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Exploring Natural Compound Pathways in Breast Cancer Therapy Through Hedgehog Signal Modulation

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ABSTRACT

With a steadily rising incidence rate, breast cancer (BC) is one of the most common malignant tumors afflicting women globally. Treatment methods for this illness have so drawn a lot of interest. Studies have shown how important the Hedgehog (Hh) signaling system is to the development and spread of BC, especially in fostering tumor growth and metastasis. Therefore, molecular targets in this pathway provide encouraging prospects for the creation of new BC treatments. Clarifying the therapeutic pathways via which natural compounds alter the Hh signaling system in BC is the goal of this work. A thorough analysis of several natural substances, including as polyphenols, terpenes, and alkaloids, revealed both common and distinct regulatory mechanisms influencing this pathway.

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1. Introduction

The unchecked proliferation of aberrant breast cells, which results in the creation of tumors, is the hallmark of breast cancer (BC). It continues to be the primary cause of cancer-related deaths in women. According to epidemiological research, the high incidence and development of BC are substantially correlated with the over-activation of the Hedgehog (Hh) signaling system [1]. The crucial significance of the Hh pathway in the pathophysiology of BC has also been highlighted by studies showing a positive association between the abnormal expression of Hh pathway genes and increased tumor growth, lymph node metastasis, and recurrence risk [2]. Natural substances have drawn a lot of attention lately as possible Hh signaling pathway modulators. It has been discovered that several natural substances, including flavonoids, polyphenols, and plant extracts, block the Hh pathway via a variety of ways, indicating their potential therapeutic efficacy. while treating BC [3e11]. These substances may have a beneficial effect on the therapy of BC by blocking the activation of the Hh signaling pathway via a variety of methods. Thus, the purpose of this research was to investigate natural substances as possible inhibitors of the Hh signaling pathway and provide a thorough evaluation of their potential for BC treatment. A key player in BC, the Hh signaling pathway influences angiogenesis, tumor behavior, and the destiny of cancer stem cells (CSCs) [5,12,13]. In particular, the transmembrane receptor-torpatched 1 (PTCH1) and the transcription factors GLI family zincfinger 1 (GLI1) and GLI family zincfinger 2 (GLI2) are overexpressed in breast cancer stem cells (BCSCs), which increases the cells' ability to self-renew [14]. As a crucial transcription factor in the Hh signaling pathway, GLIfamily zincfinger 3 (GLI3) may have a role in BCSCs, albeit its precise function is yet unknown. Although they are expressed at different phases of the development of the mammary gland in mice, Hhpathway components are usually dormant [15]. On the other hand, abnormal Hh signaling leads to increased pathway activity and aids in the onset and maintenance of BC. Hh signaling is dysregulated in many BC tissues [16]. The prognosis of BC patients may be improved by blocking this route, since Tao et al. [17] showed that abnormal Hh signaling is linked to ductal

alterations and malignant transformation in breast tissue. In BC, both ligand-dependent and ligand-independent pathways contribute to the stimulation of Hh signaling. The Hh ligand attaches itself to PTCH1 in the ligand-dependent pathway, releasing its inhibition of the transmembrane receptor smoothened (SMO). This activates SMO and initiates signal transduction, which ultimately controls genes relevant to BC [18]. The transmission of signals from the membrane receptor SMO to the transcription factor GLI is a crucial stage in the Hh signaling pathway. Numerous regulatory molecules moderate this process, which entails complex cascade processes involving different protein kinases and regulatory factors that eventually control the expression of downstream target genes. Understanding the function of Hh signaling in the onset and progression of BC requires elucidating the intricate processes and important molecular actors in this pathway [19]. Type I of the ligand-independent pathway involves PTCH1 inhibiting SMO and promoting cell death; type II involves SMO's autonomous activity driving cell migration without the involvement of GLI1; and type III involves post-translational modifications of GLI1, which are essential for transcription and intracellular transport in BC [20,21]. Therefore, a possible therapeutic approach for the treatment of BC is the development of new inhibitors that target the Hh pathway. Even though the Hh signaling pathway has a lot of promise for cancer treatment, problems including drug resistance and unfavorable side effects still exist, which emphasizes the need for innovative therapeutic approaches. Natural compounds are linked to better tolerance and reduced toxicity when compared to traditional chemotherapy [22]. Proliferation, invasion, the epithelial-mesenchymal transition (EMT), cancer stemness, angiogenesis, and drug resistance are among the functional characteristics of cancer cells that are intimately linked to aberrant activation of the Hh signaling pathway in BC. Certain natural substances alter the Hh signaling system, which impacts the activities of BC cells. This may prevent tumor development, metastasis, and drug resistance. It may also control the tumor microenvironment, offering novel approaches to treating BC [5,23e27]. According to our comparison study, natural substances may suppress BCSCs via the Hh signaling system, primarily via modifying the SMO-GLI1 axis to lower the percentage of BCSCs. It has been discovered that a wide range of natural substances block the Hh signaling pathway via different ways. Based on an investigation of these ways of action, this work finds three main inhibitory mechanisms: (1) downstream impact regulation; (2) protein activity and localization modification; and (3) direct suppression of essential protein expression. Furthermore, natural substances like polyphenols, terpenes, and alkaloids have unique chemical structures that improve their capacity to block the Hh signaling pathway. For example, various structural characteristics, such as heterocyclic rings, ben-zene rings, and ring structures, might interact with important Hh pathway molecules like SMO and GLI to impede signal transmission. Research on how natural substances affect the Hh signaling system helps to better understand the molecular processes at play, which in turn helps to identify novel therapeutic targets and provide more accurate and potent medications for the treatment of BC. We may investigate new and more effective treatment strategies, overcome present therapeutic obstacles, and provide creative avenues for the development of novel medications by comprehending how these substances alter the Hh signaling system.

2. The Hhsignaling pathway's composition and transduction The Hh Signaling Pathway's component

The GLI family of transcription factors (GLI1, GLI2, GLI3), two transmembrane receptors, PTCH1 and SMO, Hh ligands, and many downstream target genes make up the majority of the Hh signaling pathway. The Hh ligand has three structurally and functionally comparable homologs: SonicHedgehog (SHH), IndianHedgehog (IHH), and DesertHedgehog (DHH). Despite having many functional similarities, these homologs affect distinct organs [18]. As the main receptor for SHH, PTCH1 is essential for the Hh signaling pathway's inhibition. By inhibiting the action of the SMO protein, PTCH1 functions as a tumor suppressor gene that controls cellular growth. This inhibitory impact is alleviated with SHH binding to PTCH1, enabling the activation of downstream GLI-mediated signal transduction [28]. Ptch1 and Ptch2 are two homologs of the Patched gene family found in vertebrates. Under some circumstances, PTCH2 enhances PTCH1's activity, even though PTCH1 typically serves as a membrane receptor that adversely controls the Hh signaling pathway [29]. The proto-oncogene SMO encodes SMO, a G protein-coupled receptor (GPCR) that mediates extracellular ligand binding and starts intracellular signaling. As the primary transcriptional regulators of the Hh pathway, the GLI family—which includes GLI1, GLI2, and GLI3—promotes the transcription of target genes in effector cells [19]. Despite the fact that all GLI proteins have similar biochemical functions, GLI1's activity is restricted to transcriptional activation because it lacks the N-terminal repressor domain that GLI2 and GLI3 have [30]. Important biological processes, ranging from embryonic development to adult homeostasis, depend on these

signaling molecules. Consequently, the development of several disorders at different stages of life is intimately associated with abnormal expression of these genes and their downstream targets [31].

