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Original Article

Multi-omics Mendelian randomization integrating GWAS, eQTL, and mQTL data identified genes associated with breast cancer

Zhihao Zhang[1*], Tian Fang[2*], Lanlan Chen[3], Fuqing Ji[4], Jie Chen[1]

[1]Breast Center, Department of General Surgery, West China Hospital, Sichuan University, No. 37 Guo Xue Alley, Chengdu 610041, Sichuan, P. R. China; [2]Department of Medical Oncology, Cancer Center, West China Hospital, Sichuan University, No. 37 Guo Xue Alley, Chengdu 610041, Sichuan, P. R. China; [3]Department of Hepatobiliary and Pancreatic Surgery, The First Hospital of Jilin University, Changchun 130021, Jilin, P. R. China; [4]Department of Thyroid Breast Surgery, Xi'an No. 3 Hospital, The Affiliated Hospital of Northwest University, Xi'an 710018, Shaanxi, P. R. China. [*]Equal contributors.

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Abstract: Breast cancer (BC) remains a major disease posing a threat to women's health, but the underlying biological interpretation remains largely unknown. Here, we aimed to identify genes associated with breast cancer and analyze their pathophysiological mechanisms based on multi-omics Mendelian randomization (MR). Summary-data-based MR (SMR) was performed to estimate the causal effects of blood and breast mammary tissue expression quantitative trait loci (eQTLs) on BC. External validation analysis was used to validate the identified genes. Integration analyses BC GWAS summaries with eQTLs and DNA methylation QTLs (mQTLs) from the blood were conducted using SMR to prioritize putative blood genes and their regulatory elements associated with BC risk. Finally, two prior genes (ATG10 and RCCD1) from blood tissue reached significant levels in both BCAC (ATG10: OR_{BRCR} = 0.91, P_{BRCR} = 1.29 × 10^{-11}; RCCD1: OR_{BRCR} = 0.90, P_{BRCR} = 3.72 × 10^{-15}) and FinnGen cohorts (ATG10: $OR_{FinnGen}$ = 0.89, $P_{FinnGen}$ = 8.55 × 10^{-5}; RCCD1: $OR_{FinnGen}$ = 0.89, $P_{FinnGen}$ = 2.38 × 10^{-8}). Additionally, those two genes from breast tissues also replicated in both BCAC (ATG10: OR_{BRCR} = 0.95, P_{BRCR} = 1.02 × 10^{-9}; RCCD1: OR_{BRCR} = 0.87, P_{BRCR} = 4.70 × 10^{-10}) and FinnGen cohorts (ATG10: $OR_{FinnGen}$ = 0.93, $P_{FinnGen}$ = 2.38 × 10^{-4}; RCCD1: $OR_{FinnGen}$ = 0.85, $P_{FinnGen}$ = 3.81 × 10^{-6}). Sensitive analysis and external validation analysis validated those two identified genes. Multi-omics MR analysis showed that the SNP signals associated with ATG10 and RCCD1 were significant across the data from BC Genome-wide association study (GWAS), eQTL, and mQTL studies. In conclusion, we identified two priority genes that are potentially associated with BC. These findings improve our limited understanding of the mechanism of BC and shed light on the development of therapeutic agents for treating BC.

Keywords: Breast cancer, Mendelian randomization, drug targets, phenome-wide MR

Introduction

According to the global cancer statistics in 2020, breast cancer (BC) in females has exceeded lung cancer, emerging as the most frequently diagnosed cancer worldwide and the leading cause of cancer death among women. It is estimated that there were approximately 2.3 million new cases, representing 11.7% of the total cancer cases reported [1]. Since 2004, there has been a slight gradual increase in breast cancer incidence rates, with an approximate annual growth rate of 0.5% [2, 3]. In fact, in the United States, the risk of a woman dying from breast cancer is about 1 in 39 in 2021, i.e., 2.6% [1].

Despite advancements in early detection and treatment, which have partially mitigated the breast cancer crisis, the available treatment options for breast cancer, such as surgery and radiation therapy, remain limited and are associated with a high incidence of adverse effects [4, 5]. Consequently, effective management and treatment of breast cancer pose a major challenge in clinical practice. Therefore, it is

necessary to enhance the understanding of the pathogenesis of breast cancer in order to develop viable therapeutic strategies. Fortunately, the availability of comprehensive human genetic data sets offers a valuable opportunity to accelerate the development of understanding of understanding for diverse diseases [6-8]. In recent years, several extensive genome-wide association studies (GWASs) have been conducted, revealing a multitude of single-nucleotide polymorphisms (SNPs) that are linked to an increased risk of BC. However, GWAS cannot clearly identify clues regarding causal genes and therapeutic targets, as many of the identified SNPs are situated in non-coding or intergenic regions [8].

MR is a genetic epidemiological study design that offers exceptional capability in investigating the causal relationship between traits and diseases [9]. Integrating GWAS data with gene expression and methylation GWAS has facilitated the discovery of expression or methylation quantitative trait loci (eQTL or mQTL) [10]. Additionally, summary-data-based MR (SMR) has extended the conception of MR that can use the independent GWAS summary data and QTL data to discover novel therapeutic targets [11]. Such drug target SMR analyses have been used in the investigation of various several diseases, such as COVID-19 and Crohn's disease [12, 13].

In this study, we aim to identify genes associated with BC by integrating GWAS, eQTL, and mQTL data, which can enhance our comprehension of BC mechanisms and offer potential therapeutic targets for effective BC treatment.

Methods

Study design and data resources

Figure 1 summarizes the design of this study. GWAS summary statistics datasets for BC were obtained from two different publicly available databases. Breast Cancer Association Consortium (BCAC) included 122,977 breast cancer cases and 105,974 controls of European ancestry from OncoArray and ICOGS arrays [14]. Summary-level genetic data on overall BC (15,680 cases and 167,189 controls), ER+ BC (9,698 cases and 167,017 controls) and ER- BC (5,965 cases and 167,017 controls) in FinnGen study were obtained from the last publicly available R9 data release as replication data. The blood eQTL summary statistics were obtained from eQTLGen. This dataset encompassed genetic data on the peripheral blood of 31,684 individuals derived from 37 different datasets [15] (https://www.eqtlgen.org/cis-eqtls.html). Breast mammary tissue eQTL data were from the Genotype-Tissue Expression (GTEx) project (n = 396) [16]. The GTEx project, initiated in 2010, aims to construct a comprehensive catalog of the genetic effects on gene expression in various human tissues. A total of 15,201 RNA sequencing samples from 49 tissues were examined in GTEx project [16]. The blood mQTL summary data were derived from a meta-analysis of two European cohorts: the Brisbane Systems Genetics Study (n = 614) and the Lothian Birth Cohorts (n = 1366) [17] (https://yanglab.westlake.edu.cn/software/smr/#mQTLsummarydata). Another blood eQTL summary statistics were derived as external validation from the GTEx project including 670 European individuals (https://yanglab.westlake.edu.cn/software/smr/#DataResource). The primary focus of this study was limited to cis-eQTLs and cis-mQTLs, which encompassed SNPs located within a 1-Mb proximity from the gene's start and end positions.

