Original Article

Stemness-associated marker expression following hyperbaric oxygen therapy in a DMBA-induced epithelial ovarian cancer model

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Abstract

Epithelial ovarian cancer (EOC) is one of the most lethal gynecologic malignancies and is commonly associated with metastasis, recurrence, and chemotherapy resistance. Ovarian cancer stem cells (OCSCs) are thought to contribute to tumor formation, metastatic progression, treatment resistance, and recurrent disease through their ability to survive under stressful microenvironmental conditions. Hyperbaric oxygen therapy (HBOT) may influence tumor oxygenation and oxidative stress; however, its effects on ovarian cancer stemness remain unclear. The aim of this study was to evaluate the effects of HBOT on the expression profile of OCSC markers in a 7,12-dimethylbenz[a]anthracene (DMBA)-induced epithelial ovarian cancer model. This experimental post-test-only control group study included 30 female Wistar rats randomly allocated into a healthy control group, a DMBA-induced epithelial ovarian cancer group without HBOT, and a DMBA-induced epithelial ovarian cancer group treated with HBOT. HBOT was administered using 100% oxygen at 1.7 atmospheres absolute. The expression of CD44, CD133, and aldehyde dehydrogenase 1 (ALDH1) was assessed immunohistochemically using H-score analysis. Overall group differences were assessed using Kruskal–Wallis tests with Dunn–Bonferroni post hoc comparisons; inter-marker relationships and expression profiles were evaluated using Spearman correlation and principal component analysis (PCA), respectively. CD44 expression differed significantly among groups (p=0.004), and post hoc analysis showed lower CD44 expression in the HBOT-treated EOC group than in the healthy group (p=0.015) and untreated EOC group (p=0.017). CD133 expression was also significantly different among groups (p=0.032), with post hoc analysis showing higher expression in the HBOT-treated EOC group than in the healthy group (p=0.048), and no significant difference was observed between the untreated EOC and HBOT-treated EOC groups. ALDH1 expression did not differ significantly among groups (p=0.877). A strong positive correlation was observed between CD133 and ALDH1 expression (r=0.70, p<0.001). PCA demonstrated partial separation of the HBOT-treated group, suggesting changes in the overall stemness-associated expression profile. These findings suggest that HBOT may be associated with altered stemness-associated marker patterns in the DMBA-induced EOC model, although the underlying mechanisms and functional relevance require further validation.

Keywords: Epithelial ovarian cancer, ALDH1, CD44, CD133, hyperbaric oxygenation

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Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy and remains a major clinical challenge because it is frequently diagnosed at an advanced stage and is commonly associated with recurrence, metastasis, and chemoresistance [1-3]. Despite advances in molecular profiling and treatment strategies, therapeutic resistance and tumor relapse continue to limit clinical outcomes. A recent bibliometric surveillance study showed increasing research attention toward ovarian cancer and metastasis-related pathways, reflecting the ongoing concern regarding its aggressive biological behavior [4]. Co-occurrence analyses have also indicated close links between ovarian cancer, metastasis-associated keywords, and other malignancies, suggesting complex interactions among tumor progression, metastatic potential, and microenvironmental adaptation [4]. These findings highlight the importance of investigating mechanisms underlying ovarian cancer stemness and therapeutic resistance.

Tumor recurrence and persistence in EOC have been increasingly attributed to ovarian cancer stem cells (OCSCs), a small subpopulation of tumor cells with self-renewal capacity, tumor-initiating potential, and resistance to cellular stress. These cells are thought to contribute to chemotherapy resistance, metastatic progression, and tumor relapse [5-7]. Cluster of differentiation (CD)44 and CD133 are commonly used markers in OCSC studies, although their expression may vary according to tumor conditions and cellular characteristics [8-12]. In contrast, aldehyde dehydrogenase 1 (ALDH1) has been more consistently associated with stem-cell activity, chemoresistance, and poor prognosis in ovarian cancer [10,11]. In addition, hypoxic tumor microenvironments may support the survival of ALDH1-positive OCSCs by promoting metabolic reprogramming and antioxidant adaptation [13]. Therefore, evaluating multiple stem-cell markers may provide a more comprehensive understanding of stemness-related changes in EOC.

Hyperbaric oxygen therapy (HBOT) has been proposed as an oxygen-modulating intervention that may influence tumor hypoxia, oxidative stress responses, and cellular adaptation. Previous studies have demonstrated that HBOT can modify tumor oxygenation, oxidative stress response, and treatment susceptibility in several cancer models, suggesting a potential role of oxygen modulation in cancer biology [11,13-15]. However, most previous studies have focused primarily on tumor oxygenation and treatment response rather than ovarian cancer stemness-associated markers [13-15]. In particular, evidence regarding the simultaneous expression patterns of CD44, CD133, and ALDH1 following HBOT exposure is limited. Previous studies have generally evaluated individual markers rather than their combined expression profile [11,16].

Consequently, the integrated expression pattern of multiple OCSC markers following HBOT exposure remains insufficiently understood. To date, no study has comprehensively evaluated the combined expression profile of CD44, CD133, and ALDH1 after HBOT exposure in a 7,12-dimethylbenz[a]anthracene (DMBA)-induced EOC model. Therefore, the aim of this study was to evaluate the effects of HBOT on CD44, CD133, and ALDH1 expression in a DMBA-induced EOC model.

Methods

Study design and setting

An experimental study using a post-test-only control group design was conducted using DMBA-induced EOC animal models. The study was conducted at the Biomedical and Animal Research Laboratory, Faculty of Medicine, Universitas Hang Tuah, Surabaya, Indonesia. Female Wistar rats were acclimatized and then randomly allocated into three groups: healthy control group, DMBA-induced EOC group without HBOT treatment, and DMBA-induced EOC group treated with HBOT. EOC was induced using oral DMBA and HBOT was then administered. At the end of the intervention, ovarian tissue samples were collected and evaluated immunohistochemically for CD44, CD133, and ALDH1 expression using H-score analysis. Animal acclimatization, DMBA induction, HBOT exposure, and tissue processing were performed under controlled laboratory conditions.