The Hh signaling pathway's transduction Hh signaling pathway transduction during typical breast growth
The components of the Hh signaling system are expressed differently in breast tissue at various stages of development. The majority of canonical Hh signaling is suppressed during breast development. The GLI family zinc finger 3repressor (GLI3R) actively suppresses GLI1, which is essential for the development of early mammary budsinmice. The loss of mammary has been evidence of this. buds in mice whose mammary tissues lack GLI3 due to induced GLI1 expression [32]. Terminal bud elongation during puberty is facilitated by the type I atypical Hh signaling pathway, which stimulates a proliferative cascade in the luminal epithelial cells of the mammary ducts. Mature mammary glands have decreased expression levels of GLI1, GLI2, GLI3, PTCH1, and Hh ligands during puberty [33]. Therefore, type I atypical Hh signaling is implicated in ductal morphogenesis throughout puberty and is subsequently downregulated in normal adult breast tissue, while canonical Hh signaling is mostly inhibited during mammary gland development [32]. Hh signal transduction in BC: classic and non-classic
In the classical Hh signaling pathway, the HH ligand relieves the repression of SMO by binding to PTCH1 when it is plentiful. As a result, SMO is able to go to the main cilium, where it undergoes phosphorylation and undergoes a conformational shift that activates GLI. Following their translocation to the nucleus, activated GLI proteins operate as transcription factors, controlling the expression of Hh target genes. These target genes, which together drive cellular responses to Hhpathway activity [35e38], include cyclin D (CCND), cyclin E (CCNE), GLI1, PTC1, and PTC2 (Fig.1A). When the Hh ligand is not present, PTCH1 attaches itself to SMO, blocking its passage into the main cilium and so blocking the traditional Hh signaling pathway. [39] (Figure 1B). Three forms of non-canonical Hhsignaling exist outside of the canonical pathway: type I includes PTCH1-independent pathways that may suppress tumor development or encourage apoptosis [40]. TUCAN (CARD8, Cardinal) is recruited by PTCH1 in order to form a pro-apoptotic complex with the down-regulated rhabdomyosarcoma LIM protein (DRAL). Apoptosis is eventually induced by this complex's activation of caspase-9, which in turn activates caspase-3 [41]. By attaching itself to the C-terminal region of PTCH1, DRAL, sometimes referred to as four and a half LIMdomains 2 (FHL2), interacts with PTCH1 and may control its stability or function. According to reports, TUCAN, an apoptosis regulating protein, interacts with PTCH1, connecting the Hh signaling pathway to apoptotic pathways [42]. (Figure 2A). SMO activity, which functions independently of GLI1, mediates type I in non-canonical signaling [43]. Ras homolog family member A (RhoA) and Ras-related C3 botulinum toxin substrate 1 (Rac1) may be activated by this route, leading to cytoskeletal reorganization, cell migration, and axon guidance [44] (Fig. 2B). Type III includes GLI1 activation mechanisms that are not reliant on PTCH1-SMO signaling [45]. Other signaling molecules like transforming growth factor-beta (TGF-b), epidermal growth factor (EGF), and tumor necrosis factor-alpha (TNF-a) as well as their downstream effectors, including suppressor of mothers against decapentaplegic3 (SMAD3), Ras-ERK, and nuclear factor-kappaB, are the main sources of activation of GLI1 in this pathway.

