REVIEW ARTICLE

Navigating the Molecular Signaling: Deciphering Cancer Stem Cell Self-Renewal Pathways

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ABSTRACT

Cancer stem cells (CSCs) are a subset of cells within tumors that exhibit stem cell-like characteristics, including the ability to self-renew and differentiate. CSCs are the cause of carcinogenesis and tumorigenesis. The expression of cell surface markers, which varies linked to the kind of tumor, is utilized to recognize CSCs. An essential part of tumor invasion and metastasis is played by CSCs. Numerous investigations have been carried out to find distinguished markers and different phenotypes of CSCs, which are especially crucial for identifying and separating this subset of cells. It was discovered that the regulation of CSCs involves a multitude of signaling pathways. These cells are determined by their ability to self-renewal pathways such as Wnt/β-catenin, JAK/STAT3, PTEN/PI3-K/Akt, and Hedgehog, their surface biomarkers, and their resistance to many drugs. Aberrant activation of these signaling pathways is associated with cell growth. Thus, focusing on CSCs is seen to be a viable anti-cancer treatment approach. It is encouraging that CSCs' self-renewal pathways present a viable target for changing their survival tactics and limiting their capacity to proliferate tumors. This study highlights the characterization and investigation of CSC self-renewal pathways, also discusses potential targeted therapy for CSC, and gives a summary of the significant factors and pathways that adjust CSC formation.
Keywords: Cancer Stem cells, Self-renewal, Tumor recurrence, Molecular Signaling, targeted therapy

Introduction

Stem cells are characterized by their ability to self-renew and differentiate into mature cells within specific tissues. Since they are needed for tissue replacement throughout a person's lifespan, the regulation and maintenance of these cells are crucial to supporting organogenesis, embryonic development, and the homeostasis of bodily tissues (1, 2). Through asymmetric cell division, stem cells can produce a mother cell that resembles the original cell and a daughter cell capable of differentiating into various cell types. Additionally, stem cells are capable of symmetric cell division, which produces two daughter cells that can both go through differentiation (3, 4). Several human disorders, including cancer, are caused by the interruption of stem cell functions. Cancer stem cells (CSCs) are a subpopulation of cells that have the ability to self-renew, but also possess the capacity to initiate tumors and have high invasiveness (1).

As a tiny subset of neoplastic cells, CSCs have the ability to form tumors (tumorigenesis), retain the population of tumorigenic cells (self-renewal), and generate the heterogeneous cells forming the whole tumor (pluripotency) (5). CSCs have stem cell-like properties and are thought to be the source of tumor heterogeneity due to their capacity to produce many different cancer cell types (6). Surface biomarkers, multi-drug resistance pumps, and dysregulated self-renewal pathways (SRPs) are characteristics of CSCs (7). CSCs are found in the majority of liquid and solid malignancies, where they play a role in tumor initiation, extension, resistance, recurrence, and metastasis. The expression of cell surface markers, which varies depending on the tumor type, is utilized to identify CSCs (8). Research has demonstrated that cancer cells are caused by genetic alterations that happen in a cell population, and following the formation of these cells, the gene expression map in cancer cell variations (9, 10).

Variation in gene expression in the cells can mean overexpression of a group of genes and silencing of other genes. These genetic variations have led to disparate subpopulations, each of which differently respond to therapy (11). One of the key characteristics used by CSCs to sustain their multiplying capacity is self-renewal. It is suggested that epigenesis may result in the dysregulation of self-renewal pathways (SRPs) in CSCs because genetic and epigenetic modifications

may play a part in the unchecked proliferation, invasion, and resistance in cancer cells. In healthy stem cells, a variety of signaling channels work. These pathways are tightly regulated and have specific functions in early embryogenesis-like cell proliferation, differentiation, and polarity. When these SRPs are dysregulated in CSCs, significant cell proliferation results, which may be viewed as an early stage in the development of cancer (7, 12). Targeting cancer stem cell signaling pathways could revolutionize cancer therapy by improving the understanding of cancer pathology and treatment (13). In this review, an attempt has been made to further comprehend the self-renewal signaling routes and a summary of the efforts in targeting these pathways.

Self-renewal pathways of CSCs

Wnt/β-catenin

Understanding the processes of CSC self-renewal is significant for drug development and discovery. One of the essential pathways that regulates CSC self-renewal is Wnt/β-catenin signaling (14, 15). The Wnt signaling system regulates stem cells and dictates cell fate throughout development. It is an evolutionary conserved developmental mechanism. In humans, the Wnt family consists of 19 glycoproteins that have important functional and biological properties (16). In the Wnt/β-catenin signaling, the Axin/ glycogen Synthase Kinase-3 (GSK-3)/APC complex breaks down the intracellular signaling molecule β-catenin. The Axin/GSK-3/APC complex is broken apart when the Wnt ligand is activated by attachment to Frizzled and the low-density lipoprotein-related receptor (LRP). subsequently, intracytoplasmic β-catenin then becomes stable and may reach the nucleus, allowing target genes to be transcribed more easily (17, 18).

β-catenin is a principal ingredient of the canonical Wnt pathway and a significant oncogene implicated in the development of human non-small cell lung cancer (19, 20). According to a few investigations, stem cell proliferation in blast crisis leukemia is driven by aberrant Wnt/β-catenin pathway activation (12, 21). Wnt-target genes interact with the TCF/LEF transcription factor, causing Wnt-target genes, including cyclin D1, c-Jun, and c-Myc, to become activated (15, 22). In one study, high levels of β-catenin were detected in samples of leukemia patients with FLT-3 mutation (23). The Wnt/β-catenin pathway is

required for the survival of cutaneous CSCs, and knocking down the β-catenin gene leads to the loss of CD34+ CSCs and full tumor regression (24). In one investigation by Morin et al., it was shown that mutations of adenomatous polyposis coli in colorectal cancer results in disrupted downregulation of β-catenin and Tcf-4 transcriptional activity; in this study, they did genetic research in four types of adenomatous polyposis coli mutants (25).

Multiple mechanisms can cause abnormal Wnt/β-catenin signaling, many of which are particular in the way of cancer progression (26, 27). In prostate cancer stem cells, the Wnt/β-catenin signaling and its target genes c-Myc and cyclinD1 were activated, and the self-renewal of prostate CSCs was reliant on β-catenin in the nucleus, according to Zhang K et al. (28). Upregulation of β-catenin was linked to invasion and metastasis of prostate CSCs, and transplanted β-catenin ShRNA diminished invasion and metastasis, according to a paper by Luo Y et al. (29). The results of an investigation indicated that the Wnt/β-catenin pathway is recommended as a viable therapeutic target for the treatment of non-small cell lung cancer development and metastasis with CSC-like signatures and the epithelial-mesenchymal transition phenotype (30). Bisson et al. in another study revealed that activator of the Wnt/β-catenin pathway could considerably upregulate CD133 and CD44, and targeting Wnt/β-catenin signaling may ameliorate the therapeutic influence of prostate cancer (31). In human embryonic stem cells, Card et al. discovered that miR-302a reduced the productive translation of cyclin D1, a key G1 mediator (32).

Therewith, numerous studies have emphasized the significance of Wnt/ β -catenin signaling in colon CSCs (33, 34). Colon CSCs contain a substantial amount of β-catenin, which is controlled in part by the microenvironment and eventually leads to treatment resistance and metastasis. (34). Because of the considerable amount of β-catenin in the nucleus (35), the Wnt pathway has been demonstrated to be linked to epithelial-mesenchymal transition in tumors (36). This causes tumor cell division to be stopped and mesenchymal markers like fibronectin (37) to be acquired while maintaining self-renewal potential, which is a typical characteristic of CSCs. Numerous investigations have revealed the importance of the Wnt pathway in Breast Cancer; in normal breast, this pathway regulates cell fate, proliferation, and

migration, and in cancer cells, it is constitutively active (38). In nasopharyngeal cancer, dihydromyricetin's anti-tumor efficacy was discovered via inhibiting the Wnt/β-catenin pathway. Dihydromyricetin might be a promising new therapy option for nasopharyngeal cancer (39). The interaction of the Wnt/β-catenin and RAS/extracellular-signal-regulated kinase pathways is significant in malignant phenotypes, and the alterations in both β-catenin and RAS levels are linked in human colorectal cancer with adenomatous polyposis coli mutations (40, 41).

hnRNPAB and its subtypes can control the expression of Wnt/β-catenin pathway proteins (42, 43). The result of a research revealed that Irradiated-Mesenchymal stem cells might help CSCs maintain their stemness by stimulating the Wnt/β-catenin signaling (44). Niclosamide was discovered to be a Wnt/β-catenin signaling inhibitor with anti-tumor effects that targeted ovarian CSCs specifically (45). In colorectal cancer, niclosamide can lower the expression of numerous Wnt/β-catenin pathway components, as well as the self-renewal capacity and population of CSCs (46).

Ubiquitin-conjugating enzyme E2 T (UBE2T) was originally discovered in CD34+ hematopoietic stem cells, indicating that it plays a regulatory function in these cells' stemness (47). UBE2T has been revealed to control the development of stomach and nasopharyngeal cancers in part via modulating the Wnt signaling cascade (48, 49). A new UBE2T /Mule/β-catenin signaling cascade, implicated in the control of liver CSCs, was discovered in research, making it an appealing prospective therapeutic target for hepatocellular carcinoma (50). Through inhibiting the Wnt pathway, ONC201, which is in phase I/II trial for patients with advanced cancer (NCT02038699), caused substantial CSC-suppression and repressed the expression of CSC-associated genes in prostate and glioblastoma tumors (51-53).

The results of research that point to p53 as a key mediator of 5-Fluorouracil-induced CSC activation through the WNT/β-catenin, and accentuate the significance of utilizing a WNT inhibitor in combination with 5-Fluorouracil as a convincing therapeutic strategy to ameliorate the poor consequences of current 5-Fluorouracil-based therapies for colorectal cancer patients (54). By blocking Wnt/β-catenin signal transduction, trifluoperazine has been reported to reduce lung CSC spheroid formation capability and

diminution the expression of lung CSC markers (55). Through the TGF-β and Wnt/β-catenin pathways, SPTBN1 (spectrin beta chain, non-erythrocytic 1) can regulate the cell cycle and Epithelial mesenchymal transformation, therefore regulating the proliferation and migration of hepatocellular carcinoma. SPTBN1 can also have a role in cancer prevention via regulating programmed cell death, DNA damage repair, and angiogenesis (56).

miR-25 directly targets DKK3 in melanoma, and therewith diminishes its downstream signaling, the WNT/β-catenin pathway to boost melanoma cell proliferation (57). The TET family of DNA methylcytosine dioxygenases convert DNA methylation at the 5′ position of the cytosine base mainly to 5-hydroxymethylcytosine, and then to 5-formylcytosine or 5-carboxylcytosine (58, 59). In one of the investigations, the role of TET1 DNA dioxygenase in the control of Wnt Signaling and the metastasis of gastric cancer was examined. In immune-deficient mice, TET1 overexpression and TET1 knock-down enhanced and prevented metastatic dissemination to the liver, respectively. When Wnt/β-catenin Signalling was interfered with, TET1's inhibitory effects on Epithelial-mesenchymal transition and CSC, which are traits connected to metastasis, were reversed.

FOXO4 was discovered to be a direct transactivating target of TET1 by RNA-sequencing. Together, TET1/FOXO4 control of Wnt Signalling is necessary for the cellular characteristics related to metastasis, and therapies that target the TET1/FOXO4/-catenin pathway may be efficacious in preventing and treating gastric cancer metastasis (60). Palladin, an Actin-associated protein, is significantly expressed in a variety of tumor cells, including those in pancreatic, stomach, colon, and breast malignancies (61, 62). Palladin governs the organization of the actin cytoskeleton and the establishment of adhesions (62), which, in turn, contributes to the invasive and migratory character of metastatic cancer cells (63). This is noteworthy, given Palladin's function in cell assembly and maintenance (62).

