

Review

Dysregulated stemness-related genes in gynecological malignancies

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Summary. In recent years, much attention has been paid to the concept of cancer stem cells (CSC) and self-renewal related pathways in cancer biology. This review outlines the dysregulated stemness-related genes or transcription factors in gynecological cancers. Hedgehog (Hh) and Notch signaling are important pathways in tissue pattern programming and cell fate determination during embryonic development. Hyperactivation of these two pathways was frequently observed in gynecological malignancies such as ovarian, endometrial and cervical cancers. In contrast, the expression profiles of pluripotency-regulating core transcriptional circuitry: Nanog, Oct4 and Sox2 appear heterogeneous. Among these transcription factors, overexpression of Nanog was found to exert a prominent effect in gynecological tumorigenesis, while dysregulations of Oct4 and Sox2 may vary in a context dependent manner. On the other hand, the isolation of putative CSC illustrates a hierarchy model of tumor heterogeneity, in which only a subset of cells among biologically distinct populations can initiate tumor growth. Re-activation of these pluripotent transcription factors (Nanog, Oct4 and/or Sox2) in association with distinct tumorigenic properties could be found in clones isolated from gynecological tumors using various approaches. Recent understanding on the roles of Hh and Notch signaling in enhancing CSC survival may help to better understand the mechanism of carcinogenesis and identify new pharmaceutical targets for gynecological malignancies.

Key words: Hedgehog, Notch, Cancer stem cells, Stemness, Gynecological cancers

Introduction

During early embryogenesis, blastocyst at preimplantation development comprises an outer layer of cells, the trophoblast (TE), and a group of pluripotent cells, the inner cell mass (ICM). The ICM and the TE will generate distinctly different cell lineages as implantation starts and embryogenesis continues. In normal pregnancy, the placenta functions as an interface between the fetus and mother. Formation of placenta originates from a specific population of TE derived cells called trophoblast. While TE will develop into placental tissue, the ICM gives rise to all the different cell types of the embryo (ecto-, meso- and endo-derm), resulting in the various definitive structures of the fetus (Marikawa and Alarcon, 2009; Na et al., 2010). In typical fetal development, SRY (sex-determining region on the Y chromosome) gene determines gonad development. In the absence of the SRY-gene, ovaries develop, whilst the fetal gonads become testes if the SRY-gene expresses. The presence of fetal ovaries results in retention and development of the Mullerian ducts which subsequently form the internal female reproductive tract structures, including oviducts, uterus, cervix and upper vagina (Teixeira et al., 2008).

Cancers of the female reproductive system cause significant morbidity and mortality worldwide. According to GLOBOCAN 2008 database, cervical and endometrial cancers are the third and sixth most commonly diagnosed cancers in females (GLOBOCAN, 2008). Being the third most common gynecological cancer worldwide after cervical and endometrial cancer, ovarian cancer is one of the most lethal gynecological malignancies, due to late presentation, poor response to treatment and high recurrence rate. Regarding the lethality of these cancers, age-standardized mortality rates from cervical and ovarian cancers reached 7.8 and 3.8 per 100,000 women respectively.

It is generally believed that gynecological cancers originate from their corresponding progenitors after multi-step genetic changes, including activation of oncogenes and/or inactivation of tumor suppressors (Cheung, 2007; Spandidos et al., 2000). While most of the malignant lesions in the female genital tract are epithelial derived, carcinomas of different histological differentiation may occur at different parts of the genital tract, probably related to their common origin from the Mullerian epithelium (Bennett and Williamson, 2010). For instance, endometrioid adenocarcinoma can develop in the ovary and endometrium. In fact, synchronous or metachronous co-existence of endometrioid carcinomas at the ovaries and endometrium are frequently observed. Table 1 highlights the gynecological malignancies to be discussed in this review and their possible corresponding origins in the female reproductive tract.

In recent years, the similarities between tumor development and abnormal embryogenesis have raised a growing interest in investigating the possible impact of reactivation of self-renewal signal pathways in human malignancies (Clark et al., 2007; Jeter et al., 2009). It is believed that better understanding of these pathways can help to redefine the basic mechanism of carcinogenesis. This paper aimed at outlining aberrant expression profiles of "stemness" regulating machineries, including hedgehog (Hh) and Notch signaling pathways, as well as the circuitry of pluripotency transcription factors: Nanog, Sox2 and Oct4 in gynecological malignancies.

Hedgehog signaling: Overexpression of Shh and Gli1 in gynecological cancers

The Hedgehog (Hh) signaling pathway is involved in stem cell maintenance (Beachy et al., 2004). It is also critical in embryonic pattern of tissue formation in vertebrates, including the brain and spinal cord, the axial skeleton and the limbs (Beachy et al., 2004; Ingham and McMahon, 2001). Although it remains active and contributes to tissue differentiation, proliferation, and maintenance in multiple adult tissues, hyperactivation of this pathway in adult tissue can lead to the development of cancer (Evangelista et al., 2006).

