

# Targeting cancer stem cells by using the nanoparticles

In-Sun Hong<sup>1,2,\*</sup>  
Gyu-Beom Jang<sup>1,2,\*</sup>  
Hwa-Yong Lee<sup>3</sup>  
Jeong-Seok Nam<sup>1,2</sup>

<sup>1</sup>Laboratory of Tumor Suppressor, Lee Gil Ya Cancer and Diabetes Institute, <sup>2</sup>Department of Molecular Medicine, School of Medicine, Gachon University, Incheon, <sup>3</sup>The Faculty of Liberal Arts, Jungwon University, Chungbuk, Republic of Korea

\*These authors contributed equally to this work

**Abstract:** Cancer stem cells (CSCs) have been shown to be markedly resistant to conventional cancer treatments such as chemotherapy and radiation therapy. Therefore, therapeutic strategies that selectively target CSCs will ultimately lead to better cancer treatments. Currently, accessible conventional therapeutic agents mainly eliminate the bulk tumor but do not eliminate CSCs. Therefore, the discovery and improvement of CSC-targeting therapeutic agents are necessary. Nanoparticles effectively inhibit multiple types of CSCs by targeting specific signaling pathways (Wnt/ $\beta$ -catenin, Notch, transforming growth factor- $\beta$ , and hedgehog signaling) and/or specific markers (aldehyde dehydrogenases, CD44, CD90, and CD133) critically involved in CSC function and maintenance. In this review article, we summarized a number of findings to provide current information about their therapeutic potential of nanoparticles in various cancer cell types and CSCs.

**Keywords:** ALDH, Wnt/ $\beta$ -catenin, Hedgehog, Notch, TGF- $\beta$  signaling, CD44, CD133

## Introduction

Current systemic therapies for cancer such as chemotherapy and radiation are partly effective in inhibiting bulk tumor cell growth and blocking tumor formation. However, a minority of cancer patients with metastases and a quarter of those with early stage disease are at a high risk of relapse due to cancer stem cells (CSCs). The CSCs concept was first suggested to describe a small population of acute myeloid leukemia, which contribute to tumor growth, metastasis, and recurrence.<sup>1</sup> The identification of leukemic CSCs prompted further investigation into other solid tumor types. Recently, CSCs have been identified in almost all cancer types, including pancreatic,<sup>2</sup> gastric,<sup>3</sup> brain,<sup>4</sup> colon,<sup>5</sup> prostate,<sup>6</sup> and lung cancers.<sup>7</sup> CSCs are generally defined by a unique set of functional characteristics: 1) CSCs can be purified by specific biomarkers and/or signaling pathways,<sup>8–11</sup> 2) CSCs are capable of generating colonies in suspension culture conditions,<sup>12</sup> and 3) CSCs are resistant to chemotherapeutic agents<sup>13–15</sup> and radiation.<sup>15,16</sup> These CSC-specific features suggest that the majority of conventional treatments, such as chemotherapy and radiation, can kill the bulk tumor cells but may ultimately fail to induce durable clinical results because conventional approaches are not as effective at eliminating CSCs; thus, the remaining CSCs are able to form new colonies and regenerate tumors in patients. Therefore, new therapeutic strategies that selectively target CSCs will ultimately improve cancer treatments.<sup>17</sup> Currently, new treatment modalities in the form of nanoparticles (NPs)-targeting CSC-specific markers or signaling pathways are available or under investigation.<sup>18,19</sup> Hirsch et al first introduced the effects of NPs in breast cancer by using silica-gold nanoshells.<sup>20</sup> Recently, extensive research has identified various types of NP-targeting CSCs, including NP-mediated hyperthermia,<sup>21</sup> curcumin-based NPs,<sup>22</sup> and liposomes-based NPs.<sup>23</sup>

Correspondence: Jeong-Seok Nam  
Laboratory of Tumor Suppressor, Lee Gil Ya Cancer and Diabetes Institute, Gachon University, 7-45 Songdo-dong, Yeonsu-ku, Incheon 406-840, Republic of Korea  
Tel +82 32 899 6072  
Fax +82 32 899 6350  
Email namjs@gachon.ac.kr

