MALAT1 and BACH1 are prognostic biomarkers for triple-negative breast cancer

ABSTRACT

Aims: The purpose of this study was to investigate the potential correlation between metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) and the transcription factor BTB and CNC homology 1 (BACH1) and their clinicopathological significance in triple-negative breast cancer (TNBC).

Subjects and Methods: MALAT1 and BACH1 were detected by immunohistochemistry using TNBC tissue microarrays of 240 patients. The association between MALAT1 and BACH1 expression levels was statistically analyzed. Moreover, the prognostic roles as well as clinical and pathological significance of MALAT1 and BACH1 expression in TNBC were determined.

Statistical Analysis Used: Two-tailed Pearson correlation was used to examine the correlation of BACH1 and MALAT1 expression. Comparisons of clinicopathological variables between different BACH1 and MALAT1 expression groups were performed using χ² tests. Overall survival (OS) and disease-free survival (DFS) curves were plotted with the Kaplan-Meier method and the differences in OS and DFS between three groups were compared by the log-rank test. Multiple comparisons were performed using χ² tests for subsequent individual group comparisons.

Results: MALAT1 and BACH1 expression was significantly correlated with tumor-node-metastasis stage, distant metastasis, pathological stage, and survival outcomes of patients. Patients with high MALAT1 and BACH1 expression exhibited shorter overall survival and disease-free survival.

Conclusions: These findings provide further insight into the expression pattern of MALAT1 and BACH1 in TNBC and suggest them as prognostic biomarkers for TNBC.

KEY WORDS: BTB and CNC homology 1, metastasis-associated lung adenocarcinoma transcript 1, prognosis, triple-negative breast cancer

INTRODUCTION

Among females, breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death worldwide. A status report on the global burden of cancer estimated 2,088,849 (11.6%) new cases of breast cancer and 626,679 (6.6%) breast cancer deaths among women in 2018.[1] Triple-negative breast cancer (TNBC) tumors lack estrogen receptor, progesterone receptor, and human epidermal growth factor receptor-2 expression[2] and are found in 10%–20% of patients with newly diagnosed breast cancer. Patients with TNBC exhibit a dismal prognosis.[3] Moreover, effective diagnostic and prognostic markers for TNBC are lacking.

Metabolic activity can regulate tumor growth.[4] BTB and CNC homology1 (BACH1) is a heme-binding transcription factor and a member of the Cap ‘n’ Collar/basic region leucine zipper factor (CNC-bZIP) family.[5] BACH1 can reprogram metabolic pathways by targeting mitochondrial metabolism.[4,6] In addition, BACH1 expression was upregulated in the tumors of patients with TNBC.[7,8]

Long noncoding RNAs (lncRNAs) can regulate survival, proliferation, invasion, metastasis, and angiogenesis of cancer cells.[9] Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), also known as nuclear-enriched abundant transcript
MALAT1 expression has been shown to correlate with poor prognosis in patients with breast cancer.\textsuperscript{[14,15]}

In this study, we have investigated the correlation between the expression of BACH1 and MALAT1 and their clinicopathological significance in TNBC. We have also investigated the prognostic roles of BACH1 and MALAT1 in patients with breast cancer. Our study provides evidence that BACH1 and MALAT1 can serve as prognostic factors and potential therapeutic targets in TNBC.

**SUBJECTS AND METHODS**

**Ethics statement**
All procedures performed in this study involving human participants were approved by the Ethics Committee of Sun Yat-Sen University Cancer Center and were in accordance with the 1964 Helsinki Declaration and its later amendments. Written informed consent about the researchable use of the clinical data was obtained from each participant patient. All patient data were anonymous and deidentified prior to analysis. This study was conceived and presented according to the reporting recommendations for tumor marker prognostic studies guidelines.

