Identification and validation of autophagy-related lncRNA prognostic signature for breast cancer

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Abstract

Background: Long noncoding RNAs (lncRNAs) are emerging as crucial regulators to the development of breast cancer and are involved in controlling autophagy. lncRNAs are also widely known as valuable prognostic factors for breast cancer patients. It is critical to identify autophagy-related lncRNAs with prognostic value in breast cancer.

Methods: A coexpression network of autophagy-related mRNAs-lncRNAs from The Cancer Genome Atlas (TCGA) was constructed. Univariate and multivariate Cox proportional hazards analyses were used to identify an autophagy risk model with prognostic value. Kaplan-Meier analysis, univariate and multivariate Cox regression

analyses and time-dependent receiver operating characteristic (ROC) curve analysis were performed to validate the risk model. Principal component analysis (PCA) and Gene Set Enrichment Analysis (GSEA) functional annotation were conducted to analyze the risk model.

Results: In this study, autophagy-related lncRNAs in breast cancer were identified. We evaluated the prognostic value of these autophagy-related lncRNAs and eventually obtained a prognostic risk model consisting of 11 autophagy-related lncRNAs (U62317.4, LINC01016, LINC02166, C6orf99, LINC00992, BAIAP2-DT, AC245297.3, AC090912.1, Z68871.1, LINC00578 and LINC01871). The risk model was further verified as a novel independent prognostic factor for breast cancer patients based on the calculated risk score. Moreover, based on the risk model, the low risk and high risk groups displayed different autophagy and oncogenic statuses.

Conclusions: These findings suggested that the risk model of the 11 autophagy-related lncRNAs has significant prognostic value for breast cancer and might be a promising prognostic signature and therapeutic targets in clinical practice.

Background

Breast cancer is the most frequent malignancy and the leading cause of cancer-associated mortality in women worldwide [1, 2]. In clinical practice, individual specific targeted therapy has attracted more and more attention. Thus, exploring potential prognostic biomarkers and promising targets is regarded to be an essential step to achieve this goal.

Autophagy is a highly conserved process to maintain cellular homeostasis by lysosomal degradation system [3]. Autophagy plays a crucial role in many physiological processes and various pathological events, including stress and starvation adaptation, metabolism, inflammation, neurodegenerative disorders and cancer [4–7]. Over the past few years, an increasing number of studies have indicated that autophagy participates in the development and progression of breast cancer [8, 9]. Therefore, identifying key regulators of autophagy is of great importance for both theoretical basis and clinical practice.

Long noncoding RNAs (lncRNAs) are a class of transcript RNAs longer than 200 nucleotides without the capacity of protein-coding [10]. LncRNAs are considered as one of the most sensitive and specific cancer biomarkers, which participate in the development and progression of various cancers at different levels including epigenetic, transcriptional and posttranscriptional regulation [11–14]. Moreover, accumulating studies have suggested that lncRNAs promote cancer progression and predict worse prognosis in numerous cancers via regulating autophagy [15–17]. Therefore, it is valuable to identify key lncRNAs closely related to autophagy and prognosis in breast cancer.

In the present study, we analyzed a dataset of lncRNA expression in breast cancers from The Cancer Genome Atlas (TCGA) and screened out autophagy-related lncRNAs with prognostic value. We identified an eleven autophagy-related lncRNA signature with the potential to predict the survival prognosis of breast cancer patients.

Methods

Patient data sets

Clinical information and pathology records of patients with breast cancer were taken from the TCGA (<https://cancergenome.nih.gov/>). The edgeR package was used to normalize gene expression. A total of 1053 TCGA female breast cancer patients with lncRNA expression profiles were utilized in the present study.

Among them, 986 patients with complete follow-up information and survival time ≥ 30 days and 539 patients with complete clinicopathological data were applied to subsequent analyses. The clinical characteristics are detailed in Table 1.

