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Article

Increased PIEZO1 Expression Is Associated with Worse Clinical Outcomes in Hormone Receptor Negative Breast Cancer Patients

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Simple Summary: PIEZO1 is a mechanically activated ion channel connected to many important cellular functions. While implicated to various degrees in different types of cancers, the clinical significance of PIEZO1 has not been explored in breast cancer. We conducted various bioinformatic analyses on PIEZO1 in breast cancer, using publicly available online datasets from The Cancer Genome Atlas and GSE3494. Our results show that PIEZO1 expression is higher in hormone receptor (HR) negative cohorts than HR positive cohorts. We also found that high PIEZO1 expression is correlated with worse survival outcomes in HR negative patients, suggesting that PIEZO1 could be utilized as a prognostic biomarker in HR negative breast cancer. Further analysis suggests that these worse survival outcomes may be due to increased aggressive cancer pathways, including epithelial-mesenchymal transition and hypoxia, along with decreased CD8+ and CD4+ T cell infiltration in high PIEZO1 HR negative tumors.

Abstract: PIEZO1 plays a crucial role in the human body as a mechanosensory ion channel. It has been demonstrated that PIEZO1 is important in tissue development and regulating many essential physiological processes. Studies have suggested that the PIEZO1 ion channel plays a role in invasion and progression in cancer; elevated levels of PIEZO1 have been correlated with increased migration in breast cancer cells, chemo-resistance and invasion in gastric cancer cells, and increased invasion of osteosarcoma cells. In addition, high PIEZO1 expression levels were correlated with a worse prognosis in glioma patients. On the other hand, studies in lung cancer have attributed high PIEZO1 levels to better patient outcomes. However, the clinical impact of PIEZO1 in breast cancer is not well characterized. Therefore, our goal was to determine the clinical relevance of PIEZO1 in breast cancer. Analysis of breast cancer data from The Cancer Genome Atlas (TCGA) was conducted to investigate PIEZO1 expression levels and correlation to survival, followed by validation in an independent dataset, GSE3494. We also performed gene set enrichment analysis (GSEA) and analyzed immune cell levels in breast tumors from TCGA through a CIBERSORT algorithm. Our results demonstrated that PIEZO1 expression levels are higher in hormone receptor (HR) negative than HR positive cohorts. High PIEZO1 expression is correlated with a significant decrease in survival in HR negative cohorts, suggesting that PIEZO1 could be utilized as a prognostic biomarker in HR negative breast cancer. GSEA showed that various signaling pathways associated with more invasive phenotypes and resistance to treatments, including epithelial-mesenchymal transition (EMT), hypoxia, and multiple signaling pathways, are enriched in high PIEZO1 HR negative tumors. Our results also demonstrated a decrease in CD8+ and CD4+ T cell infiltration in high PIEZO1 HR negative tumors. Further investigations are necessary to elucidate the mechanistic roles of PIEZO1 in HR negative breast cancer.

1. Introduction

Breast cancer is the most abundant type of cancer globally, accounting for 12% of all cancer diagnoses in 2021 [1]. As breast cancer continues to impact millions of people worldwide, it is our goal to identify novel biomarkers and therapeutic targets to reduce breast cancer morbidity and mortality. In recent years, researchers have become increasingly interested in how mechanical forces within the tumor microenvironment (TME), including tension, shear stress, and other forces, impact the survival and propagation of cancer cells [2]. Mechanotransduction, or the sensation of mechanical stimuli and subsequent conversion to biochemical signals, occurs through various adhesion molecules, ion channels, and cytoskeletal components. Mechanosensation is essential for regulation of many downstream signaling pathways, many of which are implicated in cancer [3]. A key set of proteins involved in mechanosensation is the PIEZO proteins [4].

PIEZO proteins are evolutionarily conserved, mechanically activated ion channels [4–7]. The PIEZO family is composed of two members – PIEZO1 and PIEZO2 [6]. PIEZO1 is primarily expressed in non-excitable cell types, while PIEZO2 confers mechanosensory abilities to excitable cell types such as sensory neurons and Merkel cells [8]. PIEZO1 plays major roles in sensing various forces, including shear force, tension, and stretching, then transmitting these signals to stimulate downstream pathways important for development and homeostasis in both normal physiology and disease. PIEZO1 has been demonstrated to play critical roles in endothelial cell organization [9,10], vascular patterning and development [9,10], neural cell differentiation [9], bone formation and homeostasis [11,12], and differentiation of various cell types [8].

