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Metformin and Mitochondrial Apoptosis in Breast Cancer: Mechanistic Insights and Therapeutic Implications

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Abstract
Metformin, a first-line treatment for type 2 diabetes, has gained potential for its anti-cancer properties. Evidence suggests that metformin suppresses tumour growth and induces apoptosis, particularly through its effects on mitochondrial pathways. This review explores three interconnected mechanisms by which metformin promotes mitochondrial-induced apoptosis and inhibits tumorigenesis in breast cancer: *AMPK* activation, reduced Bcl-2 protein activity, and ROS accumulation. These processes contribute to apoptotic reactivation mechanisms such as mitochondrial outer membrane permeabilization (MOMP), cytochrome c release, and cell death, offering insight into how metformin may help overcome treatment resistance in hormone receptor-positive and triple-negative breast cancers. Breast cancer was selected for this study due to its high global incidence and marked variability in treatment response, making it an ideal framework to evaluate metformin's effects across different breast cancer subtypes. A systematic review was conducted to review metformin's role in inducing apoptosis, focusing on its effects on mitochondrial processes related to AMPK activation, reduced Bcl-2 protein activity, and ROS production. The review draws research from databases such as PubMed, ScienceDirect, and the National Institutes of Health, using keywords such as metformin, mitochondrial apoptosis, tumorigenesis, and anticancer pathways. Studies were selected based on relevance to AMPK signaling, Bcl-2 modulation, and ROS involvement, with emphasis on apoptosis markers, breast cancer subtypes, and metformin dosage-response outcomes. Metformin's role to activate AMPK, reduce Bcl-2 protein activity , and increase ROS collectively promote BAX/BAK oligomerization, mitochondrial outer membrane permeabilization (MOMP, and both caspase-dependent and caspase-independent apoptosis). These effects are expected to vary across breast cancer subtypes based on p53 status, ER expression, and metabolic profile. This review highlights metformin's potential as an anti-cancer agent by targeting various mitochondrial mechanisms, each contributing to apoptosis and inhibiting tumorigenesis. The findings can guide clinicians in integrating metformin into targeted cancer treatments to overcome cancer resistance. This research can support the development of metformin-based therapies that selectively target cancer cell vulnerabilities and guide future efforts in personalizing treatment based on tumour metabolism and genetic context.

Keywords: metformin; mitochondrial apoptosis; AMPK; Bcl-2; ROS; breast cancer; ER+; TNBC; cytochrome c; therapeutic resistance

Introduction

Breast cancer is the most common cancer affecting women globally, with 2.3 million women diagnosed with breast cancer in 2022 [1]. By 2050, diagnoses and deaths associated with breast cancer are expected to rise by 38% and 68%, respectively [2]. This jarring projection necessitates an investigation into the mechanisms underlying breast cancer to guide therapeutic endeavours. Recent research for breast cancer therapies has focused on drug repurposing as a promising strategy. Metformin, an antidiabetic drug prescribed for type 2 diabetes, has emerged as a candidate with potential anti-cancer properties [3]. Evidence from observational studies suggests that metformin use is associated with reduced breast cancer incidence and improved survival in diabetic patients. Preclinical studies demonstrate its ability to suppress tumour growth and promote apoptosis across breast cancer subtypes [4]. These effects are mediated through several interconnected mechanisms involving AMP-activated protein kinase (AMPK) activation, B-cell lymphoma 2 (Bcl-2) protein level reduction, and reactive oxygen species (ROS) generation [5].

Metformin has gained attention due to its ability to target mitochondria-induced apoptosis, a key pathway often disrupted in cancer that many cancer cells evade through overexpression of anti-apoptotic proteins like Bcl-2 [5]. Mitochondria are central regulators of intrinsic apoptosis. When the mitochondrial outer membrane is permeabilized, pro-apoptotic factors like cytochrome c are released,

triggering caspase activation and programmed cell death. Cancer cells evade this process, supporting uncontrolled growth [5]. Metformin re-engages this apoptotic pathway through three interconnected mechanisms: activation of AMPK, reduced Bcl-2 protein activity , and increased ROS production [6].

Despite promising findings, the therapeutic implications of metformin in breast cancer remain context-dependent [8]. Metformin's effectiveness varies with tumour characteristics such as p53 mutational status and estrogen receptor expression (ER+), and some cancer cells may leverage AMPK activation to support survival through autophagy [9]. Given these nuanced and contradictory findings, further research is needed to elucidate metformin's mechanisms of action and clinical utility in breast cancer. This review synthesizes current evidence on metformin's role in modulating AMPK, Bcl-2 protein expression, and ROS pathways, aiming to clarify its potential as a therapeutic adjunct and identify directions for future investigation.

