Elevated expression of LRRC15 Shows Putative Tumor-promoting Characteristics in tumor microenvironment of breast cancer

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Abstract

Background

Leucine-rich repeat containing 15 (LRRC15), belongs to the LRR superfamily and has emerged as a marker of cancer-associated fibroblasts. It was found to be particularly upregulated in breast cancer (BCa). This study aimed to investigate the correlation between LRRC15 expression and immune microenvironment and visualize its prognostic landscape in BCa.

Methods

The mRNA expression level, prognostic value, correlation of immunity, gene-gene interaction network of LRRC15 in BCa were analyzed utilizing the Oncomine, Gene Expression Profiling Interactive Analysis (GEPIA), UALCAN, Kaplan-Meier plotter, and TIMER database. We next analyzed the biological functions of LRRC15 and pathways of its co-expressed genes, and its correlation with immune system responses via the Metascape and GeneMANIA database, respectively. We validated the expression of LRRC15 in BCa via western blot and IHC assays and analyzed its correlation with clinicopathological parameters.

Results

We explored LRRC15 expression in multiple types of cancer based on the Cancer Genome Atlas (TCGA) database, with the effect being particularly pronounced in BCa. Both mRNA and protein abundance of LRRC15 were significantly elevated in BCa as compared to its non-tumor counterparts. Overexpression of LRRC15 significantly associated with reduced overall survival. LRRC15 knockdown significantly inhibited cell proliferation and cell cycle in BCa cells. There were significant positive correlations between LRRC15 expression and tumor-infiltrating immune cells (TIICs), with a particularly strong effect on macrophage infiltration. Moreover, markers of TIICs exhibited different LRRC15-related immune infiltration patterns. GSEA analysis showed that upregulated expression of LRRC15 was related to ECM receptor interaction, focal adhesion, regulation of actin cytoskeleton, and TGF Beta signaling pathway.

Conclusions

These findings revealed that LRRC15 served as a novel prognostic biomarker and putative oncogene for BCa by promoting cell proliferation, giving a novel hint for therapeutics of BCa.

Introduction

Breast cancer (BCa) is the malignancy with the highest prevalence and has the second highest cancer-related mortality rate in females worldwide [1]. BCa is a heterogeneous cancer consisting of different molecular subtypes, differs in terms of tumor biology, incidence, risk of cancer progression, response to

diverse therapies, clinical outcomes, and sites of metastases [2, 3]. Treatments for BCa range from surgery to collaborative combinations of surgery, chemotherapy, endocrine therapy, targeted therapy and radiation therapy [4, 5]. Simultaneously, despite explosive therapeutic advances, BCa exerts a substantial impact on the healthcare system and the statistics of mortality and morbidity are daunting [1, 6]. Therefore, it is urgent to identify the crucial molecular events related to the malignant transformation of BCa cells, providing a theoretical basis for the early diagnosis, monitoring progression, and molecular therapy of BCa.

Leucine-rich repeat containing 15 (LRRC15), a 581 amino acid type I transmembrane protein, belongs to the LRR superfamily and has emerged as a marker of cancer-associated fibroblasts (CAFs) recently [7]. Remarkably, LRRC15 was highly expressed on stromal fibroblasts in multiple solid tumors, such as breast, head and neck, lung and pancreas, while was lowly expressed in most normal tissues [7, 8]. The LRR family generally regulates a broad repertoire of functions, including signal transduction, cell adhesion, DNA repair, and RNA processing [9]. Augmented levels of the LRRC15+ CAF signature was linked with poor response to immune checkpoint blockade therapy, making it a determinant of patient response to cancer immunotherapy [10]. Since only a fraction of patients exhibit durable responses to cancer immunotherapy [11, 12], it is pivotal for us to have a broader view of the entire tumor microenvironment (TME) and seek potential targets.

Here, we sought to determine the expression of LRRC15 and its correlation with TME of BCa. Moreover, we explored the tumorigenic roles of LRRC15 by functional assays in vitro and revealed the critical links between LRRC15 and tumor growth in BCa. Our results offered novel insights into the functional role of LRRC15 in BCa, thereby highlighting a potential mechanistic basis whereby LRRC15 influenced tumor evolution and progression in TME of BCa.