Sustained increased endogenous expression of Hh component (ligand-dependent) or mutations of transmembrane receptor Patched or its downstream target Smoothed (Smo) (ligand-independent), could cause constitutive activation of Hh (Pasca di Magliano

and Hebrok, 2003; Evangelista et al., 2006). In the Hh signaling pathway, three Hh ligand proteins, Sonic hedgehog (Shh), Indian Hh and Desert Hh, can trigger the pathway through binding to Patched, alleviating Patched-mediated suppression of Smo. The resulting signaling cascade leads to the translocation of a zinc finger transcriptional factor called Gli. Nuclear translocation of Gli would further activate its downstream targets (Kalderon, 2002).

In ovarian cancers, up-regulation of various components in Hh signaling has been documented in both tumor samples and *in vitro* cell line models (Bhattacharya et al., 2008; Liao et al., 2009b). For instance, the expression of Shh, a crucial ligand triggering Hh signaling, was found to be elevated in almost all ovarian tumor samples examined at protein level (Liao et al., 2009b). More importantly, overexpression of Gli1 and Patched proteins in ovarian cancers correlated with poor survival. The subcellular localization of the Hh signal protein may also be important. While Gli1 expression was mainly observed in cytoplasm in ovarian epithelial tumors, a high level of Gli1 expression in invasive cancer samples was associated with scattered nuclear Gli1 immunoreactivity. These results strongly supported the potential role of aberrant activation of Hh pathways in ovarian cancer development.

In colon cancer, a reverse association between Gli1 immunoreactivity and β -catenin nuclear accumulation has been documented (Akiyoshi et al., 2006). Owing to the crucial role of nuclear β -catenin in Wnt signaling and promotion of cell proliferation, such a relationship between Gli1 and nuclear β -catenin protein expression suggested an interplay between Hh and Wnt in cancers. Indeed, a similar observation was found in endometrial cancer accompanied by correlation between Gli1 immunoreactivity and endometrial cancer development. Compared with normal endometrium, endometrial abnormalities including simple and complex hyperplasia without atypia, atypical complex hyperplasia and endometrial cancers showed significant Gli1 overexpression (Fig. 1A). Gli1 overexpression significantly correlated with β -catenin nuclear immunoreactivity in atypical complex hyperplasia and endometrial carcinoma (Liao et al., 2009a) (Fig. 1B). Overexpression of Gli1 is likely to be an early event in endometrial carcinogenesis, operating at the stage of precancerous lesion of atypical complex hyperplasia.

Table 1. Postulated origin and location of CSCs in various gynecological cancers.

Organs	Pathological conditions	Postulated origin
Ovary	Ovarian cancer (OvCa)	Ovarian surface epithelium (OSE) (Auersperg et al., 2001; Szotek et al., 2008)
Endometrium	Endometrial cancers (EmCa)	Regenerative endometrial epithelium in the basalis (Gargett, 2007)
Cervix	Cervical Squamous cell carcinoma (SCC)	Reserve cell population in squamocolumnar junction (Martens et al., 2004)
Placenta/ trophoblast	Gestational trophoblastic disease (GTD); gestational choriocarcinoma (CCA)	Cytotrophoblast (progenitor of intermediate trophoblast and syncytiotrophoblast) (Shih le, 2007)

Stemness genes in gynecological cancers

An *in vitro* immunoprecipitation study further demonstrated a complex formation between Gli1 and β -catenin protein in endometrial cancer cell lines HEC-1A and RL95-2. Ectopic overexpression of Gli1 caused a reduced expression of β -catenin in cytoplasm and an increased expression of β -catenin in the nuclei in the endometrial cancer cells, illustrating the possible cross-

talk between aberrantly activated Hh and Wnt pathways, in accordance with the clinical findings on Gli1 overexpression and nuclear localization of β -catenin with progression of endometrial cancer development.

Sustained increased endogenous expression of Hh ligand may also provide a prerequisite for the pathway hyperactivation. Strong expression of Shh protein has