There has been a growing interest in exploring PIEZO proteins and their mechanosensory abilities in the context of malignancies and the TME. Recently, we discovered that increased expression levels of PIEZO2 are correlated with worse survival outcomes in triple-negative breast cancer (TNBC) through increased AKT activation, stabilized SNAIL, and repression of E-cadherin transcription [13]. PIEZO1 expression has also been demonstrated to contribute to various physiological processes within the hallmarks of cancer, including epithelial-mesenchymal transition (EMT) [14,15], angiogenesis [15–17], hypoxia [15,16,18], metabolic alteration [19], inflammation [18], and the functional gain or loss of various signaling pathways [14,16,20–22]. The involvement of PIEZO1 within these processes and conditions results in discernable effects on cancer progression, as PIEZO1 expression has been shown to correlate with increased migration and chemo-resistance in gastric cancer cells [23,24], increased migration in breast cancer cells [25], and increased invasion of osteosarcoma cells [26]. In addition, it is well-known that these pro-cancer processes impact the immune population of the TME, either by immune suppression or immune exclusion. For example, EMT, hypoxia, and TGF-β signaling have all been shown to be inversely correlated to CD8+ and/or CD4+ T cell infiltration in various cancer types [27–29]. CD8+ T cells are major drivers of the anti-cancer immune response, with decreased levels of CD8+ infiltration correlating with worse survival outcomes in breast cancer patients [30,31]. Therefore, PIEZO1 may play a role in modulating oncogenic pathways to impact the tumor microenvironment, immune response, and patient prognosis.

From a clinical standpoint, PIEZO1 appears to be a dynamic ion channel protein, affecting varying cancers differently. Upregulated PIEZO1 is associated with worse overall survival outcomes and higher WHO grades in glioma patients [32]. On the other hand, previous studies attribute lower levels of PIEZO1 expression to increased migration in lung cancer [33] and better overall survival in non-small cell lung cancer patients with high PIEZO1 expression [34]. Though there have been some studies of PIEZO1 in breast cancer cell lines, the clinical relevance of PIEZO1 in breast cancer patients is not well characterized. Therefore, we aimed to study the involvement of PIEZO1 in breast cancer, determine how PIEZO1 levels are related to clinical outcomes, and identify possible underlying

mechanisms through a bioinformatic approach to build a foundation of knowledge concerning the PIEZO1 ion channel in breast cancer.

2. Materials and Methods

2.1. Data acquisition and pre-processing

As a discovery cohort, The Cancer Genome Atlas (TCGA) breast cancer cohort was used. There are 1100 patients in TCGA breast cancer cohort with clinical and gene expression data from RNA-sequence. Data was downloaded through cBioPortal (TCGA provisional dataset) [35,36]. Out of 1100, 1093 tumors were primary tumors. Of those, 1090 patients have overall survival (OS) data, and 999 patients have disease-free survival data. As a validation cohort, we used GSE3494 breast cancer cohort from Gene Expression Omnibus (GEO). Among 251 patients, 236 have disease-specific survival data. The patients were divided into PIEZO1 high and low expression groups with median cutoff. Since TCGA and GEO are de-identified publicly available cohorts, institutional review board approval was waived.

2.2. Gene Set Enrichment Analysis (GSEA)

GSEA was carried out comparing the PIEZO1 high and low groups using software provided by the Broad Institute (http://software.broadinstitute.org/gsea/index.jsp) using hallmark gene sets. False discovery rate (FDR) < 0.25 was considered as significant.

2.3. Statistical Analysis

Survival analysis was conducted by Kaplan-Meier curve with log-rank test. Continuous value between two groups was compared by Student's t-test, and ANOVA followed by post hoc Tukey was used for more than two groups comparison. Cell composition fraction of the tumor was estimated by CIBERSORT algorithm [37] and compared by Wilcoxon test. Two-sided $p < 0.05$ was considered as statistically significant for all tests. All statistical analyses were performed using R software (http:///www.r-project.org/) together with Bioconductor (http://bioconductor.org/)