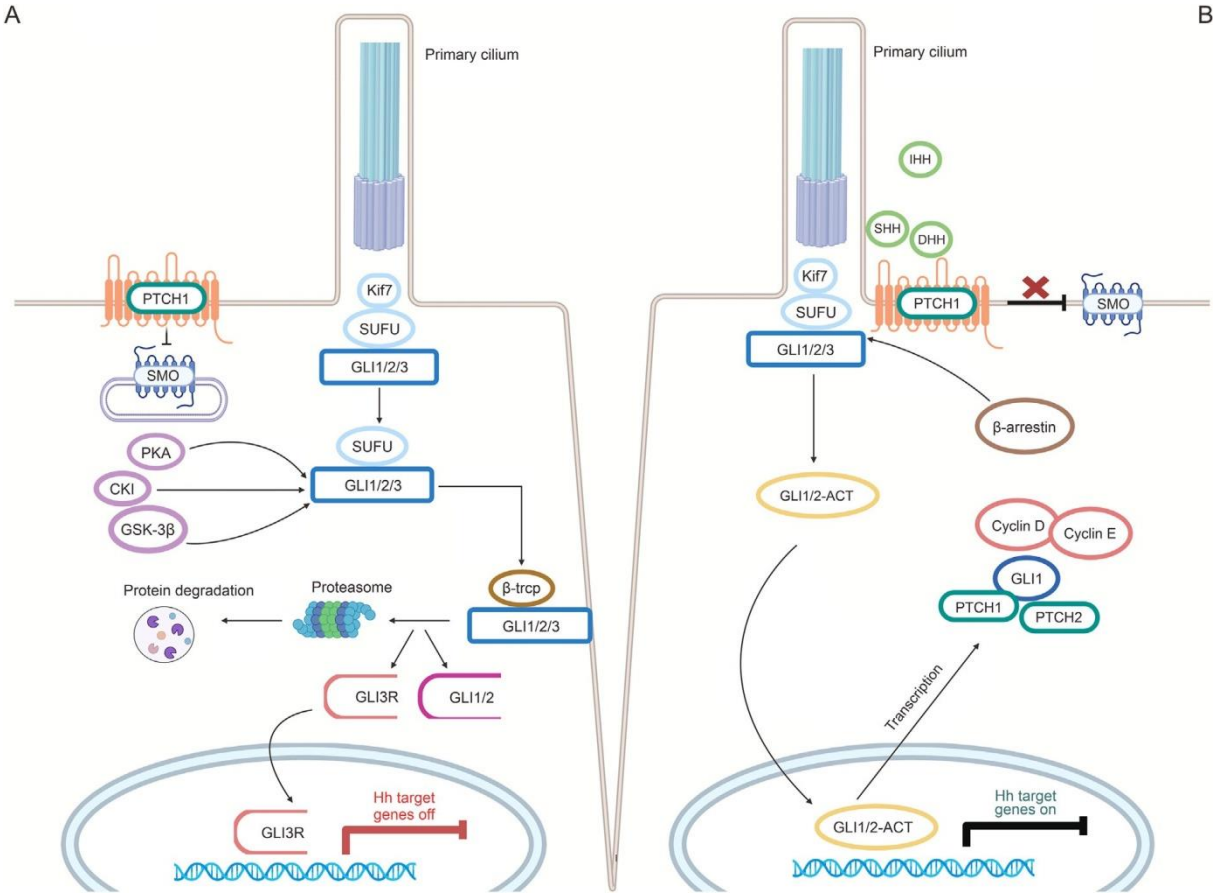

Figure 1 shows how the traditional Hedgehog (Hh) signaling pathway is transmitted. (A) Patched 1 (PTCH1) suppresses Hh target genes, GLI transcription factors are phosphorylated and degraded, and smoothened (SMO) is inhibited in the signal-off state. (B) In the signal-on state, active GLI transcription factors reach the nucleus, alleviate PTCH1 repression of SMO, and start transcription of Hh target genes. GSK-3b: glycogen synthase kinase 3 beta; Kif7: kinesin family member 7; SUFU: suppressor of fused; PKA: protein kinase A; CKI: casein kinase I; GLI1 stands for GLI family zinc finger 1, GLI2 for GLI family zinc finger 2, GLI3 for GLI3R for GLI3R repressor, b-Trcp for beta-transducin repeat-containing protein, IHH for Indian hedgehog, SHH for Sonic hedgehog, and DHH for Desert hedgehog, and GLI1/2-ACT for GLI1 and GLI2 activator forms.

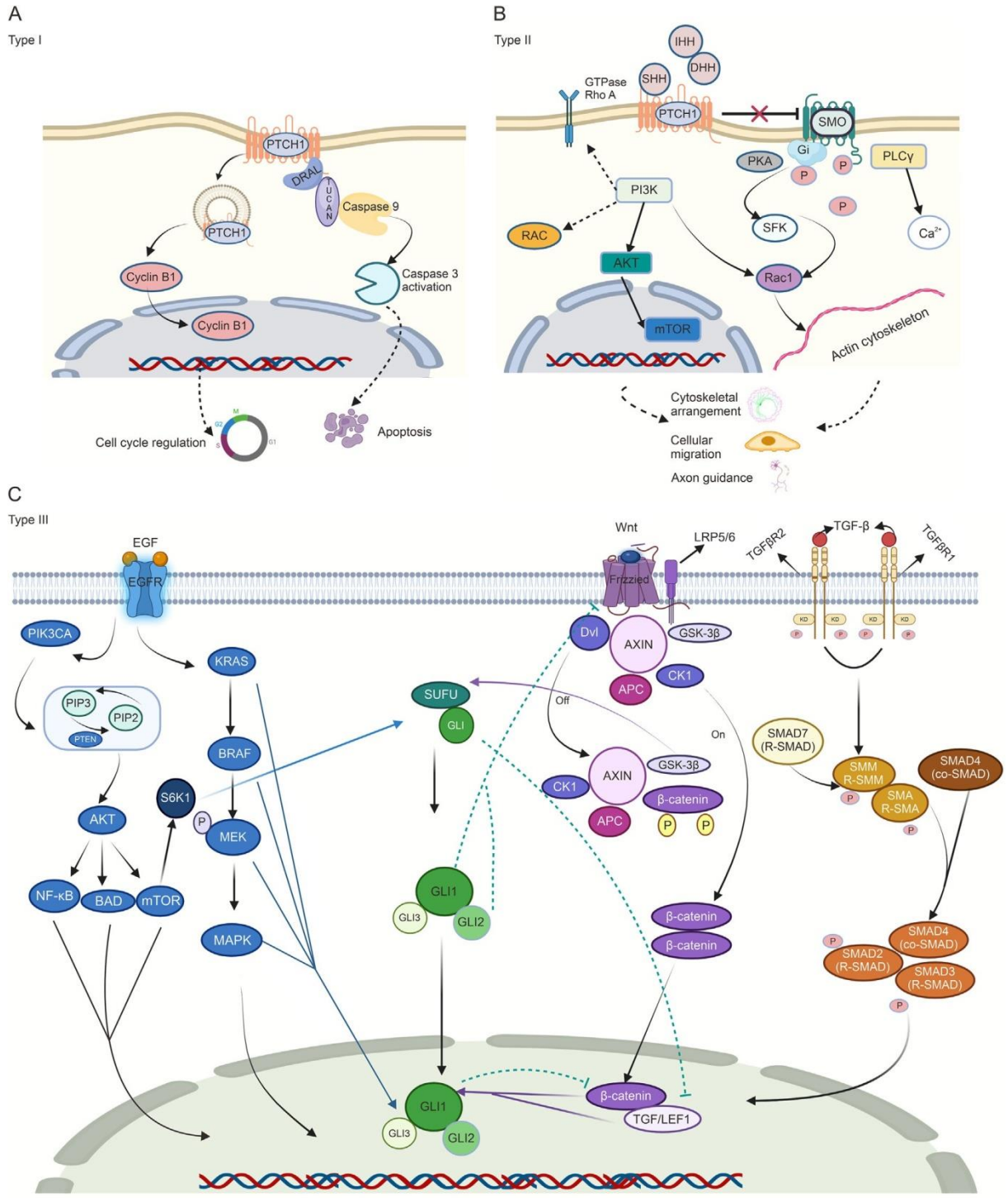

Fig. 2.The transmission of the non-classical Hedgehog (Hh) signaling pathway.