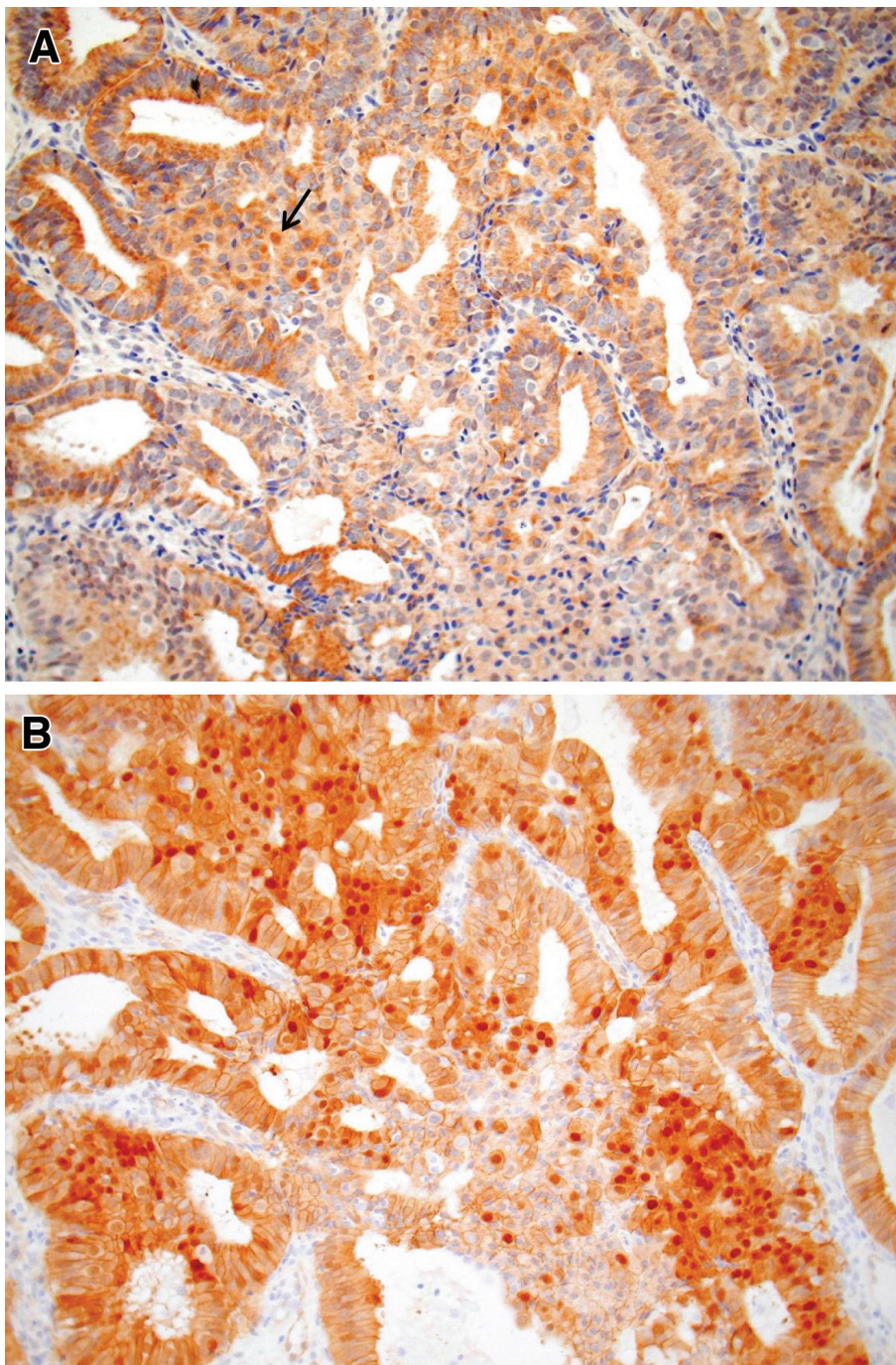


Fig. 1. Photomicrograph showing the immunoreactivities of Gli1 and β -catenin in endometrial cancer. **A.** In endometrial cancer cells, although Gli1 immunoreactivity was observed mainly in the cytoplasm, nuclear staining was sporadically present as indicated by arrow. **B.** While only membranous immunostaining of β -catenin was observed in normal endometrium (Liao et al., 2009a; with permission), distinct nuclear β -catenin could be detected in pathological endometrium, such as endometrioid type of endometrial cancer as shown in the illustration.

been demonstrated in cervical cancer and its precursor lesions, cervical intraepithelial neoplasia (CINII/III), whilst the expression was rare in normal cervical epithelium (Xuan et al., 2009). Furthermore, the expression of Shh in cervical cancer was found to be significantly related to HPV16 infection. High risk HPV infection is established to be the initial event of cervical carcinogenesis. Such findings indicate a mechanistic interaction between HPV and Hh signaling pathway in cervical carcinogenesis.

Notch signaling: Notch1, Notch3 and Notch 4

Notch signaling is an evolutionarily conserved pathway that serves as a short range communication system to direct the fate of neighboring cells, through its influences on differentiation, proliferation and apoptosis in a cellular context-dependent manner. Hence, the Notch pathway plays an important role in embryonic development and adult homeostasis (Radtke and Raj, 2003).

Activation of Notch signaling has been more extensively studied in cervical carcinogenesis. In the mid-90s, increased expression of active form of Notch1 (the intracellular form of Notch or Notch intracellular domain, NICD) was reported in high-grade precursor lesions and then invasive cervical cancers (Zagouras et al., 1995; Daniel et al., 1997). After integration of HPV genome, E6 and E7 oncoproteins encoded by the viral genome trigger the neoplastic transformation of human cervical epithelium through the modulation of gene transcriptions in the host genome (Jones and Wells, 2006). The pleiotropic nature of E6 and E7 proteins provides a mechanistic model for the interplay between HPV infection and Notch signaling reactivation. Strikingly, E6 and E7 could cooperate with Notch1 in transformation assay in HaCaT cell line, as well as generation of Xenograft tumors in nude mice (Rangarajan et al., 2001; Chakrabarti et al., 2004). Conversely, Notch signaling could also play a regulatory role on HPV transcription (Lathion et al., 2003), therefore appearing to be an intriguing self-regulatory loop in the process of cervical neoplastic transformation upon HPV viral genome integration.