All data used in our study was public, and the data source can be found in the references. All informed consent and ethical approvals were obtained in the original manuscript.

Statistical analyses

Primary MR analysis: Summary-data-based MR (SMR) method was used to estimate whether the influence of single nucleotide polymorphisms (SNPs) on the phenotype is mediated by molecular traits like gene expression and methylation quantitative trait loci [11]. Allele harmonization and analysis were performed using version 1.3.1 SMR software (https://cnsgenomics.com/software/smr/#Overview). The Heterogeneity in Dependent Instruments (HEIDI) test was employed to examine whether the observed correlation between gene expression and outcome was attributable to a linkage scenario [11]. The HEIDI test, with a significance threshold of $P < 0.05$, suggests that the observed association is likely attributable to linkage [13]. FDR-corrected *p* values were cal-

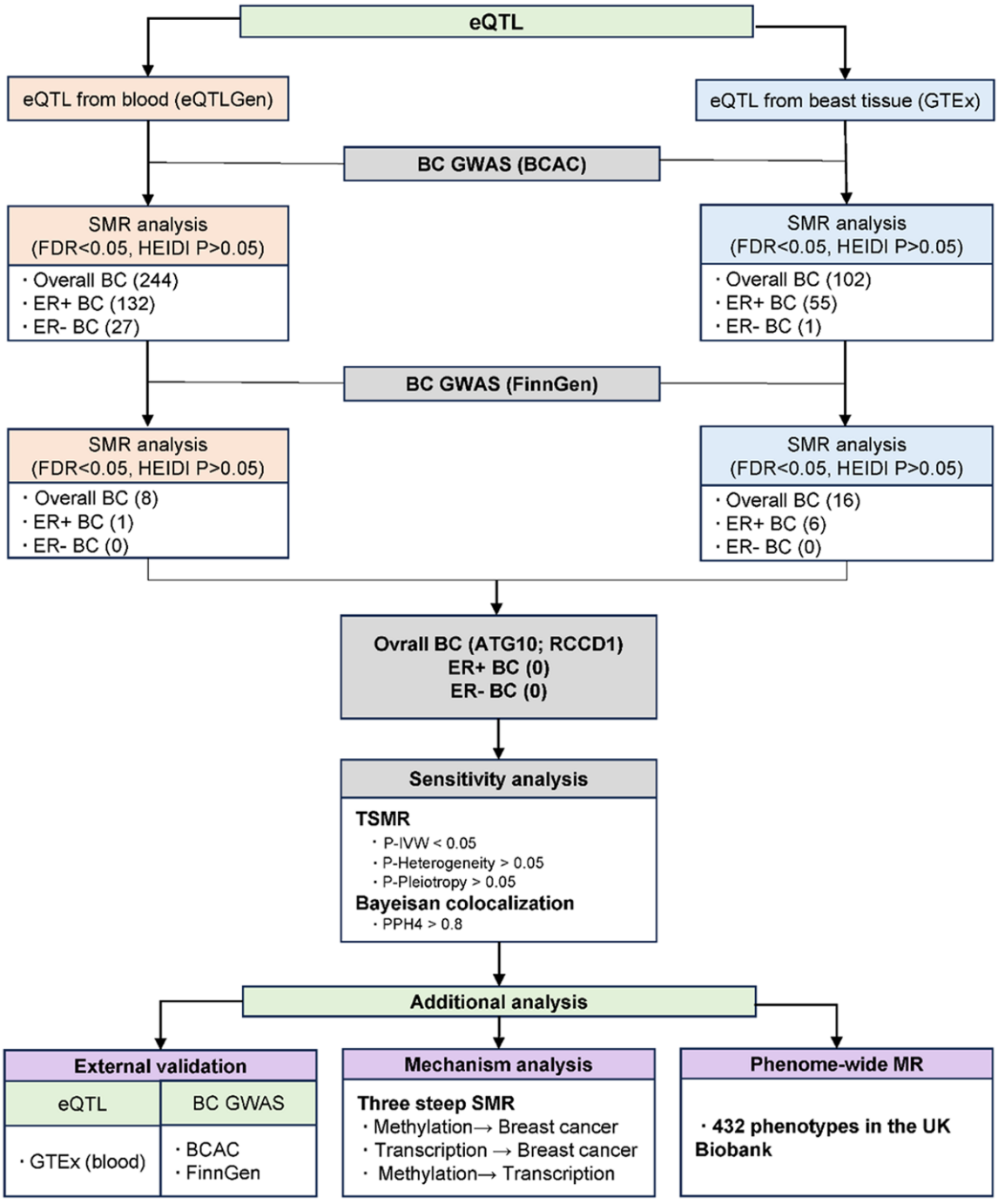

Figure 1. Workflow of the study. A series of analyses was conducted to identify candidate genes associated with breast cancer (BC). The genes that were significantly associated with BC in blood and breast mammary tissue eQTL were defined as prioritized genes (SMR FDR < 0.05; HEIDI test P > 0.05) and subjected to further analysis. Two sample (TSMR) was conducted as sensitivity analyses after the primary SMR to test the heterogeneity (Cochran Q statistic implemented in inverse variance weighting (IVW) method, P > 0.05 indicates no heterogeneity exists), pleiotropy (MR-Egger method was implemented, P > 0.05 indicates no pleiotropy exists) and causal association (IVW method was implemented, P < 0.05 indicates significant association between genes and BC). Integration of GWAS summaries and cis-eQTLs/cis-mQTLs data from the blood by using three-step SMR methods prioritized putative blood genes and their regulatory elements associated with the risk of BC (SMR FDR < 0.05; HEIDI test P > 0.05).

culated, and FDR of < 0.05 was considered significant.

Sensitivity analyses: Two sample MR (TSMR) and colocalization analysis were used as sensi-

tivity analyses to examine the robustness of our results. We obtained genes GWAS of eQTLGen results, comprising all cis regions of gene expression in whole blood, from MRCIEU data (ATG10: https://gwas.mrcieu.ac.uk/datasets/eqtl-a-ENSG00000152348/; RCCD1: https://gwas.mrcieu.ac.uk/datasets/eqtl-a-ENSG00000166965/). For TSMR, we selected independent genetic instruments with FDR of < 0.05 and within ±100 kb from each gene's transcriptional start site after linkage disequilibrium (r^2 < 0.01; kb = 1000). Finally, we obtained 18 and 16 instrument variants for ATG10 and RCCD1, respectively. The inverse variance weighting (IVW) method was applied as main analysis, MR-Egger [18] and weighted median [19] methods were applied to account for horizontal pleiotropic effects. We additionally employed MR Egger's intercept to examine pleiotropy, utilized Cochran's Q test to evaluate instrument heterogeneity, and applied F-statistics to test instrument validity. Additionally, we conducted a colocalization analysis to assess the association of BC risk and the SNPs located within ±1 Mb of each gene's transcription start site in eQTL using the 'coloc' package. The colocalization analysis was performed with P1 = 1×10^{-4}, P2 = 1×10^{-4}, and P12 = 1×10^{-5}. In terms of colocalization evidence between GWAS and QTL association, a posterior probability of H4 (PPH4) exceeding 0.8 is commonly accepted as a robust threshold [20, 21].