3. Results

3.1. Hormone receptor (HR) negative patients have increased PIEZO1 expression

First, we examined PIEZO1 expression levels in primary tumors among different subtypes of breast cancer in TCGA cohort. Based on estrogen receptor (ER) and progesterone receptor (PgR), patients were divided into either hormone receptor (HR) positive (either ER positive, PgR positive, or positive for both) or HR negative (ER negative and PgR negative) groups. Patients were also divided into groups based on human epidermal growth factor receptor 2 (HER2) expression status. We found that TNBC, which is HR negative and HER2 negative, had significantly higher levels of PIEZO1 expression than both HR positive-HER2 negative ($p < 0.001$) and HR positive-HER2 positive subtypes ($p < 0.01$) (Figure 1A). Upon analysis of patients with breast tumors classified by HR status (Figure 1B) and HER2 status (Figure 1C), we found that the HR negative patients had significantly higher levels of PIEZO1 expression than HR positive ($p < 0.001$). There was no statistically significant difference in PIEZO1 expression between HER2 negative and positive groups. These findings were validated through analysis of an independent cohort, GSE3494, where the HR negative cohort had significantly higher PIEZO1 expression than HR positive ($p = 0.006$) (Figure 1D).

Next, we investigated whether there were differences in PIEZO1 expression in primary tumors between different American Joint Committee on Cancer (AJCC) stages in the breast cancer whole cohort. As shown in Figure 1E, there were no statistically significant differences in PIEZO1 expression between any of the clinical stages within the whole cohort. There was also no significant difference in PIEZO1 expression in relation to pathological T classification (pT), reflecting primary tumor sizes (Figure SI-A). Similarly, there was no significant difference in PIEZO1 expression in relation to

pathological N classification (pN), reflecting level of tumor spread to lymph nodes (Figure SI-B), nor based on pathological M classification (pM), reflecting metastasis status (Figure SI-C). Collectively, our results demonstrate that HR negative breast cancer subtypes have increased PIEZO1 expression compared to HR positive subtypes.

3.2. HR negative breast cancer patients with high PIEZO1 tumors exhibit worse survival outcomes

Next, we explored whether PIEZO1 level is associated with breast cancer patient survival. The breast cancer patients of whole TCGA cohort were divided into high or low PIEZO1 groups based on a median cutoff. There was no significant difference in disease-free (Figure 2A) nor overall survival (Figure 2D) based on PIEZO1 expression in the whole cohort. Stemming from our results of differential PIEZO1 expression levels in different subtypes of breast cancer, we then divided the TCGA cohort into breast cancer subtypes characterized by HR status and HER2 status to examine survival. High PIEZO1 breast cancer patients exhibited significantly worse disease-free survival (p = 0.033) and overall survival (p = 0.002) compared to the low PIEZO1 group in the HR negative cohort, while there was no significant difference in disease-free nor overall survival between high and low PIEZO1 groups in the HR positive cohort (Figure 2B and Figure 2E). There was no statistically significant difference in disease-free nor overall survival outcomes between high and low PIEZO1 groups in the HER2 positive subtype (Figure 2C and Figure 2F). In the HER2 negative cohort, high PIEZO1 was correlated with worse disease-free survival (p = 0.046) (Figure 2C), however, there was no statistically significant difference in overall survival by PIEZO1 levels in the HER2 negative subtype (Figure 2F).

To validate our results from TCGA, we utilized another independent cohort, GSE3494. Upon evaluating the whole cohort of GSE3494, there was a significant difference in disease-specific survival between high and low PIEZO1 groups, with the high PIEZO1 group having decreased disease-specific survival (p = 0.01) (Figure 3A). Within the HR negative cohort of GSE3494, patients with high PIEZO1 expression exhibited worse disease-specific survival compared to the low PIEZO1 group (p = 0.003) (Figure 3C). However, there was no difference in terms of disease-specific survival in the HR positive cohort (Figure 3B). Therefore, our results highlight that high PIEZO1 expression levels correlate with worse survival outcomes in HR negative breast cancer patients.

3.3. EMT and multiple pro-tumor signaling pathways are enriched in high PIEZO1 HR negative tumors

To investigate the possible underlying mechanisms of how high PIEZO1 expression contributes to worse survival outcomes within HR negative breast cancer cohorts, we performed Gene Set Enrichment Analysis (GSEA) comparing high and low PIEZO1 HR negative tumors of the TCGA dataset. The gene sets significantly enriched in high PIEZO1 HR negative tumors are depicted in Figure 4. We found that EMT was the most enriched pathway within high PIEZO1 HR negative tumors. Other gene sets related to cancer aggressiveness, including hypoxia, apical junction, angiogenesis, and glycolysis, were also enriched in high PIEZO1 HR negative tumors. Additionally, multiple signaling pathways, including TGF-β1, TNF-α via NF-κB, and WNT/β-catenin signaling were enriched in the high PIEZO1 HR negative tumors. These results suggest that PIEZO1 may play a role to promote a hypoxic and glycolytic tumor environment with increased EMT and angiogenesis driven by TGF-β1, TNF-α via NF-κB, and WNT/β-catenin signaling to promote more aggressive features in high PIEZO1 HR negative breast tumors.

