

Cervical cancer stem cell-associated genes: Prognostic implications in cervical cancer (Review)

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Abstract. Cervical cancer is the fourth most common type of gynecological malignancy to affect females, worldwide. Although high-risk human papillomavirus (HR-HPV) infection is the primary etiologic agent associated with the development of cervical cancer, cancer stem cells (CSCs) also serve a prominent role in the development, metastasis, recurrence and prognosis of the disease. CSCs are a small subpopulation of cells that have the ability to self-renew and are present in the majority of tumors, including cervical cancer. Studies describing the phenotype of cervical CSCs (CCSCs) vary in their definition of the expression pattern of principal biomarkers, including Musashi-1, aldehyde dehydrogenase 1, Oct3/4, Sox2 and CD49f. However, these markers are not observed in all cancers, although several may be present in multiple tumor types. The present review describes the potential biomarkers of CSCs in cervical cancer. These CCSC biomarkers may serve as molecular targets to enhance the efficacy and reduce the side effects associated with chemotherapeutic treatment in HR-HPV-positive cervical cancer.

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1. Introduction

Cervical cancer is the fourth most common type of gynecological malignancy worldwide and has a high mortality rate, particularly in developing countries (1). High-risk human papillomavirus (HR-HPV) has been identified as the primary etiologic agent associated with the development of cervical cancer (2), and the most prevalent HR-HPV types are HPH-16 and HPV-18, accounting for 70% of cervical cancer cases (3). Currently, the principal challenge in clinical cancer treatment is the resistance of cancer cells to various chemotherapeutic drugs. Cervical cancers harboring HPV are known to exhibit a poor response to treatment with chemotherapeutic agents, and display impaired chemotherapy-induced apoptosis (4).

In the cervical epithelium, the transformation zone (TZ) is a niche of cells with a unique expression profile and embryonic characteristics (5-7). The target cells for HR-HPV infection are cuboidal epithelial cells within the TZ (considered to be the stem cells of the cervical epithelium), which are involved, in malignant transformation (Fig. 1) (6,7).

Cancer stem cells (CSCs) are a small subpopulation of tumor cells with self-renewal capacity, which maintain tumor growth and cell differentiation. Cell surface markers and transcription factors, including CD44, aldehyde dehydrogenase 1 (ALDH1), Nanog and Oct4, have been used to isolate and enrich CSC populations from different tumors, including cervical cancer (8-10). CSCs contribute to the tumorigenic potential of cancer, including spherogenesis, and resistance to cytotoxic drugs and ionizing radiation (11).

The strategies by which HR-HPV promotes cancer development involve the overexpression of the viral oncoproteins E6 and E7 (12). Recently, it was reported that HPV16 E7 may contribute to the transcriptional upregulation of Oct3/4 and stemness-related genes. HPV16 E7 upregulates Oct3/4, Sox2, Nanog and fibroblast growth factor 4 expression levels to maintain the self-renewal capacity of CSCs (Fig. 1) (13). Additionally, recurrence in patients with cervical cancer following treatment may be explained by the hierarchy theory of carcinogenesis, which suggests that only CSCs have tumor initiating capacity. It has also been observed that CSCs are associated with tumor metastasis, relapse and chemo/radio-resistance, resulting in unsuccessful treatment

outcomes (10,14). The present review discusses the role of cervical CSCs as it is currently understood, and how they potentially represent therapeutic targets to improve the treatment of cervical cancer.

2. HR-HPV-mediated network regulation of CSCs in cervical cancer

In the TZ of the cervical epithelium, squamous and columnar cervical neoplasia are initiated by HR-HPV infection, where the virus targets cells that express such surface markers as CD44, CD49f, CK17 and CD133 on the cell membrane; these cells are considered to be the stem cells of the cervical epithelium (8,10,15-17) (Figs. 1 and 2).

During HR-HPV infection of the target cells, viruses bind to receptors on the cell surface, resulting in virus internalization. Viral DNA is released and transported through the endosomes and endo/lysosomes to the cell nucleus (18,19). Subsequently, viral oncoprotein synthesis (including that of E6 and E7) is initiated. E7 and E6 promote the proliferation of infected stem cells by inactivation of the endogenous tumor suppressor proteins retinoblastoma-associated protein (pRb) and p53, respectively (20). The degradation of pRb terminates Sox2 and Oct3/4 repression (21). Likewise, the degradation of p53 by HR-HPV E6 leads to increased Nanog expression levels (22).

