Heliyon 9 (2023) e23162

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

SOX10 promotes the malignant biological behavior of basal-like breast cancer cells by regulating EMT process

Kai Yang [a], Fen Yun [b], Lin Shi [b], Xia Liu [b,*], Yong Feng Jia [b,**]

[a] Department of Basic Medicine College, Inner Mongolia Medical University, Inner Mongolia, China
[b] Department of Pathology, Basic Medical College, Inner Mongolia Medical University, China

ARTICLE INFO

Keywords:
SOX10
BLBC
EMT
Immunotherapy

ABSTRACT

Background: The diagnostic utility of SRY-box transcription factor 10 (SOX10) expression in basal-like breast cancer (BLBC) has been reported previously. However, the effect of SOX10 on the malignancy of BLBC cells and the underlying molecular mechanisms remain unelucidated. Here, we investigate the regulatory mechanisms and roles of SOX10 in BLBC progression.
Methods: Sequencing data from patients with BLBC were extracted from the Cancer Genome Atlas database to determine the transcriptomic levels of SOX10 across breast cancer subtypes. Subsequently, the bioinformatics relevance of SOX10 in BLBC was investigated. Immunohistochemical assays were used to corroborate the protein expression of SOX10 in clinicopathological specimens (human breast cancer paraffin tissues). RNA interference was used to downregulate SOX10 expression, and the efficiency of interference was evaluated using quantitative PCR. The expression levels of molecules related to the epithelial-mesenchymal transition (EMT) pathway were determined by western blotting. Various assays, such as transwell, colony formation, and flow apoptosis assays, were conducted to assess the malignancy of BLBC cells (MDA-MB-231).
Results: Bioinformatics analyses revealed the differential expression of SOX10 in various breast cancer subtypes. An association between SOX10 and immune checkpoint expression was observed in BLBC. Additionally, immune correlation analysis indicated a positive relationship between SOX10 expression and effector immune cells. SOX10 was identified as a potential immunotherapeutic target. Juxtaposed with non-basal-like breast cancer (N-BLBC) and breast adenosis, immunohistochemical analysis revealed the upregulated expression of SOX10 in BLBC, indicating its potential diagnostic significance. Single-gene functional enrichment analysis indicated that SOX10 is associated with EMT and the tumor inflammatory index. Experimental outcomes from cellular assays suggested that the downregulation of SOX10 inhibited multiple malignancy-associated behaviors in MDA-MB-231 cells, specifically affecting the EMT process, migration, invasion, proliferation, clone formation, and anti-apoptotic activities.
Conclusions: We concluded that SOX10 contributes to the malignancy of BLBC cells by modulating the EMT pathway. Moreover, we observed a notable correlation between SOX10 expression and immune responses, indicating the potential significance of SOX10 in immunotherapy.

* Corresponding author.
** Corresponding author.
E-mail addresses: 18547139575@163.com (X. Liu), yfjia0471@163.com (Y.F. Jia).

https://doi.org/10.1016/j.heliyon.2023.e23162
Received 15 September 2023; Received in revised form 25 November 2023; Accepted 28 November 2023
Available online 1 December 2023

1. Background

Breast cancer has recently surpassed lung cancer in the number of diagnosed cases, establishing itself as the most commonly diagnosed female-specific malignancy globally [1,2]. Concomitant with advances in molecular biology, therapeutic approaches in breast cancer have transitioned from traditional surgical methods to endocrine therapies and immunotherapies [3]. Basal-like breast cancer (BLBC) has emerged as a focal area of research and clinical concern owing to characteristics such as early onset, high invasiveness, and suboptimal response rates to conventional treatments, which lead to poor prognosis [2]. This subtype of breast cancer accounts for 15%–20 % of all breast cancer cases and is typically characterized by negative ER, PR, and HER2 expression [4].

Epithelial-mesenchymal transition (EMT) is a complex biological process involving the transformation of epithelial cells into a mesenchymal phenotype through multiple stages [5–7]. As malignant cells infiltrate healthy tissue, gene expression patterns may be altered to evade the host immune response and inhibit the proliferation of immune cells. Among the most commonly implicated cytokines in this process are the epidermal growth factor receptor, vascular endothelial growth factor, and integrin [8,9]. EMT, a key factor in malignancy, can significantly drive the distant metastasis of malignant cells originating from epithelial cells. This process is particularly pertinent to the pathogenesis of BLBC, which requires various adaptive mechanisms such as apoptosis induction and cellular proliferation for successful tissue infiltration. Dedifferentiation processes in hepatocellular carcinoma correspond to the loss of epithelial morphology, characterized by a reduction in epithelial markers during EMT. The expression of intermediate filament system components such as E-cadherin and cytokeratin are suppressed concurrently with EMT. Conversely, the upregulation of mesenchymal proteins like N-cadherin, α-smooth muscle actin, and vimentin and EMT-related transcription factors such as zinc finger transcription factors, Twist, and E-box binding zinc finger protein has also been observed [10].

SRY-box transcription factor 10 (SOX10) belongs to the SOX family of genes. The protein has a DNA-binding domain similar to the high mobility group (HMG) domain. This domain helps the SOX proteins bind to specific DNA sites, thereby inducing DNA bending and functioning as architectural transcription factors. This family of genes has similar structures across multiple species, some of which are associated with human genome-related diseases or physiological phenomena. The proteins are classified into various A-J subfamilies based on the similarity of HMG domains. SOX8, SOX9, and SOX10 are categorized under the SOXE subfamily, with a remarkable 98 % similarity in their HMG domains. These characteristics make this gene family highly suitable for investigations on the structure, function, and genetic mechanisms of the human genome. The SOX gene family has been conserved through evolutionary history, and its importance in biological processes such as stemness maintenance, cell directional differentiation, and tissue formation is significant. Mutations in SOX10 have been implicated in the development of the neural crest and peripheral nervous system as well as in the maturation and terminal differentiation of Schwann and oligodendrocyte cells. These mutations are also significant in melanocyte formation. A potential association between such mutations and the occurrence of Waardenburg-Shah and Waardenburg-Hirschsprung diseases has been suggested [11–13].

The SOX protein is known for its indispensable role in the nervous system, which aids the survival, proliferation, and differentiation of various cell types. It is also instrumental in the maintenance of stem cells and lineage differentiation during embryonic development and maturation. Recent investigations have highlighted its multifunctional properties. The aberrant expression and function of SOX proteins are closely associated with the emergence of various tumors. For instance, several SOX proteins, such as SOX2, SOX3, SOX4, and SOX5, are overexpressed in tumors and function as oncogenes. Conversely, certain SOX proteins, including SOX1, SOX7, SOX11, and SOX17, are downregulated in tumors and serve as tumor suppressor genes [14–21]. SOX10 expression has been implicated in the onset and progression of a diverse range of tumors. Immunohistochemical analyses revealed that SOX10 is predominantly expressed in tumors of neural origin, such as schwannomas and melanomas. Clinical diagnostic studies have also confirmed the presence of SOX10 in primary breast cancers, particularly those exhibiting basal-like phenotypes. However, the effects of SOX10 on the proliferation, migration, and apoptosis of BLBC cells and the underlying molecular mechanisms are yet to be elucidated [22].

