

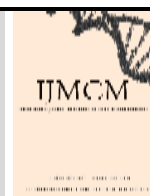


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## REVIEW ARTICLE

# 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/ $\beta$ -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

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## 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/ $\beta$ -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/ $\beta$ -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/ $\beta$ -catenin signaling, the Axin/ glycogen Synthase Kinase-3 (GSK-3)/APC complex breaks down the intracellular signaling molecule  $\beta$ -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  $\beta$ -catenin then becomes stable and may reach the nucleus, allowing target genes to be transcribed more easily (17, 18).

$\beta$ -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/ $\beta$ -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  $\beta$ -catenin were detected in samples of leukemia patients with FLT-3 mutation (23). The Wnt/ $\beta$ -catenin pathway is

required for the survival of cutaneous CSCs, and knocking down the  $\beta$ -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  $\beta$ -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/ $\beta$ -catenin signaling, many of which are particular in the way of cancer progression (26, 27). In prostate cancer stem cells, the Wnt/ $\beta$ -catenin signaling and its target genes c-Myc and cyclinD1 were activated, and the self-renewal of prostate CSCs was reliant on  $\beta$ -catenin in the nucleus, according to Zhang K et al. (28). Upregulation of  $\beta$ -catenin was linked to invasion and metastasis of prostate CSCs, and transplanted  $\beta$ -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/ $\beta$ -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/ $\beta$ -catenin pathway could considerably upregulate CD133 and CD44, and targeting Wnt/ $\beta$ -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/ $\beta$ -catenin signaling in colon CSCs (33, 34). Colon CSCs contain a substantial amount of  $\beta$ -catenin, which is controlled in part by the microenvironment and eventually leads to treatment resistance and metastasis. (34). Because of the considerable amount of  $\beta$ -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/ $\beta$ -catenin pathway. Dihydromyricetin might be a promising new therapy option for nasopharyngeal cancer (39). The interaction of the Wnt/ $\beta$ -catenin and RAS/extracellular-signal-regulated kinase pathways is significant in malignant phenotypes, and the alterations in both  $\beta$ -catenin and RAS levels are linked in human colorectal cancer with adenomatous polyposis coli mutations (40, 41).

hnRNPA and its subtypes can control the expression of Wnt/ $\beta$ -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/ $\beta$ -catenin signaling (44). Niclosamide was discovered to be a Wnt/ $\beta$ -catenin signaling inhibitor with anti-tumor effects that targeted ovarian CSCs specifically (45). In colorectal cancer, niclosamide can lower the expression of numerous Wnt/ $\beta$ -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/ $\beta$ -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/ $\beta$ -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/ $\beta$ -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- $\beta$  and Wnt/ $\beta$ -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/ $\beta$ -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/ $\beta$ -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/ $\beta$ -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/ $\beta$ -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- $\gamma$ ), and interferon-alpha (IFN- $\alpha$ ), 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 hematopoietic malignancies or solid tumors, as well as in chronic inflammatory diseases (71, 72).

