Prognostic Potential of AKR1B1 in triple-negative breast cancer

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Abstract: This research conducts a thorough bioinformatics examination to explore the possible function of Aldose Reductase (AKR1B1) in Triple - Negative Breast Cancer (TNBC). AKR1B1 displays heterogeneous expression patterns across various cancer types, with notably elevated levels in multiple malignancies such as bladder urothelial carcinoma, cholangiocarcinoma, and esophageal carcinoma, yet exhibiting lower expression in breast invasive carcinoma (BRCA). Utilizing the UALCAN and Human Protein Atlas (HPA) databases, a detailed analysis of AKR1B1 in BRCA reveals its distinct expression profile. Significantly, our results imply that AKR1B1 could act as a prospective biomarker in triple - negative breast cancer (TNBC). This is due to its connection with survival results and its co - expression network within this specific cancer subtype. Additionally, we delve into the complex interplay between AKR1B1 and the tumor immune microenvironment. We emphasize its notable link with CD8+ T cells and macrophages. Protein network analysis identifies key proteins interacting with AKR1B1, and experimental validation demonstrates the efficacy of curcumin in inhibiting the proliferation of TNBC cells, thereby suggesting its potential as a therapeutic agent targeting AKR1B1. This study establishes a foundational understanding of AKR1B1's role in TNBC and paves the way for future experimental validation and therapeutic explorations.

1 Introduction

Among women, breast cancer stands as the most prevalent form of cancer and has emerged as the primary cause of cancer - related fatalities in females [1]. In 2022, the global count of newly diagnosed female breast cancer cases exceeded 2.3 million, making up roughly 11.6% of all new cancer patients. In recent times, both the incidence and mortality rates of breast cancer in China have been rising annually [2]. In 2022, approximately 357,200 new cases of breast cancer were reported among Chinese women, accompanied by around 75,000 deaths [3]. Triple - negative breast cancer (TNBC) is a distinct subtype of breast cancer that lacks the presence of ER, PR, and HER-2[4]. Every year, there are 170,000 TNBC cases worldwide. These cases are characterized by a dismal prognosis, low overall survival (OS) rates, and high levels of tumor cell invasiveness, metastasis, and recurrence. Owing to the absence of ER, PR, and HER - 2 expression, TNBC patients do not respond effectively to traditional endocrine and targeted therapies, leading to restricted treatment effectiveness and a poor outlook. Consequently, treating TNBC is especially arduous and represents a major hurdle in current breast cancer treatment. Aldose reductase (AR) is a member of the aldehyde and ketone reductase protein superfamily. Its function is to catalyze the reduction of diverse hydrophobic and hydrophilic carbonyl - containing compounds into their corresponding alcohols [5]. In the polyol metabolism pathway, AR is tasked with converting glucose into sorbitol, a crucial rate - limiting step in polyol metabolism [6]. The aldose reductase AKR1B1, the initial enzyme in the cellular polyol pathway, transforms glucose into sorbitol, which is then oxidized into fructose by sorbitol dehydrogenase. Under hyperglycemic conditions, the activation of the polyol pathway is frequently regarded as a pivotal factor contributing to a variety of long - term diabetes complications, including diabetic retinopathy, cataracts, and diabetic nephropathy [7]. Moreover, certain studies have indicated a connection between the polyol pathway involving AKR1B1 and cancer progression. Schwab et al. found a strong association between the expression of AKR1B1 and the epithelial - mesenchymal transition (EMT) in lung cancer [8]. Nevertheless, the research regarding the mechanism of action of AKR1B1 in triple - negative breast cancer (TNBC) is still unclear and limited. Therefore, this article will concentrate on investigating the potential relationship between AKR1B1 and TNBC. This exploration will set the stage for further studies on the role and mechanism of AKR1B1 in TNBC and whether it can be a novel target for TNBC treatment.

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2 Materials and Methods

2.1 Bioinformatics analysis

2.1.1 UALCAN database.

The UALCAN database serves as a comprehensive platform for cancer analysis. Its primary functions encompass accessing the TCGA and MET500 databases. Additionally, it generates objective visual representations such as gene - expression graphs and curves. Moreover, it assesses patient survival details in relation to gene expression and examines the epigenetic regulation of gene expression through promoter methylation [9]. In the present study, the UALCAN database was employed to analyze the pathological characteristics of patients with breast cancer (BRCA) from the TCGA database.

2.1.2 HPA database.

The Human Protein Atlas (HPA) database is a proteomic, transcriptomic, and systems - based mass spectrometry database. Its function is to map every single human protein across cells, tissues, and organs. It serves as a tool for analyzing the distribution of proteins within tissues and organs and the subcellular localization in individual cells [10]. In the present study, we utilized the HPA database to examine the immunohistochemical outcomes regarding the expression of AKR1B1 in both normal breast tissues and breast invasive carcinoma tissues.

2.1.3 LinkedOmics database.

The LinkedOmics database serves as an online platform designed for the analysis of 32 multidimensional datasets associated with TCGA cancer. To examine the co - expression of AKR1B1, the Spearman correlation coefficient was employed for statistical analysis. The results of this analysis were then presented in various graphical formats, such as volcano plots, heat maps, or scatter plots.

