Antibiotic-derived approaches in cancer therapy: effectiveness of ikarugamycin in hexokinase-2 inhibition, tissue factor modulation, and metabolic regulation in breast cancer

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We aimed to explore the role of ikarugamycin (IKA) in breast cancer, its connection with hexokinase-2 (HK-2) repression, and tissue factor (TF). This study sought to extend the role of HK-2 as a TF activator in a comprehensive analysis of these interactions from the enzyme, gene, and protein levels. The investigation was performed with MDA-MB-231 and MCF-7 breast cancer lines. The oxidative stress index (OSI), lactate production, and HK activity were assessed using colorimetric assays. Western blot and quantitative PCR analyses were performed to determine HK-2 and TF expressions. Prothrombin time Tests additionally assessed the effect of IKA therapy on TF activation. Three over four significantly downregulated genes were identified after a specific analysis of the IKA's effect on HK-2 and TF in breast cancer cell lines. In the IKA treatment group, lactate production was markedly reduced ($P < 0.05$) and hexokinase activity was found to be reduced in all groups ($P < 0.05$, <0.01). Paclitaxel cytotoxicity independently causes lower OSI in all IKA-treated groups as compared to controls even though OSI is elevated in IKA groups compared to control. Molecular analysis results demonstrated significantly downregulated HK-2 and TF expressions at the protein level ($P < 0.05$, $P < 0.01$). Partial thromboplastin time results also showed that IKA-treated cells had longer TF activation duration. A potential indirect association of HK-2 inhibition and TF regulation in breast cancer cells is put forward in this study by presenting IKA's bioactivation of breast cancer in all gene, protein, and enzyme levels. *Anti-Cancer Drugs* **36: 328–337**

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Introduction

Breast cancer (BC) pathogenesis is driven by complex molecular mechanisms, with recent evidence emphasizing the critical roles of lactate production and hexokinase-2 (HK-2) expression. Elevated lactate levels, a hallmark of cancer metabolism shaped by the Warburg effect, are strongly associated with aggressive BC phenotypes [1]. The Warburg effect, which reflects the preference of cancer cells for aerobic glycolysis, even under oxygen-rich conditions, drives HK-2 overexpression. This, in turn, enhances glycolytic activity, fuels tumor growth, and contributes to therapy resistance. Understanding the dynamic relationship between lactate metabolism and HK-2 expression may provide valuable opportunities for developing targeted therapeutic strategies to combat BC progression [2,3].

Tissue factor (TF) is an important parameter of HK-2 in cancer metabolism. TF influences cancer cell proliferation, invasion, metastasis, and angiogenesis through coagulation-dependent and coagulation-independent pathways. In one of the coagulation-dependent mechanisms, TF ultimately leads to the production of fibrin and allows vascular endothelial cells to adhere to the fibrin matrix, facilitating their migration and contributing to angiogenesis [4].

TF also promotes angiogenesis through the secretion of angiogenic cytokines such as vascular endothelial growth factor and interleukin-8 (IL-8) via protease-activated receptors (PARs). Activation of PARs also leads to the generation of PAR-activated macrophages, which can misdirect the immune response, further strengthening the relationship between TF and cancer cells [5,6].

Moreover, TF creates a protective niche for tumor cells by shielding them from immune cells, including natural

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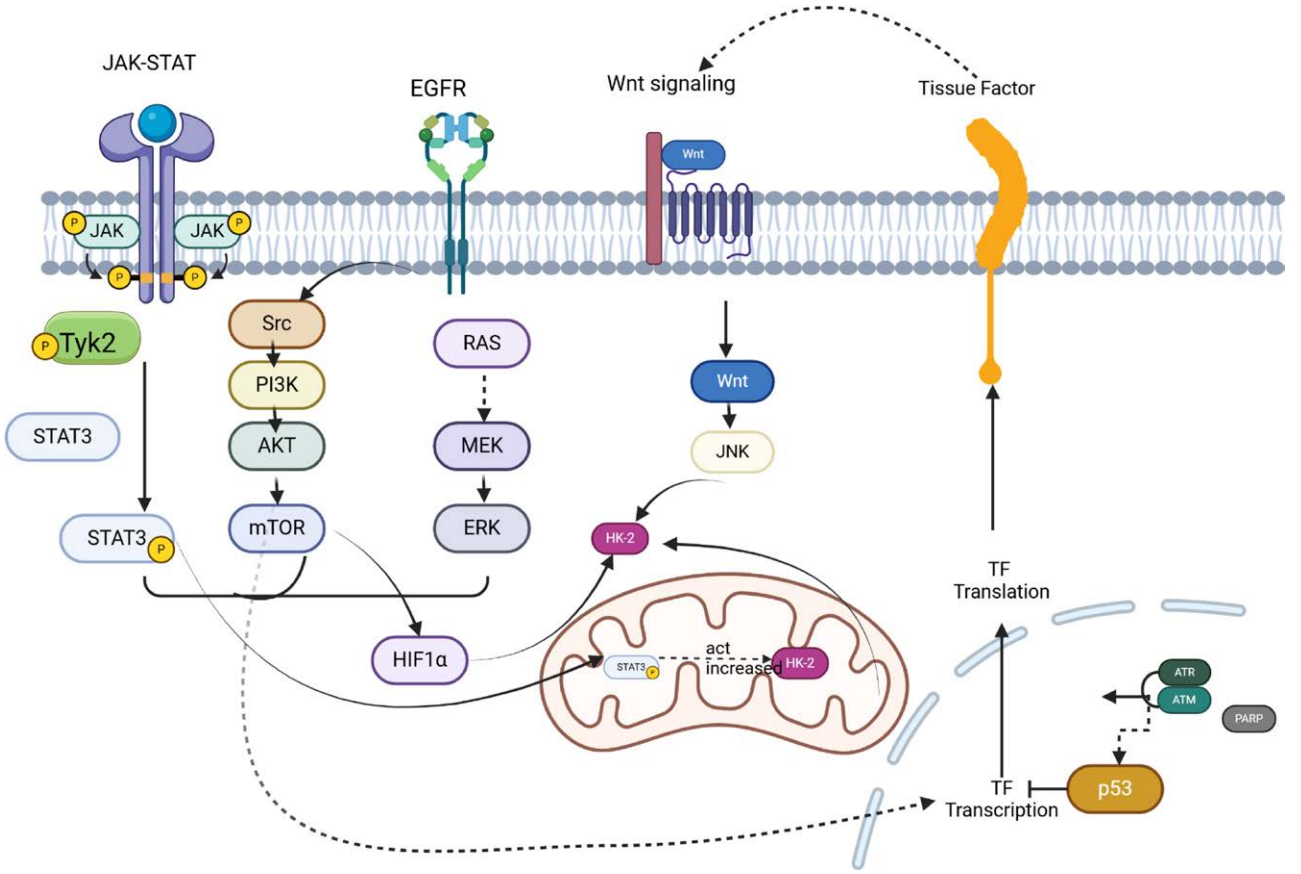
Fig. 1



