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Cervical cancer stem cells

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Abstract

The concept of cancer stem cells (CSC) has been established over the past decade or so, and their role in carcinogenic processes has been confirmed. In this review, we focus on cervical CSCs, including (1) their purported origin, (2) markers used for cervical CSC identification, (3) alterations to signalling pathways in cervical cancer and (4) the cancer stem cell niche. Although cervical CSCs have not yet been definitively identified and characterized, future studies pursuing them as therapeutic targets may provide novel insights for treatment of cervical cancer.

Introduction

Cervical carcinomas (Fig 1) are among the most common malignant tumours of women worldwide and their mortality and morbidity levels are second only to breast cancer. Gynaecological oncologists study cancer stem cells (CSC) to explore new avenues for diagnosis and therapy of cervical carcinoma.

Theory of cancer stem cells

CSC theory postulates that not all tumour cells are equal with regard to self-renewal, tumour initiation and maintenance potential (1–12). It proposes that a minority of tumour cells, with indefinite proliferation potential, unlimited capacity for self-renewal, asymmetric division, and ability to differentiate into several cell lineages (13), are stem cells that play decisive roles in oncogenesis, while the majority of tumour cells die after transient differentiation. Based on this theory, heterogeneity between multiple tumour cells provides the capacity for indefinite proliferation, continuous renewal and pluripotency. In addition, cell heterogeneity and hierarchy within tumours originates from CSCs, which give rise to daughter cells that proliferate and differentiate into the cell mass that makes up a significant portion of the bulk of a tumour (14). Furthermore, CSCs are thought to be responsible for therapy resistance, residual disease and relapse after initial successful therapy. Resistance of cancer stem cells to conventional chemotherapy and radiotherapy has been attributed to cellular mechanisms such as multidrug resistance, quiescence, enhanced DNA repair ability and anti-apoptotic mechanisms (14,15).

Direct evidence has been derived from numerous studies. Al-Hajj (1) and Clarke *et al.* (16) first succeeded in isolating cancer stem cells from human breast cancers. Results of investigations on brain tumours, including astrocytoma and medulloblastoma, on liver cancer, retinoblastoma and prostate cancer, further provided compelling evidence for cancer stem cells (17–20). CSCs have also been identified in multiple myeloma (7), amongst cancer cell lines (21,22) and in solid tumours of the breast, ovary, endometrium, prostate, brain, and lung (1–4,10,11), using experimental strategy that combines sorting tumour cell subpopulations, identified on the basis of the different expressions of surface markers, with functional transplantation into appropriate animal models.

Source of cervical cancer stem cells

There are two theories regarding the source of cancer stem cells, i.e. mutation of stem cells in normal tissue and de-differentiation of mature cells. Recent studies have indicated that stem cells exist in the centre of tumours, a region where the oncogenic effects of carcinogens are enhanced. In addition, an increasing

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number of researchers support the theory that stem cells, because of the similarities between cancer stem cells and stem cells, are normal tissue cells that have mutated. This theory has directed attention to cervical reserve cells, which are widely considered to be progenitors of cervical epithelial cells.

Cervical reserve cells

The cervix is covered by a layer of epithelial cells. These may be either squamous or columnar (also called glandular cells). Squamous cells are flat and scaly, while columnar cells appear, as indicated by their name, column-like.

Cervical carcinogenesis associated with metaplastic squamous epithelium occurs in a specific anatomical location, the transformation zone (TZ); topography and natural history of this region is complex and dynamic. In adult women, the transformation zone usually is on the vaginal surface of the cervix, an irregular line of demarcation dividing one type of epithelium from another. Microscopically, columnar epithelium lines both the endocervical canal and associated endocervical glands, whereas squamous epithelium covers the outer cervix. During cervical squamous metaplasia, foci of squamous cells are detectable among the endocervical glandular columnar epithelium. Reserve cells are pivotal in the transformation of columnar epithelium into squamous epithelium and are most likely also targeted when endocervical epithelium transforms into cervical intraepithelial neoplasia (CIN). The junction between the two migrates proximally as the endocervical columnar epithelium is replaced by metaplastic squamous epithelium as a result of increased oestrogen production and growth of vaginal bacterial flora (23). Concentration of subglandular reserve cells is highest close to the squamo-columnar junction, in the upper third of the cervix. This indicates that a subpopulation of distal reserve cells provides progenitors for squamous and columnar epithelium and that proximal reserve cells can serve as progenitor cells for columnar epithelium (24,25).

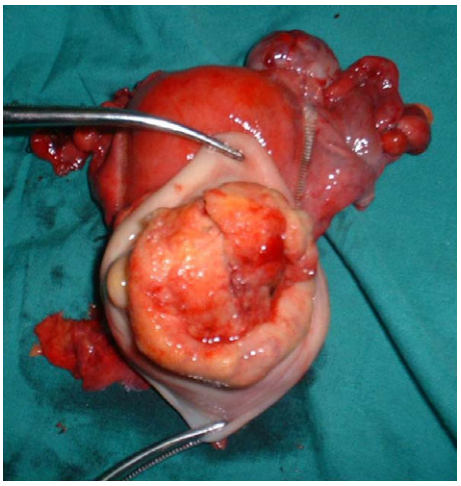

Figure 1. Image of cervical cancer lesion.

Recently, cervical cancer has been considered to originate from stem cells of the TZ by interplay between *HR-HPV* (high risk-human papilloma virus) viral oncogenes combined with cellular alterations (26–28). Stem cells from the TZ in the cervical epithelium are thought to be targets for malignant transformation because of their self-renewal and proliferativity. The TZ of the cervical epithelium is a niche for cells with a unique expression profile and embryonic characteristics (9,29) (Fig. 2).

