Expression of Malic Enzyme 3 in Breast Cancer and Precancerous Lesions: a Promising Novel Biomarker for Carcinogenesis and Prognosis

Duo You
Zhejiang University

Danfeng Du
Zhengzhou University

Xinmin Li
Zhengzhou maternal and child health hospital

Xun Hu (✉ huxun@zju.edu.cn)
Zhejiang University

Research Article

Keywords: Breast cancer, ME3 expression, carcinogenesis, prognosis

DOI: https://doi.org/10.21203/rs.3.rs-144916/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

**Purpose:** While malic enzymes 1 (ME1) was correlated with breast cancer progression and prognosis, the association of ME3 (a homologue of ME1) with breast cancer is not known. The aim of this study is to explore the potential of ME3 as a biomarker in breast cancer carcinogenesis and prognosis.

**Methods:** A total of 107 patients confirmed with breast cancer were enrolled. The ME3 expression was evaluated by IHC and correlated with clinicopathological indicators.

**Results:** The ME3 positive immunostaining rate was higher in normal breast tissues and decreased stepwise from normal (97.60%) to usual ductal hyperplasia (91.1%), atypical ductal hyperplasia (64.2%), carcinoma in situ (62.5%) and invasive carcinoma (45.5%). Similarly, the decreasing tendency was observed for ME3 positive immunostaining rate from Tis (75.0%) through T1 (62.5%) and T2 (37.5%) to T3 (33.3%) and from stag 0 (75.0%) through I (72.0%), II (44.4%) to III (24.1%). ME3 expression was related with negative lymph node metastasis. Patients with positive expression of ME3 had better outcome. By incorporating ME3 into tumor TNM staging, the area under receiver operating characteristic curve for the 5-year survival was increased from 84.0% to 87.5%.

**Conclusions:** ME3 may be a promising biomarker for better prognosis for breast cancer patients.

Introduction

Breast cancer remains the leading cause of cancer-related death among females worldwide. It accounts for 24% of all cancer cases and 15% of all cancer deaths among females. With dramatic changes in lifestyles and economy, the incidence and mortality rates of breast cancer in Chinese women have a horrendous growth, whereas the overall 5-year survival rate for Chinese women with breast cancer is much lower than American women (82% vs. 91%). Breast cancer has been a major public health problem in China.

It has been well known that changes in cell metabolism can contribute to transformation and tumor progression. Malic enzymes (MEs), including three isoforms of ME1, 2 and 3, catalyze the oxidative decarboxylation of malate to pyruvate, with a concomitant reduction of NAD(P)⁺ to NAD(P)H. The different isoforms of MEs have different cofactor specificity and sub-cellular localizations in mammals: the cytosolic NADP⁺-dependent ME1, the mitochondrial NAD(P)⁺-dependent ME2, and the mitochondrial NADP⁺-dependent ME3. MEs are important for NADPH production: while ME1 is important for the NADPH production in the cytosol, ME3 takes a part in maintaining the redox homeostasis in mitochondria.

Recently, a growing number of evidence has indicated that ME family plays an important role in different types of cancer. Jiang et al reported that up-regulation of MEs induced p53 inhibition. Lu and Murai et al demonstrated that ME1 inhibited proliferation and invasion by regulating NADPH homeostasis in...
gastric cancer and colon cancer cell lines\textsuperscript{14,15}. Ren et al showed that ME2 depletion increased reactive oxygen species (ROS) and NADP+/NADPH ratio, inhibited cell proliferation and induced cell death and differentiation in lung cancer cell\textsuperscript{16}. Woo et al reported high-expression of ME2 was associated with poorer overall survival (OS) for patients with head and neck squamous cell carcinoma\textsuperscript{17}. Dey et al found that ME3 depletion selectively killed ME2-null pancreatic cancer cells and Zhang Q et al discovered that ME3 promoted the proliferation, invasion and metastasis of pancreatic cancer cells\textsuperscript{18,19}. Moreover, Liao et al reported that ME1 expression was positively correlated with large tumor size, high grade, poor survival and chemotherapy resistance, indicating that ME1 promoted breast cancer progression and was associated with poor prognosis\textsuperscript{20}.

In this study, we sought to characterize ME3 protein expression in breast precancerous and cancerous lesions, aiming to explore the potential of ME3 as a biomarker for carcinogenesis and prognosis in breast cancer.