Methods

A comprehensive review was conducted utilizing PubMed, ScienceDirect, and the National Institutes of Health databases to identify relevant studies on metformin's role in inducing apoptosis, focusing on its effects on mitochondrial processes related to AMPK activation, reduced Bcl-2 protein activity, and ROS production. Search terms such as metformin, mitochondrial apoptosis, AMPK, Bcl-2, ROS, breast cancer, ER+, TNBC, cytochrome c, and therapeutic resistance were used to find appropriate research studies. Furthermore, eligible studies focused on metformin's impact on mitochondrial apoptotic pathways, including ROS generation, Bcl-2 protein modulation, and AMPK activation, with human-derived breast cancer cell lines or mouse models used. Research papers used were clinical trials and experimental-based studies. Key variables, such as apoptotic markers and tumour-specific responses, were extracted from the articles. This study aims to comprehensively review existing literature on metformin's role in inducing apoptosis in breast cancer and its effects on mitochondrial pathways involving AMPK activation, Bcl-2 protein suppression, and ROS production.

Results

Metformin activates AMPK, a central regulator of cellular energy homeostasis. AMPK activation enables metformin to inhibit mTORC1 signaling, suppressing cell proliferation and enhancing apoptotic signaling. Downstream of AMPK, metformin modulates the expression of Bcl-2 family proteins by downregulating the anti-apoptotic protein Bcl-2. This shift in the balance of pro- and anti-apoptotic proteins promotes mitochondrial outer membrane permeabilization (MOMP), a crucial step in apoptosis initiation. Simultaneously, metformin disrupts mitochondrial respiration by inhibiting complex I of the electron transport chain. This inhibition increases ROS production, contributing to oxidative stress within cancer cells. Elevated ROS levels destabilize the mitochondrial membrane, induce cytochrome c release, and activate caspase-dependent apoptosis. While high ROS levels drive cell death, moderate levels of ROS can paradoxically activate survival pathways, highlighting the complexity of metformin's effects [7].

AMPK Activation and the Survival of Dormant ER+ Breast Cancer Cells

AMPK helps to regulate cellular energy homeostasis, impacting cancer cell metabolism, survival, and apoptosis. AMPK activation elicits pro-apoptotic and pro-survival responses depending on the cellular context. This duality makes AMPK a complex yet promising target in breast cancer therapy. Studies have explored the effects of pharmacological AMPK activation through agents such as metformin in estrogen receptor-positive (ER+) breast cancer. These investigations revealed outcomes based on estrogen levels, whether the p53 gene is mutated or working properly, and how the cell processes energy, highlighting the importance of tailored therapeutic strategies [10, 11].

Hampsch et al. investigated the role of AMPK in the survival of ER+ breast cancer cells under estrogen deprivation, mimicking the effects of aromatase inhibitor (AI) therapy [10]. In long-term estrogen-deprived (LTED) ER+ cell lines, AMPK activity was significantly elevated compared to estrogen-supplemented controls, as shown by increased phosphorylation of AMPKα (Thr172) and its downstream target, acetyl-CoA carboxylase [10]. These metabolically adapted, dormant cells displayed increased fatty acid oxidation (FAO), evidenced by elevated CPT1A expression and oxygen consumption rates. *In vivo* metformin treatment during the estrogen-rich phase, when estrogen was supplemented to support active tumour growth, reduced tumour volume in ovariectomized NSG mice bearing ER+ tumour xenografts. However, when metformin was given during the estrogen-deprived phase (mimicking post-AI therapy), it promoted the survival of dormant tumour cells without stimulating proliferation. This led to increased residual tumour burden, an indicator that under long-term conditions, these dormant cells may contribute to recurrence [10]. These results suggest that while AMPK activation via metformin can suppress tumour growth in high-estrogen conditions, it may also support the persistence of dormant cells under estrogen-deprived conditions, cells that could later contribute to recurrence.

AMPK Activation and Apoptosis: Influence of Genetic Background

A complementary study by El-Masry et al. examined how activation of AMPK by AICAR, an AMP analog that mimics cellular energy stress, influences apoptosis and cell cycle regulation in breast cancer cells with different *TP53* mutations and ER expression levels [11]. The tumour suppressor protein p53, which plays a key role in DNA