Materials And Methods

Raw Data acquisition and processing

Transcriptome RNA-seq data of normal and cancer tissues in pan-cancer were extracted from TCGA (The Cancer Genome Atlas) database (<https://portal.gdc.cancer.gov/>). TCGA research network is a publicly funded project designed to catalog and explore major genomic alterations with the purpose of establishing an integrated 'atlas' of cancer genomic profiles [13, 14].

Oncomine database Analysis

Oncomine (www.oncomine.org), a publicly accessible online database and web-based data-mining platform, deliver plentiful cancer microarray information and genome-wide expression analysis [15]. This cancer microarray database was applied to investigate the transcriptional levels of LRRC15 in various clinical cancer specimens compared with those in normal tissues.

UALCAN database analysis

UALCAN (<http://ualcan.path.uab.edu>) web-portal is an omnibus and interactive database based on TCGA RNA-seq and clinical data of multiple cancer types [16]. We utilized this platform to elaborate the distinct expression levels of tumor and normal tissues in subtypes of BCa.

Gene Expression Profiling Interactive Analysis (GEPIA) database analysis

GEPIA (<http://gepia.cancer-pku.cn/index.html>) is a recently developed web server utilizing a standard processing pipeline and consist of 9,736 tumors and 8,587 normal samples data from TCGA and the Genotype-Tissue Expression (GTEx) projects [17]. It provides fast and customizable functionalities such as differential expression analysis, similar gene detection, patient survival analysis, profiling according to cancer types or pathological stages, correlation analysis, and dimensionality reduction analysis.

Kaplan-Meier plotter database analysis

Kaplan-Meier plotter (<http://kmplot.com/analysis/>) is a platform providing information about the correlation of gene expression level with the survival of cancer patients, including BCa, liver cancer, gastric cancer, ovarian cancer, and lung cancer [18]. Information regarding HRs, 95% CIs and P-values can be accessed at the webpage.

Human Protein Atlas (HPA) database analysis

The Human Protein Atlas (HPA) (<https://www.proteinatlas.org/>) is a public web interface that can be utilized to identify the distribution and expression of target proteins. It uses special antibodies and contains immunohistochemical expression data for 20 kinds of tumors [19]. In this study, we confirmed prognostic significance and compared the protein expression levels of LRRC15 between normal and BCa tissues.

TIMER (Tumor Immune Estimation Resource) database analysis

TIMER (<https://cistrome.shinyapps.io/timer/>) is a user-friendly web interface designed for analyzing immune cell infiltrates in diverse malignancies [20]. LRRC15 was selected to investigate via “Gene module” and generated scatterplots to visualize the relationship of its expression with immune infiltration level in BCa.

GeneMANIA database analysis

GeneMANIA algorithm (<http://genemania.org/>), an intuitive web interface, was applied to establish a biological network for LRRC15 in terms of physical interaction, prediction, co-expression, colocalization, and shared protein domains, and evaluate the functions of network components [21]. A figure where nodes symbolize genes and links represent networks was used to display interactions.

Functional And Pathway Enrichment Analyses

Genemania database has been applied to provide information regarding the predicted interaction of LRRC15. TCGA breast cancer samples were grouped as high LRRC15 expression group and low LRRC15 expression group based on the median transcriptional expression of LRRC15. Gene set enrichment analysis (GSEA) software was applied to evaluate potentially biological mechanism associated with LRRC15 mRNA expression levels. A nominal P-value <0.05 and FDR <0.25 were considered to a statistically significant gene set.

