

## REVIEW

# Signaling pathways in the regulation of cancer stem cells and associated targeted therapy

Wang Manni<sup>1,\*</sup> | Wu Min<sup>2,\*</sup>

<sup>1</sup>Department of Biotherapy, Cancer Center, West China Hospital, Sichuan University, Chengdu, P. R. China

<sup>2</sup>Department of Biomedical Sciences, School of Medicine and Health Sciences, University of North Dakota, Grand Forks, North Dakota, USA

**\*Correspondence**

Wang Manni, Department of Biotherapy, Cancer Center, West China Hospital, Sichuan University, No. 17, Block 3, Southern Renmin Road, Chengdu, Sichuan 610041, P. R. China.

Email: [wangmanni@scu.edu.cn](mailto:wangmanni@scu.edu.cn)

Wu Min, Department of Biomedical Sciences, School of Medicine and Health Sciences, University of North Dakota, Grand Forks, North Dakota, USA.

Email: [min.wu@med.und.edu](mailto:min.wu@med.und.edu)

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**Abstract**

Cancer stem cells (CSCs) are defined as a subpopulation of malignant tumor cells with selective capacities for tumor initiation, self-renewal, metastasis, and unlimited growth into bulks, which are believed as a major cause of progressive tumor phenotypes, including recurrence, metastasis, and treatment failure. A number of signaling pathways are involved in the maintenance of stem cell properties and survival of CSCs, including well-established intrinsic pathways, such as the Notch, Wnt, and Hedgehog signaling, and extrinsic pathways, such as the vascular microenvironment and tumor-associated immune cells. There is also intricate crosstalk between these signal cascades and other oncogenic pathways. Thus, targeting pathway molecules that regulate CSCs provides a new option for the treatment of therapy-resistant or -refractory tumors. These treatments include small molecule inhibitors, monoclonal antibodies that target key signaling in CSCs, as well as CSC-directed immunotherapies that harness the immune systems to target CSCs. This review aims to provide an overview of the regulating networks and their immune interactions involved in CSC development. We also address the update on the development of CSC-directed therapeutics, with a special focus on those with application approval or under clinical evaluation.

**KEYWORDS**

cancer stem cells, CAR-T therapies, inhibitors, signal pathway

## 1 | INTRODUCTION

The concept of stem cells dates back to the 18th century when scientists tried to elucidate how lower organisms developed tissues and organs.<sup>1</sup> These stem cells produce daughter cells that later undergo different biological processes, either continuous self-renewal division, or differentiation into specialized cells with a limited lifespan. Normal tissue stem cells provide a life-long source of cells for self-renewal of tissues, which leads us to speculate that

whether stem cells are capable of deriving a malignant cell population, and this lies the foundation of cancer stem cells (CSCs) theory. CSCs are defined as a subpopulation of malignant tumor cells with selective capacities for tumor initiation, self-renewal, metastasis, and unlimited growth into bulks.<sup>2</sup>

Despite decades of research on cancer treatment, it has been proved extremely challenging to achieve complete remission (CR) in cancer patients. Tumor relapse may be explained by the fact that antitumor therapeutics mainly

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target proliferative cancer cells but remain ineffective in quiescent CSCs. The role of CSC in tumor initiation was first identified in acute myeloid leukemia (AML). Since its isolation from a number of solid tumors and hematological malignancies, the CSC is believed to form the clonogenic core of these tumors.<sup>3</sup> Growing evidence now suggests that CSCs are responsible for multiple progressive tumor phenotypes, including recurrence, metastasis, and treatment failure.<sup>4,5</sup> The intrinsic treatment resistance of tumors has partially attributed to the presence of the CSC subpopulation,<sup>6,7</sup> and may also be induced by extrinsic factors, such as treatments and environments.<sup>8,9</sup>

Major signaling pathways are involved in the maintenance of stem cell properties and survival of CSCs, such as the Notch, Wnt, and Hedgehog (HH) pathways.<sup>10</sup> There is also intricate interplay network between these signal cascades and other oncogenic pathways.<sup>11-13</sup> Thus, targeting pathway molecules that regulate CSCs provides a new option for the treatment of therapy-resistant or -refractory tumors. This review aims to provide an overview of the regulating networks and their immune interactions involved in CSC development. We also summarized the update on the development of CSC-directed therapeutics, with a special focus on those with application approval or under clinical evaluation.

## 2 | CHARACTERISTICS AND IDENTIFICATION MARKERS OF CSCs

### 2.1 | Characteristics of CSCs

Over the past decades, a wide breadth of literature investigated the biological characteristics of CSCs, with the hope to develop CSC-targeted strategies that eradicate treatment-insensitive or -refractory tumor cells. The potent self-renewal ability is probably the best-characterized property of CSCs and a direct cause of tumor initiation.<sup>14</sup> CSCs divide into daughter cells in a symmetrical splitting manner and ultimately lead to excessive tumor growth.<sup>15</sup> Experimental data revealed the tumorigenic function of CSCs by forming new tumors with CSCs isolated from primary tumor tissues in immunodeficient mice.<sup>16</sup>

Another characteristic of CSCs is their differentiation ability. For instance, leukemia stem cells (LSCs), characterized by CD34-positive expression and deficient CD38 expression, were able to differentiate into multiple cell types in SCID mice.<sup>17</sup> In addition, CSCs isolated from human brains share similar surface markers CD133 and Nestin, with normal neuronal stem cells, and are thus believed to have differentiation capabilities.<sup>18</sup> In normal tissues, the balance between self-renewal and differentiation of stem cells controls the cell fate, the aberrant

regulation of which lead to tumorigenesis.<sup>19</sup> Interestingly, the trans-differentiation of CSCs into other multilineage cells may also contribute to tumor formation.<sup>20</sup> One such example is the trans-differentiation of CSCs into vascular endothelial cells, leading to oncogenesis and tumor angiogenesis of glioblastoma,<sup>21</sup> renal,<sup>22</sup> and liver cancer.<sup>23</sup>

Tumor heterogeneity is one of the key reasons for therapeutic resistance.<sup>24</sup> The theory of tumor heterogeneity concept dates back to the 1970s when tumors are believed to consist of multiple distinct tumor cell subpopulations.<sup>25</sup> CSCs are believed to contribute to tumor heterogeneity. This cell subpopulation gives rise to cancer cells with diverse differentiation levels, which then go through sporadic mutations and environmental changes for clone selection.<sup>26</sup> The aberrant differentiation programs of CSCs resemble the hierarchical compositions of normal stem cells, which ultimately lead to a hierarchical set of tumor cells.

CSCs have also been identified with surviving and expanding capacity after cytotoxic anticancer treatment, which were recently found to enrich in remaining tumor bulks following chemotherapy treatments.<sup>27,28</sup> For instance, after chemotherapy treatment, preleukemic DNMT3A mutant hematopoietic stem cells (HSCs) are able to generate a hematopoietic hierarchy that facilitates their survival and expansion.<sup>29</sup> Likewise, the transformation of differentiated tumor cells to glioma stem cells (GSCs) was often observed in temozolomide (TMZ)-treated glioma.<sup>30</sup> The underlying mechanisms for the chemoresistance caused by CSCs include epithelial-mesenchymal transition (EMT), dormancy, and tumor environment, which we will discuss in the review.<sup>31-33</sup>

### 2.2 | Identification markers of CSCs

The unique gene expression profile of the CSC makes it different from bulk tumor cells, which can be used as CSC-specific identification markers. Commonly used methodologies that evaluate CSC stemness include the detection of stemness genes, surface proteins, such as CD44 and CD133, intracellular markers, such as aldehyde dehydrogenase (ALDH), and at a more macroscopic level, phenotypic assays, such as tumorsphere formation tests.<sup>34-37</sup> Table 1 shows the key markers of CSCs in solid tumors and hematological malignancies. A number of stemness genes have been reported to modulate cell stemness in embryos and adults, including the transcription factors POU class 5 homeobox 1 (POU5F1, OCT4), Nanog homeobox (NANOG), Sex-determining region Y-box 2 (SOX2), Kruppel-like factor 4 (KLF4), and MYC proto-oncogene.<sup>38,39</sup>

**TABLE 1** Key markers of cancer stem cells in solid tumors and hematological malignancies

<b>CSC surface marker</b>	<b>Cancer types</b>
CD4	Head and neck squamous cell carcinoma
CD9	Glioblastoma
CD10	Acute myeloid leukemia, head and neck squamous cell carcinoma
CD13	Liver, pancreatic cancer
CD15	Glioblastoma
CD19	Acute myeloid leukemia
CD20	Acute myeloid leukemia, melanoma
CD24	Breast, gastric, liver, colorectal, ovarian cancer
CD25	Chronic myeloid leukemia
CD26	Chronic myeloid leukemia
CD29 ( $\beta 1$ integrin)	Breast cancer
CD33	Chronic/acute myeloid leukemia
CD34	Acute myeloid leukemia
CD36	Chronic myeloid leukemia, glioblastoma
CD44 (and its variants)	Breast, lung, gastric, liver, colorectal, prostate, bladder, esophageal, ovarian, pancreatic, cervical cancer, glioblastoma
CD47	Liver cancer
CD49f	Breast, gastric, colorectal, cervical cancer, glioblastoma
CD54	Gastric cancer
CD61	Breast cancer
CD70	Breast cancer
CD71	Acute myeloid leukemia
CD87	Lung cancer
CD90	Breast, lung, gastric, liver, esophageal, pancreatic cancer, glioblastoma
CD98	Head and neck squamous cell carcinoma
CD117	Chronic myeloid leukemia, prostate, ovarian, lung cancer
CD123	Chronic/acute myeloid leukemia
CD133	Breast, lung, gastric, liver, colorectal, prostate, ovarian, pancreatic, cervical cancer, melanoma, glioblastoma, head and neck squamous cell carcinoma
CD166	Lung, colorectal, ovarian cancer
CD206	Colorectal, liver cancer
CD271	Melanoma, head and neck squamous cell carcinoma
Ov6	Pancreatic cancer
CXCR4	Breast, gastric, prostate, renal, lung cancer
EpCAM	Breast, lung, gastric, liver, colorectal, prostate, pancreatic cancer
LGR5	Breast, gastric, colorectal cancer
ProC-R	Breast cancer
IL1RAP	Chronic myeloid leukemia
LINGO2	Gastric cancer
CLL-1	Acute myeloid leukemia
TIM3	Acute myeloid leukemia
LICAM	Glioblastoma
EGFR	Glioblastoma
ABCG2	Lung, cervical cancer
CK17	Cervical cancer

As for the surface markers that are differentially expressed on CSCs,<sup>40,41</sup> some of the markers are not unique to CSCs and may also be present on normal stem cells.<sup>42,43</sup> The first reported CSC surface markers were CD34 and CD38 proteins which are used to identify HSCs and LSCs in acute myelogenous leukemia.<sup>44</sup> ABCG2 is a phenotypic marker for CSCs<sup>45</sup> in ovarian,<sup>46</sup> hepatic,<sup>47</sup> breast,<sup>48</sup> lung cancer,<sup>48</sup> and AML.<sup>49</sup> ABCG2 is believed to associate with therapeutic resistance caused by CSC and is highly expressed in side population (SP) cells (defined as the cell fraction that excludes Hoechst DNA binding dye).<sup>50</sup> The fluorescence-activated cell sorting (FACS)-based SP sorting method is commonly used for CSCs isolation.

CD133 (Prominin 1), coded by the gene PROM1, was initially considered a surface marker for colorectal CSCs,<sup>51,52</sup> and was later confirmed of its expression on CSCs of multiple origins. However, as recent evidence suggested that CD133<sup>-</sup> cell subsets were capable of inducing tumorigenesis, CD133 positivity may not necessarily indicate CSC stemness.<sup>53</sup> In addition, CD133<sup>+</sup> cells were also found to promote tumor metastasis.<sup>54</sup> Results from a single-cell proteomic profiling analysis revealed a higher level of CD133 compared with other stemness markers, such as NANOG and ALDH1A1, in lung cancer cells with EMT phenotypes.<sup>55</sup> Given that epithelial-mesenchymal plasticity of CSCs is a potential trigger for tumor metastasis,<sup>56</sup> the coexpression of CD133 and other stemness markers may be used to identify cells with stemness characteristics.

CD44, also known as P-glycoprotein 1, is a transmembrane glycoprotein and cell surface adhesion receptor for hyaluronic acid (HA) and osteopontin (OPN).<sup>57</sup> Accumulating evidence has suggested that stemness markers, including SOX2, NANOG, and OCT4, are highly expressed in CD44<sup>+</sup> cell fraction.<sup>58</sup> Though the downstream targets of CD44 remain incompletely defined, known cancer-associated signaling pathways include Rho GTPases, Ras-MAPK, and phosphatidylinositol-3-kinase (PI3K)/AKT cascades.<sup>59</sup> The alternative splicing of the CD44 gene leads to multiple variants, and among all the variants, CD44v is expressed in epithelial cells and critical for maintaining stemness.<sup>60</sup> Although HA is a ligand for all forms of CD44, OPN only interacts with CD44v rather than CD44s.<sup>61</sup> The crosstalk between HA and CD44 regulates a number of biological processes leading to tumor cell stemness, invasion, and metastasis. For instance, HA binds to CD44 and triggers the NANOG-STAT3 pathway activation, leading to the self-renewal of ovarian cancer cells.<sup>62</sup> On the other hand, OPN, enriched in gliomas, has also been found to promote tumor cell stemness via the OPN-CD44 axis.<sup>63</sup> This evidence suggests that the downstream activities of CD44 might depend on the selection of variants by the ligands. Thus, CD44 should not be simply addressed as a marker for CSCs, the functions of which

rely on its preference of variants and ligands present in the microenvironment.

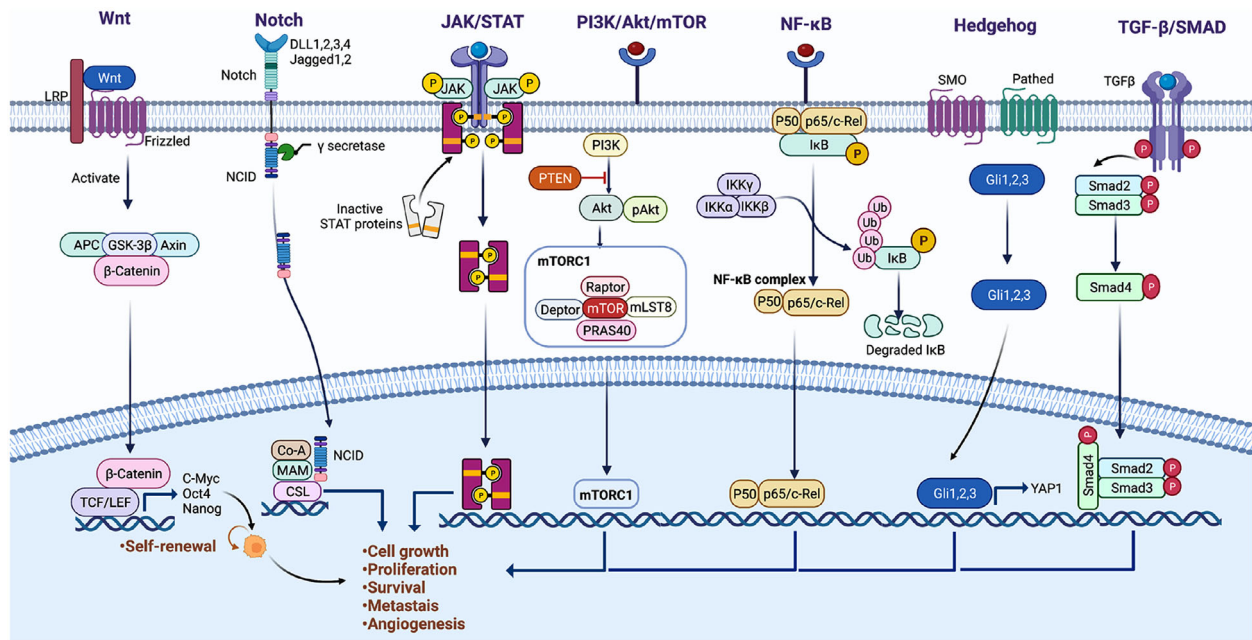
However, the surface markers of CSCs may vary according to tumor types and the cell of tumor origin, demonstrating high heterogeneity between tumors or even among cells within the same tumor.<sup>35,64</sup> Examples include breast CSCs, which frequently display different surface marker patterns, such as CD44<sup>+</sup>, CD24<sup>-</sup>, and ALDH<sup>+</sup>,<sup>65,66</sup> and melanoma stem cells that can either be CD271<sup>-</sup> or CD271<sup>+</sup>.<sup>67</sup> Such heterogeneity of CSC surface markers has also been reported in glioblastoma,<sup>68</sup> prostate cancer,<sup>69</sup> and lung cancer.<sup>70</sup> Moreover, the expression of CSC biomarkers is not constant and may change depending on the external environment. Higher CD133 expression was observed on stem cell-like pancreatic cancer cells under hypoxia<sup>71</sup> and the enzymatic dissociation alters the retention of surface CD133 on glioma cells.<sup>72</sup>

To overcome the challenge caused by constantly changing surface markers for CSCs, researchers then used nonmembrane biomarkers for CSC identification, and the ALDH represents one of its kind. The expression of ALDH is often found in normal stem cells. Highly active ALDH1 can be used to identify CSCs in breast, bladder, lung cancer, embryonal rhabdomyosarcoma, and head and neck squamous cell carcinoma.<sup>73</sup> ALDH1 expression is also related to the therapeutic resistance of cancer cells to chemotherapies.<sup>74</sup> However, subpopulations of breast cancer CSCs appeared to be ALDH-negative.<sup>75</sup>

EMT allows a polarized epithelial cell to escape from its interaction with the basement membrane and transform into mesenchymal cell phenotypes.<sup>76</sup> Thus, cancer cells that undergo EMT are more prone to invasion and metastasis.<sup>77</sup> A subpopulation of breast cancer CSCs showed both EMT and CSC markers CD44, ABCG2, and ALDH1A1/3, which might serve as identification markers for metastasis-initiating cells.<sup>78</sup> In addition, another CSC subpopulations, which are ITGA6-positive but deficient in ABCG2 and ALDH1A1/3, are referred to as non-CSC cells with metastatic abilities but no oncogenic activities.<sup>78</sup> Previous studies reported a fraction of ITGA6<sup>+</sup> cells exhibiting epithelial characteristics involved in metastasis.<sup>79</sup> In colon cancer, ITGA6<sup>+</sup> cells are included in the CD44<sup>+</sup>/CD133<sup>+</sup> cell population, representing the metastatic non-CSCs.<sup>80</sup>

### 3 | SIGNALING PATHWAYS REGULATING CSCs

The homeostasis of normal stem cells is modulated by an intricate signaling network, the aberrant activation or repression of which promotes oncogenic transformation. These aberrations lead to the self-renewal and differentiation properties of CSCs, conferring stemness



**FIGURE 1** Overview of major signaling pathways that regulate CSCs. Among an array of signaling pathways aberrantly activated in cancer Wnt, JAK/STAT, PI3k/Akt/mTOR, Notch, NF- $\kappa$ B, Hedgehog, and TGF- $\beta$ /Smad pathways are crucial for the self-renewal, cell growth, metastasis of CSCs, and angiogenesis. (Figures built with biorender.com)

to the cancers. Like their normal counterparts, CSCs also rely on these signaling pathways for survival and stemness maintenance. In this review, we classified these molecular pathways as extrinsic or intrinsic signals (Figure 1).

### 3.1 | Intrinsic signaling pathway in CSCs

#### 3.1.1 | Wnt signaling

The Wnt pathway is highly conserved and has long been identified as a key regulator of embryonic development and tissue homeostasis.<sup>81,82</sup> The abnormal activation of Wnt signaling elements, including adenomatous polyposis coli (APC), Axin,  $\beta$ -catenin, and Wnt1, is frequently found in a wide range of malignancies, which is related to cancer initiation and progression.<sup>83–87</sup> The canonical Wnt pathway is  $\beta$ -catenin dependent and the noncanonical Wnt pathway does not rely on  $\beta$ -catenin.

In the context of CSC regulation, the canonical Wnt signaling appears rather important in maintaining stem cell-like traits of tumor cells.<sup>88</sup> Canonical Wnt signaling is critical for maintaining lung CSC properties, potentially by regulating the expression of CSC marker OCT-4.<sup>89</sup> Other CSC markers stimulated by the Wnt/ $\beta$ -catenin pathway include CD24, Prom1, CD44, and ALDH1, thereby enhancing tumor stemness.<sup>90</sup> These CSC markers were also upregulated in Wnt<sup>+</sup> glioblastoma cells, indicating the

involvement of Wnt/ $\beta$ -catenin signaling in maintaining the stemness of glioblastomas.<sup>91</sup> The nuclear translocation of  $\beta$ -catenin also promoted the dedifferentiation of colorectal cancer (CRC) cells, which is a novel index for stemness.<sup>92,93</sup> Conversely, ablation of the  $\beta$ -catenin gene resulted in the loss of CSC populations and complete tumor regression of squamous cell carcinoma.<sup>94</sup>

In CRC, both microsatellite instable and microsatellite stable cells demonstrate activated Wnt cascades, especially at the intestinal crypts.<sup>95</sup> This Wnt signal activation is mainly attributed to the functional loss of a negative regulator, APC.<sup>95</sup> Another mechanism for Wnt signal activation is the function loss of RNF43 caused by gene mutations, which delays the removal of Wnt receptors in the intestinal crypt.<sup>96</sup> Though 5-fluorouracil (5-FU), the first-line treatment for CRC, is able to inhibit tumor growth, a recent study revealed that 5-FU might induce CSC activation via the WNT/ $\beta$ -catenin signaling pathway and thus cause chemoresistance in CRC patients.<sup>97</sup> Consistent with this finding, Wnt/ $\beta$ -catenin pathway activation induced by m6A modification-Sec62- $\beta$ -catenin promotes stemness and chemoresistance of CRC.<sup>98</sup>

The activated Wnt/ $\beta$ -catenin pathway is enriched in more than half of breast cancers and indicates a poor prognosis.<sup>99</sup> The expression of the activated  $\beta$ -catenin protein is also upregulated in breast CSCs. The inhibition of  $\beta$ -catenin signaling significantly prevented tumor formation and metastasis in HER2-overexpressing breast cancer cells.<sup>100</sup>

Other cancer types of c regulated by WNT signaling include renal cancer, where the proliferation and self-renewal of CSCs were significantly impaired by WNT inhibition,<sup>101</sup> lung adenocarcinoma, and were activated by Wnt/ $\beta$ -catenin and Notch signaling. Hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ )-regulated miR-1275 maintains stem cell-like phenotypes and promotes the progression of LUAD simultaneously.<sup>102</sup> These results collectively suggest the essential role of  $\beta$ -catenin in sustaining CSC phenotypes.

