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REVIEW

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Interactions between hedgehog signaling pathway and the complex tumor microenvironment in breast cancer: current knowledge and therapeutic promises

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Abstract

Breast cancer ranks as one of the most common malignancies among women, with its prognosis and therapeutic efficacy heavily influenced by factors associated with the tumor cell biology, particularly the tumor microenvironment (TME). The diverse elements of the TME are engaged in dynamic bidirectional signaling interactions with various pathways, which together dictate the growth, invasiveness, and metastatic potential of breast cancer. The Hedgehog (Hh) signaling pathway, first identified in Drosophila, has been established as playing a critical role in human development and disease. Notably, the dysregulation of the Hh pathway is recognized as a major driver in the initiation, progression, and metastasis of breast cancer. Consequently, elucidating the mechanisms by which the Hh pathway interacts with the distinct components of the breast cancer TME is essential for comprehensively evaluating the link between Hh pathway activation and breast cancer risk. This understanding is also imperative for devising novel targeted therapeutic strategies and preventive measures against breast cancer. In this review, we delineate the current understanding of the impact of Hh pathway perturbations on the breast cancer TME, including the intricate and complex network of intersecting signaling cascades. Additionally, we focus on the therapeutic promise and clinical challenges of Hh pathway inhibitors that target the TME, providing insights into their potential clinical utility and the obstacles that must be overcome to harness their full therapeutic potential.

Keywords Hedgehog signaling pathway, Breast cancer, Tumor microenvironment, Therapeutics

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Introduction

Solid tumors represent a highly complex and heterogeneous ecosystem comprising many cell types, cell states, and extracellular components. The tumor microenvironment (TME) contains a variable fraction of cancer cells, ranging from 5% to almost 100%, with the remainder consisting of other infiltrating and resident host cells, extracellular matrix, and secreted factors [1]. In this 'nest,' tumor cells interact with other cell populations by undergoing differentiation, epigenetic changes, dissemination, and immune escape [2]. The reciprocal influence that malignant cells and other cellular populations in the TME exert on each other represents a driving force that actively shapes disease progression and influences treatment outcomes [1, 3]. Therefore, various strategies for reshaping TME have become increasingly crucial for tumor therapy.

To date, the biological complexity coupled with invasion and metastatic cascades has indeed limited the research and investment in potentially valuable novel therapeutic agents [4]. The Hedgehog (Hh) signaling pathway has been recognized as one of the most intensively investigated targets in cancer therapy, playing a critical regulatory role in multiple biological processes ranging from embryo development and tissue patterning to cancer initiation, progression, and metastasis [5]. For example, the Hh pathway is quiescent in the adult mammary gland but reactivated in up to 30% of breast cancer [6, 7]. Aberrant Hh signaling activation may accelerate breast cancer growth and lead to tumor immune tolerance and drug resistance. The interaction between Hh signaling and the TME is intimately involved in these processes, such as tumor growth, tumor immune tolerance, inflammation, and drug resistance [8]. This review aims to review the current understanding of the role and mechanisms of aberrant Hh signaling activation in breast cancer TME and highlight potential therapeutic insights.

Hedgehog pathway in tumors

The Hh signaling pathway is a complex intracellular signaling mechanism associated with the development and progression of various solid malignancies due to sporadic mutations or other mechanisms of constitutive activation. In oncogenesis, the activation of the Hh signaling pathway, which can lead to tumor development, occurs through two primary mechanisms—ligand-dependent (canonical Hh signaling) and ligand-independent (non-canonical Hh signaling)(Fig. 1). Previous studies have extensively documented the two modes of aberrant activation, and in this article, we provide a brief overview of them [5, 9].

The canonical Hh pathway is initiated by the binding of Hh ligands (such as Sonic Hedgehog (Shh), or Indian Hedgehog (IHh), and Desert Hedgehog (DHh)) to their receptor Ptched (Ptch), which relieves Ptch-mediated inhibition of Smoothened (SMO) [10]. Subsequently, the activated SMO translocates to the primary cilium. where it promotes the inhibition and activation of the GLI family of transcription factors (Gli1, Gli2, and Gli3) [11] (Fig. 1). These activated transcription factors then translocate to the nucleus and regulate the expression of a series of target genes involved in various biological processes such as cell proliferation, differentiation, and survival [5, 12, 13]. The non-canonical Hh pathway operates independently of SMO and can participate in cellular signaling through various mechanisms. In this pathway, Hh signaling can directly activate non-SMO-dependent signaling molecules or interact with other signaling pathways such as Transforming growth factor β (TGF-β), Notch, and PI3K/AKT, thereby influencing cellular behavior [14]. The non-canonical Hh pathway plays a role in tissue repair, modulation of the tumor microenvironment, and maintenance of cancer stem cells [15]. Currently, our understanding of the non-canonical Hh signaling pathway and its mechanisms remains incomplete. Indeed, it seems that the non-canonical pathway serves as a backup route that comes into play when the canonical Hh pathway is inactive.

Both signaling events are essential roles in cell fate determination, tissue morphogenesis, and disease development. In cancer, aberrant activation of the canonical Hh pathway is associated with the development of various tumors, such as breast cancer, basal cell carcinoma, medulloblastoma, and certain types of lung cancer [16, 17]. Although the role of the non-canonical Hh pathway in cancer is not as well-defined as the canonical pathway, a reassessment of multiple Hedgehog network members is needed to synthesize an accurate understanding of Hedgehog network functions in a given biological context.

TME factors and conditions that modulate the hh signaling in breast cancer

It is a well-established fact that tumor development and metastasis depend on the two-way interaction between cancer cells and their environment, thereby forming the TME. Solid tumors are surrounded by a complex and dynamic microenvironment involving stromal cells, immune cells, extracellular matrix components, and a specific physical environment. As depicted in Fig. 2, the interplay between Hh signaling and the TME is multifaceted. The Hh ligands secreted by cancer cells, such as Shh, can activate the Hh pathway in both cancer and stromal cells, leading to the upregulation of target genes like Gli1 and CyclinD1, which foster cell proliferation and resistance. Furthermore, Hh activity can induce the production of angiogenic factors, such as ascular endothelial growth factor-A (VEGF-A), and recruit immune