Mechanism analysis: Furthermore, gene methylation is known to influence gene expression. Here, we perform multi-omics MR (three steps MR) to explore the association between GWAS, eQTL and mQTL [10]. Step (1): Blood eQTL was exposure, and BC GWAS was the outcome; (2) Blood mQTL was exposure, and BC GWAS was the outcome; (3) Blood mQTL was exposure, and blood geQTL was the outcome. The third step included only significant genes from steps (1) and (2), and the direction of beta in steps (1) and (2) must opposite. The significant results were defined as FDR_{SMR} < 0.05 and P_{HEIDI} > 0.05.

Phenome-wide MR: Our primary aim was to provide genetic evidence to improve success rates in clinical trials for BC drug discovery, therefore we assessed the potential side effects of these prior genes by applying phenome-wide MR. The GAWS summary analysis was obtained from UK Biobank conducted by Neale Lab (http://www.neale lab.is/uk-biobank). We excluded duplicates and other phenotypes that unlikely to reflect side effects of prior genes such as socioeconomic factors, employment, lifestyle, environmental attributes, treatment/screening, family history [22]. Binary phenotypes were classified based on International Classification of Disease (ICD)-10 chapters, i.e., circulatory, dermatologic, digestive, genitourinary, neoplasms, hematopoietic, infectious diseases, injuries and poisonings, mental health, musculoskeletal, obstetric, sense organs, respiratory, symptoms. Continuous and categorial ordered outcomes were grouped into biomarkers physical measures, and cognitive function as recommended by the UK Biobank [22].

Additional analysis: In order to explore the expression correlation among priority genes, we acquired TCGA transcriptome RNA-sequencing data for breast cancer, which includes 1093 neoplastic tissues. Additionally, the expression of priority genes in BC tissues was further examined using HPA datasets (https://www.proteinatlas.org/). The relationship between expression of priority genes and patient prognosis was also explored using HPA datasets.

Results

eQTL data analysis from the blood and breast tissues

The data of blood cis-eQTL was available from the eQTLGen Consortium, where the cis-eQTLs of 16,987 genes were obtained from healthy European-ancestry individuals. To ensure the robustness of our findings, we performed SMR analysis separately on two independent BC GWAS datasets: BACA and FinnGen study. Only genes that showed significant association in both databases (SMR FDR < 0.05) and exhibited no apparent heterogeneity (HEIDI P > 0.05) were considered as priority genes. For BACA study, there were 142, 132 and 27 genes were identified after multiple testing for overall BC, ER+ BC and ER- BC (FDR < 0.05 and HEIDI P > 0.05). Among those genes, only eight and one genes for overall BC and ER+ BC were replicable in FinnGen study (Tables S1, S2, S3, S4, S5). The data of breast mammary tissue cis-eQTL was available from the GTEx

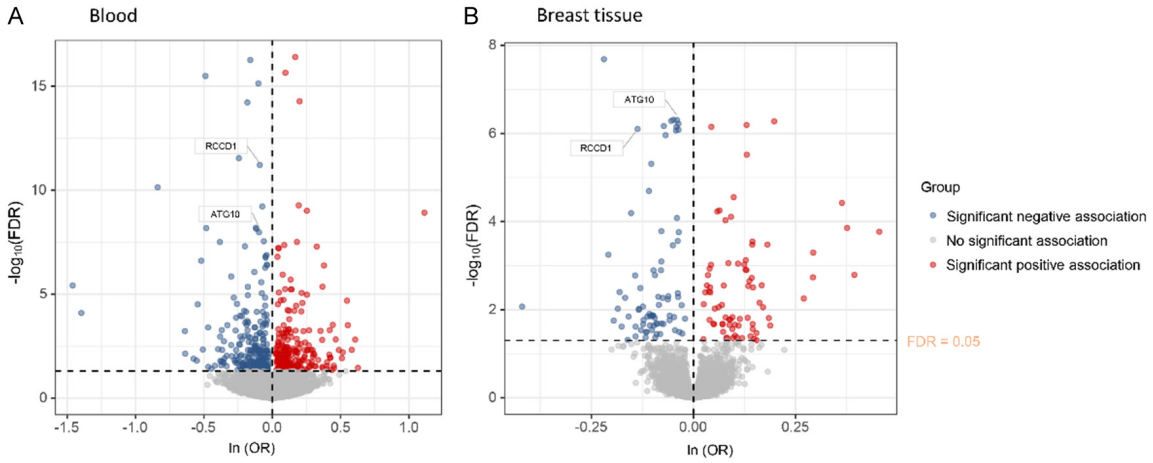

Figure 2. SMR results for blood and breast tissue eQTL and the risk of overall BC in BACA. Volcano plots of the SMR results for (A) Blood eQTL and (B) Breast tissues eQTL on the risk of overall BC.

Table 1. SMR results for blood and breast mammary tissue genes significantly associated with overall BC after Bonferroni correction

Tissue	Gene	BCAC cohort				FinnGen cohort			
		OR (95% CI)	*P* Value	FDR	P HEIDI	OR (95% CI)	*P* Value	FDR	P HEIDI
Blood	ATG10	0.91 (0.88, 0.92)	1.29E-11	1.06E-08	0.202	0.89 (0.84, 0.94)	8.22E-05	0.010	0.401
Blood	RCCD1	0.90 (0.89, 0.94)	3.72E-15	6.12E-12	0.624	0.89 (0.86, 0.93)	2.38E-08	5.83E-05	0.959
Breast mammary	ATG10	0.95 (0.93, 0.96)	1.02E-09	5.02E-07	0.342	0.93 (0.91, 0.97)	2.38E-04	4.00E-03	0.270
Breast mammary	RCCD1	0.87 (0.84, 0.91)	4.70E-10	7.94E-07	0.477	0.85 (0.79, 0.91)	3.01E-06	1.52E-04	0.388

BC, Breast cancer; CI, confidence interval; FDR, false discovery rate; OR, odds ratio; P HEIDI, *P* value for HEIDI test.

Consortium. Similarly, we conducted the SMR analysis in both BACA and FinnGen study. Finally, we obtained 102, 55 and 1 significant gens for overall BC, ER+ BC and ER- BC (FDR < 0.05 and HEIDI P > 0.05) in BACA study. Among those genes, only 16 and 6 genes for overall BC and ER+ BC were remained in FinnGen study (Tables S6, S7, S8, S9, S10).

When overlapping with the significant genes from blood and breast mammary tissue cis-eQTL, we obtained only two priority genes for overall BC, including autophagy related 10 (ATG10) and RCC1 domain containing 1 (RCCD1) (**Figure 2**).

