Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Monocarboxylate transporters in breast cancer and adipose tissue are novel biomarkers and potential therapeutic targets

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ARTICLE INFO

Article history:
Received 10 May 2018
Accepted 14 May 2018
Available online xxx

Keywords:
Monocarboxylate transporters
Breast cancer
Adipose tissue
Biomarkers

ABSTRACT

Monocarboxylate transporters (MCTs) are transmembrane proteins that control the lactate metabolism and associated with poor prognosis in solid tumours including breast cancer (BC). This study aimed to evaluate the clinical and prognostic value of MCTs used by immunohistochemistry and quantum dots-based fluorescent imaging technique in BC and surrounding stroma with emphasis on the interaction between tumour and stroma. Moreover, the data from The Cancer Genome Atlas (TCGA) was analyzed to evaluate the association between MCTs mRNA expression and prognosis of breast cancer patients. Our study found that MCT1 overexpression was observed in hormone receptor-negative and high-proliferation subtypes. High expression of MCT1 and MCT4 in tumour tissues was associated with poor patient outcome; further the correlation between MCT1 expression and poor prognosis in breast cancer was further strengthened when combined with MCT4 overexpression in the adjacent adipose tissue. These results demonstrate that MCTs tend to play a role in the aggressive BC subtypes through the dynamic interaction between breast cancer cells and adipocytes, and developing therapeutics to block this interaction will be a promising strategy in cancer therapy.

1. Introduction

Tumour progression is dominated not only by tumour cells but also by their surrounding stromal cells [1]. During breast cancer invasion and metastasis, reciprocal and dynamic communication occurs between tumour cells and the stromal compartments [1]. Specifically, tumours change the normal stroma into an advantageous microenvironment by promoting the wound-healing response [2] and are identify as metabolic parasites, seizing metabolites including lactate, pyruvate, fatty acids and ketone bodies from stromal sources [3,4].

Upon interaction with breast cancer cells, adipocytes, as the main cellular components constituting the breast cancer microenvironment, are transformed into cancer-associated adipocytes (CAAs) and promote tumour progression [5,6]. Emerging evidence indicates that adipocytes produce plenty of inflammatory factors, growth factors, and cytokines, just as endocrine cells, which stimulates receptor tyrosine kinase signalling and epithelial-mesenchymal transition (EMT) programs [4,7]. Recently, adipocytes were primarily regarded as a tremendous energy storage that also provides high-energy metabolites [8]. The metabolic reprogramming of adipocytes may be attributed to their potential tumour promoting ability and speculation that tumours may reprogram the metabolic synergy in adipocytes to promote progression through the dynamic interaction between breast cancer cells and adipocytes.

Monocarboxylate transporters (MCTs) are proteins that mediate the transport of various monocarboxylates, including lactate, pyruvate and ketones, across cell membranes [9]. The MCTs family is composed of 14 members in total, and has already been described

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https://doi.org/10.1016/j.bbrc.2018.05.091
0006-291X/

to participate in the metabolism and sustain cell homeostasis. In MCT proteins, the function of MCT1 and MCT2 are observed to uptake lactate to obtain energy while MCT4 promotes lactate efflux to maintain a steady intracellular pH [10]. Either MCT1 or MCT4 expression in both malignant tissues and normal tissues depends upon tissue type; for instance, MCT4 is universally up-regulated in prostate [11], lung [12] and colorectal [13] cancer while both MCT1 and MCT4 overexpressed in breast cancer [14,15]. Taking into consideration the features of tumour microenvironment such as high oxidative stress, the main role of MCTs would determinately control the lactate metabolism and directly impact on pH regulation.

Here, we investigated the expression level of MCT1 and MCT4 in breast cancer and stromal adipose tissue, and further examined survival outcomes to determine their prognostic impact on recurrence-free survival (RFS).

2. Material and methods

2.1. Ethics statement

Human samples were obtained from Renmin Hospital of Wuhan University, People's Republic of China. All patients included in the study wrote the informed consent, approved by the Institutional Ethics Committee of the Renmin Hospital of Wuhan University. Patients did not receive financial compensation. Clinical information was obtained from pathology reports, and the characteristics of these cases are given in Table 1. Patients with at least 5-year follow-up were included in this study. All methods were performed in accordance with the relevant guidelines and local regulations.

Table 1
Clinicopathological associations of MCT1 expression in breast cancer.