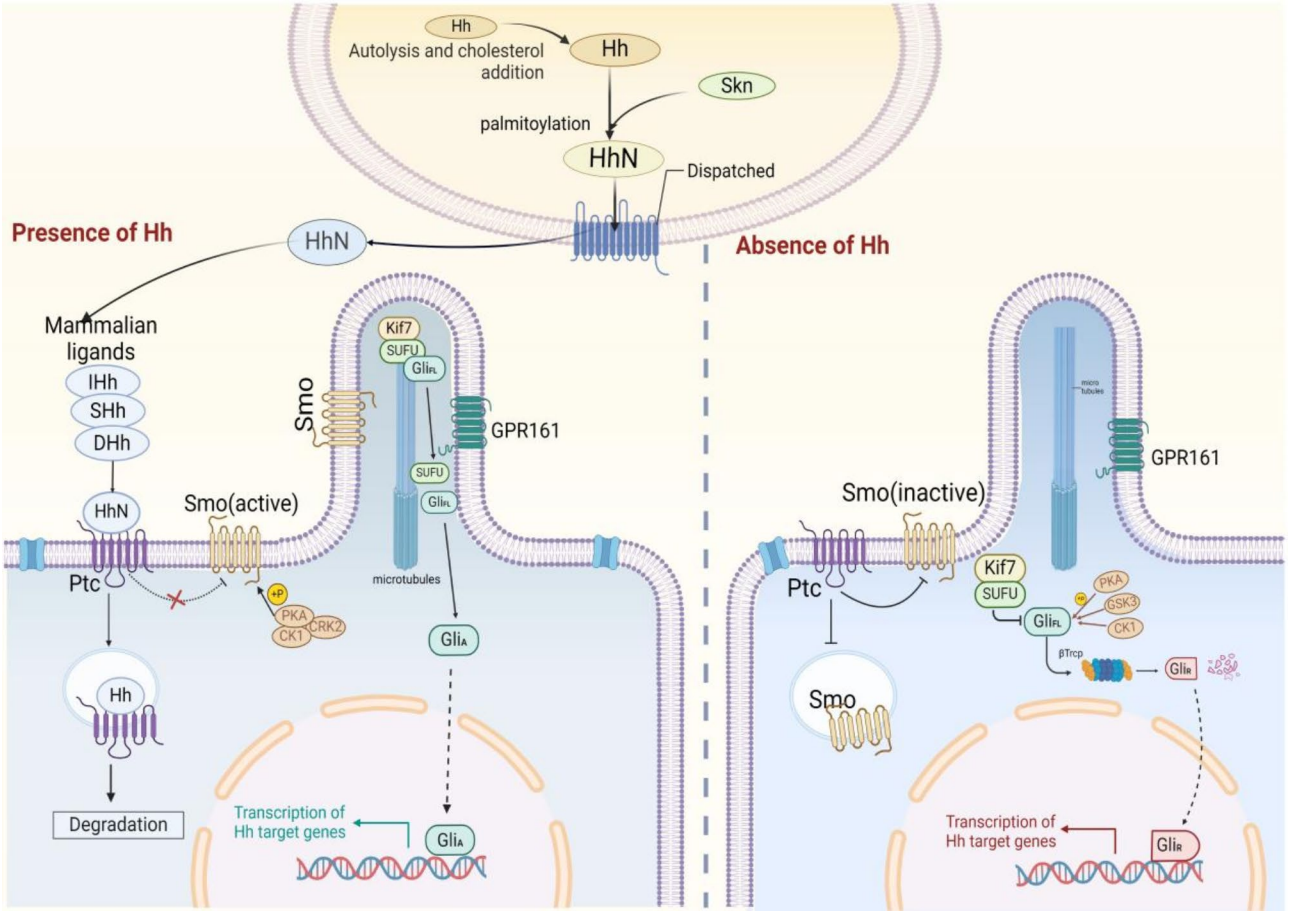

Fig. 1 Activation and regulation of Hh pathway in vertebrates. The canonical Hh signaling pathway is initiated by the binding of Hh ligands to the transmembrane protein Ptch, which inhibits the Ptch-dependent suppression of Smoothened SMO, leading to the activation of the Gli signaling pathway. Left panel: In the presence of Hh ligands, the engagement of these ligands—IHh, Shh, or DHh—with the Ptch receptor results in the alleviation of Ptch's inhibitory effect on Smo. Subsequently, the activated Smo conveys the Hh activation signal into the cytoplasm. Ultimately, the Suppressor of Fused (Sufu) releases Gli proteins from the cytoplasm, enabling their translocation to the nucleus, where they initiate the transcription and expression of their target genes, including Gli1, Gli2, and Gli3. Right panel: In the absence of Hh ligands, Ptch liberates proteins that inhibit Smo activity, thereby preventing Smo activation and shutting down the Hh signaling pathway

cells like T regulatory cells (Tregs) and tumor-associated macrophages (TAMs), creating an immunosuppressive milieu. The Hh pathway also enhances the plasticity of the extracellular matrix, promoting cancer cell invasion. These elements interact in a complex manner between Hh signaling and the TME, forming a positive feedback loop that that not only fuels the growth and invasiveness of breast cancer but also confers resistance to therapies [18].

Immune cells and hh signaling

TAMs

It is well known that macrophages are a complex and heterogeneous group of cells that exhibit high plasticity when stimulated by various signals in TME. They can respond quickly and efficiently to participate in innate and adaptive immune responses [19]. Previous efforts employed to eliminate the TAMs in breast and lung cancer have yielded successful clinical outcomes [20]. The Hh signaling pathway, a key player in driving the polarization and proliferation of TAMs, has been the subject of recent studies. In both in vivo and in vitro experiments, tumor-derived Shh has been found to promote M2 polarization of TAMs in various malignant tumors, including hepatocellular carcinoma, lung cancer, breast tumors, and multiple myeloma [21–23]. The Shh can directly act on the tumor stroma to promote transcription factor Klf4-mediated M2 polarization. Subsequently, this process further suppresses the production of CXCL9 and CXCL10 in TAMs, reducing CD8+T cell infiltration within the TME and fostering tumor growth [22]. Additionally, tumor cell-derived Shh ligand can drive the expression of PD-L1 in TAMs, causing dysfunction of CD8+T cells within the tumor and promoting an immunosuppressive environment, which ultimately propels tumor progression [24]. Ann Hanna and colleagues

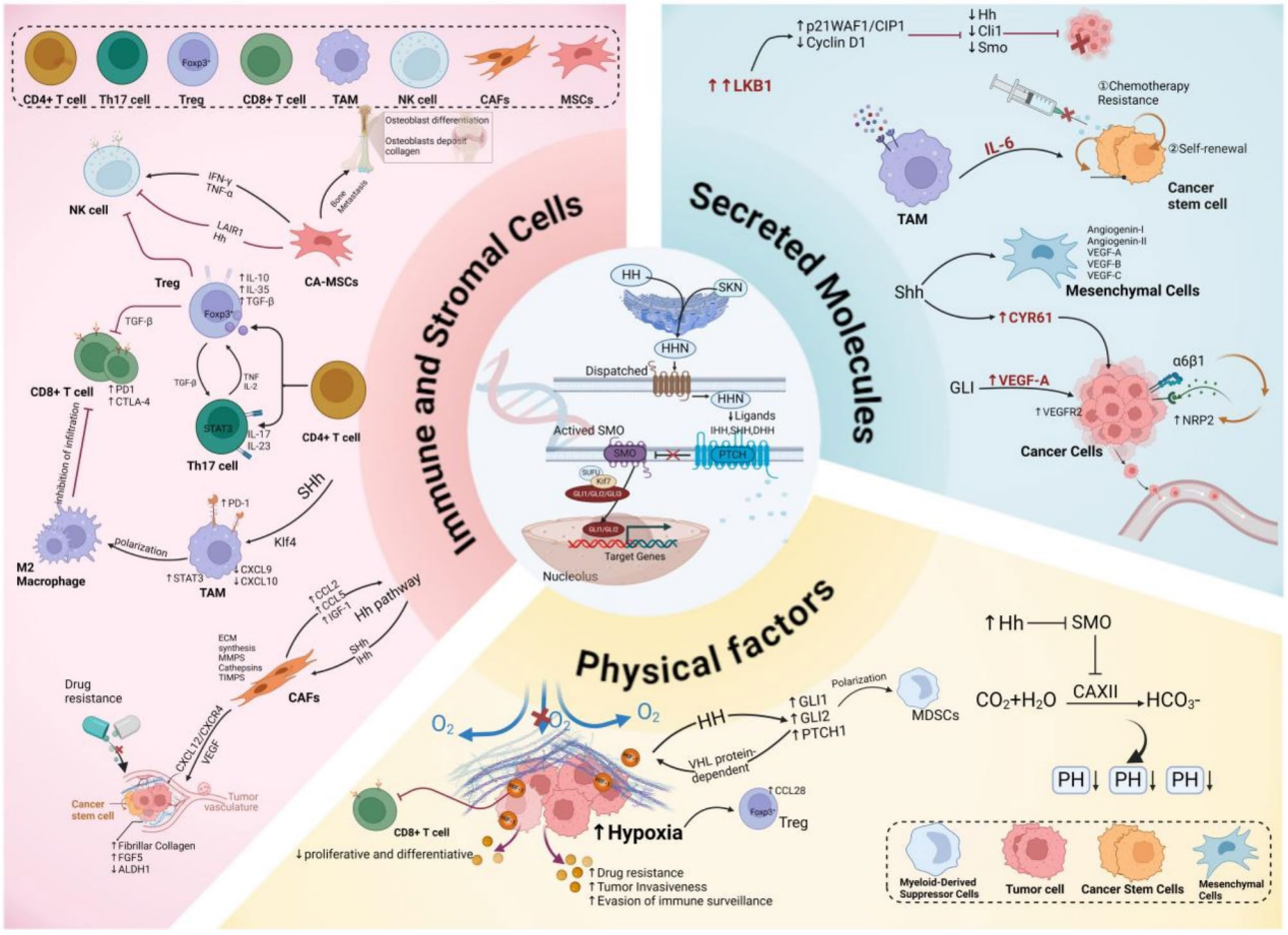

Fig. 2 Complex mechanisms of Hh pathway remodelling of TME in breast cancer. Hh signaling pathway plays a key role in the breast cancer microenvironment, affecting the function of tumor cells and immune cells. Treg cells often secrete inhibitory factors to hinder immune response. Hh signals regulate Treg/Th17 axis through metabolic remodeling, limit CD8 cell infiltration, and promote immune escape. TAMs polarize M2 under Hh signal, reduce CXCL9/10 production, inhibit CD8 T cell infiltration, and inhibit its function through PD-L1 expression. Hh signaling also reprograms CAFs to increase fibrocollagen and FGF5 expression, supporting tumor stem cell resistance. CCL2, CCL5 and IGF-1 secreted by CAFs activate the Hh signal in cancer cells, forming a vicious cycle that promotes tumor growth and drug resistance. CA-MSCs accelerate breast cancer bone metastasis by regulating immune response and promoting osteoblast differentiation. In addition, vascular endothelial growth factor (VEGF), pro-angiogenesis secretory molecules CYR61, IL-6, etc. all work synergically with Hh signaling pathway to strengthen the malignant behavior of breast tumor cells, promote the proliferation of cancer cells and enhance tumor drug resistance. Hypoxia and acidic tumor microenvironment are important factors that promote tumor invasion and metastasis and lead to poor prognosis of breast cancer patients

have further confirmed that the application of inhibitors targeting this pathway even primed the M2 macrophages to respond more potently to inflammatory stimuli and set the macrophages on a path toward reverting to M1 macrophages [21]. Considering the close relationship between TAMs and Hh signaling in breast cancer, the inhibition of the Hh signaling pathway is expected to achieve the dual benefits of directly targeting tumor cells at TAM targets and reconfiguring the tumor immune microenvironment to an immune-activated state.

