Comprehensive analysis of the \textit{NME} gene family functions in breast cancer

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\textbf{Background:} An increasing amount of research over recent years on the anti-metastasis function of the non-metastatic (\textit{NME}) gene family has been challenged, with some studies identifying its involvement in the promotion of oncogenesis. Therefore, the specific functions of the \textit{NME} gene family require redefining through a comprehensive analysis of tumor heterogeneity and survival benefit. However, the functions of \textit{NME} genes have not been comprehensively investigated in breast cancer (BC).

\textbf{Methods:} In this study, \textit{ONCOMINE}, \textit{GEPIA}, Kaplan-Meier plotter, \textit{cBioPortal}, \textit{String}, and \textit{metascape} databases were utilized for comparison of the mRNA expression, patient survival and network analysis of \textit{NME}-associated signaling pathways in BC patients.

\textbf{Results:} The mRNA expression of \textit{NME1} and \textit{NME2} was significantly increased in BC. Additionally, high N\textit{ME} 1 and N\textit{ME} 2 levels were related to poor overall survival (OS), while the upregulated expression of N\textit{ME} 3, N\textit{ME} 5, and N\textit{ME} 7 indicated prolonged survival. Moreover, increased mRNA level, amplification, or deep deletions in the \textit{NME} gene family were identified in approximately 41% (450/1098) of all included BC specimens. \textit{NME1} and \textit{NME2} genes displayed the highest correlation with genetic correlations of the human \textit{NME} genes in BC. The following pathways were regulated by \textit{NME} gene upregulation: R-HAS-380270: Recruitment of mitotic centrosome and complexes; GO:0006228: UTP biosynthetic process; R-HAS-380259: Loss of NIP from mitotic centrosomes; hsa03410: Base excision repair; and CORUM:3714: Pericenrin-GCP complex, which was significantly modulated by changes influencing the \textit{NME} genes.

\textbf{Conclusions:} Collectively, our findings revealed that the elevated expression of \textit{NME1} and \textit{NME2} could act as a biomarker and predictive tool for BC patients with poor prognosis. Furthermore, our findings indicated that \textit{NME3}, \textit{NME5}, and \textit{NME7} might play the roles of tumor suppressor genes, which require validation through further experiments.

\textbf{Keywords:} \textit{NME} gene family, breast cancer (BC); transcription factor; prognostic value; molecular functions

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**Introduction**

Breast cancer (BC) is the most prevalent female malignancy worldwide, with the highest morbidity and mortality rates (1). In 2019, approximately 268,600 new cases of invasive BC and 48,100 cases of ductal carcinoma in situ (DCIS) were reported in the female population of America. Furthermore, an estimated 41,760 females are predicted to die from BC (2). The clinical molecular subtypes of BC are based on the state of progesterone receptor (PR), estrogen receptor (ER), human epidermal growth factor receptor-2 (HER-2) and ki-67. The standard available treatments for BC include surgery, radiation, chemotherapy, hormone therapy, and targeted therapy (3), based on the particular BC molecular subtype. Although precision therapy can prolong the survival of BC patients, recurrence and metastasis remain the leading causes of death. Hence, the rapid identification of a reliable biomarker is required, which could facilitate the early diagnosis and prognosis in BC patients.

The human NME family of proteins was previously known as Nm23 proteins (4) or proteins “expressed in the non-metastatic cell.” It consists of 10 isoforms, named NME1–10, according to their subcellular localization and enzymatic activities. The conserved domain with nucleoside diphosphate kinase (NDPK) function is found in all NME family members, though not all are catalytically active (5). The human NME gene family is involved in multiple physiological and pathological activities, such as ciliary functions, proliferation, development, metastasis, and differentiation (6,7). Specifically, studies have revealed that nuclear translocation of NME1 and NME2 further modulate gene transcription via the DNA-binding abilities, which indicates potential co-regulator functions (5), despite the absence of canonical nuclear localization signal.