Cell lines, culture and reagents

A panel of BCa cell lines including MDA-MB-453, MDA-MB-468, BT-549, HS 578T, MCF7, T47D, ZR-75-1, SUM1315M02, HCC-1806, SK-BR-3, MAD-MB-231, BT-474 and human normal breast epithelial cells (MCF-10A) were obtained from Cell Bank of Type Culture Collection, Chinese Academy of Sciences (Shanghai, China). MAD-MB-231, T47D, SUM1315M02, SKBR-3, HS-578T lines were cultured in DMEM medium(KGM12800-500), BT-474, ZR-75-1, HCC-1806, BT-549 lines were cultured in 1640 medium (KGM31800N-500), MDA-MB-453, MDA-MB-468 lines were cultured in DMEM medium (KGM, KGM41300-500), MCF7(KGM12800-500,Insulin), MCF-10A (LONZA MEGM Bullet Kit(CC-3151 & CC-4136)), supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco).

Antibodies and reagents

Primary antibodies for Western blotting and immunohistochemical staining included anti-LRRC15 (Thermo Fisher Scientific, PA5-57298), anti-p63 (MXB Biotechnologies, MAB-0694), SMA (MXB Biotechnologies, MAB-0890), Calponin (MXB Biotechnologies MAB-0712), Ki-67 (MXB Biotechnologies, MAB-0672).

Tissue specimens and Immunohistochemistry (IHC) analysis

The immunohistochemistry protein expression was archived from formalin-fixed paraffin-embedded (FFPE) tumors from patients who underwent surgical treatment between February 2019 and February 2020 at Yancheng NO'1 People's Hospital. This study was approved by the Yancheng NO'1 People's Hospital Review Board. Written informed consent was obtained from these patients.

FFPE 4-μm thin tissue sections were used to perform immunohistochemistry in a Leica Bond Max automated stainer (Leica Biosystems). The tissue sections were deparaffinized and rehydrated following the Leica Bond protocol. Antigen retrieval was performed with EDTA buffer (MXB Biotechnologies, pH 9.0, MVS-0098), or citrate sodium buffer (MXB Biotechnologies, pH 6.0, MVS-0100) for 20 min. Slides were washed, blocked, and then the primary antibody was incubated overnight at 4°C temperature. The primary antibody was detected using the DAB Detection Kit (MXB Biotechnologies, Kit-0014). The slides were counterstained with haematoxylin, dehydrated and capped with coverslip.

Western blot assay

The proteins were extracted from the breast cancer specimens and cells using RIPA lysis buffer (Beyotime, Shanghai, China). BCA Protein Assay Kit was used to evaluate the protein concentration. The protein was separated from the sample buffer using SDS-PAGE, transferred into PVDF membranes and blocked with 5% skim milk for 1 h. The primary antibodies were anti-LRRC15 (Abcam, ab150376, 1:1000), anti-GAPDH (Abcam, ab82485, 1:1000). The member was incubated at 4°C overnight and then incubated with horseradish peroxidase-conjugated secondary antibodies. Immunoblots were visualized by BIO-RAD molecular imager.

SiRNA transfection

SiRNA transfection assay was performed by the transfection was performed using Lipofectamine™ 3000 (Thermofisher, L300015) according to the Lipofectamine™ 3000 Reagent protocol. MDA-MB-231 cells were transfected with siRNA Ctrl or mixture of three LRRC15 siRNAs, the final concentration of siRNA used was 50 nM. After 48 h of transfection, the cells were harvested and then subjected to the follow-up experiments. The LRRC15 siRNA sequence was designed and confirmed as 5'-GTAGGACTCACGAAGCTCA-3' (Ribobio, siG000131578A).

Cell viability assay

Cell viability assay was conducted using a CCK-8 (Cell Counting Kit-8) assay kit (X-Bio, Technology). Briefly, 8×10^3 cells/well were plated in 96-well plates and 10 μ L CCK8 solution was added into each well 2 h before detection at the indicated time points. Absorbance was measured at 450 nm by SpectraMax® Absorbance Reader.

Cell cycle assessed by flow-cytometric assay

Distribution of cells in cell cycle was analysed by flow cytometry. MDA-MB-231 cells were seeded in a 6-well cell culture plate and treated with different concentration of CoCl₂ to induce hypoxia (10, 20, 25 and 50 μ M for MDA-MB-231 cells). The cell harvested followed by fixation in 70% chilled ethanol. Staining of the cells was carried out with PI-RNase solution (1 mg/mL PI, 0.1% V/V Triton X-100 and 10 mg/mL RNase). The reading was done on a Beckman Coulter Gallios flow cytometer. A Beckman Coulter Gallios flow cytometer was used for data analysis.