(A) Patched 1 (PTCH1)-independent actions, where down-regulated in rhabdomyosarcoma LIMprotein (DRAL) and tumor-up-regulated CARD-containing antagonist of caspase-nine (TUCAN) form a pro-apoptotic complex that activates caspase-9 and caspase-3, erebyregulatingcellapoptosisandthecellcycle.(B)Smoothened(SMO)-mediatedsignalingthatisindependentofGLIfamilyzincfinger1(GLI1).SMOactivatessmallGproteinssuchasRac1andRhoA,influencingcytoskeletalrearrangementandconsequentlyregulatingcellmigrationandaxonguidance.(C)PTCH1-SMOindependentmechanismofGLI1activation.It shows how multiple signaling pathways including epidermal growth factor (EGF) , Wnt, and transforming growth factor-beta (TGF-b) converge to ultimately GLIfamilyzincfinger2(GLI2)transcriptionfactors.Thismechanisminvolvesacomplexsignalingnetwork,includingtheparticipationofvariousmoleculessuchasKirstenratsarcomaviraloncogenehomolog (KRAS), proteinkinase B(AKT),b-catenin,and suppressorofmothersagainst decapentaplegic (SMAD) proteins,ultimatelyleading tothe activation

andnucleartranslocationofGLItranscriptionfactors.IHH:IndianHedgehog;SHH:SonicHedgehog;DHH:DesertHedgehog;PKA:proteinkinaseA;PI3K:phosphatidylinositol3-kinase;SFK:Srcfamilykinases;RAC:RacfamilysmallGTPase1;PLCg:phospholipaseCgamma;mTOR:mammaliantargetofrapamycin;EGF:epidermalgrowthfactor;EGFR:epidermalgrowth 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 S6kinase beta-1; MEK: mitogen-activated protein kinase kinase; NF-kB: nuclear factor-kappa B; BAD: Bcl-2-associated death promoter; MAPK: mitogen-activated protein kinase;SUFU: suppressor of fused; GLI3: GLI family zincfinger 3; LRP5/6: low-density lipoprotein receptor-related protein 5/6; Dvl: dishevelled; AXIN: axis inhibitionprotein; APC: an-tigen-presenting cell; CK1: casein kinase 1; GSK-3b: glycogen synthase kinase 3 beta; TGF: transforming growth factor; LEF1: lymphoid enhancer-binding factor 1; TGFbR2:transforming growth factor beta receptor 2; TGFbR1: transforming growth factor beta receptor 1; SMM: smooth muscle myosin; SMA: smooth muscle actin. (NF-kB). Important cellular functions such as development, survival, and proliferation are impacted by this activation [6,46e48] (Fig. 2C). Furthermore, the intracellular transport and transcriptional activity of the SHH signaling pathway are greatly impacted by post-translational modifications of GLI1, such as phosphorylation, ubiquitination, acetylation, and O-GlcNAcylation, which regulate carcinogenesis and the advancement of cancer [49e51]. Although abnormal overexpression of SHH has been linked to tumor growth and changes within the tumor microenvironment, mutations in SHH, PTCH1, and GLI1 are uncommon in BC and do not seem to be directly implicated in the activation of the Hh pathway [2]. Even though BC does not have any mutations in the Hh gene, BC development may still be influenced by activation of the conventional Hh pathway [52]. Additionally, the overexpression of SHH has been associated with increased tumor invasiveness, lymphatic infiltration, and metastasis in addition to the upregulation of the pro-angiogenic transcription factor cysteine-rich angiogenic inducer 61 (CYR61), which promotes highly vascularized tumors [53,54]. Although the role of type I non-canonical Hh signaling in BC is still poorly understood, type I non-canonical Hh signaling has been implicated in processes like angiogenesis, cell migration, and the activation of small Rho GTPases (Rho), indicating that type II signaling may be important in the tumor stroma [55]. The primary regulatory mechanism in the Hh signaling pathway is the SMO-GLIaxis. A crucial step in controlling the expression of downstream target genes is the transmission of signals from the membrane receptor SMO to the transcription factor GLI via the HH signaling pathway [45]. Key kinases like protein kinase A (PKA), glycogen synthase kinase 3 beta (GSK3b), casein kinase I (CKI), kinesin family member 7 (KIF7), suppressor of fused (SUFU), and dual specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) are involved in a sequence of phosphorylation cascade reactions that are triggered upon SMO activation [19,56e59]. The last stage in transferring the Hh signal from the membrane to the nucleus is the regulation of GLI transcription factors' activity and subcellular localization by these cascades. In particular, PKA and GSK3b phosphorylation encourages GLI3 to change into its restrictive form, GLI3R, which inhibits the transcription of Hh target genes [56,57]. On the other hand, GLI1 and GLI2 are stabilized by CKI phosphorylation, which also promotes their nuclear translocation and activates the transcription of Hhtarget genes [60]. SUFU operates as a negative regulator of the Hh pathway by directly binding and inhibiting GLI transcription factors. KIF7 interacts with SMO, controlling its subcellular location and activity, hence impacting the activation of GLI transcription factors [58]. But when the Hh pathway is activated, SUFU becomes phosphorylated, which causes GLI to be released [19]. Furthermore, GLI1 is stabilized and phosphorylated by DYRK1A, which increases its transcriptional activity. Additionally, SUFU is phosphorylated by DYRK1A, which reduces its inhibitory impact on GLI [59]. Furthermore, b-arrestin attaches itself to SMO and participates in signal transduction and intracellular trafficking of SMO. This relationship controls SMO's activity state, which in turn influences how downstream GLI transcription factors are regulated [61]. Additionally, via additional signaling pathways such the rat sarcomaviral oncogene homolog/mitogen-activated protein kinase (RAS/MAPK) and phospha-tidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathways, SMO activation may indirectly alter GLI transcriptionfactor activity [62,63]. All things considered, this intricate regulatory system guarantees accurate control of Hh target gene expression, which is essential for a number of pathological and developmental processes. Carcinogenesis regulation by the Hh signaling pathway SHH is an essential protein in the Hh signaling pathway that binds to the cell surface receptor PTCH1 to start signal transmission [15]. In the absence of SHH binding, PTCH1 inhibits signal transduction by acting as a negative regulator of the SHH pathway [52]. Furthermore, the main effector molecules in this pathway are the GLI family of proteins. Upon activation, these proteins go to the nucleus as transcription factors, where they control target gene expression, eventually impacting cellular differentiation and proliferation [35]. Natural substances have shown significant therapeutic promise in BC in recent years by altering the Hh signaling system. Through a variety of ways, these substances may modulate the activity of the Hh signaling pathway by directly or indirectly regulating the production of important proteins such SHH, PTCH1, and GLI1. Control of important proteins in the HH signaling pathway in BC Control of SHH in BC In BC, the NF-kB transcription factor controls the expression of SHH genes. One important inflammatory signaling mediator, NF-kB, stimulates the proliferation, migration, differentiation, and self-renewal of cancer cells [64]. It has been demonstrated that NF-kB positively regulates SHH gene