damage response and apoptosis, is the most frequently mutated gene in human cancers, and its inactivation often impairs apoptotic signaling. The study compared three breast cancer cell lines: MCF-7 (*TP53* wild-type, ER+), MDA-MB-231 (*TP53* mutant, ER−), and T47D (*TP53* mutant, ER+). AMPK activation led to pro-apoptotic effects in all cell lines, but responses varied with p53 status [11]. Cells with mutant p53 (MDA-MB-231 and T47D) showed delayed or reduced PARP cleavage compared to MCF-7. Also, differences in BAX translocation and caspase dependency suggested distinct apoptosis pathways. MDA-MB-231 (*TP53* mutant, low baseline *P21* expression) showed the most robust apoptotic response, while T47D (*TP53*, high baseline *P21* expression) responded weakly, indicating that *TP53*-independent pathways might be at play. In contrast, MCF-7 cells (*TP53* wild-type) displayed early PARP cleavage and increased *P21* expression, linking AMPK activation to apoptosis and *TP53*-dependent cell cycle arrest. In fact, the high basal p21 level in T47D cells appears to bias AMPK activation toward cell cycle arrest rather than apoptosis, whereas the low p21 in MDA-MB-231 permits a stronger activation of the intrinsic apoptotic machinery, exemplifying p21 as a key switch in determining AMPK-driven outcomes in these breast cancer models [11] This study reveals that AMPK activation induces apoptosis across breast cancer subtypes, and the mechanisms, whether *TP53*-dependent or independent, are shaped by the genetic context of the cells [11]. This is further supported by findings that AMPK can stabilize p53 by phosphorylation at Ser15. In this manner, AMPK activation by metformin may reinforce p53-mediated cell death as well. [12].

Bcl-2 Family Modulation *and Mitochondrial Outer Membrane Permeabilization (*MOMP*)*

The Bcl-2 family consists of both pro-apoptotic and anti-apoptotic proteins. Anti-apoptotic proteins like Bcl-2, which bind to and inhibit pro-apoptotic proteins like BAX and BAK. When a cell receives apoptotic signals (e.g., DNA damage, oxidative stress), these signals activate pro-apoptotic proteins, such as BAX and BAK, forming pores in the mitochondrial outer membrane, leading to membrane permeabilization [9]. The formation of pores by BAX and BAK allows the release of mitochondrial contents, leading to mitochondrial-induced apoptosis [13].

In breast cancer cells, the overexpression of the Bcl-2 protein shifts cells to a pro-survival phenotype. Bcl-2 directly binds to BAK and BAX, preventing oligomerization and blocking MOMP [14]. Mutated p53 or disruptions in the Bcl-2 signaling pathway disrupt apoptotic regulation. Thus, *BCL-2* overexpression in breast cancer cells has been linked to enhanced tumour cell proliferation [15]. Targeting the Bcl-2 protein for breast cancer therapies with metformin has shown potential for modulating this pathway to restore apoptosis in breast cancer cells.

Dose-Dependent Bcl-2 Protein Suppression and Enhanced Apoptosis by Metformin

Metformin inhibits *Bcl-2* protein expression, enhancing signaling for pro-apoptotic proteins such as BAX and BAK [14]. Gao et al. (2016) determined that treatment with 5 mM metformin reduced the expression of *Bcl-2* protein in breast cancer cells. The reduction was associated with increased apoptosis, indicating metformin's role in promoting cell death through Bcl-2 protein downregulation. Higher concentrations of metformin, specifically 5 mM to 10 mM, reduced Bcl-2 levels and induced more apoptosis than lower concentrations (1 to 2 mM) [16, 17]. A marked reduction in mitochondrial membrane permeability (MMP), indicative of mitochondrial dysfunction and the initiation of apoptosis, was primarily observed at higher doses of metformin (≥5 mM), suggesting a dose-dependent effect on mitochondrial integrity [16].

Sharma and Kumar (2019) found that metformin reduced *Bcl-2* protein levels in both MCF-7 (ER+, *TP53* wild-type) and MDA-MB-231 (triple-negative breast cancer (TNBC), ER-, *TP53*mutant) breast cancer cell lines in a dose-dependent manner while increasing BAX (Bcl-2-associated X protein) and BAK expression. In MCF-7 cells, this reduction in Bcl-2 led to increased apoptosis. Similarly, in MDA-MB-231 cells, metformin's reduction of Bcl-2 promoted apoptosis despite their aggressive nature [17]. Metformin is predicted to have a minimal impact on Bcl-2 expression in HER2-positive SK-BR-3 cells since the strong association between HER2 and insulin-like growth factor 1 receptor (IGF-1R) signaling is indirectly inhibited by metformin [18]. However, the presence of HER2 may confer resistance to metformin's effects on Bcl-2 expression [18].

Bcl-2 protein expression is often higher in ER-positive breast cancers, as it is frequently co-expressed with the estrogen receptor, reflecting a positive correlation between Bcl-2 levels and ER expression across different breast cancer subtypes [19]. However, in ER– breast cancer cell lines, metformin downregulated Bcl-2 protein, promoting apoptosis independently of estrogen receptor status [16]. The variability in Bcl-2 protein expression is influenced by the genetic and molecular characteristics of the cancer cells, including mutations in key regulatory genes like p53, the presence or absence of hormone receptors, and HER2 [15]. Metformin further enhances p53 activity, downregulating Bcl-2 protein, promoting pro-apoptotic proteins such as PUMA and BAX/BAK [16]. Increasing MMP allows metformin to enhance the formation of apoptotic pores, disrupting mitochondrial integrity and promoting apoptosis [20].

