Naunyn-Schmiedeberg's Archives of Pharmacology (2025) 398:16779–16795
https://doi.org/10.1007/s00210-025-04297-3

REVIEW

PI3 K/AKT/mTOR pathway and its role in breast cancer stem cells

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Received: 13 March 2025 / Accepted: 13 May 2025 / Published online: 17 July 2025

Abstract

Cancer stem cells (CSCs) are a small subpopulation bearing self-renewal ability, mediating tumor initiation and propagation. Several molecular pathways, including the PI3K/AKT/mTOR pathway, are known to be aberrantly activated in cancers. In CSCs, PI3K/AKT/mTOR pathway has been associated with attribution of various properties to cancer cells including stemness characteristics, proliferation, migration, epithelial to mesenchymal transition, and autophagy. Thus, targeting PI3K/AKT/mTOR pathway with novel inhibitors might help to control the growth and proliferation of the breast CSC population. Though many studies have focused on PI3K/AKT/mTOR pathway in breast cancer, limited literature is available on the role of PI3K/AKT/mTOR pathway in breast CSCs. Here, in our present review, we have highlighted the role of the PI3K/AKT/mTOR signaling pathway in breast CSCs and its applications in therapeutic targeting.

Keywords Breast cancer · Cancer stem cells · Epithelial–mesenchymal transition · PI3/AKT/mTOR

Abbreviations

ALDH	Aldehyde dehydrogenase
BC	Breast cancer
BCSC	Breast cancer stem cells
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
CBP	Carboplatin
CD24	Cluster of differentiation 24
CD44	Cluster of differentiation 44
CDK1	Cyclin-dependent kinase1
CSCs	Cancer stem cells
EMT	Epithelial–mesenchymal transition
EpCAM	Epithelial cellular adhesion molecule
ER	Estrogen receptor
FOXO	Forkhead box transcription factors
GPCRs	G protein–coupled receptors
GSK-3β	Glycogen synthase kinase-3beta
HER2	Human epidermal growth factor receptor 2
mSLT8	Mammalian lethal with SEC13 protein 8
mTOR C2	MTOR complex 2
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
Oct4	Octamer-binding transcription factor 4
PDK1	Phosphoinositide-dependent protein kinase
PH	Pleckstrin homology interaction domain
PI3K	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol(4,5)-bisphosphate
PIP3	Phosphatidylinositol(3,4,5)-trisphosphate
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PR	Progesterone receptor
PTEN	Phosphatase and tensin homolog
Raptor	Regulator associated protein of mTOR
RTK	Receptor tyrosine kinase
Sin1	Stress-activated protein kinase interacting protein 1
Sox2	SRY-box transcription factor 2
TNBC	Triple-negative breast cancer
TSC	Tuberous sclerosis complex

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TKD	Tyrosine kinase domain
4EBP1	Eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1
DEPTOR	DEP domain-containing mTOR-interacting protein
RICTOR	Rapamycinin sensitive companion of mammalian target of rapamycin
PDK-1	3-Phosphoinositide-dependent kinase 1

Introduction

The Global Cancer Observatory (GLOBOCAN) reports that breast cancer (BC) ranks among the most prevalent cancers worldwide (Sung et al. 2021). The 5-year survival rate of BC exhibits considerable variation based on geographic region and stage of diagnosis. In high-income nations, the 5-year survival rate for BC frequently exceeds 90% owing to early diagnosis and sophisticated treatments; however, in low-resource countries, survival rates may be significantly poor (Sung et al. 2021; Katsura et al. 2022). Several significant risk factors including family history (Brewer et al. 2017), age (McGuire et al. 2015), race (Hill et al. 2019; Yedjou et al. 2019), reproductive status (Albrektsen et al. 2005), use of certain drugs (Vinogradova et al. 2020; Narod 2011), Body Mass Index (Wang et al. 2019), physical activity (Chen et al. 2019), alcohol consumption (Erol et al. 2019; Mostofsky et al. 2025), smoking (Jones et al. 2017), exposure to artificial light (Johns et al. 2018), and chemical exposure (Eve et al. 2020) perform a key function in the development and progression of BC.

Based on HER2 amplification, hormone receptor status, and genomic profiling, BC is molecularly divided into many subtypes that influence prognosis and therapeutic approaches. The four main subtypes are luminal A (ER/PR-positive, HER2-negative, low Ki67), luminal B (ER-positive, HER2-negative/positive with increased proliferation), HER2-enriched (HER2-positive, ER/PR-negative), and triple-negative/basal-like (ER/PR/HER2-negative) (Kinsella et al. 2013; Tsang et al. 2020). Luminal B breast cancer exhibits sensitivity to endocrine therapy; yet, certain patients may experience primary or secondary resistance to such treatment in contrast to Luminal A, which exhibits favorable responses to endocrine therapy and possesses a favorable prognosis (Yang et al. 2022; Parise et al. 2009; Park et al. 2012; DePolo J 2024). Anti-HER2 treatments improve survival for HER2-enriched subtypes (12–20%) even though their prognosis has previously been poor. Although new immunotherapies show promise, triple-negative tumors (15–20%) are aggressive and lack targeted therapeutics. They are frequently associated with BRCA1 mutations. Sensitive markers to predict chemotherapy sensitivity and whether patients can benefit from particular chemotherapy regimens are lacking (Orrantia-Borunda et al. 2022).

Breast cancer management often encompasses a combination of surgical intervention, radiation, chemotherapy, hormone therapy, and targeted therapies (Harbeck and Gnant 2017; Nicolini et al. 2018). Despite these thorough strategies, recurrence and treatment resistance persist as considerable obstacles, especially in specific subtypes (Harbeck and Gnant 2017). There is mounting evidence that these issues are largely caused by cancer stem cells (CSCs). CSCs represent a limited population of tumor cells capable of self-renewal and evading standard therapies through mechanisms including drug efflux, augmented DNA repair, and dormancy. Moreover, CSCs engage with the tumor environment to facilitate immune evasion and maintain their stem-like characteristics (Chu et al. 2024; Pan et al. 2025; Beziaud et al. 2023; Yamashina et al. 2014).

Breast cancer stem cells

In BC, CSCs were first identified and isolated by Al-Hajj et al. leading to the identification of specific CSC markers, $CD44^{+}/CD24^{-}$/EpCAM (Al-Hajj et al. 2003). Based on these markers, the authors showed that CSCs were able to induce tumor in mice indicating the role of CSC in tumor initiation and progression (Loh and Ma 2024; Ozsvari et al. 2017). BCSCs are a rare, aggressive tumor subpopulation with self-renewal and differentiation capabilities, driving metastasis and therapy resistance (Jan et al. 2025; Abreu de Oliveira et al. 2021). There are several markers specific to CSCs, including CD133, aldehyde dehydrogenase (ALDH), and SRY-box transcription factor 2 (Sox2); octamer-binding transcription factor 4 (Oct4) and Nanog have also been identified to play a significant role in its stemness characteristics (Chu et al. 2024). With regards to their role in drug resistance, CSCs are well characterized to express ATP binding cassette (ABC) drug transporters, including the ATP-binding cassette subfamily G member 2 (ABCG2) that facilitates efflux of therapeutic drugs against the concentration gradient, thereby initiating drug resistance pathways (Damiani and Tiribelli 2024; Robey et al. 2001). Compared to normal tissue samples, CSCs showed a significant upregulation of around 180 genes (Fig. 1). On the other hand, CSCs are also known to modulate several signaling pathways, including Wingless/Integrated (Wnt)/beta-catenin (β-Catenin) (Zhao et al. 2024; Lv et al. 2015), Phosphatidylinositol 3-kinase/protein kinase B (PI3 K/AKT/MTOR) (Karami Fath et al. 2022; He et al. 2019), NOTCH, Sonic Hedgehog (Berrino and Omar 2024), and Nuclear factor kappa B (NF-κB) (Guo et al. 2024; Chen et al. 2011) pathways for their growth, self-renewal, and metastatic capabilities (Yi et al. 2025).

