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Elucidating the molecular basis of ATP-induced cell death in breast cancer: construction and validation of a robust prognostic model

ATP-induced cell death in breast cancer

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Abstract

BACKGROUND

Breast cancer is a multifaceted and formidable disease with profound public health implications. Cell demise mechanisms **play** a pivotal role in breast cancer pathogenesis, with ATP-triggered cell death **attracting** mounting interest for its unique specificity and potential therapeutic pertinence.

AIM

Elucidating the Molecular Basis of ATP-Induced Cell Death in Breast Cancer: Construction and Validation of a Robust Prognostic Model

METHODS

The foundational genes orchestrating ATP-induced cell death mechanisms were **extracted** from **the** scholarly literature, underpinning the establishment of a prognostic model. Simultaneously, a miRNA prognostic model was **constructed that mirrored** the

gene-based prognostic model. Distinctions between high and low-risk cohorts within mRNA and miRNA characteristic models were scrutinized, with the aim of delineating common influence mechanisms, substantiated through enrichment analysis and immune infiltration assessment.

RESULTS

The mRNA prognostic model in this study encompassed four specific mRNAs—P2X4, PNAX1, CASP7, and CCND2. The miRNA prognostic model integrated four pivotal miRNAs—hsa-miR-615-3p, hsa-miR-519b-3p, hsa-miR-342-3p, and hsa-miR-324-3p. B cells, CD4+ T cells, CD8+ T cells, endothelial cells, and macrophages exhibited inverse correlations with risk scores across all breast cancer subtypes. Furthermore, KEGG analysis revealed that genes differentially expressed in response to mRNA risk scores significantly enriched 25 signalling pathways, while miRNA risk scores significantly enriched 29 signalling pathways, with 16 pathways being jointly enriched.

CONCLUSION

Of paramount significance, distinct mRNA and miRNA signature models were devised tailored to ATP-induced cell death, both potentially autonomous prognostic factors. This study's elucidation of the molecular underpinnings of ATP-induced cell death in breast cancer enhances the arsenal of potential therapeutic tools, offering an unparalleled window for innovative interventions. Essentially, this paper unveils the hitherto enigmatic link between ATP-induced cell death and breast cancer, potentially leading to revolutionary progress in personalized oncology.

Key Words: ATP-Induced Cell Death; mRNA; miRNA; Prognostic Model; Breast Cancer

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Core Tip: This study carefully developed and validated a prognostic model based on the intricately underlying genes associated with ATP-induced cell death. At the same time, we cleverly shaped a miRNA prognostic model that harmonizes with a gene-centric approach. This study comprehensively elucidates the complex molecular basis of ATP-induced breast cancer cell death, which not only opens up new avenues for potential therapeutic interventions, but also promotes an innovative paradigm in the field of personalized oncology. By enhancing our understanding of this complex cellular process, we anticipate that our research will facilitate the emergence of groundbreaking strategies that are transformative."

INTRODUCTION

³ ATP-Induced Cell Death (AICD) has emerged as a distinctive mode of cell death triggered by elevated extracellular adenosine triphosphate (ATP) levels and is closely associated with the progression of various cancer types^[1-4]. Cell death serves as a critical process in maintaining the normal functionality of tissues and organs, primarily presenting in two distinct forms, apoptosis, and necrosis^[5, 6]. AICD exerts a dual impact on breast cancer: firstly, ATP acts as a pro-death signal, participating in the regulation of the activation of death pathways; secondly, cell death itself leads to an escalation in extracellular ATP levels, forming an interconnected regulatory loop^[7-11]. Manipulation of ATP levels and ATP receptors in the context of breast cancer can alter biological activities such as breast cancer cell proliferation, invasion, and metastasis^[12-15]. Investigations have suggested that AICD is a pivotal mechanism regulated by both genes and microRNAs (miRNAs), contributing to the modulation of breast cancer^[16-19]. However, the precise nature of interactions requires further research. Hence, in-depth exploration of the mechanisms underlying AICD holds promising potential for its application in breast cancer treatment. Unraveling the implications of AICD in breast

cancer therapy could pave the way for novel therapeutic strategies and targeted interventions.

AICD is a multifaceted process, its intricacy being contingent upon the specific cell type and the surrounding microenvironment^[20-23]. Nonetheless, several overarching mechanisms have been revealed. Firstly, the activation of purinergic receptors, particularly the P2X7 receptor(P2X7R), emerges as a pivotal mechanism, triggering a cascade of events culminating in cell death^[8, 24]. Secondly, a significant mechanism of AICD involves the elevation of intracellular calcium ion concentration^[25, 26]. Moreover, AICD may also be linked to the activation of inflammatory responses^[8]. Finally, the disruption of mitochondrial function is crucial in AICD, with cytochrome c release intimately connected with the activation of apoptosis signalling pathways^[27-29].

Genes, and genetic factors, encompass nucleotide sequences that encode either a polypeptide chain or functional RNA, thereby supporting fundamental life processes. They serve as repositories of an organism's genetic information, encompassing data pertaining to its race, blood type, reproduction, growth, apoptosis, and other critical processes. On the other hand, MiRNAs(miRNA), which are RNA molecules approximately 21 to 23 nucleotides in length, are widely prevalent in eukaryotic organisms^[30]. MiRNAs function as key regulators of gene expression, deriving from RNA transcribed by DNA but incapable of being translated into proteins (non-coding RNAs). Through specific binding to target messenger ribonucleic acid (mRNA), miRNAs can exert post-transcriptional control over gene expression, playing pivotal roles in the regulation of gene expression, cell cycle, and developmental sequencing in organisms. Notably, in animals, a single miRNA often possesses the capacity to regulate numerous genes simultaneously.

The association between miRNAs and cancer has garnered significant recent interest^[31]. miRNAs exert a crucial impact on cancer initiation, progression, and therapeutic responses, exhibiting dichotomous effects that either facilitate tumorigenesis, growth, and metastasis, or which impede tumour formation and development^[32-35]. The functional roles of miRNAs may vary across different cancer

types, with certain miRNAs functioning as tumour suppressors to inhibit cancer growth in specific contexts, while others may function as tumour promoters, promoting cancer cell proliferation and metastasis.

The relationship between AICD and miRNA is governed by two distinct mechanisms. Firstly, certain miRNAs have the capacity to modulate cell response to ATP by targetting essential genes involved in ATP-related signalling pathways or cell death pathways. Conversely, alterations in ATP levels can prompt cells to adjust the expression patterns of miRNAs, thereby influencing cell survival and death. This reciprocal regulatory mechanism may hold particular significance in tumour cells, as it can influence tumour cell proliferation or apoptosis, consequently impacting tumour development and prognosis. Nevertheless, the current body of research concerning AICD-related miRNAs and their corresponding gene regulation in breast cancer remains limited. Although increasing evidence highlights the considerable diagnostic and prognostic value of AICD and miRNA in breast cancer^[18, 20], the precise regulatory mechanisms and associated biological processes require further exploration and elucidation.

