48, and 72 h. Cells cultured under identical conditions without treatment served as controls. At the end of each incubation period, samples were collected for immunocytochemical and gene expression analyses.

Immunocytochemistry

Cells grown on poly-D-lysine-coated coverslips were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and treated with 3% hydrogen peroxide to block endogenous peroxidase activity. After blocking, samples were incubated overnight at 4°C with the following primary antibodies: cleaved Caspase-3 (1:1000; Cat. No. bsm-61090R, Bioss, USA), TGFβR1 (1:100; Cat. No. bs-23445R, Bioss, USA), and TNF-α (1:50; Cat. No. bs-2081R, Bioss, USA). Immunoreactivity was visualized using a biotin–streptavidin-peroxidase detection system and 3,3′-diaminobenzidine (DAB) chromogen. Stained samples were examined under a light microscope[19].

Immunoreactivity was assessed semi-quantitatively according to staining intensity as negative (0), weak (1+), moderate (2+), or strong (3+).

RNA Isolation and cDNA Synthesis

Total RNA was isolated from control and boric acid-treated cells using PureZol™ RNA Isolation Reagent (Bio-Rad, USA; Cat. No. 7326890). RNA concentration and purity were assessed using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific, USA), and only samples with A260/280 ratios between 1.8 and 2.1 were included. Complementary DNA (cDNA) was synthesized from 1 µg total RNA using the RT^2 First Strand Kit (Qiagen, USA) according to the manufacturer's instructions[20].

Quantitative Real-Time PCR Analysis

Gene expression analyses were performed according to the MIQE guidelines. Expression of Hippo pathway-related genes (*YAP, TAZ, TEAD1–4*), TGF-β/SMAD pathway genes (*SMAD2 and SMAD3*), the epigenetic regulator CHD2, and extracellular matrix remodeling genes (*COL1A1, MMP2, TIMP1–3*) was evaluated using RT^2 qPCR Primer Assays (Qiagen, USA) (Table I).

Table I: RefSeq accession numbers and descriptions of target genes used for qRT-PCR analysis

Position	RefSeq Accession No.	Gene Symbol	Description
1	NM_002046	*GAPDH*	Glyceraldehyde-3-phosphate dehydrogenase
2	NM_015472	*TAZ (WWTR1)*	WW domain-containing transcription regulator 1
3	NM_139118	*YAP*	Yes-associated protein 1
4	NM_021961	*TEAD1*	TEA domain transcription factor 1
5	NM_003958	*TEAD2*	TEA domain transcription factor 2
6	NM_003214	*TEAD3*	TEA domain transcription factor 3
7	NM_003213	*TEAD4*	TEA domain transcription factor 4
8	NM_152787	*TAB3*	Transforming growth factor beta- activated kinase 1binding protein 3
9	NM_005901	*SMAD2*	SMAD family member 2
10	NM_005902	*SMAD3*	SMAD family member 3
11	NM_001271	*CHD2*	Chromodomain helicase DNA-binding protein 2
12	NM_000088	*COL1A1*	Collagen type I alpha 1 chain
13	NM_003484	*TIMP1*	Tissue inhibitor of metalloproteinases 1
14	NM_003255	*TIMP2*	Tissue inhibitor of metalloproteinases 2
15	NM_000362	*TIMP3*	Tissue inhibitor of metalloproteinases 3
16	NM_004530	*MMP2*	Matrix metalloproteinase 2

Quantitative real-time PCR was performed using RT² SYBR® Green PCR Master Mix (Qiagen, USA) on a LightCycler® 480 Real-Time PCR System (Roche Diagnostics, Germany). Reactions were carried out in a final volume of 20 µL under manufacturer-recommended cycling conditions, followed by melt curve analysis to confirm amplification specificity. All experiments were performed using at least three independent biological replicates with technical duplicates. No-template and no-reverse transcription controls were included in each run.

Relative gene expression was calculated using the $2^{-}\Delta\Delta Ct$ method. *GAPDH* was used as the reference gene after validation of expression stability. Data normalization and quality control were additionally verified using the GeneGlobe Data Analysis Center (Qiagen, USA)[21].

Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (GraphPad Software Inc., USA). Data are presented as mean ± standard deviation (SD). The normality of data distribution was evaluated using the Shapiro–Wilk test. Graphical representations and IC_{50} calculations were generated using GraphPad Prism. Gene expression analyses were performed using both LightCycler® 480 software and the GeneGlobe Data Analysis Center (Qiagen, USA). Differences were considered statistically significant at $p < 0.05$.

RESULTS

Cytotoxic Effects of Boric Acid on MDA-MB-231 Cells

The cytotoxic effects of boric acid on MDA-MB-231 cells were evaluated using the MTT assay. Boric acid treatment for 48 h reduced cell viability in a concentration-dependent manner, indicating a dose-dependent cytotoxic effect (Figure I). Nonlinear regression analysis of the dose–response curve revealed an IC50 value of approximately 240 µg/mL (~3.88 mM), which was subsequently used for immunocytochemical and gene expression analyses. These findings demonstrate that boric acid exerts significant anti-proliferative effects in this triple-negative breast cancer cell line.

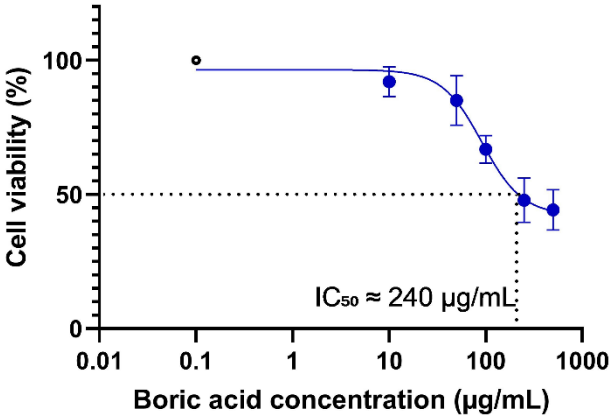

Figure I. Dose–response curve illustrating the effect of boric acid on the viability of MDA-MB-231 cells after 48 h treatment, as determined by the MTT assay. Cell viability is expressed as a percentage relative to untreated controls. Data are presented as mean ± SD (n=5). The IC50 value (~240 µg/mL) was calculated using nonlinear regression analysis.

Time-Dependent Alterations in Hippo, TGF-β/SMAD, and ECM Remodeling-Related Gene Expression

Gene expression analysis normalized to *GAPDH* revealed time-dependent alterations in Hippo signaling, TGF-β/SMAD signaling, epigenetic regulation, and extracellular matrix (ECM) remodeling-related genes following boric acid treatment (Table II).

At 24 h, significant downregulation of key Hippo pathway components was observed. *YAP* expression decreased by 2.66-fold (p=0.032), while *TEAD2* expression decreased by 2.80-fold (p=0.012), suggesting early suppression of Hippo signaling activity. Although *TAZ, TEAD1, TEAD3, TEAD4*, and TGF-β/SMAD pathway genes also exhibited reduced expression trends, these changes did not reach statistical significance (p>0.05).

At 48 h, most genes exhibited modest and non-significant expression changes. However, the

chromatin remodeling-associated gene *CHD2* was significantly downregulated (fold change:−3.33, p=0.025), suggesting the involvement of epigenetic regulatory mechanisms in the cellular response to boric acid.

More pronounced transcriptional alterations were detected at 72 h. Significant downregulation of *TAZ* (fold change:−25.52, p =0.048), *YAP* (fold change:−2.85, p=0.029), and SMAD2 (fold change:−39.76, p=0.041) was observed, indicating coordinated suppression of both Hippo and TGF-β/SMAD signaling pathways. *CHD2* expression remained significantly reduced (fold change:−9.49, p =0.010), supporting a sustained epigenetic response. In contrast, *MMP2* expression was significantly increased (fold change:2.92, p=0.034), indicating activation of ECM remodeling processes under prolonged treatment conditions (Table II).