A study reveals that Palladin may function as an oncogene by encouraging non-small-cell lung cancer (NSCLC) cell tumorigenicity and CSC-like characteristics through the Wnt/β-catenin Signalling. It has been revealed that Palladin may be utilized as a cell surface marker to identify lung cancer stem cells. These findings offer a potential target for creating putative lung cancer stem cell-targeted drugs (64). Table 1 shows a summary of some of the recent publications regarding the Wnt/β-catenin pathway in cancer. –

JAK/STAT3

Many indispensable biological processes, such as cell proliferation, differentiation, apoptosis, and immunological modulation, are regulated by the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway both in normal and transformed cells. Some studies suggest interfering with this pathway as a therapeutic strategy in malignancies (65). Cytokines and growth factors bind to their respective receptors to activate JAK through phosphorylation, which in turn causes STAT phosphorylation. Activated STAT forms a homodimer in the nucleus and binds to target genes to control transcription.

The abnormal activation of JAK/STAT3 signaling promotes cancer cell growth and survival (66, 67). Figure 1 displays the JAK/STAT pathway activation and regulation. JAK1, JAK2, JAK3, and TYK2 are the four non-receptor tyrosine kinases in the JAK family. JAK1, JAK2, and TYK2 are all expressed everywhere, while JAK3 is significantly found in hematopoietic cells (68). The JAK/STAT signaling is made up of receptor and adaptor proteins of interleukin 6 (IL-6), interferon-gamma (IFN-γ), and interferon-alpha (IFN-α), all of which exert pleiotropic effects when bound to their respective ligands (69, 70). Many human cancers rely on the IL6/JAK/STAT3 pathway for their growth and development. IL6 levels are elevated in a high percentage of individuals with hematoietic malignancies or solid tumors, as well as in chronic inflammatory diseases (71, 72).

STAT proteins are usually activated in hematological tumors, but only in a few of them, it can be shown that the signaling pathway is altered, such as myeloproliferative neoplasm (MPNs) with Negative Bcr-Abl and some subsets of acute lymphoblastic leukemia (73). Studies have demonstrated that transforming growth factor β1 (TGFβ1) causes hematopoietic stem cells to develop into myofibroblasts through the JAK/STAT3 pathway (74). STAT3 that is constitutively active is involved in tumor cell proliferation, metastasis, and programmed cell death (75-77). STAT3 is present in the cell mitochondrion, and mitochondrial STAT3 helps breast cancer cells maintain oxidative phosphorylation activity. The mitochondrial electron

transport chain is downregulated in breast cancer cells and breast tumor development in vivo when STAT3 is blocked (78, 79). In the tumor microenvironment, STAT3 can restrain IFN-γ and many other Th1 mediators (80-84). STAT3 attaches to consensus response elements in the promoters of target genes in the nucleus, causing the transcription of a large number of genes that encode cellular proliferation regulators and survival, as well as angiogenesis-promoting and immunosuppressive growth agents and cytokines like IL-6 (85, 86). STAT3 hyperactivation in tumors can be caused by a number of factors. STAT3 signaling can be induced by autocrine stimulation of growth factor receptors. Epidermal growth factor receptor (EGFR) is overexpressed or mutated to a fundamentally active form in some cancers, and JAK enzymes can be overexpressed or altered in the same way (72, 87). STAT3 is a promising target for the development of anticancer drugs. The JAK/STAT3 pathway is important in the expansion of osteosarcoma, and STAT3 might be a promising therapeutic target for human osteosarcoma medication (88-90).

Table 1. Summary of recent published papers on Cancers with WNT/β-catenin involvement.

Cancer	WNT/β-Catenin pathway	Method	Conclusion	Reference/Author
Colorectal Cancer	Activated	Using FACS, the CSCs were isolated from 3 different Human Colorectal cancer cell lines, and their proliferation ability, as well as their Chemoresistance to oxaliplatin, was measured using CCK8 assay. The Activated WNT/β-Catenin signalling was measured using Western blot.	The proliferation ability and Chemoresistance were significantly stronger than the parental cells, and the WNT/β-Catenin Signalling is activated in the CSCs of these cell lines.	(91)
Renal Cell Carcinoma (RCC)	Activated	Investigating the influences of Silibinin on RCC both in vivo and in vitro using ACHN cell line and nude mice model. The pathway analysis was done using western blot and cell survival using MTT assay.	Inhibition of WNT/β-Catenin pathway in an Autophagy dependent fashion. Furthermore, the degradation of β-Catenin molecules induced by Silibinin had ant-metastatic effects against RCC.	(92)
Breast Cancer	Deregulated	Utilizing Alpha-hederin (AH) as an inhibitor against WNT/β-Catenin Signalling in Breast cancer stem cells (BCSCs) by disrupting β-Catenin/Tcf4 interaction.	Reportedly, AH reduced the viability of bCSCs and suppressed the transcription of Wnt downstream target genes.	(93)
Cervical Cancer	Activated	Using surgical samples from normal patients and Cervical cancer patients in addition to cell culture and nude mice models, the expression of HOXB4 was examined in in vivo and in vitro. Moreover, the influence of up and down regulation of HOXB4 on β-Catenin transcription and Wnt signaling activation was studied.	The findings revealed that HOXB4 is downregulated in Cervical Cancer cells, and HOXB4 suppresses the β-Catenin transcription, consequently inhibiting the WNT/β-catenin pathway both in vivo and in vitro.	(94)
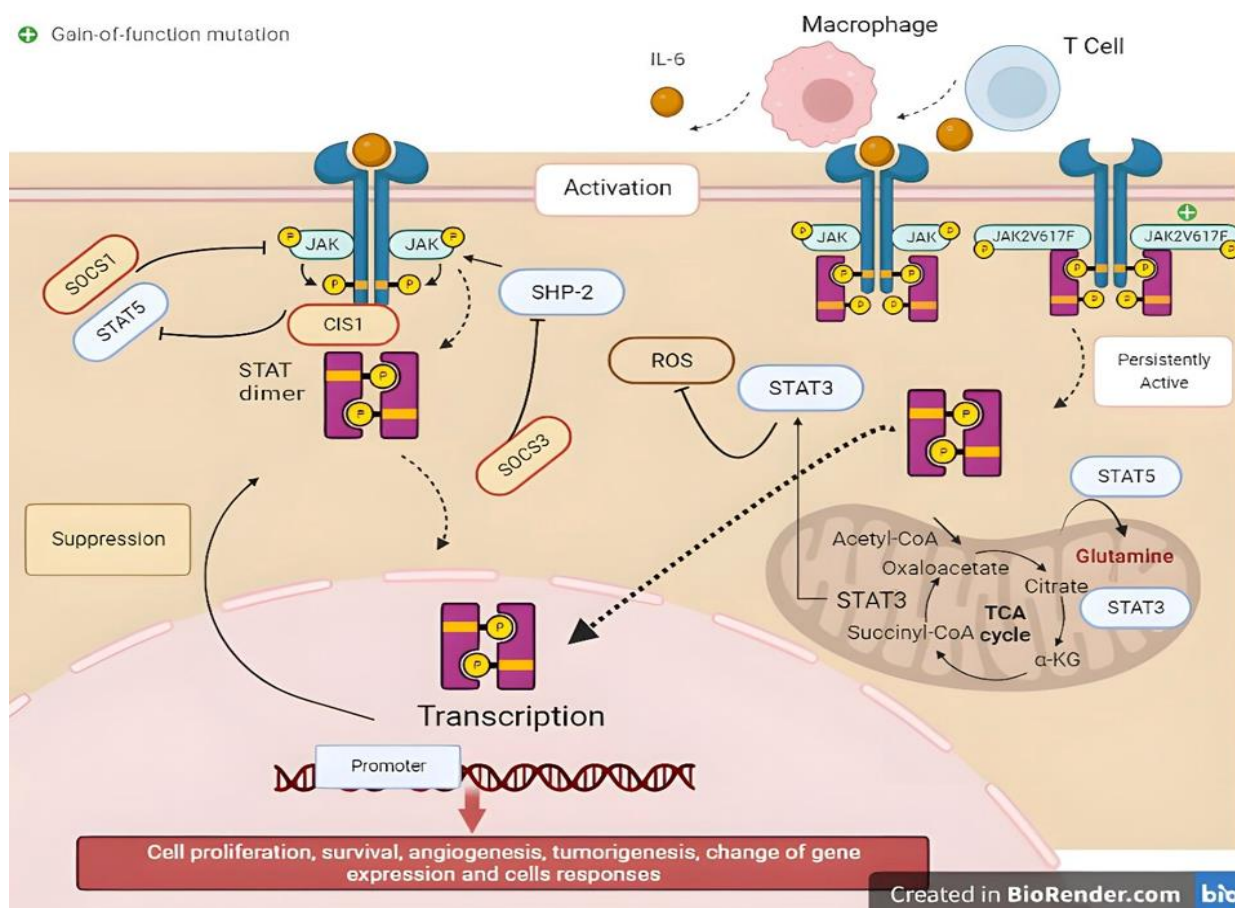
Figure 1. JAK/STAT Signalling pathway. On the left phosphorylation of JAK after activation of Cytokine receptor IL6 and homodimerization of STAT proteins leads to changes in gene expression and cellular responses, which promote cancer cell survival and proliferation. In physiological conditions, this pathway is regulated through a negative feedback, as shown above, which suppresses the excessive activation of the JAK. On the right, the JAKV617F mutated form of JAK is shown, which is in active mode regardless of cytokine-receptor binding and is resistant to SOCS3 negative regulation. This mutation is detected in Myeloproliferative neoplasm. Bottom right, Mitochondrial STAT can reduce the Radical Oxygen Species production. (Created with BioRender.com).

STAT3 is hyperactivated in tumor-infiltrating immune cells, according to new findings, and this might have a big impact on antitumor immunity (81, 95). The JAK/STAT3 signaling system has been investigated in breast and other cancer types as a potential anti-tumor therapy. JAK2 and STAT3 targeting has been shown in studies to result in more targeted and efficacious breast cancer therapy (96). The JAK/STAT pathway has been linked to gastric cancer tumorigenesis (97).

In hepatocellular carcinoma, restrain of the JAK2/STAT3 signaling has been associated to the suppression of tumors' new blood vessels formation and metastasis (98). In vitro and in vivo, inhibiting STAT3 signaling ameliorated chemobased anticancer treatment outcomes, suggesting STAT3 as an emerging pharmacological target in chemoresistance (99-111).In vivo, inhibiting JAK/STAT3 restrains CSC self-renewal and tumor development (96, 112, 113). Table 2 has demonstrated a summary of some of the recent publications regarding JAK/STAT and cancer. Disrupting constitutively active JAK/STAT signaling diminishes the number of CSCs and reduces tumorigenicity in vivo in a variety of malignancies, including ovarian cancer (114), glioblastoma (115), and prostate cancer (116). In glioblastoma, retaining tumor stem cell-like phenotypic characteristics, including sphere formation, tumorigenicity, and expression of pluripotency-linked transcription factors, requires activation of the JAK/STAT signaling

pathway (115, 117). Epithelial mesenchymal transition is a biological process in which epithelial cells acquire mesenchymal cell properties throughout development. Epithelial cancer tumorigenesis, progression, and metastasis are all linked to abnormal cell proliferation and epithelial mesenchymal transition. Epithelial mesenchymal transition, a critical step in the early stages of cancer metastasis, can be controlled by a number of pathways, including JAK/STAT3 and TGF-/Smad (118).

Table 2. Recent published papers on Cancers with JAK/STAT involvement.