A prognostic model (Signature) is a predictive model designed to estimate potential treatment outcomes based on a patient's current health status. Genetic prognostic models aim to forecast disease progression and survival by utilizing a patient's gene expression data, thereby offering more precise and personalized information to guide clinical decision-making. The miRNA prognostic model facilitates more accurate prognosis evaluation by healthcare professionals and aids in making informed treatment choices. Gene prognostic models have certain limitations, including high-dimensional data, overfitting issues, data consistency concerns, and challenges in biological interpretation. However, the miRNA prognostic model is characterized by the inherent instability of miRNA expression data, data quality problems, miRNA-gene interactions, and limitations in study samples. These factors collectively pose challenges for miRNA prognostic models to thoroughly elucidate disease mechanisms. Considering the strengths and weaknesses outlined above, the simultaneous utilization

of both gene prognostic models and miRNA prognostic models represents a promising research direction. By harnessing the advantages of both approaches, the prognostic features of cancer can be more effectively explored, providing a more robust tool for personalized treatment and prognosis assessment.

However, no studies to date have investigated the impact of AICD regulatory mechanisms in breast cancer. Therefore, the primary goal of this paper is to look into the potential prognostic importance of AICD genes in breast cancer, as well as the interaction among AICD genes and prognostic gene-associated miRNAs in breast cancer. Initially, a comprehensive literature search was conducted to identify genes associated with AICD, and subsequently, a novel AICD signature with prognostic value was constructed independently of classical clinicopathological parameters. Subsequent functional enrichment and immunoinfiltration analysis were conducted to further elucidate ¹ the role of AICD in regulating fundamental biological processes in breast cancer. The findings of this study show that AICD could be a potential candidate for breast cancer detection and therapeutic intervention, opening up a new research channel and perspective for breast cancer diagnostics and treatment. This discovery holds promise in providing valuable insights for precision treatment and accurate prognosis assessment of breast cancer.

MATERIALS AND METHODS

Literature search of AICD core genes

The literature search aimed to identify core genes associated with AICD using the following keywords: "AICD," "cell death," "apoptosis," "autophagy," "necrosis," "death," and "extracellular ATP." The search was conducted between March 10, 2023, and June 20, 2023. Databases including PubMed, Embase, Web of Science, MESH, and Scopus were employed to identify genes related to AICD. The scope of the search was expanded to encompass genes associated with AICD across all diseases, given the limited availability of literature reporting AICD specifically in breast cancer. Publications that did not mention AICD were excluded from the analysis.

Source of datasets

The miRNA and mRNA expression profiles, along with clinical information, were obtained from the UCSC Xena platform, sourced from the Cancer Genome Atlas Breast Cancer dataset (TCGA-BRCA). To establish and validate the models, the dataset was randomly partitioned into a training set (70%) and an internal validation set (30%). Furthermore, additional miRNA and mRNA expression profile data was obtained, as well as clinical information, from the meatbriic database for external validation. During the analysis, samples with missing clinical information were carefully excluded, and samples with a survival period of less than 10 days were also excluded to ensure the reliability and accuracy of the study.

Construction and validation of mRNA- and miRNA-related prognostic models

In this study, R software version 4.1.0 was used for comprehensive data analysis. Initially, the univariate Cox Proportional Hazards Model (Cox) regression, Least Absolute Shrinkage and Selection Operator (LASSO) regression, and multifactor Cox regression analyses were conducted using the glmnet (version 2.0.18) and survival (version 2.44.1.1) R packages. Univariate Cox regression enabled the assessment of the association between mRNA and miRNA expression levels with overall survival, where P-values less than 0.05 were considered statistically significant. As miRNAs were inferred from mRNAs, the P-value threshold for miRNAs was adjusted to <0.15 to indicate statistical significance.

Following this, LASSO regression analyses were performed on mRNAs and miRNAs that met the specified criteria, further refining the features. Subsequently, the prognostic effect and hazard ratio (HR) of the prediction model were evaluated using multivariate Cox regression analysis, and the 95% confidence intervals (CI) were calculated.

Using the formula $\sum (\text{expmiRNA}_n \times \beta_{\text{mirnan}})$, the prognostic risk score was computed by summing the miRNA expression values and their respective coefficients.

This risk score facilitated the stratification of samples into high-risk and low-risk groups. Kaplan-Meier analysis and bilateral log-rank testing were used with the "survminer" software package to determine the prognostic significance of the risk scores in training, internal validation, whole cohorts, and external validation, with a significance level set at $p < 0.05$.

Additionally, the "timeROC" package was used to produce time-dependent receiver operating characteristic (ROC) curves to evaluate the prediction model's performance, with the area under the curve (AUC) providing an estimate of the model's accuracy. Overall, all above-mentioned indexes achieved p-values less than 0.05, indicating significant differences. These comprehensive analyses will help validate and assess the efficacy of the constructed mRNA and miRNA prognostic models in predicting survival and risk for patients with breast cancer.

mRNAs corresponded to prediction of miRNAs and prediction of target mRNAs at miRNA binding sites

In this study, micrnas-mRNA target prediction was conducted using the miRTarBase database (https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/index.php) on July 21, 2023. Simultaneously, prognostic features of micrnas-targeted mRNA in humans were downloaded from the TargetScan databases (http://www.targetscan.org/vert_80/, accessed on July 23, 2023). Subsequently, differential gene expression analysis was performed between the high-risk and low-risk groups using the "limma" package in R.

For the mRNA prognostic model, differential genes were identified based on the criteria of adjusted p value < 0.05 and $|\text{Log}_2(\text{fold change})| > 1$. Conversely, in the miRNA prognostic model, due to the limited number of differential genes, they were defined as genes with an adjusted p value < 0.05 and $|\text{Log}_2(\text{fold change})| > 0.5$. These differential genes will undergo further analysis to evaluate their functional significance and relevance in the prognostic models.

Functional Enrichment Analysis

In this study, microbial informatics analysis tools (<https://www.bioinformatics.com.cn/>, accessed on July 24, 2023) were employed for conducting gene enrichment analysis, encompassing both gene ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. The criterion for determining statistically significant differences was set at P-value <0.05, which made it possible identify functional items and pathways that exhibited significant enrichment in the GO and KEGG analyses.

Correlation analysis of immunoinfiltration in the mRNA prognostic model

This study utilized the Tumor Immune Estimation Resource (TIMER, <http://timer.cistrome.org/>, accessed on July 24, 2023) to investigate the association between gene expression in the prognostic model and the infiltration of various immune cells in breast cancer. Concurrently, the Clinical Letter Home (https://www.aclbi.com/static/index.html#), accessed on July 24, 2023) website was used to analyze the relationship between the gene expression and breast cancer prognosis models in the context of immune cell infiltration in different classifications. This analysis enables the exploration of the correlation between differential genes in the prognostic model and the tumor immune microenvironment, thereby providing insights into the potential interplay between the prognostic model and immune cell infiltration.

RESULTS

Construct the core mRNA characteristics of AICD

A comprehensive literature search was conducted using ⁵ databases including PubMed(<https://pubmed.ncbi.nlm.nih.gov/>), Embase(Excerpta Medica Database, <https://www.embase.com/>), Web of Science(<https://www.webofscience.com/>), ²¹ MESH(Medical Subject Headings,

<https://www.nlm.nih.gov/mesh/meshhome.html>), and Scopus (<https://www.scopus.com/>), to identify a total of 35 mRNAs that are strongly associated with AICD (Table 1).