**Patients and specimens for tissue microarray**
A total of 240 female patients who were histopathologically diagnosed with TNBC at Sun Yat-Sen University Cancer Center were included in this study. TNBC tissue specimens were collected by surgery, formalin-fixed and paraffin-embedded using standard techniques, and preserved at the Department of Specimen and Resource in Sun Yat-Sen University Cancer Center. Only those patients who underwent therapeutic surgical treatment (conservative or radical surgery with axillary evaluation or mastectomy) were recruited in this study. The exclusion criteria included: male patients, inflammatory breast cancer, bilateral carcinoma, and history of malignant tumor. In addition, all of the patients included in this study had not received chemotherapy or radiation therapy previously; their complete clinical and pathological information, including age, menopause status, tumor size, lymph node status, stage, and distant metastasis, were available and can be reviewed. The histological grade of the tumor was classified based on the tumor-node-metastasis (TNM) staging system (American Joint Committee on Cancer Classification). Follow-up data were obtained by reviewing clinical records and by contacting the patient by telephone. The dates of death and relapse were used to estimate overall survival (OS) and disease-free survival (DFS), respectively.

Tissue microarrays (TMAs) were constructed as follows: briefly, histological slides were retrieved and reviewed, and representative tumor areas were selected for TMA construction. The presence of carcinoma in the tumor core was used as an inclusion criterion. We compared both hematoxylin and eosin-stained and immunostained TMA sections with the corresponding full-section slides to assess representativeness and heterogeneity of staining.

**Immunohistochemical analysis**
Immunohistochemistry (IHC) was performed on the 240 paraffin-embedded TNBC tissue sections. Briefly, the slides were deparaffinized, rehydrated, and treated with a 90% methanol/3% H\textsubscript{2}O\textsubscript{2} solution for 10 min at room temperature to block endogenous peroxidase. Thereafter, the slides were soaked in sodium citrate buffer (10 mM sodium citrate; 0.05% Tween 20, pH 6.0) at 96°C for 5 min for antigen retrieval. The slides were incubated overnight at 4°C with the following antibodies after blocking with BSA: anti-BACH1 antibody (dilution 1:100, Santa Cruz Biotechnology, Europe) and anti-MALAT1 antibody (dilution 1:500, Caiyou, Shanghai, China). The slides were subsequently incubated at room temperature with a biotinylated secondary antibody for 10 min, and finally with Horseradish Peroxidase (HRP)-streptavidin for 10 min. The results were identified as positive and negative after DAB staining.

**Statistical analysis**
All statistical analyses were performed using SPSS 25.0 statistical package (SPSS Inc., Chicago, IL, USA). A two-tailed Pearson correlation was used to examine the correlation between BACH1 expression and MALAT1 expression. The clinicopathological variables between different BACH1 and MALAT1 expression groups were compared using Chi-square tests. OS and DFS curves were plotted using the Kaplan–Meier method and the differences in OS and DFS between these groups were compared using the log-rank test. Multiple comparisons were performed using Chi-square tests for subsequent individual group comparisons. All results were statistically significant at \( P < 0.05 \).

**RESULTS**

**Metastasis-associated lung adenocarcinoma transcript 1 and BTB and CNC homology 1 expression is upregulated in triple-negative breast cancer**
The mRNA expression of MALAT1 and BACH1 was assessed by IHC using TNBC TMAs of 240 patients [Figure 1]. MALAT1 and BACH1 were both overexpressed in TNBC tissues. The characteristics of the patients with overexpressed MALAT1 and BACH1 are summarized in Table 1. This result further indicates that LDHA and AMPK were expressed synchronously in TNBC tissues.

**Coexpression of metastasis-associated lung adenocarcinoma transcript 1 and BTB and CNC homology 1 is correlated with clinicopathological parameters**
Next, we explored the potential clinicopathological implications of altered MALAT1 and BACH1 expression. The clinical tissue
specimens of 240 breast cancer patients were divided into multiple groups based on the expression scores of MALAT1 and BACH1. Of these, 65 (27%) overexpressed MALAT1 and BACH1 [Table 2]. The results showed that MALAT1 and BACH1 expression is correlated with tumor size, Lymph node metastasis (LNMET), and advanced TNM stage, suggesting that MALAT1 and BACH1 may play critical roles in carcinogenesis and progression of TNBC.