Table 1
Clinical pathological parameters of patients with breast cancer

Feature	N (539)	%
Age (years)		
> 60	227	42.1
≤ 60	312	57.9
T classification		
T1 (< 2 cm)	147	27.3
T2 (2-5cm)	323	59.9
T3 (≥ 5 cm)	55	10.2
T4 (chest wall and/or skin invasion)	14	2.6
N classification (pN)		
N0 (no metastasis)	259	48.1
N1 (1–3 metastasis)	178	33
N2 (4–9 metastasis)	64	11.9
N3 (≥ 10 metastasis)	38	7
M classification		
M0 (no distantmetastasis)	528	98
M1 (distant metastasis)	11	2
TNM stage		
I	96	17.8
II	318	59
III	114	21.2
IV	11	2
ER		
Negative	127	23.6

T: Tumor size; N: Lymph node; M: Distant metastasis;

TNM stage: According to AJCC 8th classification;

TNBC: Triple-negative breast cancer.

Feature	N (539)	%
Positive	412	76.4
PR		
Negative	175	32.5
Positive	364	67.5
HER2		
Negative	440	81.6
Positive	99	18.4
Molecular subtypes		
HER2 amplification	92	17.1
Luminal A/B	419	77.7
TNBC	28	5.2
T: Tumor size; N: Lymph node; M: Distant metastasis;		
TNM stage: According to AJCC 8th classification;		
TNBC: Triple-negative breast cancer.		

Identification Of Autophagy-related Lncrnas In Breast Cancer

A total of 395 autophagy-related encoding genes (mRNAs) were extracted from the Molecular Signatures Database of Gene Set Enrichment Analysis (GSEA; M27935, M6328 and M10281). Finally, 912 autophagy-related lncRNAs were identified by constructing autophagy-related mRNA-lncRNA coexpression networks according to the criteria of $|\text{Correlation Coefficient}| > 0.4$ and $p < 0.001$ using the Limma R package.

Identification Of Autophagy-related Lncrna Prognostic Signatures For Breast Cancer

To identify autophagy-related lncRNAs associated with survival, univariate Cox proportional hazards analysis was performed with $p < 0.01$ as the criteria and multivariate Cox analysis was subsequently performed to establish the optimal prognostic risk model based on the Akaike information criterion (AIC = 1444.62), using the Survival R package. Then, the risk score for each patient was calculated based on the following formula:

Risk score = coef (lncRNA1) × expr (lncRNA1) + coef (lncRNA2) × expr (lncRNA2) +.....+ coef (lncRNA_n) × expr (lncRNA_n)

coef (lncRNA_n) was defined as the coefficient of lncRNAs correlated with survival.

expr (lncRNA_n) was defined as the expression of lncRNAs.

Breast cancer patients in the TCGA were divided into a high risk group and a low risk group according to the median risk score. Kaplan-Meier survival analysis was conducted to evaluate the survival difference between the two groups using the Survival and survminer R packages.

Independent Prognostic Analysis And Roc Curve Plotting

Univariate and multivariate Cox regression analyses were performed to assess the relationship between survival prognosis and age; estrogen receptor (ER) expression; progesterone receptor (PR) expression; human epidermal growth factor receptor (HER2) expression; Tumor, Node, Metastasis (TNM) stage; tumor size; lymph node metastasis; distant metastasis; and risk score using the Survival R package. Time-dependent receiver operating characteristic (ROC) curves were plotted to evaluate the predictive accuracy for survival time through different clinical pathological factors and risk scores using the survivalROC R package.

