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Review paper

Probing the biological efficacy and mechanistic pathways of natural compounds in breast cancer therapy via the Hedgehog signaling pathway

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ABSTRACT

Breast cancer (BC) is one of the most prevalent malignant tumors affecting women worldwide, with its incidence rate continuously increasing. As a result, treatment strategies for this disease have received considerable attention. Research has highlighted the crucial role of the Hedgehog (Hh) signaling pathway in the initiation and progression of BC, particularly in promoting tumor growth and metastasis. Therefore, molecular targets within this pathway represent promising opportunities for the development of novel BC therapies. This study aims to elucidate the therapeutic mechanisms by which natural compounds modulate the Hh signaling pathway in BC. By conducting a comprehensive review of various natural compounds, including polyphenols, terpenes, and alkaloids, we reveal both common and unique regulatory mechanisms that influence this pathway. This investigation represents the first comprehensive analysis of five distinct mechanisms through which natural compounds modulate key molecules within the Hh pathway and their impact on the aggressive behaviors of BC. Furthermore, by exploring the structure-activity relationships between these compounds and their molecular targets, we shed light on the specific structural features that enable natural compounds to interact with various components of the Hh pathway. These novel insights contribute to advancing the development and clinical application of natural compound-based therapeutics. Our thorough review not only lays the groundwork for exploring innovative BC treatments but also opens new avenues for leveraging natural compounds in cancer therapy.

1. Introduction

Breast cancer (BC) is characterized by the uncontrolled proliferation of abnormal breast cells, leading to tumor formation. It remains the leading cause of cancer-related mortality among women. Epidemiological studies have shown that the overactivation of the Hedgehog (Hh) signaling pathway is significantly associated with the high incidence and progression of BC [1]. Moreover, research has demonstrated a positive correlation between the aberrant expression of Hh pathway genes and increased tumor size, lymph node metastasis, and recurrence risk, underscoring the pivotal role of this pathway in BC pathogenesis [2]. In recent years, natural compounds have gained considerable attention as potential modulators of the Hh signaling pathway. Certain natural compounds, such as flavonoids, polyphenols, and plant extracts, have been found to inhibit the Hh pathway through diverse mechanisms, highlighting their potential therapeutic value

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in BC treatment [3–11]. These compounds inhibit the activation of the Hh signaling pathway through various mechanisms, potentially exerting a positive impact on the treatment of BC. Therefore, this study aimed to explore natural compounds as potential inhibitors of the Hh signaling pathway and provide a detailed analysis of their potential for BC therapy.

The Hh signaling pathway plays a critical role in BC, affecting tumor behavior, angiogenesis, and the fate of cancer stem cells (CSCs) [5,12,13]. Specifically, in breast cancer stem cells (BCSCs), overexpression of components such as the transmembrane receptor patched 1 (PTCH1) and the transcription factors GLI family zinc finger 1 (GLI1) and GLI family zinc finger 2 (GLI2) enhances their self-renewal capacity [14]. Although the exact function of GLI family zinc finger 3 (GLI3) in BCSCs remains unclear, as a key transcription factor in the Hh signaling pathway, its potential role in BCSCs warrants further investigation. Components of the Hh pathway are expressed during various stages of mammary gland development in mice but generally remain inactive [15]. However, when aberrant Hh signaling is activated, it contributes to the initiation and maintenance of BC, leading to upregulated pathway activity. Many BC tissues show dysregulated Hh signaling [16]. Tao et al. [17] demonstrated that aberrant Hh signaling is associated with ductal changes and malignant transformation in breast tissue, and inhibiting this pathway can improve the prognosis of BC patients. In BC, Hh signaling activation occurs through both ligand-dependent and ligand-independent mechanisms. In the ligand-dependent pathway, theHh ligand binds to PTCH1, relieving its inhibition of the transmembrane receptor smoothened (SMO), thereby activating SMO and triggering signal transduction that ultimately regulates BC-related genes [18]. A critical step in the Hh signaling pathway is the transduction of signals from the membrane receptor SMO to the transcription factor GLI. This process is mediated by multiple regulatory molecules and involves intricate cascading reactions, including various protein kinases and regulatory factors, which ultimately govern the expression of downstream target genes. Elucidating the detailed mechanisms and key molecular players in this pathway is essential for understanding the role of Hh signaling in the development and progression of BC [19]. The ligand-independent pathway can be classified into three subtypes: type I, where PTCH1 inhibits SMO and promotes cell apoptosis; type II, in which SMO's independent activity drives cell migration without involving GLI1; and type III, which involves post-translational modifications of *GLI1*, crucial for transcription and intracellular transport in BC [20,21]. Thus, the development of novel inhibitors targeting the Hh pathway presents a promising therapeutic strategy for BC treatment.

Although the Hh signaling pathway holds significant potential for cancer therapy, challenges such as drug resistance and adverse side effects persist, underscoring the need for novel therapeutic strategies. Compared to conventional chemotherapy, natural compounds are associated with lower toxicity and improved tolerability [22]. Aberrant activation of the Hh signaling pathway in BC is closely associated with various functional phenotypes of cancer cells, including proliferation, invasion, epithelial-mesenchymal transition (EMT), cancer stemness, angiogenesis, and drug resistance. Some natural compounds modulate the Hh signaling pathway, affecting these functions of BC cells, which may inhibit tumor growth, metastasis, and drug resistance, and regulate the tumor microenvironment, providing new strategies for BC treatment [5,23–27]. Our comparative analysis revealed that natural compounds can inhibit BCSCs through the Hh signaling pathway, predominantly by modulating the SMO-GLI1 axis to reduce the proportion of BCSCs. Numerous natural compounds have been found to inhibit the Hh signaling pathway via various mechanisms. Based on an analysis of these modes of action, this study identifies three major inhibitory mechanisms: (1) direct suppression of key protein expression; (2) modulation of protein activity and localization; and (3) regulation of downstream effects. Additionally, the specific chemical structures of natural compounds, such as polyphenols, terpenes, and alkaloids, enhance their ability to inhibit the Hh signaling pathway. For instance, these structural features, including ring structures, benzene rings, and heterocyclic rings, can interact with critical molecules in the Hh pathway, such as SMO and GLI, thereby blocking signal transduction. Research on the effects of natural compounds on the Hh signaling pathway contributes to a deeper understanding of the molecular mechanisms involved, leading to the discovery of new therapeutic targets and the development of more precise and effective drugs for BC treatment. By understanding how these compounds modulate the Hh signaling pathway, we can explore new and more effective treatment approaches, overcoming current treatment challenges, and providing innovative directions for the discovery of novel drugs.

2. The composition and transduction of the Hh signaling pathway

2.1. The components of the Hh signaling pathway

The Hh signaling pathway is primarily composed of Hh ligands, two transmembrane receptors, PTCH1 and SMO, the GLI family of transcription factors (*GLI1*, *GLI2*, *GLI3*), and various down stream target genes. There are three homologs of the Hh ligand, Sonic Hedgehog (SHH), Indian Hedgehog (IHH), and Desert Hedgehog (DHH), which are structurally and functionally similar. While these homologs share a high degree of functional similarity, they exert effects on different organs [18]. PTCH1 serves as the primary receptor for SHH and plays a crucial inhibitory role in the Hh signaling pathway. As a tumor suppressor gene, PTCH1 regulates cellular proliferation by suppressing the activity of the SMO protein. Upon SHH binding to PTCH1, this inhibitory effect is relieved, allowing for the activation of downstream GLI-mediated signal transduction [28]. In vertebrates, the Patched gene family includes two homologs: *PTCH1* and *PTCH2*. While PTCH1 primarily functions as the membrane receptor that negatively regulates the Hh signaling pathway, PTCH2 complements PTCH1's role under certain conditions [29]. SMO, a G protein-coupled receptor (GPCR) encoded by the proto-oncogene *SMO*, mediates the binding of extracellular ligands and initiates intracellular signaling. The GLI family, consisting of GLI1, GLI2, and GLI3, acts as the key transcriptional regulators of the Hh pathway, promoting the transcription of target genes in effector cells [19]. Although all GLI proteins share biochemical domains, GLI1 lacks the N-terminal repressor domain found in GLI2 and GLI3, which limits its role to transcriptional activation, while GLI2 and GLI3 can function as both activators and repressors [30]. These signaling molecules are involved in crucial biological processes from embryonic development to adult homeostasis. Consequently, aberrant expression of these genes and their downstream targets is closely linked to the development of various diseases across different stages of life [31].

2.2. The transduction of the Hh signaling pathway

2.2.1. Transduction of the Hh signaling pathway during normal breast development

The expression of Hh signaling pathway components varies across different developmental stages of breast tissue. During breast morphogenesis, canonical Hh signaling is predominantly inhibited. Active repression of GLI1 by the GLI family zinc finger 3 repressor (GLI3R) is crucial for the formation of early mammary buds in mice. This has been evidenced by the loss of mammary

buds in mice with forced GLI1 expression in mammary tissues deficient in GLI3 [32]. The type I atypical Hh signaling pathway promotes a proliferative cascade in the luminal epithelial cells of mammary ducts, contributing to terminal bud elongation during puberty. After puberty, the expression levels of Hh ligands, GLI1, GLI2, GLI3, and PTCH1 decrease in mature mammary glands [33]. Thus, canonical Hh signaling is mainly suppressed during mammary gland embryogenesis, while type I atypical Hh signaling is involved in ductal morphogenesis during puberty and is subsequently downregulated in normal adult breast tissue [32].

2.2.2. Classic and non-classic Hh signal transduction in BC

In the canonical Hh signaling pathway, when the HH ligand is abundant, it binds to PTCH1, relieving the suppression of SMO. This allows SMO to translocate to the primary cilium, where it undergoes phosphorylation, leading to a conformational change that activates GLI transcription factors [34]. Activated GLI proteins then translocate to the nucleus, where they function as transcription factors, regulating the expression of Hh target genes. These target genes include cyclin D(*CCND*), cyclin E (*CCNE*), *GLI1*, *PTCH1*, and *PTCH2*, which collectively mediate cellular responses to Hh pathway activation [35–38] (Fig. 1A). In the absence of Hh ligand, PTCH1 binds to SMO, preventing its entry into the primary cilium and thereby inhibiting the classic Hh signaling pathway [39] (Fig. 1B). Beyond the canonical pathway, non-canonical Hh signaling can be categorized into three types: type I involves PTCH1-independent mechanisms, which may promote apoptosis or inhibit tumor growth [40]. PTCH1 interacts with down-regulated in rhabdomyosarcoma LIM protein (DRAL), which recruits TUCAN (CARD8, Cardinal) to form a pro-apoptotic complex. This complex activates caspase-9, which in turn activates caspase-3, ultimately inducing apoptosis [41]. DRAL, also known as four and a half LIM domains 2 (FHL2), interacts with PTCH1 by binding to its C-terminal region, potentially regulating PTCH1's function or stability. TUCAN, an apoptosis regulatory protein, has been reported to interact with PTCH1, linking the Hh signaling pathway to apoptotic pathways [42] (Fig. 2A). Type II non-canonical signaling is mediated by SMO activity that operates independently of GLI1 [43]. This pathway can activate Ras-related C3 botulinum toxin substrate 1 (Rac1) and Ras homolog family member A (RhoA), resulting in cytoskeletal rearrangement and promoting cell migration and axon guidance [44] (Fig. 2B). Type III involves mechanisms of GLI1 activation independent of PTCH1-SMO signaling [45]. In this pathway, GLI1 is primarily activated by other signaling molecules such as transforming growth factor-beta (TGF-β), epidermal growth factor (EGF), and tumor necrosis factor-alpha (TNF-α), along with their downstream effectors, such as suppressor of mothers against decapentaplegic 3 (SMAD3), Ras-ERK, and nuclear factor-kappa B