Hwa-Yong Lee  
The Faculty of Liberal Arts, Jungwon University, Chungbuk, 367-805, Republic of Korea  
Email hylee0702@gmail.com

These NP-based therapeutic approaches provide advantages over the small molecule pharmaceutical agents-based therapeutic strategies. However, there is not enough information currently available to make a conclusive statement regarding the therapeutic potential of these NPs. Therefore, in this review article, we provide an overview of the characteristics of CSC and discuss the various NPs-targeting CSC-specific signaling pathways and biomarkers involved in the development and maintenance of CSCs.

## Cancer stem cells: implications for tumorigenesis

### Identification and isolation of CSCs in various cancers

The majority of cells in bulk tumors have limited self-renewal and tumor-initiating capacity; indeed, only a small subpopulation of cancer cells retains extensive self-renewal and tumorigenic potential. These higher tumorigenic populations are called CSCs or cancer initiating cells. The CSC model of tumor development has been proposed to explain the high degree of phenotypic and functional heterogeneity among cancer cells.<sup>24</sup> In the 1960s, Bruce et al found that only 1%–4% of the total number of mouse leukemic cells transplanted *in vivo* formed colonies and initiated tumor growth in the recipient spleen.<sup>25</sup> The identification of leukemic CSCs prompted further investigation into other solid tumor types. CSCs were first identified and isolated from a solid tumor breast cancer. Breast CSCs are typically characterized with a CD44<sup>+</sup>/CD24<sup>-low</sup> phenotype and test positive for the epithelial cell adhesion molecule, also known as the epithelial-specific marker.<sup>26,27</sup> As few as 100 cells with these molecular characteristics grew rapidly and extensively *in vitro* and generated new tumors *in vivo*.<sup>26</sup> Recently, extensive research has identified CSCs in different types of solid tumors, including brain,<sup>28</sup> colon,<sup>29</sup> head and neck,<sup>30</sup> liver,<sup>31</sup> lung,<sup>32,33</sup> and other cancers.<sup>34</sup> CSCs are typically resistant to various chemotherapeutic drugs<sup>13–15</sup> and radiation therapies.<sup>15,16</sup> These CSC-specific features suggest that the majority of conventional cancer treatments, such as surgery, chemotherapy, and radiation therapy, can kill the bulk tumor cells but may ultimately fail to induce durable clinical responses because they are not as effective at killing CSCs; thus, the remaining CSCs are able to form colonies and initiate new tumors in patients.

### CSCs as a selective therapeutic target

Despite promising treatment results, current therapeutic strategies against cancers have many limitations that frequently

lead to metastatic failure and a high risk of recurrence and mortality. The most common cause of an unsatisfactory clinical response is resistance to conventional therapeutic strategies. CSC-mediated therapeutic resistance was demonstrated in different tumors, including brain,<sup>35</sup> breast,<sup>13</sup> colorectal,<sup>36</sup> leukemia,<sup>37</sup> melanoma,<sup>38</sup> and pancreatic<sup>2</sup> cancers. Furthermore, CSC-mediated radiation resistance was reported in brain<sup>28</sup> and breast<sup>39</sup> cancers. Therefore, the development of novel therapeutics and control strategies that selectively target CSCs without unduly affecting normal and healthy cells is urgently required.<sup>40–42</sup> A significantly improved therapeutic outcome could be achieved by the selective targeting of subtle differences in surface marker expression or signaling pathways when compared with bulk tumor cells. Since their identification in various cancer types, multiple CSC therapeutic strategies targeting specific stem cell surface markers and unique signaling pathways have been developed for several solid tumors. These alternative therapeutic strategies can successfully eliminate CSCs and thereby prevent tumor recurrence and metastasis.