2.1.4 String Database.

The STRING database is a resource designed for the analysis of both known and predicted protein - protein associations. As of now, this database encompasses 9,643,763 proteins originating from 2,031 different organisms. It takes into account both direct (physical) and indirect (functional) interactions. In the present study, we utilized the STRING data to examine proteins that either had a potential to interact with AKR1B1 or were already known to do so.

2.1.5 TIMER database.

The TIMER Database offers a comprehensive repository for the systematic assessment of immune infiltration in diverse cancer types. It presents multiple approaches for evaluating immune infiltration and enables researchers to generate high - quality graphics online in a dynamic manner. This allows them to explore the immunological, clinical, and genomic characteristics of different tumors. We examined the expression of AKR1B1 in breast cancer (BRCA) and the relationship between the expression of AKR1B1 and a substantial quantity of immune cell infiltrates.

2.1.6 TISIDB database.

TISIDB, a web - based platform dedicated to exploring the interplay between tumors and the immune system, consolidates diverse types of heterogeneous data. We conducted an analysis to examine the relationship between the expression level of AKR1B1 and a variety of immune signals.

We appreciate the contribution of the above databases to this research institute.

2.2 Statistical analysis

GraphPad Prism 8 was employed to carry out statistical analysis. Findings are shown as averages±SD, derived from a minimum of three separate experiments. A significance level of $p < 0.05$ was regarded as statistically meaningful.

2.3 Cell culture

The MDA - MB - 231 was obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences, which is situated in China. These cells were kept in DMEM with a high glucose content. Cultivate cells with 89% DMEM, 1% penicillin and streptomycin mixed antibiotics, and 10% premium serum, and place them in a 37 ℃ constant temperature incubator maintained at 5% CO_2 concentration.

2.4 Cell proliferation assays

Cells in the logarithmic growth phase were enzymatically detached and then re - suspended. Subsequently, the cell suspension was carefully adjusted and dispensed into a 96 - well plate, ensuring that each well contained precisely 3000 cells. Each group was set with five duplicate wells, and a circle of PBS was placed on the peripheral point. Subsequently, 100uL of complete medium and 10ul of CCK8 solution were added to each well. Following a 1.5 - hour incubation period within a 37 - degree Celsius incubator, the microplate reader was employed to measure the absorbance value at an optical density of 450 nanometers.

For the clone formation assay, cells were plated in a 6 - well plate at a density of 800 cells per well. These cells were then cultivated for a period of 14 days. Throughout this 14 - day cultivation, the culture medium was refreshed every 3 days. After the 14 - day period, the cells were immobilized using 1 mL of 4% paraformaldehyde. Following fixation, the cells were treated with a crystal violet staining solution. To conduct a live - dead cell staining analysis, a Calcein AM/PI live and dead cell

double staining kit (produced by Servicebio, China) was employed. The staining procedure was carried out strictly in accordance with the instructions provided by the kit's manufacturer.

3 Results

3.1 AKR1B1 is a potential biomarker of TNBC

Employing the TIMER database, we carried out a comparative assessment of the expression of AKR1B1 among different cancer types. The findings revealed significant variability in AKR1B1 expression. Specifically, heightened levels of AKR1B1 were detected in several cancer types, such as BLCA, CHOL, ESCA, HNSC, KIRP, LUSC, and STAD. In contrast, the expression of AKR1B1 was determined to be low in BRCA, COAD, KICH, LUAD, PRAD, READ, and THCA (Figure 1A). This distinct expression pattern hints at a complex regulatory mechanism governing AKR1B1 expression, necessitating further research efforts. Moreover, BRCA demonstrates significant variability, and TNBC stands out as the most diverse subtype. Pinpointing particular clinical subtypes is essential for attaining accurate tumor therapy. Consequently, this research intends to clarify the inherent connection among AKR1B1, BRCA, and TNBC.

Utilizing data sourced from the UALCAN and HPA databases, we conducted an analysis of AKR1B1 expression in BRCA (as depicted in Figure 1B - C). These results were in agreement with our prior findings.

Finally, we utilized the ENCORI database to investigate the relationship between AKR1B1 and ER, PR, and HER-2. The findings indicated inverse associations between AKR1B1 and these markers, as depicted in Figure 1D.

