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The role of the mTOR pathway in breast cancer stem cells (BCSCs): mechanisms and therapeutic potentials

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Abstract

Breast cancer remains the most frequently diagnosed cancer globally, exerting a profound impact on women's health and healthcare systems. Central to its pathogenesis and therapeutic resistance are breast cancer stem cells (BCSCs), which possess unique properties such as self-renewal, differentiation, and resistance to conventional therapies, contributing to tumor initiation, metastasis, and recurrence. This comprehensive review elucidates the pivotal role of the mechanistic target of rapamycin (mTOR) pathway in regulating BCSCs and its implications for breast cancer progression and treatment resistance. We explore the cellular mechanisms by which mTOR influences metastasis, metabolism, autophagy, and ferroptosis in BCSCs, highlighting its contribution to epithelial-to-mesenchymal transition (EMT), metabolic reprogramming, and survival under therapeutic stress. On a molecular level, mTOR interacts with key signaling pathways including PI3K/Akt, Notch, IGF-1R, AMPK, and TGF-β, as well as regulatory proteins and non-coding RNAs, orchestrating a complex network that sustains BCSC properties and mediates chemoresistance and radioresistance. The review further examines various therapeutic strategies targeting the mTOR pathway in BCSCs, encompassing selective PI3K/Akt/mTOR inhibitors, monoclonal antibodies, natural products, and innovative approaches such as nanoparticle-mediated drug delivery. Clinical trials investigating mTOR inhibitors like sirolimus and combination therapies with agents such as everolimus and trastuzumab are discussed, underscoring their potential in eradicating BCSCs and improving patient outcomes. Additionally, natural compounds and repurposed drugs offer promising adjunctive therapies by modulating mTOR activity and targeting BCSC-specific vulnerabilities. In conclusion, targeting the mTOR pathway presents a viable and promising avenue for enhancing breast cancer treatment efficacy by effectively eliminating BCSCs, reducing tumor recurrence,

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and improving overall patient survival. Continued research and clinical validation of mTOR-targeted therapies are essential to translate these insights into effective clinical interventions, ultimately advancing personalized cancer management and therapeutic outcomes for breast cancer patients.

Keywords Breast cancer, Cancer stem cells, mTOR pathway, Metastasis, Chemoresistance, Targeted therapy, PI3K/Akt, Autophagy, Ferroptosis, Clinical trials

Introduction

In 2022, breast cancer ranked as the second most frequently diagnosed cancer globally, with approximately 2.3 million new cases (11.6% of all cancers) and 666,000 deaths (6.9% of all cancer-related fatalities). It was the most prevalent cancer among women, representing nearly one-quarter of all female cancer diagnoses and one-sixth of cancer deaths in women worldwide [1]. Various factors, including reproductive and hormonal behaviors, lifestyle choices, and access to early detection and treatment services, contribute to the multifaceted nature of breast cancer burden [2]. Breast cancer is a highly heterogeneous disease categorized into distinct molecular subtypes: Luminal A, Luminal B, HER-2 positive, and triple-negative breast cancer (TNBC). These subtypes differ significantly in their molecular profiles, clinical outcomes, and therapeutic responses. Luminal subtypes, characterized by estrogen receptor (ER) positivity, generally respond well to antiestrogen therapies, while HER-2 positive breast cancer is driven by HER-2 gene amplification and responds to targeted therapies like trastuzumab [3, 4]. In contrast, TNBC lacks ER, progesterone receptor (PR), and HER-2 expression, exhibits a high frequency of BRCA1/2 mutations, and is highly aggressive and resistant to standard treatments [5]. Within this framework, breast cancer stem cells (BCSCs), a subpopulation with self-renewal and tumor-initiating capabilities, contribute to tumor heterogeneity, progression, and therapy resistance. The prevalence and behavior of BCSCs vary among subtypes, with TNBC harboring the highest proportion, including mesenchymal CD44+/CD24−/low cells, while luminal subtypes have fewer BCSCs, often linked to endocrine resistance [6]. BCSCs play a critical role in the treatment of breast cancer due to their unique properties and implications for disease progression. Unlike other cancer cells, BCSCs exhibit stem-like characteristics, including self-renewal and differentiation abilities, as well as resistance to conventional therapies. This resistance contributes to tumor recurrence, metastasis, and therapeutic failure. Understanding the origin and heterogeneity of BCSCs is essential for developing targeted therapies that specifically eradicate these resistant cells. Targeting BCSCs holds promise for improving treatment outcomes by preventing recurrence and metastasis, ultimately enhancing patient survival rates. Additionally, BCSC-directed therapies may provide opportunities for personalized cancer management, offering new avenues for more effective and tailored treatment approaches in breast cancer patients [7]. mTOR (mechanistic target of rapamycin) is a serine/threonine kinase that plays a crucial role in regulating various cellular processes, including protein synthesis, cell growth, metabolism, and autophagy. It exists in two distinct complexes: mTORC1 and mTORC2. mTOR signaling is frequently dysregulated in cancer, including breast cancer, leading to increased cell proliferation, survival, and tumor progression. In breast cancer therapy, mTOR inhibition has emerged as a significant strategy due to its role in promoting tumor growth. Drugs like rapamycin and its analogs (rapalogues) have been developed to target mTOR, particularly mTORC1, thereby slowing tumor growth and limiting cancer spread. Additionally, research into alternative inhibitors targeting mTOR via different mechanisms, such as ATP-competitive inhibitors and pan-PI3K inhibitors, offers potential avenues for more effective breast cancer treatments by circumventing the limitations associated with rapalogues and addressing resistance mechanisms. Thus, understanding and targeting mTOR signaling pathways hold promise for improving breast cancer therapy outcomes [8]. Activation of the mTOR pathway is pivotal for the functioning CSCs, as it fosters their self-renewal, maintenance, and tumorigenic capabilities. CSCs exhibit heightened mTOR activity, which enhances their resilience to standard cancer treatments and promotes their survival within the tumor environment. Moreover, mTOR activation in CSCs triggers epithelial-to-mesenchymal transition (EMT), a process linked to increased aggressiveness and metastasis. Targeting mTOR signaling in CSCs is a promising therapeutic avenue, as it disrupts their function, sensitizes them to conventional therapies, and impedes tumor progression and spread. Thus, comprehending the significance of mTOR activation in CSCs offers valuable insights for developing innovative treatments to combat therapy-resistant cancers and enhance patient prognosis [9]. In this review, our objective is to unravel the significance of the mTOR pathway in BCSCs for the initial time, offering an understanding of its molecular connections and its potential as a targeted approach for therapy.

Cellular mechanisms of mTOR in BCSCs

mTOR is a key regulator of cellular functions, orchestrating processes such as cell growth, proliferation, metabolism, survival, and differentiation. It senses nutrient

availability, growth factors, and cellular stress to modulate pathways involved in protein synthesis, energy metabolism, autophagy, and immune responses. Activation of mTOR promotes cell growth and survival by stimulating protein synthesis and inhibiting apoptosis, while its inhibition induces autophagy and cell death under stress conditions. Additionally, mTOR influences cell fate decisions during development and regulates immune cell function (Fig. 1) [10, 11].

Metastasis

BCSC are central to metastasis owing to their capacity for self-renewal, evasion of apoptosis, and adaptability to diverse microenvironments. They drive the initiation and maintenance of metastatic lesions by infiltrating tissues, surviving in the bloodstream, and establishing colonies in distant organs. Their resilience against standard therapies accelerates disease progression, positioning them as essential targets for advanced cancer treatment strategies [12]. The role of mTOR in metastasis is multifaceted and pivotal in various stages of the metastatic cascade. mTOR signaling is intricately involved in promoting EMT, a key process enabling cancer cells to acquire migratory and invasive properties essential for metastasis initiation. Through its downstream effectors, mTOR influences cytoskeletal rearrangement, cell motility, and invasion by modulating pathways such as RhoA and Rac1 [13]. Additionally, mTOR activation upregulates EMT transcription factors such as Snail, ZEB, and Twist, which suppress E-cadherin and promote mesenchymal traits, enhancing tumor cell invasiveness. mTOR also increases the activity of MMP-2 and MMP-9, facilitating extracellular matrix degradation and tumor dissemination. Through cytoskeletal reorganization, mTOR supports cell migration and colonization at distant sites [14]. High collagen density in the tumor microenvironment significantly impacts the behavior of ERα+ (luminal B) mammary carcinomas. This density fosters increases activity of the mTOR signaling pathway, which in turn enhances the presence of CSCs within the tumors. These CSCs are associated with aggressive tumor behavior and therapeutic resistance. Moreover, the dense collagen environment promotes

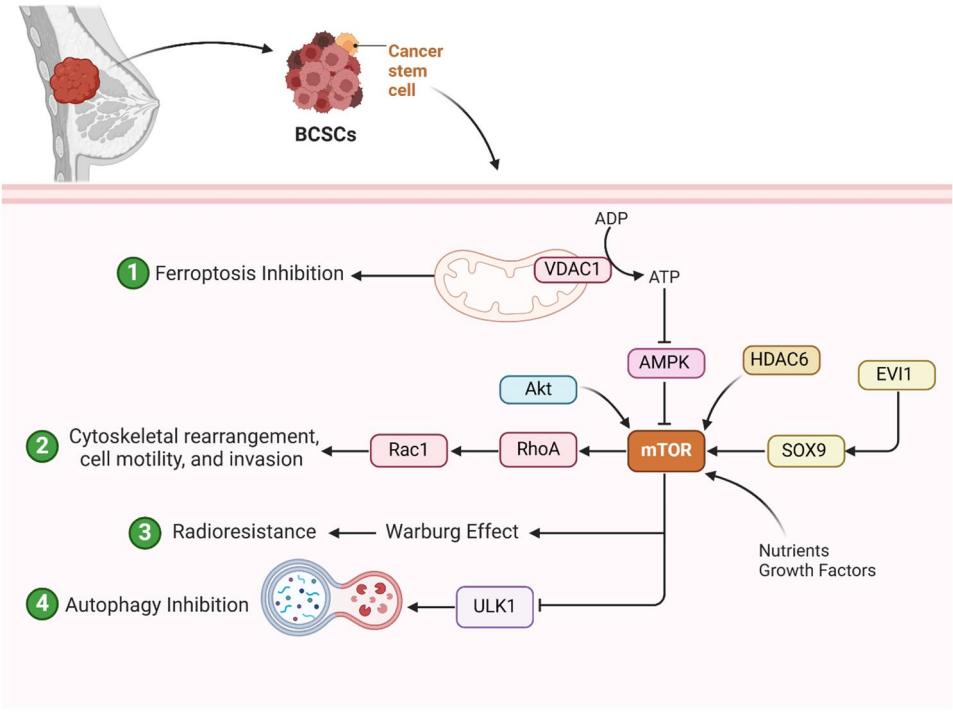

Fig. 1 Cellular Mechanisms of mTOR in BCSCs: This schematic illustrates the multifaceted roles of mTOR in regulating cellular functions in BCSCs, with implications in metastasis, metabolism, autophagy, and ferroptosis. The mTOR pathway integrates signals from nutrients, growth factors, and cellular energy levels, modulating various downstream pathways to influence cell fate. Key mechanisms are highlighted: Ferroptosis Inhibition: mTOR interacts with mitochondrial VDAC1, modulating ferroptosis pathways critical for cell survival under oxidative stress. Cytoskeletal Rearrangement, Cell Motility, and Invasion: Through the activation of Rac1 and RhoA, mTOR supports cytoskeletal changes essential for metastatic progression by promoting epithelial-mesenchymal transition (EMT) in BCSCs. Radioresistance and the Warburg Effect: mTOR's role in metabolic reprogramming enhances glycolysis (Warburg effect), supporting cancer cell survival and resistance to radiation therapy, with implications for targeting BCSCs in therapy-resistant breast cancer. Autophagy Inhibition: mTOR directly inhibits ULK1 to suppress autophagy, a process that, if activated, would degrade cellular components under nutrient-deprived conditions. Regulatory factors, including AMPK, Akt, SOX9, and EVI1, further modulate mTOR activity, enhancing BCSC survival, self-renewal, and metastatic potential. This network highlights mTOR as a therapeutic target, especially given its role in CSC resilience and therapeutic resistance. Targeting mTOR, along with auxiliary pathways like VDAC1 and HDAC6, may offer novel strategies to overcome resistance in breast cancer therapy

mTOR-independent pathways, such as those involving Yes-associated protein (YAP), further contributing to CSC activity. Consequently, tumors in this microenvironment exhibit heightened aggressiveness and metastatic potential. Despite the efficacy of mTOR inhibitors in reducing primary tumor growth and CSC activity, lung metastasis remain resistant to treatment, indicating distinct mechanisms driving metastasis compared to primary tumor growth in ERα + mammary carcinomas exposed to high collagen density [15]. Stem cell-like transcriptional reprogramming emerges as a pivotal mediator of metastatic resistance to mTOR inhibition in breast cancer. Dysregulated mTOR signaling drives a transcriptional program associated with stem cell-like properties and metastasis promotion. The proto-oncogene EVI1 plays a central role in this process, cooperating with SOX9 to sustain mTORC1 activity and enhance metastatic features. This transcriptional reprogramming leads to the emergence of a tumor cell population with increased metastatic potential and resistance to mTOR inhibitors. Depletion of EVI1 or SOX9 attenuates metastatic properties and sensitizes cells to mTOR inhibition, suggesting a crucial role for the EVI1-SOX9 axis in driving therapeutic resistance and metastasis in breast cancer. Targeting this axis may represent a promising strategy to overcome resistance to mTOR inhibitors and inhibit metastatic progression in breast cancer patients [16].

Metabolism

mTOR, a central regulator of cell growth and metabolism, plays crucial roles in coordinating various metabolic processes essential for cellular function and homeostasis. Its two distinct complexes, mTORC1 and mTORC2, integrate signals from nutrients, growth factors, and cellular energy levels to modulate protein synthesis, glucose metabolism, lipid synthesis, and glutamine metabolism. mTORC1 promotes anabolic processes such as protein synthesis by phosphorylating key effectors like S6K1 and 4EBP1, while also regulating transcription factors like HIF1α and Myc to influence glucose uptake and glycolytic metabolism. Additionally, mTORC1 stimulates lipid synthesis through activation of SREBP transcription factors and enhances glutamine metabolism by modulating enzymes involved in glutaminolysis. On the other hand, mTORC2 regulates glucose metabolism and lipid synthesis via its substrate Akt, while also impacting glutamine metabolism and purine synthesis. Together, mTOR signaling pathways orchestrate a finely tuned metabolic network essential for cell growth, proliferation, and survival, making them attractive targets for cancer therapy and metabolic disorders [17].The fasting-mimicking diet (FMD) effectively impedes the progression of TNBC and inhibits CSC escape mechanisms primarily through the modulation of the mTOR pathway. By inducing a state mimicking fasting conditions, the FMD reduces glucose levels, thereby inhibiting mTOR activation in CSCs while activating mTOR in differentiated cancer cells. This differential regulation prevents CSC survival and self-renewal while promoting the regression of tumor masses. Additionally, the FMD prevents hyperglycemia induced by mTOR inhibitors, further contributing to its anti-tumor effects [18]. Likewise, glycolytic inhibitors like 2-deoxyglucose (2DG) show promise in cancer treatment by targeting the heightened glycolytic activity in tumors, including TNBCs. 2DG disrupts glycolysis by inhibiting hexokinase, causing energy stress and indirectly downregulating the mTOR pathway, a key driver of tumor growth. In TNBCs, where mTOR is often hyperactivated, combining 2DG with agents like metformin, which directly inhibits mTOR via AMPK activation, enhances therapeutic efficacy. This dual approach exploits the metabolic vulnerabilities of TNBCs, effectively reducing cell proliferation and inducing apoptosis [19]. EMT triggers metabolic rewiring in breast cancer cells, particularly affecting glutamine metabolism. Mesenchymal breast stem cells, characterized by heightened reductive carboxylation of glutamine, exhibit altered redox homeostasis and decreased glutathione biosynthesis. These metabolic adaptations sensitize these cells to mTOR inhibition, as evidenced by their enhanced sensitivity to mTOR inhibitors compared to their epithelial counterparts. Glutamine-derived metabolites play a crucial role in mediating this sensitivity, highlighting the potential therapeutic significance of targeting glutamine metabolism in metastatic breast cancer [20]. The deregulation of cell energetics and reprogramming of energy metabolism, notably through the Warburg effect, where cancer cells upregulate glycolysis even in the presence of oxygen, are key features of cancer. Metformin, a widely prescribed anti-diabetic drug, has shown promise in cancer therapy by inhibiting aberrant glycolysis and activating the AMPK signaling pathway, which leads to mTOR inhibition and subsequent reduction in protein synthesis and cell proliferation. TNBC, characterized by its aggressive nature and lack of hormone receptors, is particularly challenging to treat, with the mTOR pathway often deregulated. Studies have suggested that metformin may have therapeutic benefits in TNBC, although its efficacy might be compromised in diabetic individuals due to elevated glucose concentrations. The impact of varying glucose concentrations on the anti-cancer effects of metformin in TNBC cells, particularly focusing on mesenchymal breast stem cells has been investigated [21]. It was found that higher glucose concentrations attenuated the efficacy of metformin, while glucose starvation enhanced its cytotoxic effects, especially in stem cell populations. Additionally, metformin effectively inhibited

the mTOR pathway under glucose-starved conditions in stem cells, suggesting a potential strategy for enhancing its therapeutic efficacy in TNBC, particularly by targeting the stem cell population. These findings underscore the importance of considering metabolic factors, such as glucose levels and stem cell populations, in optimizing the use of metformin as a therapeutic agent for TNBC [22]. Targeting the metabolic vulnerability of BCSCs with metformin has emerged as a promising strategy to enhance the efficacy of radiotherapy and selectively eradicate BCSCs. Metformin, a widely used anti-diabetic drug, disrupts mitochondrial respiration, leading to activation of AMP-activated protein kinase (AMPK) and suppression of the mTOR signaling pathway. This metabolic modulation not only sensitizes cancer cells to radiation but also preferentially targets BCSCs due to their heightened metabolic activity and reliance on mitochondrial respiration. By inhibiting mitochondrial function and altering cellular energy metabolism, metformin induces cytotoxic effects on BCSCs, reducing their clonogenic survival and impeding sphere formation, a characteristic growth pattern of BCSCs. Furthermore, metformin attenuates the radioresistance of CSCs, as evidenced by its ability to suppress the radiation-induced increase in the fraction of BCSCs. These findings highlight the potential of metformin to exploit the metabolic vulnerabilities of BCSCs, thereby enhancing the effectiveness of radiotherapy and providing a novel approach for selectively eliminating CSCs in breast cancer [23].

Autophagy

mTOR plays a crucial role in regulating autophagy, the cellular process responsible for degrading and recycling damaged organelles and proteins. In conditions of nutrient abundance or growth factor signaling, active mTOR suppresses autophagy by phosphorylating key autophagy-initiating complexes such as ULK1, preventing their association and thereby inhibiting autophagy induction. Conversely, when nutrients are scarce or cellular energy levels are low, mTOR activity is inhibited, allowing for the activation of ULK1 complex and initiation of autophagy. Additionally, mTOR phosphorylates other autophagy-related proteins like ATG14L, further regulating autophagosome formation. This dual role of mTOR in autophagy regulation enables cells to adapt to changing environmental conditions and maintain cellular homeostasis [24]. HDAC6, a regulatory enzyme, influences autophagy differently in BCSCs compared to differentiated cancer cells, largely through the mTOR signaling pathway and interactions with TSC proteins. In BCSCs, inhibiting HDAC6 paradoxically reduces autophagy, despite mTOR's known role in autophagy inhibition, a result of altered TSC1/TSC2 expression affecting mTOR activity. Conversely, in differentiated cancer cells, HDAC6 inhibition promotes autophagy through conventional mTOR pathway inhibition. This differential effect underscores the complex role of HDAC6 in cancer biology, highlighting the nuanced impact of cellular context on therapeutic targeting, particularly in treatments aimed at modulating autophagy and cell differentiation states in cancer [25]. Prolonged exposure of BCSCs to Rottlerin (Rott) leads to apoptosis, a form of programmed cell death, which is significantly associated with the suppression of the Akt/mTOR signaling pathway. Akt, also known as protein kinase B, and mTOR, the mammalian target of rapamycin, are critical for cell survival and proliferation. They play pivotal roles in various cellular processes, including growth, proliferation, and survival. In breast CSCs, the Akt/mTOR pathway is often overactivated, contributing to the cells' resistance to apoptosis and their ability to sustain cancer. Rottlerin's ability to inhibit this pathway diminishes the survival signals within the cells, thereby inducing apoptosis. This mechanism highlights a potential therapeutic approach targeting the resilience and recurrence often seen in breast cancer through the eradication of CSCs by disrupting key survival pathways [26].