This schematic illustrates the interconnected signaling pathways involving HK-2 and TF in cancer metabolism and progression. The JAK/STAT pathway influences both TF and HK-2 through direct and indirect mechanisms, including STAT3 activation, which directly interacts with HK-2 on the mitochondrial membrane. The EGFR pathway activates HK-2 via the Akt/mTOR axis and enhances TF transcription through HIF-1α-mediated mechanisms. Additionally, the mTOR pathway contributes to increased TF transcription, which remains unchecked in aggressive cancer types with p53 mutations, leading to TF overexpression. Wnt signaling is depicted as directly activating HK-2, while increased TF expression is shown to stimulate Wnt signaling, forming a feedback loop. These pathways collectively highlight the strong correlation between HK-2 and TF, which play critical roles in tumor progression, angiogenesis, and metastasis. AKT/mTOR, protein kinase B/mammalian target of rapamycin; EGFR, epidermal growth factor receptor; HK-2, hexokinase-2; JAK, Janus Kinase; STAT, Signal transducer and activator of transcription proteins; TF, tissue factor.

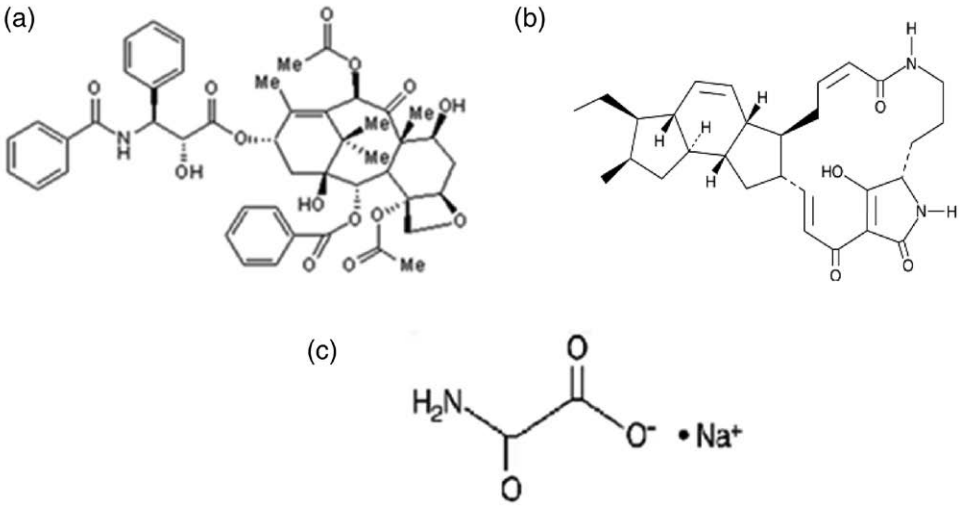
killer cells. This protection allows tumor cells to circulate freely, enhancing their invasive and metastatic potential. These multifaceted mechanisms underscore the critical role of TF in cancer progression and highlight their importance as therapeutic targets [7].

HK-2, involved in cancer energy metabolism and TF regulation, involves numerous known and unknown signaling pathways, some of which are depicted in Figure 1 [8–14]. For example, the Janus kinase (JAK)/signal transducer and activator of transcription proteins (STAT) pathway exerts both direct and indirect effects on the TF and HK-2 in cells. STAT3 molecules, upon activation, translocate to the cell membrane, where they are phosphorylated either by JAKs or tyrosine kinases, depending on the cell type and signal intensity. Activated STAT3 molecules can directly bind to and activate HK-2 on the mitochondrial membrane [15,16].

Additionally, the epidermal growth factor receptor pathway influences HK-2 activation through both positive (protein kinase B/mammalian target of rapamycin) and negative (mitogen-activated protein kinase kinase/extracellular signal-regulated kinase) subpathways. Specifically, mTOR activates HK-2 via HIF-1α while simultaneously enhancing TF transcription in the nucleus. This increased TF transcription, especially in aggressive cancer types, is accompanied by p53 mutations, leading to overexpression of TF. Similarly, Wnt signaling has been observed to directly activate HK-2. It is also noteworthy that increased TF expression can trigger Wnt signaling activation, further reinforcing the interplay between these pathways [17–19].