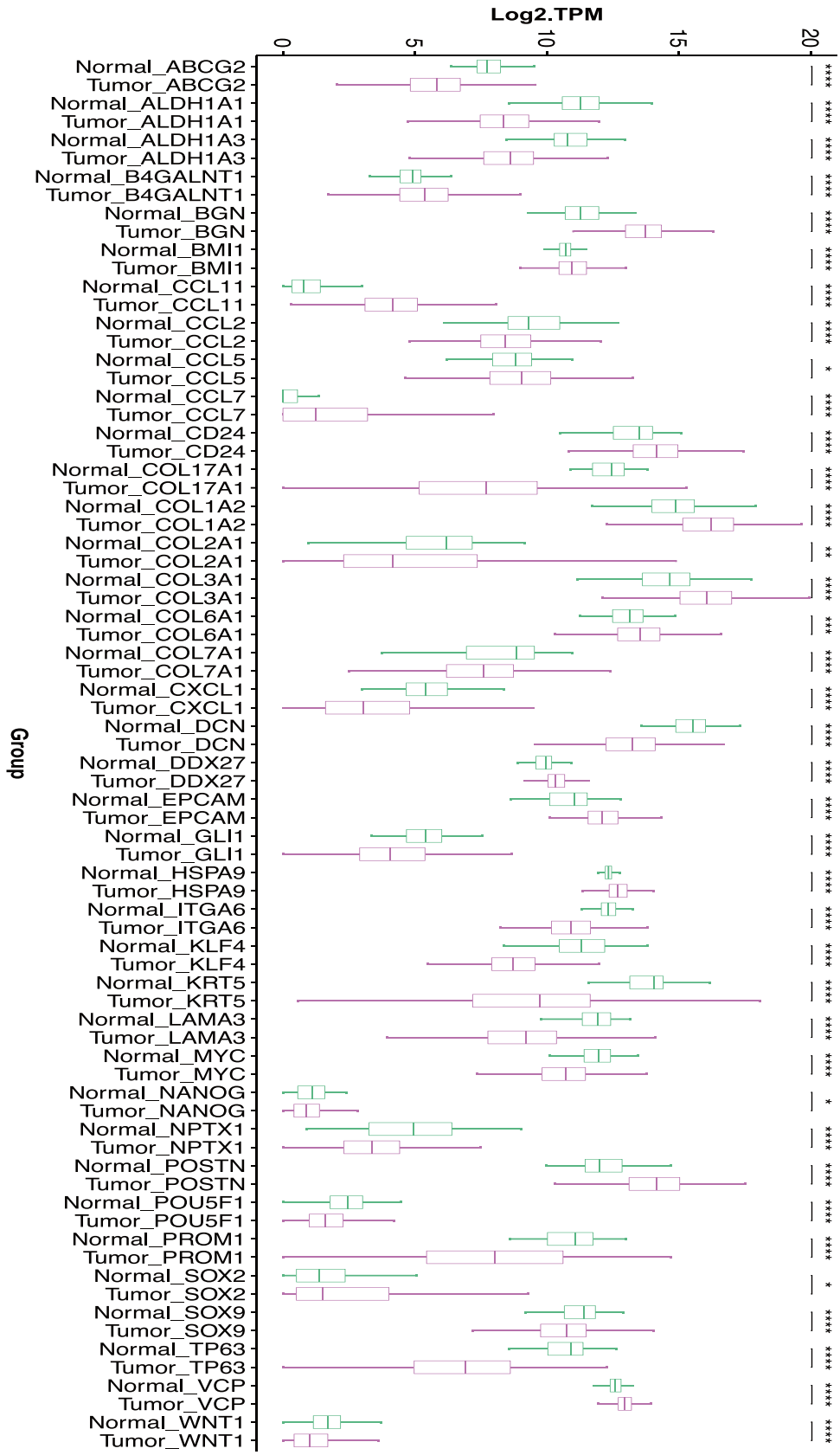


Fig. 1 Approximately 180 breast cancer stem cell (CSC) gene markers were retrieved from InnateDB (Breuer et al. 2013) along with confidence scores. Normalized expression count data of 49 high confidence CSC genes (confidence score > 0.3) was retrieved from TCGA breast cancer and normal tissues using UCSC Xena server (https://xena.ucsc.edu). Differential expressions between 114 normal and 1097 cancer tissues were calculated using Mann–Whitney *U* test and the statistically significant genes were plotted using boxplots

The aberrant PI3 K/AKT/mTOR pathway is known to be involved in cancer (Garg et al. 2025; Fanucci et al. 2024). However, limited studies on the role of CSCs in modulating PI3 K/AKT/mTOR pathways for BC progression and chemoresistance have been documented (Garg et al. 2025). Since this pathway is considered critical in cancer progression, we aim to focus this review on the role of PI3 K/AKT/mTOR signaling pathways in growth, survival, maintenance of BCSCs, and their role in therapeutic resistance. The review further aims to shed light on BCSCs as potential candidates for therapeutic targeting.

Structure, functions, and role of PI3 K/AKT/mTOR pathway

The PI3 K/AKT/mTOR pathway is an essential intracellular signaling cascade that regulates cell growth, survival, metabolism, and proliferation. The structure consists of three primary components: PI3 K (phosphoinositide 3-kinase), a heterodimeric enzyme made up of regulatory (p85) and catalytic (p110) subunits. The active PI3 K phosphorylates PIP2 to become PIP3, which subsequently activates AKT, a serine/threonine kinase with a pleckstrin homology (PH) domain that binds to PIP3 for membrane localization. The mTOR (mammalian target of rapamycin) consists of two complexes (mTORC1 and mTORC2) that are involved in nutrition and growth signaling. The activation of PI3 K by receptor tyrosine kinases (RTKs) or GPCRs triggers AKT phosphorylation, which then affects downstream effectors like GSK-3β, FOXOs, and TSC1/2. mTORC1 promotes protein synthesis via S6 K and 4EBP1, while mTORC2 regulates cytoskeletal dynamics and AKT activation. This system is essential for physiological activities like as insulin signaling and tissue homeostasis; yet, its dysregulation—caused by mutations (e.g., PIK3 CA, PTEN loss) or overactivation—leads to cancer (Glaviano et al. 2023; Asati et al. 2016).

The PI3 K/AKT/mTOR signaling pathways are highly conservative intracellular signal transduction pathways crucial for cell growth, differentiation, apoptosis, angiogenesis, and survival (Kilmister and Tan 2025; Mousavikia et al. 2025; Li et al. 2020; Mortazavi et al. 2022; Li et al. 2022; Tariq and Luikart 2021; Dworakowska et al. 2009). Figure 2 showcases crosstalk between mTOR and other signaling pathways.

Receptor tyrosine kinases

Receptor tyrosine kinases (RTKs) are a subclass of tyrosine kinases that are high-affinity cell surface receptors for several growth factors, cytokines, and hormones and are involved in mediating cell-to-cell communications (Tomuleasa et al. 2024). This receptor has identified three functional domains: an extracellular domain that binds with the ligand, a transmembrane domain spanning the plasma membrane, and an intracellular region containing a tyrosine kinase domain (TKD) and a carboxyl-terminal tail (Trenker and Jura 2020). Upon ligand binding and activation, two RTK molecules form a dimer, activating the intracellular TKD leading to autophosphorylation of these monomers (Tomuleasa et al. 2024). Autophosphorylation of TKDs foster various proteins with Src homology-2 or phosphotyrosine-binding (PTB) domains. These proteins further mediate several critical signaling pathways by interacting with different molecules controlling several cellular responses (Diop et al. 2022; Kim et al. 2023).