HR-HPV oncoproteins increase the expression of stemness-related genes (Oct3/4, Nanog, Sox2 and Notch3) and promote cell self-renewal (13,23,24). It has also been observed that overexpression of stemness-related genes promotes the formation of tumors, inhibits cancer cell apoptosis, cell migration, sphere formation and chemoresistance (25-29). These findings suggest that HR-HPV promotes self-renewal through the upregulation of Oct3/4, Sox2 and Nanog expression to maintain the cervical cancer stem cell (CCSC) population in cervical tumors (Fig. 2). The CCSC population is frequently resistant to chemotherapy, and stemness-related genes regulate numerous other genes; this includes certain ATP-binding cassette (ABC) transporters, which are known to be associated with drug resistance. Increased expression levels of Oct3/4, Nanog, Sox2 and Notch3 are reported to promote drug resistance in CSCs. Additionally, the expression of ABC transporters, ALDH1 and Musashi-1 (MSI1) is increased in cells with high expression levels of Oct3/4-Nanog, Sox2 and Notch3, respectively (29-32). These data indicate that stemness-related genes (Oct3/4, Nanog, Sox2, Notch3) are associated with the expression of ABC, ALDH1 and MSI1, which may promote the clonogenicity, proliferation, invasiveness and chemoresistance of CSCs (Fig. 2).

3. Markers of CCSCs as prognostic biomarkers

Studies to identify CSCs in cervical cancer frequently use experimental strategies that involve the sorting of tumor cell subpopulations, identification of surface markers and transplantation of these cells into the appropriate animal models (33). The surface markers CD133, CD34, CD44, CD26 and CD90 are used to identify and isolate CSCs from tumor cell populations. Additionally, these cells possess metastatic, invasive and chemoresistance abilities (10,34-37). Several

potential cervical epithelial stem cell markers, including MSI1, ALDH1, SOX2 and CD49f, have been used to identify CCSCs (10,15). In addition to MSI1, ALDH1, SOX2, CD49f and other markers, including CD44, CD133, CK-17 and Oct3/4, have also been used to identify CCSCs (10). The presence of this sub-population may help to predict the prognosis and chemoresistance of patients with cervical cancer. Additionally, CCSCs may be used as therapeutic targets for novel drugs that may increase the effectiveness of chemotherapy in patients with chemo-resistant forms of the disease.

Musashi-1. MSI1 is an RNA-binding protein expressed in the central nervous system. Mammalian MSI1 is a prominent marker of neural stem cells and progenitor cells, is expressed in several cancer types and has various cellular functions, including cell fate decision, maintenance of the stem-cell state and differentiation. It has also been reported to act as a positive regulator of cancer progression (38,39). MSI1 is a translational regulator that has been demonstrated to be overexpressed in CCSCs (15). MSI1 expression is associated with the poor prognosis of patients with cervical squamous cell carcinoma, and is significantly correlated with CD49f expression, suggesting a functional role for these 2 proteins in cervical carcinogenesis (15). The expression of MSI1 may be used to predict the prognosis of patients with cervical cancer, thus personalized therapies directed at this protein may potentially be developed.

ALDH1. ALDH1 is a detoxifying enzyme involved in the metabolism of endogenous and exogenous aldehydes, which reduces oxidative/electrophilic stress in prokaryotic and eukaryotic organisms (40). ALDH1 is involved in cellular differentiation, proliferation, mobility, embryonic development and organ homeostasis (41). ALDH expression is associated with higher rates of cell proliferation, sphere formation, migration and tumorigenesis in cervical cancer cells (42). It has also been reported that ALDH is associated with the chemo- and radio-resistance exhibited by CSCs (10). High expression of ALDH1 is associated with poor survival in patients with cervical squamous cell carcinoma that received postoperative adjuvant chemotherapy (15); as it was observed that ALDH1 expression predicts chemoresistance and poor clinical outcome in patients with cervical cancer, ALDH1 may also be a useful prognostic biomarker (43,44). Furthermore, it was observed that knockdown of ALDH1 expression reduced the migrational ability of HeLa cells, whereas augmented expression of ALDH1 increased cell migration, indicating that ALDH1 is involved in cellular migration (45). It was also observed that CCSCs in cervosphere cultures possessed increased ALDH1 activity, which is, in turn, associated with higher tumorigenic activity (10). These results provide evidence of a link between ALDH1 expression, chemoresistance and poor clinical outcome in patients with cervical cancer.

