

ALDH1 Protein as a Potential Marker of Rat Breast Cancer Stem Cells in a Preventive Model of Soybean Extract Administration

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Abstract

Objective: Breast Cancer Stem Cells (BCSCs) are associated with rapid progression, drug resistance, and recurrence, which are identified by aldehyde dehydrogenase 1 (ALDH1) expression. Soy isoflavones have been reported to interfere with stemness-related pathways. This study examined the effect of soybean extract on ALDH1 expression in a DMBA-induced rat model of breast cancer. **Materials and Methods:** Thirty female rats were divided into five groups: normal control (NoC), negative control with DMBA induction (NeC), and treatment groups receiving soybean extract at 250 mg/kg BW (SE-D250), 500 mg/kg BW (SE-D500), or 1000 mg/kg BW (SE-D1000). Breast tissues were evaluated for ALDH1 expression by immunohistochemistry and quantified using the H-index method, which uses the “ImageJ” and “IHC Profiler” software. **Results:** NeC (69.1%) exhibited significantly higher ALDH1 expression than NoC (26.7%), confirming CSC enrichment. Soybean extract intervention significantly reduced ALDH1 levels across all treatment groups compared with the NeC group. No significant difference was found between SE-D250 (49.9%) and SE-D500 (53.3%) ($p = 0.873$). SE-D1000 produced the most significant reduction (37.9%), though ALDH1 levels remained significantly higher than in NoC. **Conclusion:** Soybean extract significantly reduces ALDH1 expression in BCSC, with the most notable effect at a 1000 mg/kg BW dose. No dose-dependent pattern was observed due to genistein’s biphasic effect as the primary flavonoid. Additionally, soybean extract administration cannot restore ALDH1 expression in cancer cells to a normal level.

Keywords: ALDH1, Breast Cancer, DMBA, Isoflavones, Soybean Extract

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Introduction

Triple-negative breast cancer (TNBC) is a type of breast cancer that is known to be aggressive, relapses early, and is diagnosed in advanced stages [1, 2]. Although it covers up to 15-20% of breast cancer cases, it is challenging to manage due to its poor therapeutic response and invasiveness [3, 4]. This characteristic of TNBC resulted in a low 5-year survival rate of 8-16% [5]. The hallmark of TNBC is caused by fast cell division, alterations in DNA-repairing genes, and genetic anomalies. Those traits emerge from stem cell mutation to become cancerous, specifically in cancer stem cells

(CSCs) [6, 7]. Although other types of breast cancer may possess stemness characteristics, TNBC shows the highest expression of CSCs, explaining its aggressiveness [7-9].

The importance of CSC detection lies in its crucial role in driving the development of aggressive cancer. To this day, the presence of CSC in breast cancer (BCSC) can be detected using CD44, CD24, and Aldehyde Dehydrogenase-1 (ALDH1) as the most consistent biomarkers, among others [7]. However, compared to surface markers CD44 and CD24, which primarily reflect cell adhesion and phenotype, ALDH1 provides

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a more functional and reliable indicator of BCSC, as its enzymatic activity directly regulates stem cell proliferation and differentiation [8, 9]. Although several studies link the CD44⁺/CD24⁻ phenotype to poor breast cancer prognosis, conflicting evidence across reports highlights the need for more consistent and reliable BCSC biomarkers [10]. ALDH1 is also a known CSC biomarker that is believed better to reflect self-renewal capacity than CD44⁺/CD24⁻/low cells and is more strongly associated with poor prognosis when elevated [9].

An aggressive cancer leads to complicated procedures or treatment, which pose another concern due to the high invasiveness of the procedure, potential side effects, or their relatively high cost [11]. In response to this situation, natural substances are extensively researched as they are believed to give minimal side effects while still giving a therapeutic reaction towards the cancer [12].