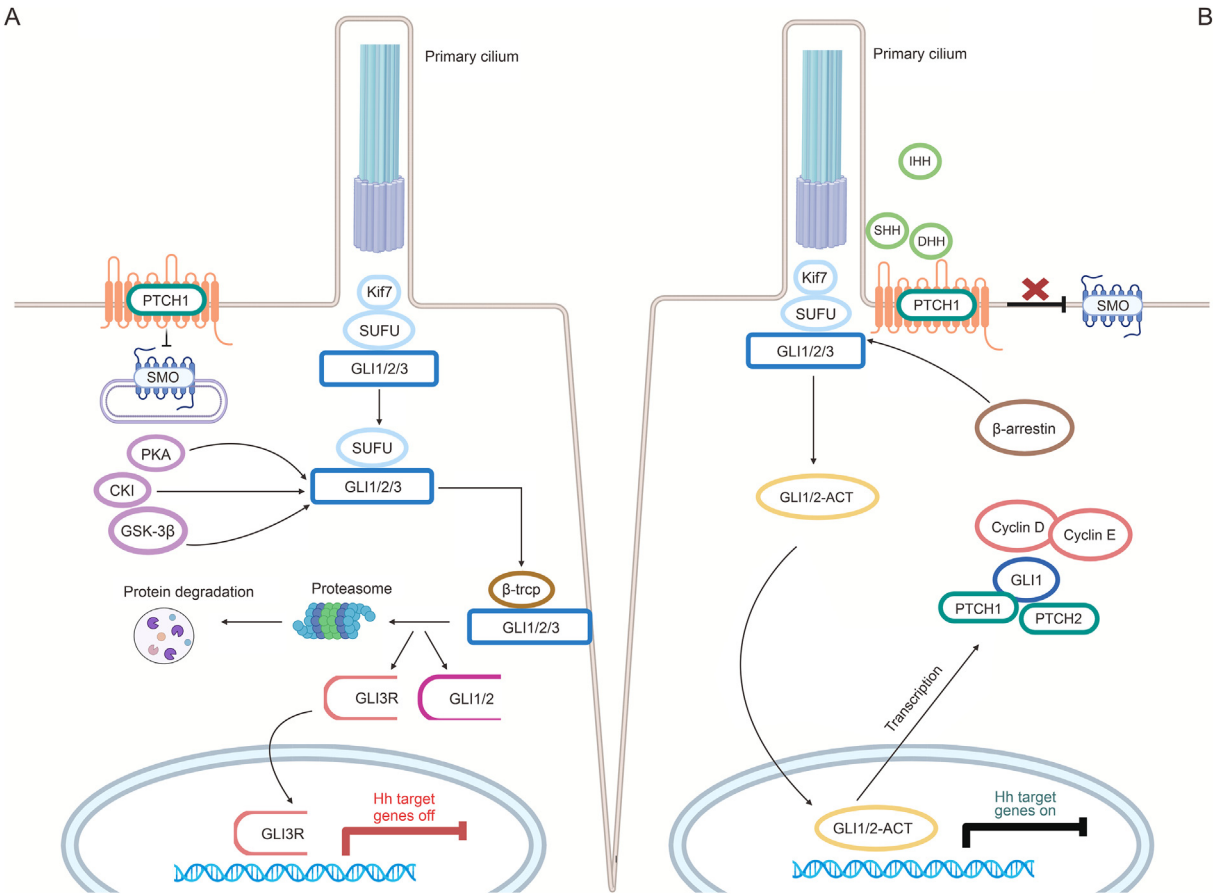


Fig. 1. The transmission of the classical Hedgehog (Hh) signaling pathway. (A) In the signal-off state, patched 1 (PTCH1) inhibits smoothened (SMO), GLI transcription factors are phosphorylated and degraded, and Hh target genes are suppressed. (B) In the signal-on state, Hh signaling relieves PTCH1 inhibition of SMO, activated GLI transcription factors enter the nucleus, and initiate transcription of Hh target genes. PKA: protein kinase A; CKI: casein kinase I; GSK-3β: glycogen synthase kinase 3 beta; Kif7: kinesin family member 7; SUFU: suppressor of fused; GLI1: GLI family zinc finger 1; GLI2: GLI family zinc finger 2; GLI3: GLI family zinc finger 3; GLI3R: GLI family zinc finger 3 repressor; β-Trcp: beta-transducin repeat-containing protein; IHH: Indian Hedgehog; SHH: Sonic Hedgehog; DHH: Desert Hedgehog; GLI1/2-ACT: GLI1 and GLI2 activator forms.

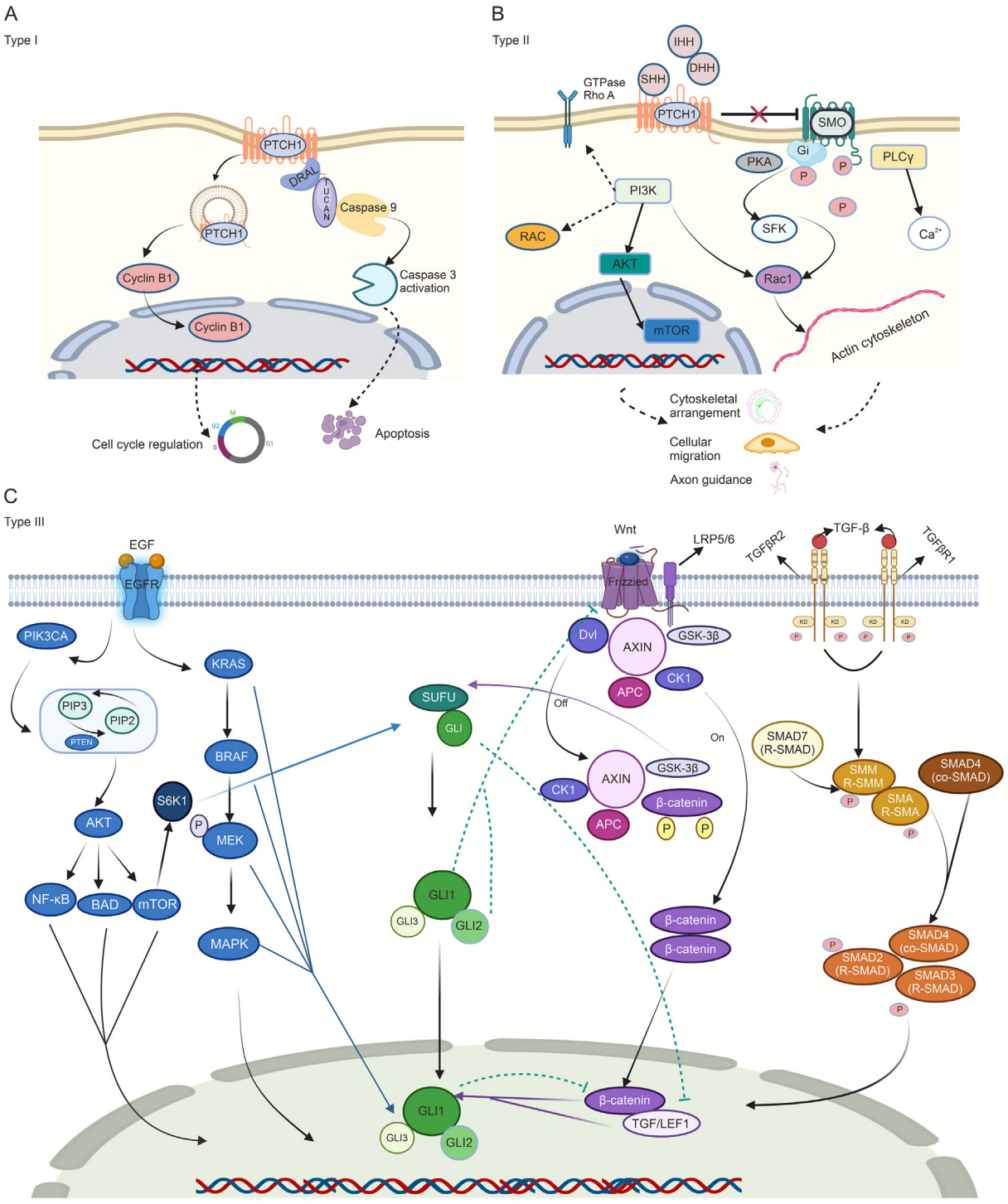


Fig. 2. The transmission of the non-classical Hedgehog (Hh) signaling pathway. (A) Patched 1 (PTCH1)-independent actions, where down-regulated in rhabdomyosarcoma LIM protein (DRAL) and tumor-up-regulated CARD-containing antagonist of caspase-nine (TUCAN) form a pro-apoptotic complex that activates caspase-9 and caspase-3, thereby regulating cell apoptosis and the cell cycle. (B) Smoothened (SMO)-mediated signaling that is independent of GLI family zinc finger 1 (GLI1). SMO activates small G proteins such as Rac1 and RhoA, influencing cytoskeletal rearrangement and consequently regulating cell migration and axon guidance. (C) PTCH1-SMO independent mechanism of GLI1 activation. It shows how multiple signaling pathways including epidermal growth factor (EGF) , Wnt, and transforming growth factor-beta (TGF-β) converge to ultimately activate GLI1 and GLI family zinc finger 2 (GLI2) transcription factors. This mechanism involves a complex signaling network, including the participation of various molecules such as Kirsten rat sarcoma viral oncogene homolog (KRAS), protein kinase B (AKT), β-catenin, and suppressor of mothers against decapentaplegic (SMAD) proteins, ultimately leading to the activation and nuclear translocation of GLI transcription factors. IHH: Indian Hedgehog; SHH: Sonic Hedgehog; DHH: Desert Hedgehog; PKA: protein kinase A; PI3K: phosphatidylinositol 3-kinase; SFK: Src family kinases; RAC: Rac family small GTPase 1; PLCγ: phospholipase C gamma; mTOR: mammalian target of rapamycin; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; PIP2: phosphatidylinositol (4,5)-bisphosphate; PIP3: phosphatidylinositol (3,4,5)-trisphosphate; PTEN: phosphatase and tensin homolog deleted on chromosome ten; BRAF: v-raf murine sarcoma viral oncogene homolog; S6K1: ribosomal protein S6 kinase beta-1; MEK: mitogen-activated protein kinase kinase; NF-κB: nuclear factor-kappa B; BAD: Bcl-2-associated death promoter; MAPK: mitogen-activated protein kinase; SUFU: suppressor of fused; GLI3: GLI family zinc finger 3; LRP5/6: low-density lipoprotein receptor-related protein 5/6; Dvl: dishevelled; AXIN: axis inhibition protein; APC: antigen-presenting cell; CK1: casein kinase 1; GSK-3β: glycogen synthase kinase 3 beta; TGF: transforming growth factor; LEF1: lymphoid enhancer-binding factor 1; TGFβR2: transforming growth factor beta receptor 2; TGFβR1: transforming growth factor beta receptor 1; SMM: smooth muscle myosin; SMA: smooth muscle actin.

(NF-κB). This activation influences critical cellular processes, including proliferation, survival, and development [6,46–48] (Fig. 2C). Additionally, post-translational modifications of GLI1, including phosphorylation, ubiquitination, acetylation, and O-GlcNAcylation, significantly impact the intracellular transport and transcriptional activity of the SHH signaling pathway, thus regulating tumorigenesis and cancer progression [49–51].