Statistical analysis

In this study, Student's t-test was used to generate a P-value and the P-value cutoff was 0.05. In oncomine database, a p value of 0.05 and a fold change of 2 were set as the significance thresholds. Axis units from TCGA data mining were Log₂ (TPM + 1). Spearman correlation analysis was adopted to evaluate the correlation of LRRC15 expression and immune markers. P-values <0.05 were considered as statistically significant.

Results

LRRC15 is Highly Upregulated in Breast cancer

We firstly assessed the expression of LRRC15 in diverse malignancies and normal tissue types using the Oncomine database, revealing that expression of this gene was elevated relative to normal tissue controls for breast particularly (Figure 1A). Using RNA expression profiling from TCGA database, we identified LRRC15 as highly expressed in multiple solid tumor indications together with limited normal tissue expression. Notably, particularly elevated abundance of LRRC15 mRNA was observed in BCa (Figure 1B). Therefore, that's why we want to study LRRC15 in BCa. Furthermore, based on data mining, we found that LRRC15 has higher expression in cancerous tissues than in normal tissues both in mRNA and protein expression level (Figure 1C-D). Consistently, it is also proven in the immunohistochemical results of BCa patients from HPA database (Figure 1G). We also analyzed LRRC15 protein levels in BCa cell lines. Through the further experiment verification, we found LRRC15 was indeed highly in BCa cell lines compared to MCF-10A cell in protein expression level. We performed Western blot analysis to examine the LRRC15 expression in clinical tissues, 7 pairs of tumor and paracancerous tissues were observed. As shown in Figure 1H, LRRC15 protein level was significantly elevated in tumor tissues compared with matched paracancerous tissues. Collectively, we concluded that LRRC15 expression was significantly elevated in BCa.

Subtype Analysis Of Lrrc15 In Bca

Further, when sorting the patients by subgroups, all subunits of the LRRC15 were still significantly up-regulated in different molecular subtypes compared with paracancerous samples. As shown in Figure 2A, the highest expression levels of LRRC15 were observed in luminal and Her-2 positive subtype samples compared with TNBC (Luminal vs TNBC $P=1.62e-12$; HER2 Positive vs TNBC $3.96e-03$). Furthermore, LRRC15 still showed obviously strong expression in luminal and Her-2 positive BCa compared with seven TNBC subtypes, including TNBC-BL1 (TNBC Basal-like 1), TNBC-BL2 (TNBC Basal-like 2), TNBC-IM (TNBC Immunomodulatory), TNBC-M (TNBC Mesenchymal), TNBC-MSL (TNBC mesenchymal stem-like), TNBC-LAR (TNBC luminal androgen receptor) and TNBC-UNS (TNBC unspecified) (Figure 2B). IHC assays were performed to observe differences in LRRC15 expression levels among different molecular subtypes of BCa, and the highest protein expressions of LRRC15 were observed in luminal B subtypes (Figure 2C-G). In addition, we also observed the expression of LRRC15 in different pathological types from oncomine database, which was highly expressed in both invasive ductal carcinoma and invasive lobular carcinoma (Figure 2H-K).

Association of mRNA expression of LRRC15 with prognosis and clinicopathological parameters in BCa patients

We used Kaplan-Meier plotter and HPA database to analyze the prognostic values of the mRNA expression of LRRC15 in BCa patients. Higher mRNA expression of LRRC15 was related to poorer OS in BCa patients (HR=1.56, 95% CI: 1.01-2.42, and $P=0.045$). We also HPA database analyze the prognostic values of the protein expression of LRRC15 in BCa patients. Overexpression of LRRC15 was significantly associated with unfavorable prognosis in BCa patients ($P=0.032$). Further, we investigated molecular

characterization of prognostic interactions. As were shown in Fig. 3D-F, higher mRNA expression of LRRC15 was significantly associated with shorter OS of BCa patients in luminal B (HR=1.57, 95% CI: 1.02-2.41, and P=0.0392), Her-2 (HR=1.52, 95% CI: 1.13-2.05, and P=0.0052) and TNBC (HR=14.07, 95% CI: 1.85->100, and P=0.0107), while LRRC15 mRNA expression showed no correlation with prognosis of luminal A BCa patients (Fig. 3C). Furthermore, we evaluate the association between LRRC15 expression and clinicopathological parameters in BCa patients, the results showed that distant metastasis was associated with high expression of LRRC15 (Fig. 3G).

