ALDH in Gynecologic Malignancies

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Gynecologic cancers cause over 600,000 deaths annually in women worldwide. The development of chemoresistance after initial rounds of chemotherapy contributes to tumor relapse and death due to gynecologic malignancies. In this regard, cancer stem cells (CSCs), a subpopulation of stem cells with the ability to undergo self-renewal and clonal evolution, play a key role in tumor progression and drug resistance. Aldehyde dehydrogenases (ALDH) are a group of enzymes shown to be robust CSC markers in gynecologic and other malignancies. These enzymes also play functional roles in CSCs, including detoxification of aldehydes, scavenging of reactive oxygen species (ROS), and retinoic acid (RA) signaling, making ALDH an attractive therapeutic target in various clinical scenarios. In this review, we discuss the critical roles of the ALDH in driving stemness in different gynecologic malignancies. We review inhibitors of ALDH, both general and isoform-specific, which have been used to target CSCs in gynecologic cancers. Many of these inhibitors have been shown to be effective in preclinical models of gynecologic malignancies, supporting further development in the clinic. Furthermore, ALDH inhibitors, including 673A and CM037, synergize with chemotherapy to reduce tumor growth. Thus, ALDH-targeted therapies hold promise for improving patient outcomes in gynecologic malignancies.

gynecologic malignancies | cancer stem cells | aldehyde dehydrogenases

1. Introduction

The first line of therapy for most gynecologic cancers includes surgery, followed by chemotherapy and radiation [1]. However, in the majority of cases, these conventional therapies do not completely eliminate the malignant cells. The primary reason for high mortality is recurrence and subsequent metastasis caused by the residual population of cancer cells [2][3]. The cells that survive after the first line of treatment and contribute to cancer recurrence are known as CSCs [4][5]. The CSC theory states that the tumor is a heterogeneous mass, and within the tumor exists a hierarchy of cells, with CSCs at the apex [6]. Lapidot et al. first proposed the idea that a set of specialized cells present within the tumor can sustain and repopulate the tumor [7]. CSCs have since been reported in gynecologic malignancies (Table 1).

Table 1. Cancer stem cells reported in gynecologic malignancies.[8][9][10][11][12][13][14][15][16][17][18][19][20]

Gynecologic Malignancy	Cancer Stem Cells	References
Cervical cancer	Reported in literature	[8–10]
Uterine cancer	Reported in literature	[11–14]
Ovarian cancer	Reported in literature	[15–19]
Vulvar cancer	Reported in literature	[20]
Vaginal cancer	No published reports	-

CSCs are resistant to conventional chemotherapy due to several mechanisms. Chemotherapeutic drugs, primarily platinum-based drugs, form DNA crosslinks, killing cells by causing DNA damage in rapidly-dividing cells [21]. However, CSCs are resistant to DNA damage due to a number of properties, including slow cycling, reduced uptake of drugs and increased drug efflux due to the high expression of a class of non-selective drug transporters called adenosine triphosphate binding cassette (ABC) ATPases [22]. Furthermore, CSCs have enhanced DNA repair due to overexpression of repair pathways such as ataxia-telangiectasia-mutated (ATM), ataxia telangiectasia and rad3-related (ATR), checkpoint kinase 1 (Chk1), poly(ADP-ribose) polymerase 1 (PARP1), and RAD51 [23] that protect CSCs from drugs designed to cause cancer cell death by inducing DNA damage. As a quiescent population [24], CSCs are further protected by platinum-induced DNA damage. Thus, it is necessary to target CSCs specifically to achieve a better prognosis in patients. Of the different CSC markers identified to date in gynecologic malignancies, ALDH is widely recognized as a highly robust CSC marker across the vast majority of cancer types, including gynecologic CSCs. Furthermore, ALDH holds the distinction of having potential functional importance in the maintenance of CSCs [25], making it an attractive target for eradicating CSC in the therapeutic maintenance setting for gynecologic malignancies such as ovarian cancer.

The ALDH superfamily comprises 19 members, all of which are involved in regulating crucial functions in normal as well as cancer stem cells. The primary role of ALDH enzymes is to metabolize reactive aldehydes produced by various biological processes [26] (Figure 1).