ROS Accumulation *and Mitochondrial Dysfunction in Apoptosis Initiation*

ROS are highly reactive oxygen derivatives that are by-products of cellular metabolism. Aerobic cells require a balance between ROS and antioxidants to ensure survival. ROS, at low doses, is necessary for normal cell functions such as cell cycle progression. When the antioxidant

detoxification systems fail to maintain tolerable levels of ROS, oxidative stress can cause damage to DNA and cellular proteins or activate apoptosis [21].

ROS play a role in mitochondrial apoptosis, triggered by intracellular stress signals. The mitochondria are a site of ROS production due to the leakage from the respiratory electron transport chain. This source of excessive intracellular ROS oxidatively damages structures such as mtDNA, resulting in apoptosis. Increased ROS triggers mitochondrial membrane hyperpolarization and cytochrome c release, further generating ROS. ROS-mediated apoptosis proceeds via both caspase-dependent and caspase-independent pathways. The caspase-dependent process involves the caspase-9-mediated activation of caspase-3, whereas the caspase-independent process involves translocating the apoptosis-inducing factor (AIF) to the nucleus. Moreover, ROS can modulate the balance between pro-apoptotic (e.g. BAX, BAK) and anti-apoptotic proteins (e.g. Bcl-2, Bcl-xL) [21, 22].

The excessive production of ROS has been associated with the development of breast cancer and modulation of the tumour microenvironment. Oshi et al. found that elevated ROS levels in breast cancer cells significantly enhanced cell proliferation and expression of pro-cancer gene sets [23]. High ROS levels were also linked to increased mutations, greater clinical aggressiveness, and poorer survival outcomes. In normal breast cells, pro-apoptotic and anti-apoptotic factors help balance cell proliferation and apoptosis [23].

Metformin-Induced ROS Generation *and Mitochondrial Apoptosis*

Gao et al. described metformin as inducing apoptosis via the mitochondrial pathway in human breast cells. They determined that a decrease in mitochondrial membrane potential increased ROS generation. Following incubation with metformin for 24 hours, cells displayed a dose-dependent increase in their extent of apoptosis. Metformin partially inhibits complex I of the mitochondrial respiratory chain. A greater flow of electrons to oxygen led to ROS accumulation within the mitochondrial matrix, which was observed. The accumulation of ROS in breast cancer cells initiated the mitochondrial pathway of apoptosis through a caspase-dependent process, as demonstrated by decreases when treated with the pan-caspase inhibitor z-VAD-FMK [16].

Schexnayder et al. performed a study demonstrating that MDA-MB-231 breast cancer cells were incubated with 100 µM metformin for 48h and measured ROS production. Metformin-exposed cells showed 40% lower ROS production compared to untreated control cells. As such, these findings support that metformin can attenuate endogenous ROS production and the subsequent stress signaling, thereby reducing metastasis [24]. Similarly, Sharma and Kumar demonstrated that metformin decreased ROS levels in a concentration-dependent manner that varied between cell types. At a 20 mmol/L concentration, ROS levels were decreased 3-, 4.31- and 3.11-fold in MCF-7, MDA-MB-231, and T47D cells, respectively [17].

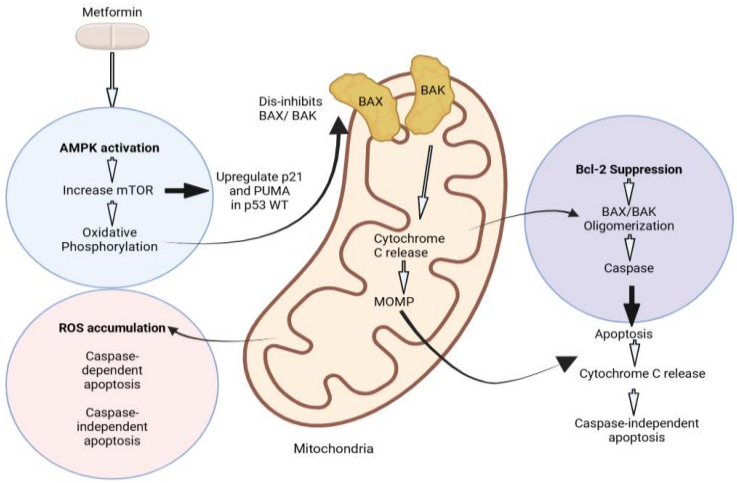

Figure 1. Metformin-Induced Mitochondrial Apoptosis in Breast Cancer Cells via AMPK Activation, Bcl-2 Protein Suppression, and ROS Accumulation. Metformin induces apoptosis in breast cancer cells through three mitochondrial pathways. AMPK activation enhances oxidative phosphorylation and ROS production, which promotes BAX/BAK oligomerization, while also upregulating p21 and PUMA in *TP53* wild-type (WT) cells (facilitating BAX/BAK activation). Concurrently, suppression of the Bcl-2 protein releases inhibition on BAX/BAK, promoting cytochrome c release and caspase-mediated apoptosis. Together, these pathways converge to drive cell death in ER+ and TNBC (triple-negative breast cancer) breast cancer subtypes. Figure created using BioRender.com.