3.4. Decreased T cell infiltration exhibited in high PIEZO1 HR negative tumors

To further explore the possible mechanisms of how PIEZO1 may contribute to worse clinical responses in HR negative breast cancer, we investigated whether there is an association of PIEZO1 with the anti-cancer immune response in HR negative breast cancer. To do so, we utilized a CIBERSORT algorithm to estimate immune cell infiltration in HR negative primary tumors. Our results showed that patients characterized by high PIEZO1 expression had decreased levels of CD8+ T cells within their tumors (p = 0.002) when compared to patients with low PIEZO1 expression (Figure

5). Similarly, patients with high PIEZO1 had lower levels of activated CD4+ memory T cells within the tumors than those with low PIEZO1 expression (p = 0.020) (Figure 5). There was no statistically significant difference in infiltration level by PIEZO1 expression for other immune cell types. These results suggest that high PIEZO1 HR negative tumors have a suppressed anti-tumor immune response in terms of T cell infiltration in comparison to low PIEZO1 HR negative tumors.

4. Discussion

To gain insight into the role of mechanosensation in breast cancer, we investigated the role of the PIEZO1 mechanosensory ion channel on breast cancer survival. Our results demonstrate that high PIEZO1 patients have worse survival outcomes than low PIEZO1 patients, specifically in HR negative cohorts. These results are clinically significant in that they suggest PIEZO1 could be utilized as a prognostic biomarker in HR negative breast cancer patients to identify patients at an increased risk for mortality from HR negative breast cancers. We did not observe a difference in survival based on PIEZO1 expression level between HER2 positive and negative patients. The survival outcomes in HR negative patients based on PIEZO1 expression were consistent between TCGA and GSE3494, whereas there was a discrepancy in the statistically significant survival outcomes of the whole cohort between the two datasets the trend was the same. The significant difference in disease-specific survival between PIEZO1 high and low patients observed in the whole cohort in GSE3494 may be due to the difference in patient population and differing methods to define ER/PgR status between datasets and follow up. We also demonstrated that HR negative subtypes, which are known to be more aggressive than HR positive subtypes [38], have increased levels of PIEZO1 expression compared to the HR positive subtype. Therefore, our results suggest that PIEZO1 primarily contributes to worse survival outcomes in HR negative patients, implying that PIEZO1 may have different functions in different subtypes of breast cancer.

As a possible mechanism, our results demonstrate that increased PIEZO1 expression levels are correlated with the upregulation of gene sets associated with cancer aggressiveness, including EMT, hypoxia, apical junction, angiogenesis, and glycolysis with multiple signaling pathways, including TGF-β1, TNF-α via NF-κB, and WNT/β-catenin, in high PIEZO1 HR negative tumors. Our results are consistent with previous studies, which implicate PIEZO1 participation in EMT [14,22], hypoxia response gene expression [18,22], angiogenesis [16], glycolytic metabolism [19], and the multiple signaling pathways [14,16,20,21,34]. EMT, hypoxia, and angiogenesis are cancer-promoting and are well-characterized to be associated with worse survival outcomes in cancer patients [39–41], thus, the enrichment of these processes in high PIEZO1 HR negative tumors may indicate the mechanistic roles of PIEZO1 in contributing to the worse survival outcomes of these patients. We also showed that PIEZO1 was highly correlated to the formation of apical junction complex (AJC) proteins. The enrichment of the AJC gene set likely relates to EMT, which was the most highly enriched gene set in high PIEZO1 HR negative tumors. AJC protein deregulation occurs in EMT to induce the loss of apical-basal polarity and cytoskeletal remodeling, which are required for the acquisition of mesenchymal and invasive characteristics [42]. Our enrichment results suggest that the PIEZO1 ion channel may respond to mechanical forces to induce cellular junction deregulation, cytoskeletal remodeling, and TME modulation, thus promoting EMT and motility of cancer cells. Based on our study, it can be implied that PIEZO1 may drive multiple signaling pathways which promote hypoxia and glycolytic metabolism, leading to EMT and angiogenesis, and ultimately worsening patient survival outcomes in the context of HR negative breast cancer.

