Exploring the mechanism of resistance to vincristine in breast cancer cells using transcriptome sequencing technology

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Abstract. Breast cancer has replaced lung cancer as the leading cancer globally, but various chemotherapy drugs for breast cancer are prone to resistance, especially in patients with distant metastases who are susceptible to multiple chemotherapy drug resistance often leading to treatment failure. Vincristine (VCR) is an alkaloid extracted from *Catharanthus roseus*, and is often used in combination with other chemotherapy drugs to treat various types of cancer, including breast cancer. Research on the development of resistance to VCR has been carried out using transcriptome sequencing technology. Firstly, gradient increase of VCR concentration was used to produce a VCR-resistant breast cancer cell line. Mechanistically, RNA was extracted from the VCR-resistant breast cancer cell line, and the transcriptome was sequenced. Further analysis showed changes in the expression levels of various genes in the aforementioned VCR-resistant breast cancer cell line. Meanwhile, the analysis of splicing events also indicated a change in variable splicing events. Further validation showed that the expression levels of multiple genes, including interleukin-1β, were altered in the VCR-resistant breast cancer cell line, and these gene expression changes were related to VCR resistance. The results of the present study provide a theoretical basis for exploring the mechanism of VCR resistance clinically.

Introduction

Breast cancer is one of the three most common cancers in the world. The World Health Organization's International Agency for Research on Cancer has released the latest global cancer statistics for 2020, revealing that breast cancer has surpassed lung cancer as the world's most prevalent cancer, with 2.26 million new cases reported globally (1). Based on the type of hormone receptor and the tumor proliferation status, breast cancer can be divided into either luminal A or B, HER2-positive or triple-negative breast cancer (2,3). Current studies show that early screening is still key to preventing breast cancer. Ultrasonography (USG) is a commonly used and convenient method for early screening of breast cancer, but it has disadvantages of low specificity (4). Magnetic Resonance Imaging (MRI) is the precise imaging of soft tissues (5). Treating with antiestrogen drugs, such as tamoxifen or raloxifene, may reduce the risk of an individual developing breast cancer (6). For patients with diagnosed breast cancer, different treatment strategies can be adopted such as targeted therapy, hormone therapy, radiation therapy, surgery and chemotherapy. For patients with distant metastasis, the goal of treatment is usually to improve their quality of life and survival rate (7). However, although an increasing number of treatment methods has been discovered for the treatment of breast cancer, and the 5-year survival rate of breast cancer increasing year by year, there are still various obstacles in treating breast cancer. For example, numerous chemotherapy drugs have serious side effects, and the development of resistance has always been an issue in the treatment of breast cancer. The treatment of most patients with distant metastases fails due to intolerance to multiple chemotherapy drugs (8,9). Therefore, identifying additional effective treatment methods with less side effects remains urgent.

Vincristine (VCR) is a biologically-derived alkaloid extracted from the periwinkle plant, often used in combination with other chemotherapy drugs to treat various types of cancer, including breast cancer (10). VCR disrupts the formation of microtubules in the mitotic spindle, leading to the arrest of cells undergoing mitosis. Resistance to VCR often occurs during the treatment of breast cancer (11). There are several ways that resistance develops during treatment with chemotherapy, including enhanced drug metabolism, reduced cellular drug uptake, altered expression of the drug target, intracellular drug sequestration and altered expression of genes involved in either cell death, cell cycle or DNA repair (12). Additionally, changes in autophagy and inflammatory pathways are often associated with resistance (13,14). However, current research has not yet provided a reasonable explanation for the development of resistance to VCR during treatment of breast cancer.

Sequencing technology has propelled the development of scientific research. With the rapid development of

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second-generation sequencing technologies, an increasing number of scientists are relying on it to solve biological issues. Whole transcriptome sequencing has become increasingly advanced, allowing for the analysis of alternative splicing, gene expression and other research (15).

RNA alternative splicing refers to the process by which different mature mRNAs can be produced from the same pre-mRNA through different splicing patterns, which can then be translated into proteins with multiple functions (16). Alternative splicing plays an important role in regulating mRNA and protein diversity (17). It is noteworthy that RNA splicing processes are strictly regulated in different tissues and developmental stages, and abnormal RNA splicing regulation is closely related to various human diseases, including breast cancer (18,19). High-throughput sequencing and functional enrichment analysis of genes with altered expression are routinely used in biological research.

Based on the background of the development of transcriptome sequencing technology, the present study investigated the development of resistance to VCR in breast cancer cells using transcriptome sequencing technology. A breast cancer cell line resistant to VCR was established by gradually increasing the concentration of VCR. We established this cell line to assist in addressing the issue of VCR resistance that arises during the clinical treatment of breast cancer. In order to explore the mechanism behind this resistance, transcriptome sequencing was performed. Gene expression analysis showed that multiple genes were deregulated in the resistant cell line. Additionally, alternative splicing events were also found to be altered in the resistant cell line. The present study focused on two genes, IL1B and VEGFA, with the aim of finding the key to drug resistance in patients with breast. The present results provide mechanistic insight into VCR resistance and a foundation for developing clinical interventions to overcome this resistance.

Materials and methods

Cell culture. The cell lines used in the present study were obtained from the American Type Culture Collection (ATCC) and were maintained in the culture medium recommended by ATCC under standard culture conditions. MCF7 cells were cultured in MEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (cat. no. FBS-Superior-L; Sinsagetech Co. Ltd.), at 37˚C in a humidified incubator with 5% CO_2.

Inhibitory concentration 50 (IC50) measurement. The IC50 value was assessed using Cell Counting Kit-8 (CCK-8; APExBIO Technology LLC). MCF7 cells were seeded into 96-well plates and treated with 1 nM VCR in 100 μl medium for 5 days, followed by 5, 10, 50, 100, 500, 1,000, 5,000 and 10,000 nM VCR for 5 days each. Eventually, the surviving MCF7 cells were expanded and screened to obtain the VCR-resistant MCF7 cell line. After 48 h of incubation, the media were removed and replaced with 100 μl of culture media containing 10 μl CCK-8 reagent, and incubated at 37˚C for 2 h. Spectrophotometric absorbance was measured at 450 nm to determine the dose-response curves, and the IC50 value of VCR was calculated using GraphPad Prism (version 8; Dotmatics).