Cancer	JAK/STAT Pathway	Method	Conclusion	Reference/Author
Cervical Cancer	Activated	JAK2 inhibition using Ruxolutinib combined with Cisplatin treatment on human papillomavirus (HPV) $^+$ Cervical cancer cells.	JAK2 inhibition reduced cell proliferation, and Ruxolutinib has synergistic effects on Cisplatin induced apoptosis.	(119)
Glioblastoma	Activated STAT3	Measuring miR-17 and miR-20a expression before and after Ruxolutinib treatment on Tumor spheres of U87 cells.	Expression of both miR-17 and miR-20a increased after treatment, and results suggest these miRs as a potential therapeutic target in glioblastoma	(120)
Gastric Cancer	Dysregulation	Suppression of cell migration, cell cycle prevent, and JAK/STAT pathway by Stigmasterol on Human Gastric Cancer cell line.	Stigmasterol has the potential to be utilized in the treatment of Gastric Cancer and inhibit the tumor growth.	(121)
Non-small lung Cancer	Activated STAT3	Ruxolutinib in Combination with VSV-IFNβ therapy to measure PDL-1 and JAK/STAT pathways in Human and Murine NSCLC cell lines (H460, A549, H2009, and H2030) and a normal NSCLC Murine model.	The combination of Virotherapy with Ruxolutinib resulted in decreased STAT1 and STAT3 phosphorylation and prevention of PDL-1 enhancement in treated cells.	(122)
Breast Cancer	Activated STAT3	Combination of "oxidation therapy" and STAT3 inhibition using novel Curcumin-BTP hybrids and measuring ROS production activity.	Compound 6b showed antitumor activity against MCF-7 and MCF-7/DOX cells and suppressed STAT3 activation.	(123)
Leukemia	Activated STAT5	Inhibition of SOCS-1 and SOCS-3 tyrosine phosphorylation in K562 leukemic cells to Target Bcr-Abl -dependent JAK/STAT5 activation.	Leukemic cells were sensitized to apoptosis, and Selective mutation of tyrosine phosphorylation sites of SOCS-1 and SOCS-3 considerably decreased Bcr-Abl-mediated carcinogenesis in nude mice and suppressed Bcr-Abl-	(124)

Cancer	JAK/STAT Pathway	Method	Conclusion	Reference/Author
			mediated murine bone marrow transformation.	
myeloproliferative neoplasm (MPN)	JAKV2617F mutation	Compared Ruxolitinib, a strong and specific Janus kinase (JAK) 1 and 2 inhibitor, to the best treatment option for Myelofibrosis patients in order to assess its effectiveness and safety.	Continuous ruxolitinib medication was linked with marked and long-lasting decreases in splenomegaly and disease-related symptoms, enhancements in role performance and quality of life, and minimal adverse effects when compared to the best accessible treatment.	(125)

The findings of a study revealed that JAK2/STAT3/cyclin D2 signaling was discovered to be a resistance mechanism for the continuous development of CSCs following radiotherapy, providing novel biomarkers and regimens for ameliorating colorectal cancer outcomes (126). Activation of the JAK/STAT signaling system or promotion of cross-talk between multiple JAK/STAT pathways to increase the generation of CSCs and medication resistance has been linked to a variety of extracellular stimuli and intracellular signaling pathways (127, 128).

Ruxolitinib is a strong and selective oral JAK 1 and JAK 2 antagonist. The JAK/STAT pathway is abnormally activated in myelofibrosis and polycythemia vera. The food and drug Administration (FDA) first authorized Ruxolitinib in 2011 for the treatment of myelofibrosis, and then in 2014 for the treatment of polycythemia vera (129).After intravenous administration, the cyclic STAT3 decoy enhanced heat and nuclease resistance, and antitumor efficacy against xenograft tumor models, and had no obvious toxicities when given at high dosages (130, 131). Curcumin has been found to suppress cancer cell growth and proliferation by targeting a variety of survival pathways, such as JAK/STAT3, PI3-kinase/AKT, Transforming Growth Factor beta, and EGFR, in a variety of malignancies (132-136).

Honokiol, a natural chemical derived from magnolia tree bark, has been shown to target Epidermal growth factor receptor signaling via STAT3 in the treatment of head/neck cancer, therefore boosting the effectiveness of Epidermal growth factor receptor-targeting treatments (137). Targeting the JAK/STAT3 signaling might be an effective cancer therapy method and further research is needed to shed light on effectiveness of these therapies.

PTEN/PI3-K/Akt

(PI3-K, phosphoinositide 3-kinase; PTEN, phosphatase, and tensin homolog)

The PTEN/PI3K/Akt signaling has been linked to CSCs in various studies (138-140). The PI3K/Akt pathway cascade is commonly disturbed in a multitude of human malignancies, and it plays a significant role in tumor survival and inhibition of apoptosis. This pathway is a promising target for therapeutic intervention since it is thought to be a significant marker for tumor aggressiveness (141). The PTEN/PI3K/AKT pathway has been related to a variety of biological activities, including apoptosis, proliferation, and growth (142-144).

The inhibition of the tumor suppressor PTEN was thought to be a typical mechanism for Akt signaling activation, and inversely, constitutive Akt activation has been revealed to be primarily responsible for PTEN-mediated carcinogenesis (145, 146). AKT1, AKT2, and AKT3 are three members of the AKT family, each of which is encoded by three disparate genes (147). PTEN, a tumor suppressor and regulator of the PI3K / AKT / mammalian target of rapamycin (mTOR) signaling antagonist, inhibits phosphorylation of PI3K, AKT, and mTOR, which was implicated in the control of different cancers, such as prostate cancer, by engaging in several tumor biological processes (148, 149). The activation and inactivation of signaling pathways in carcinogenesis, including the PI3K/AKT

pathway, is triggered by the deletion or mutation of PTEN (142).

The PI3K/AKT signaling system may be inactivated by upregulating PTEN expression, which can stop human tumor development (150). Additionally, The PTEN/PI3K/AKT pathway can be adjusted by P53 (151). The PTEN/PI3K/AKT pathway has been revealed to influence glioma tumor growth (143) and the survival of prostate cancer stem-like cells (139). Dysregulation of the PI3K pathway in prostate cancer is often associated with cancer progression, due to genetic alterations such as activating mutations or deletion of PIK3CA, AKT1, and PTEN, as well as epigenetic and post-translational modifications, making this signaling axis an appealing option for therapy in this cancer (152).

The PTEN/PI3K/Akt pathway was discovered to be strongly linked to prostate CSCs by Dubrovska and partners, suggesting that PI3K might be a beneficial therapeutic target for prostate cancer (139). The role of PTEN in the prostate has been studied in mouse models, and it has been discovered that a loss of PTEN expression is required for the initiation of prostate cancer (153, 154), and there are particular dose-dependent influences.

For example, invasive prostate cancer with a long latency period (155), and metastases (156) occurs from a full loss of PTEN expression. Moreover, MicroRNA (miRNAs) have the capability to attach to the 3′-UTR region of corresponding messenger RNAs (mRNAs) and inhibit their protein expression (157-159). Zinc finger E-box-binding homeobox 1, a zinc finger transcription factor, modulates Epithelial-mesenchymal transition progression by regulating the expression of epithelial/mesenchymal markers (160). miR-205 hampers glioblastoma cell migration and invasion by suppressing the activation of the AKT/mTOR signaling pathway by down-regulating Zinc Finger E-box-binding homeobox 1 and reverses Epithelial-mesenchymal transition (161). Intriguingly, through the PI3K/AKT pathway, certain small RNAs, such as miR-873, limit the proliferation and differentiation of pancreatic CSCs (162).

In one instance, miR-132 has been shown to have a regulatory role in antiviral innate immunity (163) and pancreatic cancer (164). By means of the PI3K/AKT pathway (165), PTEN may adjust oxidative stress (166, 167), leading to cell necroptosis, and it can also be a negative regulator of the PI3K/AKT pathway (168),

causing cell necroptosis in various species (165, 169, 170). The tumor suppressor influences of tRNA-derived fragments (tRF-5026a) are mediated via the PTEN/PI3K/AKT pathway, making it a potential biomarker for gastric cancer diagnosis (171). In fracture patients with traumatic brain damage, micro (mi)RNA-26a-5p has been found to suppress PTEN expression and enhance the bone-healing process (172).

In cervical cancer, a study found that PR domain zinc finger protein 4, a transcription factor involved in stem cell self-renewal and tumorigenesis, restrains cell proliferation and tumorigenesis by directly transactivating PTEN expression and downregulating the function of the PI3K/AKT pathway (173). AKT mutant variants with stable membrane binding are oncogenic and constitutively active. It is still up for dispute whether anchoring to PIP3 is also rate-limiting for sustaining AKT activity (174).

Wen et al. discovered PTEN mutations in 27 of 144 gastric cancer individuals, with the mutation rate being higher in advanced tumor, and metastasis stages than in poorly differentiated ones, which could explain the downregulation of PTEN expression and activation of PI3K/Akt signaling found in tumor tissues (175). The downregulation of PTEN expression in gastric cancer may be due to epigenetically silencing it by methylating 5′ CpG islands in the promoter (176). In retinoblastoma, Wan et al. discovered that PTEN was a direct target of miR-25-3p; this discovery can help researchers study this axis further as a potential target for therapy (177). Table 3 has revealed a summary of recent published papers on cancers with PTEN involvement.
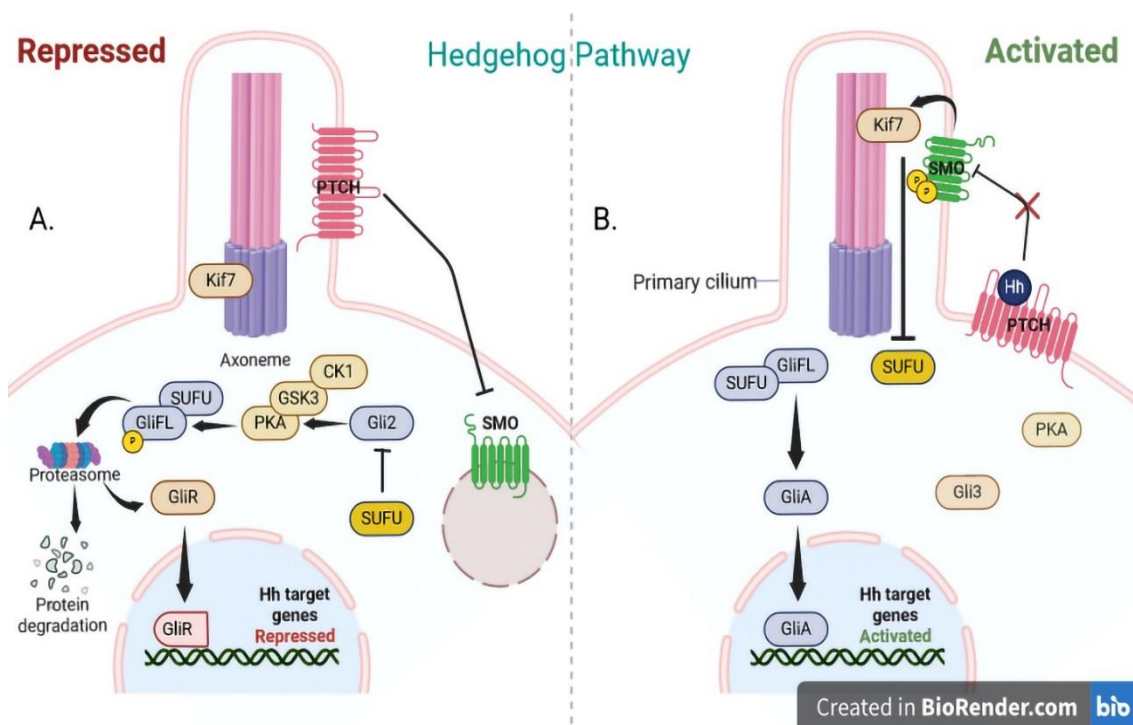
Hedgehog (Hh pathway)

Hh pathway, initially described in mutated drosophila larva (178), is crucial in regulating embryogenesis, Transition from Epithelial to Mesenchymal, and other cellular processes (179). Hh pathway is highly preserved through evolution. The most notable Hedgehog ligands consist of Sonic Hh (Shh), Indian Hh (Ihh), and Desert Hh (Dhh) (180). Emerging evidence suggests an indispensable role for the Hh signaling in stem cell homeostasis and tumor initiation, progression, and self-renewal ability (179, 181).