Cervical reserve cells and human papilloma virus (HPV)

In 1977, HPV particles were first detected in a cervical cancer biopsy by electron microscopy. Subsequently, a large number of studies indicated that HPV was the major pathogen in cervical cancer. HPV infections in different countries arise in accord with different circumstances (30). Current infections can be measured with the highest sensitivity by HPV DNA testing, which can also be combined with Pap (Papanicolaou) smears for optimizing detection of high-grade cervical intraepithelial neoplasia (31,32). Pap screening has reduced the occurrence of HPV infection and cervical cancer (33), but barriers remain in some places (34), especially in some developing countries (35,36), and Raychaudhuri *et al.* attempted to determine whether there were significant differences in context of knowledge, attitude and practice (KAP) and screening, between high income and low income countries (37).

Worldwide, the most common high-risk HR-HPVs are subtypes 16 and 18, approximately 70% cervical cancers (CC) being due to infection by these genotypes. However, persistent infection by HR-HPV is a necessary but insufficient cause of the neoplasia (38). Most infections are transient, and the virus can spontaneously disappear - continuous infection is required to produce CIN or cervical cancer. Progression of CIN-I → CIN-II → CIN-III (CIS) → invasive cancer reflects consecutive processes of cervical cancer development (Fig. 3), however, the question remains regarding how HPV infects cervical epithelium and promotes development of the malignancy. It has been suggested (32) that only after HPV has infected undifferentiated cervical stem cells can the cancer start. Initial infection of cervical

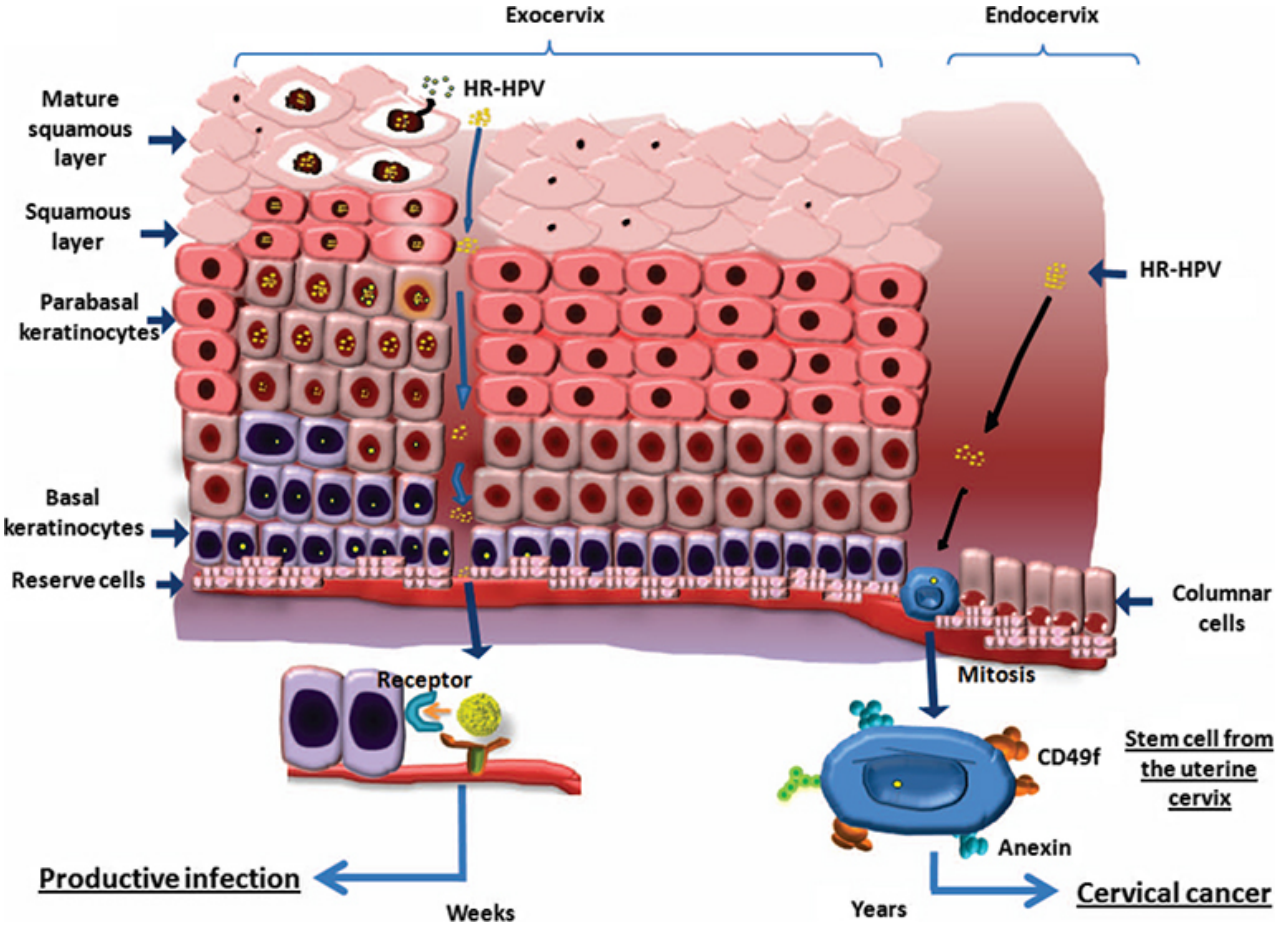

Figure 2. Transformation zone as the niche of cervical cancer stem cells.