Animals and study groups

Thirty healthy female Wistar rats aged 7–9 weeks and weighing 200–300 g were used in this study. The animals were obtained and maintained under standard laboratory conditions with free access to food and water. Before the experiment, all rats underwent a seven-day acclimatization period. Only rats with normal movement, good appetite, bright eyes, and smooth fur were included. Rats showing signs of illness, congenital abnormalities, or body weight loss of more than 10% during acclimatization were excluded. Animals that died before completion of the experimental protocol were considered dropouts.

The rats were randomly allocated into three groups, with 10 rats in each group: healthy control without DMBA induction or HBOT exposure (healthy group); DMBA-induced epithelial ovarian cancer without HBOT treatment (untreated EOC group); and DMBA-induced epithelial ovarian cancer treated with HBOT (HBOT group).

DMBA-induced carcinogenesis

Ovarian carcinogenesis was induced using DMBA, a chemical carcinogen commonly used to establish experimental EOC models in rodents [14]. Rats in the untreated EOC group and HBOT group received oral DMBA at a dose of 20 mg/kg body weight once weekly for six consecutive weeks, a dose reported to reliably induce ovarian epithelial tumorigenesis without significant systemic toxicity [15]. After the final DMBA administration, the animals were maintained without additional intervention until week 13 to allow tumor development [16]. HBOT was initiated after the tumor-induction phase in the HBOT group to ensure that oxygen exposure was applied after DMBA-induced ovarian tumor development. Rats in the healthy group did not receive DMBA induction or HBOT exposure.

Hyperbaric oxygen therapy (HBOT)

HBOT was administered after the tumor-development period in rats assigned to the HBOT group. HBOT was delivered using a custom-designed small-animal hyperbaric chamber with 100% oxygen at 1.7 atmospheres absolute (ATA). Each HBOT session lasted 30 min and was administered three times daily for five consecutive days. Rats in the healthy and untreated EOC groups did not receive HBOT exposure. The HBOT protocol was adapted from a previous study reporting that oxygen modulation can improve tissue oxygenation while remaining tolerable in rodent models [16].

Immunohistochemistry and H-score analysis

At the end of the experimental protocol, animals were anesthetized with intramuscular ketamine (20–40 mg/kg body weight) prior to tissue collection. Euthanasia was performed by exsanguination through major vascular transection. Ovarian tissue samples were harvested, fixed, processed, and subjected to immunohistochemical staining for CD44, CD133, and ALDH1. CD44 and ALDH1 immunostaining were performed using primary antibodies from MedicBio (Jakarta, Indonesia), whereas CD133 immunostaining was performed using a primary antibody from Santa Cruz Biotechnology (Dallas, TX, USA). Immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) as the chromogen and counterstained with hematoxylin according to standard immunohistochemistry protocols.

Marker expression was evaluated semi-quantitatively using the classical H-score method [17]. Three representative non-overlapping high-power fields (×400) were selected from each tissue section by two observers blinded to group allocation. Staining intensity was categorized as weak (1+), moderate (2+), or strong (3+). The H-score was calculated using the formula: H-score = (1 × percentage of weakly stained cells) + (2 × percentage of moderately stained cells) + (3 × percentage of strongly stained cells), yielding a score ranging from 0 to 300, with higher scores indicating greater marker expression. The final H-score for each animal was calculated as the mean of the three evaluated fields. Histological assessments were performed in a blinded manner, and discrepant findings were resolved by consensus.

Statistical analysis

Marker expression values were presented as mean ± standard deviation (SD). Prior to comparative analysis, data distribution was assessed using the Shapiro–Wilk test. Since the

marker expression data did not fully satisfy the assumptions required for parametric testing, differences among the three study groups were analyzed using the Kruskal–Wallis test followed by Dunn's post hoc test with Bonferroni correction for pairwise comparisons. Effect size was calculated using eta squared (η^2) to estimate the magnitude of differences between groups.

Correlations between stemness markers were evaluated using Spearman's rank correlation coefficient (r). Principal component analysis (PCA) was also performed to explore the combined expression profile of CD44, CD133, and ALDH1 and to visualize the overall distribution of samples across groups. All tests were two-tailed, and $p<0.050$ was considered statistically significant. Data were analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

Results

Immunohistochemical analysis of stemness-associated markers

Immunohistochemical (IHC) analysis was performed to evaluate CD44, CD133, and ALDH1 expression in healthy, untreated EOC and HBOT-treated EOC groups; the representative staining patterns are presented in **Figure 1**. CD44, CD133, and ALDH1 staining were observed in all groups, with differences in staining intensity between samples.