Phosphatidylinositol-3-kinase

The phosphatidylinositol 3-kinase (PI3 K) is a plasma membrane–associated heterodimer composed of the regulatory p85 and catalytic p110 subunits, which work together to mediate RTK-dependent signaling. The p85 regulatory subunit stabilizes the p110 catalytic subunit while maintaining basal inhibition, and activation occurs when phosphorylated motifs interact with active RTKs or adaptor proteins. This enzyme phosphorylates PIP2 into PIP3 and introduces AKT and 3-phosphoinositide-dependent kinase 1 (PDK1) into the plasma membrane. This spatial rearrangement enables PDK1 to phosphorylate and activate AKT, initiating downstream signaling cascades that control cell survival, growth, and metabolic processes (Li et al. 2024) (Garcia-Viloca et al. 2022; Kearney et al. 2021; Gozzelino et. al. 2020).

Protein kinase B

Protein kinase B (AKT) family of proteins includes kinases specific to serine/threonine residues of target proteins (Han et al. 2024). Three isoforms of AKT have been identified: AKT1, expressed in most of the tissues; AKT2, expressed mainly in insulin-sensitive tissues; and AKT3, expressed in the brain and testicles (Kumar et al. 2025; Adon et al. 2025). Upon their recruitment to the cell membrane by PIP3, AKT is phosphorylated partially by mammalian target of rapamycin complex 2 (mTORC2) on Ser473 in the carboxy-terminal hydrophobic motif, which imparts conformational changes to AKT (Jhanwar-Uniyal et al. 2019). This further facilitates the phosphorylation at Thr308 by PDK1, thereby accomplishing AKT activation (Zheng et al. 2023) leading to cell adhesion, proliferation, survival, and activation of its downstream targets (Singh et al. 2025; Toson et al. 2022).

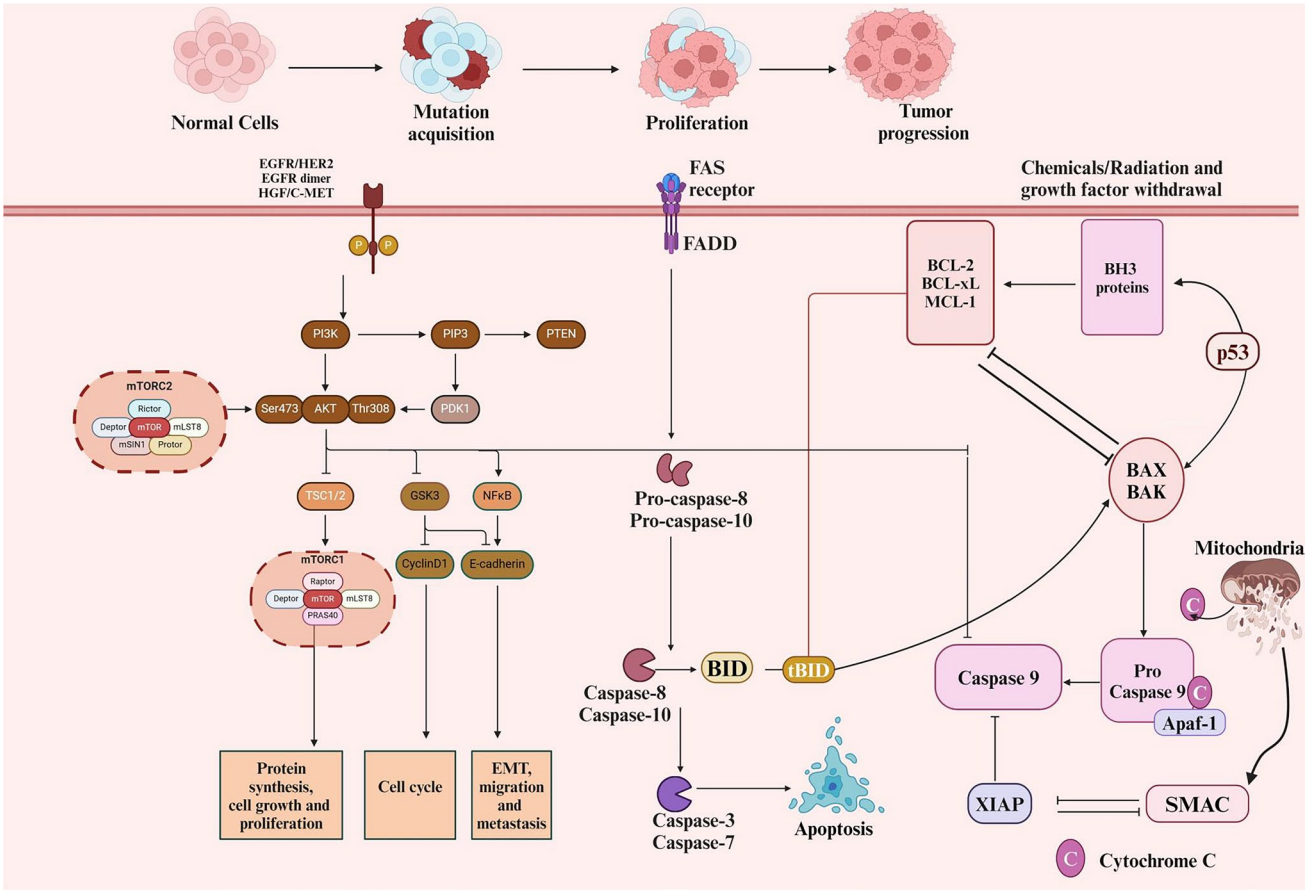


Fig. 2 Interaction of mTOR with other signaling pathways. The phosphoinositide 3-kinase (PI3 K)/mTOR pathways react to external and intracellular signals and extensively interact to modulate one another. Growth factors bind to receptor tyrosine kinases (RTK), activating the PI3 K pathway through the regulation of a phosphorylation cascade. Activated PI3 K phosphorylates PIP2 to produce membrane-associated PIP3, subsequently activating AKT. The activation of mTORC1 and mTORC2 governs cell survival, proliferation, angiogenesis, and other related functions

Mammalian target of rapamycin

Mammalian target of rapamycin (mTOR), also referred to as the mechanistic target of rapamycin, is a large serine/threonine kinase that is constitutively expressed in mammalian cells (Qiang et al. 2025). There are two mTOR complexes, mTORC1 and mTORC2, found in mammalian cells (Qiang et al. 2025). Structurally, mTORC1 contains mTOR, Raptor, and Mammalian Lethal with SEC13 protein 8 (mSLT8) that enhance the kinase activity (Qiang et al. 2025; Hua et al. 2019). Through the downstream effectors, 4EBP1 and P70S6 kinase (S6 K), it initiates the translation of mRNA into proteins promoting cell growth and metabolism. The role of mTORC1 in de novo lipid synthesis is also reported (Wu et al. 2022). mTORC2 complex encompasses six proteins, namely, DEPTOR, SIN1, RICTOR, mLST8, and mTOR, and is majorly involved in phosphorylating and activating AKT, thereby promoting cell proliferation and survival (Unni and Arteaga 2019; Ragupathi et al. 2024). Studies have also shown the role of mTOR in several other cell fate–determining events, such as apoptosis and autophagy (Khayatan 2025; Zeng et al. 2024).