Ferroptosis

Ferroptosis is a distinct form of programmed cell death characterized by iron-dependent lipid peroxidation and subsequent membrane damage, leading to cell demise [27, 28]. In the context of CSCs, targeting ferroptosis holds considerable promise as a therapeutic strategy due to its potential to selectively eliminate CSC populations, which are often implicated in cancer progression, recurrence, and treatment resistance. CSCs exhibit alterations in iron metabolism, including enhanced iron uptake, intracellular iron accumulation, and dysregulated antioxidant defense systems, rendering them particularly vulnerable to ferroptosis induction. By exploiting these vulnerabilities, therapies aimed at inducing ferroptosis in CSCs could effectively eradicate this subpopulation of cancer cells and improve treatment outcomes. Strategies to target ferroptosis in CSCs include modulating iron metabolism, enhancing lipid peroxidation, and inhibiting antioxidant defense mechanisms [29]. mTOR inhibition presents a promising strategy for targeting BCSCs due to its ability to selectively disrupt survival pathways that are hyperactive in these therapy-resistant cells. CSCs exhibit heightened metabolic plasticity, dependence on iron metabolism, and increased oxidative stress, making them particularly vulnerable to mTOR inhibition [30]. Mechanistically, mTOR inhibition plays a dual role: while it impairs key metabolic pathways such as mitochondrial oxidative phosphorylation, glycolysis, and glutaminolysis, it also prevents ferroptosis induction by stabilizing iron homeostasis and mitigating iron-catalyzed ROS production. However, this inhibition of ferroptosis by mTOR can

be overcome through the use of ferroptosis inducers such as salinomycin (Sal), which sequesters iron into lysosomes, creating oxidative stress and lipid peroxidation that triggers ferroptotic cell death. Importantly, ferroptosis selectively targets BCSCs due to their iron metabolism dysregulation and limited antioxidant defenses, while sparing normal cells, which maintain lower iron levels and stronger oxidative stress management. By carefully combining mTOR inhibitors with ferroptosis inducers, this dual mechanism can be exploited to maximize therapeutic efficacy against BCSCs, eradicating this aggressive population while preserving normal breast tissue and non-stem cancer cells (Studied by [31]). VDAC1, as the main transporter of metabolites across the mitochondrial outer membrane, influences cellular redox balance and lipid metabolism, thereby impacting susceptibility to ferroptosis. Its modulation affects the exchange of metabolites such as glutathione and NADPH, crucial components in antioxidant defense mechanisms and lipid peroxidation processes. Moreover, VDAC1-mediated calcium fluxes and interactions with pro- and anti-ferroptotic proteins regulate cellular redox homeostasis and mitochondrial function, influencing the susceptibility of cells to ferroptotic stimuli. In BCSC, silencing of mitochondrial VDAC1 triggers a cascade of metabolic rewiring events and drives the reprogramming of tumor cells into advanced differentiated states. This process involves a shift away from the Warburg effect, characterized by reduced glycolysis and altered oxidative phosphorylation, leading to decreased ATP production and mitochondrial membrane potential. Consequently, key metabolic regulators such as AMPK and mTOR signaling pathways are modulated. Additionally, VDAC1 depletion results in a marked reduction in CSC populations, as evidenced by decreased expression of CSC-associated markers like aldehyde dehydrogenase isoform 1 (ALD1HA1), SOX2, CD133, and CD44. Importantly, the downregulation of VDAC1 also induces differentiation of BCSCs, as indicated by increased expression of markers associated with mature mammary epithelial cell phenotypes, such as CD24 and prolactin receptors (PRLR), and decreased expression of stemness markers like CD44. Overall, mitochondrial VDAC1 silencing in BCSCs orchestrates a complex interplay of metabolic reprogramming, CSC depletion, and induction of cell differentiation, offering promising therapeutic avenues for breast cancer treatment [32].

EMT

EMT in breast cancer cells significantly reprograms metabolism, impacting glutamine utilization, redox homeostasis, and drug sensitivity. EMT increases reliance on mitochondrial isocitrate dehydrogenase 2 (IDH2)-mediated reductive carboxylation of glutamine for fatty acid synthesis, reducing glycolytic flux and glutathione production. This reprogramming disrupts cellular redox balance and decreases the antioxidant defense capacity, making EMT-derived cells more sensitive to mTOR inhibitors. Notably, reducing intracellular GSH levels enhances this sensitivity, highlighting a vulnerability that may be exploited therapeutically [20]. Prolonged exposure to TGF-β in breast cancer cells stabilizes EMT, thereby enhancing BCSC characteristics and drug resistance. This stabilized EMT state is associated with increased activity of the mTOR signaling pathway, which remains elevated even after TGF-β removal. mTOR inhibition using a bitopic inhibitor effectively reduced CSC populations, suppressed anchorage-independent growth, and diminished chemoresistance, highlighting mTOR's critical role in maintaining stemness and drug resistance in breast cancer cells [33]. BYL-719 (alpelisib) is a selective inhibitor of the PI3K/AKT/mTOR signaling pathway, specifically targeting the PI3K catalytic subunit p110α. In BCSCs, it disrupts key processes that drive EMT, a critical mechanism in tumor progression, metastasis, and drug resistance. EMT is modulated through the mTOR pathway, which supports BCSC stemness, survival, and plasticity. BYL-719 inhibits mTOR activity by reducing phosphorylation of downstream effectors such as p-P70S6K and p-4EBP1, leading to a decrease in EMT markers like NANOG, SOX2, and OCT3/4. This inhibition attenuates the mesenchymal phenotype of BCSCs, suppresses their self-renewal capabilities, and enhances sensitivity to apoptosis, making BYL-719 a potent therapeutic agent in targeting EMT-related resistance in breast cancer [34]. Similarly, the synergistic combination of Salinomycin and Budesonide effectively reverses EMT in BCSCs by modulating the mTOR signaling pathway. This co-treatment downregulates the PI3K/AKT/mTOR axis, reducing the phosphorylation of AKT and mTOR, which are critical for EMT induction and maintenance. As a result, the expression of mesenchymal markers like vimentin and N-cadherin decreases, while epithelial markers such as E-cadherin are upregulated, signifying EMT reversal. Furthermore, the treatment enhances autophagic activity by increasing the conversion of LC3-I to LC3-II, which contributes to the suppression of EMT-related transcription factors like Twist1. This interplay between mTOR inhibition and autophagy induction disrupts the stemness and invasive properties of BCSCs, providing a potent therapeutic strategy to combat drug resistance and tumor progression in TNBC [35].

Molecular mechanisms of mTOR in BCSCs

mTOR is intricately involved in cancer through multiple molecular interactions. Activation of the PI3K/Akt pathway, often driven by growth factor signaling or loss of tumor suppressor genes like PTEN and TSC1/2,

stimulates mTOR activity, promoting cell growth and survival. Additionally, mTOR influences hypoxia response pathways through HIF-1α, facilitating adaptation to low oxygen environments characteristic of tumors. It also regulates autophagy, impacting cellular stress responses, and affects gene expression via transcription factors like SREBP1/2 and c-Myc, influencing processes such as lipid metabolism and angiogenesis. Dysregulation of these interactions contributes to cancer development and progression, making mTOR an attractive target for therapeutic intervention in cancer treatment strategies (Fig. 2) [36–38].

PI3K/AKT

Mutations in PIK3CA, a gene that encodes catalytic subunit (p110α) of PI3K, play a crucial role in BCSCs by contributing to their self-renewal, tumorigenicity, and resistance to therapy. Activation of the PI3K/AKT/mTOR pathway resulting from PIK3CA mutations enhances the stem-like properties of BCSCs, including their ability to self-renew and initiate tumor formation. Specificzzally,

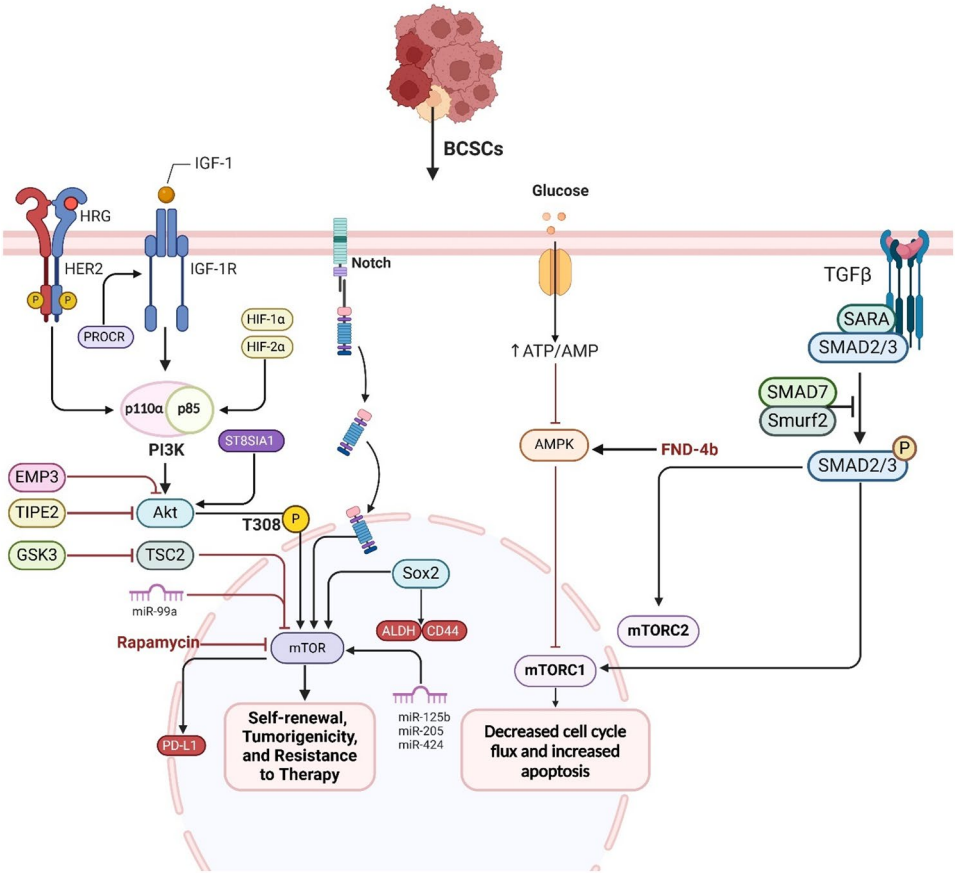

Fig. 2 Molecular Mechanisms of mTOR in BCSCs: This figure illustrates the complex interactions of the mTOR pathway within breast cancer stem cells, focusing on various upstream regulators, co-activators, and signaling pathways that sustain BCSC properties, including self-renewal, tumorigenicity, resistance to therapy, and metabolic adaptation. PI3K/AKT Pathway: PI3K/AKT activation, driven by growth factors (e.g., IGF-1R, HER2) and other proteins (e.g., EMP3, TIPE2, GSK3), stimulates mTOR signaling, which promotes BCSC survival, growth, and resistance to therapeutic interventions. Notably, nuclear localization of Akt enhances mTOR signaling, further sustaining stem-like characteristics in BCSCs. SOX2 and Notch Pathways: Overexpression of SOX2, coupled with Notch signaling activation, supports self-renewal and maintenance of BCSC populations. Notch signaling also modulates mTOR, amplifying cell survival and metabolic pathways in BCSCs.IGF-1R and TGF-β Pathways: IGF-1R signaling upregulates mTOR activity, promoting BCSC proliferation and resistance, while TGF-β influences epithelial-mesenchymal transition (EMT) and stem-like properties via SMAD pathways. This interaction contributes to resistance to anti-cancer therapies. AMPK and Energy Sensing: AMPK responds to changes in cellular energy (e.g., ATP/AMP levels), potentially antagonizing mTOR to inhibit cell cycle progression and induce apoptosis. This axis highlights the therapeutic potential of AMPK activators in targeting energy-dependent survival mechanisms in BCSCs. Non-coding RNAs and Additional Regulators: MicroRNAs (miR-99a, miR-125b) and regulatory proteins (e.g., PD-L1, HIF-1α, HIF-2α, ST8SIA1) influence mTOR activity in BCSCs. These regulators further impact metabolic and survival pathways, underscoring the therapeutic implications of targeting mTOR-related transcriptional and post-transcriptional networks. The interconnections between mTOR and these pathways highlight its central role in modulating cellular processes critical for BCSC survival and therapeutic resistance. This network provides insight into potential targets, such as mTOR inhibitors, AMPK activators, and modulators of signaling pathways like IGF-1R and Notch, which could be leveraged in breast cancer treatments to effectively target BCSCs and enhance therapeutic outcomes

PI3KCA mutations promote the expansion of BCSC populations and increase their resistance to apoptosis, leading to tumor progression and therapeutic resistance. Additionally, dysregulated PI3K/AKT/mTOR signaling in BCSCs promotes metabolic reprogramming, favoring glycolysis and providing energy for their enhanced proliferation and survival. The aberrant activation of the PI3K/AKT/mTOR pathway in BCSCs not only drives tumorigenesis but also contributes to the maintenance of their stem-like characteristics, highlighting its significance as a potential therapeutic target in breast cancer [39]. Nuclear localization of Akt not only influences the stemness of breast cancer cells but also engages the Akt/mTOR signaling pathway, further exacerbating the CSC phenotype. Akt, upon translocation to the nucleus, demonstrates increased phosphorylation at T308, indicative of heightened kinase activity, leading to enhanced mTOR activation. The Akt/mTOR axis plays a pivotal role in regulating various cellular processes, including protein synthesis, metabolism, and cell proliferation, all of which contribute to CSC maintenance and expansion. Activation of mTOR downstream of Akt fosters the expression of pluripotency factors and promotes cell cycle progression, ultimately fueling the growth and survival of CSCs. This intricate interplay between nuclear Akt and mTOR signaling underscores the multifaceted mechanisms by which Akt potentiates the stem-like characteristics of breast cancer cells, highlighting its significance as a potential therapeutic target for disrupting CSC-driven tumorigenesis [40]. Targeting mTOR rather than PI3K has been shown to be more effective in reducing cell proliferation, tumor growth, cell migration, and stemness in breast cancer for several reasons. Firstly, mTOR functions as a central hub downstream of PI3K/AKT signaling, integrating inputs from various upstream signaling pathways beyond PI3K alone. Therefore, directly inhibiting mTOR may provide a more comprehensive blockade of downstream signaling compared to targeting PI3K alone. Additionally, mTOR exists in two distinct complexes, mTORC1 and mTORC2, each with unique functions in regulating cell growth, survival, and metabolism. Inhibition of mTORC1, particularly with drugs like rapamycin, has been shown to exert potent anti-proliferative effects in breast cancer cells. Moreover, mTOR inhibition can suppress key processes involved in cancer progression, such as protein synthesis and cell cycle progression, thereby reducing tumor growth and metastatic potential. Importantly, mTOR inhibitors have demonstrated efficacy independent of the PIK3CA mutation status, suggesting that mTOR inhibition may effectively target other dysregulated pathways or compensatory mechanisms involved in breast cancer pathogenesis and resistance [41].

Stem cell markers

SOX2

Nuclear reprogramming of luminal-like breast cancer cells using Yamanaka factors initiates a process leading to the emergence of Sox2-overexpressing cancer stem-like cellular states. This reprogramming induces morphological changes reminiscent of human embryonic stem cells and significantly increases the expression of pluripotency marker SSEA-4, indicating an intermediate pluripotent state. While incomplete, the reprogramming results in the overexpression of Sox2, a crucial transcription factor associated with stemness. These Sox2-overexpressing cells exhibit characteristics of CSCs, including heightened ALDH activity and CD44 expression, without undergoing epithelial-to-mesenchymal transition. Importantly, transcriptional analysis reveals significant alterations in the mTOR pathway, with downregulation of mTOR inhibitors PRKAA1, DDIT4, and DEPTOR, alongside upregulation of the insulin receptor INSR. Furthermore, downstream targets of mTOR signaling, such as p70S6K1, show increased activity, indicating heightened mTOR pathway activation. This upregulation of the mTOR pathway coincides with the acquisition of CSC-like properties, suggesting a potential link between Sox2 overexpression and mTOR pathway activation in driving the emergence of CSC-like states in luminal-like breast cancer cells [42]. In addition, Nguyen et al. have discovered a link between SOX2 and mTOR in TNBC cells, mediated by ST8SIA1. They demonstrated that ST8SIA1 regulates SOX2 expression via the activation of the FAK–AKT–mTOR signaling pathway in BCSCs [43]. Monensin, an ionophoric antibiotic, disrupts cellular ion gradients, while erlotinib, an EGFR inhibitor, blocks key signaling in cancer cells. Together, these agents effectively inhibit the PI3K/AKT/mTOR pathway, which are critical for maintaining cancer stem-like properties, including SOX2 expression. The downregulation of SOX2 upon mTOR inhibition highlights mTOR's role in sustaining TNBC stem-like characteristics and regulating SOX2-dependent pathways [44].

NANOG

mTOR and its inhibitors significantly influence the regulation of NANOG expression BCSCs through translational reprogramming. mTOR inhibition, commonly aimed at reducing tumor growth, paradoxically promotes the accumulation of BCSCs by enabling the selective translation of NANOG mRNA isoforms with specific 5′UTRs. Under mTOR suppression, global protein synthesis decreases; however, certain stress-responsive NANOG isoforms escape this repression, driving increased NANOG protein levels. This process is mediated by the Integrated Stress Response (ISR), marked by eIF2α phosphorylation, which facilitates selective

translation. ISRIB, an ISR inhibitor, effectively mitigates this effect by preventing eIF2α phosphorylation, reducing NANOG-driven BCSC plasticity and enhancing therapeutic outcomes. Thus, while mTOR inhibitors suppress tumor proliferation, they unintentionally foster stem-cell-like phenotypes [45].

Notch

Activation of Notch1 by its ligand Jagged1 triggers IKKα, which phosphorylates Rictor, a component of mTORC2, leading to the activation of AKT (phosphorylation at Ser473). This Notch1-IKKα-mTORC2-AKT axis regulates mitochondrial oxidative phosphorylation and supports BCSC survival, highlighting a non-canonical (RBP-Jκ-independent) signaling mechanism. The interaction is particularly relevant in PTEN wild-type TNBC cells, with no significant role identified for other Notch paralogs like Notch4 in this context. Targeting this pathway through γ-secretase inhibitors, AKT inhibitors, or IKK inhibitors disrupts CSC survival and metabolism, making it a promising therapeutic strategy for TNBC [46].

