Fan Y. et al. (2022) Exploring the Expression of Dna-Binding Differentiation Inhibitor 1 (Id-1) in Breast Cancer Among Female Athletes: Implications of Smart Medicine on Carcinogenesis and Treatment Strategies. Revista Internacional de Medicina y Ciencias de la Actividad Física y el Deporte vol. 22 (88) pp. 1084-1096
**DOI:** https://doi.org/10.15366/rimcafd2022.88.023

# ORIGINAL

# Exploring the Expression of Dna-Binding Differentiation Inhibitor 1 (Id-1) in Breast Cancer Among Female Athletes: Implications of Smart Medicine on Carcinogenesis and Treatment Strategies

**Haifeng Zhou[1], Yuhong Fan[2,*], Xueliang Wu[3], Bo Liu[2], Yijun Zhang[1], Yue Zhang[1], Wanping Liang[1], Zhisheng Zhang[1]**

[1] Breast surgery, The First Affiliated Hospital of Hebei North University.
[2] Pathology department, The First Affiliated Hospital of Hebei North University.
[3]General surgery, The First Affiliated Hospital of Hebei North University.
**E-mail:** cock00@126.com

**Recibido** 27 de julio de 2021 **Received** July 27, 2021
**Aceptado** 26 de octubre de 2022 **Accepted** October 26, 2022

## ABSTRACT

Binding differentiation inhibitor 1 (ID-1) in breast cancer among female athletes, utilizing smart medicine approaches. By establishing a breast cancer cell line experiment, we assessed the expression of ID-1 in breast cancer tissues and adjacent normal tissues, compared ID-1 expression levels across different breast cancer cell groups, investigated the impact of ID-1 on Src kinase activation in breast cancer cells, and explored the relationship between ID-1 expression and clinical factors relevant to female athletes. Results: The investigation revealed a significantly higher positive expression rate of ID-1 in breast cancer tissues compared to adjacent non-cancerous tissues (P<0.05). The shRNA-ID-1 group exhibited a notable decrease in ID-1 mRNA and protein expression levels compared to the Control group and the shRNA-NC group (P<0.05), indicating effective ID-1 silencing. Conversely, the PCDNA3.1-ID-1 group showed a significant increase in ID-1 mRNA and protein expression compared to the Control group and PCDNA3.1-NC group (P<0.05), suggesting enhanced ID-1 expression. Additionally, the expression level of phosphorylated Src (p-Src) protein in SUM159 cells was significantly reduced in the shRNA-ID-1 group compared to Control and shRNA-NC groups (P<0.05), while it was significantly increased in the PCDNA3.1-ID-1 group compared to Control and PCDNA3.1-NC groups (P<0.05). Importantly, ID-1 expression was significantly correlated with tumor stage in female

athletes (P<0.05). Conclusion: ID-1 plays a pivotal role in enhancing the stem cell characteristics of breast cancer cells and promoting angiogenesis within breast cancer, potentially through the activation of the Src kinase pathway. The findings suggest that ID-1 could be a critical factor in the development and progression of breast cancer in female athletes, underlining the importance of smart medicine in identifying and targeting molecular pathways for effective breast cancer treatment and prevention.

**KEYWORDS:** Breast cancer, DNA-binding differentiation suppressor protein, Smart health care, carcinogenesis

## 1. INTRODUCTION

Breast cancer remains one of the most prevalent and devastating diseases affecting women worldwide, with its incidence rising among female athletes, a group traditionally considered to be at a lower risk due to their active lifestyle and healthier overall health profiles. Recent advancements in molecular biology and smart medicine have begun to unravel the complex etiology of breast cancer, highlighting the role of genetic and molecular factors in its development and progression(Chen et al., 2020). Among these, the DNA-binding protein inhibitor ID-1 (Inhibitor of Differentiation or DNA Binding 1) has emerged as a significant player in the carcinogenesis of breast cancer. ID-1, a member of the helix-loop-helix protein family, is implicated in the regulation of cell differentiation and proliferation, and its overexpression has been associated with various cancers, including breast cancer(Morales, Rodríguez, Cruz, & Teran, 2020).

The objective of this study is to delve into the expression and oncogenic effects of ID-1 in breast cancer among female athletes, employing smart medicine approaches to understand the potential pathways through which ID-1 contributes to breast cancer progression. Smart medicine, encompassing precision medicine and advanced molecular diagnostics, offers a novel perspective on cancer treatment by targeting specific molecular alterations within cancer cells. This approach is particularly pertinent to breast cancer, where heterogeneity in tumor biology necessitates tailored therapeutic strategies(Bell, 2020; Hosonaga, Saya, & Arima, 2020).