### Targeting CSC-specific markers Aldehyde dehydrogenase activity

Aldehyde dehydrogenases (ALDHs) are a superfamily of enzymes that play a key role in the metabolism of various aldehyde derivatives. These enzymes were first described and identified as conferring resistance to cyclophosphamide and other alkylating agents in hematopoietic stem/progenitor cells.<sup>43</sup> Recent studies have demonstrated that high levels of ALDH activity are associated with enhanced tumorigenicity and chemoresistance in CSCs.<sup>44–46</sup> Indeed, higher ALDH activity has been observed in the highly tumorigenic colon CSC subpopulations with an EpCAM<sup>high</sup>/CD44<sup>+</sup> phenotype.<sup>47</sup> Moreover, ALDH activity was used to predict a poor clinical outcome in breast cancer patients due to its correlation with tumorigenicity and chemoresistance.<sup>45</sup> Ma et al have found that ALDH was preferentially expressed in the CD133-positive subpopulation and could be used to better characterize the tumorigenic CD133-positive CSC population in liver cancers.<sup>46</sup> The hierarchical tumorigenic potential was determined as CD133<sup>+</sup>ALDH<sup>+</sup> > CD133<sup>+</sup>ALDH<sup>-</sup> > CD133<sup>-</sup>ALDH<sup>-</sup>.<sup>46</sup> Therefore, it is reasonable that ALDH can act as a potential prognostic marker and therapeutic target for the treatment of various cancer types. NPs loaded with low-dose decitabine, a DNA hypermethylation inhibitor, that significantly reduced clonogenic growth and ALDH-positive stem-like population in malignant breast cancer by inhibiting cancer cell growth and stem cell phenotypes.<sup>48</sup> Chenna et al have also developed polymeric NP from

poly(lactic-*co*-glycolic acid) conjugated with polyethylene glycol encapsulating hedgehog signaling inhibitor (HPI).<sup>49</sup> This HPI-incorporated polymeric NP showed a remarkably increased apoptotic effects in secondary mutational pancreatic cells by suppressing the growth of ALDH-positive CSCs in the orthotopic Pa03C xenograft.<sup>49</sup>

## CD44

CD44 is a transmembrane receptor for hyaluronic acid and has recently been identified in CSCs from numerous solid tumors, including breast,<sup>26</sup> bladder,<sup>50</sup> cervical,<sup>51</sup> colon,<sup>52</sup> gastric,<sup>53</sup> lung,<sup>54</sup> ovarian,<sup>55</sup> pancreatic,<sup>56</sup> and prostate cancers.<sup>57</sup> Enhanced CD44 expression in CSCs suggests that CD44 is an attractive new target for the treatment of multiple cancer types. Yang et al demonstrated that CD44<sup>+</sup> subpopulations were more tumorigenic than their CD44<sup>-</sup> counterparts in nude mice.<sup>58</sup> Therefore, there is an urgent need for the development of effective CD44-targeted therapeutic strategies. Polymeric micelles have amphiphilic block copolymers with a spherical inner core and outer shell. Hydrophobic inner core serves as a container for hydrophobic drug, while hydrophilic outer core provides structural stability and extends the circulation time.<sup>59</sup> Shah et al have reported that paclitaxel-incorporated micelles showed a remarkably increased therapeutic efficacy and specificity in CD44-positive metastatic ovarian cancer cells isolated from patients.<sup>60</sup> In addition, liposomal NP can potentially enhance the delivery of suicide gene or chemotherapeutic drugs to the breast and colon cancers.<sup>61,62</sup> Wang et al have reported that anti-CD44 antibody-incorporated liposomal NP delivery system loaded with suicide gene or doxorubicin could specifically target the CD44-positive hepatocellular carcinoma cells and effectively induce apoptotic cell death.<sup>23</sup> A more recent report suggests that a hyaluronic acid-coated chitosan NPs loaded with 5-fluorouracil (5-FU)/oxaliplatin showed a significantly enhanced cytotoxicity compared with either 5-FU or oxaliplatin alone in human colorectal cancer cells, which overexpress CD44.<sup>63,64</sup> Although several NPs for targeting CD44-positive cells are recently developed, their therapeutic effects in vivo have not yet been demonstrated convincingly.