Together, these mechanisms converge to trigger mitochondrial-induced apoptosis. Figure 1 below illustrates how metformin activates AMPK, suppresses Bcl-2, and increases ROS levels to promote apoptosis in ER+ and TNBC cells.

Discussion

Hampsch et al. (2020) observed that metformin-activated AMPK promoted survival in dormant ER+ breast cancer cells under estrogen deprivation by enhancing fatty acid oxidation [10]. El-Masry et al. (2019) demonstrated that AMPK activation via AICAR induces apoptosis and cell cycle arrest dependent on p53 protein and ER presence [11]. Complementary studies by Gao et al. (2016) and Sharma and Kumar (2019) revealed that metformin downregulates anti-apoptotic Bcl-2 protein and enhances BAX/BAK-mediated mitochondrial permeabilization, particularly in ER+ and TNBC cells [16, 17]. The associated loss of MMP and cell death was partially reversed by caspase inhibition, indicating that metformin's pro-apoptotic effects involve both caspase-dependent and caspase-independent mitochondrial pathways [16]. By regulating survival and apoptotic pathways, metformin presents a potential strategy to exploit metabolic vulnerabilities, specifically in ER+ and TNBC cells.

The observation that metformin's effects vary across different breast cancer cell lines highlights the complexity of its therapeutic impact and the necessity for personalized treatment approaches. Metformin has been shown to induce apoptosis in ER+ MCF-7 and p53 mutant MDA-MB-231 TNBC cells while having minimal effects on HER2+ SK-BR-3 cells [17]. In ER+ MCF-7 cells, metformin's activation of AMPK inhibits the mTOR pathway, which is essential for cell growth and proliferation, leading to apoptosis [14]. In addition, p53 mutant MDA-MB-231 TNBC cells have a compromised DNA repair mechanism, which makes them more susceptible to metformin-induced oxidative stress [11]. However, HER2-+ SK-BR-3 cells, which rely on the HER2 signaling pathway for growth and survival rather than AMPK, may not be significantly affected by AMPK activation alone, explaining the minimal impact of metformin on these cells [18]. This variability highlights the importance of understanding genetic mutation and metabolic differences among different cell lines to determine their response to metformin. Accounting for patient-specific molecular and metabolic profiles is essential when evaluating metformin's potential as a therapeutic agent.

Furthermore, extending the diversity observed in cell lines to a patient context is crucial for translating preclinical findings into clinical use. The genetic and metabolic heterogeneity in cell lines mirrors the diversity in patient tumours, which can shape therapeutic responsiveness. Specific mutations, such as those in the TP53 gene, or variations in the expression of pro-apoptotic and anti-apoptotic proteins, can influence a patient's tumour response to metformin [15, 25]. Additionally, the tumour microenvironment, including factors such as hypoxia, immune cell infiltration, and stromal interactions, plays a significant role in modulating drug efficacy [26-28]. Understanding these complex interactions is essential for developing personalized treatment strategies and improving clinical outcomes.

One major limitation is the potential for inconsistent responses with patients, complicating the development of standardized treatment protocols [25]. Clinical trials must account for this diversity in potential patient health outcomes by including various breast cancer subtypes and patient demographics to ensure the findings are broadly applicable. The tumour microenvironment, consisting of various factors, can affect metformin's activity and needs to be considered in clinical studies. By addressing the individual genetic profiles and considering the metabolic diversity of tumours, researchers can develop more effective and personalized treatment approaches that maximize the clinical benefits of metformin.