Phosphoinositide-dependent kinase 1

Phosphoinositide-dependent kinase 1 (PDK1), a 63-kDa serine-threonine kinase, functions as a key regulator of the AGC kinase superfamily (PKA, PKG, PKC), predominantly activating AKT via phosphorylation at Thr308. The pleckstrin homology (PH) domain binds PIP2 and PIP3, facilitating membrane localization and substrate activation (Mousavikia et al. 2025; Xiang et al. 2025; Wang et al. 2022; Levina et al. 2022; Sacerdoti et al. 2023). Overexpression of PDK1 accelerates tumor growth in several malignancies, with continuing activation of the PI3 K/PDK1/AKT pathway (Tao et al. 2024; Glaviano et al. 2023; Agrawal et al. 2025; Hao et al. 2025; Watt and Goel 2022; Ippen et al. 2019). In breast cancer (BC), PDK1 is genomically amplified and overexpressed, correlating with advanced tumor stage and poor prognosis. In comparison to benign

lesions, BC demonstrates elevated phosphorylation at Ser-241, indicating PDK1 activation. Functional studies demonstrate PDK1's critical role in BC formation and metastasis by facilitating cell proliferation, survival, and glycolytic reprogramming. In BC, PDK1 is genomically amplified and overexpressed, correlating with advanced tumor stage and poor prognosis 19,602,588 (Wang et al. 2022). BC shows increased phosphorylation at Ser-241, a marker of PDK1 activation, in comparison to benign lesions (Levina et al. 2022; Wang et al. 2022). In addition to oncogenic signaling, PDK1 engages with cyclin-dependent kinase 1 (Cdk1) to modulate stem cell self-renewal and pluripotency during cellular reprogramming. This interaction indicates PDK1's dual function in sustaining malignant and stem-like characteristics. In BCSC-enriched tumors, therapeutic targeting of PDK1 holds promise for overcoming resistance, especially when combined with PI3 K/AKT inhibitors (Wang et al. 2017; Varzideh et al. 2023; Martin 1981).

Phosphatase and tensin homolog

As a negative regulator of the PI3 K/AKT/mTOR signaling pathway, phosphatase and tensin homolog (PTEN) catalyzes the dephosphorylation of PIP3 to PIP2 (Luongo et al. 2019; Maehama and Dixon 1998; Liu et al. 2024). One tumor suppressor gene that is often changed in malignancies is PTEN, and it controls the process by which stem cells regenerate themselves (Abdelaziz et al. 2023; Zhang et al. 2018). Several studies have shown that PTEN has a crucial impact on normal stem cells as well as CSC homeostasis wherein loss of PTEN has been reported to promote the growth and survival of CSCs (Korkaya et al. 2009; Al-Dhfyan et al. 2017). It has been shown that PTEN inhibits epithelial–mesenchymal transition (EMT) and cancer stem cell activity by reducing the expression of Abi1 (Qi et al. 2020). Overexpression of Abi1 in non-tumorigenic mammary epithelial cells leads to the induction of the EMT and an increase in the activity of stem/progenitor cells. However, when Abi1 is depleted in BC, it hinders the process of EMT and reduces the activity of CSCs, which is like the effect of reintroducing PTEN (Qi et al. 2020). PTEN suppression results in the accumulation of both normal and malignant mammary stem cells and induces a substantial elevation in the levels of AKT phosphorylation. This, in turn, leads to the activation of β-catenin through GSK3β-dependent mechanisms, which promotes the progression of malignant transformation. Consistently, the administration of perifosine (an inhibitor of AKT) or Ly294002 (an inhibitor of PI3 K), either alone or in conjunction with chemotherapy, decreases the population of mammary stem cells and inhibits the formation of tumors in BC xenografts (Korkaya et al. 2009; Wylaz et al. 2023).

PI3 K/AKT/mTOR mediated regulation of cancer stem cell markers

Interactions between mTOR and CSC genes are depicted in a gene regulatory network. By utilizing this network, we may better understand the intricate relationship between these genes and draw conclusions about potential treatments and cause disease (Fig. 3). In addition, there has been evidence linking cancer metastasis and chemo-resistance to markers specific to CSCs, including CD44, CD24, ALDH1, and CD133 (Sheridan et al. 2006; Fraszczak and Barczynski 2023; Izycka et al. 2023). Moreover, circulating BCSC markers, EpCAM, CD44, CD24, ALDH1, CD133, and PIWIL2 have also been identified as critical determinants of prognosis, diagnosis, and prediction of response (Table 1) (Mansoori et al. 2017; Kong et al. 2018; Kehoe et al. 2015). Patients with BC who exhibit these CSC markers at high levels have a very low chance of overall survival (Figs. 4–5). CD44 is the most common tumor biomarker used in BC stratification. It cooperates with RTK and regulates BCSC proliferation, adhesion, and migration (Yousefnia et al. 2020; Liu et al. 2014).

Several signaling pathways including PI3 K/AKT pathways are known to be stimulated by CD44 (Herishanu et al. 2011; Vadhan et al. 2022; Ahmad et al. 2023). Bai et al. showed that the downregulation of hypoxia-inducible factor 2 alpha (HIF-2α) expression inhibits the stemness of BC cells and promotes apoptosis mediated by CD44/PI3 K/AKT/mTOR signaling pathway (Bai et al. 2020). Studies

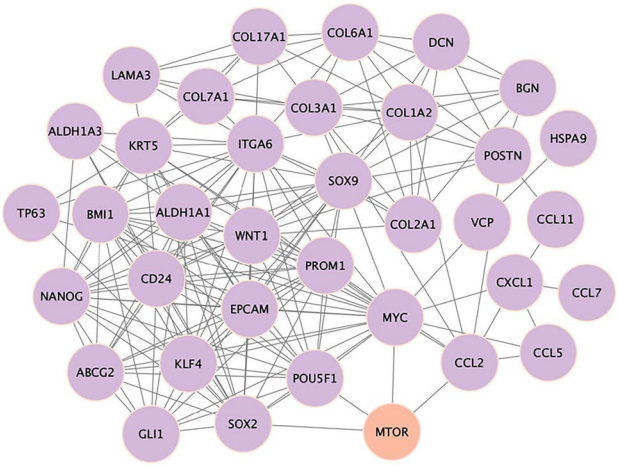


Fig. 3 A gene regulatory network, showing interactions between MTOR with CSC genes. Network analysis of high confidence dysregulated CSC genes analyzed using STRING and visualized using Cytoscape. The network shows direct and indirect connections of CSC genes with *MTOR*. Each node represents a gene, and the lines between them indicate regulatory relationships or interactions. This network aids in illustrating the complex interplay between these genes and can be utilized to explore implications in disease mechanisms or therapeutic strategies