Statistical analysis

All statistical analyses were performed using R software (version 3.6.2). The correlation between the expression of autophagy-related lncRNAs and clinicopathological factors was analyzed by ggpubr R package. Principal component analysis (PCA) was performed for effective dimension reduction, pattern recognition, and exploratory visualization of high-dimensional data of the whole-genome, autophagy-related encoding genes and the risk model of the autophagy-related lncRNAs expression profiles, respectively [18, 19]. Gene Set Enrichment Analysis (GSEA) was used for functional annotation. GSEA (<https://www.gsea-msigdb.org/gsea/index.jsp>) is a powerful analytical approach for interpreting genome-wide expression profiles [20]. GSEA focuses on gene sets rather than just high scoring genes, which can detect biological processes such as several cancer-related pathways, metabolic pathways, transcriptional programs, and stress responses, and so on. GSEA tends to be more reproducible and more interpretable to analyze molecular profiling data. Two-tailed $p < 0.05$ was considered statistically significant.

Results

Identification of autophagy-related lncRNAs with significant prognostic value in breast cancer

A total of 912 autophagy-related lncRNAs were obtained by constructing the coexpression networks with 395 autophagy-related encoding genes (mRNAs). Among them, 24 autophagy-related lncRNAs were significantly associated with the survival of breast cancer patients from the TCGA ($p < 0.01$) by Cox proportional-hazards analysis, including 19 lncRNAs with low risk (hazard ratio (HR) < 1) and 5 lncRNAs with high risk (hazard ratio (HR) > 1) (Fig. 1a). Subsequently, multivariate Cox analysis further screened 11 lncRNAs from the above 24 BCSCs-related lncRNAs with prognostic significance, namely, U62317.4, LINC01016, LINC02166, C6orf99, LINC00992, BAIAP2-DT, AC245297.3, AC090912.1, Z68871.1, LINC00578 and LINC01871 (Table 2). These 11 lncRNAs were constructed into the optimal prognostic risk model of autophagy-related lncRNAs. As shown in Fig. 1b, a visualization coexpression network of autophagy-related lncRNAs prognostic risk model with autophagy-related mRNAs was established. According to the risk score formula and the calculated median risk score, breast cancer patients were divided into a high risk group and a low risk group. Kaplan-Meier survival analysis showed that the high risk group presented worse overall survival (OS) than the low risk group ($p = 3.048E-10$) (Fig. 2a), suggesting that the risk score had prognostic value. The risk curve and scatterplot were made to illustrate the risk score and the corresponding survival status of breast cancer patients. The results showed that the higher the risk score was, the more mortality occurred (Fig. 2b-c). The heatmap of the expression of these 11 autophagy-related lncRNAs expression in breast cancer samples showed that C6orf99, LINC00992, Z68871.1 and LINC00578 were upregulated in the high risk group, while U62317.4, LINC01016, LINC02166, BAIAP2-DT, AC245297.3, AC090912.1 and LINC01871 were highly expressed in the low risk group (Fig. 2d). Therefore, these above studies identified 11 autophagy-related lncRNAs with prognostic significance for breast cancer.

Table 2

The risk model of selected 11 autophagy-related lncRNAs with prognostic value for breast cancer by multivariate Cox proportional-hazards analysis

ID	coef	HR	HR.95L	HR.95H	p-value	risk
U62317.4	-0.308	0.735	0.488	1.106	0.140	low
LINC01016	-0.173	0.842	0.678	1.045	0.119	low
LINC02166	-0.596	0.551	0.292	1.041	0.066	low
C6orf99	0.284	1.329	1.017	1.738	0.038	high
LINC00992	0.188	1.207	0.988	1.473	0.065	high
BAIAP2-DT	-0.385	0.680	0.525	0.882	0.004	low
AC245297.3	-0.381	0.683	0.520	0.897	0.006	low
AC090912.1	-0.475	0.622	0.342	1.131	0.119	low
Z68871.1	0.573	1.774	1.133	2.778	0.012	high
LINC00579	0.146	1.158	0.979	1.369	0.087	high
LINC01871	-0.417	0.659	0.495	0.878	0.004	low

Evaluation of the risk model of the 11 autophagy-related lncRNAs as an independent prognostic factor for breast cancer patients