Correlation Analysis Between Lrrc15 Expression And Immunity In Bca

We primarily aimed to investigate the relationship between LRRC15 expression and immunity in TME of BCa. We analyzed the correlation between LRRC15 expression and 6 types of infiltrating immune cells from TIMER database (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells). The results showed that LRRC15 expression levels had a significantly negative correlation with tumor purity ($r = -0.206$, $P = 5.05e-11$) and a significantly positive correlation with infiltrating levels of B cells ($r = 0.101$, $P = 1.58e-03$), CD8+ T cells ($r = 0.248$, $P = 4.28e-15$), CD4+ T cells ($r = 0.2$, $P = 3.55e-10$), macrophages ($r = 0.436$, $P = 7.60e-47$), neutrophils ($r = 0.262$, $P = 2.17e-16$), and dendritic cells ($r = 0.287$, $P = 1.54e-19$) in breast cancer (Figure 4A). Furthermore, we investigated whether LRRC15 expression was correlated with immune infiltration levels in different BCa subtypes, and the correlations were not all the same (Figure 4B–4D). Analogously, in luminal BCa, LRRC15 expression levels were negatively correlated with tumor purity and positively correlated with infiltrating levels of these 6 types of infiltrating immune cells. In TNBC, LRRC15 expression levels were positively correlated with infiltrating levels of 4 types of infiltrating immune cells, including CD4+ T cells, macrophages, neutrophils, and dendritic cells. Divertingly, in HER2+ BCa, LRRC15 expression levels had no significant correlations with CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells. In addition, copy number of LRRC15 expression was linked with infiltrating levels of CD4+ T cells (Figure 4E). Expression of LRRC15 was negatively correlated with TMB in BCa, whereas no significant correlation with MSI (Figure 4F-G). LRRC15 expression was positively correlated with CD274, CTLA4, HAVCR2, PDCD1LG2 and SIGLEC15, and negatively correlated with LAG3. Expression of LRRC15 had significantly positive correlation with follicular helper T cell and activated NK cell, and had moderately positive correlation with CD8+T cell, memory activated CD4+ T cell, activated myeloid dendritic cell, resting NK cell, plasma B cell and memory B cell. Expression of LRRC15 was negatively correlated with CD4+ memory resting T cell, active mast cell and M2 macrophage. We next further explored the link between LRRC15 expression and sets of immunological markers in GEPIA. These markers were used to characterize immune cells, including CD8+ T cell, T cell (general), B cell, monocyte, TAM, M2 Macrophage, neutrophils, natural killer cell, dendritic cell, Th1, Th2, Tfh, Th17, Treg, T cell exhaustion in BCa. LRRC15 was positively correlated with Treg markers (FOXP3, CCR8, STAT5B, TGF β) in BCa tissues. Detailed findings in BCa and normal tissues are compiled in Table 1. Hence, these results suggests that LRRC15 was a vital factor in tumor immune

microenvironment of BCa, potentially playing a significant role in governing immune cell infiltration and thus representing a meritorious prognostic biomarker in BCa patients.