Cell proliferation assay. Cell proliferation was assessed using a CCK-8 assay. Cells were initially counted and 2,000 cells were seeded onto 96-well plates, followed by incubation in a CO_2 incubator at 37˚C for 24 h. Cells were then treated with VCR at various concentrations in 100 μl of medium. After a 48-h incubation period, the media were replaced with 100 μl of culture media containing 10 μl of CCK-8 reagent, and incubated at 37˚C for 2 h. Absorbance was measured at 450 nm using a microplate reader (DNM-9602; Perlong Medical Equipment Co., Ltd.), and the dose-response curve was plotted using GraphPad Prism (version 8; Dotmatics).

Colony formation assay. The relevant cells (MCF7 or VCR-resistant MCF7 cells) were initially counted and 1,000 cells were seeded into a 6-cm dish and cultured in an incubator at 37˚C. Over a 2-week period, the growth media were refreshed every 4 days. The cell colonies were fixed with paraformaldehyde in room temperature for 15 min, followed by washing with PBS 3 times and staining with 0.1% crystal violet for 20 min. A gel imaging system (Tanon-2500R; Tanon Science and Technology Co., Ltd.) was used to capture images of the cell colonies, which were subsequently quantified using ImageJ v.1.50i (National Institutes of Health). Deep color visible to the naked eye was counted as clones.

RNA-sequencing (RNA-seq) analysis. The RNA of both MCF7-wild type (WT) and VCR-resistant VCR/MCF7 cells was extracted using the TRIzol™ Reagent (Ambion; Thermo Fisher Scientific, Inc.), followed by RNA-seq analysis performed by Haplox Co. Ltd. [Platform: GPL20795 HiSeq X Ten (*Homo sapiens*)]. Each cell line was analyzed once. To identify enriched pathways, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted using Metascape (20) and KOBAS-3.0 (http://kobas.cbi.pku.edu.cn/). Additionally, functional interaction networks were generated by analyzing the functional association of VCR-induced targets using protein interaction data from the STRING database (https://cn.string-db.org/).

Reverse transcription-quantitative (RT-q)PCR. RT was carried out using the Evo M-MLV Plus cDNA Synthesis Kit (cat. no. AG11705; Accurate Biology) according to the manufacturer's instructions. Mixed genomic DNA (gDNA) was removed from the RNA template using gDNA Clean Reagent (included in the above-mentioned kit) at 42˚C for 2 min. The RT solution was added and incubated at 37˚C for 15 min, followed by 85˚C for 5 sec. qPCR was conducted using High-specificity Chemically-colored Quantitative PCR Premix (Low ROX) (cat. no. MQ00601S; Monad Biotech Co., Ltd.) according to the manufacturer's instructions. The thermocycling conditions were as follows: 95˚C/10 min for initial denaturation, followed by 40 cycles of 95˚C/10 sec for denaturation, 60˚C/10 sec for primer annealing and 72˚C/30 sec for extension. Dissociation curve was used as the default option of QuantStudio3 (Thermo Fisher Scientific, Inc.). Expression was normalized to GAPDH and quantified using the $2^{-\Delta\Delta Cq}$ method (21). Primer sequences can be found in Table SI.

Public datasets. The functional association networks of genes related to VCR resistance were analyzed using the

STRING database. TCGA data analysis of IL1B/VEGFA expression in BRCA and normal tissues was based on sample types, individual cancer stages and nodal metastasis status. Kaplan-Meier survival curves showing overall survival of patients with BRCA bearing either high or low IL1B/VEGFA were generated.

ELISA. Human interleukin-1β (IL-1β) ELISA Kit (cat. no. E-EL-H0149c; Elabscience Biotechnology Inc.) was used to study the protein expression of IL-1β in the VCR-resistant breast cancer cell line. The experimental procedures were performed according to manufacturer's instructions.

Western blot analysis. Total protein was extracted from cells using RIPA lysis buffer (cat. no. R0020; Solarbio) containing 1 mM Cocktail. The protein concentration was determined using the BCA assay. A total of 30 μg protein sample was denatured by heating at 95˚C for 5 min in 1X SDS sample buffer, and then separated by 12% SDS-PAGE. The separated proteins were subsequently transferred onto a PVDF membrane (cat. no. 10600023; GE Healthcare Life Science Co. Ltd.). 10% milk was used for blocking for 1 h in room temperature. The membrane was then incubated with primary antibody at 4˚C overnight. PBS with 0.05% Tween-20 was used to wash the PVDF membrane 3 times. Subsequently, membranes were incubated with secondary antibody with HRP at room temperature for 1 h. Finally, MINICHEMI (MiniChemi® 580; Sinsagetech Co. Ltd.) was used to visualization, and NcmECL (NCM Biotech cat. no. P10300B) was used as visualisation reagent. The following antibodies were used: vascular endothelial growth factor A (VEGFA; 1:1,000 dilution; cat. no. 19003-1-AP; Proteintech Group, Inc.) and GAPDH (1:5,000 dilution; cat. no. 10494-1-AP; Proteintech Group, Inc.).

Statistical analysis. The experiments were conducted using three biologically independent repeats, and the data are presented as mean ± SD. Statistical significance was determined using a 2-tailed, unpaired Student's t-test, as well as one-way or two-way ANOVA followed by Tukey's post-hoc test in GraphPad Prism (version 8; Dotmatics). Kaplan-Meier plots were used to investigate patient survival (http://kmplot.com/analysis/index.php?p=background). Fisher's exact test was used in KEGG.