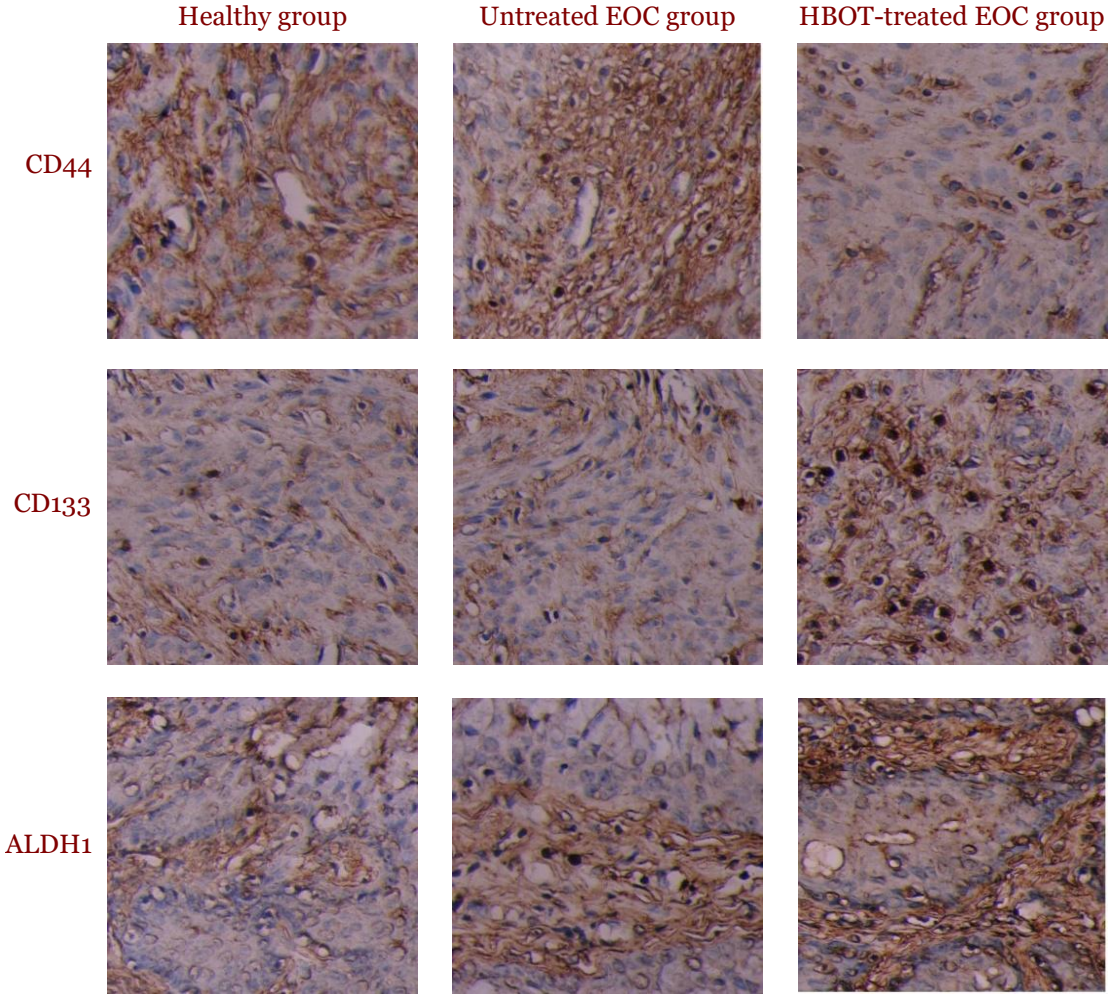

Figure 1. Representative immunohistochemical staining for CD44, CD133, and ALDH1 in the healthy, untreated EOC, and HBOT-treated EOC groups. Brown staining indicates positive expression. All images were obtained under ×400 magnification.

Quantitative analysis showed detectable expression of all markers in all groups. Compared with the healthy group, the untreated EOC group showed higher overall expression values for all

markers. CD44 expression in the untreated EOC group varied between specimens, and CD133 staining also appeared uneven across samples. In contrast, ALDH1 expression appeared more consistent than that of CD44 and CD133.

Comparisons of quantitative expression analysis of CD44, CD133, and ALDH1

The comparison of CD44, CD133, and ALDH1 H-score expression among the healthy, untreated EOC, and HBOT-treated EOC groups is presented in **Table 1**. CD44 expression differed significantly among the three groups (p=0.004), with the highest mean H-score observed in the untreated EOC group and the lowest in the HBOT-treated EOC group. A significant difference was also observed for CD133 expression (p=0.032), with the highest mean H-score found in the HBOT-treated EOC group. The effect size was larger for CD44 (η^2=0.34) than for CD133 (η^2=0.19), indicating a stronger group-related difference for CD44 expression. In contrast, ALDH1 expression showed no significant difference among the groups (p=0.877), suggesting relatively comparable ALDH1 H-score expression across healthy, untreated EOC, and HBOT-treated EOC tissues (**Table 1**).

Table 1. Comparison of CD44, CD133, and ALDH1 H-score expression among healthy, untreated EOC, and HBOT-treated EOC groups

Stemness-markers	H-scores (mean±SD)			Kruskal–Wallis H	p-value[b]
	Healthy group	Untreated EOC group	HBOT-treated EOC group		
	n=10	n=10	n=10		
CD44	33.83±12.94	37.32±20.37	17.16±5.00	10.93	0.004*
CD133	27.99±12.66	36.32±9.36	41.26±8.31	6.90	0.032*
ALDH1	25.31±10.93	30.00±13.57	26.35±17.58	0.26	0.877

ALDH1: aldehyde dehydrogenase 1; CD133: cluster of differentiation 133; CD44: cluster of differentiation 44; EOC: epithelial ovarian cancer; HBOT: hyperbaric oxygen therapy; SD: standard deviation.
[a] H-scores were calculated from the average of three representative non-overlapping high-power fields (×400) per animal
[b] Group comparisons were performed using the Kruskal–Wallis test
* Statistically significant at p<0.05

Post hoc pairwise analyses using Dunn's test with Bonferroni correction were conducted for markers demonstrating significant overall differences in the Kruskal–Wallis test (**Table 2**). For CD44, the HBOT-treated EOC group exhibited significantly lower H-score expression compared with both the healthy group and the untreated EOC group, with p=0.015 and p=0.017, respectively. In contrast, CD44 expression did not differ significantly between the healthy group and the untreated EOC group (p=1.000) (**Table 2**).

For CD133, a significant difference was observed only between the healthy group and the HBOT-treated EOC group (p=0.048), indicating altered CD133 expression following HBOT exposure relative to healthy tissue (**Table 2**). No significant differences were detected between the healthy group and the untreated EOC group or between the untreated EOC group and the HBOT-treated EOC group (**Table 2**). Pairwise comparisons were not performed for ALDH1 because the overall Kruskal–Wallis test did not indicate a significant difference among groups.