**Table 1: Patient characteristics (n=240)**

| Variables                  | Number of patients, n (%) |
|----------------------------|---------------------------|
| Age (years)                |                           |
| <50                        | 136 (56.7)                |
| ≥36                        | 104 (43.3)                |
| Menopause                  |                           |
| No                         | 144 (60.0)                |
| Yes                        | 96 (40.0)                 |
| Tumor size                 |                           |
| <2                         | 66 (27.5)                 |
| ≥6                         | 174 (72.5)                |
| LNMET                      |                           |
| No                         | 123 (51.2)                |
| Yes                        | 117 (48.8)                |
| Distant metastasis         |                           |
| No                         | 236 (98.3)                |
| Yes                        | 4 (1.7)                   |
| TNM stage                  |                           |
| I-II                       | 187 (77.9)                |
| III-IV                     | 53 (22.1)                 |
| pathological stages        |                           |
| I                          | 3 (1.3)                   |
| II                         | 141 (58.8)                |
| III                        | 96 (40.0)                 |

%=Percentage within the row, TNM=Tumor-node-metastasis, *Statistically significant (P<0.05)

Coexpression of metastasis-associated lung adenocarcinoma transcript 1 and BTB and CNC homology 1 is correlated with poor clinical outcome of patients with breast cancer

To analyze the significance of MALAT1 and BACH1 expression for the clinical prognosis of TNBC, Kaplan–Meier survival analysis was performed using OS [Figure 2a] and DFS [Figure 2b]. The results showed that patients overexpressing MALAT1 and BACH1 exhibit shorter OS and DFS than those with downregulated expression of MALAT1 and BACH1 [P < 0.0001 for both OS and DFS; Figure 2a and b]. These results indicated that coexpression of LDHA and AMPK is significantly associated with shorter survival of TNBC patients.

**DISCUSSION**

LncRNAs are a class of noncoding RNAs of more than 200 nucleotides but lack protein-coding potential. They are emerging as a new class of indispensable molecules for the development and promotion of malignant diseases. Therefore, it is reasonable that abnormal expression of lncRNAs results in dysregulation of normal biological and pathological processes of disease. From a clinical perspective, lncRNA can serve as a potential therapeutic target, especially for TNBC because of the constant failure of chemotherapy and other conventional treatment options. MALAT1 is one of the earliest identified lncRNAs. Several studies have shown that MALAT1 plays a pivotal role in the development and progression of various cancers and promotes proliferation, migration, metastasis,
High expression of MALAT1 is associated with increased tumor size and advanced stage of breast cancer, as well as poor outcome, especially in TNBC, suggesting that MALAT1 could be an important prognostic factor and a therapeutic target in TNBC.

BACH1 is a transcriptional factor that forms a heterodimer with the small Maf family proteins. BACH1 can repress Maf recognition element expression in vivo. According to recent studies, BACH1 is primarily involved in the physiological regulation of oxidative stress, heme oxidation, and senescence. Alvarez et al. (2011) have predicted that BACH1 might be a regulator of the prostate cancer marker ACPP; although, this hypothesis has not been experimentally verified. Furthermore, Han et al. (2019) found that BACH1 could promote EMT gene expression by recruiting HMGA2.