Results

VCR-resistant cells have stronger proliferation and cloning formation abilities. In order to investigate the specific mechanism underlying the development of VCR resistance in breast cancer treatment, the VCR-resistant breast cancer cell line VCR/MCF7 was generated. First, the IC50 of MCF7-WT and VCR/MCF7 cells to VCR was measured using a CCK-8 assay, and it was found to be 7.371 nM (Fig. 1A) and 10,574 nM (Fig. 1B), respectively. The tolerance of the two cell lines to VCR treatment was then investigated and it was shown that the VCR/MCF7 cells were more resistant to VCR compared with the MCF7-WT cells (Fig. 1C). MCF7-WT and VCR/MCF7 cells were treated with 0.5 and 50 nM VCR, respectively, while DMSO was used as a control. It was shown that the number of colonies of VCR/MCF7 cells after VCR treatment was significantly higher than that of MCF7-WT cells ($P<0.0001$; Fig. 1F). These results indicated that a VCR-resistant cell line was successfully constructed.

The tumor phenotype of the VCR-resistant cells was further investigated. It was shown that the VCR/MCF7 cells had stronger proliferation ability than the MCF7-WT cells through a growth curve experiment (Fig. 1D). Moreover, by performing a colony-formation assay, it was revealed that VCR/MCF7 cells had stronger colony formation ability compared with that of MCF7-WT cells (Fig. 1E). This suggested that VCR-resistant breast cancer cells have a more aggressive tumor phenotype than MCF7-WT cells.

VCR-resistant breast cancer cells have a broad change in gene expression levels. To investigate the mechanism of VCR resistance in breast cancer cells, RNA was extracted from both the VCR/MCF7 and MCF7-WT cells, and RNA-seq was performed after purification. According to the analysis of RNA-seq data, it was revealed that the expression levels of 263 genes were altered (log2fold change >1; $P<0.05$) in the VCR-resistant breast cancer cells compared with those in the MCF7-WT cells; more specifically, the expression levels of 94 and 169 genes increased and decreased, respectively (Fig. 2A). A GO analysis was then performed on the genes of the sequencing data and it was found that in terms of biological processes, these genes were mainly concentrated in 'angiogenesis' and 'positive regulation of cell motility', while in terms of cellular component, these genes were mainly concentrated in 'actin cytoskeleton'. In terms of molecular function, these genes mainly concentrated in 'actin binding' (Fig. 2B). These results indicated that the genes the expression of which was altered in the VCR-resistant breast cancer cell line mainly concentrate on genes related to microtubules. This suggested that there might be changes in microtubule-related functions in the VCR-resistant cells. Furthermore, through KEGG analysis, it was shown that VCR-resistant is related to the pathways including 'MAPK signaling pathway', 'human papillomavirus infection' and others (Fig. 2C). Through STRING analysis, it was shown that the gene expression, which changed in VCR-resistant breast cancer cells, was functionally related (Fig. 2D). These results revealed that there were extensive changes in the expression levels of certain genes in the VCR-resistant breast cancer cells, which may associated with VCR resistance.

Drug-resistant VCR/MCF7 cells undergo extensive changes at the level of gene splicing. In order to further explore the possible mechanism of drug resistance in VCR-resistant breast cancer cells, the splicing changes in the sequencing data were further analyzed. The results showed that compared with the MCF7-WT breast cancer cells, VCR-resistant breast cancer cells undergo various alternative splicing events. By analyzing the splicing types of these events, it was found that the cassette type was the main splicing event type that changed in the VCR-resistant breast cancer cells (Fig. 3A). KEGG analysis of these splicing events showed that the genes undergoing variable splicing changes in the VCR-resistant breast cancer cells were mainly concentrated on the autophagy signaling pathway