Table 2. Pairwise comparisons of CD44 and CD133 H-score expression among study groups

Stemness-markers	Group comparison[a]	Adjusted p-value[b]
CD44	G0 vs G2	0.015*
	G1 vs G2	0.017*
	G0 vs G1	1.000
CD133	G0 vs G2	0.048*
	G0 vs G1	0.200
	G1 vs G2	1.000

ALDH1: aldehyde dehydrogenase 1; CD133: cluster of differentiation 133; CD44: cluster of differentiation 44; EOC: epithelial ovarian cancer; G0: healthy group; G1: untreated epithelial ovarian cancer group; G2: hyperbaric oxygen therapy-treated epithelial ovarian cancer group; HBOT: hyperbaric oxygen therapy
[a] Pairwise comparisons were performed only after a significant overall Kruskal–Wallis test result
[b] Adjusted p-values were calculated using Bonferroni correction
* Statistically significant at p<0.05

Effect-size analysis supported the comparative findings by indicating that the strongest group-level separation occurred for CD44 (η^2=0.34), followed by a smaller separation for CD133 (η^2=0.19), whereas ALDH1 showed minimal separation across groups. Thus, CD44 represented the clearest marker response after HBOT exposure, while CD133 and ALDH1 reflected a more heterogeneous stemness-associated expression pattern.

Correlation among CD44, CD133, and ALDH1 H-score expression

Spearman correlation analysis was performed to assess the inter-marker relationships of CD44, CD133, and ALDH1 H-score expression across all study animals (**Table 3**). A strong positive correlation was observed between CD133 and ALDH1 expression (r=0.70, p<0.001), indicating coordinated expression of these two stemness-associated markers across the study population. In contrast, CD44 showed no significant correlation with CD133 (r=−0.09, p=0.630) or ALDH1 (r=0.30, p=0.120). These findings suggest that CD133 and ALDH1 may reflect a more closely related expression pattern, whereas CD44 expression appears to vary independently of the other two markers in this experimental setting.

Table 3. Correlation between CD44, CD133, and ALDH1 H-score expression in all study animals (n=30)

Stemness-markers[a]	Spearman's rank correlation coefficients (r)[b]		
	CD44	CD133	ALDH1
CD44	1.00	−0.09 (p=0.630)	0.30 (p=0.120)
CD133		1.00	0.70 (p<0.001)*
ALDH1			1.00

ALDH1: aldehyde dehydrogenase 1; CD133: cluster of differentiation 133; CD44: cluster of differentiation 44

[a] Marker expression values were calculated as H-scores derived from the average of three representative microscopic fields per animal

[b] Corresponding p-values are shown in parentheses

* Statistically significant at p<0.05

Distribution and multivariate profiling of stemness-marker H-scores

Violin plot analysis demonstrated marker-specific distribution patterns across the healthy, untreated EOC, and HBOT-treated EOC groups (**Figure 2**). CD44 H-scores were markedly shifted downward in the HBOT-treated EOC group, with a narrower distribution than those observed in the healthy and untreated EOC groups. CD133 H-scores showed an upward shift in the EOC groups, particularly in the HBOT-treated EOC group, although substantial overlap remained across groups. In contrast, ALDH1 H-scores displayed broad and overlapping distributions, indicating substantial inter-animal variability without a clear group-specific shift.

Unsupervised PCA was performed to evaluate whether the combined expression profiles of CD44, CD133, and ALDH1 discriminated the study groups (**Figure 3**). The first two principal components accounted for 91.8% of the total variance, with PC1 and PC2 explaining 63.0% and 28.8%, respectively. The HBOT-treated EOC group showed partial separation from the healthy and untreated EOC groups, primarily along PC1, whereas the healthy and untreated EOC groups showed greater overlap. These findings suggest that HBOT was associated with a shift in the overall stemness-marker expression profile, although residual overlap between groups indicates persistent biological heterogeneity.

Discussion

A main finding of this study was a decrease in CD44 expression after HBOT exposure. CD44 is involved in cell adhesion, migration, and interaction with the tumor microenvironment, and its expression may change under hypoxic conditions [18,19]. In the present study, CD44 showed the clearest response after HBOT exposure. These findings are consistent with the possibility that oxygen modulation affects stemness-related pathways associated with tumor microenvironment interactions. Similar observations have been reported in ovarian cancer stem cell studies, showing that stemness-associated signaling may change in response to alterations in oxygen availability and cellular stress [11,20].

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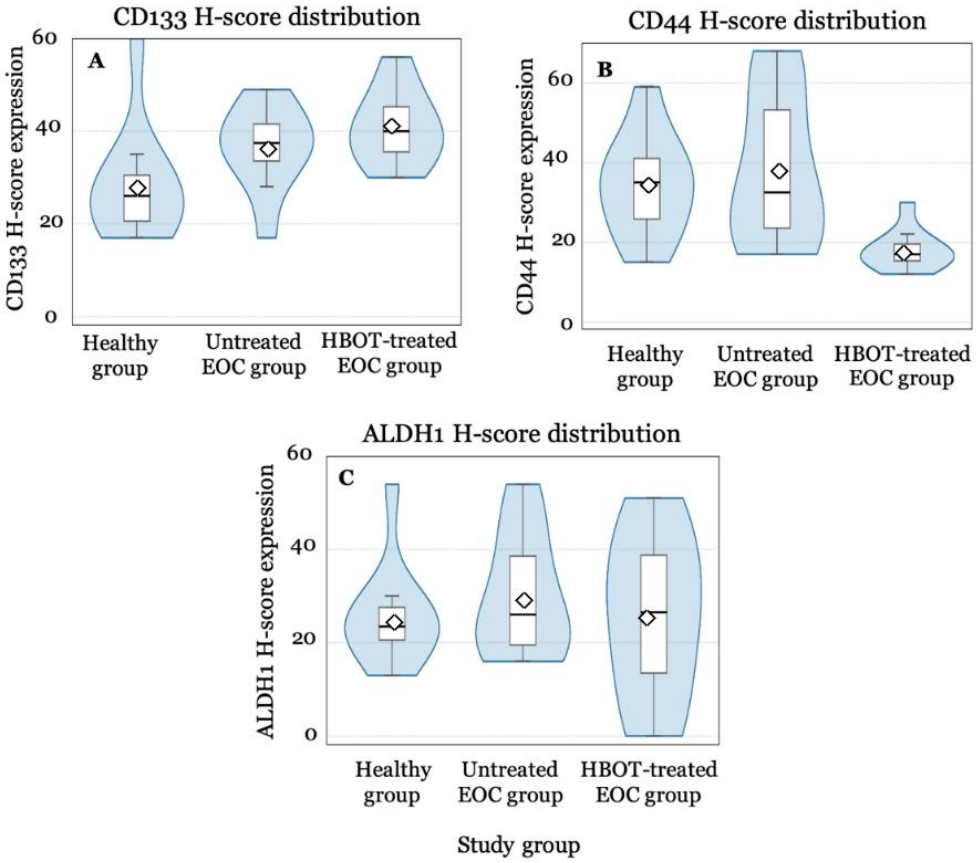