Table 2: Clinicopathological variables and LDHA and AMPK expression in 240 breast cancer patients

| Characteristics                        | Both negative (n=88), n (%) | One positive (n=87), n (%) | Both negative (n=65), n (%) | P      |
|----------------------------------------|----------------------------|----------------------------|----------------------------|--------|
| OS                                     |                            |                            |                            |        |
| Present                                | 87 (98.9)                  | 77 (88.5)                  | 38 (58.5)                  | 0.000* |
| Absent                                 | 1 (1.1)                    | 10 (11.5)                  | 27 (41.5)                  |        |
| DFS                                    |                            |                            |                            |        |
| Present                                | 87 (98.9)                  | 76 (87.4)                  | 35 (53.8)                  | 0.000* |
| Absent                                 | 1 (1.1)                    | 11 (12.6)                  | 30 (46.2)                  |        |
| Age (years) <50                        | 54 (61.4)                  | 48 (55.2)                  | 34 (52.3)                  | 0.503  |
| ≥50                                    | 34 (38.6)                  | 39 (44.8)                  | 31 (47.7)                  |        |
| Menopause                              |                            |                            |                            |        |
| No                                     | 55 (62.5)                  | 53 (60.9)                  | 36 (55.4)                  | 0.658  |
| Yes                                    | 33 (37.5)                  | 34 (39.1)                  | 29 (44.6)                  |        |
| Tumor size (cm) <2                     | 34 (38.6)                  | 25 (28.7)                  | 7 (10.8)                   | 0.001* |
| ≥2                                     | 54 (61.4)                  | 62 (71.3)                  | 58 (89.2)                  |        |
| LNMET                                  |                            |                            |                            |        |
| No                                     | 57 (64.8)                  | 45 (51.7)                  | 21 (32.3)                  | 0.000* |
| Yes                                    | 31 (35.2)                  | 42 (48.3)                  | 44 (67.7)                  |        |
| Distant metastasis                     |                            |                            |                            |        |
| No                                     | 87 (98.9)                  | 85 (97.7)                  | 64 (98.5)                  | 0.831  |
| Yes                                    | 1 (1.1)                    | 2 (2.3)                    | 1 (1.5)                    |        |
| TNM stage                              |                            |                            |                            |        |
| I-II                                   | 76 (86.4)                  | 70 (80.5)                  | 41 (63.1)                  | 0.002* |
| III-IV                                 | 12 (13.6)                  | 17 (19.5)                  | 24 (36.9)                  |        |
| Pathological stage                     |                            |                            |                            |        |
| I                                      | 1 (1.1)                    | 2 (2.3)                    | 0 (0)                      | 0.779  |
| II                                     | 51 (58.0)                  | 50 (57.5)                  | 40 (61.5)                  |        |
| III                                    | 36 (40.9)                  | 35 (40.2)                  | 25 (38.5)                  |        |

*Statistically significant (P<0.05). %=Percentage within the row, OS=Overall survival, DFS=Disease-free survival, TNM=Tumor-node-metastasis, LNMET= Lymph node metastasis

Table 3: Multiple comparisons of clinicopathological variables between different expression groups

| Characteristics                        | Both negative versus one positive | One positive versus both positive | Both negative versus both positive |
|----------------------------------------|----------------------------------|----------------------------------|----------------------------------|
| OS                                     | 0.005*                           | 0.000*                           | 0.000*                           |
| DFS                                    | 0.003*                           | 0.000*                           | 0.000*                           |
| TNM stage                              | 0.294                            | 0.170                            | 0.001*                           |
| Tumor size (cm)                        | 0.166                            | 0.007*                           | 0.000*                           |
| LNMET                                  | 0.080                            | 0.017                            | 0.000*                           |

*Statistically significant (P<0.05). OS=Overall survival, DFS=Disease-free survival, TNM=Tumor-node-metastasis, LNMET= Lymph node metastasis

Figure 2: Coexpression of metastasis-associated lung adenocarcinoma transcript 1 and BTB and CNC homology 1 were correlated with poor clinical outcomes of breast cancer. (a) Overall survival curves for 240 studied patients. (b) Disease-free survival curves for 240 studied patients...
in epithelial ovarian cancer cells. HMGA2 plays a role in BACH1-induced EMT of epithelial ovarian tumor cells.\cite{6}
Yun et al. have indicated that BACH1 can upregulate metastatic genes such as CXCR4 and MMP1 and promote bone metastasis of breast cancer.\cite{23,24} These evidence indicate that BACH1 could function as cancer development and migration factor and is often associated with poor outcomes.\cite{25,26} Hence, in this study, we attempted to explore the feasibility of BACH1 as a prognostic factor for TNBC.