Table 1. List of core mRNAs of AICD

According to the univariate Cox proportional hazard regression analysis, 4 of the 35 mRNAs in the training cohort exhibited significant p-values <0.05 (Figure 1A). Utilizing the LASSO-Cox regression analysis, the selection was further narrowed down to 4 mRNAs, which were incorporated into the prognostic model for the training cohort (Figure 1B, C). The construction of the new risk scoring formula based on these selected mRNA features is as follows:

$$\text{Risk score} = 0.344 \times \text{PNAX1} - 0.223 \times \text{P2RX4} - 0.274 \times \text{CASP7} - 0.198 \times \text{CCND2}.$$

Figure 1 illustrates the results obtained from the analyses. In (A), four AICD-related mRNAs are presented with p<0.05 along with their corresponding risk ratios derived from the univariate Cox proportional hazard regression analysis. In (B), the process of tuning parameter (λ) selection for OS-related mRNAs in the LASSO model is shown. Finally, (C) shows the four chosen mRNAs' LASSO coefficient spectra, and the vertical line shows the coefficient values that the LASSO algorithm chose.

mRNA characteristics significantly distinguished between high-risk and low-risk groups

Each patient's risk score was calculated using the accepted formula (Figure 2). Subsequently, these features' robustness was evaluated using training and internal validation cohorts (Figure 3,4). Patients in the high-risk group had shorter overall survival (OS) than those in the low-risk group, according to Kaplan-Meier survival analysis (KM) and bilateral log-rank tests performed on the complete dataset (p<0.001) (Figure 5A).

The prognostic efficacy of this risk score was further validated in the test set and training set cohorts, wherein similar and significant OS differences were observed

between the high-risk and low-risk groups ($p < 0.05$) (Figure 5B,C). Additionally, for the 5-year OS prediction in the training and internal validation populations, the time-varying ROC curve analysis produced AUC values of 0.64 and 0.72, respectively (Figure 5C,D).

Figure 2. Illustrates the TCGA-BRCA entire dataset's risk score distribution and expression heat map. In panel (A), the risk score distribution is illustrated, with pink dots representing the low-risk group and red dots representing the high-risk group. The vertical dotted line represents the median risk score cut-off point. Panel (B) displays the survival time and survival status of all patients. Furthermore, the heat maps in panel (C) show the expression patterns of the four selected genes from the AICD signature.

Figure 3. The remarks are the same as in Figure 2.

Figure 4. The remarks are the same as in Figure 2.

Figure 5 displays time-varying ROC curves and KM survival analyses. In the complete dataset, the KM curve revealed a substantial difference in overall survival among the low-risk and high-risk groups (A), as well as in the training (B) and validation (C) cohorts. The time-varying ROC curve AUC values for the total dataset (A), training group (E), and validation group (F) at 5 years of overall survival were 0.67, 0.64, and 0.72, respectively.

The prognostic significance of 4 mRNA features was verified in the METABRIC dataset

Further validation was conducted in the METABRIC dataset, comprising 1,978 cases of primary breast cancer. The established algorithm was used to compute the risk score for each patient. Subsequently, the robustness of the features was assessed in the validation queue of the METABRIC dataset (Figure 6).

In the METABRIC dataset, significant differences were found in overall survival between the high-risk and low-risk groups, as had been seen in earlier cohorts ($p < 0.001$) (Figure 7A). Additionally, at 1, 3, and 5 years, the time-varying ROC curve AUC values were 0.68, 0.66, and 0.61, respectively (Figure 7B).

Figure 6 illustrates the TCGA-BRCA entire dataset's ² risk score distribution and expression heat map. In panel (A), the risk score distribution is illustrated, with pink dots representing the ² low-risk group and red dots representing the high-risk group. The vertical dotted line represents the median risk score cut-off point. Panel (B) displays the survival time and survival status of all patients. Furthermore, the heat maps in panel (C) show the expression patterns of the four selected genes from the AICD signature.

Figure 7. A: Kaplan-Meier survival analysis and B: ROC curve over time.

Construction of miRNA corresponding to the mRNA characteristics of the AICD core prognosis model

In this investigation, micrnas-mRNA target prediction was conducted utilizing the miRTarBase database as of July 21, 2023. A comprehensive collection of 213 miRNAs was retrieved, after eliminating duplicate entries. Subsequently, through an intersection analysis with the TCGA-BRCA and METABRIC databases, a refined set of 21 miRNAs was obtained (Table 2).

Table 2. List of miRNAs corresponding to mRNA of prognostic model

Construction of mRNA corresponding to the miRNA prognostic model of AICD

A total of 1019 cases of primary breast cancer were extracted from the TCGA-BRCA dataset *via* the UCSC Xena website, after excluding duplicate cases and those with incomplete OS data. Subsequently, these cases were randomly ¹⁰ divided into a training set comprising 70% of the samples ($n = 714$) and an internal validation set comprising 30% of the samples ($n = 305$).

Six of the 21 miRNAs in the training group had univariate Cox proportional hazard regression analysis results with p-values < 0.15 (Figure 8A). Four of the miRNAs were subsequently chosen for inclusion in the training cohort's prognostic model using LASSO-Cox regression analysis (Figure 8B, ¹ C). The resulting risk scoring formula based on these features is as follows:

$$\text{Risk score} = 0.181 \times (\text{hsa-miR-615-3p}) + 0.195 \times (\text{hsa-miR-324-3p}) - 0.333 \times (\text{hsa-miR-342-3p}) - 1.496 \times (\text{hsa-miR-519b-3p})$$

Figure 8 displays the results of the analysis on AICD-related miRNAs. In panel A, four miRNAs with p-values less than 0.05 are provided, along with the risk ratios calculated using univariate Cox proportional hazard regression. Panel B shows the selection process of tuning parameters (λ) for the OS-related miRNA using the LASSO model. Lastly, in panel C, the four miRNAs' LASSO coefficient spectra are illustrated, with the vertical line reflecting the coefficient chosen using LASSO.

miRNA profiles significantly distinguished between high-risk and low-risk groups

A risk score was calculated for each patient using the established formula (Figure 9). To evaluate the dependability of these features, KM survival analysis and bilateral log-rank tests were performed on the training and internal validation cohorts (Figure 10 and Figure 11). The examination of the entire dataset cohort indicated an important distinction in OS among the high-risk and low-risk groups, with the high-risk group having a shorter OS ($p < 0.001$) (Figure 12A). Furthermore, the predictive significance of this risk score was subsequently evaluated in both the test set and training set cohorts, revealing similarly substantial OS variations among the high-risk and low-risk groups ($p < 0.05$) (Figure 12B,C).

The AUC (Area Under Curve) for the time-varying ROC curve for the 5-year OS in the training and internal validation groups was 0.69 and 0.75, respectively (Figure 12C,D).

Figure 9 illustrates the TCGA-BRCA entire dataset's risk score distribution and expression heat map. (A) The risk score distribution is illustrated, with pink dots representing the low-risk group and red dots representing the high-risk group. The vertical dotted line represents the median risk score cut-off point. (B) Shows the patient's survival time and status. (C) Presents the heat maps depicting the expression levels of four selected AICD signature genes.

Figure 10. : The remarks are the same as in Figure 9.

Figure 11: The remarks are the same as in Figure 9.

The prognostic significance of 4 miRNA features was verified in the METABRIC dataset

In order to further validate the prognostic model, validation was conducted using the METABRIC dataset, which comprised 1,282 cases of primary breast cancer. A risk score was calculated for each subject using the standard formula. The reliability and performance of the model were then assessed in the METABRIC dataset validation cohort (Figure 13). Consistent with prior findings, there were substantial differences in overall survival among high-risk and low-risk groups ($p < 0.001$) (Figure 14A). For 1 year, 3 years, and 5 years of overall survival, the time-varying ROC curve AUC values were 0.59, 0.57, and 0.521, respectively (Figure 14B). These results affirm the robustness and prognostic value of the model in predicting patient outcomes in an independent dataset.