Figure 2. Distribution of CD133, CD44, and ALDH1 H-score expression across the study groups. Violin plots show the distribution of H-score values for CD133 (A), CD44 (B), and ALDH1 (C) in the healthy, untreated EOC, and HBOT-treated EOC groups. Embedded box plots indicate the median and interquartile range, while white diamonds represent group means.

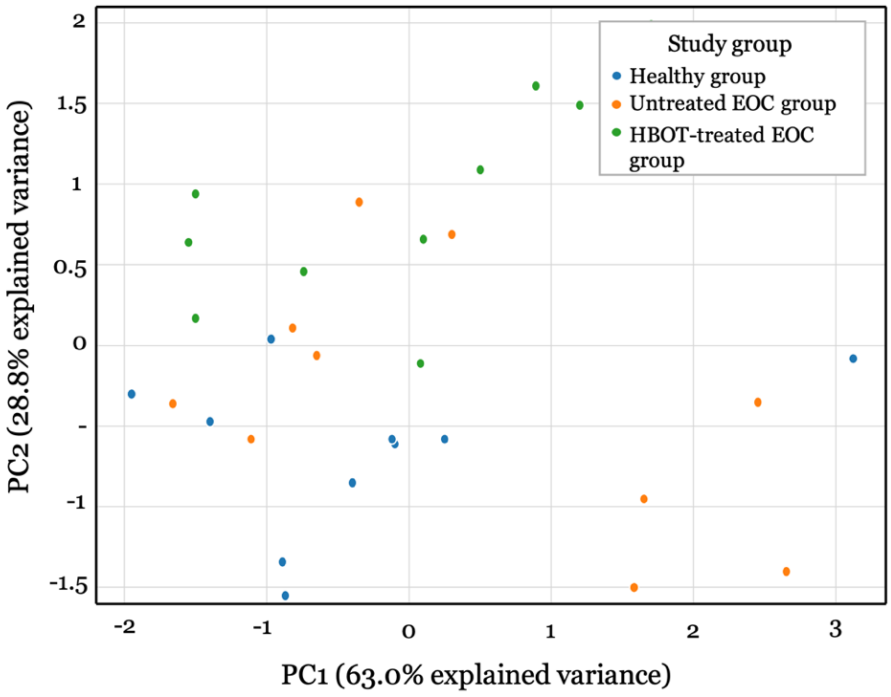

Figure 3. Principal component analysis (PCA) of CD44, CD133, and ALDH1 H-score expression profiles. Each point represents one animal. Colors denote the healthy, untreated EOC, and HBOT-treated EOC groups. PC1 and PC2 explained 63.0% and 28.8% of the total variance, respectively, indicating that the first two components captured most of the variability in combined marker expression.

CD133 showed a different response pattern after HBOT treatment. Increased expression in the HBOT-treated group may reflect adaptive cellular responses to environmental stress. Similar findings have been reported in studies on OCSCs under hypoxic conditions [5,21]. The increase in CD133 expression observed after HBOT may represent a temporary adaptive response rather than expansion of a stable cancer stem cell population. OCSCs may exhibit dynamic changes in marker expression depending on microenvironmental conditions [6,22].

ALDH1 expression levels varied across samples after HBOT exposure. Previous studies have shown that ALDH1-positive cells may survive oxidative stress more effectively because of their metabolic and antioxidant adaptability [8,10,21]. In our data, ALDH1 expression did not decrease consistently after HBOT treatment, suggesting that some tumor cell populations may retain adaptive survival mechanisms despite increased oxygen exposure. Earlier studies have also associated ALDH1 expression with chemoresistance, metabolic flexibility, and persistent stem-like behavior in ovarian cancer [8,10].

An important finding of this study was the strong positive correlation between CD133 and ALDH1 expression (r=0.70, p<0.001). This observation suggests that both markers may identify overlapping ovarian cancer stem-cell subpopulations. Previous studies have reported that CD133 and ALDH1 are associated with stemness-related phenotypes characterized by enhanced self-renewal capacity, chemoresistance, and tumor progression. Co-expression of these markers has been linked to increased tumor aggressiveness and resistance to therapy in ovarian cancer. Although the present correlation analysis does not establish a direct biological interaction, the findings support the possibility that CD133 and ALDH1 reflect related aspects of ovarian cancer stem-cell biology and adaptive responses to microenvironmental stress [1,8,10].