### 3.1.2 | Notch signaling

Notch signaling is a genetically conserved pathway involved in the embryonic development of the central nervous system, heart, and multiple other organs.<sup>103,104</sup> Notch signal is also important to the initiation and progression of cancer. In mammalian cells, four Notch transmembrane receptors (Notch 1–4) and five membrane-bound cell surface ligands (JAG 1 and 2, DLL 1, 3, and 4) have been reported.<sup>105</sup> Following the ligand-receptor interaction, the active fragment of Notch receptors, the Notch intracellular domain (NICD), is released via proteolysis. Upon activation, the NICD-CSL complex was formed following the nuclear translocation of NICD, which then recruits MAML and p300 coactivators, activating the downstream genes of Notch signals Hes-1, c-Myc, HER2, NF- $\kappa$ B, cyclin-D1, and p21.<sup>106</sup>

Hyperactivation of Notch signaling is significantly correlated with the maintenance of CSC characteristics in various cancer types, including breast,<sup>107,108</sup> colon,<sup>109</sup> pancreatic,<sup>110</sup> hepatic,<sup>111</sup> cervical,<sup>112</sup> and ovarian cancer.<sup>113</sup> This theory was first established in medulloblastomas, where the tumor-initiating ability of the CD133<sup>+</sup> CSC population largely relied on Notch signal activation.<sup>114</sup> Likewise, the inhibition of Notch2 activation dramatically reduced the number of CSCs, whereas the better-differentiated cells remained unaffected.<sup>115</sup>

In hepatocellular carcinoma (HCC), higher expression of TACE/ADAM17 and Notch1 was found to predict a worse prognosis.<sup>111</sup> Besides, inducible nitric oxide synthase enhances the TACE/ADAM17-mediated Notch1 signaling, leading to the enrichment of CD24<sup>+</sup>CD133<sup>+</sup> liver CSCs.<sup>111</sup> In breast and pancreatic cancer, there was an increase in the expression levels of JAG1, JAG2, Notch1, Notch3, and Hes1, a downstream gene of Notch signaling.<sup>116,117</sup> The hypoxic microenvironment in breast cancer upregulated the expression of HIF-2 $\alpha$ , which then stimulated Notch signaling molecules NICD and promoted stem cell phenotypes, thereby facilitating chemoresistance of breast cancer cells.<sup>107</sup> The hypoxic environment also upregulated Notch1 signaling in glioblastoma and increased its stem cell marker CD133 on the cell surface.<sup>118</sup> Moreover, the

heterogeneous metabolic signature of glioblastoma stem cells was also modulated by Notch signaling.<sup>119</sup> Similar findings were obtained from the analyses of CSC populations in pancreatic cancer<sup>120</sup> and myeloid leukemias,<sup>121</sup> which displayed Notch-mediated chemoresistance. Thus, Notch signaling plays a critical role in promoting therapy-resistant CSC populations across malignancies. Owing to the significant impact of Notch signaling in CSCs leading to tumor initiation and therapy-resistance, targeting Notch pathway molecules may be a promising strategy in the wide spectrum of cancers.

### 3.1.3 | JAK/STAT signaling

The JAK/STAT signaling pathway comprises various types of ligands, including interleukins, interferons, and hormones, and their corresponding receptors.<sup>122</sup> Upon the binding of ligands to receptors, JAK proteins (JAK1-3 and TYK2) are activated via phosphorylation, which then phosphorylates the cytoplasmic domain of receptors, recruiting the STAT family proteins.<sup>123</sup> The dimerization and translocation of STATs lead to transcription regulation of downstream target genes.

Similar to Notch and Wnt signaling, JAK/STAT axis is also evolutionarily conserved and facilitates hematopoiesis, neurogenesis, and self-renewal of normal embryonic stem cells. CSCs from hematological malignancies, such as AML, demonstrated aberrant activation of JAK/STAT signaling.<sup>124</sup> The tumor-formation ability of AML CSCs was potently inhibited in immunodeficient mice following JAK1/2 inhibitor treatment,<sup>125</sup> reinforcing the promoting effect of JAK/STAT signaling on CSC stemness across a wide panel of cancers.<sup>126</sup>

The role of the JAK/STAT pathway in CSC regulation is best characterized by its tumor-initiating effect in glioblastoma.<sup>127</sup> In a glioblastoma model, the transforming growth factor (TGF)- $\beta$ -activated JAK/STAT pathway induced the self-renewal capacity and prevented the differentiation of glioma-initiating cells derived from patient tumors, thereby facilitating tumor formation.<sup>127</sup> Besides, inhibition of STAT3 in CSCs reduced the tumorsphere formation and increased the expression of the neuronal differentiation genes.<sup>128</sup> Similar results were observed in breast CSCs, where STAT3 inhibitors decreased tumor growth and the abundance of CSCs.<sup>129</sup> Recently, JAK-STAT signaling was reported to modulate stemness and chemoresistance of myxoid liposarcoma.<sup>130</sup> Likewise, JAK2/STAT3/CCND2 signals also control the radioresistance of CRC by regulating its stem cell persistence.<sup>131</sup>

The role of STAT3 in determining cell fate is well established.<sup>132</sup> JAK proteins activate STAT3 via phosphorylation at Tyr705 residues. The downstream target genes

of STAT3 nuclear translocation include cyclin D1, c-Myc, and Bcl-2. STAT3 holds profound importance in governing embryonic and adult stem cells in mice and humans.<sup>133</sup> Apart from its modulation of self-renewal of both embryonic and CSCs as an element of JAK signaling, STAT3 interacts with Notch ligands DLL1 (Delta-like 1) to facilitate neocortical development in infancy.<sup>134</sup> STAT3 also interacts with NF- $\kappa$ B and HIF-1 $\alpha$  to enrich CD133<sup>+</sup> cell populations.<sup>135</sup> The activated form of STAT3 (p-STAT3-Tyr705) was enriched in ALDH<sup>+</sup> and CD44<sup>+</sup>/CD24<sup>+</sup> CSCs, the inhibition of which subsequently reduced stem cell phenotypes of this CSC population.<sup>136</sup>

### 3.1.4 | HH signaling

The HH signaling pathway was identified by the Nobel prize winner team in 1980.<sup>137</sup> The HH pathway is critical to the development of multiple organs during embryogenesis.<sup>138</sup> Interestingly, HH signaling remains inactive in all postnatal tissues except central nervous system (CNS), skin, hair, and teeth.<sup>139</sup> The HH pathway is composed of three secreted ligand isoforms—Sonic hedgehog (Shh), Desert hedgehog, and Indian hedgehog, with their corresponding receptors being—Patched, Smoothed (SMO), and three Gli transcription factors (Gli1–3).<sup>140</sup>

The aberrant activation of HH signaling has been reported to support the proliferation and stemness maintenance of CSC in various cancer types, such as multiple myeloma, glioma, HCC, and chronic myeloid leukemia (CML).<sup>141–143</sup> HH pathway activation is heterogeneous in multiple myeloma CSCs, with overexpression of the SMO gene and high Gli1 transcriptional activity.<sup>144</sup> The SMO gene encodes Smoothed protein, the chemical inhibition of which reduced stemness and proliferation of multiple myeloma CSCs.<sup>144</sup> In CML, the activation of HH signaling is with early disease progression.<sup>145</sup> The restoration of SMO expression in SMO-deficient CML animal models promoted tumor growth and led to a four-fold increase in CSC proportions.<sup>146</sup> Likewise, the overexpression of the HH signaling genes Gli1, SHH, and PATCHED1 was also present in glioma CSCs.<sup>147</sup> HH signaling supports glioma tumor growth in animal models by inducing SMO-expressing gliomasphere formation.<sup>147</sup> A recent study showed that GLI1 inhibition reduced mammosphere formation of breast cancer cells. Interestingly, GLI1 inhibition resulted in a decrease in expression of YAP1, a Hippo pathway effector, suggesting a regulation activity of HH signaling on the Hippo pathway.<sup>148</sup>

The development of treatment resistance to cancer requires the functional support of CSC-related HH sig-

aling. GLI-1 regulates oncogenesis and therapeutic resistance of colon rectal cancer,<sup>149</sup> with significantly higher GLI-1 expression observed in 5-FU resistant CRC cells than in nonresistant cells.<sup>150–152</sup> The expression of stem cell markers of CRC cells was significantly decreased by GLI-1 inhibition, and the cell response to 5-FU, Irinotecan, and Oxaliplatin was also resumed.<sup>153</sup> In gastric adenocarcinoma, the forkhead box C1 (FOXC1) gene mediates the CSC phenotypes and tumor response to chemotherapy by regulating HH signaling.<sup>154</sup>

### 3.1.5 | TGF $\beta$ /SMAD signaling

TGF- $\beta$  is a bifunctional regulator in cancer that represents a differentiation signal that potentially inhibits tumor initiation at an early stage.<sup>155–157</sup> In contrast to tumor initiation, TGF- $\beta$  promotes the CSC-like phenotypes of cancer cells by inducing EMT.<sup>158</sup> TGF- $\beta$  binds to TGF- $\beta$  type I receptor kinase (ALK5) and triggers the Smad-dependent canonical TGF- $\beta$  pathway.<sup>159</sup> TGF- $\beta$ -ALK5 activates Smad2/3 via phosphorylation which then forms a complex with Smad4 and regulates gene transcription following nucleus translocation.<sup>160</sup> The crosstalk between TGF- $\beta$  and the bioactive lipid mediator sphingosine-1-phosphate, a regulator of CSC expansion, is essential for cancer migration and the proliferation of breast CSCs.<sup>161–163</sup>

The TGF- $\beta$ -SMAD signaling is implicated in the regulation of CSC-like properties of CD44<sup>+</sup> gastric cancer cells,<sup>164</sup> HCC cells,<sup>165</sup> and cervical cancer cells.<sup>166</sup> In addition, CD44<sup>+</sup> breast cancer cells, which are referred to as breast cancer CSCs, are frequently accompanied by activated TGF- $\beta$  signaling.<sup>167</sup> TGF- $\beta$  induced the expression of EMT-associated genes Snail and Twist in breast cancer CSCs.<sup>168</sup> A recent study identified the role of TGF- $\beta$ -SMAD signaling in maintaining CSCs in the bone microenvironment.<sup>169</sup>

TGF- $\beta$  is able to switch non-CSCs into CSC states via activating ZEB1 and Snail.<sup>170–172</sup> The response of CSCs to chemotherapy is associated with TGF- $\beta$ /Smad pathway activation, the suppression of which sensitizes CSCs to chemotherapy.<sup>173</sup> According to a previous study, TGF- $\beta$ -induced chemoresistance is a downstream reaction of the Hh signaling, suggesting the interaction of TGF- $\beta$  with Hh signaling.<sup>174</sup> In addition, TGF- $\beta$  also demonstrated crosstalk with Notch signaling, with the synergistic promoting effect of TGF- $\beta$  and Notch1 on CSC proliferation.<sup>175</sup>

The hypoxic microenvironment is a positive regulator of TGF- $\beta$  activities in CSC stemness and chemoresistance.<sup>176</sup> HIF-1 $\alpha$  induces the expression and activation of TGF- $\beta$  and COX-2, thereby promoting CSC enrichment.<sup>177</sup> The

positive feedback loop between Snail and TGF- $\beta$  is also regulated by hypoxia, which together promotes EMT and recruits CSCs.<sup>178</sup>

### 3.1.6 | NF- $\kappa$ B signaling

There are five members in the NF- $\kappa$ B protein family: p65 (RelA), RelB, c-Rel, NF- $\kappa$ B1 (p105/p50), and NF- $\kappa$ B2 (p100/p52).<sup>179,180</sup> NF- $\kappa$ B family proteins are present in the cytoplasm of both differentiated cells and stem cells.<sup>181</sup> At the inactive state, NF- $\kappa$ B are bound to inhibitory I $\kappa$ B proteins, which prevents its nuclear localization.<sup>182</sup> Upon activation by various stimuli, such as lipopolysaccharide, the I $\kappa$ B kinase (IKK) complex (IKK $\alpha$ , IKK $\beta$ , and IKK $\gamma$ ) phosphorylates I $\kappa$ B proteins, resulting in their degradation.<sup>183</sup> NF- $\kappa$ B then translocates into the nucleus and activates the transcription of target genes involved in multiple biological processes.

Abnormal activation of NF- $\kappa$ B signaling is implicated in the progression of various cancers. The role of the NF- $\kappa$ B pathway in regulating CSCs was first identified in AML, where the primitive AML cells aberrantly expressing NF- $\kappa$ B were referred to as potential leukemic stem cells.<sup>184</sup> NF- $\kappa$ B pathway participates in the viability and self-renewal of AML stem-like cells.<sup>185</sup> Since then, growing evidence has shown elevated or constitutive NF- $\kappa$ B activity in other cancer types. For instance, increased expression of total p65 and downregulation of I $\kappa$ B $\alpha$  expression were found in prostate CSCs.<sup>186</sup> In addition, the CD44<sup>+</sup> fraction of ovarian cancer cells displayed higher expression of major stemness genes and NF- $\kappa$ B signal genes, including RelA, RelB, and IKK $\alpha$ .<sup>187</sup> The loss of the APC gene represents a canonical alteration during tumorigenesis, which promotes the activation of NF- $\kappa$ B signaling, allowing the expansion of Lgr5<sup>+</sup> CSCs.<sup>188</sup>

NF- $\kappa$ B activation mediates the tumorigenesis of glioma.<sup>189</sup> Both adherent and spheroid glioma CSCs exhibited constitutive activation of the STAT3/NF- $\kappa$ B signaling.<sup>190</sup> Gliomasphere-forming cells showed increased phosphorylation of p65 and sustained oncogenic activation of NF- $\kappa$ B signaling.<sup>191</sup> In Her2-driven breast cancer models, the inactivation of NF- $\kappa$ B pathways by I $\kappa$ B $\alpha$ -SR decreased the tumorigenesis of luminal epithelial tumors.<sup>192</sup> A genome-wide expression analysis suggested that I $\kappa$ B $\alpha$ -SR impaired stem cell expansion in breast cancer and CSC markers in transgenic tumors.<sup>193</sup> IKK $\alpha$  activity is required for Her2-induced oncogenesis, providing self-renewal signals that maintain mammary tumor-initiating cells.<sup>194</sup> The underlying mechanism may be the phosphorylation of p27 by IKK $\alpha$  leading to its nuclear export in Her2 breast cancer cells.<sup>195</sup> The expression of Dll1,

a Notch ligand, promotes the tumor-initiating abilities of breast cancer cells. It has been recently reported that NF- $\kappa$ B activation is a downstream target of Dll1, which collectively contributes to a chemoresistant phenotype of breast cancer CSCs.<sup>196</sup>

### 3.1.7 | PI3K/AKT/mammalian target of rapamycin signaling

PI3K is an intracellular phosphatidylinositol kinase composed of a regulatory subunit p85 and a catalytic subunit p110.<sup>197,198</sup> AKT is a downstream effector of PI3K and has three isoforms: AKT1, AKT2, and AKT3.<sup>199</sup> As a key member of the PI3K-associated kinase protein family, mammalian target of rapamycin (mTOR) functions as a nutritional signal sensor and a regulating factor for cell proliferation.<sup>200</sup> There are two protein complexes formed by mTOR. The mTORC1 is composed of mTOR, raptor, mLST8, and two negative regulators, PRAS40 and DEPTOR,<sup>199,201</sup> which controls cell growth in response to metabolism and nutrition signals.<sup>202,203</sup> The mTORC2 (mTOR complex 2) consists of mTOR, Rictor, mSin1, and mLST8. It is well established that mTORC2 activates Akt via phosphorylation at serine residue 473 and modulates stem-like properties,<sup>204</sup> whereas mTORC1 and its downstream cascades directly correlate with stem-like properties.<sup>205</sup>

The activation of the PI3K/Akt/mTOR pathway is important to cancer cell growth and its therapeutic resistance.<sup>206</sup> The PI3K/Akt/mTOR pathway can be activated through multiple mechanisms, such as the insulin-like growth factor (IGF)/IGFR, ErbB, and fibroblast growth factor (FGF)/FGFR signaling.<sup>206</sup> PTEN is known for its negative regulation on PI3K/AKT cascades. Data from several cancer models showed that PTEN depletion resulted in CSC expansion and increased tumor growth in mice.<sup>207,208</sup> Importantly, PI3K/AKT/mTOR is critical to maintaining the CSC population in various cancers, including nasopharyngeal carcinoma,<sup>209</sup> glioma,<sup>210</sup> pancreatic cancer,<sup>211</sup> lung cancer,<sup>212</sup> prostate,<sup>213</sup> and breast cancer.<sup>214</sup>

In breast cancer, PI3K/Akt/mTOR pathway is required for the colony-formation capacity of tumor cells and the maintenance of stem-like properties.<sup>214</sup> One underlying mechanism may be the HIF-2 $\alpha$ -induced CD44 alteration that promotes CSC activation in triple-negative breast cancer (TNBC) via PI3K/AKT/mTOR pathway.<sup>215</sup> The transcriptional suppression of negative regulators of mTOR is intrinsic in luminal-like breast cancer cells, leading to the development of CSC-like properties.<sup>216</sup> Likewise, the inhibition of mTOR in CRC cells suppressed cell



stemness represented by decreased ALDH1 activity.<sup>37,217</sup> PI3K/AKT/mTOR signaling pathway also enhances the angiogenesis of CRC and recruitment of tumor-associated macrophages (TAMs).<sup>218</sup>

The chemoresistance of hepatoma is also related to the Akt/mTOR signaling by promoting the expansion of hepatic tumor-initiating cells.<sup>219</sup> The inhibition of the PI3K/Akt/mTOR pathway overcame the chemoresistance of ovarian cancer by decreasing CSC marker expression.<sup>220</sup> The radioresistance of prostate cancer is significantly associated with PI3K/Akt/mTOR signaling activation via maintaining CSC phenotypes.<sup>221</sup> Moreover, prostate cancer CSCs present a feedback inhibition on AKT signaling through HIF1 $\alpha$ , which impairs CSC metabolism and growth.<sup>222</sup>

It was previously suggested that CD133 expression was upregulated by mTOR signaling in gastrointestinal cancer.<sup>223</sup> Similar results were obtained from hepatic cancer cells, where mTOR promotes the conversion of CD133<sup>-</sup> to CD133<sup>+</sup> cells.<sup>224</sup> Moreover, aberrant activation of the PI3K/Akt/mTOR pathway facilitates the stemness maintenance of NSCLC (non small cell lung cancer) cells by upregulating chemokine (C-X-C motif) receptor 4 (CXCR4) and the subsequent CXCR4-stimulated STAT3 signaling.<sup>225</sup>

On the other hand, in gliomas, Akt but not mTOR regulates ATP binding cassette transporters (ABCG2) activity, which is referred to as stemness hallmark.<sup>226</sup> Besides, PI3K inhibition restored the sensitivity to nilotinib of CML stem cells, whereas mTOR inhibition demonstrated no effect on CML.<sup>227</sup>

### 3.1.8 | Peroxisome-proliferator-activated receptor signaling

The peroxisome-proliferator-activated receptor (PPAR) pathway is activated following the binding of the G protein-coupled receptor with its ligand, stimulating a cascade of signal transducers, such as adenylyl cyclase, cyclic adenosine monophosphate, and protein kinase A, which then induces the translocation of PPAR, a nuclear receptor protein that regulates the target gene expression.<sup>228–230</sup> PPAR $\alpha$ , PPAR $\delta$ , and PPAR $\gamma$  are the three subtypes of PPAR with respective functions. In the context of a tumor, PPARs are involved in the modulation of cell proliferation, apoptosis, and survival of multiple cancers, including prostate cancer, breast cancer, glioblastoma, neuroblastoma, pancreatic cancer, hepatic cancer, leukemia, bladder cancer, and thyroid tumors, with either promoting or inhibitory effects on cancer development.<sup>231</sup> PPARs have also been reported to regulate the EMT process and stem cell-like properties of CSCs.<sup>232</sup>

CPT1A (Carnitine palmitoyl transferase I) and CPT2 (Carnitine palmitoyl transferase II) are two known target genes of PPAR $\alpha$ , which increase fatty acid oxidation (FAO) required for the cell metabolism in radioresistant breast cancer cells and radiation-derived breast CSCs.<sup>233</sup> SCD1 (stearoyl-CoA desaturase 1) is another functional downstream molecule of PPAR $\alpha$ , and the activation of the PPAR $\alpha$ -SCD1 axis is important to the maintenance of CSCs of HCC.<sup>234</sup> PPAR $\alpha$ , on the other hand, is considered a downstream molecule of lipid droplet-derived signaling, which was highly abundant in pancreatic and colorectal CSCs than non-CSCs. The inhibition of PPAR $\alpha$  decreases stemness characteristics of pancreatic and colorectal CSCs.<sup>235</sup> Likewise, PPAR $\delta$  is involved in the maintenance of HSCs by regulating the FAO pathway. The inhibition of PPAR- $\delta$  or mitochondrial FAO reduced the stemness of HSCs, whereas PPAR- $\delta$  agonists enhanced HSC maintenance.<sup>236</sup>

On the contrary, PPAR $\gamma$  is considered a tumor suppressor that reduces the CD49<sup>high</sup>/CD24<sup>+</sup> mesenchymal stem cells (MSCs) and inhibits tumor angiogenesis of breast cancer.<sup>237</sup> The existence of quiescent LSCs may contribute to treatment failure in CML patients. PPAR $\gamma$  agonist glitazones decrease the expression of STAT5 and its downstream targets HIF2 $\alpha$  and CITED2, two key genes maintaining the quiescence and stemness of CML LSCs.<sup>238</sup> PPAR $\gamma$  activation decreases the stem cell-like characteristics of bladder CSCs and accelerates the differentiation of adipocytes.<sup>239</sup> PPAR $\gamma$  also induces the differentiation in osteosarcoma stem cells and melanoma cells by suppressing the transcriptional activity of YAP.<sup>240,241</sup> Notably, PTEN is a target gene of PPAR $\gamma$  activation, which in turn blocks the PI3K/Akt/mTOR pathway and prevents the self-renewal, tumorigenicity, and metastasis of cervical, hepatic, and glioblastoma CSCs.<sup>242,243</sup>

## 3.2 | Extrinsic signaling pathways that regulate CSCs

The “seed and soil” theory was first brought up in the 19th century, which describes the metastasis of tumor cells to sites with a favorable microenvironment.<sup>244</sup> In this theory, the “seed” refers to the metastatic tumor cells, and “fertile soil” refers to the sites with a microenvironment that favors tumor colonization and growth. In accordance with the “seed and soil” theory, it is widely accepted that CSCs dwell in such “soil,” a specific tumor microenvironment (TME) composed of stroma, immune cells, microvessels, and external regulating signals.<sup>245</sup> This system provides CSCs with a conducive environment via the action of paracrine factors or direct contact with immune cells.<sup>8,246</sup>

### 3.2.1 | Vascular microenvironments that regulate CSCs

The theory of tumor vascular microenvironment dates back to the 1940s when glioblastoma cells were found to grow into the blood vessels-enriched sites.<sup>247</sup> The multilineage differentiation capacity of CSCs may allow them to take part in tumor angiogenesis or forming the vascular mimicry (VM) in the TME. Recent research has shed light on the relationship between CSCs and the vascular microenvironment. A typical example is glioblastoma CSC, where the expression of surface marker Nestin is positively related to microvessel density.<sup>248</sup> The vascular endothelium of glioblastoma has similar genomic alterations to CSCs.<sup>21</sup> Neural stem cells cocultured with epithelial cells demonstrated increased self-renewal and impaired differentiation ability via paracrine signaling, including the Notch pathway and the chemokine axis CXCL12/CXCR4.<sup>249–251</sup>

Recently, the CSC surface marker CD44 was reported to promote the VM generation in oral squamous cell carcinoma.<sup>252</sup> The CD44/c-Met signaling has also been identified as the key regulator for VM in Ewing sarcoma and breast cancers.<sup>253</sup> The presence of VM is also associated with ALDH1 expression in breast cancer.<sup>254,255</sup> ALDH<sup>+</sup> TNBC cells isolated from FACS initiated VM on matrigel.<sup>256</sup> The increased expression level of VM-related genes, such as MMP-2 and MMP-9, was observed in CD133<sup>+</sup> breast cancer cells.<sup>257</sup>