expression in a variety of cancers, including BC [65]. Additionally, CpG island methylation in the SHH promoter region is linked to the suppression of SHH gene expression. Experimental research shows that using DNAmethylation inhibitors to treat breast cancer cell lines lowers SHH promoter methylation, which in turn increases SHH expression [66]. In stomach cancer, a similar effect has been seen [67]. Furthermore, DNA methylation inhibitors further enhance SHH gene upregulation in BC cells stimulated with NF-kB activators, an effect not seen when NF-kB inhibitors are applied [65,68]. These findings imply that NF-kB co-regulates SHH gene expression in BC at both the transcriptional and epigenetic levels. It is still mostly unknown how natural substances might prevent BC metastases. Nevertheless, it has been shown that natural substances such as cordycepin and sinomenine restrict SHH activation by obstructing NF-kB signaling in the non-canonical Hh pathway, which in turn suppresses BC metastasis [64,69]. PTCH1 expression in BC cells PTCH1 is a known surrogate marker for Hh pathway activation because, despite its role as a negative regulator of Hh signaling, its expression is elevated by GLI-dependent transcription [45]. However, PTCH1's ac-curated identification in BC tumors is difficult because to its generally low expression and the limits of commercial antibodies. Research has demonstrated that in MCF7BC cells, the downregulation of PTCH1mRNA is connected to increased promoter methylation [70]. After attaching to radiolabeled SHH proteins, another research showed enhanced PTCH1 expression in a number of BC cell lines [71]. However, SHH binds to various receptors, making it more difficult to interpret these findings. Therefore, further investigation is required to resolve the disparities across studies and ascertain if BC characteristics are connected to dysregulation of PTCH1 expression. According to some research, some elements of traditional Chinese medicine may alter signaling pathways, providing possible approaches to control the dysregulation of PTCH1 expression. For example, in triple-negative breast cancer (TNBC), the administration of natural compounds like curcumin or cordycepin, either alone or in combination, dramatically lowers the levels of SHH, GLI1, and PTC1 mRNA and protein, which in turn inhibits the proliferation of BC cells [9,72]. GLI1 expression in BC Proliferation, survival, migration, invasion, EMT, angiogenesis, and bone metastasis are among the important biological processes in BC that are closely associated with GLI1 and GLI2 expression [3e7]. In BC, GLI3 plays a variety of roles. Through interactions with estrogen receptors, it stimulates the proliferation of BC cells. Through different signaling pathways, it also contributes to the advancement of cancer in triple-negative and HER2-positive BC. A worse prognosis is associated with high GLI3 expression [73]. Estro-gen receptor (ER)-positive cell lines have lower GLI1 expression than TNBC and basal-like breast cancer (BLBC) cell lines [2,18]. It has been shown that the truncated GLI1mRNA variation, or tGLI1, increases BC cells' capacity to spread. In conclusion, GLI1 and tGLI1 seem to play important roles in BC [74]. GLI proteins (GLI1, GLI2, and GLI3) are transcription factors that control the expression of downstream target genes [2,75] and are crucial mediators in both canonical and non-canonical Hh signaling. Almost every natural substance that has been investigated for its connection to the Hh signaling pathway has some inhibitory effects on GLI proteins. By lowering GLI activity, cordycepin, for example, inhibits tumor development along the non-canonical Hh pathway, blocking the Hh-Notch-EMT axis and suppressing tumor spread [69]. Additionally, cordycepin inhibits BC development and metastasis by downregulating the expression of important canonical Hh pathway components, including as SHH, PTCH1, SMO, GLI1, and GLI2 [72]. These results provide possible therapeutic choices with minimal toxicity and excellent tolerance, opening up new therapy options for BC. As a result, we investigate further how natural substances impact breast cancer cells via the Hhsignaling pathway, offering vital information for creating innovative treatment approaches. 2. Natural substances used in BC intervention that target the Hh pathway The regulation of the Hh signaling pathway by natural products has demonstrated broad potential in BC, as these compounds affect various biological behaviors of breast cancer cells through multiple mechanisms, such as invasion, proliferation, EMT, drug resistance, reduction of BCSC populations, and tumor angiogenesis [5,7,12,64]. In particular, 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 drugresistance, regulate Hh pathway activity, and influence the proliferation, self-renewal, and metastatic

capacity of BCxenografts. Cyclopamine is the most extensively studied Hh pathway inhibitor to date. 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 like polyphenols, terpenes, alkaloids, and other miscellaneous compounds can also regulate the Hh signaling pathway [4,9,13,14,78]. It does this by suppressing the expression and translocation of GLIfactors and inhibiting the activity of the SMO receptor [76]. Interfering with BC's EMTs

EMT and the Hh signaling pathway interact in a reciprocal manner. EMT is induced as a consequence of the Hh signaling pathway's regulation of EMT-related genes, such as SNAIL and TWIST. EMT-related morphological and molecular alterations might then modify the Hh signaling pathway by changing the expression levels of important molecules, which will affect signal transmission. Research has shown that Hh-mediated activation of GLI1 can induce EMT by upregulating the expression of vimentin (VIM) and SNAIL, while downregulating the expression of CDH1 (E-cadherin) [79]. Natural drugs typically suppress BC cell migration, inva-sion, and EMT by targeting the SHH/GLI1 axis. Through the non-canonical Hh signaling pathway, cananginone, a naturally occurring compound from the Annonaceae family, exhibits potent cytotoxicity. It does this by upregulating phosphorylated GSK3b, which encourages the proteasomal degradation of GLI1 protein and, in turn, modulates the Hh signaling transduction. Additionally, it suppresses the expression of E-cadherin, which prevents EMT and helps to prevent BC metastases [80]. Similar to this, cordycepin affects the downstream Notch signaling system, which is essential for EMT, by working via the non-canonical Hh route. In cells when GLI is knocked out, cordycepin does not block the expression of NOTCH1 and NOTCH3, but NOTCH1 may indirectly control EMT. Cordycepin also downregulates the expression of NOTCH1, NOTCH3, JAGGED1, and HES1 in the Notch signaling pathway. Consequently, cordycepin suppresses tumor development by decreasing GLI activity and limiting the Hh-Notch-EMT axis, which in turn suppresses tumor metastasis [69]. Simoenine and cordycepin both influence EMT via the non-canonical Hh route by preventing SHH activation through the blockage of the NF-kB signaling cascade. Since NF-kB is required for EMT, its suppression interfereswith the EMT process, therefore reducing BC metastasis [64]. Key proteins including SHH, PTCH1, SMO, GLI1, and GLI2 are often elevated in the Hh signaling pathway, and cordycepin largely inhibits their expression in the canonical Hh pathway. Treatment with cordycepin dramatically reduces the expression of these factors, which in turn limits the growth and metastasis of BC [72]. In GLI-overexpressing BCSCs, the plant polyphenol curcumin inhibits the protein levels of GLI1, GLI2, SMO, CDH1, and VIM. It also lowers the protein levels of stemness markers octamer-binding transcription factor 4 (OCT4) and SRY-box transcription factor 2 (SOX2) while lowering GLI1 nuclear expression, indicating that curcumin inhibits GLI1 translocation to the nucleus, thereby blocking the Hh pathway. Additionally, VIM interacts with GLI1, demonstrating its function as a crucial gene that GLI1 induces in the control of stemness and EMT [9]. By suppressing the protein production of GLI transcriptional activators and Patch receptors, Sinomenine, a quin-oline alkaloid, inhibits the Hh signaling pathway and reverses EMT [64] (Fig.3A). Stopping angiogenesis TNBCtumorsexpresshigherlevelsofvascularendothelialgrowth factor (VEGF)comparedtonon-TNBC tumorsanddisplayincreasedmicrovesseldensity,indicatingarelianceonangiogen-esis[81].TheHhsignalingpathwaycontributestotumor-associatedangiogenesis.ActivationoftheHhpathwayincreasesvasculardensityin BC[53].Additionally,Hhsignalingregulatesangiopoietin-1(Ang-