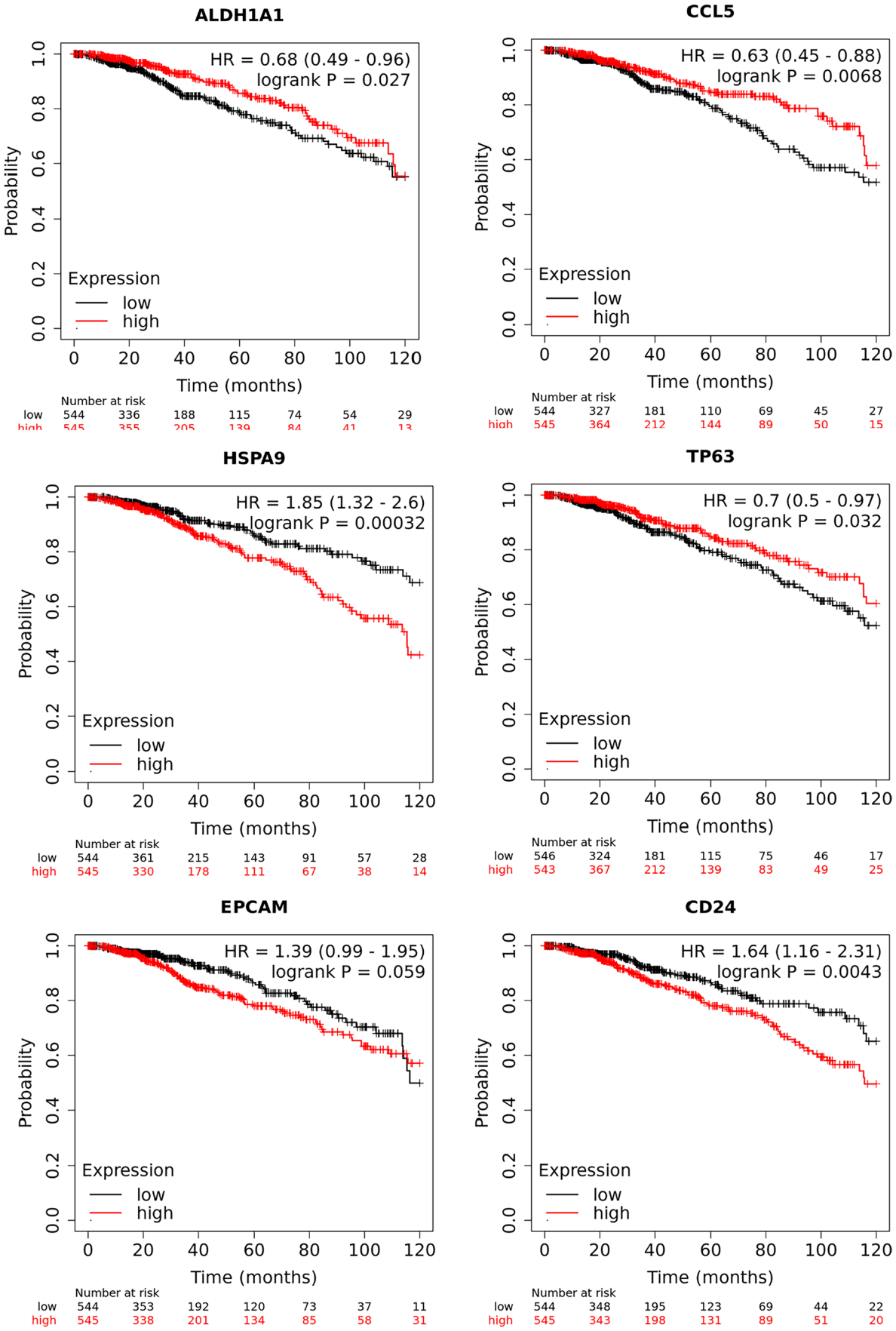
Table 1 Major stem cell marker and function in breast cancer

Markers	Functions	Reference
CD44 and CD24	Supports tumor growth, epithelial–mesenchymal transition (EMT), and drug resistance	(Li et al. 2017; Xu et al. 2016)
Aldehyde dehydrogenase 1	Promotes angiogenesis Drives epithelial–mesenchymal transition (EMT) via TWIST1 and MUC1-C pathways Modulates the tumor microenvironment and tumor vascularization	(Althobiti et al. 2020; Ciccone et al. 2018; Wei et al. 2022; Raghunathan et al. 1988)
ABCG2	Confers multidrug resistance, enhances sphere-forming ability	(Nakanishi and Ross 2012; Sicchieri et al. 2015)
CD133	Plays a major role in tumor progression and metastasis, and confers treatment resistance	(Li et al. 2017; Brugnoli et al. 2019)
CD49f	Stemness maintenance, tumor initiation and progression Metastasis promotion	(Barbieri et al. 2017; Lee et al. 2019; Xia and Xu 2015; Mohammed et al. 2013)
LGR5	Has the ability to self-renew spheres and tumorigenicity, activate Wnt/β-catenin signaling, and increase the stemness of breast cancer cells	(Lee et al. 2021; Montazer et al. 2023; Yang et al. 2015)
CD70	Metastasis promotion, enhanced tumorigenicity, and marker for aggressive phenotype	(Kitajima et al. 2018; Liu et al. 2018)
EpCAM (CD326)	Facilitates proliferation, differentiation, and cell signaling; linked to tumor progression	(Lehr 1996)
Sox2	Regulates pluripotency and is necessary for mammosphere formation	(Johnson et al. 2019; Leis et al. 2012; Shim 2014)
Oct4	Maintains stemness and is associated with tamoxifen resistance	(Gwak et al. 2017)
Nanog	Enhances self-renewal and correlates with tumor size, grade, stage, and poor survival	(Emadian Saravi et al. 2019; Shan et al. 2012; Wang et al. 2014)
ZEB1	Drives epithelial-to-mesenchymal transition (EMT), increases $CD44^{+}/CD24^{-}$ population	(Feldker et al. 2020; Murray et al. 2016; Shivhare et al. 2023)

have shown that inhibition of CD44 alters BCSCs' properties including tumor initiation, adhesion, metastasis, and treatment resistance (Al-Othman et al. 2020; Yang et al. 2020). PI3 K/AKT/mTOR signaling pathway influences CSCs' properties and EMT phenotype associated with aggressive BC in vitro and in vivo. The first cohort study indicated that tumor samples with *PIK3 CA* mutations were associated with high PI3 K/AKT/mTOR signaling and stemness scores (Madsen et al. 2021). Because of their limited expression, the small tissue samples available, and the fact that BCSCs are not always easy to identify, indicators mediated by the PI3 K/AKT/mTOR pathway continue to provide a challenge to their identification.

PI3 K/AKT/mTOR mediated regulation of epithelial-to-mesenchymal transition markers

During EMT, epithelial cells change from an epithelial phenotype to a fibroblastic phenotype, leading the cancer cells to lose epithelial markers (E-cadherin, α-catenin, and γ-catenin) and to gain mesenchymal markers (N-cadherin fibronectin, and vimentin) (Zhang et al. 2025; Kanwal et al. 2025; Blaszczak et al. 2025). EMT regulates metastasis, invasion, chemoresistance, and immunosuppression in BCSCs (Blaszczak et al. 2025; Yuan et al. 2024). BCSCs are characterized by a more dormant/quiescent mesenchymal-like state and $CD44^{+}CD24^{-}$/low expression, which are placed at the tumor edge and form micro-metastases at distant sites. The other type of BCSCs is characterized by a proliferative/epithelial-like state and ALDH activity which is situated at the tumor center and restored epithelial-like state (Bushnell et al. 2021). BCSCs can induce EMT in human mammary epithelial cells (Mani et al. 2008; Liaghat et al. 2024). Moreover, Hennessy et al. indicated that basal B/claudin-low BC cells expressed both EMT and stem cell surface markers (Hennessy et al. 2009). Another study showed that overexpression of serine/threonine kinase (PIM1) induced EMT and BCSC properties; however, knockdown of PIM1 downregulated the expression of EMT-related transcription factors (Snail, TWIST) and mesenchymal marker N-cadherin (Gao et al. 2019), thereby clearly indicating the importance of EMT and BCSCs in synergistically promoting BC metastasis. EMT markers are associated with reduced immune recognition and increased expression of immune