## CD90

Various CD cell surface proteins were used as a marker to identify CSCs in human liver cancer cell lines and clinical samples. CD90 is a glycosyl phosphatidylinositol-anchored membrane glycoprotein of the immunoglobulin superfamily expressed mainly on the surface of leukocytes.<sup>65</sup> This marker is involved in cell-cell and cell-adhesive matrix interactions. Yang et al found that the CD90-positive

subpopulation showed a distinct higher tumorigenicity and metastatic potential in a mouse xenograft model when compared with CD90-negative counterparts.<sup>58,66</sup> All clinical tumor specimens and ~90% of blood samples from liver cancer patients contained a CD45<sup>-</sup>/CD90<sup>+</sup> subpopulation capable of initiating and maintaining tumor formation in an immunodeficient mouse model.<sup>58,66</sup> Based on the aforementioned findings, CD90 can be used to identify potential hepatic CSCs from tumor specimens and blood samples of liver cancer patients. Thus, CD90 may be an important prognostic marker and effective therapeutic target for the treatment of various hepatic cancer types. Wang et al have reported that anti-CD90 antibody-mediated water-soluble CdSe core nanocrystals loaded with photosensitizers such as trifluoperazine could specifically target the CD90-positive leukemia CSCs and sensitize leukemia CSCs to UV irradiation and promote apoptotic cell death.<sup>67</sup>

## CD133

The stem cell marker CD133, also known as prominin-1, is a transmembrane glycoprotein.<sup>68</sup> CD133 is highly expressed in immature hematopoietic stem cells and endothelial progenitor cells.<sup>69</sup> In glioblastoma, CD133-positive subpopulations were shown to be considerably more tumorigenic than CD133-negative compartments, which form the bulk tumor. Moreover, a poor clinical outcome is associated with increased CD133 expression in glioblastoma patients.<sup>70</sup> In addition, the protein is overexpressed in various cancer types, including metastatic colorectal cancer,<sup>71</sup> ovarian cancer,<sup>72</sup> glioblastoma,<sup>73</sup> and gastric carcinoma.<sup>74</sup> Recent evidence suggests that a subpopulation of CD133-positive cancer cells have a significant key molecule that confers resistance to conventional chemotherapeutic agents.<sup>75</sup> Moreover, radiation-exposed CD133-positive liver cancer cells induce enhanced radioresistance via MAPK/ERK signaling activation and tumor development in a xenograft model compared with CD133-negative cells, suggesting that the CD133-positive populations confer radioresistance in hepatocellular carcinoma.<sup>76</sup> Therefore, the surface marker CD133 should be a potential target to improve the efficiency of treating CSCs. NanoCurc™, a recently developed polymeric NP encapsulating curcumin, significantly reduced clonogenic growth and CD133-positive stem-like population in malignant brain tumors.<sup>22</sup> In addition, NPs conjugated with PEGylated poly(lactic-*co*-glycolic acid) receive extensive attention and are used widely because of their safety in the clinical trials.<sup>77</sup> Interestingly, salinomycin-loaded NPs conjugated with CD133 aptamers selectively inhibit CD133-positive osteosarcoma both in vitro and in vivo.<sup>78</sup>