IGF-1R

Insulin-like Growth Factor 1 Receptor (IGF-1R) is a cell surface receptor protein that plays a crucial role in mediating the effects of insulin-like growth factors (IGFs), particularly IGF-1 and IGF-2. It belongs to the family of receptor tyrosine kinases and is involved in regulating various cellular processes such as cell growth, proliferation, survival, and differentiation. Activation of IGF-1R initiates downstream signaling cascades, including the PI3K/Akt/mTOR pathway, which are essential for normal growth and development. Elevated IGF-1R activity, evidenced by increased phosphorylation levels and expression in BCSCs compared to non-BCSCs, has been linked to enhanced tumor initiation, growth, and the expression of stem cell markers. This activity correlates with higher tumorigenicity in vivo and an increased capacity for mammosphere formation in vitro. Importantly, IGF-1R signaling is implicated in the activation of the PI3K/Akt/mTOR pathway, a key regulator of cell survival, proliferation, and differentiation, which further supports the survival and stem-like qualities of BCSCs. Inhibition of IGF-1R, either by specific inhibitors like picropodophyllin or by knockdown approaches, significantly reduces these cancer stem cell properties, leading to decreased tumor growth, diminished mammosphere formation, and alterations in markers indicative of EMT. Collectively, these findings highlight the pivotal role of IGF-1R signaling in sustaining BCSC characteristics and suggest that targeting IGF-1R could be a promising strategy in treating breast cancer by eradicating the stem-like cell population within tumors [47]. The IGF-1R/PI3K/AKT/mTOR and Hippo pathways play critical roles in regulating BCSCs and their tumorigenic properties. The IGF-1R/PI3K/AKT/mTOR pathway is implicated in maintaining BCSC properties, including self-renewal and tumorigenicity. Activation of this pathway promotes cell proliferation and survival in BCSCs. Conversely, the Hippo pathway, with its core components MST1/2 and LATS1/2, regulates BCSCs by inhibiting the activity of YAP and TAZ, key transcriptional co-activators. Inhibition of YAP/TAZ leads to reduced stemness features and tumorigenic potential in BCSCs. Importantly, there is an interplay between these pathways, as evidenced by the regulatory impact of IGF-1R signaling on YAP expression and localization. Additionally, YAP regulate the expression of IGF-1, suggesting a feedback loop between these pathways [48].

AMPK

Breast cancer, particularly TNBC, presents significant challenges due to its aggressive nature and limited treatment options. AMP-activated protein kinase (AMPK) activation has emerged as a promising therapeutic avenue, given its role in inhibiting oncogenic pathways and promoting apoptosis. The effects of the AMPK activator FND-4b have been investigated in TNBC and estrogen receptor-positive breast cancer (ER + BC) cells. Analysis revealed no significant difference in AMPK expression between TNBC and ER + BC cells. Treatment with FND-4b activated AMPK and downstream signaling pathways, leading to decreased cell cycle flux and increased apoptosis in both subtypes, as evidenced by changes in phosphorylated AMPK, acetyl-CoA carboxylase, ribosomal protein S6, cyclin D1, and cleaved PARP levels. Furthermore, FND-4b exhibited dose-dependent growth inhibition across all breast cancer subtypes, with ER + BC cells showing slightly higher sensitivity. Importantly, FND-4b treatment increased apoptosis, particularly in MCF-7 and T-47D cells, underscoring its potential as a therapeutic agent for breast cancer. Notably, FND-4b-mediated activation of AMPK also led to attenuated mTOR signaling, further contributing to its anti-tumor effects. Although further studies are warranted to elucidate its full clinical potential and underlying mechanisms of action, these findings highlight the promising role of AMPK activation, coupled with mTOR inhibition, in breast cancer therapy [49].

TGF-β

Chronic exposure to TGF-β induces and stabilizes epithelial-mesenchymal transition (EMT) in mammary epithelial cells, leading to the acquisition of mesenchymal features and the maintenance of a stem cell-like state even after the removal of TGF-β. This prolonged exposure correlates with enhanced tumor stemness, as evidenced by

increased expression of stem cell markers and enhanced mammosphere formation capacity. Additionally, chronic TGF-β exposure confers resistance to anticancer drugs, a phenomenon associated with increased mTOR signaling. The activation of mTOR, including both mTORC1 and mTORC2, plays a crucial role in mediating drug resistance and maintaining the mesenchymal and stem cell-like characteristics induced by chronic TGF-β exposure. Inhibition of mTOR signaling effectively reduces the stem cell phenotype and increases sensitivity to anticancer drugs, highlighting the therapeutic potential of targeting mTOR to disrupt these processes and improve treatment outcomes in breast cancer [33].

Non-coding RNAs

Non-coding RNAs (ncRNAs) have emerged as key regulators of cellular processes, including the modulation of the mTOR pathway. Various types of ncRNAs, such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), exert regulatory control over mTOR signaling at multiple levels. miRNAs can directly target mTOR or its upstream regulators, inhibiting their expression and consequently suppressing mTOR activity. Additionally, lncRNAs can act as scaffolds or decoys, sequestering regulatory proteins or RNA molecules involved in mTOR signaling, thereby modulating its activity. Furthermore, circRNAs have been implicated in regulating mTOR signaling through diverse mechanisms, including acting as miRNA sponges or interacting with mTOR-associated proteins. Collectively, the intricate interplay between ncRNAs and the mTOR pathway underscores the complexity of cellular regulation and highlights the importance of non-coding transcripts in fine-tuning essential cellular processes [50–53]. In BCSCs, miR-99a directly targets the mTOR signaling pathway. Through computational prediction programs and luciferase assays, a putative binding site for miR-99a was identified in the 3'UTR of the mTOR gene. Restoration of miR-99a expression suppressed luciferase activity in CSCs, confirming the direct interaction between miR-99a and mTOR mRNA. Additionally, overexpression of miR-99a decreased both mRNA and protein levels of mTOR in CSCs. This downregulation of mTOR led to reduced expression of its downstream target, HIF-1α, as well as downstream transcription factors Oct-4 and c-Myc, which are implicated in CSC progression. These findings elucidate a mechanistic link between miR-99a and the mTOR signaling pathway, underscoring the role of miR-99a in modulating CSC characteristics in breast cancer [54]. The deregulated expression of miR-125b, miR-205, and miR-424 is shown to be adequate in inducing resistance to aromatase inhibitors (AIs), activating the AKT/mTOR pathway, and triggering the emergence of a subset of cells exhibiting stem-like characteristics. This finding underscores the pivotal role these microRNAs play in driving resistance to AI therapy, as well as in promoting aggressive cancer phenotypes. Notably, their ability to activate the AKT/mTOR pathway, a key signaling cascade implicated in cancer cell survival and proliferation, further elucidates the mechanistic underpinnings of AI resistance. Additionally, the observed emergence of cells with stem-like properties suggests a potential link between miRNA dysregulation and the acquisition of traits associated with tumor-initiating cells, which are known to contribute to therapy resistance and disease recurrence in breast cancer [55].

Other Regulator proteins

TIPE2

Tumor necrosis factor-α-induced protein-8-like-2 (TIPE2) plays a crucial role in regulating BCSCs properties through the mTOR signaling pathway. In breast cancer cells, TIPE2 overexpression leads to the inhibition of BCSC markers such as OCT4, Nanog, and Sox2, thereby suppressing self-renewal abilities and migratory potential. Mechanistically, TIPE2 modulates the mTOR pathway, which is intricately involved in BCSC maintenance. Specifically, TIPE2 promotes the phosphorylation of AKT and mTOR, leading to downstream signaling that inhibits CSC characteristics. By regulating mTOR activation, TIPE2 effectively attenuates the stemness of breast cancer cells, offering a promising avenue for targeting BCSCs and potentially overcoming chemoresistance in breast cancer therapy [56].

EMP3

Epithelial membrane protein 3 (EMP3), a member of the PMP22 gene family, exhibits context-dependent roles in cancer, including breast cancer. In breast cancer stem cells, EMP3 plays crucial functions related to chemoresistance and stem-like properties. Through in silico analyses and experimental validations, EMP3 is implicated as a tumor suppressor in breast cancer, correlating with favorable patient outcomes such as improved survival and reduced recurrence. Mechanistically, EMP3 inhibits S-phase entry and DNA replication while enhancing sensitivity to chemotherapy drugs like Adriamycin. Furthermore, EMP3 interferes with stem-like properties by downregulating stem-related markers and inhibiting mammosphere formation, characteristic of cancer stem cells. EMP3 also modulates signaling pathways such as Akt-mTor, inducing autophagy and influencing downstream effectors like YTHDC1, thereby impacting DNA damage repair and chemosensitivity. Overall, EMP3 emerges as a promising target for combating chemoresistance and suppressing stem-like properties in breast cancer cells [57].

Heregulin

Heregulin, also known as neuregulin, is a ligand that activates HER3 and HER4 receptors, members of the HER family. In breast cancer, heregulin plays a pivotal role in regulating CSC populations and the mTOR pathway. Studies have shown that heregulin treatment increases the proportion of CSCs within breast cancer cell lines, as evidenced by upregulation of CSC markers such as CD44+/CD24 – and NANOG/KLF4 expression. Additionally, heregulin has been implicated in attenuating the growth inhibitory effects of mTOR inhibitors, such as rapamycin and everolimus, suggesting a mechanism for resistance to anti-mTOR therapy. Through its actions on CSC regulation and mTOR signaling, heregulin contributes to the aggressiveness and therapeutic resistance of breast cancer, highlighting its significance as a potential target for novel therapeutic interventions aimed at overcoming endocrine resistance and improving patient outcomes [58].

PD-L1

Programmed death-ligand 1 (PD-L1) is a protein expressed on the surface of various cells, including cancer cells, that plays a crucial role in regulating the immune response. Its interaction with programmed cell death protein 1 (PD-1) on immune cells suppresses the immune system's ability to recognize and attack cancer cells, leading to immune evasion and tumor progression [59]. PD-L1 expression is associated with poor prognosis in many cancers, including breast cancer. Notably, BCSCs are implicated in tumor growth and resistance to therapy. Recent research has revealed a mechanism by which BCSCs repress PD-L1 expression through the Notch3/mTOR axis, thus aiding immune evasion and promoting tumor survival. This insight underscores the intricate interplay between cancer stem cells, immune regulation, and therapeutic resistance in breast cancer, offering potential targets for therapeutic intervention [60].

HIF-1α

Hypoxia-inducible factor 1-alpha (HIF-1α) is a transcription factor that plays a crucial role in cellular response to low oxygen levels (hypoxia). In breast cancer, HIF-1α has been implicated in various processes, including the acquisition of CSC properties. One pathway through which HIF-1α influences CSC characteristics is via the mTOR pathway. Activation of mTOR signaling has been linked to cancer progression and stemness in breast cancer cells. HIF-1α can promote CSC properties by activating mTOR signaling, leading to enhanced cell survival, proliferation, and resistance to therapy. This interaction between HIF-1α and mTOR underscores the importance of targeting these pathways in cancer therapy to inhibit CSC properties and improve treatment outcomes [61].

HIF-2α

Hypoxia-inducible factor-2α (HIF-2α) is a transcription factor that plays a crucial role in cellular responses to low oxygen levels. In the context of TNBC, HIF-2α is highly expressed and has been shown to significantly impact the behavior of CSCs. A study highlighted the connection between HIF-2α and CD44, a stem cell marker highly expressed in CSCs. HIF-2α under hypoxic conditions enhances the stemness features of breast cancer cells, including their tumorigenic and metastatic potential, by upregulating CD44 expression. This upregulation is involved in the activation of the PI3K/AKT/mTOR signaling pathway, promoting cell survival, proliferation, and resistance to chemotherapy. Therefore, HIF-2α plays a pivotal role in sustaining breast CSC populations, partly through its regulation of CD44 expression, thereby contributing to the aggressive nature and treatment resistance of TNBC [62].

ST8SIA1

Identifying specific molecular markers for BCSCs is crucial for targeted therapy development. While markers like $CD44^{high}$, $CD24^{low,}$ and aldehyde dehydrogenase activity have been identified, ganglioside GD2 has recently emerged as a surface marker for BCSCs. GD2 expression, regulated by GD3 synthase ST8SIA1, is elevated in basal-like breast cancer, correlating with poor patient outcomes. Knockdown of ST8SIA1 inhibits BCSC function, suggesting its role in tumor progression. Mechanistically, ST8SIA1 positively correlates with p53 mutations and genes associated with stem cell phenotypes, while negatively correlating with GATA3 mutations. Further, ST8SIA1 regulates downstream signaling pathways, including FAK-AKT-ERK-mTOR, critical for BCSC functions. Inhibition of FAK or mTOR signaling suppresses BCSC activities in vitro. Moreover, ST8SIA1 knockout impedes tumor growth and metastasis in vivo, highlighting its potential as a therapeutic target in TNBC. These findings underscore the importance of ST8SIA1 in BCSC biology and its potential as a target for TNBC therapy [43].

SALL1

SALL1, a member of the SALL gene family, is a zinc finger transcription factor implicated in the development of various mammalian organs and the regulation of stem cell pluripotency. Recent studies have identified SALL1 as a tumor suppressor in breast cancer, with downregulation observed in breast cancer cell lines and tissues, particularly in aggressive subtypes like TNBC. SALL1 overexpression inhibits tumor cell growth, proliferation, and induces cell cycle arrest and senescence in breast cancer cells. Mechanistically, SALL1 exerts its tumor-suppressive effects by recruiting the NuRD chromatin

remodeling complex, thereby modulating gene expression. Additionally, SALL1 expression activates the mTOR signaling pathway, known for its role in tumor cell proliferation and senescence induction. In breast cancer cells, SALL1-induced senescence involves the activation of ATM-associated DNA damage response and selective modulation of MAPK p38 and ERK1/2 signaling pathways. Together, these findings highlight the multifaceted role of SALL1 in breast cancer pathogenesis, shedding light on potential therapeutic targets for combating breast cancer progression and metastasis [63].

PROCR

The protein C receptor (PROCR) plays a crucial role in BCSCs through its activation of various signaling pathways, particularly impacting the mTOR pathway. PROCR activates multiple intracellular signaling cascades including the ERK, PI3K–Akt–mTOR, and RhoA–ROCK pathways. In breast cancer, PROCR activates ERK and PI3K–Akt–mTOR pathways independently of the traditional surface receptor F2R or epidermal growth factor receptor (EGFR). Instead, PROCR engages insulin-like growth factor 1 receptor (IGF-1R) through Src kinase activation, leading to the stimulation of ERK and PI3K–Akt–mTOR signaling. Additionally, PROCR's interaction with F2R activates the RhoA–ROCK pathway. These signaling events collectively contribute to the maintenance of stemness in breast cancer cells, as evidenced by the inhibition of PROCR or its downstream signaling pathways resulting in decreased colony-forming ability of breast cancer cells. Therefore, PROCR serves as a critical regulator of BCSCs, orchestrating intricate molecular mechanisms that modulate mTOR signaling and ultimately influence cancer stem cell behavior in breast cancer [64].

GSK3

Glycogen synthase kinase 3 (GSK3) plays a pivotal role in regulating the mTOR pathway, thereby influencing critical cellular processes such as proliferation, survival, and metabolism. GSK3 exerts its regulatory effects on mTOR through intricate signaling mechanisms involving phosphorylation events and protein interactions. Specifically, GSK3 has been shown to phosphorylate key components of the mTOR pathway, including tuberous sclerosis complex 2 (TSC2), which inhibits mTOR activity. Additionally, GSK3 can modulate mTOR signaling by regulating the phosphorylation status of upstream kinases such as Akt and AMP-activated protein kinase (AMPK), which are known regulators of mTOR. Dysregulation of GSK3-mTOR signaling axis has been implicated in various diseases, including cancer. In BCSCs, aberrant activation of the GSK3-mTOR pathway contributes to the maintenance of stemness properties, enhanced proliferation, and resistance to therapy. Targeting GSK3-mediated regulation of mTOR represents a promising therapeutic strategy for disrupting BCSC maintenance and overcoming therapy resistance in breast cancer [65].

mTOR in chemoresistance of BCSCs

mTOR, an essential regulator of cell growth and metabolism, emerges as a pivotal player in the perplexing phenomenon of chemoradioresistance exhibited by CSCs. As a signaling hub, mTOR orchestrates intricate cellular processes, including protein synthesis, autophagy, and cell survival, all of which are implicated in the resistance mechanisms of CSCs against chemotherapy and radiotherapy. Through its intricate interplay with various molecular pathways, mTOR not only promotes CSC survival and self-renewal but also confers resistance to cytotoxic agents and radiation, thereby fostering tumor progression and treatment failure. Understanding the intricate crosstalk between mTOR signaling and the resistance mechanisms of CSCs holds promise for developing targeted therapeutic strategies to overcome chemoradioresistance and improve cancer treatment outcomes [66].

Radioresistance

Inhibition of the PI3K/mTOR pathway exerts significant impacts on BCSCs, particularly in terms of sensitizing them to radiotherapy. By targeting this pathway, which plays a crucial role in regulating cellular processes such as growth, proliferation, and survival, inhibitors like PKI-402 demonstrate the potential to overcome radiation resistance exhibited by CSCs. Notably, inhibition of PI3K/mTOR signaling reduces the colony formation ability of CSCs and enhances radiation-induced apoptosis in luminal A breast cancer cells, highlighting its efficacy in disrupting CSC-mediated resistance mechanisms. Moreover, the combination of PI3K/mTOR inhibition with ionizing radiation leads to increased levels of DNA double-strand breaks (DSBs) in triple-negative breast cancer cells, further underscoring the ability of this approach to sensitize CSCs to radiation therapy. Overall, targeting the PI3K/mTOR pathway holds promise in overcoming therapy resistance in breast cancer, particularly by disrupting the resilient nature of CSCs and enhancing the effectiveness of conventional treatments [67].Inhibition of mTOR with rapamycin sensitizes BCSCs to the effects of radiation therapy, effectively reducing their self-renewal capacity. This sensitization is mediated through various pathways, including the suppression of MnSOD (manganese superoxide dismutase) and the Akt pathway. Furthermore, rapamycin-induced inhibition of mTOR leads to increased ROS (reactive oxygen species) activity and asymmetric cell division in BCSCs, ultimately enhancing the efficacy of radiation therapy by reducing mammosphere formation. Understanding the intricate interplay

between mTOR signaling and radiotherapy response in BCSCs holds promise for developing more effective treatment strategies, particularly for aggressive forms of breast cancer [68].

Chemoresistance

mTOR plays a pivotal role in mediating chemoresistance in BCSCs through various mechanisms. Firstly, mTOR signaling promotes BCSC survival and self-renewal, contributing to the maintenance of a stem cell-like phenotype that confers resistance to chemotherapy [69]. Activation of mTOR promotes the expression of drug efflux pumps, such as ATP-binding cassette (ABC) transporters, leading to increased drug efflux and reduced intracellular drug accumulation in BCSCs [70]. The mTOR pathway plays a pivotal role in regulating ABC transporters, particularly ABCC1 and ABCB1, in breast cancer [71, 72]. Activation of mTOR signaling enhances the transcription and translation of these transporters, thereby increasing their expression on the cell surface. ABCC1 primarily mediates the efflux of glutathione-conjugated drugs, while ABCB1 actively pumps out hydrophobic chemotherapeutics, such as paclitaxel and doxorubicin, reducing intracellular drug concentrations. By promoting the expression of these transporters, mTOR contributes to drug resistance in breast cancer cells, making them less responsive to chemotherapy [73, 74]. Targeting mTOR could, therefore, mitigate the overexpression of ABCC1 and ABCB1, enhancing chemosensitivity and improving therapeutic outcomes. Additionally, mTOR signaling enhances DNA repair mechanisms and anti-apoptotic pathways, further bolstering BCSC survival in the face of chemotherapy-induced damage [57]. Furthermore, mTOR activation in BCSCs contributes to the evasion of immune surveillance and the establishment of a supportive tumor microenvironment, fostering chemoresistance [75]. Overall, targeting mTOR signaling in BCSCs represents a promising therapeutic strategy to overcome chemoresistance and improve treatment outcomes in breast cancer [41].