### Results

#### The clinicopathological characteristics of the patients

Table 1 summarized the clinicopathological characteristics of the 107 breast cancer patients. All the patients were confirmed with breast cancer, and were predominately at the invasive stage (92.5%), only 7.5% at CIS stage. Pathologically, there were 101 cases with ductal carcinoma, 3 cases with invasive lobular carcinoma, 1 case with medullary carcinoma, 1 case with mucinous carcinoma, and 1 case with mixed carcinoma. Based on the T staging, more than half of the patients were in T2 (59.8%), followed by T1 (29.9%), Tis (7.5%) and T3 (2.8%). About half of the patients (42.1%) had positive lymph node metastasis when they were diagnosed. 71.9% of the patients were in early stage based on TNM staging, including 7.5% patients in stage 0, 23.4% in stage I, 42.1% in stage II, and the rest (27.1%) in advanced stage III.
Table 1
Clinicopathological characteristics of 107 breast cancer patients.

| Variables                        | No. of patients examined |
|----------------------------------|--------------------------|
|                                  | N (%)                    |
| **Age**                          |                          |
| < 50 years                       | 64 (59.8)                |
| ≥ 50 years                       | 43 (40.2)                |
| **Gender**                       |                          |
| Male                             | 0 (0)                    |
| Female                           | 107 (100.0)              |
| **T staging**                    |                          |
| Tis                              | 8 (7.5)                  |
| T1                               | 32 (29.9)                |
| T2                               | 64 (59.8)                |
| T3                               | 3 (2.8)                  |
| **Lymph node metastasis**        |                          |
| No                               | 62 (57.9)                |
| Yes                              | 45 (42.1)                |
| **Pathological staging**         |                          |
| 0                                | 8 (7.5)                  |
| I                                | 25 (23.4)                |
| II                               | 45 (42.1)                |
| III                              | 29 (27.1)                |
| **Vascular cancer embolus**      |                          |
| No                               | 87 (81.3)                |
| Yes                              | 20 (18.7)                |
| **ER status**                    |                          |
| Negative                         | 38 (35.5)                |
| Positive                         | 69 (64.5)                |
| Variables          | No. of patients examined |
|--------------------|--------------------------|
|                    | N (%)                    |
| PR status          |                          |
| Negative           | 42 (39.3)                |
| Positive           | 65 (60.7)                |
| Her-2 status       |                          |
| Negative           | 45 (42.1)                |
| Positive           | 39 (36.4)                |
| Not available      | 23 (21.5)                |
| Ki67 index         |                          |
| Low expression (≤14%) | 27 (25.2)            |
| High expression (>14%) | 80 (74.8)            |

**ME3 positive expression is decreased with the progression from precancerous to cancerous lesions**

The immunohistochemical staining showed that the immunoreactivity for ME3 protein was located in the cytoplasm both in breast cancerous and precancerous tissue (Fig. 1). The ME3 positive expression is defined by semiquantitative scoring system as described in Materials and Methods. The ME3 positive immunostaining rate was higher in the normal breast tissue (97.60%, 82/84), and then the positive rate decreased from UHP (91.1%, 72/79) to AHP (64.2%, 43/67), CIS (62.5%, 40/64) and invasive carcinoma (45.5%, 45/99). The decreasing tendency was inversely correlated with the progression from precancerous to cancerous lesions ($R^2 = 0.9414$, $P = 0.006$, linear regression analysis, Table 2, Fig. 2).

**Table 2**

| Pathological classification | No. of lesions examined | Expression of ME3 protein | $\chi^2$ | P     |
|-----------------------------|-------------------------|---------------------------|---------|-------|
|                             |                         | Positive N (%) | Negative N (%) |       |       |
| Normal                      | 84                      | 82 (97.6)       | 2 (2.4)      | 195.89| 0.000 |
| Usual hyperplasia           | 79                      | 72 (91.1)       | 7 (8.9)      |       |       |
| Atypical hyperplasia        | 67                      | 43 (64.2)       | 24 (35.8)    |       |       |
| Carcinoma in situ           | 64                      | 40 (62.5)       | 24 (37.5)    |       |       |
| Invasive carcinoma          | 99                      | 45 (45.5)       | 54 (54.5)    |       |       |
**ME3 is associated with better prognosis in breast cancer patients**

The median follow-up of the entire cohort was 73.7 months (range 11.7–94.8 months) and 21 (19.6%) patients died because of the deteriorated breast cancer during the follow-up. The survival rates of 1-, 3- and 5-year were 98.1%, 87.9%, and 82.2%, respectively. It was noteworthy that the median survival time of the 21 dead patients was only 36.6 months, ranging from 11.7 to 67.2 months. Of the 21 died cases, one third (33.3%) was in early stage I and II, and the rest (66.7%) was in advanced stage III. In this study, the younger patients with ages ≤ 40 years accounted 16.8% (18/107) and about half of the patients (44.4%, 8/18) were in advanced stage III.