Figure 12 displays the Kaplan-Meier survival analysis as well as the ROC curve over time. In the total data set (A), training cohort (B), and validation cohort (C), the Kaplan-Meier curve shows that the low-risk group had longer overall survival than the high-risk group. Furthermore, the time-varying ROC curve AUC for 5-year overall survival was 0.70 for the total data set (A), 0.69 for the training group (E), and 0.75 for the validation group (F), respectively. These findings illustrate the prognostic model's power in predicting patient outcomes.

Figure 13 : The remarks are the same as in Figure 9.

Figure 14. A: KM survival analysis and B ROC curve over time.

Figure 15. Correlation between mRNA and immunoinfiltrated population.

Immunoinfiltration analysis of prognostic model genes

The validation of risk scores derived from the tissue-resident macrophage (TRM) characteristics was confirmed by their strong correlation with the abundance of tumor-associated macrophage (TAM) subtypes within the dataset. Utilizing the CIBERSORT

algorithm to estimate the immunoinfiltrating population, it was observed that macrophages exhibited a positive correlation with all four selected mRNAs. Conversely, purity cells displayed the highest negative correlation with CASP7, CCND2, and PNAX1, with consistent correlations observed between these genes and immune infiltration (Figure 15).

mRNA differentiation between high and low risk differential gene expression and gene set enrichment analysis

As indicated in Figure 16 below, 169 genes were up-regulated and 200 genes were down-regulated in the high-low-risk group compared to the low-risk group (adjusted p value 0.05 and $|\text{Log}_2(\text{fold change})| > 0.5$). The GO was used to conduct a representative analysis of differentially expressed genes, and the BP enrichment results revealed that they were primarily involved in the control of systemic arterial blood pressure, amine transport, and neuropeptide signalling pathways (Figure 17). The results of CC enrichment showed that it was involved in terminal bouton, GABA receptor complex and ion channel complex. The results of MF enrichment showed that it was involved in neuropeptide hormone activity and neuropeptide receptor binding. The KEGG pathway gene set enrichment analysis revealed that it was mostly engaged in Neuroactive ligand receptor interaction, Retinol metabolism, Drug metabolism cytochrome P450, and xenobiotic metabolism *via* cytochrome P450.

Figure 16. Volcano map of differential gene expression.

Figure 17. GO and KEGG enrichment analysis: (A.D) BP enrichment results; (B.E) CC enrichment results; (C.F) MF enrichment results; (D.H) KEGG enrichment results;

miRNA differentiating high-low risk differential gene expression and gene set enrichment analysis

In contrast with the low-risk group, as shown in Figure 18 below, differential gene expression revealed that 113 genes were up-regulated, and 74 genes were down-regulated (adjusted p value 0.05 and $|\text{Log}_2(\text{fold change})| > 0.5$). GO was used to

conduct a representative analysis of differentially expressed genes. The BP enrichment results showed that they were involved in antimicrobial humoral response, regulation of membrane potential, and neuron fate commitment. Meanwhile, CC enrichment results showed that it was mainly involved in GABA receptor complex, synaptic membrane, and postsynaptic membrane, while MF enrichment was shown to be the main participant in extracellular ¹⁴ ligand-gated ion channel activity, neurotransmitter receptor activity, and transmitter-gated ion channel activity. Lastly, the KEGG pathway gene set enrichment study revealed that it was mostly involved in Neuroactive ligand receptor interaction, Nicotine addiction, and Morphine addiction (Figure 19).

Figure 18. Volcano map of differential gene expression.

Immunoinfiltration analysis of risk scores in different breast cancer subtypes

Within the immunoinfiltrating population ¹⁵ estimated by the CIBERSORT algorithm, B cells, CD4+ T cells, CD8+ T cells, endothelial cells, and macrophages exhibited a consistent negative correlation with the risk score across all breast cancer types (see Figure 20 below). Furthermore, the HER2-enriched subtype showed a negative correlation with risk scores. Conversely, a positive connection was found between the risk score and Natural Killer (NK) cell infiltration in the Her2+, PR+, Luminal A, and Normal-like subtypes. These results suggest that the risk score derived from the prognostic model is associated with distinct immune cell populations, indicating potential immunological implications for breast cancer progression and prognosis.

Figure 19. GO and KEGG enrichment analysis: (A.D) ⁶ BP enrichment results; (B.E) CC enrichment results; (C.F) MF enrichment results; (D.H) KEGG enrichment results;

Figure 20. Immunoinfiltration analysis of risk scores in different breast cancer types: (A) Her2+, (B) Her2-, (C) ER+, (D) ER-, (E) PR+. (F) PR-, (G) Luminal A, (H) Luminal B, (I) HER2-enriched, (J) Basal like, (K) Normal like.

DISCUSSION

The subsets of cells that exhibited higher sensitivity to ATP expressed a greater number of P2X7 receptors (P2X7Rs) compared to the subsets of cells that were less sensitive. Consequently, interfering with the P2X7R reduced AICD, showing that it performs a significant role in this process. The primary mechanism of AICD appears to be necrosis, as evidenced by cellular atrophy with membrane permeabilization. However, there was no evidence of apoptosis, as defined by phosphatidylserine externalization and caspase activity^[22]. These findings highlight the importance of P2X7 in AICD and cast light on its role in tumour growth. In many inflammatory conditions, elevated levels of extracellular ATP function as mediators to activate the P2X7R. However, a distinct study demonstrated that ATP treatment induced apoptosis and autophagy-mediated cell death in the HCT8 cell line, rather than necrosis^[1]. In healthy human intestinal epithelial tissue explants, extracellular ATP may be the cause of caspase-3 activation and apoptosis, which would explain the underlying process. This further stresses that AICD is a multifaceted process influenced by cell type and the specific microenvironment.

In modern society, breast cancer has become a major public health concern. Under the impact of different carcinogenic causes, the pathophysiology of this illness involves the unchecked proliferation of mammary epithelial cells, which is linked to cell death processes. Several classical modes of cell death, such as autophagy, apoptosis, and necrosis, as well as iron and copper death, have been extensively studied. AICD exhibits a degree of specificity while also displaying interplay with other cell death pathways. This phenomenon may result in the release and disturbance of iron and copper ions, consequently activating core pathways of autophagy, apoptosis, and necrosis, thereby augmenting the activation and execution of other cell death pathways. In turn, activation of cell death pathways such as autophagy, apoptosis, and necrosis may alter ATP release and metabolic processes, influencing the outcome of AICD. However, the precise processes behind the key role of AICD are still unknown.

Hence, this paper proposes a hypothesis positing that AICD serves as a fundamental logic of cell demise, contributing to a deeper comprehension of disease

treatment and progression. To support this hypothesis, a comprehensive review of relevant literature was carried out, focusing on the basic mechanisms and core genes involved in AICD. Additionally, functional mechanisms are explored within the context of breast cancer. This research endeavour has aimed to shed light on the intricacies of AICD and its potential implications in breast cancer management.