Mutations in SHH, PTCH1, and GLI1 are rare in BC and do not appear to be directly involved in the activation of the Hh pathway. However, aberrant upregulation of SHH has been associated with tumor progression and modifications within the tumor microenvironment [2]. Despite the absence of Hh gene mutations in BC, activation of the canonical Hh pathway can still contribute to BC development [52]. Moreover, overexpression of SHH has been linked to increased tumor invasiveness, lymphatic infiltration, and metastasis, as well as the upregulation of the pro-angiogenic transcription factor cysteine-rich angiogenic inducer 61 (CYR61), which promotes highly vascularized tumors [53,54]. While the role of type I non-canonical Hh signaling in BC remains understudied, type II non-canonical Hh signaling has been implicated in processes such as angiogenesis, cell migration, and the activation of small Rho GTPases (Rho), suggesting that type II signaling may play a significant role in the tumor stroma [55].

2.2.3. SMO-GLI axis: the key regulatory mechanism in Hh signaling pathway

The Hh signaling pathway transmits signals from the membrane receptor SMO to the transcription factor GLI, a critical step in the regulation of downstream target gene expression [45]. Upon SMO activation, a series of phosphorylation cascade reactions are triggered, involving key kinases such as protein kinase A (PKA), glycogen synthase kinase 3 beta (GSK3β), casein kinase I (CKI), kinesin family member 7 (KIF7), suppressor of fused (SUFU), and dual specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) [19,56–59]. These cascades ultimately regulate the activity and subcellular localization of GLI transcription factors, a crucial step in relaying the Hh signal from the membrane to the nucleus.

Specifically, the phosphorylation of PKA and GSK3β promotes the conversion of GLI3 to its repressive form, GLI3R, thereby inhibiting the transcription of Hh target genes [56,57]. In contrast, the phosphorylation of CKI stabilizes GLI1 and GLI2, facilitating their nuclear translocation and activating the transcription of Hh target genes [60]. KIF7 interacts with SMO, regulating its subcellular localization and activity, thus influencing the activation of GLI transcription factors [58]. SUFU functions as a negative regulator of the Hh pathway by directly binding and inhibiting GLI transcription factors. However, upon Hh pathway activation, SUFU undergoes phosphorylation, leading to the release of GLI [19]. Additionally, DYRK1A phosphorylates and stabilizes GLI1, enhancing its transcriptional activity. DYRK1A also phosphorylates SUFU, diminishing its inhibitory effect on GLI [59].

Additionally, β-arrestin binds to SMO, playing a role in SMO's intracellular transport and signal transduction. This interaction regulates the activity state of SMO, consequently affecting the regulation of downstream GLI transcription factors [61]. Furthermore, SMO activation can indirectly modulate GLI transcription factor activity via other signaling pathways, such as the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) and rat sarcoma viral oncogene homolog/mitogen-activated protein kinase (RAS/MAPK) pathways [62,63].

Overall, this complex regulatory network ensures precise control of Hh target gene expression, which is crucial for various developmental and pathological processes.

3. Regulation of carcinogenesis by the Hh signaling pathway

SHH is a crucial protein within the Hh signaling pathway, responsible for initiating signal transduction by binding to the cell surface receptor PTCH1 [15]. PTCH1 functions as a negative regulator of the SHH pathway, inhibiting signal transduction in the absence of SHH binding [52]. Moreover, the GLI family of proteins serves as the core effector molecules within this pathway. As transcription factors, upon activation, these proteins translocate to the nucleus, where they regulate the expression of target genes, ultimately influencing cellular proliferation and differentiation [35]. In recent years, natural compounds have demonstrated significant therapeutic potential in BC by modulating the Hh signaling pathway. These compounds can directly or indirectly regulate the expression of key proteins such as SHH, PTCH1, and GLI1, thereby modulating the activity of the Hh signaling pathway through multiple mechanisms.

3.1. Regulation of key proteins in the Hh signaling pathway in BC

3.1.1. Regulation of SHH in BC

In BC, *SHH* gene expression is regulated by the NF-κB transcription factor. NF-κB, a key mediator of inflammatory signaling, promotes cancer cell growth, migration, differentiation, and self-renewal [64]. NF-κB has been shown to positively regulate *SHH* gene expression in various cancers, including BC [65]. Furthermore, CpG island methylation in the SHH promoter region is associated with the suppression of *SHH* gene expression. Experimental studies indicate that treating breast cancer cell lines with DNA methylation inhibitors reduces SHH promoter methylation, leading to increased SHH expression [66]. A similar phenomenon has been observed in gastric cancer [67]. Moreover, in BC cells stimulated with NF-κB activators, DNA methylation inhibitors further enhance *SHH* gene upregulation, an effect not observed when NF-κB inhibitors are applied [65,68]. These findings suggest that *SHH* gene expression in BC is co-regulated by NF-κB at both the transcriptional and epigenetic levels. The role of natural compounds in inhibiting BC metastasis remains relatively understudied. However, natural compounds like sinomenine and cordycepin have been shown to inhibit SHH activation by blocking NF-κB signaling in the non-canonical Hh pathway, thereby suppressing BC metastasis [64,69].

3.1.2. The expression of PTCH1 in BC cells

Although PTCH1 functions as a negative regulator of Hh signaling, its expression is upregulated by GLI-dependent transcription, making it a recognized surrogate marker for Hh pathway activation [45]. However, the typically low expression of PTCH1, coupled with limitations in commercial antibodies, renders its accurate detection in BC tumors challenging. Studies have shown that in MCF7 BC cell lines, the downregulation of *PTCH1* mRNA is linked to increased promoter methylation [70]. Another study demonstrated increased PTCH1 expression in several BC cell lines following binding to radiolabeled SHH proteins [71]. However, SHH binds to multiple receptors, complicating the interpretation of these results. Thus, further research is necessary to reconcile the discrepancies among studies and to determine whether BC characteristics are linked to PTCH1 expression dysregulation. Some studies suggest that specific components of traditional Chinese medicine may modulate signaling pathways, offering potential mechanisms for regulating PTCH1 expression dysregulation. For instance, the combined or individual application of natural compounds such as curcumin or cordycepin significantly reduces *SHH,GLI1*, and *PTCH1* mRNA and protein levels in triple-negative breast cancer (TNBC), thereby inhibiting BC cell proliferation [9,72].

3.1.3. The expression of GLI1 in BC

In BC, the expression of GLI1 and GLI2 is strongly linked to key biological processes, including proliferation, survival, migration, invasion, EMT, angiogenesis, and bone metastasis [3–7]. The role of GLI3 in BC is multifaceted. It promotes the growth of BC cells through interactions with estrogen receptors and contributes to cancer progression in triple-negative and HER2-positive BC through distinct signaling pathways. High GLI3 expression is linked to poor prognosis [73]. GLI1 expression is elevated in TNBC and basal-like breast cancer (BLBC) cell lines, but lower in estrogen receptor (ER)-positive cell lines [2,18]. The truncated *GLI1* mRNA variant, tGLI1, has been shown to enhance the metastatic potential of BC cells. In summary, both GLI1 and tGLI1 appear to play critical roles in BC [74]. As central mediators in both canonical and non-canonical Hh signaling, GLI proteins (GLI1, GLI2, GLI3) function as transcription factors regulating the expression of downstream target genes [2,75]. Nearly all natural compounds studied for their relevance to the Hh signaling pathway exhibit some inhibitory effects on GLI proteins. For instance, cordycepin inhibits tumor growth within the non-canonical Hh pathway by reducing GLI activity, which blocks the Hh-Notch-EMT axis and suppresses tumor metastasis [69]. Cordycepin also decreases the expression of key canonical Hh pathway components, including SHH, PTCH1, SMO, GLI1, and GLI2, thereby inhibiting BC growth and metastasis [72]. These findings open new avenues for BC treatment, offering potential therapeutic options with low toxicity and high tolerability. Therefore, we further explore how natural compounds affect breast cancer cells through the Hh signaling pathway, providing critical insights for developing novel therapeutic strategies.

4. Natural compounds targeting the Hh pathway in BC intervention

The regulation of the Hh signaling pathway by natural products has shown broad potential in BC, as these compounds affect various biological behaviors of breast cancer cells through multiple mechanisms, including proliferation, invasion, EMT, drug resistance, reduction of BCSC populations, and tumor angiogenesis [5,7,12,64]. Specifically, natural compounds can modulate the expression of key factors, inhibit the transition of tumor cells to invasive phenotypes, disrupt tumor angiogenesis, limit the blood and nutrient supply to the tumor, affect drug resistance, regulate Hh pathway activity, and influence the proliferation, self-renewal, and metastatic capacity of BC xenografts. For example, cyclopamine is the most extensively studied Hh pathway inhibitor to date. It regulates the Hh pathway through various mechanisms, including inhibiting the activity of the SMO receptor and suppressing the expression and translocation of GLI factors [76]. Numerous studies have confirmed that cyclopamine can inhibit the proliferation, invasion, and migration of breast cancer cells while also reversing drug resistance [77]. In addition to cyclopamine, other natural compounds such as polyphenols, terpenes, alkaloids and other miscellaneous compounds can also regulate the Hh signaling pathway [4,9,13,14,78].

4.1. Interfering with EMTs in BC

There is a reciprocal interaction between EMT and the Hh signaling pathway. Upon activation, the Hh signaling pathway regulates EMT-related genes, including *SNAIL* and *TWIST*, resulting in the induction of EMT. Morphological and molecular changes occurring during EMT can, in turn, modulate the Hh signaling pathway by altering the expression levels of key molecules, thereby impacting signal transduction. Studies have demonstrated that Hh-mediated activation of GLI1 can promote EMT by upregulating the expression of *SNAIL* and vimentin (*VIM*), while downregulating E-cadherin (*CDH1*) expression [79].

Natural compounds frequently inhibit BC cell migration, invasion, and EMT by targeting the SHH/GLI1 axis. Cananginone, a natural compound from the Annonaceae family, exhibits potent cytotoxicity through the non-canonical Hh signaling pathway. Cananginone upregulates phosphorylated GSK3β, which promotes the proteasomal degradation of GLI1 protein, thereby modulating the Hh signaling transduction through a non-canonical pathway. It also downregulates E-cadherin expression, inhibiting EMT and contributing to the suppression of BC metastasis [80]. Similarly, cordycepin acts through the non-canonical Hh pathway, particularly impacting the downstream Notch signaling pathway, which plays a crucial role in EMT. Cordycepin downregulates the expression of NOTCH1, NOTCH3, JAGGED1, and HES1 in the Notch signaling pathway. In cells where GLI is knocked out, cordycepin fails to inhibit NOTCH1 and NOTCH3 expression. However, NOTCH1 can indirectly regulate EMT. Therefore, cordycepin inhibits tumor growth by reducing GLI activity and blocking the Hh-Notch-EMT axis, consequently suppressing tumor metastasis [69]. Sinomenine and cordycepin similarly affect EMT through the non-canonical Hh pathway by inhibiting SHH activation via blockade of the NF-κB signaling cascade. Since NF-κB is essential for EMT, its inhibition interferes with the EMT process, thus suppressing BC metastasis [64].

In the canonical Hh pathway, cordycepin primarily inhibits the expression of key factors such as SHH, PTCH1, SMO, GLI1, and GLI2, which are typically upregulated in the Hh signaling pathway. Treatment with cordycepin significantly suppresses the expression of these factors, thereby inhibiting the growth and metastasis of BC [72]. The plant polyphenol curcumin inhibits the protein levels of GLI1, GLI2, SMO, CDH1, and VIM in GLI-overexpressing BCSCs. Additionally, curcumin reduces the protein levels of stemness markers octamer-binding transcription factor 4 (OCT4) and SRY-box transcription factor 2 (SOX2) while decreasing GLI1 nuclear expression, suggesting that curcumin inhibits GLI1 translocation to the nucleus, thereby blocking the Hh pathway. Furthermore, VIM interacts with GLI1, highlighting its role as a critical gene induced by GLI1 in EMT and stemness regulation [9]. Sinomenine, a quinoline alkaloid, inhibits the Hh signaling pathway by downregulating the protein expression of GLI transcriptional activators and Patch receptors, resulting in the reversal of EMT [64] (Fig. 3A).