The regulation of CSC phenotype by endothelial cells (ECs) can be based on the secretion of soluble factors by ECs.<sup>248</sup> In acute leukemia, bone marrow stromal cells derived from CD133<sup>+</sup>/CD34<sup>+</sup> stem cells secrete IGF-1, leading to the formation of capillary-like structures.<sup>258</sup> Shh is a soluble factor secreted by ECs, which enhances CSC properties and stimulates the Hh signaling.<sup>259–261</sup> Hh signaling facilitates the acquisition of CSC self-renewal in thyroid cancer, via regulating Snail expression.<sup>260</sup> Interestingly, CD133<sup>+</sup> GSCs were identified in areas surrounding Shh-expressing ECs. The depletion of Shh in ECs prevents of promoting effect of ECs on CSC-like phenotype maintenance.<sup>259</sup>

In addition to HH signaling, Notch signal cascades also take an active part in the EC-mediated regulation of CSCs.<sup>262–265</sup> In CRCs, the promotion of CSC phenotypes by ECs is dependent on Notch signaling and independent of Shh or Wnt signaling. The knockout of Jagged-1, a Notch signaling element, in EC impairs its angiocrine effect.<sup>263</sup> The nitric oxide derived from ECs is able to trigger Notch signaling, leading to increased stemness of GSCs and glioma initiation in mice.<sup>264</sup> Furthermore, in SHH-driven medulloblastomas, the EC-induced promotion of CSC

characteristics additionally requires PI3K/AKT/mTOR signals.<sup>266</sup>

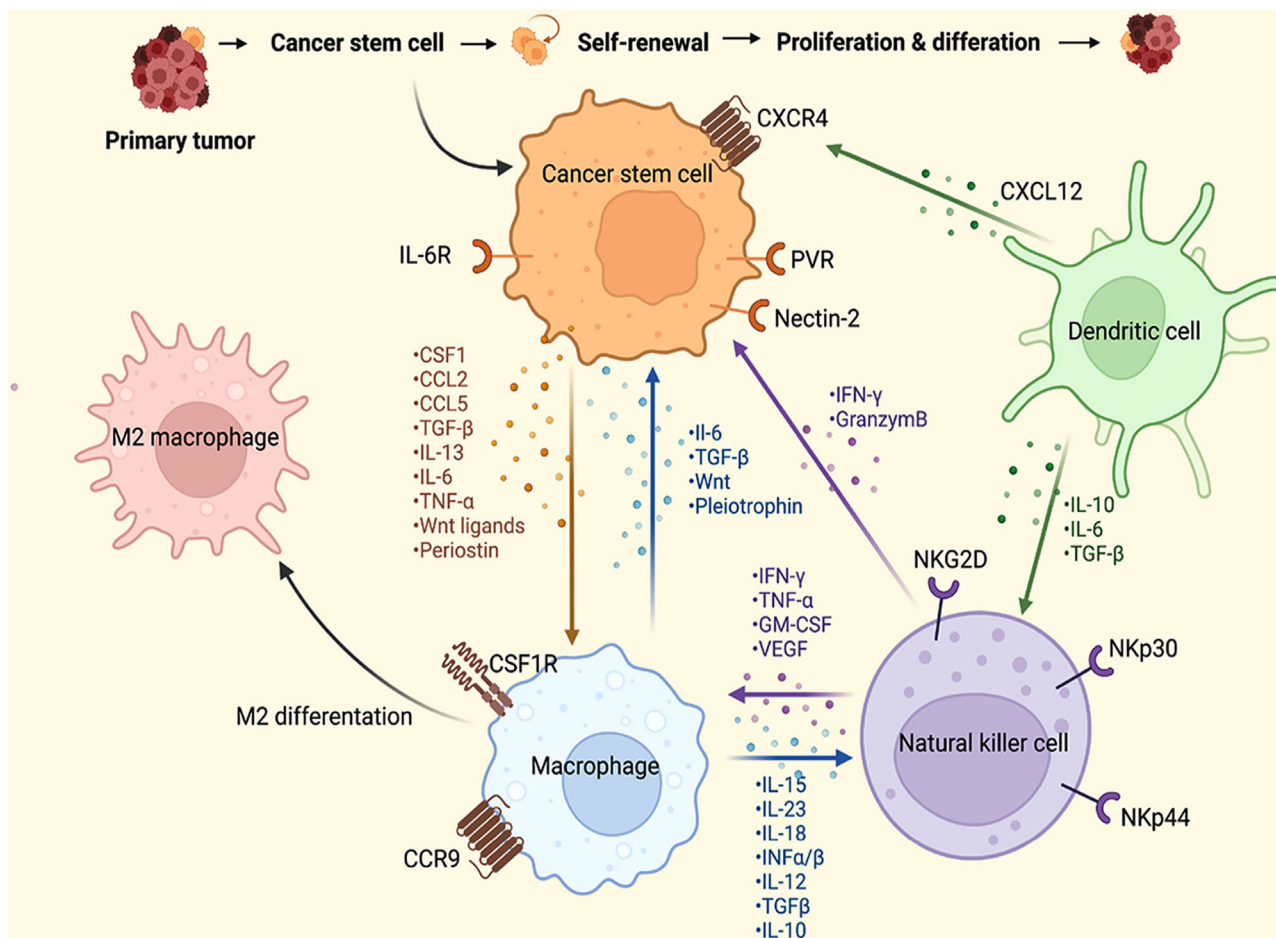
### 3.2.2 | Hypoxic microenvironments that regulate CSCs

Hypoxia is a common hallmark of TME in solid tumors.<sup>267,268</sup> In solid tumors, the rapidly proliferating tumor cells require a high level of oxygen to meet the expanding demands, resulting in relative hypoxia.<sup>269,270</sup> In this sense, an extreme hypoxic environment appears as the natural selection for tumor cells, where aggressive CSCs are more likely to survive and proliferate.<sup>271,272</sup> It is thus not surprising that CSCs are more resistant to conventional cancer therapies.<sup>273</sup>

Hypoxia leads to the acquisition of CSC phenotypes in breast tumors, which is primarily mediated by HIFs.<sup>274</sup> HIFs (HIF-1, HIF-2, and HIF-3) are key sensors of intracellular oxygen alterations and modulate the transcription of multiple genes at low oxygen levels.<sup>275–277</sup> The Notch signaling is a key regulating pathway for hypoxia response, which can be activated by HIF-1 $\alpha$  and HIF-2 $\alpha$  for the maintenance of CSC stemness.<sup>278,279</sup> For instance, the HIF-2 $\alpha$ -mediated Notch pathway activation promotes the phenotypic transformation of breast cancer cells into breast CSCs and cell resistance to paclitaxel treatment.<sup>107</sup> Likewise, hypoxia-induced AKT activation contributes to gemcitabine-induced stemness of pancreatic cancer cells by enhancing downstream Notch1 activity.<sup>280</sup> Another downstream element of PI3K/AKT signaling, mTOR, regulates HIF-1 $\alpha$  activity via phosphorylation of p70 S6Kinase (S6K).<sup>281</sup> However, the inactive state of mTOR also facilitates the maintenance of CSC characteristics, which explains the suboptimal efficacy of mTOR inhibitors in clinical evaluation.<sup>222</sup> The agonist of PTEN, a negative regulator of the PI3K/AKT pathway, provides a new clue to inhibit HIF-1 $\alpha$  activities and thus reduce CSC stemness.<sup>282</sup>

Fibroblasts are major components of tumor stroma, which are able to produce various extracellular matrix proteins and growth factors, such as TGF- $\beta$ .<sup>283</sup> Hypoxia induces the upregulation in TGF- $\beta$ 3 expression by promoting the binding of HIF-1 to the TGF- $\beta$ 3 gene promoter.<sup>284</sup> It has also been reported that hypoxia increased TGF- $\beta$ 1 expression in MSCs.<sup>285</sup> The hypoxia-induced secretion of TGF- $\beta$ 1 by MSCs in turn enhances tumor progression,<sup>286</sup> potentially by promoting the stabilization of HIFs.<sup>8</sup> Thus, the concomitant inhibition of HIF-1 $\alpha$  and TGF- $\beta$  delays tumor initiation and blocks the activity of CSCs.<sup>287</sup>

Fibroblasts-directed CSC reprogramming includes the stimulation of COX-2 and nuclear factor of  $\kappa$ B (NF- $\kappa$ B).<sup>8</sup> On one hand, hypoxia-mediated downregulation of dual



**FIGURE 2** The crosstalk between cancer stem cells (CSCs) and immune cells in the CSC niche via soluble mediators or juxtacrine signals, which regulate CSC stemness. (Figures built with biorender.com)

specificity phosphatase 2 (DUSP2) upregulates COX-2, leading to increased cancer stemness.<sup>288</sup> On the other hand, HIF-1 $\alpha$  induces COX-2 expression, which in turn upregulates HIF-2 $\alpha$  expression and enhances treatment resistance of cancer cells.<sup>289</sup>

### 3.2.3 | Immune cells that regulate CSCs

As immune evasion and CSCs both substantially contribute to tumor progression, it is widely accepted that there is potential crosstalk between CSCs and immune cells in the TME. A significantly high stemness signature was identified in 21 solid malignancies with a poor immunogenic response.<sup>290</sup> It is thus of paramount importance to elucidate the CSC-immune cell interactions in cancer, which will facilitate the identification of immunotherapies to eliminate tumor-promoting CSCs. Figure 2 presents the crosstalk between CSCs and immune cells in the CSC niche, which regulates CSC stemness.

#### *Tumor-associated macrophage*

The critical role of CSCs in monocyte recruitment to tumor sites has been well established as various protumorigenic macrophage factors were increased in supernatant collected from CSC sphere culture, including IL-13, TGF- $\beta$ , and WNT-induced signaling protein 1.<sup>291–293</sup> Incubation of macrophages with such sphere culture leads to macrophage polarization toward an immunosuppressive phenotype.<sup>294–296</sup>

On the other hand, TAMs in turn influence CSC phenotypes by secreting soluble mediators, such as IL-6, TGF- $\beta$ , and WNT ligands, or through juxtacrine signaling.<sup>297,298</sup> The direct interactions of CSCs with TAMs activates NF- $\kappa$ B in CSCs, which stimulates the secretion of cytokines to sustain the stem cell state of breast CSCs.<sup>299</sup> In the pleiotrophin (PTN)-PTPRZ1 paracrine signaling, which supports glioma progression, PTN released by TAMs binds to its receptor PTPRZ1 on GSCs, suggesting the significance of TAMs as important components of the CSC niche.<sup>300</sup>

IL-6 produced by TAMs promotes the expansion of hepatic CSCs, and the inhibition of IL-6 with tocilizumab prevents TAM-stimulated generation of CD44<sup>+</sup> cells.<sup>301</sup> In breast cancer, TAM-produced IL-6 induces and maintains the CSC characteristics through STAT3.<sup>302</sup> In addition, STAT3 is a transcription factor that could also modulate CSC maintenance in an IL-6-independent manner. For instance, the self-renewal and tumorigenicity of bladder CSCs are regulated by the KMT1A-GATA3-STAT3 circuit, which is independent of IL-6.<sup>303</sup> The STAT3 blockade decreased the expression of PD-L1 on CD44<sup>+</sup> cells in squamous cell carcinoma of the head and neck, a well-characterized cell population with CSC characteristics, resuming T-cell-mediated immunity.<sup>304</sup> These results further justify the development of IL-6<sup>-</sup> or STAT3-targeting strategies in cancer treatment.

#### *Natural killer cell*

Though CSCs were previously believed as less immunogenic than non-CSCs due to decreased MHC class-I (MHC I) expression,<sup>305,306</sup> growing evidence now suggests that CSCs are preferentially susceptible to natural killer (NK) cell activities.<sup>307,308</sup> This vulnerability may be attributed to the activated natural cytotoxicity receptors, particularly NKp30 and NKp44. Though glioblastoma CSCs express deficient MHC I molecules, various ligands that activate NK cell receptors were found on these CSCs, such as PVR and Nectin-2.<sup>308</sup> Interestingly, CSCs were resistant to NK cells freshly isolated from tumor specimen, but were sensitive to the activities of both allogeneic and autologous IL-2 or IL-15-activated NK cells.<sup>308</sup> In melanoma, both CD133<sup>-</sup> and CD133<sup>+</sup> subpopulations are susceptible to the cytotoxicity of IL-2-activated allogeneic NK cells.<sup>309</sup> Likewise, the increased sensitivity of breast CSCs to IL-2 or IL-15-activated NK cells, which is potentially mediated by the upregulation of NKG2D ligands ULBP1, ULBP2, and MICA on CD44<sup>+</sup>CD24<sup>-</sup> breast CSCs.<sup>310</sup> Similar results were observed in ovarian cancer<sup>311</sup> and CCR7<sup>+</sup> melanoma.<sup>312</sup>

Notably, an increased frequency of CSCs is often observed following cytotoxic treatments for primary cancers.<sup>313,314</sup> A study reported a novel mechanism for the immune escape of breast CSCs from NK cell attack, due to decreased expression of ligands that stimulate NKG2D.<sup>315</sup> The upregulation of the NKG2D stress ligands MICA/B on surviving CSCs following cytotoxic treatments, such as radiotherapy, sensitizes CSCs to NK cell killing. NK cells were recruited to the tumor-adjacent areas but lost their cytotoxic efficacy in breast tumors due to the altered ligand expression ligands on radioresistant breast CSCs.<sup>316</sup> This evidence further provides a rationale for combining the NK cell-stimulating factors with conventional therapies.

#### *Cancer-associated fibroblasts and MSCs*

The oncogenic effect of cancer-associated fibroblasts (CAFs) is mostly based on their secretion of a number of paracrine factors, including proinflammatory cytokines, chemokines, prostaglandins, growth factors, and proteases, which collectively promote tumor growth, angiogenesis, and invasion.<sup>317–319</sup> CAFs are also believed to create an immunosuppressive TME by potentiating regulatory T cells,<sup>320</sup> or induce M2-polarized macrophages.<sup>321</sup> Moreover, CAFs-derived exosomes lead to treatment resistance of cancer.<sup>322,323</sup>

Notably, one of the key mechanisms for the CAFs-mediated tumor promotion is based on their regulation of CSC stemness.<sup>324</sup> The paracrine factors produced by specific CAF subpopulations accelerate the transformation of cancer cells into CSCs and help maintain the stemness properties of existing CSCs.<sup>325</sup> Under the cell stimuli, such as chemotherapy, CAFs acquire a senescence-like secretory phenotype, and their secretion of prostemness chemokines is further increased, resulting in CSC-associated chemoresistance.<sup>326</sup>

Both resident and recruited MSCs within TME can acquire CAF-like phenotypes, suggesting that CAFs can be derived from MSC transformation.<sup>327</sup> In pancreatic ductal adenocarcinoma (PDAC) and gastric cancer models, bone marrow-derived MSCs are recruited to TME in a TGF- $\beta$  and CXCL-12-dependent manner and differentiate into CAFs.<sup>328</sup> This transformation may be attributed to tumor-secreted factors, such as the TGF- $\beta$ , which activate MSCs into CAFs, further enhancing the cell heterogeneity of the CSC microenvironment.<sup>329</sup> MSCs are stromal cells with multipotent differentiation abilities and can migrate to tumor sites and promote tumor EMT via the secretion of various factors.<sup>330</sup> For example, in gastric cancer, MSCs secrete VEGF, macrophage inflammatory protein-2, TGF- $\beta$ 1, and the proinflammatory cytokines interleukin IL-6 and IL-8, which collectively facilitate tumor growth and angiogenesis.<sup>331</sup>

## 4 | THERAPIES TARGETING SIGNALING PATHWAYS OF CSC-s

Given that CSCs are a major contributing factor to progressive phenotypes of cancer, targeting CSCs in the tumor now appears as a promising strategy against cancer. Numerous efforts have been undertaken these years to identify such therapies, such as kinase inhibitors and antibodies that block CSC-associated signaling pathway elements, and some of these approaches have already entered the clinical phase.<sup>332,333</sup> The ongoing and completed clinical trials on therapies targeting signaling pathways of

CSCs are presented in Table 2. Immunotherapies targeting CSCs include MHC-restricted killing, such as checkpoint inhibitors, and MHC-unrestricted killing, such as the chimeric antigen receptor (CAR) T-cell approach.<sup>334,335</sup>

## 4.1 | Targeting Notch signaling

Tumors with NOTCH1 mutations represent a distinct tumor phenotype with increased activation in Notch1 signaling. NOTCH1-mutant tumors are often associated with metastasis, poor prognosis, and potential responsiveness to brontictuzumab.<sup>336</sup>

### 4.1.1 | Gamma-secretase inhibitors

A number of Notch-pathway inhibitors have been developed with different action mechanisms, some of which are currently under clinical evaluation. Gamma-secretase inhibitors (GSIs) have long been identified as a large family of Notch-targeted small molecule inhibitors, by blocking the proteolytic cleavage of Notch receptors. GSI PF-03084014 inhibited tumor growth in a mouse xenograft model of T-cell acute lymphoblastic leukemia.<sup>337,338</sup> GSI MRK-003 works synergistically with trastuzumab in HER2-positive breast cancer mouse model.<sup>339</sup> Likewise in NSCLC models, BMS-906024 has demonstrated potent antitumor efficacy in combination with chemotherapies, such as cisplatin, paclitaxel, docetaxel, and target therapies, such as crizotinib.<sup>340,341</sup>

The ability of GSIs to block Notch signaling and subsequently reduce CSC burden in preclinical studies has spurred clinical assessment of GSIs in clinical trials. A well-studied GSI RO4929097 substantially reduced the expression level of stem cell markers on primary melanoma cells and inhibited tumor formation in melanoma xenograft transplants.<sup>342</sup> In a phase II trial, RO4929097 was well tolerated in patients previously treated with PDA, with 25% of patients achieving stable disease.<sup>343</sup> In a phase I trial, four of 24 melanoma patients were reported with clinical benefits from RO4929097 treatments, with one patient achieving a complete response.<sup>344</sup> This encouraged the following phase II trial of RO4929097 in patients with metastatic melanoma with monotherapy.<sup>345</sup> Besides, the combinational treatment of GSIs with other cancer treatments further improved clinical outcomes. One such example is the combination of RO4929097 with bevacizumab in patients with malignant gliomas.<sup>346</sup> These clinical results suggest that GSIs can effectively cross the blood-brain barrier and reach therapeutic concentrations at tumor sites. However, RO4929097 monotherapy displayed minimal inhibition

of neurosphere formation in recurrent glioblastoma samples.<sup>347</sup> Similarly, in metastatic CRCs, the antitumor activity of GSIs, including RO4929097,<sup>344</sup> LY900009,<sup>348</sup> MK-0752,<sup>349</sup> and BMS-986115<sup>350</sup> as monotherapies, is suboptimal.<sup>351</sup> A recent phase Ib/II trial evaluated the treatment combination of RO4929097 with vismodegib, an HH inhibitor, in advanced sarcoma, providing a rationale for the synergy of GSIs in this patient population.<sup>352</sup>

PF-03084014 and MK-0752 are two GSIs frequently used in clinical studies, both of which have been used to treat advanced-stage solid tumors but failed to reach evident clinical efficacy in patients with lung cancer, breast cancer, or pancreatic cancer as monotherapies.<sup>353</sup> As such, these failures further encouraged combinatorial regimens of GSIs with other anticancer therapies, as evidenced by the fact that PF-03084014 enhanced the antitumor effect of DOX in prostate cancer stem-like cells.<sup>354</sup> Moreover, the concomitant use of RO4929097 and cediranib has prolonged disease stabilization in 11 out of 20 patients with advanced solid tumors.<sup>355</sup>

The most common dose-limiting toxicities (DLTs) of GSIs occur in the gastrointestinal system, with secretory diarrhea accounting for 30–60% of all reported DLTs in cancer patients and grade  $\geq 3$  diarrhea accounting for around 11%. This may be explained by the fact that inhibition of Notch1 and Notch2 prevents the proliferation of crypt progenitors leading to goblet-cell metaplasia of the small-intestinal epithelium.<sup>356</sup> The addition to glucocorticoids and antiestrogens in the GSI treatment regimens significantly relieved GSI-induced gastrointestinal toxicities.<sup>338,357</sup> Hypophosphatemia is another GSIs-induced toxicity, which is potentially caused by abnormal gastrointestinal function and can be relieved by oral administration of phosphate replacement.<sup>358</sup>

### 4.1.2 | Pan-Notch small molecule inhibitor

Though Notch1 is the most common activated oncogene in tumors, the coexpression of Notch1 and Notch4 is frequently observed in breast cancer. Moreover, accumulating evidence suggests the Notch3-mediated progression of cancer. These results collectively reveal the requirement for pan-Notch inhibition to achieve a broader spectrum of antitumor efficacy. A previous study evaluated the structure–activity relationships in a series of (2-oxo-1,4-benzodiazepin-3-yl)-succinamides as pan-Notch inhibitors.<sup>359</sup> Among these GSIs, MS-906024 displayed the broadest spectrum efficacy in multiple in-vivo tumor models and thus advanced into clinical trials (NCT01292655).<sup>360</sup> BMS-906024 sensitizes NSCLC to paclitaxel treatment, and patients with wild-type KRAS and BRAF tumors may

TABLE 2 Ongoing and completed clinical trials on therapies targeting signaling pathways of CSCs

Agents (targets)	Condition	Cootherapy	Phase	NCT number
<b>Gamma-secretase inhibitors (GSIs)</b>				
RO4929097 (Notch, A $\beta$ 40, secretase)	Metastatic pancreas cancer		II	NCT01232829
	Advanced solid tumors		I	NCT01145456
	Advanced solid tumors	Cediranib maleate	I	NCT01131234
	Advanced solid tumors		I	NCT01096355
	Refractory NSCLC		II	NCT01070927
	Metastatic epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer		II	NCT01175343
	Advanced sarcoma		I/II	NCT01154452
	Advanced solid tumors	Capecitabine	I	NCT01158274
	Advanced renal cell carcinoma after VEGF/VEGFR therapy failure		II	NCT01141569
	Advanced solid tumors		I	NCT0121862
Advanced solid tumors		Temsirolimus	I	NCT01198184
	Malignant glioma	Temozolomide and radiation therapy	I	NCT01119599
	Metastatic melanoma	Cisplatin, vinblastine, and temozolomide	I/II	NCT01196416
	Metastatic colorectal cancer		II	NCT01116687
PF-03084014 (secretase)	Desmoid tumors		II	NCT01981551
	Advanced cancer and leukemia		I	NCT00878189
MK-0752 (secretase)	Advanced breast cancer	Docetaxel	I/II	NCT00645333
	Early-stage breast cancer	Tamoxifen/letrozole	IV	NCT00756717
	Pancreatic cancer	Gemcitabine hydrochloride	I	NCT01098344
	Advanced cancer	Ridaforolimus	I	NCT01295632
	Advanced breast cancer		I	NCT00106145
<b>Pan-Notch small molecule inhibitor</b>				
BMS-906024 ( $\gamma$ -Secretase and Notch)	Advanced solid tumors		I	NCT01292655
	Advanced solid tumors	Chemotherapy	I	NCT01653470
	Acute T-cell lymphoblastic leukemia or T-cell lymphoblastic lymphoma		I	NCT01363817
CB-103 (Notch)	Luminal advanced breast cancer	Nonsteroidal aromatase inhibitor	II	NCT04714619
	Advanced solid tumors and hematological malignancies		I/II	NCT03422679
<b>Monoclonal antibodies (mAbs) targeting Notch</b>				
MEDI0639 (Dll4)	Advanced solid tumors		I	NCT01577745
SIBP-03 (HER3)	Advanced solid tumors		I	NCT05203601
OMP-52M51 (Notch1)	Metastatic colorectal cancer		I	NCT03031691
	Advanced solid tumors		I	NCT01778439
	Refractory lymphoid malignancies		I	NCT01703572
	Adenoid cystic carcinoma		NA	NCT02662608