There is limited literature on the association between AICD and breast cancer, but research on the pivotal signalling receptor P2X7 in this context is more extensive. P2X7 is a purinergic ligand-gated ion channel receptor that functions as a non-selective cationic channel that is activated by ATP. This receptor is essential for signalling, growth regulation, cytokine release, and tumour cell formation^[9]. Recent research has demonstrated that overexpression of the P2X7R in breast cancer controls the V-Akt Murine Thymoma Viral Oncogene Homolog(AKT) signalling pathway, the Ca²⁺-activated Potassium Channel Subfamily K Member 3(SK3) potassium channel, and the epithelial-mesenchymal transition (EMT), as well as controlling extracellular vesicle release. Consequently, these pathways drive breast cancer cell invasion and migration, and their modulation is influenced by variables such as hypoxia and ATP. Furthermore, microRNAs have been found to bind to the P2X7 receptor's 3' untranslated region, influencing the occurrence and progression of breast cancer by either upregulating or downregulating the receptor's expression. In response to these discoveries, novel P2X7R inhibitors like emodin and Unicell have demonstrated the ability to hinder P2X7 receptor-mediated breast cancer invasion, thus holding promise for potential clinical applications^[3]. Additionally, P2X7 has been found to be overexpressed in tamoxifen (TAM)-resistant breast cancer cells, and functional investigations have been undertaken using selective P2X7 antagonists to study its effects^[50].

MicroRNAs are important regulators of post-transcriptional gene expression, and they play a significant part in cellular processes including differentiation, proliferation, and metastasis. Notably, miR-150 has been observed to be upregulated in both breast cancer cell lines and tissues. Inhibiting miR-150 function resulted in cell death in several breast cancer cell lines, whereas ectopic expression resulted in increased cell

proliferation^[18]. These striking findings highlight the potential of microRNAs and mRNAs as novel therapeutic targets for breast cancer.

However, it is noteworthy that the current study did not include P2X7 in the predictive model, suggesting that P2X7 may not be a significant predictor of patient outcomes in breast cancer. Interestingly, the predictive model incorporated the P2X4 receptor instead, which unveils new avenues for the treatment of breast cancer.

The mRNA prognostic model utilized in this study encompassed four specific mRNAs, namely P2X4, PNAX1, CASP7, and CCND2. P2X4 is a P2 receptor located on the cell membrane, which becomes activated when there is an excess of extracellular ATP. This activation then activates other P2 receptors, such as P2X7, as well as the PNAX1 channel, resulting in the extrusion of intracellular ATP from the cell *via* the PNAX1 channel. Consequently, this process promotes AICD. Additionally, the activation of P2 receptors on the cell membrane can also stimulate immune-inflammatory factors, CASP7 apoptosis factors, CCND2, and other factors that influence the apoptosis and autophagy processes in cells. This perspective reinforces the concept of AICD as the fundamental basis of cellular demise, thereby distinguishing it from other modes of cell death. Nevertheless, it is worth noting that existing studies have yet to establish a direct relationship between AICD and cancer, especially in the context of breast cancer prognostic models.

The P2X4 receptor is a member of the P2X receptor family that generates a highly Ca^{2+} permeable non-selective cation channel on the plasma membrane in response to exogenous ATP. However, the P2X4 receptor is distinguished from other subtypes by its preference for late endosomal, lysosomal, and/or lysosomal related organelles. The inclusion of tyrosine and dileucine transport motifs in the C-terminal and N-terminal sections, respectively, aids in this particular localization. Recent studies have shown that the P2X4 receptor regulates ion flux on lysosomal membranes, modulating lysosomal membrane fusion and desensitization of receptors exposed to external ATP^[51].

Recent investigations have highlighted the upregulation of P2X4 purinergic receptors in breast cancer patient biopsies, with a predominant localization in endolysosomes. Moreover, these investigations have shed light on the involvement of the P2X4 receptor in boosting tumour development and metastasis *in vivo* and enhancing breast cancer cell invasion *in vitro*. The P2X4 receptor's pro-malignant impact is linked to its control of lysosomal acidity, promotion of autophagy, and facilitation of cell survival. Additionally, a correlation was observed between autophagy activity and EMT, with the influence of P2X4 being particularly pronounced under conditions of metabolic challenges. P2X4 gene silencing and pharmacological intervention inhibited autophagy and EMT, whereas restoring P2X4 expression in knocked-out cells restored the aggressive phenotype^[52]. These recent discoveries contribute valuable insights to our understanding of the underlying mechanisms involving the P2X4 receptor in breast cancer and its regulatory role in autophagy and EMT processes.

The Pannexin protein channel family represents a class of channels characterized by large pore size, serving as crucial mediators of intercellular communication with fundamental implications for cell development and maintenance of cellular homeostasis. Among these, Pannexin 1 has been extensively studied and participates in regulating the permeability of ATP and ions within and outside cells, playing a pivotal role in various physiological processes and pathological mechanisms. Mutations in the Pannexin 1 gene can impact protein glycosylation and accelerate ATP release, leading to cell death.

In breast cancer cells, the Pannexin 1 channel exhibits a dual role in purinergic signalling, contributing to both cell survival and the induction of cytotoxic effects. This function is mediated by the amplified opening of the P2X4/P2X7-gated Pannexin-1 channel, triggering cell death characterized by caspase-1 activation and exhibiting a mixed pattern of apoptosis and necrosis, consistent with pyroptosis. Studies have revealed that cancer cell death is reliant on ATP release and downstream death signalling mediated by the P2X7R, which can be reversed by inhibiting Nicotinamide

adenine dinucleotide phosphate(NADPH) oxidase-produced Reactive Oxygen Species(ROS), ¹³ Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), or the mitochondrial permeability transition pore (MPTP). Enhanced P2X4/P2X7 signalling may also be related with the ATP-rich tumour microenvironment, providing a molecular basis for purinergic receptor-mediated selective control of breast cancer and serving as a possible platform for comprehensive breast cancer immunotherapy^[7].

CASP7, a member of the cysteine aspartate protease (caspase) family, plays a crucial role in the execution stage of apoptosis, wherein sequential caspase activation is vital. Caspase exists as an inactive proenzyme, and proteolytic cleavage of conserved aspartic acid residues generates two large and small subunits, which then dimerize to form active enzymes. The precursors encoding for CASP7 protein are cleaved by caspase 3 and caspase 10, and they become activated upon stimulation by cell death signals, subsequently inducing apoptosis.

Recent research has investigated the expression of CASP7 in breast cancer patients, confirming its involvement with regulating tumorigenicity in breast cancer cells. Moreover, CASP7 expression was discovered to be higher in breast cancer tissues than in normal tissues. Ectopic expression of CASP7 has a significant connection with estrogen receptor α (ER α) expression and demonstrated progressive rise across various stages of breast tumour grading. According to a Kaplan-Meier analysis, increased levels of CASP7 expression were related with a better outcome in breast cancer patients following systemic endocrine therapy^[53]. Furthermore, according to the findings, both the precursor and active forms of CASP7 are mostly found outside of the cytoplasmic region of breast cancer cells, primarily within the nucleus.

When compared to comparable normal tissues and samples from healthy people, hypermethylation of the CCND2 promoter was seen in 40.9% of breast tumour patients and 44.4% of circulating cell-free DNA in plasma. Furthermore, the study has shown that in female breast cancer patients, CCND2 promoter hypermethylation acts as a distinctly poor prognostic factor. Experiments using cell models clarified the functional involvement of CCND2 in breast cancer. Specifically, cancer cell proliferation and

migration were reported to be inhibited by the expression of CCND2. Moreover, the demethylating drug antroquinonol D caused an increase in CCND2 expression, leading to cell cycle arrest and suppression of cancer cell growth and migration^[54].