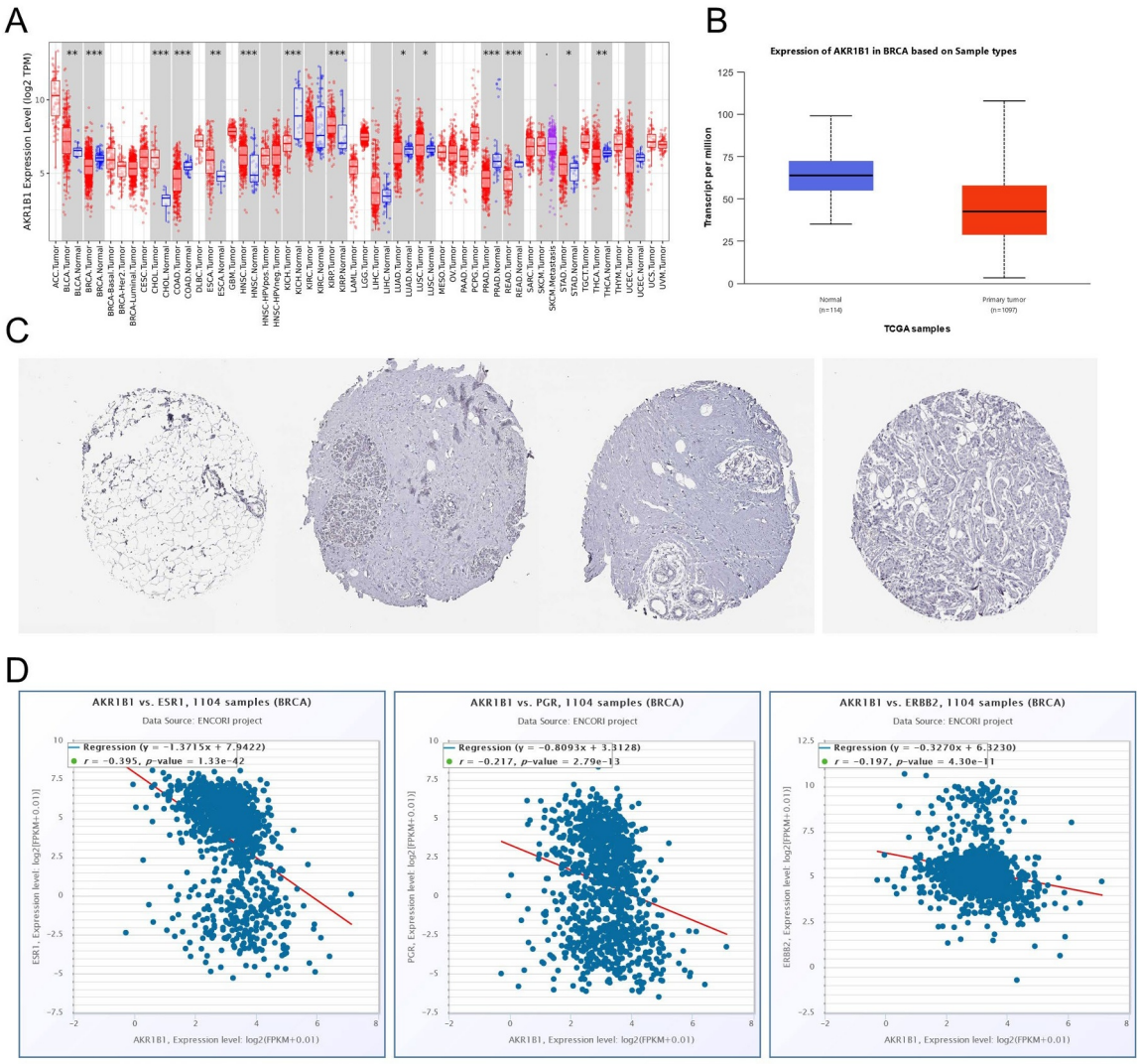

Figure 1. Transcription level of AKR1B1 in BRCA. (A) Investigation of the expression pattern of AKR1B1 from a pan - cancer viewpoint. (B) The expression of AKR1B1 in tumor tissue and the neighboring normal tissue was presented. (C) Utilizing the HPA database, an analysis was conducted on the expression levels of AKR1B1 in pathological tissues. From left to right, they are adipocytes, glandular cells, epithelial cells, and tumor cells. (D) Analyzing the relative expression relationship between AKR1B1 and three hormone receptor genes using the ENCORI database.

3.2 Expression of AKR1B1 is Associated with Survival

We utilized the Kaplan - Meier Plotter database to conduct an analysis that sought to explore the association between the expression levels of AKR1B1 in triple - negative breast cancer (TNBC) and the survival prognoses of patients. The findings indicated that patients with elevated AKR1B1 expression experienced lengthier intervals of recurrence - free survival (RFS), overall survival (OS), and distant metastasis - free survival (DMFS) compared to those with reduced expression levels (Figure 2A - C). These results imply that specific activation strategies for AKR1B1 may warrant consideration in the therapeutic management of TNBC.