Figure 2 indicates the Hh pathway in CSCs. Hh pathway is responsible for the number of cancers and

CSCs "Stemness" this pathway is involved in many tumors such as Odontogenic Keratocyst (182, 183), Uterine Sarcoma (184, 185), Medulloblastoma (186, 187), Nevoid basal Cell Carcinoma (188, 189), Cervical (190), Renal (190), Breast (188), Colorectal (191), Small-Cell lung (192), Skin (193), Stomach (194) and Ovarian (195) Cancer. In a study by Zhou et al., it was indicated that a novel lncRNA-cCSC1 is aberrantly expressed in Colorectal Cancer cells and may regulate Cancer Stem cell properties through the Hh pathway (196). Another lncRNA named lncRNA-Hh has been shown to increase glioma-associated oncogene -1 (Gli-1), SOX2, and OCT4 expression levels and subsequently maintain CSC properties (197). It is suggested that Mutations in a number of genes, such as KMT2D(MLL2) may have a role in Cancer cell fate and metastasis in Sonic hedgehog-driven medulloblastoma (SHH-MB) patients (198).

Table 3. Recent published papers on Cancers with PTEN involvement.

Cancer	Method	Conclusion	Reference/Author
Ovarian Cancer	Silencing PTEN in Fallopian tube epithelial cells to understand the role of this pathway in regulating CSC like properties in ovarian cancer using RNAseq data, mouse models, and multispheroid matrigel assay.	Two distinct cell subpopulations with distinct patterns of chemoresistance, tumorigenicity, and CSC marker expression were formed as a consequence of PTEN deficiency. Fallopian tube epithelium (FTE) cells respond to PAX2 lack by reprogramming towards a more stem-like phenotype when PTEN is deficient, and this may be used as a model to examine initial processes in the formation of FTE-driven ovarian tumors.	(199)
Glioblastoma	Using immunohistochemical analysis, data from, The Cancer Genome Atlas, and the orthotropic GBM model, the role of Smurf1 in PTEN activity and tumor growth was investigated.	It is suggested that Smurf1 is associated with a poor prognosis in GBM. Smurf1 stimulates cell proliferation by speeding up the cell cycle and disrupting signaling networks. Furthermore, they demonstrate that Smurf1 degrades PTEN. Results further show that Smurf1's oncogenic function is reliant on PTEN. Smurf1 overexpression impairs PTEN activity, resulting in persistent activation of the PI3K/Akt/mTOR signaling pathway and Smurf1 depletion.	(200)
Non-small lung Cancer	Using Aldefluor assay, NSCLC cells, and flow cytometry, they targeted therapy-resistant lung cancer stem cells by disrupting the AKT/TSPYL5/PTEN positive feedback loop.	According to the results, TSPYL5 can be removed by blocking TSPYL5-pT120, which can stop abnormal AKT/TSPYL5/PTEN cyclic signaling and TSPYL5-mediated cancer stemness regulation. The results further demonstrate TSPYL5 might be a useful target for therapy-resistant lung tumors.	(201)
Breast Cancer	Investigating Notch-1-PTEN-ERK1/2 signaling axis in promotion of HER2+ breast cancer cell proliferation and stem cell survival.	The outcomes reveal that Notch-1 induced inhibition of PTEN is crucial in the survival of Breast Cancer Stem Cells. Furthermore, results show that Trastuzumab resistance in breast cancer is through Notch-1 mediated PTEN suppression.	(202)

Figure 2. Hedgehog pathway in CSCs. A. In the repressed state, the absence of the Hh ligand causes PTCH induced inhibition of SMO. Conversely, SUFU acts as an inhibitor promoting GliFL degradation, which results in the formation of GliR and repression of of Hh target genes. B. In the activated state, PTCH and SUFU inhibition is removed by Hh ligand binding and Kif7 activation, respectively, forming GliA which results in Hh target genes activation (GliFL: s, GliA: , GliR: , PTCH:, SMO:, SUFU: , GSK3: , CK1: and kif7:). (Created with biorender.com).

The formation of tissues, homeostasis, epithelial-mesenchymal transition, and cell proliferation and differentiation are all dependent on the HH signalling pathway (179, 203, 204). HH signaling's primary function is restricted to the development of the embryo, especially to organogenesis. Neuronal development is mostly dependent on SHH, gonadal development and steroidogenesis are influenced by DHH, and bone growth, ovarian steroidogenesis, and folliculogenesis are all regulated by IHH (205-207).

Defective development of the brain, face, and other midline organs is caused by dysregulation of HH signalling (208-211). In certain target tissues, HH molecules function alone, whereas in others, they play overlapping functions. Thus, "the loss of function" phenotypes resulting from the loss of a single HH are distinct from those resulting from the loss of many HH molecules together (207, 212, 213). When SHH and IHH are lost, early embryos lack a membrane bound activator (SMO) expression, which is linked to fatal abnormalities in extraembryonic vasculogenesis and

heart development (212, 213). Conversely, ovarian granulosa cells (GCs) express both IHH and DHH, which are crucial for steroidogenesis and follicle formation (207).

According to reports, female mice that lack both DHH and IHH in GC experience infertility, impaired steroidogenesis, and failure in the formation of theca cells (TCs) (207). Over the past decade, there has been an interest in altering the Hh pathway using drugs and plant based chemical compounds; for instance, it was demonstrated in a study that berberine may downregulate the Hh pathway in colorectal cancer and lead to tumor suppression (214). Hedgehog ligand processing in secretory cells results in the formation of a dual lipid-modified amino-terminal polypeptide of the autocatalytically cleaved precursor protein, which is the basis for the start of Hedgehog signalling (179, 215).

Signal reception is carried out via conserved receptors on the cell membrane, such as the 12-pass transmembrane protein Patched (Ptch) and the 7-pass

transmembrane protein Smoothened (Smo). Pathway inhibition results from Ptch's suppression of Smo activity in the absence of hedgehog ligand. The inhibition of Smo is removed when the hedgehog ligand attaches to Ptch, which keeps downstream signalling active. Furthermore, co-receptors—which may also be implicated in Ptch inactivation—are necessary for the activation of hedgehog signalling (179, 216). The Hh pathway is known to play a considerable role in CSC maintenance, yet the mechanisms are not fully understood. Therefore, more research needs to be done on the Hh pathway to find novel strategies in the ever-going battle treating cancer. Below, there has been a table summarizing some of the recent publications on the Hh pathway in different tumors. Below, table 4 reveals some of the recent publications on the Hh pathway in different tumors.

Cross-talk Between CSCs Self-Renewal Pathways

Recent research has pointed to the critical importance of cross-talk between Wnt/β-catenin, JAK/STAT3, PTEN/PI3-K/Akt, and Hedgehog in the activation of CSC self-renewal and tumorigenesis-promoting pathways. The constitutive activation of these signaling pathways often reciprocally potentiates each other, which serves as the basis for resistance to conventional therapies. Understanding these intricate interactions is essential for developing effective anti-cancer therapies that can target CSCs and improve patient outcomes.

An increase in AKT and MTORC activity can cause GLI activation (217) while AKT activity inhibits GSK which in turn influences the WNT pathway, this inhibitory effect is thought to be done via N-terminal serine residue phosphorylation (218). Another cross-talk is between SMO, an activator of the Hh pathway, and β-catenin activation in cancer cells especially in tumorigenesis of intestine (219). GSK-3β can degrade glioma-associated oncogene-3 (Gli3) and inhibit Gli1 activity (218-220). Figure 3 demonstrates summary and the connection between self-renewal pathways of CSCs.

Table 4. Recent publications on the Hedgehog pathway in different cancers.

Entity	Method	Conclusion	Reference/Author
Cervical Tumor	Using cyclopamin and HPVE6/E7siRNA treatment on SiHa and C33a Cell lines on HPV⁺ and HPV⁻ Cervical Cancer Stem Cells and Side Population (analysis by flow cytometry using DCV labeling) to investigate a possible interaction between Hh pathway (GLI) and HPV oncoproteins.	Reportedly, it was found that there is a synergistic effect on Cervical cancer stem cell's viability and their self-renewal ability, specifically on HPV⁺ Cell lines. The results also show absence of either one of GLI or HPVE6 causes Cell programmed death.	(221)
Renal Cancer Stem Cell	Investigating Hh pathway involvement in RCSC induced by cigarette smoke (CS) using tumorsphere formation assay, Immunohistochemistry (IHC), immunofluorescence staining, and flowcytometery.	The results demonstrated the effect of CS on activating the Hh pathway and contributing to RCSC Stemness and suppression of the Hh pathway reduced the effects of CS on enriched human kidney cancer cell lines 786-O and ACHN.	(222)
Breast Cancer	a transcription factor (ETV4) may cause Breast Cancer Cell Stemness by promotion of glycolytic enzymes and enhancement of CXCR4 expression, resulting in the activation of the Hh pathway on human breast cancer Cell lines. This was investigated via tumor xenograft assay, dual luciferase reporter assay, measuring lactate	It was concluded that ETV4 exhaustion may possess an inhibitory effect on CXCR4 expression, Shh pathway signaling, and tumor growth. ETV4 could be responsible for maintaining BCSC and glycolytic shift promotion.	(223)

| Entity	Method	Conclusion	Reference/Author
	production, glucose consumption, and flow cytometry.		
Colorectal Carcinoma	Investigation of a possible inhibitory effect of RUNX3 tumor suppressor on Hedgehog signaling and colorectal cancer Cells self-renewal ability through methods such as plasmid construction, co-immunoprecipitation, self-renewal assay, wound healing assay and Matrigel invasion assay on tissues collected from patients.	It is suggested that the ability of E3 ligase β-transducin repeat-containing E3 ubiquitin protein (**β**-TrCP) to Ubiquitinated GLI1 is augmented, creating a RUNX3-independent regulatory loop, especially in the early stages of Tumor.	(224)
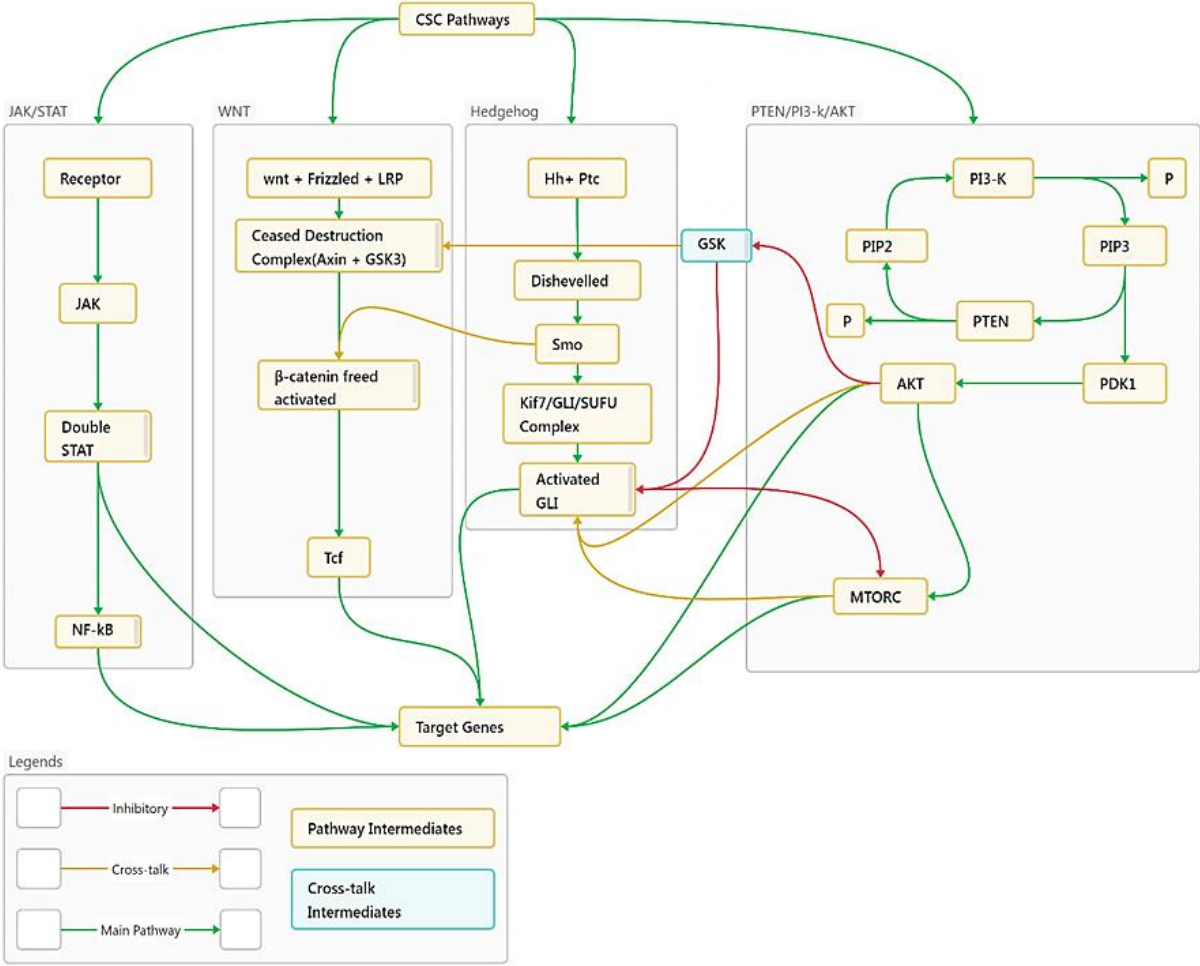
Figure 3. Cross-talk Between CSCs Self-Renewal Pathways.