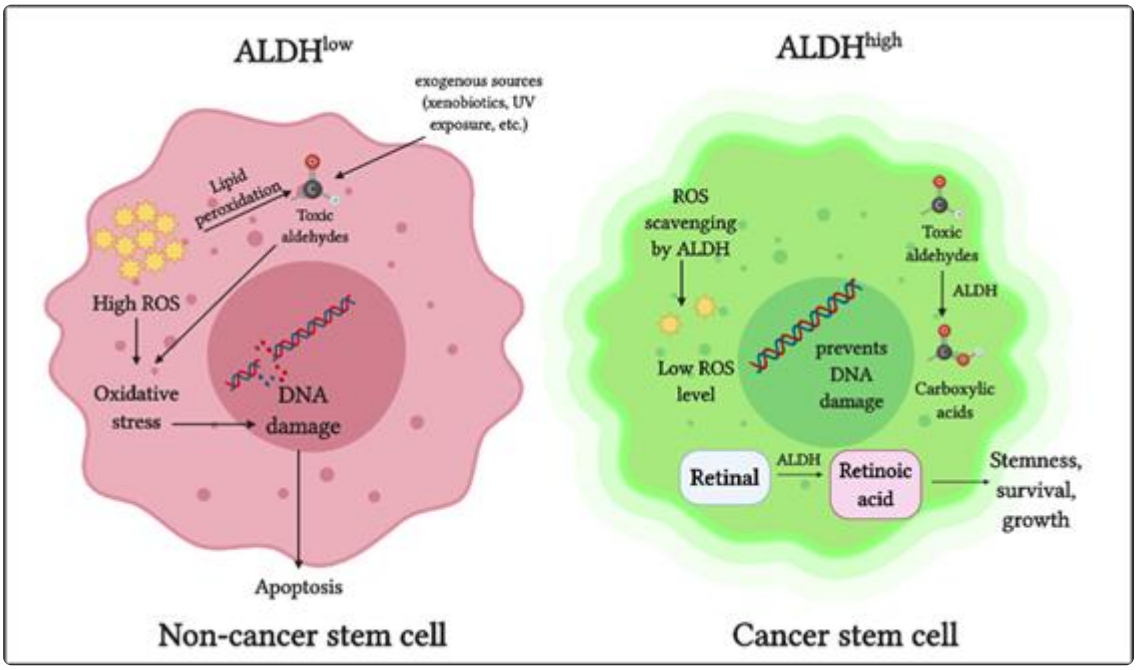

Figure 1. Role of aldehyde dehydrogenases (ALDH) in cancer stem cells: ALDH detoxifies toxic aldehydes (endogenous and exogenous) into less toxic carboxylic acids. ALDH maintains intracellular reactive oxygen species (ROS) at a low level thus preventing oxidative stress and DNA damage. ALDH oxidizes retinaldehyde into retinoic acid, which promotes stemness, growth, and survival in cancer stem cells.

Detoxification of aldehydes is critical for cellular health, as aldehyde toxicity can lead to DNA damage, impaired cellular homeostasis, and cell death [27]. Another vital role of ALDH is in retinoic acid metabolism, which is crucial for gene expression and morphogenesis during embryonic development growth, cellular differentiation, and homeostasis of vertebrates [28][29][30]. Cytosolic class I ALDH enzymes catalyze the NAD-dependent oxidation of both all-trans-retinal and 9-cis-retinal to all-trans-retinoic acid and 9-cis-retinoic acid [31][32]. ALDH also plays a role in reactive oxygen species (ROS) scavenging and thereby reducing oxidative stress in stem cells [33] (Figure 1).

In cancer cells, ALDH contributes to chemoresistance via different mechanisms [34]. ALDH isoforms, ALDH1A1, and ALDH3A1 are both involved in the metabolism of the cancer drug cyclophosphamide, metabolizing the active compound to a less active form and contributing to drug resistance [35]. When combined with cyclophosphamide in ALDH3A1^{high} cell lines, ALDH3A1 inhibitors have been shown to increase sensitivity to the mafosphamide (cyclophosphamide analog) [34]. Another mechanism by which ALDH protects cancer cells is by reducing ROS-mediated oxidative stress [36] (Figure 1). As a consequence of this, ALDH^{high} CSCs have a lower baseline ROS level and oxidative damage than the ALDH^{low} counterparts [33].

Clinically, high ALDH expression is associated with poor outcomes in several gynecologic malignancies, including ovarian cancer (OC) [37][38][39][40], endometrial [41], and cervical cancer (CC) [42][43], as well as other solid tumors including breast [44][45][46], lung adenocarcinoma [47], rectal [48], esophageal squamous adenocarcinoma [49], gastric [50], colorectal [51], prostate [52], and neuroblastoma [53]. To our knowledge, there are no published reports correlating ALDH and prognosis in vulvar or vaginal cancer. Of the 19 ALDH isoforms, ALDH1 is the primary isoform implicated in CSCs of solid tumors [54]. The advent of the Aldefluor assay has stimulated research on CSCs expressing high ALDH. Briefly, the Aldefluor assay can be used to detect cells expressing high levels of ALDH. The Aldefluor reagent Bodipy- aminoacetaldehyde (BAAA) is converted into BODIPY-aminoacetate (BAA), in the presence of ALDH, and retained inside the cells. ALDH activity of the cells is directly proportional to the fluorescence intensity [55]. The assay can detect nine ALDH isoforms, with ALDH1 as the predominant isoform contributing to ALDH^{high} cells [56]. These provide a strong rationale for targeting ALDH to eliminate CSCs in gynecologic malignancies. A recent review by Dinavahi et al. elegantly highlights the inhibitors developed to target ALDH from a pharmacologic perspective in different cancer types [57].