In this study, we investigated the expression pattern of BACH1 and MALAT1 in TNBC. We found that BACH1 and MALAT1 expression was synchronously upregulated in TNBC tissues. These results indicated that BACH1 and MALAT1 could be used as biomarkers for TNBC. Next, we explored the potential clinicopathological implications of altered LDHA and AMPK expression. We found that patients with upregulated BACH1 and MALAT1 expression exhibited large tumors, lymph node metastasis, and advanced TNM stage. Moreover, the patients with upregulated BACH1 and MALAT1 expression were associated with shorter OS and DFS. Our results are consistent with those reported previously, suggesting that BACH1 and MALAT1 together play critical roles in breast cancer. Hence, BACH1 and MALAT1 could be important prognostic factors and promising therapeutic targets in TNBC.

In summary, this study demonstrated that BACH1 and MALAT1 are upregulated synchronously in patients with TNBC who had poor clinical outcomes. Our results indicate that BACH1 and MALAT1 could be important prognostic factors and potential therapeutic targets in TNBC. The simultaneous detection and targeting of BACH1 and MALAT1 should be performed in the clinical management of TNBC.

CONCLUSION

In summary, this study demonstrated that BACH1 and MALAT1 were up-regulated synchronously in TNBC, which was associated with poor clinical outcomes. Our findings provide significant evidence that BACH1 and MALAT1 could be prognostic factors and potential therapeutic targets in TNBC. Detecting and targeting BACH1 and MALAT1 at the same time should be recommended in clinical management of TNBC.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Jia H, Truica CI, Wang B, Wang Y, Ren X, Harvey HA, et al. Immunotherapy for triple-negative breast cancer: Existing challenges and exciting prospects. Drug Resist Updat 2017;32:1-5.
3. Saha P, Nanda R. Concepts and targets in triple-negative breast cancer: Recent results and clinical implications. Ther Adv Med Oncol 2016;8:351-9.
4. van der Heiden MG, DeBerardinis RJ. Understanding the intersections between metabolism and cancer biology. Cell 2017;168:657-69.
5. Davudian S, Mansoori B, Shajari N, Mohammadi A, Baradaran B. BACH1, the master regulator gene: A novel candidate target for cancer therapy. Gene 2016;588:30-7.
6. De Berardinis RJ, Chandel NS. Fundamentals of cancer metabolism. Sci Adv 2016;2:e1600200.
7. Lee J, Yesilkanal AE, Wynne JP, Frankenberger C, Liu J, Yan J, et al. Effective breast cancer combination therapy targeting BACH1 and mitochondrial metabolism. Nature 2019;568:254-8.
8. Han W, Zhang Y, Niu C, Guo J, Li J, Wei X, et al. BTB and CNC homology 1 (Bach1) promotes human ovarian cancer cell metastasis by HMG2-mediated epithelial-mesenchymal transition. Cancer Lett 2019;445:45-56.
9. Rodriguez Bautista R, Ortega Gómez A, Hidalgo Miranda A, Zentella Dehesa A, Villarreal-Garza C, Ávila-Moreno F, et al. Long non-coding RNAs: Implications in targeted diagnoses, prognosis, and improved therapeutic strategies in human non- and triple-negative breast cancer. Clin Epigenetics 2018;10:88.
10. Amodio N, Raimondi L, Juli G, Stamato MA, Caracciolo D, Tagliaferri P, et al. MALAT1: A druggable long non-coding RNA for targeted anti-cancer approaches. J Hematol Oncol 2018;11:63.
11. Sun Q, Hao Q, Prasanth KV. Nuclear long noncoding RNAs: Key regulators of gene expression. Trends Genet 2018;34:142-57.
12. Yang J, Xia Z, Wu J, Guo L, Li J, et al. MALAT1 as a metastasis driver in ER negative lymph node negative breast cancer. Oncotarget 2016;7:40418-36.
13. Zhang X, Hamblin MH, Yin KJ. The long non-coding RNA malat1: Its physiological and pathophysiological functions. RNA Biol 2017;14:1705-14.
14. Hamoudi OA, Huang WC, Lee WH, Wu A, Wang LS, Hsiao M, et al. Aberrant KDM5B expression promotes aggressive breast cancer through MALAT1 overexpression and downregulation of hsa-miR-448. BMC Cancer 2016;16:160.
15. Chen W, Xu XK, Li JL, Kong KK, Li H, Chen C, et al. MALAT1 is a prognostic factor in glioblastoma multiforme and induces chemoresistance to temozolomide through suppressing miR-203 and promoting thymidylate synthase expression. Oncotarget 2017;8:22783-99.
16. Jadaliha M, Zong X, Malakar P, Ray T, Singh DK, Freier SM, et al. Functional and prognostic significance of long non-coding RNA MALAT1 as a metastasis driver in ER negative lymph node negative breast cancer. Oncotarget 2016;7:40418-36.
17. Shi X, Liu Z, Liu Z, Feng X, Hua F, Hu X, et al. Long noncoding RNA PCAT6 functions as an oncogene by binding to EZH2 and suppressing LAT52 in non-small-cell lung cancer. EBioMedicine 2018;37:177-87.
18. Chandra Gupta S, Nandan Tripathi Y. Potential of long non-coding RNAs in cancer patients: From biomarkers to therapeutic targets. Int J Cancer 2017;140:1955-67.
19. Lin J, Xu YE, Jiang YZ, Liu YR, Sun W, Guo YJ, et al. The endogenous retrovirus-derived long noncoding RNA TRGJAN promotes triple-negative breast cancer progression via ZMYND8 degradation. Sci Adv 2019;5:eaa19820.
20. Amodio N, D’Aquilo P, Passarino G, Tassone P, Bellizzi D. Epigenetic modifications in multiple myeloma: Recent advances on the role of DNA and histone methylation. Expert Opin Ther Targets 2017;21:91-101.
21. Sun Y, Ma L. New insights into long non-coding RNA MALAT1 in cancer and metastasis. Cancers (Basel) 2019;11: pii: E216.
22. Dong P, Xiong Y, Yue J, J B Hanley S, Kobayashi N, Todo Y, et al.
Exploring lncRNA-mediated regulatory networks in endometrial cancer cells and the tumor microenvironment: Advances and challenges. Cancers (Basel) 2019;11. pii: E234.