Targeting mTOR in BCSCs

Targeting the mTOR pathway in CSCs is crucial due to its central role in regulating various cellular processes essential for CSC maintenance and survival. CSCs, characterized by their ability to self-renew and differentiate into heterogeneous tumor cell populations, contribute to tumor initiation, progression, and therapy resistance. The mTOR pathway controls key functions such as cell growth, proliferation, metabolism, and survival, making it a promising target for cancer therapy. Inhibition of mTOR signaling can disrupt CSC maintenance and self-renewal, leading to reduced tumor growth and recurrence. Additionally, CSCs often exhibit heightened mTOR activity, which is associated with aggressive tumor behavior and resistance to conventional therapies. By targeting the mTOR pathway in CSCs, it may be possible to selectively eradicate these treatment-resistant cells, ultimately improving cancer treatment outcomes and reducing the risk of relapse (Table 1) [76].

Selective PI3K/Akt/mTOR inhibitors

Everolimus

Everolimus is an oral inhibitor of the mTOR, a key serine-threonine kinase in the PI3K/Akt/mTOR signaling pathway, which plays a critical role in regulating cell growth, proliferation, and survival. Everolimus has significant antitumor activity in TNBC cell lines, particularly those of the basal-like subtype, characterized by the reduced expression of EGFR or CK5/6. The drug effectively inhibited cell growth at low nanomolar concentrations in five out of nine TNBC cell lines tested. However, its efficacy was less pronounced in TNBC cell lines exhibiting characteristics of cancer stem cells, such as decreased E-cadherin expression and increased expression of Snail or Twist, which are associated with resistance. In vivo, everolimus showed a pronounced antitumor effect in a basal-like breast cancer xenograft model, underscoring its potential as a therapeutic option for this particularly aggressive and difficult-to-treat breast cancer subtype [77]. The combination of Everolimus (Ever) and Letrozole (Let) has demonstrated a potent inhibitory effect on human breast cancer stem cells, as evidenced by various experiments. This combined therapy significantly reduced the proliferation of breast cancer stem cells, as shown by the higher sensitivity of these cells to the combination treatment compared to either drug alone, with a notable decrease in IC50 values indicating increased effectiveness. The combination therapy also significantly increased early apoptosis rates and a more substantial accumulation of cells in the G0-G1 phase of the cell cycle, highlighting its ability to halt cell division and promote cell death. Additionally, in soft agar colony formation assays and in vivo xenograft tumor models, the combined use of Ever and Let led to a marked reduction in colony formation and tumor volume, respectively, underscoring its potential to inhibit the oncogenic capabilities of breast cancer stem cells. Immunohistochemical analyses further confirmed the combination's efficacy, with significant downregulation of markers associated with cell proliferation and survival pathways, including Ki67, AKT1, phospho-AKT (Thr308), and mTOR, indicating a profound impact on signaling pathways critical for breast cancer stem cell maintenance and resistance (Fig. 3) [78].

VS-5584

VS-5584, a potent inhibitor targeting the PI3K and mTOR pathways, demonstrates a multifaceted approach

Table 1 Therapeutics targeting mTOR signaling pathway in breast cancer stem cells

Treatment	Cancer type	Drug type	Target genes	Model	Highlights	Ref.
Everolimus	Triple-negative breast cancer cells (basal-like)	mTOR inhibitor	PTEN, p-AKT, Akt, p-mTOR, mTOR, p-S6, S6, p-4EBP1, and 4EBP1	In vivo and In vitro	"Resistant cell lines tended to show characteristics of cancer stem cells, with decreased E-cadherin expression and the increased expression of Snail or Twist." Reduced Tumor Volume	[77]
Everolimus + Letrozole	Breast cancer MCF-7/Aro stem cells	mTOR inhibitor + Aromatase inhibitor	KI67, CD31, AKT1, phospho-AKT (Thr308), and mTOR	In vivo and In vitro	Increase in G1 cell cycle arrest and increases in early apoptosis "Everolimus has effective inhibition on aromatase-overexpressing stem cell in vitro and in vivo"	[78]
VS-5584	MDA-MB-231 triple-negative breast cancer	PI3K/mTOR Dual Inhibitor	PI3K/mTOR	In vivo and In vitro	By inhibiting PI3K and mTOR, VS-5584 disrupts critical signaling pathways involved in cell growth, survival, and self-renewal, thus selectively depleting CSC populations in breast cancer models.	[79]
PF-04691502	Patient derived BCSC cultures	PI3K/mTOR Dual Inhibitor	p-AKT, p-S6K1, p-S6RP, p-4E-BP1	In vitro	- Tamoxifen activated genes related to ribosome synthesis and mRNA translation in CSCs. - Tamoxifen induced mTOR signaling in CSCs, potentially promoting their growth. - mTOR inhibitors, especially PF-04691502, suppressed tamoxifen-induced mTOR activation and reduced CSC growth. - Targeting mTOR signaling may offer a promising strategy to overcome endocrine resistance in breast cancer.	[80]
B591	BCSCs	Pan-PI3K inhibitor	PI3K, Akt and mTOR, mTORC1 (S6K1, ribosomal S6 protein), eIF4E and 4E-BP1.	In vivo and In vitro	In mouse models, B591 reduces tumor burden, eliminates CSCs, and delays tumor recurrence. B591 shows synergistic effects with chemotherapy, suggesting promise for combination therapy.	[81]
NVP-BEZ235	BCSCs	PI3K/AKT/mTOR dual inhibitor	PI3K/AKT/mTOR	In vitro	IL-6 pretreatment enhanced the efficacy of NVP-BEZ235 in decreasing cell viability. NVP-BEZ235 sensitized TNBC cells to radiotherapy, especially when combined with SRT1720 and IL-6 pretreatment,	[82]
NVP-BEZ235	Basal-like breast cancer	PI3K/AKT/mTOR dual inhibitor	PI3K/AKT/mTOR	In vitro	In combination with JAKi, fails to suppress stem gene expression in BCSCs, including markers such as OCT4, SOX2, NANOG, and ALPL.	[83]
BEZ235 and MLN128	TNBC and CSCs	PI3K/AKT and mTOR inhibitors	PI3K as well as mTORC1 and mTORC2	In vivo and In vitro	BEZ235 and MLN128 treatment led to the enrichment of a cell population with cancer stem cell-like properties in TNBC. The treatments inadvertently activated Notch1 signaling in TNBC cells.	[84]
PKI-402	MCF-7 and BCSCs	Dual inhibitor of the PI3K/mTOR	GSK-3b, PDK1, PRAS40, AMPKa, and mTOR	In vitro	PKI-402 combined with IR increases double-strand breaks (DSBs) in triple-negative BC cells (MDA-MB-231), as indicated by elevated levels of c-H2AX, suggesting enhanced efficacy against this aggressive subtype.	[67]
Trastuzumab	BT474 human breast cancer cell lines	Anti-HER2 monocolonal antibody	Ki67, CD31, and AKT1	In vivo and In vitro	Combining everolimus with trastuzumab significantly enhances the inhibition of breast CSC growth compared to either drug alone. This combination therapy demonstrates superior efficacy in reducing tumor size in a xenograft animal model. The combination treatment induces cell cycle arrest and apoptosis in breast CSCs, effectively reducing their clonogenicity in vitro.	[85]

Table 1 (continued)

Treatment	Cancer type	Drug type	Target genes	Model	Highlights	Ref.
Dinutuximab	TNBC and BCSCs	Anti-GD2 monocolonal antibody	GD2 and mTOR	In vivo and In vitro	Dinutuximab treatment inhibits adhesion, migration, and mammosphere formation in GD2-positive BCSCs. Dinutuximab inhibits the mTOR signaling pathway in GD2-positive cells, disrupting cellular signaling mechanisms associated with proliferation and survival.	[87]
BIBR1532	BCSCs	Selective telomerase inhibitor	hTERT and mTOR	In vitro	BIBR1532 induces cell cycle arrest, particularly in the G2/M phase, in BCSCs, indicating its ability to disrupt cell proliferation and potentially halt tumor growth. BIBR1532 modulates the expression of genes associated with the mTOR pathway, a key regulator of cell proliferation, metabolism, and drug resistance.	[97]
AZD4547	MaSCs	FGFRs inhibitor	FGFRs, Akt, mTOR, ERKs, and Wnt/β-catenin	In vivo and In vitro	AZD4547 suppresses FGFR, Akt, Erk1/2, mTOR, and Wnt/β-catenin pathways, indicating multiple mechanisms for its anti-cancer effects. AZD4547 induces architectural changes in mammary ducts, reduces ductal growth and density, and decreases cell proliferation.	[98]
Metformin + Hyperthermia	MCF-7 BCSCs	Antidaibetic	mTOR, AMPK, ErbB2	In vitro	Metformin in combination with hyperthermia reduces CSC populations, as evidenced by decreased CD44high/CD24low cell proportions and inhibition of sphere formation. Enhanced cytotoxicity against breast cancer cells, including BCSCs, is observed with the combined treatment, attributed to AMPK/mTOR modulation.	[114]
Metformin	TICs	Antidaibetic	ErbB2, PI3K/Akt pathway, IGF-1R, mTOR, and Stat3	In vivo and In vitro	Metformin downregulates ErbB2/PI3K/Akt pathway and inhibits activation of IGF-1R, mTOR, and Stat3 in mammary tissues. Metformin pretreatment effectively suppresses the initiation and growth of ErbB2-overexpressing tumors in a syngeneic graft mouse model. ErbB2 signaling is critical for maintaining self-renewal and proliferation of CSCs, and metformin preferentially targets this pathway in CSCs, disrupting their growth and survival mechanisms.	[101]
Metformin	MCF-7 and FSall	Antidaibetic	AMPK, mTOR, S6K1, and 4EBP1 and	In vitro	By targeting both the bulk tumor cells and the CSCs, metformin holds the promise of improving patient outcomes by not only shrinking tumors but also reducing the likelihood of recurrence and metastasis.	[23]
Buformin	Mice SKBR3 and BT474 cells	Antidaibetic	mTOR, erbB-2/PI3K/Akt, ER, and Wnt/β-catenin	In vivo and In vitro	Buformin has been shown to effectively reduce the 'stemness' characteristics of cancer cells, which include the ability to initiate tumors, proliferate, and resist conventional therapies.	[102]

Table 1 (continued)

Treatment	Cancer type	Drug type	Target genes	Model	Highlights	Ref.
Thioridazine+ Carboplatin	CSCs and TNBC	Antipsychotic + Chemotherapy	PI3K/Akt/mTOR, ER, GRP78, and CHOP	In vivo and In vitro	THZ targets CSCs by antagonizing dopamine receptors, leading to CSC destruction. The combination therapy reduces lung metastasis and inhibits tumor vascularization, further enhancing its anti-tumor efficacy. THZ and CBP combination therapy inhibits the PI3K/mTOR pathway and induces endoplasmic reticulum (ER) stress-mediated apoptosis. THZ and CBP combination therapy effectively reduces the population of CSCs within tumors	[103]
SB-699,551	Breast tumor initiating cells	Antagonists of the serotonin receptor 5 A	AKT, PRAS40, CREB, and FOXO1	In vivo and In vitro	SB-699,551 reduces tumorsphere formation and ex vivo tumor initiation, indicating a decrease in BTIC activity. Disrupts signaling pathways downstream of 5-HT5A, particularly the Gαi/o-coupled pathway and the PI3K/AKT/mTOR axis.	[104]
Ursolic Acid	BCSC	Pentacyclic triterpene	AGO2, FAK/PI3K/Akt/mTOR, PTEN and c-Myc s	In vitro	UA reduces the fraction of BCSCs by decreasing the expression of key stemness markers such as ABCG2, Nanog, Oct4, and CD133. Treatment with UA suppresses the migration and invasion capabilities of breast cancer cells, attributed to downregulation of microRNAs miR-9 and miR-221, which promote EMT and metastasis.	[89]
Pentadecanoic Acid	MCF – 7/SCs	Odd – chain saturated fatty acid	EGFR, mTOR, Ras, and MAPK	In vivo and In vitro	Research indicates that pentadecanoic acid inhibits stemness characteristics and induces apoptosis in human breast carcinoma MCF-7 stem cells. Pentadecanoic acid inhibits mTOR signaling, leading to suppression of cell proliferation and induction of apoptosis in MCF-7/SCs.	[90]
Dioscin	BCSC	Steroidal glucoside saponin	p53, p21, CDK4, cyclin D, CDK2, and cyclin E + Akt/mTOR	In vitro	Dioscin inhibits BCSC proliferation, migration, invasion, and colony formation, potentially hindering metastasis and recurrence.	[91]
Sophoridine	BCSCs	Natural alkaloid	Bax, Bcl-2, caspase-3, PARP, LC3, p62, Beclin 1, AKT, 4EBP1, and p70S6K	In vivo and In vitro	These compounds induce apoptosis, arrest cell cycle at G1-phase, and stimulate autophagy in cancer cells via mTOR Pathway Inhibition	[92]
Ginsenoside Rg3	BCSCs	Panax ginseng deriviates	AKT/mTOR, including 4E-BP1, RPS6, P70S6K, PRAS40, Raf-1, and RSK1	In vivo and In vitro	Molecular studies revealed their ability to reduce phosphorylation of key proteins downstream of the AKT/mTOR pathway, including 4E-BP1, RPS6, P70S6K, PRAS40, Raf-1, and RSK1. These effects contribute to the inhibition of TNBC cell migration, reduction of BCSC characteristics, and suppression of tumor growth observed both in vitro and in vivo.	[93]
Matcha green tea	BCSCs	Green tea catechins	PI3K/Akt/mTOR- p53 and RB1-	In vitro	MGT treatment reduces mammosphere formation, indicating its ability to hinder CSC-mediated tumorigenesis. MGT induces metabolic reprogramming in cancer cells, leading to decreased mitochondrial metabolism and glycolysis.	[94]

Table 1 (continued)

Treatment	Cancer type	Drug type	Target genes	Model	Highlights	Ref.
Extra-virgin olive oil (EVOO)	BCSCs	Olea europaea L.	mTOR, DNMTs, ALDH1A1	In vivo and In vitro	At the cellular level, DOA suppresses CSCs' ability to form tumorspheres. Molecularly, DOA acts as an ATP-competitive inhibitor of mTOR, a crucial pathway in CSC maintenance. DOA competes with S-adenosyl methionine (SAM) to inhibit DNA methyltransferases (DNMTs), disrupting epigenetic regulation associated with CSC phenotypes. DOA synergistically interacts with other drugs like rapamycin and 5-azacytidine, enhancing its anti-CSC effects.	[95]
Quercetin	BCSCs (CD44+/CD24−)	Fruits, vegetables, nuts, and seeds	PI3K/Akt/mTOR/ERα, CyclinD1, Bax, and Bcl-2	In vivo and In vitro	Quercetin treatment significantly inhibited the viability, proliferation, and self-renewal capacity of BCSCs in vitro. In vivo experiments using mouse models demonstrated that quercetin reduced tumor growth and metastatic potential of CD44+/CD24 − BCSCs.	[96]
Aqueous Nyctanthes arbortristis and doxorubicin	BCSCs paclitaxel-resistant	Conjugated gold nanoparticles	mTOR and NCOA4	In vivo and In vitro	Cellular ROS levels were significantly increased in treated cells. Significant increases in apoptotic cell populations were observed upon treatment. RT-PCR and immunofluorescence analyses demonstrated the induction of ferritinophagy, evidenced by the suppression of mTOR and upregulation of LC-3B, leading to ferritin degradation and potentially enhancing cell death mechanisms.	[108]
Pseudolaric acid B	TNBC BCSCs	Conjugated ferritin nanoparticles	EGFR/mTOR- TfR	In vivo and In vitro	Inhibition of mTOR pathway to enhance ferroptosis and reduce TNBC cell viability. Upregulation of transferrin receptor (TfR) expression by PAB to increase intracellular iron accumulation and induce ferroptotic cell death. Selective targeting of TNBC cells, leading to inhibition of tumor growth both in vitro and in vivo. Minimal toxicity to normal cells and organs, highlighting the potential of L/P@Ferritin nanoparticles as a safe and effective therapeutic strategy for TNBC.	[109]
QAuNP + NIR	BCSCs	Hybrid nanoparticle	PI3K/AKT/mTOR- HSP-70/TGF-β	In vivo and In vitro	QAuNP + NIR treatment in P-BCSCs resulted in downregulation of the PI3K/AKT/mTOR pathway in HUVECs, accompanied by reduced nitric oxide (NO) production. In vivo studies using patient-derived xenograft (PDX) mouse models showed a significant decrease in tumor volume and downregulation of angiogenic markers, including HSP-70, TGF-β, ANG-1, and ANG-2, upon QAuNP + NIR treatment.	[110]

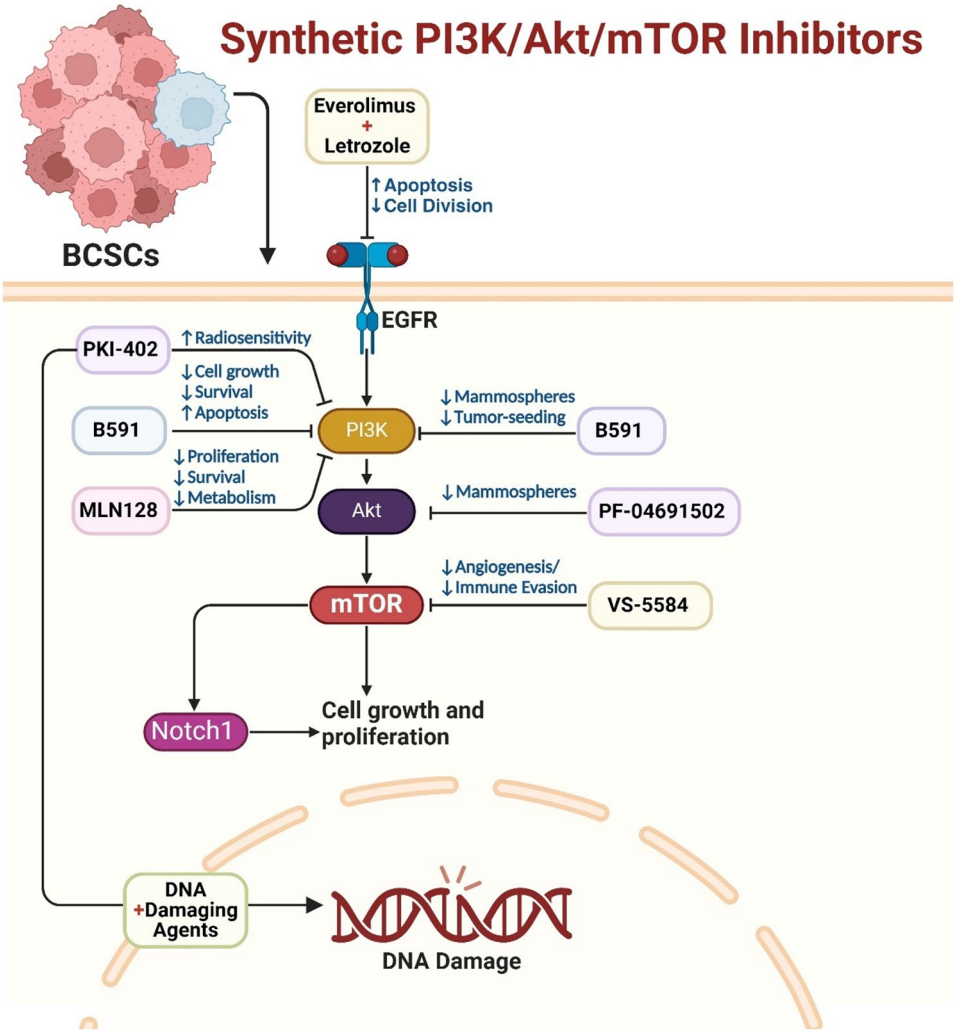

Fig. 3 Targeting the PI3K/Akt/mTOR Pathway in BCSCs with synthetic drugs: This figure depicts synthetic inhibitors targeting the PI3K/Akt/mTOR pathway in BCSCs to reduce survival, proliferation, and resistance. Key agents include Everolimus (mTOR inhibitor) and dual inhibitors (e.g., VS-5584, NVP-BEZ235) that block both PI3K and mTOR, disrupting mammosphere formation and self-renewal. Other inhibitors, like PKI-402 and B591, enhance radiosensitivity and apoptosis in BCSCs. These targeted therapies inhibit essential pathways for BCSC maintenance, aiming to overcome tumor initiation, therapeutic resistance, and recurrence

in targeting breast CSCs and influencing tumor dynamics. By inhibiting PI3K, it disrupts crucial signaling cascades involved in cell proliferation and survival while targeting mTOR further impairs cell growth and survival mechanisms. This dual inhibition extends to CSCs, a subpopulation notoriously resistant to conventional therapies, thus addressing a critical challenge in cancer treatment. By disrupting the self-renewal and survival pathways vital for CSC maintenance, VS-5584 selectively depletes CSC populations in breast cancer models. Additionally, its potential impact on the tumor microenvironment, including angiogenesis and immune evasion, further contributes to its therapeutic efficacy. This comprehensive molecular mechanism positions VS-5584 as a promising candidate for breast cancer therapy, with implications for overcoming resistance and improving treatment outcomes (Fig. 3) [79].