While this review demonstrates metformin's potential in promoting mitochondrial-induced apoptosis through AMPK activation, Bcl-2 modulation, and ROS accumulation, its translational relevance is a challenge. The findings are based on preclinical xenograft models and *in vitro* studies, where metformin concentrations often exceed clinically achievable levels [10, 29]. This raises concerns about whether similar effects can be observed in human patients. Non-clinically safe doses used in preclinical studies may not accurately reflect the drug's efficacy and safety in humans, leading to potential difficulties when translating these findings to clinical settings [30]. High concentrations in these studies result in off-target effects not observed at therapeutic doses, complicating the interpretation of results and their applicability to patient care [31]. Conducting clinical trials using established, clinically safe doses of metformin, already well-characterized from its decades-long use in diabetes management, is critical to determine whether its observed anti-cancer effects can be replicated in patient populations [32]. Furthermore, few cell lines were compared, leading to inconclusive data regarding the correlation between non-TNBC or ER+ cell lines and metformin [24]. Alternatively, emerging evidence suggests that long-term metformin may induce resistance in ER^+ breast cancer cells. In one instance, chronic metformin treatment to ER^+ MCF-7 cells developed resistance to metformin and tamoxifen via ERα suppression and constitutive activation of Akt/Snail1 signaling [33]. These implications can manifest in future studies through preemptively monitoring molecular markers such as Snail1 as well as refining patient stratification for those predisposed to metformin resistance [34]. Future research should refine dosing strategies and assess metformin's pharmacokinetics in combination with targeted therapies. Investigating co-administration with Bcl-2 inhibitors like venetoclax could help optimize therapeutic efficacy while minimizing potential resistance mechanisms. Studies have indicated that combining metformin with venetoclax enhanced anti-tumour activity by exploiting metabolic vulnerabilities in cancer cells, suggesting a

promising avenue for combination therapy [31]. A study by Haikala et al. found that metformin and venetoclax effectively killed breast cancer cells in culture and suppressed tumour growth in mice models [3]. Another study highlighted the potential of combining metformin with histone deacetylase inhibitors (HDACi) to enhance anti-tumour effects in triple-negative breast cancer [36]. Long-term *in vivo* studies using patient-derived xenograft models will be critical in bridging the gap between preclinical findings and clinical applications [37]. Unlike MCF-7, MDA-MB-231 and T47D cultures - which, although they are established breast cancer cell lines, cannot capture the full spectrum of genetic, epigenetic, and microenvironmental heterogeneity seen in patient tumours - PDX and organoid systems more universally recapitulate human breast cancer biology. Future studies employing patient-derived xenografts or organoid systems are essential to validate these AMPK-mediated effects across diverse tumour contexts and to better inform clinical translation. [32]. This ensures that metformin's potential as an adjunct therapy in breast cancer treatment is fully explored.

Conclusions

Overall, metformin induces mitochondrial apoptosis through AMPK activation, Bcl-2 protein suppression, and ROS accumulation; however, its effects vary depending on p53 status, ER expression, and tumour metabolism. Key gaps include limited long-term in vivo data, inconsistent responses across subtypes like HER2+ cells, and reliance on supra-physiological dosing. Addressing these gaps through subtype-specific trials and dose optimization could better define metformin's role in breast cancer therapy.

List of Abbreviations Used

AIF: apoptosis-inducing factor
AMPK: AMP-activation protein kinase
Bcl-2: B-cell lymphoma 2
MMP: mitochondrial membrane permeability
MOMP: mitochondrial outer membrane permeabilization
ROS: reactive oxygen species
TNBC: triple-negative breast cancer

Conflicts of Interest

The author(s) declare that they have no conflicts of interest.

Ethics Approval and/or Participant Consent

This study is a systematic review of previously published literature and did not involve any new experiments with human participants or animals. Therefore, ethics approval and participant consent were not required.

Authors' Contributions

HH: contributed to the conception and design of the study, conducted literature review and data analysis, drafted and revised the manuscript, and approved the final version for publication.
SC: contributed to the conception and design of the study, conducted literature review and data analysis, drafted and revised the manuscript, and approved the final version for publication.
YX: contributed to the conception and design of the study, conducted literature review and data analysis, drafted and revised the manuscript, and approved the final version for publication.

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References

[1] Breast cancer [Internet]. [cited 2025 Apr 6]. Available from: https://www.who.int/news-room/fact-sheets/detail/breast-cancer
[2] Breast cancer cases projected to rise by nearly 40 per cent by 2050, [Internet] WHO warns | UN News. [cited 2025 Apr 6]. Available from: https://news.un.org/en/story/2025/02/1160391
[3] Camacho L, Dasgupta A, Jiralerspong S. Metformin in breast cancer - an evolving mystery. Breast Cancer Research. 2015 Jun 26 ;17(1):88, s13058-015-0598–8. https://doi.org/10.1186/s13058-015-0598-8
[4] Corleto KA, Strandmo JL, Giles ED. Metformin and Breast Cancer: Current Findings and Future Perspectives from Preclinical and Clinical Studies. Pharmaceuticals. 2024 Mar 19 ;17(3):396. https://doi.org/10.3390/ph17030396
[5] Cejuela M, Martin-Castillo B, Menendez JA, Pernas S. Metformin and Breast Cancer: Where Are We Now? International Journal of Molecular Sciences. 2022 Feb 28 ;23(5):2705. https://doi.org/10.3390/ijms23052705
[6] Queiroz EAIF, Puukila S, Eichler R, Sampaio SC, Forsyth HL, Lees SJ, et al. Metformin Induces Apoptosis and Cell Cycle Arrest Mediated by Oxidative Stress, AMPK and FOXO3a in MCF-7 Breast Cancer Cells. Katoh M, editor. PLoS ONE. 2014 May 23 ;9(5):e98207. https://doi.org/10.1371/journal.pone.0098207
[7] Pollak M. Metformin and other biguanides in oncology: advancing the research agenda. Cancer Prevention Research. 2013;11(9):594–5. https://doi.org/10.1158/1940-6207.capr-10-0175

