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Original article

High expressions of LDHA and AMPK as prognostic biomarkers for breast cancer

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ABSTRACT

Objectives: The purpose of this study was to investigate the potential correlation between lactate dehydrogenase A (LDHA) and AMP-activated protein kinase (AMPK) and their clinicopathologic significance in breast cancer.

Materials and Methods: Western blot and qRT-PCR were used to detect the expression levels of LDHA and AMPK in eight breast cancer lines and eight breast cancer tissues. In addition, LDHA and AMPK were detected by immunohistochemistry (IHC) using breast cancer tissue microarrays (TMAs) of 112 patients. The association between LDHA and AMPK expression levels was statistically analyzed. So were the prognostic roles and clinicopathologic significances in breast cancer.

Results: The expression levels of LDHA and AMPK were relatively higher in triple-negative breast cancer (TNBC) cell lines than in non-triple-negative breast cancer (NTNBC) cell lines. LDHA and AMPK were also further up-regulated in TNBC tissues than in NTNBC tissues. Correlation analysis showed a positive correlation between LDHA and AMPK expression levels. Expression of LDHA and AMPK were significantly correlated with TNM stage, distant metastasis, Ki67 status and survival outcomes of patients. Patients with both positive expression of LDHA and AMPK showed shorter overall survival (OS) and disease-free survival (DFS).

Conclusions: These findings improve our understanding of the expression pattern of LDHA and AMPK in breast cancer and clarify the role of LDHA and AMPK as promising prognostic biomarkers for breast cancer.

Introduction

Breast cancer is one of the highest incidences of cancers in women worldwide and is the first and second cause of cancer death among women in more developed and less developed countries, respectively. It is estimated that there were 1,676,600 new cases and 521,900 deaths of breast cancers in women worldwide in 2012 [1]. According to its molecular subtype, breast cancer is classified into different categorizations, including luminal A, luminal B, Her-2 neu and basal like subtypes. Among them, the prognosis of triple-negative breast cancer (TNBC) patients remains poor. And there is still lack of effective diagnostic and prognostic markers for TNBC [2].

Reprogramming of metabolism is very common in cancer cells. Due to the Warburg effect, cancer cells increase metabolic intermediates to better support rapid cell growth [3]. Aberrant tumor metabolism has been proved to help the progression and metastasis of tumors [4]. A series of enzymes have been identified to play vital roles in regulation of Warburg effect. Among them, lactate dehydrogenase A (LDHA) and AMP-activated protein kinase (AMPK) have been reported to be of great importance in tumor metabolism [5–7]. It's been reported that LDHA is commonly up-

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regulated and is correlated with worse survival outcomes of many cancers [8–12]. We previously found a high expression of LDHA in breast cancer, which was associated with worse clinical outcomes [13]. As for AMPK, its dysfunction has recently been highlighted in several cancers, including breast cancer [14,15]. In our previous study we found that AMPK was significantly up-regulated in breast cancer cell lines and tissues and predicted shorter OS and DFS [16]. What's more, increasing number of researches show that LDHA and AMPK promoted glycolysis and cell proliferation in cancers [8,9,11,12,15,16]. These findings indicate that LDHA and AMPK could be prognostic factors and therapeutic targets in breast cancer patients.

In this study, we explored the expression pattern of LDHA and AMPK in breast cancer, especially in TNBC. Then we investigated the prognostic roles of LDHA and AMPK in breast cancer patients. Our study provides evidence that LDHA and AMPK could be prognostic factors and potential therapeutic targets in breast cancer, especially in TNBC.

Materials and methods

This study was conceived and presented with regard to the reporting recommendations for tumor marker prognostic studies (REMARK) guidelines [17].