Additionally, NME1, NME5, NME7, and NME8 harbor the 3′–5′ exonuclease activity (8), indicating their involvement in DNA recombination and repair. NME1 and NME3 may participate in repairing both single- and double-stranded breaks in DNA, contributing to the genomic instability and promoting malignant tumor progression. The subcellular localization of the NME gene family varies from the nucleus, cell membrane, mitochondria, and cytoplasm with different extents. Interestingly, extracellular NME protein has also been found in normal and tumor environments and consistently exhibits NDPK activity. Interestingly, the underlying mechanism which facilitates the nuclear translocation of NME proteins remains unclear due to the absence of the canonical nuclear localization signal. Indeed, clinical data also indicate that the NME gene family may be a critical prognostic indicator of several malignancies, such as gastric, neuroblastoma, and hematological malignancies, without definitive mechanisms. Traditionally, high levels of NME1 protein expression in melanoma, liver cancer, ovarian, lung, and BC are correlated with low metastatic potential, while the opposite phenomenon was observed in hematopoietic malignancies, neuroblastoma, and osteosarcoma. Additionally, the effects of NME1 on OS have not been established (7,9,10). Several therapeutic strategies using NME have been explored in tumor models over the past two decades, including re-expression of NME (11), gene therapy using NME delivery method (12,13), protein-based therapy to deliver cell-permeable NME protein (14), and lysoosphatidic acid (LPA) inhibitor (15).

The mechanism of Janus-faces of NME genes family oncoprotein interactions has not yet clarified (16). These extracellular NME proteins have previously been reported to show oncogenic functions or anti-differentiation capacities, which contradicts their known anti-metastatic function (17). The latest report has revealed that NME1 could enhance tumor growth and metastases in melanoma (18).

Clarifications on the precise functions of the NME gene family as oncogenes or tumor suppressor genes in BC patients are therefore critical. However, the diverse transcriptional expression, molecular functions, biological processes, and prognostic significance of most NME genes family require further elucidation in BC. In this study, a comprehensive analysis of the correlation of NME isoforms with the pathogenesis and progression of BC was performed via the incorporation of several widely-established databases, which could contribute to our current knowledge on the pathology, molecular significance and clinical predictive value in BC.

We present the following article in accordance with the MDAR checklist (available at http://dx.doi.org/10.21037/tcr-20-1712).

**Methods**

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

**Oncomine database**

The transcriptional levels of NME in diverse types of
malignancies were analyzed using the online cancer microarray database and data-mining platform (https://www.oncomine.org), ONCOMINE (19). ONCOMINE database contains 715 datasets and 86,733 samples covering most common cancer types. The mRNA expression of NME was compared between cancer and normal samples before determining the P values via a Students’ t-test. The P value and fold change (FC) were set at 0.01 and 2, respectively.

The Gene Expression Profiling Interactive Analysis (GEPIA) Dataset

GEPIA (http://gepia.cancer-pku.cn) is a newly developed web-based tool that facilitates expression analyses at the subtype level, based on TCGA and GTEx data (20). Gene and isoform expression can be analyzed by GEPIA, including general information, differential analysis, expression analysis, survival analysis, profiling plotting, similar genes detection, etc. Herein, the differential expression of NME genes in BRCA and normal samples was validated by the pathological stage. The P values via a Students’ t-test, and P<0.05 was considered statistically significant.

The Kaplan-Meier Plotter

Kaplan-Meier (KM) Plotter (www.kmplot.com) is a web-based tool used to assess the effect of various genes on survival across different cancers. It contained gene expression and survival data from the largest dataset on BC patients (21) and was employed to validate the prognostic value of the mRNA expression of the NME gene family. In order to evaluate the effects of NME gene expression on the OS of BC patients, samples were categorized into two groups based on the median gene expression (high versus low expression), followed by assessment using the KM survival plot, with a hazard ratio (HR) and corresponding 95% confidence intervals (CIs) as well as log rank P value. KM curves were plotted only on the JetSet best probe set of NMEs.

The cBioPortal for cancer genomics

The open-access and open-source cBioPortal (http://www.cbioportal.org/) for cancer genomics were developed at Memorial Sloan-Kettering Cancer Center (MSKCC) to explore multidimensional cancer genomics interactively (22,23). Functionally, it supports and stores data associated with DNA methylation, DNA copy-number, nonsynonymous mutations, enrichment, mRNA and miRNA expression, co-expression, and clinical parameters. The online cBioPortal tool rendered the calculation of the frequency of NME gene family alterations, including mutation, amplification, deletion, and mRNA expression z-scores (RNA Seq V2 RSEM). The heatmap demonstrated that the correlation of NME genes was procured by using the online tool (https://chart-studio.plot.ly). The counting data were expressed as rate (%). The correlation of the different genes was tested by Pearson’s correlation.

Functional enrichment and bioinformatics analysis

Metascape (http://metascape.org) is an online platform designed to provide a comprehensive gene list annotation and analysis resource. The Metascape database integrates over 40 independent bioinformatics bases, which could be utilized to extract abundant annotations, perform functional enrichment, interactome analysis, gene annotation, and establish PPI networks from lists of genes and proteins (25). Herein, the most frequently-changed related genes were identified via the analysis of gene lists containing the NME gene family by using Gene Ontology (GO) as well as the Kyoto Encyclopedia of Genes and Genomes (KEGG) tools within Metascape. Pearson’s correlation was determining the correlation. The correlation of the different genes was tested by Pearson’s correlation.