4.2. Inhibiting angiogenesis

TNBC tumors express higher levels of vascular endothelial growth factor (VEGF) compared to non-TNBC tumors and display increased microvessel density, indicating a reliance on angiogenesis [81]. The Hh signaling pathway contributes to tumor-associated angiogenesis. Activation of the Hh pathway increases vascular density in BC [53]. Additionally, Hh signaling regulates angiopoietin-1 (Ang-1) in bone marrow-derived pro-angiogenic cells, promoting neovascularization [82], while canonical Hh signaling induces VEGF expression to facilitate tumor angiogenesis. tGLI1, a splice variant of GLI1, enhances the human VEGF (h-VEGF) gene promoter, leading to the upregulation of VEGF in BC cells [74].

Wogonoside, a major active component of the traditional Chinese medicine *Scutellaria baicalensis* (Huangqin), primarily exerts its effects on BC via the non-canonical Hh signaling pathway. Wogonoside, derived from the roots of *S.baicalensis*, is a natural flavonoid compound. It exhibits a range of pharmacological effects, including anti-inflammatory, anti-angiogenic, antioxidant, neuroprotective, and anti-tumor properties [5,83,84]. Wogonoside suppresses angiogenesis in TNBC by downregulating the expression of the pro-angiogenic factor VEGF or by directly binding to SMO, thereby inhibiting the nuclear translocation and transcriptional

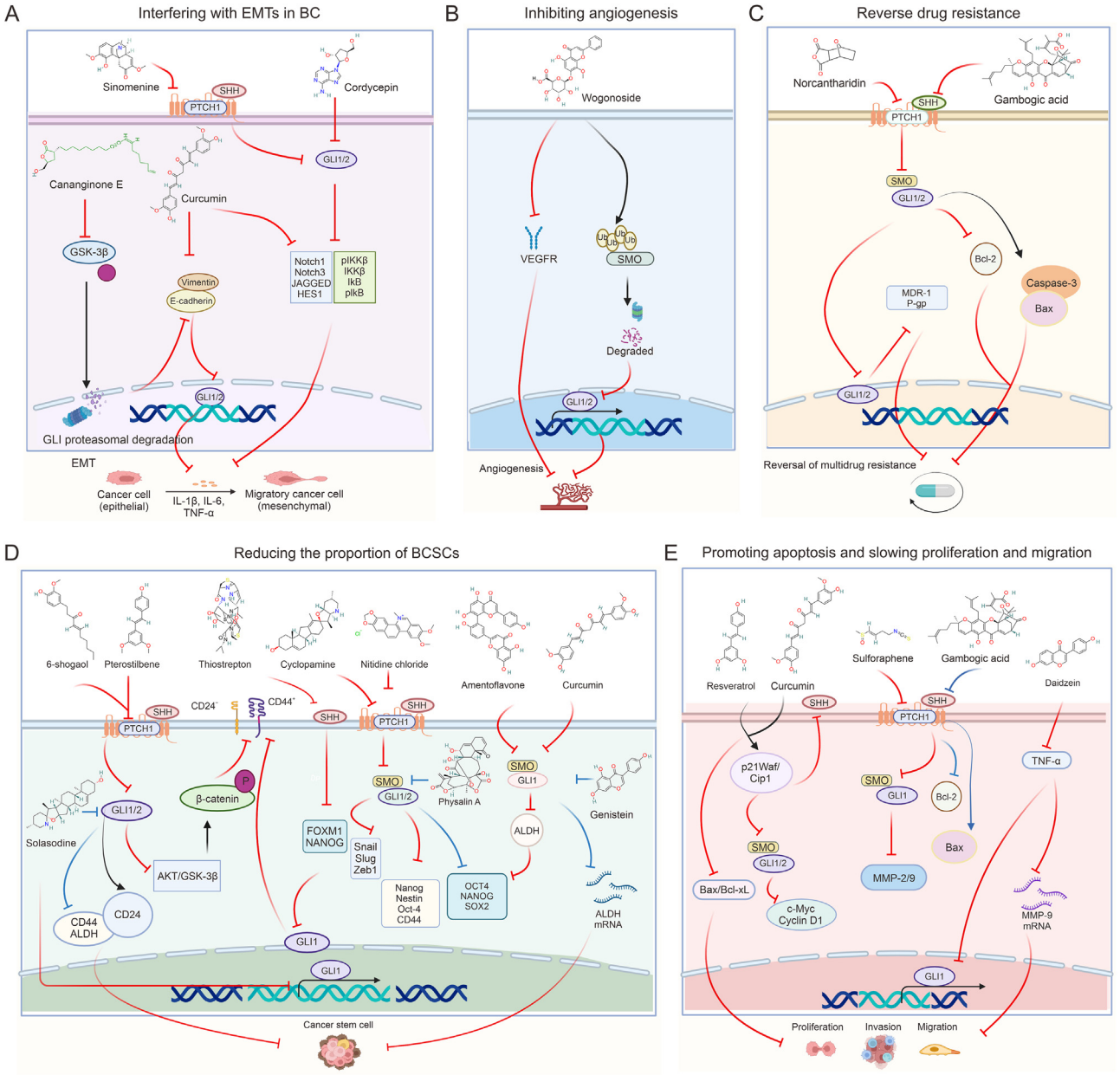


Fig. 3. Natural compounds act on breast cancer (BC) through the Hedgehog (Hh) signaling pathway. (A) Natural compounds primarily inhibit the activation of key components in the Hh signaling pathway, thereby down-regulating the expression of epithelial-mesenchymal transition (EMT)-related proteins and suppressing the activation of the neurogenic locus notch homolog protein (Notch)/nuclear factor-kappa B (NF-κB) pathway. This mechanism ultimately leads to the inhibition of the EMT process. (B) Natural compounds inhibit angiogenesis by directly inhibiting the expression of vascular endothelial growth factor (VEGF) or inhibiting the activation of the Hh signaling pathway by promoting ubiquitination-dependent degradation of smoothened (SMO). (C) Natural compounds overcome multidrug resistance in human BC cells by inhibiting Sonic Hedgehog (SHH) signaling and its downstream multidrug resistance protein 1 (MDR-1)/P-gp expression. (D) Natural compounds can inhibit BC stemness by suppressing the Hh/protein kinase B (AKT)/glycogen synthase kinase 3 beta (GSK-3β) signaling pathway, promoting the phosphorylation of β-catenin, which in turn reduces the expression of $CD44^{+}$ and $CD24^{-}$. Phosphorylated β-catenin can decrease the downstream targets c-Myc and cyclin D1, thereby lowering the stemness of breast cancer stem cells (BCSCs). Alternatively, these compounds can inhibit key components of the Hh signaling pathway, leading to a reduction in cancer stem cell markers such as aldehyde dehydrogenase (ALDH), octamer-binding transcription factor 4 (OCT4), nanog homeobox (NANOG), and sex determining region Y-box 2 (SOX2), which also contributes to the decrease in BC stemness. (E) Natural compounds can inhibit the proliferation of BC cells by either suppressing the cell cycle-dependent kinase inhibitor wild-type p53-activated fragment 1 (p21Waf)/CDK-interacting protein 1 (Cip10 or inhibiting the anti-apoptotic protein B-cell lymphoma (Bcl-2), while promoting the expression of the pro-apoptotic protein Bcl-2-associated X protein (Bax). Additionally, they can suppress the invasion of BC cells by downregulating matrix metalloproteinases matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9). Furthermore, these compounds can enhance the activity of cysteine-aspartic acid protease 3 (caspase-3) and caspase-8, thereby inhibiting the migration of BC cells. PTCH1: patched 1; GLI1/2: GLI family zinc finger 1/2; JAGGED: jagged canonical Notch ligand; HES1: Hes family bHLH transcription factor 1; pIKKβ: phosphorylated IκB kinase β; IKKβ: iinhibitor of nuclear factor kappa-B kinase subunit beta; IκB: inhibitor of nuclear factor kappa-B; pIκB: phosphorylated IκB; IL-1β: interleukin-1 beta; IL-6: interleukin-6; TNF-α: tumor necrosis factor-alpha; VEGFR: vascular endothelial growth factor receptor; MDR-1: multidrug resistance protein 1; P-gp: P-glycoprotein; CD44: cluster of differentiation 44; CD24: cluster of differentiation 24; ALDH: aldehyde dehydrogenase; FOXM1: forkhead box protein M1; Snail: zinc finger protein SNAI1; Slug: zinc finger protein SNAI2; Zeb1: zinc finger E-box-binding homeobox 1; OCT4: octamer-binding transcription factor 4; NANOG: nanog homeobox; SOX2: sex determining region Y-box 2; Bcl-xL: B-cell lymphoma-extra large.

activity of GLI1. Furthermore, wogonoside specifically promotes the ubiquitination-dependent degradation of SMO in TNBC cells [5] (Fig. 3B).

4.3. Reverse drug resistance

The role of Hh signaling in promoting resistance to cancer chemotherapy can be summarized into five primary mechanisms: (1) Regulation of DNA repair mechanisms: Hh signaling enhances the expression and activity of DNA repair enzymes, promotes the activation of DNA damage response pathways, and regulates cell cycle checkpoints, thereby enabling cancer cells to repair and tolerate DNA damage induced by chemotherapeutic drugs [85]. (2) Regulation of transporter proteins: Hh signaling increases the efflux of chemotherapeutic drugs, reducing their intracellular concentration [86]. (3) Induction of EMT: This process enables tumor cells to acquire more invasive and drug-resistant properties [9]. (4) The formation of a CSC ecological niche enhances the resistance to chemotherapeutic drug [27]. (5) Regulation of autophagy, drug inactivation, and the tumor microenvironment: These factors collectively influence the sensitivity and resistance of tumor cells to chemotherapeutic agents [87–89].

In the non-classical Hh signaling pathway, norcantharidin (NVTD) enhances doxorubicin (DOX) accumulation in the DOX-resistant MCF-7R breast cancer cell line. Upon up-regulation of SHH in the MCF-7R breast cancer cell line, the expression of *Abcb1* (*P-gp*), *Mdr1* (*mdr-1*) mRNA, and breast cancer resistance protein (BCRP)were also increased, suggesting that SHH operates upstream and P-gp functions downstream in the mechanism. NVTD may reverse drug resistance in BC by inhibiting SHH and its downstream targets, such as Mdr-1 or P-gp, which mediate multidrug resistance [23].

In the classical Hh signaling pathway, the combination of gambogic acid and paclitaxel significantly reduced both mRNA and protein expression levels of SHH, GLI1, and PTCH1 in TNBC cells. This treatment combination also significantly increased the expression of cleaved caspase-3 and Bcl-2-associated X protein (BAX), while decreasing the expression of Bcl-2 in xenograft tumors. These findings demonstrate that gambogic acid inhibits proliferation and induces apoptosis in paclitaxel-resistant TNBC cells by suppressing the SHH signaling pathway. However, this effect was only observed in TNBC [7] (Fig. 3C).