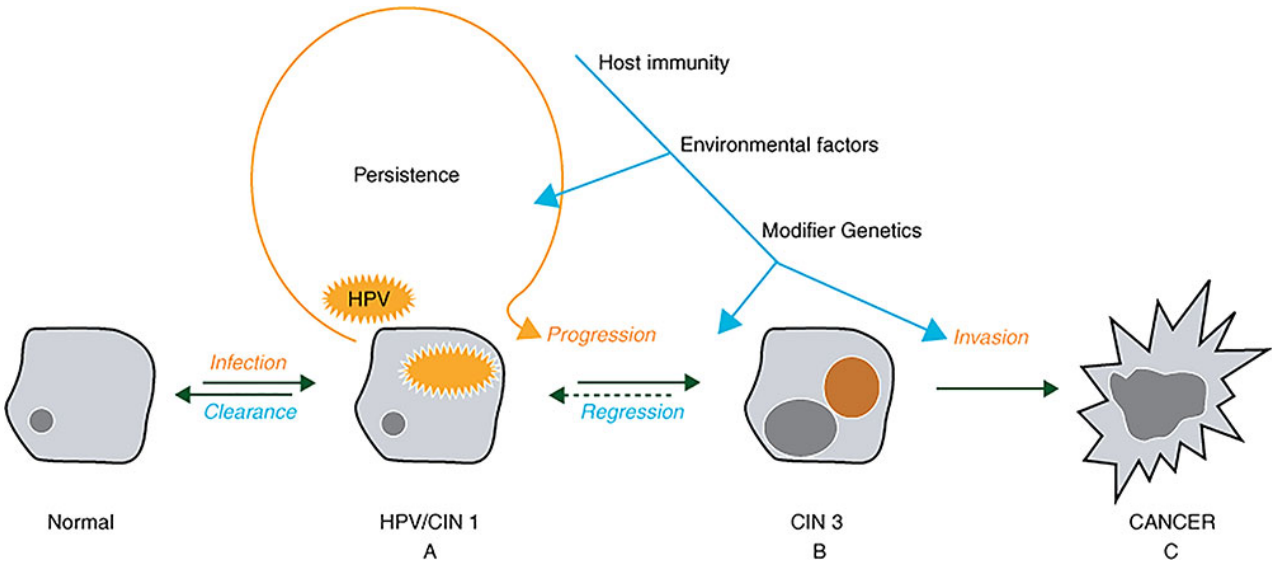

Figure 3. Illustration of the two shocks of cervical cancer stem cells.

epithelial cells with HPV does not lead diretly to malignant pathology. After a second infection, carcinogenic *E6/E7* gene can be integrated into cervical epithelial cell DNA, disturbing modulation of proliferation and differentiation, and finally resulting in transformation. HPV oncoprotein E6 binds and degrades wild-type p53 protein product. Transfection with HPV E6 and E7 oncogenes in YD8(HPV-negative, p53-mutated oropharyngeal cell lines) reduces abundance of proteins encoded by tumour suppressor genes, such as *p53* and *pRb* (Fig 4).

Thus, HPV may infect cervical stem cells, cervical reserve cells, as only reserve cells are undifferentiated and exist long enough to be infected a second time. Martens *et al.* measured expression of CK17 and p63 in CIN and cervical cancer and concluded that cervical reserve cells were, indeed, the target cells of HPV (39). Furthermore, one study by Regauer *et al.* indicated that HR-HPV infection of cervical reserve cells resulted in occurrence of CIN-II and increased p63 expression. There is considerable evidence that HPV-infected target cells are cervical reserve cells, thus the marker profile of HPV infected cells is crucially important for treatment and prevention (40).

Markers of cervical cancer stem cells

Currently, isolation of cervical cancer stem cells (CCSCs) from tumours requires knowledge of their specific surface markers, but current knowledge regarding the markers is incomplete.

Embryonic stem cell transcription factor

Nanog is a unique homeobox transcription factor that is expressed mainly in the inner cell mass (ICM) of blastocysts (41). It is highly expressed in early embryonic stem (ES) cells, and its expression reduces with their differentiation (42). Nanog plays a critical role in regulating pluripotent ICM cells in embryonic development, preventing ES cells from differentiation and maintaining their undifferentiated state (43). Recent studies have shown that Nanog is highly related to tumourigenesis. For example, it has been detected in certain tumours in which existence of cancer stem cells has already been acknowledged, such as gastric carcinoma (44), breast cancer, glioblastoma (45), lung cancer (46) and various human sarcomas (47). In one study, it was observed that Nanog is strongly expressed in CIN-II–III and cervical carcinoma tissues, in contrast to its low expression in CIN-I cells and normal cervical epithelia (48). Furthermore, cells of cervical epithelial tissues that overexpress Nanog are more capable of forming tumours (49).

Cytokeratin (CK)

Cytokeratins (CK) are cytoskeletal intermediate filament proteins; there are 20 subtypes expressed in various types of human epithelial cells. CK isotype depends on cell type and its localization. It has been reported CaM kinase-like-1 (DCAMKL-1), Lgr5, CD133, α-fetoprotein, cytokeratin-19 (CK19), Lin28 and c-Myc are up-regulated in hepatocellular carcinoma CSCs, DCAMKL-1 possibly being a specific marker of CSCs (50).

Differences between CKs expressed in normal cervical epithelial cells and reserve cells in the uterine cervix have been analysed. CK8 and CK17 are important subtypes expressed in CIN and cervical cancer tissues, and have been investigated as valuable markers of cervical cancer stem cells. CK8 is expressed in reserve cells, cervical gland epithelial cells and immature squamous metaplastic cells in the cervix, but not in squamous cells in a part of or mature squamous metaplastic cells. CK17 is expressed in reserve cells and immature metaplastic cells but not in cervical gland epithelial cells, squamous

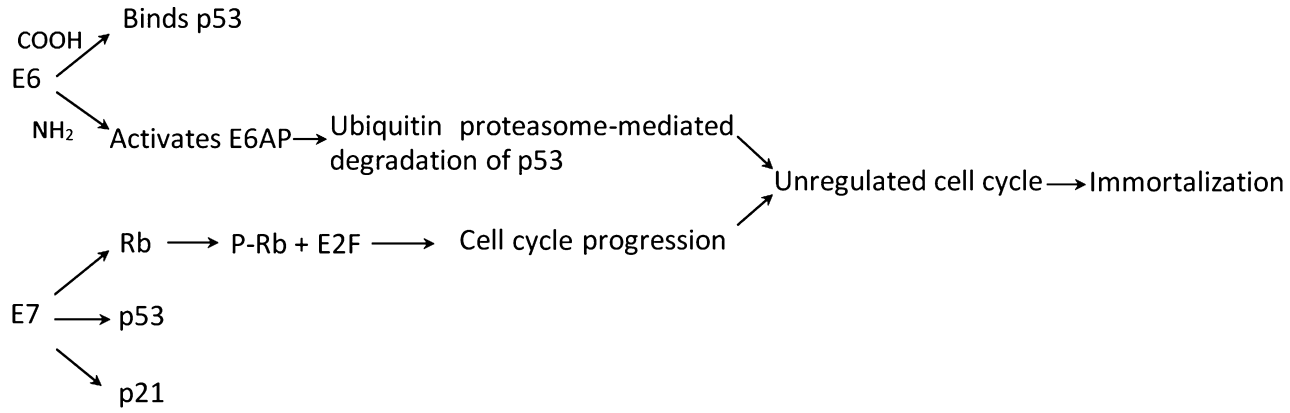

Figure 4. HPV E6 and E7 oncoproteins and p53, p21 and Rb tumour suppressor proteins.