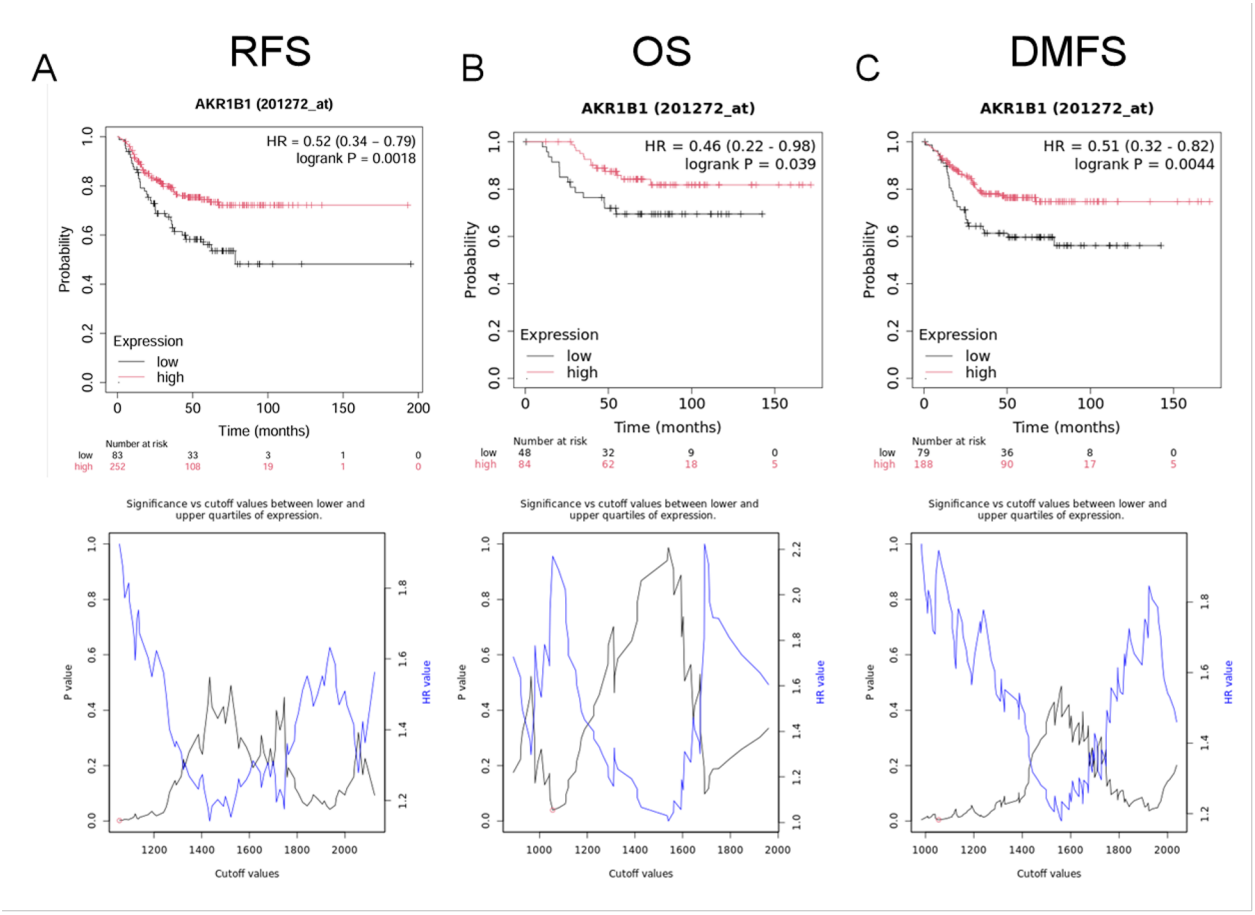

Figure 2. The survival outcomes were found to be associated with AKR1B1. In the TCGA triple - negative breast cancer (TNBC) cohort, the relapse - free survival (RFS) is illustrated in panel (A), the overall survival (OS) is demonstrated in panel (B), and the distant metastasis - free survival (DMFS) is presented in panel (C).

3.3 AKR1B1 Co-expression Network in TNBC

We utilized the LinkedOmics database to conduct a comprehensive assessment and selection of the gene set associated with AKR1B1 in TNBC. Figures 3A - B presented the genes with the top rankings. Next, we refined the gene set we had acquired. We established a significance threshold with $p < 0.01$ and a false discovery rate (FDR) < 0.05, resulting in the generation of a refined gene set. Subsequently, we applied the DAVID database to perform a functional enrichment analysis on this refined gene set. The results indicated that AKR1B1 is primarily associated with the cell proliferation function in TNBC (Figure 3E), which will be the primary focus of our subsequent experimental investigations. Furthermore, we explored the relationship between AKR1B1 and human immune subtypes (illustrated in Figure 3C) and molecular subtypes (depicted in Figure 3D) by making use of the TISDIB database. The findings revealed that AKR1B1 is most significantly correlated with breast invasive carcinoma (BRCA), further corroborating the pivotal role of AKR1B1 in BRCA.