Soybean extract, rich in bioactive flavonoids, has shown potential in preventing carcinogenesis and serving as an adjuvant in breast cancer treatment. It induces apoptosis, inhibits oncogenesis, and reduces the risk of metastasis by downregulating Epithelial-to-Mesenchymal Transition (EMT) markers and modulating molecular pathways [13–17]. However, the specific effects of flavonoid content on ALDH1 remain unclear [18, 19]. Therefore, this study aims to investigate the effect of soybean extract administration on ALDH1 expression in a DMBA-induced rat model of breast cancer to evaluate its potential in targeting BCSCs

Materials and Methods

Study Design and Subjects

This true-experimental in vivo study used a preserved pathology specimen from a previous study by Maulana AM, et al [20]. conducted between September 2024 and September 2025, and received ethical approval from the reviewing committee. The animal treatments, tissue collection, and initial specimen preparation were carried out as part of that original experiment. For this study, additional immunohistochemistry (IHC) staining targeting ALDH1 expression was performed specifically on the preserved specimen to evaluate the relevant biomarker. The sample consisted of 7-week-old female Sprague-Dawley (SD) rats weighing 120–150 grams. If a rat could no longer participate in the experiment, it was excluded and ethically euthanized. These rats were chosen for their peripubertal stage, when mammary glands are most susceptible to carcinogenesis. Table 1 shows the distribution of the rats into five groups. The rats were divided into five groups: a normal control (NoC), a negative control (NeC), and three soybean extract-treated groups with different dosages (SE-D250/500/1000), assigned with simple random allocation. A total of 30 rats were used, with 6 rats per group. Using Federer's Formula, only five rats per group were initially needed, but an additional rat was included in each group to account for potential exclusions during the experiment. At the end of the study, no rats were excluded.

Acquiring the Soybean Extract

Soybean extract in this study was prepared by Maulana et al [20]. using Grobogan soybean seeds (*Glycine max* (L.) Merr), verified at Balitkabi, Malang. Seeds were washed, ground (AEG typ AMEB 80 FX® grinder, 3 mm sieve), wrapped in gauze (10 packs), and pressed at 100–150 atm for 30 minutes at 48.9°C to form plates. The plates were then re-grounded, blended (Miyako®), sieved (40 mesh), and stored at 4°C. The powder was macerated using PBS (pH 7.4) prepared by dissolving PBS tablets in distilled water to improve soybean protein solubility. A 5:1 solvent-to-solute ratio was used, and the mixture was stirred and macerated for 60 minutes. It was then filtered (Whatman™ paper) and stored at 4°C. Finally, the filtrate was dried using a rotary evaporator (Eyela®) to obtain a dry extract, and the yield was calculated. Furthermore, its content biochemical compounds were screened with the Q-TOF LC/MS method.

Induction of Breast Cancer in the Rats

Breast cancer induction in female Sprague-Dawley (SD) rats followed the protocol of Maulana AM, et al [20]. Rats were acclimated for one week with free access to food and water, and any animal showing distress was ethically euthanized. DMBA was dissolved in corn oil (2 mg/mL) and administered via an intragastric probe at 20 mg/kg body weight, 11 times over two weeks. Tumor development was monitored through regular palpation and body weight measurements 1–2 times weekly.

Tissue staining with Haematoxylin and Eosin (HE)

HE staining was performed to confirm tumor growth. Paraffin-embedded tissue sections (4 µm) were deparaffinized with xylol, rehydrated through graded alcohols, and rinsed with water. Slides were stained with haematoxylin for 7 minutes, blued in lithium carbonate, and counterstained with eosin for 1–2 minutes. After dehydration and clearing with alcohol and xylene, specimens were mounted with Entellan™ and a cover glass. The stained sections were examined under an optical microscope at 400× magnification. The analysis revealed invasive ductal carcinoma (IDC) formation with a 74% incidence rate [20].