(Continues)

TABLE 2 (Continued)

Agents (targets)	Condition	Cotherapy	Phase	NCT number
OMP-59R5 (Notch 2/3)	Stage IV pancreatic cancer	Nab-paclitaxel and gemcitabine	I/II	NCT01647828
	Advanced solid tumors		I	NCT01277146
SMO inhibitor				
BMS-833923 (SMO)	Chronic myeloid leukemia		I/II	NCT01218477
LEQ506 (SMO)	Advanced solid tumors		I	NCT01106508
Vismodegib (SMO)	Prostate cancer		I	NCT02115828
	Metastatic colorectal cancer	Chemotherapy	II	NCT00636610
	Ovarian cancer		II	NCT00739661
	Keratocystic odontogenic tumor		II	NCT02366312
	Advanced pancreatic cancer	Gemcitabine hydrochloride	I/II	NCT01064622
	Advanced Solid Tumors		II	NCT01195415, NCT01088815
		I	NCT01546519, NCT03878524, NCT00878163, NCT00607724, NCT01209143, NCT00968981, NCT01537107	
		II	NCT05159245, NCT05238831, NCT05159245, NCT02091141, NCT00959647	
	Basal cell carcinoma		I	NCT01631331, NCT02639117, NCT03158389
			II	NCT01543581, NCT03035188, NCT00833417, NCT01700049, NCT02667574, NCT01201915, NCT02371967, NCT01367665, NCT01556009, NCT01815840, NCT04416516
		IV	NCT03610022, NCT02436408	
Head/neck basal cell carcinoma	Radiation therapy	II	NCT01835626	
Advanced gastric adenocarcinoma		II	NCT03052478	
Advanced stomach cancer or gastroesophageal junction cancer	Chemotherapy	II	NCT00982592	
Basal cell skin cancer		I/II	NCT02690948	
Small cell lung carcinoma	Cisplatin and etoposide	II	NCT00887159	
Advanced sarcoma		I/II	NCT01154452	
Multiple myeloma		I	NCT01330173	
Recurrent glioblastoma		II	NCT00980343	
Advanced urothelial carcinoma		II	NCT02788201	
Advanced malignancies		II	NCT02465060	
Advanced chondrosarcomas		II	NCT01267955	
Refractory medulloblastoma		II	NCT01239316, NCT00939484, NCT01878617	
Progressive meningiomas		I	NCT00822458	
		II	NCT02523014	

(Continues)

TABLE 2 (Continued)

Agents (targets)	Condition	Cootherapy	Phase	NCT number	
Sonidegib (SMO)	Basal cell carcinoma		NA	NCT01529450	
			II	NCT00961896, NCT03534947, NCT01327053, NCT04806646, NCT01350115, NCT00961896	
	Advanced solid tumors			I	NCT00880308, NCT01208831,
		Paclitaxel		I	NCT01954355
		BKM120		I	NCT01576666
		Pembrolizumab		I	NCT04007744
	Myeloid leukemia	Nilotinib		I	NCT01456676
	Prostate cancer			I	NCT02111187
	Triple-negative (TN) advanced breast cancer (ABC)	Docetaxel		I	NCT02027376
	Recurrent ovarian cancer	Paclitaxel		I	NCT02195973
	Extensive stage small cell lung cancer (ES-SCLC)	Etoposide and cisplatin		I	NCT01579929
	Pancreatic cancer	Gemcitabine and nab paclitaxel		I/II	NCT02358161
				I	NCT01487785
		Chemotherapy		I	NCT01485744
		Esophageal cancer	Everolimus		I
	Myeloid malignancies	Azacitidine		I	NCT02129101
	Recurrent brain tumors			I	NCT03434262
	Multiple myeloma			II	NCT02086552
	Medulloblastoma			II	NCT01708174, NCT04402073
	Hepatocellular carcinoma			I	NCT02151864
Acute leukemias			II	NCT01826214	
Chronic myelogenous leukemia	Nilotinib		I	NCT01456676	
Glasdegib (SMO)	Acute myeloid leukemia, chronic myelomonocytic leukemia	Azacitidine	I	NCT02367456	
			II	NCT01546038, NCT01841333, NCT03226418	
	Acute myeloid leukemia			II	NCT01546038, NCT01841333, NCT03226418
	Soft tissue sarcoma			III	NCT03784014
Glioblastoma	Temozolomide and radiotherapy		I/II	NCT03529448	
<b>Inhibitors of WNT pathway elements</b>					
DKN-01 (DKK1)	Advanced biliary tract cancer	Nivolumab		II	NCT04057365
	Prostate cancer	Docetaxel		I/II	NCT03837353
	Multiple myeloma or advanced solid tumors			I	NCT01457417
	Epithelial endometrial or epithelial ovarian cancer	Paclitaxel		II	NCT03395080
	Gastric or gastroesophageal cancer	Tislelizumab ± chemotherapy		II	NCT04363801
		Paclitaxel or pembrolizumab		I	NCT02013154
	Advanced liver cancer			I/II	NCT03645980
	Cancer of hepatic biliary system or gallbladder	Gemcitabine + cisplatin		I	NCT02375880
	Multiple myeloma	Lenalidomide/ dexamethasone		I	NCT01711671

(Continues)



TABLE 2 (Continued)

Agents (targets)	Condition	Cootherapy	Phase	NCT number	
Vantictumab (FZD receptors)	Metastatic breast cancer		I	NCT01973309	
	Pancreatic cancer	Nab-paclitaxel and gemcitabine	I	NCT02005315	
	NSCLC	Docetaxel	I	NCT01957007	
Cirmtuzumab (ROR1)	Metastatic castration-resistant prostate cancer		II	NCT05156905	
	Breast cancer	Cirmtuzumab + paclitaxel	I	NCT02776917	
	B-cell lymphoid malignancies	Ibrutinib	I/II	NCT03088878	
	Refractory chronic lymphocytic leukemia			I	NCT02222688
				II	NCT04501939

Clinical trial data sources: clinicaltrials.gov.

have improved response to the BMS-906024 + paclitaxel combination.<sup>340</sup> It was later reported that BMS-906024 significantly enhanced the delay in NSCLC tumor spheroid growth delay caused by etoposide and crizotinib, and the most prominent delay in spheroid growth was observed in cells treated with BMS-906024 + chemoradiation triple combination.<sup>341</sup>

CB-103 is an oral pan-Notch inhibitor that specifically targets protein–protein interaction by suppressing the Notch transcriptional complex.<sup>361</sup> Another small molecule inhibitor, IMR-1, inhibits the recruitment of MAML1 to the notch transcriptional complex, thereby preventing its activation.<sup>362</sup> This approach has the advantage of acting downstream of aberrant Notch receptor activation by blocking the assembly of the transcription complex and thereby inhibiting the expression of Notch target genes. A phase I/IIa study is under way to investigate the safety and efficacy of CB-103 in patients with advanced solid tumors and hematological malignancies (NCT03422679). In a phase II trial, patients with advanced breast cancer will receive the combinational treatment of CB-103 with NSAI therapy (letrozole or anastrozole, continuing prior therapy) to evaluate the efficacy (NCT04714619).

#### 4.1.3 | Monoclonal antibodies targeting Notch signaling

Brontictuzumab (OMP-52M51) is a humanized monoclonal antibody (mAb) that selectively targets Notch1 juxtamembrane negative regulatory region and thus inhibits Notch signaling. In a phase II trial of brontictuzumab, six of total 36 (17%) patients with refractory solid tumors demonstrated clinical benefits, with four patients displaying prolonged disease stabilization (NCT01778439).<sup>363</sup> A

functional assay evaluated the efficacy of brontictuzumab in a series of glioma stem-like cell models, supporting brontictuzumab as a promising drug candidate for CNS tumors.<sup>364</sup>

Tarextumab (OMP-59R5) is a human IgG2 antibody with inhibition on both Notch2 and Notch3 and has shown encouraging antitumor efficacy in small cell lung cancer (SCLC). An overall response rate (ORR) of 84% was reported in phase I/II trial when patients received the combination of tarextumab with etoposide and platinum-based therapies. Meanwhile, tarextumab leads to potent inhibition in Notch signaling, and tarextumab-induced diarrhea was dose-limiting above 2.5 mg weekly and 7.5 mg/kg every third week (NCT01277146). The triple combination of tarextumab in combination with gemcitabine plus nab-paclitaxel resulted in increased inhibition of tumor growth and tumor-initiating cell frequency compared with the combination of tarextumab with gemcitabine alone.<sup>365</sup> However, a randomized phase II trial found that the addition of tarextumab to nab-paclitaxel and gemcitabine failed to induce a prolonged overall survival (OS), progression-free survival (PFS), or ORR in patients with metastatic PDAC.<sup>366</sup> The specific role of Notch signaling in PDAC remains unclear with evidence supporting both its oncogenic and tumor-suppressive roles.<sup>366</sup> Further research using individual Notch inhibitors and agonists may facilitate the clinical evaluation of Notch-targeting agents in pancreatic cancer.

## 4.2 | Targeting HH signaling

The pharmacological inhibition targeting the HH pathway in cancer is an active research field, some of which have received regulatory approvals. Major HH pathway

antagonists investigated so far include SMO inhibitors and GLI inhibitors.<sup>367</sup>

#### 4.2.1 | SMO inhibitor

Two SMO antagonists, vismodegib and sonidegib, have been approved by the Food and Drug Administration (FDA) for the treatment of advanced basal cell carcinoma (BCC). Vismodegib (GDC-0449) was first granted approval following success in clinical trials in 2012, where the independently assessed response rate was 30% in 33 patients with metastatic BCC (NCT00833417).<sup>368</sup> In the subsequent phase II trial, a total number of 1215 patients with advanced BCC were treated with vismodegib (NCT01367665). The response rate of patients with metastatic disease was 36.9% and that in patients with locally advanced disease was 68.5% (29073584). However, no benefits were obtained from the additional use of vismodegib in standard treatment regimens for metastatic CRC,<sup>368</sup> PDAC,<sup>369</sup> gastric cancer, and gastroesophageal junction cancer.<sup>370</sup> Besides, the combinations of erlotinib or chemotherapy with vismodegib were well tolerated but induced no improved outcome in metastatic PDAC or PDA, respectively.<sup>371</sup> Vismodegib also failed to deliver clinical benefits compared with placebo as maintenance therapy for ovarian cancer patients.<sup>372</sup> Vismodegib improved PFS in patients with SHH-subtype medulloblastoma but not in those with non-SHH disease subtypes.<sup>373–376</sup> Recent evidence, however, suggested that the addition of vismodegib to TMZ did not improve PFS even in SHH refractory medulloblastoma.<sup>377</sup> A recent phase II trial suggested that the histopathologic subtypes of BCC had no significant impact on patient response to vismodegib.<sup>378</sup>

Sonidegib (LDE225) was approved by FDA in 2015 based on the promising results from a randomized phase II study, where sonidegib at 200 and 800 mg daily induced similar ORR (58% vs. 44%) in patients with locally advanced BCC.<sup>135</sup> In the following analyses, sonidegib demonstrated long-term efficacy and safety profile (30<sup>379</sup> and 42 months<sup>380</sup>) in patients with advanced BCC. A meta-analysis showed that ORRs of vismodegib and sonidegib were comparable in locally advanced BCC (69% vs. 57% respectively), whereas the complete response rates of the two drugs were different (31% vs. 3% respectively).<sup>381</sup> Likewise, BCC patients resistant to vismodegib similarly developed resistance with sonidegib.<sup>382</sup> In both mouse models and phase I clinical trial of TNBC, sonidegib decreased CSC markers expression and sensitizes cancer cells to docetaxel chemotherapy.<sup>383</sup>

Another key SMO inhibitor, glasdegib (PF-04449913), received FDA approval in 2018 as a combination partner for cytarabine for the treatment of AML. The addition of glas-

degib to low-dose cytarabine (LDAC) increased the median OS of newly diagnosed AML patients from 4.9 to 8.8 months.<sup>384</sup> Glasdegib + LDAC continued to induce long-term survival benefits in patients with AML, especially with secondary AML (NCT01546038).<sup>385</sup> In phase II clinical trial, 46.4% of patients achieved CR after glasdegib + cytarabine and daunorubicin treatment (NCT01546038).<sup>386</sup> The subsequent phase III trial of glasdegib in combination with chemotherapy (7 + 3 schedules) to treat AML patients is currently under way (NCT03416179).<sup>384</sup>

#### 4.2.2 | Inhibitors of GLI transcriptional activity

GLI-mediated transcription constitutes the final step of the HH pathway and the inhibition of GLI transcription factors is thus a promising strategy that reduces tumor cell proliferation.<sup>387</sup> Inhibitors of GLI-mediated transcription, such as GANT58 and GANT61, were first designed to overcome tumor resistance to SMO inhibitors. A wide breadth of literature has described the antitumor activity of these agents in various cancers, including NSCLC, breast cancer, prostate cancer, and rhabdomyosarcoma.<sup>388–392</sup> However, neither of these two agents have advanced into clinical trials.

Arsenic trioxide (ATO), an FDA-approved drug widely accepted in treating acute promyelocytic leukemia, is also a potent inhibitor of GLI1 and GLI2 and inhibits cancer growth by blocking GLI transcription.<sup>392</sup> With its inhibition of GLI transcription activities, ATO inhibits the viability and maintenance of CSCs derived from SCLC<sup>393</sup> and pancreatic cancer.<sup>394</sup> ATO also prevents osteosarcoma growth via DNA damage accumulation.<sup>395</sup> In a phase II study, the concomitant use of ATO and itraconazole was tested in BCC patients who were resistant to SMO inhibitors. Significant alterations in mRNA levels of GLI1 were observed.<sup>396</sup> Given that none of the participants had tumor shrinkage though they experienced SD for 3 months, continuous dosing was later recommended to achieve a better clinical response.<sup>396</sup> Currently, multiple clinical trials of ATO, alone or in synergy with standard therapies in cancer patients, are under way.

### 4.3 | Targeting Wnt signaling

The Wnt pathway inhibitor family is mainly comprised of agents targeting Wnt pathway molecules, Porcupine inhibitors that diminish the ability to secrete Wnt ligands, and inhibitors of downstream  $\beta$ -catenin-TCF-LEF-dependent transcription. Many of these agents have been extensively studied and are currently under clinical evaluation.

### 4.3.1 | Inhibitors of Wnt pathway elements

DKN-01 is an IgG4 mAb targeting Dkk1 that suppresses canonical Wnt signaling via negative feedback.<sup>397</sup> Some studies addressed the direct antitumor effects of DKK1 inhibition,<sup>398</sup> whereas some recently reported its indirect antitumor effects via stimulation of immune responses in cancers, including ovarian cancer<sup>399</sup> and prostate cancer.<sup>400</sup> The murine version of DKN-01 overcomes the DKK1-mediated immune suppression and improves the efficacy of PD-1 blockade.<sup>401</sup> Similarly, inhibiting Wnt/ $\beta$ -catenin signaling by DKN-01 enhances the antitumor immune infiltration into tumors and improves the response of ovarian tumors to immune checkpoint inhibitors.<sup>399</sup>

Multiple clinical trials of DKN-01 are now carried out across a wide range of cancer types. In a phase I trial, the combination of DKN-01 with paclitaxel is well tolerated in patients with DKK1-positive esophageal or gastroesophageal junction tumors (NCT02013154).<sup>402</sup> In a following phase II trial, the combination of DKN-01 with pembrolizumab was well tolerated in patients with a gastroesophageal junction or gastric cancer, and especially effective in anti-PD-1/PD-L1-naïve patients with DKK1-high tumors.<sup>403</sup> A biomarker analysis revealed that DKN-01 in combination with chemotherapies potentially led to reduced angiogenesis and inflammation markers in patients with biliary tract cancer (NCT02375880).<sup>404</sup>

Vantictumab is a fully human mAb that inhibits Wnt pathway signaling by targeting FZD1, 2, 5, 7, and 8 receptors. Vantictumab decreases the enrichment of CSCs in various tumor types, either alone or in synergy with a chemotherapeutic.<sup>405</sup> A phase I study evaluated the combination of vantictumab with nab-paclitaxel and gemcitabine in metastatic PDA patients. However, this trial was ultimately terminated due to bone-related cytotoxicity.<sup>406</sup> Another phase I study assessed the efficacy and safety of the combination of vantictumab with paclitaxel metastatic breast cancer and the further use of this combination was restricted by the frequently occurred fractures.<sup>407</sup>

Cirmtuzumab is a humanized mAb that inhibits the activity of ROR1, an oncoembryonic orphan receptor for Wnt5a in CSCs.<sup>408</sup> The antitumor activities of cirmtuzumab are mostly documented in chronic lymphocytic leukemia (CLL), where it inhibits the activation of both NF- $\kappa$ B and STAT3 in patients.<sup>409</sup> Results from a phase I trial showed that cirmtuzumab is effective in suppressing tumor cell ROR1 signaling in CLL (NCT02222688).<sup>410</sup> Targeting ROR1 with cirmtuzumab may also improve the response of breast cancer patients to chemotherapies.<sup>411</sup> Cirmtuzumab could work synergistically with the Bruton tyrosine kinase inhibitor ibrutinib to treat patients with CLL or other ROR1<sup>+</sup> B-cell malignancies.<sup>412</sup> Currently, a

phase Ib/II study is under way to evaluate this combination in patients with CLL, small lymphocytic lymphoma, or mantle cell lymphoma (NCT03088878).

## 5 | CSC-DIRECTED IMMUNOTHERAPIES

As promising CSC-directed immunotherapy, CSC-based dendritic cell (DC) vaccines facilitate tumor cell recognition and eradication by potentiating antigen-specific T-cell responses against CSCs.<sup>413</sup> The CSC-specific T cells can also be produced by CSC priming. CSC lysate-pulsed DCs stimulate CD8<sup>+</sup> T cells, and the generated CSC-specific T cells induce antitumor immunity by directly targeting CSCs in tumors.<sup>414,415</sup> Table 3 summarizes the ongoing and completed clinical trials on CSC-directed immunotherapies. Bispecific antibodies (BiAbs) targeting CSC-specific antigens represent another candidate for CSC-directed immunotherapies. For instance, a BiAb composed of CD133 mAb monomer and a single chain of humanized muromonab-CD3 targets CD133-expressing tumor cells by arming activated T cells.<sup>416</sup>

One of the most studied CSC-directed immunotherapies that enter clinical trials is the CAR T-cell transfer, based on the identification of CSC surface antigens by CAR T cells.<sup>417</sup> Though a wide range of CSC-related antigens are used to design CAR T-cell therapies,<sup>417</sup> CSC-targeting CAR T cells to date have been approved by FDA. The largest concern about CAR T-cell treatment could be its safety profile, cytokine release syndrome, and soluble tumor syndrome.<sup>417</sup> The application of well-characterized CSC markers in CAR T-cell design is a promising approach to eliminate CSCs in many cancers, which, however, still requires further investigations to advance CAR T cells into the clinic.

A typical example of CAR T therapies is the CAR T cocktail immunotherapy composed of successive infusions of CART cells targeting epidermal growth factor receptor (EGFR) and CD133, which specifically target CSCs in cholangiocarcinoma (NCT01869166 and NCT02541370).<sup>418</sup> CART-133 cell therapy patients demonstrate promising antitumor activity. In HCC patients, CAR-133 cell therapy demonstrated promising efficacy with manageable toxicity.<sup>419</sup> This study also revealed potential biomarkers that predicted patient response to CART-133 cells (NCT02541370). GBM CSCs are characterized as EGFRVIII<sup>+</sup>/CD133<sup>+</sup> cells with self-renewal as well as cancer initiation abilities.<sup>420</sup> In the first clinical trial of EGFRVIII-specific CAR T-cell infusions, patients with EGFRVIII<sup>+</sup> recurrent GBM did not obtain noticeable tumor regression according to MRI.<sup>421</sup> Meanwhile, an additional study suggested that the CAR T-EGFRVIII cell

TABLE 3 Ongoing and completed clinical trials on CSC-directed immunotherapies

Immunotherapy	Condition	Phase	NCT number
Dendritic cell (DC) vaccines			
Cancer stem cells vaccine	Ovarian cancer	I/II	NCT02178670
Tumor lysate-pulsed DC vaccine	High-risk solid tumor	II	NCT00405327
Minor histocompatibility antigens (MiHA)-loaded PD-L-silenced DC vaccination	Hematological malignancies	I/II	NCT02528682
Total tumor RNA (TTRNA)-loaded-DCs	Recurrent medulloblastoma and primitive neuroectodermal tumor	II	NCT01326104
CD34 <sup>+</sup> -derived DCs	Breast neoplasms	I	NCT00197522
Dendritic cell vaccine with mRNA from tumor stem cells	Glioblastoma	I/II	NCT00846456
Dendritic cell fusion vaccine	Multiple myeloma	II	NCT01067287
Autologous DCs with glioma stem-like cells associated antigens	Glioblastoma	II	NCT01567202
Bispecific antibodies			
Anti-CD3 × anti-CD20 bispecific antibody-armed activated T cells	Multiple myeloma and plasma cell neoplasm	I	NCT00938626
Elranatamab (B-cell maturation antigen [BCMA] CD3-targeted bispecific antibody)	Multiple myeloma	III	NCT05317416
Anti-CD3 × anti-CD20 bispecific antibody (CD20Bi)-activated T cells (ATC)	Non-Hodgkin lymphoma	I	NCT00244946

Clinical trial data sources: clinicaltrials.gov.

therapy failed to induce clinical benefits in patients with recurrent GBM.<sup>422</sup> Ongoing and completed clinical trials on CSC-directed CAR T-cell therapy are presented in Table 4.

The CSC characteristics are associated with an increased level of CD44,<sup>168</sup> which requires the transformation of CD44v to CD44s isoform.<sup>423,424</sup> It was reported that 50% of pancreatic cancer tissues were CD44v6-positive, which indicated a poorer survival in this group of patients.<sup>425</sup> Currently, two phase I/II clinical trials are ongoing to assess the safety and efficacy of CD44v6 CAR-T-cell therapy in patients with breast cancer and other CD44v6-positive tumors. (NCT04430595 and NCT04427449) Recently, a highly specific CAR against CD44v6 was established aiming to eliminate CD44v6-expressing HNSCC cells.<sup>426</sup>

## 6 | CONCLUSION AND FUTURE PERSPECTIVES

In conclusion, CSCs are a subpopulation of malignant tumor cells with selective capacities for tumor initiation, self-renewal, metastasis, and unlimited growth into bulks. There is intricate signaling network within CSCs that regulates stemness and biological functions. Thus, targeting pathway molecules that regulate CSCs provides a new option for the treatment of therapy-resistant or -refractory tumors. Meanwhile, extracellular regulating

factors, including angiogenic microenvironment, hypoxic microenvironment, TAM, fibroblasts, and a series of protumor paracrine factors, collectively provide a fertile soil that favors CSC growth. Numerous efforts have been undertaken these years to identify such therapies, such as kinase inhibitors and antibodies that block CSC-associated signaling pathway elements, and some of these approaches have already entered the clinical phase. Furthermore, vaccines, antibodies, and CAR T cells have also expanded the range of CSC-target therapies.