The research focuses on establishing a breast cancer cell line experiment to assess the expression of ID-1 in cancerous tissues and adjacent non-cancerous tissues (Qiu, Gu, Ni, & Li, 2020), comparing ID-1 expression across different groups of breast cancer cells, and examining the influence of ID-1 on the activation of Src kinase, a key signaling molecule in cancer cell proliferation and survival. Additionally, the study aims to elucidate the relationship between ID-1 expression and clinically relevant factors among female athletes, a group for whom the interplay between intense physical

activity and cancer risk factors presents unique challenges and opportunities for intervention(Min Khine Maw, Fujimoto, & Tamaya, 2008; Zhang et al., 2007).

Given the role of ID-1 in promoting cell proliferation and inhibiting differentiation, its study is crucial for understanding the mechanisms of breast cancer initiation and progression. Furthermore, the activation of Src kinase by ID-1 suggests a possible pathway through which ID-1 may enhance the malignant phenotype of breast cancer cells, including increased stem cell characteristics and angiogenesis, critical factors in tumor growth and metastasis (Min K Maw, Fujimoto, & Tamaya, 2010). By investigating these mechanisms in the context of female athletes, this study aims to contribute valuable insights into the role of physical activity and athletic lifestyle in modulating breast cancer risk and progression, potentially leading to the development of targeted prevention and treatment strategies for this unique population(Wu et al., 2019).

## 2. Materials and Methods

### 2.1 Clinical Data

From March 2021 to March 2022, a total of 56 samples of cancer tissues and adjacent tissues of female breast invasive carcinoma (not specifically indicated) patients who underwent surgical resection in our hospital were collected. The mean age of the patients was (50.07±9.59) years (range, 33-67 years). The clinical and pathological data of all patients were complete. None of them had received radiotherapy, chemotherapy or immunotherapy before surgery, and no other tumors or major diseases occurred. According to the 2019 WHO classification of breast neoplasms (Tan et al., 2020), the patients were divided into grade I (7 cases), grade II (37 cases), and grade III (12 cases). TNM staging included 6 cases of stage I, 40 cases of stage II, and 10 cases of stage III. There were 38 cases with lymph node metastasis and 18 cases without lymph node metastasis.

### 2.2 Main materials and reagents

Human breast cancer cell line SUM159 and human umbilical vein endothelial cell line HUVECs(Shanghai Cell Bank, Chinese Academy of Sciences), Ham's F-12K medium, penicillin, streptomycin and trypsin (HyClone Company, USA), Lipofectamine transfection 2000 kits and Trizol reagent kit (Invitrogen companies in the United States), SensiFast cDNA synthesis kits and Sensi FastTM SYBR Hi - ROX kit (Meridian companies in the United States), Tumor cell sphere-inducible factor (Sigma, USA), cell culture plate and Matrigel glue (Corning, USA), PVDF membrane (Roche, USA), crystal violet solution, DAB immunohistochemical staining kit and BeyoECL Plus kit (Biyuntian Biological Research Institute, Shanghai),

Immunofluorescence staining kit (Nanjing Born Biological Company), horseradish peroxidase labeled goat anti-rabbit and fluorescein isothiocyanate labeled mouse anti-rabbit (Beijing Boson Company), ID-1, P-Src, Src and GAPDH antibodies (Abcam Company, UK), Shrna-nc, shrNA-ID-1, PCDNA3.1-NC and PCDNA3.1-ID-1 plasmids and primer sequences were constructed and synthesized by Guangzhou Ruibo Biological Co., LTD.

### 2.3 Methods

#### 2.3.1 Immunostaining methods

The collected breast cancer tissues and adjacent tissues were placed in 4% paraformaldehyde and fixed overnight at room temperature. Wax blocks were prepared and cut into sections with a thickness of 4μm. The antigen was repaired by boiling in citrate buffer for 15 min. After cooling, the endogenous catalase activity was removed by deionizing 3% hydrogen peroxide at water temperature for 10 min. Then the non-specific antigen was blocked with 10% goat serum at 37℃ for 30 min. Join a working fluid resistance, 4 ℃ incubation in the night, the next day, put to a working fluid resistance, to join the resistance corresponding to two, 37 ℃ for 40 min incubation, PBS after cleaning, use of DAB chromogenic liquid color, wood grain redyeing, 1% hydrochloric acid alcohol separation 3 s, gradient alcohol dehydration, transparent, xylene neutral rubber seal, ordinary optical microscope and taking pictures. Id-1 was mainly expressed in the cytoplasm, and the staining showed brownish yellow to brown particles indicating positive. Positive cell rate score: ≤5% was 0 points, 6%-25% was 1 point, 26%-50% was 2 points, 51%-75% was 3 points, ≥76% was 4 points; The staining intensity score was 0 for colorless, 1 for yellow, 2 for brown-yellow, and 3 for brown to tan. The product of the two index scores is 0 to 4 points for negative results, and 5 to 12 points for positive results.