Immunohistochemistry Process

IHC staining was performed to assess ALDH1 protein expression. Unstained slides were first heated at 56°C for 30 minutes, deparaffinized in xylol I–III, and rehydrated through graded alcohols (absolute, 90, 80, 70%) before being rinsed in water. Antigen retrieval was carried out in a decloaking chamber with citrate buffer at 95°C for 20 minutes, then cooled and washed with PBS. After drying, PAPEN was applied to the tissue sections, followed by 3% hydrogen peroxide (H₂O₂) to block endogenous peroxidase activity that could cause false positives. The slides were washed and then treated with a protein block for 15 minutes. Subsequently, the slides were incubated with the primary antibody (ALDH1) for one hour in a humidity chamber, using N-Universal as a negative control, then washed with PBS and incubated with the secondary

antibody for another hour. Next, they were incubated with HRP-conjugated streptavidin for 30 minutes, and protein expression was visualized using 3,3'-diaminobenzidine (DAB) for 10 minutes. After counterstaining with Harris Hematoxylin, the slides were washed, dipped in lithium carbonate, dehydrated through graded alcohols, cleared in xylol, and mounted with Entellan™ and a cover glass. [13, 20, 21]

IHC Result observation

After IHC staining, the specimens were observed under an optical microscope with a built-in camera at 400× magnification. Blinding was done to the author and the physicians who aided in the IHC procedure to minimize measurement bias. Five random fields from each specimen were documented, and ALDH1-positive (brown) and negative (blue) cells were counted using “IHC Profiler” software by Varghese et al.[22] and “ImageJ”. The percentage of ALDH1-expressing cells was recorded for analysis. Interpretation of ALDH1 expression was based on a weighted histoscore (H-Index), which combined the proportion and staining intensity of positive cells. Intensity was graded on a 0–3 scale (0 = none, 1 = weak, 2 = moderate, 3 = strong), and H-scores were calculated by multiplying the percentage of positive cells by their respective intensity values. Therefore, the formula is stated as $H\text{-Index} = (1 \times \% \text{ Weak}) + (2 \times \% \text{ Moderate}) + (3 \times \% \text{ Strong})$.

Statistical Analysis

ALDH1 expression was presented as medians with [min-max] ranges due to non-normal distribution in some groups (NoC & NeC) based on the Shapiro-Wilk analysis. The author evaluated significant differences in ALDH1 expression across all groups using the Kruskal-Wallis test, followed by the Mann-Whitney test to compare two groups. Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 30.0.

Results

Figure 1 shows the IHC result of ALDH1 expression in mammary tumor cells from each group. The normal control (A) has minimal brown staining, indicating baseline ALDH1 activities, whereas the negative control (B) showed the most intense staining. The SE-D250 group (C) still showed prominent ALDH1 expression, but at a lower intensity. The SE-D500 group (D) also gave a less prominent brown color compared to the negative control. The SE-D1000 group (E) visualized the least intensity of brown staining compared to C and D.

Table 2 presents the distribution of the ALDH1 H-Index score in all groups. Compared with other groups induced with DMBA, the NoC groups have the lowest median of 26.7 (range, 24.7 – 39.1). In contrast, the highest median ALDH1 expression is observed in the NeC group, with a score of 69.1 (range, 65.4 – 115). It is a reasonable number since this is the group that was only given DMBA, without receiving soybean extract (SE). All SE-treated groups have medians that are lower compared

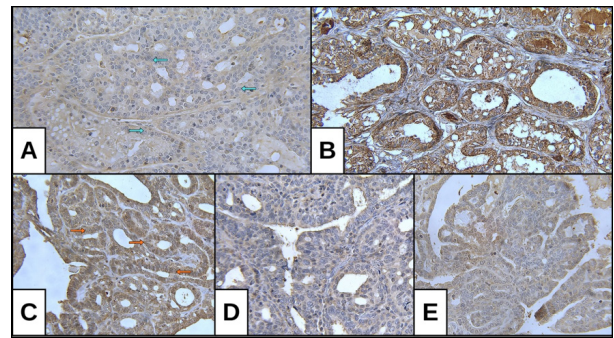


Figure 1. Representative IHC Staining of All Groups. Representative IHC staining of ALDH1 expression in normal control (A), negative control (B), SE-D250 (C), SE-D500 (D), and SE-D1000 (E). The blue arrow indicates epithelial cells with negative ALDH1 expression, whereas the orange arrow indicates epithelial cells with positive ALDH1 expression.

to NeC. The SE-D250 group exhibited a median of 49.9 (range, 41.6 – 73.2), whereas the SE-D500 group showed a slight increase, with a median of 53.3 (range, 45.8 – 59.6). At the maximum dose intervention, the SE-D1000 group achieved a median of 37.9 (range, 33.1 – 48.5).