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1)inbonemarrow-derivedpro-angiogeniccells,promotingneovascularization[82],whilecanonicalHhsignalinginducesVEGFexpressiontofacilitatetumorangiogenesis.tGLI1,asplicevariantofGLI1,enhancesthehumanVEGF(h-VEGF)genepromoter,leadingtotheupregulationofVEGFinBCcells[74].Wogonoside,amajor active componentofthetraditionalChinesemedicineScutellariabaicalensis(Huangqin),primarilyexertsitseffectsonBCviathenon-canonicalHhsignalingpathway.Wogonoside,derivedfromtherootsofS.baicalensis,isanaturalflavonoidcompound.Itexhibitsarangeofpharmacologicaleffects,including anti-inflammatory,anti-angiogenic,antioxidant,neuro-protective, and anti-tumor properties [5,83,84]. By downregulating the production of the pro-angiogenic protein VEGFor and directly binding to SMO, wogonoside suppresses angiogenesis in TNBC by preventing nuclear translocation and transcriptional

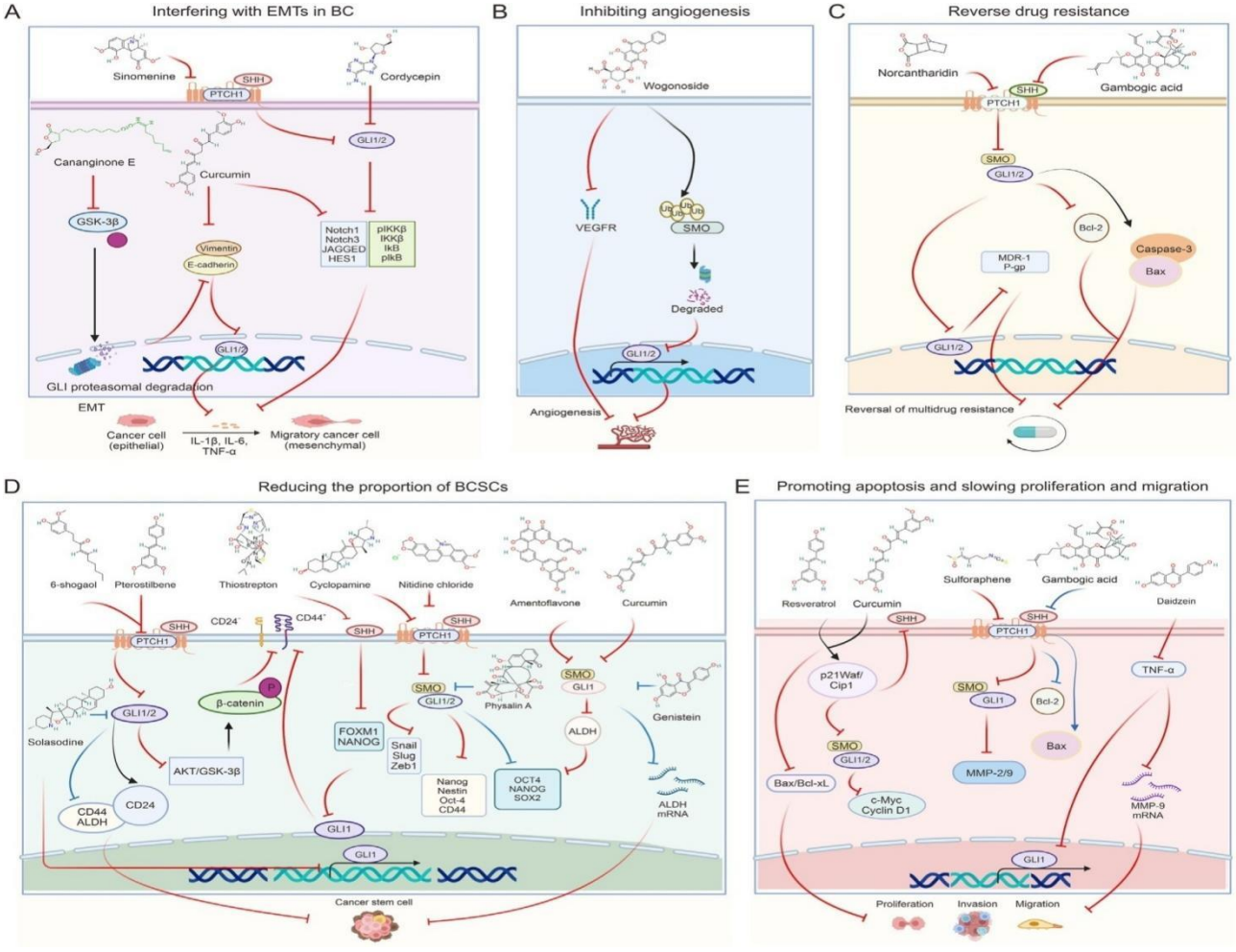
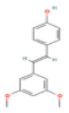
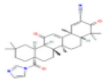
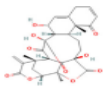
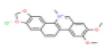
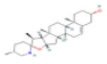
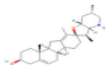
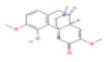
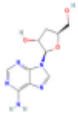
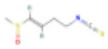


Table 1 (*continued*)

Group	Name	Chemical structure[a]	Mode of action	Effect	Cell type/mode	Refs.
	Pterostilbene					
Terpenoid	CDDO-IM		↓*Gli1* mRNA, *Gli3* 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, *Myc* 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, *Snai2* mRNA, *Zeb1* mRNA,*Cdh2* mRNA, *Vim* mRNA, *Gli1* mRNA, *Smo* mRNA ↑E-cadherin ↑*Cdh1* (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]