Table 1
Correlation analysis between LRRC15 and genes markers of immune cells in
GEPIA

Description	Gene markers	Tumor		Normal	
		cor	P	cor	P
CD8+ T cell	CD8A	-0.039	0.19	0.0014	0.99
	CD8B	-0.097	**	-0.04	0.67
T cell (general)	CD3D	-0.072	*	0.071	0.45
	CD3E	0.045	0.14	-0.091	0.34
	CD2	-0.022	0.47	0.11	0.23
B cell	CD19	-0.077	*	0.13	0.17
	CD79A	-0.086	**	0.15	0.11
Monocyte	CD86	0.2	***	0.00048	0.32
	CD115	0.24	***	0.29	**
TAM	CCL2	0.039	0.2	0.26	**
	CD68	0.23	***	0.15	0.13
	IL10	0.1	***	0.2	*
	IRF5	-0.0047	0.88	0.22	*
M2 Macrophage	CD163	0.036	0.24	0.16	0.092
	VSIG4	0.15	***	0.19	*
	MS4A4A	0.12	***	0.23	*
Neutrophils	CD66b	-0.0087	0.78	0.069	0.47
	CD11b	0.24	***	0.16	0.09
	CCR7	-0.057	0.062	0.15	0.11
Natural killer cell	KIR2DL1	-0.032	0.29	0.099	0.3
	KIR2DL3	-0.068	*	0.23	*
	KIR2DL4	-0.014	***	0.13	0.16
	KIR3DL1	-0.053	0.083	0.18	0.062
	KIR3DL2	-0.069	*	0.29	**

	KIR3DL3	0.041	*	-0.025	0.79
	KIR2DS4	-0.071	*	0.06	0.53
Dendritic cell	HLA-DPB1	0.14	***	0.06	0.53
	HLA-DQB1	0.043	0.15	0.15	0.1
	HLA-DPA1	0.19	***	0.26	**
	BDCA-1	0.013	0.68	0.28	**
	BDCA-4	0.44	0	0.16	0.084
	CD11c	0.19	***	0.1	0.28
Th1	T-bet	-0.071	*	0.23	*
	STAT4	0.0082	0.79	0.13	0.17
	STAT1	0.0074	0.81	0.041	0.67
	IFN- γ	-0.088	**	0.18	0.053
	TNF- α	-0.046	0.13	0.16	0.096
Th2	GATA3	0.023	0.44	-0.013	0.89
	STAT6	0.14	***	0.28	**
	STAT5A	0.075	*	-0.14	0.13
	IL13	-0.0012	0.97	0.24	**
Tfh	BCL6	0.092	**	0.0012	0.99
	IL21	-0.049	0.1	0.12	0.21
Th17	STAT3	0.22	***	0.22	*
	IL17A	-0.042	0.17	0.15	0.12
Treg	FOXP3	0.067	*	0.051	0.6
	CCR8	0.12	**	0.16	0.083
	STAT5B	0.14	***	0.055	0.57
	TGF β	0.39	***	0.3	**
T cell exhaustion	PD-1	-0.012	0.69	0.27	**
	CTLA4	-0.051	0.095	0.21	0.029
	LAG3	-0.13	***	0.23	*
	TIM-3	0.33	***	0.18	0.056

The cancer-promoting role of LRRC15 in tumor microenvironment of BCa

The above results strongly showed that LRRC15 might have a tumor-promoting effect in BCa. We then focused on its potential oncogenic roles by conducting siRNA-mediated loss-of-function approach. We selected MDA-MB-231 and BT-474 cells for knockdown experiments for the relatively higher endogenous LRRC15 in these cell lines (Figure 1D). We then explored the effects of LRRC15 on proliferation abilities of BCa cells using cell cycle assay and CCK-8 assay in vitro. CCK-8 assay demonstrated that significant impairment in cell proliferation at different time points in LRRC15-silenced MDA-MB-231 and BT-474 cells (Figure 5C). We also carried out flow cytometric analysis to access whether LRRC15 affected cell cycle progression. depletion of LRRC15 in MDA-MB-231 cells notably inhibited cell proliferation and tumorigenicity by modulating G1/S phase transition in vitro. In line with this, IHC assays demonstrated that LRRC15 was found mainly in BCa cell membrane and cytoplasm, and was rarely expressed in normal tissues. As shown in Fig. 2d, we observed a significant positive correlation between LRRC15 and Ki67. The direct association with rapid proliferation in BCa tissues (high numbers of Ki67-positive cells) highlighted its vital role in human BCa progression. Therefore, these functional data indicated that LRRC15 greatly contributed to proliferative ability of BCa cells, thereby further enhancing tumor progression.