Table II: Time-dependent gene expression changes in MDA-MB-231 cells following boric acid treatment (GAPDH-normalized)

Gene	24 h	48 h	72 h
TAZ	↓1.45 (p = 0.218)	↓1.87 (p = 0.146)	**↓25.52 (p = 0.048)***
YAP	**↓2.66 (p = 0.032)***	↓1.42 (p = 0.211)	**↓2.85 (p = 0.029)***
TEAD1	↓1.58 (p = 0.174)	↓1.21 (p = 0.356)	↓1.09 (p = 0.412)
TEAD2	**↓2.80 (p = 0.012)***	↓1.36 (p = 0.188)	↓1.75 (p = 0.094)
TEAD3	↓1.33 (p = 0.267)	↓1.18 (p = 0.321)	↓1.41 (p = 0.203)
TEAD4	↓1.71 (p = 0.145)	↓1.92 (p = 0.109)	↓1.12 (p = 0.398)
TGF-β	↓1.22 (p = 0.284)	↓1.09 (p = 0.367)	↓1.35 (p = 0.190)
SMAD2	↓1.54 (p = 0.173)	↓1.66 (p = 0.118)	**↓39.76 (p = 0.041)***
SMAD3	↓1.29 (p = 0.241)	↓1.11 (p = 0.338)	↓1.47 (p = 0.221)
CHD2	↓1.21 (p = 0.301)	**↓3.33 (p = 0.025)***	**↓9.49 (p = 0.010)***
COL1A1	↓1.34 (p = 0.229)	↓1.42 (p = 0.201)	↑1.18 (p = 0.334)
TIMP1	↑1.88 (p = 0.144)	↑2.31 (p = 0.098)	↑2.67 (p = 0.072)
TIMP2	↓1.17 (p = 0.311)	↑1.22 (p = 0.276)	↑1.31 (p = 0.241)
TIMP3	↓1.56 (p = 0.182)	↓1.08 (p = 0.347)	↓1.22 (p = 0.289)
MMP2	↑1.36 (p = 0.265)	↓1.22 (p = 0.233)	**↑2.92 (p = 0.034)***

Relative mRNA expression levels of Hippo pathway (*YAP, TAZ, TEAD1–4*), TGF-β/SMAD pathway (*SMAD2, SMAD3*), chromatin remodeling (CHD2), and ECM remodeling-related genes (*COL1A1, MMP2, TIMP1–3*) following boric acid treatment for 24, 48, and 72 h. Gene expression levels were normalized to *GAPDH* and calculated using the $2^{-}\Delta\Delta Ct$ method. Data are presented as fold change relative to the control group. Statistically significant differences ($^*p<0.05$) are indicated by an asterisk.

Overall, these findings indicate that boric acid exerts time-dependent regulatory effects on multiple signaling pathways in MDA-MB-231 cells, characterized by suppression of proliferative and transcriptional regulators together with selective activation of ECM remodeling-associated genes.

Immunocytochemical Evaluation of Apoptotic, Inflammatory, and TGF-β Signaling Markers

Immunocytochemical analysis demonstrated time-dependent alterations in the expression of Cleaved Caspase-3, TNF-α, and TGFβR1 following boric acid treatment (Figure II, Table III).

Cleaved Caspase-3 immunoreactivity increased progressively in treated cells compared with controls. Staining intensity was scored as moderate positive (2+) at 24 and 48 h and strong positive (3+) at 72 h, indicating enhanced apoptotic activity over time.

TNF-α immunoreactivity exhibited weak-to-moderate staining and showed a mild increase following treatment, particularly at 48 and 72 h, suggesting activation of inflammatory signaling pathways. Staining patterns were heterogeneous among treated cells.

In contrast, TGFβR1 expression showed a progressive decline after boric acid treatment. While control cells consistently exhibited strong immunoreactivity (3+), treated cells displayed moderate staining intensity (2+) at later time points, indicating suppression of TGF-β signaling at the protein level.

Overall, semi-quantitative immunocytochemical evaluation demonstrated a time-dependent increase in Cleaved Caspase-3 expression together with reduced TGFβR1 immunoreactivity, whereas TNF-α exhibited mild-to-moderate alterations during the treatment period. These protein-level findings were generally consistent with the gene expression results.