[8] Xu JX, Zhu QL, Bi YM, Peng YC. New evidence: Metformin unsuitable as routine adjuvant for breast cancer: a drug-target mendelian randomization analysis. BMC Cancer. 2024 Jun 6 ;24(1):691. https://doi.org/10.1186/s12885-024-12453-w

[9] Hua Y, Hua Y, Zheng Y, Yao Y, Jia R, Ge S, Zhuang A. Metformin and cancer hallmarks: shedding new lights on therapeutic repurposing. Journal of Translational Medicine. 2023 Jun 21 ;21(1):403. https://doi.org/10.1186/s12967-023-04263-8

[10] Hampsch RA, Wells JD, Traphagen NA, McCleery CF, Fields JL, Shee K, et al. AMPK Activation by Metformin Promotes Survival of Dormant ER+ Breast Cancer Cells. Journal of Clinical and Translational Research. 2020 Jul 15 ;26(14):3707–19. https://doi.org/10.1158/1078-0432.CCR-20-0269

[11] El-Masry OS, Brown BL, Dobson PRM. AMPK Activation of Apoptotic Markers in Human Breast Cancer Cell Lines with Different p53 Backgrounds: MCF-7, MDA-MB-231 and T47D Cells. Asian Pacific Journal of Cancer Prevention. 2019 Dec 1 ;20(12):3763–70. https://doi.org/10.31557/APJCP.2019.20.12.3763

[12] Li M, Li X, Zhang H, Lu Y. Molecular Mechanisms of Metformin for Diabetes and Cancer Treatment. Frontiers in Physiology. 2018;9:1039. Published 2018 Jul 31. http://doi.org/10.3389/fphys.2018.01039

[13] Dewson G, Kluck RM. Mechanisms by which Bak and Bax permeabilise mitochondria during apoptosis. Journal of Cell Science. 2009 Aug 15 ;122(16):2801–8. https://doi.org/10.1242/jcs.038166

[14] Wang Y, Xu W, Yan Z, Zhao W, Mi J, Li J, et al. Metformin induces autophagy and G0/G1 phase cell cycle arrest in myeloma by targeting the AMPK/mTORC1 and mTORC2 pathways. Journal of Experimental and Clinical Cancer Research. 2018 ;37(1):63. https://doi.org/10.1186/s13046-018-0731-5

[15] Honma N, Horii R, Ito Y, Saji S, Younes M, Iwase T, et al. Differences in clinical importance of Bcl-2 in breast cancer according to hormone receptors status or adjuvant endocrine therapy. BMC Cancer. 2015 ;15(1):698. https://doi.org/10.1186/s12885-015-1686-y

[16] Gao ZY, Liu Z, Bi MH, Zhang JJ, Han ZQ, Han X, et al. Metformin induces apoptosis via a mitochondria-mediated pathway in human breast cancer cells in vitro. Experimental and Therapeutic Medicine. 2016;11(5):1700–6. https://doi.org/10.3892/etm.2016.3143

[17] Sharma P, Kumar S. Metformin inhibits human breast cancer cell growth by promoting apoptosis via a ROS-independent pathway involving mitochondrial dysfunction: pivotal role of superoxide dismutase (SOD). Cellular Oncology. 2018 ;41(6):637–50. Available from: https://doi.org/10.1007/s13402-018-0398-0

[18] Bashraheel SS, Kheraldine H, Khalaf S, Moustafa AEA. Metformin and HER2-positive breast cancer: Mechanisms and therapeutic implications. Biomedicine & Pharmacotherapy. 2023 ;162:114676. https://doi.org/10.1016/j.biopha.2023.114676

[19] Williams MM, Cook RS. Bcl-2 family proteins in breast development and cancer: could Mcl-1 targeting overcome therapeutic resistance? Oncotarget. 2015 Feb 28 ;6(6):3519–30. https://doi.org/10.18632/oncotarget.2792

[20] Sovilj D, Kelemen CD, Dvorakova S, Zobalova R, Raabova H, Kriska J, et al. Cell-specific modulation of mitochondrial respiration and metabolism by the pro-apoptotic Bcl-2 family members Bax and Bak. Apoptosis. 2024 ;29(3–4):424–38. https://doi.org/10.1007/s10495-023-01917-2

[21] Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 2016 ;1863(12):2977–92. https://doi.org/10.1016/j.bbamcr.2016.09.012