Co-expression And Pathway Enrichment Analyses Of Lrrc15

A gene-gene interaction network for LRRC15 was constructed, and their functions were analyzed using the GeneMANIA database (Fig. 6A). The 12 central nodes representing LRRC15 were surrounded by 20 nodes representing genes that greatly correlated with the family in terms of predictions, physical interactions, co-expression, genetic interactions, and co-localization. The top five genes displaying the greatest correlations with the LRRC15 included TARDBP, ANXA5, KHDRBS2, USP15 and DDIT4L. LRRC15 was correlated with these genes mainly in terms of physical interactions. Further GSEA analysis revealed that high expression of LRRC15 showed the greatest correlation with ECM receptor interaction, focal adhesion, regulation of actin cytoskeleton, and TGF Beta signaling pathway. Besides, low expression of LRRC15 showed the greatest correlation with DNA replication, homologous recombination, oxidative phosphorylation and ribosome. Additionally, we further performed transcriptome RNA sequencing to identify the DEGs (differentially expressed genes) between si-LRRC15 and si-NC MDA-MB-231 cells. GO (gene ontology) analysis results showed DEGs were enriched in extracellular space, protein heterodimerization and immune response. In addition, KEGG (Kyoto Encyclopedia of Gene and Genome) analysis showed DEGs were enriched in alcoholism, systemic lupus erythematosus and transcriptional misregulation in cancer.

Discussion

Herein, we conducted a comprehensive assessment of the correlation between LRRC15 and TME of BCa. LRRC15 primarily had effects on regulation tumor growth and immunity of BCa. ABBV-085, a novel antibody-drug conjugate (ADC) targeting LRRC15, is conjugated to the tubulin inhibitor monomethyl auristatin E (MMAE) and has indicated distinct anti-tumor activity in multiple tumors [22–24]. LRRC15 played a vital role in regulating immune cell infiltration in BCa, and the degree of immune infiltration remarkably influenced cancer survival. Previous studies demonstrated that immune cells in the TME could be used in the prognostic assessment of multiple malignancies, such as BCa, glioblastoma, and melanoma [25–27]. Tang et.al reported that LRRC15 acted in collusion with macrophages in the TME of glioblastoma to prompt poor prognosis, especially in mesenchymal subtype [28]. This was consistent with our analysis results: macrophages were most associated with LRRC15 among various immune infiltration cells. It indicated targeting LRRC15 to enhance macrophages-based immunosuppressive effects might be promising strategies for cancer treatments. Furthermore, other MMAE-linked ADCs have been assumed to augment immunogenic cell death and have displayed evidence of enhance response rates when combined with immunomodulatory anti-PD-1 antibody therapy [29]. Therefore, a promising hypothesis for future research is to combine ABBV-085 with anti-PD-1 treatment to augment tumor responses.

LRRC15 is capable of binding collagen, which sustains its role in tissue structure and cell-to-cell adhesion [7]. It has been proposed that extracellular matrix (ECM) proteins generated by CAF prevent the effective uptake of traditional chemotherapeutics, and contribute to the immunosuppressive environment and chemoresistance in most solid tumors [8, 30, 31]. As a biomarker of CAF, LRRC15 might be an important contributor, and also an effective therapeutic target of immunosuppression and chemoresistance. LRRC15 was also an essential mediator for osteogenesis of mesenchymal stem cells (MSCs) through repressing the NF- κ B pathway in a p65-dependent manner, giving a novel hint for MSC-mediated bone regeneration [32].

Conclusions

In summary, our results provided insights into understanding the potential role of LRRC15 mRNA in tumor immunology, prognostic value and enrichment pathways. LRRC15 mRNA level was correlated with immune infiltrating levels, indicating that it might participate in tumor progression and exert immunotherapeutic effects on BCa. The potential for LRRC15 inhibitors to interfere with tumor immune microenvironment deserved further and deepgoing investigations.

Declarations

Author Contributions

Concept, drafting: LL and XWX. Figure making: FYJ, LMX, and WGQ. Figure making: SXY. Revision CW and LJY. Supervision: TJH. All authors contributed to the article and approved the submitted version.