T lymphocytes

T lymphocytes are the most effective adaptive anti-tumor immune response mediators, and their abundance, functional activity, and spatial distribution are critical determinants of tumor prognosis and prediction [25]. Mature T lymphocytes are predominantly categorized into two types based on molecular markers: CD8+and CD4+cells. These two cell types work in concert to maintain the effective functioning of the immune system [26]. There is increasing evidence that Hh signaling plays a vital role in T cell production under physiological conditions by modulating tumor antigen-specific T cell receptors (TCRs). Increased Hh activity impacts TCR-induced calcium inflow and critical components of the TCR signaling pathway, thereby impacting TCR's ability to transmit signals to T cells for activation and proliferation [27, 28]. Recent studies have indicated that Hh/Gli1 signaling has been shown to regulate the expression of immune checkpoint proteins such as PD1, CTLA-4, IL-10, etc. in

T cells, as well as PD-L1/2, CD80/86, OX40L, CD137L, IDO, and CCL22 in cancer cells [29, 30]. Many studies have reported the correlation between the Hh signal and PD-L1 regulation, and PD-L1 on tumor-associated macrophages induced by the Hh signal can inhibit the function of tumor-infiltrating CD8+T cells [24]. For example, Gli2 expression was found to be positively correlated with PD-L1 and negatively correlated with CD8+T cell infiltration in gallbladder and gastric cancer tissues [31]. In the tumor immune microenvironment, Gli1 can directly bind the promoter of the transcription factor STAT3 and induce its expression, promote the transcription of PD-L1, and thus inhibit the effector function of CD8+T cells [32]. In conclusion, the Hh signaling pathway can stimulate the expression of PD-L1 in various cell types, and increasing Hh activity may be a potential biomarker for predicting the therapeutic response of PD-1 inhibitors. Moreover, the combination of checkpoint inhibitors and Hh inhibitors will have more therapeutic advantages than treatment alone.

Tregs

Tregs are an immunosuppressive subpopulation of CD4+T cells that maintain self-tolerance and protect against autoimmunity. Among them, the delicate balance between Tregs and Th17 has become essential in regulating immune homeostasis in breast cancer [33]. Hedgehog signaling regulates breast cancer microenvironment regulatory T/Th17 differentiation through metabolic remodeling and provides a means of immune evasion by breast cancer cells. Hh signaling promotes O-GlcNAc modifications of critical Tregs and Th17 transcription factors, Foxp3 and STAT3, respectively, that orchestrated the transition between Tregs and Th17. Moreover, recent experimental evidence has confirmed that when Hh signaling is inhibited, Tregs undergo a phenotypic shift towards a Th17-like state, accompanied by an enhanced inflammatory profile. Therefore, the blockade of the Hh pathway to reprogram Tregs and promote their conversion into pro-inflammatory Th17 cells may facilitate the creation of an immunological microenvironment conducive to tumor eradication in breast cancer [34]. The involvement of Hh signaling in the interplay between tumors and Tregs has also been demonstrated in other malignancies [35, 36]. In summary, the dynamic interplay between the Hh pathway and Tregs reveals the complexity of modulating the immune microenvironment in treating breast cancer.

Stromal cells and Hh signaling

CAFs

As an essential part of the tumor microenvironment, tumor-associated fibroblasts (CAFs) play an important role in the genesis and development of tumors. Activated CAFs reshape TME and influence malignant biological processes in cancer cells by altering transcriptional and secretory properties, and this regulation depends in part on the regulation of signaling cascades. With knowledge of the association between Hh and CAFs, the Hh signaling pathway activated in CAFs has been extensively studied [37]. It was found that CAFs infiltration was positively correlated with Hh activity, and the two had related biological functions [38]. As an essential signaling pathway in stem cells, the Hh signaling pathway in CAFs plays a vital role in regulating Breast Cancer Stem Cells (BCSCs). In a mouse model of triple-negative breast cancer (TNBC), Hh ligands produced by cancer cells are able to reprogram CAFs, providing support for the drug-resistant phenotype of Cancer Stem Cells (CSCs) through FGF5 expression and fibrous collagen production [39]. CAFs also activate Hh signaling in cancer cells by producing soluble mediators such as CCL2, CCL5, and IGF-1. They can all contribute to tumor progression in many ways, including enhancing proliferation, migration, and stem cell phenotypes [40, 41]. In addition to affecting CSCs, Hh signaling in CAFs also influences the development of other malignant biological behaviors. In gastric cancer, Galectin1 induces epithelial-mesenchymal transition (EMT), migration, and invasion of cancer cells by binding to the carbohydrate structure of integrin β1, activating Hh signaling, and up-regulating Gli1 expression [42]. Interestingly, Hh-mediated CAFs also engage in crosstalk with cancer cells that promote breast cancer progression and impact the efficacy of clinical antitumor therapies. Maria Peiris-Pages et al. have discovered that standard chemotherapy for cancer can induce the production of CAFs, leading to the establishment of a highly glycolytic phenotype, autophagy, and a pro-inflammatory microenvironment. This catabolic microenvironment activates stemness, antioxidant, and immune responses in breast cancer cells through paracrine Hh-Gli signaling [43]. Steele et al. have demonstrated that inhibition of the Hh signaling pathway reduces the number of myofibroblast CAFs while concurrently increasing the population of inflammatory CAFs [44]. Moreover, Aurelie S. Caazet et al. have demonstrated that treatment with a small molecule inhibitor (SMOi) of the Hh pathway can lead to a reduction in the expression of aldehyde dehydrogenase 1 (ALDH1) in cancer cells and an enhancement of the body's sensitivity to docetaxel. This strategy has been shown to improve survival rates and reduce the metastatic burden in patients with invasive TNBC [39]. As researchers continue to explore CAFs in the tumor microenvironment, targeted CAFs therapies are expected to become an indispensable tool in the oncologists' arsenal in the next decade.

MSCs

Mesenchymal stem cells (MSCs), endowed with stem cell characteristics and diverse regulatory activities on other cells, have become a focal point of interest due to their immunosuppressive and tumor-promoting mechanisms in cancer [45, 46]. Research has shown that MSCs facilitate the progression of breast cancer by activating signaling cascades involving intracellular transduction nodes [47, 48]. In the context of bone metastasis in breast cancer, the activation of the Hh signaling pathway in MSCs has been shown to promote osteoblast differentiation, collagen deposition by osteoblasts, and the suppression of natural killer (NK) cells via the inhibitory leukocyte associated immunoglobulin like receptor 1 (LAIR1) signaling pathway, thereby facilitating tumor colonization [49]. This suggests that targeted therapy against the Hh signaling pathway may present a novel treatment option for disorders of bone homeostasis. Future research is warranted to further elucidate the intricate relationship between the Hh signaling pathway and MSCs within the breast TME, and to understand how they collectively influence the onset and progression of breast cancer.

