Toxicology Letters 223 (2013) 205–210

Contents lists available at ScienceDirect

Toxicology Letters

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

LncRNA-DQ786227-mediated cell malignant transformation induced by benzo(a)pyrene

Liyun Gao[a], Ai Mai[a], Xun Li[a], Yandong Lai[a], Jingli Zheng[a], Qiaoyuan Yang[a], Jianjun Wu[a], Aruo Nan[a], Sheng Ye[b,**], Yiguo Jiang[a,*]

[a] Institute for Chemical Carcinogenesis, State Key Laboratory of Respiratory Disease, Guangzhou Medical University, Guangzhou 510182, PR China
[b] Department of Medical Oncology, First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, PR China

HIGHLIGHTS

- LncRNA-DQ786227 is overexpressed in transformed BEAS-2B cells.
- Overexpression of this lncRNA increases cell proliferation and inhibits apoptosis.
- LncRNA-DQ786227 acts as an oncogene in transformed cells.

ARTICLE INFO

Article history:
Received 5 February 2013
Received in revised form
18 September 2013
Accepted 20 September 2013
Available online 29 September 2013

Keywords:
Benzo(a)pyrene
Cell transformation
LncRNA

ABSTRACT

It has recently been found that the new class of transcripts, long non-coding RNAs (lncRNAs), are pervasively transcribed in the genome. LncRNAs are a large family of non-coding RNAs and regulate many protein-coding genes. Growing evidence indicates that lncRNAs may play an important functional role in cancer biology. Emerging data have shown that lncRNAs are closely related to the occurrence and development of lung cancer. However, the function and mechanism of lncRNAs in lung cancer remain elusive. Here, we investigated the role of a novel lncRNA in transformed human bronchial epithelial cells induced by benzo(a)pyrene. After establishing the transformed cell model using the BEAS-2B cell line *in vitro*, we found that expression of lncRNA-DQ786227 was high and changed during the transformation of BEAS-2B cells. Silencing of lncRNA-DQ786227 expression in malignant transformed BEAS-2B cells led to inhibition of cell proliferation and colony formation, and increased apoptosis. LncRNA-DQ786227 dramatically promoted the ability of BEAS-2B-T cells to form colonies *in vitro* and develop tumors in nude mice. These findings revealed that lncRNA-DQ786227 acts as an oncogene in malignantly transformed BEAS-2B cells induced by benzo(a)pyrene. The identification of lncRNA could provide new insight into the molecular mechanisms of chemical carcinogenesis.

© 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Some long non-coding RNAs (lncRNAs) have been discovered ranging from 200 up to several kilobases (Pastori and Wahlestedt, 2012). LncRNAs do not show protein-coding potential (Derrien et al., 2012), and are pervasively transcribed throughout eukaryotic genomes (Nie et al., 2012). LncRNAs have diverse roles in human biology (Martin and Chang, 2012). Dysfunction of lncRNAs is associated with a wide range of diseases, including cancer (Chen et al., 2013). Therefore, the discovery of lncRNAs has provided new opportunities to study the molecular mechanisms of cancer. However, the molecular mechanisms involved in chemical carcinogenesis are complex and poorly understood. In particular, the carcinogenic mechanisms of lncRNAs induced by chemical carcinogens are rarely reported.

Benzo(a)pyrene (B[a]P) is one of the many compounds found in tobacco smoke, and it is a ubiquitous environmental carcinogen. B[a]P exposure can increase the risk of human lung cancer (Friesen et al., 2007), which is the leading cause of cancer deaths worldwide in both men and women (Esposito et al., 2010). B[a]P is also one of the most potent pro-carcinogens, and it may cause lung tumor formation in a multistep manner. B[a]P is a representative polycyclic aromatic hydrocarbon and metabolically activated by three enzymatic pathways (Jiang et al., 2007). The immortalized human bronchial epithelial cell line (BEAS-2B) possesses a high metabolic

* Corresponding author at: Chemical Carcinogenesis, Guangzhou Medical University, 195 Dongfeng Road West, Guangzhou 510182, PR China. Tel.: +86 20 81340186; fax: +86 20 81340724.
** Corresponding author at: Department of Medical Oncology, First Affiliated Hospital, Sun Yat-sen University, 58 Zhongshan No. 2 Road, Guangzhou 510080, PR China. Tel.: +86 20 87332200.
E-mail addresses: yes20111212@163.com (S. Ye), jiangyiguo@vip.163.com (Y. Jiang).