4.4. Reducing the proportion of BCSCs

CSCs are capable of forming tumors, even when transplanted in small quantities into experimental animals like non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice, and they maintain their self-renewal ability over multiple passages. Additionally, CSCs can thrive under low-adhesion conditions and demonstrate increased invasive and metastatic capabilities [61]. Several studies have demonstrated high expression levels of PTCH1, GLI1, GLI2, and SMO in CSC fractions, with these levels decreasing following stem cell differentiation [11]. Interestingly, CD24, a protein that is either absent or expressed at low levels in BCSCs, reduces the stem cell phenotype by inhibiting SHH and GLI1 expression and deactivating Hh signaling [3,4]. Overall, activation of the Hh signaling pathway promotes stemness through SHH signal transduction, regulates self-renewal, and contributes to the CSC-driven progression and metastasis of BC.

In the non-canonical Hh pathway, the combination of 6-gingerol (6-S) and pterostilbene (PTE) has been shown to reduce CD44 expression, promote β-catenin phosphorylation, and thereby diminish the stemness of BCSCs by inhibiting the Hh/AkT/GSK3β signaling pathway [11]. Polyphenolic compounds, including genistein, amentoflavone, resveratrol, and curcumin, exhibit inhibitory effects on BCSCs via the classical Hh signaling pathway. For instance, genistein effectively reduces the population of cells expressing the CSC-specific markers $CD44^+$ and $CD24^-$ in breast spheres. It also inhibits the mRNA expression and protein levels of the BCSC marker aldehyde dehydrogenase (ALDH), primarily by downregulating the expression of SMO and GLI1, thus suppressing the Hh pathway [3]. Likewise, amentoflavone exhibits inhibitory effects by suppressing the expression of CD44, CD24, and ALDH1 in BCSCs and downregulating SMO and GLI1. This leads to reduced expression of pluripotent transcription factors OCT4 and nanog homeobox (NANOG), which are key regulators of BCSCs [4].

2-Cyano-3,12-dioxooleana-1,9-dien-28-oic acid-imidazolide (CDDO-Im) and physalin A, both triterpenoid compounds, have demonstrated significant inhibitory effects on BCSCs via the classical Hh signaling pathway. Among these compounds, CDDO-Im shows particularly potent therapeutic effects, effectively inhibiting $CD24^-/EpCAM^+$ cells and reducing sphere formation efficiency as well as tumor sphere size in both primary and secondary cultures. Beyond suppressing the Hh pathway, CDDO-Im also downregulates key molecules involved in other critical stem cell signaling pathways, including Notch, TGF-β/SMAD, and Wnt [12]. In TNBC, GLI1 is the key regulatory factor in the Hh signaling pathway, and CDDO-Im shows promise as a targeted therapy by significantly reducing the mRNA and protein levels of GLI1 and its negative regulator, SUFU [12]. Physalin A reduces the expression of BCSC biomarkers (CD44 high/CD24 low and ALDH1) and decreases the transcription levels of CSC-specific genes (*Oct4*, *Cd44*, *Sox2*, *c-Myc*, and *Nanog*). The mechanism of action primarily involves downregulating GLI1 and GLI2 expression in breast spheres and reducing *GLI1* transcription levels. Furthermore, physalin A reduces the transcriptional and protein levels of YAP1 in the Hippo signaling pathway. This suggests that physalin A targets BCSCs through the Hh/Hippo signaling pathway, showing potential as a therapeutic agent [13].

Alkaloid compounds, including huaier aqueous extract, nitidine chloride, solasodine, and cyclopamine, demonstrate significant inhibitory effects on BCSCs through the classical Hh signaling pathway. Huaier aqueous extract significantly inhibits activation of the Hh pathway by downregulating GLI1 expression, exhibiting strong therapeutic effects on BCSCs in a time- and dose-dependent manner. Interestingly, the inhibitory concentration of huaier aqueous extract has minimal impact on most breast cancer cell lines, indicating its preferential targeting of tumor stem cells [24]. Nitidine chloride reduces the cancer stem cell-like phenotype, primarily reflected in decreased mammosphere formation ability. Mechanistically, nitidine chloride inhibits key components of the Hh signaling pathway, leading to reduced expression of EMT-related transcription factors (Snail, Slug, and Zeb1) and changes in EMT markers (increased E-cadherin, decreased N-cadherin, and vimentin). Furthermore, nitidine chloride suppresses the expression of cancer stem cell-related factors (*Nanog*, *Nestin*, *Oct-4*, and *CD44*) through the canonical Hh pathway. Notably, nitidine chloride reverses EMT and cancer stem cell properties induced by transforming growth factor-β1 (TGF-β1) [25]. Solasodine's mechanism of action involves inhibiting nuclear entry and promoting GLI degradation. Solasodine's cytotoxicity is strongly correlated with GLI levels in breast cancer cells, particularly in cases of GLI overexpression. It demonstrates significant inhibitory activity against MCF7 CSCs and enhances the efficacy of tamoxifen [26]. Cyclopamine, another steroidal alkaloid, inhibits cell proliferation or induces apoptosis in MCF7 cells across various concentrations, exhibiting a dose-dependent effect. Cyclopamine exerts its cytotoxic effects on breast cancer cells by inhibiting the expression of the key SHH signaling molecule GLI and reducing the expression of

the anti-apoptotic protein survivin. Additionally, cyclopamine inhibits key proteins in the classical Hh signaling pathway, including PTCH, SMO, GLI1, and GLI2, thereby reducing the formation of BCSCs. It also enhances the sensitivity of breast cancer cells to paclitaxel [14,27].

Thiostrepton, an antibiotic with a thiazole ring structure, was first isolated from *Streptomyces azureus*. It is commonly used in veterinary medicine to treat mastitis and skin infections caused by Gram-negative bacteria. Research indicates that thiostrepton inhibits BCSC by downregulating critical components of the Hh signaling pathway, including SHH, PTCH1, SMO, and GLI*1*. This leads to a reduction in $CD44^{+}/CD24^{-}$ stem cell-like populations and decreases sphere-forming capacity [90] (Fig. 3D).

4.5. Promoting apoptosis and slowing proliferation and migration of BC cells

In BC, activation of the Hh signaling pathway is commonly associated with the inhibition of apoptosis and promotion of cell proliferation, primarily through the regulation of genes such as *Bcl-2* and *Cyclin D*, which support cancer cell survival and growth. Furthermore, the Hh signaling pathway may regulate BC cell migration and invasion through modulation of factors such as matrix metalloproteinases (MMPs), which enhance cancer cell migratory capabilities. In summary, the Hh signaling pathway plays a complex and critical role in BC progression, affecting cancer advancement by regulating key processes such as apoptosis, proliferation, and migration.

In the non-canonical Hh pathway, combined application of the non-flavonoid polyphenol resveratrol and the polyphenolic compound curcumin demonstrated synergistic anti-tumor activity in both *in vitro* and *in vivo* models. This therapy inhibited the Hh-GLI signaling cascade by downregulating key components, including SMO, GLI1, and SHH, while also increasing p21Waf/Cip1 levels, suggesting a link between Hh-GLI pathway inhibition and p21Waf/Cip1 activation. These findings suggest that resveratrol and curcumin inhibit the Hh-GLI pathway cascade through p21Waf/Cip1, exerting synergistic apoptotic effects on BCSC transformation [10]. In the canonical Hh pathway, sulforaphene effectively inhibits MMP expression, specifically MMP-2 and MMP-9, significantly reducing cell migration and invasiveness without affecting cell viability. Sulforaphene suppresses downstream effectors SMO and GLI1 in the Hh pathway, leading to a concurrent reduction in MMP-2 and MMP-9 protein levels. These findings indicate that MMP-2 and MMP-9 expression is regulated by Hh/GLI1 signaling [78]. Ginger extract primarily inhibits *GLI1* and *PTCH1* gene expression to modulate Hh signaling feedback, thereby reducing BC cell proliferation and migration while promoting apoptosis. While it modulates Hh signaling, this effect appears to act at the transcriptional level rather than through typical protein-level interactions [91] (Fig. 3E).

Among the 23 natural compounds discussed, mechanisms of action on the Hh signaling pathway vary significantly. These compounds can be broadly categorized into four types: polyphenols, terpenoids, alkaloids, and miscellaneous compounds, including thiostrepton, sulforaphene, cananginone E, and ginger extract. Polyphenolic compounds can indirectly suppress Hh signaling pathway activation and the EMT process in BC, either by modulating Hh-GLI1 and HH-SMO-GLI cascade reactions or by inhibiting TNF-α activity, thus curbing cancer cell invasion and migration. Additionally, they can induce apoptosis in BC cells or sustain the stemness of BCSCs by inhibiting cascade reaction activity involving the Hh pathway and associated kinases [3–7]. Terpenoid compounds disrupt BCSC formation and maintenance and can reverse drug resistance in BC by inhibiting Hh pathway activity while upregulating *Abcb1*, *Mdr1* mRNA, and BCRP protein expression [12,13,23]. Alkaloids inhibit proliferation in BC cells and stem cells by downregulating *GLI1* overexpression, and they also induce apoptosis by targeting *SHH* and suppressing survivin expression [24–27,64,69]. Sulforaphene, categorized among miscellaneous compounds, primarily reduces BC cell migration and invasiveness by modulating Hh pathway signaling and suppressing downstream effectors SMO and GLI, leading to a reduction in MMP-2 and MMP-9 expression [78].

To summarize, these compounds can be classified into three distinct mechanisms of action: (1) Direct inhibition of key protein expression: Compounds reduce the expression of SMO, GLI, PTCH1, SUFU, and SHH at both the mRNA and protein levels. (2) Modulation of protein activity and localization: Direct binding to SMO promoting its ubiquitination, thereby diminishing its activity; inhibiting the nuclear translocation of GLI, reducing its transcriptional activity. (3) Regulation of downstream effects: Downregulation of MMP-9 and MMP-2 protein expression through SMO and GLI inhibition, impacting tumor invasion and metastasis; Reduced GLI levels altering crosstalk with other signaling pathways, including Hh/Hippo, SHH/P-gp (mdr-1), and Hh/AkT/GSK3β pathways (Fig. 3).

5. A comprehensive analysis of the structure-activity relationship of natural compounds in regulating the Hh signaling pathway and their potential applications in BC treatment

5.1. Polyphenolic compounds

Polyphenolic compounds play a critical role in modulating the Hh signaling pathway. The presence of benzene ring structures and multiple hydroxyl groups enables these compounds to interact strongly with key proteins in the Hh pathway, including SMO and GLI transcription factors (GLI1, GLI2, and GLI3). By binding to SMO, polyphenolic compounds induce conformational changes, closing SMO's active site and preventing interaction with downstream signaling molecules [3,4]. Specifically, resveratrol is a stilbene compound containing two phenolic rings linked by a double bond [92]. The benzene ring and hydroxyl groups of resveratrol bind to SMO's active site: the benzene ring forms hydrophobic interactions with amino acid residues in the binding pocket, while the hydroxyl groups form hydrogen bonds with aspartate and glutamate residues, causing the pocket to close and inhibit SMO activation [93–96]. Additionally, rosmarinic acid (a caffeic acid ester consisting of two phenolic rings connected by carboxylic acid groups) contains abundant hydroxyl groups. These hydroxyl groups may form hydrogen bonds with critical residues on the SMO surface, such as serine and threonine, thereby stabilizing the inactive conformation of SMO and effectively inhibiting signal transduction [97,98].

Curcumin contains two phenol rings connected by an α, β-unsaturated β-diketone moiety, thus forming a polyphenolic structure [99]. Through its diketone structure, it forms covalent bonds with cysteine residues within the DNA-binding domain of GLI1. This covalent bonding causes steric hindrance and disrupts GLI1's DNA-binding ability, thereby preventing its nuclear translocation and transcriptional activation [9,100,101]. Additionally, curcumin's benzene ring structure interacts with hydrophobic residues in GLI1, such as phenylalanine and leucine, through van der Waals forces, further inhibiting GLI1's function [9,102]. Notably, polyphenolic compounds can reduce intracellular reactive oxygen species (ROS) levels due to their antioxidant properties, thereby indirectly inhibiting Hh signaling pathway activity [103,104].