Secreted molecules and Hh signaling

VEGF

Angiogenesis represents the formation of new blood vessels and is a principal pathogenetic action in breast cancer. Vascular endothelial growth factor (VEGF) is a major angiogenesis regulator that modulates the maintenance and function of mature vascular networks [50, 51]. While the active hh signal in tumors has been extensively studied due to its conduction role in angiogenesis [52]. Shh has been found to induce the expression of angiopoietins and the VEGF family in mesenchymal cells to promote angiogenesis. And stimulate the production of secretory factors [53, 54]. At the same time, Concetta Di Mauro et al. found that Gli1-overexpressed cancer cells can not only induce the secretion of VEGF-A but also establish a secreted factor/receptor autocrine circuit to coordinate the vascularization of TNBC by upregulating VEGFR2 on the cancer cell surface [55]. Although many anti-angiogenic studies have focused on VEGF, it is Lillianne G. Harris and colleagues who identified a novel VEGF-independent transcriptional target of the Hh signaling pathway, the pro-angiogenic secretory molecule cysteine-rich angiogenic inducer 61 (CYR61), playing a significant role in breast cancer progression. CYR61 works in concert with the Hh ligand Shh to intensify the malignancy of breast cancer cells, stimulates robust tumor vascularization in vivo, and facilitates the spread of these cells through the bloodstream [56]. In addition, Hira Lal Goel et al. demonstrated that Gli1 regulates VEGF/neuropilin-2 (NRP2) and α6β1 integrated into mediated novel autocrine signaling pathways by enhancing the expression of VEGF receptor NRP2 to drive the initiation of TNBC [57]. Given the dynamic regulatory processes played by the Hh pathway in breast cancer angiogenesis, targeting the Hh signaling pathway offers new possibilities for replacing and mitigating resistance to conventional anti-angiogenic therapies, which has significant implications for clinical breast cancer management.

IL-6

Interleukin-6 (IL-6) is a pleiotropic cytokine involved in immune regulation that has an important impact on cancer progression through the activation of signaling cascades [58, 59]. In breast cancer, TAMs increase tumor initiation ability and chemotherapy resistance of tumor stem cells by secreting IL-6 [60], while also up-regulating circulating VEGF in breast cancer patients and promoting tumor angiogenesis and metastasis [61]. Xiao et al.'s study has elucidated that the intricate relationship between the Hh pathway and IL-6 expression in breast cancer. They discovered that the inhibition of the Hh pathway not only leads to a significant up-regulation of IL-6 but also revealed a novel role for M2 macrophage-derived IL-6. This IL-6 acts as a protective mechanism for breast cancer cells against Hh inhibitors, suggesting its integral role in the maintenance and enhancement of the malignant potential of these cells [62]. In the future, antagonizing IL-6 and Hh pathway inhibitors may be a new therapeutic strategy for breast cancer.

Physical factors influencing Hh signaling

Hypoxic microenvironment

The hypoxic microenvironment is an essential driver of tumor progression, and hypoxia makes breast cancer more aggressive, metastatic, and drug-resistant than other tumors [63]. Hypoxia-inducing factor 1 (HIF-1), a principal regulator of the cellular response to hypoxic stress is intimately involved in nearly all critical steps of the metastatic process in breast cancer, including EMT, invasion, intravasation, extravasation, and the formation of a metastatic niche [64, 65]. Research has discovered that signaling pathways and molecules that remain inactive under normoxic conditions are often activated under hypoxic environments [66]. As a prominent feature of tumor growth, hypoxia affects several signaling circuits, including the Hh signaling, that converge during malignant progression. The Hh signaling pathway is often abnormally activated in breast cancer, promoting tumor progression and metastasis [67]. Tshering D.Lama-Sherpa et al. found that breast cancer with Hh activity can establish a hypoxic environment, which up-regulates the transcription levels of Hh pathway target genes Gli1, Gli2, and PTCH1 in breast tumor cells [68]. This hypoxic milieu, in turn, enhances HIF signaling in a Von Hippel-Lindau (VHL)-dependent manner, which is recognized

for driving processes integral to breast cancer progression and metastasis, such as angiogenesis, metabolic reprogramming, and the acquisition of a more aggressive phenotype [68–70]. In addition, hypoxia enhances the ability of tumor cells to fight immune attacks, prompting tumors to adapt to evade immune surveillance [71]. It is well known that TME is closely related to hypoxia and Hh signaling activation. Therefore, the function of immune cells is also related to hypoxia and Hh signaling pathway. Studies have shown that the hypoxic areas in tumors contain more Myeloid-derived suppressor cells (MDSCs), TAMs, and Tregs [72, 73]. Hypoxic stress increases the cytotoxicity of CD8+T cells while decreasing their proliferation and differentiation ability [73]. HIF-1α plays a crucial role in macrophage-mediated T-cell inhibition under hypoxic conditions [74]. The proliferation of activated lymphocytes was reduced under hypoxic conditions, but their migration did not change significantly. Under hypoxia in vitro, cell proliferation, migration, and cytotoxicity of activated TNK lymphocytes were significantly inhibited by Hh inhibitor treatment, and IFN-γ secretion was significantly increased [75]. The Hh signaling pathway plays a key role in activating TNK lymphocyte function. Hypoxia has been reported to induce overexpression of CCL28 to recruit Treg cells [76]. Hypoxia also enhanced immunosuppression by promoting treg activity [77]. It has been established that the function and differentiation of MDSCs in hypoxic TME are regulated by HIF-1α [78]. The polarization of aggressive bone marrow cells to MDSCs requires induction of the Hh pathway transcription factor Gli1, creating a microenvironment conducive to tumor transformation [79]. In conclusion, the association between hypoxia and Hh signaling may affect immune cell function. In the clinic, hypoxia often leads to radiation and chemotherapy resistance and immunosuppression, causing changes in the TME, which is a significant obstacle to cancer treatment effectiveness [78]. At the cellular level, inhibiting Hh signaling can effectively improve tumor hypoxic landscape and reduce tumor metastasis potential. At the molecular level, blocking the Hh signaling pathway can significantly weaken the ability of tumor cells to adapt to an anoxic environment. Based on this, the study of Hh signaling pathway inhibitors provides a new strategy and perspective for clinical treatment in preventing hypoxic-mediated breast cancer progression and metastasis.

Tumor acidification

The tumor microenvironment plays a vital role in tumor formation and development, and acidity is one of the significant characteristics of the tumor microenvironment [80]. Aerobic glycolysis-mainly attributable to chronic activation of HIF-1-and tumor hypoxia, are chiefly responsible for this phenomenon [81]. This particular microenvironment promotes gene reprogramming of tumor cells, promotes tumor invasion and metastasis, and is an essential factor leading to poor prognosis in breast cancer patients [82]. Carbonic anhydrases (CAs) constitute a family of enzymes integral to the regulation of pH homeostasis in living organisms. They modulate the extracellular pH by facilitating the reversible interconversion between carbon dioxide and bicarbonate [83]. In breast cancer cells, the expression and activity of CAs are dysregulated, contributing to the exacerbation of the tumor acidification [84]. Giuditta Guerrini and colleagues have elucidated the role of the Hh signaling pathway in the regulation of Carbonic Anhydrase isoform XII (CAXII) expression.By targeting the SMO component of the Hh pathway, it is possible to diminish CAXII expression at both the mRNA and protein levels, subsequently impeding the migration and invasion of breast tumor cells [85]. This discovery implicates the Hh pathway as a promising therapeutic target for the treatment of breast cancer. Tumor cells engineer their microenvironment by creating an acidic milieu that is non-toxic to malignant cells, thereby facilitating local invasion. Consequently, from a therapeutic standpoint, identifying strategies to reduce the pH surrounding the tumor could potentially offer a valuable adjunct or alternative to the traditional approach of exclusively targeting tumor cell eradication in the future.

Crosstalk between Hh signaling and other pathways in breast cancer

The intricate cellular cascades play a pivotal role in the evolution of cancer, with an increasing body of evidence highlighting the Hh pathway as a central player within the breast cancer microenvironment. Herein, we elucidate the collaborative mechanisms of Hh signaling with Wnt/TGF-β/Notch/RAS/MAPK/NF-κβ/PI3K pathways to govern a spectrum of functions, including cell proliferation, apoptosis, migration, and epithelial-mesenchymal transition (Fig. 3).