Notch is a large transmembrane receptor protein which consists of an intracellular domain (NICD) and an extracellular domain. The extracellular domain contains multiple epidermal growth factor (EGF)-like repeats for ligand binding. In mammalian, four Notch genes (Notch1, Notch2, Notch3, and Notch4) encoding protein isoforms in similar organization and structure have been isolated and identified (Radtke and Raj, 2003; Weinmaster, 1997). Upon ligand binding to the receptor Notch, simultaneous proteolytic cleavage activates NICD by releasing it from the membrane. The resulting activated cytosolic fragment translocates to the nucleus where it activates transcription.

Heterogeneous expression patterns of these different Notch isoforms have been demonstrated in various

gynecological malignancies compared with their normal counterparts. In pathological endometrium, Cobellis and co-workers summarized that Notch1 expression increased from polyps to carcinoma, whereas Notch4 expression decreased (Cobellis et al., 2008). On the other hand, frequent amplification of Notch3 was found in ovarian tumors. More than half of ovarian cancers exhibit Notch3 expression in both cytoplasm and nucleus by immunohistochemistry, while normal ovarian epithelial cells did not show immunoreactivity for Notch3 (Park et al., 2006).

Although both cervical and endometrial cancers showed elevated Notch1 expression, Notch1 mRNA was found to be downregulated in ovarian tumors. However, Hopfer et al. demonstrated in their comprehensive experiments that the increased Notch1 protein expression was produced by an increase in the cleavage of transmembrane Notch (NICD) in ovarian tumors, but not by direct increased transcription. They found that the expression of a downstream target of NICD, the HES-1 protein, was higher in the malignant ovarian tumors compared with benign cases. Transfection of active NICD also resulted in a proliferative advantage over cells transfected with empty vector control in the ovarian carcinoma cell line A2780 (Hopfer et al., 2005). In summary, hyperactivation of Notch signaling, particularly though elevated Notch1 and its active cleaved form, is a common event in gynecological cancers.

Master transcription factors: Nanog, Sox2 and Oct4

In early embryo development, Nanog, Sox2 and Oct4 are three key transcription factors responsible for pluripotency induction and regulation. As mentioned earlier in this article, the blastocyst comprises an outer layer trophectoderm (TE), and a group of pluripotent cells, the ICM at preimplantation development. While the TE will develop into placental tissues, the ICM gives rise to the various tissue types of the embryo (Na et al., 2010). The term "stemness" refers to the unique abilities of stem cells, such as pluripotency, as well as an ability to self renew (Wong et al., 2008).

Oct4 and Nanog are the two of the earliest factors known to regulate the formation of pluripotent ICM cells (Nichols et al., 1998; Chambers et al., 2003). On the other hand, Sox2 closely works with Oct4 to regulate the transcription of key pluripotency genes, including Oct4, Sox2 and Nanog as complex reciprocal regulatory circuits (Masui et al., 2007). Since Nanog, Oct4 and Sox2 form a core regulatory network that coordinately determines embryonic stem cells self renewal and differentiation, these three transcription factors are postulated to conceptually contribute to tumorigenesis (Jeter et al., 2009).

Nanog

Expression of Nanog is reported to be restricted to

the ICM but not TE, although Oct4 was detected in both TE and ICM cells during early embryogenesis (Harvey et al., 2009). The trophoblast population in placenta derived from TE lineage is composed of trophoblastic sub-populations: cytotrophoblast, syncytiotrophoblast and villous intermediate trophoblast. Abnormal growth of trophoblast leads to the development of several subtypes of gestational trophoblastic disease (GTD), including premalignant hydatidiform moles, as well as classical neoplastic malignancies such as choriocarcinoma (Cheung et al., 2009).

In first trimester and term placenta, Siu et al. also found that Nanog expression was barely detectable by quantitative PCR and Nanog protein expression was scarce and confined to the nucleus of the cytotrophoblast as shown by immunohistochemistry (Siu et al., 2008) (Fig. 2). These findings suggest that expression of Nanog should be tightly controlled for proper trophoblast physiology. Evidently, significantly higher Nanog mRNA and protein expressions were demonstrated in GTD including, hydatidiform moles and choriocarcinoma. Overexpression of Nanog was also found to correlate with clinical outcome of GTD patients. *In vitro*, stable knockdown of Nanog impaired aggressive behaviors in choriocarcinoma cells, including decreased cell motility and increased apoptosis. Hence, these findings in GTD highlight the prominent role of Nanog in neoplastic transformation once it was aberrantly overexpressed.