PF-04691502

PF-04691502 is a dual inhibitor targeting both mTOR and PI3K, two key signaling pathways implicated in cancer progression and therapeutic resistance. In the context of BCSCs, PF-04691502 has been shown to significantly reduce mammosphere formation, which is indicative of BCSC self-renewal capacity. Additionally, PF-04691502 effectively attenuates the tamoxifen-induced activation of mTOR signaling in BCSCs, suggesting its potential as a therapeutic agent to counteract the deleterious effects of tamoxifen on BCSC expansion and endocrine resistance. This dual inhibitor presents a promising strategy for targeting BCSCs and overcoming resistance mechanisms in

breast cancer, offering new avenues for improved therapeutic interventions (Fig. 3) [80].

B591

B591 is a newly developed pan-PI3K inhibitor with high selectivity against Class I isoforms of PI3K, identified for its potent inhibition of the PI3K/Akt/mTOR signaling pathway critical for CSC maintenance and viability. Through its mechanism of action, B591 effectively suppresses downstream effectors involved in cell survival, proliferation, and CSC maintenance. Specifically, it inhibits the phosphorylation of Akt at Thr308, leading to subsequent suppression of mTORC1 and mTORC2 signaling cascades. B591 exhibits preferential targeting of CSCs over bulk tumor cells, as evidenced by its ability to inhibit mammosphere formation, reduce expression of CSC markers (such as ALDH, CD44, BMI1, SOX2, and Nanog), and suppress tumor-seeding ability both in vitro and in vivo. Moreover, B591 demonstrates efficacy in reducing tumor burden, eliminating CSCs, and delaying tumor recurrence in mouse xenograft models of breast cancer. This dual action of B591, targeting both CSCs and the mTOR pathway, holds significant promise for improving cancer therapy outcomes, particularly in addressing tumor relapse and metastasis. Further research is warranted to fully elucidate its therapeutic potential and explore its clinical applicability in cancer treatment strategies (Fig. 3) [81].

NVP-BEZ235

NVP-BEZ235 is a compound known as a dual PI3K and mTOR inhibitor. It works by blocking signaling pathways involved in cell growth and survival, particularly those mediated by PI3K/AKT/mTOR. In the context of breast cancer, NVP-BEZ235 has shown promise as a therapeutic agent, particularly in TNBC, which is known for its aggressiveness and resistance to conventional therapies. One notable aspect of NVP-BEZ235's mechanism is its ability to affect BCSCs. Studies have demonstrated that NVP-BEZ235 can decrease the percentage of CSCs within breast cancer cell populations. This effect is crucial as CSCs are believed to contribute significantly to tumor recurrence and metastasis. By targeting CSCs, NVP-BEZ235 holds potential for improving treatment outcomes in breast cancer, particularly in TNBC, where CSCs are thought to play a significant role in therapy resistance and disease progression [82]. NVP-BEZ235, demonstrates notable efficacy in reducing cell growth and survival in BCSCs, as evidenced by decreased cell numbers in both growth and recovery assays. Moreover, Western blot analysis confirms its ability to attenuate AKT activation, a key downstream effector of the PI3K pathway. Importantly, NVP-BEZ235 treatment induces apoptosis in BCSCs, as indicated by the upregulation of apoptotic genes and the observed increase in apoptotic cells via flow cytometry. However, despite its cytotoxic effects and ability to induce apoptosis, NVP-BEZ235 fails to suppress stem gene expression in BCSCs, as markers such as OCT4, SOX2, NANOG, and ALPL remain unaffected. This persistence of stem gene expression suggests that while NVP-BEZ235 effectively targets the proliferative capacity of BCSCs, additional strategies may be necessary to specifically target the stem cell population and mitigate the risk of recurrence (Fig. 3) [83].

MLN128

BEZ235 and MLN128 are targeted anticancer agents designed to inhibit specific pathways crucial for cancer cell survival and proliferation. BEZ235 is a dual inhibitor of PI3K and mTOR, affecting both the PI3K/AKT/mTOR pathway and directly targeting the kinase domains of mTORC1 and mTORC2 complexes. This broad targeting approach allows BEZ235 to potently suppress tumor growth by inhibiting critical signaling pathways involved in cell proliferation, survival, and metabolism. MLN128 appears to similarly target the mTOR pathways, specifically inhibiting the TORC1/2 complexes, which are critical components of the mTOR signaling cascade. In the BCSCs, both BEZ235 and MLN128 influence their survival and proliferation. These compounds, through the inhibition of mTORC1/2, inadvertently promote the survival of CSCs in TNBC by activating Notch1 signaling, a pathway known for its role in maintaining CSC properties and promoting drug resistance. Thus, while BEZ235 and MLN128 effectively target and inhibit key growth and survival pathways in cancer cells, their impact on TNBC also involves the unintended consequence of enriching a CSC-like population, highlighting the complexity of targeting such deeply integrated signaling networks in cancer therapy (Fig. 3) [84].

PKI-402

PKI-402 is a small molecule ATP-competitive dual inhibitor of the PI3K/mTOR survival pathway, known for its ability to suppress specific mutations in the PI3K-a enzyme and mTOR, thereby inhibiting downstream effector proteins like phosphorylated Akt (p-Akt; T308) responsible for tumor cell growth. In breast cancer, PKI-402 demonstrates significant inhibitory effects on cell proliferation across various cell lines, including MCF-7 and MDA-MB-231. Notably, it exhibits stronger inhibition in non-stem cancer cells compared to BCSCs. However, despite its differential impact on proliferation, PKI-402 enhances the radiosensitivity of both MCF-7 and BCSCs, reducing their colony formation ability when combined with ionizing radiation (IR). This suggests that while PKI-402 may not directly target BCSCs as effectively as other cancer cells, it can sensitize them

to radiation therapy, potentially by modulating survival pathways and enhancing the efficacy of DNA damage-inducing treatments (Fig. 3) [67].

Monoclonal antibodies

Monoclonal antibodies (mAbs) are lab-made molecules designed to mimic the immune system's ability to target specific cells or molecules involved in disease processes. These antibodies, called "monoclonal" because they are produced by identical immune cells derived from a single parent cell, are engineered to bind to precise targets on cells, such as proteins or other molecules. Trastuzumab, is an example of a monoclonal antibody used in cancer treatment, particularly for HER2-positive breast cancer. Trastuzumab targets and blocks HER2, which is overexpressed in HER2-positive breast cancer cells. The therapeutic potential of combining everolimus, an mTOR inhibitor, with trastuzumab, an anti-HER2 monoclonal antibody, in treating HER2-positive breast cancer, particularly in targeting BCSCs has been investigated. HER2-positive breast cancer is known for its aggressiveness and resistance to conventional therapies, including trastuzumab. Everolimus, as an mTOR inhibitor, has shown promise in cancer treatment by inhibiting cell proliferation and survival. Researchers explored whether combining everolimus with trastuzumab could enhance the efficacy of targeting CSCs, which are believed to play crucial roles in tumor initiation, metastasis, and resistance to therapies. The results demonstrate that both everolimus and trastuzumab exhibit inhibitory effects on breast CSC growth, with everolimus being less effective than trastuzumab alone. However, combining everolimus with trastuzumab significantly enhances the inhibition of breast CSC growth compared to either drug alone. This combination therapy also induces cell cycle arrest and apoptosis in breast CSCs and effectively reduces their clonogenicity in vitro. Moreover, in a xenograft animal model, the combination treatment demonstrates superior efficacy in reducing tumor size compared to individual drug treatments. Immunohistochemical analysis reveals alterations in markers associated with cell proliferation and angiogenesis in response to the combination therapy, further supporting its therapeutic potential [85]. Combination therapy involving trastuzumab with either BKM120, a pan-class I PI3K inhibitor, or RAD001, an mTOR inhibitor, demonstrates significant efficacy in targeting CSCs in breast cancer. Trastuzumab, when combined with BKM120, exhibits synergistic effects in inhibiting CSC growth and reducing the generation of drug-resistant CSCs. Additionally, the combination of trastuzumab with RAD001 shows similar synergistic effects in suppressing CSC proliferation and inhibiting CSC-mediated tumor formation. These combinations not only inhibit the PI3K/Akt/mTOR signaling pathway, which is crucial for CSC survival and proliferation, but also demonstrate enhanced efficacy in suppressing CSC-derived tumor growth in xenograft models. These findings highlight the potential of combination therapies involving trastuzumab with BKM120 or RAD001 as promising strategies for targeting CSCs and overcoming therapy resistance in breast cancer [86]. Similarly, dinutuximab is a monoclonal antibody that targets GD2, a ganglioside expressed on the surface of certain cancer cells, particularly BCSCs. This antibody binds specifically to GD2-positive cancer cells, inhibiting their adhesion, migration, and mammosphere formation, which are characteristic features associated with BCSCs. Additionally, dinutuximab treatment leads to the inhibition of the mTOR signaling pathway within GD2-positive cells, thereby disrupting their cellular signaling mechanisms involved in proliferation and survival. Furthermore, when combined with activated natural killer (NK) cells, dinutuximab enhances its therapeutic efficacy by promoting antibody-dependent cell-mediated cytotoxicity (ADCC), inducing apoptosis in GD2-positive cancer cells. This dual mechanism of action suggests dinutuximab as a promising therapeutic agent for targeting and inhibiting cancer stem cells, particularly in TNBC, offering potential benefits for improved treatment outcomes (Fig. 4) [87].

Natural products

Research into the potential of natural products for targeting the mTOR pathway in cancer treatment has gained momentum. Natural compounds such as salidroside, oridonin, curcumin, and others have been investigated for their ability to modulate mTOR activity with promising results. These compounds offer potential advantages, including fewer adverse effects compared to conventional mTOR inhibitors. Studies have shown that natural products can inhibit mTOR signaling and exert anticancer effects through various mechanisms, including regulation of upstream regulators like PTEN and Akt, as well as direct inhibition of mTOR complexes. Additionally, researchers are exploring combinatorial approaches involving natural compounds and conventional therapies to enhance efficacy and mitigate potential side effects. While clinical trials involving natural products targeting mTOR are limited, ongoing preclinical studies demonstrate the potential of these compounds as alternative or adjunct therapies for cancer treatment, warranting further investigation into their safety and efficacy in clinical settings (Fig. 5) [88].

Ursolic acid

Ursolic acid (UA) is a pentacyclic triterpene derived from medicinal herbs, fruits, and vegetables, renowned for its diverse biological activities, including potential

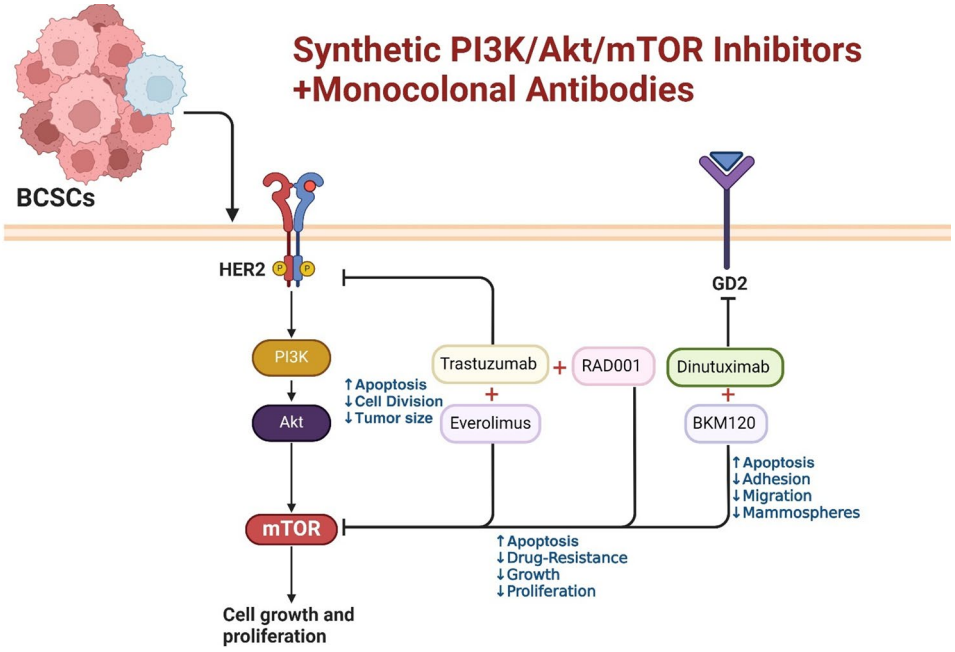

Fig. 4 Targeted PI3K/Akt/mTOR Pathway inhibition using Monoclonal Antibody Therapy in BCSCs. This schematic illustrates the use of synthetic inhibitors and monoclonal antibodies to target the PI3K/Akt/mTOR signaling pathway in breast cancer stem cells. The HER2 receptor, overexpressed in HER2-positive breast cancers, activates PI3K/Akt signaling, leading to mTOR pathway activation, which promotes cell growth and proliferation. Trastuzumab, a monoclonal antibody targeting HER2, combined with RAD001 (an mTOR inhibitor) or everolimus, enhances apoptosis, reduces cell division, and decreases tumor size. Combining trastuzumab with PI3K inhibitors like BKM120 shows synergistic effects in reducing CSC growth, drug resistance, and proliferation. Additionally, dinutuximab, an antibody targeting GD2, disrupts CSC functions by inhibiting adhesion, migration, and mammosphere formation, further enhancing apoptosis. This figure highlights the potential therapeutic efficacy of monoclonal antibody combinations in inhibiting CSC-driven tumor growth and overcoming therapy resistance in aggressive breast cancers, including triple-negative and HER2-positive subtypes

anti-cancer properties. In the context of breast cancer, UA demonstrates significant inhibitory effects on CSCs, particularly in TNBC, a subtype associated with aggressiveness and limited treatment options. UA treatment leads to a reduction in the fraction of BCSCs, as evidenced by decreased expression of key stemness markers such as ABCG2, Nanog, Oct4, and CD133. Moreover, UA suppresses the migration and invasion capabilities of breast cancer cells, attributed to its ability to downregulate microRNAs miR-9 and miR-221, which promote EMT and metastasis. Mechanistically, UA enhances the expression of PTEN while inhibiting the AGO2-mediated FAK/PI3K/Akt/mTOR signaling pathway, thereby impeding CSC characteristics and EMT progression [89].

Pentadecanoic acid

Pentadecanoic acid (C15:0) is an odd-chain saturated fatty acid found naturally in certain plants, fish, dairy fats, and meats. It has shown promising anticancer effects, particularly in human breast carcinoma MCF-7 stem cells (MCF-7/SCs). Research indicates that pentadecanoic acid inhibits stemness characteristics and induces apoptosis in MCF-7/SCs via modulation of the mTOR signaling pathway. Pentadecanoic acid's inhibition of mTOR signaling leads to suppression of cell proliferation, induction of apoptosis, and inhibition of tumor growth in MCF-7/SCs. This suggests that pentadecanoic acid may serve as a potential therapeutic agent for targeting cancer stem cells in breast carcinoma through its modulation of the mTOR pathway, offering a novel approach for breast cancer treatment [90].

Dioscin

Dioscin, a steroidal glucoside saponin found in various plants, marine organisms, and bacteria, exhibits significant anticancer properties against breast cancer, particularly targeting BCSCs. Dioscin demonstrates inhibition of BCSC proliferation, migration, invasion, and colony formation, indicative of its potential to hinder metastasis and recurrence. Moreover, it induces cell cycle arrest in BCSCs, impacting key regulatory proteins such as p53, p21, CDK4, cyclin D, CDK2, and cyclin E, thus disrupting cell cycle progression. Additionally, dioscin inhibits the AKT/mTOR signaling pathway, essential for cancer cell growth and survival, further contributing to its anticancer effects. These findings suggest that dioscin holds promise as a therapeutic agent for breast cancer by targeting BCSCs and crucial signaling pathways involved in cancer progression, offering potential avenues for improved treatment strategies [91].

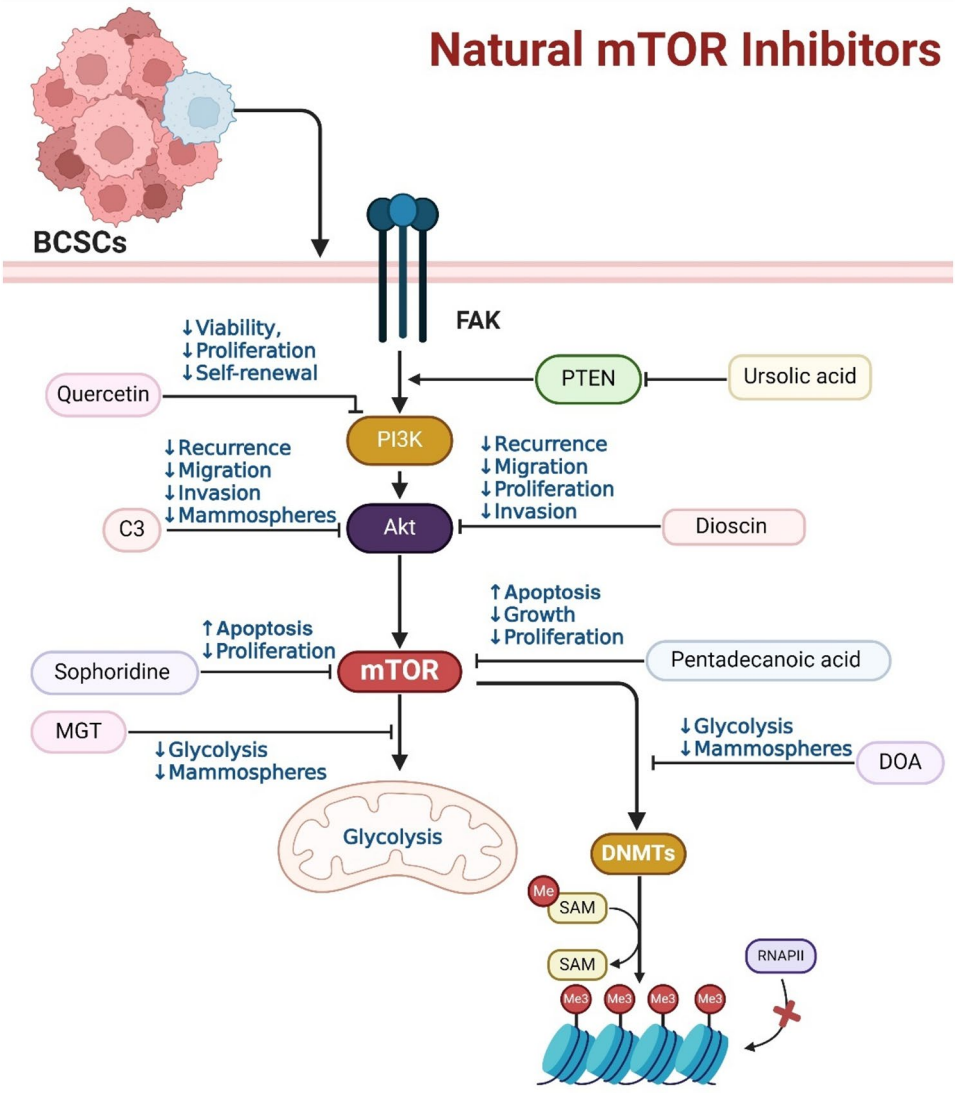

Fig. 5 Naturally occurring products that inhibit mTOR in BCSCs. This figure illustrates the inhibitory effects of various natural compounds on the mTOR pathway in breast cancer. Compounds like quercetin, C3 (combination of SRg3 and RRg3), ursolic acid, dioscin, sophoridine, pentadecanoic acid, matcha green tea (MGT), and decarboxymethyl oleuropein aglycone (DOA) derived from extra-virgin olive oil inhibit mTOR activity through modulation of upstream regulators, including PTEN and Akt. These compounds reduce cancer cell viability, proliferation, migration, invasion, and self-renewal of BCSCs by disrupting key processes such as glycolysis and DNA methylation, inducing apoptosis, and reducing mammosphere formation. Mechanistic pathways involve inhibition of the PI3K/Akt/mTOR axis, highlighting their potential as adjunctive or alternative therapies for breast cancer, particularly in aggressive subtypes like TNBC

Sophoridine

Sophoridine derivatives are synthesized compounds based on sophoridine, a natural alkaloid with diverse biological activities, including anti-inflammatory, antivirus, and antitumor effects. In the context of breast cancer, these derivatives have been investigated for their potential to target BCSCs and modulate the mTOR pathway. Sophoridine derivatives have demonstrated promising cytotoxic activity against BCSCs, inducing apoptosis and inhibiting cell proliferation through various mechanisms, including modulation of apoptotic proteins (e.g., Bax, Bcl-2), cell cycle arrest, and induction of autophagy. Additionally, these derivatives have been found to inhibit the mTOR signaling pathway, which plays a crucial role in regulating cell growth, proliferation, and survival. By targeting both BCSCs and mTOR signaling, sophoridine derivatives hold potential as effective therapeutic agents for breast cancer treatment, particularly against aggressive subtypes such as TNBC [92].