In this study, we used realistic clinicopathological diagnostic criteria, results of bioinformatics analyses, and findings from a literature review to elucidate the notable diagnostic and immunotherapeutic potential of SOX10 in BLBC. The expression of SOX10 transcripts in various subtypes of breast cancer was determined using bioinformatics analyses, particularly highlighting its elevated levels in BLBC. Furthermore, functional enrichment ($P < 0.05$) indicates that SOX10 may modify EMT to influence BLBC progression and modulate immune responses in BLBC. A detailed examination of immune infiltration in BLBC as compared to that in non-basal-like breast cancer (N-BLBC) revealed disparities in the distribution of various immune cells. Significant correlations between SOX10 expression and multiple immune-related indicators, as well as various immune cells, were identified. In this study, we identified potential for diagnostic guidance based on SOX10 protein expression levels across 44 cases of BLBC, 67 cases of N-BLBC, and 22 cases of breast adenosis. The diagnostic relevance of SOX10 expression levels was subsequently evaluated. The regulatory effect of SOX10 expression on BLBC cell MDB-MA-231 was investigated using siRNA interference. We also explored the subsequent impact on EMT processes as well as on the proliferation, migration, and invasion of BLBC cells. Our findings contribute to a more comprehensive understanding of BLBC pathogenesis and offer insights into potential diagnostic and therapeutic targets in BLBC.

2. Materials and methods

2.1. Materials

2.1.1. Clinical samples

Pathological breast tissues were obtained from 111 patients with breast cancer who were diagnosed in the Pathology Department of the First Affiliated Hospital of Inner Mongolia Medical University between 2017 and 2019. The samples were collected from 44

patients with BLBC and 67 patients with N-BLBC. Additionally, 22 tissue samples of breast adenosis were collected in the same period. Patients who had undergone immunotherapy, chemotherapy, or radiotherapy before surgical intervention were excluded. Ethical clearance for the use of these samples in the retrospective study was granted by the Medical Ethics Committee of Inner Mongolia Medical University, with informed consent obtained from the patients. The license number for this study is YKD2019255.

2.1.2. Reagents

The immunohistochemical antibody for SOX10 was procured from Fuzhou Maixin Biotechnology Development Co., Ltd. Other reagents such as TRIzol, Mir-X miRNA First-Strand Synthesis, and SYBR Green PCR kits were purchased from TAKARA in Japan. A 0.8 μm Transwell chamber was sourced from Corning (USA), and Matrigel was obtained from BD (USA). Monoclonal antibodies specific to E-cadherin, N-cadherin, and Vimentin were purchased from Wuhan Sanying Company in China. Fetal bovine serum (FBS) was acquired from Israel BI Company, and DMEM medium was supplied by Gibco (USA).

2.2. Methods

2.2.1. Bioinformatics analysis

mRNA data and the relevant clinical information were extracted from the Cancer Genome Atlas (TCGA) database. Disparities in mRNA expression between SOX10 and tissues related to human breast cancer were scrutinized and verified using web-based tools reliant on the TCGA database. R language packages, such as "survival," "ggplot," "limma," "ggpubr," and "BiocManager," were used to examine the relationship between SOX10 mRNA transcriptome data and clinical parameters. The study also focused on the differential expression of SOX10 and the functional enrichment of associated differentially expressed genes. Furthermore, the correlation between gene expression and clinicopathological characteristics such as clinical stage, prognosis time, and tumor mutation index was assessed.

2.2.2. Immunohistochemical analysis

Immunohistochemical analysis was conducted using the EnVision method. The process involved a series of specific steps: dewaxing, antigen retrieval, 3 % hydrogen peroxide blocking, goat serum blocking, primary antibody treatment, secondary antibody treatment, DAB coloration, hematoxylin staining, dehydration, transparency, and mounting. EDTA microwave heating was used for SOX10 repair, and ready-to-use antibodies were used.

2.2.3. Cell culture and transfection

For cell culture and transfection, MDA-MB-231 human BLBC cells were sourced from the Chinese Academy of Medical Sciences. The cells were cultured in a medium composed of 90 % DMEM high-glucose and 10 % FBS in an incubator set at 37 °C and 5 % CO_2. Cells were seeded in 6-well plates and were subjected to transfection upon reaching approximately 70 % confluence. The transfection was facilitated using ribo FECT™ CP reagent for miRNA mimics/inhibitors and their corresponding negative controls. In this particular study, an experimental group designated as the siSOX10 group (siSOX10) was transfected with SOX10 interference siRNA (si-SOX10) in the MDB-MA-231 cells. No treatments were administered to the MDB-MA-231 cells to serve as the blank negative control group (Blank). Meanwhile, a negative control group (NC) was established by transfecting negative control siRNA (si-NC) into MDB-MA-231 cells.

2.2.4. Western blotting

In the present study, western blotting was performed using the following methodology. Cells were treated with a specified volume of RIPA lysate for 3–5 min. Following this, the cells were transferred to EP tubes using cell scrapers. To initiate lysis, the tubes were chilled on ice for 30 min. Centrifugation was conducted at 12,000 r/min for 10 min at 4 °C, following which the supernatant was collected. Protein concentrations were measured in accordance with the BCA protein assay kit manual. A mixture of protein loading buffer and protein solution in a 1:4 ratio was heated to 100 °C for 15 min to denature the proteins. Following gel preparation and sample electrophoresis, bromophenol blue reaction was carried out to complete electrophoresis. The membrane was subsequently blocked by treating with skimmed milk for 30 min. Primary antibodies for E-cadherin, N-cadherin, Vimentin, and GAPDH were added at specified dilutions, and the membrane was incubated overnight at 4 °C. The membrane was then washed and treated with secondary antibodies at room temperature for 30 min. After an additional wash with TBST, a contrasting agent was added to the ECL chemiluminescence solution for detection.

2.2.5. Transwell assay

For the Transwell migration assay, cells were treated with trypsin for digestion and subjected to transfection for 24 h. Following centrifugation, the cells were resuspended in a serum-free medium, and their concentration was adjusted accordingly. The serum-free medium was used to dilute Matrigel at a ratio of 1:8, which was then applied to the chamber. A 100 μL aliquot of the cell suspension was introduced into the upper layer of the chamber, while 600 μL of complete medium containing serum was added to the lower layer. The cells were incubated in a cell incubator for an additional 24 h. After fixation in formaldehyde for 30 min, the cells were stained with 0.1 % Giemsa stain for 20 min. The cells were then rinsed twice with PBS, and the upper surface of the chamber cells was carefully wiped clean for microscopic observation.

2.2.6. Plate cloning experiment

In the plate cloning experiment, cells were transfected for a 24 h period before being distributed into a 12-well culture plate.

