Self-Renewal Signalling Pathway Inhibitors: Perspectives on Therapeutic Approaches for Cancer Stem Cells

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Abstract: The poor survival and prognosis of individuals with cancer are often attributed to tumour relapse and metastasis, which may be due to the presence of cancer stem cells (CSCs). CSCs have the characteristics of self-renewal, differentiation potential, high carcinogenicity, and drug resistance. In addition, CSCs exhibit many characteristics similar to those of embryonic or tissue stem cells while displaying persistent abnormal activation of self-renewal pathways associated with development and tissue homeostasis, including the Wnt, Notch, Hedgehog (Hh), TGF- β , JAK/STAT3, and NF- κ B pathways. Therefore, we can eliminate CSCs by targeting these self-renewal pathways to constrain stem cell replication, survival and differentiation. At the same time, we cannot neglect the ping-pong effect of the tumour microenvironment, which releases cytokines and promotes self-renewal pathways in CSCs. Recently, meaningful progress has been made in the study of inhibitors of self-renewal pathways in tumours. This review primarily summarizes several representative and novel agents targeting these self-renewal signalling pathways and the tumour microenvironment and that represent a promising strategy for treating refractory and recurrent cancer.

Keywords: cancer stem cells, signalling pathway inhibitors, targeted therapy, small-molecule chemicals, tumour microenvironment

Introduction

A growing collection of evidence has demonstrated that cancer is the primary threat to human health. To date, there are 18.1 million newly diagnosed cases and 9.6 million cancer-related deaths.¹ Due to its high fatality rate, cancer remains one of the toughest health challenges human beings face.

Cancer therapy is primarily hindered by recurrence and chemoresistance. Accumulating evidence has suggested that cancer stem cells (CSCs), which initiate and maintain tumour growth, are a small subset of tumour cells. CSCs are thought to cause tumour relapse, metastasis, and chemo-resistance.^{2,3} In 1994, Lapidot et al first isolated human acute myeloid leukaemia stem cells (LSCs) using specific cell surface markers. Their research revealed that only LSCs possessed the high self-renewal capacity necessary to maintain the malignant phenotype, strongly supporting the objective existence of CSCs.⁴ Subsequently, CSCs were identified in many types of solid tumours, including pancreatic,⁵ breast,⁶ lung,⁷ and liver tumors.⁸ Traditional chemotherapy eliminates the bulk of tumour cells but cannot eradicate CSCs, which have enhanced repair and renewal abilities.^{9,10} Due to their self-renewal ability and

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therapy resistance, CSCs are considered the root cause of tumorigenesis, progression, drug resistance and recurrence.¹¹ Previous studies have found that CSCs are enriched after chemotherapy.¹² Multiple signalling pathways are abnormally activated in CSCs.¹³ Furthermore, changes in the tumour microenvironment (TME) after treatment, such as anti-angiogenic tumour tube neonatal drug treatment, can cause tumour tissue to become hypoxic, which induces tumour stem cell proliferation.¹⁴ Therefore, targeting CSCs is a more effective approach for treating cancer. Numerous studies have shown that abnormalities in different signalling pathways exist in CSCs, including the Notch, Hedgehog (Hh), and Wnt pathways, which play vital roles in embryonic development and differentiation of normal stem cells.¹⁵ In addition, the TME releases cytokines that increase activation of these signalling pathways to enhance the cancer stem cell population.¹⁶ Therefore, targeting these pathways and the TME represents a promising therapy to suppress CSC self-renewal and proliferation and thus the tumour development promoted by CSCs.

Herein, we focus on six key self-renewal CSC signalling pathway inhibitors for the Wnt, Hh, Notch, TGF- β , JAK/STAT3 and NF- κ B pathways as well as the TME, with the hope that this discussion may provide new insight for advances in clinical oncology.

Signalling Pathway Inhibitors

Tumours are prone to recurrence and metastasis due to the existence of CSCs, which convey a poor prognosis. CSCs demonstrate persistent abnormal activation of self-renewal pathways. Hence, targeting these dysregulated signalling pathways is expected to be useful for cancer treatment.¹⁷ It has been hypothesized that cancer can be eliminated or perpetually inhibited by inhibiting CSC signalling pathways while avoiding serious impacts on normal tissue renewal.¹⁸ Therefore, signalling pathway inhibitors are a promising strategy for cancer therapy.¹⁹

Targeting the Wnt Signalling Pathway

The evolutionarily conserved Wnt pathway regulates the pluripotency of stem cells²⁰ and plays a crucial role in self-renewal and differentiation of cells.^{21,22} In this signalling pathway, the Axin/GSK-3 β /APC complex promotes degradation of the intracellular signalling molecule β -catenin. However, when the Wnt ligand is activated by binding to Frizzled and the low-density lipoprotein-related receptor (LRP), the Axin/GSK-3 β /APC complex decomposes. Then, intracytoplasmic β -catenin becomes stable and can enter the

nucleus to facilitate transcription of target genes^{21,23} (Figure 1). Abnormal activation of Wnt signalling is thought to promote CSC development, leading to malignant transformation.²⁴ Therefore, many small-molecule inhibitors that specifically target these key factors in the pathway, such as Frizzled, Dishevelled, Porcupine, or Tankyrase, can be designed via drug development.²⁵

