Gene 587 (2016) 173–177

Contents lists available at ScienceDirect

Gene

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

Research paper

MTUS1 tumor suppressor and its miRNA regulators in fibroadenoma and breast cancer

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ARTICLE INFO

Article history:
Received 29 February 2016
Received in revised form 25 April 2016
Accepted 2 May 2016
Available online 4 May 2016

Keywords:
Breast cancer
Fibroadenoma
MTUS1
miRNA
miR-183-5p
let-7

ABSTRACT

Breast cancer is major public health problem predominantly effects female population. Current therapeutic approaches to deal with breast cancer are still lack of effectiveness. Thus, identifying/developing novel strategies to fight against breast cancer is very important. The frequent deletions at 8p21.3-22 chromosomal location nearby D8S254 marker enabled the discovery of a novel tumor suppressor gene, *MTUS1*. Subsequently, *MTUS1* was demonstrated to be less expressed in a variety cancer types including breast cancer. Also, it is obvious that gene expression is widely regulated by miRNAs. Here, we aimed to report differential expression of *MTUS1* and its regulatory miRNAs in breast cancer and fibroadenoma tissues. Dynamic analysis of *MTUS1* expression levels and its miRNAs regulators were attained by Fluidigm 96 × 96 Dynamic Array Expression chips and reactions were performed in Fluidigm BioMark™ HD System qPCR. Consequently, *MTUS1* mRNA levels were significantly diminished in breast cancer tissues and elevated in fibroadenoma tissues. Also, among *MTUS1* targeting miRNAs, miR-183-5p was identified to be overexpressed in breast cancer and down-regulated in fibroadenoma tissues. Also, expression levels of *MTUS1* and miR-183-5p were well correlated with clinical parameters. In particular, *MTUS1* expression was found to be diminished and miR-183-5p expression was elevated with the advancing stage. In conclusion, as a potential therapeutic target, miR-183-5p can be a chief regulator of *MTUS1* and *MTUS1*-miR-183-5p axis may have significant influence in the pathology of breast cancer.

1. Introduction

Breast cancer is a heterogeneous lethal malignant disease predominantly affecting women all over the world (Ergun et al., 2015). Recent findings in targeted therapy by gene profiling assays was improved disease free survival rates in breast cancer (Ergun et al., 2015). However, current strategies to fight against breast cancer are still limited and many patients develop resistance to therapeutic agents. Therefore, proposing probable novel molecular biomarkers with prognostic, diagnostic and therapeutic potential is still high priority.

MTUS1 (Microtubule-associated tumor suppressor 1) is a recently identified gene with its significant tumor suppressor functions (Kara et al., 2015). *MTUS1* was revealed to be frequently down-regulated in numerous types of cancers such as pancreas (Seibold et al., 2003), colorectal (Kara et al., 2015; Zuern et al., 2010), ovarian (Pils et al., 2005), head and neck (Ye et al., 2007) and breast cancers (Frank et al., 2007; Rodrigues-Ferreira et al., 2009). More importantly, reduced expression of *MTUS1* was shown to be associated with the advanced proliferation of breast, ovarian and oral tongue squamous cell carcinoma cells (Pils et al., 2005; Frank et al., 2007; Ding et al., 2012).

Moreover, the level of gene expression is widely regulated by the small RNA molecules, called miRNAs (Bartel, 2004). miRNAs are approximately 22 nucleotides tiny non-coding RNAs that either interfere with the translation of mRNAs or trigger mRNA degradation by forming complementary base pairing at post-transcriptional level (Bartel, 2004). miRNAs are accepted as fine-tuners of gene expression (Sevignani et al., 2006). Thus, they are small players with big jobs. Studies reported that miRNAs are highly involved in the processes of carcinogenesis by affecting target gene expression in tumor tissues (Calin and Croce, 2006). Several miRNAs were identified with their oncogenic and tumor suppressor potentials (Calin and Croce, 2006).

Abbreviation: ATIP, AT2 receptor interacting protein; BC, breast cancer; CRC, colorectal cancer; FA, fibroadenoma; FFPE, formalin fixed paraffin-embedded; mRNA, messenger RNA; miRNA, micro RNA; *MTUS1*, Microtubule-associated tumor suppressor 1.

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http://dx.doi.org/10.1016/j.gene.2016.05.006
0378-1119/

To imagine the whole story of a gene's functions in a pathogenic state like cancer, determining the miRNA-target interrelations are very crucial.

Accordingly, in the present study, the role of *MTUS1* gene and its potential miRNA regulators were assessed in fibroadenoma and breast cancer cases through using a high-throughput qPCR technology. In particular, expression levels of *MTUS1* and its miRNA regulators were evaluated by using Fluidigm BioMark™ HD System qPCR.

2. Material and methods

2.1. Study population and collection of FFPE tissue samples

For the present study, 25 breast cancer and 24 fibroadenoma patients and 25 control subjects were enrolled. The control subjects were pathologically confirmed normal breast tissues of independent individuals. The present work was ethically accepted by the local ethics committee of Mugla Sitki Kocman University (Decision Number: 13) in agreement with Declaration of Helsinki. Individuals who accepted to be included in the study were asked to give a written informed consent prior to inclusion to study. Demographic and clinical characteristics of study participants were presented in Table 1. 5–20 μm thick FFPE (formalin fixed paraffin-embedded) tissue slices were obtained from the library of pathology department and kept at −20 °C until RNA extraction.