cells in the portio or mature squamous metaplastic cells (51). In normal cervical epithelial cells, CK17 is only expressed at borders, while in cervical cancer cells, CK17 is overexpressed. In addition, cervical cancer cells with higher expression of CK17 have greater tendency to metastasise and are thus, more highly malignant (24,52,53); CK8 and CK17 have great significance in diagnosis of CIN and uterine cervical cancer. However, recent studies have attached importance to CK19 in distinguishing cervical cancer stem cells. A large part of CK19 mRNA is detectable by RT-PCR in breast, prostate and gastric cancers and some studies have shown that it is highly expressed in sentinel lymph nodes in cervical cancer but has low expression in benign lesion groups (54). Moreover, expression of CK19 is related to lymph node metastasis (55); however, understanding the exact function of CK19 in cervical cancer stem cells requires further research.

Cell adhesion molecules (CAMs)

CD44 is a transmembrane glycoprotein that participates in many cellular processes, including growth, survival, differentiation and motility, and exists as a standard isoform (CD44s) and a range of variant isoforms (CD44v), generated by alternative splicing (56,57). CD44 has been reported in many types of cancer and has been shown to play a role in cancer cell migration and matrix adhesion in response to cell microenvironment, thus enhancing aggregation and tumour cell growth (58). Recent studies have demonstrated that CD44 can be used to isolate cancer stem cells, in neoplasms of breast, prostate, pancreas and colorectal CSCs (1,39,59–61).

Cells isolated from uterine cervical cancer tissues have fibroblast-like morphology and grow in colonies. Immunophenotyping by flow cytometry has revealed that isolated cells are positive for CD13, CD29, CD44, CD105 and HLA-I, while not expressing CD10, CD14, CD31, CD34, CD38 and HLA-DR (62). Sphere-forming cells (SFCs) from cervical cancer cell lines have been used to successfully isolate or enrich CSCs from cancers (63–65). HeLa-SFCs are resistant to multiple chemotherapeutic drugs and are more tumourigenic, as evidenced by growth of tumours in immunodeficient mice following injection with 1×10^4 cells, compared to 1×10^6 parental HeLa cells, required to grow tumours of similar size in the same time frame. These cells have been found to exhibit an expression pattern of CD44 (high)/CD24 (low) that resembles CSC surface biomarker pattern of breast cancer. It has also been demonstrated that HeLa-SFCs express higher levels (6.9-fold) of human papillomavirus oncogene E6 compared to parental HeLa cells (66).

Octamer-binding transcription factor 4 (Oct4)

Oct4, the POU domain transcription factor, is important for maintenance of self-renewal, regulation of pluripotency and differentiation into specific lineages in the early mammalian embryo (67). Normally, low amounts of Oct4 protein are expressed in somatic, but not germ line, cells; however, expression of Oct4 is up-regulated in several tumours and tumour cell lines (68,69), which contributes to malignant transformation of normal stem cells and proliferation and therapeutic resistance of cancer cells. Down-regulation of Oct4 leads to cancer cell apoptosis (70). Thus, Oct4 is also considered as a hallmark of cancer stem-like cells (CSC). Recent studies indicate that it is highly expressed in several particular types of cancer tissues in which existence of cancer stem cells has been affirmed, such as lung cancer (71), hepatocellular carcinoma (72) and prostate cancer (70). It has been shown that Oct4 is strongly expressed in primary tumour cells and cancer cells in cervical cancer tissues but has relatively low level of expression in normal cervical epithelial cells (73). One recent study confirmed that cervical cancers contain a subpopulation of stem-like cancer cells expressing Oct4 protein (74). Previous studies have also confirmed that levels of both Oct4 mRNA and protein in HPV16-positive cervical cancer cells (Caski cells) were higher than in HPV-negative cervical cancer cells (C-33A cells) (75). These results suggest that HPV16 infection might be associated with up-regulation of Oct4 expression in the process of cervical carcinogenesis.

Aldehyde dehydrogenase 1 (ALDH1)

The aldehyde dehydrogenase (ALDH) family consists of cytosolic isoenzymes responsible for oxidizing intracellular aldehydes contributing to oxidation of retinol to retinoic acid in early stem cell differentiation. *ALDH1* gene is located on chromosome 9q21 and contains 13 exons encoding a 501 amino acid polypeptide. The human ALDH1 family includes ALDH1A1, ALDH1A2, ALDH1A3, ALDH1B1 and ALDH1L1; gene sequences of ALDH1A2, ALDH1A3, ALDH1B1 and ALDH1L1 are currently unknown. Human ALDH-1 plays a role in biological synthesis of retinoic acid and is involved in early differentiation of stem cells by mediating oxidation of retinol into retinoic acid. One previous study indicated that retinoic acid is involved in cell differentiation and proliferation *via* binding to the retinoic acid receptor. Increased ALDH1 activation has been found in myelomatosis and acute myelocytic leukaemia; a clinical study has also indicated that some breast cancer cells also express ALDH1. Prognosis in patients with positive

ALDH1 was poor, and ALDH1 expression was closely correlated to tumour grade, state of oestrogen receptor (ER)/progesterone receptor (PR), overexpression of ERBB2 and formation of CK (76,77). Our group found that higher ALDH1 expression correlates with significantly higher rates of cell proliferation, microsphere formation and migration. We also demonstrated that SiHa-ALDH1-positive cells are significantly more tumourigenic compared to SiHa-ALDH1-negative cells. Similarly, SiHa cells that overexpress ALDH1 are significantly more tumourigenic and exhibit higher rates of cell proliferation and migration compared to SiHa cells, when ALDH1 expression was knocked down using a lentivirus vector. Our data suggested ALDH1 to be a marker of cervical cancer stem cells, which expands our understanding of its functional role (78,79).