Since the Kruskal-Wallis test showed a significant difference of $p < 0.001$, the Mann-Whitney test was conducted. From the data analysis above (Figure 2), the most prominent differences among groups can be seen between the normal and negative control groups. The Mann-Whitney test revealed a significant difference between the two groups ($p = 0.004$). This result showed that the ALDH1 level is different between normal rats compared to those that were induced with DMBA to promote cancer development. ALDH1 levels in all groups treated with soybean extract, while still induced with DMBA, are lower compared to the negative control group. Statistical analysis revealed that SE-D250 ($p = 0.025$), 500 ($p = 0.004$), and 1000 ($p = 0.004$) differed significantly from the NeC group as indicated by the asterisks. These results mean that soybean extract administration, regardless of dosage, led to a reduction in the H-index of ALDH1 level compared with the negative control.

To assess whether different doses exhibit different expression of ALDH1, a Mann-Whitney test was also conducted to compare differences within SE-treated groups (Figure 3). The result showed that there is no significant difference in ALDH1 expression between groups that are given soybean extract at a dose of 250 mg/kg BW and 500 mg/kg BW ($p = 0.873$). Meanwhile, the SE-D1000 group, which received the highest dose in this experiment, exhibited a significant decrease in ALDH1 expression compared to the SE-D250 group ($p = 0.025$). If the comparison were to be brought closer to the SE-D500 dose, the result still showed a significant decrease ($p = 0.006$).

The bar chart in Figure 4 compares the H-index of the ALDH1 level between NoC and SE-D1000. This analysis of the Mann-Whitney test aims to determine whether the lowest expressing ALDH1 level among treated groups can achieve a level comparable to that of normal rats. The results showed a significant difference between these

Table 1. Group Allocation of Rats. This table presents each of the group treatments for this study.

Group Name	Group Definition	Treatment	Number of Rats (N)
NoC	Normal control	Treated with corn oil	6
NeC	Negative control	Induced with DMBA 20 mg/kg BW without soybean extract administration	6
SE-D250	Soybean extract with a dose of 250 mg/kg BW	Given with soybean extract one week before, and during DMBA induction, until the end of treatment	6
SE-D500	Soybean extract with a dose of 500 mg/kg BW	Given with soybean extract one week before, and during DMBA induction, until the end of treatment	6
SE-D1000	Soybean extract with a dose of 1000 mg/kg BW	Given with soybean extract one week before, and during DMBA induction, until the end of treatment	6

groups ($p = 0.025$), suggesting that the data between them are not the same.

Discussion

ALDH1 Expression in Normal Cells and Cancer Stem Cells

The first indication of a promising biomarker should be tested for its ability to differentiate between normal and pathological cells. This characteristic was examined by comparing the standard control and negative control groups. Results showed significant differences between them ($p = 0.004$). Specifically, ALDH1 H-scores in NoC are approximately 26.7, whereas in NeC, they are marked at 69.1. This aligns with the fact that ALDH1 expression exists in the first place due to stem cell differentiation and detoxification. Normal breast cells are quiescent and have a reduced metabolic function compared to malignant cells. Studies also support that differentiated cells tend to show low levels of biomarkers associated with stemness, such as ALDH1, for its role in retinoid metabolism and regulating pathways for cellular proliferation and differentiation [23, 24].