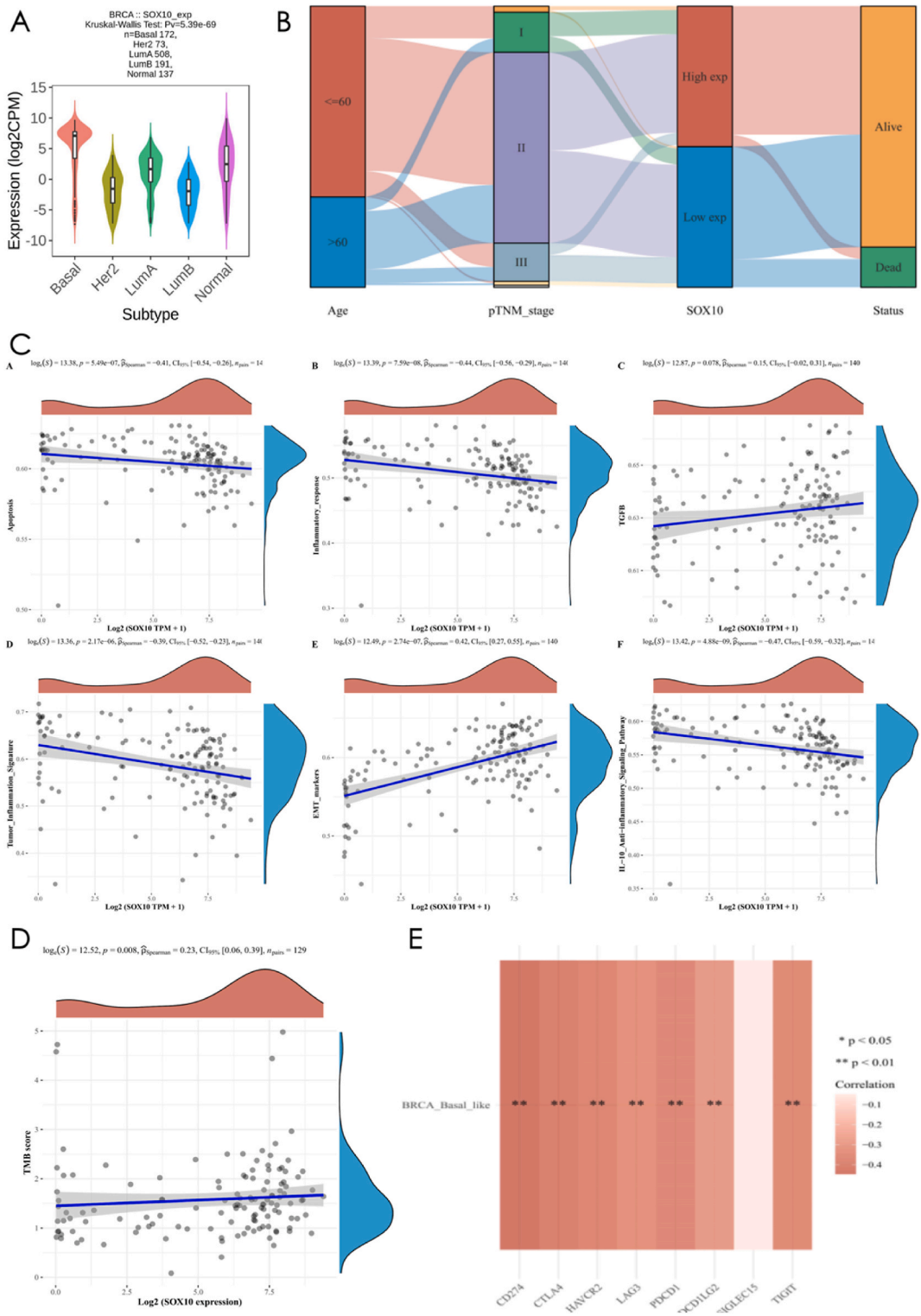

Fig. 1. Single gene bioinformatics analysis of SOX10 expression based on BLBC data. (A) SOX10 expression in different subtypes of breast cancer and normal breast tissues; (B) Correlation between SOX10 expression levels in BLBC and clinical information; (C) Enrichment analysis of functional pathways associated with SOX10 expression levels in BLBC; (D) Correlation between SOX10 expression levels in BLBC and tumor mutation load; (E) Correlation between SOX10 expression levels in BLBC and immune checkpoint expression levels in BLBC; E. Correlation between SOX10 expression levels and immune checkpoint expression levels in BLBC. (*P < 0.05, **P < 0.01, ***P < 0.001).

Specifically, MDA-MB-231 cells were added to three wells per group at a density of 400 cells per 1000 μl in each well. The culture was paused once the cell clusters were visible. The medium was subsequently discarded, and the wells were washed thrice with PBS and then fixed with methanol. The cells were counted after Giemsa staining.

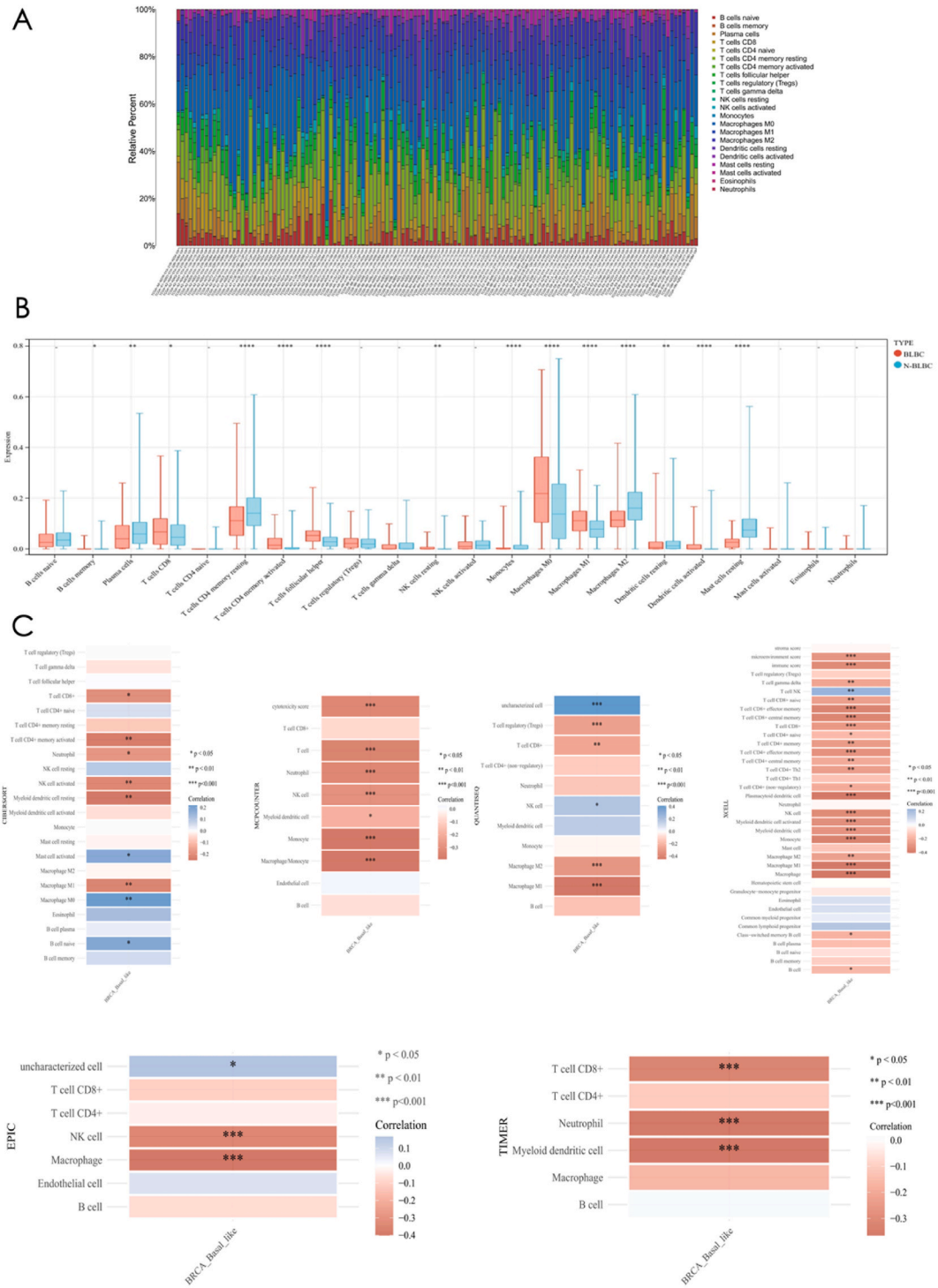

Fig. 2. Infiltration of immune cells in BLBC, and correlation between SOX10 expression and the degree of immune cell infiltration. (A) BLBC immune cell infiltration; (B) Differences in BLBC and N-BLBC immune cell infiltration comparisons; (C) Correlation of SOX10 with immune cell infiltration in different assessment methods. (*P < 0.05, **P < 0.01, ***P < 0.001).