Nucleostemin and Musashi1

Nucleostemin (NS) is a putative GTPase that binds to p53 and is highly expressed in nucleoli of ES cells and cancer cell lines. Musashi1 (Msil1) is an RNA-binding protein and is highly expressed in neural progenitor cells. These two proteins also play important roles in carcinogenesis of embryonic malignancies. A study by Ye *et al.* also demonstrates that NS and Msil1 are abundantly expressed in CIN-II–III and cervical carcinoma tissues (48).

p16

p16 is an important tumour suppressor gene first identified in 1993. It is located on chromosome 9 its full length being 8.5 kb. Encoded products of p16, $p16^{INK4A}$ and $p14^{ARF}$, play significant roles in oncogenesis. $p16^{INK4A}$ is a cyclin-dependent kinase inhibitor that negatively regulates cell proliferation by inhibiting hyperphosphorylation of the retinoblastoma tumour suppressor (Rb) by cyclin D–CDK4/CDK6 complexes (80). $p14^{ARF}$ is a small basic arginine-rich nuclear/nucleolar protein that can arrest cell growth in G1–S and G2–M in a p53-dependent manner; its expression is increased after oncogenic stress or cell cycle deregulation (81). As a tumour suppressor gene, *p16* silenced by any mechanism causes promotion of carcinogenesis.

Some studies have indicated that p16 is closely related to cervical carcinoma (82–84) and further study has demonstrated that $p16^{INK4A}$ and $p14^{ARF}$ are overexpressed in high-grade CIN and invasive cancer, relative to low-grade CIN or normal epithelium (85). Moreover, previous investigations have shown that methylation of *p16* is a frequent event in cervical carcinoma samples, and early promoter methylation of the *p16* gene may be a crucial event in malignant progression in the cervix (86). It has been reported $p16^{INK4A}$ is required for DDP resistance in cervical carcinoma SiHa cells (87). Thus, p16 is suggested to be a potential utility in diagnosis and prognosis (88–90).

p63

p63, a homologue of tumour suppressor gene *p53*, has been described to be a transcription factor operating mainly in the embryonic stage of development (91). Existence of p63 has recently been demonstrated in bronchial, prostate and cervical reserve tissues using immunohistochemical techniques, and is believed to play a role in regulation and maturation of epithelium in the adult phase (92–95). Several studies have shown (by immunostaining) that p63 is strongly expressed in the basal layer of ectocervical epithelia and in basal layers of CIN lesions, irrespective of their grade (39,53). Nevertheless, whether p63 can be a biomarker of cervical stem cells remains questionable. Some researchers have suggested that p63 can be detected in all cancer cells in the uterine cervix and are not unique to cervical cancer stem cells (73).

However, there are shortcomings and difficulties in using several sorting markers addressed to isolate CCSCs. For some inconsistencies present in published data, the difficulties can be ascribed to i) differential glycosylation, ii) epigenetic modifications and iii) tumour microenvironment.

The CSC pathway

SHH signalling pathway

Hh (Hedgehog) is one of the core developmental signalling pathways in which mutation during development causes congenital malformations (96). During embryology, this pathway controls cell proliferation and differentiation and can be activated by binding sonic hedgehog (SHH), Indian hedgehog (IHH) desert Hedgehog, and patched hedgehog (PTCH). In the absence of the Hh ligand, PTCH functions as an inhibitor of smoothened (SMO), a transmembrane protein with homology to G-coupled protein. Activation of SMO may initiate processes during which transcription factors belonging to the Gli family, modify transcription of Ptc, WNT and Noggin, an inhibitor of BMP-4 (bone morphogenetic protein) belonging to the TGF-β superfamily (97,98). In mammals, SHH regulates proliferation of primitive haematopoietic cells with BMP-4 (98). Errors in the pathway can be a pathogenic factor for development of medulloblastoma and basal cell carcinoma (17).

SHH signalling has also been implicated in regulation of self-renewal by the finding that populations highly enriched for human HSCs (haematopoietic stem cell) exhibit increased self-renewal in response to SHH stimulation *in vitro*, albeit in combination with other growth factors (98) (Fig. 5).

There are two different scenarios in which this pathway may be involved in carcinogenesis. One is by mutation of pathway components, as exemplified by mutation of PTCH1 and SMO; the other is autocrine requirement for an Hh ligand. It has previously been reported that components of Hh signalling pathway are gradually upregulated as normal epithelium progresses to squamous cell carcinoma (99), suggesting a role for Hh pathway molecules in this development**.** Based on this, Samarzija *et al.* found that an autocrine Hh signalling loop could exist in cervical cancer cell lines (100)**.**

Concerning HPV, there are two contrary results. One indicates that Hh signalling is not induced directly by HPV-encoded proteins but rather that Hh-activating mutations are selected in cells initially immortalized by HPV (100). The other is activation of the Hedgehog pathway being due to PTCH1 inactivation, along with HPV infection as important in CACX development (101).

The Hedgehog signalling pathway is associated with poor outcome in women with node negative cervical cancer, treated with radiation (102). Thus some researchers support a role for the Hh pathway in repopulation after chemoradiation and suggest that SMO may be a valid therapeutic target (103). Recently, miRNAs have become a focus; Gli3 may be a target of miR-506 exerting its anti-proliferative function in cervical neoplasia (104).