Our results also show that PIEZO1 plays a role in the immune cell infiltration into HR negative tumors. There is an inverse correlation of PIEZO1 expression level to CD8+ and activated CD4+ memory T cell infiltration in HR negative cohorts, with those characterized by high PIEZO1 levels having lower CD8+ and activated CD4+ memory T cell infiltration. Thus, PIEZO1 may play a role in CD8+ and CD4+ T cell exclusion from HR negative tumors. As a mechanosensory protein, PIEZO1 may respond to external forces and participate in modulation of the TME and cytoskeletal reorganization to establish an environment that physically inhibits CD8+ and CD4+ T cells from penetrating the tumor.

In addition, we showed that PIEZO1 is correlated to EMT, hypoxia, and TGF-β signaling, processes which have all been shown to be correlated to decreased CD8+ and/or CD4+ T cell infiltration in various cancer types [27–29]. PIEZO1 may participate in these processes to reduce T cell infiltration into the tumor. CD8+ T cells are major drivers of the anti-cancer immune response, utilizing granzymes and perforins to kill the target cancer cells, while CD4+ T cells contribute to the anti-cancer immune response through CD8+ T cell priming and activation of innate immune cells [30,43]. In breast cancer, we have shown that higher levels of CD8+ T cell infiltration and increased CD4+ activity are correlated with a better patient prognosis [31]. Due to the importance of CD8+ and CD4+ T cells in the anti-cancer immune response, exclusion of CD8+ T cells and activated CD4+ memory T cells from high PIEZO1 HR negative tumors enables cancer progression and likely contributes to the worse survival outcomes that we see in HR negative breast cancer patients with high PIEZO1 expression levels. Interestingly, while we saw decreased levels of these effector cells in high PIEZO1 groups, this same observation was not seen for other immune cell types. Macrophages, NK cells, dendritic cells, and other immune cells showed no significant difference in infiltration levels between high and low PIEZO1 HR negative tumors. As a mechanosensory ion channel, PIEZO1 may participate in the physical modulation of the TME to lead to immune cell exclusion, but our results suggest that this isn't the only function of PIEZO1 within the HR negative tumors. If this was the case, we would expect to see lower levels of all immune cell types in high PIEZO1 tumors, rather than exclusion of only CD8+ and CD4+ T cells. CD8+ and CD4+ T cells are well-established as the most prominent mediators of anti-cancer immunity [44], so our results suggest that PIEZO1 may also have a molecular role that reflects cancer aggressiveness and suppression of anti-tumor immunity in HR negative breast cancer.

Our study, in conjunction with past studies, exemplifies the importance of mechano-signaling, and specifically the PIEZO1 ion channel, in the clinical outcomes of breast cancer patients. Decreased levels of CD8+ and CD4+ T cell infiltration, along with the enrichment of important cancerous processes like EMT, hypoxia, and angiogenesis, may explain why high PIEZO1 patients within the HR negative cohort exhibit worse survival outcomes than those with low PIEZO1 expression. Further research is needed to determine how PIEZO1 mechanistically acts to worsen patient prognosis in HR negative cohorts, and if PIEZO1 could be utilized as a viable therapeutic target.

This study has limitations. First, this was an observational study based on bioinformatic analyses in publicly available patient data with known bias. To validate our bioinformatic results, we are conducting further experimental studies to identify the mechanistic role of PIEZO1 and confirm the involvement of PIEZO1 in highlighted pro-cancer pathways and T cell infiltration. Another caveat is that we analyzed primary tumors only, so the role of PIEZO1 in metastatic sites is undetermined.

5. Conclusions

Our study demonstrated that HR negative breast cancer patients have higher PIEZO1 ion channel expression levels than HR positive patients, and that high PIEZO1 expression is attributed to worse survival in HR negative breast cancer cohorts. Our study suggests that worse survival outcomes in high PIEZO1 HR negative patients may be due to the promotion of EMT, hypoxia, angiogenesis, and glycolysis through multiple signaling pathways, such as TGF-β1, TNF-α via NF-κB, and WNT/β-catenin signaling. It may also be due to PIEZO1-mediated modulation of the TME, resulting in exclusion of CD8+ and CD4+ T cells from HR negative tumors. Our results establish a foundation of knowledge regarding the clinical relevance of the PIEZO1 ion channel in breast cancer and highlight the importance of PIEZO1 on patient survival, ultimately suggesting that PIEZO1 could potentially be utilized as a prognostic biomarker in HR negative breast cancer patients. Further studies investigating the mechanistic roles of PIEZO1 in breast cancer are warranted.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: PIEZO1 expression and AJCC TNM stage in TCGA breast cancer cohort.

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