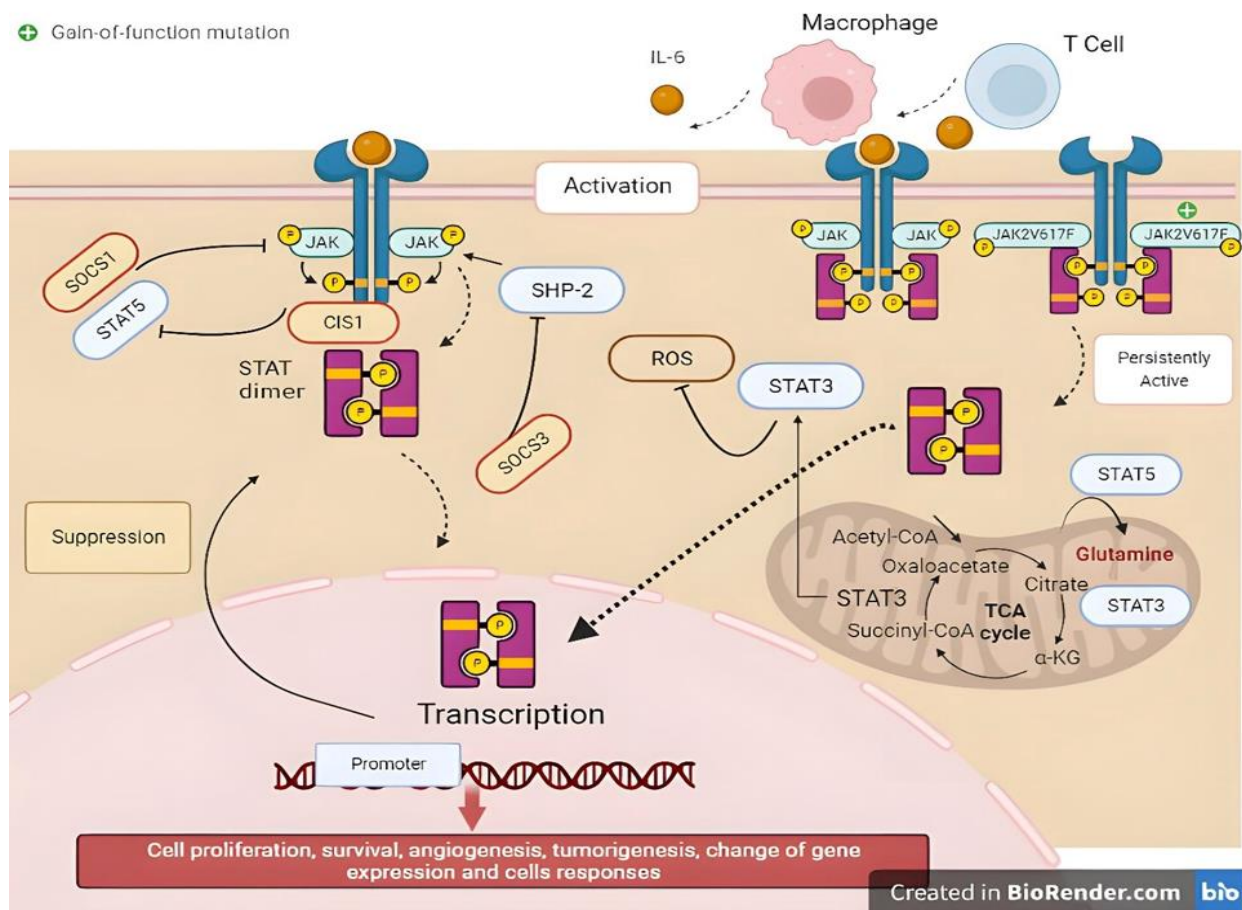
STAT proteins are usually activated in hematological tumors, but only in a few of them, it can be shown that the signaling pathway is altered, such as myeloproliferative neoplasm (MPNs) with Negative Bcr-Abl and some subsets of acute lymphoblastic leukemia (73). Studies have demonstrated that transforming growth factor  $\beta$ 1 (TGF $\beta$ 1) 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- $\gamma$  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/ $\beta$ -catenin involvement**

| Cancer                            | WNT/ $\beta$ -Catenin pathway | Method                                                                                                                                                                                                                                                                                                                       | Conclusion                                                                                                                                                                                                                | Reference/Author |
|-----------------------------------|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| <b>Colorectal Cancer</b>          | 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/ $\beta$ -Catenin signalling was measured using Western blot.                                     | The proliferation ability and Chemoresistance were significantly stronger than the parental cells, and the WNT/ $\beta$ -Catenin Signalling is activated in the CSCs of these cell lines.                                 | (91)             |
| <b>Renal Cell Carcinoma (RCC)</b> | 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/ $\beta$ -Catenin pathway in an Autophagy dependent fashion. Furthermore, the degradation of $\beta$ -Catenin molecules induced by Silibinin had anti-metastatic effects against RCC.                   | (92)             |
| <b>Breast Cancer</b>              | Deregulated                   | Utilizing Alpha-hederin (AH) as an inhibitor against WNT/ $\beta$ -Catenin Signalling in Breast cancer stem cells (BCSCs) by disrupting $\beta$ -Catenin/Tcf4 interaction.                                                                                                                                                   | Reportedly, AH reduced the viability of bCSCs and suppressed the transcription of Wnt downstream target genes.                                                                                                            | (93)             |
| <b>Cervical Cancer</b>            | 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 vivo and in vitro. Moreover, the influence of up and down regulation of HOXB4 on $\beta$ -Catenin transcription and Wnt signaling activation was studied. | The findings revealed that HOXB4 is downregulated in Cervical Cancer cells, and HOXB4 suppresses the $\beta$ -Catenin transcription, consequently inhibiting the WNT/ $\beta$ -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, restraint 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- $\beta$ /Smad (118).