The heterogeneous response observed across stemness markers may reflect the biological complexity of OCSC populations. OCSCs are not considered a uniform cell population but rather a dynamic and adaptable cellular hierarchy influenced by oxygen availability, oxidative stress, and microenvironmental signaling [15,22]. Differences in CD44, CD133, and ALDH1 expression after HBOT exposure may therefore indicate distinct adaptive pathways rather than a single uniform response to oxygen modulation. This may also explain why reductions in one marker were not consistently accompanied by decreases in other stemness-associated markers. Previous studies have suggested that OCSCs may shift between different phenotypic states depending on environmental stress and metabolic conditions, contributing to therapeutic resistance and tumor persistence [6,15]. The relatively broad distribution observed in several groups may reflect biological heterogeneity among tumor samples within the DMBA-induced model. Under altered oxygen tension, CSC populations may undergo metabolic reprogramming and activate distinct survival pathways rather than exhibiting a uniform biological response [23,24].

The reduction in CD44 expression observed after HBOT exposure may reflect alterations in the tumor microenvironment associated with increased oxygen availability. Previous studies have suggested that oxygen modulation can influence stemness-associated signaling and cellular adaptation in ovarian cancer. However, because hypoxia-related pathways and oxidative stress markers were not directly measured in the present study, the mechanisms underlying the observed reduction in CD44 expression remain uncertain and should be interpreted cautiously. CD44 participates in cellular adhesion, migration, epithelial-mesenchymal transition, and metastatic behavior through the activation of multiple downstream pathways associated with cancer progression. Increased oxygen availability during HBOT may be associated with alterations in the tumor microenvironment, potentially contributing to reduced adaptive responses of CD44-positive ovarian cancer stem cell populations [25]. In contrast, the relatively stable ALDH1 expression observed in this study may indicate that ALDH1-positive OCSCs possess stronger antioxidant defense mechanisms against HBOT-induced oxidative stress. ALDH1 is associated with aldehyde detoxification, reactive oxygen species scavenging, and maintenance of intracellular redox homeostasis. Several recent studies have suggested that ALDH1-positive cancer stem cells can survive under oxidative stress conditions because of enhanced metabolic flexibility and resistance to ROS-mediated cellular injury. This adaptive antioxidant capacity may explain why ALDH1 expression remained variable despite increased oxygen exposure [26,27].

The increase in CD133 expression after HBOT exposure may represent a transient adaptive response rather than the expansion of a stable cancer stem cell population. Because functional

stemness assays were not performed, it remains unclear whether the observed increase reflects enhanced stem-cell activity or transient phenotypic adaptation. Previous studies have shown that CD133 expression may fluctuate according to environmental stress, oxygen modulation, and metabolic conditions within the local microenvironment. OCSCs are increasingly recognized as a heterogeneous and plastic cellular hierarchy capable of shifting between stem-like states in response to oxidative stress and therapeutic pressure. Therefore, the increased CD133 expression observed after HBOT may reflect temporary phenotypic adaptation aimed at maintaining survival under altered redox conditions [24,28]. Previous studies have suggested that ROS may contribute to HBOT-associated cellular responses. However, because oxidative stress markers were not measured in the present study, the involvement of ROS-related mechanisms remains speculative. Alterations in oxidative balance have previously been linked to ovarian cancer stem cell maintenance and therapeutic resistance [29,30].

Recent studies have highlighted the potential role of oxygen modulation in improving the therapeutic susceptibility of resistant ovarian cancer cell populations. Alterations in tumor oxygenation may influence cellular stress adaptation and therapeutic responsiveness by modulating oxidative stress and stemness-associated pathways. Therefore, HBOT may have greater therapeutic value when combined with conventional chemotherapy or targeted therapy rather than as a single treatment modality [30-32].

Overall, each marker showed a different response after HBOT exposure. CD44 demonstrated the clearest reduction after HBOT exposure, whereas ALDH1 expression remained heterogeneous, and CD133 expression showed a significant increase following treatment. These findings are consistent with the possibility that HBOT influences stemness-associated marker expression through microenvironmental modulation rather than direct cytotoxic activity. HBOT may therefore be more useful when combined with other therapies than when used alone [16]. Based on the observed changes in stemness marker expression, a proposed biological mechanism underlying the effects of HBOT on ovarian cancer stem cell behavior is illustrated in **Figure 4**. Increased oxygen exposure may contribute to alterations in stemness-associated cellular behavior. These findings further support the relevance of oxygen modulation in ovarian cancer stemness biology. Modulation of oxygen availability through HBOT could influence stemness-associated pathways and may enhance the susceptibility of resistant ovarian cancer cell populations to conventional therapy. However, further studies are warranted to determine whether these molecular changes translate into improved therapeutic outcomes [9,20,33].

Principal component analysis suggested partial separation of the HBOT-treated group from the other groups, suggesting differences in overall expression patterns. Therefore, these findings should be interpreted as evidence of pattern variation rather than mechanistic confirmation. These findings support the possibility that HBOT primarily influences tumor biology through microenvironmental and stress-related modulation rather than direct cytotoxic activity [12,23,34].

DMBA exposure may increase hypoxic conditions and affect the ALDH1-positive cell populations. HBOT exposure may contribute to decreased CD44 expression and heterogeneous ALDH1 expression across samples. CD133 expression may increase as part of an adaptive cellular response following oxygen exposure.

This study has some strengths. The use of a DMBA-induced ovarian cancer model enabled evaluation of stemness-associated marker expression within an in vivo tumor context. In addition, effect size estimation was performed to complement statistical significance testing and to quantify the magnitude of between-group differences. The incorporation of distributional and multivariate approaches, including violin plots and principal component analysis, further supported visualization of inter-sample heterogeneity and group-level expression patterns.

Nevertheless, several limitations should be acknowledged. Stemness was inferred from immunohistochemical expression of CD44, CD133, and ALDH1, without functional validation using assays such as tumorsphere formation, self-renewal capacity, tumor-initiation assays, or chemoresistance testing. Intratumoral oxygenation, hypoxia-related signaling, and oxidative stress markers were not directly measured; therefore, mechanistic interpretations regarding oxygen-mediated modulation of stemness remain exploratory.