ALDH1A1
HR = 0.68 (0.49 - 0.96)
logrank P = 0.027
Probability
Expression
low
high
Time (months)
Number at risk
low 544 336 188 115 74 54 29
high 545 355 205 139 84 41 13
CCL5
HR = 0.63 (0.45 - 0.88)
logrank P = 0.0068
Probability
Expression
low
high
Time (months)
Number at risk
low 544 327 181 110 69 45 27
high 545 364 212 144 89 50 15
HSPA9
HR = 1.85 (1.32 - 2.6)
logrank P = 0.00032
Probability
Expression
low
high
Time (months)
Number at risk
low 544 361 215 143 91 57 28
high 545 330 178 111 67 38 14
TP63
HR = 0.7 (0.5 - 0.97)
logrank P = 0.032
Probability
Expression
low
high
Time (months)
Number at risk
low 546 324 181 115 75 46 17
high 543 367 212 139 83 49 25
EPCAM
HR = 1.39 (0.99 - 1.95)
logrank P = 0.059
Probability
Expression
low
high
Time (months)
Number at risk
low 544 353 192 120 73 37 11
high 545 338 201 134 85 58 31
CD24
HR = 1.64 (1.16 - 2.31)
logrank P = 0.0043
Probability
Expression
low
high
Time (months)
Number at risk
low 544 348 195 123 69 44 22
high 545 343 198 131 89 51 20

◂**Fig. 4** Overall survival Kaplan–Meier curves of statistically significant ($P < 0.05$) and borderline significance ALDH1 A1, CCL5, HSPA9, TP63, EPCAM, and CD24 CSC genes analyzed using KMplot (Lanczky and Gyorffy 2021). This data is based on RNA-seq data of breast cancer patients from the TCGA cohort. Patients are categorized into high and low expression based on the median expression scores, and a *P*-value of less than 0.05 is considered as statistically significant

checkpoint proteins, while CSCs exhibit resistance to immune-mediated destruction. CSCs evade immune detection through PI3 K/AKT/mTOR pathway activation, which downregulates MHC molecules, promotes immunosuppressive cytokines (e.g., TGF-β, IL-10), and upregulates immune checkpoints like PD-L1 (Pan et al. 2025; Wu et al. 2023). Targeting this pathway may disrupt CSC-mediated immune escape, suggesting potential synergy with immunotherapy (Ibrahim et al. 2025; Hashemi et al. 2024).

The PI3 K/AKT/mTOR (PAM) pathway has been implicated to play a pivotal role in BC progression via governing the maintenance of BCSCs and their EMT properties (Chang et al. 2013; Hong et al. 2024). A previous study on HER2-positive BC reported that HER2 is involved in inducing the expression of stem cell–related genes (OCT3/4, NOTCH1, NOTCH2, JAG1), thus leading to activation of the PI3 K/AKT pathway (Korkaya et al. 2008; DiNatale et al. 2022). Therefore, the dysregulation of HER2 leads to improved AKT phosphorylation in the ALDH-positive population of BCSCs (Alanazi and Khan 2016). PI3 K/AKT pathway activates the runt-related transcription factor 2, leading to carcinogenicity, progression, and EMT in BCSCs (Fritz et al. 2020). However, understanding the exact mechanisms of PI3 K/AKT/mTOR-mediated EMT signaling in BCSCs is a challenge.

Therapeutic approaches for targeting PI3 K/AKT/mTOR pathway in BCSCs

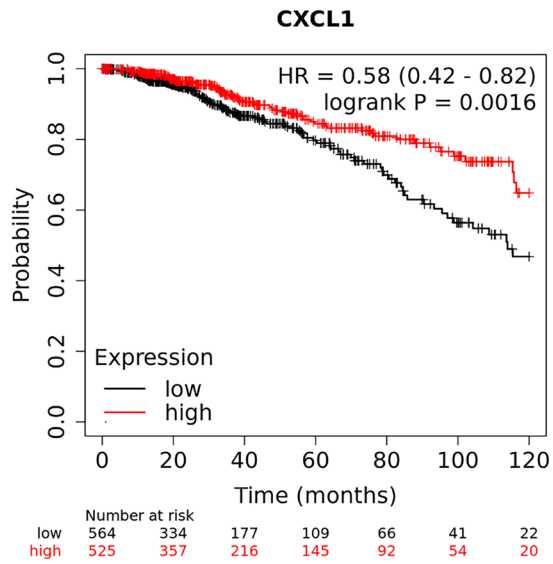
Deregulation of the PI3 K/AKT/mTOR molecular pathway is associated with the progression of various cancers (Hussain et al. 2015; Ahmed et al. 2014; Du FY et al. 2019); thus, agents such as inhibitors/antagonists/agonists were structurally designed and formulated to target the above cascade in multiple cancers and CSCs, including BC. Although a small percentage of small molecule inhibitors targeting PI3 K/AKT/mTOR are under investigation, a handful has been used in clinics. Here in the segment below, we have summarized the molecules that have the potential to control the growth of CSCs and may find a special place in translational research.

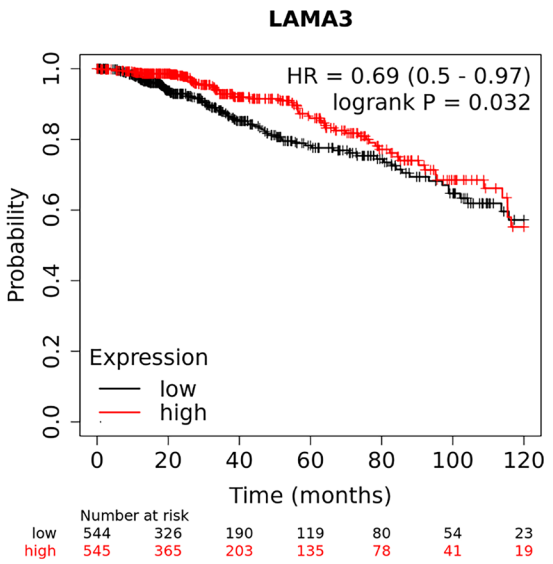
The pan-PI3 K inhibitor dihydro benzofuran-imidazolium salt, B591, was reported to have a strong inhibitory effect on class I PI3 K isoforms by preventing the activation of PI3 K/AKT/mTOR signaling pathways in MDA-MB-231 and SUM-159PT cell lines. In addition, this inhibitor proved to be more effective at reducing CSCs survival and eradicating CSCs than bulk tumor cell populations and significantly eradicated CSCs when combined with paclitaxel. In an in vivo mouse xenograft model of human BC, B591 dramatically decreased tumor-initiating capacity, demonstrating that it primarily lowered CSC levels (Zhou et al. 2019) 30,635,656.

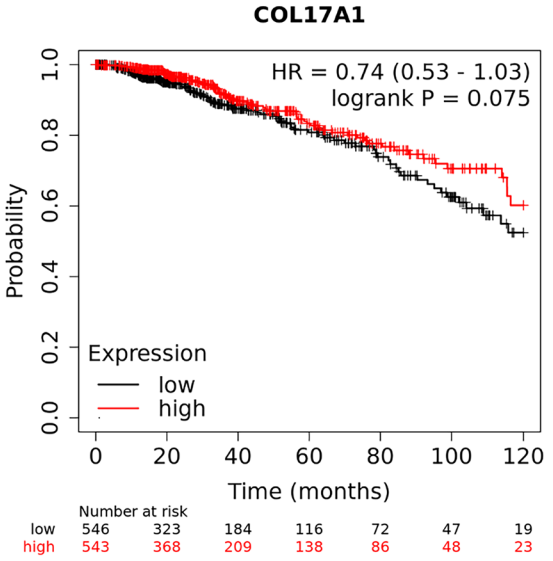
Yu et al. (Yu et al. 2016) found that when NVP-BKM120, a PI3 K inhibitor, was combined with trastuzumab or RAD001 (everolimus), the potential to target BCSCs increased. The results were subsequently corroborated using xenograft mouse models, which showed that combining BKM120 with trastuzumab or RAD001 reduced the mammosphere-forming efficiency (MFE) (Yu et al. 2016; Maira et al. 2012). Consistent with the findings above, another study revealed that NVP-BKM120 (BKM120) significantly reduced PI3 K, AKT1, and S6 expression, inhibiting BCSCs-CYP19 growth, migration, and colony formation (Hu et al. 2015). In conjunction with letrozole, PI3 K, AKT1, and S6 expression levels were reduced, impacting the development and proliferation of BCSCs-CYP19 (Liu et al. 2019; Chae and Kim 2021; Hakeem et al. 2024).