ATG10

ATG10 showed a negative estimate effect in the SMR results, showing a relationship between decrease ATG10 expression and increased overall BC risk (**Table 1**). Additionally, TSMR and colocalization analysis were performed as sensitivity analyses to assess the robustness of this association. We obtained 18 independent SNPs as genetic instruments for ATG10 (Table S11). The TSMR indicated that the association remained in BACA (IVW OR = 0.96; 95% CI = 0.93-0.99; P = 0.013) and FinnGen study (IVW OR = 0.93; 95% CI = 0.89-0.97; P = 0.002). There is no evidence indicating the existence of horizontal pleiotropy and heterogeneity (Table S12). The association between ATG10 and BC was confirmed by colocalization analysis (PPH4 > 0.80, Table S12), which was in line with the SMR results.

Furthermore, the blood eQTL from GTEx Consortium was used to replicate the primary findings, ATG10 was also found to be negative associated with BC in both the BACA and FinnGen cohorts (SMR P < 0.05, HEIDI *P* > 0.05, Table S13). Our three-step SMR showed that the SNP signals associated with ATG10 were significant across the data from BC GWAS, eQTL, and mQTL studies (Tables S14, S15). The DNAm probe cg17942617 located in the 92 kbp upstream of ATG10. The methylation level of cg17942617 showed a positively association on ATG10 expression ($beta_{SMR}$ =

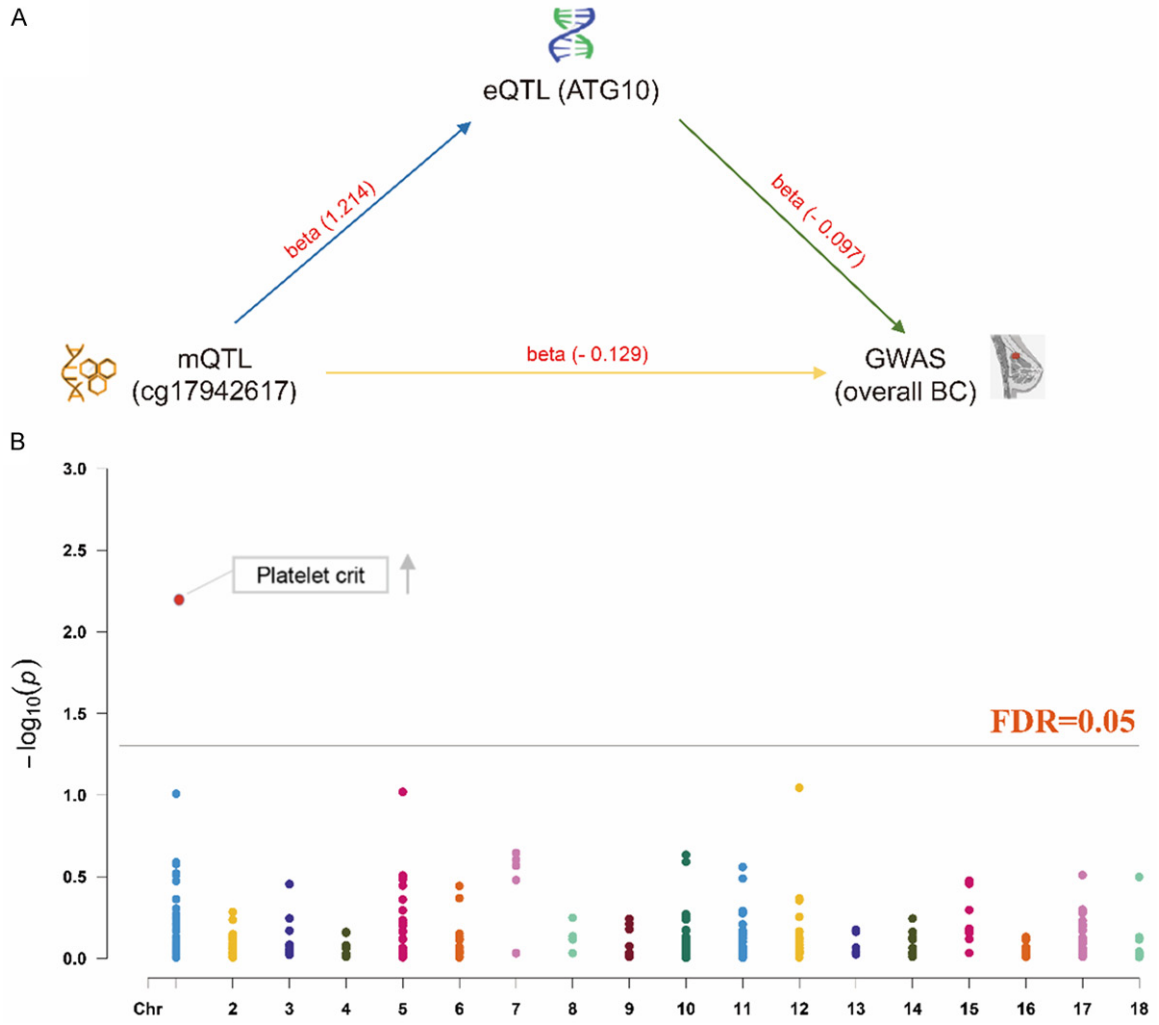

Figure 3. Three-step SMR analysis and phenome-wide MR results of blood ATG10. A. Three-step SMR indicating significant causal relationships between gene expressions and BC risk mediated by methylation (all three-step SMR FDR < 0.05; HEIDI test P > 0.05). B. Manhattan plot for phenome-wide TSMR results of blood ATG10.

1.21) and negative effect on BC ($beta_{SMR}$ = -0.13), while the ATG10 expression level was negative effect on BC. Together, our findings indicate a putative mechanism wherein a higher DNAm level of cg17942617 upregulates the expression of ATG10 and subsequently decrease BC risk (**Figure 3A**).

Nevertheless, in studies focused on drug development, it is crucial to take into account potential adverse effects and alternative indications. Therefore, a phenome-wide TSMR screening of 432 diseases or traits were performed using data from the UK Biobank. Overall, we didn't find significant side effects after multiple testing (**Figure 3B**; Table S16). Higher blood ATG10 levels may potentially benefit platelet crit (FDR < 0.05).

Additional analysis shown that there were no significant relationships between ATG10 and RCCD1 (P = 0.246; Figure S1). HPA immunohistochemical staining also showed downregulation of ATG10 expression in breast cancer tissues (Figure S2), which was consistent with our research findings. Although the *p*-value from the survival analysis is not statistically significant (P = 0.120), it can be observed from the Kaplan-Meier survival curve that breast cancer patients with high expression of ATG10 have better survival (Figure S3).