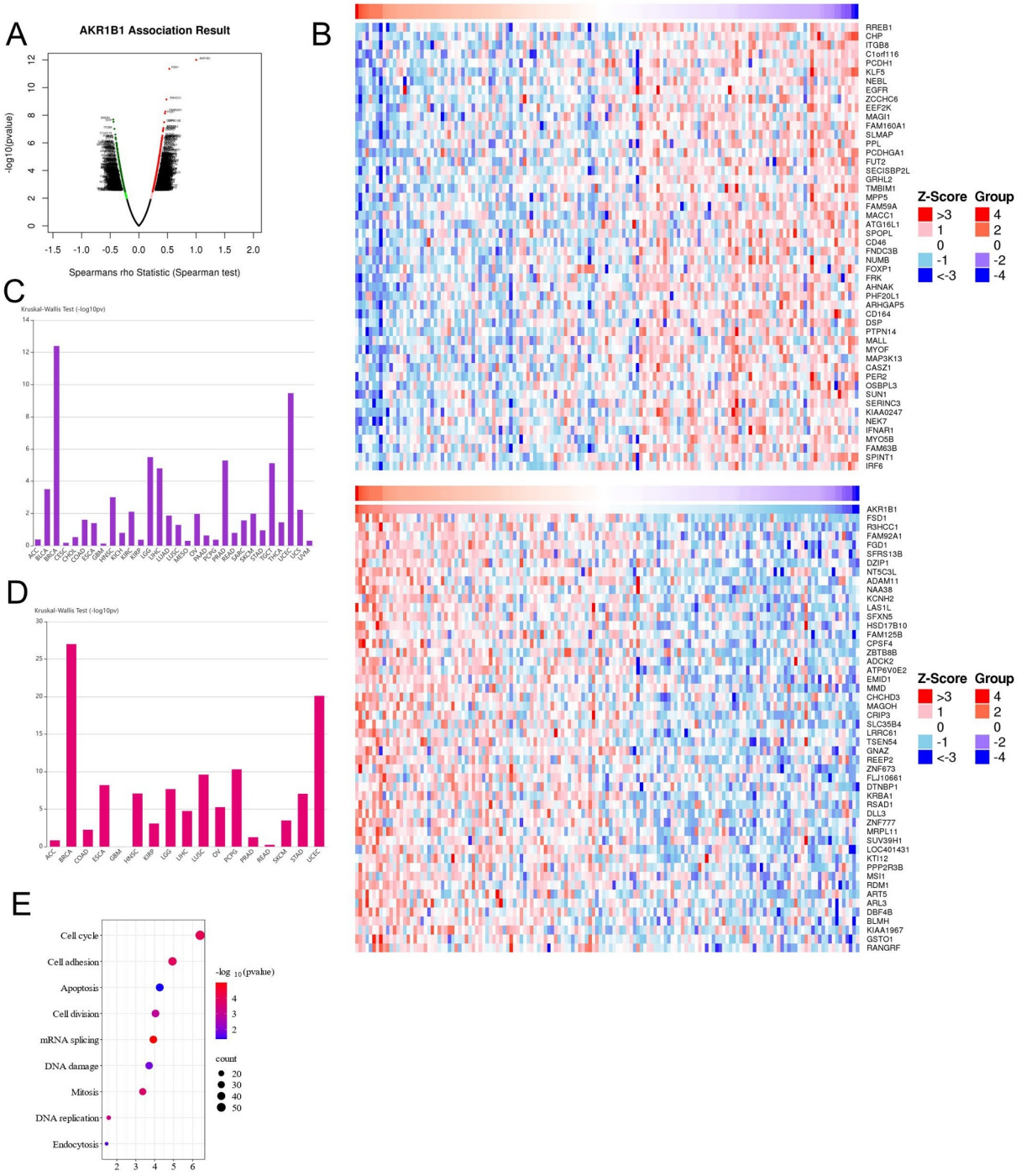

Figure 3. Genes co - expressed with AKR1B1 in triple - negative breast cancer (TNBC). (A) Using the Spearman test, genes that were highly correlated with AKR1B1 were determined within the TNBC cohort. (B) A heatmap was used to display the top 50 genes in TNBC that had positive or negative associations with AKR1B1. Genes with a positive correlation were denoted by red, while those with a negative correlation were shown in blue. (C) The relationship between AKR1B1 and human immune subtypes. (D) The relationship between AKR1B1 and molecular subtypes. (E) A functional enrichment map depicting AKR1B1 and its associated genes within triple - negative breast cancer (TNBC).

3.4 AKR1B1 is Associated with the Level of immunologic Invasion in TNBC

An increasing amount of research has demonstrated that tumors are not isolated entities. On the contrary, they are intricate systems where the tumor microenvironment plays a crucial role in affecting tumor initiation and progression. Consequently, we conducted a study to elucidate the relationship between AKR1B1 and immune cell populations within the tumor microenvironment, utilizing the TIMER database (Figure 4A). Our findings revealed a significant correlation between AKR1B1 and both CD8+ T cells and macrophages (Figure 4B), suggesting a robust interaction between AKR1B1 and the tumor immune microenvironment. This association may imply that AKR1B1 facilitates tumor malignancy by modulating CD8+ T cells and macrophages. To further

enhance our understanding, we employed the TISDIB database to conduct an analysis of the correlation between the expression levels of AKR1B1 and various immune cell populations (Figure 4C), immunosuppressive factors (Figure 4D), immune activators (Figure 4E), and MHC molecules (Figure 4F) among different types of cancer.