Notch signaling pathway

The Notch signaling pathway is frequently deregulated in several human malignancies, which has been described as a primary culprit (one of the three members of the so-called axis of evil) in breast cancer; the other two are HER2 and cancer stem cells [86]. In a manner analogous to Hh signaling, the activity of the Notch pathway has increasingly become a focus of research within the subpopulation of BCSCs [87, 88]. Genetic evidence in mice, as well as molecular biological studies in human cells, clearly indicate that the balance between Notch and Hh signaling networks is important for the maintenance of homeostasis among stem and progenitor cells [89]. Furthermore, the dysregulated Hh signaling, in conjunction

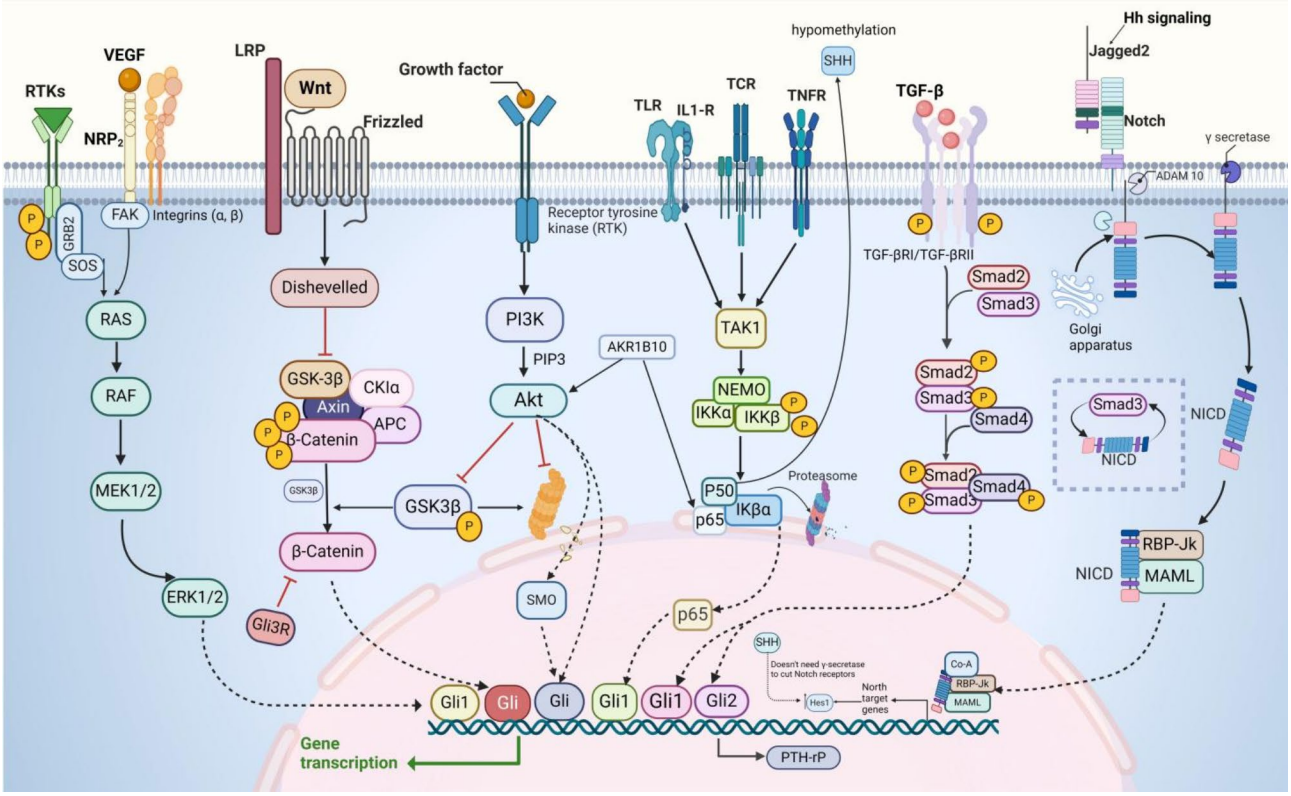

Fig. 3 Communication between the Hh signalling pathway and other pathways in breast cancer TME. The regulatory role of Hh signaling pathway in breast cancer is complex, involving a variety of signaling pathways, including Wnt/TGF-β/Notch/RAS/MAPK/NF-κβ/PI3K pathways. They have direct effects on target genes or activation of transcription factors through various mechanisms to affect cell behavior. For example, in the crosstalk with the North pathway, Hh promotes tumor cell proliferation and inhibits apoptosis by regulating its key target gene jagged2. In addition, Shh does not require γ-secretase to cut Notch receptor, and can directly act on Hes1 to promote tumor cell development. In endocrine-resistant breast cancer, activation of PI3K/AKT pathway receptors such as EGFR and IGF1-R stabilizes SMO and Gli1 proteins by affecting GSK3β-mediated phosphorylation and proteasome degradation. In the NF-κB pathway, various stimuli (such as TNF-α) activate the IκB kinase (IKK) complex and phosphorylate the IκB inhibitory protein, leading to ubiquitination and subsequent degradation of the IκB protein, thereby releasing proteins of the NF-κB family (such as P65, P50, etc.), mediating the up-regulation of Gli1 and SHh expression. In TGF-β pathway, the formation of complexes between phosphorylated Smad2/3 and Smad4 can effectively induce Gli1, Gli2 and their target gene PTH-rP (osteoclast formation regulator), and promote osseolytic metastasis of breast cancer. The RAS pathway phosphorylates and activates MEK (MAPKK) and ERK (MAPK) in sequence through integrin conduction and downstream effector Raf activation in the absence of Hh ligand, and finally the activated ERK enters the nucleus to activate Hh signaling pathway ligand GLI. In the Wnt pathway, the key molecule β-catenin enhances the transcriptional activity of GLI and participates in the Hh signaling pathway. In the absence of Hh signaling, the transcription factor Gli3 (Gli3R) interacts with the carboxy-terminal domain of β-catenin, blocking classical Wnt signaling. This mechanism may provide reference for the development of cancer treatment drugs

with the Notch and Wnt pathways, is involved in the maintenance of embryonic stem cell self-renewal and is regulated during the self-renewal and differentiation of adult stem cells [90]. During carcinogenesis, the Hh signals induce Notch pathway ligand JAG2 to promote tumor cell proliferation and inhibit cell apoptosis [91]. Similarly, Hes1, a target protein of the Notch signaling pathway, is upregulated by the Shh through a mechanism that bypasses the canonical Notch signaling pathway. This finding underscores the complexity of the crosstalk between the Hh and Notch signaling pathways [92]. Given the complexity of the specific interplays between the Hh and Notch pathways, the mechanisms underlying their crosstalk are currently under intensive investigation. A study assessing the combined inhibition of Hh and Notch in advanced breast cancer (NCT01071564) is underway, which may pave the way for establishing the safety of a combined therapeutic approach [93].

PI3K/AKT/mTOR signaling pathway

Endocrine therapy resistance is a predominant clinical challenge in the management of estrogen receptor (ER)-positive breast cancer, with one of the significant hurdles in treating this illness being great crosstalk in tumor pathways. Among these pathways, the PI3K/AKT/mTOR pathway has been recognized as a substantial contributor to endocrine resistance [94]. Contemporary research has revealed crosstalk between the PI3K/AKT and the Hh pathways that can contribute to the development of tamoxifen resistance. Specifically, in tamoxifen-resistant

cells, the activation of the PI3K/AKT pathway has been shown to attenuate the proteasomal degradation of key components of the Hh pathway, Gli1 and SMO, leading to the atypical activation of the Hh signaling cascade [95]. Gli1, a positive transcriptional effector of the Hh signaling pathway, has been demonstrated to be significantly upregulated under conditions of serum starvation, where activation of the PI3K/AKT pathway enhances the expression of the Gli1 gene. This upregulation of Gli1 is associated with a prosurvival role in breast cancer [96]. Additionally, a multitude of studies have indicated that PI3K/Akt/mTOR inhibitors and Hh inhibitors have synergistic effects, suggesting that the concurrent use of these two classes of signaling modulators can delay the emergence of drug resistance and curb cancer metastasis in breast cancer [97–99]. For example, the combination therapy using sonidegib (SMO inhibitor) and buparlisib (PI3K kinase inhibitor) treating metastatic breast cancer has been in phase Ib study (NCT01576666). These discoveries have furnished evidence for the interplay between the Hh signaling and PI3K/Akt/mTOR signal transduction in breast cancer. Therefore, the concurrent targeting of these two pathways is imperative for addressing the issue of endocrine resistance in patients with advanced hormone-refractory breast cancer.

NF-κB signaling pathway

Nuclear factor-kappa B (NF-κB) is a crucial transcription factor, the activation of which is a significant common mechanism in the metastasis and therapeutic progression of breast cancer [100, 101]. Numerous reports have described a bidirectional regulation between Notch and NF-κB through various context-dependent mechanisms. Clinical studies have identified elevated levels of Shh in breast cancer tissues with positive nuclear staining for NF-κB and hypomethylation of the promoter region [102]. Further in vitro experiments have elucidated the relationship between Shh, NF-κB, and promoter methylation. Specifically, hypomethylation within the Shh promoter facilitates the binding of NF-κB to its cognate sites, subsequently cooperating to induce the transcription of Shh [103]. Overexpression of the Shh gene continually enhances the self-renewal and migratory capabilities of breast cancer cells, providing new insights into the role of Shh in promoting malignant behavior in breast cancer. In addition, Sierra A Colavito et al. offered direct evidence of the binding of NF-κB subunit p65 to Gli1 promoters in EMT and claudin-low cell lines, revealing crosstalk between NF-κB and Gli1 signaling. They suggest that targeting these pathways may be effective against the claudin-low breast cancer subtype [104]. Sinomenine suppressed the activation of Shh by blocking the NF-κB pathway and alleviated the progression of breast cancer lung metastasis in mice in vivo [105]. Hh pathway-specific small molecule inhibitors also suppress the invasive capabilities of breast cancer cell lines by inhibiting the expression levels of NF-κB, MMP2, and MMP9 proteins [106]. These findings indirectly substantiate the entangled crosstalk between the two pathways. Elucidating the molecular mechanisms underlying the cross-regulation between Hh and NF-κB may pave the way for developing synergistic therapeutic strategies to enhance the efficacy of breast cancer treatment in the future.