LGK974 (Wnt974)

LGK974 is a compound that specifically targets Porcupine,²¹ which performs posttranslational Wnt acylation that is necessary for Wnt secretion.²⁶ LGK974 significantly blocks Wnt signalling²⁷ and is well tolerated in rodent tumour models. It has been reported that treatment with LGK974 significantly inhibits the proliferation of breast cancer stem cells (BCSCs).²⁸ LGK974 is a new strategy for effectively targeting glioblastoma stem-like cells.²⁷ The latest research suggests that cutaneous squamous cell carcinoma (cSCC) mice given LGK974 exhibit differentiation of cancer cells and an obvious reduction in CSCs. Therefore, the Wnt signalling pathway is crucial for initiation, progression and maintenance of the CSC niche in cSCC. Thus, interfering with Wnt secretion is a promising therapeutic approach.²⁹ LGK-974 is currently undergoing phase I clinical trials.³⁰

ICG-001 (PRI 724)

ICG-001 is a small-molecule inhibitor that selectively targets cAMP-response element-binding (CREB) binding protein (CBP). The CBP/ β -catenin/FOXM1 transcriptional complex leads to increased CSC numbers, drug resistance and a poor prognosis. Therefore, CBP may represent a novel therapeutic target for eliminating CSCs.³¹ In light of its ability to suppress the Wnt signalling pathway, ICG-001 has anti-tumour effects in certain tumour types. It was shown that ICG-001 represents a potential therapeutic strategy for gastric cancer because of its ability to restrain cell growth, metastasis, gastric cancer stem cell-like properties and chemoresistance.³² Moreover, ICG-001 effectively suppressed tumour metastasis of aggressive subpopulations of oral squamous cell carcinoma stem-like cells in zebrafish embryos.³³ By disrupting Wnt signalling, ICG-001 also exerts potent effects against leukaemia stem cells.³⁴ Using clinical databases and patient tumour samples, dual inhibition of Wnt and YAP using ICG-001 and simvastatin, respectively, powerfully suppressed CSC populations in triple negative breast cancer (TNBC).³⁵ Collectively, these data suggest that ICG-001 is a potential agent for eliminating or inhibiting CSCs in various cancers.

Figure 1 Schematic representation of the key CSC signalling pathways Notch, Wnt, and Hedgehog. Some of the current drugs that target these pathways in CSCs are shown. **Abbreviations:** NICD, Notch intracellular domain; CBF-1, C-promoter binding factor-1; TCF/LEF, T cell factor/lymphoid enhancer factor; LRP, Low-density lipoprotein-related receptor; GSK-3 β , glycogen synthase kinase 3 β ; APC, adenomatous polyposis coli; CBP, cAMP-response element-binding protein; Gli, glioma-associated oncogene.

Ipafricept (OMP-54F28)

Ipafricept is a recombinant fusion protein comprising the ligand-binding domain of frizzled receptor 8 and an immunoglobulin G1 (IgG1) Fc fragment. Ipafricept performs the role of a decoy receptor that prevents the Wnt receptor from binding to Wnt ligands, thereby inhibiting Wnt signalling.³⁶ Recent studies have shown that the Wnt inhibitor Ipafricept can suppress the invasiveness, sphere formation and growth of CSCs in head and neck squamous cell carcinoma (HNSCC).³⁷ Ipafricept is being used in phase I clinical trials for treatment of cancer patients.³⁸

Targeting the Notch Signalling Pathway

The Notch signalling pathway is an evolutionarily conserved pathway that influences the survival and differentiation of CSCs.^{39,40} It also plays an important role in tumour initiation and progression.⁴¹ When the ligand protein binds to the Notch receptor, the Notch intracellular domain (NICD) is

released into the nucleus through three cleavages mediated by γ -secretase, activating transcription of Notch target genes⁴² (Figure 1). The Notch pathway is composed of a series of signalling molecules, including NICD, Hes1 and Hey1, which play a vital role in cell development and maintenance of stemness.⁴³ Activation of Notch signalling promotes tumour proliferation and metastasis, while inhibition of this pathway can eliminate CSCs and increase sensitivity to drugs. Thus, genes in the Notch signalling pathway may represent potential cancer therapeutic targets.^{44,45} Notch inhibitors can be used singly or in combination with chemotherapy agents to treat cancer and prevent recurrence.⁴⁶ For example, chemotherapeutic drugs combined with NOTCH1 inhibitors synergistically impair chemotherapy-enriched CSC populations both in vitro and in vivo. Therefore, Notch signalling pathway inhibitors exhibit anti-proliferative tumour effects and represent a novel treatment for cancer.⁴⁷