The Kaplan–Meier survival analysis showed that the patients with positive expression of ME3 had significant better outcome than those with negative expression ($\chi^2 = 8.233, P = 0.004$, Fig. 4).

In this study, lymph node metastasis ($\chi^2 = 12.284, P = 0.000$), TNM staging progression ($\chi^2 = 18.232, P = 0.000$) and high expression of Ki67 index ($\chi^2 = 5.155, P = 0.024$) were also negatively associated with survival (Supplementary Table S1). However, no influence was observed for age, T staging, vascular cancer embolus, ER status, PR status and Her-2 status on survival (Supplementary Table S1).

**ME3 is inversely associated with the progression of breast cancer**

Furthermore, linear regression analysis showed that ME3 expression decreased from Tis (75.0%, 6/8) to T1 (62.5%, 20/32), T2 (37.5%, 24/64) and T3 (33.3%, 1/3), ($R^2 = 0.9394, P = 0.031$, Table 3, Fig. 3A). The analysis on TNM staging and ME3 expression showed ME3 positive immunostaining rate was much higher in stage 0 (75.0%, 6/8) and stage I (72.0%, 18/25) than in stage II (44.4%, 20/45) and stage III (24.1%, 7/29) ($\chi^2 = 14.953, P = 0.002$, Table 3). Similarly, the decreasing tendency was also significant from stage 0 to I, II and III ($R^2 = 0.9283, P = 0.037$, linear regression analysis, Fig. 3B). ME3 positive expression in breast cancer tissue was related with negative lymph node metastasis ($\chi^2 = 6.393, P = 0.011$). No significant difference was observed for ME3 expression with age, vascular cancer embolus, ER status, PR status, Her-2 status, or Ki67 index (Table 3)
Table 3
Relationship between ME3 expression and clinicopathological characteristics in breast cancer.

| Variables                        | No. of patients examined | Expression of ME3 protein | \( \chi^2 \) | \( P \) |
|----------------------------------|--------------------------|---------------------------|--------------|-------|
|                                  |                          | Positive (n = 51)         | Negative (n = 56) |       |
|                                  |                          | N (%)                     | N (%)         |       |
| Age                              |                          |                           | 1.914         | 0.166 |
| < 50 years                       | 64                       | 27 (42.2)                 | 37 (57.8)     |       |
| ≥ 50 years                       | 43                       | 24 (55.8)                 | 19 (44.2)     |       |
| T staging                        |                          |                           | 8.117         | 0.044 |
| Tis                              | 8                        | 6 (75.0%)                 | 2 (25.0%)     |       |
| T1                               | 32                       | 20 (62.5%)                | 12 (37.5%)    |       |
| T2                               | 64                       | 24 (37.5%)                | 40 (62.5%)    |       |
| T3                               | 3                        | 1 (33.3%)                 | 2 (66.7%)     |       |
| Lymph node metastasis           |                          |                           | 6.393         | 0.011 |
| No                               | 62                       | 36 (58.1)                 | 26 (41.9)     |       |
| Yes                              | 45                       | 15 (33.3)                 | 30 (66.7)     |       |
| Pathological staging             |                          |                           | 14.953        | 0.002 |
| 0                                | 8                        | 6 (75.0)                  | 2 (25.0)      |       |
| I                                | 25                       | 18 (72.0)                 | 7 (28.0)      |       |
| II                               | 45                       | 20 (44.4)                 | 25 (55.6)     |       |
| III                              | 29                       | 7 (24.1)                  | 22 (75.9)     |       |
| Vascular cancer embolus          |                          |                           | 0.070         | 0.791 |
| No                               | 87                       | 42 (48.3)                 | 45 (51.7)     |       |
| Yes                              | 20                       | 9 (45.0)                  | 11 (55.0)     |       |
| ER status                        |                          |                           | 0.730         | 0.393 |
| Negative                         | 38                       | 16 (42.1)                 | 22 (57.9)     |       |
| Positive                         | 69                       | 35 (50.7)                 | 34 (49.3)     |       |
| PR status                        |                          |                           | 2.537         | 0.111 |
| Negative                         | 42                       | 16 (38.1)                 | 26 (61.9)     |       |
| Positive                         | 65                       | 35 (53.8)                 | 30 (46.2)     |       |
| Variables          | No. of patients examined | Expression of ME3 protein | $\chi^2$ | P  |
|--------------------|--------------------------|---------------------------|---------|----|
|                    |                          | Positive(n = 51) | Negative(n = 56) |      |    |
|                    |                          | N (%) | N (%)           |      |    |
| Her-2 status       |                          |       |                 | 1.439 | 0.487|
| Negative           | 45                       | 22 (48.9) | 23 (51.1)         |      |    |
| Positive           | 39                       | 16 (41.0) | 23 (59.0)         |      |    |
| Not available      | 23                       | 13 (56.5) | 10 (43.5)         |      |    |
| Ki67 index         |                          |                 |                 | 3.389 | 0.066|
| Low expression ($\leq$ 14%) | 27                       | 17 (63.0) | 10 (37.0)         |      |    |
| High expression (> 14%) | 80                       | 34 (42.5) | 46 (57.5)         |      |    |