These observations strongly support the hypothesis of a robust correlation between HK-2 and TF through multiple mechanisms. Understanding these interconnected pathways provides critical insights into their roles in

Fig. 2



(a) Paclitaxel molecule (2α,4α,5β,7β,10β,13α)-4,10-Bis(acetyloxy)-13-{[(2R,3S)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl]oxy}-1,7-dihydroxy-9-oxo-5,20-epoxytax-11-en-2-yl benzoate. (b) Ikarugamycin molecule; (2R,3R,7Z,14S,19E)-3-ethyl-2,3,3aS,5aR,5bS,6,10,11,12,13,14,15,20aS,21,21aR,21bR-hexadecahydro-22-hydroxy-2-methyl-14,17-metheno-17H-as-indaceno [3,2-k][1,6]diazacycloheptadecine-9,16,18(1H)-trione. (c) Sodium oxamate molecule (oxalic acid monoamide, oxamic acid, CAS Number: 565-73-1) | Cayman chemical).

cancer progression and highlights potential therapeutic targets.

Oxidative stress plays a critical role in cancer development and progression by promoting oncogenic transformation and supporting tumor survival. It arises from an imbalance between pro-oxidants and antioxidants, leading to cellular damage, including impaired DNA, proteins, and membranes. Reactive oxygen species disrupt essential cellular functions, particularly DNA integrity, and unrepaired damage increases the likelihood of mutations in genes that regulate cell growth, thereby promoting cancer development. Furthermore, oxidative stress is linked to treatment response and resistance. Evaluating both total oxidant levels and total antioxidant capacity together is crucial for gaining a comprehensive understanding of their role in cancer therapy [20,21].

Our research established the bioactivation of the anticancer antibiotic drug IKA in MCF-7 and MDA-MB-231 breast cancer cell lines, along with its association with TF. IKA has been shown to act as a HK-2 inhibitor in pancreatic cancer cells, and our team recently demonstrated that IKA also decreases the proliferation of breast cancer cells. In this study, the inhibitory effect of HK-2 on IKA and its correlation with TF were planned to be demonstrated at the enzyme, gene, and protein levels in the currently used cell types. Additionally, this study was designed to compare their effects with those of paclitaxel (Ptx), a widely used chemotherapeutic agent in clinical settings, and sodium oxamate (NaOx), an lactate dehydrogenase (LDH)-A inhibitor commonly used in preclinical cancer research.

Methods

Materials

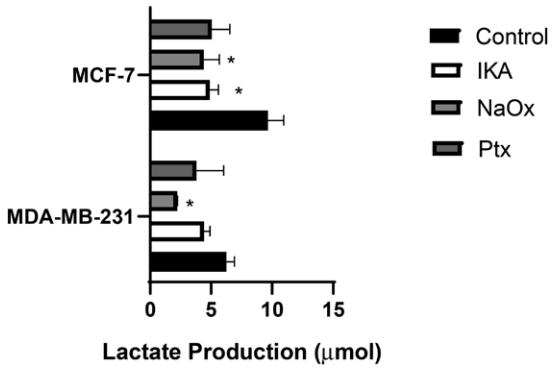
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), hepes-buffered dulbecco's modified Eagle's medium/high glucose, fetal bovine serum, PBS, penicillin-streptomycin (10 000 U/mL), L-glutamine, and ethanol. The HK-2 inhibitor, Ikarugamycin (IKA) (CAYMAN;15386), Ptx, and NaOx (Fig. 2) were also utilized.

Methods

The breast cancer cell lines MDA-MB-231 and MCF-7 were cultured under standard conditions in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% L-glutamine. The cells were maintained in a humidified incubator at 37 °C with 5% CO_2. For consistent growth, the cells were subcultured in T-25 flasks once they reached confluency, and further experiments were conducted using cells grown in T-75 flasks. Uniform cell passage techniques were adhered to throughout. Half-maximal inhibitory concentration (IC_{50}) values were used across all treatment groups [22].

To prepare cell lysates, cells were harvested through trypsinization and centrifuged, followed by washing with cold PBS. Homogenization was performed with the aid of metal beads, and a subsequent centrifugation step yielded supernatants containing lysates. These lysates were appropriately dissolved in PBS, radioimmunoprecipitation assay buffer, or ribosomal protein L buffer, depending on whether they were intended for western blotting or quantitative PCR (qPCR) analyses.

Fig. 3



Lactate production in MDA-MB-231 and MCF-7 cell lines across treatment groups. Lactate production was measured in MDA-MB-231 and MCF-7 breast cancer cell lines treated with Control, IKA, NaOx, and Ptx. (*$P < 0.01$, **$P < 0.001$,). Data are presented as mean ± SD from three independent experiments. IKA, ikarugamycin; NaOx, sodium oxamate; Ptx, paclitaxel.

Total antioxidant capacity (TAC) and total oxidant status (TOS) were assessed colorimetrically based on Erel's established method, with absorbance measurements taken at 658 nm. The oxidative stress index (OSI) was calculated using the formula (TOS/TAC) × 100 [23].