DAPT (GSI-IX)

DAPT (GSI-IX) is a novel γ -secretase inhibitor that suppresses A β with an IC₅₀ of 20 nM in HEK 293 cells. Currently, it is primarily used to treat Alzheimer's disease, but a study found that it had potential as an anti-CSC drug. In fact, the Notch signalling pathway is significant in the self-renewal of osteosarcoma stem cells (OSCs). DAPT is a cell-permeable dipeptide that blocks ligand-induced Notch proteolysis and inhibits Notch activity, targeting OSCs and increasing tumour sensitivity to platinum.⁴⁸ A previous study demonstrated that DAPT suppresses LSC growth by inhibiting proliferation in colony formation assays and regulates LSC self-renewal, potentially providing a novel agent for cancer therapy.⁴⁹ In addition, DAPT suppresses the self-renewal ability and stemness of ovarian cancer cells.⁵⁰ Therefore, DAPT is a prospective anti-tumour agent due to its high specificity, minimal side effects and attenuated toxicity. DAPT has currently entered phase II clinical trials.

MK-0752

MK-0752 is a moderately effective γ -secretase inhibitor that targets A β 40 with an IC₅₀ of 5 nM. CSCs can be targeted by MK-0752 combined with chemotherapy agents to treat recurrent, metastatic and refractory cancer. Studies have shown that MK-0752 combined with tocilizumab significantly reduces BCSC numbers and suppresses tumour growth.⁵¹ For patients with advanced solid tumours, treatment with MK-0752 combined with dalotuzumab was shown to inhibit cell proliferation, angiogenesis and stem cell propagation in a phase I trial.⁵² Likewise, patients with advanced breast cancer were treated with MK-0752 and docetaxel in clinical trials, and tumours were serially biopsied. This study found that the index of CSCs, which identified CD44⁺/CD24⁻ and ALDH⁺ cells, and mammosphere formation ability were significantly reduced.⁵³ These studies suggest that the combination of MK-0752 and chemotherapy may represent a novel approach to treating various cancers. At present, MK-0752 has completed a phase I clinical trial.

Demcizumab (OMP-21M18)

Demcizumab is an IgG2 humanized monoclonal antibody agent that targets the Notch ligand DLL4.⁵⁴ DLL4 is an important ligand in the Notch signalling pathway and plays a critical role in the maintenance and proliferation of CSCs as well as tumour angiogenesis.⁵⁵ Thus, the anti-CSC and anti-angiogenesis activities of Demcizumab contribute to its

anti-tumour efficacy. Furthermore, Demcizumab is the first antibody agent targeting the Notch signalling pathway that has entered the clinical trial phase. The anti-tumour activity of Demcizumab monotherapy was demonstrated in phase I trials in patients previously treated for solid tumours, and doses of ≤ 5 mg per week were generally well tolerated.⁵⁴ Demcizumab combined with pemetrexed and carboplatin as first-line treatment for metastatic non-squamous non-small cell lung cancer has undergone a phase IB trial.⁵⁶

Targeting the Hedgehog Signalling Pathway

The classical Hh signalling pathway is responsible for embryonic development, regulating stem cell renewal and tissue homeostasis in cancer.⁵⁷ Hh has three gene homologs: Sonic Hh (SHh), Desert Hh (DHH), and Indian Hh (IHH).^{58,59} When extracellular Hh ligands (SHh, IHH, DHH) bind to PTCH, the inhibitory effect of PTCH on Smoothened (Smo) is relieved, allowing Gli to translocate to the nucleus and induce transcription of target genes.¹⁵ (Figure 1). Abnormal activation of Hh signalling is a key driver of tumorigenesis in breast, prostate, small cell lung cancer, stomach and haematopoietic malignancies.^{60,61} Hh signalling pathway inhibitors have demonstrated efficacy in early clinical trials. Furthermore, the development of Hh inhibitors has aroused great interest in the pharmaceutical exploitation field.^{15,30} Recently, inhibition of Hh signalling was reported to suppress self-renewal and chemoresistance in pancreatic CSCs.⁶² The Hh pathway also regulates self-renewal of BCSCs via Bmi-1.⁶³ In addition, treatment with Smo inhibitors downregulated the expression of CSC markers and sensitized tumours to docetaxel treatment in a patient-derived xenograft model, with significantly improved survival and reduced metastasis.⁶⁴ Therefore, Smo receptor and Gli family proteins are useful as molecular targets for suppressing CSC self-renewal by inhibiting the Hh pathway.