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None.

Competing interests

The authors declare no potential conflict of interest. All the datasets were retrieved from the published literature, so it was confirmed that all written informed consent was obtained.

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Figures

Figure 1

LRRC15 was overexpressed in BCa cells and tissues (A) LRRC15 mRNA expression level in data sets of diverse cancers compared with normal tissues from the Oncomine database. The numbers in the colored

squares display the number of the involved studies. The color intensity is directly proportional to the high or low expression level, respectively. Red indicates high expression, while blue indicates low expression. (B) Relative mRNA expression of LRRC15 in pan-cancer based on TCGA data mining. (C) mRNA expression of LRRC15 in from GEPIA database. (D) Protein expression of LRRC15 in BCa from UALCAN database. (E-F) Endogenous expression of LRRC15 in MCF-10A and multiple BCa cell lines were detected by Western blotting. (G) Representative immunohistochemistry images of LRRC15 in BCa and normal tissues derived from the HPA database. (H) LRRC15 protein levels in 7 paired BCa tissues and their adjacent noncancerous tissues were examined by Western blotting.

Figure 2

Expression levels of LRRC15 in BCa tissues was from different subtypes (A) LRRC15 mRNA expression in four major molecular subtypes of BCa from UALCAN database. (B) LRRC15 mRNA expression in four major molecular subtypes of BCa from UALCAN database. Representative images of immunohistochemical staining in (C) normal tissues (D) luminal A (E) luminal B (F) HER2 (G)TNBC BCa

tissues. LRRC15 mRNA expression in (H) invasive ductal BCa (I) invasive lobular BCa (J) invasive ductal and lobular BCa (K) invasive BCa from oncomine database.

Figure 3

Association of mRNA expression of LRRC15 with prognosis and clinicopathological parameters in BCa patients (A) Relationship between LRRC15 mRNA expression and the OS of BCa patients. (B) Relationship between LRRC15 protein expression and the OS of BCa patients. Prognosis of LRRC15 mRNA expression in different molecular subtypes of BCa: (C) luminal A (D) luminal B (E) HER2 (F) TNBC

BCa tissues. (G) Association between LRRC15 expression and clinicopathological parameters in BCa patients.

Figure 4

Correlation of LRRC15 expression with immunity in BCa. Immune infiltration analysis based on TIMER database in (A) Breast invasive carcinoma, (B) TNBC, (C) HER2 and (D) luminal subtypes. (E) Effect of CNA of LRRC15 expression on immune infiltration level in BCa. Correlation of LRRC15 expression with (F)

MSI (G) TMB, (H) immune checkpoints and (I) 22 immune cell subtypes. TMB, tumor mutational burden; MSI, microsatellite instability.

Figure 5

LRRC15 was positively correlated with cell proliferation of BCa. Cell viability was examined CCK-8 assay in (A) MDA-MB-231 cells and (B) BT-474 cells. (C) Representative examples of LRRC15 immunohistochemistry (IHC) staining in BCa tissues. IHC analysis of LRRC15 and Ki-67 levels in BCa tissues with low or high expression. It revealed that LRRC15 was positively correlated with Ki-67 in situ. (D-F) Cell cycle analyzed by flow cytometry in BCa.

Figure 6

Gene-gene interaction network and enrichment pathway of LRR15 in BCa patients (A) Gene-gene interaction network of LRR15 from Genemania database. Each node represents a gene, and the inter-node connection lines represent the types of gene-gene interactions. The node size represents the strength of interactions. The node color represents the possible functions of respective genes. (B) The enriched KEGG pathway sets by the high LRR15 expression. (C) The enriched KEGG pathway sets by the low LRR15 expression. (D) GO enrichment analysis of DEGs between si-LRR15 and si-NC MDA-MB-231 cells. (E) KEGG enrichment analysis of DEGs between si-LRR15 and si-NC MDA-MB-231 cells. GO: gene ontology; KEGG: Kyoto Encyclopedia of Gene and Genome; DEGs: Differentially expressed genes.