Hexokinase activity (ab136957, Abcam, Cambridge, Cambridgeshire, UK) and lactate production (Sigma, St. Louis, Missouri, USA, MAK064) were measured using commercial assay kits, where cell lysates were prepared in lysis buffer and clarified by centrifugation. The assays involved incubation with an enzyme mix and subsequent absorbance readings at 450 nm for HK activity and 470 nm for lactate production.

For RNA analysis, total RNA was extracted from cells using TRIzol reagent, and purity was evaluated spectrophotometrically. cDNA was synthesized from the RNA samples, and qPCR analysis was performed using SYBR Green (Bio-Rad, California, USA). The housekeeping genes β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used for normalization.

Western blotting was performed to analyze protein expression. Proteins were separated by SDS-PAGE, transferred to polyvinylidene fluoride membranes, and probed with specific antibodies targeting TF, HK-2, GAPDH, and β-actin. Immunoreactive bands were detected using an Odyssey Scanning System (LI-COR Inc., Lincoln, Nebraska, USA).

The study was conducted with the approval of the Istanbul Medipol University Non-Interventional Medical Practice Clinical Research Ethics Committee.

Statistical analysis

The study employed GraphPad Prism software was used for data analysis, with variables characterized by standard deviations. Student's *t*-test was used to compare means exhibiting a normal distribution. Two-way analysis of variance was used to compare groups and elucidate differences among subgroups in variables that demonstrated variations. *P* values <0.001 (***), <0.01 (**), and <0.05 (*) were considered significant.

Results

HK-2 inhibition is known to halt cell proliferation and glycolytic activity, leading to apoptosis in neoplastic cells. IKA, which is primarily used to treat bacterial infections, has been found to exhibit in-vitro bioactivation in breast cancer cell lines by our team. The IC_{50} values for IKA were determined to be 24.1 μM for MDA-MB-231 and 19.25 μM for MCF-7. For NaOx, the IC_{50} values were established as 60 mM for MCF-7 cells and 80 mM for MDA-MB-231 cells. Ptx was used at 16.9 μM for each cell line.

Hexokinase activity was quantitatively measured, and the results are shown in Figure 3. In the MCF-7 cell line, the hexokinase activity in the IKA-treated group (23.9 ± 0.17) was significantly reduced compared to that in the control group (100.5 ± 2.5) and the Ptx-treated group (53.79 ± 8.9) ($P < 0.01$). Similarly, in the MDA-MB-231 cell line, the hexokinase activity in the IKA-treated group (35.83 ± 1.31) was significantly lower than in the control group (99.5 ± 2.5) and the Ptx-treated group (71.2 ± 2.04), with statistical significance observed at $P < 0.05$ and $P < 0.01$, respectively. A comparison of the statistical significance between the two cell lines revealed that IKA treatment led to greater inhibition of hexokinase activity in the MCF-7 cell line than in MDA-MB-231 cells. Additionally, an analysis of the NaOx-treated groups further confirmed that the MCF-7 cell line exhibited a higher response to treatment than MDA-MB-231, with statistical significance calculated as $P < 0.05$ and $P < 0.01$, respectively.

The analysis of lactate production in cell lysates obtained from the MCF-7 and MDA-MB-231 cell lines is presented in Figure 4. In the MCF-7 cell line, the lactate levels in the control group (9.67 ± 0.9) were significantly higher than those in the IKA (4.89 ± 0.55) and NaOx (3.91 ± 0.89) groups ($P < 0.05$). In the MDA-MB-231 cells, a significant reduction in lactate levels was observed only in the NaOx-treated group (2.23 ± 0.04).

The TAC, TOS, and OSI values were calculated to assess the effects of the treatment groups on oxidative stress. The TOS and OSI values are shown in Figure 5. In the MCF-7 cell line, TOS levels showed a statistically significant increase in all treatment groups compared with the control group. Similarly, in the MDA-MB-231 cell line, a significant increase in TOS levels was observed in the IKA- and Ptx-treated groups compared with that in the control group. Among all groups in both cell lines, the Ptx-treated group exhibited the highest TOS levels in both cell lines.

Using the obtained TOS and TAC values, the OSI, a parameter employed to measure oxidative stress at the cellular level, was calculated (Fig. 5b). Increased OSI values indicate insufficient defense mechanisms, making them an important parameter for evaluating tissue damage and its role in disease pathogenesis, including breast cancer. In both cell lines, all treatment groups showed statistically significant increases in OSI levels compared with the control group.

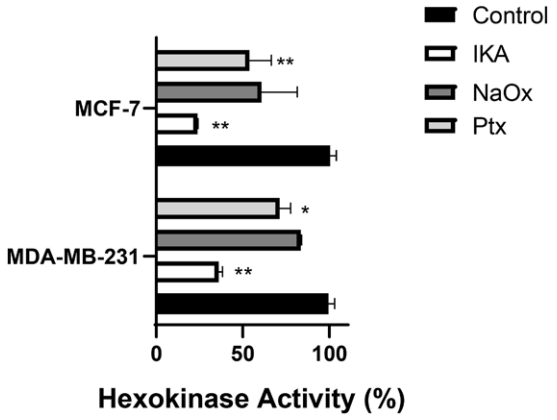
In the MCF-7 cell line, the OSI values significantly increased compared to the control group in the following ranges: Ptx-treated group (10.32 ± 1.02; $P > 0.001$), IKA-treated group (5.69 ± 0.58; $P < 0.001$), and NaOx-treated group (3.78 ± 0.34; $P < 0.05$). Similarly, in the MDA-MB-231 cell line, all treatment groups exhibited significant increases in OSI values, specifically the Ptx-treated group (9.56 ± 0.97; $P < 0.001$), IKA-treated group (4.16 ± 0.52; $P < 0.01$), and NaOx-treated group (3.16 ± 0.16; $P < 0.05$).