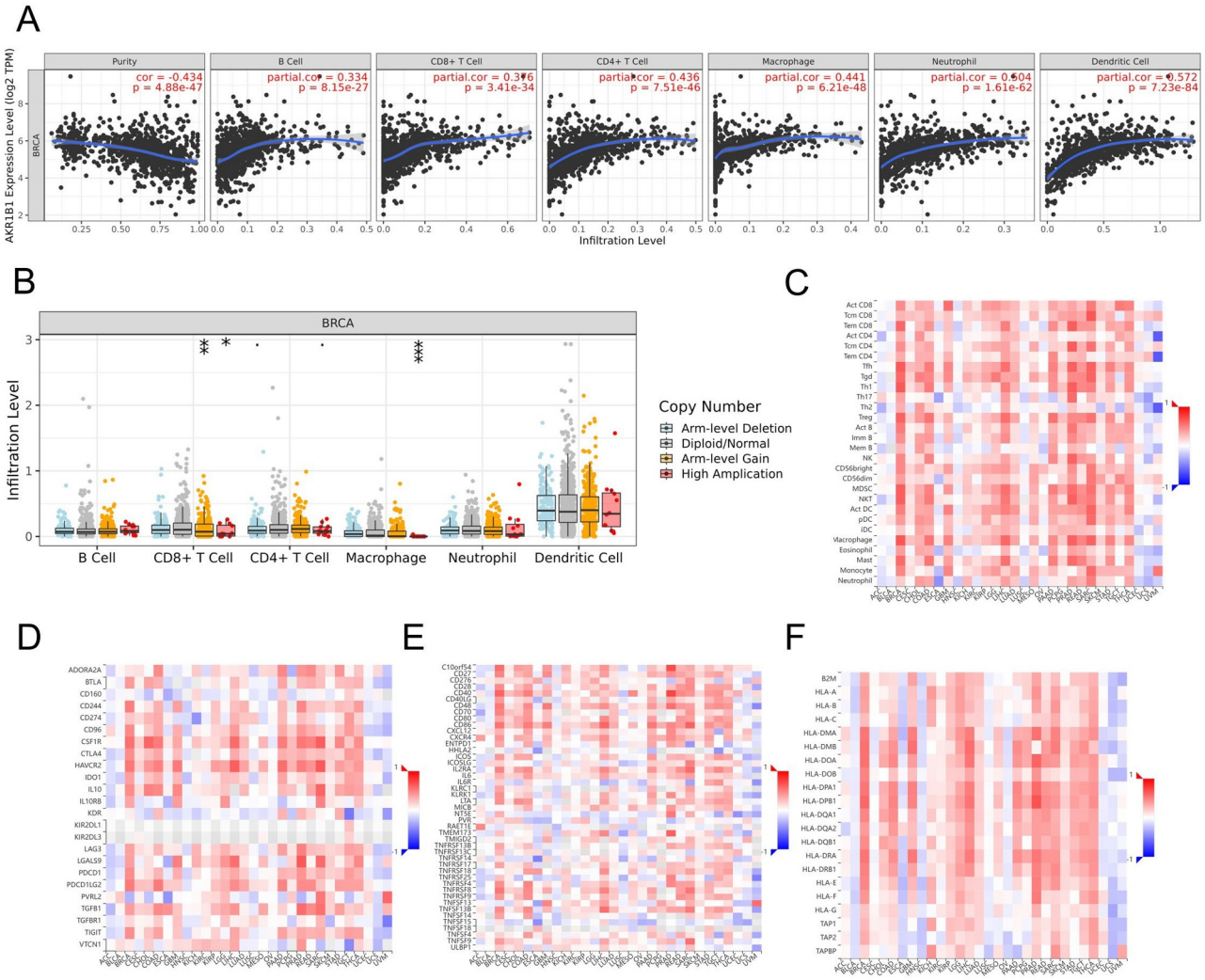

Figure 4. Association between the expression of AKR1B1 and the degree of immunological infiltration in triple - negative breast cancer (TNBC). (A) Examination of the link between AKR1B1 expression and the extent of immunological infiltration in TNBC. (B) Analysis of the correlation between the expression level of AKR1B1 and the quantity of immune cells in TNBC. (C) Investigation of the correlation between AKR1B1 and the abundance of tumor - infiltrating lymphocytes (TILs). (D) Assessment of the correlation between AKR1B1 and immunoinhibitory factors. (E) Evaluation of the correlation between AKR1B1 and immunostimulatory agents. (F) Study of the correlation between AKR1B1 and major histocompatibility complex (MHC) molecules.

3.5 Protein Network Interacting with AKR1B1.

Subsequently, we conducted an analysis of the key proteins that interact with AKR1B1 using the STRING database, with AKR1B1 serving as the central node (Figure 5A). Furthermore, we employed the ENCORI database to examine the association between these ten essential proteins and AKR1B1 (Figure 5B). Additionally, we screened for potential chemical substances that could interact with AKR1B1 in the Comparative Toxicogenomics Database (CTD) (Figure 5C). The research results indicated that curcumin can act as an efficient agent for targeting AKR1B1 when treating TNBC. This has the possibility of making notable contributions to the advancement of clinical cancer therapy.