TGF-β signaling pathway

TGF-β is a pleiotropic cytokine and multifunctional growth factor that plays a complex role in the pathogenesis of breast cancer [107, 108]. TGF-β acts as a tumor suppressor in the early stages of the disease and as a tumor promoter in its later stages [109]. Its intercommunication and crosstalk with diverse signaling pathways augment the potential for exploring it as an integrative biomarker. The TGF-β signaling pathway crosstalk with Hh signaling, resulting in the acquisition of mesenchymal phenotypes, enhanced growth, and TNBC invasion [110]. The TGF-β/SMAD cascade is often considered a potent inducer of Gli2 [111]. In the pathological state of TGF-β induced cancer fibrosis, reactivation of the Hh signal will lead to more fibrosis [112]. Jitesh Pratap and colleagues have demonstrated that Gli2 and its target gene PTHrP, a regulator of osteoclast formation, are upregulated in response to TGF-β signaling, and this process plays a pivotal role in the osteolytic metastasis of TNBC [113]. Consequently, this exciting discovery opens up new possibilities for treatment, as targeting the TGF-β and Hh signaling pathways in breast cancer bone metastasis may inhibit osteoclast activation and bone erosion, effectively delaying cancer progression.

RAS/MAPK signaling pathway

A growing body of preclinical evidence supports the targeting of the Ras/MAPK signaling pathway in the TNBC subtype, despite the infrequency of atypical mutations in this pathway as indicated by large-scale genomic surveys, such as The Cancer Genome Atlas [114, 115]. However, studies have identified that the Ras signaling pathway is capable of activating GLI target genes downstream of SMO in a manner independent of Hedgehog ligand engagement [116]. Arthur M. Mercurio and colleagues have revealed a novel autocrine signaling pathway in TNBC mediated by VEGF/NRP2 and α6β1 signaling. This signaling cascade activates a FAK-dependent RAS/MEK signaling enhancement, leading to increased expression of Gli1. Significantly, Gli1, in turn, stimulates the expression of NRP2, establishing a positive feedback loop that sustains this autocrine pathway [57]. In vitro, human recombinant Shh has been shown to directly

upregulate the expression of MAPK and activate the p38/MAPK-activated protein kinase (MK2), thereby exerting a novel role in promoting glycolysis and proliferation in breast cancer cells [117]. Additionally, the natural Hh pathway inhibitor cyclopamine has been shown to induce a robust G1 cell cycle arrest and significantly impact the expression of the cell cycle protein cyclin D1 through modulation of the MAPK/ERK signaling pathway [106]. Cancerous and non-mutational abnormal RAS signaling has been found to enhance the function of Gli1 in pancreatic, gastric, and breast cancers through multiple mechanisms. These include enhancing its transcriptional activity, promoting its nuclear translocation, inhibiting Sufu-mediated cytoplasmic sequestration, and suppressing proteasome-mediated degradation [57, 118, 119]. These findings suggest that RAS and Hh signaling pathways may have a synergistic role in tumor formation or maintenance. KRAS, a particular member of the RAS gene family, is frequently mutated in pancreatic cancer and has been found to activate the function of GLI downstream of SMO significantly. However, this effect is independent of Hedgehog ligands, as the Hh inhibitor cyclopamine does not reverse the overactivation of GLI induced by oncogenic KRAS [120, 121]. In addition, Morton et al. reported a positive interaction between Hh and KRAS, suggesting that cotransduction of Shh and KRAS significantly enhanced cell proliferation and tumor growth in non-cancerous pancreatic duct epithelial cells compared to single gene transduction [122]. It is noteworthy that the interplay between RAS and GLI may be more complex than what current research has revealed. The existence of this intriguing mechanistic effect in breast cancer warrants further exploration.

Wnt signaling pathway

The Wnt signaling pathway is a developmental signaling cascade that plays a crucial role in regulating normal mammary gland development. Aberrant activation of this pathway can lead to hyperplasia of the breast and tumorigenesis [123, 124]. In recent years, the crosstalk between the Hh and Wnt signaling pathways has been established as having significant clinical implications in the progression of breast cancer. β-catenin, a vital molecule of the Wnt signaling pathway, participates in the Hh signaling pathway by enhancing GLI transcriptional activity [125]. It is noteworthy that the Hh-regulated transcription factor Gli3, produced in the absence of Gli3R, can interact with the C-terminal domain of β-catenin to inhibit the canonical Wnt signaling pathway [126]. Researchers such as Arnold, K. M. et al. found that co-activation of Hh and Wnt signaling pathways is associated with poorer prognosis in patients with TNBC and that this crosstalk may lead to an increased risk of early cancer recurrence and shorter overall survival [127]. Therefore, the joint activation status of Hh and Wnt signaling pathways may serve as an effective indicator for predicting the risk of early recurrence in TNBC patients. Monitoring their activation status can help identify patients who are resistant to treatment and at risk for early relapse, and this combined analysis may hold more valuable prognostic information than considering either pathway alone.

Inhibitors of the hedgehog signalling pathway targeting TME in breast cancer

Due to the essential and multiple roles of Hedgehog signaling in the TME, novel molecular therapies targeting this pathway have shown unlimited prospects. A variety of Hedgehog pathway inhibitors have been developed and can be divided into different classes based on their targets in the pathway, including SMO inhibitors (e.g. Cyclopamine, GDC-0449), HH inhibitors (e.g. 5E1, RU-SKI 43) and GLI inhibitors (e.g. GANT61, Arsenic trioxide). Figure 4 highlights current Hh signaling pathway inhibitors targeting TME in breast cancer.

The first class is SMO inhibitors. Small molecule SMO antagonists target Hh signaling by binding to the pockets within the extracellular domain (ECD) and the transmembrane domain (TMD) of SMO [128]. Cyclopamine, recognized as the inaugural Hh signaling pathway inhibitor, has revealed its multifaceted potential in targeting the TME. It potently suppresses the SMO-GLI signaling cascade, consequently downregulating the expression of GLI target genes, such as cyclin D1 and BCL2. This downregulation is crucial for inhibiting EMT in breast cancer cells, a process fundamental to the metastatic cascade. Additionally, cyclopamine attenuates the secretion of matrix metalloproteinases (MMP-9 and MMP-2), enzymes that are significantly implicated in tumor invasiveness and metastasis. Through these mechanisms, cyclopamine exerts a synergistic effect on the inhibition of anchorage-independent growth in oncogenic breast cancer cells, markedly reducing tumor proliferation and promoting apoptosis [129, 130]. In the modulation of the tumor's acidic microenvironment, cyclopamine also plays a constructive role. It diminishes the expression of carbonic anhydrase II (CAXII), an enzyme pivotal to establishing the TME's acidic milieu. By mitigating the acidic status of the TME, cyclopamine impairs the migratory capacity of breast cancer cells, thereby curtailing their propensity for proliferation and metastasis [85]. Moreover, cyclopamine has demonstrated significant efficacy in inhibiting the Hh signaling pathway within CSCs. It prevents the overexpression of GLI proteins and the upregulation of the BMI-1 gene, factors that are intimately associated with the self-renewal and tumorigenic potential of CSCs. Through these actions, cyclopamine suppresses the number and proliferation rate of CSCs and diminishes the expression of the stemness marker