When HK-2 gene expression was analyzed in the MCF-7 cell line, a statistically significant decrease was observed in all treatment groups (IKA, Ptx, and NaOx) compared to that in the control group ($P < 0.05$, $P < 0.01$). In the MDA-MB-231 cell line, HK-2 gene expression was significantly reduced in the IKA- and Ptx-treated groups compared with that in the control group ($P < 0.05$). Although a reduction in HK-2 expression was noted in the NaOx-treated group, this decrease was not statistically significant ($P > 0.05$) (Fig. 6). Protein expression levels in the cell lines were determined using western blotting (Fig. 6). Analysis of HK-2 protein expression in MCF-7 cells revealed a statistically significant decrease in the IKA-treated group, which showed the greatest reduction compared to the control group ($P < 0.01$). A significant decrease was observed in the Ptx-treated group ($P < 0.01$, $P < 0.05$).

In the MDA-MB-231 cell line, HK-2 protein expression analysis demonstrated a statistically significant reduction in the IKA- and Ptx-treated groups compared to that in the control group ($P < 0.05$; $P < 0.01$). Although a decrease in HK-2 protein expression was observed in both cell lines in the NaOx-treated group, this reduction was not statistically significant (Fig. 6).

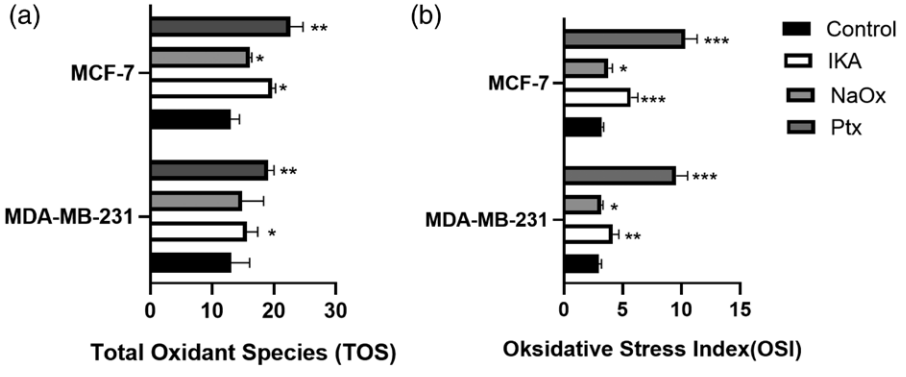
Analysis of TF gene expression in MCF-7 cells revealed that the IKA-treated group exhibited the most significant reduction compared to the control group ($P < 0.01$). Statistically significant decreases were observed in the NaOx- and Ptx-treated groups ($P < 0.05$). In the MDA-MB-231 cell line, a quantitative reduction in TF

Fig. 4



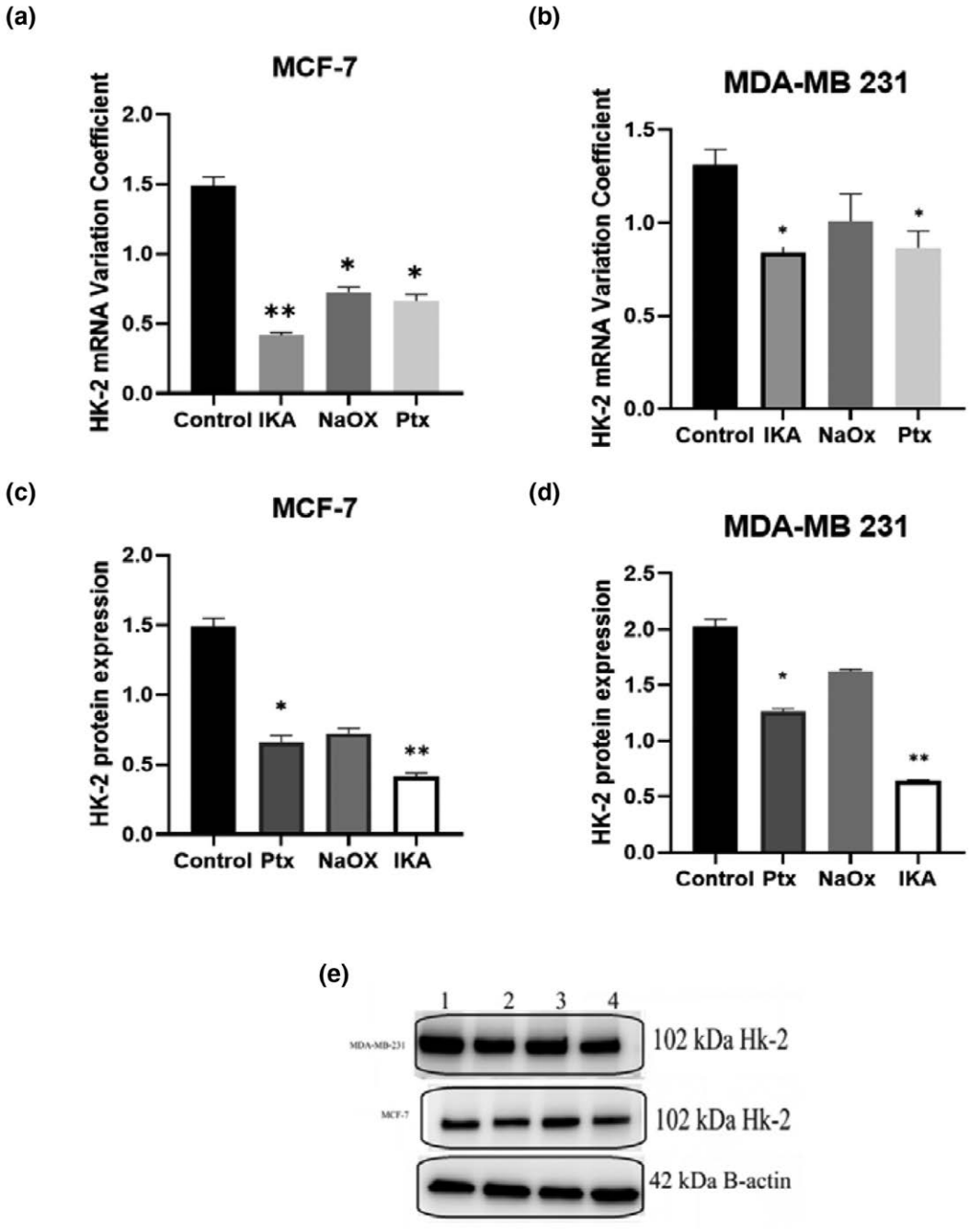
Normalized hexokinase activity in MDA-MB-231 and MCF-7 cell lines across treatment groups. HK activity was assessed in MDA-MB-231 and MCF-7 breast cancer cell lines treated with Control, IKA, NaOx, and Ptx. (*$P < 0.01$, **$P < 0.001$,). Data are presented as mean ± SD from three independent experiments. HK, hexokinase; IKA, ikarugamycin; NaOx, sodium oxamate; Ptx, paclitaxel.