22. Arun G, Spector DL. MALAT1 long non-coding RNA and breast cancer. RNA Biol 2019;16:860-3.

23. Kobayashi M, Kato H, Hada H, Itoh-Nakadai A, Fujiwara T, Muto A, et al. Iron-heme-bach1 axis is involved in erythroblast adaptation to iron deficiency. Haematologica 2017;102:454-65.

24. Alam J, Igarashi K, Immenschuh S, Shibahara S, Tyrrell RM. Regulation of heme oxygenase-1 gene transcription: Recent advances and highlights from the international conference (Uppsala, 2003) on heme oxygenase. Antioxid Redox Signal 2004;6:924-33.

25. Liang Y, Wu H, Lei R, Chong RA, Wei Y, Lu X, et al. Transcriptional network analysis identifies BACH1 as a master regulator of breast cancer bone metastasis. J Biol Chem 2012;287:33533-44.

26. Yun J, Frankenberg CA, Kuo WL, Boelens MC, Eves EM, Cheng N, et al. Signalling pathway for RKIP and let-7 regulates and predicts metastatic breast cancer. EMBO J 2011;30:4500-14.

27. Dangi-Garimella S, Yun J, Eves EM, Newman M, Erkeland SJ, Hammond SM, et al. Raf kinase inhibitory protein suppresses a metastasis signalling cascade involving LIN28 and let-7. EMBO J 2009;28:347-58.