Ginsenosides

SRg3 and RRg3 are epimers of ginsenoside Rg3 extracted from Panax ginseng, each exhibiting distinct anti-cancer properties. In the context of breast cancer, SRg3 and

RRg3 have been investigated for their potential to target BCSCs via the mTOR pathway. SRg3 selectively inhibits proliferation and blocks aquaporin 1 (AQP1) water channel, crucial for cancer cell proliferation, migration, and invasion. On the other hand, RRg3 inhibits the invasion of breast cancer cells. Through response surface methodology (RSM) modeling, an optimal combination of SRg3 and RRg3 (C3) was determined to effectively inhibit TNBC cell migration. This combination treatment significantly reduced mammosphere formation efficiency (MFE) and the proportion of BCSCs expressing CD44+/CD24 – phenotype in TNBC cell lines. Furthermore, molecular studies revealed that C3 treatment reduced phosphorylation of key proteins downstream of the AKT/mTOR signaling pathway, including 4E-BP1, RPS6, P70S6K, PRAS40, Raf-1, and RSK1, suggesting its potential to disrupt mTOR-mediated pathways crucial for BCSC self-renewal and tumor progression. These effects collectively contribute to the inhibition of TNBC cell migration, reduction of BCSC characteristics, and suppression of tumor growth observed both in vitro and in vivo [93].

Matcha green tea

Matcha green tea (MGT) is a type of powdered green tea known for its rich antioxidant content, particularly epigallocatechin-3-gallate (EGCG), which has been implicated in various health benefits. In the context of breast cancer, MGT has attracted attention for its potential therapeutic effects. Recent studies have demonstrated that MGT can effectively inhibit the propagation of BCSCs. MGT treatment significantly reduces the formation of mammospheres, a hallmark of CSC activity, indicating its ability to hinder BCSCs-mediated tumorigenesis. Furthermore, MGT exerts its effects on breast cancer cells by modulating the mTOR signaling pathway. Through the downregulation of mTOR pathway components and related proteins, MGT induces metabolic reprogramming in cancer cells, leading to decreased mitochondrial metabolism and glycolysis. These findings suggest that MGT holds promise as a natural product for breast cancer therapy, targeting BCSCs and disrupting mTOR-mediated cellular processes to impede tumor progression [94].

Extra-virgin olive oil

Phenol-conjugated oleosidic secoiridoids found in extra virgin olive oil (EVOO) represent a group of bioactive phytochemicals with potential anti-cancer properties, particularly against BCSCs. These compounds, such as decarboxymethyl oleuropein aglycone (DOA), have been identified as potent inhibitors of BCSCs function through various cellular and molecular mechanisms. At the cellular level, DOA suppresses the functional traits of CSCs, including their ability to form tumorspheres, thereby hindering their self-renewal capacity. Molecularly, DOA acts as an ATP-competitive inhibitor of mTOR, a key signaling pathway involved in CSC maintenance, and competes with S-adenosyl methionine (SAM) to inhibit DNA methyltransferases (DNMTs), thereby disrupting epigenetic regulation associated with BCSCs phenotypes. Additionally, DOA synergistically interacts with other drugs like rapamycin and 5-azacytidine, further enhancing its anti-BCSCs effects. Overall, phenol-conjugated oleosidic secoiridoids from EVOO offer a promising avenue for targeting BCSCs, providing new insights into cancer therapy [95].

Quercetin

Quercetin, a flavonoid compound abundant in fruits and vegetables, exhibits a spectrum of pharmacological activities, including antioxidant and anti-cancer properties. Particularly significant in breast cancer research is its potential to target BCSCs, notably those characterized by the CD44+/CD24 – phenotype, which are implicated in tumor initiation, progression, and therapy resistance. Studies have demonstrated that quercetin treatment inhibits the viability, proliferation, and self-renewal capacity of BCSCs, leading to reduced tumor growth and metastatic potential in preclinical in vivo models. Mechanistically, quercetin exerts its anti-BCSC effects by modulating key signaling pathways such as the PI3K/Akt/mTOR pathway. These findings underscore the promise of quercetin as a novel therapeutic strategy for breast cancer, specifically targeting CD44+/CD24 – BCSCs, with potential implications for improving treatment outcomes and reducing the risk of cancer recurrence and metastasis [96].

Other drugs

Telomerase inhibitors

BIBR1532 is a selective telomerase inhibitor that demonstrates cytotoxic effects on BCSCs. BIBR1532 inhibits the catalytic subunit of telomerase, an enzyme overexpressed in cancers, which plays a crucial role in maintaining chromosome stability and promoting cell immortality. Treatment with BIBR1532 induces apoptosis and cell cycle arrest, particularly in the G2/M phase, in BCSCs. Additionally, BIBR1532 downregulates the expression of genes associated with the mTOR pathway, which is implicated in cell proliferation, metabolism, and drug resistance. This dual mechanism of action suggests that BIBR1532 holds promise as a potential therapeutic agent for targeting BCSCs and combating breast cancer progression [97].

FGFR inhibitor

AZD4547 is a potent inhibitor of fibroblast growth factor receptors (FGFRs) and has shown promise as a

preventative and therapeutic agent for ErbB2-overexpressing breast cancer. It exerts its effects by targeting the FGFR signaling pathway, which is crucial for cell proliferation, survival, and differentiation. In breast cancer, aberrant FGFR expression can lead to oncogenic consequences, including the reprogramming of mammary stem cells (MaSCs) with tumorigenic potential. AZD4547 effectively inhibits FGFR activity, suppressing downstream signaling pathways such as Akt, Erk1/2, and mTOR. Consequently, AZD4547 reduces the percentage of CSCs/tumor-initiating cells (TICs) within breast cancer cell populations, as evidenced by a decrease in acetaldehyde dehydrogenase-positive (ALDH+) cells, a marker of TICs, and inhibition of tumorsphere formation, which reflects the self-renewal capacity of CSCs/TICs. This ability of AZD4547 to target stemness in breast cancer cells suggests its potential as a therapeutic strategy to mitigate tumor initiation, progression, and recurrence [98].

Antidiabetic drugs

Metformin, a widely used anti-diabetic drug, has shown promise in targeting BCSCs, which play crucial roles in tumor initiation, metastasis, and therapy resistance. Metformin affects BCSCs through multiple mechanisms, primarily by modulating AMPK and mTOR signaling pathways. Activation of AMPK and inhibition of mTOR by metformin disrupt cellular processes essential for CSC maintenance, such as self-renewal and differentiation. This disruption leads to decreased CSC population, impairing tumorigenic growth and enhancing sensitivity to anti-cancer treatments. Additionally, metformin's ability to induce cell cycle arrest and apoptosis in breast cancer cells further contributes to its anti-CSC effects. These findings underscore the potential of metformin as an adjunctive therapy in breast cancer treatment strategies, particularly in targeting CSCs and overcoming tumor heterogeneity and therapeutic resistance [99]. Conversely, hyperthermia induces cellular stress and disrupts cell membrane integrity, resulting in cell death. While traditional hyperthermia methods may exhibit limited efficacy against CSCs, newer approaches, combined with nanoparticles or optically activated agents, have shown promise in sensitizing CSCs to therapy. When metformin is combined with hyperthermia, there is a synergistic enhancement of cytotoxicity against both breast cancer cells and CSCs, potentially due to the complementary mechanisms of action targeting mTOR signaling, ultimately leading to improved therapeutic outcomes [100]. Metformin exerts its preventive effects on ErbB2-mediated breast cancer development primarily by targeting CSCs. Through systemic administration in a mouse model of ErbB2 overexpression, metformin selectively inhibits CSC subpopulations in preneoplastic mammary glands by downregulating the ErbB2/PI3K/Akt pathway and inhibiting activation of IGF-1R, mTOR, and Stat3. Moreover, metformin pretreatment effectively suppresses the initiation and growth of ErbB2-overexpressing tumors in a syngeneic graft mouse model. In vitro studies further support these findings, demonstrating that metformin inhibits proliferation and signaling in ErbB2-overexpressing breast cancer cells, with a pronounced effect on CSCs at low concentrations. These results underscore the pivotal role of CSCs in ErbB2-mediated breast cancer and highlight metformin's potential as a preventive agent by targeting CSCs and disrupting key signaling pathways associated with their maintenance and growth [101]. Furthermore, metformin has shown a remarkable capability to target CSCs, which are known for their resistance to conventional therapies and their role in tumor initiation and recurrence. By reducing the proportion of CSCs and sensitizing cancer cells to radiation, metformin enhances the efficacy of cancer treatments, potentially leading to improved patient outcomes by targeting both the bulk of the tumor cells and the CSCs responsible for relapse and metastasis [23]. Similarly, Buformin, a biguanide antidiabetic agent, has been identified as a potential therapeutic agent against erbB-2-overexpressing breast cancer cells and premalignant mammary tissues in MMTV-erbB-2 transgenic mice. Its mode of action involves the inhibition of the mitochondrial complex I, leading to a decrease in cellular energy levels (ATP) and the activation of AMP-activated protein kinase (AMPK). This activation subsequently downregulates the mTOR pathway, which is crucial for protein synthesis and cell growth. By targeting these metabolic and signaling pathways, Buformin effectively reduces the 'stemness' of cancer cells—characteristics that enable the cells to initiate tumors, proliferate, and resist conventional therapies. Additionally, Buformin's ability to selectively target cancer cells while sparing normal cells makes it a promising candidate for the development of targeted therapies against erbB-2-positive breast cancer, offering a potential strategy for preventing the progression of premalignant tissues to invasive cancer [102].

Psychiatric drugs

Thioridazine (THZ) is an antipsychotic drug that has garnered attention for its inherent anti-tumor effects, particularly in targeting CSCs. When combined with carboplatin (CBP), a platinum-based chemotherapy commonly used in treating TNBC, THZ demonstrates synergistic inhibition of TNBC. THZ has been found to specifically target breast CSCs by antagonizing dopamine receptors, leading to CSC destruction. Additionally, THZ has been shown to induce apoptosis in TNBC cells by promoting caspase-mediated apoptotic pathways and inducing endoplasmic reticulum (ER) stress. When combined with CBP, THZ enhances the anti-tumor activity,

resulting in significant inhibition of TNBC proliferation, induction of apoptosis, and suppression of tumor growth both in vitro and in vivo. This combination therapy not only targets TNBC cells but also effectively reduces the population of CSCs within the tumor, thereby inhibiting their tumorigenic potential. The synergistic effects of THZ and CBP are mediated through the PI3K/mTOR pathway and ER stress, highlighting the potential of this combination as an efficient therapeutic approach for TNBC treatment [103]. SB-699,551 is a selective antagonist of the serotonin receptor 5-HT5A, identified as a potential target for inhibiting breast tumor-initiating cells (BTICs), which are a subset of cells within breast tumors with stem cell-like properties. These BTICs possess the ability to self-renew and differentiate and are resistant to traditional chemotherapy, contributing to tumor recurrence. SB-699,551 disrupts signaling pathways downstream of 5-HT5A, particularly the Gαi/o-coupled pathway and the PI3K/AKT/mTOR axis, leading to decreased phosphorylation of proteins involved in cell survival and proliferation. This disruption ultimately reduces the activity of BTICs, as evidenced by functional assays demonstrating a decrease in tumorsphere formation and ex vivo tumor initiation. Additionally, genetic manipulation experiments using CRISPR-Cas9-mediated knockout of HTR5A, the gene encoding 5-HT5A, further confirmed its role in regulating BTIC activity. In preclinical studies, SB-699,551 demonstrated efficacy in reducing tumor growth rates and mass in breast tumor xenograft models, both alone and in combination with traditional chemotherapy agents like docetaxel [104].

Nanoparticles

Nanoparticles revolutionize cancer therapy by enabling precise drug delivery, reducing side effects, and enhancing the efficacy of treatment at the molecular level [105–107]. Gold nanoparticles (AuNPs) are nanoscale particles made of gold atoms. Due to their unique properties such as high surface area to volume ratio, biocompatibility, and ease of surface functionalization, AuNPs have gained attention in various biomedical applications including cancer therapy. In the context of paclitaxel-resistant breast cancer stem cells, AuNPs can be utilized as drug delivery vehicles to overcome drug resistance. By encapsulating therapeutic agents such as Nyctanthes arbortristis (NAT) and doxorubicin, AuNPs can target cancer cells, including the resistant stem cell population. The molecular and cellular mechanisms underlying their efficacy involve several pathways. Firstly, the combination therapy induces iron-dependent cell death, possibly through the induction of ferritinophagy, a process involving the degradation of ferritin. This leads to increased cellular ROS levels, causing mitochondrial disruption and sensitizing cancer cells to apoptosis. Furthermore, AuNP-loaded drugs inhibit the mTOR pathway, a key regulator of cell growth and proliferation, contributing to the suppression of cancer cell survival and proliferation. Overall, AuNPs offer a multifaceted approach to combating paclitaxel-resistant breast cancer stem cells by targeting multiple pathways involved in cancer progression and resistance, including the regulation of mTOR signaling [108]. Similarly, co-loaded lapatinib and pseudolaric acid B (PAB) within ferritin nanoparticles (L/P@Ferritin) were developed to target EGFR and induce ferroptosis in triple-negative breast cancer (TNBC) cells, particularly those in extracellular matrix (ECM)-detached clusters. The L/P@Ferritin nanoparticle selectively targeted TNBC cells, leading to enhanced ferroptosis and inhibition of tumor growth both in vitro and in vivo. Mechanistically, EGFR inhibition by L/P@Ferritin suppressed TNBC cell viability and reduced cancer stemness by modulating mTOR pathway, leading to increased intracellular lipid peroxidation and ferroptotic cell death. This approach highlights the potential of L/P@Ferritin nanoparticles as a novel therapeutic strategy for eliminating ECM-detached TNBC clusters by targeting EGFR and modulating the mTOR pathway to induce ferroptosis [109]. Another study investigated the antiangiogenic potential of quinacrine-gold hybrid nanoparticles (QAuNP) irradiated with near-infrared (NIR) light in breast cancer. Using ex vivo models of primary breast cancer stem cells (P-BCSCs) and human umbilical vein endothelial cells (HUVECs), as well as in vivo chick chorioallantoic membrane (CAM) assay and patient-derived xenograft (PDX) mice, the researchers demonstrated that QAuNP + NIR treatment significantly reduced P-BCSC proliferation and viability, inhibited angiogenesis, and decreased the secretion of key cytokines involved in angiogenesis within the tumor microenvironment. Mechanistically, QAuNP + NIR treatment downregulated the PI3K/AKT/mTOR pathway in HUVECs and reduced nitric oxide (NO) production, contributing to its antiangiogenic effects. In PDX mouse models, QAuNP + NIR treatment led to reduced tumor volume and downregulation of angiogenic markers. These findings suggest that QAuNP + NIR treatment holds promise as a therapeutic strategy for inhibiting tumor angiogenesis and progression in breast cancer [110].

MicroRNA based therapies

The involvement of miRNAs in modulating the PI3K/AKT/mTOR pathway in breast cancer influences cell proliferation, survival, metastasis, and therapy resistance (Reviewed in [111]). Anti-microRNA therapies targeting BCSCs focus on inhibiting oncogenic miRNAs (oncomiRs) that drive BCSC characteristics such as self-renewal, chemoresistance, and metastasis. Key oncomiRs like miR-155, miR-181, miR-29a, miR-9, and

miR-221 enhance stemness and tumor aggressiveness through pathways such as Wnt/β-catenin, Notch, PI3K/AKT/mTOR, and STAT3. Anti-miRNA strategies aim to restore normal regulatory mechanisms by silencing these oncomiRs, which can suppress tumor progression, reduce BCSC populations, and enhance sensitivity to chemotherapy (Reviewed in [112]). The role of miR-100 as a critical regulator in BCSCs has been highlighted, demonstrating its ability to suppress stemness, induce luminal markers, and enhance endocrine responsiveness through its downstream effects. A significant finding is miR-100's role in downregulating mTOR, a key driver of proliferation and therapy resistance in luminal breast cancer. High levels of miR-100 were associated with better endocrine therapy response and overall survival, suggesting its therapeutic potential. These results imply that targeting miR-100 pathways, particularly through anti-miR-100 therapy, could strategically reactivate mTOR in specific contexts, disrupting BCSC resistance mechanisms and sensitizing tumors to existing treatments. This approach warrants further exploration as a precision therapy to mitigate endocrine resistance in luminal breast cancer [113]. Similarly, targeting miR-125b can effectively counteract resistance to aromatase inhibitors (AIs) in breast cancer by inhibiting the AKT/mTOR pathway. Specifically, silencing miR-125b in letrozole-resistant cells (Res-Let) significantly reduced the constitutive activation of the AKT/mTOR pathway and restored sensitivity to letrozole treatment. Furthermore, combining an AKT inhibitor (MK-2206) with letrozole in cells overexpressing miR-125b also successfully re-sensitized them to the AI, demonstrating that the miR-125b-driven activation of the AKT/mTOR pathway is a critical mechanism for resistance [55].