Furthermore, in pursuit of enhancing the prognostic model's precision to accurately predict patients' survival times and identify potential therapeutic targets, this study extended its investigation to model the miRNAs that correspond to the predictive genes identified after establishing the mRNA prognostic model. This iterative approach enabled the narrowing down of potential targets for future anti-cancer interventions, consequently augmenting the model's predictive accuracy. The miRNA prognostic model integrated four key miRNAs, namely hsa-miR-615-3p, hsa-miR-519b-3p, hsa-miR-342-3p, and hsa-miR-324-3p. These miRNAs were found to exert considerable influence on the prognosis of breast cancer, offering promising avenues for novel therapeutic targets and approaches in future breast cancer treatments.

Recent investigations have demonstrated a significant upregulation of miR-615-3p expression in both breast cancer cells and tissues, particularly in those with metastatic properties. Notably, stable overexpression of miR-615-3p in breast cancer cell lines resulted in notable enhancements in cellular motility in vitro, along with a consequential increase in lung metastasis in vivo. The expression of mesenchymal markers increased along with these effects, and epithelial markers decreased. Further investigation found that reintroducing miR-615-3p resulted in increased downstream signalling of the Transforming Growth Factor (TGF)- pathway, *via* targetting the 3'-untranslated region (3'-UTR) of Protein Interacting with C Kinase 1(PICK1). PICK1 inhibits the interaction of Dicer 1, Ribonuclease III(DICER1) and Mothers [IS THIS CORRECT???] against decapentaplegic homolog 2/3(Smad2/3), as well as the processing of pre-miR-615-3p to its mature miRNA form, within breast cancer cells. This regulatory mechanism establishes a negative feedback loop^[55].

Furthermore, the investigation revealed a noteworthy upregulation of HOXA transcript at the distal tip (HOTTIP) expression and a significant downregulation of miR-615-3p expression in breast cancer patients when compared to healthy individuals,

as well as patients from non-breast cancer groups ($p < 0.001$ for all comparisons). Importantly, in the serum of breast cancer patients, there was a substantial negative connection between the expression levels of HOTTIP and miR-615-3p^[56]. Additionally, by inhibiting CUGBP Elav-like family member 2 (CELF2) expression, miR-615-3p was discovered to increase cancer cell motility and proliferation^[57].

Although research on the effect of miR-519b-3p in breast cancer is limited, it appears to have a major impact on cancer cell proliferation and invasion. Previous research has found that miR-519b-3p expression is significantly downregulated in colorectal cancer tissues and cell lines. Overexpression of miR-519b-3p inhibited proliferation and invasion in the colorectal cancer cell lines, the Rous sarcoma oncogene-transformed rat kidney cell line (RKO) and Duke's Type B colorectal Adenocarcinoma cell Line 1 (DLD-1). On the other hand, low expression had the reverse effect.

Notably, miR-519b-3p was discovered to suppress colorectal cancer cell proliferation and invasion by targetting the uMtCK/Wnt signalling pathway^[58]. Furthermore, LINC01419 has been found to negatively regulate miR-519b-3p, with Renal Cell Carcinoma Differentiation 1 (RCCD1) identified as a downstream target of miR-519b-3p and its expression being negatively associated with that of miR-519b-3p. Conversely, the expression of LINC01419 showed a positive correlation with RCCD1 expression. Later studies showed that the effects of LINC01419 silencing on cell proliferation, migration, and invasion could be mitigated by overexpressing miR-519b-3p inhibitors or RCCD1. These findings suggest that LINC01419 enhances RCCD1 expression through sequestering miR-519b-3p, thereby promoting carcinogenesis in lung adenocarcinoma. As a result, LINC01419 holds potential therapeutic value in the management of lung adenocarcinoma^[59].

MiR-342-3p has been linked to a poor prognosis and decreased expression in triple-negative cancers, according to research. Experimental findings show that the proliferation, viability, and motility of several triple-negative tumour cells *in vitro* are greatly decreased when miR-342-3p is overexpressed. MiR-342-3p regulates lactic acid

and glucose fluxes by directly targetting monocarboxylate transporter 1 (MCT1), causing tumour cells' metabolic equilibrium to be disrupted. Additionally, cells with elevated miR-342-3p levels demonstrate higher optical Reduction-Oxidation (REDOX) rates ¹⁷[60]. The expression of miR-342-3p in breast cancer cells is significantly reduced in patients with metastatic disease. Inhibiting miR-342-3p inhibits aggressive and drug-resistant activity in breast cancer tumour cells. Further investigations have confirmed the presence of binding sites between miR-342-3p and Inhibitor of DNA binding 4(ID4). Notably, ID4 reduces the effects of miR-342-3p on chemotherapy resistance. ID4 siRNA has also been shown *in vivo* to have tumour-suppressive properties. MiR-342-3p suppresses metastasis and chemotherapy resistance in breast cancer cells by targetting ID4, implying its potential as a tumour suppressor^[61]. This discovery identifies miR-342-3p as a possible therapeutic target for breast cancer treatment.

¹⁸Metformin causes iron mortality in MDA-MB-231 cells *via* increasing the expression of miR-324-3p. Overexpression of miR-324-3p leads to the inhibition of cancer cell growth activity, while its inhibition promotes cell growth activity. The regulatory action of MiR-324-3p involves direct targetting of glutathione peroxidase 4 (GPX4) *via* binding to the 3'-UTR region of GPX4, resulting in the downregulation of GPX4 expression ^[62]. MiR-324-3p may exert its regulatory role through multiple pathways, including iron death and AICD. LINC00963 works by blocking the inhibitory activity of miR-324-3p on activated Cdc42-associated kinase 1(ACK1) expression. Clinically, miR-324-3p has a negative connection with LINC00963 expression in breast cancer tissues. The inhibitory effect of miR-324-3p on the growth and radiosensitivity of breast cancer cells can be reduced by overexpressing LINC00963 or ACK1^[63]

¹⁹Additionally, in the tissues and cells of triple-negative breast cancer, Small Nucleolar RNA Host Gene 22(SNHG22) is abundantly expressed. Triple-negative breast cancer cells are prevented from proliferating, migrating, and invading when SNHG22 is silenced. Furthermore, SNHG22 acts as a sponge for miR-324-3p, lowering its expression in triple-negative breast cancer cells. However, miR-324-3p upregulation suppresses the proliferation and migration of triple-negative breast cancer cells^[64].

Notably, miR-324-3p inhibition, or Suppressor of Defective Silencing 3(SUDS3) overexpression can reverse the anticancer effect of SNHG22 silencing on the malignant phenotype of triple-negative breast cancer cells.

The CIBERSORT algorithm was used to quantify immune infiltration, and there was a substantial association between immune infiltrating populations and risk scores in distinct breast cancer subtypes. B cells, CD4+ T cells, CD8+ T cells, endothelial cells, and macrophages exhibited an inverse association with risk scores across all breast cancer subtypes. Moreover, the HER2-enriched subtype showed a negative correlation with risk scores. These findings suggest a potential protective effect or involvement of these immune cell populations in an immune response, leading to a reduced risk and improved prognosis in breast cancer patients. Conversely, in the Her2+, PR+, Luminal A, and Normal-like classifications, there was a positive correlation with the infiltration of NK cells. This shows that NK cells may have a role in or contribute to the disease's genesis and progression, significantly impacting patient outcomes. Furthermore, macrophages showed a positive correlation with the expression of P2X4, PNAX1, CASP7, and CCND2. This association may indicate a potential link between macrophage infiltration and the activation of specific genes associated with breast cancer.