Oct3/4. Oct3/4 is a transcription factor encoded by the POU domain class 5 transcription factor 1 gene that is involved in embryonic development, stem cell maintenance, tumor growth and metastasis (46,47). Oct3/4 has been used as a biomarker of CSCs, though it cannot be used alone without considering other genes involved in the complex CSC phenotype, including Sox2, Nanog and ALDH1 (23,48,49). Overexpression of Oct3/4

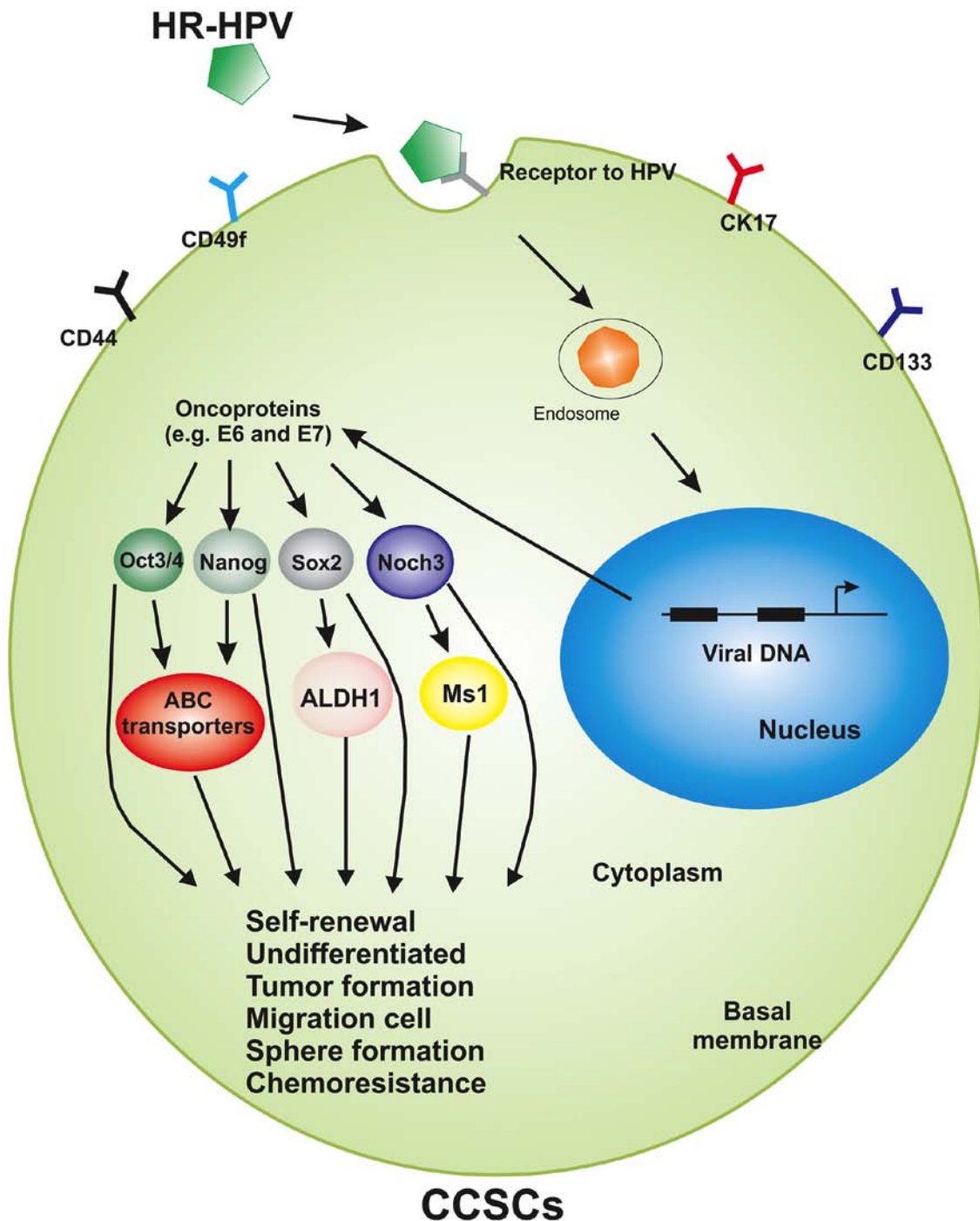


Figure 1. HR-HPV oncoprotein-mediated network regulation of CCSCs. HR-HPV targets stem cells of the cervical epithelium with the following surface markers: CD44, CD49f, CK17 and CD133. HR-HPV binds to receptors on the cell surface and the viral DNA is internalized and transported to the cell nucleus. Subsequently, the E6 and E7 oncogenes are transcribed and translated, giving rise to the oncoproteins E6 and E7. E6 and E7 have a number of molecular targets, including stemness-related genes Oct3/4, Nanog, Sox2 and Notch3; overexpression of these genes promotes the formation of tumors, inhibits cancer cell apoptosis, cell migration and sphere formation. Oct3/4, Nanog, Sox2 and Notch3 promote drug resistance via the upregulated expression of ABC transporters, ALDH1 and MSI1, which promotes the clonogenicity, proliferation, increased invasiveness and chemoresistance of CCSCs. HR-HPV, high risk-human papillomavirus; CCSCs, cervical cancer stem cells; ABC transporter, ATP-binding cassette transporter; ALDH1, aldehyde dehydrogenase 1; MSI1, Musashi-1.

has been used to determine its role in chemoresistance to multiple drugs. For example, transfection of Oct3/4 increases cisplatin resistance in cervical cancer cell lines (50). Although there have been limited studies on the association between Oct3/4 and the prognosis of patients with cervical cancer, Shen *et al* (51) reported that Oct3/4 expression was associated with radiation-resistance and poor survival in squamous cell carcinoma. Likewise, Yang *et al* (52) and Liu *et al* (53)

demonstrated that Oct3/4 was highly expressed in CCSCs, is associated with biological behavior, and is a prognostic factor in cervical cancer. The expression of Oct3/4 in tumor cells is also associated with resistance to radiotherapy, which is an important predictor of poor survival in patients with cervical squamous cell carcinoma (51,54). These results suggest that Oct3/4 expression is associated with poor prognosis in patients with cervical cancer.