Variables	Negative N = 76	Positive N = 70	P value*
Survival time (months)	71	61	
Age at diagnosis, y			0.06
≤64	62 (81.6)	56 (80.0)	
≥65	14 (18.4)	14 (20.0)	
Grade			0.445
Well	4 (5.3)	4 (5.7)	
Moderately	20 (26.3)	10 (14.3)	
Poorly	52 (68.4)	56 (80.0)	
Tumour size(cm)			**0.028**
≤2	28 (36.8)	10 (14.3)	
>2	48 (63.2)	60 (85.7)	
lymph node metastasis			0.872
Negative	34 (44.7)	30 (42.9)	
Positive	42 (55.3)	40 (57.1)	
Vascular invasion			0.103
Negative	66 (86.8)	50 (71.4)	
Positive	10 (13.2)	20 (28.6)	
ER			**0.027**
Negative	18 (23.7)	34 (48.6)	
Positive	58 (76.3)	36 (51.4)	
PR			**0.007**
Negative	18 (23.7)	38 (54.3)	
Positive	58 (76.3)	32 (45.7)	
HER2			0.103
Negative	66 (86.8)	50 (71.4)	
Positive	10 (13.2)	20 (28.6)	
Ki67			**<0.01**
<14%	58 (76.3)	24 (34.4)	
≥14%	18 (23.7)	46 (65.7)	
Recurrence			**0.021**
No	70 (92.1)	50 (71.4)	
Yes	6 (7.9)	20 (28.6)	

*P values calculated by Log-rank testing; Bold if statistically significant, P < 0.05.
ER: estrogen receptor, PR: progesterone receptor, HER2: human epithelial growth factor receptor-2.

2.2. Immunohistochemistry

A cohort of 146 paraffin-embedded human breast cancer specimens was diagnosed by histopathology at Renmin Hospital of Wuhan University from 2011 to 2012. The detailed clinicopathologic characteristics of the patients with breast cancer are shown in Table 1. Immunohistochemistry (IHC) staining was performed and the staining results were scored by two independent pathologists based on both the proportion of positively stained tumour cells and the intensity of staining. The proportion of tumour cells was scored as: 0 (less than 10% positive cells), 1 (10%–20% positive cells), 2 (21%–50% positive cells) and 3 (more than 50% positive cells). The intensity of protein expression was determined as: 0 (no staining), 1 (weak staining, light brown), 2 (moderate staining, brown) and 3 (strong staining, dark brown). The protein staining positivity was determined using the following formula: overall score = percentage score × intensity score. The receiver operating characteristic analysis (ROC) was used to determine the optimal cut-off values of all proteins expression levels for survival rate.

2.3. Quantum dots (QDs)-based fluorescent imaging technique

QDs-based fluorescent imaging technique (QD-IIQAS) has been established at our cancer center with detailed procedures reported previously [16]. The QD-conjugated streptavidin (QD-SA) probe (1:200; QDs-605–goat F (ab)2 anti-rabbit immunoglobulin G conjugate; Wuhan Jiayuan Quantum Dots Co. Ltd.) was used as the secondary antibody in the QD-based immunofluorescent imaging. Briefly, the sequence of the procedure was as follows: deparaffinizing, antigen retrieval, blocking (2% bovine serum albumin, 37 °C for 30 min), incubation with primary antibody (dilution 1:100, 37 °C for 2 h), washing, blocking, incubation with biotinylated secondary antibody (dilution 1:500, 37 °C for 30 min), washing, blocking, application of QD-SA 605 probes (dilution 1:200, 37 °C for 30 min, emitting red light), washing, mounting, and observation (Olympus BX51 fluorescence microscope; Olympus Corporation) with a blue light (wavelength of 450–490 nm) excitation.

2.4. Analysis of gene expression data

The data from The Cancer Genome Atlas (TCGA) was analyzed to evaluate the association between MCTs mRNA expression and clinicopathological features of breast cancer patients. In addition, the association between MCTs mRNA level and survival outcomes of patients with breast cancer was analyzed.

2.5. Statistical analysis

All statistical analyses and all charts of survival probabilities were performed with SPSS 22.0 (IBM Corporation, Armonk, NY, USA). The relationships between MCTs and the baseline clinical characteristics of patients with breast cancer were evaluated by Chi-square test. The Kaplan-Meier method was used to calculate the patient survival probability and the log-rank test was used to assess the heterogeneity in the survival data for each prognostic factor. Multivariate Cox proportional hazard regressions were used to obtain hazard ratios (HRs) and their respective 95% confidence intervals to show the strength of the estimated relative risks. Pearson's correlation was used to evaluate the correlation between MCT1 and MCT4 expression levels. Significance levels were set at P value < 0.05. All tests were two-sided.

Please cite this article in press as: Z. Li, et al., Monocarboxylate transporters in breast cancer and adipose tissue are novel biomarkers and potential therapeutic targets, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.091