Discussion

Our analysis of the literature reveals some of CSC's unique capacities for self-renewal and differentiation, which are critical for cancer recurrence, development, and metastasis. Crucially, the identification of CSCs is dependent on certain biomarkers that have not yet been expanded or globalized. An enhancing number of investigations have shown that targeting and utilizing CSCs has the potential to improve new anticancer agents. The

investigation of signaling pathways has provided promising solutions for the treatment and prevention of cancer progression. Understanding the biology of the pathways and using a variety of genes, drugs, and biomarkers to eliminate them is a difficult field of study that offers hope for the future of cancer treatment by eliminating CSCs. Bridging the gap between basic research on CSC self-renewal pathways and their clinical applications is a critical challenge. To fully understand the significance of self-renewal and signaling pathways as effective therapeutic targets for a variety of illnesses, with malignancies receiving particular attention, further research must be done.

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References

1. Sarabia-Sánchez MA, Moreno-Londoño AP, Castañeda-Patlán MC, et al. Non-canonical Wnt/Ca2+ signaling is essential to promote self-renewal and proliferation in colon cancer stem cells. Frontiers in Oncology. 2023;13:1121787.

2. Sugimura R, Li L. Noncanonical Wnt signaling in vertebrate development, stem cells, and diseases. Birth Defects Research Part C: Embryo Today: Reviews. 2010;90(4):243-56.

3. Saeedi M, Nezhad MS, Mehranfar F, et al. Biological aspects and clinical applications of mesenchymal stem cells: key features you need to be aware of. Current Pharmaceutical Biotechnology. 2021;22(2):200-15.

4. Giebel B, Beckmann J. Asymmetric cell divisions of human hematopoietic stem and progenitor cells meet endosomes. Cell Cycle. 2007;6(18):2201-4.

5. Abbaszadegan MR, Bagheri V, Razavi MS, et al. Isolation, identification, and characterization of cancer stem cells: A review. Journal of cellular physiology. 2017;232(8):2008-18.

6. De Francesco EM, Sotgia F, Lisanti MP. Cancer stem cells (CSCs): metabolic strategies for their identification and eradication. Biochemical Journal. 2018;475(9):1611-34.

7. Borah A, Raveendran S, Rochani A, et al. Targeting self-renewal pathways in cancer stem cells: clinical implications for cancer therapy. Oncogenesis. 2015;4(11):e177-e.

8. Najafi M, Farhood B, Mortezaee K. Cancer stem cells (CSCs) in cancer progression and therapy. Journal of cellular physiology. 2019;234(6):8381-95.

9. Csermely P, Hódsági J, Korcsmáros T, et al., editors. Cancer stem cells display extremely large evolvability: alternating plastic and rigid networks as a potential mechanism: network models, novel therapeutic target strategies, and the contributions of hypoxia, inflammation and cellular senescence. Seminars in cancer biology; 2015: Elsevier.

10. Meacham CE, Morrison SJ. Tumour heterogeneity and cancer cell plasticity. Nature. 2013;501(7467):328-37.

11. Rapin N, Bagger FO, Jendholm J, et al. Comparing cancer vs normal gene expression profiles identifies new disease entities and common transcriptional programs in AML patients. Blood. 2014;123(6):894-904.

12. Jamieson CH, Ailles LE, Dylla SJ, et al. Granulocyte–macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. New England Journal of Medicine. 2004;351(7):657-67.

13. Borlongan MC, Wang H. Profiling and targeting cancer stem cell signaling pathways for cancer therapeutics. Frontiers in Cell and Developmental Biology. 2023;11:1125174.

14. Kawaguchi-Ihara N, Murohashi I, Nara N, et al. Promotion of the self-renewal capacity of human acute leukemia cells by Wnt3A. Anticancer research. 2008;28(5A):2701-4.

15. Liu S, Dontu G, Wicha MS. Mammary stem cells, self-renewal pathways, and carcinogenesis. Breast cancer research. 2005;7:1-10.

16. Komiya Y, Habas R. Wnt signal transduction pathways. Organogenesis. 2008;4(2):68-75.

17. Clevers H, Nusse R. Wnt/β-catenin signaling and disease. Cell. 2012;149(6):1192-205.

18. Fleming HE, Janzen V, Celso CL, et al. Wnt signaling in the niche enforces hematopoietic stem cell quiescence and is necessary to preserve self-renewal in vivo. Cell stem cell. 2008;2(3):274-83.

19. Mazieres J, He B, You L, et al. Wnt signaling in lung cancer. Cancer letters. 2005;222(1):1-10.

20. Anastas JN, Moon RT. WNT signalling pathways as therapeutic targets in cancer. Nature Reviews Cancer. 2013;13(1):11-26.

21. Abrahamsson AE, Geron I, Gotlib J, et al. Glycogen synthase kinase 3β missplicing contributes to leukemia stem cell generation. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(10):3925-9.

22. Katoh M, Katoh M. WNT signaling pathway and stem cell signaling network. Clinical cancer research. 2007;13(14):4042-5.

23. Ruan Y, Kim HN, Ogana H, et al. Wnt signaling in leukemia and its bone marrow microenvironment. International Journal of Molecular Sciences. 2020;21(17):6247.

24. Malanchi I, Peinado H, Kassen D, et al. Cutaneous cancer stem cell maintenance is dependent on β-catenin signalling. Nature. 2008;452(7187):650-3.

25. Morin PJ, Sparks AB, Korinek V, et al. Activation of β-catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC. Science. 1997;275(5307):1787-90.

26. Iwao K, Nakamori S, Kameyama M, et al. Activation of the β-catenin gene by interstitial deletions involving exon 3 in primary colorectal carcinomas without adenomatous polyposis coli mutations. Cancer research. 1998;58(5):1021-6.

27. Sparks AB, Morin PJ, Vogelstein B, et al. Mutational analysis of the APC/β-catenin/Tcf pathway in colorectal cancer. Cancer research. 1998;58(6):1130-4.

28. Zhang K, Guo Y, Wang X, et al. WNT/β-catenin directs self-renewal symmetric cell division of hTERThigh prostate cancer stem cells. Cancer research. 2017;77(9):2534-47.

29. Luo Y, Lan L, Jiang Y-G, et al. Epithelial-mesenchymal transition and migration of prostate cancer stem cells is driven by cancer-associated fibroblasts in an HIF-1α/β-catenin-dependent pathway. Molecules and Cells. 2013;36:138-44.

30. Liu L, Zhu H, Liao Y, et al. Inhibition of Wnt/β-catenin pathway reverses multi-drug resistance and EMT in Oct4+/Nanog+ NSCLC cells. Biomedicine & Pharmacotherapy. 2020;127:110225.

31. Bisson I, Prowse DM. WNT signaling regulates self-renewal and differentiation of prostate cancer cells with stem cell characteristics. Cell research. 2009;19(6):683-97.

32. Greer Card DA, Hebbar PB, Li L, et al. Oct4/Sox2-regulated miR-302 targets cyclin D1 in human embryonic stem cells. Molecular and cellular biology. 2008;28(20):6426-38.

33. Tanaka H, Kawaguchi M, Shoda S, et al. Nuclear accumulation of β-catenin in cancer stem cell radioresistance and stemness in human colon cancer. Anticancer Research. 2019;39(12):6575-83.

34. Vermeulen L, De Sousa E Melo F, Van Der Heijden M, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nature cell biology. 2010;12(5):468-76.

35. Jung A, Schrauder M, Oswald U, et al. The invasion front of human colorectal adenocarcinomas shows co-localization of nuclear β-catenin, cyclin D1, and p16INK4A and is a region of low proliferation. The American journal of pathology. 2001;159(5):1613-7.

36. Brabletz T, Jung A, Reu S, et al. Variable β-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. Proceedings of the National Academy of Sciences. 2001;98(18):10356-61.

37. Kirchner T, Brabletz T. Patterning and nuclear β-catenin expression in the colonic adenoma-carcinoma sequence: analogies with embryonic gastrulation. The American journal of pathology. 2000;157(4):1113-21.

38. Mukherjee N, Panda CK. Wnt/β-Catenin signaling pathway as chemotherapeutic target in breast cancer: an update on pros and cons. Clinical breast cancer. 2020;20(5):361-70.

39. Ye L, Yin G, Jiang M, et al. Dihydromyricetin exhibits antitumor activity in nasopharyngeal cancer cell through antagonizing Wnt/β-catenin signaling. Integrative Cancer Therapies. 2021;20:1534735421991217.

40. Lee S-K, Hwang J-H, Choi K-Y. Interaction of the Wnt/β-catenin and RAS-ERK pathways involving co-stabilization of both β-catenin and RAS plays important roles in the colorectal tumorigenesis. Advances in biological regulation. 2018;68:46-54.

41. Jeong W-J, Ro EJ, Choi K-Y. Interaction between Wnt/β-catenin and RAS-ERK pathways and an anti-cancer strategy via degradations of β-catenin and RAS by targeting the Wnt/β-catenin pathway. NPJ Precision oncology. 2018;2(1):5.

42. Stockley J, Villasevil MEM, Nixon C, et al. The RNA-binding protein hnRNPA2 regulates β-

catenin protein expression and is overexpressed in prostate cancer. RNA biology. 2014;11(6):755-65.

43. Meng X, Cui J, Wang Y, et al. Heterogeneous nuclear ribonucleoprotein A1 interacts with microRNA-34a to promote chondrogenic differentiation of mesenchymal stem cells. American Journal of Translational Research. 2017;9(4):1774.

44. Hou J, Zhao N, Zhu P, et al. Irradiated mesenchymal stem cells support stemness maintenance of hepatocellular carcinoma stem cells through Wnt/beta-catenin signaling pathway. Cell Biosci. 2020;10:93.

45. Lin C-K, Bai M-Y, Hu T-M, et al. Preclinical evaluation of a nanoformulated antihelminthic, niclosamide, in ovarian cancer. Oncotarget. 2016;7(8):8993.

46. Park S-Y, Kim J-Y, Choi J-H, et al. Inhibition of LEF1-mediated DCLK1 by niclosamide attenuates colorectal cancer stemness. Clinical Cancer Research. 2019;25(4):1415-29.

47. Zhang Q-H, Ye M, Wu X-Y, et al. Cloning and functional analysis of cDNAs with open reading frames for 300 previously undefined genes expressed in CD34+ hematopoietic stem/progenitor cells. Genome research. 2000;10(10):1546-60.

48. Luo C, Yao Y, Yu Z, et al. UBE2T knockdown inhibits gastric cancer progression. Oncotarget. 2017;8(20):32639.

49. Hu W, Xiao L, Cao C, et al. UBE2T promotes nasopharyngeal carcinoma cell proliferation, invasion, and metastasis by activating the AKT/GSK3β/β-catenin pathway. Oncotarget. 2016;7(12):15161.