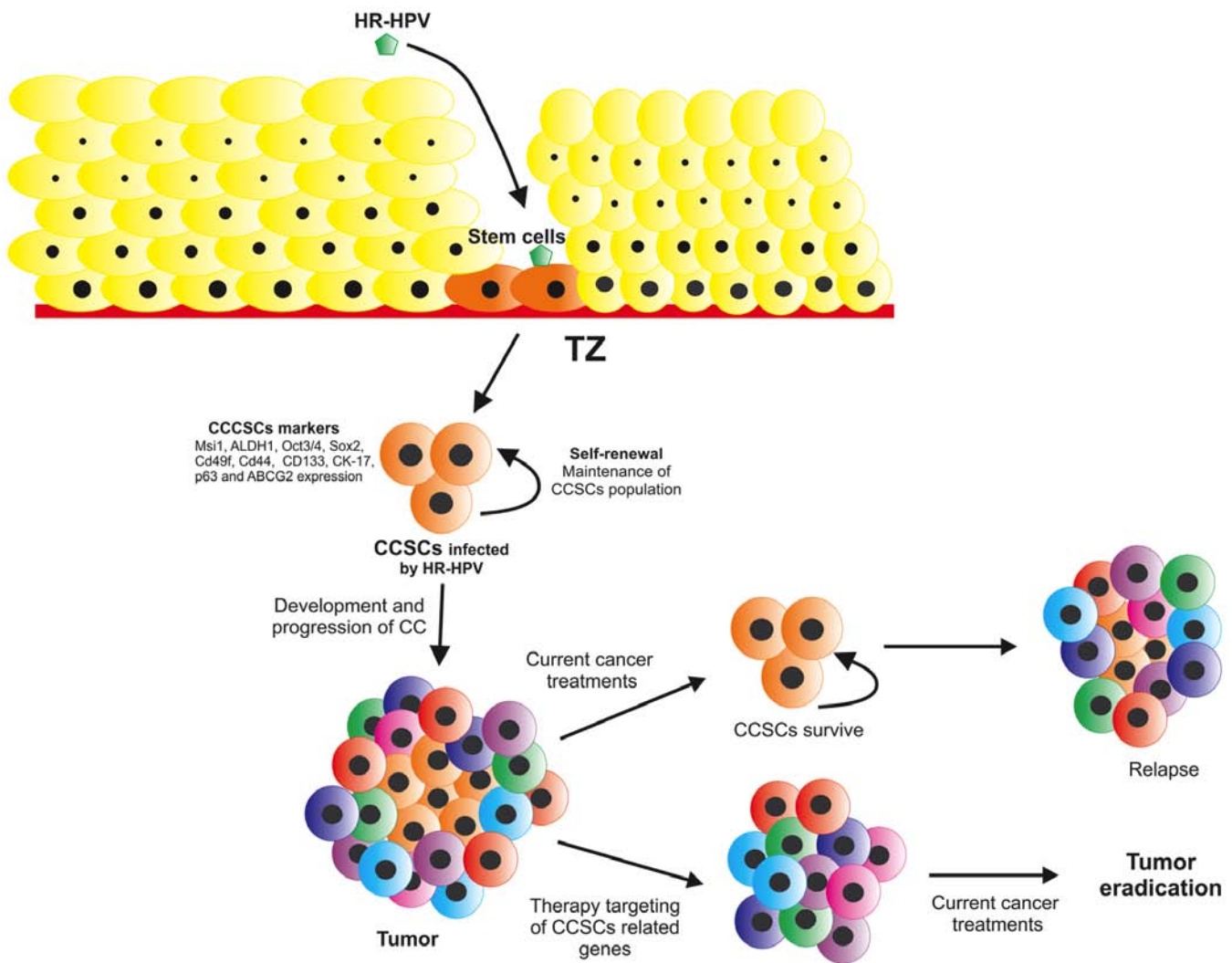


Figure 2. Model of the potential role of HR-HPV infection in CCSCs, and the implications for the treatment of cervical cancer. HR-HPV infects stem cells of the squamous epithelium, and HR-HPV oncoproteins HPV16 E6 and E7 are involved in the upregulation of Oct3/4, Sox2, Nanog and Fgf4 expression, which enhances the self-renewal and proliferation of cancer stem cells in cervical cancer. Current cancer treatments do not destroy CCSCs, leading to tumor recurrence and relapse. The combination of current cancer treatments with therapy directed at CCSC-associated genes may eradicate CCSCs, and tumor cells derived or transformed from CCSC. HR-HPV, high risk-human papillomavirus; CCSCs, cervical cancer stem cells.

Sox2. Sox2, a member of the SRY-related HMG-box family of transcription factors, serves a principal role in tissue development and cellular differentiation, and is associated with the stem cell phenotype. Sox2 maintains stem cell-like properties in cancer cells by interacting with other stem cell markers, including Nanog and Oct3/4 (25,55,56). The increased expression of Sox2 has been observed in a variety of tumors; however, this is not a universal finding (57,58). Sox2 expression was reported to be higher in cervical cancer cells than in normal cervical cells. It was also observed to be strongly associated with poor prognosis in patients with cervical cancer (15,51,58). Additionally, Sox2-positive cells exhibit a greater capacity for self-renewal, differentiation and tumor formation (29,59). Furthermore, Sox2 overexpression is associated with poor survival and chemo-resistance (51,58), suggesting that it may be a valuable prognostic biomarker in patients with cervical cancer.