**Receiver operating characteristic curve**

Using multivariate logistic regression and receiver operating characteristic curve (ROC), we explored the potential of ME3 expression in the classification of 5-year survival outcome. Logistics regression analysis showed that TNM staging was the influencing factor for 5-year survival outcome in the available clinical information in this study. Using TNM alone, the 5-year survival model based on the prediction probability showed that the area under ROC curve was 84.0% (Fig. 5A). Using TNM combined with ME3 expression, the area under ROC curve increased to 87.5% (Fig. 5B), suggesting a potential role of the ME3 in prediction of patients at risk of breast cancer death.

**Discussion**

To the best of our knowledge, this is the first report to investigate the potential of ME3 as a biomarker in breast cancer carcinogenesis and progression. The present study demonstrated a stepwise decrement for ME3 protein expression at the different stages of breast malignant progression, indicating that ME3 aberrant loss of expression may be involved in breast carcinogenesis.

Furthermore, ME3 positive immunostaining rate was decreasing from Tis through T1, T2 to T3 and from stag 0 through I, II to III. In addition, ME3 expression was strongly related with lymph node status. The close association between ME3 expression and pathological staging suggests that ME3 may involve in the progression of in-situ invasion and lymph node metastasis of breast cancer. Meanwhile, patients with ME3 positive expression have a better survival than the negative expression of ME3.

Breast cancer is the most frequent cancer among female and TNM staging at diagnosis is still one of the most important factors affecting prognosis so far. Breast cancer has a heterogeneous prognosis of the 5-year survival rate varies substantially among patients, range from 27% for distant metastasis to 86% for
regional breast cancer and 99% for localized breast cancer in the report from the American Cancer Society. In this study, addition of ME3 expression into TNM staging has a stronger predictive value of 5-year survival outcome than TNM staging alone. Hence, ME3 could be a potential biomarker for better outcome of breast cancer patients.

It has not been well characterized for the ME3 and the associated metabolic pathway in carcinogenesis, especially in breast cancer. The mechanism of ME3 aberrant expression in breast cancer is largely unknown. Biologically, ME3 catalyzes a reaction by oxidizing malate to pyruvate and concomitantly reducing NADP⁺ to NADPH and this reaction is crucial for maintaining the NADPH pool in mitochondria. As NAPDH is the essential molecule for antagonizing mitochondrial ROS (mROS), ME3 downregulation may enhance the accumulation of mROS. It has been well recognized that mROS overproduction would promote carcinogenesis and tumor progression by inducing genetic instability, modifying gene expressions, and activating diverse signaling. Therefore, ME3 downregulation may take a part in breast carcinogenesis and breast cancer progression via perturbing the homeostasis of NADPH/NADP⁺ in mitochondria, leading to accumulation of mROS. Further investigation is required to validate the hypothesis.