#### 2.3.2 Cell culture and transfection methods

SUM159 cells were resuscitated and then cultured in Ham 'SF-12K medium containing 10% fetal bovine serum and 1% streptomycin in an incubator with 5%CO2 at 37 ° C. When the growth of the cells was observed to 80%~90%, the cells were digested with 0.25% trypsin and subcultured.

The cells were randomly divided into Control group, the shRNA NC group, the group, the pcDNA3.1 shRNA - ID- 1 - NC group, pcDNA3.1 - ID - 1, Lipofectamine 2000 was used to transfect shrNA-NC, shrNA-ID-1, PCDNA3.1-NC and PCDNA3.1-ID-1 plasmids into SUM159 cells of the corresponding group, respectively. According to the instructions of the kit, 48 h after transfection, cells and culture supernatants of each group were collected. In subsequent experiments, cells in the Control group were cultured normally without transfection.

### 2.3.3 Quantitative real-time polymerase chain reaction (qRT-PCR) method

Trizol reagent solution was added into the cells of each group to fully lysate the cells. Total RNA was extracted according to the instructions of the kit. RNA content and purity were determined by nucleic acid analyzer. Reverse transcription experiments were performed by one-step qRT-PCR according to the SensiFast cDNA Synthesis Kit instructions. The obtained cDNA was used as the template, and the relative expression of ID-1 mRNA was detected by qRT-PCR system. The operation was performed according to the SensiFastTM SYBR Hi-ROx kit instruction, and β-actin was used as the reference gene. Amplification conditions :95℃ for 2 min; A total of 40 cycles were performed at 95℃ for 5 s and 60℃ for 10 s. Primer sequences: ID-1 upstream sequence 5' -ACGGCTGTTactcacGCCTC-3', downstream sequence 5' -GCTGGAGaATCTCCaccTTGC-3'; Beta actin upstream sequence of 5'- TGCGTGACATGAGAAG-3', downstream sequence 5'- GCTCG TAGCTTCTCCA - 3'. The relative expression of ID-1 mRNA was calculated by $2^{-\Delta\Delta Ct}$.

### 2.3.4 Tumor sphere formation experiment

SUM159 cells of each group were seeded in 6-well plates with low adhesion cells at a density of 1×104/ well, supplemented with culture medium containing 10 ng/mL bFGF, 5μg/mL insulin, 4 g/L BSA, 1%B27 and 20 ng/mL EGF, and incubated at 37℃ with 5%CO2 for 1 week. The formation and growth of tumor spheroids were observed under an inverted microscope, and the images were obtained and the number of spheroids was counted.

### 2.3.5 Detection of relative expression by Western blot

SUM159 cells in each group were lysed by adding PIPES buffer containing NP-40 to obtain total cell lysates, and the protein concentration was detected by BCA method. After preparation of 12%SDS-PAGE, 40μg protein sample was taken for loading and separated by electrophoresis. The separated protein was transferred to PVDF membrane and blocked with 5% nonfat milk powder at room temperature for 1 h. The membrane was washed with TBST and then immersed in the primary antibody working solution and incubated overnight at 4℃. After TBST was washed again, Beyo ECL was used for color development, and GAPDH was used as the reference protein. Image J software was used to analyze the gray value of each protein band, and the ratio of gray value of target protein and reference protein was used as the relative expression level of target protein.

### 2.4 Statistical Analysis

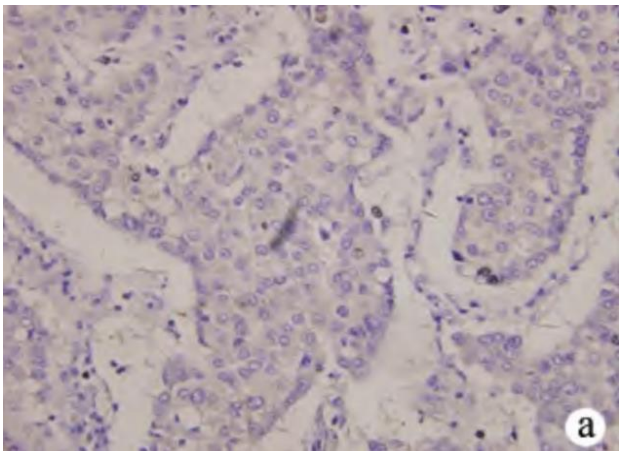
In this study, measurement data were expressed as mean ± standard deviation. Graph pad Prism 9 statistical software was used to analyze data

and draw statistical charts. Comparison between multiple groups was performed by ANOVA, comparison between groups was performed by LSD-T test, count data were expressed as (*n*, %), and $P<0.05$ was considered statistically significant.