50. Ho NPY, Leung CON, Wong TL, et al. The interplay of UBE2T and Mule in regulating Wnt/β-catenin activation to promote hepatocellular carcinoma progression. Cell Death & Disease. 2021;12(2):148.

51. Prabhu VV, Lulla AR, Madhukar NS, et al. Cancer stem cell-related gene expression as a potential biomarker of response for first-in-class imipridone ONC201 in solid tumors. PLoS One. 2017;12(8):e0180541.

52. Lev A, Lulla AR, Ross BC, et al. ONC201 targets AR and AR-V7 signaling, reduces PSA, and synergizes with everolimus in prostate cancer. Molecular Cancer Research. 2018;16(5):754-66.

53. Arrillaga-Romany I, Odia Y, Prabhu VV, et al. Biological activity of weekly ONC201 in adult recurrent glioblastoma patients. Neuro-oncology. 2020;22(1):94-102.

54. Cho Y-H, Ro EJ, Yoon J-S, et al. 5-FU promotes stemness of colorectal cancer via p53-mediated WNT/β-catenin pathway activation. Nature communications. 2020;11(1):5321.

55. Yeh C-T, Wu AT, Chang PM-H, et al. Trifluoperazine, an antipsychotic agent, inhibits cancer stem cell growth and overcomes drug resistance of lung cancer. American journal of respiratory and critical care medicine. 2012;186(11):1180-8.

56. Chen S, Li J, Zhou P, et al. SPTBN1 and cancer, which links? Journal of cellular physiology. 2020;235(1):17-25.

57. Huo J, Zhang Y, Li R, et al. Upregulated microRNA-25 mediates the migration of melanoma cells by targeting DKK3 through the WNT/β-catenin pathway. International journal of molecular sciences. 2016;17(11):1124.

58. Ito S, D'Alessio AC, Taranova OV, et al. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature. 2010;466(7310):1129-33.

59. Tahiliani M, Koh KP, Shen Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009;324(5929):930-5.

60. Qi J, Cui D, Wu Q-N, et al. Targeting Wnt/β-catenin signaling by TET1/FOXO4 inhibits metastatic spreading and self-renewal of cancer stem cells in gastric cancer. Cancers. 2022;14(13):3232.

61. Mykkänen O-M, Gronholm M, Ronty M, et al. Characterization of human palladin, a microfilament-associated protein. Molecular biology of the cell. 2001;12(10):3060-73.

62. Gilam A, Conde J, Weissglas-Volkov D, et al. Local microRNA delivery targets Palladin and prevents metastatic breast cancer. Nature Communications. 2016;7(1):12868.

63. Goicoechea S, Garcia-Mata R, Staub J, et al. Palladin promotes invasion of pancreatic cancer cells by enhancing invadopodia formation in cancer-associated fibroblasts. Oncogene. 2014;33(10):1265-73.

64. Shu X, Chen M, Liu SY, et al. Palladin promotes cancer stem cell- like properties in lung cancer by activating Wnt/B- Catenin signaling. Cancer Medicine. 2023;12(4):4510-20.

65. Resemann HK, Watson CJ, Lloyd-Lewis B. The Stat3 paradox: a killer and an oncogene. Molecular and cellular endocrinology. 2014;382(1):603-11.

66. Zhu Q, Shen Y, Chen X, et al. Self-renewal signalling pathway inhibitors: perspectives on therapeutic approaches for cancer stem cells. OncoTargets and therapy. 2020:525-40.

67. Liu L, Nam S, Tian Y, et al. 6-Bromoindirubin-3′-oxime inhibits JAK/STAT3 signaling and induces apoptosis of human melanoma cells. Cancer research. 2011;71(11):3972-9.

68. Haan C, Kreis S, Margue C, et al. Jaks and cytokine receptors—an intimate relationship. Biochemical pharmacology. 2006;72(11):1538-46.

69. Taga T, Hibi M, Hirata Y, et al. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell. 1989;58(3):573-81.

70. Darnell Jr JE, Kerr lM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science. 1994;264(5164):1415-21.

71. Kumari N, Dwarakanath B, Das A, et al. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumor Biology. 2016;37:11553-72.

72. Johnson DE, O'Keefe RA, Grandis JR. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. Nature reviews Clinical oncology. 2018;15(4):234-48.

73. Vainchenker W, Constantinescu SN. JAK/STAT signaling in hematological malignancies. Oncogene. 2013;32(21):2601-13.

74. Tang L-Y, Heller M, Meng Z, et al. Transforming growth factor-β (TGF-β) directly activates the JAK1-STAT3 axis to induce hepatic fibrosis in coordination with the SMAD pathway. Journal of Biological Chemistry. 2017;292(10):4302-12.

75. Chun J, Li R-J, Cheng M-S, et al. Alantolactone selectively suppresses STAT3 activation and exhibits potent anticancer activity in MDA-MB-231 cells. Cancer letters. 2015;357(1):393-403.

76. Devarajan E, Huang S. STAT3 as a central regulator of tumor metastases. Current molecular medicine. 2009;9(5):626-33.

77. Zhang X, Sun Y, Pireddu R, et al. A novel inhibitor of STAT3 homodimerization selectively suppresses STAT3 activity and malignant transformation. Cancer research. 2013;73(6):1922-33.

78. Tammineni P, Anugula C, Mohammed F, et al. The import of the transcription factor STAT3 into mitochondria depends on GRIM-19, a component of the electron transport chain. Journal of Biological Chemistry. 2013;288(7):4723-32.

79. Zhang Q, Raje V, Yakovlev VA, et al. Mitochondrial localized Stat3 promotes breast cancer growth via phosphorylation of serine 727. Journal of Biological Chemistry. 2013;288(43):31280-8.

80. Herrmann A, Kortylewski M, Kujawski M, et al. Targeting Stat3 in the myeloid compartment drastically improves the in vivo antitumor functions of adoptively transferred T cells. Cancer research. 2010;70(19):7455-64.

81. Kortylewski M, Kujawski M, Wang T, et al. Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. Nature medicine. 2005;11(12):1314-21.

82. Kortylewski M, Xin H, Kujawski M, et al. Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment. Cancer cell. 2009;15(2):114-23.

83. Yue C, Shen S, Deng J, et al. STAT3 in CD8+ T cells inhibits their tumor accumulation by downregulating CXCR3/CXCL10 axis. Cancer immunology research. 2015;3(8):864-70.

84. Wang T, Fahrmann JF, Lee H, et al. JAK/STAT3-regulated fatty acid β-oxidation is critical for breast cancer stem cell self-renewal and chemoresistance. Cell metabolism. 2018;27(1):136-50. e5.

85. Bournazou E, Bromberg J. Targeting the tumor microenvironment: JAK-STAT3 signaling. Jak-Stat. 2013;2(2):e23828.

86. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nature reviews cancer. 2009;9(11):798-809.

87. Grandis JR, Drenning SD, Chakraborty A, et al. Requirement of Stat3 but not Stat1 activation for epidermal growth factor receptor-mediated cell growth In vitro. The Journal of clinical investigation. 1998;102(7):1385-92.

88. Angulo P, Kaushik G, Subramaniam D, et al. Natural compounds targeting major cell signaling pathways: a novel paradigm for osteosarcoma therapy. Journal of hematology & oncology. 2017;10(1):1-13.

89. Ryu K, Choy E, Yang C, et al. Activation of signal transducer and activator of transcription 3 (Stat3) pathway in osteosarcoma cells and overexpression of phosphorylated- Stat3 correlates with poor prognosis. Journal of Orthopaedic Research. 2010;28(7):971-8.

90. Wang W, Li J, Ding Z, et al. Tanshinone I inhibits the growth and metastasis of osteosarcoma via suppressing JAK/STAT3 signalling pathway. Journal of Cellular and Molecular Medicine. 2019;23(9):6454-65.

91. Zhou F, Qi Y, Geng Z, et al. Activation of Wnt/β-Catenin signaling in EpCAMhigh/CD44+ cells endow colorectal cancer with tumor proliferation and oxaliplatin chemoresistance. Combinatorial Chemistry & High Throughput Screening. 2023.

92. Fan Y, Hou T, Dan W, et al. Silibinin inhibits epithelial- mesenchymal transition of renal cell carcinoma through autophagy- dependent Wnt/β- catenin signaling. International Journal of Molecular Medicine. 2020;45(5):1341-50.

93. Saliu TP, Seneviratne NN, Faizan M, et al. In silico identification and in vitro validation of alpha-hederin as a potent inhibitor of Wnt/beta-catenin signaling pathway in breast cancer stem cells. In Silico Pharmacol. 2024;12(1):31.

94. Lei D, Yang W-T, Zheng P-S. HOXB4 inhibits the proliferation and tumorigenesis of cervical cancer cells by downregulating the activity of Wnt/β-catenin signaling pathway. Cell Death & Disease. 2021;12(1):105.

95. Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nature reviews immunology. 2007;7(1):41-51.

96. Marotta LL, Almendro V, Marusyk A, et al. The JAK2/STAT3 signaling pathway is required for growth of CD44+ CD24–stem cell–like breast cancer cells in human tumors. The Journal of clinical investigation. 2011;121(7):2723-35.

97. Xiao C, Hong H, Yu H, et al. MiR-340 affects gastric cancer cell proliferation, cycle, and apoptosis through regulating SOCS3/JAK-STAT signaling pathway. Immunopharmacology and immunotoxicology. 2018;40(4):278-83.

98. Shi L, Zheng H, Hu W, et al. Niclosamide inhibition of STAT3 synergizes with erlotinib in human colon cancer. Onco Targets Ther. 2017;10:1767-76.

99. Han Z, Feng J, Hong Z, et al. Silencing of the STAT3 signaling pathway reverses the inherent and induced chemoresistance of human ovarian cancer cells. Biochemical and biophysical research communications. 2013;435(2):188-94.

100. Liu S, Sun J, Cai B, et al. NANOG regulates epithelial-mesenchymal transition and chemoresistance through activation of the STAT3 pathway in epithelial ovarian cancer. Tumor Biology. 2016;37:9671-80.

101. Wu X, Tang W, Marquez RT, et al. Overcoming chemo/radio-resistance of pancreatic cancer by inhibiting STAT3 signaling. Oncotarget. 2016;7(10):11708.

102. Zhao C, Xiao H, Wu X, et al. Rational combination of MEK inhibitor and the STAT3 pathway modulator for the therapy in K-Ras mutated pancreatic and colon cancer cells. Oncotarget. 2015;6(16):14472.

103. Yar Saglam A, Alp E, Elmazoglu Z, et al. Treatment with cucurbitacin B alone and in combination with gefitinib induces cell cycle inhibition and apoptosis via EGFR and JAK/STAT pathway in human colorectal cancer cell lines. Human & experimental toxicology. 2016;35(5):526-43.

104. Shi L, Zheng H, Hu W, et al. Niclosamide inhibition of STAT3 synergizes with erlotinib in human colon cancer. OncoTargets and therapy. 2017:1767-76.

105. Dave B, Landis MD, Dobrolecki LE, et al. Selective small molecule Stat3 inhibitor reduces breast cancer tumor-initiating cells and improves recurrence free survival in a human-xenograft model. PLoS One. 2012;7(8):e30207.

106. Tao L, Huang G, Wang R, et al. Cancer-associated fibroblasts treated with cisplatin facilitates chemoresistance of lung adenocarcinoma through IL-11/IL-11R/STAT3 signaling pathway. Scientific reports. 2016;6(1):38408.

107. Lou W, Chen Y, Zhu K-y, et al. Polyphyllin I overcomes EMT-associated resistance to erlotinib in lung cancer cells via IL-6/STAT3 pathway

inhibition. Biological and Pharmaceutical Bulletin. 2017;40(8):1306-13.

108. Wang J, Wang Y, Zheng C, et al. Tyrosine kinase inhibitor- induced IL- 6/STAT3 activation decreases sensitivity of EGFR- mutant non- small cell lung cancer to icotinib. Cell Biology International. 2018;42(10):1292-9.