## Targeting CSC-specific signaling pathways

### Wnt/ $\beta$ -catenin signaling pathway

The Wnt/ $\beta$ -catenin signaling pathway is an evolutionarily well-conserved pathway that regulates various physiologic processes, including development, growth, regeneration, and self-renewal.<sup>79</sup> The Wnt/ $\beta$ -catenin pathway is activated when a Wnt ligand binds to the transmembrane complex comprising the Frizzled receptor, leading to binding of the low-density lipoprotein-related receptor. This leads to the suppression of glycogen synthase kinase-3 $\beta$ -binding protein, thereby improving the stability of  $\beta$ -catenin, which then accumulates and is translocated to the nucleus. Consequently,  $\beta$ -catenin forms a complex with the transcription factor/lymphocyte enhancer factor and activates the expression of Wnt target gene such as c-myc and cyclin D1.<sup>80–82</sup> Altered activation of Wnt/ $\beta$ -catenin signaling is a key feature of various cancer types and is considered to be critical for epithelial–mesenchymal transitions that favor tumor metastasis.<sup>83</sup> Aberrant activation of the Wnt/ $\beta$ -catenin signaling has recently been implicated in several types of cancers, including ovarian,<sup>84</sup> colon,<sup>85</sup> and breast cancer.<sup>86</sup> Interestingly, this signaling pathway was initially reported as a key CSC signaling pathway in acute myeloid leukemia<sup>87</sup> and was reported to be involved in the maintenance and function of CSCs from breast,<sup>88</sup> colon,<sup>89</sup> liver,<sup>90</sup> and lung<sup>91</sup> cancers. Therefore, selective targeting of Wnt/ $\beta$ -catenin signaling may be considered as an effective therapeutic strategy for the treatment of various types of cancer. Yallapu et al have developed curcumin-loaded poly(lactic acid-co-glycolic acid) (PLGA) NP to provide increased bioavailability of curcumin in the blood circulation.<sup>92</sup> These curcumin-incorporated PLGA NPs showed a remarkably increased apoptotic effects in cisplatin-resistant ovarian cancer cells by suppressing Wnt/ $\beta$ -catenin signaling component  $\beta$ -catenin.<sup>92</sup> Similarly, Tang et al loaded 5-FU to NPs, and demonstrated that these 5-FU NPs can effectively inhibit the peritoneal dissemination of colorectal cancer cells,<sup>93</sup> which overexpress Wnt/ $\beta$ -catenin signaling components.<sup>94,95</sup>

### Notch signaling pathway

The Notch signaling pathway is an evolutionarily conserved developmental pathway governing a broad spectrum of events, such as cell differentiation decisions and the formation of precise tissue patterns.<sup>96</sup> Notch signaling also plays an important role in regulating stem cell maintenance and differentiation.<sup>24,97–99</sup> This signaling pathway is activated through four Notch receptors (Notch 1–4) that can interact with five Jagged family ligands.<sup>100,101</sup> It has been suggested

that Notch 1 and 2 share the highest degree of sequence and structural similarities and are ubiquitously distributed in a wide variety of tissues. In contrast, Notch 2 and 4 are expressed in a limited range of cell types (eg, vascular endothelial and smooth muscle cells). While the oncogenic effects of Notch signaling in several types of tumors are well-documented, its potential role in CSCs has only recently emerged. A recent study suggested that inhibition of Notch signaling sharply decreased self-renewal, clonogenic, and the tumorigenic potential of glioblastoma CSCs.<sup>102</sup> In addition, inhibition of Notch signaling led to a decrease of the CSC-like subpopulation and increased the susceptibility of CSCs to radiation-induced apoptosis in glioblastomas.<sup>103</sup> Aberrant activation of Notch signaling has been observed in CD133<sup>+</sup> liver CSC subpopulations when compared with CD133<sup>-</sup> subpopulations.<sup>104</sup> Consequentially, targeting Notch signaling pathway may provide an effective therapeutic approach in the treatment of various cancers. Despite the inhibition of Notch signaling can be achieved by  $\gamma$ -secretase inhibitors, inhibitory peptides,<sup>105</sup> and antibodies.<sup>106</sup> Clinical application of these inhibitory agents are currently restricted by their considerable side effects.<sup>107,108</sup> Rosenholm et al have developed mesoporous silica NPs (MSNPs) as drug-delivery systems with high selectivity and demonstrated that these drug-conjugated NPs are suitable for targeted delivery of hydrophobic drugs in vitro.<sup>109,110</sup> They demonstrate that these drug-conjugated MSNPs have significantly enhanced cytotoxicity by selective targeting the Notch signaling in various cancer cell types, such as cervical and breast cancer cells.<sup>111</sup> In addition, Steg et al have developed Jagged1 (Notch ligand) siRNAs-loaded chitosan NPs, which selectively inhibit ovarian cancer both in vitro and in vivo by selectively targeting Notch ligand Jagged1.<sup>112</sup> Despite the number of in vitro studies' evidences for the therapeutic efficiency and selectivity of drug-conjugated NPs targeting Notch signaling, valid in vivo models are still largely lacking.