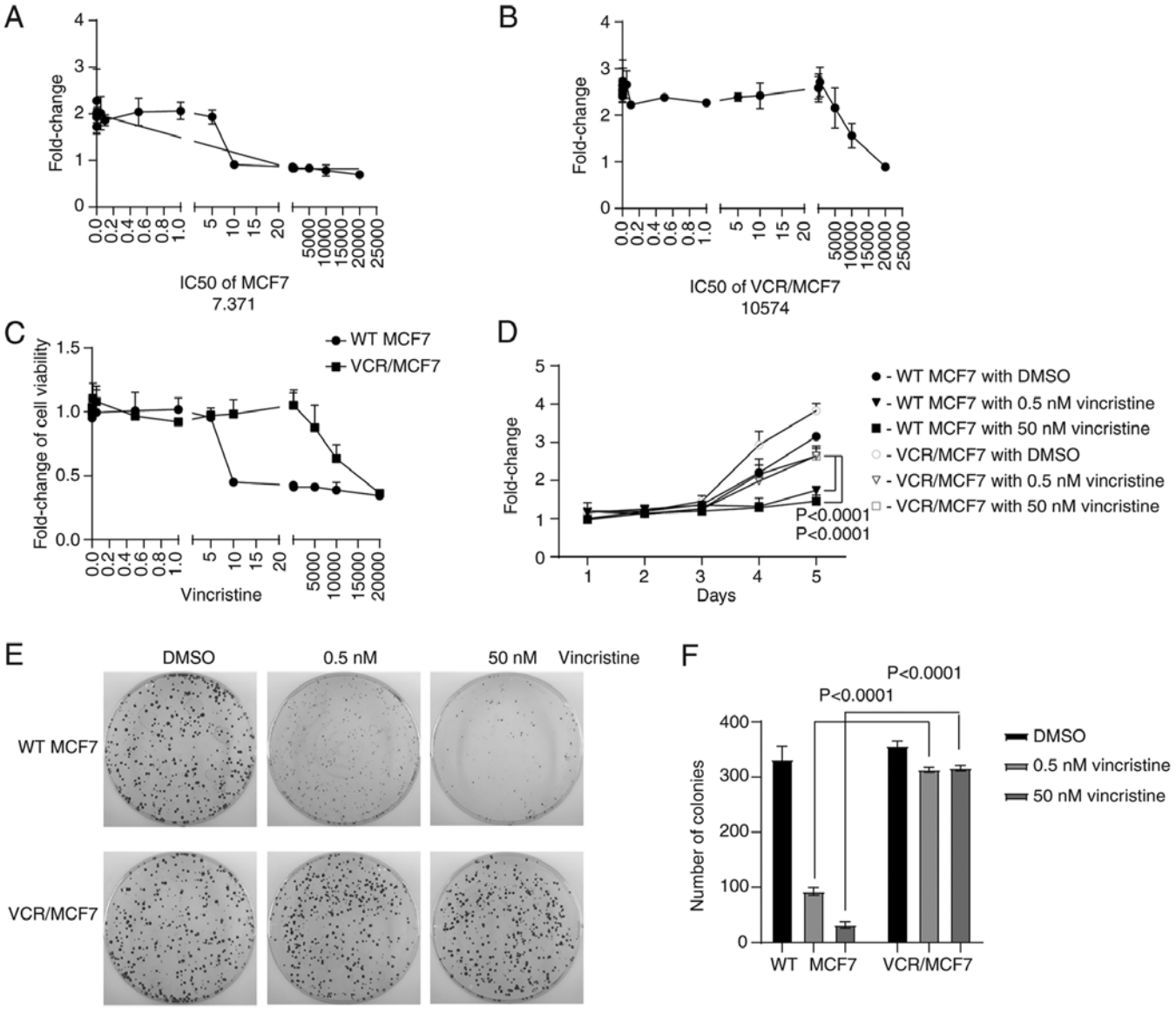

Figure 1. VCR-resistant cells have stronger proliferation and cloning formation abilities. (A and B) Cell viability was assessed using a CCK-8 assay, and the relative cell viability was determined as the ratio of VCR treatment to blank control. the IC50 of MCF7-WT and VCR/MCF7 cells were found to be (A) 7.371 nM and (B) 10,574 nM. (C) The cell viabilities of the treated cells were measured distinctly with a CCK-8 assay. The percentages of cell viability were compared to concentration 0. It was shown that the OD of VCR/MCF7 cells were more than the MCF7-WT cells. Data shown as mean ± SD from three experiments. (D) Growth curve assays were carried out to evaluate the effects of VCR on the proliferation of MCF7 cells. P-values were calculated using two-way ANOVA followed by Tukey's post-hoc test. (E) Colony-formation assays were conducted in MCF7-WT and VCR/MCF7 cells, which were treated with different concentrations of VCR for 24 h. It was shown that the VCR/MCF7 cells had more clones than the MCF7-WT cells. (F) Quantitative results of three experiments; the obtained data were normalized to the control and presented as the mean ± SD. P-values were determined using one-way ANOVA with Tukey's post-hoc test. WT, wild type; VCR, vincristine; CCK-8, Cell Counting Kit-8; IC50, 50% inhibitory concentration.

(Fig. 3B). This prompted the hypothesis that the autophagy pathway could act as a possible compensatory pathway for VCR resistance. Further GO analysis showed that the genes the splicing of which changed in drug-resistant VCR/MCF7 breast cancer cells, were mainly focused on 'organelle localization' and other functions (Fig. 3C). Analysis using the STRING website revealed that the genes the splicing of which changed in the VCR-resistant breast cancer cells, were functionally related (Fig. 3D). The aforementioned results indicated that the VCR-resistant breast cancer cells had extensive changes at the gene splicing level, which suggested that the variable splicing process may be involved in the development of VCR resistance.

The expression levels of genes such as VEGFA and IL-1β are altered in VCR-resistant breast cancer cells. In order to validate the genes, the expression of which changed in the sequencing data, some genes were selected for validation. qPCR primers were designed for these genes and details of the qPCR primer sequences are shown in Table SI. The results showed that the expression levels of several genes in the VCR-resistant breast cancer cells were consistent with the aforementioned sequencing results ($P<0.01$; Fig. 4A-J). The genes with increased expression levels included VEGFA, the functions of which include inducing endothelial cell proliferation, promoting cell migration, inhibiting apoptosis and inducing permeabilization of blood vessels. The genes with