Similarly, overexpression of Nanog is involved in cervical and endometrial tumors. Nanog expression level was significantly higher in cervical squamous cell

carcinoma (SCC) than CIN. The Nanog expression in normal cervical epithelia was even lower than that of CIN. Nanog expression levels also vary with different tumor sizes (Ye et al., 2008). Recently, overexpression of Nanog in endometrial adenocarcinoma suggests Nanog to be a potential therapeutic target and a useful biomarker for endometrial adenocarcinoma, and the latter may be useful to differentiate between endometrial adenocarcinoma and benign endometrial tissues (Zhou et al., 2011)

Oct4 and Sox2

Peng and co-workers analyzed 14 human ovarian tumor samples of various histological subtypes by immunohistochemistry. They found that six out of seven (85.7%) poorly differentiated tumors showed positive Oct4 immunostaining, whereas four of the five (80%) moderately or well-differentiated tumors, and two of the two benign tumors (100%) were negative for Oct4 staining (Peng et al., 2010). Similarly, expression of Oct4 was not detectable in normal endometrial tissues, while overexpression of Oct4 was found in poorly differentiated endometrial adenocarcinoma samples, at a higher level compared with that in well-differentiated samples (Wu et al., 2011). Oct4 activity is essential for pluripotency of ICM cells (Nichols et al., 1998). Overexpression of Oct4, particularly in those poorly differentiated tumor cells, appears to resemble the property of undifferentiated stem cells for unlimited proliferation and self-renewal.

However, Oct4 may express differently in cervical

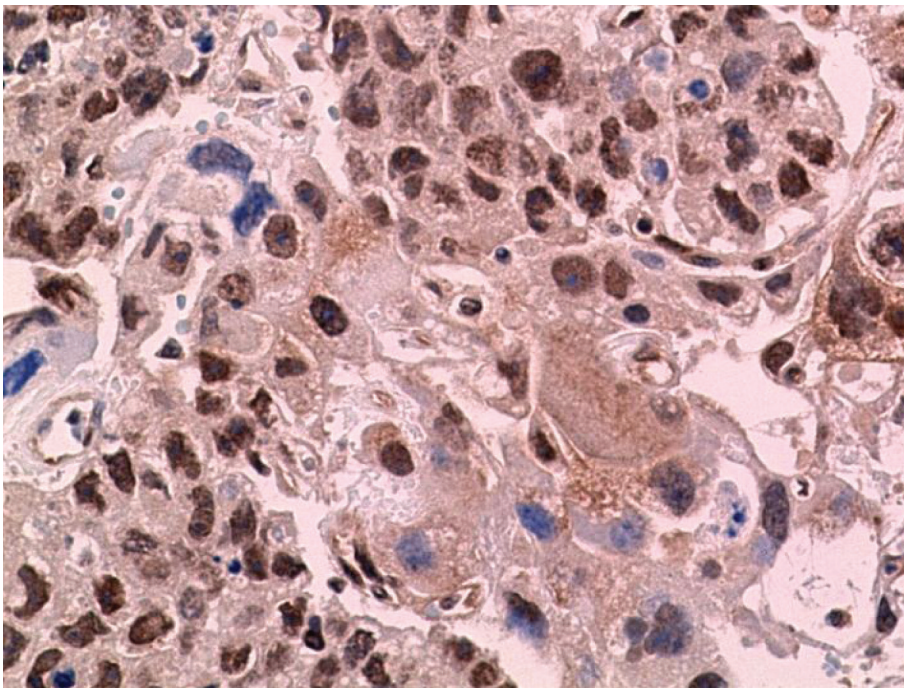


Fig. 2. Immunoreactivity of Nanog in choriocarcinoma. Histologically, choriocarcinoma is composed of a mononucleated cytotrophoblast and multi-nucleated syncytiotrophoblast. Immunohistochemistry showed that the Nanog immunoreactivity was mainly detected in nucleus of cytotrophoblast. Overexpression of Nanog has been demonstrated in choriocarcinoma in comparison to normal placenta.

cancers. Oct4 expression was almost absent in somatic cervical cell line Hela accompanied by promoter hypermethylation (Cantz et al., 2008). Likewise, while Oct4 is already epigenetically regulated by methylation at transcriptional level in trophoblast cells of normal placenta, Oct4 promoter was further hypermethylated and down-regulated in GTD (Zhang et al., 2008a). Similarly, hypermethylation of Sox2 was also demonstrated in GTD with significant correlation with mRNA expression. A significant reduction in Sox2 mRNA expression was found in the hydatidiform moles when compared with that in the placentas (Li et al., 2008)

In addition, epigenetic mechanisms may also play a

crucial role in the transcriptional down-regulation of Sox2 in endometrial carcinogenesis (Wong et al., 2010). On the contrary, a significant increase of nuclear Sox2 staining was reported compared with normal cervix (Ji and Zheng, 2010). Hence, re-activation or inactivation of Sox2 and Oct4 may be tissue specific and therefore different in the context of corresponding gynecological cancers.