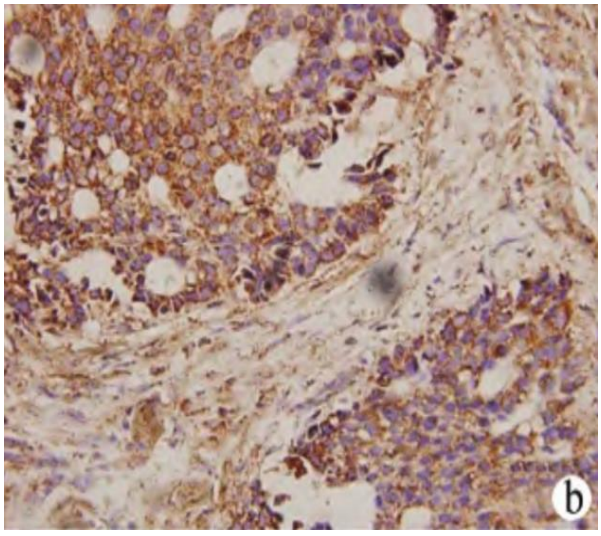
## 3. The results

### 3.1 The expression of ID-1 in cancer tissues and adjacent tissues was observed

Immuno histochemical staining results applied in this study showed that the positive expression rate of ID-1 in breast cancer tissues was 83.93%(47/56), and the positive expression rate of ID-1 in adjacent tissues was 7.14%(4/56). The positive expression rate of ID-1 in breast cancer tissues increased significantly than that in adjacent tissues ($P<0.05$), as shown in Figure 1.




**a:** adjacent tissue




**b:** Breast cancer tissue

**Figure 1:** Expression of ID-1 in breast cancer tissues and adjacent tissues

### 3.2 Comparison of ID-1 expression in breast cancer cells of each group

The breast cancer cell lines selected in this study were transfected and detected by qRT-PCR and Western blot. The results showed that compared with the Control group and the shRNA-NC group, the expression levels of ID-1 mRNA and protein in the shRNA-ID-1 group showed a trend of low expression ($P$<0.05). Compared with Control group and PCDNA3.1-NC group, the mRNA and protein expression of ID-1 in PCDNA3.1-ID-1 group were significantly higher ($P$<0.05), as shown in Figure 2. These results indicated that ID-1 was successfully inhibited or overexpressed in SUM159 cells.

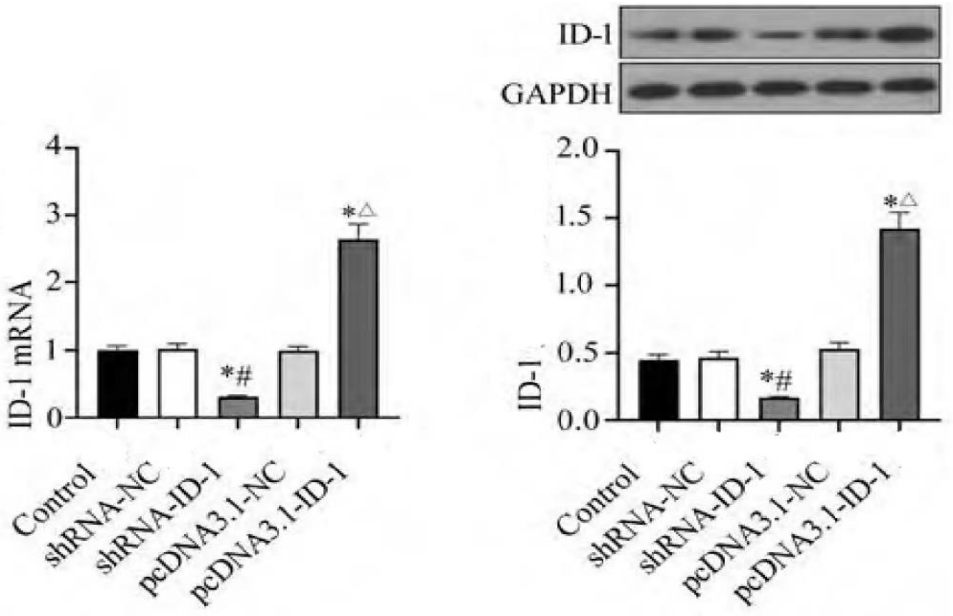



**Figure 2:** Comparison of ID-1 mRNA and protein expression in SUM159 cells of each group

***Note:*** **: Compared with Control group, P<0.05; #: Compared with shRNA-NC group, P<0.05; △: Compared with PCDNA3.1-NC group, P<0.05.*