On the other hand, studies have shown supporting evidence that inducing DMBA promotes stemness characteristics, resulting in an increased level of ALDH1 expression [13, 25]. The effect of DMBA in activating the Ahr/CYP1A1 results in the expansion of CSCs,

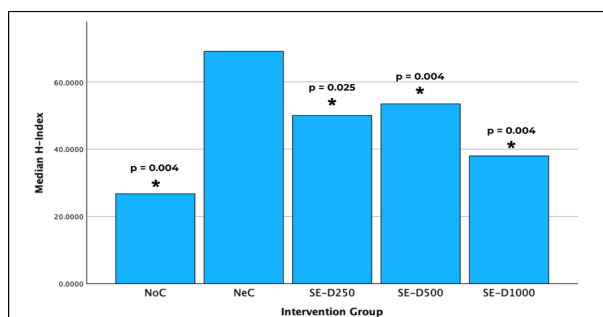


Figure 2. Follow-up Mann-Whitney Test of NeC Compared with All Groups. * = Groups compared with NeC showed significant differences (Mann-Whitney test, cut-off $p < 0.05$). NoC: Normal control, NeC: Negative control, SE-D250: Soybean extract treated group of 250 mg/kg BW dose, SE-500: Soybean extract treated group of 500 mg/kg BW dose, SE-D1000: Soybean extract treated group of 1000 mg/kg BW dose.

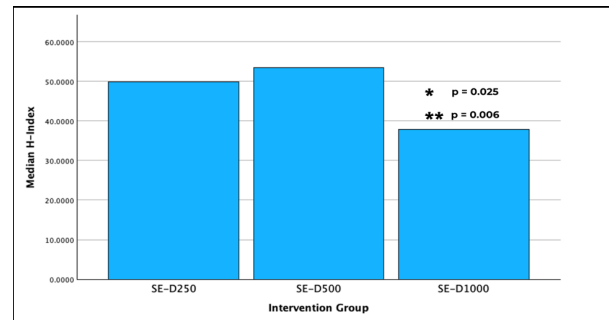


Figure 3. Follow-up Mann-Whitney Test for Dose-Related Differences. * = Groups compared with SE-D250 showed significant differences (Mann-Whitney test, cut-off $p < 0.05$). ** = Groups compared with SE-D500 showed significant differences (Mann-Whitney test, cut-off $p < 0.05$). NoC: Normal control, NeC: Negative control, SE-D250: Soybean extract treated group of 250 mg/kg BW dose, SE-500: Soybean extract treated group of 500 mg/kg BW dose, SE-D1000: Soybean extract treated group of 1000 mg/kg BW dose.

characterized by specific markers such as ALDH1 [25]. Furthermore, Ahr/CYP1A1 suppressed PTEN/PI3K/Akt, a tumor suppressor pathway, that increases Akt expression. The upregulated Akt contributes to TME alterations and anti-differentiation growth, thereby increasing ALDH1 expression and enhancing mammosphere formation [25, 26].

In addition, BCSC is strongly correlated with TNBC, indicating that high ALDH1 expression contributes to its aggressive behavior and poor prognosis due to its stemness properties [27]. This is linear towards the pathological mechanism of ALDH1 in CSCs, in which it causes a molecular pathway alteration that activates c-MYC, cyclin D1, or PDK-1/Akt pathway, resulting in anti-apoptotic and anti-differentiation growth [28]. Overall, the tumors in the NeC group exhibited significantly high ALDH1 expression, induced by DMBA, which triggers various molecular pathways and manifests in CSC characteristics.

ALDH1 Expression in Soybean Extract-Treated Groups

All SE-treated groups exhibited significantly lower ALDH1 expression compared to NeC ($p < 0.001$). While NeC has the highest ALDH1 H-Index of 69.1, the treated groups exhibit reduced ALDH1 H-Index at 49.9 (SE-D250), 53.3 (SE-D500), and 37.9 (SE-D1000). This result demonstrated that soybean extract administration