### Transforming growth factor- $\beta$ signaling pathway

Transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling provides important regulatory signals during the initial phase of normal development and regeneration<sup>113,114</sup> and exerts promoting effects in the initiation and progression of multiple cancer types including breast,<sup>115</sup> colon,<sup>116</sup> liver,<sup>117</sup> lung,<sup>118</sup> and ovary.<sup>119</sup> TGF- $\beta$  ligands bind to a type II receptor, which constitutively recruits and phosphorylates a type I receptor. Next, the type I receptor triggers the phosphorylation of receptor-regulated SMADs and results in ligand-induced transcription.<sup>120,121</sup> In addition, cancer patients with enhanced

TGF- $\beta$  levels in urine and serum samples had shorter survival periods when compared with patients with normal TGF- $\beta$  levels.<sup>122–124</sup> Constitutively elevated levels of TGF- $\beta$  are closely correlated with an advanced disease state and poorer prognosis in cancer patients.<sup>125,126</sup> These studies indicate that TGF- $\beta$  signaling is an important prognostic marker in various types of cancer. While the oncogenic effects of TGF- $\beta$  in several types of tumors are well-documented, its potential role in CSCs has only recently emerged. Interestingly, this signaling pathway has been reported to be involved in the maintenance and function of CSCs from breast,<sup>127</sup> colon,<sup>128</sup> liver,<sup>129</sup> and lung<sup>130</sup> cancers. Thus, selective targeting TGF- $\beta$  signaling may be considered as an effective therapeutic strategy for the treatment of various types of cancer. In order to improve the drug delivery for breast cancer treatment, a polyethyleneimine/polyethylene glycol-conjugated MSNPs were developed by Meng et al to load a small molecule TGF- $\beta$  inhibitor, LY364947.<sup>131</sup> This approach provided significantly improved therapeutic efficiency in tumor xenograft models compared to the treatment with free LY364947 alone.<sup>131</sup> In addition, gold NPs (AuNPs) are widely used as carriers for therapeutic and diagnostic agents because of their great biocompatibility and unique physiochemical properties.<sup>132</sup> In this context, Tsai et al found that AuNPs could selectively capture TGF- $\beta$ 1 through S–Au binding between cysteine and disulfides residues resulting in deactivation of TGF- $\beta$  signaling pathway.<sup>133</sup> Interestingly, they also found that the immunosuppressive function of TGF- $\beta$  was significantly attenuated by AuNPs and resulted in an increased number and frequency of tumor-infiltrating T lymphocytes.<sup>133</sup> These results demonstrate that AuNPs may be a promising immune modulator by inhibiting immunosuppressive function of TGF- $\beta$ 1 signaling pathway.

## Hedgehog signaling pathway

The Hedgehog (Hh) signaling pathway was first identified as a critical regulator of pattern formation during early development and regeneration, and it also regulates differentiation, growth, and migration in a temporal-, spatial-, and concentration-dependent manner.<sup>134–137</sup> The functional significance of this signaling pathway is illustrated by an increase in birth defects and malignancies associated with aberrant activation of this normally quiescent pathway in adults.<sup>138,139</sup> Three Hh homologs with different spatial and temporal distribution patterns have been identified in humans: Desert hedgehog (Dhh), Indian hedgehog (Ihh), and Sonic hedgehog (Shh).<sup>140</sup> The Hh signaling cascade is initiated by Hh binding to the 12-transmembrane receptor Patched 1, which relieves its inhibition on Smoothed (Smo),