Cyclopamine

Cyclopamine, a natural compound, is a Hh signalling pathway inhibitor that specifically targets Smo.⁶⁵ Hh signalling, which is overactivated in CSCs, is involved in embryonic development and tumorigenesis. A previous study demonstrated that cyclopamine is an effective method to reverse gemcitabine resistance in pancreatic cancer cells, significantly suppressing the expression of CSC markers and certain Hh members.⁶⁶ Treatment with cyclopamine downregulated the expression of stemness and epithelial-mesenchymal transition (EMT) markers, as

well as sHh downstream gene expression, in HCT-116 spheres.⁶⁷ A previous study showed that the SHh inhibitor cyclopamine has potent antitumour activity against bladder tumorigenesis and inhibits self-renewal of bladder cancer stem cells.⁶⁸ Increasing evidence indicates that cyclopamine has anti-cancer activity against a variety of human cancer types by suppressing CSC proliferation, emphasizing the druggability of the Hh pathway and providing new opportunities for discovery of novel anticancer drugs.

Vismodegib (GDC-0449)

Vismodegib (GDC-0449) is a first-in-class small molecular antagonist of the Hh pathway that binds to Smo and inhibits abnormal activation of the Hh pathway.^{69,70} Vismodegib was the first agent to target the Hh pathway and received US Food and Drug Administration (FDA) approval for treatment of advanced basal cell carcinoma (BCC).⁷¹ Vismodegib promotes differentiation of tumour cells and inhibits a hair follicle-like fate, thereby mediating regression of BCC.⁷² Currently, vismodegib has been used as a monotherapy or in combination with other chemotherapeutic drugs in clinical trials for many cancer types, including small-cell lung cancer, glioblastoma, basal cell carcinoma, metastatic pancreatic cancer and prostate cancer.⁷³ The biological activities of pancreatic CSCs that are involved in conveying malignancy, such as cell viability, Gli-DNA binding and transcriptional activities, are suppressed by vismodegib. Furthermore, vismodegib activates caspase 3 and induces PARP cleavage, promoting apoptosis of pancreatic CSCs. Therefore, by targeting pancreatic CSCs, vismodegib can be applied for effective inhibition of pancreatic cancer.⁷⁴ In addition, vismodegib has several effects on BCSCs, such as anti-proliferative, anti-invasive, pro-apoptotic and inhibitory effects. In vivo studies have also found that treatment with this drug leads to pronounced inhibition of tumour formation and growth.⁷⁵ Although vismodegib is effective in therapy against BCC, its clinical application has been impeded by severe side effects, low selectivity for CSCs, and drug resistance.⁷³ Drug-resistant Smo mutations and abnormal activation of Hh signalling downstream of Smo have been observed clinically, suggesting that alternative approaches are urgently needed.⁷⁶

GANT61

GANT61 was the first small molecular inhibitor of glioma-associated oncogene homologue 1 (Gli1).⁷⁷ Meanwhile, it is the most extensively used antagonist that targets Gli proteins to specifically block Hh signalling.⁷⁶ GANT61 has a strong

inhibitory influence on cell proliferation and migration, which has been confirmed in both in vivo and in vitro experiments in breast cancer, pancreatic cancer and other malignancies.^{78–80} Because basal cell carcinoma patients were previously reported to develop resistance to Smo inhibitors, the Gli inhibitor GANT61 is one of the most promising drugs for inhibiting Hh signalling and has been studied in various cancers.⁸¹ Accumulating experimental evidence supports the use of GANT61 as an anti-cancer agent to suppress cell proliferation, survival, apoptosis, DNA damage repair, CSCs, autophagy, EMT, and immune responses in multiple malignant tumour types.⁸¹ GANT61 not only effectively inhibited the growth of paclitaxel-resistant cells but also markedly reduced the proportion of CSCs in TNBC cells.⁸² In addition, combining GANT61 with mTOR inhibition provides an effective method for inhibiting pancreatic CSCs, thereby improving pancreatic cancer treatment⁸³ (the chemical structures of Notch, Wnt, and Hh inhibitors are shown in Figure 2).

Targeting the TGF- β Signalling Pathway

TGF- β represents a family of polypeptides involved in various pathophysiological processes, including cell proliferation, migration, viability, angiogenesis, immunosurveillance, and regulation of stem cell pluripotency and differentiation.^{84–86} Furthermore, TGF- β plays a significant role in cancer development.⁸⁷ The TGF- β ligand binds to the type II receptor, which recruits and phosphorylates the type I receptor. The type I receptor then phosphorylates the SMAD protein (R-SMAD), and R-SMAD and coSMAD subsequently combine. The R-SMAD/coSMAD complex then translocates to the nucleus, where it activates transcription of various target genes^{88,89} (Figure 3). Studies have demonstrated that the TGF- β signalling pathway may be involved in regulation of cancer stem cell-like properties. Inhibition of TGF- β signalling prevents cancer cell proliferation and EMT and reduces the CSC population, impeding cancer progression.⁹⁰ TGF- β was shown to possess anticancer effects, which are mediated by inhibition of CSC survival, and may represent a novel treatment for hepatocellular carcinoma.⁹¹ Furthermore, use of TGF- β signalling pathway inhibitors reinforces tumour penetration by nanoparticles, thereby effectively killing CSCs.⁹² Therefore, TGF- β pathway inhibitors represent a novel treatment paradigm against CSCs.