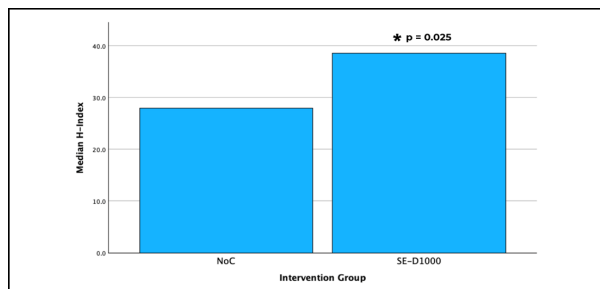


Figure 4. Follow-up Mann-Whitney Test for NoC and SE-D1000. * = Groups compared with NoC showed significant differences (Mann-Whitney test, cut-off $p < 0.05$). NoC: Normal control, NeC: Negative control, SE-D250: Soybean extract treated group of 250 mg/kg BW dose, SE-500: Soybean extract treated group of 500 mg/kg BW dose, SE-D1000: Soybean extract treated group of 1000 mg/kg BW dose.

affects DMBA-induced tumor cells, lowering the expression of the CSC marker. Genistein, a component of soy isoflavones, exerts its anti-cancer effect by inhibiting the NF- κ B and PI3K/Akt signaling pathways. This downregulation suppresses the stemness properties, resulting in lower ALDH1 expression [15–17].

In addition to its direct effect on CSC signalling, genistein also promotes the differentiation of tumor cells by suppressing MEK/ERK activation and activating tumor-suppressing genes such as PTEN and E-cadherin [16, 17]. Genistein also downregulates EMT mediators such as Snail, Slug, MMPs, and Vimentin, thereby halting the transition from epithelial to mesenchymal cells and reducing the risk of invasiveness [16, 28].

Dose-Dependent Effects of Soybean Extract on ALDH1 Expression

The effect between SE-D250 and SE-D500 did not have a significant difference ($p = 0.873$), as reflected by their similar distribution (SE-D250: 49.9; SE-D500: 53.3). However, SE-D1000 showed a significant decrease in ALDH1 expression compared to the previous two groups (37.9). This result showed a threshold effect of soybean extract, where a 1000mg/BW dosage created a greater decrease in ALDH1 level. This phenomenon is consistent with the evidence that genistein also achieved a greater suppressive effect of CSC features at a higher dose for CD44+/CD24- subpopulations [15]. This phenomenon of genistein was also observed in inhibiting cell proliferation and simultaneously lowering ALDH1 expression in head and neck carcinoma tumor-initiating cells (HNC-TIC) [29].

The molecular mechanism behind genistein's dose-dependent effect in HNC-TIC lies in the role of miR-34a. The higher the dose of genistein, the higher the expression of miR-34a. The increased expression also inhibits EMT-associated factors, which results in a lower risk of invasion. Activated miR-34a also increases oxidative stress and inhibits the RNA 2',3'-cyclic phosphate (RTCB), which reduces cell proliferation and self-renewal, respectively. This is essential because miR-34a also exists in breast tissue, which regulates the differentiation of mammary cells. In breast cancer,

miR-34a is downregulated, and the practical effect at high dosage of genistein observed in this study suggests a similar phenomenon [16, 29].

However, this study did not demonstrate a specific dose-dependent decrease in ALDH1 expression. This might be because genistein also plays a biphasic role due to its nature as a phytoestrogen. At low doses, it might act as a mild estrogenic modulator by activating estrogen receptors. The ER α activation leads to a pro-tumorigenic effect, although ER β has the opposite impact and possesses a much higher affinity. In higher doses, genistein produces its anti-cancer effects by inhibiting oncogenic signaling [17, 30]. Other studies also supported this mechanism, where low concentrations of genistein (0.1–10 μ M) stimulate proliferation of ER-positive breast cancer cells (MCF-7), whereas higher concentrations (>10–20 μ M) support tumor growth [31, 32]. These findings suggest that low-to-moderate dose administration of SE may not be sufficient to strongly suppress CSC pathways (SE-D250 & SE-D500), whereas high exposure (SE-D1000) is.

Overall, genistein interferes with multiple common pathways, such as ALDH1, which explains the significance in this study. Therefore, we proposed a molecular mechanism in which these pathways are interconnected, involving cellular components that are upregulated and/or downregulated by the effects of DMBA, ALDH1, and genistein (Figure 5).