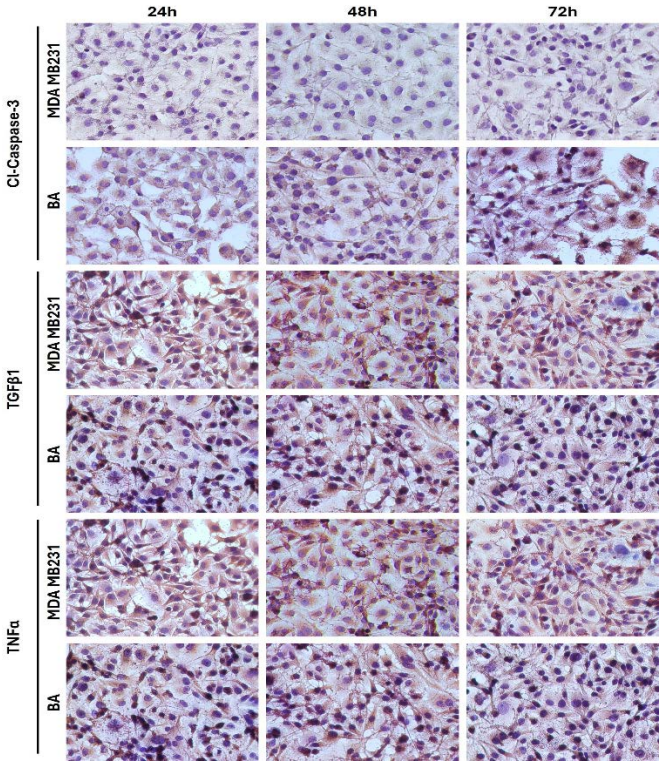

Figure II. Immunocytochemical evaluation of Cleaved Caspase-3, TNF-α, and TGFβR1 expression in MDA-MB-231 cells following boric acid treatment. Representative photomicrographs show control and boric acid-treated cells at 24, 48, and 72 h. (DAB chromogen, hematoxylin counterstain, ×400 magnification).

Table III: Semi-quantitative immunocytochemical scoring of Cleaved Caspase-3, TGFβR1, and TNF-α expression in MDA-MB-231 cells following boric acid treatment

Marker	Group	24h	48h	72h	Overall Interpretation
Cleaved Caspase-3	Control	1+	1+	1+	Minimal staining
	Boric acid	2+	2+	3+	Time-dependent increase in apoptotic activity
TGFβR1	Control	3+	3+	3+	Strong basal expression
	Boric acid	3+	2+	2+	Progressive reduction in immunoreactivity following treatment
TNF-α	Control	1+	1+	2+	Basal inflammatory expression
	Boric acid	1+	2+	2+	Mild-to-moderate increase with heterogeneous staining pattern

Semi-quantitative immunocytochemical evaluation demonstrated a time-dependent increase in Cleaved Caspase-3 immunoreactivity and a decrease in TGFβR1 expression following boric acid treatment. TNF-α showed mild-to-moderate and heterogeneous staining patterns throughout the experimental period.

DISCUSSION

In the present study, we examined the time-dependent effects of boric acid on the viability of MDA-MB-231 triple-negative breast cancer cells, as well as on their gene expression and protein signalling levels. We paid special attention to the Hippo and TGF-β/SMAD pathways. Our results demonstrate the multifactorial biological effects of boric acid, including the inhibition of key proliferative signalling molecules such as *YAP, TAZ* and *TEAD2*, and the downregulation of *SMAD2* and the epigenetic regulator *CHD2*. These transcriptional modifications were associated with increased apoptotic activity and an inflammatory response, as indicated by elevated levels of Caspase-3 and TNF-α immunoreactivity, as well as modulation of extracellular matrix remodelling markers such as MMP2. Overall, our findings suggest that boric acid induces coordinated, time-dependent reprogramming of oncogenic signalling networks in MDA-MB-231 cells.

The somewhat high IC50 value in the present investigation (240 µg/mL; ~3.88 mM) should be taken into account when contemplating the translational implications of our findings. This value was determined under in vitro experimental circumstances and should not be directly compared to therapeutically attainable systemic amounts in vivo. Boric acid is rapidly absorbed and mainly excreted unchanged by the kidneys. The elimination half-life has been estimated to be approximately[21] hours in humans. This implies that systemic exposure is greatly dependent on absorption and renal clearance[22]. However, prior investigations with MDA-MB-231 cells have indicated concentration-dependent suppression of cell growth and induction of apoptotic responses after boric acid exposure, which supports the biological relevance of the present in vitro findings[23]. Thus, the IC_{50} obtained in our work should be more considered as an experimental concentration that defines the cellular response, instead of a dose to be immediately used in the clinic. Further pharmacokinetic and in vivo research are needed to evaluate if safe physiologically effective amounts can be attained in vivo.