LY2109761

As a novel TGF- β receptor type I/II (T β RI/II) dual inhibitor, LY2109761 exhibits an inhibitory spectrum that targets SMAD2 and exerts antitumour activity in various tumour

LGK974

ICG-001

DAPT

MK-0752

Cyclopamine

Vismodegib

GANT61

Figure 2 Chemical structures of key inhibitors of CSC signalling pathways, including the Notch, Wnt, and Hedgehog pathways.

models, including pancreatic cancer,⁹³ colorectal cancer,⁹⁴ and hepatocellular carcinoma.⁹⁵ Previous experiments indicated that LY2109761 is an effective therapeutic method to reduce clonogenicity and enhance radiation sensitivity in glioblastoma multiforme cancer stem-like cells.⁹⁶ Recent studies have demonstrated that HSP47 promotes glioblastoma multiforme (GBM) stem-like cell survival, while blocking the TGF- β pathway using LY2109761 inhibits HSP47-induced tumorigenesis and stemness, providing a broad spectrum for GBM treatment.⁹⁷

Galunisertib (LY2157299)

Galunisertib (LY2157299) is a TGF β RI/ALK5 inhibitor with highly selective activity and is the only TGF- β pathway inhibitor currently undergoing clinical studies in patients with

hepatocellular carcinoma (HCC). It was reported that galunisertib decreases clonogenic ability, liver spheroid formation and the invasive ability of HCC cells in vitro. Recent studies have suggested that downregulation of CD44 in response to galunisertib may represent one of the mechanisms by which the agent mediates its effects on stemness and invasion of HCC cells. Galunisertib may have great prospects for clinical application⁹⁸ and is currently in phase I and phase II clinical trials.

Targeting the JAK/STAT3 Signalling Pathway

The JAK-STAT signalling pathway is involved in many important biological processes, such as cell proliferation, differentiation, apoptosis and immune regulation. Certain cytokines and

Figure 3 Schematic representation of the key CSC signalling pathways TGF- β , JAK/STAT3, and NF- κ B. Some of the current drugs targeting these pathways in CSCs are shown. **Abbreviations:** TGF- β , transforming growth factor- β ; R-Smad, receptor-regulated Smad; Co-Smad, common Smad; JAK, Janus kinase; STAT, signal transducer and activator of transcription; NF- κ B, nuclear factor kappa-B; I κ B, inhibitor of NF- κ B; IKK, inhibitor of NF- κ B kinase.

growth factors bind to the receptor, which induces STAT phosphorylation via JAK activation. Activated STAT translocates into the nucleus as a dimer and binds to target genes to regulate transcription (Figure 2). Abnormal activation of JAK/STAT3 signalling facilitates proliferation and survival of cancer cells.⁹⁹ Therefore, the JAK/STAT3 signalling pathway is a potential anti-tumour target that has been extensively studied in breast and other cancer types. Previous studies have demonstrated that targeting JAK2 and STAT3 may result in more specific and effective breast cancer treatment.¹⁰⁰ The IL-6/JAK2/STAT3 pathway is activated in BCSCs, and inhibition of JAK2 reduced this cell population and suppressed the growth of xenograft tumours. Recently, studies have shown that leptin-JAK/STAT3 facilitates breast cancer cell stemness and chemoresistance, while its inhibition leads to chemosensitization and a reduction in BCSCs *in vivo*.¹⁰¹ Thus, targeting the JAK/STAT3 signalling pathway may represent an efficient cancer treatment strategy.

Ruxolitinib (INCB018424)

Ruxolitinib, previously referred to as INCB018424 or INC424, is a selective and potent oral antagonist of Janus kinase (JAK) 1 and JAK2. In myelofibrosis (MF) and polycythemia vera (PV), abnormal activation of the JAK/STAT pathway is observed. In 2011, ruxolitinib was approved by the FDA for treatment of MF and was subsequently approved for treatment of PV in 2014.¹⁰² In addition, as an inhibitor of JAK, ruxolitinib suppresses the growth and activity of CSCs. It was demonstrated that myxoid liposarcoma (MLS) is a subpopulation of cells with CSC characteristics. Rosobinib combined with doxorubicin to treat proliferating cells and CSCs provides a new method for evading chemoresistance in patients with MLS.¹⁰³ Ruxolitinib is currently undergoing phase II clinical trials.

Napabucasin

Napabucasin (BBI608) is an oral CSC inhibitor that targets Stat3.^{104,105} In both preclinical toxicology studies and

clinical trials, napabucasin was well tolerated and showed no signs of adverse effects on haematopoiesis or other normal adult stem cells. Napabucasin effectively down-regulated stemness gene expression and eliminated cancer cells exhibiting high stemness marker expression, leading to inhibition of cancer cell spherogenesis. In addition, napabucasin had strong anti-metastasis activity in a model of spontaneous liver metastasis of colorectal cancer and inhibited cancer recurrence in a model of pancreatic cancer.¹⁰⁶ Recent studies have also shown that napabucasin significantly reduces colon cancer cell growth and tumoursphere formation, decreasing the expression of CSC markers at both the mRNA and protein level.¹⁰⁷ In summary, napabucasin may prove useful in targeting CSCs, with the potential to attenuate cancer relapse and metastasis in patients with various cancer types.¹⁰⁵ Napabucasin is currently being investigated in a phase III clinical trial.