Restorative Effect is Unlikely

Among the groups treated with soybean extract, the SE-D1000 showed the lowest ALDH1 expression levels, indicating the strongest anti-CSC characteristics compared to other dosages. However, this group still exhibited significant differences when compared to NoC ($p = 0.025$). Specifically, the expression of SE-D1000 reached as low as 37.9%, which is insufficient to be categorized as NoC, as it possesses a score of 26.7%. This result suggests that the maximum dosage of soybean extract cannot fully restore cellular functions related to stemness characteristics to the levels of normal cells.

Cancer cells are known to have a low rate of differentiation. Among their types, CSC has the slowest rate, which allows it to maintain its pluripotency [7, 33]. The exposure of soybean extract to CSC promotes differentiated growth through various signalling pathways,

Table 2. Distribution of ALDH1 H-Scores. Data distribution of ALDH1 expression as H-scores, presented in median [min-max]. Groups: list of all groups used in the experiment. n: number of rats per group.

Group	n	ALDH1 H-Scores (Median [min-max])
NoC	6	26.7 [24.7 – 39.1]
NeC	6	69.1 [65.4 – 115]
SE-D250	6	49.9 [41.6 – 73.2]
SE-D500	6	53.3 [45.8 – 59.6]
SE-D1000	6	37.9 [33.1 – 48.5]

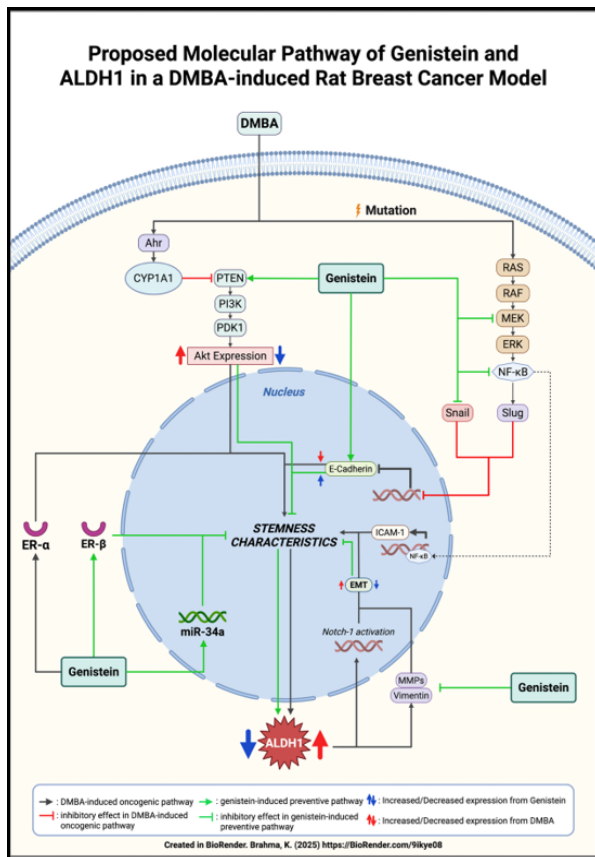


Figure 5. Proposed Molecular Pathway of Genistein and ALDH1. Legends are already embedded within the figure.

meaning that cancer cells are pushed out of their “stemness” state, thereby increasing the differentiation rate. There may be some irreversible mutations in CSC that render the cells incapable of reverting to normal differentiation rates, as indicated by the persistent expression of ALDH1 at higher levels [34, 35].

CSC is sustained through the modulation of epigenetic programs, enabling them to adapt to various environmental conditions and therapeutic interventions. Tumor heterogeneity in genes and morphology also presents a challenge in delivering targeted therapy. Within the same tumor, the CSC subpopulations exhibit unique gene expression and metabolic dependencies, which may respond differently to a particular treatment. Due to the nature of CSC, soybean extract alone is unable to eradicate the stemness features in cancer cells [30, 33].