109. Kandala PK, Srivastava SK. Diindolylmethane suppresses ovarian cancer growth and potentiates the effect of cisplatin in tumor mouse model by targeting signal transducer and activator of transcription 3 (STAT3). BMC medicine. 2012;10:1-18.

110. Liu T, Liu P, Li Y, et al. Inhibition of STAT3 with shRNA enhances the chemosensitization of cisplatin in laryngeal carcinoma stem cells. Int J Exp Pathol. 2017;10(6):6512-9.

111. Wu X, Xiao H, Wang R, et al. Persistent GP130/STAT3 signaling contributes to the resistance of doxorubicin, cisplatin, and MEK inhibitor in human rhabdomyosarcoma cells. Current cancer drug targets. 2016;16(7):631-8.

112. Herrmann A, Cherryholmes G, Schroeder A, et al. TLR9 is critical for glioma stem cell maintenance and targeting. Cancer research. 2014;74(18):5218-28.

113. Schroeder A, Herrmann A, Cherryholmes G, et al. Loss of androgen receptor expression promotes a stem-like cell phenotype in prostate cancer through STAT3 signaling. Cancer research. 2014;74(4):1227-37.

114. Abubaker K, Luwor RB, Zhu H, et al. Inhibition of the JAK2/STAT3 pathway in ovarian cancer results in the loss of cancer stem cell-like characteristics and a reduced tumor burden. BMC cancer. 2014;14(1):1-22.

115. Ashizawa T, Miyata H, Iizuka A, et al. Effect of the STAT3 inhibitor STX-0119 on the proliferation of cancer stem-like cells derived from recurrent glioblastoma. International journal of oncology. 2013;43(1):219-27.

116. Kroon P, Berry PA, Stower MJ, et al. JAK-STAT blockade inhibits tumor initiation and clonogenic recovery of prostate cancer stem-like cells. Cancer research. 2013;73(16):5288-98.

117. Jensen KV, Cseh O, Aman A, et al. The JAK2/STAT3 inhibitor pacritinib effectively inhibits patient-derived GBM brain tumor initiating cells in vitro and when used in combination with temozolomide increases survival in an orthotopic xenograft model. PloS one. 2017;12(12):e0189670.

118. Liu R-Y, Zeng Y, Lei Z, et al. JAK/STAT3 signaling is required for TGF-β-induced epithelial-mesenchymal transition in lung cancer cells. International journal of oncology. 2014;44(5):1643-51.

119. Morgan EL, Macdonald A. JAK2 inhibition impairs proliferation and sensitises cervical cancer cells to cisplatin-induced cell death. Cancers. 2019;11(12):1934.

120. Delen E, Doganlar O, Doganlar ZB, et al. Inhibition of the invasion of human glioblastoma U87 cell line by ruxolitinib: a molecular player of miR-17 and miR-20a regulating JAK/STAT pathway. Turk Neurosurg. 2020;30(2):182-9.

121. Li K, Yuan D, Yan R, et al. Stigmasterol exhibits potent antitumor effects in human gastric cancer cells mediated via inhibition of cell migration, cell cycle arrest, mitochondrial mediated apoptosis and inhibition of JAK/STAT signalling pathway. J BUON. 2018;23(5):1420-5.

122. Patel MR, Dash A, Jacobson BA, et al. JAK/STAT inhibition with ruxolitinib enhances oncolytic virotherapy in non-small cell lung cancer models. Cancer gene therapy. 2019;26(11-12):411-8.

123. Zhang W, Guo J, Li S, et al. Discovery of monocarbonyl curcumin-BTP hybrids as STAT3 inhibitors for drug-sensitive and drug-resistant breast cancer therapy. Scientific reports. 2017;7(1):46352.

124. Qiu X, Guo G, Chen K, et al. A requirement for SOCS-1 and SOCS-3 phosphorylation in Bcr-Abl-induced tumorigenesis. Neoplasia. 2012;14(6):547-IN22.

125. Harrison C, Kiladjian J-J, Al-Ali HK, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. New England Journal of Medicine. 2012;366(9):787-98.

126. Park S-Y, Lee C-J, Choi J-H, et al. The JAK2/STAT3/CCND2 Axis promotes colorectal Cancer stem cell persistence and radioresistance. Journal of Experimental & Clinical Cancer Research. 2019;38(1):1-18.

127. Shibue T, Weinberg RA. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nature reviews Clinical oncology. 2017;14(10):611-29.

128. Dongre A, Weinberg RA. New insights into the mechanisms of epithelial–mesenchymal transition and implications for cancer. Nature reviews Molecular cell biology. 2019;20(2):69-84.

129. Ajayi S, Becker H, Reinhardt H, et al. Ruxolitinib. Recent results in cancer research. 2018;212:119-32.

130. Sen M, Thomas SM, Kim S, et al. First-in-human trial of a STAT3 decoy oligonucleotide in head and neck tumors: implications for cancer therapy. Cancer discovery. 2012;2(8):694-705.

131. Sen M, Paul K, Freilino ML, et al. Systemic administration of a cyclic signal transducer and activator of transcription 3 (STAT3) decoy oligonucleotide inhibits tumor growth without inducing toxicological effects. Molecular medicine. 2014;20:46-56.

132. Ghasemi F, Shafiee M, Banikazemi Z, et al. Curcumin inhibits NF-kB and Wnt/β-catenin pathways in cervical cancer cells. Pathology, research and practice. 2019;215(10):152556.

133. Kuttikrishnan S, Siveen KS, Prabhu KS, et al. Curcumin induces apoptotic cell death via inhibition of PI3-kinase/AKT pathway in B-precursor acute lymphoblastic leukemia. Frontiers in oncology. 2019;9:484.

134. Seo SU, Woo SM, Lee H-S, et al. mTORC1/2 inhibitor and curcumin induce apoptosis through lysosomal membrane permeabilization-mediated autophagy. Oncogene. 2018;37(38):5205-20.

135. Wan Mohd Tajuddin WNB, Lajis NH, Abas F, et al. Mechanistic understanding of curcumin's therapeutic effects in lung cancer. Nutrients. 2019;11(12):2989.

136. Liu Y, Wang X, Zeng S, et al. The natural polyphenol curcumin induces apoptosis by suppressing STAT3 signaling in esophageal squamous cell carcinoma. Journal of Experimental & Clinical Cancer Research. 2018;37(1):1-12.

137. Hale AJ, Smith CA, Sutherland LC, et al. Apoptosis: molecular regulation of cell death. European Journal of Biochemistry. 1996;236(1):1-26.

138. Xia P, Xu X-Y. PI3K/Akt/mTOR signaling pathway in cancer stem cells: from basic research to clinical application. American journal of cancer research. 2015;5(5):1602.

139. Dubrovska A, Kim S, Salamone RJ, et al. The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations. Proceedings of the National Academy of Sciences. 2009;106(1):268-73.

140. Daya HA, Kouba S, Ouled-Haddou H, et al. Orai3-mediates cisplatin-resistance in non-small cell lung cancer cells by enriching cancer stem cell population through PI3K/AKT pathway. Cancers. 2021;13(10):2314.

141. Mitsiades CS, Mitsiades N, Koutsilieris M. The Akt pathway: molecular targets for anti-cancer drug development. Current cancer drug targets. 2004;4(3):235-56.

142. Carnero A, Blanco-Aparicio C, Renner O, et al. The PTEN/PI3K/AKT signalling pathway in cancer, therapeutic implications. Current cancer drug targets. 2008;8(3):187-98.

143. Bleau A-M, Hambardzumyan D, Ozawa T, et al. PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. Cell stem cell. 2009;4(3):226-35.

144. Gallardo A, Lerma E, Escuin D, et al. Increased signalling of EGFR and IGF1R, and deregulation of PTEN/PI3K/Akt pathway are related with trastuzumab resistance in HER2 breast carcinomas. British journal of cancer. 2012;106(8):1367-73.

145. Vara JÁF, Casado E, de Castro J, et al. PI3K/Akt signalling pathway and cancer. Cancer treatment reviews. 2004;30(2):193-204.

146. Li VSW, Wong CW, Chan TL, et al. Mutations of PIK3CA in gastric adenocarcinoma. BMC cancer. 2005;5:1-6.

147. Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. Genes & development. 1999;13(22):2905-27.

148. Dastmalchi N, Hosseinpourfeizi MA, Khojasteh SMB, et al. Tumor suppressive activity of miR-424-5p in breast cancer cells through targeting PD-L1 and modulating PTEN/PI3K/AKT/mTOR signaling pathway. Life Sciences. 2020;259:118239.

149. Bi X, Lv X, Liu D, et al. METTL3-mediated maturation of miR-126-5p promotes ovarian cancer progression via PTEN-mediated PI3K/Akt/mTOR pathway. Cancer Gene Therapy. 2021;28(3-4):335-49.

150. Carnero A, Paramio JM. The PTEN/PI3K/AKT pathway in vivo, cancer mouse models. Frontiers in oncology. 2014;4:252.

151. Stambolic V, MacPherson D, Sas D, et al. Regulation of PTEN transcription by p53. Molecular cell. 2001;8(2):317-25.

152. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004;304(5670):554-.

153. Carracedo A, Pandolfi P. The PTEN–PI3K pathway: of feedbacks and cross-talks. Oncogene. 2008;27(41):5527-41.

154. Carver BS, Pandolfi PP. Mouse modeling in oncologic preclinical and translational research. Clinical cancer research. 2006;12(18):5305-11.

155. Wang S, Gao J, Lei Q, et al. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer cell. 2003;4(3):209-21.

156. Freeman D, Lesche R, Kertesz N, et al. Genetic background controls tumor development in PTEN-deficient mice. Cancer research. 2006;66(13):6492-6.

157. Lai EC. Micro RNAs are complementary to 3′ UTR sequence motifs that mediate negative post-transcriptional regulation. Nature genetics. 2002;30(4):363-4.

158. Stark A, Brennecke J, Bushati N, et al. Animal MicroRNAs confer robustness to gene expression and have a significant impact on 3′ UTR evolution. Cell. 2005;123(6):1133-46.

159. Lee I, Ajay SS, Yook JI, et al. New class of microRNA targets containing simultaneous 5′-UTR and 3′-UTR interaction sites. Genome research. 2009;19(7):1175-83.

160. Wellner U, Schubert J, Burk UC, et al. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nature cell biology. 2009;11(12):1487-95.

161. Chen W, Kong K-K, Xu X-K, et al. Downregulation of miR- 205 is associated with glioblastoma cell migration, invasion, and the epithelial-mesenchymal transition, by targeting ZEB1 via the Akt/mTOR signaling pathway. International Journal of Oncology. 2018;52(2):485-95.

162. Yang X-L, Ma Y-S, Liu Y-S, et al. microRNA-873 inhibits self-renewal and proliferation of pancreatic cancer stem cells through pleckstrin-2-dependent PI3K/AKT pathway. Cellular Signalling. 2021;84:110025.

163. Lagos D, Pollara G, Henderson S, et al. miR-132 regulates antiviral innate immunity through suppression of the p300 transcriptional co-activator. Nature cell biology. 2010;12(5):513-9.

164. Park J-K, Henry JC, Jiang J, et al. miR-132 and miR-212 are increased in pancreatic cancer and target the retinoblastoma tumor suppressor. Biochemical and biophysical research communications. 2011;406(4):518-23.

165. Xin C, Guangliang S, Qing Z, et al. Astilbin protects chicken peripheral blood lymphocytes from cadmium-induced necroptosis via oxidative stress and the PI3K/Akt pathway. Ecotoxicology and Environmental Safety. 2020;190:110064.

166. Ke J, Bian X, Liu H, et al. Edaravone reduces oxidative stress and intestinal cell apoptosis after burn through up-regulating miR-320 expression. Molecular Medicine. 2019;25(1):1-10.

167. Chen S, Yang S, Wang M, et al. Curcumin inhibits zearalenone-induced apoptosis and oxidative stress in Leydig cells via modulation of the PTEN/Nrf2/Bip signaling pathway. Food and Chemical Toxicology. 2020;141:111385.