### 3.3 Effect of ID-1 on Src kinase activation in breast cancer cells

The expression of p-Src in SUM159 cells of each group was detected by immunofluorescence staining. Green fluorescence expression was observed in Control group, shRNA-NC group and PCDNA3.1-NC group, while no obvious green fluorescence expression was observed in shRNA-ID-1 group. Pcdna3.1-ID-1 cells showed strong green fluorescence expression, indicating that overexpression of ID-1 promoted Src kinase activation. Western blot showed that compared with Control group and shRNA-NC group, the expression level of p-Src protein in SUM159 cells in shRNA-ID-1 group was significantly down-regulated ($P$<0.05).

Compared with Control group and PCDNA3.1-NC group, the expression level of p-Src protein in PCDNA3.1-ID-1 group was significantly up-regulated ($P$<0.05), as shown in Figure 3.

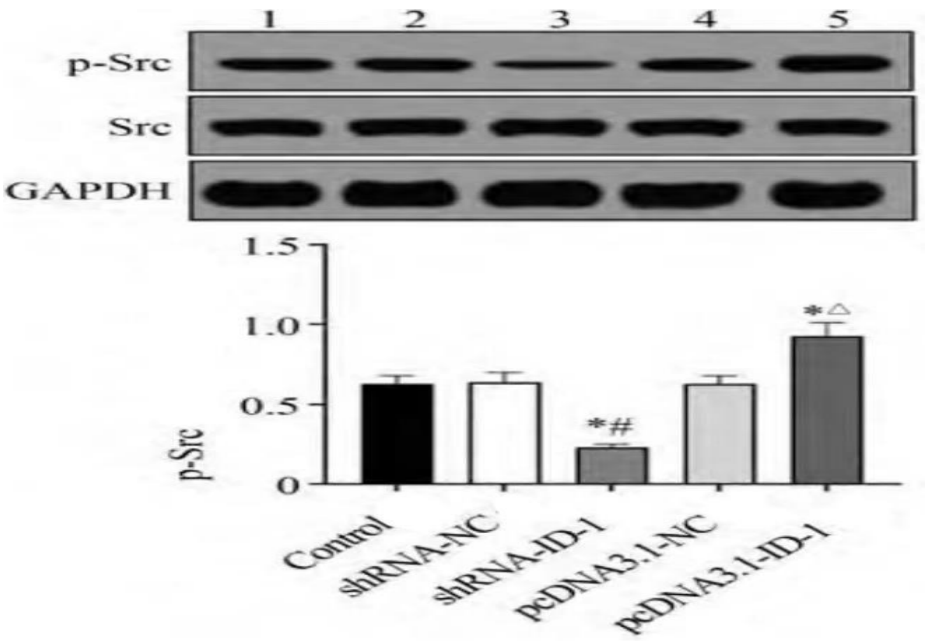



**Figure 3:** Comparison of P-Src and Src protein expression in SUM159 cells of each group

***Note:*** **: Compared with Control group, P<0.05; #: Compared with shRNA-NC group, P<0.05; △: Compared with PCDNA3.1-NC group, P<0.05.*

### 3.4 Analyze the relationship between ID-1 expression and clinical related factors in breast cancer

The results of this study showed that ID-1 expression was significantly correlated with tumor stage (*P*<0.05), as shown in Table 1.