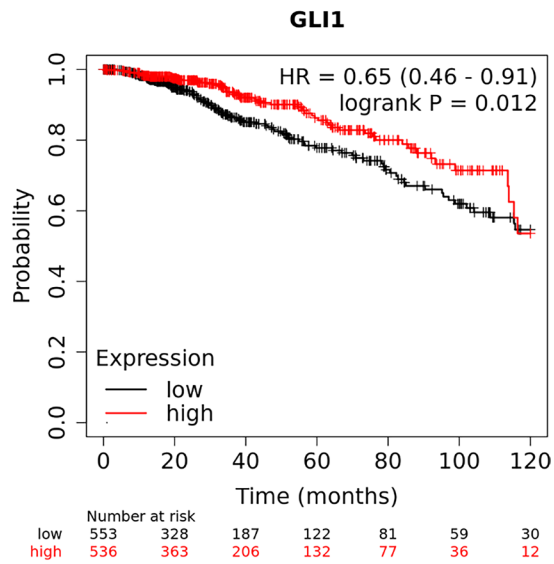
AKT/mTOR and p38 MAPK signaling pathways are both modulated by the steroid saponin dioscin, which inhibits the development of BC cells (Chae and Kim 2021). It specifically targets BCSCs in MDA-MB-231 and MCF-7 models, resulting in G2/M and G0/G1 cell cycle arrest, respectively (Chae and Kim 2021). By blocking the PI3 K/Akt/mTOR axis and reducing cancer stem cell indicators such as ALDH, piperine and doxorubicin in combination helps to overcome chemoresistance in triple-negative breast cancer (TNBC) (Hakeem et al. 2024). Concurrently, wortmannin—a broad-spectrum inhibitor of PI3 K/Akt/mTOR—enhances the antiproliferative effects of mesenchymal stem cell–conditioned medium (MSC-CM) in BC cells. This combination inhibits the PI3 K/AKT/mTOR signaling pathways, inducing apoptosis and autophagy-mediated cell death (Ismail et al. 2025).

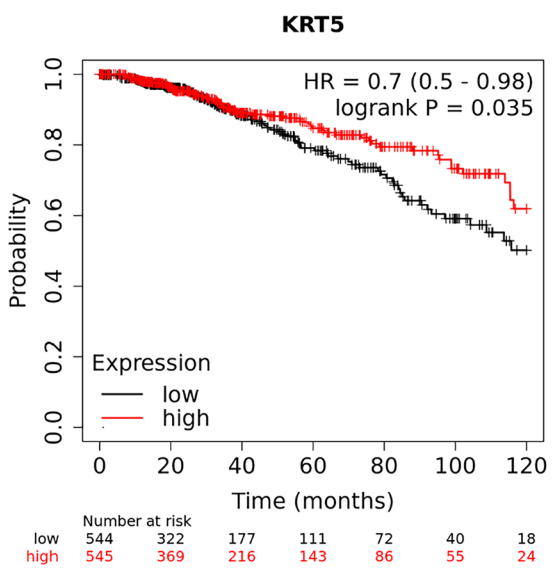
Anindita Chakrabarty and colleagues (Chakrabarty et al. 2013) investigated the effects of trastuzumab alone and in combination with the pan-PI3 K inhibitor XL147. The combination group lowered proliferation and pAKT levels, resulting in the death of trastuzumab-resistant HR5 and HR6 cell lines. The combination outperformed XL147 in anticancer activity in a trastuzumab-resistant tumor xenograft model. Trastuzumab, in combination with XL147, also decreased CSCs in trastuzumab-resistant tumors (Chakrabarty et al. 2013). Treating trastuzumab-resistant cell lines (MDA-MB-453 and JIMT-1) with the PI3 K inhibitor LY-294002 reduced PI3 K/AKT signaling in a HER2-dependent manner, resulting in a significant reduction in the number of ALDH + BCSCs (Korkaya et al. 2008). Similar


CXCL1
HR = 0.58 (0.42 - 0.82)
logrank P = 0.0016
Probability
Expression
low
high
Time (months)
Number at risk
low 564 334 177 109 66 41 22
high 525 357 216 145 92 54 20


LAMA3
HR = 0.69 (0.5 - 0.97)
logrank P = 0.032
Probability
Expression
low
high
Time (months)
Number at risk
low 544 326 190 119 80 54 23
high 545 365 203 135 78 41 19


COL17A1
HR = 0.74 (0.53 - 1.03)
logrank P = 0.075
Probability
Expression
low
high
Time (months)
Number at risk
low 546 323 184 116 72 47 19
high 543 368 209 138 86 48 23


GLI1
HR = 0.65 (0.46 - 0.91)
logrank P = 0.012
Probability
Expression
low
high
Time (months)
Number at risk
low 553 328 187 122 81 59 30
high 536 363 206 132 77 36 12


KRT5
HR = 0.7 (0.5 - 0.98)
logrank P = 0.035
Probability
Expression
low
high
Time (months)
Number at risk
low 544 322 177 111 72 40 18
high 545 369 216 143 86 55 24

◂**Fig. 5** Overall survival Kaplan–Meier curves of statistically significant ($P < 0.05$) and borderline significance CXCL1, LAMA3 CoL17 A1, GLI1, and KRT5 CSC genes analyzed using KMplot (Lanczky and Gyorffy 2021). This data is based on RNA-seq data of breast cancer patients from the TCGA cohort. Patients are categorized into high and low expression based on the median expression scores, and a *P*-value of less than 0.05 is considered as statistically significant

results were observed with LY-294002 against trastuzumab-resistant cell lines (Korkaya et al. 2008).

Quercetin, a naturally occurring chemical, reduced clone formation and mammosphere development in $CD44^{+}$/CD24 CSCs. Furthermore, quercetin reduced BCL2 and cyclin D1 expression levels via blocking the PI3/AKT/mTOR pathway (Li et al. 2018). A study by Yi Wang et al. (Wang et al. 2022) discovered that the combination of carboplatin (CBP) and thioridazine (THZ), an antipsychotic medication, inhibited CSC proliferation by inhibiting the PI3 K/AKT/mTOR pathway. Another study found that perifosine, an AKT inhibitor, effectively altered the stemness phenotype in tamoxifen-resistant BC cells (Farahmand et al. 2018). While there are a few targeted therapeutic compounds that show promise, these candidates are still in the early stages and require further investigation through in vitro experiments to comprehend their full potential.