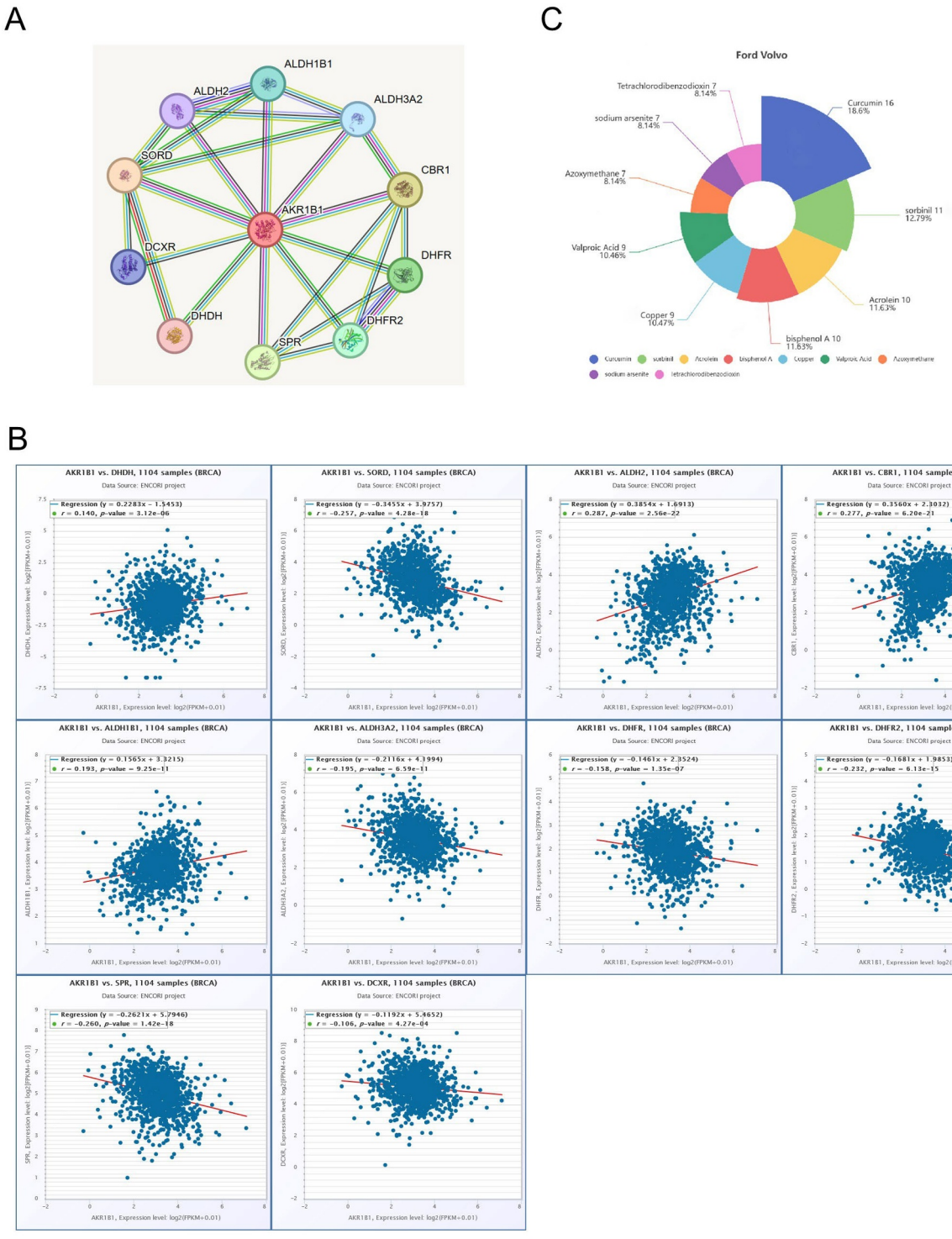

Figure 5. Investigations were carried out on the protein interactions and RNA interactions of AKR1B1. (A) A gene network was constructed, showing AKR1B1 and the proteins that collaborate with it. (B) An analysis was conducted to examine the correlation between the expression of AKR1B1 and the expression of other genes. (C) The CTD database indicates potential drugs that could exert an influence on AKR1B1.

3.6 Curcumin Inhibits Proliferation of MDA-MB-231 Cells

Subsequent to our previous analysis, we subjected the MDA - MB - 231 cell line to 20 μM of curcumin for a duration of two days. A live - dead cell staining test revealed the potential cytotoxic effects of curcumin on MDA - MB - 231 cells (Figure 6C). Subsequently, we employed the CCK-8 assay to quantify changes in cell viability, and the results demonstrated a significant inhibition of cell viability following treatment (Figure 6B). Additionally, we conducted clone formation experiments (Figure 6A), which yielded results that were

in accordance with our previous findings.