2.2. Determination of predicted and validated MTUS1 regulators

To determine, putative and confirmed miRNA regulators of *MTUS1* gene, three databases of Targetscan, DianaTools, Mirtarbase databases were used. Validated miRNAs of *MTUS1* gene was updated by literature scanning of recent studies (Helwak et al., 2013). Predicted and validated miRNAs that target *MTUS1* gene was presented in Fig. 1.

2.3. Isolation and quantification of RNA from FFPE samples

Isolation of total RNA samples including miRNAs were attained by using miRNeasy FFPE Kit (QIAGEN Sample & Assay Technologies, Germany) according to the instructions of the supplier. Quantity and quality of RNA samples were determined by using NanoDrop ND-100 spectrophotometer (Thermo Fisher Scientific Inc.,Wilmington, USA).

Table 1
Demographic and clinical characteristics of breast cancer patients. Some demographic characteristics of fibroadenoma patients and controls were also presented. Some of the missing information about breast cancer patients was indicated in the table.

Clinical and demographic characteristics of patients and controls		
Age	Control (n = 25)	38.24 ± 4.42
	Fibroadenoma (n = 24)	30.58 ± 11.85
	Breast cancer (n = 25)	61.2 ± 16.73
Clinical Characteristics		
Parameter	Breast cancer	n = 25 (%)
Stage	I	2 (8)
	II	8 (32)
	III	8 (32)
	IV	7 (28)
Bone metastasis	Yes	4 (16)
	No	21 (84)
ER	Positive	23 (92)
	Negative	2 (8)
PR	Positive	18 (72)
	Negative	7 (28)
HER2	Positive	7 (28)
	Negative	18 (72)
ECAD	Positive	10 (40)
	Negative	2 (8)
	Missing	13 (52)
P53	Positive	11 (44)
	Negative	14 (56)

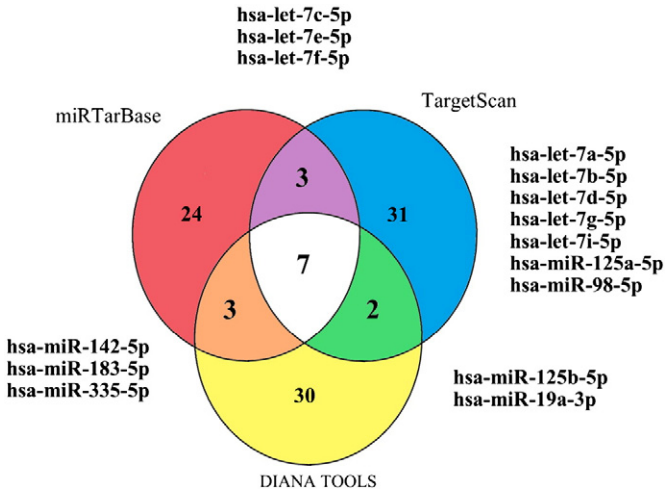

Fig. 1. Graphical representation of the predicted and validated miRNA regulators of *MTUS1* gene. For the determination of MTUS1 targeting miRNAs, Targetscan, DianaTools, Mirtarbase databases were used.

RNA samples were stored as equal aliquots at −80 °C until further experiments.

2.4. Synthesis of cDNA from RNA samples

Qiagen miScript II RT Kit (QIAGEN Sample & Assay Technologies, Germany) were used to synthesize cDNA from RNA samples. Synthesis was achieved according to manufacturer's supplied protocol and cDNA samples were stored in equal aliquots at −80 °C for further analysis.

2.5. Expression analysis by high-throughput Fluidigm BioMark™ HD System

For the expression analysis a high- throughput gene expression screening system was used. Dynamic analysis of *MTUS1* expression levels and its miRNAs regulators were attained using Fluidigm 96 × 96 Dynamic Array Expression chips (Fluidigm, South San Francisco, Calif., USA) and reactions were performed in Fluidigm BioMark™ HD System qPCR (Fluidigm, South San Francisco, Calif., USA). For the qPCR reactions, TaqMan Universal Master Mix (Life Technologies) and Sample Loading Reagent (Fluidigm) were used. For the dilution of cDNA samples Tris-EDTA (TE) buffer was used.

2.6. Statistical analysis

The resulting expression records were first examined with Fluidigm Real Time PCR Analysis software (Fluidigm, South San Francisco, Calif., USA) and expression signals were converted to obtain Ct values. For the normalization of mRNA expression levels were achieved using *Beta-2-microglobulin* (*B2M*) gene. In addition, SNORD61, SNORD68, SNORD72, and SNORD95 small RNA molecules were used for the normalizations of miRNA expressions. Quantitative expression levels were calculated using the formula: $2 - \Delta Ct$ (ΔCt = Target gene − reference gene). In the statistical analysis, SPSS (version 20) program was used and Wilcoxon signed rank test was performed. In the fold change analysis, RT2 Profiler PCR Array Data Analysis Version 3.5 (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php?target=upload) online software was used. In all statistics analysis, p values were two-tailed, and $p < 0.05$ were accepted as statistically significant.