According to these results, downregulation of *YAP, TAZ* and members of the TEAD family found after boric acid treatment is in line with the known oncogenic involvement of the Hippo pathway in breast cancer. The Hippo signaling cascade is a major regulator of cell proliferation, apoptosis and tumor advancement, mainly thru its downstream effectors YAP and *TAZ. YAP* and *TAZ* are transcriptional co-activators which regulate genes involved in cell growth, survival and tumor progression[7]. Increased *YAP/TAZ* activity has been associated with increased cellular proliferation, invasion, stemness and tumor aggressiveness in breast cancer 8. Overall, the downregulation of *YAP/TAZ* and *TEAD2* expression in the early phase and the more dramatic inhibition at later time points suggest that boric acid may exert its anti-proliferative effects, at least in part, by modulating Hippo signaling activity.

Importantly, recent studies have highlighted the Hippo pathway as a promising therapeutic target, with several natural and bioactive compounds shown to inhibit *YAP/TAZ* activity and thereby suppress tumor growth[24]. In this context, although the direct effects of boric acid on Hippo signaling have not been extensively characterized, our findings align with the broader concept that small bioactive molecules can interfere with *YAP/TAZ*-mediated

transcriptional programs. The significant downregulation of *YAP* and *TAZ* observed in this study, particularly at 72 hours, supports the hypothesis that boric acid may function as a modulator of oncogenic transcriptional networks.

Mechanistically, *YAP/TAZ* activity inhibition is known to decrease nuclear *TEAD* mediated gene transcription and decrease expression of genes involved in proliferation, survival and metastasis[25]. This is consistent with our observation of reduced *TEAD2* expression at early time points, further supporting the concept of coordinated Hippo pathway suppression. These data collectively suggest that boric acid may have a role in inhibiting tumor-promoting signaling via targeting the *YAP/TAZ–TEAD* transcriptional axis, providing a novel link between boron compounds and Hippo pathway regulation in breast cancer cells that has not been previously investigated.

Significantly, in this study, we observed the downregulation of *YAP/TAZ* and *SMAD2*, which supports the functional cross-talk between the Hippo and TGF-β/SMAD signaling pathways. *YAP/TAZ* have been shown to physically interact with *SMAD2/3* proteins to regulate their nuclear localization and transcriptional activity, and thus modulate TGF-β-dependent gene expression programs[26]. These interactions allow the coupling of mechanical and biochemical signals to modulate processes such as epithelial-mesenchymal transition (EMT), invasion and metastasis in cancer cells[27]. In this context, the marked suppression of both *YAP/TAZ* and *SMAD2* at later time points in our study suggests that boric acid may impair this cooperative signaling axis, potentially impacting SMAD-mediated transcriptional outputs. Moreover, previous studies have shown that *YAP/TAZ* activity can modulate SMAD signaling dynamics, such as nuclear retention and transcriptional efficiency, further highlighting the interdependence of these pathways[28]. Thus, simultaneous dampening of Hippo and TGF-β signaling components described here might be a concerted mechanism by which boric acid inhibits tumor-promoting signaling pathways.

In addition to the transcriptional changes, immunocytochemistry analyses provided further insight into the cellular response induced by boric acid treatment. In treated MDA-MB-231 cells, a time-dependent increase in Cleaved Caspase-3 immunoreactivity was observed, indicating the activation of apoptotic pathways. This result is in agreement with the known function of Caspase-3 as a major executioner protease in apoptosis and supports the anti-proliferative effects seen in the viability assays[27]. In parallel, TNF-α immunoreactivity showed mild-to-moderate increases following boric acid exposure, particularly at later time points, suggesting the involvement of inflammatory signaling mechanisms. Although the magnitude of TNF-α induction was limited, previous studies have highlighted the importance of cytokine-mediated inflammatory signaling, including TNF-α, in regulating tumor progression, immune modulation, and the breast cancer microenvironment[28].