Univariate and multivariate Cox regression analyses were performed to assess whether the risk model of the above 11 autophagy-related lncRNAs was an independent prognostic factor for breast cancer. The hazard ratio (HR) of the risk score and 95% CI were 1.507 and 1.308–1.736 ($p < 0.001$) in univariate Cox regression analysis (Fig. 3a), and 1.489 and 1.280–1.732 ($p < 0.001$) in multivariate Cox regression analysis (Fig. 3b), respectively, indicating that the risk model of the 11 autophagy-related lncRNAs was the most significant prognostic factor for breast cancer, independent of clinicopathological parameters such as age, ER expression, PR expression, HER2 expression, molecular subtypes, TNM stage, tumor size, lymph node metastasis and distant metastasis. To evaluate the predictive specificity and sensitivity of the risk score on the prognosis of breast cancer patients, the area under the ROC curve (AUC) of the risk score was estimated. The AUC of the risk score was 0.774, similar to the AUC of tumor size, followed by the AUC of age and more than the AUCs of other clinicopathological factors (Fig. 3c), suggesting that the prognostic risk model of the 11 autophagy-related lncRNAs for breast cancer was considerably reliable. All of the above results indicated that the 11 autophagy-related lncRNAs were significant independent prognostic factors for breast cancer patients.

Correlation of the expression of the 11 autophagy-related lncRNAs expression with clinicopathological factors

To further investigate whether the 11 autophagy-related lncRNAs were involved in the development of breast cancer, we assessed the association of the expression of the 11 autophagy-related lncRNAs with clinicopathological factors. There was a significant correlation between the 11 autophagy-related lncRNAs and ER expression, PR expression, HER2 expression and molecular subtypes, as shown in Fig. 4.

Different stemness statuses in the low risk and high risk groups

PCA was performed to compare the difference between low-risk and high-risk groups based on the risk model of the 11 autophagy-related lncRNAs, 395 autophagy-related encoding genes and the whole-genome expression profiles from the TCGA, respectively (Fig. 5). The results showed that the low risk and high risk groups were distributed in distinct directions, prior to the other two patterns, suggesting that the risk model could divide breast cancer patients into two parts and that the autophagy status of breast cancer patients in the high risk group differed from those in the low risk group. Functional annotation was further conducted using GSEA, and the results displayed that the differentially expressed genes between the high risk and low risk groups based on the risk model of the 11 autophagy-related genes were enrichment in autophagy processes and oncogenic signatures (Fig. 6). Above these, it all indicated that the low risk and high risk groups showed different autophagy and oncogenic statuses.

Discussion

In the field of clinical treatment, although the OS of breast cancer patients has gained great improvements, metastasis and recurrence of breast cancer have constantly grown, which were the major causes of breast cancer mortality. Considerable evidence has revealed that the role of autophagy in the development of cancer is a double-edged sword. That is, autophagy may serve as either a pro-survival or pro-death mechanism under different circumstances [21, 22]. Consistent with the theory, autophagy functioned dual roles in breast cancer. Autophagy induced the recurrence of metastatic breast cancer by elevating the survival of dormant breast cancer cells [8]. Cytostatic autophagy suppressed the proliferation and metastasis of triple-negative breast cancer cells [9]. Furthermore, numerous researches indicated the crucial role of lncRNAs in autophagy-inducing progression or inhibition of various cancers, such as hepatocellular carcinoma, lung cancer, breast cancer [23–25]. Based on the above, it led us to find potential specific lncRNAs associated with autophagy and the survival prognosis. In this study, we identified the risk model of the 11 autophagy-related lncRNAs as an independent prognostic factor for breast cancer. So far, among these 11 autophagy-related lncRNAs, only LINC01016 and LINC00578 have been studied in breast cancer or other cancers. LINC01016-miR-302a-3p/miR-3130-3p/NFYA/SATB1 axis plays a crucial role in the occurrence of endometrial cancer [26]. In addition, LINC01016 is a direct target of ER α , associated with positive survival prognosis of breast cancer [27]. It has been reported that LINC00578 is associated with poor OS in pancreatic cancer and lung adenocarcinoma [28, 29]. In accordance with our results, LINC01016 was low-risk autophagy-related lncRNA and LINC00578 was high-risk autophagy-related lncRNA, both with prognostic value in breast cancer patients. Breast cancer is a complex disease and highly heterogeneous tumor [30]. To assess survival prognosis and guide individual therapeutic decisions, breast cancer has been divided into distinct molecular subtypes based