NK cells are critical components of the anti-tumour surveillance system, and in animal models, they serve a significant part in suppressing tumour growth and metastasis. The reduction in natural killing activity has been positively associated with the extent of tumour dissemination^[65]. This bears substantial relevance in the context of tumours, as transformation events may lead to the downregulation of auto ligands and stress-induced ligands that are recognized by NK cells. Additionally, NK cell activation results in the secretion of stimulatory cytokines, which participate in cancer elimination through various direct mechanisms and by stimulating the adaptive immune system^[65].²⁰ The Janus kinase/signal transducer and activator of transcription(JAK/STAT) pathway remains active during metastasis; therefore, the impact of JAK inhibitors (JAKi) on NK

cells and the potential value of immunostimulants to enhance weakened tumour immune surveillance merit consideration in the clinical management of breast cancer^[66].

Macrophages exhibit distinct activation states and functions, and can be categorized into M1 type (classically activated macrophages) and M2 type (alternatively activated macrophages). Tumour-associated macrophages (TAMs) bear similarities to M2 macrophages. Clinicopathological studies have demonstrated that TAMs are associated with poor clinical prognosis in tumours. In the context of human breast cancer, a high density of TAMs is linked to unfavourable prognosis. Extensive research has unveiled the role of TAMs in breast cancer progression. This encompasses the capacity of TAMs to induce angiogenesis, promote invasion through remodelling the tumour extracellular matrix, mimic breast cancer cells to evade the host immune system, and to recruit immunosuppressive white blood cells to the tumour microenvironment. In breast cancer, TAMs can also enhance cancer cell invasion and suppress the anti-tumour function of tumour-suppressing T cells. This leads to a compromised anti-tumour immune response and subsequently impacts patient prognosis^[67].

In addition, KEGG analysis showed that the genes differentiated by mRNA risk score significantly enriched 25 signalling pathways. Also, those genes differentiated by miRNA risk score significantly enriched 29 signalling pathways, and the two genes jointly enriched 16 signalling pathways. These include neuroactive ligand-receptor interaction, retinol metabolism, drug metabolism - cytochrome P450, pancreatic secretion, metabolism of xenobiotics by cytochrome P450, nicotine addiction, porphyrin metabolism, chemical carcinogenesis-DNA adducts, the cAMP signalling pathway, ascorbate and aldarate metabolism, gamma-Aminobutyric Acid(GABA) ergic synapse, steroid hormone biosynthesis, Pentose and glucuronate interconversions, protein digestion and absorption, nitrogen metabolism and drug metabolism - other enzymes. The enrichment analysis results show that the model has satisfactory performance.

The Neuroactive ligand-receptor interaction signalling pathway represents the highest enrichment in both models. This pathway encompasses the intricate interactions between neuroactive substances within the nervous system, such as dopamine,

acetylcholine, glutamate, serine, GABA, and their corresponding receptors. These interactions elicit a range of regulatory mechanisms, including protein kinase cascades, G-protein-coupled receptor signalling, and modulation of intracellular calcium concentration.

Based on the findings, increased usage of dopamine antagonists for the treatment of mental disorders may be linked to the development and promotion of breast cancer. The use of antipsychotic dopamine antagonists demonstrated a dose-response relationship with cumulative dose, resulting in a 16% increased risk of breast cancer^[68]. Extracellular ATP has recently emerged as a crucial factor in regulating pathological cell death. Exposure to ATP induces cell death characterized by cell swelling, disruption of endoplasmic reticulum integrity, formation of large cytoplasmic vacuoles, and ultimately cell lysis and DNA release.

Additionally, ATP processing triggers the cleavage of caspase-3, a hallmark of apoptosis. This suggests that the release of extracellular ATP from damaged tissues may induce dopaminergic cell death by accelerating necrosis of P2X7Rs^[69]. PNAX1 participates in the release of ATP and glutamate in neurons and astrocytes. Extracellular ATP activates PNAX1 at the resting membrane potential through the P2X7R. Studies have indicated that the P2X7R-PNAX1 complex may function as a negative regulator mediated by acetylcholine receptors *in vivo*^[70]. A recent study demonstrated the promising potential of using low-concentration muscarinic receptor agonists in combination with conventional chemotherapy agents for breast cancer treatment^[71]. In summary, extracellular ATP plays a role in the neuroactive ligand-receptor interaction signalling pathway, influencing the invasion, survival, and migration of breast cancer cells.

In general, the interpretation of the data in this study may be subject to certain limitations, warranting the need for prospective experimental validation to enhance the reliability of the research findings. Initially, an extensive literature search was conducted to identify the key mechanisms of AICD that have been experimentally verified. Building upon this foundation, the correlation between AICD and breast

cancer prognosis has been elucidated for the first time. Consequently, a prognostic model has been developed that integrates mRNA and miRNA, effectively narrowing down potential therapeutic targets for future anticancer interventions and augmenting the precision of the predictive model.

CONCLUSION

This study conducted a comprehensive investigation into the primary mechanism underlying AICD and performed an in-depth analysis of the associated mRNA expression patterns. Notably, mRNA and miRNA characteristic models were successfully established and specifically tailored to AICD, both of which exhibit potential as independent prognostic factors. Leveraging these two models achieved heightened precision in estimating patient survival status and simplified the decision-making process regarding relevant therapeutic interventions. Consequently, the findings offer a robust scientific foundation for comprehending the fundamental logic governing cell death. Moreover, the clinical implications of this research are highly significant, as they shed light on the regulatory mechanisms of cell death and provide valuable guidance for the treatment and prognosis evaluation of breast cancer.

Future investigations need to allocate greater focus on the examination, analysis, and discourse of discrete cancer cells, in order to reach more exacting insights. Recognizing the fact that the domain of cancer research is inherently rooted in single-cell substrates, it is imperative to note that a dearth of single-cell analyses for individual patients could potentially undermine the comprehensive nature of these studies. Subsequent to this, the primary objectives include giving precedence to functional insights at the single-cell level, thereby ensuring that later research provides actionable and targetted insights.

ARTICLE HIGHLIGHTS

Research background

ATP-Induced Cell Death (AICD) has emerged as a distinctive mode of cell death triggered by elevated extracellular adenosine triphosphate (ATP) levels and is closely associated with the progression of various cancer types^[1-4]. Cell death serves as a critical process in maintaining the normal functionality of tissues and organs, primarily presenting in two distinct forms, apoptosis, and necrosis^[5, 6]. AICD exerts a dual impact on breast cancer: firstly, ATP acts as a pro-death signal, participating in the regulation of the activation of death pathways; secondly, cell death itself leads to an escalation in extracellular ATP levels, forming an interconnected regulatory loop^[7-11]. Manipulation of ATP levels and ATP receptors in the context of breast cancer can alter biological activities such as breast cancer cell proliferation, invasion, and metastasis^[12-15]. Investigations have suggested that AICD is a pivotal mechanism regulated by both genes and microRNAs (miRNAs), contributing to the modulation of breast cancer^[16-19]. However, the precise nature of interactions requires further research. Hence, in-depth exploration of the mechanisms underlying AICD holds promising potential for its application in breast cancer treatment. Unraveling the implications of AICD in breast cancer therapy could pave the way for novel therapeutic strategies and targeted interventions.