RCCD1

RCCD1 was another priority gene that passed the significance threshold in the blood and breast tissues eQTL SMR analysis (**Table 1**),

and TSMR indicated that the negative association between RCCD1 expression and overall BC risk still remained in BACA (IVW OR = 0.93; 95% CI = 0.91-0.96; P = 1.99 × 10^{-7}) and FinnGen study (IVW OR = 0.88; 95% CI = 0.81-0.95; P = 0.001). Cochran's Q method did not reveal any sign of heterogeneity and MR-Egger regression didn't examine the exited of horizontal pleiotropy (Table S12). RCCD1 also reached significant levels in the blood in the further colocalization analysis (PPH4 > 0.80, Table S12).

We further investigated the association using another external validation data. Using blood eQTL, we found that RCCD1 expression levels were consistently negatively associated with overall BC risk (Table S13), which was in line with our primary results. Additionally, we also found that DNAm probe cg01710897 and cg04851675 were causally positively associated with RCCD1 expression ($beta_{SMR}$ = 1.80; $beta_{SMR}$ = 2.01, respectively). Consistently, higher RCCD1 expression and higher methylation levels potentially decreased the BC risk. Thus, the putative mechanism could be that the genetic variants upregulate RCCD1 expression by influencing the promoter DNAm status, showing a protective effect on BC risk (**Figure 4A** and **4B**). Furthermore, no significant association was identified in the phenome-wide TSMR analysis, although we observed some trends. RCCD1 gene expression levels may be positively association with red blood cell distribution width, mean platelet volume and negatively association with platelet count levels (**Figure 4C**; Table S17), which indicated a few potential safety concerns.

HPA immunohistochemical staining also showed down-regulation of RCCD1 expression in breast cancer tissues (Figure S4), which was consistent with our research findings. In addition, the results of the survival analysis indicate that breast cancer patients with high expression of RCCD1 benefit from prolonged survival (P = 0.010; Figure S5), this makes it a potential new target for the treatment of breast cancer in the future.

Discussion

To the best of our knowledge, this study pioneers the application of a multi-omics integration methodology in the detection of candidate causal genes and exploration of the fundamental mechanisms implicated in breast cancer using blood and breast mammary tissues. At last, our study provides robust evidence that two genes (ATG10 and RCCD1) are causally associated with overall BC based on blood and breast mammary tissues, and passed the external validation and colocalization analysis. Furthermore, the integration of GWAS with the eQTLs and mQTLs have identified two methylation-mediated pathways that impact gene expression and influence the risk of BC. Phenome-wide MR revealed additional beneficial indications of those two therapeutics targeting genes and indicated a few potential safety concerns.

Autophagy-related 10 (ATG10) is a gene that encodes an E2-like enzyme involved in the process of autophagosome formation, also mediated formation of the autophagy-essential Atg12-Atg5 conjugate, therefore, ATG10 plays a critical role in the formation of autophagosomes [23-25]. The role of autophagy in cancer development is dual, contingent upon the cancer type, stage, or genetic context [26]. It is widely acknowledged that autophagy inhibition suppresses tumor initiation in the early stages of cancer [27]. The downregulation of ATG10 in colorectal cancer facilitates cancer progression by depleting epithelial-mesenchymal transition (EMT) associated proteins [28]. Another study indicated that rs1864182 and rs10514231 localized to the intron of ATG10 were significantly correlated with a reduced risk of BC [29]. The expression levels of certain transcription factors were significantly increased in ATG10-depleted cells; however, no alterations were observed in the expression levels of invasion-associated proteases such as MMP. The experimental study conducted by Flanagan et al. demonstrated the crucial involvement of ATG10 in cell cycle progression in *Saccharomyces cerevisiae*, independent of autophagy [30]. Consequently, further investigation is warranted to determine the specific role of ATG10 in epithelial-mesenchymal transition (EMT) and autophagy. The role of ATG10 in tumor carcinogenesis was rarely examined in the past studies, our results extended previous findings by adding evidence for the directionally consistent effects of ATG10 on overall BC outcomes in the blood or breast mammary tissue base on multi-omics MR study. The asso-