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

| <b>Cancer</b>                | <b>JAK/STAT Pathway</b> | <b>Method</b>                                                                                                                                                                                       | <b>Conclusion</b>                                                                                                                                                                                                      | <b>Reference/Author</b> |
|------------------------------|-------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|
| <b>Cervical Cancer</b>       | 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)                   |
| <b>Glioblastoma</b>          | 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)                   |
| <b>Gastric Cancer</b>        | 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)                   |
| <b>Non-small lung Cancer</b> | Activated STAT3         | Ruxolutinib in Combination with VSV-IFN $\beta$ 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)                   |
| <b>Breast Cancer</b>         | 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)                   |
| <b>Leukemia</b>              | 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 Dubrovskaya 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).

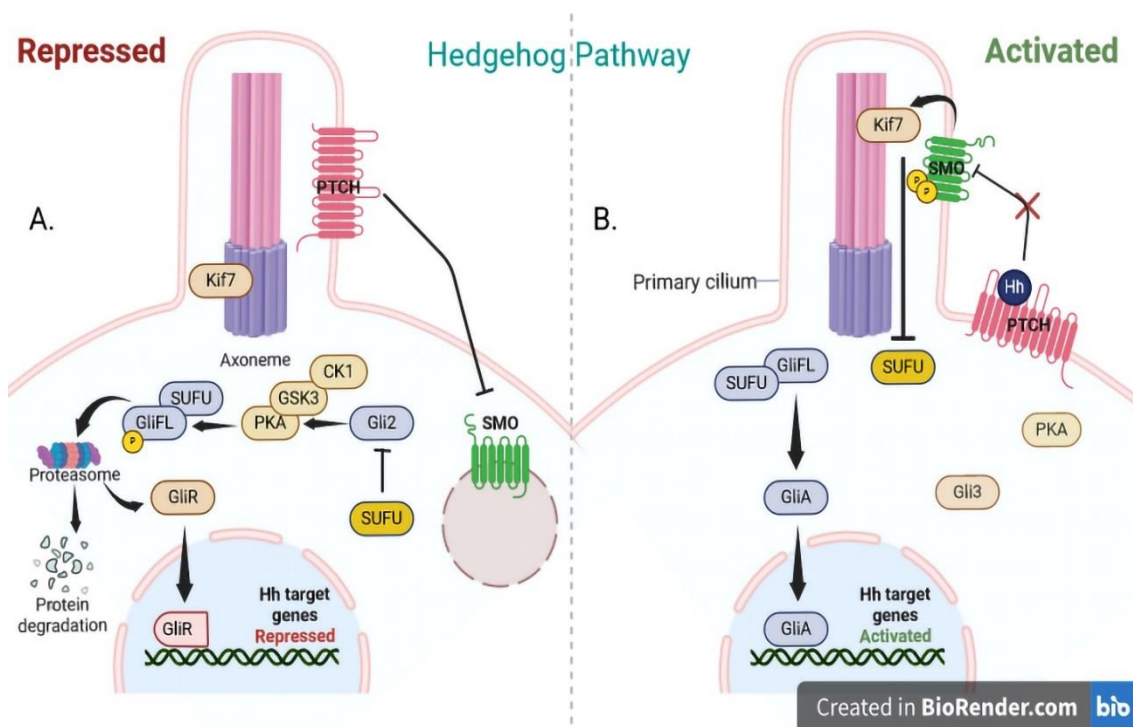
Figure 2 indicates the Hh pathway in CSCs. Hh pathway is responsible for the number of cancers and

CSCs "Stemness" this pathway is involved in many tumors such as Odontogenic Keratocyst (182, 183), Uterine Sarcoma (184, 185), Medulloblastoma (186, 187), Nevroid 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 orthotopic 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 Smoothed (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/ $\beta$ -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  $\beta$ -catenin activation in cancer cells especially in tumorigenesis of intestine (219). GSK-3 $\beta$  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 <sup>+</sup> and HPV <sup>-</sup> 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 <sup>+</sup> 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 flowcytometry.                                                                                                                                     | 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.<br>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 $\beta$ -transducin repeat-containing E3 ubiquitin protein ( $\beta$ -TrCP) to Ubiquitinated GLI1 is augmented, creating a RUNX3-independent regulatory loop, especially in the early stages of Tumor. | (224)            |