AICD is a multifaceted process, its intricacy being contingent upon the specific cell type and the surrounding microenvironment^[20-23]. Nonetheless, several overarching mechanisms have been revealed. Firstly, the activation of purinergic receptors, particularly the P2X7 receptor(P2X7R), emerges as a pivotal mechanism, triggering a cascade of events culminating in cell death^[8, 24]. Secondly, a significant mechanism of AICD involves the elevation of intracellular calcium ion concentration^[25, 26]. Moreover, AICD may also be linked to the activation of inflammatory responses^[8]. Finally, the disruption of mitochondrial function is crucial in AICD, with cytochrome c release intimately connected with the activation of apoptosis signalling pathways^[27-29].

Genes, and genetic factors, encompass nucleotide sequences that encode either a polypeptide chain or functional RNA, thereby supporting fundamental life processes. They serve as repositories of an organism's genetic information, encompassing data

pertaining to its race, blood type, reproduction, growth, apoptosis, and other critical processes. On the other hand, MiRNAs(miRNA), which are RNA molecules approximately 21 to 23 nucleotides in length, are widely prevalent in eukaryotic organisms^[30]. MiRNAs function as key regulators of gene expression, deriving from RNA transcribed by DNA but incapable of being translated into proteins (non-coding RNAs). Through specific binding to target messenger ribonucleic acid (mRNA), miRNAs can exert post-transcriptional control over gene expression, playing pivotal roles in the regulation of gene expression, cell cycle, and developmental sequencing in organisms. Notably, in animals, a single miRNA often possesses the capacity to regulate numerous genes simultaneously.

The association between miRNAs and cancer has garnered significant recent interest^[31]. miRNAs exert a crucial impact on cancer initiation, progression, and therapeutic responses, exhibiting dichotomous effects that either facilitate tumorigenesis, growth, and metastasis, or which impede tumour formation and development^[32-35]. The functional roles of miRNAs may vary across different cancer types, with certain miRNAs functioning as tumour suppressors to inhibit cancer growth in specific contexts, while others may function as tumour promoters, promoting cancer cell proliferation and metastasis.

The relationship between AICD and miRNA is governed by two distinct mechanisms. Firstly, certain miRNAs have the capacity to modulate cell response to ATP by targetting essential genes involved in ATP-related signalling pathways or cell death pathways. Conversely, alterations in ATP levels can prompt cells to adjust the expression patterns of miRNAs, thereby influencing cell survival and death. This reciprocal regulatory mechanism may hold particular significance in tumour cells, as it can influence tumour cell proliferation or apoptosis, consequently impacting tumour development and prognosis. Nevertheless, the current body of research concerning AICD-related miRNAs and their corresponding gene regulation in breast cancer remains limited. Although increasing evidence highlights the considerable diagnostic and prognostic value of AICD and miRNA in breast cancer^[18, 20], the precise regulatory

mechanisms and associated biological processes require further exploration and elucidation.

A prognostic model (Signature) is a predictive model designed to estimate potential treatment outcomes based on a patient's current health status. Genetic prognostic models aim to forecast disease progression and survival by utilizing a patient's gene expression data, thereby offering more precise and personalized information to guide clinical decision-making. The miRNA prognostic model facilitates more accurate prognosis evaluation by healthcare professionals and aids in making informed treatment choices. Gene prognostic models have certain limitations, including high-dimensional data, overfitting issues, data consistency concerns, and challenges in biological interpretation. However, the miRNA prognostic model is characterized by the inherent instability of miRNA expression data, data quality problems, miRNA-gene interactions, and limitations in study samples. These factors collectively pose challenges for miRNA prognostic models to thoroughly elucidate disease mechanisms. Considering the strengths and weaknesses outlined above, the simultaneous utilization of both gene prognostic models and miRNA prognostic models represents a promising research direction. By harnessing the advantages of both approaches, the prognostic features of cancer can be more effectively explored, providing a more robust tool for personalized treatment and prognosis assessment.

Research motivation

No studies to date have investigated the impact of AICD regulatory mechanisms in breast cancer. Therefore, the primary goal of this paper is to look into the potential prognostic importance of AICD genes in breast cancer, as well as the interaction among AICD genes and prognostic gene-associated miRNAs in breast cancer.

Research objectives

This study conducted a comprehensive investigation into the primary mechanism underlying AICD and performed an in-depth analysis of the associated mRNA

expression patterns. Notably, mRNA and miRNA characteristic models were successfully established and specifically tailored to AICD, both of which exhibit potential as independent prognostic factors. Leveraging these two models achieved heightened precision in estimating patient survival status and simplified the decision-making process regarding relevant therapeutic interventions. Consequently, the findings offer a robust scientific foundation for comprehending the fundamental logic governing cell death. Moreover, the clinical implications of this research are highly significant, as they shed light on the regulatory mechanisms of cell death and provide valuable guidance for the treatment and prognosis evaluation of breast cancer.

Research methods

The foundational genes orchestrating ATP-induced cell death mechanisms were extracted from the scholarly literature, underpinning the establishment of a prognostic model. Simultaneously, a miRNA prognostic model was constructed that mirrored the gene-based prognostic model. Distinctions between high and low-risk cohorts within mRNA and miRNA characteristic models were scrutinized, with the aim of delineating common influence mechanisms, substantiated through enrichment analysis and immune infiltration assessment.

Research results

The mRNA prognostic model in this study encompassed four specific mRNAs—P2X4, PNAX1, CASP7, and CCND2. The miRNA prognostic model integrated four pivotal miRNAs—hsa-miR-615-3p, hsa-miR-519b-3p, hsa-miR-342-3p, and hsa-miR-324-3p. B cells, CD4+ T cells, CD8+ T cells, endothelial cells, and macrophages exhibited inverse correlations with risk scores across all breast cancer subtypes. Furthermore, KEGG analysis revealed that genes differentially expressed in response to mRNA risk scores significantly enriched 25 signalling pathways, while miRNA risk scores significantly enriched 29 signalling pathways, with 16 pathways being jointly enriched.

Research conclusions

This study conducted a comprehensive investigation into the primary mechanism underlying AICD and performed an in-depth analysis of the associated mRNA expression patterns. Notably, mRNA and miRNA characteristic models were successfully established and specifically tailored to AICD, both of which exhibit potential as independent prognostic factors. Leveraging these two models achieved heightened precision in estimating patient survival status and simplified the decision-making process regarding relevant therapeutic interventions. Consequently, the findings offer a robust scientific foundation for comprehending the fundamental logic governing cell death. Moreover, the clinical implications of this research are highly significant, as they shed light on the regulatory mechanisms of cell death and provide valuable guidance for the treatment and prognosis evaluation of breast cancer.

Research perspectives

Future investigations need to allocate greater focus on the examination, analysis, and discourse of discrete cancer cells, in order to reach more exacting insights. Recognizing the fact that the domain of cancer research is inherently rooted in single-cell substrates, it is imperative to note that a dearth of single-cell analyses for individual patients could potentially undermine the comprehensive nature of these studies. Subsequent to this, the primary objectives include giving precedence to functional insights at the single-cell level, thereby ensuring that later research provides actionable and targetted insights.

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