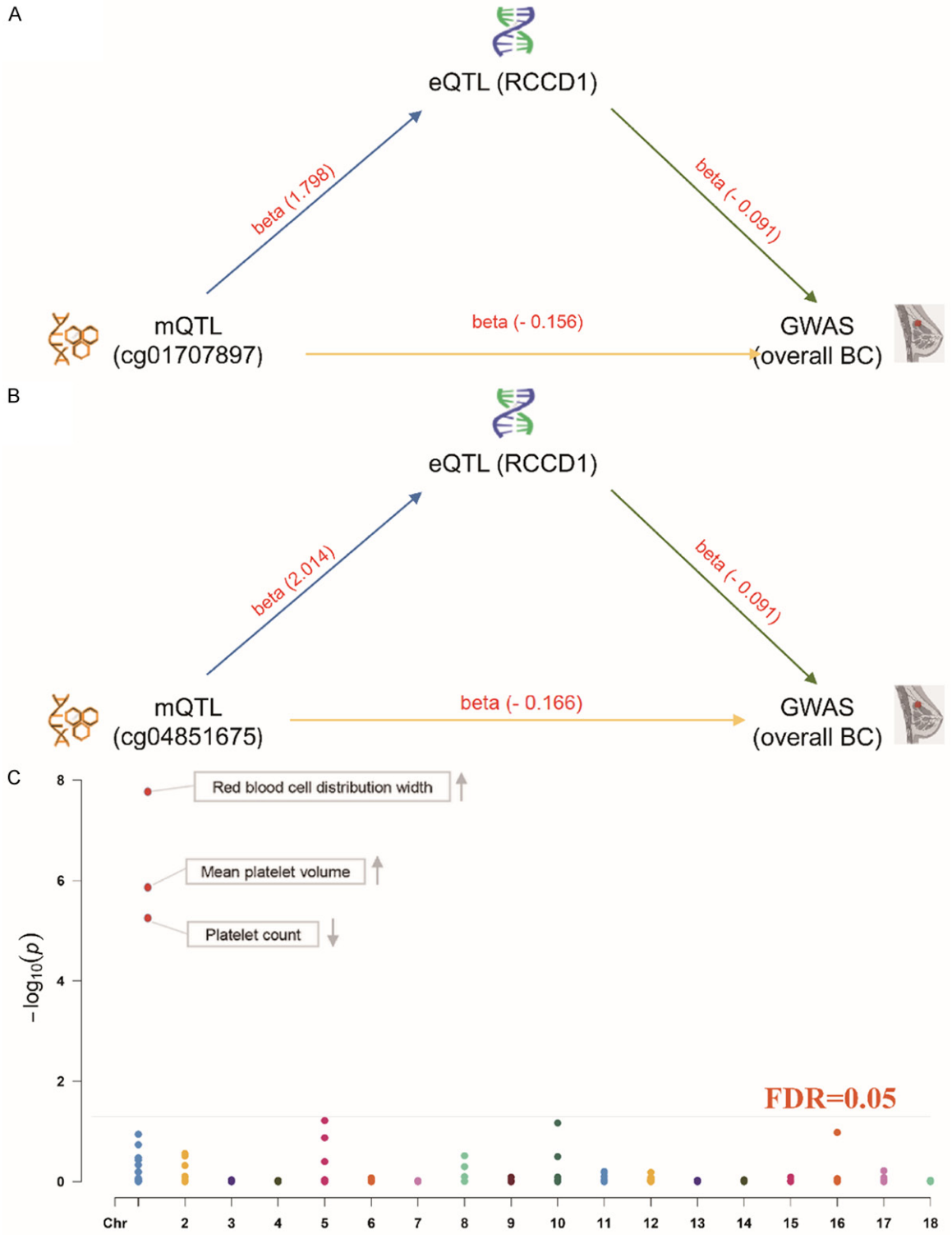

Figure 4. Three-step SMR analysis and phenome-wide MR results of blood RCCD1. A, B. Three-step SMR indicating significant causal relationships between gene expressions and BC risk mediated by two DNAm sites (all three-step SMR FDR < 0.05; HEIDI test P > 0.05). C. Manhattan plot for phenome-wide TSMR results of blood RCCD1.

ciation between ATG10 and BC was confirmed by colocalization in BCAC cohort (HHP4 = 0.907) and FinnGen cohort (HHP4 = 0.850). Furthermore, multi-omics analysis indicated a

putative mechanism wherein a lower DNAm level of ATG10 upregulates the expression of ATG10 and subsequently increases BC risk. In addition, the results of the K-M curve analysis indicate that breast cancer patients with high ATG10 expression tend to have better prognosis, although the statistical significance is not clear. We think that the insufficient number of participants included is an important reason for the lack of statistical significance. More importantly, we also performed a phenome-wide MR analysis of GWAS for ATG10, and the ATG10 expression was not strongly associated with other risks, indicating a few potential safety concerns.

RCCD1 encodes RCC1 domain containing 1 that plays a central role in enzyme inhibition, guanine nucleotide exchange, protein interaction and binding to lipids [31]. A cross-ethnic meta-analysis suggests that RCCD1 exhibits high expression in blood and breast tissues and is associated with a decreased risk of breast cancer, which is consistent with our findings [32]. In a genome-wide association analysis conducted in 2014 on East Asian women, a significant association was identified between the genetic variant rs2290203, which is 5 kbp downstream of RCCD1, and an increased risk of BC [33]. Another recent genome-wide association analysis found an association between rs8037137, another SNP in moderate LD with rs2290203, and risk of BC [34]. Evidence suggests that the effect alleles of both rs2290203 and rs8037137 demonstrate a consistent decrease in the expression of RCCD1 [33, 34], which aligns with our finding that elevated RCCD1 expression is concomitant with an increased risk of BC. In Bayeisan colocalization analysis, RCCD1 also produced significant results in BCAC cohort (HHP4 = 0.989) and FinnGen cohort (HHP4 = 0.970). Additionally, three step SMR analysis confirmed that DNAm negatively regulated RCCD1 expression, indicating a link between DNAm, RCCD1 expression, and BC risk. The results of the survival analysis indicate that breast cancer patients with high expression of RCCD1 benefit from prolonged survival. In addition, our application of phenome-wide MR analysis demonstrated that the blood RCCD1 did not yield statistically significant adverse impacts on other physiological systems. However, the mechanism of RCCD1 on breast tumor cells remains uncertain. Previous studies on RCCD1 have been limited, necessitating additional experimental investigations to explore its underlying mechanisms and determine its potential as a therapeutic agent.

Investigations into tissue- and cell-specific gene expression have consistently revealed distinct biological molecular processes [12, 35]. Therefore, it may be of greater significance to explore the correlation between genes and BC specifically in breast tissue using mammary eQTLs. Moreover, considering our main research goal of advancing the success rates in clinical trials for BC drug discovery, we opted to prioritize the reduction of false positive results and provide robust genetic evidence. Therefore, we conducted two-stage MR in blood and mammary tissues and used colocalization analysis and TSMR to identify robust druggable genes. At last, we prioritized two genes and DNAm sites for mechanism analysis using the largest BC GWAS. Considering the predominant mechanism of drug action through systemic blood circulation, we assessed whether those two BC-associated gene expressions in the blood had strongly side effects on other systems. Therefore, we conducted a phenome-wide MR analysis screening of 432 diseases or traits in the UK Biobank.

This study also has some limitations. First, despite we employed the largest BC GWAS and conducted validation in other databases to increase statistical power, the restricted number of cases in the subgroup of BC remains a significant obstacle in the identification of target genes within this particular subgroup. Second, considering the limitations of the dataset, it was not possible to replicate these findings at the protein level. Third, identifying the optimal tissue for discovering BC poses challenges [8]. Genes exhibiting significant expression levels in both blood and breast tissues may present more compelling evidence. Forth, it is important to note that our analysis specifically targeted the cis-regions for eQTL, with limited consideration for the potential broader impact of trans-eQTL on regulatory networks. Fifth, the GWAS analyses performed in our study were limited to individuals of European descent, caution should be exercised when extrapolating our findings to other racial

and ethnic groups. In addition, it is important to note that our research specifically examined the adverse effects associated with ATG10 and RCCD1 eQTL in diseases found within the European descent. It is essential to consider the extensive range of effects that drugs can have on their targets, as there are often numerous off-target effects that cannot be adequately assessed using MR. Furthermore, due to the limitations of GWAS data, we were only able to obtain subtypes based on estrogen receptor traits. Breast cancer is a highly heterogeneous solid tumor, and the treatment approaches for different subtypes of breast cancer vary significantly. Therefore, we believe that future multi-omics studies focusing on comprehensive breast cancer subtyping are necessary. Additionally, considering the multifactorial nature of genes expression, we believe that the integration of omics data from various molecular levels, including proteins and metabolites, with extensive sample sizes, holds the potential to uncover novel insights and enhance our understanding of the putative causal mechanisms underlying target genes in BC.

Conclusion

Our SMR analysis indicated that high level of ATG10 and RCCD1 expression showed a decreased risk of BC. These findings provided important leads to a better understanding of the mechanism of ATG10 and RCCD1 in BC and revealed potential therapeutic targets for the prevention and effective treatment of BC.

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Written informed consent for publication was obtained from all participants.

Disclosure of conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Address correspondence to: Jie Chen, Breast Center, Department of General Surgery, West China Hospital, Sichuan University, No. 37 Guo Xue Alley, Chengdu 610041, Sichuan, P. R. China. Tel: +86-18682757565; E-mail: chenjiedoctor@wchscu.cn

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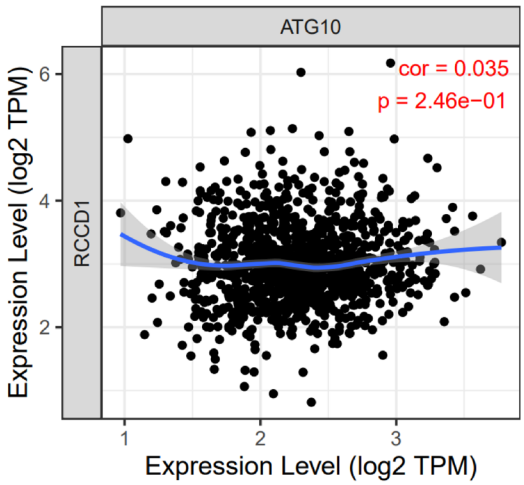

Figure S1. The relationship between expression of ATG10 and RCCD1 based on TCGA data.