SMO: smoothed aldehydedehydrogenase (ALDH); glioma-associated oncogenehomolog1 (GLI1); OCT4: octamer-binding transcription factor4; SUFU: suppressor offused; VEGF: vascular endothelial growth factor; CD44: cluster of differentiation44; CD24: cluster of differentiation24; Hip:hedgehog-interacting protein; CCDN2:CyclinD2 Gene; Gas1: growtharrest-specific1; tumor necrosis factor-alpha (TNF-a); Sonic hedgehog (SHH); matrixmetalloproteinase-9 (MMP-9); BCL-2: B-cell lymphoma 2; CASP3: caspase-3; BAX: BCL2-associated X protein; PTCH1:patched 1; Glioma-associated oncogene homolog 2 (GLI2), epithelial cadherin (E-cadherin), G1/S-Specific SOX2: sry-box transcription factor 2; p21: cyclin-dependent kinase inhibitor 1A Cyclin-D1: G1/S-specific Cyclin-D1; MYC: mycproto-oncogene, bHLHtranscriptionfactor; b-catenin: cateninbeta1; p-AKT: phosphorylated AKT; p-GSK3b: phosphorylated GSK3 beta; p-b-catenin: phosphorylated B-catenin; Neurogenic locus notch homolog protein 1 is known as NOTCH1, while neurogenic locus notch homolog protein 3 is known as NOTCH3. TGFBR 2: changing growth factor into a receptor; TGFBR 3: changing growth factor into a receptor; p-SMAD2: mothers against decapentaplegichomolog2; p-SMAD3: mothers against decapentaplegichomolog3; C-NOTCH1:cleavednotch1intracellulardomain;
Nanog:nanoghomeoboxprotein;
POU5F1:pouclass5homeobox1; P-glycoprotein: P-gp; Breast Cancer Resistance Protein, or BCRP NES: Nestin N-cadherin: neural cadherin; ZEB1: zincfinger e-box binding homeobox 1; SNAIL1: snail family transcriptional repressor 1; SNAIL2: snail family transcriptional repressor 2; NESTIN: neuroepithelial stem cell marker protein; POU5F1: pou class 5 homeobox 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; Slug: snail family transcriptional repressor2; CYCS: cytochromec; FAS:fas cell surface death receptor;
TNFRSF10A:TNFreceptorsuperfamilymember10A;
TNFRSF10B:TNFreceptorsuperfamilymember10B;
JAAGED1: jagged canonical notch ligand1; Hes1: hesfamilybhlh transcriptionfactor1; 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; FOXM1: forkhead box protein M1; MMP-2: matrix metalloproteinase-2; MMP-9: matrix metalloproteinase-9; and EpCAM: epithelial cell adhesion molecule. The teroid structure of solasonine suppresses GLI1 function by forming hydrogen bonds with certain surface residues on GLI1, designating it for ubiquitination and pro-teasomal degradation [121,122]. In conclusion, natural compounds, such as cyclic frameworks, benzene, and heterocyclic elements found in polyphenolic, terpenoid, and alkaloid compounds, influence the Hh signaling pathway through distinct structural features. These structures modulate signal transduction and pathway regulation by facilitating interactions with important proteins or regulatory elements within the Hh signaling pathway. By targeting key molecules in the Hh signaling system, interfering with cancer cells' dependence on this route, and exhibiting molecular selectivity, these drugs also show selective inhibitory effects on BC stem cells (Table 1) [3e13,23e27,64,69,72,76,78,80,91].

2. The present state of studies on inhibitors of the Hh signaling system and its therapeutic uses

The Hh signaling pathway has been thoroughly examined in relation to the development of cancer and is becoming more widely acknowledged as a promising target for the development of anticancer drugs. Hhligand inhibitors, SMO inhibitors, and downstream SMO target inhibitors, which include GLI1 antagonists, are the three main kinds of Hh pathway inhibitors now on the market (Table 2). Inhibitors that target the HHL

Hh protein inhibitors and Hhacyltransferase inhibitors are the two main kinds of ligand inhibitors that target the Hh signaling pathway [132,133]. One of the SHH-specific inhibitors of the Hh protein is the monoclonal antibody 5E1, which has been shown to decrease tumors in pancreatic cancer models [134,135]. Although they may be linked to increased cytotoxicity, another family of drugs, known as RU-SKI compounds, block the palmitoylation of SHH via hedgehog acyltransferase (HHAT) [136]. The connection between SHH and PTCH1 is disrupted by the macrocyclic peptide HL2-m5, which effectively inhibits SHH-mediated signaling and gene transcription [137]. These inhibitors, however, are not yet well studied in non-canonical Hh signaling and mainly target the traditional Hhligand route. To address these issues and broaden the field of therapeutic action, further research is required. SMO-targeting inhibitors

One important mechanism for cellular regulation is the Hh signaling system. Vismodegib, sonidegib, and glasdegib are common SMO inhibitors that have shown significant effectiveness in treating basal cell carcinoma [133,138]. Long-term use, however, often results in resistance, and adverse consequences pose a serious problem for inclined applications. Novel inhibitors like taladegib, BMS-833923, and saridegib are now undergoing clinical trials to address these problems and may provide more powerful therapy alternatives [139].

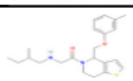
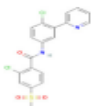
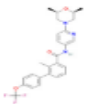
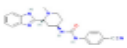
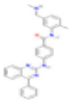
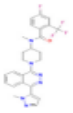
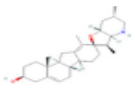
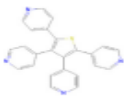
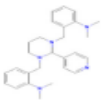
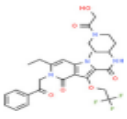
Furthermore, cyclopamine, a naturally occurring SMO inhibitor, has garnered a lot of attention from researchers. New asymmetric synthesis techniques for cyclop-amine have been revealed in recent publications, opening the door for further investigation and advancement. Even though SMO inhibitors have potential for treating cancer, further study is necessary to address issues with resistance and managing adverse effects [140]. GLI-targeting Inhibitors