2.2.7. Flow cytometry experiment

In the flow cytometry experiment, cells were collected from each group 24 h post-transfection, subjected to trypsin digestion, and resuspended in pre-chilled DPBS. The cells were counted, and 1×10^6 cells were obtained. Experimental reagents were prepared according to the guidelines specified in the Annexin V-FITC/PI apoptosis kit manual. Flow cytometry was used for the analysis.

2.3. Statistical analysis

SPSS 22.0 software was used for statistical evaluation. Multiple analytical methods were used, including Pearson's chi-squared test, Spearman's correlation analysis, variance analysis, and Fisher's exact probability method. The level of statistical significance was predetermined at $P = 0.05$. Significance levels were further categorized as follows: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.

3. Results

3.1. Results of SOX10 bioinformatics analysis

For SOX10 bioinformatics analysis, we used the TCGA database to investigate variations in SOX10 expression across multiple subtypes of breast cancer tissues. A dataset of 1222 breast cancer cases, along with associated clinical data, was procured from the TCGA database. The expression of SOX10 in various breast cancer subtypes was confirmed using the TISIDB web tool. A significant discrepancy in SOX10 expression was observed across the subtypes of breast cancer compared to that in normal tissue ($P < 0.05$) (Fig. 1A). Moreover, differential SOX10 expression was more pronounced in BLBC than in other subtypes and normal tissues. We further analyzed the relationship between SOX10 expression and various clinical parameters such as STAGE and TMN stages; however, we did not observe a substantial correlation (Fig. 1B). Upon conducting differential expression analysis and functional enrichment analysis of the differentially expressed genes, genes with varying expression levels in comparison to SOX10 levels were found to be associated with apoptosis, inflammatory response, TGFB, tumor inflammation signature, tumor EMT, and the IL-10 anti-inflammatory signaling pathway ($P < 0.05$) (Fig. 1C). Additionally, a correlation was identified between SOX10 expression and the tumor mutation burden (TMB) ($P < 0.05$) (Fig. 1D). Further investigation was conducted to analyze the correlation between SOX10 expression and immune checkpoint factors. The findings indicated a degree of correlation between SOX10 expression and various immune checkpoints ($P < 0.05$) (Fig. 1E). Additional bioinformatic analysis was conducted to explore the infiltration of immune cells in patients diagnosed with BLBC; these findings were combined with those from patients with N-BLBC (Fig. 2A). We observed varying levels of infiltration by different immune effector cells ($P < 0.05$) (Fig. 2B). To evaluate the effect of SOX10 on immune cell infiltration in BLBC cases, we used several methods to assess immune cell infiltration, including the CIBERSORT score, MCP-counter score, QUANTISEQ score, XCELL score, EPIC score, and TIMER score. We observed a significant association between SOX10 expression and the abundance of effector immune cells such as $CD8^+$ T cells, $CD4^+$ T cells, NK T cells, and macrophages using the different analytical methods ($P < 0.05$) (Fig. 2C).

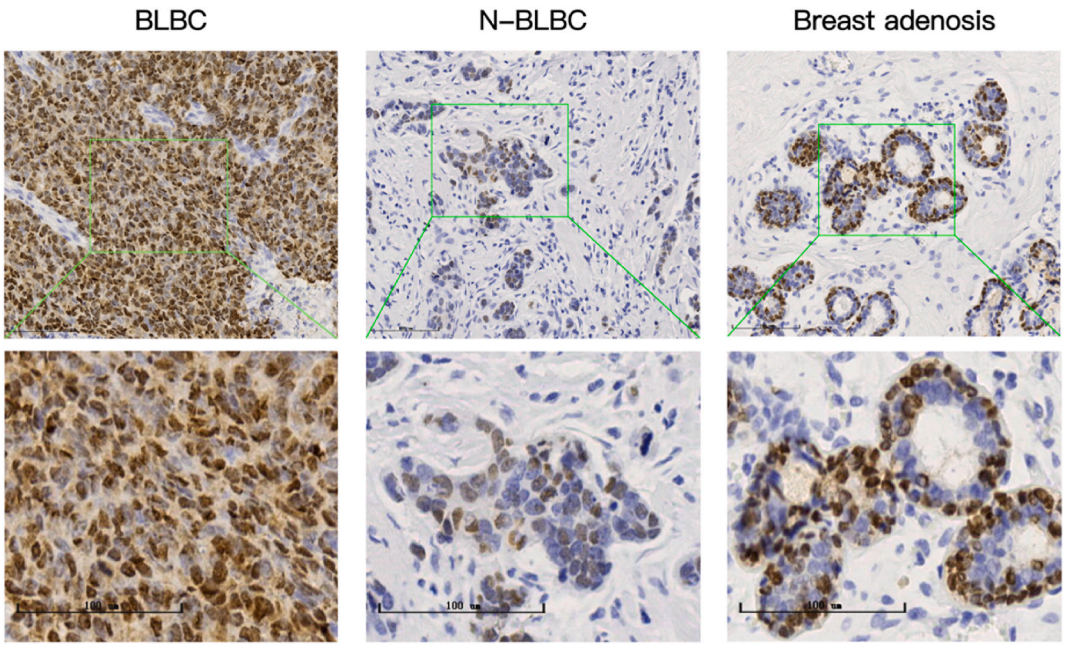

Fig. 3. Representative images depicting SOX10 protein expression in BLBC, N-BLBC, and breast adenosis tissues.

3.2. Elevated expression of SOX10 in BLBC tissues relative to that in other breast cancer subtypes and breast adenosis tissues

To quantify SOX10 protein expression in breast cancer tissues, we performed immunohistochemical analysis on 44 BLBC, 67 N-BLBC, and 22 breast adenosis tissues. Immunohistochemical staining showed that SOX10 was primarily localized in the cell nucleus (Fig. 3). Moderate-to-high levels of diffuse SOX10 expression were observed in tumor cells within the affected BLBC tissues. In contrast, in most N-BLBC tissues, negative staining for SOX10 was predominant, with only a minority of intraductal carcinoma myoepithelial cells showing positive staining results. Mild SOX10 expression was observed in breast adenosis tissues, with SOX10 primarily localized in follicular, ductal, and myoepithelial cells and only sporadically in glandular epithelial cells.

3.3. SOX10 knockdown inhibits the EMT pathway

After transfection with siRNA and the establishment of a control group, alterations in the expression levels of EMT marker proteins in MDA-MB-231 cells were assessed using western blotting. GAPDH was used as the internal reference. Once the cells were transfected with either si-NC or si-SOX10, protein extraction was conducted after 48 h. As illustrated in Fig. 4A, a significant increase in the expression level of the epithelial marker, E-cadherin, was observed in the siSOX10 group (Fig. 4B), whereas the expression levels of mesenchymal markers, such as N-cadherin and Vimentin, were significantly lower than those in the Blank group and the NC group (Fig. 4C and D) ($P < 0.05$). These results demonstrate that suppressing SOX10 expression significantly inhibits EMT in MDA-MB-231 cells.

3.4. Decreased SOX10 expression hinders the invasion and migration of MDA-MB-231 cells

Transwell invasion assays were performed on MDA-MB-231 cells 24 h after transfection with either si-SOX10 or si-NC or no treatment (control group). The assays showed that the number of cells passing through the membrane in the siSOX10 group was significantly lesser than that in both the NC group and the blank group (Fig. 5) ($P < 0.05$). These results indicate that the suppression of SOX10 expression significantly impairs the invasive and migratory potential of MDA-MB-231 cells.

3.5. Decreased SOX10 expression hinders the proliferation and colony formation potential of MDA-MB-231 cells

Plate cloning assays were performed using MDA-MB-231 cells, which were transfected with si-SOX10 and si-NC for 24 h and included in the blank group. After 2–3 weeks of culture, a significant reduction in the number of cell clones in the siSOX10 group was observed compared to that in the blank group and the NC group (Fig. 6) ($P < 0.05$). The data indicate that SOX10 suppression significantly affects the proliferative and colony-forming potential of MDA-MB-231 cells.