Results

The transcription expression of NME genes in BC patients

The identification of the NME gene family has been
reported in the BC genome. ONCOMINE database analysis of NME transcriptional levels between tumor and normal samples in different cancer types (Figure 1) revealed upregulated mRNA expression of NME1, NME2, NME3, NME4, NME5, NME8, NME9, and NME10 in BC patients (Table 1). Moreover, the mRNA expression of NME1 increased from 2.006 to 3.309-fold in different types of BC compared to normal tissues (26-32). Curtis et al. revealed an upregulated mRNA expression of NME2 in Mucinous Breast Carcinoma (FC = 2.004) (28). Moreover, the TCGA dataset showed that NME3 was elevated in Intraductal Cribriform Breast Adenocarcinoma, Invasive Ductal and Lobular Breast Carcinoma, and Mixed Lobular and Ductal Breast Carcinoma compared to the control samples, with FC of 2.783, 2.179 and 2.313 respectively. Meanwhile, they also demonstrated the overexpression of NME4 in Mucinous Breast Carcinoma (FC = 2.190) and the overexpression of NME5 in Mucinous Breast Carcinoma (FC = 2.493). According to the datasets from Ma Breast 4 and TCGA (30), NME8 expression was higher in tumor tissues than normal tissues as follows: (I) Ductal Breast Carcinoma in Situ Stroma (FC = 2.640); (II) Invasive Ductal Breast Carcinoma (FC = 2.434); (III) Invasive Lobular Breast Carcinoma (FC = 2.017). Moreover, Finak et al. revealed the upregulated expression of NME9 (FC = 3.482) in Invasive Breast Carcinoma Stroma than normal tissue (33). According to the dataset by Ma et al., NME10 was elevated in Invasive Ductal Breast Carcinoma (FC = 2.018) (30). However, ONCOMINE analysis revealed no significant difference in mRNA levels of NME6 and NME7 between BC and normal samples.

The GEPIA dataset was subsequently adopted to compare NME mRNA expression between BC and normal samples. The expression of NME1 and NME2 was significantly elevated in BC tissues compared to normal specimens (P<0.05), while NME3-10 expression was not statistically significant (Figure 2). Additionally, the expression levels of NME1-10 were not significantly different among different stages of BC (Figure 3).

Furthermore, the KM Plotter was employed to study

![Figure 1](image_url) The transcriptional levels of NME genes in different types of cancer.
Table 1 The transcriptional alterations of NME expression between different types of breast carcinoma and normal specimens (Oncomine Database)