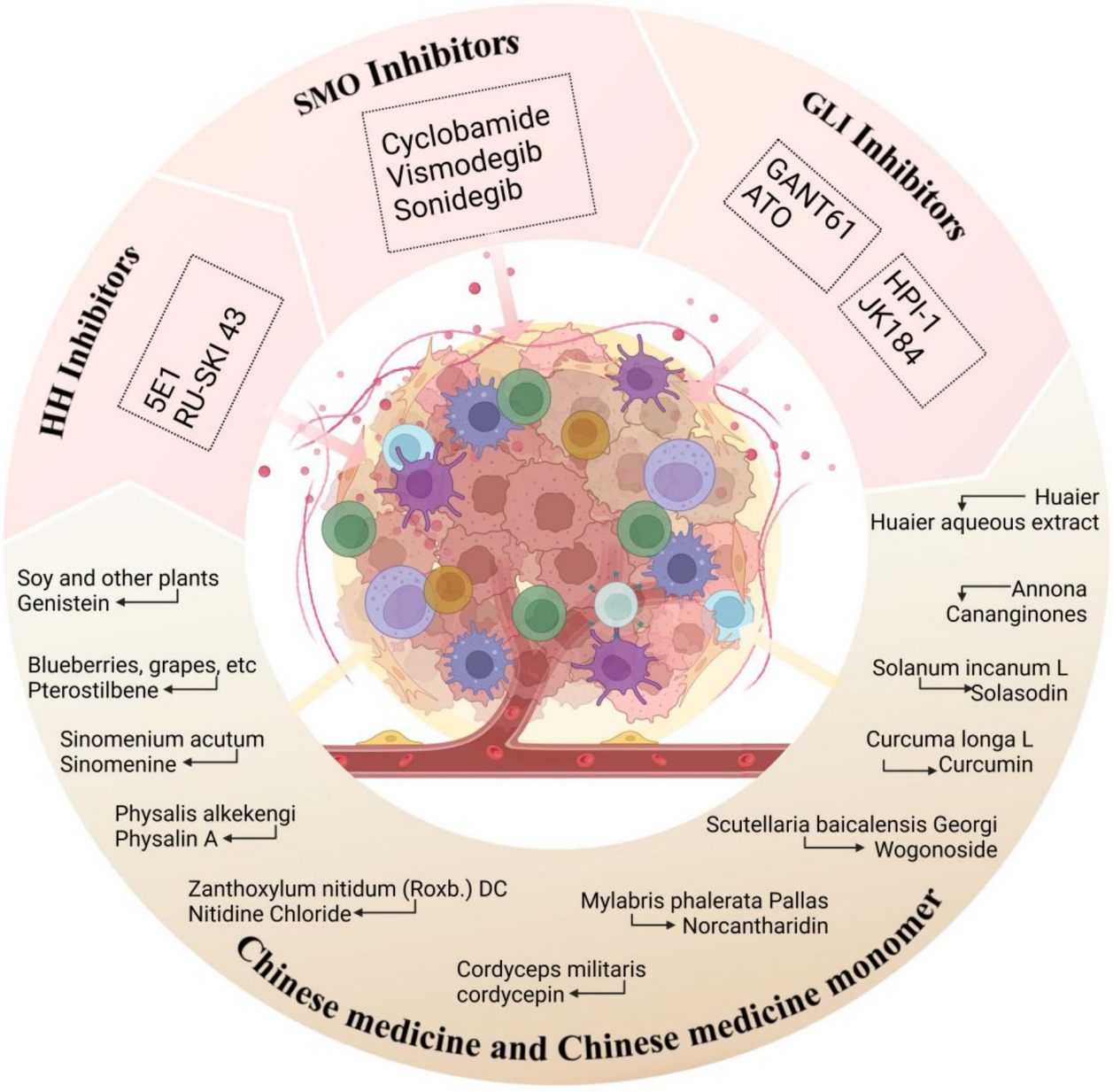

Fig. 4 Hh signalling pathway inhibitors targeting TME in breast cancer. Utilizing a multitude of promising approaches to inhibit the Hh signaling pathway may offer significant contributions to therapeutic strategies for breast cancer treatment. Current inhibitors targeting Hh signaling include small molecule inhibitors and natural compounds. These small molecule inhibitors are broadly categorized into: SMO inhibitors (e.g., Cyclopamine, Vismodegib, Sonidegib), HHh inhibitors (e.g., 5E1, RU-SKI 43), and GLI inhibitors (e.g., GANT61, Arsenic trioxide [ATO], HPI-1, JK184). In addition, several naturally occurring compounds have been identified as inhibitors of the Hh signaling pathway, such as Huaier aqueous extract, Genistein from soy and other plants, Cananginones from Annona, Pterostilbene from blueberries and grapes, Solasodin from Solanum incanum L, Sinomenine from Sinomenium acutum, Curcumin from Curcuma longa L, Physalin A from Physalis alkekengi, Wogonoside from Scutellaria baicalensis Georgi, Nitidine Chloride from Zanthoxylum nitidum (Roxb.) DC, Norcantharidin from Mylabris phalerata Pallas, and Cordycepin from Cordyceps militaris

ALDH1, which is of paramount importance for the management of tumor recurrence and metastasis [131]. GDC-0449 or Vismodegib, was the first FDA-approved small molecule as an SMO inhibitor for use in the treatment of Basal Cell Carcinoma (BCC) and is currently included in a clinical trial of the combination in TNBC [67, 132]. Studies have confirmed that treatment with Vimodegib enhances the efficacy of breast cancer chemotherapy by normalizing the TME through a reduction in proliferative CAFs and, in some cases, the levels of collagen and hyaluronan [133]. Meanwhile, the administration of Vismodegib induces changes in the portfolio of breast cancer tumor-infiltrating immune cells, reprogramming the dysfunctional immune microenvironment [21].

Sonidegib, another potent and selective oral SMO inhibitor, significantly reduced alkaline phosphatase (ALP) activity in a mouse model of breast cancer metastasis and restored NK cell infiltration at the site of bone metastasis, thereby inhibiting ER-positive breast cancer colonization [49]. In addition, the antifungal drug itraconazole has been reassessed as an inhibitor of the Hh signaling pathway as it prevents the ciliary accumulation of SMO and acts on the Hh pathway [134]. It was found that itraconazole effectively inhibited angiogenesis and reduced tumor blood perfusion by selectively inhibiting VEGF signaling in MCF-7 cells in the breast cancer microenvironment [135, 136].

While the majority of Hh pathway inhibitors target the transmembrane protein SMO, some small molecules that target the signaling cascades upstream and downstream of SMO to remodel the TEM have also attracted particular interest from researchers.

The most prominent drug candidate that works upstream of SMO is the 5E1 monoclonal antibody. Related studies have highlighted the importance of the stroma in Hh-mediated tumor growth and that the monoclonal antibody 5E1 can inhibit breast cancer growth by affecting Hh signaling in the stroma [137]. Palmitoylation is a post-translational modification essential for Hedgehog signaling. Hedgehog acyltransferase (Hhat), a member of the membrane-bound O-acyltransferase (MBOAT) family, achieves palmitoylation modification of Shh by catalyzing the covalent attachment of palmitate to the N-terminus of Hedgehog proteins [138]. Notably, RU-SKI 43, a small molecule inhibitor, can target the activity of Hhat to directly inhibit shh palmitoylation, thereby reshaping the breast cancer microenvironment andsuppressing tumor proliferation [139, 140].

Compared to targeting SMO upstream targets, downstream GLI inhibitors have demonstrated broader applicability in intervening in TME treatment strategies. In particular, GANT61, the first GLI inhibitor to be discovered, has been investigated in various tumor types in recent years [141]. It was found that GANT61 interferes with TME reprogramming by inhibiting FGF5 activation and collagen fiber secretion in CAFs, thereby reducing the number of CSCs in TNBC and achieving tumor stem cell plasticity [142]. It was confirmed that EMT-transcription factors are closely associated with activated Hedgehog/GLI signaling in human breast tumors [143]. Of interest, EMT cells can promote breast cancer metastasis through non-cell-autonomous activation of the GLI transcriptional program in neighboring epithelial tumor cells. However, applying GANT61 blocked this effect and inhibited growth in the PDX model [143]. Furthermore, treatment with GANT61 in immunocompetent mouse models of breast cancer induces significant alterations in the TME, characterized by an increase in cytotoxic immune cells and a decrease in immunosuppressive innate and adaptive cells [21]. In pharmacological experiments, GANT61 not only targets and inhibits the proliferation and growth of inflammatory breast cancer cells but also effectively reduces the formation of tumor emboli, lowering the risk of tumor metastasis and the likelihood of embolism [144]. It is evident that GANT61 is one of the most compelling antagonists. Unfortunately, current evidence of efficacy is limited to the preclinical drug development stage [145].

Arsenic trioxide (ATO), a drug that has been approved by the FDA for the treatment of acute promyelocytic leukaemia (APL), has been shown to participate in preclinical trials as a GLI destabiliser [146]. This is attributed to the fact that by blocking ciliary transport and promoting destabilisation of the Gli2 protein, ATO effectively inhibits its downstream components of the Hedgehog signalling pathway [147].