Notch signalling

Notch is a transmembrane receptor tethered to the plasma membrane as a heterodimeric protein. There are four mammalian Notch receptors, Notch 1–4 and they bind to two distinct families of Notch ligands, Delta-like (DLL1, DLL3 and DLL4) and Jagged-like (JAG1, JAG2). Signals are transduced by the Notch intracellular domain (NICD) produced by ligand-induced cleavage, which translocates to the nucleus and directly induces transcription of downstream targets by forming a transactivation complex with several cofactors (105) (Fig. 6).

Notch is a cell to cell signalling pathway communication system, known to play a critical role in regulating cell proliferation and differentiation, during embryogenesis and in normal adult stem cells (106). Notch signalling determines which progenitor cells will be committed to a certain developmental route and which remain uncommitted and capable of differentiating into various cell types (107,108). It is also involved in regulation of a set of neural stem cells (106).

Notch pathways are known to be abnormal in several cancer subtypes (109–114) and its activation in HSCs in culture by Jagged-1 ligand, consistently increases amounts of primitive progenitor activity observed *in vitro* and *in vivo*, suggesting that Notch activation promotes stem cell self-renewal, or at least maintenance of multipotentiality (98,115). It has also been suggested that interference in function of the Notch system is responsible for T-cell leukaemia and breast cancers (107).

Notch signalling may have different roles during early and late stages of cervical cancer development (116). Reduction in Notch1 expression directs basal cells

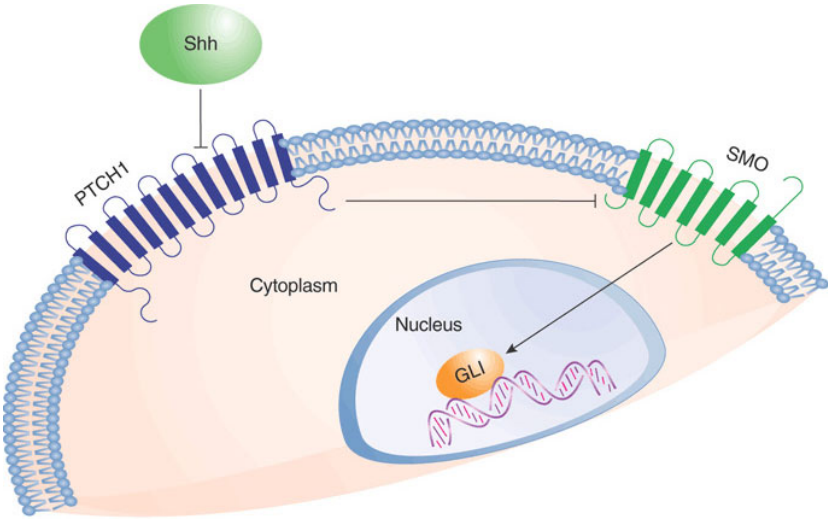

Figure 5. SHH pathway (cited from Sheridan C (2009) Genentech obtains proof of concept for hedgehog inhibition. Nat. Biotechnol. **27**, 968–969).

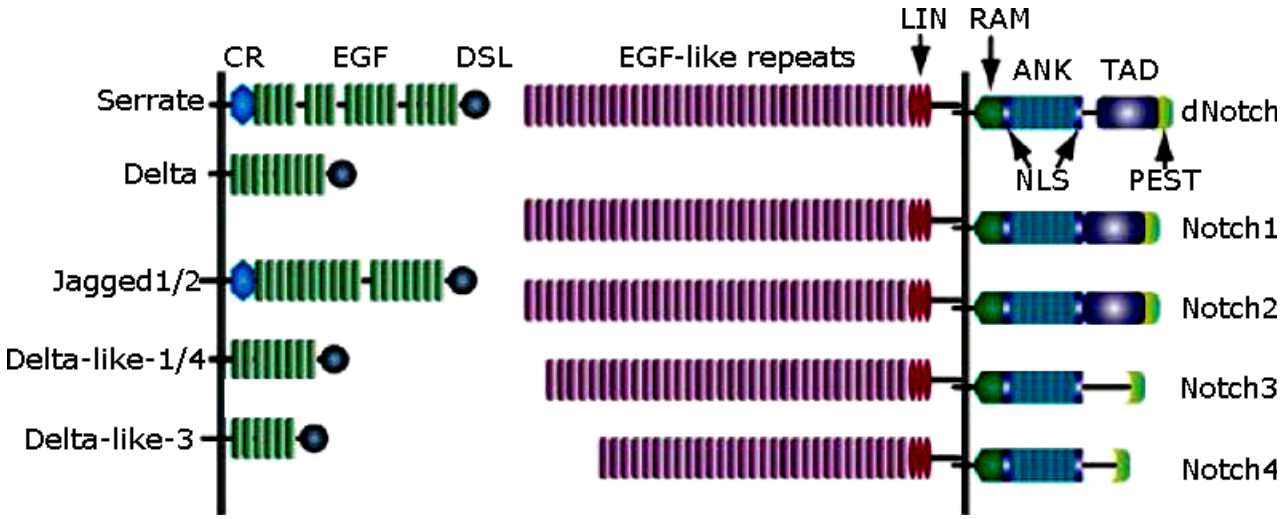

Figure 6. Receptors and ligands of Notch pathway (cited from Maillard I, Fang T, Pear WS (2005) Regulation of lymphoid development, differentiation, and function by the Notch pathway. Annu. Rev. Immunol. **23**, 945–974).

to cease terminal differentiation and to form immature epithelium, thereby playing a major role in histopathogenesis of epithelial dysplasia (117). Furthermore, down-regulation of Notch1 expression seems to be an inherent mechanism for switching epithelium from the normal mature state to an activated immature one, suggesting its essential role in maintaining epithelial integrity (118). Notch-1 receptor expression increases with progression of cervical cancer (119). Regulation of the Notch signalling pathway by HPV E6 has been demonstrated in cervical cancer cell lines. At late passage of W12 cell line, Jagged-1 is up-regulated, while Manic Fringe is reduced (120); silencing Jagged-1 inhibits tumourigenicity of CaSki. However, a controversial report has shown that Notch 1 is low in HPV-positive cervical cancer cell lines HeLa, C40I, C4-II, SiHa and Caski compared to HPV-negative C33a and primary keratinocytes (121,122).