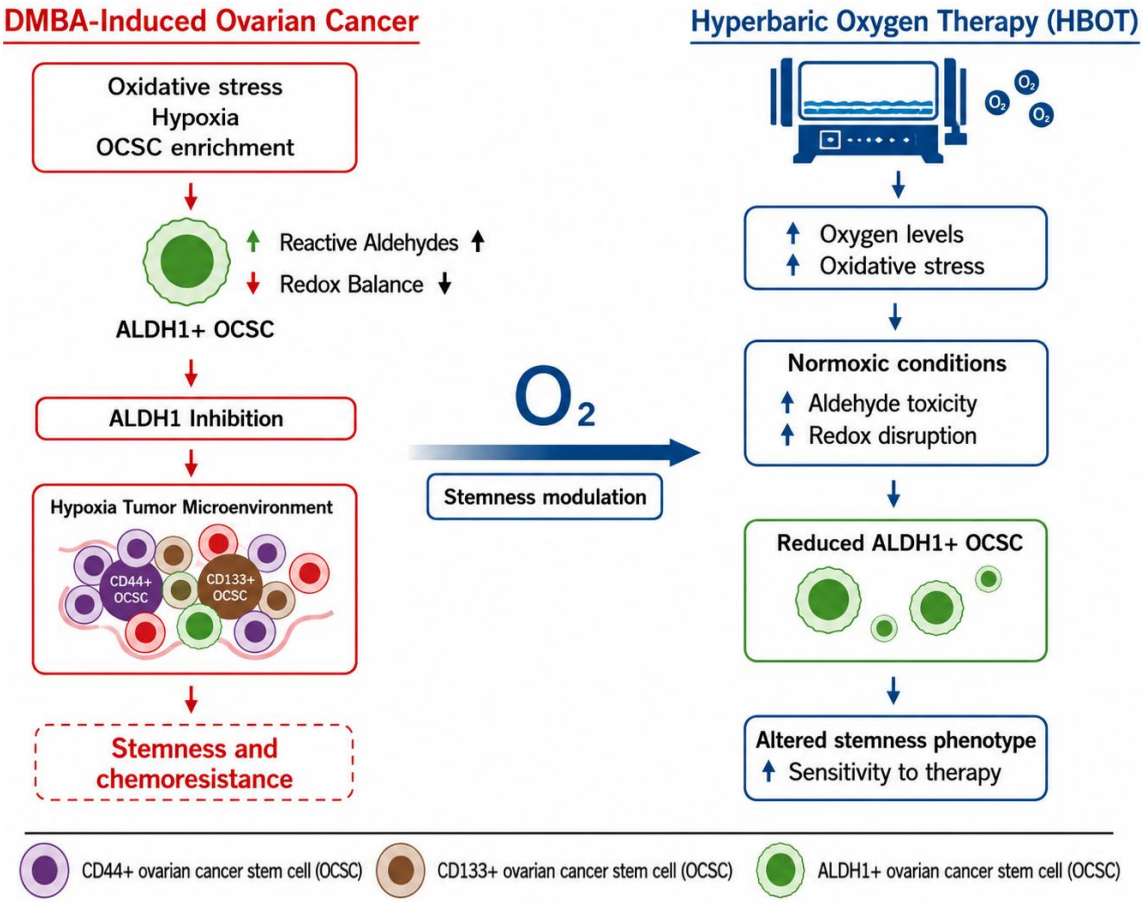

Figure 4. Conceptual schematic of hyperbaric oxygen therapy (HBOT)-associated changes in stemness-associated marker expression in a DMBA-induced epithelial ovarian cancer model.

HBOT was evaluated as a single treatment modality and was not tested in combination with chemotherapy or targeted agents. In addition, the long-term effects of HBOT were not assessed, and the use of an animal model limits direct extrapolation to human ovarian cancer. Accordingly, further studies integrating functional stemness assays, molecular pathway analyses, direct assessment of hypoxia and oxidative stress, and combination-treatment models are needed to clarify the mechanisms by which HBOT may influence ovarian cancer stem-cell phenotypes and therapeutic responsiveness.

Conclusion

HBOT exposure was associated with differential stemness-associated marker expression in the DMBA-induced EOC model. CD44 H-score expression was significantly reduced in the HBOT-treated EOC group, whereas CD133 expression was increased and ALDH1 expression remained statistically unchanged. These findings suggest that HBOT may alter the stemness-associated expression profile in ovarian cancer, potentially through oxygen-dependent remodeling of the tumor microenvironment. However, because functional stemness assays and direct measurements of hypoxia or oxidative stress were not performed, these findings should be interpreted as exploratory and require further mechanistic validation.

Ethics approval

The study protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Hang Tuah, Surabaya, Indonesia (IACUC approval number: I/031/UHT. KEPK.03/VII/2025) and conducted in accordance with institutional international guidelines.

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procedures. The authors also acknowledge the institutional facilities that made this study possible.

Competing interests

All authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declaration of artificial intelligence use

This study used ChatGPT (OpenAI, San Francisco, CA, USA) only to assist with English-language refinement. The tool was not used to generate primary data, perform statistical analysis, or determine scientific interpretation. Authors confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were made solely by the authors.