CD49f. Integrin $\alpha 6$, also known as CD49f, is a biomarker commonly expressed in >30 different populations of stem

cells, including certain CSC populations (60-62). CD49f has also been used for the characterization of CCSC populations from cervical cancer cell lines (HeLa, SiHa, Ca Ski and C-4 I). Additionally, CD49f-positive cells exhibit a greater capacity for self-renewal, enhanced tumorigenic capabilities and increased resistance to ionizing radiation, compared with CD49f-negative cells (9,10). A high level of CD49f expression was also demonstrated in cervical tumor tissues, and is associated with the poor prognosis of patients with cervical cancer (15). Ammothumkandy *et al* (63) reported that CD49f expression is associated with overall survival and progression in cervical cancer. Nevertheless, further investigation is required to gain a more complete understanding of the prognostic potential of CD49f expression in cervical cancer.

CD44. CD44 is a primary adhesion molecule expression in the extracellular matrix, and is involved in various biological processes. CD44 has been reported as a stem cell and CSC marker, and demonstrated to be involved in tumor progression

and metastasis (64). Furthermore, CD44 expression in cervical cancer tissues was higher compared with that in normal, non-tumorous tissues (65). CD44-positive cells exhibit a greater capacity for self-renewal in subpopulations from various cervical cancer cell lines (9,10), and CD44/CD24-positive cells exhibit radiation-resistance and possess stem cell characteristics (8); these studies suggest that CD44 expression has value as a predictive biomarker for radiation-resistance in patients with cervical cancer. Nevertheless, further research is required to confirm the prognostic significance of CD44 expression in cervical cancer.