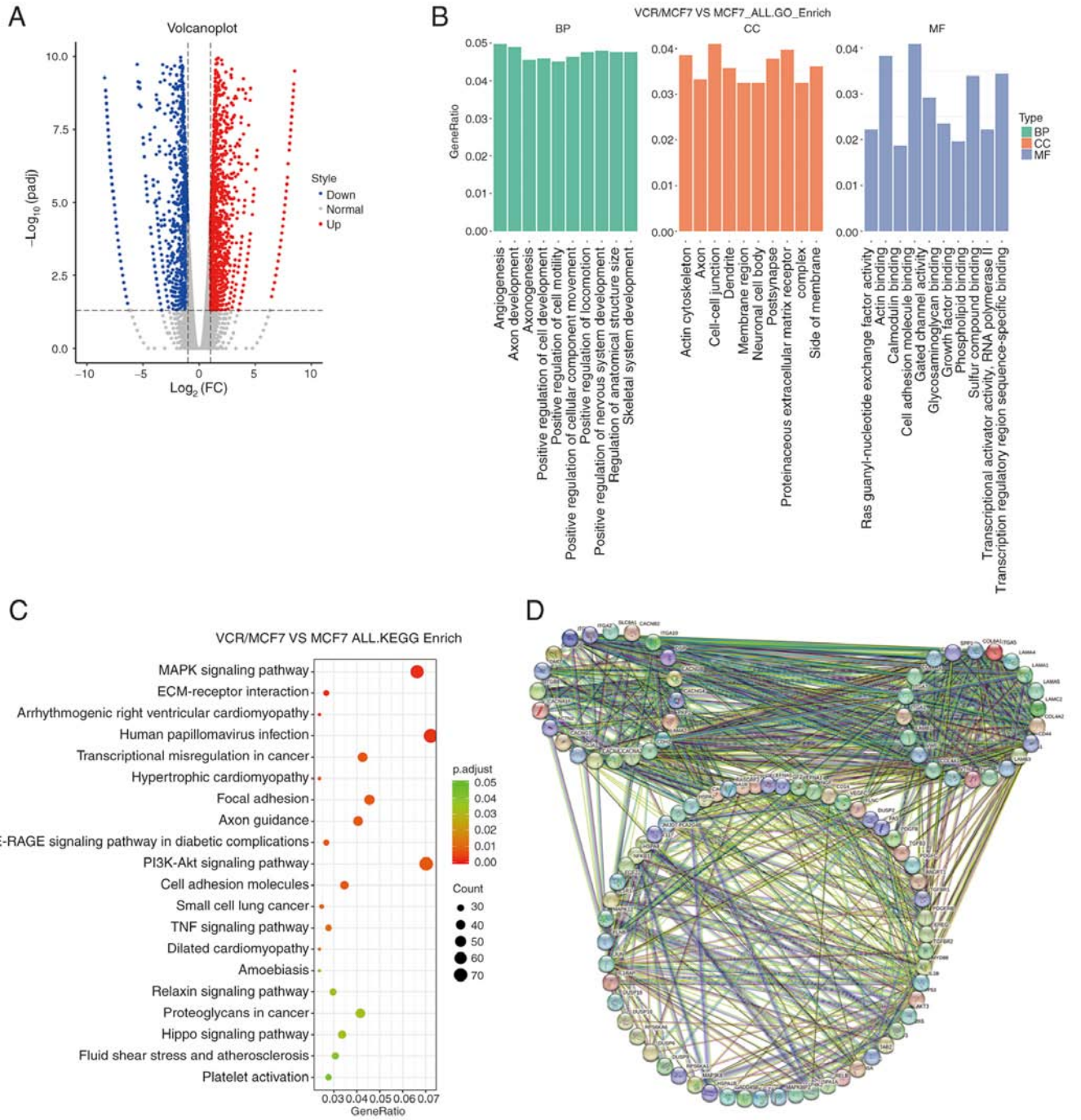

Figure 2. VCR-resistant breast cancer cells have a broad change in gene expression levels. (A) Volcano plot of differentially expressed genes induced by VCR resistance. (B) GO analysis of gene expression events. (C) KEGG analysis of gene expression events. Fisher's exact P-values were plotted for each category. (D) The functional association networks of genes related to VCR resistance were analyzed using the STRING database, with subgroups distinguished based on their respective functions. VCR, vincristine; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cellular component; MF, molecular function.

decreased expression levels included IL1B, which encodes the IL-1β protein and its main functions include prostaglandin synthesis, neutrophil influx and activation, T-cell activation and cytokine production, B-cell activation and antibody production, fibroblast proliferation and collagen production. Changes in autophagy and inflammatory pathways are often associated with drug resistance (13,14). VEGFA can promote the autophagy process (22), while IL1B is related to inflammatory pathways (23), and thus, the study focused on these two genes.

VCR resistance may be due to changes in the expression of either VEGFA or IL-1β. In order to validate the impact of VEGFA and IL1B on tumors, TCGA data were analyzed, and all the selected patients were VCR-resistant. It was shown that the expression of IL1B was significantly lower in breast cancer tissues than in normal tissues (Fig. 5A), while the expression of VEGFA was higher in breast cancer tissues (Fig. 5F). Further analysis showed that the expression levels of IL1B and VEGFA were abnormally expressed at various clinical stages and nodal metastasis states, and the expression