primarily on the expression status of hormonal receptors such as ER, PR, HER2 and Ki67 (tumor proliferation index) as follows: luminal A/B (ER and/or PR positive), HER2 enriched (HER2 positive) and triple-negative breast cancer (ER, PR and HER2 negative) [31]. It is also well known that ER and/or PR positive indicates an effective endocrine therapy outcome and better survival prognosis; HER2 positive represents a more aggressive phenotype but is sensitive to HER2-targeted therapy; triple-negative is enriched with breast cancer stem cells and associated with a worse prognosis due to the lack of effective therapeutic targets [32, 33]. Thus, distinct ER, PR and HER2 statuses indicated different biological processes of breast cancer and survival outcomes. The significant correlations of the 11 autophagy-related lncRNAs with ER expression, PR expression, HER2 expression and molecular subtypes indicated that the 11 autophagy-related lncRNAs might be involved in regulating ER, PR and HER2 gene expression, further affecting molecular subtype formation. More interestingly, there were no significant relationship between most of the 11 autophagy-related lncRNAs and tumor size (T), lymph node (N) status, and TNM stage, indicating that the risk model of the 11 autophagy-related lncRNAs has no close correlation with the sooner or later for finding and diagnosis of breast cancer, but only strongly linked to the intrinsic biological characteristics of distinct subtypes of breast cancer. Our results also showed that the risk model of the 11 autophagy-related lncRNAs was the most statistically significant prognostic signature compared with other clinicopathological factors, and the ROC result confirmed that the risk model is reliable. Combined with the AUC of the risk model score, these results all indicated that the risk model of the 11 autophagy-related lncRNAs had superior prognostic value to other clinicopathological factors. The results of PCA and GSEA functional annotation illustrated that the high risk and low risk groups showed different distribution directions and aggregation centers based on the risk model, rather than the whole-genome profiles and autophagy genes expression, indicating that the significant difference in OS between the high risk and low risk groups might result from different autophagy and oncogenic statuses induced by the risk model of the 11 autophagy-related lncRNAs. These results also suggested that the risk model of the 11 autophagy-related lncRNAs represented more significant autophagy characteristics than autophagy-related genes. Taken together, these results suggested that the prognostic signature of the 11 autophagy-related lncRNAs might be a feasible independent prognostic factor for breast cancer in clinical practice. To date, a key challenge of precision genomic medicine is to make reliable and accurate predictions of clinical outcomes from high-dimensional molecular data [34]. To solve this problem, there have been some advances in the Cox regression with prognostic value in recent years. A Cox elastic net has been used in objective and data-driven feature selection with time-to-event data [35]. Cox-nnet is an artificial neural network approach that has been utilized in predicting low-dimensional survival prognosis [36]. Bayesian-optimized deep survival models (SurvivalNet models) have successfully improved the accuracy of prognostic prediction for high-dimensional cancer genomic profiles [37]. In addition, Cox-nnet produces a better performance than SurvivalNet models, and SurvivalNet models provide comparable performance to the Cox elastic net [37, 38]. Moreover, Cox-PASNet, which is a novel pathway-based sparse deep neural network for survival analysis that integrates high-dimensional genomic data and clinical data, has been applied to identify significant prognostic factors [38]. However, our study has some limitations. We applied univariate and multivariate Cox proportional-hazards analysis to establish and estimate the prognostic value of the risk model of 11 autophagy-related lncRNAs. Although the

method has been approved and employed in many researches, it is necessary to improve our further study with more advanced methodologies and technologies in future. To further validate our bioinformatics prediction results, in-depth studies on the 11 autophagy-related lncRNAs, including functional experiments and molecular mechanisms, are needed.