3.6. Decreased SOX10 expression triggers the apoptosis of MDA-MB-231 cells

The apoptosis of MDA-MB-231 cells was assessed using flow cytometry and Annexin V-FITC/PI double staining. A statistically significant increase in the number of apoptotic cells in the siSOX10 group was observed compared to that in the blank group and the NC group (Fig. 7) ($P < 0.05$). These findings suggest that SOX10 inhibition is associated with higher levels of apoptosis in MDA-MB-231 cells.

4. Discussion

Owing to the increase in invasive characteristics and limited responsiveness to existing pharmacological treatments, BLBC presents a formidable challenge in clinical settings [23]. However, despite advancements in our understanding of tumor biology, the efficacy of

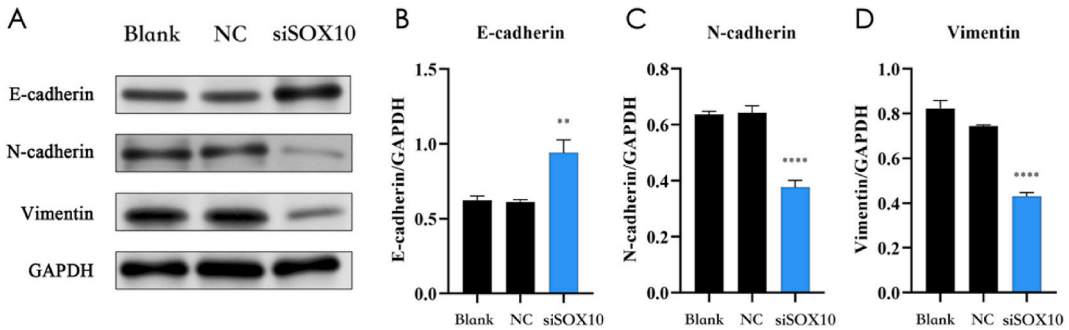

Fig. 4. Interference with SOX10 expression inhibits the EMT pathway of MDA-MB-231 cells. (A) Expression of EMT marker protein in MDA-MB-231 cells after SOX10 knockdown. (B) Statistical results of E-cadherin. (C) Statistical results of N-cadherin. (D) Statistical results of Vimentin. (**$P < 0.01$; ****$P < 0.0001$).

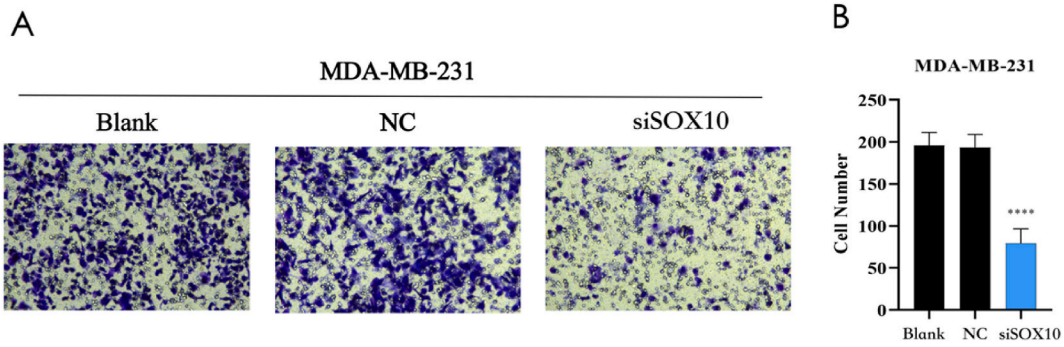

Fig. 5. Interference with SOX10 expression inhibits the invasion of MDA-MB-231 cells. (A) The invasion of MDA-MB-231 cells after SOX10 knockdown. (B) Statistical results. (****P < 0.0001).

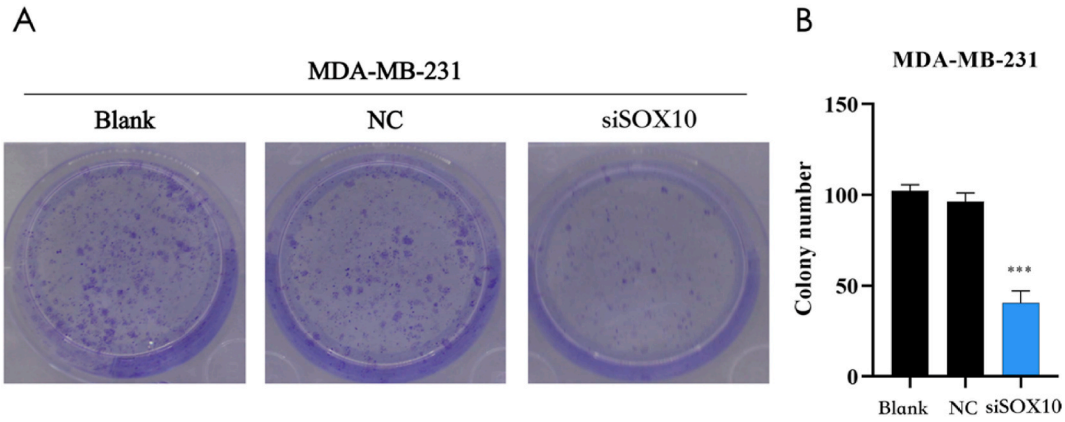

Fig. 6. Interference with SOX10 inhibits the proliferation and colony formation ability of MDA-MB-231. (A) The clone formation of MDA-MB-231 cells after SOX10 knockdown. (B) Statistical results. (***P < 0.001).

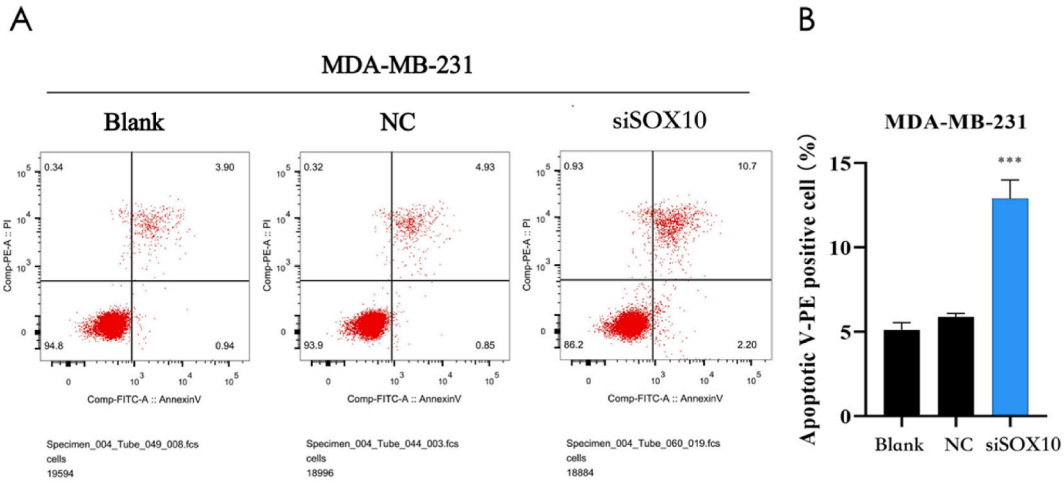

Fig. 7. Decreased SOX10 expression triggers the apoptosis of MDA-MB-231 cells. (A) The apoptosis of MDA-MB-231 cells after SOX10 knockdown. (B) Statistical results. (***P < 0.001).