It is interesting to note that ME3 and ME1 showed opposite effect on diagnosis on the survival of breast cancer patients. While it has been extensively studied that higher ME1 expression is associated with worse prognosis of breast cancer patients than the lower ME1 expression, the higher ME3 expression was associated with a better prognosis of breast cancer patients than the lower ME3 expression. Because ME1 and ME3 share the same catalytic function catalyzing the oxidative decarboxylation of malate, the different effect of the enzymes on prognosis may emanate from their different locations. ME1 is located in cytoplasm, whereas ME3 is located in mitochondria. Because it is well recognized that the vicious cycle of ROS in mitochondria is important for carcinogenesis and cancer progression, it is conceivable that this vicious cycle is to some extent associated with ME3 activity. The lower ME3 expression may enhance the vicious cycle and hence may be correlated with the progression from normal to precancerous tissue and to cancerous tissue as well as the progression from early to later stage of breast cancer.

In conclusion, our data supported ME3 as a potential biomarker for breast carcinogenesis as well as for the progression of breast cancer patients. ME3 positive expression may be a promising biomarker for better outcome for breast cancer patients.

Materials And Methods

Patients

107 breast cancer patients were retrieved in this study from a database, which has been established by The State Key Laboratory of Esophageal Cancer Prevention & Treatment and Henan Key Laboratory of Esophageal Cancer Research in The First Affiliated Hospital of Zhengzhou University. All the patients had
been underwent mastectomy from August 2012 to December 2014 without any radiation therapy or chemotherapy prior to surgery before operation. All patients were females with a mean age of 48.41±11.18 years and a median age of 46 years (range from 26 to 82 years). Staging for breast cancer were based on American Joint Committee on Cancer (AJCC) TNM staging system in 2017. The follow-up was performed with hospital medical records, either through telephone or through home interview to each patient yearly until death. The last follow-up was May 2020. Overall survival time was calculated from the day of mastectomy to death or to the last follow-up date. The study protocol was approved by the Medical Ethics Committee of Zhengzhou University.

**Tissue collection and processing**

A total of 107 breast cancer tissues and 87 matched adjacent non-cancerous tissues were collected from surgically resected specimens from the patients with primary breast cancer. The tissues were routinely 10% neutral formalin fixed, paraffin-embedded and then sectioned. Serial 4 µm sections were prepared for histopathological analysis (hematoxylin and eosin stain) and immunohistochemical staining.

**Histopathological analysis**

Histopathological diagnoses and staging for breast cancer and adjacent non-cancerous lesions were made based on WHO criteria in 2012. The epithelial proliferative lesions in mammary gland acini and duct were categorized as "normal", usual hyperplasia (UHP), atypical hyperplasia (AHP) and carcinoma in situ (CIS). Histopathologically, UHP was usually found adjacent to the tumor tissue; AHP and CIS were frequently observed as isolated lesions in the surgically resected breast specimens. Pathologically, of the 87 cases with adjacent cancer tissue, there were 84 cases identified with normal tissue (96.6%, 84/87), 79 with UHP (90.8%, 79/87), 67 with AHP (77.0%, 67/87) and 64 with CIS (73.6%, 64/87). Histological examination was carried out by two pathologists independently.

**Immunohistochemical staining**

Follow the manufacturer's instructions, tissue sections were deparaffinized, rehydrated, subjected to antigen retrieval. Polyclonal rabbit anti-human ME3 antibody was purchased from Sigma (HPA038473, USA). The samples were incubated with primary antibody (ME3, 1:400) overnight at 4 °C. The secondary antibody was conjugated to horseradish peroxidase. The immunoreactivity for ME3 was visualized through adding diaminobenzidine and subsequently counterstained with hematoxylin. The sections were evaluated under microscope by two pathologists independently who were blinded to the patients' clinical information.

According to immunohistochemical scoring system previously with some modification, ME3 immunostaining score (S) was determined semiquantitatively by multiplying the percentage of positive cells (P) with intensity (I), according to the formula: S= P×I. The range score for percentage of positive cells is 0 to 4 (0, 0%; 1, 1%-10%; 2, 11%-50%; 3, 51%-75%; 4, 76%-100%). The range score for intensity is 0 to 3 (0, no staining; 1, weak; 2, moderate; 3, strong.). The index score was obtained a range from 0 to 12.
The final immunostaining results were denoted as negative expression of ME3 (0-1 scores) and positive expression of ME3 (≥2 scores).