GLI proteins are key targets for preventing Hhpathway activation because they are terminal transcription factors in the Hhsignaling pathway. Direct or indirect inhibition of GLI may effectively decrease the Hh signaling pathway, especially when non-canonical Hh signaling pathways are activated or SMO inhibitor resistance occurs. The primary use of the first class of GLI inhibitors, which includes substances like GANT-58 and GANT-61, is in drug development [141]. GLI-inhibiting medications, such FN1-8 and TAK-441, have shown promise in cancer treatment [142]. No GLI inhibitors have been licensed expressly for commercial distribution, despite the fact that several substances, such as arsenic trioxide, have been approved and shown to block GLI transcriptional activity [142,143]. GLI inhibitors avoid the resistance problem that comes with SMO inhibitors by avoiding upstream aberrations in the Hhpathway. Although preclinical investigations employing a variety of small compounds have shown promise, no drug has shown clear benefits in terms of potency and selectivity. Researchers are becoming more aware of the variety of Hh signaling pathway anomalies in carcinogenesis, particularly those resulting from SMO mutations and the activation of non-canonical Hh signaling. It is known that one inhibitor could not be enough to completely stop carcinogenesis, and that it might result in chemoresistance and other negative consequences [133,144,145]. Therefore, it is now essential to create inhibitors that precisely target the Hh signaling system. Natural chemicals have the ability to reduce toxicity and adverse effects because of their improved biocompatibility and structural variety. However, studies on natural compounds as Hh pathway inhibitors are still in the experimental stage, therefore further study is required to evaluate their safety and therapeutic potential. Although all 23 of the above mentioned natural substances have inhibitory effects on the Hh signaling pathway, we believe that two well-known polyphenolic compounds, curcumin and resveratrol, have the most promising potential. First, polyphenolic chemicals are beneficial because of their low toxicity profiles and anti-inflammatory, anti-cancer, and antioxidant qualities. Furthermore, curcumin and resveratrol have both shown effectiveness in in vitro and in vivo models for a variety of malignancies. Interestingly, resveratrol increases the absorption of curcumin in BC cells in a dose-dependent manner [10]. By reducing the expression of GLI and EMT-related genes, namely CDH1 and VIM, in BCSCs, curcumin has also been shown to decrease cancer cell stemness [9]. Both compounds have therapeutic promise, however their clinical use is limited by poor bioavailability and structural instability. Therefore, in order to improve their therapeutic value, further research is needed to maximize their biological activity and stability.

3.Conversation

The formation and progression of BC are significantly influenced by the Hh signaling system, underscoring its importance in carcinogenesis. The Hh pathway may control the proliferation and differentiation of BC cells and is implicated in a number of activities, including apoptosis, organ growth, and cell proliferation [15,16]. According to studies, the Hh signaling system may influence EMT and drug-induced dedifferentiation pathways, which might lead to BC development and drug resistance [7,146]. Furthermore, additional biological processes as flexible cellular metabolism and traits of persistent tumor cells may be linked to the Hh pathway in BC [147,148]. Clarifying the mechanisms of Hh pathway modulation is becoming a more important focus of research on the therapeutic potential of natural substances in BC. By focusing on both canonical and non-canonical Hh signaling pathways, these natural substances affect the biological characteristics of BC cells [79]. These natural compounds' inhibitory mechanisms can be divided into three main categories: (1) Direct inhibition of important protein expression, including lowering 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		$C_{19}H_{14}C_{12}N_2O_3S$	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

GLI: Glioma-associated oncogenehomolog; SMO: Smoothened. of important molecules such as SMO, GLI, PTCH1, SUFU, and SHH, which effectively block Hh signaling at its source; (2) modification of protein activity and localization, wherein specific compounds either directly bind to SMO, promote its ubiquitination to reduce its activity, or inhibit GLI nuclear translocation, which diminishes transcriptional activity and weakens signal transduction; (3) regulation of downstream effects, whereby these compounds inhibit the activation of SMO and GLI, which in turn downregulates MMP-9 and MMP-2 expression to prevent tumor invasion and metastasis, while also reducing crosstalk between Hh and other pathways (Hh/Hippo, SHH/mdr-1, and Hh/AKT/GSK3bpathways). We identified similar structural characteristics linked to the inhibitory action of natural substances that are known to inhibit the Hh signaling pathway. Inhibitor efficacy, for instance, is closely correlated with the presence of aromatic rings, and distinctive amino acid residues found in natural chemicals are also essential for the construction of inhibitors, offering a clear indication for the creation of more specific inhibitors in further research [149,150]. The structure-activity relationships of various natural compound types are the primary determinants of the inhibitory effects of natural compounds on the Hh signaling pathway. In polyphenolic substances, hydroxybenzoic acid, flavonoid structures, and benzene rings often interact with important Hhsignaling pathway proteins, such GLI proteins, to modify signal transduction. These substances may also alter the conformation of the Hh pathway receptor SMO, which might disrupt regular signal transmission [91,96]. Interpenoids' effects on the Hh pathway are mostly dependent on their cyclic structures and functional groups. Terpe-noids might influence the signaling process by interacting with important proteins or molecular targets in the Hhpathway via particular interactions including hydrogen bonding, van der Waals forces, or hydrophobic contacts [104,107e109]. By interacting with important proteins within the Hh pathway, alkaloids, which have a variety of chemical structures such as alicyclic rings, benzene rings, heterocycles, and distinct

functional groups, alter downstream gene expression and signal transmission. The potential of natural substances to suppress BC cells via the Hhpathway, namely via the effect of the SMO-GLI1 axis on the BCSC population, is methodically summarized in this work. Prior studies have shown that the Hh route contributes to the maintenance of cancer cell self-renewal and proliferation, with the SMO-GLI1axis acting as a crucial regulatory hub, particularly in the regulation of stem cell populations [44]. Natural substances may dramatically reduce the survival and self-renewal capacities of BCSCs by focusing on this axis, which lowers the risk of tumor invasiveness and recurrence [3,4]. These results support guidelines for BC therapy and the creation of individualized therapeutic methods, and they provide evaluable insights for creating therapeutic strategies against BCSCs.
In contrast to conventional chemotherapeutic agents Natural compounds often have greater tolerance and lower toxicity, which is particularly beneficial for cancer patients. Significant obstacles to use still exist, nevertheless, including limited bioavailability and poor stability in vivo [22]. In order to improve treatment effects, future research may examine techniques like chemical modification and nanocarrier technology to increase these drugs' in vivostability and targeting capability. Moreover, the use of synergists to increase in vivoactivity or high-throughput screening to find and optimize natural substances and their derivatives are viable remedies. Future research might examine the combination use of natural chemicals with additional anticancer medications to further enhance therapeutic effectiveness. In order to give a diverse approach against BC, this multi-targeted combination method may use the specificity of other medicines while capitalizing on the inhibitory effects of natural drugs on the SMO-GLI1axis.
In conclusion, natural chemicals show significant therapeutic promise in controlling BCSC populations and blocking the Hh signaling pathway, providing a useful guide for creating individualized treatment plans that specifically target BCSCs.

2.Conclusion

From the standpoint of natural substances, this work offers a thorough analysis of the regulatory function of the Hh signaling pathway in BC therapy. We clarified the distinct and overlapping impacts of several natural substances on the Hh signaling pathway by investigating their mechanisms of action. Notably, this study provides the first thorough overview of the main molecular routes by which natural compounds alter the Hh signaling system, emphasizing how they mitigate the aggressive traits of BC. Additionally, the study of the structure-activity connection between natural compounds and their targets provides new information for the creation of new drugs and the use of natural compounds in clinical settings. These results provide intriguing avenues for using natural chemicals in cancer therapy and lay a crucial basis for the creation of novel B therapeutic approaches.

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