Fig. 5



Oxidative stress parameters in MDA-MB-231 and MCF-7 cell lines across treatment groups. (a) TOS levels were measured in MDA-MB-231 and MCF-7 breast cancer cell lines treated Control, IKA, NaOx, and Ptx. (*$P < 0.01$, **$P < 0.05$,). Data are presented as mean ± SD from three independent experiments. (b) Oxidative Stress Index (OSI): OSI values, calculated as TOS/TAC × 100, were used to quantify oxidative stress in both cell lines. OSI levels were significantly elevated in Ptx treatment group. Data are presented as mean ± SD from three independent experiments. IKA, ikarugamycin; NaOx, sodium oxamate; Ptx, paclitaxel; TAC, Total antioxidant capacity; TOS, total oxidant status.

Fig. 6

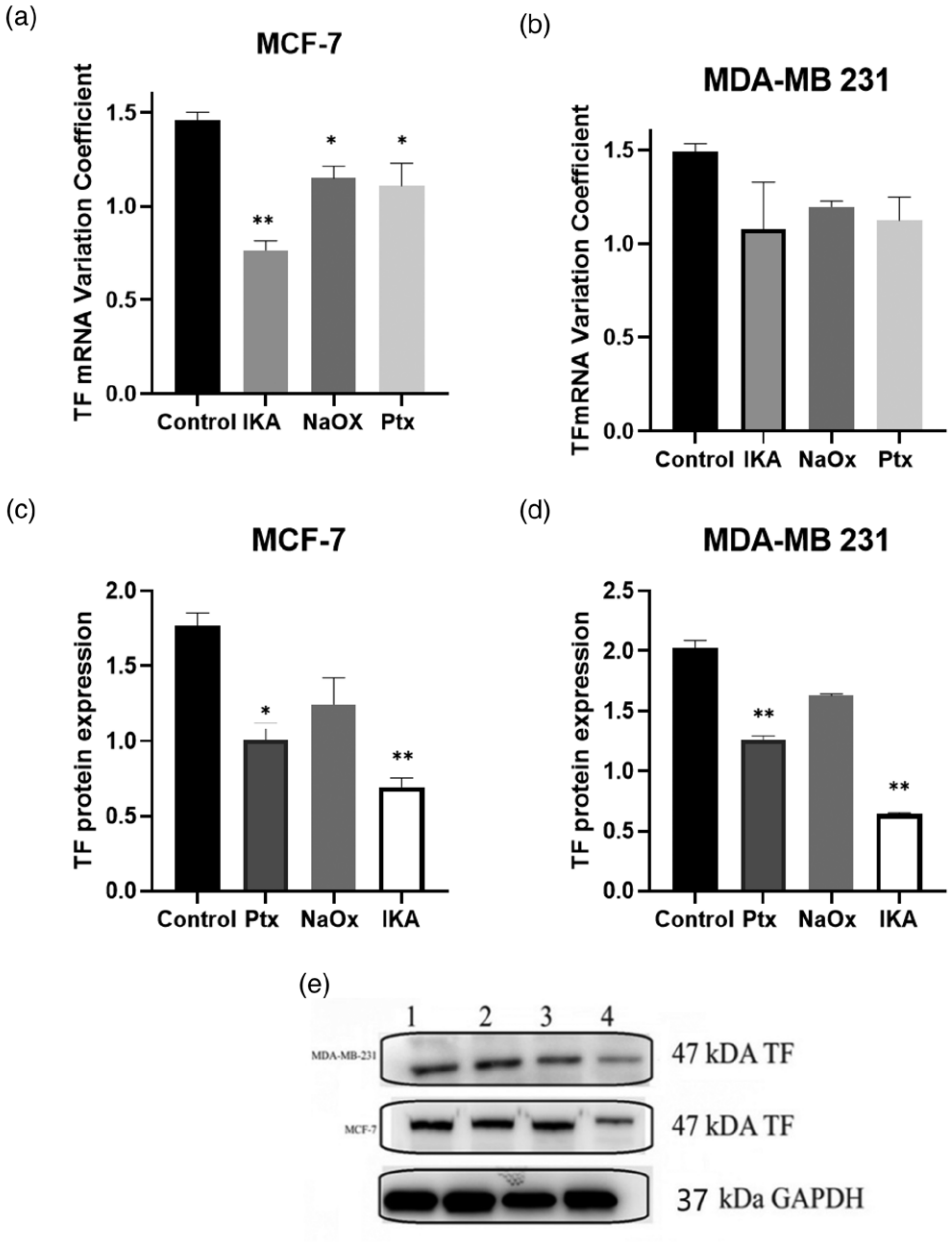


Gene and protein expression analysis of HK-2 in MCF-7 and MDA-MB-231 cell lines across treatment groups by qPCR and western blot. (a) HK-2 mRNA expression in MCF-7 cells: mRNA expression of HK-2 in the MCF-7 cell line as determined by qPCR. (b) HK-2 mRNA expression in MDA-MB-231 cells: mRNA expression of HK-2 in the MDA-MB-231 cell line as determined by qPCR. (c) HK-2 protein expression in MCF-7 cells: quantified protein expression levels of HK-2 in the MCF-7 cell line as determined by western blot analysis. (d) HK-2 protein expression in MDA-MB-231 cells: quantified protein expression levels of HK-2in the MDA-MB-231 cell line as determined by western blot analysis. (e) Western blot gel images for HK-2 expression: representative gel images for HK-2 protein expression in MCF-7 and MDA-MB-231 cell lines across treatment groups (Control, IKA, Ptx, and NaOx). β-actin was used as a loading control. HK, hexokinase; IKA, ikarugamycin; NaOx, sodium oxamate; Ptx, paclitaxel; qPCR, quantitative PCR.

gene expression was noted across all treatment groups compared with the control group; however, this reduction was not statistically significant ($P > 0.05$) (Fig. 7).

TF protein expression levels in both cell lines were determined using western blotting (Fig. 7). In the MCF-7 cell line, quantitative reductions in TF protein expression

Fig. 7



Gene and protein expression analysis of HK-2 in MCF-7 and MDA-MB-231 cell lines across treatment groups by qPCR and western blot. (a) TF mRNA expression in MCF-7 cells: mRNA expression of TF in the MCF-7 cell line as determined by qPCR. (b) TF mRNA expression in MDA-MB-231 Cells: mRNA expression of TF in the MDA-MB-231 cell line as determined by qPCR. (c) TF protein expression in MCF-7 cells: quantified protein expression levels of TF in the MCF-7 cell line as determined by western blot analysis. (d) TF protein expression in MDA-MB-231 cells: quantified protein expression levels of TF in the MDA-MB-231 cell line as determined by western blot analysis. (e) Western blot gel images for TF expression: representative gel images for HK-2 protein expression in MCF-7 and MDA-MB-231 cell lines across treatment groups (Control, IKA, Ptx, and NaOx). GAPDH was used as a loading control. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HK, hexokinase; IKA, ikarugamycin; NaOx, sodium oxamate; Ptx, paclitaxel; qPCR, quantitative PCR.

were observed across all treatment groups compared with the control group. Statistically significant differences were noted in the Ptx-treated group ($P < 0.05$) and IKA-treated group ($P < 0.01$), although no significant differences were detected between the treatment groups ($P > 0.05$).

In the MDA-MB-231 cell line, TF protein expression analysis showed significant reductions in the Ptx- and IKA-treated groups compared with the control group ($P < 0.01$). Additionally, the NaOx-treated group exhibited statistically significant reductions in expression levels compared to both the IKA- and Ptx-treated groups ($P < 0.05$) (Fig. 7).

Discussion

Inhibition of HK-2 inhibits cell proliferation and glycolytic activity, inducing apoptosis in neoplastic cells [24,25]. IKA, primarily employed for bacterial infections, has demonstrated in-vitro bioactivation by inhibiting HK-2 in pancreatic (BxPC-3 PANC-1) and breast cancer (MDA-MB-231 and MCF-7) cell lines [26].

Several analyses were conducted to validate the anticancer properties of IKA in MDA-MB-231 and MC7 cells. Through calorimetric lactate analysis, it was observed that IKA, in conjunction with the NaOx group, significantly reduced lactate formation in MCF-7 cells ($P < 0.05$), reinforcing our hypothesis. Given that HK-2 is one of the enzymes at the onset of glycolysis, its influence on the conversion of pyruvate to lactate in the subsequent stages of the reactions will also be significant. However, direct inhibition of LDH-A by NaOx yielded statistically more significant results in the MDA-MB-231 group. This observation may be attributed to several factors including metabolic plasticity, resistance to HK-2 inhibition, and the ability of MDA-MB-231 cells to adapt to hypoxic conditions. As a triple-negative breast cancer subtype, MDA-MB-231 is characterized by its aggressive nature, which enhances its survival capabilities and renders effective treatment more challenging. Nevertheless, the ability of IKA to inhibit HK-2 and reduce lactate production with efficacy comparable to or approaching that of NaOx makes it a more favorable option, as it suggests a dual mechanism of action [27,28].