Cancer stem cells (CSCs)

Although the general expression pattern of pluripotent transcription factors, especially Oct4 and Sox2, appears to be varied in various gynecological

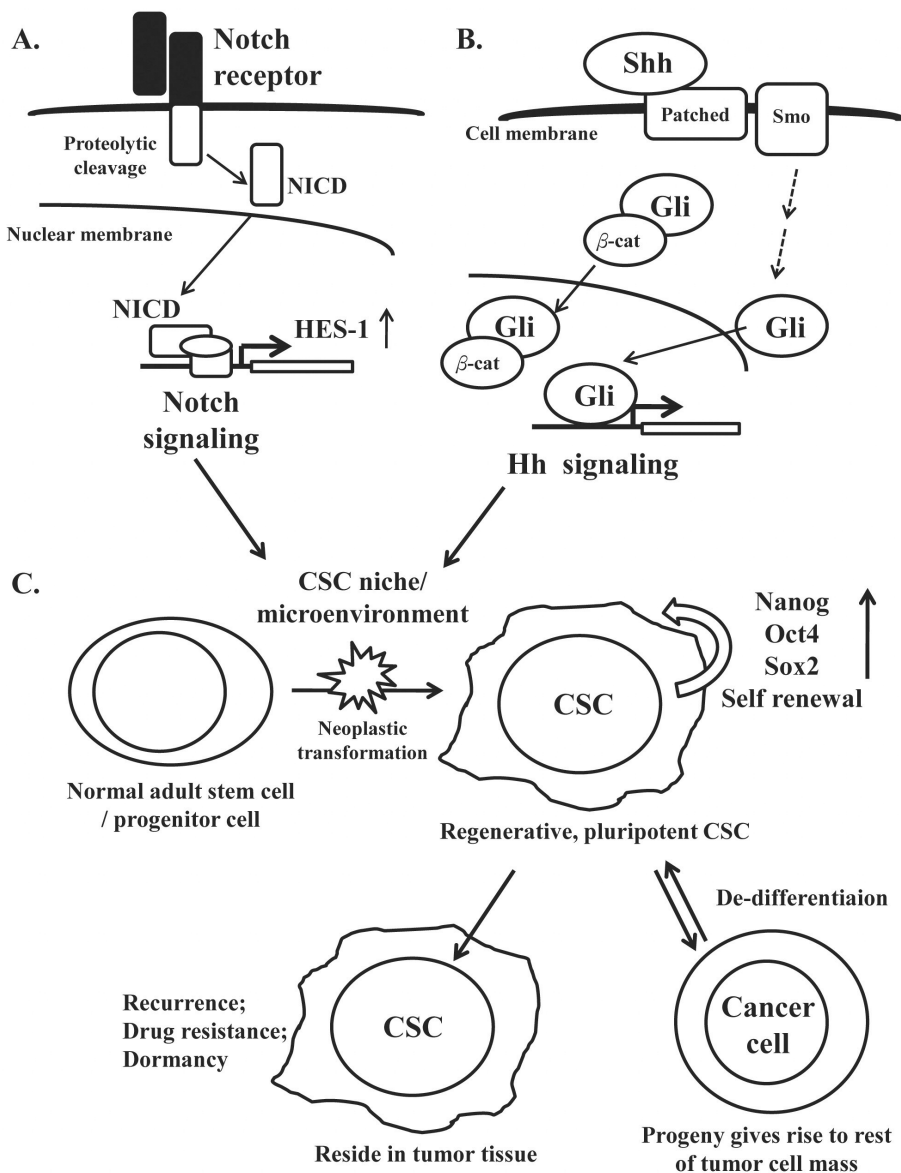


Fig. 3. Schematic diagram of dysregulated stemness-related pathways and cancer stem cell (CSC) theory. Stemness-related signaling pathways, Notch and hedgehog (Hh), are frequently hyperactivated in gynecological tumors. **A.** Notch signal transduction is mediated by the transmembrane Notch receptors (Notch1 to 4). Activation of Notch upon ligand binding leads to proteolytic cleavage that releases an intracellular domain of Notch (NICD), which in turn translocates to the nucleus as a transcription activator on target genes (Radtke and Raj, 2003). Overexpression of Notch pathway components, as well as downstream effector HES-1, results in hyperactivation of this pathway in cervical, endometrial and ovarian cancers (see text). **B.** Constitutive activation of Hh signaling in gynecological cancers is usually caused by increased expression of ligand Shh and transcription factor Gli (Evangelista et al., 2006). Signal cascade triggered by ligand binding of Shh subsequently leads to translocation of Gli from cytoplasm to nucleus for its function. Overexpressed Gli also promotes increased β -catenin (β -cat) nuclear accumulation through protein-protein interaction, suggesting a possible cross-talk with Wnt signaling (Akiyoshi et al., 2006). **C.** In tumor hierarchy model, cancer stem cells (CSCs) recapitulate the expression of core regulatory genes like Nanog, Oct4 and Sox2, which are important in self-renewal and pluripotency. Hence, regenerative CSCs possess the potential to give rise to progenies which in turn generate a bulk of tumor mass. CSC residing in the tumor tissue therefore implicates tumor dormancy, drug resistance and recurrence (Bapat, 2010). Recent hypothesis on CSC niche or microenvironment suggests that short-range communicating Notch and Hh signaling maintain stemness of CSCs in a paracrine manner (LaBarge, 2010). These models may help to explain the neoplastic transformation of CSC from normal adult stem cell or progenitor cells in gynecological cancers under frequent activation of Notch and Hh pathways as discussed in this review.