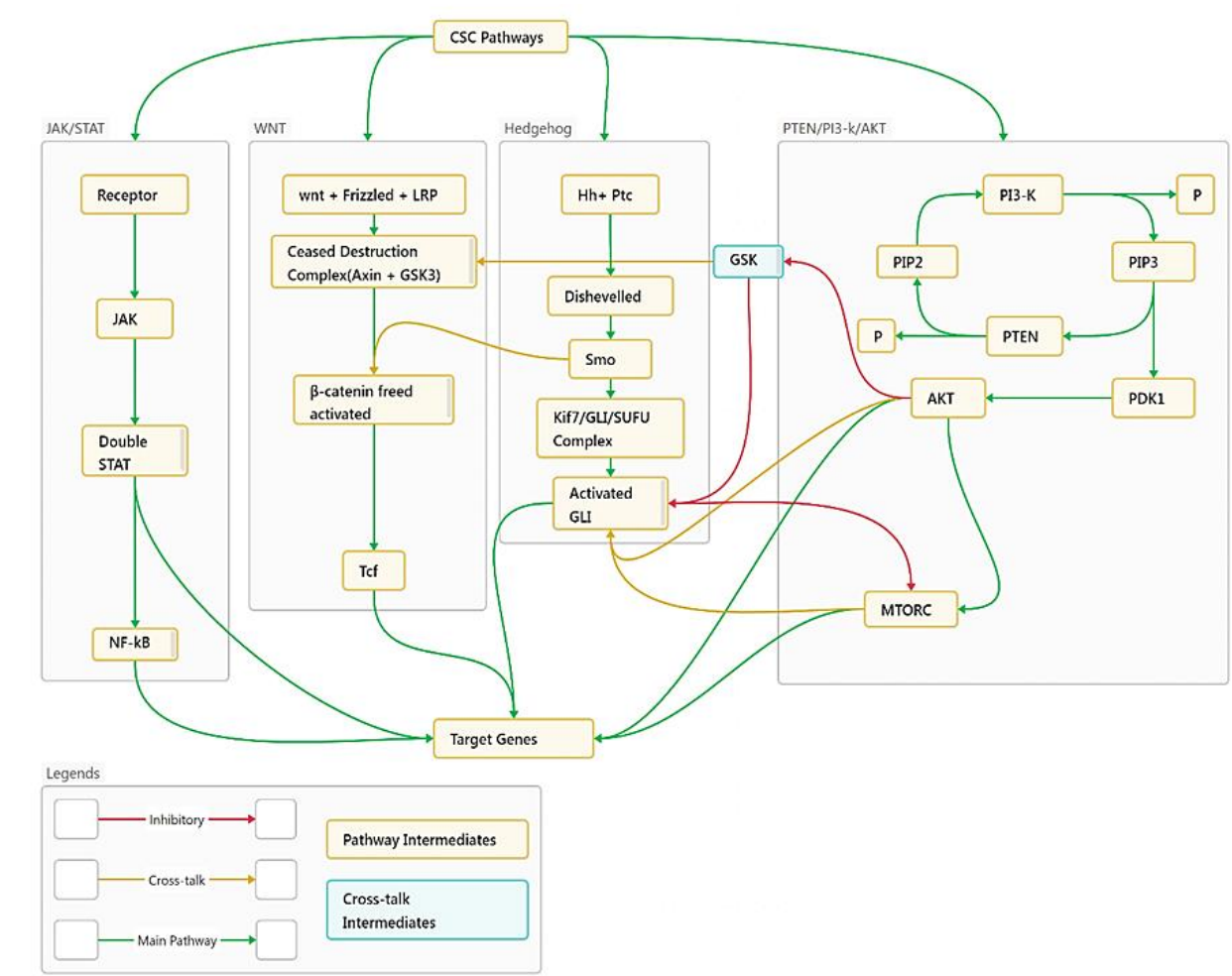


Figure 3. Cross-talk Between CSCs Self-Renewal Pathways.

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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