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References

1. Kossaï M, Leary A, Scoazec JY, *et al.* Ovarian cancer: A heterogeneous disease. Pathobiology 2018;85(1-2):41-49.
2. Bowtell DDL, Böhm S, Ahmed AA, *et al.* Rethinking ovarian cancer II: Reducing mortality from high-grade serous ovarian cancer. Nat Rev Cancer 2015;15(11):668-679.
3. Patch AM, Christie EL, Etemadmoghadam D, *et al.* Whole-genome characterization of chemoresistant ovarian cancer. Nature 2015;521(7553):489-494.
4. Iqhrammullah M, Refin RY, Rasmi RI, *et al.* Cancer in Indonesia: A bibliometric surveillance. Narra X 2023;1(2):e86.
5. Keyvani V, Farshchian M, Esmaeili SA, *et al.* Ovarian cancer stem cells and targeted therapy. J Ovarian Res 2019;12(1):120.
6. Ma H, Tian T, Cui Z. Targeting ovarian cancer stem cells: A new way out. Stem Cell Res Ther 2023;14(1):28.
7. Roy-Chowdhuri S. The updated College of American Pathologists principles of analytic validation of immunohistochemical assays: A step forward for cytopathology. Cancer Cytopathol 2024;132(9):547-548.
8. Królewska-Daszczyńska P, Wendlocha D, Smycz-Kubańska M, *et al.* Cancer stem cells markers in ovarian cancer: Clinical and therapeutic significance (Review). Oncol Lett 2022;24(6):465.
9. Wilczyński JR, Wilczyński M, Paradowska E. Cancer stem cells in ovarian cancer: A source of tumor recurrence and a challenging target for novel therapies. Int J Mol Sci 2022;23(5):2496.
10. Izycka N, Rucinski M, Andrzejewska M, *et al.* The prognostic value of cancer stem cell markers (CSCs) expression-ALDH1A1, CD133, CD44- for survival and long-term follow-up of ovarian cancer patients. Int J Mol Sci 2023;24(3):2400.
11. Jing X, Yang F, Shao C, *et al.* Role of hypoxia in cancer therapy by regulating the tumor microenvironment. Mol Cancer 2019;18(1):157.
12. Abba MC, Zhong Y, Lee J, *et al.* DMBA induced mouse mammary tumors display high incidence of activating Pik3caH1047 and loss of function Pten mutations. Oncotarget 2016;7(39):64289-64299.
13. Seo EJ, Kim DK, Jang IH, *et al.* Hypoxia-NOTCH1-SOX2 signaling is important for maintaining cancer stem cells in ovarian cancer. Oncotarget 2016;7(34):55624-55638.
14. Anggraeni TD, Thiono J, Pratiwi IW, *et al.* The presence of stem cells in ovarian cancer: A review. Bali Med J 2023;12(1):1001-1008.
15. Song Y, Pan S, Li K, *et al.* Insight into the role of multiple signaling pathways in regulating cancer stem cells of gynecologic cancers. Semin Cancer Biol 2022;85:219-233.

16. Peña-Villalobos I, Casanova-Maldonado I, Lois P, *et al.* Hyperbaric oxygen increases stem cell proliferation, angiogenesis and wound-healing ability of WJ-MSCs in diabetic mice. Front Physiol 2018;9:995.
17. Fedchenko N, Reifenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue: A review. Diagn Pathol 2014;9:221.
18. Frąszczak K, Barczyński B. The role of cancer stem cell markers in ovarian cancer. Cancers 2024;16(1):40.
19. Motohara T, Katabuchi H. Ovarian cancer stemness: Biological and clinical implications for metastasis and chemotherapy resistance. Cancers 2019;11(7):907.
20. Roy L, Cowden Dahl KD. Can stemness and chemoresistance be therapeutically targeted via signaling pathways in ovarian cancer? Cancers 2018;10(8):241.
21. Prager BC, Xie Q, Bao S, *et al.* Cancer stem cells: The architects of the tumor ecosystem. Cell Stem Cell 2019;24(1):41-53.
22. Yang W, Kim D, Kim DK, *et al.* Therapeutic strategies for targeting ovarian cancer stem cells. Int J Mol Sci 2021;22(10):5059.
23. Alizadeh H, Akbarabadi P, Dadfar A, *et al.* A comprehensive overview of ovarian cancer stem cells: Correlation with high recurrence rate, underlying mechanisms, and therapeutic opportunities. Mol Cancer 2025;24(1):135.
24. Lee H, Kim B, Park J, *et al.* Cancer stem cells: Landscape, challenges and emerging therapeutic innovations. Signal Transduct Target Ther 2025;10(1):248.
25. Markowska A, Kojs Z, Twardawa D, *et al.* Selected markers of ovarian cancer and their relation to targeted therapy (Review). Exp Ther Med 2024;27(5):236.
26. Datta N, Snijesh VP, Parvathy K, *et al.* ALDH1A1 as a marker for metastasis initiating cells: A mechanistic insight. Exp Cell Res 2024;442(1):114213.
27. Wei Y, Li Y, Chen Y, *et al.* ALDH1: A potential therapeutic target for cancer stem cells in solid tumors. Front Oncol 2022;12:1026278.
28. Tan J, Tang L, Zhang Q. The metabolic landscape of ovarian cancer stem cells: How do they survive? Front Oncol 2026;15:1738742.
29. Nigam M, Punia B, Dimri DB, *et al.* Reactive oxygen species: A double-edged sword in the modulation of cancer signaling pathway dynamics. Cells 2025;14(15):1207.
30. Meng Y, Zhou L, Xia Y, *et al.* Breaking the hypoxia barrier: Advances and challenges of hyperbaric oxygen therapy in cancer treatment. Biomed Pharmacother 2025;193:118703.
31. Wang P, Wang XY, Man CF, *et al.* Advances in hyperbaric oxygen to promote immunotherapy through modulation of the tumor microenvironment. Front Oncol 2023;13:1200619.
32. Vaupel P, Multhoff G. Revisiting the Warburg effect: Historical dogma versus current understanding. J Physiol 2021;599(6):1745-1757.
33. Capó X, Monserrat-Mesquida M, Quetglas-Llabrés M, *et al.* Hyperbaric oxygen therapy reduces oxidative stress and inflammation, and increases growth factors favouring the healing process of diabetic wounds. Int J Mol Sci 2023;24(8).
34. Memar MY, Yekani M, Alizadeh N, *et al.* Hyperbaric oxygen therapy: Antimicrobial mechanisms and clinical application for infections. Biomed Pharmacother 2019;109:440-447.