Of particular importance is the critical parameter evaluated, which was the activity of HK-2. The result strongly supported our hypothesis, as there was a significant decrease in HK-2 activity in the IKA-treated group ($P < 0.01$ and <0.001). Hence, it is important to note that IKA is a potent inhibitor of HK-2 activity in breast cancer and may be one of the main factors behind its anticancer effects. Additionally, both findings are consistent with the current knowledge of breast cancer treatment and suggest that IKA may be a potent therapeutic agent for this difficult-to-treat pathology [26].

Assessment of TOS and OSI in the context of cancer is essential for elucidating the role of oxidative stress in the pathogenesis of cancer and identifying therapeutic options to ameliorate its impact. It is important to assess the ratio and magnitude of these parameters [29].

The increase in TOS values in tumor cells originates mainly from an increase in metabolism in these cells. Such a phenomenon can be observed in the MCF-7 group, where TOS is elevated but OSI is lower, which could be explained by the high TAC of these cells. The MDA-MB231 cell line, characterized as a triple-negative subtype, is more aggressive and treatment-resistant, which may contribute to a greater oxidative stress burden. Another noteworthy observation was the significantly higher OSI levels in the Ptx-treated group. While TOS levels across all treatment groups appeared comparable, the marked increase in OSI for the Ptx group suggests that this increase is not solely treatment-related, but also indicative of the cytotoxic effects of Ptx. From this perspective, metabolic inhibitors appear to offer a more efficient therapeutic approach, potentially minimizing the unintended cytotoxicity observed with conventional chemotherapeutics [21].

When examining TF protein and gene expression in the MCF-7 cell line and analyzing their correlation with partial thromboplastin time (PTT), it was observed that TF expression significantly decreased across all treatment groups. However, in the NaOx group, the prolongation of TF activation time in PTT was less than expected. In the MDA-MB-231 cell line, although no statistically significant differences were observed in TF gene expression, reductions in TF protein levels and prolonged activation times were evident in PTT analysis. Among the treatment groups, the Ptx-treated group was particularly notable for this cell type [30].

The discrepancy between gene-level changes and subsequent effects, or vice versa, where no changes at the gene level are observed but significant reductions or increases occur later, may arise from several factors. These include transcriptional regulation, post-translational modifications, and chaperone interactions [31,32].

To the best of our knowledge, this is the first study to demonstrate the potential correlation between HK-2 and TF in cancer pathophysiology, both in terms of signaling and molecular mechanisms, from theoretical and practical perspectives. This finding highlights the role of this correlation as a biomarker associated with oncogenic events and therapeutic targets. Both TF and HK-2 function as critical mediators of tumor progression and angiogenesis, thereby underscoring their significant potential for use not only in diagnosis but also as a basis for therapeutic applications [4].

There are limitations to our study with the use of IKA. One major limitation is the reliance on IKA's anticancer effects of IKA for HK-2 inhibition. Although efforts have been made to develop selective approaches targeting HK-2, this enzyme plays a vital role in energy metabolism within healthy cells, raising concerns about potential off-target toxicity [33].

A final aspect to consider is that, similar to bactericidal agents, tumor cells can develop resistance to IKA. Natural strategies are shared by tumor cells and bacteria to develop resistance. While bacteria form biofilms to protect themselves from external threats, tumor cells exploit their microenvironment to protect themselves from drugs, thus reducing drug efficacy. Moreover, they can use drug efflux pumps, genetic mutations to change

drug targets, and metabolic adaptation to harsh environments to take different scenarios to live on. Such mechanisms of resistance underscore the importance of further studies on the optimization and expanded use of IKA in cancer medicine [34].

Evidence of an association between chemotherapy resistance and glycolytic pathways has been well documented. This mechanism is bidirectional; in resistant cells, metabolic enzymes, such as LDH-A, HK-2, and pyruvate dehydrogenase, are overexpressed, and conversely, blockage of these enzymes has been demonstrated to disrupt mechanisms for resistance. Intervention in glycolytic pathways can attenuate the Warburg effect, leading to an energy crisis in tumor cells and increasing drug sensitivity [1,2].

In a recent study, Lin *et al*. [35] identified HK-2 as a mediator of Ptx resistance in ovarian cancer. They found that HK-2 expression in Ptx-resistant ovarian cancer cells was significantly higher than that in nonresistant ovarian cancer cells and healthy ovarian cells. Moreover, targeting HK-2 with PROTAC technology induced the effective degradation of HK-2 and successfully disrupted the resistance mechanism, restoring the sensitivity of cells to chemotherapy. Similarly, the combined administration of gemcitabine and IKA showed remarkable effects both *in vitro* and *in vivo* by reducing cancer cell viability, decreasing tumor size, and increasing apoptosis compared to chemotherapy [35].

Based on these results, we propose that the IKA-Ptx combination may serve as an effective treatment strategy. This combination can create synergistic effects with lower doses, which may reduce side effects and provide a promising avenue for cancer treatment. Further studies are needed to clarify the detailed molecular basis of the observed synergistic interactions, as well as to determine the optimal dosing regimens and sequences to achieve clinical benefits. Furthermore, this combination therapy needs to be validated *in vivo* and evaluated in a variety of cancer models to investigate its cine and efficacy. To ultimately augment the clinical relevance of this strategy, investigating the ability to overcome resistance mechanisms or the relevance of the tumor microenvironment in modulating the response to IKA and Ptx will further enhance the translational potential of this approach.

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Conflicts of interest

There are no conflicts of interest.

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