Obstacles remain regarding the design of CSC-targeted therapies. Though our understanding of CSCs surface biomarkers has been largely improved in recent years, the surface markers of CSCs may vary according to tumor types and the cell of tumor origin, demonstrating high heterogeneity between tumors or even among cells within one tumor. This heterogeneity has highlighted the challenges in identifying and isolating CSC subpopulations from tumors. Thus, functional assays are recommended to more specifically identify CSCs, including sphere formation capacity in vitro and tumor-initiation of after transplantation in vivo.

Accumulating evidence now suggests that quiescent CSCs contribute to the refractoriness of cancers to chemotherapies. Thus, therapeutic approaches that merely inhibit CSC stemness might not be sufficient to suppress postchemotherapy recurrence. The TME plays a critical role in CSC regulation, which not only maintains CSC

**TABLE 4** Ongoing and completed clinical trials on CSC-directed chimeric antigen receptor (CAR) T-cell therapy

Chimeric antigen receptor (CAR) T cell	Condition	Phase	NCT number
CAR T-cell therapy	Hematologic neoplasms	NA	NCT04691284
PSCA-targeted CAR T cells (BPX-601)	Advanced solid tumors	I/II	NCT02744287
Anti-CD19 CAR T cells	B-cell non-Hodgkin lymphoma	I/II	NCT01318317
	B-cell malignancies	I/II	NCT01475058, NCT01087294, NCT02659943
	Relapsed malignant lymphoma	I	NCT05239676
CD19CAR/virus-specific T cells	CD19 <sup>+</sup> malignancies	I	NCT00840853
	CD19 <sup>+</sup> acute lymphoblastic leukemia or non-Hodgkin lymphoma	I	NCT03768310
Ciltacabtagene autoleucel (BCMA-directed CAR T)	Multiple myeloma	III	NCT04923893, NCT05257083
	B-cell lymphoma	II	NCT04531046
Anti-CD19 allo-CAR T cells	Relapsed B-cell malignancies	I	NCT04516551
Genetically modified T cells (CMV-specific CD19-CAR T cells)	B-cell non-Hodgkin lymphoma	I	NCT05432635
CD19 <sup>+</sup> CD22 CAR-T	B acute lymphoblastic leukemia	I	NCT04626726
CD19CAR-CD28-CD3zeta-EGFRt-expressing TCM-enriched T cells	Non-Hodgkin lymphoma	I	NCT01815749
	B-cell non-Hodgkin lymphoma	I	NCT02051257
CD19CAR-CD28Z T cells	B-cell lymphoblastic leukemia	I	NCT02050347
CAR T directed against CD19 <sup>+</sup> B cells	B-cell non-Hodgkin lymphoma	I	NCT01840566
Anti-CD133-CAR T cells	Advanced malignancies	I/II	NCT02541370
	Malignant gliomas	I	NCT03423992
Sarcoma-specific CAR T cells	Sarcoma	I/II	NCT03356782
CD44v6-specific CAR T cells	CD44v6-positive cancers	I/II	NCT04427449
4SCAR T cells	Breast cancer	I/II	NCT04430595

Clinical trial data sources: [clinicaltrials.gov](https://clinicaltrials.gov).

characteristics via various signals but also facilitates the transition of nonstem cells to stem cell states.<sup>427</sup> It is thus conceivable that targeting TME components may be more effective in overcoming treatment resistance than directly inhibiting CSCs stemness. However, the heterogeneity of immune cells across cell types has made it difficult to identify the precise CSC-immune cell interactions. Recently, single-cell RNA sequencing is extensively used to identify the altering states of CSCs and immune cells, as well as their interactions under different tumor contexts. Moreover, BiAbs that act on both intrinsic regulating factors of CSCs and the CSC-immune cell crosstalk are recommended.<sup>31</sup>

Finally, in addition to TAMs that have long been identified for their activities in CSC maintenance, recent reports highlight the significance of NK cells in suppressing cancer cell stemness.<sup>290</sup> CSCs are mostly sensitive to NK cell killing, but in some cases, such as GBM, AML, and breast cancer, CSCs may be resistant to activated NK cells.<sup>428</sup> However, the anti-CSC functions of NK cells are suppressed by TAMs, MDSCs (myeloid-derived suppressor cells), and T-reg cells.<sup>429</sup> Future research is required to address the crosstalk between these immune cells in the TME, thereby facilitating the development of more effective CSC-targeted immunotherapies.

Future research is required to address the crosstalk between these immune cells in the TME, thereby facilitating the development of more effective CSC-targeted immunotherapies.

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## CONFLICT OF INTERESTS

The authors declared no conflict of interests.

## AUTHOR CONTRIBUTIONS

Wu Min offered the main direction and significant guidance of this manuscript. Wang Manni drafted the manuscript and illustrated the figures for the manuscript. All authors have read and approved the final manuscript.

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Not applicable.

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Not applicable.

## REFERENCES

- Dinsmore CE. Animal regeneration—from fact to concept. *BioScience*. 1995;45(7):484-492.
- Frank NY, Schatton T, Frank MH. The therapeutic promise of the cancer stem cell concept. *J Clin Invest*. 2010;120(1):41-50.
- Vermeulen L, Sprick MR, Kemper K, Stassi G, Medema JP. Cancer stem cells—old concepts, new insights. *Cell Death Differ*. 2008;15(6):947-958.
- Chang JC. Cancer stem cells: role in tumor growth, recurrence, metastasis, and treatment resistance. *Medicine (Baltimore)*. 2016;95(1 Suppl 1):S20-S25.
- Chen K, Huang YH, Chen JL. Understanding and targeting cancer stem cells: therapeutic implications and challenges. *Acta Pharmacol Sin*. 2013;34(6):732-740.
- Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006;444(7120):756-760.
- Li X, Lewis MT, Huang J, et al. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. *J Natl Cancer Inst*. 2008;100(9):672-679.
- Plaks V, Kong N, Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? *Cell Stem Cell*. 2015;16(3):225-238.
- Wiechert A, Saygin C, Thiagarajan PS, et al. Cisplatin induces stemness in ovarian cancer. *Oncotarget*. 2016;7(21):30511-30522.
- Saygin C, Matei D, Majeti R, Reizes O, Lathia JD. Targeting cancer stemness in the clinic: from hype to hope. *Cell Stem Cell*. 2019;24(1):25-40.
- Espinoza I, Miele L. Deadly crosstalk: Notch signaling at the intersection of EMT and cancer stem cells. *Cancer Lett*. 2013;341(1):41-45.
- Nishio M, Otsubo K, Maehama T, Mimori K, Suzuki A. Capturing the mammalian Hippo: elucidating its role in cancer. *Cancer Sci*. 2013;104(10):1271-1277.
- Pelullo M, Zema S, Nardozza F, Checquolo S, Screpanti I, Bellavia D. Wnt, Notch, and TGF-beta pathways impinge on hedgehog signaling complexity: an open window on cancer. *Front Genet*. 2019;10:711.
- Bjerkvig R, Tysnes BB, Aboody KS, Najbauer J, Terzis AJ. Opinion: the origin of the cancer stem cell: current controversies and new insights. *Nat Rev Cancer*. 2005;5(11):899-904.
- Hill RP. Identifying cancer stem cells in solid tumors: case not proven. *Cancer Res*. 2006;66(4):1891-1895. discussion 1890.
- Huntly BJ, Gilliland DG. Leukaemia stem cells and the evolution of cancer-stem-cell research. *Nat Rev Cancer*. 2005;5(4):311-321.
- Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature*. 1994;367(6464):645-648.
- Hemmati HD, Nakano I, Lazareff JA, et al. Cancerous stem cells can arise from pediatric brain tumors. *Proc Natl Acad Sci U S A*. 2003;100(25):15178-15183.
- Jin X, Jin X, Kim H. Cancer stem cells and differentiation therapy. *Tumour Biol*. 2017;39(10).
- Huang Z, Wu T, Liu AY, Ouyang G. Differentiation and transdifferentiation potentials of cancer stem cells. *Oncotarget*. 2015;6(37):39550-39563.
- Ricci-Vitiani L, Pallini R, Biffoni M, et al. Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. *Nature*. 2010;468(7325):824-828.
- Bussolati B, Bruno S, Grange C, Ferrando U, Camussi G. Identification of a tumor-initiating stem cell population in human renal carcinomas. *FASEB J*. 2008;22(10):3696-3705.
- Xiong YQ, Sun HC, Zhang W, et al. Human hepatocellular carcinoma tumor-derived endothelial cells manifest increased angiogenesis capability and drug resistance compared with normal endothelial cells. *Clin Cancer Res*. 2009;15(15):4838-4846.
- Huang T, Song X, Xu D, et al. Stem cell programs in cancer initiation, progression, and therapy resistance. *Theranostics*. 2020;10(19):8721-8743.
- Nowell PC. The clonal evolution of tumor cell populations. *Science*. 1976;194(4260):23-28.
- van Niekerk G, Davids LM, Hattingh SM, Engelbrecht AM. Cancer stem cells: a product of clonal evolution? *Int J Cancer*. 2017;140(5):993-999.
- Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer*. 2008;8(10):755-768.
- Quinn HM, Vogel R, Popp O, et al. YAP and beta-catenin cooperate to drive oncogenesis in basal breast cancer. *Cancer Res*. 2021;81(8):2116-2127.
- Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature*. 2014;506(7488):328-333.
- Auffinger B, Tobias AL, Han Y, et al. Conversion of differentiated cancer cells into cancer stem-like cells in a glioblastoma model after primary chemotherapy. *Cell Death Differ*. 2014;21(7):1119-1131.
- Chen P, Hsu WH, Han J, Xia Y, DePinho RA. Cancer stemness meets immunity: from mechanism to therapy. *Cell Rep*. 2021;34(1):108597.
- Chen K, Zhang C, Ling S, Wei R, Wang J, Xu X. The metabolic flexibility of quiescent CSC: implications for chemotherapy resistance. *Cell Death Dis*. 2021;12(9):835.
- Ponomarev A, Gilazieva Z, Solovyeva V, Allegrucci C, Rizvanov A. Intrinsic and extrinsic factors impacting cancer stemness and tumor progression. *Cancers (Basel)*. 2022;14(4):970.
- Ajani JA, Song S, Hochster HS, Steinberg IB. Cancer stem cells: the promise and the potential. *Semin Oncol*. 2015;42(1):S3-S17.
- Tang DG. Understanding cancer stem cell heterogeneity and plasticity. *Cell Res*. 2012;22(3):457-472.
- Boman BM, Wicha MS. Cancer stem cells: a step toward the cure. *J Clin Oncol*. 2008;26(17):2795-2799.
- Huang EH, Hynes MJ, Zhang T, et al. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. *Cancer Res*. 2009;69(8):3382-3389.
- Boyer LA, Lee TI, Cole MF, et al. Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell*. 2005;122(6):947-956.

39. Neph S, Stergachis AB, Reynolds A, Sandstrom R, Borenstein E, Stamatoyannopoulos JA. Circuitry and dynamics of human transcription factor regulatory networks. *Cell*. 2012;150(6):1274-1286.
40. Sangiolo D, Mesiano G, Gammaitoni L, et al. Cytokine-induced killer cells eradicate bone and soft-tissue sarcomas. *Cancer Res*. 2014;74(1):119-129.
41. Guerra-Rebollo M, Garrido C, Sanchez-Cid L, et al. Targeting of replicating CD133 and OCT4/SOX2 expressing glioma stem cells selects a cell population that reinitiates tumors upon release of therapeutic pressure. *Sci Rep*. 2019;9(1):9549.
42. Yang L, Shi P, Zhao G, et al. Targeting cancer stem cell pathways for cancer therapy. *Signal Transduct Target Ther*. 2020;5(1):8.
43. Walcher L, Kistenmacher AK, Suo H, et al. Cancer stem cells—origins and biomarkers: perspectives for targeted personalized therapies. *Front Immunol*. 2020;11:1280.
44. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*. 1997;3(7):730-737.
45. Moserle L, Ghisi M, Amadori A, Indraccolo S. Side population and cancer stem cells: therapeutic implications. *Cancer Lett*. 2010;288(1):1-9.
46. Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, et al. Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian inhibiting substance responsiveness. *Proc Natl Acad Sci U S A*. 2006;103(30):11154-11159.
47. Chiba T, Kita K, Zheng YW, et al. Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. *Hepatology*. 2006;44(1):240-251.
48. Wang M, Wang Y, Zhong J. Side population cells and drug resistance in breast cancer. *Mol Med Rep*. 2015;11(6):4297-4302.
49. Feuring-Buske M, Hogge DE. Hoechst 33342 efflux identifies a subpopulation of cytogenetically normal CD34(+)CD38(-) progenitor cells from patients with acute myeloid leukemia. *Blood*. 2001;97(12):3882-3889.
50. Montanaro F, Liadaki K, Schianda J, Flint A, Gussoni E, Kunkel LM. Demystifying SP cell purification: viability, yield, and phenotype are defined by isolation parameters. *Exp Cell Res*. 2004;298(1):144-154.
51. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature*. 2007;445(7123):106-110.
52. Ricci-Vitiani L, Lombardi DG, Pilozzi E, et al. Identification and expansion of human colon-cancer-initiating cells. *Nature*. 2007;445(7123):111-115.
53. Shmelkov SV, Butler JM, Hooper AT, et al. CD133 expression is not restricted to stem cells, and both CD133<sup>+</sup> and CD133<sup>-</sup> metastatic colon cancer cells initiate tumors. *J Clin Invest*. 2008;118(6):2111-2120.
54. Min DW, Kim HP, Kim J, et al. Phenotype-based single cell sequencing identifies diverse genetic subclones in CD133 positive cancer stem cells. *Biochem Biophys Res Commun*. 2021;558:209-215.
55. Taverna JA, Hung CN, DeArmond DT, et al. Single-cell proteomic profiling identifies combined AXL and JAK1 inhibition as a novel therapeutic strategy for lung cancer. *Cancer Res*. 2020;80(7):1551-1563.
56. Celia-Terrassa T, Jolly MK. Cancer stem cells and epithelial-to-mesenchymal transition in cancer metastasis. *Cold Spring Harb Perspect Med*. 2020;10(7):a036905.
57. Underhill C. CD44: the hyaluronan receptor. *J Cell Sci*. 1992;103(Pt 2):293-298.
58. Gzil A, Zarebska I, Bursiewicz W, Antosik P, Grzanka D, Szyberg L. Markers of pancreatic cancer stem cells and their clinical and therapeutic implications. *Mol Biol Rep*. 2019;46(6):6629-6645.
59. Al-Othman N, Alhendi A, Ihabaisha M, Barahmeh M, Alqaraleh M, Al-Momany BZ. Role of CD44 in breast cancer. *Breast Dis*. 2020;39(1):1-13.
60. Negi LM, Talegaonkar S, Jaggi M, Ahmad FJ, Iqbal Z, Khar RK. Role of CD44 in tumour progression and strategies for targeting. *J Drug Target*. 2012;20(7):561-573.
61. Katagiri YU, Sleeman J, Fujii H, et al. CD44 variants but not CD44s cooperate with beta1-containing integrins to permit cells to bind to osteopontin independently of arginine-glycine-aspartic acid, thereby stimulating cell motility and chemotaxis. *Cancer Res*. 1999;59(1):219-226.
62. Fang D, Kitamura H. Cancer stem cells and epithelial-mesenchymal transition in urothelial carcinoma: possible pathways and potential therapeutic approaches. *Int J Urol*. 2018;25(1):7-17.
63. Lamour V, Henry A, Kroonen J, et al. Targeting osteopontin suppresses glioblastoma stem-like cell character and tumorigenicity in vivo. *Int J Cancer*. 2015;137(5):1047-1057.
64. Immervoll H, Hoem D, Sakariassen PO, Steffensen OJ, Molven A. Expression of the "stem cell marker" CD133 in pancreas and pancreatic ductal adenocarcinomas. *BMC Cancer*. 2008;8:48.
65. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A*. 2003;100(7):3983-3988.
66. Ginestier C, Hur MH, Charafe-Jauffret E, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell*. 2007;1(5):555-567.
67. Quintana E, Shackleton M, Foster HR, et al. Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. *Cancer Cell*. 2010;18(5):510-523.
68. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. *Nature*. 2004;432(7015):396-401.
69. van den Hoogen C, van der Horst G, Cheung H, et al. High aldehyde dehydrogenase activity identifies tumor-initiating and metastasis-initiating cells in human prostate cancer. *Cancer Res*. 2010;70(12):5163-5173.
70. Zhang WC, Shyh-Chang N, Yang H, et al. Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. *Cell*. 2012;148(1-2):259-272.
71. Hashimoto O, Shimizu K, Semba S, et al. Hypoxia induces tumor aggressiveness and the expansion of CD133-positive cells in a hypoxia-inducible factor-1alpha-dependent manner in pancreatic cancer cells. *Pathobiology*. 2011;78(4):181-192.
72. Lv D, Ma QH, Duan JJ, et al. Optimized dissociation protocol for isolating human glioma stem cells from tumorspheres via fluorescence-activated cell sorting. *Cancer Lett*. 2016;377(1):105-115.

73. Tomita H, Tanaka K, Tanaka T, Hara A. Aldehyde dehydrogenase 1A1 in stem cells and cancer. *Oncotarget*. 2016;7(10):11018-11032.
74. Huang CP, Tsai MF, Chang TH, et al. ALDH-positive lung cancer stem cells confer resistance to epidermal growth factor receptor tyrosine kinase inhibitors. *Cancer Lett*. 2013;328(1):144-151.
75. Luo M, Brooks M, Wicha MS. Epithelial-mesenchymal plasticity of breast cancer stem cells: implications for metastasis and therapeutic resistance. *Curr Pharm Des*. 2015;21(10):1301-1310.
76. Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest*. 2003;112(12):1776-1784.
77. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009;119(6):1420-1428.
78. Akrap N, Andersson D, Bom E, Gregersson P, Stahlberg A, Landberg G. Identification of distinct breast cancer stem cell populations based on single-cell analyses of functionally enriched stem and progenitor pools. *Stem Cell Rep*. 2016;6(1):121-136.
79. Ammothumkandy A, Maliekal TT, Bose MV, et al. CD66 and CD49f expressing cells are associated with distinct neoplastic phenotypes and progression in human cervical cancer. *Eur J Cancer*. 2016;60:166-178.
80. Haraguchi N, Ishii H, Mimori K, et al. CD49f-positive cell population efficiently enriches colon cancer-initiating cells. *Int J Oncol*. 2013;43(2):425-430.
81. Kahn M. Can we safely target the WNT pathway? *Nat Rev Drug Discov*. 2014;13(7):513-532.
82. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. *Oncogene*. 2017;36(11):1461-1473.
83. Pramanik KC, Fofaria NM, Gupta P, Ranjan A, Kim SH, Srivastava SK. Inhibition of beta-catenin signaling suppresses pancreatic tumor growth by disrupting nuclear beta-catenin/TCF-1 complex: critical role of STAT-3. *Oncotarget*. 2015;6(13):11561-11574.
84. Li J, Ji L, Chen J, Zhang W, Ye Z. Wnt/beta-catenin signaling pathway in skin carcinogenesis and therapy. *Biomed Res Int*. 2015;2015:964842.
85. Stewart DJ. Wnt signaling pathway in non-small cell lung cancer. *J Natl Cancer Inst*. 2014;106(1):djt356.
86. Li Y, Hu J, Song H, Wu T. Antibiotic anisomycin selectively targets leukemia cell lines and patient samples through suppressing Wnt/beta-catenin signaling. *Biochem Biophys Res Commun*. 2018;505(3):858-864.
87. Chen Z, Zhou L, Wang L, et al. HBO1 promotes cell proliferation in bladder cancer via activation of Wnt/beta-catenin signaling. *Mol Carcinog*. 2018;57(1):12-21.
88. Prasetyanti PR, Kimberlin CD, Bots M, Vermeulen L, Melo Fde S, Medema JP. Regulation of stem cell self-renewal and differentiation by Wnt and Notch are conserved throughout the adenoma-carcinoma sequence in the colon. *Mol Cancer*. 2013;12(1):126.
89. Teng Y, Wang X, Wang Y, Ma D. Wnt/beta-catenin signaling regulates cancer stem cells in lung cancer A549 cells. *Biochem Biophys Res Commun*. 2010;392(3):373-379.
90. de Sousa EMF, Vermeulen L. Wnt signaling in cancer stem cell biology. *Cancers (Basel)*. 2016;8(7):60.
91. Liu S, Kin Pong U, Zhang J, et al. R-spondin2 enhances canonical Wnt signaling to maintain the stemness of glioblastoma cells. *Cancer Cell Int*. 2018;18:156.
92. Liu D, Du L, Chen D, et al. Reduced CD146 expression promotes tumorigenesis and cancer stemness in colorectal cancer through activating Wnt/beta-catenin signaling. *Oncotarget*. 2016;7(26):40704-40718.
93. Malta TM, Sokolov A, Gentles AJ, et al. Machine learning identifies stemness features associated with oncogenic dedifferentiation. *Cell*. 2018;173(2):338-354.e15.
94. Malanchi I, Peinado H, Kassen D, et al. Cutaneous cancer stem cell maintenance is dependent on beta-catenin signalling. *Nature*. 2008;452(7187):650-653.
95. Vermeulen L, De Sousa EMF, van der Heijden M, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol*. 2010;12(5):468-476.
96. Loregger A, Grandl M, Mejias-Luque R, et al. The E3 ligase RNF43 inhibits Wnt signaling downstream of mutated beta-catenin by sequestering TCF4 to the nuclear membrane. *Sci Signal*. 2015;8(393):ra90.
97. Cho YH, Ro EJ, Yoon JS, et al. 5-FU promotes stemness of colorectal cancer via p53-mediated WNT/beta-catenin pathway activation. *Nat Commun*. 2020;11(1):5321.
98. Liu X, Su K, Sun X, et al. Sec62 promotes stemness and chemoresistance of human colorectal cancer through activating Wnt/beta-catenin pathway. *J Exp Clin Cancer Res*. 2021;40(1):132.
99. Khramtsov AI, Khramtsova GF, Tretiakova M, Huo D, Olopade OI, Goss KH. Wnt/beta-catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome. *Am J Pathol*. 2010;176(6):2911-2920.
100. Schade B, Lesurf R, Sanguin-Gendreau V, et al. Beta-catenin signaling is a critical event in ErbB2-mediated mammary tumor progression. *Cancer Res*. 2013;73(14):4474-4487.
101. Fendler A, Bauer D, Busch J, et al. Inhibiting WNT and NOTCH in renal cancer stem cells and the implications for human patients. *Nat Commun*. 2020;11(1):929.
102. Jiang N, Zou C, Zhu Y, et al. HIF-1a-regulated miR-1275 maintains stem cell-like phenotypes and promotes the progression of LUAD by simultaneously activating Wnt/beta-catenin and Notch signaling. *Theranostics*. 2020;10(6):2553-2570.
103. Clara JA, Monge C, Yang Y, Takebe N. Targeting signalling pathways and the immune microenvironment of cancer stem cells - a clinical update. *Nat Rev Clin Oncol*. 2020;17(4):204-232.
104. Dandawate PR, Subramaniam D, Jensen RA, Anant S. Targeting cancer stem cells and signaling pathways by phytochemicals: novel approach for breast cancer therapy. *Semin Cancer Biol*. 2016;40-41:192-208.
105. Wang Z, Ahmad A, Li Y, Azmi AS, Miele L, Sarkar FH. Targeting notch to eradicate pancreatic cancer stem cells for cancer therapy. *Anticancer Res*. 2011;31(4):1105-1113.
106. Fortini ME. Notch signaling: the core pathway and its post-translational regulation. *Dev Cell*. 2009;16(5):633-647.
107. Yan Y, Liu F, Han L, et al. HIF-2alpha promotes conversion to a stem cell phenotype and induces chemoresistance in breast cancer cells by activating Wnt and Notch pathways. *J Exp Clin Cancer Res*. 2018;37(1):256.
108. Barnawi R, Al-Khaldi S, Majed Sleiman G, et al. Fascin is critical for the maintenance of breast cancer stem cell pool predominantly via the activation of the notch self-renewal pathway. *Stem Cells*. 2016;34(12):2799-2813.