Clinical trials on the role of mTOR inhibitors in BCSCs

A clinical trial utilizing sirolimus presented a compelling avenue for combatting BCSCs, particularly given its demonstrated efficacy in modulating mammary epithelial populations and inhibiting key markers associated with early-stage breast cancer progression. Sirolimus, through its inhibition of the mTOR signaling pathway, offers a targeted approach to disrupt CSC activity, which is crucial for tumor initiation, progression, and recurrence. The observed reduction in mammary stem cell (MaSC) activity and self-renewal capacity of luminal progenitors underscores sirolimus' potential to directly impede the cellular origins and maintenance of breast cancer. By specifically targeting CSCs, sirolimus may disrupt the hierarchical organization of tumors, preventing the generation of heterogeneous cell populations that drive tumor growth and therapeutic resistance. Furthermore, the downregulation of proliferation markers like Ki67 and the increase in cell cycle inhibitor p21 expression suggest a shift towards quiescence and reduced replicative potential in CSCs, further hampering their ability to propagate and contribute to tumor growth. Importantly, sirolimus treatment also mitigates the expression of senescence-associated secretory phenotype (SASP) factors, such as IL-6 and TNFα, which are implicated in creating a pro-tumorigenic microenvironment. By dampening the SASP, sirolimus may attenuate the inflammatory milieu that fosters CSC survival and expansion. Thus, the comprehensive effects of sirolimus on CSC activity, tumor proliferation, and tumor microenvironment suggest its potential as a promising therapeutic strategy against breast cancer, particularly in targeting the roots of tumor initiation and progression represented by CSCs. Continued investigation and clinical validation of sirolimus as a CSC-targeted therapy hold promise for improving outcomes in breast cancer patients, potentially offering a novel approach to prevent recurrence and enhance overall survival rates [69]. Another clinical trial exploring the combination of the bromodomain inhibitor OTX015 (MK-8628) with everolimus presents a promising strategy to target the stemness of cancer cells, particularly in the context of TNBC. The rationale behind this combination lies in the complementary mechanisms of action of these two agents and their potential to disrupt key pathways involved in CSC maintenance and tumor progression. OTX015, as a bromodomain inhibitor, targets BET proteins (BRD2, BRD3, BRD4) involved in epigenetic regulation, thereby influencing the expression of genes crucial for CSC self-renewal and survival. By disrupting the BET-mediated transcriptional program, OTX015 has shown promising preclinical activity in various cancer models, including TNBC, by downregulating stemness markers and impairing CSC function. On the other hand, everolimus, an mTOR inhibitor, acts on the PI3K-Akt-mTOR pathway, which is frequently dysregulated in TNBC and implicated in CSC maintenance and tumor progression. By inhibiting mTOR signaling, everolimus can suppress CSC proliferation and survival, potentially sensitizing them to other therapeutic agents. Therefore, the combination of OTX015 and everolimus offers a dual-targeted approach to tackle CSCs in TNBC, synergistically disrupting key pathways essential for their maintenance and survival. This combination therapy holds promise for overcoming therapeutic resistance and improving outcomes in TNBC patients by targeting the root cause of tumor heterogeneity and recurrence. Further clinical evaluation of this combination regimen is warranted to validate its efficacy and safety in TNBC patients [115].

Conclusion

This comprehensive review highlights the critical role of the mTOR pathway in the biology and treatment resistance of BCSCs. Breast cancer remains the most frequently diagnosed cancer globally, significantly impacting women's health and imposing a substantial burden on healthcare systems. BCSCs are pivotal in driving tumor initiation, metastasis, recurrence, and resistance to conventional therapies, largely due to their ability to self-renew and adapt to various treatment modalities. Central to these processes is the mTOR pathway, which regulates essential cellular functions such as growth, metabolism, autophagy, and survival. The activation of mTOR signaling in BCSCs promotes their maintenance, enhancing their resilience against standard treatments and contributing to aggressive tumor behavior. Mechanistically, mTOR facilitates EMT, thereby increasing the invasiveness and metastatic potential of BCSCs. In addition, mTOR plays a subtype-specific role in regulating BCSCs across the molecular subtypes of breast cancer. In luminal breast cancer, mTOR activation is often linked to increased resistance to endocrine therapies by promoting cell survival and metabolic adaptation in BCSCs. In HER-2 positive breast cancer, mTOR interacts with HER-2 signaling to enhance the survival and self-renewal of BCSCs, often driving resistance to HER-2-targeted therapies such as trastuzumab. In TNBC, which harbors the highest proportion of BCSCs, mTOR activation promotes EMT, metabolic reprogramming, and survival under therapeutic stress, contributing to the aggressive nature of TNBC. These distinct roles highlight the importance of targeting mTOR to manage therapy resistance and disease progression in each subtype. Additionally, mTOR orchestrates metabolic reprogramming, favoring glycolysis and glutamine metabolism, which are crucial for the survival and proliferation of these stem cells. This metabolic flexibility not only supports the energetic and biosynthetic demands of BCSCs but also underpins their ability to thrive in diverse tumor microenvironments. Furthermore, mTOR's regulation of autophagy and ferroptosis underscores its dual role in maintaining cellular homeostasis and mediating programmed cell death, presenting opportunities to manipulate these processes for therapeutic gain. The interplay between mTOR and various molecular pathways, including PI3K/Akt, Notch, IGF-1R, AMPK, and TGF-β, reveals a complex network that sustains BCSC properties and promotes chemoresistance. Non-coding RNAs and regulatory proteins further modulate mTOR activity, adding additional layers of regulation that influence BCSC behavior and therapeutic outcomes. mTOR's contribution to chemoresistance and radioresistance is particularly noteworthy, as it enhances survival pathways, drug efflux mechanisms, and DNA repair processes in BCSCs, thereby fostering tumor progression and treatment failure. Targeting the mTOR pathway emerges as a promising therapeutic strategy to disrupt the maintenance and survival of BCSCs. Synthetic inhibitors such as Everolimus, VS-5584, and NVP-BEZ235 have demonstrated efficacy in reducing BCSC viability and tumor growth, especially when used in combination with other agents like Trastuzumab. Monoclonal antibodies, including Trastuzumab and Dinutuximab, show synergistic effects when paired with mTOR inhibitors, effectively diminishing BCSC populations and inhibiting tumor progression. Additionally, natural products like Ursolic acid, Pentadecanoic acid, and Quercetin offer promising alternatives or adjuncts to conventional therapies by modulating mTOR activity and targeting BCSC-specific pathways. Other therapeutic agents, including telomerase inhibitors, FGFR inhibitors, antidiabetic drugs like Metformin, psychiatric drugs, and innovative nanoparticle-based approaches, provide diverse strategies to disrupt mTOR-mediated survival and proliferation of BCSCs. The intricate role of mTOR in BCSCs underscores its potential as a central therapeutic target in breast cancer treatment. However, the complexity of mTOR signaling and its interactions with multiple pathways necessitate a multifaceted approach to effectively target BCSCs. Combination therapies that simultaneously inhibit mTOR and other complementary pathways offer the most promising strategy to overcome resistance and achieve sustained therapeutic outcomes. Future research should focus on personalized medicine approaches, tailoring mTOR-targeted therapies based on individual patient profiles and specific molecular characteristics of their tumors. Additionally, developing strategies to counteract resistance mechanisms to mTOR inhibitors and expanding clinical evaluations of combination therapies and novel agents are essential for translating preclinical findings into effective treatment protocols. Identifying reliable biomarkers to monitor mTOR activity and BCSC populations will also facilitate early detection of therapeutic responses and resistance.

In conclusion, targeting the mTOR pathway presents a viable and promising avenue for improving breast cancer treatment by effectively eradicating BCSCs, reducing tumor recurrence, and enhancing overall patient survival. Continued exploration of mTOR's multifaceted roles and the development of innovative therapeutic strategies are essential to translate these insights into clinical success, ultimately leading to better treatment outcomes for breast cancer patients.

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References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global Cancer statistics 2022: GLOBOCAN estimates of incidence and Mortality Worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74:229–63.
2. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast Cancer—epidemiology, risk factors, classification, prognostic markers, and current treatment strategies—an updated review. Cancers (Basel). 2021;13:4287.
3. Al-thoubaity FK. Molecular classification of breast Cancer: a retrospective cohort study. Ann Med Surg. 2020;49:44–8.
4. Zeng Q, Chen C, Chen C, Song H, Li M, Yan J, Lv X. Serum Raman Spectroscopy combined with Convolutional Neural Network for Rapid Diagnosis of HER2-Positive and triple-negative breast Cancer. Spectrochim Acta Part Mol Biomol Spectrosc. 2023;286:122000.
5. Pal SK, Childs BH, Pegram M. Triple negative breast Cancer: Unmet Medical needs. Breast Cancer Res Treat. 2011;125:627–36.
6. Zhou J, Chen Q, Zou Y, Chen H, Qi L, Chen Y. Stem cells and Cellular origins of breast Cancer: updates in the Rationale, controversies, and therapeutic implications. Front Oncol. 2019;9:820.
7. Zhang L, Chen W, Liu S, Chen C. Targeting breast Cancer stem cells. Int J Biol Sci. 2023;19:552.
8. Hare SH, Harvey AJ. MTOR function and therapeutic targeting in breast Cancer. Am J Cancer Res. 2017;7:383.
9. Ebrahimi M, Nourbakhsh E, Hazara AZ, Mirzaei A, Shafieyari S, Salehi A, Hoseinzadeh M, Payandeh Z, Barati G. PI3K/Akt/MTOR signaling pathway in Cancer Stem cells. Pathol Pract. 2022;237:154010.
10. Hung C-M, Garcia-Haro L, Sparks CA, Guertin DA. MTOR-Dependent cell survival mechanisms. Cold Spring Harb Perspect Biol. 2012;4:a008771.
11. Yu G, Ding J, Yang N, Ge L, Chen N, Zhang X, Wang Q, Liu X, Zhang X, Jiang X, Geng Y, Zhang C, Pan J, Wang X, Gao W, Li Z, Zhang H, Ni W, Xiao J, Zhou K, Yang L. Evaluating the Pro-survival potential of apoptotic bodies derived from 2D- and 3D- cultured adipose stem cells in ischaemic flaps. J Nanobiotechnol. 2024;22:333.
12. Velasco-Velázquez MA, Popov VM, Lisanti MP, Pestell RG. The role of breast Cancer stem cells in Metastasis and therapeutic implications. Am J Pathol. 2011;179:2–11.
13. Murugan AK. *MTOR: Role in Cancer, Metastasis and Drug Resistance* In: Proceedings of the Seminars in cancer biology; Elsevier, 2019; Vol. 59, pp. 92–111.
14. Karimi Roshan M, Soltani A, Soleimani A, Rezaie Kahkhaie K, Afshari AR, Soukhtanloo M. Role of AKT and MTOR Signaling Pathways in the induction of epithelial-mesenchymal transition (EMT) process. Biochimie. 2019;165:229–34.
15. Shea MP, O'Leary KA, Wegner KA, Vezina CM, Schuler LA. High collagen density augments MTOR-Dependent Cancer stem cells in ERα + mammary carcinomas, and increases MTOR-Independent lung metastases. Cancer Lett. 2018;433:1–9.
16. Mateo F, Arenas EJ, Aguilar H, Serra-Musach J, De Garibay GR, Boni J, Maicas M, Du S, Iorio F, Herranz-Ors C. Stem cell-like Transcriptional Reprogramming Mediates Metastatic Resistance to MTOR Inhibition. Oncogene. 2017;36:2737–49.
17. Magaway C, Kim E, Jacinto E. Targeting MTOR and metabolism in Cancer: lessons and innovations. Cells. 2019;8:1584.
18. Salvadori G, Zanardi F, Iannelli F, Lobefaro R, Vernieri C, Longo VD. Fasting-mimicking Diet blocks Triple-negative breast Cancer and Cancer stem cell escape. Cell Metab. 2021;33:2247–59.
19. Samuel SM, Varghese E, Satheesh NJ, Triggle CR, Büsselberg D. Metabolic heterogeneity in TNBCs: a potential determinant of therapeutic efficacy of 2-Deoxyglucose and Metformin Combinatory Therapy. Biomed Pharmacother. 2023;164:114911.
20. Karvelsson ST, Sigurdsson A, Seip K, Grinde MT, Wang Q, Johannsson F, Mælandsmo GM, Moestue SA, Rolfsson O, Halldorsson S. EMT-Derived alterations in glutamine metabolism sensitize mesenchymal breast cells to MTOR Inhibition. Mol Cancer Res. 2021;19:1546–58.
21. Lyu Z, Xin M, Oyston DR, Xue T, Kang H, Wang X, Wang Z, Li Q. Cause and consequence of heterogeneity in human mesenchymal stem cells: challenges in clinical application. Pathol Pract. 2024;260:155354.
22. Varghese S, Samuel SM, Varghese E, Kubatka P, Büsselberg D. High glucose represses the anti-proliferative and pro-apoptotic effect of Metformin in Triple negative breast Cancer cells. Biomolecules. 2019;9:16.
23. Song CW, Lee H, Dings RPM, Williams B, Powers J, Santos T, Dos; Choi B-H, Park HJ. Metformin kills and Radiosensitizes Cancer cells and preferentially kills Cancer Stem cells. Sci Rep. 2012;2:362.
24. Xu Z, Han X, Ou D, Liu T, Li Z, Jiang G, Liu J, Zhang J, Targeting. PI3K/AKT/MTOR-Mediated autophagy for Tumor Therapy. Appl Microbiol Biotechnol. 2020;104:575–87.
25. Sharif T, Martell E, Dai C, Ghassemi-Rad MS, Hanes MR, Murphy PJ, Margam NN, Parmar HB, Giacomantonio CA, Duncan R. HDAC6 differentially regulates autophagy in stem-like versus differentiated Cancer cells. Autophagy. 2019;15:686–706.
26. Kumar D, Shankar S, Srivastava RK. Rottlerin-Induced Autophagy leads to the apoptosis in breast Cancer stem cells: Molecular mechanisms. Mol Cancer. 2013;12:1–15.
27. Lou J-S, Zhao L-P, Huang Z-H, Chen X-Y, Xu J-T, Tai WC-S, Tsim KWK, Chen Y-T, Xie T. Ginkgetin Derived from Ginkgo Biloba leaves enhances the therapeutic effect of cisplatin via ferroptosis-mediated disruption of the Nrf2/HO-1 Axis in EGFR Wild-Type Non-small-cell Lung Cancer. Phytomedicine. 2021;80:153370.
28. Zhang Z, Wang L, Guo Z, Sun Y, Yan J. A PH-Sensitive Imidazole Grafted Polymeric Micelles Nanoplatform based on ROS amplification for ferroptosis-enhanced chemodynamic therapy. Colloids Surf B Biointerfaces. 2024;237:113871.
29. Cosialls E, El Hage R, Dos Santos L, Gong C, Mehrpour M, Hamaï A. Ferroptosis: Cancer Stem cells rely on Iron until to die for it. Cells. 2021;10:2981.
30. Son B, Lee W, Kim H, Shin H, Park HH. Targeted therapy of Cancer Stem cells: inhibition of MTOR in Pre-clinical and Clinical Research. Cell Death Dis. 2024;15:696.
31. Cosialls E, Pacreau E, Duruel C, Ceccacci S, Elhage R, Desterke C, Roger K, Guerrera C, Ducloux R, Souquere SMTOR. Inhibition suppresses