5.2. Terpenoid compounds

Terpenoids inhibit the Hh signaling pathway through their distinctive cyclic structures and various functional groups. By activating adenylyl cyclase, these compounds increase intracellular cyclic adenosine monophosphate (cAMP) levels, which indirectly inhibit the Hh pathway. Increased cAMP promotes PKA activation, leading to the phosphorylation and inhibition of GLI transcription factors (GLI1, GLI2, and GLI3), thus preventing their nuclear translocation and transcriptional activation [105,106]. Forskolin, a diterpenoid compound with a distinct structure, consists of a fused double ring system and a tetrahydropyran-derived heterocyclic ring [107]. Its molecular structure includes a unique tetracyclic diterpene skeleton, enabling a strong fit with the catalytic site of adenylate cyclase and providing the structural basis for precise localization of its functional groups. Research indicates that forskolin directly binds two hydrophobic pockets in the catalytic region of adenylate cyclase C2, forming a dimer structure. Within this central cleft, forskolin's hydroxyl group forms hydrogen bonds with specific charged amino acid residues, while its hydrophobic skeleton interacts with adjacent hydrophobic amino acids via van der Waals forces. These interactions stabilize the active conformation of adenylate cyclase, facilitating adenosine triphosphate (ATP) substrate binding and significantly enhancing cAMP synthesis, which can subsequently inhibit the Shh signaling pathway [105,108–110]. CDDO-Im (a synthetic triterpenoid compound characterized by a pentacyclic triterpenoid skeleton and an imidazole ring [111]) functions as a bifunctional synthetic triterpenoid with an imidazolide reactive site that can covalently bind to amino acids beyond cysteine, specifically targeting proteins such as glutathione S-transferase pi (GSTP), serum albumin, and Keap1. This interaction suggests that CDDO-Im may affect GLI proteins, with its imidazolide structure facilitating binding and subsequent inhibition of GLI1 activity [12,112]. Additionally, physalin A is a steroid lactone with a complex structure, featuring a unique 13,14-seco-16,24-cyclosteroid skeleton. Previous research indicates that lactone compounds can alter the fluidity of lipid rafts [113]. Therefore, we hypothesize that physalin A may indirectly regulate the Hh signaling pathway by modifying lipid raft fluidity, thereby influencing the distribution and function of the SMO protein on the cell membrane [13].

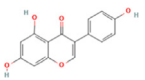
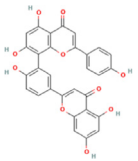
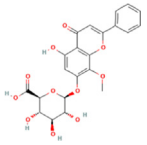
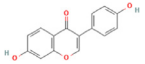
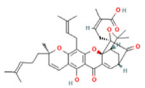
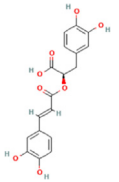
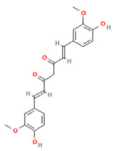
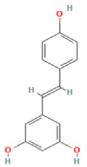
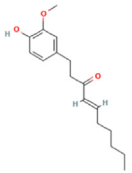
5.3. Alkaloids

Alkaloids demonstrate notable inhibitory effects on the Hh signaling pathway, largely due to their unique chemical structures. These compounds, featuring diverse cyclic configurations such as steroidal backbones and heterocycles, are capable of specifically interacting with SMO or GLI proteins, thereby modulating their activity [114–116]. Cyclopamine, for instance, has a complex six-ring structure that includes a piperidine ring and a fundamental steroid skeleton composed of four interconnected rings (three six-membered and one five-membered) [117]. This steroid backbone can embed within the Smo binding pocket, forming robust hydrophobic interactions with residues such as leucine and isoleucine, which stabilizes Smo in its inactive conformation and effectively halts signal transduction [118,119]. Furthermore, alkaloids can enhance the ubiquitination and degradation of GLI proteins, impeding their transcriptional activation functions. Solasonine, another example, possesses a steroid skeleton made of four linked rings (three six-membered and one five-membered) with a trisaccharide chain attached at the 3-hydroxyl position [120]. The glycosyl group of solasonine interacts via hydrogen bonds with specific residues on GLI1, such as serine and threonine, marking it for ubiquitination and proteasomal degradation, thus inhibiting GLI1 activity [121,122]. Solasodine is the aglycone structure of solasonine, comprising a six-ring arrangement that includes five six-membered rings and one five-membered ring, resulting in a steroid skeleton structurally analogous to cholesterol [123]. Studies have demonstrated that solasodine can bind with high affinity to the zinc finger domain of GLI1, primarily through hydrogen bonding with the amino acid residues THR243 and ASP216 [26]. Given that hydroxyl groups (−OH) can generally serve as both hydrogen bond donors and acceptors, the side chain hydroxyl of THR can act as either, while the carboxyl side chain of aspartic acid (ASP) typically functions as a hydrogen bond acceptor, we infer that hydrogen bonds formed between the hydroxyl group of solasodine and the residues THR243 and ASP216 contribute significantly to this high affinity for GLI1's zinc finger structure [124]. Additionally, the glycosyl group of solasodine can establish further hydrogen bonds with other polar residues on GLI1, such as aspartic acid and glutamic acid, thereby enhancing its inhibitory effects [125–127]. Alkaloids may also influence the subcellular localization of Hh pathway-associated proteins, including SMO, PTCH1, and GLI family members (GLI1, GLI2, GLI3), by modulating cytoskeletal dynamics, which further inhibits signal transduction [128–131].

In summary, natural compounds modulate the Hh signaling pathway primarily through interactions involving their distinct chemical structures, such as cyclic formations, benzene rings, and heterocycles, found in polyphenolic, terpenoid, and alkaloid compounds. These structures enable specific binding to key proteins or regulatory elements of the Hh pathway, thereby influencing signal transduction and pathway regulation.

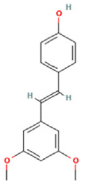
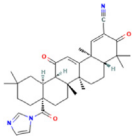
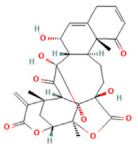
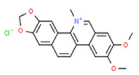
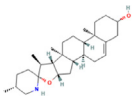
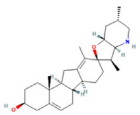
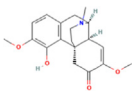
Polyphenolic compounds interact strongly with key proteins in the Hh signaling pathway, such as SMO and GLI transcription factors, through their benzene ring structures and multiple hydroxyl groups, which influence signal transduction. For instance, the benzene ring and hydroxyl groups of resveratrol bind to the active site of SMO, forming hydrogen bonds and a hydrogen bond network that induces conformational changes in SMO, thereby affecting its interaction with downstream signaling molecules [93–96]. Furthermore, polyphenolic compounds can indirectly inhibit the Hh pathway by reducing intracellular ROS levels due to their antioxidant properties [103,104]. Terpenoid compounds inhibit the Hh signaling pathway through their unique cyclic structures and diverse functional groups, which interact with key proteins or molecular targets, thus modulating signal transduction. For example, the molecular structure of forskolin features a unique tetracyclic diterpene skeleton, allowing it to bind tightly to the active site of adenylyl cyclase, promoting cAMP synthesis and consequently negatively regulating the SHH signaling pathway [105,109,110,132]. Additionally, CDDO-Im, with its pentacyclic triterpenoid skeleton and imidazole ring, can specifically interact with GLI proteins through its distinctive structure, thereby inhibiting their activity [12,112]. Alkaloid compounds demonstrate notable inhibitory effects on the Hh signaling pathway, attributable to their distinct chemical structures. These compounds, with diverse cyclic configurations such as steroidal backbones and heterocycles, can selectively interact with SMO or GLI proteins, modulating their activity. For instance, the steroid backbone of cyclopamine embeds within the binding pocket of SMO, establishing strong hydrophobic interactions with hydrophobic amino acid residues, which stabilizes SMO's inactive conformation, effectively locking it in an inactive state and blocking signal transduction [118,119]. Furthermore, alkaloid compounds can promote the ubiquitination and subsequent degradation of GLI proteins, inhibiting their transcriptional activation. For example, the

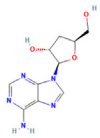
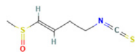
Table 1
Research progress of natural compounds regulating Hedgehog signaling pathway associated with breast cancer.

Group	Name	Chemical structure[a]	Mode of action	Effect	Cell type/mode	Refs.
Flavonoids	Genistein		↓SMO, GLI1, ALDH ↓*Smo* mRNA,*Gli1* mRNA, *Aldh* mRNA ↓$CD44^{+}CD24^{-}$/low cell population in MCF-7 cells	↓stemness of BCSCs	MCF-7/nude mice inoculated with MCF-7 cells	[3]
	Amentoflavone		↓GLI1, SMO, CD44, ALDH1, NANOG, OCT4 ↑ SUFU, PTCH1, CD24	↓the stemness of basal-like BCSCs	SUM159	[4]
	Wogonoside		↓ VEGF, GLI1, SMO ↓*Vegf* mRNA,*Ccnd2*mRNA,*Hip* mRNA, *Gas1* mRNA ↑ubiquitination and proteasomal degradation of SMO	↓cell migration, tube formation, and aorta microvessel out growth ↓formation of blood vessels	MDAMB-231, MDA-MB-46, HUVECs/ nudemice inoculated with MDAMB-231 and MDA-MB-468 cells	[5]
	Daidzein		↓TNF-α, GLI1 ↓*Mmp*-9 mRNA,*Gli1* mRNA	↓mammary tumor cell migration and invasion	ER-negative MCF10DCIS. comMCF10DCIS	[6]
	Gambogic acid		↓SHH, GLI1, PTCH1, Bcl-2 ↑CASP3, BAX	↓MDA-MB-231R (paclitaxel-resistant TNBC cells) proliferation ↑apoptosis, reversed drug resistance in TNBC cells	MDA-MB-231, MDA-MB-468/ female BALB/c-nu/numice inoculated with MDAMB-231R	[7]
Polyphenols	Rosmarinic acid		↓SMO, GLI2, Bcl-2 ↓*Smo* mRNA,*Gli2* mRNA,*Bcl2* mRNA ↑BAX ↑*Bax* mRNA	↓migration viability and migration of MDA-MB-231 and its BCSCs ↑BCSCs apoptotic rate	MDA-MB-231, MCF-10A cell	[8]
	Curcumin		↓GLI1, GLI2, PTCH1, E-cadherin,VIM(Vimentin), OCT-4, SOX2. ↓*Gli1* mRNA, *Gli2* mRNA, *Ptch1* mRNA	↓ proliferation, invasion and migration of MDA-MB-231and MDA-MB-468 cells ↓the formation of BCSCs	MDA-MB-231 and MDA-MB-468/ emale BALB/c-nu/nu mice were inoculated with MDA-MB-231 adherent cells and mammospheres	[9]
	Resveratrol		↑p21. ↑BAX/BCL2L1(Bcl-xL) expression ratio ↓GLI1, SMO, SHH, Cyclin D1, MYC	↓cell viability and proliferation ↑true apoptotic cells	MCF-10A (normal breastepithelial) and MCF-10A-Tr (cigarette smoke condensate mediated transformed breastepithelial) cells	[10]
	6-Shogaol		↓SHH, β-catenin, MYC (c-Myc), Cyclin D1, p-AKT, p-GSK3β, CD44 ↑p-β-catenin	↓BCSCs survival rate ↓size and number of mammospheres ↑auxiliary effects on paclitaxel to reduce the survival of that cell population	BCSCs from MCF-7 human breast adenocarcinoma cell line	[11]