Cell lines and culture

Human breast cancer cell lines MCF-7, T47D, BT-474, SKBR3, MDA-MB-453, MDA-MB-468, MDA-MB-231 and BT-549 were obtained from the American Type Culture Collection (Manassas, VA, USA) and were passaged in our laboratory for less than six months after resuscitation of frozen aliquots. The breast cancer cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, CA, USA) supplemented with 10% fetal bovine serum (FBS, GIBCO, Cappinas, Brazil), in a humidified incubator at 37 °C containing 5% CO_2. All cell lines were re-authenticated by short tandem repeat DNA profiling every 6 months after used.

Patients and specimens for tissue microarray

A total of 112 female breast cancer patients who were diagnosed by histopathology from October 2001 to September 2006 in Sun Yat-Sen University Cancer Center were obtained. Specimens were formalin-fixed and embedded in paraffin by standard methodology after obtained during surgery and were stored in the Department of Specimen and Resource in Sun Yat-Sen University Cancer Center. IHC of ER, PR, HER-2, P53, Ki67 and VEGF status were performed in the Pathology Department of Sun Yat-Sen University Cancer Center. The scoring systems for ER, PR, HER-2 and Ki67 staining were described before [18]. So was scoring for P53 [19] and VEGF [20]. Only patients who underwent curative surgical treatment (mastectomy or breast-conserving surgery with axillary evaluation) were recruited in our study. The exclusion criteria included male patients, inflammatory breast carcinomas, bilateral carcinomas, and history of any malignant tumor. Besides, all the patients included in this study did not receive any chemotherapy and radiation therapy before, and their complete clinicopathological data, including age, menopause status, tumor size, lymph nodes status, stage, distant metastasis, ER status, PR status and HER-2 status, P53 status, VEGF status and Ki67 status were available and reviewed. Stage of tumor was based on the TNM staging system (American Joint Committee on Cancer classification). Characteristics of 112 patients are summarized in Table 1. Follow-up was updated by review of records and telephone calls. The median follow up was 69 months. The date of death and the date of relapse were used to calculate estimate overall survival (OS) and disease-free survival (DFS). Tissue microarrays (TMAs) were constructed as follow: briefly, histological slides were retrieved and reviewed and representative tumor areas were selected for TMA construction using the Beecher microarrayer with 1-mm cores. The presence of carcinoma in the core was used as an inclusion criterion. We compared both haematoxylin and eosin (HE) stained and immunostained TMA sections with the corresponding full-section slides to assess representativeness and heterogeneity of staining. This study was approved by the Ethics Committees of Sun Yat-Sen University Cancer Center, and conducted in accordance with the Helsinki Declaration. Informed consents were obtained from all patients included in the study.

Table 1
Patient characteristics.

Variables	No. of patients (n = 112)	%
Age (years)		
<50	67	59.8
>=50	45	40.2
Menopause		
Yes	55	49.1
No	57	50.9
Tumor size (cm)		
=<2	31	72.3
>2	81	27.7
LNMET		
Yes	66	58.9
No	46	41.1
TNM stage		
I–II	59	52.7
III–IV	53	47.3
Distant metastasis		
Yes	29	25.9
No	83	74.1
ER status		
Positive	42	37.5
Negative	70	62.5
PR status		
Positive	45	40.2
Negative	67	59.8
HER-2 status		
Positive	16	14.3
Negative	96	85.7
TNBC		
Yes	54	48.2
No	58	51.8
P53 status		
Positive	68	60.7
Negative	44	39.3
VEGF status		
Positive	17	16.5
Negative	86	83.5
Ki67 status		
>14%	38	55.9
<=14%	30	44.1

% Means percentage within the row.

Western blot

Expression levels of LDHA, AMPK and pAMPK were detected in both breast cancer cell lines and tissues by Western blot. Four NTNBC tissues and four TNBC tissues from patients diagnosed in our hospital were collected unintentionally. The breast cancer cell lines mentioned above were also included. Total protein was extracted from the cell lines and tissues using RIPA lysis buffer with a proteinase inhibitor. The protein concentrations in the lysates were measured with the Protein BCA Assay Kit (Bio-Rad, USA), and 30 μg of protein mixed with 5× SDS loading buffer was loaded per lane. The proteins in the lysates were separated by 10% SDS-