| Gene   | Types of breast cancer vs. normal                                      | Fold change | P value     | t-test   | Reference and/or Source |
|--------|-----------------------------------------------------------------------|-------------|-------------|----------|-------------------------|
| NME1   | Lobular Breast Carcinoma vs. Normal                                   | 2.166       | 3.59E-4     | 5.080    | Sorlie T (26)            |
|        | Ductal Breast Carcinoma vs. Normal                                   | 2.826       | 5.98E-6     | 10.849   | Sorlie T (26)            |
|        | Lobular Breast Carcinoma vs. Normal                                  | 2.303       | 0.003       | 4.567    | Perou CM (27)            |
|        | Ductal Breast Carcinoma vs. Normal                                   | 3.309       | 6.05E-4     | 8.640    | Perou CM (27)            |
|        | Medullary Breast Carcinoma vs. Normal                                | 2.876       | 1.88E-17    | 13.844   | Curtis C (28)            |
|        | Mucinous Breast Carcinoma vs. Normal                                 | 2.217       | 1.09E-20    | 12.957   | Curtis C (28)            |
|        | Tubular Breast Carcinoma vs. Normal                                  | 2.021       | 3.23E-27    | 13.770   | Curtis C (28)            |
|        | invasive Ductal and Invasive Lobular Breast Carcinoma vs. Normal     | 2.188       | 1.32E-29    | 14.086   | Curtis C (28)            |
|        | Breast Carcinoma vs. Normal                                          | 2.101       | 3.99E-5     | 5.460    | Curtis C (28)            |
|        | Ductal Breast Carcinoma in Situ vs. Normal                          | 2.530       | 0.002       | 3.878    | Curtis C (28)            |
|        | Lobular Breast Carcinoma vs. Normal                                  | 2.006       | 0.006       | 3.748    | Sorlie T (29)            |
|        | Ductal Breast Carcinoma vs. Normal                                  | 2.841       | 1.25E-4     | 8.456    | Sorlie T (29)            |
|        | Invasive Ductal Breast Carcinoma Epithelia vs. Normal                | 2.209       | 5.03E-6     | 5.959    | Ma XJ (30)               |
|        | Ductal Breast Carcinoma in Situ Epithelia vs. Normal                | 2.527       | 0.004       | 3.275    | Ma XJ (30)               |
|        | Ductal Breast Carcinoma vs. Normal                                  | 2.825       | 6.59E-11    | 8.351    | Richardson AL (31)       |
|        | Invasive Ductal Breast Carcinoma vs. Normal                          | 2.133       | 0.002       | 3.862    | Radanyi L (32)           |
|        | NME2                                                                  |             |             |          |                         |
| NME3   | Mucinous Breast Carcinoma vs. Normal                                 | 2.004       | 2.00E-11    | 8.537    | Curtis C (28)            |
|        | Intraductal Cribriform Breast Adenocarcinoma vs. Normal              | 2.783       | 6.14E-36    | 26.856   | TCGA                     |
|        | Invasive Ductal and Lobular Breast Carcinoma vs. Normal              | 2.179       | 1.76E-8     | 15.396   | TCGA                     |
|        | Mixed Lobular and Ductal Breast Carcinoma vs. Normal                 | 2.313       | 2.01E-5     | 8.713    | TCGA                     |
| NME4   | Mucinous Breast Carcinoma vs. Normal                                 | 2.190       | 0.004       | 5.008    | TCGA                     |
|        | Intraductal Cribriform Breast Adenocarcinoma vs. Normal              | 2.493       | 2.58E-5     | 7.006    | TCGA                     |
| NME5   | Mixed Lobular and Ductal Breast Carcinoma vs. Normal                 |             |             |          |                         |
| NME6   | NA                                                                    | NA          | NA          | NA       | NA                       |
| NME7   | NA                                                                    | NA          | NA          | NA       | NA                       |
| NME8   | Ductal Breast Carcinoma in Situ Stroma vs. Normal                    | 2.640       | 0.002       | 3.45     | Ma XJ (30)               |
|        | Invasive Ductal Breast Carcinoma Stroma vs. Normal                   | 2.434       | 0.008       | 2.958    | Ma XJ (30)               |
|        | Invasive Lobular Breast Carcinoma vs. Normal                         | 2.017       | 6.27E-8     | 6.022    | TCGA                     |
| NME9   | Invasive Breast Carcinoma Stroma vs. Normal                          | 3.84        | 2.52E-15    | 13.022   | Finak G (33)             |
| NME10  | Invasive Ductal Breast Carcinoma vs. Normal                          | 2.018       | 0.003       | 3.120    | Ma XJ (30)               |

NA, not available.
the effects of NME genes in BC survival. As a result, upregulated NME1 and NME2 levels were significantly associated with poor OS (P<0.05) in BC patients (Figure 4). Hence, the overexpression of NME1 and NME2 potentially indicates poor prognosis in BC patients, while NME3, NME5, and NME7 displayed effects opposite to NME1 and NME2.

**Genetic alteration and correlation of NME genes in BC**

The cBioportal online tool and analysis of the TCGA database revealed NME genetic alterations, followed by the identification of any genetic associations. Consequently, genetic alterations of NME genes were detected in 450/1,098 (41%) of BC samples (Figure 5), including mRNA and protein expression, mutation, amplification as well as homozygous deletion. The cBioPortal was additionally adopted to investigate the interactions between specific NME genes (including Pearson’s correlation) (Figure 6).

**Predicted functions and pathway enrichment analysis of NME genes in BC**

The PPI network for the NMEs gene family revealed that the 30 most frequently changed neighboring genes (Figure 7). A list of the expressed NME genes was compiled along with the most frequently changed associated genes before GO and KEGG analysis using Metascape (Figure 8). NME gene alterations influenced the following processes: R-HAS-380270: Recruitment of mitotic centrosome and complexes; GO:0006228: UTP biosynthetic process; R-HAS-380259: Loss of NlP from mitotic centrosomes; hsa03410: Base excision repair; CORUM:3714: Pericentrin-GCP complex; GO:1902850: microtubule cytoskeleton organization involved in mitosis; GO:0045454: cell redox homeostasis; GO:0031346: positive regulation of cell projection organization.