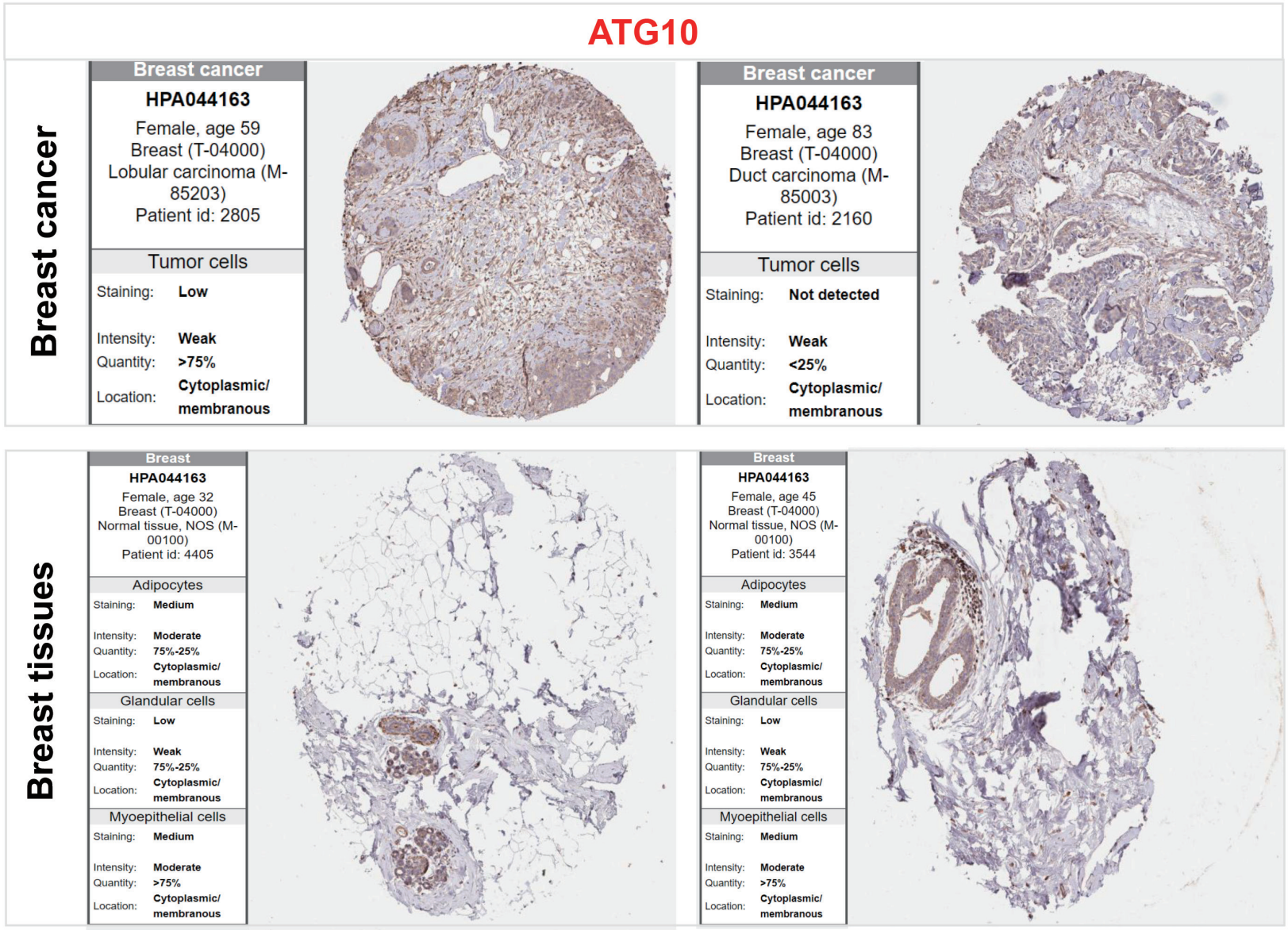

Figure S2. The expression of ATG10 in breast tumor and normal tissues based on HPA.

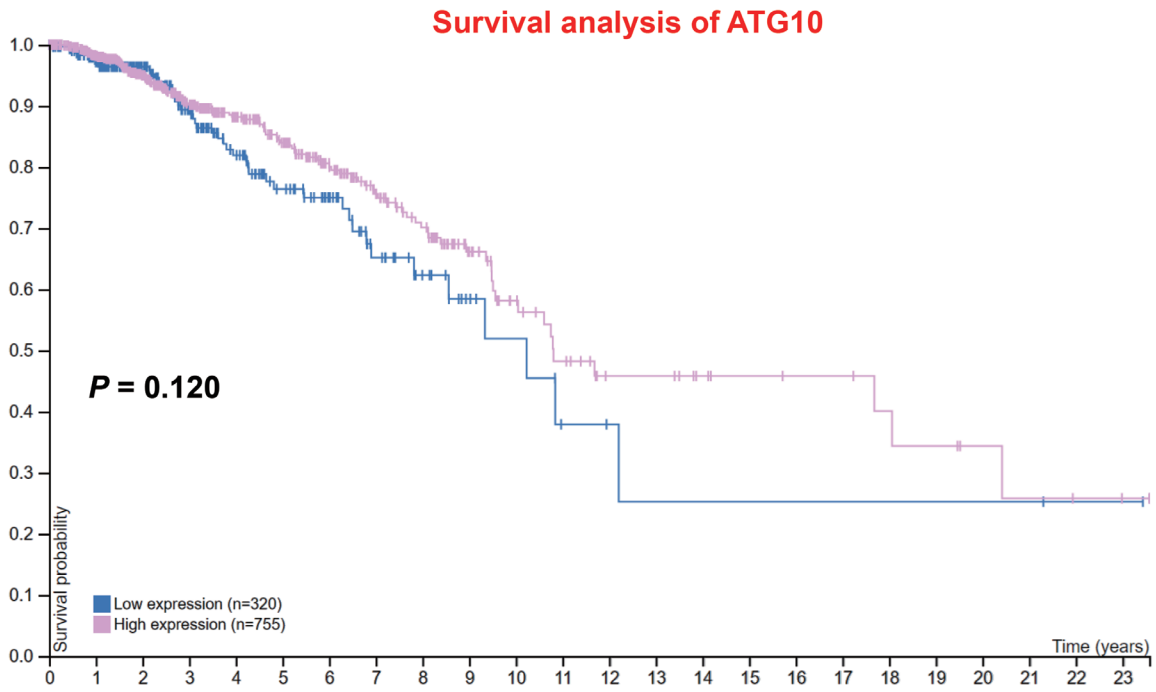

Figure S3. The relationship between expression of ATG10 and patient prognosis based on HPA.