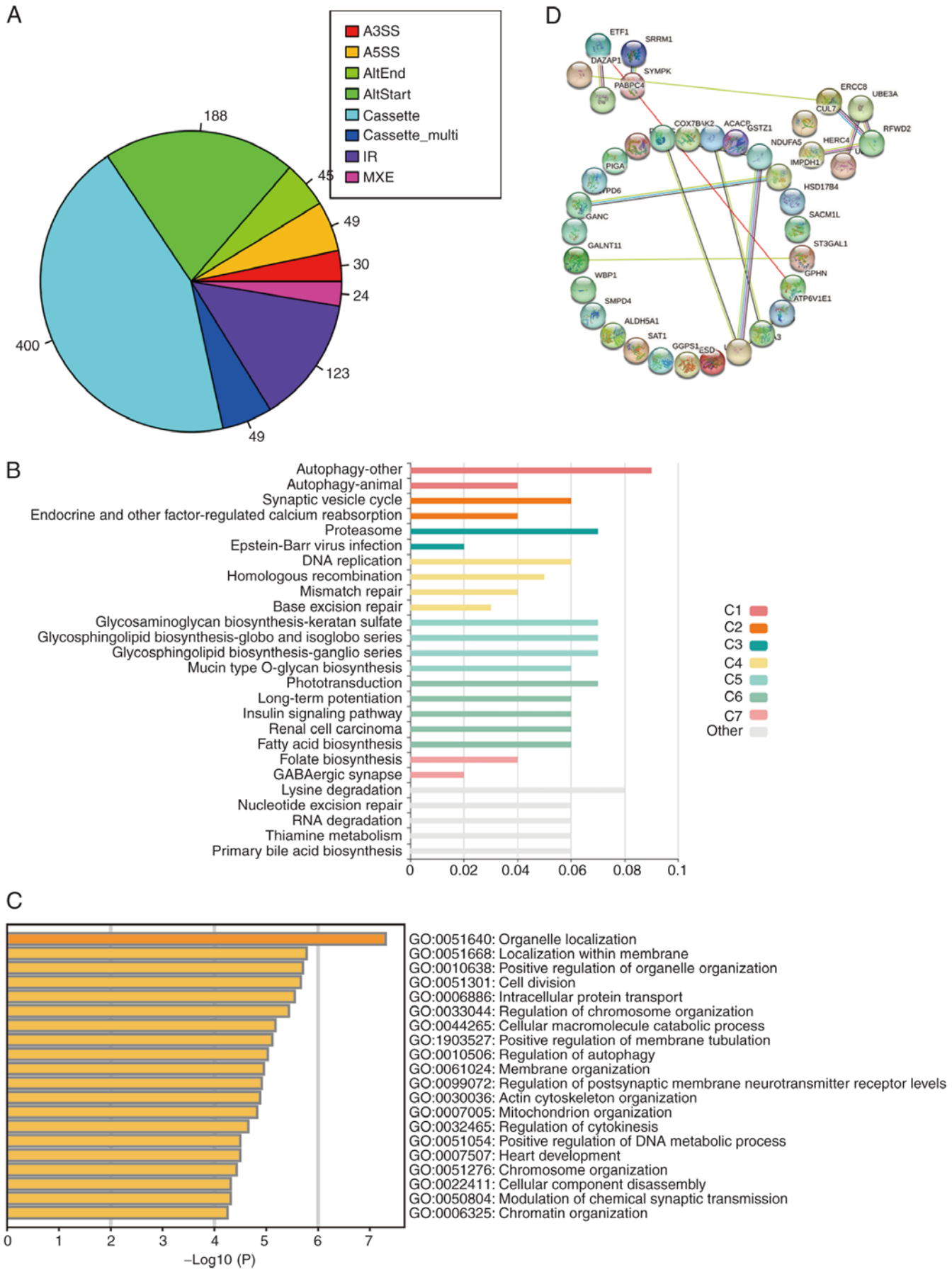

Figure 3. Drug-resistant VCR/MCF7 breast cancer cells undergo extensive changes at the level of gene splicing. (A) Quantitative statistics of alternative splicing events. (B) KEGG analyses of gene alternative splicing events. (C) GO analyses of gene alternative splicing events. (D) The functional association networks of VCR resistance alternative splicing events were analyzed through STRING database, with subgroups marked by their function. VCR, vincristine; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

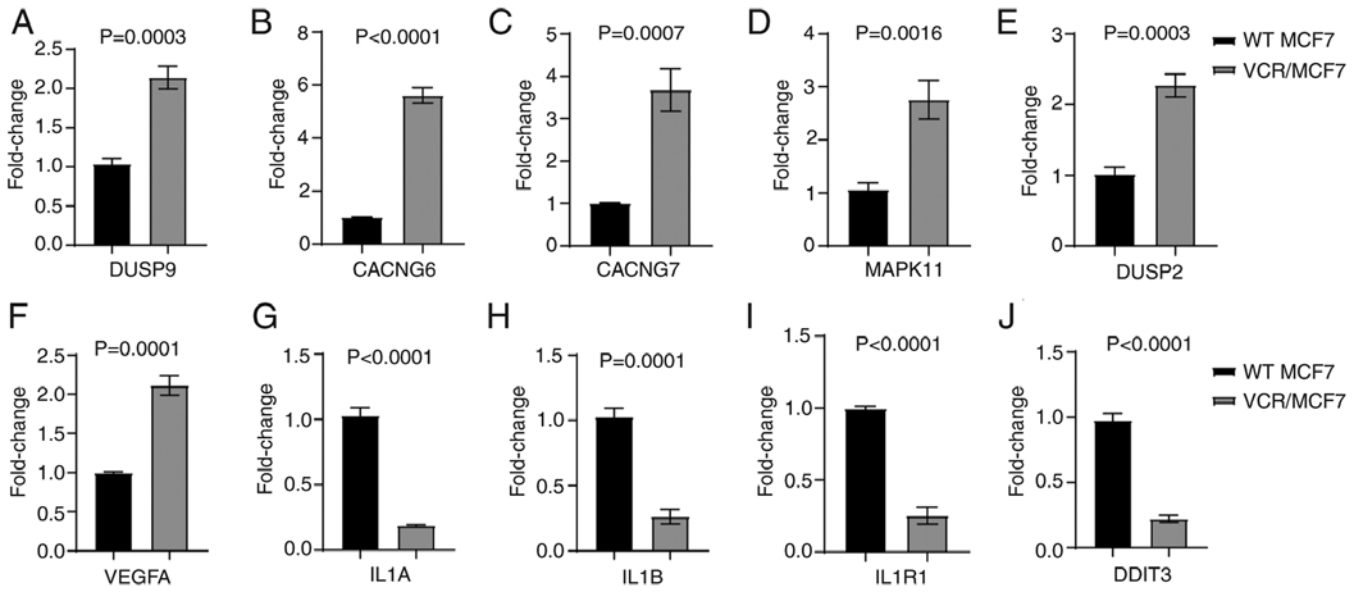

Figure 4. Expression levels of genes such as VEGFA and IL-1β are altered in drug-resistant VCR/MCF7 cells. (A-J) The changes in expression levels of (A) DUSP9, (B) CACNG6, (C) CACNG7, (D) MAPK11, (E) DUSP2, (F) VEGFA, (G) IL-1A, (H) IL1B, (I) IL1R1 and (J) DDIT3 confirmed via reverse transcription-quantitative PCR. The mean ± SD of the relative fold changes obtained from triplicate experiments were plotted, and P-values were calculated using an unpaired Student's t-test. VEGFA, vascular endothelial growth factor A; IL, interleukin; VCR, vincristine; WT, wild-type; DUSP9, dual specificity protein phosphatase 9; CACNG6, voltage-dependent calcium channel gamma-6 subunit; MAPK11, mitogen-activated protein kinase 11; VEGFA, vascular endothelial growth factor A, long form; IL1B: Interleukin-1β; IL1R1, interleukin-1 receptor type 1; DDIT3, DNA damage-inducible transcript 3 protein.

of VEGFA was positively correlated with the clinical stage, and IL1B was negatively correlated with the clinical stage (Fig. 5B-C and G-H). Next, Kaplan-Meier analysis was used to analyze the relationship between VEGFA and IL1B, and breast cancer survival, and Kaplan-Meier survival curves were generated. The analysis revealed that high expression of VEGFA was associated with low breast cancer survival rates (Fig. 5D), while low expression of IL1B was associated with high breast cancer survival rates (Fig. 5I). The protein expression of VEGFA and IL-1β in the VCR-resistant breast cancer cell line was then verified, and it was found that IL-1β expression was significantly lower in the VCR-resistant breast cancer cells as shown by ELISA (P<0.0001; Fig. 5E), while VEGFA protein expression was higher in VCR-resistant breast cancer cells than MCF7-WT breast cancer cells as shown by western blotting (Fig. 5J). This suggested that VCR resistance may be due to changes in the expression of either VEGFA or IL-1β.