**Table 1:** Analysis of the relationship between Id-1 expression and clinical related factors in breast cancer

| Factors | Id-1negative | Id-1positive | $X^2$ | *P* |
|---|---|---|---|---|
| Age (years) | | | 0.359 | 0.549 |
| ≤50(*n*=30) | 4(13.33) | 26(86.67) | | |
| >50(*n*=26) | 5(19.23) | 21(80.77) | | |
| Tumor diameter(cm) | | | 0.336 | 0.562 |
| ≤5cm(*n*=39) | 7(17.95) | 32(82.05) | | |
| >5cm(*n*=17) | 2(11.76) | 15(88.24) | | |
| Tnm | | | 9.452 | 0.002 |
| I(*n*=6) | 2(33.33) | 4(66.67) | | |
| Ii(*n*=40) | 6(15.00) | 34(85.00) | | |
| Iii(*n*=10) | 1(10.00) | 9(90.00) | | |
| The degree of differentiation | | | | |
| Poorly differentiated(*n*=19) | 4(21.05) | 15(78.95) | 2.134 | 0.223 |
| In the differentiation(*n*=28) | 4(14.29) | 24(85.71) | | |
| High differentiation(*n*=9) | 1(11.11) | 8(88.89) | | |

## 4. Discussion

At present, the most critical problem in the clinical treatment of breast cancer is the lack of sensitive and effective molecular targets. Most breast cancer patients are already in advanced stage when diagnosed, with poor prognosis and high mortality (Barbato, Bocchetti, Di Biase, & Regad, 2019). The pathological process of breast cancer is complex, involving a variety of cellular activities and the interaction of related signaling pathways. With the progress of medical technology and medical wisdom sharing performance boost, scholars have devoted to study the pathogenesis of breast cancer and constantly updated at the same time, relevant data, accurate understanding the molecular mechanisms of breast cancer clinical, clear valuable biological markers for early diagnosis and predicting clinical outcome has a positive effect.

ID-1 is overexpressed in multiple solid tumors and is closely related to the high aggressiveness of tumors and adverse clinical outcomes of patients. In addition, ID-1 also plays an important role in maintaining self-renewal and pluripotency of embryonic neural stem cells (Walcher et al., 2020) (De Francesco, Sotgia, & Lisanti, 2018). We applied the intelligent medical system to review the literature and professional data and found that domestic scholars had established researches around ID-1 and maintained the stem cell characteristics of colorectal cancer cells by activating Wnt/β-catenin, Shh and ID-1-C-MYC-PLAC8 signaling pathways. And promote epithelial-mesenchymal transition of tumor cells (Sun et al., 2019). Other scholars in the study progress in mechanism of gastric cancer by using small interference RNA on low ID 1 expression in gastric cancer cells, key Nanog related factors found that tumor stem cells and the expression of Oct - 4 have been suppressed, and weakened the self-renewal capacity of stem cells in gastric cancer cells, but also reduce the gastric cancer cell proliferation and resistance to cisplatin chemotherapy (Li et al., 2017).

In this study, shRNA-ID-1 and PCDNA3.1-ID-1 were transfected into breast cancer cell line SUM159, and the inhibitory or overexpression efficiency was verified at the gene and protein levels. It was found that the number of cell colony formation was significantly reduced after the inhibition of ID-1 expression in SUM159 cells. The volume and number of tumor spheres were significantly smaller, indicating that inhibition of ID-1 expression weakened the stem cell characteristics of SUM159 cells. However, in SUM159 cells overexpressing ID-1, the effect was completely opposite to that after inhibition, and the number of cell colony formation and tumor spheroids were significantly increased. Therefore, it is speculated that inhibition of ID-1 expression can reduce the stem cell characteristics of breast cancer cells.

Our follow-up survey of previous breast cancer patients through smart

medical system showed that tumor metastasis was the main cause of treatment failure and death in cancer patients. Tumor angiogenesis is a necessary condition for tumor progression, and its mechanism may be that the increase of tumor blood vessels provides a guarantee for tumor growth and metastasis by providing nutrients and oxygen. Angiogenesis is a highly regulated process, in which the interaction between pro-angiogenic factors and anti-angiogenic factors mediates the transformation of vascular endothelium in different stages of angiogenesis.

Through continuous exploration of tumor angiogenesis, many drugs targeting angiogenesis have been put into clinical use (Cook et al., 2016). At present, on the basis of surgery or radiotherapy/chemotherapy in breast cancer patients, supplemented with therapies targeting angiogenesis, may play a further role in the control of tumor metastasis. Previous studies have shown that ID-1 can enhance the angiogenesis of ovarian cancer endothelial cells by mediating the activation of PI3K/Akt and NF-κB/MMP-2 signaling pathways, so ID-1 and its downstream effectors can be used as potential targets for the treatment of ovarian cancer (Cook et al., 2016). These results suggest that ID-1 can be used as a therapeutic target against tumor angiogenesis.