Targeting the NF- κ B Pathway

The NF- κ B family consists of five proteins: p65 (RelA), RelB, c-Rel, NF- κ B1 (p105/p50), and NF- κ B2 (p100/p52).¹⁰⁸ The biological roles of NF- κ B involve immune responses, proliferation, development, survival, invasion and metastasis.¹⁰⁹ In general, the NF- κ B dimer binds to I κ B (inhibitory protein of NF- κ B), which inactivates NF- κ B. IKK acts as a kinase that phosphorylates I κ B, leading to its degradation. NF- κ B dimers can expose nuclear localization signals (NLS), rapidly enter the nucleus from the cytoplasm, and bind to specific DNA sequences, inducing transcription of target genes.¹¹⁰ (Figure 2). Abnormal activation of NF- κ B is found in CSCs in various cancers and may be involved in maintenance, proliferation, expansion and survival of CSCs.¹¹¹ The NF- κ B pathway reportedly activates pathways in pancreatic CSCs, whereas stem cell-like properties of cells are observably decreased in response to treatment with NF- κ B pathway inhibitors.¹¹² Therefore, inhibition of NF- κ B may provide a promising strategy to eliminate CSCs and improve anti-cancer therapies.

GTCpFE

GTCpFE is an ASA prodrug for which fumarate is the ancillary pharmacophore. This prodrug potently suppresses NF- κ B activity with no congenital cytotoxicity. GTCpFE displays apparent anti-CSC activity by inhibiting mammosphere formation and impairing the CSC-associated CD44⁺/CD24⁻ immunophenotype. These findings established a viable strategy for development of improved anti-inflammatory agents

for chemoprevention and cancer treatment.¹¹³ To improve drug efficacy and minimize toxicity, one study found that the fumarate-based ASA prodrug GTCpFE represents a prototype for development of novel anti-inflammatory and anti-CSC drugs for treatment of potentially aggressive breast cancers.¹¹⁴

BMS-345541

BMS-345541 is an IKK complex inhibitor that has been used to inactivate NF- κ B in a variety of cancer types, including melanoma and leukaemia.^{115,116} BMS-345541 effectively inhibits NF- κ B activity at concentration ranges from 0.4 μ M to 10.0 μ M. It was reported that BMS-345541 is a potential therapeutic agent for restraining breast tumorigenesis and metastasis through targeting of CSCs.¹¹⁷ Recent studies have also suggested that BMS-345541 potently inhibits stemness and self-renewal of lung CSCs. In addition, the expression of EMT-related genes and apoptosis resistance were significantly decreased in response to treatment, indicating that NF- κ B inhibition is sufficient to both induce apoptosis and prevent EMT in lung CSCs.¹¹⁸ These findings suggest that BMS-345541 decreases the CSC population within the tumour, potentially reducing cancer progression, recurrence, and chemoresistance (the chemical structures of TGF- β , JAK/STAT3, and NF- κ B inhibitors are presented in Figure 4).

Cross-Talk Between Signalling Pathways

Due to the complexity of tumour development, most tumours do not rely on a single signalling pathway to maintain their growth and survival, and there is cross-talk between signalling pathways. Multipathway inhibitors can achieve dual functions for synergistic therapy and overcome drug resistance by inhibiting multiple signalling pathways.

EC-70124

EC-70124, a novel chimeric indolocarbazole compound, acts as a broad-spectrum multikinase inhibitor, displaying strong anti-proliferative effects. Because it targets many elements of the NF- κ B, JAK/STAT, PI3K/AKT/mTOR and FLT3 pathways, EC-70124 shows antitumour activity in various solid tumours, including breast, colorectal and prostate tumours, and in acute myeloid leukaemia (AML).^{119–122} Accordingly, the effect of EC-70124 on CSCs has been detected in other cancer types. Accumulating evidence indicates that EC-70124 exerts an inhibitory effect on the NF- κ B and STAT3 signalling pathways, reversing the characteristics of tumorigenesis in prostate CSCs. EC-70124 provides an innovative

Figure 4 Chemical structures of key CSC signalling pathway inhibitors, including TGF- β , JAK/STAT3, and NF- κ B inhibitors.

approach for advanced prostate cancer treatment by interfering with CSCs in prostate tumours.¹²⁰ EC-70124 has currently completed safety assessments in preclinical models and is beginning clinical development.¹²³