Discussion

Breast cancer is the most common cancer in women, second only to lung cancer in the number of annual deaths (24,25). Epidemiological studies show that the incidence range of breast cancer worldwide is 22-26% and the mortality rate is ~18% (26,27). During the treatment of breast cancer, metastasis and chemotherapy resistance are often the main reasons for treatment failure. Chemotherapy resistance is often the result of multiple factors, including mutations in the tubulin protein (28), alternative tubulin expression (29,30), changes in other cytoskeletal proteins, activation of autophagy or intracellular detoxification systems, and changes in drug transport protein expression, which lead to a decrease in intracellular drug concentration (31-33). Changes in apoptosis-related proteins are also often associated with cell resistance (34,35).

Through growth curve and clone formation experiments, it was shown that the VCR-resistant breast cancer cell line became less sensitive to VCR treatment. RNA-seq showed that multiple gene expression levels, including microtubule assembly, were altered in the VCR-resistant breast cancer cell line. Analysis of splicing events also revealed that the variable splicing of numerous genes, including autophagy-related genes, was altered in the VCR-resistant breast cancer cell line. Further verification showed that the expression levels of certain genes, including VEGFA and IL1B, were altered in the VCR-resistant breast cancer cell line, and these gene expression changes were related to VCR resistance. The results of the present study indicated that the production of the VCR-resistant cell line VCR/MCF7 may be caused by changes in microtubule proteins or drug metabolism pathways such as autophagy, providing new avenues for the development of VCR resistance.

The sequencing data were then validated and it was found that the expression levels of several genes in the VCR-resistant breast cancer cells were consistent with the aforementioned sequencing results. The further analysis focused on VEGFA and IL1B1.

Most cells in the human body can produce VEGFA and express it at a higher level under hypoxia (36). In the development of tumors, VEGFA is mainly produced by tumor cells in low oxygen environments, as well as by endothelial cells and tumor-associated macrophages (37). Its function is mainly related to angiogenesis, and the regulation of VEGFA on endothelial cell proliferation and invasive properties is strictly controlled. The ERK and PI3K/Akt pathways are the primary regulators of endothelial cell proliferation (38,39), whereas endothelial cell invasion is facilitated by the release