targeted therapies for BLBC remains suboptimal [24]. This shortcoming has led to concerns among medical practitioners, patients, and investigators alike. Distinct molecular phenotypes make BLBC particularly resistant to both conventional chemotherapy agents and immunotherapies commonly effective against N-BLBC. Traditional postoperative adjuvant radiotherapy and chemotherapy yield

unsatisfactory outcomes in BLBC. After these treatments, a significant number of sub-static lesions have been observed to exhibit tumor recurrence. The average survival duration for patients with metastatic BLBC is estimated at approximately 18 months [25]. Furthermore, accumulating evidence suggests that the synergistic application of existing targeted therapies and immune checkpoint inhibitors can only lead to limited improvements [26]. As innovations in treatment strategies continue to be integrated into clinical protocols, a novel consensus has emerged with respect to the malignant biological behavior of tumors. Factors such as molecular heterogeneity, accumulation of gene mutations, and clonal evolution are now known to contribute to individual variations in tumor characteristics as well as treatment resistance. Given that precision medicine and next-generation sequencing are being increasingly integrated into cancer diagnostic protocols, current research endeavors are largely contingent on the development of reliable predictive biomarkers based on molecular markers. Concurrently, efforts are being directed toward the synthesis of novel pharmaceutical agents targeting recognized molecular pathways, to improve therapeutic outcomes for patients with BLBC.

SOX10 is a member of the SOX family. It is characterized by the presence of an HMG domain and its association with the testicular determinant SRY [27]. Initial investigations have identified the role of SOX10 in the differentiation of neural crest cells [28,29]. Mutations in the human SOX10 gene have been linked to specific neural crest diseases, such as Waardenburg-Shah syndrome types II E, II, and IV, which manifest as hypopigmentation, cochlear nerve sensory deafness, and intestinal ganglion hyperplasia [30–34]. Several studies have further established the involvement of SOX10 in various forms of cancer [35–38]. For instance, SOX10 overexpression has been observed in hepatocellular carcinoma, wherein it exerts oncogenic effects through the activation of the Wnt/double-stranded protein/TCF4 cascade [39]. Conversely, in digestive system cancers, SOX10 has been identified as a tumor suppressor that inhibits the Wnt/beta-catenin signaling pathway [40]. The roles, clinical significance, and biological functions of SOX10 in BLBC are yet to be elucidated, which indicates the need for additional research to understand the mechanisms through which SOX10 influences tumorigenesis and development.

The differential transcript levels of SOX10 across breast cancer subtypes were observed through data analysis. Immunohistochemical assays have revealed a marked elevation in the expression of SOX10 in BLBC. Concurrently, a correlation between SOX10 and immune checkpoint expression was also observed in BLBC, suggesting the potential utility of SOX10 as a diagnostic and immunotherapeutic marker for BLBC. Single-gene functional enrichment analysis further revealed that SOX10 expression is correlated with the inflammation index in BLBC tumors. Moreover, immunoreactivity assays demonstrated a positive association between SOX10 and effector immune cell infiltration. These findings support the hypothesis that SOX10 may influence the formation of the immune microenvironment in BLBC, reinforcing its potential as an immunotherapeutic marker.

Functional enrichment analysis has revealed a link between SOX10 expression and the EMT process in BLBC. This finding has led to the hypothesis that SOX10 can influence the malignant biological behavior of BLBC cells by regulating EMT. EMT is characterized by the loss of epithelial cell polarity and detachment from the basement membrane, which subsequently leads to the structural loosening of the cells. Such alterations facilitate the transition of cells from a low-differentiation state to heightened invasiveness and disorganized proliferation [41–43]. Various tumor microenvironment inhibitors, gene signaling pathways, and cytokine regulatory proteins are implicated in EMT. The expression of E-cadherin, a hallmark of epithelial cells, reduces, whereas the expression of mesenchymal cell markers, such as N-cadherin and Vimentin, increases. These shifts increase tumor cell motility, migration potential, and the acquisition of anti-apoptotic properties as well as the ability to degrade the extracellular matrix [44]. The activation of EMT has been identified as a critical step that helps cancerous cells originating from epithelial cells acquire migratory and invasive capabilities, which eventually leads to metastasis. Research has indicated a significant role of SOX10 in the EMT process of papillary thyroid carcinoma as well as in EMT-related pathways in chronic kidney disease-associated chronic fibrosis [45]. Further investigations are warranted to ascertain whether SOX10 can serve as a therapeutic target of BLBC. Additionally, whether the inherent functions of SOX10, such as sustaining cell proliferation and stabilizing cell differentiation, influence EMT is yet to be investigated.

While examining the function of SOX10 protein, the protein was found to directly influence the structural integrity of cells, a factor critical for cell proliferation and differentiation, as well as for the regulation of cell differentiation. Western blot assays were used to assess the impact of SOX10 expression on EMT in BLBC cells. Results from these assays indicated an increase in the expression level of the epithelial marker E-cadherin in the siSOX10 group, in contrast to that in the blank and NC groups. Concurrently, a reduction was observed in the expression levels of the mesenchymal markers Vimentin and N-cadherin. The suppression of SOX10 disrupts EMT. Therefore, it is speculated that SOX10 overexpression can facilitate the invasion and migration of MDA-MB-231 cells by promoting EMT.

To elucidate the influence of SOX10 on other malignant biological properties of MDA-MB-231 cells, we conducted the Transwell assay and measured the invasion and migration potential of the cells under different treatment conditions. The data obtained indicated a significant correlation between the high invasion and migration potential of MDA-MB-231 cells and SOX10 overexpression. Interference with SOX10 expression markedly influenced the invasion and migration potential of these tumor cells. Collectively, these findings suggest that SOX10 plays a role in facilitating the metastasis of MDA-MB-231 cells by enhancing their invasion and migration potential.

The findings from the colony proliferation assay demonstrated a marked reduction in the proliferation ability of MDA-MB-231 cells when SOX10 expression levels reduced compared to that in control groups in response to gene interference. Subsequently, flow cytometry assays were conducted to assess the impact of SOX10 expression interference on apoptosis. In summary, the experimental results suggest that interference with SOX10 expression can attenuate the proliferative potential of MDA-MB-231 cells by inducing apoptosis. To put this differently, within MDA-MB-231 cells, SOX10 may contribute to tumor progression by disrupting the standard process of apoptosis. This effect could be related to the intrinsic functions of the SOX10 housekeeping gene, which is known to regulate cell stemness to maintain a normal cellular survival state. SOX10 overexpression in MDA-MB-231 cells was found to be associated with elevated cell proliferation in experimental settings. The interruption of SOX10 expression was found to influence the proliferation

potential of these cells negatively. The experimental results were consistent with existing scientific hypotheses.

The aforementioned experimental data indicate that elevated SOX10 expression is correlated with the initiation of EMT in MDA-MB-231 cells. The expression was also found to influence various cellular activities, including movement, invasion, migration, and proliferation, in these cells. Consequently, it is considered that such an expression pattern could potentially amplify the malignant biological characteristics of BLBC cells.

In summary, the elevated levels of SOX10 in BLBC cells were found to facilitate EMT. This was further observed to affect malignant biological functions, including but not limited to the invasion, migration, and proliferation of cancer cells. This phenomenon is considered to be a significant risk factor and merits consideration in the assessment of tumor progression. Resultantly, SOX10 is proposed as a potential diagnostic and prognostic biomarker and may aid the management of BLBC progression. The outcomes of bioinformatics analysis revealed a significant correlation between increased SOX10 expression in BLBC and various functional pathways related to tumor progression, such as apoptosis and EMT. Elements of the tumor immune microenvironment, including the inflammatory response, tumor inflammatory index, and IL-10 anti-inflammatory response, were also found to be related. Additionally, SOX10 expression was found to correlate with the expression of diverse immune-related markers and the infiltration of specific immune cells in BLBC. Based on these observations, SOX10 may be considered to contribute to BLBC progression by modifying the tumor immune microenvironment to facilitate immune evasion. This implication further strengthens the potential role of SOX10 as a crucial node for immunotherapy in patients with BLBC. Based on the findings from our cell-based experiments, we hypothesize that SOX10 could be a potential dual target for both solid tumor therapy and immunotherapy. However, the molecular mechanisms underlying the role of SOX10 in tumorigenesis and tumor development require further investigation.