(*continued on next page*)

Table 1 (*continued*)

Group	Name	Chemical structure[a]	Mode of action	Effect	Cell type/mode	Refs.
	Pterostilbene					
Terpenoid	CDDO-IM		↓*Gil1* mRNA, *Gil3* mRNA, *Notch1* mRNA,*Notch3* mRNA,*Tgfbr2* mRNA,*Tgfbr3* mRNA ↓ C-NOTCH1, NOTCH1, NOTCH3, GLI1, SHH, SUFU, p-SMAD2/3 ↑ NOTCH2	↓growth of both SUM159 and MDA-MB-231 cells ↑apoptotic cell death ↓$CD24^{+}$/EpCAM cancer stem cell subpopulation in sphere ↓number and size of tumorspheres in cells,the spher forming efficiency	SUM159 and MDA-MB-231	[12]
	Physalin A		↓SHH, SMO, GLI1, GLI2, CD44, ALDH1 ↓*Pou5f1*mRNA, *Cd44* mRNA, *Sox2* mRNA, M*yc* mRNA, *Nanog* mRNA	↓ proliferation, migration and colony formation of breast cancer cell ↓number and size of the mammospheres ↓frequency of BCSC populations, $CD44^{+}/CD24^{-}$, and ALDH1-expressing subpopulations	MDA-MB-231, MDA-MB-453, HCC-1937, MCF-7	[13]
	Norcantharidin		↑p-β-catenin ↓SHH, SMO, GLI1, P-gp,BCRP ↓*Mdr*-1 mRNA	↓growth of both DOX-sensitive (MCF-7S) and DOX-resistant (MCF-7R) breast cancer celllines; reversed the cells' resistance to chemotherapeutic drugs	MCF-7, BT-474 and MDA-MB-231 cells	[23]
Alkaloids	Huaier aqueous extract	(No details)	↓SHH, SMO, GLI1, GLI2, CD44, ALDHA1, C-NOTCH1 , NOTCH1, NOTCH3, p-SMAD2/3 ↓*Pou5f1* mRNA, *Nes* mRNA, *Nanog* mRNA. ↑NOTCH2	↓formation of primary spheres and the number and size of spheres ↓clonogenic ability of breast cancer cells ↓ $CD44^{+}/CD24^{-}$ cells and stemness gene signatures	MCF-7 cells	[24]
	Nitidine chloride		↓SNAI1, SNAI2, ZEB1, N-cadherin, VIM, CD44, NANOG, NESTIN,POU5F1, PTCH1, GLI1, GLI2, SMO ↓*Snai1* mRNA, S*nal2* mRNA, *Zeb1* mRNA,*Cdh2* mRNA, V*im* mRNA, G*il1* mRNA, *Smo* mRNA ↑E-cadherin ↑C*dh1* (E-cadherin) mRNA	↓proliferation of breast cancer parental cells and mammospheres ↓migration and invasion of breast cancer cells ↓CSCs-like properties of breast cancer cells; TGF-β1 induced EMT and CSC of breast cancer cells	MCF-7, MDA-MB-468	[25]
	Solasodine		↓GLI1, CD44, ALDH1 ↑CD24 ↑*Cd24* mRNA ↓*Gli1* mRNA, *Cd44* mRMA	↑solasodine was enhanced by hyperactivation of GLI1 in breast cancer cells ↓Hh/GLI1 axis, which significantly suppressed MCF7 stem-like cells; the formation of MCF7 tumorsphere	MCF7, MCF10CA1a, MCF10DCIS	[26]
	Cyclopamine		↓PTCH1, SMO, GLI1, GLI2	↑paclitaxel-induced cytotoxicity and inhibition of mammosphere formation in MCF-7 MS cells	MCF7, BT474, T47D, MDA-MB-231, SKBR3, MCF10A, MCF12A	[27]
	Sinomenine		↓*Ccnd1* mRNA,*Bcl2* mRNA ↓MMP2, VIM, IL11 IKBKB , SHH, IκBα	↓effect on the proliferation and migration of MDA-MB-231 breast cancer cells ↓ progression of lung metastasis of breast cancercells	MDA-MB-231/breast cancer-lung metastasis mouse model	[64]

	Cordycepin		↑*Snail, Slug, Zeb1,Cdh2* ↑CYCS, FAS, TNFRSF10A(DR4), TNFRSF10B(DR5) ↓*Notch1,Notch3,Jagged1,Hes1* ↓Bcl-2, XIAP, PDGFRA, E-cadherin, NOTCH1, NOTCH3, JAGGED1,HES1,SMO, GLI1, GLI2	↓apoptosis in BC cells ↓motility, migration, invasion and EMT markers in BC cells	HMECs, MDA-MB-231, MDA-MB-468 and MCF-7	[69]
			↓SHH, PTCH1, SMO, GLI1, GLI2, Cyclin D1, PCNA, Ki-67, N-cadherin, SNAI1, ZEB1, MMP2, MMP9, BCL2. ↑BAX, CASP3, E-cadherin ↓*Shh* mRNA,*Ptch1*mRNA,*Smo.* mRNA,*Gli1* mRNA,*Gli2* mRNA	↓MDA-MB-231 tumor growth, the proliferation of MDA-MB-231 Xenografts; Invasion and metastasis of MDA-MB-231 xenograft ↑apoptosis in MDA-MB-231 xenografts	MDA-MB-231/20 nude mice to establish a human breast cancer MDA-MB-231 xenograft model	[72]
Others	Thiostrepton		↓NANG, SHH, FOXM1, GLI1 ↓*Foxm1* mRNA.-	↓$CD44^{+}/CD24^{-}$ stem-like population, sphere-forming capacity	MDA-MB-231, BT549, T47D	[76]
	Sulforaphene		↓MMP-2, MMP-9, GLI1 ↓*Gli1* ↓*Gli1*mRNA	↓invasion and migration in triple-negative SUM159 human breast cancer cells	MCF7 and T47D, MDA-MB-231, SUM149, SUM159, MCF10 series celllines, MCF10A, MCF10AT1,MCF10DCIS.com MCF10DCIS, MCF10CA1a	[78]
	Cananginone E		↓GLI1, NANOG, EpCAM, SOX2, c-Myc, VIM, N-cadherin, Twist1, SNAIL, MMP9, BCL2 ↑GSK3β, E-cadherin, BAX ↓$CD44^{+}CD24^{-}$/low cell population	↓viability of MCF-7 breast cancer cells ↓migratory and invasive potential of MCF7 cells ↑apoptosis and cell cycle arrestin MCF-7 cells	ShhL2 cells (NIH3T3 cell line with a GLI-dependent); Hep G2, PC3, A549, and MCF7 cells	[80]
	Ginger extract	(No details)	↓*Gli1* mRNA ↑PTCH1n(low concentration inhibits, high concentration promotes) ↓GLI1 ↑ *Ptch1* mRNA	↑apoptosis in BC cells	ER positive cells, MCF7	[91]

[a] PubChem (nih.gov).SMO: smoothened; GLI1: glioma-associated oncogene homolog 1; ALDH: aldehyde dehydrogenase; CD44: cluster of differentiation 44; CD24: cluster of Differentiation 24; OCT4: octamer-binding transcription factor 4; SUFU: suppressor of fused; VEGF: vascular endothelial growth factor; CCDN2: Cyclin D2 Gene; Hip: hedgehog-interacting protein; Gas1: growth arrest-specific 1; TNF-α: tumor necrosis factor-alpha; MMP-9: matrix metalloproteinase-9; SHH: sonic hedgehog; PTCH1:patched 1; BCL-2: B-cell lymphoma 2; CASP3: caspase-3; BAX: BCL2-associated X protein; GLI2: glioma-associated oncogene homolog 2; E-cadherin: epithelial cadherin; SOX2: sry-box transcription factor 2; p21: cyclin-dependent kinase inhibitor 1A; G1/S-Specific Cyclin-D1: G1/S-specific cyclin-D1; MYC: myc proto-oncogene, bHLH transcription factor; β-catenin:catenin beta 1;p-AKT: phosphorylated AKT; p-GSK3β: phosphorylated glycogen synthase kinase 3 beta; p-β-catenin: phosphorylated β-catenin; NOTCH1: neurogenic locus notch homolog protein 1; NOTCH3: neurogenic locus notch homolog protein 3; TGFBR 2: transforming growth factor beta receptor 2;TGFBR 3: transforming growth factor beta receptor 3; C-NOTCH1: cleaved notch1 intracellular domain; p-SMAD2: mothers against decapentaplegic homolog 2; p-SMAD3: mothers against decapentaplegic homolog 3; POU5F1: pou class 5 homeobox 1; Nanog: nanog homeobox protein; P-gp: P-glycoprotein; BCRP: Breast Cancer Resistance Protein; NES: Nestin; SNAIL1: snail family transcriptional repressor 1; SNAIL2: snail family transcriptional repressor 2; ZEB1: zinc finger e-box binding homeobox 1; N-cadherin: neural cadherin; POU5F1: pou class 5 homeobox 1; NESTIN: neuroepithelial stem cell marker protein; IKBKB: inhibitor of nuclear factor kappa B kinase subunit beta; IκBα: inhibitor of nuclear factor kappa B alpha; Slug: snail family transcriptional repressor 2; CYCS: cytochrome c; FAS: fas cell surface death receptor; TNFRSF10A: TNF receptor superfamily member 10A; TNFRSF10B: TNF receptor superfamily member 10B; Notch3: neurogenic locus notch homolog protein 3; JAAGED1: jagged canonical notch ligand 1; Hes1: hes family bhlh transcription factor 1; XIAP: x-linked inhibitor of apoptosis protein; PDGFRA:platelet-derived growth factor receptor alpha; PCNA: proliferating cell nuclear antigen; Ki-67: nuclear proliferation antigen Ki - 67; MMP-2: matrix metalloproteinase-2; MMP-9:matrix metalloproteinase-9; FOXM1:forkhead box protein M1; EpCAM: epithelial cell adhesion molecule.

steroid skeleton of solasonine forms hydrogen bonds with specific surface residues on GLI1, marking it for ubiquitination and proteasomal degradation, thereby suppressing GLI1 activity [121,122].

In conclusion, natural compounds influence the Hh signaling pathway through distinct structural features, including cyclic frameworks, benzene rings, and heterocyclic elements found in polyphenolic, terpenoid, and alkaloid compounds. These structures enable interactions with critical proteins or regulatory elements within the Hh signaling pathway, thereby modulating signal transduction and pathway regulation. These compounds also exhibit selective inhibitory effects on BC stem cells by targeting essential molecules in the Hh signaling pathway, disrupting cancer cells' reliance on this pathway, and demonstrating molecular selectivity (Table 1) [3–13,23–27,64,69,72,76,78,80,91].

6. The current status of research on Hh signaling pathway inhibitors and their clinical applications

The Hh signaling pathway is extensively studied in the context of cancer development and is increasingly recognized as a promising target for antitumor drug development. Current inhibitors of the Hh pathway are primarily categorized into three classes: Hh ligand inhibitors, SMO inhibitors, and downstream SMO target inhibitors, which include GLI1 antagonists (Table 2).

6.1. Inhibitors targeting the Hh ligand

Ligand inhibitors targeting the Hh signaling pathway are primarily divided into two classes: Hh protein inhibitors and Hh acyltransferase inhibitors [132,133]. Within the Hh protein inhibitors, SHH-specific inhibitors include the monoclonal antibody 5E1, which demonstrates tumor-suppressive effects in pancreatic cancer models [134,135]. Another class, RU-SKI compounds, inhibit Hedgehog acyltransferase (HHAT)-mediated palmitoylation of SHH, although they may be associated with higher cytotoxicity [136]. The macrocyclic peptide HL2-m5 disrupts the interaction between SHH and PTCH1, effectively inhibiting SHH-mediated signaling and gene transcription [137]. However, these inhibitors primarily target the classical Hh ligand pathway and are not yet widely explored in non-canonical Hh signaling. Further research is necessary to expand the scope of drug action and to address associated challenges.