Aberrant activation of PI3/AKT/mTOR causes activation or suppression of various protein molecules in numerous

Table 2 Therapeutic agents in clinical trials that target PI3 K/AKT/mTOR pathway

Therapeutic agent	Target	Mechanism of action	Status/clinical trials	Clinical trial number
Buparlisib (BKM120)	PI3 K (pan-PI3 K inhibitor)	Inhibits all class I PI3 K isoforms, reducing BCSC survival and self-renewal	Phase II/III trials in breast cancer	NCT01610284, NCT01633060, NCT01790932
Alpelisib (BYL719	PI3 Kα (p110α isoform)	Selective inhibition of PI3 Kα, suppressing BCSC proliferation and tumor growth	FDA approved for PIK3 CA-mutated breast cancer	NCT02437318
MK-2206	AKT	Allosteric AKT inhibitor, reduces BCSC stemness and enhances chemotherapy sensitivity	Phase II trials in breast cancer	NCT01277757
Ipatasertib (GDC-0068)	AKT	ATP-competitive AKT inhibitor, targets BCSC survival and tumor progression	Phase II/III trials in breast cancer	NCT02301988, NCT04464174
Everolimus (RAD001)	mTORC1	mTORC1 inhibitor, reduces BCSC self-renewal and tumorigenicity	FDA approved for hormone receptor–positive breast cancer	NCT00863655
Temsirolimus (CCI-779)	mTORC1	mTORC1 inhibitor, suppresses BCSC proliferation and survival	FDA approved for advanced breast cancer	NCT00062751
Dactolisib (BEZ235)	PI3 K/mTOR (dual inhibitor)	Dual inhibition of PI3 K and mTOR, targeting BCSC stemness and tumor growth	Phase I/II trials in breast cancer	NCT01288092, NCT01508104
Gedatolisib (PF-05212384)	PI3 K/mTOR (dual inhibitor	Dual inhibition of PI3 K and mTOR, targeting BCSC stemness and tumor growth	Phase I/II trials in breast cancer	NCT02626507, NCT01920061
Capivasertib (AZD5363)	PI3 K/mTOR (dual inhibitor)	Dual inhibitor of PI3 K and mTOR, reduces BCSC survival and tumor progression	Phase II trials in breast cancer	NCT01992952, NCT01625286, NCT03375880
Capivasertib (AZD5363)	AKT	Selective AKT inhibitor, targets BCSC stemness and enhances therapy response	Phase III trials in breast cancer	NCT04862663
LY294002	PI3 K	Broad PI3 K inhibitor, reduces BCSC self-renewal and tumorigenicity	Preclinical studies	Not applicable

Table 3 Combinatorial approaches under clinical trial for treatment of breast cancer

Clinical trials	Status	Combination therapy components
NCT02246621	Active, not recruiting—phase III	Abemaciclib + fulvestrant/anastrozole/letrozole
NCT03155997	Active, not recruiting—phase III	Abemaciclib + tamoxifen/anastrozole/letrozole
NCT01231659	Completed—phase II	Everolimus + letrozole
NCT01082068	Completed—phase II	Pilaralisib/voxtalisib + letrozole
NCT01491737	Completed	Anastrozole/letrozole + pertuzumab/trastuzumab
NCT02734004	Active, not recruiting—phase II	Olaparib + durvalumab
NCT03036488	Active, not recruiting, phase III	Pembrolizumab + nab-paclitaxel/paclitaxel/gemcitabine + carboplatin
NCT02425891	Completed—phase III	Atezolizumab + nab-paclitaxel
NCT02614794	Completed—phase II	Trastuzumab + capecitabine + tucatinib
NCT01992952	Unknown status	AZD5363 + fulvestrant
NCT01942135	Completed—phase III	Palbociclib + fulvestrant
NCT02422615	Completed—phase III	Ribociclib + fulvestrant
NCT02456857	Completed—phase II	Doxorubicin/bevacizumab/everolimus
NCT01281696	Completed—phase II	Bevacizumab + etoposide/cisplatin
NCT02000622	Active, not recruiting, phase III	Olaparib + capecitabine/vinorelbine/eribulin
NCT01698918	Completed—phase II	Everolimus + letrozole
NCT02657889	Completed—phase II	Niraparib + pembrolizumab
NCT03036488	Active, not recruiting, phase III	Pembrolizumab + chemotherapy
NCT03125902	Active, not recruiting, phase III	Atezolizumab + chemotherapy
NCT01942135	Completed, phase III	Palbociclib + fulvestrant
NCT01958021	Completed, phase III	Letrozole + ribociclib
NCT02657889	Completed, phase II	Niraparib + pembrolizumab
NCT01584648	Completed, phase III	Dabrafenib + trametinib
NCT02993523	Active, not recruiting, phase III	Venetoclax + azacitidine
NCT03840200	Completed, phase II	Ipatasertib + rucaparib
NCT0430549	Active, not recruiting, phase III	Capivasertib + fulvestrant

cancers, representing it as attractive therapeutic targets in multiple malignancies. Despite available targeted therapies and clinical experience, the risk for therapeutic resistance, side effects, and associated toxicities cannot be neglected, thereby limiting its clinical application. In addition, many cross-talks occurring between the PI3 K/AKT/mTOR signaling pathway and other pathways must be considered while designing effective therapeutic molecules. The drugs used in combination therapy for targeting multiple ways in solid malignancies need to have thorough follow-up and observation. Therefore, further in-depth studies are warranted to identify precise and safe therapeutic targeted agent/s to ascertain clinical benefits further.

Preclinical validation of an anticancer candidate takes place before the enrollment of participants in clinical trials to assess safety and efficacy in human subjects. Unfortunately, positive outcomes in preclinical studies may not necessarily indicate a positive outcome in clinical trials. It is common practice in preclinical in vitro investigations to subject the candidate of interest to continuous high-concentration exposure. Furthermore, compared to preclinical studies, clinical trials are somewhat costly. Immunotherapy has been used to treat different types of cancer, but not as often as surgery or more standard treatments like chemotherapy or radiation therapy. Although immunotherapy may yield positive outcomes, not every patient exhibits a response to treatment. Additionally, certain patients receiving a combination of immunotherapeutic agents may manifest immune-related disorders (Wang and Minden 2022). Table 2 displays a list of the therapeutic candidates and Table 3 summarizes combinatorial approaches that have been used in clinical trials.

Conclusion

Breast cancer stem cells (BCSCs) constitute a vital subset of tumor-initiating cells that accelerate tumor development, metastasis, resistance, and recurrence. These cells exhibit strong self-renewal and differentiation abilities, facilitated by dysregulated signaling pathways, notably the

PI3 K/AKT/mTOR axis, which promotes their survival, proliferation, and EMT.

Targeting the PI3 K/AKT/mTOR pathway has become a viable approach to eradicate BCSCs; nonetheless, difficulties like intrinsic and acquired resistance remain. Integrating PI3 K/AKT/mTOR inhibitors with drugs that target alternative cancer stem cell–related pathways, epithelial–mesenchymal transition regulators, or the tumor microenvironment may enhance treatment effectiveness. Clinical trials are now being conducted on a number of PI3 K/AKT/mTOR inhibitors for breast cancer, both alone and in combination. In addition to creating next-generation inhibitors with enhanced selectivity and less toxicity, future research should concentrate on finding predictive biomarkers to stratify individuals who are most likely to benefit from these treatments. Targeting PI3 K/AKT/mTOR signaling in breast cancer stem cells presents considerable potential to enhance clinical outcomes and long-term survival in breast cancer patients.

Author contribution KPS – Wrote original draft of the manuscript, prepared illustrations and tables; SK- helped in preparing of illustrations, revieing & editing the manuscript; ZM,UH,AJP- helped in preparing of illustrations, reviewing & editing the manuscript; TM—TCGA data mining, helped in writing the manuscript, and prepared illustrations, KJ -, wrote, reviewed, and edited the manuscript; SU- conceptualized, supervised, wrote, edited the manuscript. The authors confirm thatno paper mill and artificial intelligence was used.

Funding Open Access funding provided by the Qatar National Library. This work was supported by Medical Research Centre, HMC, Doha, Qatar (IRGC-05-SI-18–307).The authors acknowledge Qatar National Library for the open access fund support.

Data availability All source data for this work (or generated in this study) are available upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Name of principal scientist Shahab Uddin.

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