RESEARCH COMMUNICATION

DLC-1 Expression Levels in Breast Cancer Assessed by qRT-PCR are Negatively Associated with Malignancy

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Abstract

Objective: The aim of this study was to explore the expression of DLC-l in breast carcinoma and any association with tumor metastasis. Methods: 51 surgical specimens of human breast carcinoma, divided into high invasive and low invasive groups according to their clinicopathological features, 30 cases of adjacent normal tissue and 28 benign breast lesions were examined by qRT-PCR for expression of DLC-1. Results: Expression level of DLC-1 in adjacent normal tissue and benign breast lesion specimens was higher than that in breast carcinoma (P<0.0001); the values in the high invasive group with synchronous metastases were also lower than in the low invasive group (P=0.0275). The correlation between DLC-1 expression level and tumor progression and metastasis of breast cancer was negative. Conclusion: As an anti-oncogene, DLC-1 could play an important part in breast carcinoma occurrence, progression, invasiveness and metastasis. Detecting the changes of the expression of DLC-1 in the breast carcinoma may contribute to earlier auxiliary diagnosis of invasiveness, metastasis and recrudescence.

Keywords: DLC-1 - breast carcinoma - qRT-PCR - malignancy - negative correlation

Asian Pacific J Cancer Prev, **13**, 1231-1233

Introduction

Breast cancer is one of the most common malignancies in female. The morbidity was 7-10% of all kinds of malignant tumor. It's related to genetic factor, the high incidence often occur the women ageing 40 to 60 years old, before and after menopause. Only about 1-2% of breast cancer patients are male. Breast cancer is one of the most common malignancies that affect women's physical and mental health and even life-threatening. 5-year survival rate of after eradicative operation is about 50%, and no positive nodes disease survival rate is about 35%. So early detection and early predictions to the metastasis of recurrence, and giving timely prevention and treatment of patients with breast cancer, plays a vital role.

DLC-1 is considered as a kind of important tumor inhibitory factor, gene heterozygous lost or DNA methylation can cause DLC-1 expression decreasing or deletion in liver cancer, lung cancer, prostate cancer and nasopharyngeal carcinoma cancer tissues (Kim et al., 2003; Guan et al., 2006; Kim et al., 2007). As a kind of GAPs protein, can regulate the enzyme activity of Rho GTP family, GTP enzyme activity is usually in low state, GAPs can make its activity enhanced obviously (Durkin, 2002). It also participates in gene transcription, cell cycle regulation and signaling pathways regulation, through the RhoA regulating actin reaction to the signal of the cells, make monomer actin filaments form mitoplast actin and polymerizes to form stress fiber and focal adhesion, make the cells shape change and apoptosis (Yin and Janmey, 2003). Research also shows that DLC-1 protein has RhoGAP activity, START structure domain and elastin combined activity, its most important functions is to guide DLC-1 to stress fiber and focal adhesion (Sekimata et al., 1999; Wong et al., 2005; Yam et al., 2006), to maintain the cell shape and endow cells tenacity and strength, which affect the cytoskeleton building, cells movement and migration, DLC-1 is of great important to the tumor invasion and metastasis. In the current study, DLC-1 gene expression level of breast cell carcinoma lesion was detected using qRT-polymerase chain reaction (PCR), o explore the relationship between the tumor-suppressor genes DLC-1 expression and tumor metastasis in breast cancer.

Materials and Methods

Specimens

51 cases of breast cancer tissue samples were obtained by resection from the Affiliated Hospital of Guangdong Medical College from March 2008 to October 2008. With reference to the international cancer society TNM classification and combining with the history, the specimen was divided into low invasion N0M0 group (no local lymph nodes and distant metastasis, n=18), and high invasion group N + M + (with local lymph nodes and/or

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distant metastasis, n=33).

In addition, 30 cases cancer adjacent tissue that distance was more than 5 cm from the cancer focal were collected as a normal control group (no breast carcinoma tissue), 28 cases of breast benign hyperplasia tissues were also collected. All the specimens are diagnosed by pathological examination. Specimens were taken from the body 5 min, quick-frozen in liquid nitrogen for 5 min and stored at -70 °C.

Primers

DLC-I primers were provided by Guangzhou Laideer Biological Co., LTD China. DLC-I primer sequences were as follows: 5'-GAGCCGATGTCGTAATTC-3' (forward) 5'-TCCAACAG-GTCTACATCC-3' (reverse), he product was 324 bp; the inter control (18SrRNA): 5'-CCT GGA TACCGCAGCTAGGA-3' (forward), 18SrRNA-R 5'-GCGGCGCAATACGAATGC CCC-3' (reverse), the product was 112bp.

Quantitative PCR

100 mg of normal breast tissue, mammary gland hyperplasia tissue and breast cancer tissues were collected, respectively. 1 ml Trizol (Invitrogen, SA) solution was added into each tissue. RNA was extracted according to kit instructions (Invitrogen, SA). RNA purity and integrity were detected by the OD value and electrophoresis.