168. Parsons R. Discovery of the PTEN Tumor Suppressor and Its Connection to the PI3K and AKT Oncogenes. Cold Spring Harbor Perspectives in Medicine. 2020;10(8):a036129.

169. Chen Z, Gu D, Zhou M, et al. Regulatory role of miR-125a/b in the suppression by selenium of cadmium-induced apoptosis via the mitochondrial pathway in LLC-PK1 cells. Chemico-biological interactions. 2016;243:35-44.

170. Chen Q, Chen X, Han C, et al. FGF-2 transcriptionally down-regulates the expression of BNIP3L via PI3K/Akt/FoxO3a signaling and inhibits necrosis and mitochondrial dysfunction induced by high concentrations of hydrogen peroxide in H9c2 cells. Cellular Physiology and Biochemistry. 2016;40(6):1678-91.

171. Zhu L, Li Z, Yu X, et al. The tRNA-derived fragment 5026a inhibits the proliferation of gastric cancer cells by regulating the PTEN/PI3K/AKT signaling pathway. Stem Cell Research & Therapy. 2021;12(1):1-13.

172. Xiong Y, Cao F, Hu L, et al. miRNA-26a-5p accelerates healing via downregulation of PTEN in fracture patients with traumatic brain injury. Molecular Therapy-Nucleic Acids. 2019;17:223-34.

173. Yang W-T, Chen M, Xu R, et al. PRDM4 inhibits cell proliferation and tumorigenesis by inactivating the PI3K/AKT signaling pathway through targeting of PTEN in cervical carcinoma. Oncogene. 2021;40(18):3318-30.

174. Ebner M, Lučić I, Leonard TA, et al. PI (3, 4, 5) P3 engagement restricts Akt activity to cellular membranes. Molecular cell. 2017;65(3):416-31. e6.

175. Wen Y-G, Wang Q, Zhou C-Z, et al. Mutation analysis of tumor suppressor gene PTEN in patients with gastric carcinomas and its impact on PI3K/AKT pathway. Oncology reports. 2010;24(1):89-95.

176. Kang Y-H, Lee HS, Kim WH. Promoter methylation and silencing of PTEN in gastric carcinoma. Laboratory investigation. 2002;82(3):285-91.

177. Wan W, Wan W, Long Y, et al. MiR-25-3p promotes malignant phenotypes of retinoblastoma by regulating PTEN/Akt pathway. Biomedicine & Pharmacotherapy. 2019;118:109111.

178. Ingham PW. Drosophila segment polarity mutants and the rediscovery of the hedgehog pathway genes. Current topics in developmental biology. 2016;116:477-88.

179. Jing J, Wu Z, Wang J, et al. Hedgehog signaling in tissue homeostasis, cancers, and targeted therapies. Signal Transduction and Targeted Therapy. 2023;8(1):315.

180. Skoda AM, Simovic D, Karin V, et al. The role of the Hedgehog signaling pathway in cancer: A comprehensive review. Bosnian journal of basic medical sciences. 2018;18(1):8.

181. Zhu R, Gires O, Zhu L, et al. TSPAN8 promotes cancer cell stemness via activation of sonic Hedgehog signaling. Nature communications. 2019;10(1):2863.

182. Zhai J, Zhang H, Zhang J, et al. Effect of the sonic hedgehog inhibitor GDC-0449 on an in vitro isogenic cellular model simulating odontogenic keratocysts. International journal of oral science. 2019;11(1):4.

183. Hoyos Cadavid AM, Kaminagakura E, Rodrigues M, et al. Immunohistochemical evaluation of Sonic Hedgehog signaling pathway proteins (Shh, Ptch1, Ptch2, Smo, Gli1, Gli2, and Gli3) in sporadic and syndromic odontogenic keratocysts. Clinical oral investigations. 2019;23:153-9.

184. Punjabi LS, Goh CHR, Sittampalam K. Expanding the spectrum of GLI1- altered mesenchymal tumors—A high- grade uterine sarcoma harboring a novel PAMR1:: GLI1 fusion and literature review of GLI1- altered mesenchymal neoplasms of the gynecologic tract. Genes, Chromosomes and Cancer. 2023;62(2):107-14.

185. Momeni-Boroujeni A, Mohammad N, Wolber R, et al. Targeted RNA expression profiling identifies high-grade endometrial stromal sarcoma as a clinically relevant molecular subtype of uterine sarcoma. Modern Pathology. 2021;34(5):1008-16.

186. Infante P, Malfanti A, Quaglio D, et al. Glabrescione B delivery by self-assembling micelles efficiently inhibits tumor growth in preclinical models of Hedgehog-dependent medulloblastoma. Cancer letters. 2021;499:220-31.

187. Azatyan A, Zhang S, Darabi A, et al. Circular RNAs in hedgehog signaling activation and hedgehog-mediated medulloblastoma tumors. Cancers. 2021;13(20):5138.

188. Eibenschutz L, Caputo S, Camera E, et al. Evaluation of Hedgehog Pathway Inhibition on Nevoid Basal Cell Carcinoma Syndrome Fibroblasts and Basal Cell Carcinoma-Associated Fibroblasts: Are Vismodegib and Sonidegib Useful to Target Cancer-Prone Fibroblasts? Cancers. 2021;13(22):5858.

189. Martinez MF, Romano MV, Martinez AP, et al. Nevoid basal cell carcinoma syndrome: PTCH1 mutation profile and expression of genes involved in the hedgehog pathway in Argentinian patients. Cells. 2019;8(2):144.

190. Zhang X, Wang Y, Wang X, et al. Extracellular vesicles-encapsulated microRNA-10a-5p shed from cancer-associated fibroblast facilitates cervical squamous cell carcinoma cell angiogenesis and tumorigenicity via Hedgehog signaling pathway. Cancer Gene Therapy. 2021;28(5):529-42.

191. Geyer N, Gerling M. Hedgehog signaling in colorectal cancer: all in the stroma? International Journal of Molecular Sciences. 2021;22(3):1025.

192. Lim S, Lim SM, Kim M-J, et al. Sonic hedgehog pathway as the prognostic marker in patients with extensive stage small cell lung cancer. Yonsei medical journal. 2019;60(10):898-904.

193. Trieu K. Understanding Genetic Factors that Modulate Hedgehog-Driven Skin Cancer: University of Michigan; 2022.

194. Xu Y, Song S, Wang Z, et al. The role of hedgehog signaling in gastric cancer: molecular mechanisms, clinical potential, and perspective. Cell Communication and Signaling. 2019;17:1-10.

195. Londero AP, Orsaria M, Viola L, et al. Survivin, sonic hedgehog, krüppel-like factors, and p53 pathway in serous ovarian cancer: an immunohistochemical study. Human pathology. 2022;127:92-101.

196. Zhou H, Xiong Y, Peng L, et al. LncRNA- cCSC1 modulates cancer stem cell properties in colorectal cancer via activation of the Hedgehog signaling pathway. Journal of cellular biochemistry. 2020;121(3):2510-24.

197. Zhou M, Hou Y, Yang G, et al. LncRNA-Hh strengthen cancer stem cells generation in twist-positive breast cancer via activation of hedgehog signaling pathway. Stem cells. 2016;34(1):55-66.

198. Sanghrajka RM, Koche R, Medrano H, et al. KMT2D suppresses Sonic hedgehog-driven medulloblastoma progression and metastasis. Iscience. 2023;26(10).

199. Russo A, Colina JA, Moy J, et al. Silencing PTEN in the fallopian tube promotes enrichment of cancer stem cell-like function through loss of PAX2. Cell Death & Disease. 2021;12(4):375.

200. Xia Q, Zhang H, Zhang P, et al. Oncogenic Smurf1 promotes PTEN wild-type glioblastoma growth by mediating PTEN ubiquitylation. Oncogene. 2020;39(36):5902-15.

201. Kim I-G, Lee J-H, Kim S-Y, et al. Targeting therapy-resistant lung cancer stem cells via disruption of the AKT/TSPYL5/PTEN positive-feedback loop. Communications Biology. 2021;4(1):778.

202. Baker A, Wyatt D, Bocchetta M, et al. Notch-1-PTEN-ERK1/2 signaling axis promotes HER2+ breast cancer cell proliferation and stem cell survival. Oncogene. 2018;37(33):4489-504.

203. Ingham PW. Hedgehog signaling. In: editor.^editors. Current Topics in Developmental Biology. ed.: Elsevier; 2022: 1-58.

204. Hooper JE, Scott MP. Communicating with hedgehogs. Nature reviews Molecular cell biology. 2005;6(4):306-17.

205. Dilower I, Niloy AJ, Kumar V, et al. Hedgehog Signaling in Gonadal Development and Function. Cells. 2023;12(3):358.

206. St-Jacques B, Hammerschmidt M, McMahon AP. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. Genes & development. 1999;13(16):2072-86.

207. Liu C, Rodriguez KF, Brown PR, et al. Reproductive, physiological, and molecular outcomes in female mice deficient in Dhh and Ihh. Endocrinology. 2018;159(7):2563-75.

208. Abramyan J. Hedgehog signaling and embryonic craniofacial disorders. Journal of developmental biology. 2019;7(2):9.

209. Belloni E, Muenke M, Roessler E, et al. Identification of Sonic hedgehog as a candidate gene responsible for holoprosencephaly. Nature genetics. 1996;14(3):353-6.

210. Roessler E, Belloni E, Gaudenz K, et al. Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. Nature genetics. 1996;14(3):357-60.

211. Roper RJ, Baxter LL, Saran NG, et al. Defective cerebellar response to mitogenic Hedgehog signaling in Down's syndrome mice. Proceedings of the National Academy of Sciences. 2006;103(5):1452-6.

212. Astorga J, Carlsson P. Hedgehog induction of murine vasculogenesis is mediated by Foxf1 and Bmp4. Development. 2007;134:3753-61.

213. Zhang XM, Ramalho-Santos M, McMahon AP. Smoothened mutants reveal redundant roles for Shh and Ihh signaling including regulation of L/R asymmetry by the mouse node. Cell. 2001;105(6):781-92.

214. Leavitt E, Lask G, Martin S. Sonic hedgehog pathway inhibition in the treatment of advanced basal cell carcinoma. Current Treatment Options in Oncology. 2019;20:1-12.

215. Chen X, Tukachinsky H, Huang C-H, et al. Processing and turnover of the Hedgehog protein in the endoplasmic reticulum. Journal of Cell Biology. 2011;192(5):825-38.

216. Lum L, Beachy PA. The Hedgehog response network: sensors, switches, and routers. science. 2004;304(5678):1755-9.

217. Larsen LJ, Møller LB. Crosstalk of hedgehog and mTORC1 pathways. Cells. 2020;9(10):2316.

218. Thornton TM, Pedraza-Alva G, Deng B, et al. Phosphorylation by p38 MAPK as an alternative pathway for GSK3β inactivation. Science. 2008;320(5876):667-70.

219. Bertrand FE, Angus CW, Partis WJ, et al. Developmental pathways in colon cancer: crosstalk between WNT, BMP, Hedgehog and Notch. Cell cycle. 2012;11(23):4344-51.

220. Ryan KE, Chiang C. Hedgehog secretion and signal transduction in vertebrates. Journal of Biological Chemistry. 2012;287(22):17905-13.

221. Vishnoi K, Mahata S, Tyagi A, et al. Cross-talk between human papillomavirus oncoproteins and hedgehog signaling synergistically promotes stemness in cervical cancer cells. Scientific reports. 2016;6(1):34377.

222. Qian W, Kong X, Zhang T, et al. Cigarette smoke stimulates the stemness of renal cancer stem cells via Sonic Hedgehog pathway. Oncogenesis. 2018;7(3):24.

223. Zhu T, Zheng J, Zhuo W, et al. ETV4 promotes breast cancer cell stemness by activating glycolysis and CXCR4-mediated sonic Hedgehog signaling. Cell death discovery. 2021;7(1):126.

224. Kim BR, Na YJ, Kim JL, et al. RUNX3 suppresses metastasis and stemness by inhibiting Hedgehog signaling in colorectal cancer. Cell Death & Differentiation. 2020;27(2):676-94.