Niclosamide

Niclosamide is an FDA-approved anthelmintic drug^{124,125} that suppresses multiple CSC signalling pathways, such as the Wnt, Notch, STAT3 and NF- κ B pathways.¹²⁶ In addition to targeting mitochondria to induce cell cycle arrest, growth suppression, and apoptosis in cancer cells, niclosamide inhibits CSCs, providing further evidence that it represents a promising agent for cancer therapy. Previous reports have indicated that niclosamide inhibits breast and ovarian CSCs.^{127,128} Recently, niclosamide was shown to inhibit the CSC population and their self-renewal activity in colorectal cancer (CRC) cells, leading to irreversible destruction of tumour-initiating potential *in vivo*.¹²⁴ In a trial of cisplatin-induced oral CSC enrichment, application of niclosamide suppressed this process. Consequently, ALDH⁺ OSCC cells are more sensitive to cisplatin therapy.¹²⁹ Presently, niclosamide is undergoing phase I/II clinical trials (the chemical structures of EC-70124 and niclosamide are presented in Figure 5).

Targeting the Tumour Microenvironment

CSCs can regulate and control tumour development and metastasis through the interaction between abnormally activated self-renewal signalling pathways and cytokines in the TME.¹³⁰ The TME releases certain cytokines that increase activation of these signalling pathways to enhance the cancer stem cell population. Studies have found that microenvironmental cytokines, such as IL-6, can promote Notch-Jagged signalling, increasing the proportion of CSCs.¹⁶ Studies have found that CAFs can secrete hepatocyte growth factor (HGF) to activate the Wnt signalling pathway and regulate stemness in colon cancer cells.¹³¹ Tumour-associated macrophages can secrete IL-6 and other cytokines to activate the STAT3 and Hh signalling pathways, thereby affecting stem cell self-renewal and chemoresistance.¹³² In turn, the cytokines produced by CSCs recruit and regulate various cells in the TME. For example, CSCs can regulate the expression of cytokines (such as mip-1 α /CCL3 and icam-1) through SOX2, activate the NF- κ B and Stat signalling pathways, regulate the TME, recruit tumour-related macrophages (TAMs), and create an environment for tumour development.¹³³ Simultaneous treatment is needed when inhibiting CSCs. It is possible to use drugs that inhibit the TME and self-renewal pathways or

EC-70124

Niclosamide

Figure 5 Chemical structures of the multipathway inhibitors EC-70124 and niclosamide.

a combination of these drugs, which plays an important role in inhibiting the development of CSCs and tumour recurrence and metastasis. Studies have shown that ZL170 specifically targets the TGF β /BMP signalling pathway and may produce an inhibitory TME characterized by decreased ECM deposition and impaired recruitment and proliferation of stromal cells (tumour-associated fibroblasts, endothelial cells, and immune cells), thereby inhibiting stemness, EMT and metastasis of TNBC cells.¹³⁴ One study showed that CAF secretory factors enhance Wnt signalling and that the Wnt inhibitor Ipafricept (OMP-54f28) suppresses proliferation of patient-derived xenografts (PDXs) and inhibits CSC tumour initiation by inhibiting Wnt signalling involved in the cancer-TME interaction in head and neck squamous cell carcinoma (HNSCC).³⁷ Other research on breast cancer indicates that paclitaxel treatment of breast cancer cells leads to relatively high levels of ROR1 expression and that accessory cells in the TME induce activation of the Wnt5a signalling pathway by expressing ROR1, thereby promoting the stemness of breast cancer cells. Furthermore, the humanized anti-ROR1 drug cirmtuzumab inhibits activation of the ROR1 signalling pathway, which hinders the development and metastasis of breast cancer.¹³⁵ Therefore, targeting the TME to block CSC proliferation holds great therapeutic potential.

Current and Future Developments

Cancer is a major medical problem throughout the world, and CSCs have been confirmed to be an important cause of tumour resistance and tumorigenesis. Successful targeting

of CSCs is expected to cure cancer. CSCs exhibit many characteristics similar to those of embryonic or tissue stem cells while demonstrating persistent abnormal activation of self-renewal pathways associated with development and tissue homeostasis, which can cause cancer. Therefore, signalling pathway inhibitors can be developed for this small subpopulation of cells. Distinct inhibitors targeting key elements of these signalling pathways are currently in different clinical stages of development for the treatment of human malignancies (Table 1). However, the development of signalling pathway inhibitors comes with several challenges. First, these pathways contain too many elements that are difficult to control. Second, some signalling pathways are highly expressed in normal tissues, and their inhibition will affect normal biological functions. Therefore, studies on how to effectively eradicate CSCs without destroying normal stem cell signals are needed. The possible reason for the relative success of some signalling pathway inhibitors, such as Hh CSC inhibitors, is that Hh pathway-related genes are barely expressed in normal cells; thus, blocking this pathway has little effect on the biological function of normal tissues. Therefore, pathway inhibitors with high selectivity for tumour tissues are preferred. At the same time, we can also strengthen the development of multipathway inhibitors, such as niclosamide and EC-70124, which simultaneously act on multiple overactivated signalling pathways in cancer, producing effects superior to those of single pathway inhibitors. Furthermore, the use of single pathway inhibitors can