culminating in the nuclear localization of DNA-binding Gli transcription factors in target cells.<sup>141</sup> Several research groups reported that Hh signaling is aberrantly activated in various cancer types, including colon,<sup>142</sup> brain,<sup>143</sup> and breast,<sup>144</sup> liver,<sup>145</sup> lung,<sup>146</sup> and ovarian<sup>147</sup> cancer. Therefore, targeting Hh signaling pathway may provide an effective therapeutic approach in the treatment of various cancers. Almost currently available Hh small-molecule inhibitors approved for clinical trials for cancer therapy are Smo antagonists.<sup>148</sup> However, clinical applications of these inhibitors are currently restricted by their limited binding ability to Smo and poor systemic bioavailability. Therefore, Xu et al developed and characterized Gli antagonist (HPI-1)-conjugated polymeric nanoparticle (NanoHHI).<sup>49</sup> NanoHHI significantly inhibited the growth and invasion of CD133-positive cells, which are implicated as CSCs in liver cancers.<sup>149</sup> In addition, Verma et al found that Anthothocol (a limonoid derived from plant *Khayaanthotheca*)-conjugated PLGA NPs effectively inhibited cell proliferation and colony formation and induced apoptosis in pancreatic CSCs by modulating Hh signaling pathway.<sup>150</sup> These studies suggest that Hh signaling targeting NPs might be effective therapeutic approaches in patients with recurrence following curative surgical resection.

## Conclusion

The CSC was first discovered in acute myeloid leukemia >40 years ago. The identification of leukemic CSCs prompted further investigation into other types of solid tumors. Interestingly, CSCs are markedly resistant to conventional cancer treatments, such as chemotherapy and radiation. Therefore, identifying and selectively targeting markers and signaling pathways of CSCs are feasible therapeutic strategies for treating various cancer types, regardless of the underlying cause. Currently, new treatment modalities in the form of NPs-targeting CSC-specific markers or signaling pathways are available or under investigation. Various NPs-targeting CSC-specific surface markers or signaling pathways are listed in Table 1. Despite the number of in vitro and in vivo studies evidences for the therapeutic efficiency and selectivity of chemodrugs- or antibodies-conjugated NPs-targeting CSC-specific markers or signaling pathways, there is not enough information currently available to make a conclusive statement regarding the clinical therapeutic effects of NPs. Therefore, more detailed information about the effects of NPs-targeting CSCs on various cancer types will undoubtedly lead to more effective clinical therapy in the future. Moreover, the majority of the currently available information on CSCs is markedly influenced by the biological characteristics of normal stem/progenitor cells, in terms of

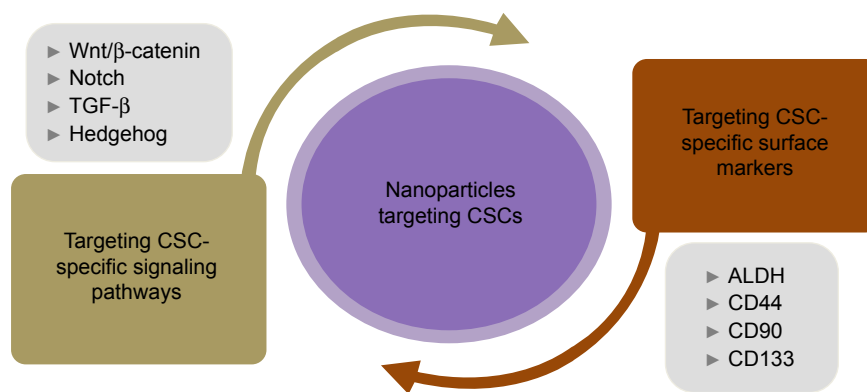
**Table 1** The list of nanoparticles targeting CSC-specific markers or signaling pathways for cancer therapy