Salinomycin-Induced ferroptosis in breast Cancer stem cells by ironing out mitochondrial dysfunctions. Cell Death Dis. 2023;14:744.
32. Arif T, Paul A, Krelin Y, Shteinfer-Kuzmine A, Shoshan-Barmatz V. Mitochondrial VDAC1 silencing leads to metabolic rewiring and the reprogramming of Tumour Cells into Advanced Differentiated States. Cancers (Basel). 2018;10:499.
33. Katsuno Y, Meyer DS, Zhang Z, Shokat KM, Akhurst RJ, Miyazono K, Derynck R. Chronic TGF-β exposure drives stabilized EMT, Tumor Stemness, and Cancer Drug Resistance with vulnerability to Bitopic MTOR Inhibition. Sci Signal. 2019;12:eaau8544.
34. Yu L, Zang C, Ye Y, Liu H, Eucker J. Effects of BYL-719 (Alpelisib) on human breast Cancer stem cells to Overcome Drug Resistance in human breast Cancer. Front Pharmacol, 2024, *15*.
35. Sarkar S, Ghosh SS. Synergistic effect of Salinomycin with Budesonide on TNBC Regression via EMT reversal and Autophagy induction. J Biochem Mol Toxicol. 2024;38:e70045.
36. Gargalionis AN, Papavassiliou KA, Basdra EK, Papavassiliou AG. MTOR Signaling Components in Tumor Mechanobiology. Int J Mol Sci. 2022;23:1825.
37. Chu H, Du C, Yang Y, Feng X, Zhu L, Chen J, Yang F. MC-LR aggravates liver lipid metabolism disorders in obese mice Fed a High-Fat Diet via PI3K/AKT/MTOR/SREBP1 signaling pathway. Toxins (Basel). 2022;14:833.
38. Tong G, Peng T, Chen Y, Sha L, Dai H, Xiang Y, Zou Z, He H, Wang S. Effects of GLP-1 receptor agonists on Biological Behavior of Colorectal Cancer cells by regulating PI3K/AKT/MTOR signaling pathway. Front Pharmacol. 2022;13:901559.
39. Madsen RR, Erickson EC, Rueda OM, Robin X, Caldas C, Toker A, Semple RK, Vanhaesebroeck B. Positive correlation between Transcriptomic Stemness and PI3K/AKT/MTOR signaling scores in breast Cancer, and a counterintuitive relationship with PIK3CA genotype. PLoS Genet. 2021;17:e1009876.
40. Jain MV, Jangamreddy JR, Grabarek J, Schweizer F, Klonisch T, Cieślar-Pobuda A, Łos MJ. Nuclear localized akt enhances breast Cancer stem-like cells through Counter-regulation of P21Waf1/Cip1 and P27kip1. Cell Cycle. 2015;14:2109–20.
41. Rodriguez MJ, Perrone MC, Riggio M, Palafox M, Salinas V, Elia A, Salgueiro ND, Werbach AE, Marks MP, Kauffman MA. Targeting MTOR to overcome resistance to hormone and CDK4/6 inhibitors in ER-Positive breast Cancer models. Sci Rep. 2023;13:2710.
42. Corominas-Faja B, Cufí S, Oliveras-Ferraros C, Cuyàs E, López-Bonet E, Lupu R, Alarcón T, Vellon L, Manuel Iglesias J, Leis O. Nuclear reprogramming of luminal-like breast Cancer cells generates Sox2-Overexpressing Cancer stem-like Cellular States harboring Transcriptional activation of the MTOR pathway. Cell Cycle. 2013;12:3109–24.
43. Nguyen K, Yan Y, Yuan B, Dasgupta A, Sun J, Mu H, Do K-A, Ueno NT, Andreeff M, Battula VL. St8SIA1 regulates Tumor Growth and Metastasis in TNBC by activating the FAK–AkT–MTOR signaling pathway. Mol Cancer Ther. 2018;17:2689–701.
44. Fang T, Hu S, Song X, Wang J, Zuo R, Yun S, Jiang S, Guo D. Combination of Monensin and Erlotinib synergistically inhibited the growth and Cancer Stem Cell properties of Triple-negative breast Cancer by simultaneously inhibiting EGFR and PI3K Signaling pathways. Breast Cancer Res Treat. 2024;207:435–51.
45. Jewer M, Lee L, Leibovitch M, Zhang G, Liu J, Findlay SD, Vincent KM, Tandoc K, Dieters-Castator D, Quail DF. Translational control of breast Cancer plasticity. Nat Commun. 2020;11:2498.
46. Hossain F, Sorrentino C, Ucar DA, Peng Y, Matossian M, Wyczechowska D, Crabtree J, Zabaleta J, Morello S. Del Valle, L. Notch Signaling regulates mitochondrial metabolism and NF-KB activity in Triple-negative breast Cancer cells via IKKα-Dependent non-canonical pathways. Front Oncol. 2018;8:575.
47. Chang W-W, Lin R-J, Yu J, Chang W-Y, Fu C-H, Lai AC-Y, Yu J-C, Yu AL. The expression and significance of insulin-like growth Factor-1 receptor and its pathway on breast Cancer Stem/Progenitors. Breast cancer Res. 2013;15:1–16.
48. Chan Y-T, Lin R-J, Wang Y-H, Hung T-H, Huang Y, Yu J, Yu J-C, Yu AL. The interplay between IGF-1R signaling and Hippo-YAP in breast Cancer stem cells. Cell Commun Signal. 2023;21:81.
49. Johnson J, Rychahou P, Sviripa VM, Weiss HL, Liu C, Watt DS, Evers BM. Induction of AMPK activation by N, N′-Diarylurea FND-4b decreases growth and increases apoptosis in Triple negative and estrogen-receptor positive breast cancers. PLoS ONE. 2019;14:e0209392.
50. Moafian Z, Maghrouni A, Soltani A, Hashemy SI. Cross-talk between non-coding RNAs and PI3K/AKT/MTOR pathway in Colorectal Cancer. Mol Biol Rep. 2021;48:4797–811.
51. HajiEsmailpoor Z, Fayazi A, Teymouri M, Tabnak P. Role of long non-coding RNA ELFN1-AS1 in carcinogenesis. Discov Oncol. 2024;15:74.
52. Pu X, Sheng S, Fu Y, Yang Y, Xu G. Construction of CircRNA–MiRNA–MRNA CeRNA Regulatory Network and Screening of diagnostic targets for tuberculosis. Ann Med. 2024;56:2416604.
53. Zhu Q, Sun J, An C, Li X, Xu S, He Y, Zhang X, Liu L, Hu K, Liang M. Mechanism of LncRNA Gm2044 in germ cell development. Front Cell Dev Biol. 2024;12:1410914.
54. Yang Z, Han Y, Cheng K, Zhang G, Wang X. MiR-99a directly targets the MTOR signalling pathway in breast Cancer Side Population cells. Cell Prolif. 2014;47:587–95.
55. Vilquin P, Donini CF, Villedieu M, Grisard E, Corbo L, Bachelot T, Vendrell JA, Cohen PA. MicroRNA-125b Upregulation confers aromatase inhibitor resistance and is a novel marker of poor prognosis in breast Cancer. Breast cancer Res. 2015;17:1–20.
56. Hu W, Zheng W, Du J, Tian Z, Zhao Y, Zhao P, Li J. TIPE2 sensitizes breast Cancer cells to Paclitaxel by suppressing Drug-Induced Autophagy and Cancer Stem Cell Properties. Hum Cell. 2023;36:1485–500.
57. Zhou K, Sun Y, Dong D, Zhao C, Wang W. EMP3 negatively modulates breast Cancer cell DNA replication, DNA damage repair, and stem-like Properties. Cell Death Dis. 2021;12:844.
58. Fukui F, Hayashi S, Yamaguchi Y. Heregulin controls ERα and HER2 signaling in Mammospheres of ERα-Positive breast Cancer cells and interferes with the efficacy of molecular targeted therapy. J Steroid Biochem Mol Biol. 2020;201:105698.
59. Chen L, He Y, Zhu J, Zhao S, Qi S, Chen X, Zhang H, Ni Z, Zhou Y, Chen G. The roles and mechanism of M6A RNA methylation regulators in Cancer Immunity. Biomed Pharmacother. 2023;163:114839.
60. Mansour FA, Al-Mazrou A, Al-Mohanna F, Al-Alwan M, Ghebeh H. PD-L1 is overexpressed on breast Cancer stem cells through Notch3/MTOR Axis. Oncoimmunology. 2020;9:1729299.
61. Kazi AA, Gilani RA, Schech AJ, Chumsri S, Sabnis G, Shah P, Goloubeva O, Kronsberg S, Brodie AH. Nonhypoxic regulation and role of Hypoxia-Inducible factor 1 in aromatase inhibitor resistant breast Cancer. Breast Cancer Res. 2014;16:1–18.
62. Bai J, Chen W-B, Zhang X-Y, Kang X-N, Jin L-J, Zhang H, Wang Z-Y. HIF-2α regulates CD44 to Promote Cancer Stem Cell activation in Triple-negative breast Cancer via PI3K/AKT/MTOR signaling. World J Stem Cells. 2020;12:87.
63. Ma C, Wang F, Han B, Zhong X, Si F, Ye J, Hsueh EC, Robbins L, Kiefer SM, Zhang Y. SALL1 functions as a tumor suppressor in breast Cancer by regulating Cancer Cell Senescence and Metastasis through the NuRD Complex. Mol Cancer. 2018;17:1–21.
64. Wang D, Liu C, Wang J, Jia Y, Hu X, Jiang H, Shao Z, Zeng YA. Protein C receptor stimulates multiple signaling pathways in breast Cancer cells. J Biol Chem. 2018;293:1413–24.
65. Tu C, Xu R, Koleti M, Zoldan J. Glycogen synthase Kinase-3 Inhibition Sensitizes Human Induced Pluripotent Stem cells to Thiol-Containing antioxidants Induced apoptosis. Stem Cell Res. 2017;23:182–7.
66. Andrade NP, Warner KA, Zhang Z, Pearson AT, Mantesso A, Guimaraës DM, Altemani A, Mariano FV, Nunes FD, Nör JE. Survival of salivary gland Cancer stem cells requires MTOR Signaling. Cell Death Dis. 2021;12:108.
67. Gasimli R, Kayabasi C, Ozmen Yelken B, Asik A, Sogutlu F, Celebi C, Yilmaz Susluer S, Kamer S, Biray Avci C, Haydaroglu A. The effects of PKI-402 on breast tumor models' Radiosensitivity via Dual Inhibition of PI3K/MTOR. Int J Radiat Biol. 2023;99:1961–70.
68. Lai Y, Yu X, Lin X, He S. Inhibition of MTOR sensitizes breast Cancer stem cells to Radiation-Induced repression of Self-Renewal through the regulation of MnSOD and Akt. Int J Mol Med. 2016;37:369–77.
69. Bouamar H, Broome LE, Lathrop KI, Jatoi I, Brenner AJ, Nazarullah A, Gorena KM, Garcia M, Chen Y, Kaklamani VMTOR. Inhibition abrogates human mammary stem cells and early breast Cancer progression markers. Breast Cancer Res. 2023;25:131.
70. Zhou J, Wulfkuhle J, Zhang H, Gu P, Yang Y, Deng J, Margolick JB, Liotta LA, Petricoin E, Zhang Y. Activation of the PTEN/MTOR/STAT3 Pathway in Breast Cancer Stem-like Cells Is Required for Viability and Maintenance. *Proc. Natl. Acad. Sci.*, 2007, *104*, 16158–16163.
71. Kim S-J, Seo I, Kim MH, Park J-W, Kim S, Park W-J. Ceramide synthase 4 overexpression exerts oncogenic properties in breast Cancer. Lipids Health Dis. 2023;22:183.
72. Abd El-Aziz YS, Spillane AJ, Jansson PJ, Sahni S. Role of ABCB1 in Mediating Chemoresistance of Triple-negative breast cancers. Biosci Rep. 2021;41:BSR20204092.

73. Marques AVL, Ruginsk BE, Prado L, de Lima O, Daniel DE, Moure IW, Valdameri VR. The Association of ABC Proteins with Multidrug Resistance in Cancer. Biochim Biophys Acta - Mol Cell Res. 2025;1872:119878.
74. Begicevic R-R, Falasca MABC. Transporters in Cancer Stem cells: beyond Chemoresistance. Int J Mol Sci, 2017, *18*.
75. Mukherjee P, Bagchi A, Banerjee A, Roy H, Bhattacharya A, Biswas A, Chatterji U. PDE4 inhibitor eliminates breast Cancer stem cells via Noncanonical activation of MTOR. J Cell Biochem. 2022;123:1980–96.
76. Alhasan BA, Gordeev SA, Knyazeva AR, Aleksandrova KV, Margulis BA, Guzhova IV, Suvorova II. The MTOR pathway in pluripotent stem cells: lessons for understanding Cancer Cell Dormancy. Membr (Basel). 2021;11:858.
77. Yunokawa M, Koizumi F, Kitamura Y, Katanasaka Y, Okamoto N, Kodaira M, Yonemori K, Shimizu C, Ando M, Masutomi K. Efficacy of Everolimus, a novel m TOR inhibitor, against basal-like Triple-negative breast Cancer cells. Cancer Sci. 2012;103:1665–71.
78. Liu Y, Zhang X, Liu J, Hou G, Zhang S, Zhang J. Everolimus in Combination with Letrozole Inhibit human breast Cancer MCF-7/Aro stem cells via PI3K/MTOR pathway: an experimental study. Tumor Biol. 2014;35:1275–86.
79. Kolev VN, Wright QG, Vidal CM, Ring JE, Shapiro IM, Ricono J, Weaver DT, Padval MV, Pachter JA, Xu Q. PI3K/MTOR dual inhibitor VS-5584 preferentially targets Cancer Stem cells. Cancer Res. 2015;75:446–55.
80. Karthik G-M, Ma R, Lövrot J, Kis LL, Lindh C, Blomquist L, Fredriksson I, Bergh J, Hartman JMTOR. Inhibitors counteract tamoxifen-Induced activation of breast Cancer stem cells. Cancer Lett. 2015;367:76–87.
81. Zhou H, Yu C, Kong L, Xu X, Yan J, Li Y, An T, Gong L, Gong Y, Zhu H. B591, a Novel specific Pan-PI3K inhibitor, preferentially targets Cancer Stem cells. Oncogene. 2019;38:3371–86.
82. Fatehi D, Soltani A, Ghatrehsamani M. SRT1720, a potential sensitizer for radiotherapy and cytotoxicity effects of NVB-BEZ235 in metastatic breast Cancer cells. Pathol Pract. 2018;214:889–95.
83. Vazquez N, Lopez A, Cuello V, Persans M, Schuenzel E, Innis-Whitehouse W, Keniry M. NVP-BEZ235 or JAKi Treatment leads to decreased survival of examined GBM and BBC Cells. Cancer Treat Res Commun. 2021;27:100340.
84. Bhola NE, Jansen VM, Koch JP, Li H, Formisano L, Williams JA, Grandis JR, Arteaga CL. Treatment of Triple-negative breast Cancer with TORC1/2 inhibitors sustains a drug-resistant and notch-dependent Cancer Stem Cell Population. Cancer Res. 2016;76:440–52.
85. Zhu Y, Zhang X, Liu Y, Zhang S, Liu J, Ma Y, Zhang J. Antitumor Effect of the MTOR inhibitor Everolimus in Combination with Trastuzumab on human breast Cancer stem cells in Vitro and in vivo. Tumor Biol. 2012;33:1349–62.
86. Yu F, Zhao J, Hu Y, Zhou Y, Guo R, Bai J, Zhang S, Zhang H, Zhang J. The combination of NVP-BKM120 with Trastuzumab or RAD001 synergistically inhibits the growth of breast Cancer stem cells in vivo. Oncol Rep. 2016;36:356–64.
87. Ly S, Anand V, El-Dana F, Nguyen K, Cai Y, Cai S, Piwnica-Worms H, Tripathy D, Sahin AA, Andreeff M. Anti-GD2 Antibody Dinutuximab Inhibits Triple-Negative Breast Tumor Growth by Targeting GD2 + Breast Cancer Stem-like Cells. *J. Immunother. cancer*, 2021, *9*.
88. Tewari D, Patni P, Bishayee A, Sah AN, Bishayee A. *Natural Products Targeting the PI3K-Akt-MTOR Signaling Pathway in Cancer: A Novel Therapeutic Strategy* In: Proceedings of the Seminars in cancer biology; Elsevier, 2022; Vol. 80, pp. 1–17.
89. Liao W-L, Liu Y-F, Ying T-H, Shieh J-C, Hung Y-T, Lee H-J, Shen C-Y, Cheng C-W. Inhibitory effects of Ursolic Acid on the stemness and progression of human breast Cancer cells by modulating Argonaute-2. Int J Mol Sci. 2022;24:366.
90. To NB, Truong VN-P, Ediriweera MK, Cho SK. Effects of combined pentadecanoic acid and tamoxifen treatment on tamoxifen resistance in MCF− 7/SC breast Cancer cells. Int J Mol Sci. 2022;23:11340.
91. Ock CW, Kim GD. Dioscin decreases breast Cancer stem-like cell proliferation via cell cycle arrest by modulating P38 Mitogen-Activated Protein Kinase and AKT/MTOR signaling pathways. J cancer Prev. 2021;26:183.
92. Dai L, Wang L, Tan C, Cai J, Shen H, Zhang T, Zhi S, Yang Z, Hu Y, Zhao X. Sophoridine Derivatives Induce Apoptosis and autophagy to suppress the growth of Triple-negative breast Cancer through inhibition of MTOR Signaling. ChemMedChem. 2022;17:e202100434.
93. Nakhjavani M, Smith E, Palethorpe HM, Tomita Y, Yeo K, Price TJ, Townsend AR, Hardingham JE. Anti-cancer effects of an optimised combination of Ginsenoside Rg3 epimers on Triple negative breast Cancer models. Pharmaceuticals. 2021;14:633.
94. Bonuccelli G, Sotgia F, Lisanti MP. Matcha Green Tea (MGT) inhibits the propagation of Cancer Stem cells (CSCs), by targeting mitochondrial metabolism, Glycolysis and multiple cell signalling pathways. Aging. 2018;10:1867.
95. Corominas-Faja B, Cuyàs E, Lozano-Sánchez J, Cufí S, Verdura S, Fernández-Arroyo S, Borrás-Linares I, Martin-Castillo B, Martin ÁG, Lupu R. Extra-virgin Olive Oil contains a metabolo-epigenetic inhibitor of Cancer Stem cells. Carcinogenesis. 2018;39:601–13.
96. Li X, Zhou N, Wang J, Liu Z, Wang X, Zhang Q, Liu Q, Gao L, Wang R. Quercetin suppresses breast Cancer stem cells (CD44+/CD24–) by inhibiting the PI3K/Akt/MTOR-Signaling pathway. Life Sci. 2018;196:56–62.
97. Doğan F, Özateş NP, Bağca BG, Abbaszadeh Z, Söğütlü F, Gasımlı R, Gündüz C, Biray Avcı Ç. Investigation of the effect of telomerase inhibitor BIBR1532 on breast Cancer and breast Cancer stem cells. J Cell Biochem. 2019;120:1282–93.
98. Zhao Q, Parris AB, Howard EW, Zhao M, Ma Z, Guo Z, Xing Y, Yang XFGFR, Inhibitor. AZD4547, impedes the stemness of mammary epithelial cells in the premalignant tissues of MMTV-ErbB2 transgenic mice. Sci Rep. 2017;7:11306.
99. Lee K-M, Lee M, Lee J, Kim SW, Moon H-G, Noh D-Y, Han W. Enhanced Anti-tumor Activity and Cytotoxic Effect on Cancer Stem Cell Population of Metformin-Butyrate compared with metformin HCl in breast Cancer. Oncotarget. 2016;7:38500.
100. Cui P, Liu Z, Wang G, Ma J, Qian Y, Zhang F, Han C, Long Y, Li Y, Zheng X, Sun D, Zhang J, Cai S, Jiao S, Hu Y. Risk factors for Pneumonitis in patients treated with anti-programmed Death-1 therapy: a case-control study. Cancer Med. 2018;7:4115–20.
101. Zhu P, Davis M, Blackwelder AJ, Bachman N, Liu B, Edgerton S, Williams LL, Thor AD, Yang X. Metformin selectively targets tumor-initiating cells in ErbB2-Overexpressing breast Cancer models. Cancer Prev Res. 2014;7:199–210.
102. Parris AB, Zhao Q, Howard EW, Zhao M, Ma Z, Yang X. Buformin inhibits the stemness of ErbB-2-Overexpressing breast Cancer cells and premalignant mammary tissues of MMTV-ErbB-2 transgenic mice. J Exp Clin Cancer Res. 2017;36:1–15.
103. Wang Y, Xia L, Lin J, Gong L, Xia Y, Xu Y, Liu L, Bao J, Zhang C, Chai Y. Thioridazine Combined with Carboplatin results in synergistic inhibition of Triple negative breast Cancer by Targeting Cancer Stem cells. Transl Oncol. 2022;26:101549.
104. Gwynne WD, Shakeel MS, Girgis-Gabardo A, Kim KH, Ford E, Dvorkin-Gheva A, Aarts C, Isaac M, Al-Awar R, Hassell JA. Antagonists of the serotonin receptor 5A Target Human breast tumor initiating cells. BMC Cancer. 2020;20:1–17.
105. Cheng Z, Li H, Chen C, Lv X, Zuo E, Xie X, Li Z, Liu P, Li H, Chen C. Application of serum SERS Technology based on thermally annealed silver nanoparticle composite substrate in breast Cancer. Photodiagnosis Photodyn Ther. 2023;41:103284.
106. Ma X, Cheng H, Hou J, Jia Z, Wu G, Lü X, Li H, Zheng X, Chen C. Detection of breast Cancer based on Novel Porous Silicon Bragg Reflector Surface-enhanced raman spectroscopy-active structure. Chin Opt Lett. 2020;18:51701.
107. Wang Y, Xu Y, Song J, Liu X, Liu S, Yang N, et al. Tumor Cell-Targeting and Tumor Microenvironment–Responsive nanoplatforms for the Multimodal Imaging-guided Photodynamic/Photothermal/Chemodynamic treatment of Cervical Cancer. Int J Nanomed, 2024;19:5837–58.
108. Chittineedi P, Pandrangi SL. Aqueous nyctanthes Arbortristis and Doxorubicin Conjugated Gold Nanoparticles synergistically Induced MTOR-Dependent autophagy-mediated Ferritinophagy in Paclitaxel-resistant breast Cancer stem cells. Front Pharmacol. 2023;14:1201319.
109. Wu X, Sheng H, Zhao L, Jiang M, Lou H, Miao Y, Cheng N, Zhang W, Ding D, Li W. Co-loaded Lapatinib/PAB by Ferritin Nanoparticles Eliminated ECM-Detached cluster cells via modulating EGFR in Triple-negative breast Cancer. Cell Death Dis. 2022;13:557.
110. Dash SR, Das B, Das C, Sinha S, Paul S, Pradhan R, Kundu CN. Near-Infrared enhances antiangiogenic potentiality of quinacrine-gold hybrid nanoparticles in breast Cancer stem cells via Deregulation of HSP-70/TGF-β. Nanomedicine. 2023;18:19–33.
111. Abu-Alghayth MH, Khan FR, Belali TM, Abalkhail A, AlshaghdaliK, Nassar SA, Almoammar NE, Almasoudi HH, Hessien KBG, aldossari Msaad, Binshaya AS. The emerging role of noncoding RNAs in the PI3K/AKT/MTOR signalling pathway in breast Cancer. Pathol - Res Pract. 2024;255:155180.
112. Niu T, Zhang W, Xiao W. MicroRNA Regulation of Cancer Stem cells in the pathogenesis of breast Cancer. Cancer Cell Int. 2021;21:31.
113. Petrelli A, Bellomo SE, Sarotto I, Kubatzki F, Sgandurra P, Maggiorotto F, Di Virgilio MR, Ponzone R, Geuna E, Galizia D, Nuzzo AM, Medico E, Miglio U, Berrino E, Venesio T, Ribisi S, Provero P, Sapino A, Giordano S, Montemurro F. MiR-100 is a predictor of endocrine responsiveness and prognosis in patients with operable luminal breast Cancer. ESMO Open, 2020, *5*.

114. Lee H, Park HJ, Park C-S, Oh E-T, Choi B-H, Williams B, Lee CK, Song CW. Response of breast Cancer cells and Cancer Stem cells to metformin and hyperthermia alone or combined. PLoS ONE. 2014;9:e87979.
115. Vázquez R, Riveiro ME, Astorgues-Xerri L, Odore E, Rezai K, Erba E, Panini N, Rinaldi A, Kwee I, Beltrame L. The bromodomain inhibitor OTX015 (MK-8628) exerts Anti-tumor Activity in Triple-negative breast Cancer models as single Agent and in combination with Everolimus. Oncotarget. 2017;8:7598.

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