To date, the underlying mechanisms by which ID-1 functions in tumors have not been fully elucidated. Is a kind of non-receptor tyrosine kinase Src kinase family, as a kind of multiple effect activator, can be mediated by the G protein coupled receptors, beta coupling protein and growth factor receptor 1 - the start of many signal transduction pathways (Lien et al., 2020), plays a key role in the development of tumor, involved in regulating tumor cell proliferation, migration, invasion and transformation of epithelial mesenchymal process (Yu et al., 2019) (De Kock & Freson, 2020). After activation, Src kinase can interact with downstream target factors to promote tumor progression. In this study, it was found that there was no obvious green fluorescence expression of p-Src marker and p-Src protein expression was inhibited in SUM159 cells with inhibition of ID-1 expression, while there was strong green fluorescence expression and increased expression of p-Src protein in cells with overexpression of ID-1, indicating that, ID-1 may play a role in promoting Src kinase activation. In conclusion, through the detection of breast cancer tissues and cells in vitro, this study found that ID-1 is highly expressed in breast cancer tissues, which can increase the characteristics of breast cancer cells and promote tumor angiogenesis, and the mechanism of action may be related to the activation of Src kinase pathway. Therefore, it is speculated that ID-1 is expected to become a new target for breast cancer treatment (Lee et al., 2015).

However, further studies are needed to clarify how ID-1 regulates Src kinase expression and whether this pathway involves other molecular

mechanisms. The results of this study showed that the expression of ID-1 in breast cancer was significantly correlated with tumor stage, but not with patient age, tumor size, and tissue differentiation. It is suggested that tumor staging is a prognostic factor for breast cancer, and that reducing the expression of ID-1 can be used as one of the strategies for the treatment of breast cancer metastasis. In the future, the follow-up investigation of breast cancer patients can be improved by smart medical system for further analysis and research.

## 5. Conclusion

In conclusion, our investigation into the expression of DNA-binding differentiation inhibitor 1 (ID-1) in breast cancer among female athletes, utilizing smart medicine techniques, has provided significant insights into the molecular mechanisms underpinning breast cancer carcinogenesis. The study revealed that ID-1 is overexpressed in breast cancer tissues compared to adjacent non-cancerous tissues, and this overexpression is closely associated with the activation of the Src kinase pathway, which plays a pivotal role in enhancing the stem cell characteristics of breast cancer cells and promoting tumor angiogenesis. The differential expression of ID-1 and its impact on p-Src protein levels underscore the importance of ID-1 as a potential biomarker for breast cancer progression and as a target for therapeutic intervention, especially in the context of female athletes. This demographic may have unique physiological and metabolic profiles that influence cancer progression pathways, including those mediated by ID-1. Furthermore, the correlation between ID-1 expression and tumor stage highlights the potential of ID-1 as a prognostic indicator, providing valuable information for tailoring treatment strategies for female athletes diagnosed with breast cancer. The findings advocate for the integration of smart medicine approaches in cancer research and treatment, emphasizing the need for targeted therapies that address specific molecular alterations. Future research should focus on exploring the complex interplay between physical activity, ID-1 expression, and breast cancer development, aiming to develop preventive and therapeutic strategies that can mitigate the risk and impact of breast cancer in female athletes. By harnessing the insights gained from studies like ours, the medical community can move closer to achieving personalized medicine, optimizing treatment outcomes for patients based on their unique genetic and lifestyle factors.

## Data Availability

The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the

publication of this paper.

**Acknowledgments**

NONE

**FUNDING**

Project Name: Medical Science Research Key Project of Hebei Province. Research Topic: Study on the expression, clinical significance and prognosis of DNA binding differentiation inhibitory protein 1 in breast cancer. Project No: 20180817.

**REFERENCES**

Barbato, L., Bocchetti, M., Di Biase, A., & Regad, T. (2019). Cancer stem cells and targeting strategies. *Cells, 8*(8), 926.

Bell, R. (2020). Mammographic density and breast cancer screening. *Climacteric, 23*(5), 460-465.

Chen, J., Zhang, F., Wang, D., Yang, Z., Liu, S., & Dong, Z. (2020). Prognostic ability of DNA-binding protein inhibitor ID-1 expression in patients with oral squamous cell carcinoma. *Oncology Letters, 19*(6), 3917-3922.

Cook, P. J., Thomas, R., Kingsley, P. J., Shimizu, F., Montrose, D. C., Marnett, L. J., . . . Benezra, R. (2016). Cox-2-derived PGE2 induces Id1-dependent radiation resistance and self-renewal in experimental glioblastoma. *Neuro-oncology, 18*(10), 1379-1389.

De Francesco, E. M., Sotgia, F., & Lisanti, M. P. (2018). Cancer stem cells (CSCs): metabolic strategies for their identification and eradication. *Biochemical Journal, 475*(9), 1611-1634.