Table 1 Summary of the Signalling Pathway Inhibitors and Their Characteristics

Signalling pathway	Drug name (or alias)	Primary targets and (IC50, in vitro trial)	Cancer type	Phase	Formula	Ref.
Wnt signalling pathway	LGK-974 (WNT974)	Porcn (0.4 nM)	breast cancer; cutaneous squamous cell carcinoma, glioblastoma	Phase I	C ₂₃ H ₂₀ N ₆ O	(27-30)
	ICG-001 (PRI-724)	CBP (3 µM)	melanoma, gastric cancer, leukaemia oral squamous cell carcinomas, TNBC	Phase I	C ₃₃ H ₃₂ N ₄ O ₄	(32-35)
	Ipafricept (OMP-54F28)	Wnt ligand	solid tumours, hepatocellular carcinoma, ovarian cancer, pancreatic cancer	Phase I	-	(36-38)
Notch signalling pathway	DAPT (GSI-IX)	γ-secretase (Aβ) (20 nM)	osteosarcoma, leukaemia, ovarian cancer	Phase II	C ₂₃ H ₂₆ F ₂ N ₂ O ₄	(48-50)
	MK-0752	γ-secretase (Aβ40) (5 nM)	breast cancer	Phase I	C ₂₁ H ₂₁ ClF ₂ O ₄ S	(51)
	Demcizumab (OMP-21M18)	Notch ligand	head and neck squamous cell carcinoma	Phase I	-	(54-56)
Hh signalling pathway	Cyclopamine	Smoothed (Smo) (46 nM)	pancreatic cancer, bladder tumour	Phase I	C ₂₇ H ₄₁ NO ₂	(66-68)
	Vismodegib (GDC-0449)	Hedgehog (3.0 µM)	basal cell carcinoma, pancreatic cancer, small-cell lung cancer; prostate cancer; glioblastoma	Phase IV	C ₁₉ H ₁₄ Cl ₂ N ₂ O ₂ S	(71, 73, 75)
	GANT61	GLI1/GLI2 (5 µM)	breast cancer; pancreatic cancer	preclinical	C ₂₇ H ₃₅ N ₅	(78-80)
TGF-β signalling pathway	LY2109761	TβRI/II (38 nM/300 nM)	pancreatic cancer colorectal cancer; hepatocellular carcinoma, glioblastoma	preclinical	C ₂₈ H ₂₇ N ₅ O ₂	(93-96)
	Galunisertib (LY2157299)	TβRI (56 nM)	hepatocellular carcinoma	Phase I/II	C ₂₂ H ₁₉ N ₅ O	(98)
	Ruxolitinib (INC018424)	JAK1/2 (3.3 nM/2.8 nM)	myelofibrosis, polycythemia vera, myxoid liposarcoma	Phase II	C ₁₇ H ₁₈ N ₆	(102, 103)
NF-κB signalling pathway	Napabucasin (BB1608)	Stat3 (0.291~1.19 µM)	gastrointestinal malignancies, biliary tract cancer, colorectal cancer	Phase III	C ₁₄ H ₈ O ₄	(105-107)
	GTCpFE	IKKα/β phosphorylation (20 µM)	breast cancer	preclinical	-	(113, 114)
	BMS-345541	IKK-2/IKK-1 (0.3 µM/4 µM)	leukaemia, lung cancer, breast cancer	preclinical	C ₁₄ H ₁₇ N ₅	(116-118)
Multiple pathways	EC-70124 Niclosamide	- STAT3 (0.7 µM)	breast, colorectal, prostate tumours, acute myeloid leukaemia breast cancer; colorectal cancer; oral cancer	preclinical Phase I/II	C ₂₆ H ₁₉ N ₃ O C ₁₃ H ₈ Cl ₂ N ₂ O ₄	(119-122) (124, 126-129)

Abbreviations: CBP, cAMP-response element-binding (CREB) binding protein; TβR, TGFβ receptor; TNBC, Triple negative Breast Cancer.

activate compensation mechanisms, resulting in the development of drug resistance. More importantly, multipathway inhibitors have a wider range of effects and serum concentrations than single-pathway inhibitors, leading to reduced toxicity and side effects. In addition, we cannot neglect the ping-pong effect of the TME, which releases cytokines and promotes activation of self-renewal pathways in CSCs. Simultaneous treatment is needed when inhibiting CSCs. It is possible to use drugs that inhibit the TME and self-renewal pathways or a combination of them, which plays an important role in inhibiting the development of CSCs and tumour recurrence and metastasis. Recently, an increasing number of studies have demonstrated that targeting of CSCs has potential for development of novel anticancer agents. As self-renewal signalling pathway inhibitors continue to be refined, cancer patients are expected to have improved treatment options in the future.

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The authors report no conflicts of interest in this work.

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