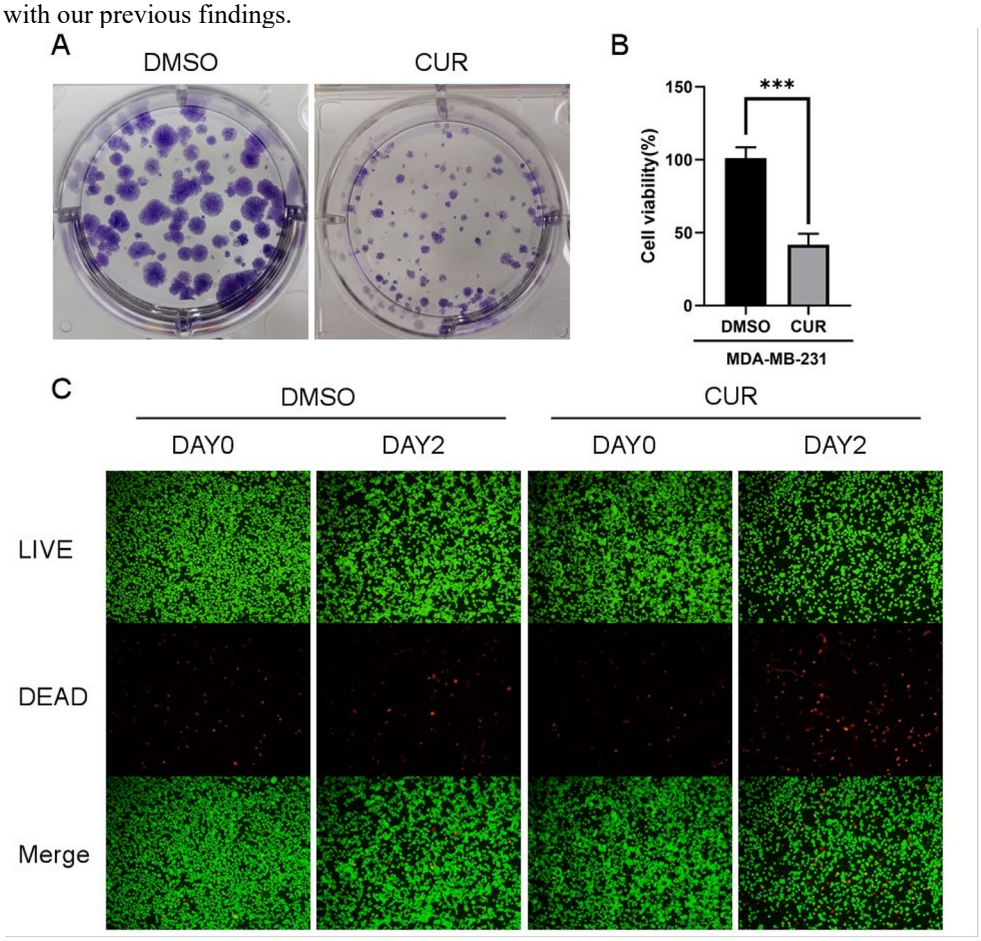

Figure 6. Curcumin significantly inhibited the proliferation of MDA-MB-231 cell line. To assess this inhibition, a variety of assays have been used. (A) Colony formation experiments, to measure the cell proliferation ability of MDA-MB-231 cells treated with curcumin (B) the cell viability of MDA-MB-231 cells treated with curcumin was measured by CCK8 method (C) The effect of curcumin on the cell viability was evaluated by live and dead cell staining methods.

4 Discussion

The present study offers valuable insights into the role of AKR1B1 in TNBC. This is accomplished through a comprehensive bioinformatics analysis that utilizes multiple databases. Our findings underscore AKR1B1 as a potential biomarker in TNBC, exhibiting heterogeneous expression patterns across various cancer types, with notably low levels observed in TNBC. This distinctive expression pattern highlights the existence of complex regulatory mechanisms and underscores its potential therapeutic relevance.

The results of our research demonstrate a positive correlation between the expression of AKR1B1 and recurrence - free survival (RFS), overall survival (OS), and distant metastasis - free survival (DMFS) in patients diagnosed with TNBC. This suggests that strategies aimed at AKR1B1 activation may be beneficial in the management of TNBC. The AKR1B1 co-expression network revealed key proliferative genes, reinforcing its functional role. Furthermore, significant associations between AKR1B1 and immune cell populations, particularly CD8+ T cells and macrophages, indicate its intricate interplay with the tumor immune microenvironment.

In comparison to previous research, our study provides a nuanced understanding of AKR1B1's function and therapeutic implications in TNBC by integrating multiple dimensions. We identify AKR1B1 as a potential therapeutic target and propose curcumin as a promising agent. However, it is essential to stress that our findings are based solely on bioinformatics assessments and require experimental validation. Additional research is necessary to elucidate the mechanisms underlying the associations between AKR1B1 and immune cells.

To surmount these limitations, we propose conducting in vitro and in vivo experiments to validate the functional roles of AKR1B1 and the efficacy of curcumin in TNBC. Furthermore, exploring the molecular mechanisms of AKR1B1's immune interactions may yield additional insights. Based on our findings, we hypothesize that AKR1B1 serves as a critical regulatory node in TNBC progression and immune response. This suggests the need for further investigation into AKR1B1-targeted therapies, including curcumin, as a novel treatment approach for TNBC. Our study establishes a foundation for future experimental validation and enhances the understanding of AKR1B1's role in TNBC.

5 Conclusions

This investigation presents valuable insights into the role of AKR1B1 in TNBC. It achieves this by performing a comprehensive bioinformatics analysis that utilizes multiple databases. Our findings highlight AKR1B1 as a potential biomarker in TNBC, exhibiting heterogeneous expression across various cancer types. Notably, the expression of AKR1B1 is reduced in TNBC. However, elevated levels of AKR1B1 are associated with improved survival outcomes for patients. These favorable results include an extended period of recurrence - free survival, a longer overall survival time, and a greater duration of distant metastasis - free survival. Furthermore, AKR1B1 is primarily associated with cell proliferation in TNBC and significantly correlated with immune cell populations within the TME, particularly $CD8^+$ T cells and macrophages. An examination of protein networks reveals significant proteins that engage with AKR1B1. Additionally, curcumin has been identified as a potential candidate for targeting AKR1B1 in the management of TNBC. Experimental validation has demonstrated that curcumin can efficiently inhibit the growth of TNBC cells. This study lays the foundation for future experimental investigations and enhances our understanding of the pivotal role that AKR1B1 plays in TNBC progression and the immune reaction. As a result, it suggests that further investigation into therapies aimed at AKR1B1 could offer a new strategy for treating TNBC.

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