Wnt signalling

Wnts compose a family of 19 glycoproteins that act as ligands for the Frizzled (Fz) transmembrane receptor. Binding of Wnt ligands to Fz receptors activates two distinct signal transduction pathways, known as the 'canonical' and 'non-canonical' Wnt pathways. The canonical pathway causes accumulation of β-catenin (a downstream activator of the Wnt signalling pathway) in the cell nucleus and consequent transcription of Wnt target genes. Ordinarily, β-catenin levels are maintained at low levels in the cytoplasm, but activation of the Wnt/β-catenin pathway causes translocation and accumulation of β-catenin in the nucleus, thereby promoting transcription of Wnt target genes (Fig. 7).

The Wnt signalling pathway is known to play key roles in controlling cell proliferation and differentiation in both embryogenesis and in regulating homoeostasis in normal adult tissues (123–125). Reya *et al.* demonstrated that overexpression of activated β-catenin in long-term cultures of transplantable HSCs, and ectopic expression of axin, lead to inhibition of HSC proliferation, their increased death and reduced reconstitution *in vivo* (126). In separate studies, soluble Wnt proteins from conditioned supernatants have also been shown to influence proliferation of hematopoietic progenitors from mouse foetal liver and human bone marrow (127,128), and studies of epidermal and gut progenitors suggest that the Wnt signalling pathway may contribute to regulation of stem cell/progenitor cell self-renewal in other tissues. Cultured human keratinocytes with higher proliferative potential have higher levels of β-catenin compared to keratinocytes, which have a lower proliferative capacity. Moreover, retroviral transduction of activated β-catenin results in increased epidermal stem cell self-renewal and reduced differentiation (129). *In vivo* data from transgenic mice suggest that activation of the Wnt signalling pathway in epidermal stem cells can give rise to epithelial cancers (130). Furthermore, mice lacking TCF-4, one of the transcriptional mediators of the Wnt signalling pathway, quickly exhaust undifferentiated progenitors in crypts of gut epithelium during foetal development (131), suggesting that this pathway is required for maintenance or self-renewal of gut epithelial stem cells.

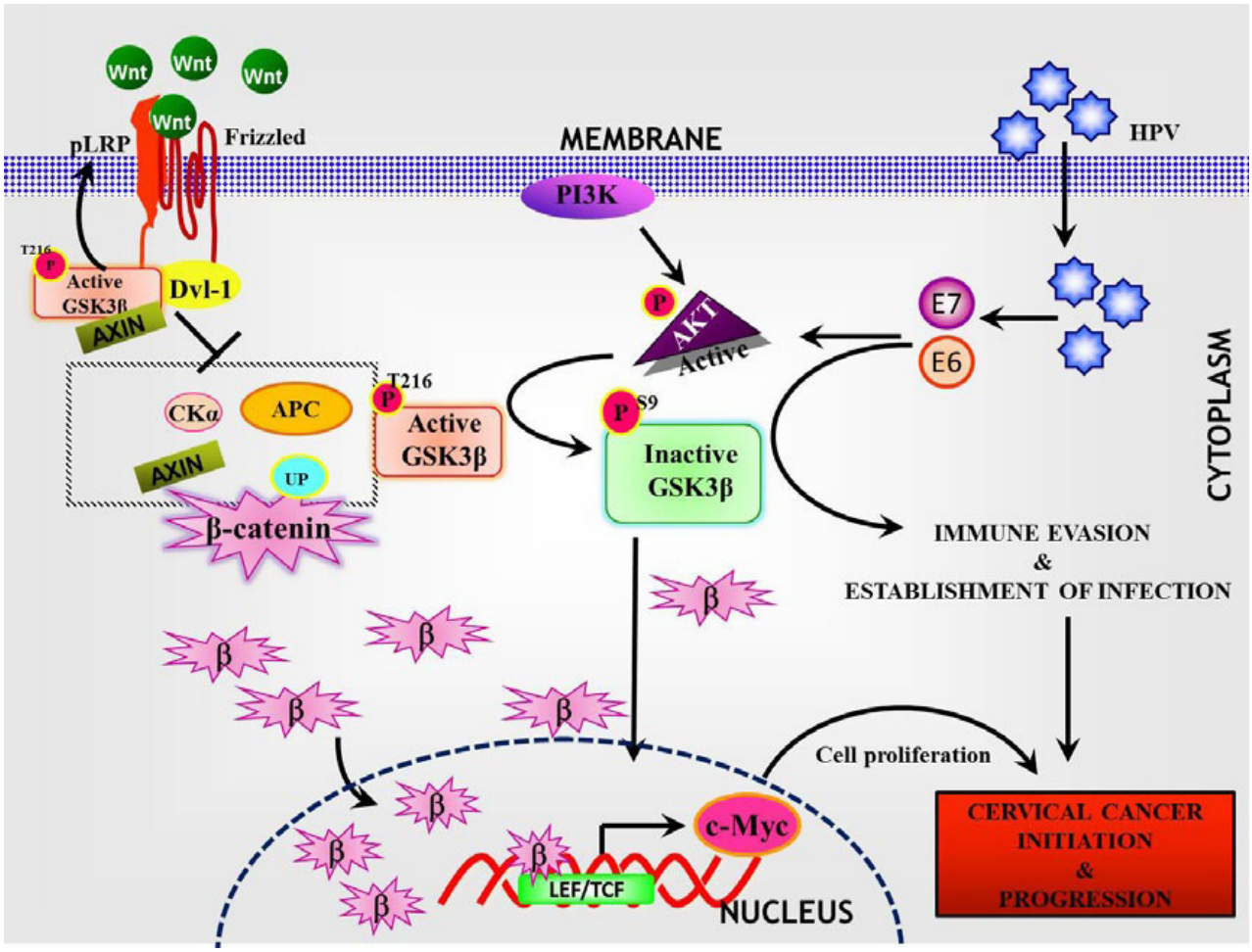

Figure 7. Relationship between cervical cancer and Wnt pathway (cited from Rath G, Jawanjal P, Salhan S, Nalliah M, Dhawan I (2015) Clinical significance of inactivated glycogen synthase kinase 3beta in HPV-associated cervical cancer: Relationship with Wnt/beta-catenin pathway activation. Am. J. Reprod. Immunol. **73**, 460–478).