2. ALDH in Gynecologic Cancers

2.1. ALDH and Cervical Cancer

In tissue specimens from patients with cervical SCC or cervical intraepithelial neoplasia (CIN) II-III, high ALDH expression was observed immunohistochemically . Interestingly, peripheral blood (plasma) samples from the same

patients had increased ALDH1A1 expression when compared with samples from healthy patients . In patients with invasive SCC, ALDH1 expression correlated with lymph nodal metastasis and disease recurrence [58]. These data indicate that ALDH1 can be used as a reliable biomarker for the identification of cervical CSCs [59]. The $ALDH^{high}$ cells isolated from these cancers showed high gene and protein expression of stemness transcription factors Nanog, sex-determining region Y-box2 (Sox2), octamer-binding transcription factor (Oct4), and twist-related protein 1 (Twist1). However, the exact mechanism by which ALDH regulates stemness in cervical cancer remains incompletely understood.

2.2. ALDH and Uterine Cancer

In EC, the most prevalent type of uterine cancer, altered stemness-related pathways, including Wnt and β-catenin, support a role for CSCs [60]. Furthermore, $ALDH^{high}$ subpopulation of cells has been demonstrated to be CSCs in EC [60]. The $ALDH^{high}$ cells isolated from primary endometrial tumors were highly tumorigenic and resistant to chemotherapeutic drugs and showed increased invasive ability compared to the $ALDH^{low}$ cells [61].

In patients with uterine endometrioid carcinosarcoma, high ALDH1 expression predicted poor prognosis, lymphatic invasion, recurrence, and low overall survival [62]. $ALDH^{high}$ CSCs cells have distinct stem-like properties, such as high expression of stem-cell markers BMI1, HEY1, HES1, and adhesive molecule CD44 [63][64]; in addition, reduced expression of differentiation markers, enhanced migration, high tumorigenicity, and self-renewal ability was reported [65]. When EC cells were sorted using flow cytometry and cultured in vitro, $ALDH^{high}$ cells yielded both $ALDH^{high}$ and $ALDH^{low}$ cells, whereas $ALDH^{low}$ cells only yielded $ALDH^{low}$ cells. These results demonstrated that endometrial CSCs divide asymmetrically, in agreement with the CSC hypothesis. Furthermore, when injected into mice subcutaneously, $ALDH^{high}$ endometrial cells formed larger tumors more rapidly than the $ALDH^{low}$ cells, demonstrating that CSCs were able to repopulate the entire endometrial tumor mass. Based on these studies ALDH1 serves as both a marker for identifying endometrial CSCs and a therapeutic target, based in its functional importance in the disease.

2.3. ALDH and Ovarian Cancer

Cancer relapse after surgery and chemotherapy is common in OC, and CSCs are strongly associated with OC relapse [66]. $ALDH^{high}$ cells are widely accepted as CSCs in OC, as demonstrated by us and others [67][68][69][70][71][72]. $ALDH^{high}$ ovarian cancer stem cells (OCSCs) exhibit classic stem cell characteristics, such as being highly chemoresistant and enriched in residual xenografts after platinum therapy [72]. Upregulation of stemness genes such as Sox2, Kruppel like factor 4 (Klf4), Nanog, and downregulation of differentiation genes such as homeobox A10 (HOXA10) and homeobox A11 (HOXA11), were reported in $ALDH^{high}$ cells [72]. $ALDH^{high}$ cells demonstrated enhanced ability to form spheroids in low attachment conditions in vitro.

In OCSCs, of the 19 ALDH isoforms, ALDH1A1 was highly correlated with chemotherapy resistance [73][74]. Expression of ALDH1A1 was 100-fold higher in OC cells selected for taxane-resistance in vitro, and ALDH1A1 knockdown sensitized the resistant cells to chemotherapy. ALDH1A1 expression was higher in residual tumors

after the first round of chemotherapy compared to tumors from untreated patients [75], demonstrating enrichment of OCSC post-treatment. In addition to ALDH1A1, CD133 serves as a robust marker for OCSC when used in combination with ALDH. In this regard, Silva et al. observed that as low as 11 ALDH and CD133 double-positive cells resulted in tumor induction in mice [17]. Moreover, in tumors harvested during debulking surgeries, $ALDH^{high}CD133+$ cells correlated with reduced disease-free and overall survival in OC patients [17]. $ALDH^{high}$ cells were highly metastatic with the enhanced invasive ability and were resistant to apoptosis [76]. These data provide a strong rationale for targeting $ALDH^{high}$ cells in OC.

2.4. ALDH and Vulvar Cancer

Vulvar cancer is an uncommon type of tumor in women. To date, one study on ALDH in this gynecologic cancer has been reported. ALDH1 expression in vulvar squamous cell carcinoma, normal vulvar epithelium, and stromal tissues in a cohort of 154 patients was studied [77]. Based on clinicopathological studies, high ALDH1 expression correlated with a favorable prognosis and can be considered a potential marker for differentiated vulvar cells [77]. However, this report is contradictory to the correlation of ALDH with poor prognosis in other gynecologic cancers, suggesting that ALDH expression could be a tissue-specific marker for CSCs.

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