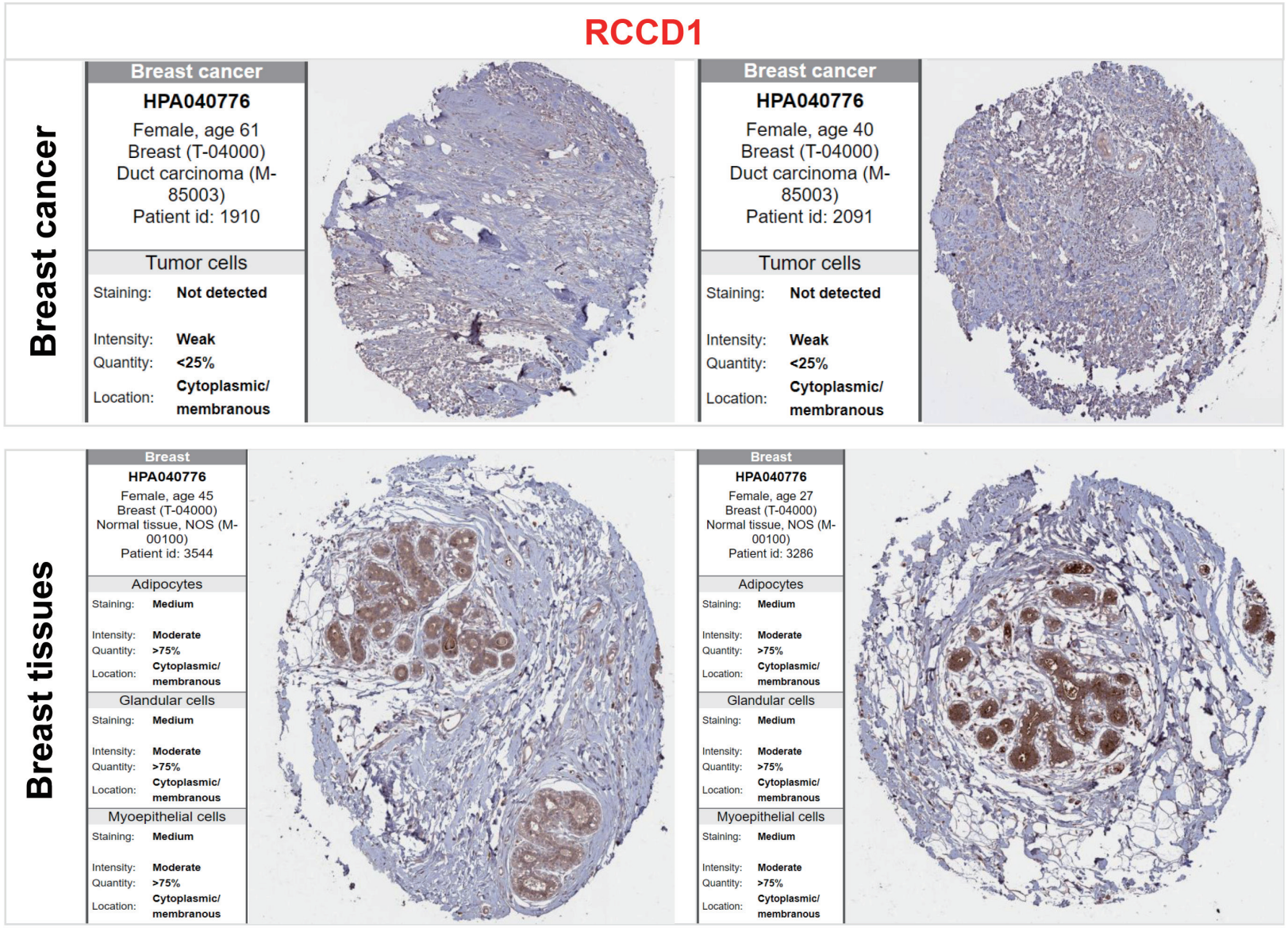

Figure S4. The expression of RCCD1 in breast tumor and normal tissues based on HPA.

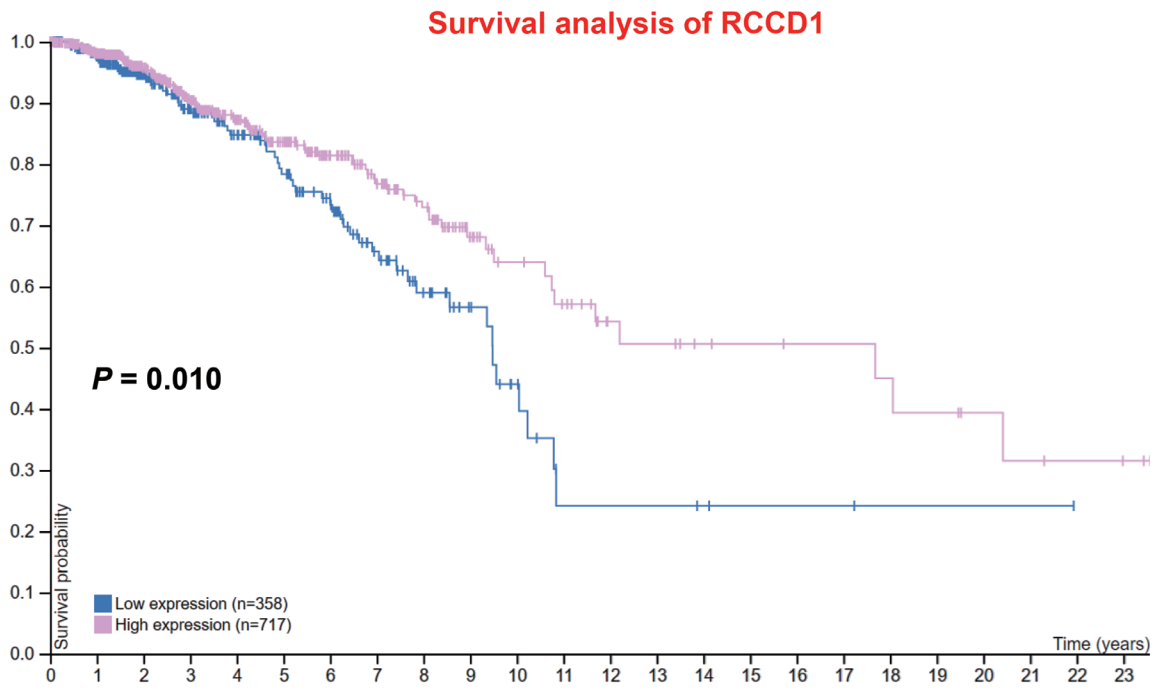

Figure S5. The relationship between expression of RCCD1 and patient prognosis based on HPA.