109. Qiu L, Yang X, Wu J, Huang C, Miao Y, Fu Z. HIST2H2BF potentiates the propagation of cancer stem cells via notch signaling to promote malignancy and liver metastasis in colorectal carcinoma. *Front Oncol.* 2021;11:677646.
110. Yu JB, Jiang H, Zhan RY. Aberrant notch signaling in glioblastoma stem cells contributes to tumor recurrence and invasion. *Mol Med Rep.* 2016;14(2):1263-1268.
111. Wang R, Li Y, Tsung A, et al. iNOS promotes CD24(+)CD133(+) liver cancer stem cell phenotype through a TACE/ADAM17-dependent notch signaling pathway. *Proc Natl Acad Sci U S A.* 2018;115(43):E10127-E10136.
112. Bajaj J, Maliekal TT, Vivien E, et al. Notch signaling in CD66<sup>+</sup> cells drives the progression of human cervical cancers. *Cancer Res.* 2011;71(14):4888-4897.
113. Hopfer O, Zwahlen D, Fey MF, Aebi S. The Notch pathway in ovarian carcinomas and adenomas. *Br J Cancer.* 2005;93(6):709-718.
114. Hallahan AR, Pritchard JI, Hansen S, et al. The SmoA1 mouse model reveals that notch signaling is critical for the growth and survival of sonic hedgehog-induced medulloblastomas. *Cancer Res.* 2004;64(21):7794-7800.
115. Fan X, Matsui W, Khaki L, et al. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. *Cancer Res.* 2006;66(15):7445-7452.
116. Abel EV, Kim EJ, Wu J, et al. The Notch pathway is important in maintaining the cancer stem cell population in pancreatic cancer. *PLoS One.* 2014;9(3):e91983.
117. Koury J, Zhong L, Hao J. Targeting signaling pathways in cancer stem cells for cancer treatment. *Stem Cells Int.* 2017;2017:2925869.
118. Zeng F, Chen H, Zhang Z, et al. Regulating glioma stem cells by hypoxia through the Notch1 and Oct3/4 signaling pathway. *Oncol Lett.* 2018;16(5):6315-6322.
119. Bayin NS, Frenster JD, Sen R, et al. Notch signaling regulates metabolic heterogeneity in glioblastoma stem cells. *Oncotarget.* 2017;8(39):64932-64953.
120. Fox RG, Lytle NK, Jaquish DV, et al. Image-based detection and targeting of therapy resistance in pancreatic adenocarcinoma. *Nature.* 2016;534(7607):407-411.
121. Ito T, Kwon HY, Zimdahl B, et al. Regulation of myeloid leukaemia by the cell-fate determinant Musashi. *Nature.* 2010;466(7307):765-768.
122. Rane SG, Reddy EP. Janus kinases: components of multiple signaling pathways. *Oncogene.* 2000;19(49):5662-5679.
123. Stine RR, Matunis EL. JAK-STAT signaling in stem cells. *Adv Exp Med Biol.* 2013;786:247-267.
124. de Freitas RM, da Costa Maranduba CM. Myeloproliferative neoplasms and the JAK/STAT signaling pathway: an overview. *Rev Bras Hematol Hemoter.* 2015;37(5):348-353.
125. Cook AM, Li L, Ho Y, et al. Role of altered growth factor receptor-mediated JAK2 signaling in growth and maintenance of human acute myeloid leukemia stem cells. *Blood.* 2014;123(18):2826-2837.
126. Chambers I. The molecular basis of pluripotency in mouse embryonic stem cells. *Cloning Stem Cells.* 2004;6(4):386-391.
127. Penuelas S, Anido J, Prieto-Sanchez RM, et al. TGF-beta increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. *Cancer Cell.* 2009;15(4):315-327.
128. Sherry MM, Reeves A, Wu JK, Cochran BH. STAT3 is required for proliferation and maintenance of multipotency in glioblastoma stem cells. *Stem Cells.* 2009;27(10):2383-2392.
129. Zhou J, Wulfkuhle J, Zhang H, et al. Activation of the PTEN/mTOR/STAT3 pathway in breast cancer stem-like cells is required for viability and maintenance. *Proc Natl Acad Sci U S A.* 2007;104(41):16158-16163.
130. Dolatabadi S, Jonasson E, Linden M, et al. JAK-STAT signalling controls cancer stem cell properties including chemotherapy resistance in myxoid liposarcoma. *Int J Cancer.* 2019;145(2):435-449.
131. Park SY, Lee CJ, Choi JH, et al. The JAK2/STAT3/CCND2 axis promotes colorectal cancer stem cell persistence and radioresistance. *J Exp Clin Cancer Res.* 2019;38(1):399.
132. Thomas SJ, Snowden JA, Zeidler MP, Danson SJ. The role of JAK/STAT signalling in the pathogenesis, prognosis and treatment of solid tumours. *Br J Cancer.* 2015;113(3):365-371.
133. Galoczova M, Coates P, Vojtesek B. STAT3, stem cells, cancer stem cells and p63. *Cell Mol Biol Lett.* 2018;23:12.
134. Yoshimatsu T, Kawaguchi D, Oishi K, et al. Non-cell-autonomous action of STAT3 in maintenance of neural precursor cells in the mouse neocortex. *Development.* 2006;133(13):2553-2563.
135. Gupta PB, Chaffer CL, Weinberg RA. Cancer stem cells: mirage or reality? *Nat Med.* 2009;15(9):1010-1012.
136. Lin L, Jou D, Wang Y, et al. STAT3 as a potential therapeutic target in ALDH<sup>+</sup> and CD44<sup>+</sup>/CD24<sup>+</sup> stem cell-like pancreatic cancer cells. *Int J Oncol.* 2016;49(6):2265-2274.
137. Nusslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in *Drosophila*. *Nature.* 1980;287(5785):795-801.
138. Petrova R, Joyner AL. Roles for hedgehog signaling in adult organ homeostasis and repair. *Development.* 2014;141(18):3445-3457.
139. Matsui WH. Cancer stem cell signaling pathways. *Medicine (Baltimore).* 2016;95(1 Suppl 1):S8-S19.
140. Kinzler KW, Bigner SH, Bigner DD, et al. Identification of an amplified, highly expressed gene in a human glioma. *Science.* 1987;236(4797):70-73.
141. Li L, Zhao J, Zhang Q, et al. Cancer cell-derived exosomes promote HCC tumorigenesis through Hedgehog pathway. *Front Oncol.* 2021;11:756205.
142. Merchant AA, Matsui W. Targeting Hedgehog—a cancer stem cell pathway. *Clin Cancer Res.* 2010;16(12):3130-3140.
143. Hutchin ME, Kariapper MS, Grachtchouk M, et al. Sustained hedgehog signaling is required for basal cell carcinoma proliferation and survival: conditional skin tumorigenesis recapitulates the hair growth cycle. *Genes Dev.* 2005;19(2):214-223.
144. Peacock CD, Wang Q, Gesell GS, et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. *Proc Natl Acad Sci U S A.* 2007;104(10):4048-4053.
145. Ghia EM, Rassenti LZ, Neuberg DS, et al. Activation of hedgehog signaling associates with early disease progression in chronic lymphocytic leukemia. *Blood.* 2019;133(25):2651-2663.
146. Zhao C, Chen A, Jamieson CH, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature.* 2009;458(7239):776-779.

147. Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. *Curr Biol*. 2007;17(2):165-172.
148. Ko YC, Choi HS, Liu R, Lee DS. Physalin A, 13,14-seco-16, 24-cyclo-steroid, inhibits stemness of breast cancer cells by regulation of Hedgehog signaling pathway and yes-associated protein 1 (YAP1). *Int J Mol Sci*. 2021;22(16):8718.
149. Zhou H, Xiong Y, Peng L, Wang R, Zhang H, Fu Z. LncRNA-cCSC1 modulates cancer stem cell properties in colorectal cancer via activation of the Hedgehog signaling pathway. *J Cell Biochem*. 2020;121(3):2510-2524.
150. Yang Z, Cui Y, Ni W, Kim S, Xuan Y. Gli1, a potential regulator of esophageal cancer stem cell, is identified as an independent adverse prognostic factor in esophageal squamous cell carcinoma. *J Cancer Res Clin Oncol*. 2017;143(2):243-254.
151. Palle K, Mani C, Tripathi K, Athar M. Aberrant GLI1 activation in DNA damage response, carcinogenesis and chemoresistance. *Cancers (Basel)*. 2015;7(4):2330-2351.
152. Tolba MF, Abdel-Rahman SZ. Pterostilbene, an active component of blueberries, sensitizes colon cancer cells to 5-fluorouracil cytotoxicity. *Sci Rep*. 2015;5:15239.
153. Usui T, Sakurai M, Umata K, et al. Hedgehog signals mediate anti-cancer drug resistance in three-dimensional primary colorectal cancer organoid culture. *Int J Mol Sci*. 2018;19(4):1098.
154. Lu J, Cao LL, Xu Y, et al. FOXC1 modulates stem-like cell properties and chemoresistance through hedgehog and EMT signaling in gastric adenocarcinoma. *Mol Ther*. 2021.
155. Huang F, Shi Q, Li Y, et al. HER2/EGFR-AKT signaling switches TGFbeta from inhibiting cell proliferation to promoting cell migration in breast cancer. *Cancer Res*. 2018;78(21):6073-6085.
156. Battle E, Clevers H. Cancer stem cells revisited. *Nat Med*. 2017;23(10):1124-1134.
157. Najafi M, Farhood B, Mortezaee K. Cancer stem cells (CSCs) in cancer progression and therapy. *J Cell Physiol*. 2019;234(6):8381-8395.
158. Nakano M, Kikushige Y, Miyawaki K, et al. Dedifferentiation process driven by TGF-beta signaling enhances stem cell properties in human colorectal cancer. *Oncogene*. 2019;38(6):780-793.
159. Sheen YY, Kim MJ, Park SA, Park SY, Nam JS. Targeting the transforming growth factor-beta signaling in cancer therapy. *Biomol Ther (Seoul)*. 2013;21(5):323-331.
160. Massague J, Wotton D. Transcriptional control by the TGF-beta/Smad signaling system. *EMBO J*. 2000;19(8):1745-1754.
161. Hirata N, Yamada S, Yanagida S, Ono A, Yasuhiko Y, Kanda Y. Transforming growth factor beta promotes the expansion of cancer stem cells via S1PR3 by ligand-independent Notch activation. *Biol Pharm Bull*. 2022;45(5):649-658.
162. Miller AV, Alvarez SE, Spiegel S, Lebman DA. Sphingosine kinases and sphingosine-1-phosphate are critical for transforming growth factor beta-induced extracellular signal-regulated kinase 1 and 2 activation and promotion of migration and invasion of esophageal cancer cells. *Mol Cell Biol*. 2008;28(12):4142-4151.
163. Hirata N, Yamada S, Shoda T, Kurihara M, Sekino Y, Kanda Y. Sphingosine-1-phosphate promotes expansion of cancer stem cells via S1PR3 by a ligand-independent Notch activation. *Nat Commun*. 2014;5:4806.
164. Yu D, Shin HS, Lee YS, Lee YC. miR-106b modulates cancer stem cell characteristics through TGF-beta/Smad signaling in CD44-positive gastric cancer cells. *Lab Invest*. 2014;94(12):1370-1381.
165. Jiang F, Mu J, Wang X, et al. The repressive effect of miR-148a on TGF beta-SMADs signal pathway is involved in the glabridin-induced inhibition of the cancer stem cells-like properties in hepatocellular carcinoma cells. *PLoS One*. 2014;9(5):e96698.
166. Wu L, Han L, Zhou C, et al. TGF-beta1-induced CK17 enhances cancer stem cell-like properties rather than EMT in promoting cervical cancer metastasis via the ERK1/2-MZF1 signaling pathway. *FEBS J*. 2017;284(18):3000-3017.
167. Shipitsin M, Campbell LL, Argani P, et al. Molecular definition of breast tumor heterogeneity. *Cancer Cell*. 2007;11(3):259-273.
168. Mani SA, Guo W, Liao MJ, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*. 2008;133(4):704-715.
169. Futakuchi M, Lami K, Tachibana Y, Yamamoto Y, Furukawa M, Fukuoka J. The effects of TGF-beta signaling on cancer cells and cancer stem cells in the bone microenvironment. *Int J Mol Sci*. 2019;20(20):5117.
170. Shao T, Song P, Hua H, et al. Gamma synuclein is a novel Twist1 target that promotes TGF-beta-induced cancer cell migration and invasion. *Cell Death Dis*. 2018;9(6):625.
171. Moon H, Ju HL, Chung SI, et al. Transforming growth factor-beta promotes liver tumorigenesis in mice via up-regulation of snail. *Gastroenterology*. 2017;153(5):1378-1391.e6.
172. Chaffer CL, Marjanovic ND, Lee T, et al. Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity. *Cell*. 2013;154(1):61-74.
173. Brown JA, Yonekubo Y, Hanson N, et al. TGF-beta-induced quiescence mediates chemoresistance of tumor-propagating cells in squamous cell carcinoma. *Cell Stem Cell*. 2017;21(5):650-664.e8.
174. Gencer S, Oleinik N, Kim J, et al. TGF-beta receptor I/II trafficking and signaling at primary cilia are inhibited by ceramide to attenuate cell migration and tumor metastasis. *Sci Signal*. 2017;10(502).
175. Natsuzaka M, Whelan KA, Kagawa S, et al. Interplay between Notch1 and Notch3 promotes EMT and tumor initiation in squamous cell carcinoma. *Nat Commun*. 2017;8(1):1758.
176. Tang YA, Chen YF, Bao Y, et al. Hypoxic tumor microenvironment activates GLI2 via HIF-1alpha and TGF-beta2 to promote chemoresistance in colorectal cancer. *Proc Natl Acad Sci U S A*. 2018;115(26):E5990-E5999.
177. Hashemi Goradel N, Najafi M, Salehi E, Farhood B, Mortezaee K. Cyclooxygenase-2 in cancer: a review. *J Cell Physiol*. 2019;234(5):5683-5699.
178. Lee JH, Jung SM, Yang KM, et al. A20 promotes metastasis of aggressive basal-like breast cancers through multi-monoubiquitylation of Snail1. *Nat Cell Biol*. 2017;19(10):1260-1273.
179. Napetschnig J, Wu H. Molecular basis of NF-kappaB signaling. *Annu Rev Biophys*. 2013;42:443-468.
180. Oeckinghaus A, Ghosh S. The NF-kappaB family of transcription factors and its regulation. *Cold Spring Harb Perspect Biol*. 2009;1(4):a000034.
181. Widera D, Kaus A, Kaltschmidt C, Kaltschmidt B. Neural stem cells, inflammation and NF-kappaB: basic principle of mainte-

- nance and repair or origin of brain tumours? *J Cell Mol Med.* 2008;12(2):459-470.
182. Hoessel B, Schmid JA. The complexity of NF-kappaB signaling in inflammation and cancer. *Mol Cancer.* 2013;12:86.
  183. Schmid JA, Birbach A. IkappaB kinase beta (IKKbeta/IKK2/IKBKB)-a key molecule in signaling to the transcription factor NF-kappaB. *Cytokine Growth Factor Rev.* 2008;19(2):157-165.
  184. Guzman ML, Neering SJ, Upchurch D, et al. Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. *Blood.* 2001;98(8):2301-2307.
  185. Cheng JH, Zhang WJ, Zhu JF, et al. CaMKIIgamma regulates the viability and self-renewal of acute myeloid leukaemia stem-like cells by the Alox5/NF-kappaB pathway. *Int J Lab Hematol.* 2021;43(4):699-706.
  186. Rajasekhar VK, Studer L, Gerald W, Socci ND, Scher HI. Tumour-initiating stem-like cells in human prostate cancer exhibit increased NF-kappaB signalling. *Nat Commun.* 2011;2:162.
  187. Gonzalez-Torres C, Gaytan-Cervantes J, Vazquez-Santillan K, et al. NF-kappaB participates in the stem cell phenotype of ovarian cancer cells. *Arch Med Res.* 2017;48(4):343-351.
  188. Myant KB, Cammareri P, McGhee EJ, et al. ROS production and NF-kappaB activation triggered by RAC1 facilitate WNT-driven intestinal stem cell proliferation and colorectal cancer initiation. *Cell Stem Cell.* 2013;12(6):761-773.
  189. Xu J, Zhang Z, Qian M, et al. Cullin-7 (CUL7) is overexpressed in glioma cells and promotes tumorigenesis via NF-kappaB activation. *J Exp Clin Cancer Res.* 2020;39(1):59.
  190. Garner JM, Fan M, Yang CH, et al. Constitutive activation of signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappaB signaling in glioblastoma cancer stem cells regulates the Notch pathway. *J Biol Chem.* 2013;288(36):26167-26176.
  191. Song L, Liu L, Wu Z, et al. TGF-beta induces miR-182 to sustain NF-kappaB activation in glioma subsets. *J Clin Invest.* 2012;122(10):3563-3578.
  192. Rinkenbaugh AL, Baldwin AS. The NF-kappaB pathway and cancer stem cells. *Cells.* 2016;5(2):16.
  193. Liu M, Sakamaki T, Casimiro MC, et al. The canonical NF-kappaB pathway governs mammary tumorigenesis in transgenic mice and tumor stem cell expansion. *Cancer Res.* 2010;70(24):10464-10473.
  194. Cao Y, Luo JL, Karin M. IkappaB kinase alpha kinase activity is required for self-renewal of ErbB2/Her2-transformed mammary tumor-initiating cells. *Proc Natl Acad Sci U S A.* 2007;104(40):15852-15857.
  195. Zhang W, Tan W, Wu X, et al. A NIK-IKKalpha module expands ErbB2-induced tumor-initiating cells by stimulating nuclear export of p27/Kip1. *Cancer Cell.* 2013;23(5):647-659.
  196. Kumar S, Nandi A, Singh S, et al. Dll1(+) quiescent tumor stem cells drive chemoresistance in breast cancer through NF-kappaB survival pathway. *Nat Commun.* 2021;12(1):432.
  197. Tasian SK, Teachey DT, Rheingold SR. Targeting the PI3K/mTOR pathway in pediatric hematologic malignancies. *Front Oncol.* 2014;4:108.
  198. Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, Bilanges B. The emerging mechanisms of isoform-specific PI3K signalling. *Nat Rev Mol Cell Biol.* 2010;11(5):329-341.
  199. Wang Q, Chen X, Hay N. Akt as a target for cancer therapy: more is not always better (lessons from studies in mice). *Br J Cancer.* 2017;117(2):159-163.
  200. Wei X, Luo L, Chen J. Roles of mTOR signaling in tissue regeneration. *Cells.* 2019;8(9):1075.
  201. Sancak Y, Thoreen CC, Peterson TR, et al. PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell.* 2007;25(6):903-915.
  202. Duzgun Z, Eroglu Z, Biray Avci C. Role of mTOR in glioblastoma. *Gene.* 2016;575(2 Pt 1):187-190.
  203. Yip CK, Murata K, Walz T, Sabatini DM, Kang SA. Structure of the human mTOR complex I and its implications for rapamycin inhibition. *Mol Cell.* 2010;38(5):768-774.
  204. Knowles MA, Platt FM, Ross RL, Hurst CD. Phosphatidylinositol 3-kinase (PI3K) pathway activation in bladder cancer. *Cancer Metastasis Rev.* 2009;28(3-4):305-316.
  205. Matsubara S, Tsukasa K, Kuwahata T, Takao S. Prevention of Akt phosphorylation is a key to targeting cancer stem-like cells by mTOR inhibition. *Hum Cell.* 2020;33(4):1197-1203.
  206. Ghayad SE, Cohen PA. Inhibitors of the PI3K/Akt/mTOR pathway: new hope for breast cancer patients. *Recent Pat Anticancer Drug Discov.* 2010;5(1):29-57.
  207. Dubrovskaya 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. *Proc Natl Acad Sci U S A.* 2009;106(1):268-273.
  208. Korkaya H, Paulson A, Charafe-Jauffret E, et al. Regulation of mammary stem/progenitor cells by PTEN/Akt/beta-catenin signaling. *PLoS Biol.* 2009;7(6):e1000121.
  209. Yang CF, Yang GD, Huang TJ, et al. EB-virus latent membrane protein 1 potentiates the stemness of nasopharyngeal carcinoma via preferential activation of PI3K/AKT pathway by a positive feedback loop. *Oncogene.* 2016;35(26):3419-3431.
  210. Li X, Wu C, Chen N, et al. PI3K/Akt/mTOR signaling pathway and targeted therapy for glioblastoma. *Oncotarget.* 2016;7(22):33440-33450.
  211. Wolin EM. PI3K/Akt/mTOR pathway inhibitors in the therapy of pancreatic neuroendocrine tumors. *Cancer Lett.* 2013;335(1):1-8.
  212. Tan AC. Targeting the PI3K/Akt/mTOR pathway in non-small cell lung cancer (NSCLC). *Thorac Cancer.* 2020;11(3):511-518.
  213. Shorning BY, Dass MS, Smalley MJ, Pearson HB. The PI3K-AKT-mTOR pathway and prostate cancer: at the crossroads of AR, MAPK, and WNT signaling. *Int J Mol Sci.* 2020;21(12):4507.
  214. Miricescu D, Totan A, Stanescu S, II, Badoiu SC, Stefani C, Greabu M. PI3K/AKT/mTOR signaling pathway in breast cancer: from molecular landscape to clinical aspects. *Int J Mol Sci.* 2020;22(1):173.
  215. Bai J, Chen WB, Zhang XY, et al. HIF-2alpha regulates CD44 to promote cancer stem cell activation in triple-negative breast cancer via PI3K/AKT/mTOR signaling. *World J Stem Cells.* 2020;12(1):87-99.
  216. Corominas-Faja B, Cufi S, Oliveras-Ferraro C, et al. Nuclear reprogramming of luminal-like breast cancer cells generates Sox2-overexpressing cancer stem-like cellular states harboring