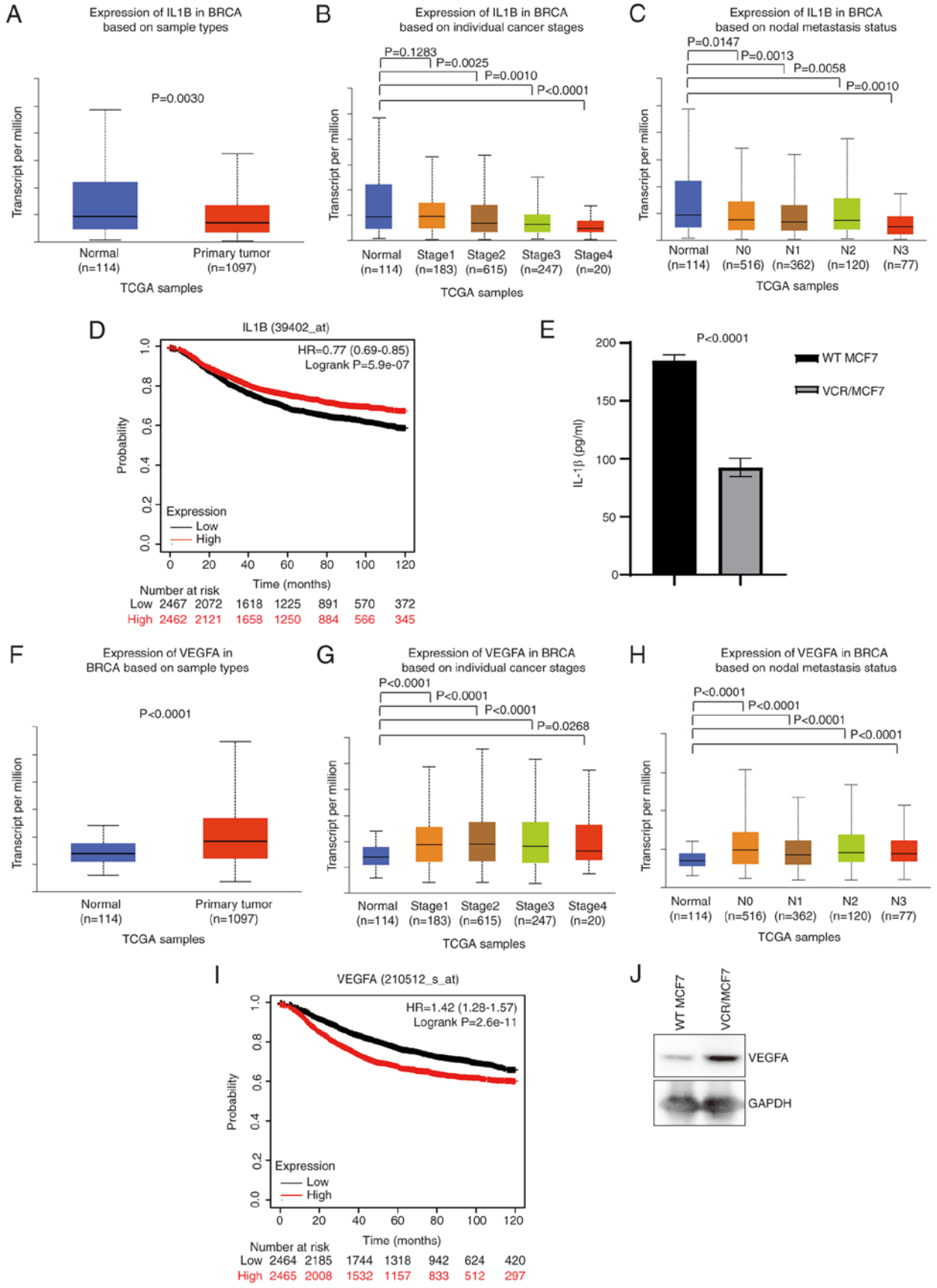

Figure 5. VCR resistance may be due to changes in the expression of VEGFA or IL-1β. (A-C) TCGA data analysis of IL1B expression in BRCA and normal tissues based on (A) sample types, (B) individual cancer stages and (C) nodal metastasis status. (D) Kaplan-Meier survival curve showing overall survival of patients with BRCA bearing either high or low IL1B. (E) ELISA was used to verify IL-1β level expression. TCGA data analysis of VEGFA expression in BRCA and normal tissues based on (F) sample types, (G) individual cancer stages and (H) nodal metastasis status. (I) Kaplan-Meier survival curves showing overall survival of patients with BRCA bearing either high or low VEGFA. (J) Western blotting was used to verify VEGFA protein level expression. HR, hazard ratio; IL-1β, interleukin-1β; TCGA, The Cancer Genome Atlas; BRCA, breast cancer; N, nodal; VEGFA, vascular endothelial growth factor A; WT, wild type; VCR, vincristine.

of matrix metalloproteinases that break down the basement membrane and extracellular matrix, promoting the migration of new endothelial cells and the development of capillary buds. VEGFA has been shown to regulate the activity of proteins such as MMP-2 and -9 through the activation of β-catenin and NF-κB dependent on Akt (39,40).

IL-1β mainly regulates the inflammatory signaling pathway, and is mainly produced by blood monocytes, tissue macrophages, skin dendritic cells and brain microglia (41). IL-1β is produced in the form of a precursor peptide, which is cleaved and activated under the stimulation of PAMPs and DAMPs, and is a cellular stress response mechanism induced by invading pathogens and other danger signals, such as mycobacterium tuberculosis components (42,43). IL-1β is activated after being cleaved by caspase-1, which is in turn activated by the NLRP3 inflammatory body complex. Once IL-1β is activated, it is released into the extracellular space and causes an inflammatory response by activating the IL-1R1 receptor (44). The expression of IL-1β in breast cancer cells is often closely related to the development of breast cancer (45).

Through TCGA data analysis, it was shown that the expression of IL-1β in breast cancer tissue was significantly lower than that in normal tissue, while the expression of VEGFA was higher in breast cancer tissue. Survival analysis showed that high expression of VEGFA was related to low survival rates in breast cancer, while low expression of IL-1β was related to high survival rates in breast cancer. These results indicate that VEGFA and IL-1β may participate in the process of drug resistance in breast cancer cells. VEGFA is known to mediate vasculogenesis and angiogenesis by regulating the activity of endothelial cells (46). Accumulating evidence suggests that VEGFA is expressed at high levels in a range of human cancers, including liver, ovary, kidney and colon cancers, and is associated with tumor progression and poor prognosis (47-50). It has been shown that patients with metastatic breast cancer have higher circulating VEGFA levels than those without metastasis (51). In addition to regulating angiogenesis, VEGFA also promotes tumor growth, metastasis and survival directly (46). In ovarian cancer, VEGFA upregulation is associated with poor survival, and it has been proposed as a biomarker for subsets of advanced ovarian tumors (52). Various therapies against VEGFA have been used for anticancer treatment (53,54). However, the role of VEGFA in chemotherapy resistance is still not clear. As an inflammatory mediator, IL-1β is frequently upregulated in a variety of cancers, which is different from the experimental findings of the present study, and its production is associated with poor prognosis (55,56). Some studies suggest that IL-1β induces neoangiogenesis and regulates the expression of soluble mediators in stromal cells to enhance tumor cell survival and metastasis (56,57).

Activation of autophagy leads to a decrease in intracellular drug concentration, which often associated with cell resistance (13). Changes in apoptosis-related proteins are also often associated with cell resistance. Based on a study that showed that VEGFA can promote the autophagy process (22), it was hypothesized that VEGFA may reduce the intracellular drug concentration by promoting autophagy, leading to the generation of drug-resistant cells. The decreased expression of IL-1β may play a role in the formation of drug-resistant cells by participating in the process of apoptosis (23).

There are still some deficiencies and limitations in the current study: It only included cellular experiments without involving animal models or analysis of clinical specimens; sequencing of drug-resistant cell lines often has a large number of genes with expression or splicing changes, but only a small number of these genes are typically involved in the generation of drug resistance. Therefore, it is important to identify those genes that are truly related to drug resistance among others. This requires the analysis and the validation processes of the present study to be further optimized.

The present study showed that the development of resistance to VCR may be related not only to changes in microtubule proteins or drug metabolism pathways such as autophagy, but also to changes in the expression of VEGFA and IL-1β, providing a theoretical basis for exploring the mechanism of VCR resistance clinically.

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Availability of data and materials

The data generated in the present study may be found in the GEO database (accession no. GSE241356).

Authors' contributions

YC and CMW conceived and designed the study. LLY and CW were involved in data collection. YC performed statistical analysis and prepared the figures. All authors have read and approved the final manuscript. YC and CMW confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of the First Affiliated Hospital of Dalian Medical University in accordance with the Declaration of Helsinki (approval no. PJ-KS-KY-2021-145). Written informed consent was obtained from each participant.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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