| Target                          | Anticancer agent      | Type of nanoparticle           | Type of cancer   | References                 |              |
|---------------------------------|-----------------------|--------------------------------|--|----------------------------|--------------|
| CSC-specific markers            | ALDH                  | Decitabine                     | Copolymer of poly(ethylene glycol) with poly(D,L-lactide)                        | Breast cancer              | 48           |
|                                 |                       | Hedgehog inhibitor             | Poly(lactic-co-glycolic acid) conjugated with polyethylene glycol                | Pancreatic cancer          | 49           |
|                                 | CD44                  | Paclitaxel                     | Polymeric micelles   | Ovarian cancer             | 60           |
|                                 |                       | Suicide gene or doxorubicin    | Anti-CD44 antibody-incorporated liposomal  | Hepatocellular carcinoma   | 23           |
|                                 | CD90                  | 5-FU/oxaliplatin               | Hyaluronic acid-coated chitosan  | Colorectal cancer          | 63, 64       |
| CSC-specific signaling pathways | CD90                  | Trifluoperazine                | Anti-CD90 antibody-mediated water-soluble CdSe core nanocrystals                 | Leukemia                   | 67           |
|                                 |                       | CD133                          | Curcumin   | Polymeric nanoparticle     | Brain cancer |
|                                 | Wnt/ $\beta$ -catenin | Salinomycin                    | PEGylated poly(lactic-co-glycolic acid)  | Osteosarcoma               | 78           |
|                                 |                       | Curcumin                       | Poly(lactic acid-co-glycolic acid)   | Ovarian cancer             | 92           |
|                                 | Notch                 | 5-FU                           | Poly(lactic-co-glycolic acid)-polyesters and poly(ethylene glycol)               | Colorectal cancer          | 93           |
|                                 |                       | $\gamma$ -secretase inhibitors | Mesoporous silica nanoparticle   | Cervical and breast cancer | 111          |
|                                 | TGF- $\beta$          | Jagged 1 siRNA                 | Chitosan nanoparticles   | Ovarian cancer             | 112          |
|                                 |                       | LY364947                       | Polyethyleneimine/polyethylene glycol-conjugated mesoporous silica nanoparticles | Brain cancer               | 131          |
|                                 | Hedgehog              | TGF- $\beta$ 1                 | Gold nanoparticles   | Bladder cancer             | 133          |
|                                 |                       | HPI-1                          | Polymeric nanoparticle   | Hepatocellular carcinoma   | 149          |
|                                 |                       | Anthothecol                    | Poly(lactic acid-co-glycolic acid)   | Pancreatic cancer          | 150          |

**Abbreviations:** CSCs, cancer stem cells; ALDH, aldehyde dehydrogenases; 5-FU, 5-fluorouracil; TGF- $\beta$ , transforming growth factor- $\beta$ ; HPI-1, hedgehog pathway inhibitor-1.

their specific surface markers and distinct signaling pathways. Therefore, targeting these markers and/or aberrantly activated signaling pathways to selectively eliminate CSCs may reduce normal stem/progenitor cells and prevent the normal tissue regeneration processes, thus causing tissue or organ damages. Consequently, it remains unclear whether CSCs in various cancers can be selectively eliminated without significantly inhibiting all other normal stem/progenitor cells

in the organs or tissues. Therefore, further characteristics related to CSC-specific signaling pathways and surface markers need to be elucidated. These conclusions warrant future studies aimed at providing adequate and unique diagnostic and therapeutic strategies for cancer patients with fewer side effects. Schematic diagram summarizes the potential roles of NPs-targeting CSC-specific markers and signaling pathways in cancer treatments (Figure 1).



**Figure 1** Schematic diagram summarizing the potential roles of nanoparticles targeting CSC-specific signaling pathways or surface markers.

**Notes:** Nanoparticles effectively inhibit multiple types of CSCs by targeting specific signaling pathways (Wnt/ $\beta$ -catenin, Notch, TGF- $\beta$ , and hedgehog signaling) and/or specific markers (ALDH, CD44, CD90, and CD133) critically involved in CSC function and maintenance.

**Abbreviations:** CSCs, cancer stem cells; TGF- $\beta$ , transforming growth factor- $\beta$ ; ALDH, aldehyde dehydrogenases.

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## Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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