6.2. Inhibitors targeting SMO

The Hh signaling pathway plays a crucial role in cellular regulation. Common SMO inhibitors, including vismodegib, sonidegib, and glasdegib, have shown considerable efficacy in treating basal cell carcinoma [133,138]. However, prolonged treatment often leads to resistance, and side effects present a significant challenge in clinical applications. To address these issues, novel inhibitors such as saridegib, BMS-833923, and taladegib are currently in clinical trials, with the potential to offer more effective treatment options [139]. Additionally, the naturally occurring SMO inhibitor cyclopamine has attracted considerable research interest. Recent studies have reported novel asymmetric synthetic methods for cyclopamine, paving the way for further research and development. Although SMO inhibitors show promise in cancer therapy, continued research is essential to overcome challenges related to resistance and side effect management [140].

6.3. Inhibitors targeting GLI

GLI proteins serve as terminal transcription factors in the Hh signaling pathway, making them strategic targets for inhibiting Hh pathway activation. Inhibiting GLI, either directly or indirectly, can effectively suppress the Hh signaling pathway, particularly in cases of SMO inhibitor resistance or activation of non-canonical Hh signaling pathways. The first class of GLI inhibitors, including compounds like GANT-58 and GANT-61, are primarily utilized as tools in drug development [141]. GLI-inhibiting drugs, such as TAK-441 and FN1-8, have shown potential in cancer therapy [142]. Although certain compounds, like arsenic trioxide, have been approved and demonstrated to inhibit GLI transcriptional activity, no GLI inhibitors have been approved specifically for market release [142,143]. GLI inhibitors bypass upstream abnormalities in the Hh pathway, thereby circumventing the issue of resistance associated with SMO inhibitors. Despite promising preclinical studies involving various small molecules, no compound has demonstrated distinct advantages in terms of potency and selectivity.

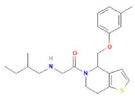
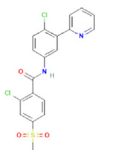
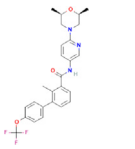
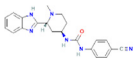
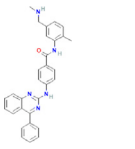
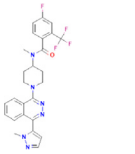
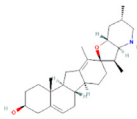
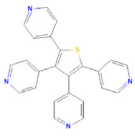
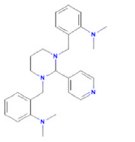
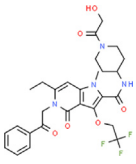
The diversity of Hh signaling pathway abnormalities in tumorigenesis, especially those arising from *SMO* mutations and the activation of non-canonical Hh signaling, is increasingly recognized by researchers. It is understood that a single inhibitor may be insufficient to fully suppress tumorigenesis and may lead to chemoresistance and other adverse effects [133,144,145]. Consequently, developing inhibitors that specifically target the Hh signaling pathway has become imperative. Due to their structural diversity and enhanced biocompatibility, natural compounds offer promising advantages, potentially minimizing toxicity and side effects. However, research into natural compounds as Hh pathway inhibitors remains in experimental stages, necessitating further investigation to assess their clinical potential and safety.

While all 23 natural compounds previously discussed exhibit inhibitory effects on the Hh signaling pathway, we posit that resveratrol and curcumin, prominent polyphenolic compounds, demonstrate the most promising potential. First, polyphenolic compounds are advantageous due to their antioxidant, anti-inflammatory, and anti-cancer properties, alongside low toxicity profiles. Moreover, both resveratrol and curcumin have shown efficacy in both *in vivo* and *in vitro* models across various cancers. Notably, resveratrol enhances curcumin uptake in BC cells in a dose-dependent manner [10]. Additionally, curcumin has been shown to reduce cancer cell stemness by lowering the expression of GLI and EMT-related genes, such as *CDH1* and *VIM*, in BCSCs [9]. Despite their therapeutic potential, the clinical application of both compounds is limited by factors such as low bioavailability and structural instability. Therefore, further studies to optimize their biological activity and stability are crucial to advance their clinical utility.

7. Discussion

The Hh signaling pathway plays a crucial biological role in the development and progression of BC, highlighting its significance in tumorigenesis. The Hh pathway is involved in various processes, such as cell proliferation, apoptosis, and organ growth, and may regulate the proliferation and differentiation of BC cells [15,16]. Studies indicate that the Hh signaling pathway may contribute to BC progression and drug resistance, potentially by modulating EMT and drug-induced dedifferentiation mechanisms [7,146]. Furthermore, the Hh pathway in BC may be associated with other biological processes, such as flexible cellular metabolism and characteristics of persistent tumor cells [147,148]. Research on the therapeutic role of natural compounds in BC shows an increasing focus on elucidating mechanisms of Hh pathway regulation. These natural compounds influence the biological properties of BC cells by targeting both canonical and non-canonical Hh signaling pathways [79]. The inhibitory mechanisms of these natural compounds can be categorized into three primary types: (1) Direct inhibition of key protein expression, such as decreasing mRNA and protein levels

Table 2
Summary of chemical structures of Hedgehog (Hh) signaling pathway inhibitor.

Inhibitor class	Compound name[a]	Chemical structure	Molecular formula	Molecular weight (g/mol)	Therapeutic applications
Hh ligand inhibitors	RU-SKI		$C_{22}H_{30}N_2O_2S$	386.6	(No details)
SMO target inhibitors	Vismodegib		$C_{19}H_{14}Cl_2N_2O_3S$	421.3	Basal cell carcinoma
	Sonidegib		$C_{26}H_{26}F_3N_3O_3$	485.5	Basal cell carcinoma
	Glasdegib		$C_{21}H_{22}N_6O$	374.4	Chronic myelomonocytic leukemia
	BMS-833923		$C_{30}H_{27}N_5O$	473.6	Solid tumor/cancer
	Taladegib		$C_{26}H_{24}F_4N_6O$	512.5	Ovarian cancer
	Cyclopamine		$C_{27}H_{41}NO_2$	411.6	Solid tumor/cancer
GLI target inhibitors	GANT-58		$C_{24}H_{16}N_4S$	392.5	(No details)
	GANT-61		$C_{27}H_{35}N_5$	429.6	Solid tumor/cancer
	TAK-441		$C_{28}H_{31}F$	576.564	Solid tumor/cancer

[a] PubChem (nih.gov). SMO: Smoothened; GLI: glioma-associated oncogene homolog

of critical molecules like SMO, GLI, PTCH1, SUFU, and SHH, effectively blocking Hh signaling at the source; (2) Modulation of protein activity and localization, where certain compounds directly bind to SMO, promote its ubiquitination to reduce its activity, or inhibit GLI nuclear translocation, thereby diminishing transcriptional activity and weakening signal transduction; (3) Regulation of downstream effects, whereby these compounds inhibit the activation of SMO and GLI, subsequently downregulating MMP-9 and MMP-2 expression to curb tumor invasion and metastasis, while also reducing crosstalk between Hh and other pathways (Hh/Hippo, SHH/mdr-1), and Hh/AkT/GSK3β pathways). By analyzing natural compounds known to inhibit the Hh signaling pathway, we identified common structural features associated with their inhibitory activity. For example, the presence of aromatic rings is directly related to inhibitor potency, and unique amino acid residues in natural compounds are also critical in constructing inhibitors,

providing a clear direction for designing more targeted inhibitors in future studies [149,150].

The inhibitory effects of natural compounds on the Hh signaling pathway are primarily determined by the structure-activity relationships of different types of natural compounds. Generally, hydroxybenzoic acid, flavonoid structures, and benzene rings in polyphenolic compounds interact with key proteins within the Hh signaling pathway, such as GLI proteins, to modulate signal transduction. These compounds may also interfere with normal signal transmission by affecting the conformation of the Hh pathway receptor SMO [91,96]. The cyclic structures and functional groups in terpenoids are crucial for their effects on the Hh pathway. Terpenoids can interact with key proteins or molecular targets in the Hh pathway through specific interactions, such as hydrogen bonding, van der Waals forces, or hydrophobic interactions, influencing the signaling process [104,107–109]. Alkaloids, with diverse chemical structures that include alicyclic rings, benzene rings, heterocycles, and unique functional groups, modulate signal transduction and downstream gene expression by interacting with key proteins within the Hh pathway. This study systematically summarizes the potential of natural compounds to inhibit BC cells via the Hh pathway, particularly through the SMO-GLI1 axis's impact on the BCSC population. Previous research has shown that the Hh pathway plays a role in maintaining the self-renewal and proliferation of cancer cells, with the SMO-GLI1 axis serving as a critical regulatory hub, especially influential in controlling stem cell populations [44]. By targeting this axis, natural compounds can significantly inhibit the survival and self-renewal abilities of BCSCs, thereby reducing tumor invasiveness and recurrence risk [3,4]. These findings provide valuable insights for developing therapeutic strategies against BCSCs and inform recommendations for BC treatment and the design of personalized therapeutic approaches.

Compared to traditional chemotherapeutic agents, natural compounds generally exhibit low toxicity and higher tolerability, which is especially advantageous for cancer patients. However, significant challenges in clinical application remain, such as low bioavailability and poor stability *in vivo* [22]. Future research may explore methods such as nanocarrier technology and chemical modification to enhance these compounds' *in vivo* stability and targeting capacity, thus improving therapeutic outcomes. Additionally, high-throughput screening to identify and optimize natural compounds and their derivatives, or the use of synergists to boost *in vivo* activity, are potential solutions. To further improve therapeutic efficacy, future studies could investigate the combined application of natural compounds with other anticancer drugs. This multi-targeted combination strategy can capitalize on the inhibitory effects of natural compounds on the SMO-GLI1 axis, while leveraging the specificity of other therapies to provide a multifaceted approach against BC.

In summary, natural compounds demonstrate substantial therapeutic potential in inhibiting the Hh signaling pathway and regulating BCSC populations, offering a valuable reference for developing personalized treatment strategies targeting BCSCs.

8. Conclusion

This study provides a detailed examination of the regulatory role of the Hh signaling pathway in BC treatment through the perspective of natural compounds. By exploring the mechanisms of action of various natural compounds, we elucidated both their unique and overlapping effects on the Hh signaling pathway. Notably, this research offers the first comprehensive summary of the principal molecular pathways through which natural compounds modulate the Hh signaling pathway, highlighting their mechanisms for mitigating the aggressive characteristics of BC. Furthermore, the investigation of the structure-activity relationship between natural compounds and their targets yields novel insights for drug development and the clinical application of natural compounds. These findings establish a critical foundation for the development of innovative B treatment strategies and suggest promising directions for leveraging natural compounds in cancer therapy.

CRediT authorship contribution statement

Yining Cheng: Writing – original draft, Investigation, Data curation, Conceptualization. **Wenfeng Zhang:** Writing – original draft, Investigation, Data curation, Conceptualization. **Qi Sun:** Investigation, Data curation. **Xue Wang:** Investigation, Data curation. **Qihang Shang:** Writing – review & editing. **Jingyang Liu:** Writing – review & editing. **Yubao Zhang:** Writing – review & editing. **Ruijuan Liu:** Writing – review & editing, Validation, Supervision. **Changgang Sun:** Writing – review & editing, Writing – original draft, Validation, Supervision, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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