About 20 μL of amplification reactions contained 5 μL of cDNA, 10 μL of UltraSYBR Mixture with Rox, and 0.5 μL of primers. Following the initial steps at 95 °C (10 min), PCR was carried out for 40 cycles at 95 °C (15 s), and then at 60 °C (1 min)

Statistical analysis

SPSS 13.0 software was used for statistical analysis, and comparison of DLC-1 mRNA level among different groups was analyzed using nonparametric Wilcoxon Signed Ranks test.

Results

DLC-1 expression

The DLC-1 cDNA template onset quantity was determined by comparison of Ct value (△△Ct) relative quantitative method. 18 s rRNA was used as inter gene, normal breast tissue was as control, DLC-1 cDNA expression in breast cancer gene, benign breast lesions tissue were analyzed. The results showed that DLC-1 in breast cancer gene expression was significantly lower than that normal breast tissue and benign breast lesions tissue (Table 1), that of highly infiltration breast cancer group was significantly lower than that of the low infiltration group (Table 2).

Table 1. Statistical Analysis qRT-PCR Results

Group	Cases	△-2△△median value	Z value	P
Normal tissue	28	2.75		
Benign tumor	30	2.8	-0.8404	0.4007
breast cancer	50	1.09	6.3914	<0.0001

Note: Comparison between lesions and normal group, Z=6.6296, P <0.0001

Table 2. qRT-PCR Results of High and Low Invasion Groups

Group	Cases	△-2△△median value	Z value	P
Low Invasion	18	1.47		
High Invasion	32	1.01	2.204	0.0275

Discussion

As a new candidate suppressor gene, DLC-1 is located in 8 p21. 3 ~ 22 area, this region occur missing or down-regulation frequently in the liver cell cancer, breast cancer, lung cancer and prostate cancer and so on (Yuan and Burkin, 2003). Park et al. (2003) considered that DLC-1 gene inactivation will cause biological process change and lead to malignant phenotype, and DLC-1 over-expression can inhibit tumor cell growth in vitro. RhoGTP enzyme signaling pathways plays an important role in the invasion and metastasis of HCC, DLC-1 gene expression products are specific GTP enzyme activating protein of RhoA and Cdc42, related closely with regulation of cell proliferation and adhesion of signaling pathways, mainly through the down-regulation RhoA activity to inhibit tumor (Wong et al., 2003). Liver cancer cell lines without endogenous DLC-1 gene expression was transfected by DLC-1 cDNA, confirmed DLC-1 gene expression has an inhibitory effect on liver cancer cells growth (Zhou et al., 2004). In addition, the same results were also obtained from the lung cancer, breast cancer cell lines and nasopharyngeal carcinoma (NPC) (Yuan et al., 2004; Plaumann et al., 2003; Qian et al., 2007; Seng et al., 2007). Yuan et al. (2003) transfected DLC-1 into two DLC-1 negative breast cancer cell lines, and found cell proliferation rate declined obviously, and further the DLC-1 negative cells and DLC-1 trasfected cells were injected into the nude mouse for 3 weeks, respectively, the former gradually developed into tumor, its histopathological was same as mammary gland adenocarcinoma, the latter had no tumor formation, therefore, DLC-1 play an important inhibition role in the breast cancer formation.

This study found that DLC-1 mRNA expression in normal breast tissue, breast benign lesions tissue and low invasion breast cancer tissues had no obvious difference, but DLC-1 mRNA expression of high invasion group breast cancer tissue and normal breast tissue had obvious difference. These results are consistent with the previous report (Yuan et al., 2003). Our results showed that DLC-1 the expression in breast cancer was negatively correlated with cancer progress and metastasis. DLC expression was high in normal breast tissue-1, while less significantly in the breast cancer tissues. These results are consistent with the results of Paumann et al. (2003). DLC-1 may closely related with the growth of primary tumor, differentiation and the metastasis of tumor cells, its mechanism may be DLC-1 proteins participate in actin skeleton formation, adjust the cell shape and movement, thus affecting the metastasis of tumor cells and invasion (Yuan et al., 2003). RNAi technology experiments have also confirmed the results (Liu et al., 2008; Shang et al., 2008).

DLC-1 expression was over-regulated when Non-hodgkin's lymphoma (Mocellin et al., 2004), leukemia (Sacha and Elizabeth, 2008) cell lines was treated using

trichostatin A and 5-nitrogen-2-deoxidizing cytidine. It may be new targets for testing breast cancer cell proliferation, metastasis and gene therapy. DLC-1 mRNA expression level detected by QRT-polymerase chain reaction (PCR), can reflect the disseminated degree of the tumor cells, the level decreasing or lacking indicates that distant metastasis of patients may occur. Through the dynamic detection, to observe curative effect, provide the reference for drawing up individualized treatment and prognosis assessment.

Acknowledgements

This study was supported by Industrial Technology Research and Development Funds, Guangdong Province (No: 2010 B031600289).

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