De Kock, L., & Freson, K. (2020). The (patho) biology of SRC kinase in platelets and megakaryocytes. *Medicina, 56*(12), 633.

Hosonaga, M., Saya, H., & Arima, Y. (2020). Molecular and cellular mechanisms underlying brain metastasis of breast cancer. *Cancer and Metastasis Reviews, 39*, 711-720.

Lee, H.-P., Lin, C.-Y., Shih, J.-S., Fong, Y.-C., Wang, S.-W., Li, T.-M., & Tang, C.-H. (2015). Adiponectin promotes VEGF-A-dependent angiogenesis in human chondrosarcoma through PI3K, Akt, mTOR, and HIF-α pathway. *Oncotarget, 6*(34), 36746.

Li, L., Wei, X., Wu, B., Xiao, Y., Yin, M., & Yang, Q. (2017). siRNA-mediated knockdown of ID1 disrupts Nanog-and Oct-4-mediated cancer stem cell-likeness and resistance to chemotherapy in gastric cancer cells. *Oncology Letters, 13*(5), 3014-3024.

Lien, M.-Y., Chang, A.-C., Tsai, H.-C., Tsai, M.-H., Hua, C.-H., Cheng, S.-P., . . . Tang, C.-H. (2020). Monocyte chemoattractant protein 1 promotes VEGF-A expression in OSCC by activating ILK and MEK1/2 signaling and downregulating miR-29c. *Frontiers in oncology, 10*,

592415.

Maw, M. K., Fujimoto, J., & Tamaya, T. (2008). Expression of the inhibitor of DNA-binding (ID)-1 protein as an angiogenic mediator in tumour advancement of uterine cervical cancers. *British journal of cancer, 99*(10), 1557-1563.

Maw, M. K., Fujimoto, J., & Tamaya, T. (2010). Role of inhibitor of DNA binding-1 protein is related to angiogenesis in the tumor advancement of uterine endometrial cancers. *Experimental and therapeutic medicine, 1*(2), 351-356.

Morales, M. A. G., Rodríguez, R. B., Cruz, J. R. S., & Teran, L. M. (2020). Overview of new treatments with immunotherapy for breast cancer and a proposal of a combination therapy. *Molecules, 25*(23).

Qiu, X., Gu, Y., Ni, Y., & Li, Q. (2020). ID1 As a Prognostic Biomarker and Promising Drug Target Plays a Pivotal Role in Deterioration of Clear Cell Renal Cell Carcinoma. *BioMed Research International, 2020*.

Sun, Y., Lai, X., Yu, Y., Li, J., Cao, L., Lin, W., . . . Li, C. (2019). Inhibitor of DNA binding 1 (Id1) mediates stemness of colorectal cancer cells through the Id1-c-Myc-PLAC8 axis via the Wnt/β-catenin and Shh signaling pathways. *Cancer management and research*, 6855-6869.

Tan, P. H., Ellis, I., Allison, K., Brogi, E., Fox, S. B., Lakhani, S., . . . Salgado, R. (2020). The 2019 WHO classification of tumours of the breast. *Histopathology*.

Walcher, L., Kistenmacher, A.-K., Suo, H., Kitte, R., Dluczek, S., Strauß, A., . . . Kossatz-Boehlert, U. (2020). Cancer stem cells—origins and biomarkers: perspectives for targeted personalized therapies. *Frontiers in immunology, 11*, 1280.

Wu, X. L., Wang, L. K., Xue, J., Dai, Y. J., Yang, D. D., Qu, M., . . . Sun, G. Y. (2019). A study of the impact of inhibitors of DNA binding-1 on proliferation and migration in human colon carcinoma cells. *The Kaohsiung Journal of Medical Sciences, 35*(4), 209-213.

Yu, X., Hu, L., Li, S., Shen, J., Wang, D., Xu, R., & Yang, H. (2019). Long non-coding RNA Taurine upregulated gene 1 promotes osteosarcoma cell metastasis by mediating HIF-1α via miR-143-5p. *Cell death & disease, 10*(4), 280.

Zhang, X., Ling, M.-T., Wang, Q., Lau, C.-K., Leung, S. C., Lee, T. K., . . . Wang, X. (2007). Identification of a novel inhibitor of differentiation-1 (ID-1) binding partner, caveolin-1, and its role in epithelial-mesenchymal transition and resistance to apoptosis in prostate cancer cells. *Journal of Biological Chemistry, 282*(46), 33284-33294.