**Discussion**

Continuous advances in precision treatment have prolonged
the survival of BC patients and enhanced their quality of life. However, tumor recurrence and metastasis are still the main causes of death in BC patients. The underlying mechanisms remain poorly understood, highlighting a critical need to explore and identify more efficient molecular markers for the early diagnosis and treatment of BC. Currently, the significance of NME gene family expression in the initiation and prognosis of BC remains largely unknown. The NME
Figure 4 The prognostic significance of NME gene expression in patients with breast cancer.
gene family display NDPK functions that share DNA binding, DNA damage repair, class switch recombination, and transcription (34), highlighting their potential in tumor progression (5). Essentially, the NME gene family is involved in multiple functions across three major cellular processes, namely protein histidine phosphorylation, nucleotide channeling and membrane remodeling, tumor progression, and metastasis (35).

Our study showed alterations of NME genes in ~41% (450/1,098) of BC specimens in the form of increased
mRNA levels, deep deletion, or amplification. We also revealed that increased mRNA expression of NME1 and NME2 was significantly correlated with poor prognosis in BC patients. Amongst the NME genes family, NME1 and NME2 are the most commonly investigated in BC. Several types of research have demonstrated that NME1 not only suppresses metastasis, but enhances cancer cell growth and metastatic properties, including melanoma (18), BC (36), lung adenocarcinoma (37), and neuroblastoma (38). In agreement with the previous study, NME1 is characterized by the capacity to inhibit the metastatic phenotype of tumor cells, without affecting primary tumor growth (5,16). This positive correlation in NME1 and NME2 is attributed to the 88% sequential homology, as well as the similar

Figure 7 Genes significantly associated with NME gene family alterations in breast cancer.
Conery et al. revealed that the loss of NME1, suspected to enhance metastasis, might also trigger the acquisition of chromosomal instability at an earlier stage of tumor development by in vitro assay in HBE125 E6/E7 and 293T cells (39). Meanwhile, another study confirmed that NME1, as a novel nonhomologous end-joining (NHEJ) factor, promoted genome stability (40).

It is worth noting that high expression of NME3, NME5, and NME7 was correlated with long-term survival, despite contradictory findings in transcriptional expression between ONCOMINE and GEPIA dataset. Similarly, one study confirmed NME3 as a positive downstream regulator of flagellin-mediated NF\(\kappa\)B signaling, which is highly correlated with the expression of toll-like receptor 5 (TLR5) in ovarian, lung and BCs, and increased OS. Interestingly, they further recognized the proinflammatory effects of NME3, a signaling downstream target of TLR5, which might facilitate tumor immunotherapies (41).

By using expression microarrays and array comparative genomic hybridization, Parris et al. have revealed 97 invasive diploid breast tumors with changes in DNA copy number and transcriptional expression. The integrative analysis also showed that downregulated NME5 and other genes play vital roles in BC carcinogenesis and progression, highlighting their potential as novel therapeutic targets (42).

NME6 and NME7 are critically involved in the renewal of embryonic stem cells and oncogenesis (43). NME7, a poorly characterized member of the NME family, is a component of the \(\gamma\)-tubulin ring complex (\(\gamma\)TuRC) and regulates the microtubule-nucleating activity (44).

Although a small number of studies have suggested that NME4, NME8, NME9, and NME10 are highly expressed in BC; the comprehensive analysis undertaken in our study showed no correlation with clinical diagnosis and prognosis. NME4, also known as a moonlighting protein, is transported to the mitochondria through the mitochondria-specific targeting signal. Unlike the other NME family members, NME4 requires cleavage for its catalytic activity (45).

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In this study, we constructed and explored the PPI network for the NMEs family. Furthermore, the association of NME gene expression and the most frequently changed correlated genes with BC carcinogenesis and prognosis was assessed by GO and KEGG analysis. The gene families with the highest frequencies were NIPA, TUBG, TUBGCP, etc. As a result, we conclude that the following pathways should be further investigated, including R-HAS-380270: Recruitment of mitotic centrosome proteins and complexes; GO:0006228: UTP biosynthetic process; R-HSA-380259: Loss of NIP from mitotic centrosomes; hsa03410: Base excision repair; and CORUM:3714: Pericentrin-GCP complex.

Collectively, our research suggested that the elevated expression of NME1 and NME2 could potentially act as a molecular marker for the identification of BC patients with poor prognosis, while NME3, NME5, and NME7 may still play tumor-suppressive roles. Furthermore, NME1 and NME2 are likely to be a strong prognostic biomarker and potential therapeutic target to enhance the diagnosis and therapy of BC. Moreover, large-scale studies are needed to validate the functions of NME3, NME5, and NME7.
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**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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