**Statistical analysis**

All statistical analyses were conducted using IBM SPSS version 21.0. Data was represented as the mean ± standard deviation for continuous variables and frequency (number-percent) for categorical data. Relationships of ME3 expression and clinicopathological features were evaluated by the chi-square (χ²-value) and linear regression analysis (R²-value). The Kaplan–Meier survival analysis was used to estimate the association between eligible variables and survival. Cox proportional hazards regression models were used for multivariate survival analysis in stepwise regression manner. Receiver operating characteristic curve was based on the prediction probability of 5-year survival outcome from the logistic regression model, and the area under ROC curve was used to assess the classification performance of the model. Any result with a P-value of less than 0.05 was considered as statistically significant.

**Declarations**

**Acknowledgments**

This work has been supported in part by the China National 973 project (2013CB911303), China Natural Sciences Foundation projects (81470126), a key project (2018C03009) funded by Zhejiang Provincial Department of Sciences and Technologies, and the Fundamental Research Funds for the Central Universities, National Ministry of Education, China, to XH.

**Authors' contributions**

Conception and design of the study: XH and DY; Sample acquisition and analysis: DD, and DY; Histopathological diagnosis: XML and DD; Manuscript preparation: All authors; Approval of the final version: All authors.

**Competing interests**

The authors declare that they have no conflicts of interest with the contents of this article.

**Availability of data and material**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics declarations**

Informed consent was obtained from patients in this study. The human study protocol was in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans were approved by the Medical Ethics Committee of Zhengzhou University.
Consent to participate

Not applicable.

References

1. Bray, F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68, 394-424, doi:10.3322/caac.21492 (2018).

2. Li, T., Mello-Thoms, C. & Brennan, P. C. Descriptive epidemiology of breast cancer in China: incidence, mortality, survival and prevalence. Breast Cancer Res Treat 159, 395-406, doi:10.1007/s10549-016-3947-0 (2016).

3. Zeng, H. et al. Changing cancer survival in China during 2003–15: a pooled analysis of 17 population-based cancer registries. The Lancet Global Health 6, e555-e567, doi:10.1016/s2214-109x(18)30127-x (2018).

4. Miller, K. D. et al. Cancer treatment and survivorship statistics, 2019. CA Cancer J Clin 69, 363-385, doi:10.3322/caac.21565 (2019).

5. Chen, W. et al. Cancer statistics in China, 2015. CA Cancer J Clin 66, 115-132, doi:10.3322/caac.21338 (2016).

6. Fan, L. et al. Breast cancer in China. The Lancet Oncology 15, e279-e289, doi:10.1016/s1470-2045(13)70567-9 (2014).

7. Pavlova, N. N. & Thompson, C. B. The Emerging Hallmarks of Cancer Metabolism. Cell Metab 23, 27-47, doi:10.1016/j.cmet.2015.12.006 (2016).

8. Vander Heiden, M. G. & DeBerardinis, R. J. Understanding the Intersections between Metabolism and Cancer Biology. Cell 168, 657-669, doi:10.1016/j.cell.2016.12.039 (2017).

9. Chang, G.-G. & Tong, L. Structure and function of malic enzymes, a new class of oxidative decarboxylases. Biochemistry 42, 12721-12733 (2003).

10. Xu, Y., Bhargava, G., Wu, H., Loeber, G. & Tong, L. Crystal structure of human mitochondrial NAD (P)+-dependent malic enzyme: a new class of oxidative decarboxylases. Structure 7, 877-889 (1999).

11. Yao, P. et al. Evidence for a direct cross-talk between malic enzyme and the pentose phosphate pathway via structural interactions. J Biol Chem 292, 17113-17120, doi:10.1074/jbc.M117.810309 (2017).

12. Hasan, N. M., Longacre, M. J., Stoker, S. W., Kendrick, M. A. & MacDonald, M. J. Mitochondrial malic enzyme 3 is important for insulin secretion in pancreatic beta-cells. Mol Endocrinol 29, 396-410, doi:10.1210/me.2014-1249 (2015).

13. Jiang, P., Du, W., Mancuso, A., Wellen, K. E. & Yang, X. Reciprocal regulation of p53 and malic enzymes modulates metabolism and senescence. Nature 493, 689-693, doi:10.1038/nature11776 (2013).
14. Lu, Y. X. et al. ME1 Regulates NADPH Homeostasis to Promote Gastric Cancer Growth and Metastasis. Cancer Res 78, 1972-1985, doi:10.1158/0008-5472.CAN-17-3155 (2018).