Funding statement

This article was supported by the Science and Technology Major Project of Inner Mongolia, China (No. 2019GG083).

Data availability statement

Data included in article.

CRediT authorship contribution statement

Kai Yang: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Fen Yun:** Validation, Supervision, Software, Resources. **Lin Shi:** Software, Resources, Project administration, Methodology. **Xia Liu:** Visualization, Supervision, Software, Resources. **Yong Feng Jia:** Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23162.

References

[1] S.A. Miraghel, N. Ebrahimi, L. Khani, A. Mansouri, A. Jafarzadeh, A. Ahmadi, A.R. Aref, Crosstalk between non-coding RNAs expression profile, drug resistance and immune response in breast cancer, Pharmacol. Res. 176 (2022 Feb), 106041.
[2] S. Bekeschus, F. Saadati, S. Emmert, The potential of gas plasma technology for targeting breast cancer, Clin. Transl. Med. 12 (8) (2022 Aug), e1022.
[3] M.A. Medina, G. Oza, A. Sharma, L.G. Arriaga, J.M. Hernández, V.M. Rotello, J.T. Ramirez, Triple-negative breast cancer: a review of conventional and advanced therapeutic strategies, Int. J. Environ. Res. Publ. Health 17 (6) (2020 Mar 20) 2078.
[4] E.M. Grasset, M. Dunworth, G. Sharma, M. Loth, J. Tandurella, A. Cimino-Mathews, M. Gentz, S. Bracht, M. Haynes, E.J. Fertig, A.J. Ewald, Triple-negative breast cancer metastasis involves complex epithelial-mesenchymal transition dynamics and requires vimentin, Sci. Transl. Med. 14 (656) (2022 Aug 3), eabn7571.
[5] T. Jiang, L. Xie, S. Zhou, Y. Liu, Y. Huang, N. Mei, F. Ma, J. Gong, X. Gao, J. Chen, Metformin and histone deacetylase inhibitor based anti-inflammatory nanoplatform for epithelial-mesenchymal transition suppression and metastatic tumor treatment, J. Nanobiotechnol. 20 (1) (2022 Aug 31) 394.
[6] M. Hashemi, S. Hajimazdarany, C.D. Mohan, M. Mohammadi, S. Rezaei, Y. Olyaee, Y. Goldoost, A. Ghorbani, S.R. Mirmazloomi, N. Gholinia, A. Kakavand, S. Salimimoghadam, Y.N. Ertas, K.S. Rangappa, A. Taheriazam, M. Entezari, Long non-coding RNA/epithelial-mesenchymal transition axis in human cancers: tumorigenesis, chemoresistance, and radioresistance, Pharmacol. Res. 186 (2022 Dec), 106535.
[7] M. Sen, R.M. Hausler, K. Dulmage, T.A. Black, W. Murphy, C.H. Pletcher Jr., L. Wang, C. Chen, S.S. Yee, S.J. Bornheimer, K.N. Maxwell, B.Z. Stanger, J.S. Moore, J.C. Thompson, E.L. Carpenter, Transcriptional profiling of single tumour cells from pleural effusions reveals heterogeneity of epithelial to mesenchymal transition and extra-cellular matrix marker expression, Clin. Transl. Med. 12 (7) (2022 Jul) e888.
[8] X. Chen, Y. Huang, H. Chen, Z. Chen, J. Chen, H. Wang, D. Li, Z. Su, Augmented EPR effect post IRFA to enhance the therapeutic efficacy of arsenic loaded ZIF-8 nanoparticles on residual HCC progression, J. Nanobiotechnol. 20 (1) (2022 Jan 15) 34.