In addition, Wnt/β-catenin signalling has been shown to be of importance in CSCs in a number of malignancies, such as colon cancer (132) and cutaneous squamous cell carcinoma (133).

Multiple therapeutic interventions have been proposed related to cervical cancer and the Wnt pathway. Activation of canonical Wnt at multiple levels (plasma, membrane, cytoplasm or nucleus) specifically supports transformation of HPV-infected primary human keratinocytes (134). Wnt inhibitory factor 1 induces apoptosis and inhibits cervical cancer growth, invasion and angiogenesis *in vivo* (135,136), and expression of Wnt7A (137), SOX1 (138),Wnt-11 (139),Wnt5A (140) and nuclear β-catenin (141) are related to cervical cancer survival and chemo-/ radioresistance. Generation of HPV-16-associated cervical tumourigenesis synergizes with overactivation of the Wnt/ β-catenin pathway (142). The mechanism of this has been associated with inactivation of negative regulators such as APC or axin (143), and overexpression of Dishevelled (144), frizzled receptors and Wnt ligands (145,146).

These reports demonstrate that Wnt and Notch signalling are frequently co-activated in human CC cells and specimens, and resveratrol can inhibit signalling activation, meanwhile leading to cervical squamous cell carcinoma and adenocarcinoma cells growth arrest and apoptosis (147).

The cancer stem cell niche

The ‘stem cell niche’ was proposed by Schofield in 1978 during research on processes of HSC self-renewal and differentiation. The neighbouring physiological microenvironment of specialized cells, physically anchors stem cells and provides factors necessary for maintenance of stem cell characteristics (148).

Anatomical structure of the stem cell niche was first described in the study of germline stem cell (GSC) niches in adult *Drosophila* testis and ovary (149). In the testis, GSCs adhering to niche components receive signals supporting self-renewal, while those further away are initiated to differentiation (150). Niches have also been identified in mammalian stem cells in the intestinal, neural, epidermal and hematopoietic systems. It has been widely acknowledged that HSCs may have two niches, osteoblast and vascular (151). The major signal pathways that have been verified in niches are Hedgehog, Wnt, BMPs, FGF (fibroblast growth factor) and Notch.

In the light of findings of stem cell niches, existence of cancer stem cell niches has been proposed. Putative existence of a cancer stem cell niche consisting of bi-directional stromal and stem cell secreting factors that trigger cancer stem cell growth and proliferation, has been hypothesized in the nervous and hematopoietic systems (152,153). Interactions between cancer stem cells and their niches may initiate some typical attributes of tumour cells. The surrounding microenvironment of cancer stem cells is composed of stromal fibroblasts, adipocytes, endothelial cells, extracellular matrix (ECM) and the immune system.

The microenvironment of cancer stem cells may be the key to maintaining their self-renewal. Specialized microenvironments of bone marrow endothelial cells have proved to be crucial to homing and engraftment of both normal HSCs and leukaemic cells (148,154). Extracellular matrix components and signalling molecules in the HSC microenvironment promote cell survival in AML, providing resistance to chemotherapeutic treatments (148,155).

A more significant example comes from the glioma stem cell and its microenvironment. It has been verified that intrinsic properties of glioma stem cells are tightly regulated by specific signals from the niches (156). The signals help to maintain number of glioma stem cells and their undifferentiated state. Hypoxia is a major characteristic of the glioma stem cell niche. A number of studies has demonstrated that a hypoxic niche may play an important role in maintaining stemness of glioma stem cells and in promoting the glioma stem cell pool. Moreover, relationships between cancer stem cells and their niches can be bi-directional. Glioma stem cells do not simply exploit pre-existing microenvironments, but are also actively involved in shaping and generation of their niches through intricate crosstalk with various components of both surrounding and distant tissues (156).

Components of cancer stem cell niches may be highly related to metastatic potential of cancer stem cells (148). For example, MMP9 is induced in clusters of pre-metastatic lung endothelial cells through VEGF Receptor 1 (VEGFR1) signalling from distant primary tumours (151). In addition, adhesion molecules and integrins may be associated with migration of CSCs (157).

At the moment, the transformation zone of cervix is considered to be a baseline lymphangiogenic niche in the cervical neoplastic process (157).

Cancer stem cell-directed therapies

Resistance of CSCs to conventional cancer treatments such as chemotherapy and radiatiotherapy is considered to be a formidable problem as residual CSCs presumably trigger relapse after termination of treatment. Development of new therapeutic strategies based on the CSC model has therefore become a key goal in the challenge to achieve complete eradication of cancer. To date, four strategies have been considered.

The first is to attack CSCs directly. CSCs can be targeted by agents that kill them specifically, or that promote their differentiation into non-CSCs, which in turn undergo apoptosis, senescence or terminal differentiation.The second strategy for CSC eradication is to promote their exit from the quiescent state and entry into the cycling state, and the third is to attack the CSC niche, which provides the supportive environment for maintenance of CSCs.The fourth strategy is to inhibit transitions between CSCs and non-CSCs (158).

Considering cervical carcinoma, sorting markers or signalling pathway has not been applied in clinical practice. In addition, gene silencing of *E6* with a lentiviral short hairpin RNA (shRNA) was found to profoundly inhibit HeLa-SFC sphere formation and cell growth (65).

Conclusion

Study of cervical cancer stem cells is still at an early stage, and many problems remain to be solved. For example, there are abundant matrix and fibrous components of cervical tissue, which render it difficult to isolate tumour cells. The study of cervical stem cells is limited at present, and there are no internationally recognized markers of cervical stem cells. Finally, cervical cancer stem cells have not been isolated, and identifying their specific membrane markers is key to their isolation.

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Conflicts of interest

The authors declare no conflicts of interest.

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