cancers compared to their normal counterparts, re-expression of these key transcription factors indeed help to identify a limited number of tumor cells that potentially initiate a heterogeneous tumor. In this hierarchy model, only a subset of cells, so-called cancer stem cells (CSCs) that possess self-renewal ability, can initiate tumour growth. They can in turn give rise to non-tumorigenic progeny, making up the bulk of the tumour (Dick, 2008).

The origin of CSC remains controversial. It has been hypothesized that CSCs may be derived from normal adult stem cells or progenitor cells that maintain homeostasis of normal tissue, but evades physiological regulatory mechanisms; or from remodeling of fully mature cells by aberrant de-differentiation (Lapidot et al., 1994; Singh et al., 2004; Song and Miele, 2007). CSCs are considered particularly important in drug resistance, tumor dormancy, minimal residual disease, and relapse of malignancy (Bapat, 2010). Their basic characteristics define mechanisms of quiescence that enable them to resist and evade therapy, and even exert their regenerative capabilities through self-renewal under optimal conditions.

Isolation of cancer stem cells (CSCs) in gynecological cancers

As CSCs only contribute to a small fraction of the total tumor cell mass, special isolating and enriching approaches have been developed. Some of the CSC isolating approaches make use of properties that are shared between the putative CSCs and normal embryonic/ adult stem cells. The following are three commonly used approaches that have been applied in the study of CSC in gynecological cancers to date.

Clonogenicity

Classical cell culture allows establishment of single-cell derived clones having stem cell characteristics. Based on this approach, Bapat and co-workers isolated long term surviving tumorigenic clones among a mixed population of cells derived from ascitic fluid of a patient with advanced ovarian cancer. These transformed clones differentiate to grow in an anchorage-independent manner as spheroids *in vitro* (Bapat et al., 2005). Further analysis showed that these clones expressed specific markers associated with stem and/or progenitor cells, including Nestin, Oct4 and Nanog, suggesting the maintenance of an undifferentiated state. Interestingly, these three markers were distinctly expressed in the transformed clones in monolayer form, but the expressions were then reduced or absent on differentiation into spheroids, indicating a possible multipotent nature of the isolated clones from ovarian cancers. Using a similar approach in cervical cancers, most tumorsphere cells isolated from fresh cervical cancer tissues also showed Sox2 expression, but the differentiated tumorsphere cells did not (Ji and Zheng,

2010).

Another study in endometrial cancer with a similar isolating approach showed that 25 out of 28 endometrial carcinoma samples contained a small population of clonogenic cells (on average 0.24%). This small population of proposed CSC demonstrated no significant difference in cloning efficiency between different grades of endometrial carcinoma or between endometrial carcinoma and normal endometrial epithelial counterpart. Clonally derived endometrial carcinoma cells also expressed the self-renewal genes, such as Nanog, Sox2, and Bmi-1. The clonogenic, self-renewing, differentiating, and tumorigenic properties of these cells suggest that such a CSC population may be responsible for production of endometrial carcinoma tumor cells (Hubbard et al., 2009).

Side population identified by Hoechst dye

Side population (SP) is a small subpopulation of cells having putative cancer stem cells properties accompanied by the capacity of active expulsion of the dye Hoechst 33342, resulting in the isolation of a low and high fluorescing side population. Gao et al. identified and isolated the SP cells from ovarian cancer cell line OVCAR-3. In this study, only 0.9 % of the whole OVCAR-3 population was sorted as SP cells which were capable of holoclone formation. Besides having a higher colony formation efficiency, these SP cells also express a higher level of self-renewal marker Oct4 than the non-SP cells (Gao et al., 2009). *In vivo*, Szotek et al. demonstrated that their isolated SP cells from ovarian cancer cell lines could regenerate tumors with lower latency and at a higher frequency than the non-SP cells. In addition to the capacity for self-renewal, these SP cells could produce heterologous non-SP descendent (Szotek et al., 2006).

Kato and colleagues characterized the SP cells in human endometrial cancer cells and in rat endometrial cells expressing oncogenic human K-Ras protein. Besides characteristics that resembled the putative CSCs such as self-renewal, long term proliferative capacity, enhanced tumorigenicity as well as reduction in the expression levels of differentiation markers, these endometrial SP cells also showed enhancement of migration, lamellipodia, and uropodia formation (Kato et al., 2010).