From the perspective of the CSC biomarker, ALDH1 only reflects a portion of CSC biology. Other known BCSC biomarkers include CD44/CD24, which exhibit stemness characteristics through various signaling pathways, such as JAK2/STAT3, that are influenced by the epidermal growth factor receptor (EGFR). Therefore, targeted therapy using genistein effectively reduces ALDH1+ CSC populations but may not eliminate ALDH1-negative CSC clones [33, 34].

In a chemically induced model, such as DMBA, cells can sustain irreversible damage and gene mutations related to stemness, especially in BCSC. The primary mechanism of DMBA is to damage DNA, thereby increasing the risk of mutations and activating oncogenes, which promote

stemness features. Furthermore, the primary mechanism of genistein is to increase gene expression, which reduces stemness characteristics through multiple signaling pathways, rather than necessarily diminishing existing gene mutations [13, 36]. From existing studies, it can be inferred that soybean extract administration is unable to restore ALDH1 expression in BCSC to that of normal cells due to the nature of CSC and the preventive effect of soybean extract itself.

Implications for Cancer Prevention and Therapy

The findings from this study could lead to a new approach for breast cancer prevention. Administration of soybean extract may lower the risk of recurrence by decreasing ALDH1+ cells, as high stemness is strongly linked to this phenotype. Additionally, it has the potential to serve as a chemopreventive agent in populations at risk for BCSC. This study was conducted in a rat model whose physiology is most like that of humans. However, clinical trials are still needed as additional factors in humans could alter the results. Outcomes may vary depending on agent factors (dosage, mixtures, duration) or host factors (menopausal state, incidence of breast cancer, and the host’s ability to metabolize isoflavones [17, 35].

Although SE-administration is effective at lowering ALDH1+ cells, it is not as great a standalone therapeutic approach. It has been shown in recent studies that, when combined with breast cancer endocrine treatments, genistein showed a significant effect on mitigating bone loss due to its nature as a phytoestrogen [30]. As one of the main challenges of BCSC was its resistance to chemotherapy, genistein also showed a promising effect of reducing chemoresistance when combined with doxorubicin in MCF-7/adr cells by increasing the intracellular concentration of the drug [17, 30]. Its effect of reducing chemoresistance and stemness properties was also studied in prostate and gastric cancers by inhibiting ABCG2 expression and ERK 1/2 activity [29].

ALDH1 also has the potential to be applied clinically as a prognostic marker to identify breast cancer subtypes that are suspected of having high stemness features. A clinical study demonstrated that ALDH1, used in IHC, can act as an independent prognostic factor for axillary lymph node metastases in biopsy specimens. Knowing that ALDH1+ cells are associated with increased risk of relapse and a lower rate of overall survival, IHC using ALDH1 for breast biopsies may also be a potential study to determine the course of disease early [37].

Limitations

Although the hypothesis is shown to have significant meaning, it also has limitations. This study mainly focused on the expression of ALDH1 as a marker for BCSC. At the same time, the broader mechanism of how soybean extract exerts its therapeutic effect during tumor development was not addressed. Studies on the impact of SE on other CSC protein markers, such as CD44+/CD24-, in animal models should be carried out to address this knowledge gap.

In conclusion, the results showed that ALDH1 expression differed significantly between normal and

cancer cells, as well as between the negative control group and soybean extract (SE)-treated cancer cells. However, no dose-dependent effect was observed, and even the highest SE dose (SE-D1000) did not restore ALDH1 expression to levels comparable to normal cells.

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Statements and Declarations

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Competing Interest

The author declares no competing interests in this study.

Author contributions

All authors contributed to the study conception and design. Keanvito Abyaz Brahma, Kusmardi Kusmardi, and Andi Muh Maulana performed material preparation, data collection, and analysis. The first draft of the manuscript was written by Keanvito Abyaz Brahma, and all authors commented on earlier versions. All authors read and approved the final manuscript.

Data availability

The datasets generated and/or analysed during the current study are available to access in the university repository with the link of <https://lib.ui.ac.id/unggah/skripsi/181634>.

Ethics Approval

This study was approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia (Approval number: KET-1201/UN2.F1/ETIK/PPM.00.02/2021.).

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