[9] L. Zhang, Y. Zhan, L. Li, H. Deng, J. Wang, Z. Zhu, X. Zhang, CircOMA1 promotes tumour growth and metastasis of bladder cancer by modulating IGF-IR/MAPK/EMT pathway, Clin. Transl. Med. 12 (8) (2022 Aug) e983.
[10] C.H. Österreicher, M. Penz-Österreicher, S.I. Grivennikov, M. Guma, E.K. Koltsova, C. Datz, R. Sasik, G. Hardiman, M. Karin, D.A. Brenner, Fibroblast-specific protein 1 identifies an inflammatory subpopulation of macrophages in the liver, Proc. Natl. Acad. Sci. U.S.A. 108 (1) (2011 Jan 4) 308–313.
[11] M.L. Harris, L.L. Baxter, S.K. Loftus, W.J. Pavan, Sox proteins in melanocyte development and melanoma, Pigment Cell Melanoma Res 23 (2010) 496–513.
[12] C.S. Hong, J.P. Saint-Jeannet, Sox proteins and neural crest development, Semin. Cell Dev. Biol. 16 (2005) 694–703.
[13] K. Kuhlbrodt, C. Schmidt, E. Sock, V. Pingault, N. Bondurand, M. Goossens, M. Wegner, Functional analysis of Sox10 mutations found in human Waardenburg-Hirschsprung patients, J. Biol. Chem. 273 (1998) 23033–23038.
[14] Y. Chen, L. Shi, L. Zhang, R. Li, J. Liang, W. Yu, L. Sun, X. Yang, Y. Wang, Y. Zhang, Y. Shang, The molecular mechanism governing the oncogenic potential of SOX2 in breast cancer, J. Biol. Chem. 283 (2008) 17969–17978.
[15] K. Li, R.W. Wang, Y.G. Jiang, Y.B. Zou, W. Guo, Overexpression of Sox3 is associated with diminished prognosis in esophageal squamous cell carcinoma, Ann. Surg Oncol. 20 (Suppl 3) (2013) S459–S466.
[16] P. Liu, S. Ramachandran, M. Ali Seyed, C.D. Scharer, N. Laycock, W.B. Dalton, H. Williams, S. Karanam, M.W. Datta, D.L. Jaye, C.S. Moreno, Sex-determining region Y box 4 is a transforming oncogene in human prostate cancer cells, Cancer Res. 66 (2006) 4011–4019.
[17] J.L. Kopp, G. von Figura, E. Mayes, F.F. Liu, C.L. Dubois, JPt Morris, F.C. Pan, H. Akiyama, C.V. Wright, K. Jensen, M. Hebrok, M. Sander, Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma, Cancer Cell 22 (2012) 737–750.
[18] W. Zhang, S.C. Glockner, M. Guo, E.O. Machida, D.H. Wang, H. Easwaran, L. Van Neste, J.G. Herman, K.E. Schuebel, D.N. Watkins, N. Ahuja, S.B. Baylin, Epigenetic inactivation of the canonical Wnt antagonist SRY-box containing gene 17 in colorectal cancer, Cancer Res. 68 (2008) 2764–2772.
[19] L. Guo, D. Zhong, S. Lau, X. Liu, X.Y. Dong, X. Sun, V.W. Yang, P.M. Vertino, C.S. Moreno, V. Varma, J.T. Dong, W. Zhou, Sox7 Is an independent checkpoint for beta-catenin function in prostate and colon epithelial cells, Mol. Cancer Res. 6 (2008) 1421–1430.
[20] E. Gustavsson, S. Sernbo, E. Andersson, D.J. Brennan, M. Dictor, M. Jerkeman, C.A. Borrebaeck, S. Ek, SOX11 expression correlates to promoter methylation and regulates tumor growth in hematopoietic malignancies, Mol. Cancer 9 (2010) 187.
[21] Shakhova O, Zingg D, Schaefer SM, Hari L, Civenni G, Blunschi J, Claudinot S, Okoniewski M, Beermann F, Mihic-Probst D, Moch H, Wegner M, Dummer R, Barrandon Y, Cinelli P, Sommer L. Sox10 promotes the formation and maintenance of giant congenital naevi and melanoma. Nat. Cell Biol.; 14:882-890..
[22] G.H. Tozbikian, D.L. Zynger, A combination of GATA3 and SOX10 is useful for the diagnosis of metastatic triple-negative breast cancer, Hum. Pathol. 85 (2019 Mar) 221–227.
[23] F. Heitz, P. Harter, H.J. Lueck, et al., Triple-negative and HER2-overexpressing breast cancers exhibit an elevated risk and an earlier occurrence of cerebral metastases[J], Eur. J. Cancer 45 (16) (2009) 2792–2798.
[24] L. Zhang, C. Hao, G. Dong, et al., Analysis of clinical features and outcome of 356 triple-negative breast cancer patients in China[J], Breast Care 7 (1) (2012) 13–17.
[25] R. Dent, M. Trudeau, K.I. Pritchard, W.M. Hanna, H.K. Kahn, C.A. Sawka, L.A. Lickley, E. Rawlinson, P. Sun, S.A. Narod, Triple-negative breast cancer: clinical features and patterns of recurrence, Clin. Cancer Res. 13 (15 Pt 1) (2007 Aug 1) 4429–4434.
[26] X. Bai, J. Ni, J. Beretov, P. Graham, Y. Li, Triple-negative breast cancer therapeutic resistance: where is the Achilles' heel? Cancer Lett. 497 (2021 Jan 28) 100–111.
[27] J. Gubbay, J. Collignon, P. Koopman, B. Capel, A. Economou, A. Munsterberg, et al., A gene mapping to the sex-determining region of the mouse Y chromo some is a member of a novel family of embryonically expressed genes, Nature 346 (6281) (1990) 245–250.
[28] Y. Watanabe, F. Broders-Bondon, V. Baral, P. Paul-Gilloteaux, V. Pingault, et al., Sox10 and Itgb1 interaction in enteric neural crest cell migration, Dev. Biol. 379 (1) (2013) 92–106.
[29] K. Miyahara, Y. Kato, H. Koga, R. Dizon, G.J. Lane, R. Suzuki, et al., Visualization of enteric neural crest cell migration in SOX10 transgenic mouse gut using time-lapse fluorescence imaging, J. Pediatr. Surg. 46 (12) (2011) 2305–2308.
[30] R. Mollaaghababa, W.J. Pavan, The importance of having your SOX on ：role of SOX10 in the development of neural crest-derived melanocytes and glia, Oncogene 22 (20) (2003) 3024–3034.
[31] K. Inoue, Y. Tanabe, J.R. Lupski, Myelin deficiencies in both the central and the peripheral nervous systems associated with a SOX10 mutation, Ann. Neurol. 46 (3) (1999) 313–318.
[32] Y. Izumi, I. Musha, E. Suzuki, M. Iso, T. Jinno, R. Horikawa, et al., Hypogonadotropic hypogonadism in a female patient previously diagnosed as having Waardenburg syndrome due to a sox10 mutation, Endocrine 49 (2) (2015) 553–556.
[33] K. Okamura, N. Oiso, G. Tamiya, S. Makino, D. Tsujioka, Y. Abe, et al., Waardenburg syndrome type IIE in a Japanese patient caused by a novel missense mutation in the SOX10 gene, J. Dermatol. 42 (12) (2015) 1211–1212.
[34] H. Wenzhi, W. Ruijin, L. Jieliang, M. Xiaoyan, L. Haibo, W. Xiaoman, et al., Heterozygous deletion at the SOX10 gene locus in two patients from a Chinese family with Waardenburg syndrome type II, Int. J. Pediatr. Otorhinolaryngol. 79 (10) (2015) 1718–1721.
[35] A. Panaccione, M.T. Chang, B.E. Carbone, Y. Guo, C.A. Moskaluk, R.K. Virk, et al., NOTCH1 and SOX10 are essential for proliferation and radiation resistance of cancer stem-like cells in adenoid cystic carcinoma, Clin. Cancer Res. 22 (8) (2016) 2083–2095.
[36] A.Y. Kwon, I. Heo, H.J. Lee, G. Kim, H. Kang, J.H. Heo, et al., Sox10 expression in ovarian epithelial tumors is associated with poor overall survival, Virchows Arch. 468 (5) (2016) 597–605.
[37] A.C. Schmitt, C. Cohen, M.T. Siddiqui, Expression of SOX10 in salivary gland oncocytic neoplasms: a review and a comparative analysis with other immunohistochemical markers, Acta Cytol. 59 (5) (2015) 384–390.
[38] C. Lopez-Anido, G. Sun, M. Koenning, R. Srinivasan, H.A. Hung, B. Emery, et al., Differential Sox10 genomic occupancy in myelinating glia, Glia 63 (11) (2015) 1897–1914.
[39] D. Zhou, F. Bai, X. Zhang, M. Hu, G. Zhao, Z. Zhao, et al., SOX10 is a novel oncogene in hepatocellular carcinoma through Wnt/beta-catenin/TCF4 cascade, Tumor Biol. 35 (10) (2014) 9935–9940.
[40] X. Tong, L. Li, X. Li, L. Heng, L. Xhong, X. Su, et al., SOX10, a novel HMG-boxcontaining tumor suppressor, inhibits growth and metastasis of digestive cancers by suppressing the Wnt/beta-catenin pathway, Oncotarget 5 (21) (2014) 10571–10583.
[41] M. Hashemi, M.S. Moosavi, H.M. Abed, M. Dehghani, M. Aalipour, E.A. Heydari, M. Behroozaghdam, M. Entezari, S. Salimimoghadam, E.S. Gunduz, A. Taheriazam, S. Mirzaei, S. Samarghandian, Long non-coding RNA (lncRNA) H19 in human cancer: from proliferation and metastasis to therapy, Pharmacol. Res. 184 (2022 Oct), 106418.
[42] M. Sadrkhanloo, M. Entezari, S. Orouei, M. Ghollasi, N. Fathi, S. Rezaei, E.S. Hejazi, A. Kakavand, H. Saebfar, M. Hashemi, M.A.S.B. Goharrizi, S. Salimimoghadam, M. Rashidi, A. Taheriazam, S. Samarghandian, STAT3-EMT axis in tumors: modulation of cancer metastasis, stemness and therapy response, Pharmacol. Res. 182 (2022 Aug), 106311.
[43] X. Shi, Q. Li, C. Zhang, H. Pei, G. Wang, H. Zhou, L. Fan, K. Yang, B. Jiang, F. Wang, R. Zhu, Semiconducting polymer nano-radiopharmaceutical for combined radio-photothermal therapy of pancreatic tumor, J. Nanobiotechnol. 19 (1) (2021 Oct 24) 337.
[44] J. Banyard, D.R. Bielenberg, The role of EMT and MET in cancer dissemination, Connect. Tissue Res. 56 (5) (2015) 403–413.
[45] A. Slusser, C.S. Bathula, D.A. Sens, S. Somji, M.A. Sens, X.D. Zhou, S.H. Garrett, Cadherin expression, vectorial active transport, and metallothionein isoform 3 mediated EMT/MET responses in cultured primary and immortalized human proximal tubule cells, PLoS One 10 (3) (2015 Mar 24), e0120132.