- transcriptional activation of the mTOR pathway. *Cell Cycle*. 2013;12(18):3109-3124.
217. Douville J, Beaulieu R, Balicki D. ALDH1 as a functional marker of cancer stem and progenitor cells. *Stem Cells Dev*. 2009;18(1):17-25.
  218. Tan X, Zhang Z, Yao H, Shen L. Tim-4 promotes the growth of colorectal cancer by activating angiogenesis and recruiting tumor-associated macrophages via the PI3K/AKT/mTOR signaling pathway. *Cancer Lett*. 2018;436:119-128.
  219. Wen W, Han T, Chen C, et al. Cyclin G1 expands liver tumor-initiating cells by Sox2 induction via Akt/mTOR signaling. *Mol Cancer Ther*. 2013;12(9):1796-1804.
  220. Deng J, Bai X, Feng X, et al. Inhibition of PI3K/Akt/mTOR signaling pathway alleviates ovarian cancer chemoresistance through reversing epithelial-mesenchymal transition and decreasing cancer stem cell marker expression. *BMC Cancer*. 2019;19(1):618.
  221. Chang L, Graham PH, Hao J, et al. Acquisition of epithelial-mesenchymal transition and cancer stem cell phenotypes is associated with activation of the PI3K/Akt/mTOR pathway in prostate cancer radioresistance. *Cell Death Dis*. 2013;4:e875.
  222. Marhold M, Tomasich E, El-Gazzar A, et al. HIF1alpha regulates mTOR signaling and viability of prostate cancer stem cells. *Mol Cancer Res*. 2015;13(3):556-564.
  223. Matsumoto K, Arao T, Tanaka K, et al. mTOR signal and hypoxia-inducible factor-1 alpha regulate CD133 expression in cancer cells. *Cancer Res*. 2009;69(18):7160-7164.
  224. Yang Z, Zhang L, Ma A, et al. Transient mTOR inhibition facilitates continuous growth of liver tumors by modulating the maintenance of CD133<sup>+</sup> cell populations. *PLoS One*. 2011;6(12):e28405.
  225. Jung MJ, Rho JK, Kim YM, et al. Upregulation of CXCR4 is functionally crucial for maintenance of stemness in drug-resistant non-small cell lung cancer cells. *Oncogene*. 2013;32(2):209-221.
  226. Bleau AM, Hambarzumyan 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):226-235.
  227. Airiau K, Mahon FX, Josselin M, Jeanneteau M, Belloc F. PI3K/mTOR pathway inhibitors sensitize chronic myeloid leukemia stem cells to nilotinib and restore the response of progenitors to nilotinib in the presence of stem cell factor. *Cell Death Dis*. 2013;4:e827.
  228. You M, Jin J, Liu Q, Xu Q, Shi J, Hou Y. PPARalpha promotes cancer cell Glut1 transcription repression. *J Cell Biochem*. 2017;118(6):1556-1562.
  229. Hou Y, Moreau F, Chadee K. PPARgamma is an E3 ligase that induces the degradation of NFkappaB/p65. *Nat Commun*. 2012;3:1300.
  230. Zhang W, Xu Y, Xu Q, Shi H, Shi J, Hou Y. PPARdelta promotes tumor progression via activation of Glut1 and SLC1-A5 transcription. *Carcinogenesis*. 2017;38(7):748-755.
  231. Yousefnia S, Momenzadeh S, Seyed Foroootan F, Ghaedi K, Nasr Esfahani MH. The influence of peroxisome proliferator-activated receptor gamma (PPARgamma) ligands on cancer cell tumorigenicity. *Gene*. 2018;649:14-22.
  232. Zhang Y, Zhang X, Wang J, et al. Expression and function of PPARs in cancer stem cells. *Curr Stem Cell Res Ther*. 2016;11(3):226-234.
  233. Han S, Wei R, Zhang X, et al. CPT1A/2-mediated FAO enhancement—a metabolic target in radioresistant breast cancer. *Front Oncol*. 2019;9:1201.
  234. Ma XL, Sun YF, Wang BL, et al. Sphere-forming culture enriches liver cancer stem cells and reveals Stearoyl-CoA desaturase 1 as a potential therapeutic target. *BMC Cancer*. 2019;19(1):760.
  235. Kuramoto K, Yamamoto M, Suzuki S, et al. Inhibition of the lipid droplet-peroxisome proliferator-activated receptor alpha axis suppresses cancer stem cell properties. *Genes (Basel)*. 2021;12(1):99.
  236. Ito K, Carracedo A, Weiss D, et al. A PML-PPAR-delta pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. *Nat Med*. 2012;18(9):1350-1358.
  237. Kramer K, Wu J, Crowe DL. Tumor suppressor control of the cancer stem cell niche. *Oncogene*. 2016;35(32):4165-4178.
  238. Prost S, Relouzat F, Spentchian M, et al. Erosion of the chronic myeloid leukaemia stem cell pool by PPARgamma agonists. *Nature*. 2015;525(7569):380-383.
  239. Wang Y, Tan H, Xu D, et al. The combinatory effects of PPAR-gamma agonist and survivin inhibition on the cancer stem-like phenotype and cell proliferation in bladder cancer cells. *Int J Mol Med*. 2014;34(1):262-268.
  240. Basu-Roy U, Han E, Rattanakorn K, et al. PPARgamma agonists promote differentiation of cancer stem cells by restraining YAP transcriptional activity. *Oncotarget*. 2016;7(38):60954-60970.
  241. Giampietri C, Petrunaro S, Cordella M, et al. Lipid storage and autophagy in melanoma cancer cells. *Int J Mol Sci*. 2017;18(6):1271.
  242. Bigoni-Ordóñez GD, Ortiz-Sánchez E, Rosendo-Chalma P, Valencia-González HA, Aceves C, García-Carranca A. Molecular iodine inhibits the expression of stemness markers on cancer stem-like cells of established cell lines derived from cervical cancer. *BMC Cancer*. 2018;18(1):928.
  243. Liu L, Yang Z, Xu Y, et al. Inhibition of oxidative stress-elicited AKT activation facilitates PPARgamma agonist-mediated inhibition of stem cell character and tumor growth of liver cancer cells. *PLoS One*. 2013;8(8):e73038.
  244. Paget S. The distribution of secondary growths in cancer of the breast. *Cancer Metastasis Rev*. 1989;8(2):98-101.
  245. Liu Q, Zhang H, Jiang X, Qian C, Liu Z, Luo D. Factors involved in cancer metastasis: a better understanding to “seed and soil” hypothesis. *Mol Cancer*. 2017;16(1):176.
  246. Adjei IM, Blanka S. Modulation of the tumor microenvironment for cancer treatment: a biomaterials approach. *J Funct Biomater*. 2015;6(1):81-103.
  247. Scherer HJ. Structural development in gliomas. *Am J Cancer*. 1938;34(3):333-351.
  248. Calabrese C, Poppleton H, Kocak M, et al. A perivascular niche for brain tumor stem cells. *Cancer Cell*. 2007;11(1):69-82.
  249. Androutsellis-Theotokis A, Rueger MA, Park DM, et al. Angiogenic factors stimulate growth of adult neural stem cells. *PLoS One*. 2010;5(2):e9414.
  250. Shen Q, Wang Y, Kokovay E, et al. Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. *Cell Stem Cell*. 2008;3(3):289-300.
  251. Kokovay E, Goderie S, Wang Y, et al. Adult SVZ lineage cells home to and leave the vascular niche via differential responses to SDF1/CXCR4 signaling. *Cell Stem Cell*. 2010;7(2):163-173.

252. Irani S, Dehghan A. The expression and functional significance of vascular endothelial-cadherin, CD44, and vimentin in oral squamous cell carcinoma. *J Int Soc Prev Community Dent.* 2018;8(2):110-117.
253. Paulis YW, Huijbers EJ, van der Schaft DW, et al. CD44 enhances tumor aggressiveness by promoting tumor cell plasticity. *Oncotarget.* 2015;6(23):19634-19646.
254. Zhu B, Zhou L, Yu L, et al. Evaluation of the correlation of vasculogenic mimicry, ALDH1, KAI1 and microvessel density in the prediction of metastasis and prognosis in colorectal carcinoma. *BMC Surg.* 2017;17(1):47.
255. King P, Dong H, Liu Q, et al. ALDH1 expression and vasculogenic mimicry are positively associated with poor prognosis in patients with breast cancer. *Cell Physiol Biochem.* 2018;49(3):961-970.
256. Izawa Y, Kashii-Magaribuchi K, Yoshida K, et al. Stem-like human breast cancer cells initiate vasculogenic mimicry on matrigel. *Acta Histochem Cytochem.* 2018;51(6):173-183.
257. Liu TJ, Sun BC, Zhao XL, et al. CD133<sup>+</sup> cells with cancer stem cell characteristics associates with vasculogenic mimicry in triple-negative breast cancer. *Oncogene.* 2013;32(5):544-553.
258. Mirshahi P, Rafii A, Vincent L, et al. Vasculogenic mimicry of acute leukemic bone marrow stromal cells. *Leukemia.* 2009;23(6):1039-1048.
259. Yan GN, Yang L, Lv YF, et al. Endothelial cells promote stem-like phenotype of glioma cells through activating the Hedgehog pathway. *J Pathol.* 2014;234(1):11-22.
260. Heiden KB, Williamson AJ, Doscas ME, et al. The sonic hedgehog signaling pathway maintains the cancer stem cell self-renewal of anaplastic thyroid cancer by inducing snail expression. *J Clin Endocrinol Metab.* 2014;99(11):E2178-E2187.
261. Yu D, Shin HS, Lee YS, Lee D, Kim S, Lee YC. Genistein attenuates cancer stem cell characteristics in gastric cancer through the downregulation of Gli1. *Oncol Rep.* 2014;31(2):673-678.
262. Zhu TS, Costello MA, Talsma CE, et al. Endothelial cells create a stem cell niche in glioblastoma by providing NOTCH ligands that nurture self-renewal of cancer stem-like cells. *Cancer Res.* 2011;71(18):6061-6072.
263. Lu J, Ye X, Fan F, et al. Endothelial cells promote the colorectal cancer stem cell phenotype through a soluble form of Jagged-1. *Cancer Cell.* 2013;23(2):171-185.
264. Charles N, Ozawa T, Squatrito M, et al. Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells. *Cell Stem Cell.* 2010;6(2):141-152.
265. Hovinga KE, Shimizu F, Wang R, et al. Inhibition of notch signaling in glioblastoma targets cancer stem cells via an endothelial cell intermediate. *Stem Cells.* 2010;28(6):1019-1029.
266. Hambardzumyan D, Becher OJ, Rosenblum MK, Pandolfi PP, Manova-Todorova K, Holland EC. PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma in vivo. *Genes Dev.* 2008;22(4):436-448.
267. Chouaib S, Noman MZ, Kosmatopoulos K, Curran MA. Hypoxic stress: obstacles and opportunities for innovative immunotherapy of cancer. *Oncogene.* 2017;36(4):439-445.
268. Kim H, Lin Q, Glazer PM, Yun Z. The hypoxic tumor microenvironment in vivo selects the cancer stem cell fate of breast cancer cells. *Breast Cancer Res.* 2018;20(1):16.
269. Comerford KM, Wallace TJ, Karhausen J, Louis NA, Montalto MC, Colgan SP. Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (MDR1) gene. *Cancer Res.* 2002;62(12):3387-3394.
270. McIntyre A, Hulikova A, Ledaki I, et al. Disrupting hypoxia-induced bicarbonate transport acidifies tumor cells and suppresses tumor growth. *Cancer Res.* 2016;76(13):3744-3755.
271. Storci G, Bertoni S, De Carolis S, et al. Slug/beta-catenin-dependent proinflammatory phenotype in hypoxic breast cancer stem cells. *Am J Pathol.* 2013;183(5):1688-1697.
272. Csermely P, Hodsagi J, Korcsmaros T, et al. 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. *Semin Cancer Biol.* 2015;30:42-51.
273. Jeong H, Kim S, Hong BJ, et al. Tumor-associated macrophages enhance tumor hypoxia and aerobic glycolysis. *Cancer Res.* 2019;79(4):795-806.
274. Semenza GL. The hypoxic tumor microenvironment: a driving force for breast cancer progression. *Biochim Biophys Acta.* 2016;1863(3):382-391.
275. Barnhart BC, Simon MC. Metastasis and stem cell pathways. *Cancer Metastasis Rev.* 2007;26(2):261-271.
276. Hung SC, Deng WP, Yang WK, et al. Mesenchymal stem cell targeting of microscopic tumors and tumor stroma development monitored by noninvasive in vivo positron emission tomography imaging. *Clin Cancer Res.* 2005;11(21):7749-7756.
277. Nishi H, Nakada T, Kyo S, Inoue M, Shay JW, Isaka K. Hypoxia-inducible factor 1 mediates upregulation of telomerase (hTERT). *Mol Cell Biol.* 2004;24(13):6076-6083.
278. Schoning JP, Monteiro M, Gu W. Drug resistance and cancer stem cells: the shared but distinct roles of hypoxia-inducible factors HIF1alpha and HIF2alpha. *Clin Exp Pharmacol Physiol.* 2017;44(2):153-161.
279. Seo EJ, Kim DK, Jang IH, et al. Hypoxia-NOTCH1-SOX2 signaling is important for maintaining cancer stem cells in ovarian cancer. *Oncotarget.* 2016;7(34):55624-55638.
280. Zhang Z, Han H, Rong Y, et al. Hypoxia potentiates gemcitabine-induced stemness in pancreatic cancer cells through AKT/Notch1 signaling. *J Exp Clin Cancer Res.* 2018;37(1):291.
281. Qiang L, Wu T, Zhang HW, et al. HIF-1alpha is critical for hypoxia-mediated maintenance of glioblastoma stem cells by activating Notch signaling pathway. *Cell Death Differ.* 2012;19(2):284-294.
282. Yang L, Lin C, Wang L, Guo H, Wang X. Hypoxia and hypoxia-inducible factors in glioblastoma multiforme progression and therapeutic implications. *Exp Cell Res.* 2012;318(19):2417-2426.
283. Zeisberg M, Strutz F, Muller GA. Role of fibroblast activation in inducing interstitial fibrosis. *J Nephrol.* 2000;13(3):S111-S120.
284. Nishi H, Nakada T, Hokamura M, et al. Hypoxia-inducible factor-1 transactivates transforming growth factor-beta3 in trophoblast. *Endocrinology.* 2004;145(9):4113-4118.
285. Hung SP, Ho JH, Shih YR, Lo T, Lee OK. Hypoxia promotes proliferation and osteogenic differentiation potentials of human mesenchymal stem cells. *J Orthop Res.* 2012;30(2):260-266.

286. Hung SP, Yang MH, Tseng KF, Lee OK. Hypoxia-induced secretion of TGF-beta1 in mesenchymal stem cell promotes breast cancer cell progression. *Cell Transplant*. 2013;22(10):1869-1882.
287. Liu Y, Chen C, Qian P, et al. Gd-metallofullerenol nanomaterial as non-toxic breast cancer stem cell-specific inhibitor. *Nat Commun*. 2015;6:5988.
288. Hou PC, Li YH, Lin SC, et al. Hypoxia-induced downregulation of DUSP-2 phosphatase drives colon cancer stemness. *Cancer Res*. 2017;77(16):4305-4316.
289. Dong XF, Liu TQ, Zhi XT, et al. COX-2/PGE2 axis regulates HIF2alpha activity to promote hepatocellular carcinoma hypoxic response and reduce the sensitivity of sorafenib treatment. *Clin Cancer Res*. 2018;24(13):3204-3216.
290. Miranda A, Hamilton PT, Zhang AW, et al. Cancer stemness, intratumoral heterogeneity, and immune response across cancers. *Proc Natl Acad Sci U S A*. 2019;116(18):9020-9029.
291. Raggi C, Correnti M, Sica A, et al. Cholangiocarcinoma stem-like subset shapes tumor-initiating niche by educating associated macrophages. *J Hepatol*. 2017;66(1):102-115.
292. Tao W, Chu C, Zhou W, et al. Dual role of WISP1 in maintaining glioma stem cells and tumor-supportive macrophages in glioblastoma. *Nat Commun*. 2020;11(1):3015.
293. Guo X, Pan Y, Gutmann DH. Genetic and genomic alterations differentially dictate low-grade glioma growth through cancer stem cell-specific chemokine recruitment of T cells and microglia. *Neuro Oncol*. 2019;21(10):1250-1262.
294. Zhou W, Ke SQ, Huang Z, et al. Periostin secreted by glioblastoma stem cells recruits M2 tumour-associated macrophages and promotes malignant growth. *Nat Cell Biol*. 2015;17(2):170-182.
295. Guo X, Zhao Y, Yan H, et al. Single tumor-initiating cells evade immune clearance by recruiting type II macrophages. *Genes Dev*. 2017;31(3):247-259.
296. Wu A, Wei J, Kong LY, et al. Glioma cancer stem cells induce immunosuppressive macrophages/microglia. *Neuro Oncol*. 2010;12(11):1113-1125.
297. Fan QM, Jing YY, Yu GF, et al. Tumor-associated macrophages promote cancer stem cell-like properties via transforming growth factor-beta1-induced epithelial-mesenchymal transition in hepatocellular carcinoma. *Cancer Lett*. 2014;352(2):160-168.
298. Jinushi M, Chiba S, Yoshiyama H, et al. Tumor-associated macrophages regulate tumorigenicity and anticancer drug responses of cancer stem/initiating cells. *Proc Natl Acad Sci U S A*. 2011;108(30):12425-12430.
299. Lu H, Clauser KR, Tam WL, et al. A breast cancer stem cell niche supported by juxtacrine signalling from monocytes and macrophages. *Nat Cell Biol*. 2014;16(11):1105-1117.
300. Shi Y, Ping YF, Zhou W, et al. Tumour-associated macrophages secrete pleiotrophin to promote PTPRZ1 signalling in glioblastoma stem cells for tumour growth. *Nat Commun*. 2017;8:15080.
301. Wan S, Zhao E, Kryczek I, et al. Tumor-associated macrophages produce interleukin 6 and signal via STAT3 to promote expansion of human hepatocellular carcinoma stem cells. *Gastroenterology*. 2014;147(6):1393-1404.
302. Korkaya H, Liu S, Wicha MS. Breast cancer stem cells, cytokine networks, and the tumor microenvironment. *J Clin Invest*. 2011;121(10):3804-3809.
303. Yang Z, He L, Lin K, et al. The KMT1A-GATA3-STAT3 circuit is a novel self-renewal signaling of human bladder cancer stem cells. *Clin Cancer Res*. 2017;23(21):6673-6685.
304. Lee Y, Shin JH, Longmire M, et al. CD44<sup>+</sup> cells in head and neck squamous cell carcinoma suppress T-cell-mediated immunity by selective constitutive and inducible expression of PD-L1. *Clin Cancer Res*. 2016;22(14):3571-3581.
305. Guillerey C, Huntington ND, Smyth MJ. Targeting natural killer cells in cancer immunotherapy. *Nat Immunol*. 2016;17(9):1025-1036.
306. Codony-Servat J, Rosell R. Cancer stem cells and immunoresistance: clinical implications and solutions. *Transl Lung Cancer Res*. 2015;4(6):689-703.
307. Tallero R, Todaro M, Di Franco S, et al. Human NK cells selective targeting of colon cancer-initiating cells: a role for natural cytotoxicity receptors and MHC class I molecules. *J Immunol*. 2013;190(5):2381-2390.
308. Castriconi R, Daga A, Dondero A, et al. NK cells recognize and kill human glioblastoma cells with stem cell-like properties. *J Immunol*. 2009;182(6):3530-3539.
309. Pietra G, Manzini C, Vitale M, et al. Natural killer cells kill human melanoma cells with characteristics of cancer stem cells. *Int Immunol*. 2009;21(7):793-801.
310. Yin T, Wang G, He S, Liu Q, Sun J, Wang Y. Human cancer cells with stem cell-like phenotype exhibit enhanced sensitivity to the cytotoxicity of IL-2 and IL-15 activated natural killer cells. *Cell Immunol*. 2016;300:41-45.
311. Patankar MS, Jing Y, Morrison JC, et al. Potent suppression of natural killer cell response mediated by the ovarian tumor marker CA125. *Gynecol Oncol*. 2005;99(3):704-713.
312. Cristiani CM, Turdo A, Ventura V, et al. Accumulation of circulating CCR7(+) natural killer cells marks melanoma evolution and reveals a CCL19-dependent metastatic pathway. *Cancer Immunol Res*. 2019;7(5):841-852.
313. Ames E, Canter RJ, Grossenbacher SK, et al. NK cells preferentially target tumor cells with a cancer stem cell phenotype. *J Immunol*. 2015;195(8):4010-4019.
314. Ames E, Canter RJ, Grossenbacher SK, et al. Enhanced targeting of stem-like solid tumor cells with radiation and natural killer cells. *Oncoimmunology*. 2015;4(9):e1036212.
315. Wang B, Wang Q, Wang Z, et al. Metastatic consequences of immune escape from NK cell cytotoxicity by human breast cancer stem cells. *Cancer Res*. 2014;74(20):5746-5757.
316. Jin H, Kim HJ. NK cells lose their cytotoxicity function against cancer stem cell-rich radiotherapy-resistant breast cancer cell populations. *Int J Mol Sci*. 2021;22(17):9639.
317. Song M, He J, Pan QZ, et al. Cancer-associated fibroblast-mediated cellular crosstalk supports hepatocellular carcinoma progression. *Hepatology*. 2021;73(5):1717-1735.
318. Yoon H, Tang CM, Banerjee S, et al. TGF-beta1-mediated transition of resident fibroblasts to cancer-associated fibroblasts promotes cancer metastasis in gastrointestinal stromal tumor. *Oncogenesis*. 2021;10(2):13.
319. Chen Z, Yan X, Li K, Ling Y, Kang H. Stromal fibroblast-derived MFAP5 promotes the invasion and migration of breast