[22] Pallepati P, Averill-Bates DA. Mild thermotolerance induced at 40°C protects HeLa cells against activation of death receptor-mediated apoptosis by hydrogen peroxide. Free Radical Biology and Medicine . 2011 ;50(6):667–79. https://doi.org/10.1016/j.freeradbiomed.2010.11.022

[23] Oshi M, Gandhi S, Yan L, Tokumaru Y, Wu R, Yamada A, et al. Abundance of reactive oxygen species (ROS) is associated with tumor aggressiveness, immune response, and worse survival in breast cancer. Breast Cancer Research Treatment. 2022 ;194(2):231–41. https://doi.org/10.1007/s10549-022-06633-0

[24] Schexnayder C, Broussard K, Onuaguluchi D, Poché A, Ismail M, McAtee L, et al. Metformin Inhibits Migration and Invasion by Suppressing ROS Production and COX2 Expression in MDA-MB-231 Breast Cancer Cells. International Journal of Molecular Sciences. 2018 Nov 21 ;19(11):3692. https://doi.org/10.3390/ijms19113692

[25] Tan K, Naylor MJ. Tumour Microenvironment-Immune Cell Interactions Influencing Breast Cancer Heterogeneity and Disease Progression. Frontiers in Oncology. 2022 May 13 ;12:876451. https://doi.org/10.3389/fonc.2022.876451

[26] Chen Z, Han F, Du Y, Shi H, Zhou W. Hypoxic microenvironment in cancer: molecular mechanisms and therapeutic interventions. Signal Transduction and Targeted Therapy. 2023 Feb 17 ;8(1):70. https://doi.org/10.1038/s41392-023-01332-8

[27] Dzobo K, Senthebane DA, Dandara C. The Tumor Microenvironment in Tumorigenesis and Therapy Resistance Revisited. Cancers. 2023 Jan 6 ;15(2):376. https://doi.org/10.3390/cancers15020376

[28] McMillin DW, Negri JM, Mitsiades CS. The role of tumour–stromal interactions in modifying drug response: challenges and opportunities. Nature Reviews Drug Discovery. 2013 ;12(3):217–28. https://doi.org/10.1038/nrd3870

[29] Chaudhari K, Wang J, Xu Y, Winters A, Wang L, Dong X, et al. Determination of metformin bio-distribution by LC-MS/MS in mice treated with a clinically relevant paradigm. Kanzaki M, editor. PLoS ONE. 2020 Jun 11 ;15(6):e0234571. https://doi.org/10.1371/journal.pone.0234571

[30] Mohammed I, Hollenberg MD, Ding H, Triggle CR. A Critical Review of the Evidence That Metformin Is a Putative Anti-Aging Drug That Enhances Healthspan and Extends Lifespan. Frontiers in Endocrinology. 2021 Aug 5 ;12:718942. https://doi.org/10.3389/fendo.2021.718942

[31] Kajbaf F, De Broe ME, Lalau JD. Therapeutic Concentrations of Metformin: A Systematic Review. Clinical Pharmacokinetics. 2016 ;55(4):439–59. https://doi.org/10.1007/s40262-015-0323-x

[32] Roberts AW. Therapeutic development and current uses of BCL-2 inhibition. Hematology . 2020 Dec 4 ;2020(1):1–9. https://doi.org/10.1182/hematology.2020000154

[33] Scherbakov AM, Sorokin DV, Tatarskiy VV Jr, et al. The phenomenon of acquired resistance to metformin in breast cancer cells: The interaction of growth pathways and estrogen receptor signaling. IUBMB Life. 2016;68(4):281-292. https://doi.org/10.1002/iub.1481

[34] Oliveras-Ferraros C, Vazquez-Martin A, Cuyàs E, et al. Acquired resistance to metformin in breast cancer cells triggers transcriptome reprogramming toward a degradome-related metastatic stem-like profile. Cell Cycle. 2014;13(7):1132-1144. https://doi.org/10.4161/cc.27982

[35] ScienceDaily. Combination treatment, diabetes drug and immunotherapy, may help to fight breast cancer. https://doi.org/10.1038/s41467-019-08541-2

[36] Gu Z, Ye F, Luo H, Li X, Gong Y, Mao S, et al. Metformin sensitizes triple-negative breast cancer to histone deacetylase inhibitors by targeting FGFR4. Journal of Biomed Sci. 2025 Mar 17 ;32(1):36. https://doi.org/10.1186/s12929-025-01129-7

[37] Abdolahi S, Ghazvinian Z, Muhammadnejad S, Saleh M, Asadzadeh Aghdaei H, Baghaei K. Patient-derived xenograft (PDX) models, applications and challenges in cancer research. Journal of Translational Medicine. 2022 May 10 ;20(1):206. https://doi.org/10.1186/s12967-022-03405-8

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