Surface markers

Based on the premise that CSCs can either be derived from normal stem cells by neoplastic transformation or from de-differentiated cells that have acquired stemness restoration, CSCs may display similar cell surface immunophenotype as normal stem cells in the organ (Lapidot et al., 1994; Singh et al., 2004). Therefore, cell surface markers may also help to identify CSCs from tumors.

CD44 is a multi-functional cell surface marker of

'normal' stem cells with adhesion and signaling roles (Tang et al., 2007). Isolation of stem-like populations using CD44 has been reported from various cell lineages (Sales et al., 2007). Expression of CD44 is likely to be a supportive marker for ovarian CSCs, as CD44 and CD117 have been identified in normal OSE cells (Parrott et al., 2000). After enrichment of ovarian CSCs from tumor samples making use of their capability of anchorage-independent growth to give rise to multilayered spheroids in culture, Zhang et al. screened the developing spheroids for expression of CD44 and CD117 (Zhang et al., 2008b). These ovarian CSCs showed CD44+/CD117+ phenotypes in association with enhanced chemoresistance to the ovarian cancer chemotherapeutics cisplatin or paclitaxel, as well as up-regulation of stem cell markers (e.g. Bmi-1, Notch1, Nanog, nestin, and Oct4) compared with parental tumor cells. Interestingly, CD44 expression is also observed in hyperplastic and malignant endometrial tissue (Afify et al., 2005), but analysis of cultured endometrial cancer cell lines revealed highly variable CD44 expression (Friel et al., 2008). The presence of CD133+ cells has been reported in normal endometrium (Schwab et al., 2008), and the role of CD133 as a CSC cell marker in endometrial cancer has been investigated. Whereas sorted CD133+ endometrial cancer cells were capable of generating both CD133+ and CD133- cells, CD133+ cells showed more aggressive proliferative potential *in vitro* and increased tumorigenicity *in vivo* than CD133- cells. These findings suggest that CD133 can serve as a marker for endometrial CSC (Nakamura et al., 2010)

Crosstalk between the Hh and Notch signals in CSCs

While putative CSCs reside in the bulk of tumor tissue, neighboring cells contribute to the niche microenvironments of CSCs and exert crucial control of their stem-like activities. The importance of Notch, Hh and Wnt pathways in determining the biological properties of CSCs in a paracrine manner have been lately projected (LaBarge, 2010). Under such a specialized environment, the stem cell niche, stem cell maintenance and self-renewal can be regulated. Indeed, Hh and Notch signaling can act as short-range communication system to direct the fate of neighboring cells (Artavanis-Tsakonas et al., 1999; Christian, 2000). Recently, Steffensen and co-workers found that CD44+ epithelial ovarian cancer stem cells localized in clusters surrounded by differentiated ovarian cancer cells and in close proximity with the stroma. Alterations to the stroma may thus affect the control of self-renewal (Steffensen et al., 2011).

Zbinden and colleagues found that Gli-nanog axis could promote stemness and growth. They showed in gliomas that Nanog modulating gliomasphere clonogenicity, stem cell behavior and proliferation are regulated by Hh signaling. Interestingly, Gli1 also requires Nanog activity to form a positive regulatory loop (Zbinden et al., 2010). Moreover, involvement of

the hedgehog pathway in regulating growth of cancer spheroid-forming cells was also recently reported in ovarian cancer *in vitro*. Ovarian cancer spheroid-forming cells (SFCs) were treated with Hh agonists (Shh and Ihh) as well as an Hh inhibitor cyclopamine to determine changes in spheroid growth and survival. Results showed that all ovarian cancer cell lines readily formed spheroids in non-adherent growth conditions, while IOSE80, a normal cell line, failed to form SFCs (Ray et al., 2011). Similarly, Notch blockade by a γ -secretase inhibitor, (DAPT), markedly inhibited self-renewal and proliferation of ovarian cancer stem-like cells (OCSCs), significantly downregulated the expression of OCSC-specific surface markers, and reduced protein and mRNA expression of Oct4 and Sox2 in OCSC-like cells (Jiang et al., 2011). This suggested that Notch signaling might be useful for the stemness maintenance of OCSCs and the γ -secretase inhibitor could be a promising treatment targeting OCSCs. Owing to these newly proposed potential regulatory roles of Hh and Notch pathways on stemness of CSCs, the effects and underlying molecular mechanisms of these pathways in the carcinogenesis of gynecological malignancies are still not fully understood. Complete eradication of CSCs may be possible if CSCs can be specifically targeted through manipulation of these signaling pathways.

Conclusion

In this review, we highlighted the expression pattern of dysregulated Hh, Notch and master transcription factors in gynecological cancers. One of the implications of CSC is its crucial roles in tumor dormancy, drug resistance and recurrence. Given that Hh and Notch are frequently activated in gynecological cancers, understanding the interplay between these components and pluripotent transcription regulators, in particular Nanog, may promote better women's health through refining the mechanism of carcinogenesis. This can eventually help in the development of effective treatments targeting, for example, CSC, for gynecological malignancies.

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