- cancer cells via Notch1/slugg signaling. *Clin Transl Oncol.* 2020;22(4):522-531.
320. Costa A, Kieffer Y, Scholer-Dahirel A, et al. Fibroblast heterogeneity and immunosuppressive environment in human breast cancer. *Cancer Cell.* 2018;33(3):463-479.e10.
  321. Chen S, Morine Y, Tokuda K, et al. Cancer-associated fibroblast-induced M2-polarized macrophages promote hepatocellular carcinoma progression via the plasminogen activator inhibitor-1 pathway. *Int J Oncol.* 2021;59(2):59.
  322. Zhang H, Deng T, Liu R, et al. CAF secreted miR-522 suppresses ferroptosis and promotes acquired chemo-resistance in gastric cancer. *Mol Cancer.* 2020;19(1):43.
  323. Liu J, Ren L, Li S, et al. The biology, function, and applications of exosomes in cancer. *Acta Pharm Sin B.* 2021;11(9):2783-2797.
  324. Nallasamy P, Nimmakayala RK, Karmakar S, et al. Pancreatic tumor microenvironment factor promotes cancer stemness via SPPI-CD44 axis. *Gastroenterology.* 2021;161(6):1998-2013.e7.
  325. Li Y, Wang R, Xiong S, et al. Cancer-associated fibroblasts promote the stemness of CD24(+) liver cells via paracrine signaling. *J Mol Med.* 2019;97(2):243-255.
  326. Zhao Q, Huang L, Qin G, et al. Cancer-associated fibroblasts induce monocytic myeloid-derived suppressor cell generation via IL-6/exosomal miR-21-activated STAT3 signaling to promote cisplatin resistance in esophageal squamous cell carcinoma. *Cancer Lett.* 2021;518:35-48.
  327. Atiya H, Frisbie L, Pressimone C, Coffman L. Mesenchymal stem cells in the tumor microenvironment. *Adv Exp Med Biol.* 2020;1234:31-42.
  328. Barcellos-de-Souza P, Comito G, Pons-Segura C, et al. Mesenchymal stem cells are recruited and activated into carcinoma-associated fibroblasts by prostate cancer microenvironment-derived TGF-beta1. *Stem Cells.* 2016;34(10):2536-2547.
  329. Oya Y, Hayakawa Y, Koike K. Tumor microenvironment in gastric cancers. *Cancer Sci.* 2020;111(8):2696-2707.
  330. Wang Y, Lan W, Xu M, et al. Cancer-associated fibroblast-derived SDF-1 induces epithelial-mesenchymal transition of lung adenocarcinoma via CXCR4/beta-catenin/PPARdelta signalling. *Cell Death Dis.* 2021;12(2):214.
  331. Goulet CR, Champagne A, Bernard G, et al. Cancer-associated fibroblasts induce epithelial-mesenchymal transition of bladder cancer cells through paracrine IL-6 signalling. *BMC Cancer.* 2019;19(1):137.
  332. Shibata M, Hoque MO. Targeting cancer stem cells: a strategy for effective eradication of cancer. *Cancers (Basel).* 2019;11(5):732.
  333. Desai A, Yan Y, Gerson SL. Concise reviews: cancer stem cell targeted therapies: toward clinical success. *Stem Cells Transl Med.* 2019;8(1):75-81.
  334. Zhang D, Tang DG, Rycak J. Cancer stem cells: regulation programs, immunological properties and immunotherapy. *Semin Cancer Biol.* 2018;52(Pt 2):94-106.
  335. Pan Q, Li Q, Liu S, et al. Concise review: targeting cancer stem cells using immunologic approaches. *Stem Cells.* 2015;33(7):2085-2092.
  336. Ferrarotto R, Mitani Y, Diao L, et al. Activating NOTCH1 mutations define a distinct subgroup of patients with adenoid cystic carcinoma who have poor prognosis, propensity to bone and liver metastasis, and potential responsiveness to Notch1 inhibitors. *J Clin Oncol.* 2017;35(3):352-360.
  337. Samon JB, Castillo-Martin M, Hadler M, et al. Preclinical analysis of the gamma-secretase inhibitor PF-03084014 in combination with glucocorticoids in T-cell acute lymphoblastic leukemia. *Mol Cancer Ther.* 2012;11(7):1565-1575.
  338. Wei P, Walls M, Qiu M, et al. Evaluation of selective gamma-secretase inhibitor PF-03084014 for its antitumor efficacy and gastrointestinal safety to guide optimal clinical trial design. *Mol Cancer Ther.* 2010;9(6):1618-1628.
  339. Pandya K, Meeke K, Clementz AG, et al. Targeting both Notch and ErbB-2 signalling pathways is required for prevention of ErbB-2-positive breast tumour recurrence. *Br J Cancer.* 2011;105(6):796-806.
  340. Morgan KM, Fischer BS, Lee FY, et al. Gamma secretase inhibition by BMS-906024 enhances efficacy of paclitaxel in lung adenocarcinoma. *Mol Cancer Ther.* 2017;16(12):2759-2769.
  341. Sosa Iglesias V, Theys J, Groot AJ, et al. Synergistic effects of NOTCH/gamma-secretase inhibition and standard of care treatment modalities in non-small cell lung cancer cells. *Front Oncol.* 2018;8:460.
  342. Huynh C, Polisenio L, Segura MF, et al. The novel gamma secretase inhibitor RO4929097 reduces the tumor initiating potential of melanoma. *PLoS One.* 2011;6(9):e25264.
  343. De Jesus-Acosta A, Laheru D, Maitra A, et al. A phase II study of the gamma secretase inhibitor RO4929097 in patients with previously treated metastatic pancreatic adenocarcinoma. *Invest New Drugs.* 2014;32(4):739-745.
  344. Tolcher AW, Messersmith WA, Mikulski SM, et al. Phase I study of RO4929097, a gamma secretase inhibitor of notch signaling, in patients with refractory metastatic or locally advanced solid tumors. *J Clin Oncol.* 2012;30(19):2348-2353.
  345. Lee SM, Moon J, Redman BG, et al. Phase 2 study of RO4929097, a gamma-secretase inhibitor, in metastatic melanoma: SWOG 0933. *Cancer.* 2015;121(3):432-440.
  346. Pan E, Supko JG, Kaley TJ, et al. Phase I study of RO4929097 with bevacizumab in patients with recurrent malignant glioma. *J Neurooncol.* 2016;130(3):571-579.
  347. Peereboom DM, Ye X, Mikkelsen T, et al. A phase II and pharmacodynamic trial of RO4929097 for patients with recurrent/progressive glioblastoma. *Neurosurgery.* 2021;88(2):246-251.
  348. Pant S, Jones SF, Kurkjian CD, et al. A first-in-human phase I study of the oral Notch inhibitor, LY900009, in patients with advanced cancer. *Eur J Cancer.* 2016;56:1-9.
  349. Krop I, Demuth T, Guthrie T, et al. Phase I pharmacologic and pharmacodynamic study of the gamma secretase (Notch) inhibitor MK-0752 in adult patients with advanced solid tumors. *J Clin Oncol.* 2012;30(19):2307-2313.
  350. Aung KL, El-Khoueiry AB, Gelmon K, et al. A multi-arm phase I dose escalating study of an oral NOTCH inhibitor BMS-986115 in patients with advanced solid tumours. *Invest New Drugs.* 2018;36(6):1026-1036.
  351. Strosberg JR, Yeatman T, Weber J, et al. A phase II study of RO4929097 in metastatic colorectal cancer. *Eur J Cancer.* 2012;48(7):997-1003.
  352. Gounder MM, Rosenbaum E, Wu N, et al. A phase Ib/II randomized study of RO4929097, a gamma-secretase or Notch

- inhibitor with or without vismodegib, a Hedgehog inhibitor, in advanced sarcoma. *Clin Cancer Res.* 2022;28(8):1586-1594.
353. Messersmith WA, Shapiro GI, Cleary JM, et al. A phase I, dose-finding study in patients with advanced solid malignancies of the oral gamma-secretase inhibitor PF-03084014. *Clin Cancer Res.* 2015;21(1):60-67.
  354. Wang L, Zi H, Luo Y, et al. Inhibition of Notch pathway enhances the anti-tumor effect of docetaxel in prostate cancer stem-like cells. *Stem Cell Res Ther.* 2020;11(1):258.
  355. Sahebjam S, Bedard PL, Castonguay V, et al. A phase I study of the combination of ro4929097 and cediranib in patients with advanced solid tumours (PJC-004/NCI 8503). *Br J Cancer.* 2013;109(4):943-949.
  356. Kappes DJ, He X, He X. CD4-CD8 lineage commitment: an inside view. *Nat Immunol.* 2005;6(8):761-766.
  357. Yun J, Pannuti A, Espinoza I, et al. Crosstalk between PKC $\alpha$  and Notch-4 in endocrine-resistant breast cancer cells. *Oncogenesis.* 2013;2:e60.
  358. Kummar S, O'Sullivan Coyne G, Do KT, et al. Clinical activity of the gamma-secretase inhibitor PF-03084014 in adults with desmoid tumors (aggressive fibromatosis). *J Clin Oncol.* 2017;35(14):1561-1569.
  359. Gavai AV, Quesnelle C, Norris D, et al. Discovery of clinical candidate BMS-906024: a potent pan-Notch inhibitor for the treatment of leukemia and solid tumors. *ACS Med Chem Lett.* 2015;6(5):523-527.
  360. Chan D, Kaplan J, Gordon G, Desai J. Activity of the gamma secretase inhibitor ALI01 in desmoid tumors: a case report of 2 adult cases. *Curr Oncol.* 2021;28(5):3659-3667.
  361. Lehal R, Zaric J, Vigolo M, et al. Pharmacological disruption of the Notch transcription factor complex. *Proc Natl Acad Sci U S A.* 2020;117(28):16292-16301.
  362. Astudillo L, Da Silva TG, Wang Z, et al. The small molecule IMR-1 inhibits the Notch transcriptional activation complex to suppress tumorigenesis. *Cancer Res.* 2016;76(12):3593-3603.
  363. Ferrarotto R, Eckhardt G, Patnaik A, et al. A phase I dose-escalation and dose-expansion study of brontictuzumab in subjects with selected solid tumors. *Ann Oncol.* 2018;29(7):1561-1568.
  364. Herrera-Rios D, Li G, Khan D, et al. A computational guided, functional validation of a novel therapeutic antibody proposes Notch signaling as a clinical relevant and druggable target in glioma. *Sci Rep.* 2020;10(1):16218.
  365. Yen WC, Fischer MM, Axelrod F, et al. Targeting Notch signaling with a Notch2/Notch3 antagonist (tarextumab) inhibits tumor growth and decreases tumor-initiating cell frequency. *Clin Cancer Res.* 2015;21(9):2084-2095.
  366. Hu ZI, Bendell JC, Bullock A, et al. A randomized phase II trial of nab-paclitaxel and gemcitabine with tarextumab or placebo in patients with untreated metastatic pancreatic cancer. *Cancer Med.* 2019;8(11):5148-5157.
  367. Yang L, Xie G, Fan Q, Xie J. Activation of the Hedgehog-signaling pathway in human cancer and the clinical implications. *Oncogene.* 2010;29(4):469-481.
  368. Sekulic A, Migden MR, Oro AE, et al. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. *N Engl J Med.* 2012;366(23):2171-2179.
  369. Catenacci DV, Junttila MR, Karrison T, et al. Randomized phase Ib/II study of gemcitabine plus placebo or vismodegib, a Hedgehog pathway inhibitor, in patients with metastatic pancreatic cancer. *J Clin Oncol.* 2015;33(36):4284-4292.
  370. Cohen DJ, Christos PJ, Kindler HL, et al. Vismodegib (V), a Hedgehog (HH) pathway inhibitor, combined with FOLFOX for first-line therapy of patients (pts) with advanced gastric and gastroesophageal junction (GEJ) carcinoma: a New York Cancer Consortium led phase II randomized study. *J Clin Oncol.* 2013;31(15):4011.
  371. McCleary-Wheeler AL, Carr RM, Palmer SR, et al. Phase 1 trial of vismodegib and erlotinib combination in metastatic pancreatic cancer. *Pancreatol.* 2020;20(1):101-109.
  372. Kaye SB, Fehrenbacher L, Holloway R, et al. A phase II, randomized, placebo-controlled study of vismodegib as maintenance therapy in patients with ovarian cancer in second or third complete remission. *Clin Cancer Res.* 2012;18(23):6509-6518.
  373. Rudin CM, Hann CL, Laterra J, et al. Treatment of medulloblastoma with Hedgehog pathway inhibitor GDC-0449. *N Engl J Med.* 2009;361(12):1173-1178.
  374. Robinson GW, Orr BA, Wu G, et al. Vismodegib exerts targeted efficacy against recurrent sonic hedgehog-subgroup medulloblastoma: results from phase II pediatric brain tumor consortium studies PBTC-025B and PBTC-032. *J Clin Oncol.* 2015;33(24):2646-2654.
  375. Gajjar A, Stewart CF, Ellison DW, et al. Phase I study of vismodegib in children with recurrent or refractory medulloblastoma: a pediatric brain tumor consortium study. *Clin Cancer Res.* 2013;19(22):6305-6312.
  376. Petrirena GJ, Masliah-Planchon J, Sala Q, et al. Recurrent extraneural sonic hedgehog medulloblastoma exhibiting sustained response to vismodegib and temozolomide monotherapies and inter-metastatic molecular heterogeneity at progression. *Oncotarget.* 2018;9(11):10175-10183.
  377. Frappaz D, Barritault M, Montane L, et al. MEVITEM—a phase I/II trial of vismodegib + temozolomide vs temozolomide in patients with recurrent/refractory medulloblastoma with sonic hedgehog pathway activation. *Neuro Oncol.* 2021;23(11):1949-1960.
  378. Fosko SW, Chu MB, Armbrecht E, et al. Efficacy, rate of tumor response, and safety of a short course (12–24 weeks) of oral vismodegib in various histologic subtypes (infiltrative, nodular, and superficial) of high-risk or locally advanced basal cell carcinoma, in an open-label, prospective case series clinical trial. *J Am Acad Dermatol.* 2020;82(4):946-954.
  379. Lear JT, Migden MR, Lewis KD, et al. Long-term efficacy and safety of sonidegib in patients with locally advanced and metastatic basal cell carcinoma: 30-month analysis of the randomized phase 2 BOLT study. *J Eur Acad Dermatol Venereol.* 2018;32(3):372-381.
  380. Dummer R, Guminski A, Gutzmer R, et al. Long-term efficacy and safety of sonidegib in patients with advanced basal cell carcinoma: 42-month analysis of the phase II randomized, double-blind BOLT study. *Br J Dermatol.* 2020;182(6):1369-1378.
  381. Xie P, Lefrancois P. Efficacy, safety, and comparison of sonic hedgehog inhibitors in basal cell carcinomas: a systematic review and meta-analysis. *J Am Acad Dermatol.* 2018;79(6):1089-1100.e17.
  382. Danial C, Sarin KY, Oro AE, Chang AL. An investigator-initiated open-label trial of sonidegib in advanced basal cell



- carcinoma patients resistant to vismodegib. *Clin Cancer Res.* 2016;22(6):1325-1329.
383. Cazet AS, Hui MN, Elsworth BL, et al. Targeting stromal remodeling and cancer stem cell plasticity overcomes chemoresistance in triple negative breast cancer. *Nat Commun.* 2018;9(1):2897.
  384. Cortes JE, Heidel FH, Hellmann A, et al. Randomized comparison of low dose cytarabine with or without glasdegib in patients with newly diagnosed acute myeloid leukemia or high-risk myelodysplastic syndrome. *Leukemia.* 2019;33(2):379-389.
  385. Heuser M, Smith BD, Fiedler W, et al. Clinical benefit of glasdegib plus low-dose cytarabine in patients with de novo and secondary acute myeloid leukemia: long-term analysis of a phase II randomized trial. *Ann Hematol.* 2021;100(5):1181-1194.
  386. Cortes JE, Douglas Smith B, Wang ES, et al. Glasdegib in combination with cytarabine and daunorubicin in patients with AML or high-risk MDS: phase 2 study results. *Am J Hematol.* 2018;93(11):1301-1310.
  387. Lauth M, Bergstrom A, Shimokawa T, Toftgard R. Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists. *Proc Natl Acad Sci U S A.* 2007;104(20):8455-8460.
  388. Huang L, Walter V, Hayes DN, Onaitis M. Hedgehog-GLI signaling inhibition suppresses tumor growth in squamous lung cancer. *Clin Cancer Res.* 2014;20(6):1566-1575.
  389. Didiasova M, Singh R, Wilhelm J, et al. Pirfenidone exerts antifibrotic effects through inhibition of GLI transcription factors. *FASEB J.* 2017;31(5):1916-1928.
  390. Polydorou C, Mpekris F, Papageorgis P, Voutouri C, Stylianopoulos T. Pirfenidone normalizes the tumor microenvironment to improve chemotherapy. *Oncotarget.* 2017;8(15):24506-24517.
  391. Zou WJ, Huang Z, Jiang TP, et al. Pirfenidone inhibits proliferation and promotes apoptosis of hepatocellular carcinoma cells by inhibiting the Wnt/beta-catenin signaling pathway. *Med Sci Monit.* 2017;23:6107-6113.
  392. Beauchamp EM, Ringer L, Bulut G, et al. Arsenic trioxide inhibits human cancer cell growth and tumor development in mice by blocking Hedgehog/GLI pathway. *J Clin Invest.* 2011;121(1):148-160.
  393. Chang KJ, Yin JZ, Huang H, Li B, Yang MH. Arsenic trioxide inhibits the growth of cancer stem cells derived from small cell lung cancer by downregulating stem cell-maintenance factors and inducing apoptosis via the Hedgehog signaling blockade. *Transl Lung Cancer Res.* 2020;9(4):1379-1396.
  394. Han JB, Sang F, Chang JJ, et al. Arsenic trioxide inhibits viability of pancreatic cancer stem cells in culture and in a xenograft model via binding to SHH-Gli. *Onco Targets Ther.* 2013;6:1129-1138.
  395. Nakamura S, Nagano S, Nagao H, et al. Arsenic trioxide prevents osteosarcoma growth by inhibition of GLI transcription via DNA damage accumulation. *PLoS One.* 2013;8(7):e69466.
  396. Ally MS, Ransohoff K, Sarin K, et al. Effects of combined treatment with arsenic trioxide and itraconazole in patients with refractory metastatic basal cell carcinoma. *JAMA Dermatol.* 2016;152(4):452-456.
  397. Wall JA, Klempner SJ, Arend RC. The anti-DKK1 antibody DKN-01 as an immunomodulatory combination partner for the treatment of cancer. *Expert Opin Investig Drugs.* 2020;29(7):639-644.
  398. Betella I, Turbitt WJ, Szul T, et al. Wnt signaling modulator DKK1 as an immunotherapeutic target in ovarian cancer. *Gynecol Oncol.* 2020;157(3):765-774.
  399. Wall JA, Meza-Perez S, Scalise CB, et al. Manipulating the Wnt/beta-catenin signaling pathway to promote anti-tumor immune infiltration into the TME to sensitize ovarian cancer to ICB therapy. *Gynecol Oncol.* 2021;160(1):285-294.
  400. Wise DR, Schneider JA, Armenia J, et al. Dickkopf-1 can lead to immune evasion in metastatic castration-resistant prostate cancer. *JCO Precis Oncol.* 2020;4:PO.20.00097.
  401. Haas MS, Kagey MH, Heath H, Schuerpf F, Rottman JB, Newman W. mDKN-01, a novel anti-DKK1 mAb, enhances innate immune responses in the tumor microenvironment. *Mol Cancer Res.* 2021;19(4):717-725.
  402. Ryan DP, Murphy JE, Mahalingam D, et al. Current results of a phase I study of DKN-01, an anti-DKK1 antibody, in combination with paclitaxel (P) in patients (pts) with advanced DKK1+ esophageal cancer (EC) or gastro-esophageal junction tumors (GEJ). *J Clin Oncol.* 2016;34(15):e15525.
  403. Klempner SJ, Bendell JC, Villaflor VM, et al. Safety, efficacy, and biomarker results from a phase Ib study of the anti-DKK1 antibody DKN-01 in combination with pembrolizumab in advanced esophagogastric cancers. *Mol Cancer Ther.* 2021;20(11):2240-2249.
  404. Goyal L, Sirard C, Schrag M, et al. Phase I and biomarker study of the Wnt pathway modulator DKN-01 in combination with gemcitabine/cisplatin in advanced biliary tract cancer. *Clin Cancer Res.* 2020;26(23):6158-6167.
  405. Gurney A, Axelrod F, Bond CJ, et al. Wnt pathway inhibition via the targeting of Frizzled receptors results in decreased growth and tumorigenicity of human tumors. *Proc Natl Acad Sci U S A.* 2012;109(29):11717-11722.
  406. Davis SL, Cardin DB, Shahda S, et al. A phase 1b dose escalation study of Wnt pathway inhibitor vantictumab in combination with nab-paclitaxel and gemcitabine in patients with previously untreated metastatic pancreatic cancer. *Invest New Drugs.* 2020;38(3):821-830.
  407. Diamond JR, Becerra C, Richards D, et al. Phase Ib clinical trial of the anti-frizzled antibody vantictumab (OMP-18R5) plus paclitaxel in patients with locally advanced or metastatic HER2-negative breast cancer. *Breast Cancer Res Treat.* 2020;184(1):53-62.
  408. Karvonen H, Perttala R, Niininen W, et al. Wnt5a and ROR1 activate non-canonical Wnt signaling via RhoA in TCF3-PBX1 acute lymphoblastic leukemia and highlight new treatment strategies via Bcl-2 co-targeting. *Oncogene.* 2019;38(17):3288-3300.
  409. Chen Y, Chen L, Yu J, et al. Cirmtuzumab blocks Wnt5a/ROR1 stimulation of NF-kappaB to repress autocrine STAT3 activation in chronic lymphocytic leukemia. *Blood.* 2019;134(13):1084-1094.
  410. Choi MY, Widhopf GF 2nd, Ghia EM, et al. Phase I trial: cirmtuzumab inhibits ROR1 signaling and stemness signatures in patients with chronic lymphocytic leukemia. *Cell Stem Cell.* 2018;22(6):951-959.e3.
  411. Zhang S, Zhang H, Ghia EM, et al. Inhibition of chemotherapy resistant breast cancer stem cells by a ROR1 specific antibody. *Proc Natl Acad Sci U S A.* 2019;116(4):1370-1377.
  412. Yu J, Chen L, Cui B, et al. Cirmtuzumab inhibits Wnt5a-induced Rac1 activation in chronic lymphocytic

- leukemia treated with ibrutinib. *Leukemia*. 2017;31(6):1333-1339.
413. Xu Q, Liu G, Yuan X, et al. Antigen-specific T-cell response from dendritic cell vaccination using cancer stem-like cell-associated antigens. *Stem Cells*. 2009;27(8):1734-1740.
  414. Luo H, Zeng C, Fang C, Seeruttun SR, Lv L, Wang W. A new strategy using ALDHhigh-CD8<sup>+</sup>T cells to inhibit tumorigenesis. *PLoS One*. 2014;9(8):e103193.
  415. Visus C, Wang Y, Lozano-Leon A, et al. Targeting ALDH(bright) human carcinoma-initiating cells with ALDH1A1-specific CD8(+) T cells. *Clin Cancer Res*. 2011;17(19):6174-6184.
  416. Zhao L, Yang Y, Zhou P, et al. Targeting CD133high colorectal cancer cells in vitro and in vivo with an asymmetric bispecific antibody. *J Immunother*. 2015;38(6):217-228.
  417. Guo Y, Feng K, Wang Y, Han W. Targeting cancer stem cells by using chimeric antigen receptor-modified T cells: a potential and curable approach for cancer treatment. *Protein Cell*. 2018;9(6):516-526.
  418. Feng KC, Guo YL, Liu Y, et al. Cocktail treatment with EGFR-specific and CD133-specific chimeric antigen receptor-modified T cells in a patient with advanced cholangiocarcinoma. *J Hematol Oncol*. 2017;10(1):4.
  419. Dai H, Tong C, Shi D, et al. Efficacy and biomarker analysis of CD133-directed CAR T cells in advanced hepatocellular carcinoma: a single-arm, open-label, phase II trial. *Oncoimmunology*. 2020;9(1):1846926.
  420. Emler DR, Gupta P, Holgado-Madruga M, et al. Targeting a glioblastoma cancer stem-cell population defined by EGF receptor variant III. *Cancer Res*. 2014;74(4):1238-1249.
  421. O'Rourke DM, Nasrallah MP, Desai A, et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci Transl Med*. 2017;9(399):eaaa0984.
  422. Goff SL, Morgan RA, Yang JC, et al. Pilot trial of adoptive transfer of chimeric antigen receptor-transduced T cells targeting EGFRvIII in patients with glioblastoma. *J Immunother*. 2019;42(4):126-135.
  423. Brown RL, Reinke LM, Damerow MS, et al. CD44 splice isoform switching in human and mouse epithelium is essential for epithelial-mesenchymal transition and breast cancer progression. *J Clin Invest*. 2011;121(3):1064-1074.
  424. Zhao S, Chen C, Chang K, et al. CD44 expression level and isoform contributes to pancreatic cancer cell plasticity, invasiveness, and response to therapy. *Clin Cancer Res*. 2016;22(22):5592-5604.
  425. Gotoda T, Matsumura Y, Kondo H, et al. Expression of CD44 variants and its association with survival in pancreatic cancer. *Jpn J Cancer Res*. 1998;89(10):1033-1040.
  426. Haist C, Schulte E, Bartels N, et al. CD44v6-targeted CAR T-cells specifically eliminate CD44 isoform 6 expressing head/neck squamous cell carcinoma cells. *Oral Oncol*. 2021;116:105259.
  427. de Sousa e Melo F, Kurtova AV, Harnoss JM, et al. A distinct role for Lgr5(+) stem cells in primary and metastatic colon cancer. *Nature*. 2017;543(7647):676-680.
  428. Sultan M, Coyle KM, Vidovic D, Thomas ML, Gujar S, Marcato P. Hide-and-seek: the interplay between cancer stem cells and the immune system. *Carcinogenesis*. 2017;38(2):107-118.
  429. Ghiringhelli F, Menard C, Martin F, Zitvogel L. The role of regulatory T cells in the control of natural killer cells: relevance during tumor progression. *Immunol Rev*. 2006;214:229-238.

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