It is well known that metabolic reprogramming is essential for the establishment of TME in breast cancer [148]. ATO was found to reduce the protective effect of CAFs on breast cancer cells by uncoupling metabolism between epithelial cancer cells and fibroblasts in the TME, which is an essential strategy for preventing resistance to anti-estrogenic drugs. At the same time, ATO increased ROS production and was able to inhibit the mitochondrial electron transport chain, thereby increasing glycolytic processes and optimizing the TME [149]. Furthermore, ATO also promotes the release of high mobility group protein B1 (HMGB1), transforming “cold” tumors, which are unattractive to immune cells, into “hot” tumors, which are more attractive to immune cells, thus promoting immune cell infiltration and identification of new targets [150].

Another class of potent antagonists of the Hh transcription factor Gli1, HPI-1, disrupt GLI transport to primary cilia and ciliogenesis in a different manner, functioning independently of upstream components of the pathway [151, 152]. HPI-1 has been reported to significantly reduce the percentage of cancer stem cells in breast cancer cells and is a candidate for targeting breast cancer stem cells [153]. In recent years, JK184 has shown great promise in cancer therapy by virtue of its specific inhibition of GLI in the Hedgehog pathway. On the one hand, in the 3D tumor spheroid assay, JK184 was able to significantly reduce the size and number of tumor spheroids formed by SUM149 cells, suggesting that JK184 may have an inhibitory effect on tumor cell aggregation and tumor embolus formation [144]. On the other hand, JK184 was more effective in inhibiting the proliferation of EMT cells, which was associated with the fact that EMT cells exhibited high Gli1 expression [104].

Over the past few years, natural products have provided an unrivaled supply of anti-cancer drugs. The use

of natural compounds and their derivatives to modify the TME and Hh signaling pathways offers an attractive approach to treating tumors. Here, we emphasize the potential of phytochemicals to target the Hh signaling pathway and remodel TME in breast cancer therapy (Table 1).

Conclusion and prospect

Breast cancer is the leading cause of cancer morbidity and mortality in women worldwide, characterized by poor prognosis, early recurrence, high invasiveness, and a high degree of metastasis [166, 167]. These adverse outcomes can be partly attributed to complex TME [168, 169]. Within this tumor nest, the interrelations among various components constitute a three-dimensional network. The Hh signaling pathway, a pivotal modulator of developmental processes and stem cell fate, resides at the epicenter of this network. Collective evidence to date indicates that aberrant activation of the Hh signaling pathway exerts regulatory control over the cellular constituents within the breast cancer TME and is engaged in interactions with physical factors. Beyond the interplays above, the Hh signaling cascade also induces crosstalk among numerous signaling pathways, culminating in the formation of a complex regulatory network, which further extends the proliferative potential of this pathway in human cancers. This complex interplay results in the hallmark invasive characteristics of breast cancer, such as the development and maintenance of cancer stem cells, EMT, therapeutic resistance, and immune evasion. Therefore, gaining a more profound understanding of the crosstalk between the Hh signaling and the TME may pave the way for developing novel therapeutic strategies to halt breast cancer progression.

Over the past decade, the targeting of specific signaling pathways has revolutionized the management of certain cancers. The critical oncogenic role of the Hh signaling pathway has spurred efforts to identify small molecule inhibitors of this pathway. Inhibitors targeting the Hh signaling pathway have been demonstrated to exhibit significant efficacy in the treatment of advanced and metastatic basal cell carcinoma [170, 171]. That said, they still carry the risk of drug resistance, which is mainly attributed to point mutations in the drug binding pocket of the SMO protein or downstream molecules, as well as the activation of compensatory pathways such as PI3K/AKT [172–175]. Furthermore, unfavorable solubility and suboptimal pharmacokinetic (PK) properties also constrain the therapeutic success of Hh antagonists [176]. Fortunately, researchers have developed several drug delivery systems to overcome many of these challenges [177, 178]. For example, the superparamagnetic iron oxide nanoparticle formulation of curcumin enhances gemcitabine therapeutic response via suppression of the Shh pathway and an oncogenic CXCR4/CXCL12 signaling axis that inhibits bidirectional tumor-stromal cell interaction [179]. Concurrently, targeted therapies that act on the Hh pathway must be approached cautiously due to the potential for inducing numerous side effects and drawbacks. GDC-0449 and Vismodegib have demonstrated the ability to elicit objective responses in patients with locally advanced and metastatic basal cell carcinoma. However, adverse events, including taste disturbances, muscle spasms, and alopecia, have led to treatment discontinuation in most patients [171, 180]. In a phase I clinical

Table 1 Summary of phytochemicals targeting hedgehog signalling to intervene in TME in breast cancer

Drugs	Natural sources	Proposed Mechanism	Reference
Genistein	Soy and other plants	Decrease Breast cancer stem cells (BCSCs) by downregulating the Hedgehog-Gli1 Signaling Pathway	[154]
Pterostilbene	Blueberries, grapes, etc.	Reduce BCSCs stemness by inhibiting the hedgehog/Akt/GSK3β signaling	[155]
Curcumin	Curcuma longa L	Inhibit EMT and characteristics of BCSCs by targeting the Hedgehog/Gli1 pathway	[156]
Nitidine Chloride	Zanthoxylum nitidum (Roxb.) DC	Suppresse breast cancer EMT and CSCs-like properties by inhibiting Hedgehog signaling pathway	[157]
Cananginones	Annona	Abrogate cancer stem cell renewal as well as EMT and increase the ROS level by inhibiting Gli1 in a non-canonical pathway	[158]
cordycepin	Cordyceps militaris	Block EMT by inhibiting expression of Hh pathway components and GLI transcriptional activity	[159, 160]
Sinomenine	Sinomenium acutum	Suppress the activation of NF-κB and Shh signaling pathways	[105]
Wogonoside	Scutellaria baicalensis Georgi	Attenuate growth and angiogenesis by inhibiting Gli1 nuclear translocation and transcriptional activities associated with Hedgehog signaling	[161]
Solasodine	Solanum incanum L	Target CSCs suppressing Hh/Gli1 signaling	[162]
Norcantharidin	Mylabris phalerata Pallas	Overcome multidrug resistance by inhibiting Shh signaling and expression of its downstream mdr-1/P-gp expression	[163]
Physalin A	Physalis alkekengi	Reduce CSC marker genes by reducing Gli1 gene expression	[164]
Huaier aqueous extract	Huaier	Eliminate the breast cancer stem cells through inactivation of the Hh pathway	[165]

study, the combination of sonidegib plus docetaxel (fixed dose at 75 mg/m^2) for the treatment of TNBC patients also resulted in a series of Grade 3 adverse events [181]. Therefore, there is an urgent need to elucidate the specific conditions and processes underlying the activation of the Hh pathway and to identify additional and superior strategies for inhibiting oncogenic Hh signaling.

Abbreviations

TME	Tumor microenvironment
Hh	Hedgehog
TNBC	Triple-negative breast cancer
SMO	Smoothened
Shh	Sonic Hedgehog
IHh	Indian Hedgehog
DHh	Desert Hedgehog
TAMs	Tumor-associated macrophages
TCRs	T cell receptors
Tregs	T regulatory cells
CAFs	Tumor-associated fibroblasts
BCSCs	Breast Cancer Stem Cells
MSCs	Mesenchymal stem cells
NK	Natural killer
VEGF	Vascular endothelial growth factor
CYR61	Cysteine-rich angiogenic inducer 61
NRP2	Neuropilin-2
IL-6	Interleukin-6
HIF-1	Hypoxia-inducing factor 1
EMT	Epithelial-mesenchymal transition
MDSCs	Myeloid-derived suppressor cells
CAs	Carbonic anhydrases
CAXII	Carbonic Anhydrase isoform XII
ER	Estrogen receptor
NF-κB	Nuclear factor-kappa B
TGF-β	Transforming growth factor β
MK2	P38/MAPK-activated protein kinase
ECD	Extracellular domain
TMD	Transmembrane domain
BCC	Basal Cell Carcinoma
ALP	Alkaline phosphatase
Hhat	Hedgehog acyltransferase
MBOAT	Membrane-bound O-acyltransferase
ATO	Arsenic trioxide
APL	Acute promyelocytic leukaemia
HMGB1	High mobility group protein B1
LAIR1	Leukocyte associated immunoglobulin like receptor 1
VHL	Von Hippel-Lindau
ALDH1	Aldehyde dehydrogenase 1

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Author contributions
Ruijuan Liu, Yang Yu and Qingyang Wang designed the framework of this article. Ruijuan Liu and Qianxiang Zhao are coresponsible for the collection, and writing of the original manuscript. Yan Yao and Mengxuan Sun revised the details of the manuscript and created visualizations. Jing Zhuang, Changgang Sun and Yuanfu Qi responsible for the review of the manuscript. All authors contributed to the article and approved the submitted version.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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