Interestingly, the concomitant increase in TNF-α immunoreactivity and suppression of *YAP/TAZ* expression may suggest a functional link between inflammatory and Hippo signaling pathways[28]. Together, these findings indicate that boric acid modulates multiple interconnected pathways involved in cell survival, inflammation, and tumor progression.

Another notable finding of this study was the significant downregulation of *CHD2* following boric acid treatment, suggesting the involvement of epigenetic regulatory mechanisms. *CHD2* is a chromatin-remodeling ATPase that contributes to transcriptional regulation by maintaining chromatin accessibility and proper nucleosome organization[29,30]. Previous studies have

demonstrated that *CHD2* depletion is associated with reduced expression of actively transcribed genes and the establishment of a more repressive chromatin environment[30]. In this context, the sustained suppression of *CHD2* observed in our study may have contributed to the downregulation of oncogenic signaling components, including members of the Hippo and TGF-β/SMAD pathways. Although direct mechanistic links between CHD2 and these pathways remain poorly defined, our findings suggest that epigenetic modulation may represent an additional mechanism through which boric acid exerts its anti-tumoral effects in breast cancer cells.

CONCLUSION

In summary, the current study shows that boric acid has significant time-dependent anti-proliferative effects on MDA-MB-231 triple-negative breast cancer cells via the modulation of several interconnected signalling pathways. Boric acid treatment down-regulated the epigenetic regulator *CHD2* and suppressed the key components of the Hippo and TGF-β/SMAD pathways including *YAP, TAZ, TEAD2* and *SMAD2*. These molecular changes were correlated with increased apoptotic activity as indicated by increased Caspase-3 immunoreactivity, modulation of inflammatory signalling as indicated by TNF-α expression and changes in extracellular matrix remodelling including up-regulation of *MMP2*.

Taken together, these data suggest a concerted reprogramming of oncogenic signalling networks at the transcriptional, protein and epigenetic levels induced by boric acid. The multiple effects of its biological actions in triple-negative breast cancer cells are explained by the simultaneous regulation of Hippo, TGF-β/SMAD, apoptotic/inflammatory/extracellular matrix remodelling pathways. There remains a need for additional mechanistic and in vivo validation studies, but our findings offer novel evidence in support of the potential for boric acid as a modulator of critical regulatory networks in aggressive breast cancer subtypes, in particular, triple-negative breast cancer.

Ethics Committee Approval: This study did not require ethical approval, as it was based solely on commercially available established cell lines and did not involve human participants, human-derived samples or experimental animals.

Conflict of Interest: The author(s) declare that there is no financial conflict of interest related to this article.

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REFERENCES

1. Jemal A, Siegel R, Sung H, et al. Cancer statistics, 2025. CA Cancer J Clin. 2025; 75(1): 10-45.

2. Pratelli G, Carlisi D, Di Liberto D, et al. MCL1 Inhibition Overcomes the Aggressiveness Features of Triple-Negative Breast Cancer MDA-MB-231 Cells. Int J Mol Sci. 2023; 24(13): 11149.

3. Arroyo-Crespo JJ, Armiñán A, Charbonnier D, et al. Characterization of triple-negative breast cancer preclinical models provides functional evidence of metastatic progression. Int J Cancer. 2019; 145(8): 2267-81.

4. Cailleau R, Young R, Olive M, et al. Breast tumor cell lines from pleural effusions. J Natl Cancer Inst. 1974; 53(3): 661-74.

5. Huang Z, Yu P, Tang J. Characterization of Triple-Negative Breast Cancer MDA-MB-231 Cell Spheroid Model. Onco Targets Ther. 2020; 13: 5395-405.

6. Pan D. The Hippo signaling pathway in development and cancer. Dev Cell. 2010; 19(4): 491-505.

7. Yu FX, Zhao B, Guan KL. Hippo pathway in organ size control, tissue homeostasis, and cancer. Cell. 2015; 163(4): 811-28.

8. Zanconato F, Cordenonsi M, Piccolo S. YAP/TAZ at the roots of cancer. Cancer Cell. 2016; 29(6): 783-803.

9. Harvey, K. F., Zhang, X., & Thomas, D. M. The Hippo pathway and human cancer. Nature reviews. Cancer. 2013; 13(4), 246–57.