Conclusions

In conclusion, we identified an autophagy-related lncRNAs signature consisting of 11 lncRNAs (U62317.4, LINC01016, LINC02166, C6orf99, LINC00992, BAIAP2-DT, AC245297.3, AC090912.1, Z68871.1, LINC00578 and LINC01871), which can act as a novel independent prognostic factor for breast cancer. In the future, with prospective validation, the 11 autophagy-related lncRNAs signature may improve the predictive accuracy and guide the individual specific therapy for patients with breast cancer.

Abbreviations

lncRNAs

Long noncoding RNAs; TCGA: The Cancer Genome Atlas; ROC: Receiver operating characteristics; PCA: Principal components analysis; GSEA: Gene Set Enrichment Analysis; OS: Overall survival; HR: Hazard ratio; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2.

Declarations

Acknowledgement

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Authors' Contributions

Xiaoying Li, Feng Jin and Yang Li designed the study and conceived the project. Xiaoying Li acquired the data and analyzed the data. Xiaoying Li drafted the manuscript. Yang Li and Feng Jin revised the manuscript. All authors approved the final version to be published.

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Availability of data and materials

All data utilized in this study are included in this article and all data supporting the findings of this study are available on reasonable request from the corresponding author.

Competing interests

The authors declare no conflicts of interest.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

Figure 1

Identification of autophagy-related lncRNAs with significant prognostic value in breast cancer. a The forest showed the HR (95%CI) and p-value of selected lncRNAs by univariate Cox proportional-hazards

analysis. b A visualization coexpression network of the prognostic risk model of the 11 autophagy-related lncRNAs with autophagy-related mRNAs.

Figure 2

The prognostic value of the risk model of the 11 autophagy-related lncRNAs in the TCGA cohort. a Kaplan-Meier survival analysis of the high risk and low risk groups based on the risk model and median risk score. b The risk curve based on the risk score of each sample. c The scatterplot based on the survival status of each sample. The green and red dots represent survival and death, respectively. d The heatmap displayed the expression levels of autophagy-related lncRNAs in the high risk and low risk groups.

Figure 3

Assessment of the prognostic risk model of the 11 autophagy-related lncRNAs for breast cancer. a The univariate and b multivariate Cox regression analysis of risk model score and clinical features regarding prognostic value. c The AUC for risk model score and clinical features according to the ROC curves. Clinical features: age, ER, PR, HER2, Subtypes (molecular subtypes), TNM stage, T (tumor size), N (lymph node metastasis) and M (distant metastasis).

Figure 4

The correlation of the expression of the 11 autophagy-related lncRNAs with clinicopathological factors. a ER expression. b PR expression. c HER2 expression. d Subtypes (LuminalA/B; HER2 amplification; TNBC: triple-negative breast cancer). e TNM stage. f Tumor size (T1: <2cm; T2: $\geq$ 2cm and <5cm; T3: $\geq$ 5cm; T4: invasion of chest wall and/or skin). g N classification (N0: no lymph node metastasis; N1: 1-3 lymph node metastasis; N2: 4-9 lymph node metastasis; N3: $\geq$ 10 lymph node metastasis). h M classification

(M0: no distant metastasis; M1: distant metastasis). ns: no statistical significance, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Figure 5

The low risk and high risk groups displayed different autophagy statuses. a-c Principal components analysis (PCA) between low-risk and high-risk groups based on the whole-genome, autophagy-related encoding genes and the risk model of the 11 autophagy-related lncRNAs expression profiles.

Figure 6

Functional enrichment analysis based on the risk model of the 11 autophagy-related lncRNAs by GSEA. Significantly enriched autophagy of Gene Ontology (GO), KEGG pathways and oncogenic signatures in the low risk group.