15. Murai, S. et al. Inhibition of malic enzyme 1 disrupts cellular metabolism and leads to vulnerability in cancer cells in glucose-restricted conditions. Oncogenesis 6, e329, doi:10.1038/oncsis.2017.34 (2017).

16. Ren, J. G. et al. Knockdown of malic enzyme 2 suppresses lung tumor growth, induces differentiation and impacts PI3K/AKT signaling. Sci Rep 4, 5414, doi:10.1038/srep05414 (2014).

17. Woo, S. H. et al. Down-regulation of malic enzyme 1 and 2: Sensitizing head and neck squamous cell carcinoma cells to therapy-induced senescence. Head Neck 38 Suppl 1, E934-940, doi:10.1002/hed.24129 (2016).

18. Dey, P. et al. Genomic deletion of malic enzyme 2 confers collateral lethality in pancreatic cancer. Nature 542, 119-123, doi:10.1038/nature21052 (2017).

19. Zhang, Q., Li, J., Tan, X. & Zhao, Q. Effects of ME3 on the proliferation, invasion and metastasis of pancreatic cancer cells through epithelial-mesenchymal transition. Neoplasma 66, 896-907 (2019).

20. Liao, R. et al. ME1 promotes basal-like breast cancer progression and associates with poor prognosis. Sci Rep 8, 16743, doi:10.1038/s41598-018-35106-y (2018).

21. Society, A. C. Breast cancer facts & figures 2019–2020. Am. Cancer Soc, 1-44 (2019).

22. Fan, J. et al. Quantitative flux analysis reveals folate-dependent NADPH production. Nature 510, 298-302, doi:10.1038/nature13236 (2014).

23. Lewis, C. A. et al. Tracing compartmentalized NADPH metabolism in the cytosol and mitochondria of mammalian cells. Mol Cell 55, 253-263, doi:10.1016/j.molcel.2014.05.008 (2014).

24. Sabharwal, S. S. & Schumacker, P. T. Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles' heel? Nat Rev Cancer 14, 709-721, doi:10.1038/nrc3803 (2014).

25. DeBerardinis, R. J. & Chandel, N. S. Fundamentals of cancer metabolism. Science advances 2, e1600200 (2016).

26. Liu, C. et al. Malic enzyme 1 indicates worse prognosis in breast cancer and promotes metastasis by manipulating reactive oxygen species. OncoTargets and therapy 13, 8735 (2020).

27. Birch-Machin, M. The role of mitochondria in ageing and carcinogenesis. Clinical and Experimental Dermatology: Experimental dermatology 31, 548-552 (2006).

28. Ježek, J., Cooper, K. F. & Strich, R. Reactive oxygen species and mitochondrial dynamics: the yin and yang of mitochondrial dysfunction and cancer progression. Antioxidants 7, 13 (2018).

29. Yang, Y. et al. Mitochondria and mitochondrial ROS in cancer: novel targets for anticancer therapy. Journal of cellular physiology 231, 2570-2581 (2016).

30. Amin, M. B. et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin 67, 93-99, doi:10.3322/caac.21388 (2017).
31. Frank, G. A., Danilova, N. V., Andreeva, I. & Nefedova, N. A. [WHO classification of tumors of the breast, 2012]. *Arkhiv patologii* **75**, 53-63 (2013).

32. Fedchenko, N. & Reifenrath, J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review. *Diagn Pathol* **9**, 221, doi:10.1186/s13000-014-0221-9 (2014).

33. Fitzgibbons, P. L. *et al.* Template for reporting results of biomarker testing of specimens from patients with carcinoma of the breast. *Arch Pathol Lab Med* **138**, 595-601, doi:10.5858/arpa.2013-0566-CP (2014).

**Figures**

**Figure 3**

Linear regression of ME3 positive immunostaining rate in T staging and TNM staging. (A) ME3 positive immunostaining rate in Tis, T1, T2 and T3, (R2=0.9394, P=0.031); (B) ME3 positive immunostaining rate in stage 0 to I, II and III (R2=0.9283, P=0.037). Data are from table 3.
Figure 5

Receiver operating characteristic curves for prediction of breast cancer 5-year survival outcome. (A) 5-year overall survival rate based on tumor TNM staging (the area under ROC curve =84.0%); (B) 5-year overall survival rate based on tumor TNM staging combined with ME3 expression (the area under ROC curve =87.5%).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.docx