10. Massague J. TGFbeta in cancer. Cell. 2008; 134(2): 215-30.

11. Ikushima H, Miyazono K. TGFbeta signalling: a complex web in cancer progression. Nat Rev Cancer. 2010; 10(6): 415-24.

12. Varelas X. The Hippo pathway effectors TAZ and YAP in development, homeostasis and disease. Development. 2014; 141: 1614-26.

13. Liu H, Sun M, Wu N, et al. TGF-β/Smads signaling pathway, Hippo-YAP/TAZ signaling pathway, and VEGF: their mechanisms and roles in vascular remodeling related diseases. Immun Inflamm Dis. 2023; 11:e1060.

14. Zhang J, Wu H, Ren X, et al. Hippo/YAP signaling's multifaceted crosstalk in cancer. Front Cell Dev Biol. 2025; 13:1595362.

15. Li J, Chen B, Huang Y, et al. TNF-α modulates cell proliferation via SOX4/TGF-β/Smad signaling in benign prostatic hyperplasia. Cell Death Dis. 2025; 16:472.

16. Pizzorno L. Nothing Boring About Boron. Integr Med (Encinitas). 2015; 14(4): 35-48.

17. Scorei RI, Popa R Jr. Boron-containing compounds as preventive and chemotherapeutic agents for cancer. Anticancer Agents Med Chem. 2010; 10(4): 346-51.

18. Marfella CG, Imbalzano AN. The CHD family of chromatin remodelers. Mutat Res. 2007; 618 (1-2): 30-40.

19. Sarıbaş GS, Turna Saltoğlu G, Yalçın Azarkan S, et al. Effects of irinotecan (CPT-11), ellagic acid and combination of both on HeLa cells in 2D and 3D culture. Cyprus J Med Sci. 2023; 8: 173-83.

20. Akalın H, Bilgiç B, Avcil S, et al. Low expression levels of MAOA and TPH1 genes may represent risk factors in boys with autism spectrum disorder-a case-control study. Int J Dev Neurosci. 2025; 85: e70073.

21. Bustin SA, Benes V, Garson JA, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009; 55: 611-22.

22. Schou JS, Jansen JA, Aggerbeck B. Human pharmacokinetics and safety of boric acid. Arch Toxicol Suppl. 1984; 7: 232-5.

23. Bayram D, Özgöçmen M, Çelik DA, et al. Does boric acid inhibit cell proliferation on MCF-7 and MDA-MB-231 cells in monolayer and spheroid cultures by using apoptosis pathways? Biol Trace Elem Res. 2024; 202: 2008-21.

24. Elsori D, Pandey P, Alshaikh K, et al. Targeting Hippo-YAP/TAZ signaling pathway: an updated review demonstrating the therapeutic potential of key plant derived anticancer compounds. Front Pharmacol. 2026; 16: 1743102.

25. Li X, Zhuo S, Zhuang T, et al. YAP inhibits ERα and ER+ breast cancer growth by disrupting a TEAD-ERα signaling axis. Nat Commun. 2022; 13: 3075.

26. Labibi B, Bashkurov M, Wrana JL, et al. Modeling the control of TGF-β/Smad nuclear accumulation by the Hippo pathway effectors TAZ/YAP. iScience. 2020; 23(8): 101416.

27. Dou H, Yu PY, Liu YQ, et al. Recent advances in caspase-3, breast cancer, and traditional Chinese medicine: a review. J Chemother. 2024; 36(5): 370-88.

28. Ciurescu S, Buciu V, Serban D, et al. Role of cytokines in breast cancer: a systematic review and meta-analysis. Biomedicines. 2025; 13(9): 2203.

29. Liu JC, Ferreira CG, Yusufzai T. Human CHD2 is a chromatin assembly ATPase regulated by its chromo- and DNA-binding domains. J Biol Chem. 2015; 290(1): 25-34.

30. Siggens L, Cordeddu L, Ronnerblad M, et al. Transcription-coupled recruitment of human CHD1 and CHD2 influences chromatin accessibility and histone H3 and H3.3 occupancy at active chromatin regions. Epigenetics Chromatin. 2015; 8: 4.