CD133. CD133 is a glycoprotein with 5 transmembrane domains, that was initially identified in human hematopoietic stem cells (66). CD133 expression is not restricted to normal stem cells, as it has also been detected in tumors and used as a CSC biomarker (67). CD133-positive cells exhibit increased self-renewal properties, and proliferation and differentiation abilities (67,68). Recently, it was reported that CD133-positive cells exhibited increased sphere-formation capacity compared with CD133-negative cells, providing further evidence to support its use as a biomarker of CCSCs. Additionally, high CD133 expression levels were detected in cervical carcinoma tissue biopsies (69), and CD133-positive CSCs were increased in radiation-resistant patients compared with those that were sensitive to radiotherapy; these data suggest that CD133 is a phenotypic marker of CSCs in cervical carcinoma (69), and that CD133 may be a prognostic biomarker in cervical cancer.

CK-17. CK17 is a keratinocyte marker and has been observed in the basal cells of the epithelium. It is also considered to be a biomarker for cervical stem cells and CSCs (16,70). High expression of CK17 has been reported in patients with cervical cancer and is associated with the development of cervical lesions; it was also reported that CK17 expression is critical for maintaining stem cell properties (10,16,17,71). Wu *et al* (72) reported that the altered regulation of CK17 expression affects the initiation and tumor chemoresistance of cervical cancer, suggesting that CK17 may have value as a prognostic biomarker in patients with cervical cancer.

ABC transporters. ABC transporters are one of the largest families of transmembrane proteins. These proteins use energy derived from ATP hydrolysis to transport numerous, chemically diverse compounds, including xenobiotics, antibiotics, toxins, vitamins, steroids, lipids, ions, polysaccharides, peptides and proteins, across the plasma membrane (73-75). However, these efflux mechanisms may protect cancer cells from first line cytotoxic drugs, and be responsible for resistance to chemotherapy. The most extensively characterized transporter within the ABC protein family is ATP-binding cassette sub-family B member 1 (ABCB1), which is associated with resistance to doxorubicin, paclitaxel and vincristine (75). ABCG2 is implicated in resistance to camptothecin analogues and mitoxantrone (76), and ABCC1 confers resistance to folate-based antimetabolites, anthracyclines, vinca-alkaloids and anti-androgens (77). ABC transporters are frequently overexpressed in CSCs (73,74,78). As an efflux transporter on the cell membrane, ABCG2 has been reported to confer drug

resistance by expelling chemotherapeutic agents out of cancer cells, and the increased expression of this transporter has been demonstrated in CCSCs (53,79,80). These observations suggest that ABC transporters serve an important role in the maintenance of CCSCs and in chemo-resistance in cervical cancer.

On the other hand, it has been revealed that a number of compounds, including salinomycin (81), curcumin (82,83) and sulforaphane (84,85), are not affected by resistance, and reduce tumor recurrence by destroying cancer cells and CSCs. Salinomycin, an antibiotic isolated from *Streptomyces albus*, has been shown to destroy CSCs in different types of human cancers, most likely by interfering with ABC drug transporters, the Wnt/ β -catenin signaling pathway and other pathways active in CSCs (81,86). Due to its suppression of CSC-stimulating cytokines (including IL-6, -8 and -1) curcumin has numerous cytotoxic effects on CSCs, and effects on the Wnt, Notch, Hedgehog and focal adhesion kinase signaling pathways (82,83). Sulforaphane also exhibits anticarcinogenic effects on CSCs, reducing proliferation and stimulating apoptosis of cancer cells, and interfering with numerous cell signaling pathways, including Keap1-Nrf2 signaling, the mitogen-activated protein kinase pathway and NK- κ B signaling (84,85).

4. Conclusions

Stem cells from the TZ of the cervical epithelium are targets for HR-HPV infection, which results in a unique expression profile that promotes the transformation of these cells into CCSCs (Fig. 1). CCSCs are involved in tumor development, metastasis, recurrence, resistance to multiple chemotherapeutic drugs and poor survival in patients with cervical cancer. Markers expressed by CCSCs include MSI1, ALDH1, Oct3/4, Sox2, CD49f, CD44, CD133, CK17 and ABC transporters. These biomarkers have potential to be used as prognostic indicators and therapeutic targets in patients with cervical cancer. Development of specific drugs and/or molecules to target CCSCs may provide the basis for an innovative treatment approach for the elimination of CSCs in cervical cancer (Fig. 1). Novel therapies based on the characteristics of CSCs, making them a target within the tumor, are crucial for improving clinical responses. Therefore, understanding the biology of CSC and the mechanism of CSC-targeted therapies may facilitate the development of effective treatments for patients with cervical cancer.

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JON, YGG and OLGC collated the references and wrote this review. JON, MALV and BIA reviewed and edited the manuscript.

Ethics approval and consent to participate

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Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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