0378-4274/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2013.09.015

capacity by expressing several biotransformation enzymes (Mace et al., 1994). We used transformed BEAS-2B cells induced by chemical carcinogen B[a]P to study the role of lncRNA involved in lung carcinogenesis *in vitro*.

In a lncRNA expression microarray analysis, lncRNA-DQ786227 was found overexpressed in human bronchial epithelial cells transformed by anti-benzo[a]pyrene-trans-7,8-diol-9,10-epoxide (anti-BPDE), the most important carcinogenic metabolite of B[a]P. In this study, we explored the function of lncRNA-DQ786227 in transformed BEAS-2B cells. We found that expression of lncRNA-DQ786227 was high and changed during the malignant transformation of BEAS-2B cells. Silencing of lncRNA-DQ786227 reduced cell viability and dramatically suppressed the ability of BEAS-2B-T cells to form colonies *in vitro* and suppressed tumor development in nude mice. LncRNA-DQ786227 may play a role as an oncogene. This is believed to be a novel study concerning the role of lncRNA-DQ786227 in carcinogenesis. Our results increase the available knowledge regarding lncRNAs in B[a]P-induced malignant transformation.

2. Materials and methods

2.1. Cell culture

BEAS-2B cell line was kindly provided by Lijin Zhu (Institute of Hygiene, Zhejiang Academy of Medical Sciences, Hangzhou, China). BEAS-2B cells were cultured as described previously (Azad et al., 2010; Jing et al., 2012; Son et al., 2012) in H1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Sijiqing, Hangzhou, China), 100 U/ml penicillin and 100 μg/ml streptomycin, and incubated at 37 °C in a humidified chamber with 5% CO_2. BEAS-2B cells were passaged 2–3 times per week. The lung cancer cell lines A549 and QG56 were purchased from the Chinese Academy of Sciences Cell Bank of Type Culture Collection (Shanghai, China) and also cultured in H1640 medium.

2.2. Cell transformation by B[a]P exposure

B[a]P (96% purity, Sigma–Aldrich, St. Louis, MO, USA) was dissolved in dimethylsulfoxide (DMSO; Sigma–Aldrich). BEAS-2B cells were treated with five concentrations of B[a]P (4, 20, 100, 500, and 1000 nM) dissolved in DMSO. The final concentration of DMSO did not exceed 0.1% of total incubation volume. B[a]P 100 nM appeared to be the optimal concentration for induction of cell transformation. This was consistent with the study by van Agen et al. (1997). After BEAS-2B cells were cultured in H1640 medium supplemented with 10% heat-inactivated FBS for 24 h, the medium was discarded. Then the cells were exposed to 100 nM B[a]P. After 24 h, the cells were washed three times with phosphate-buffered saline (PBS; Hyclone, Logan, UT, USA). Finally, the cells were cultured under normal conditions. In order to simulate repeated low-dose exposure to environmental carcinogens in humans, these procedures were repeated nine times. When confluence reached 80–90%, the cells in each dish were split into two dishes. After the ninth B[a]P treatment, the morphology of the cells was observed every day, and the 45th passage cells designated BEAS-2B-T.

2.3. Soft agarose assay

Soft agarose assays were used to identify the colony forming ability of malignant transformed cells and shRNA-transfected cells. Soft agar plates were prepared in six-well plates with a bottom layer of 0.6% low-melting-point agarose (Sigma–Aldrich) in H1640 medium containing 20% FBS. After solidification, 1000 cells per well of each group were suspended in 2 ml 0.3% (w/v) low-melting-point agarose with 20% FBS supplemented H1640 medium, and then seeded in the plate (3 plates per group). The six-well plates were incubated at 37 °C in a humidified 5% CO_2 atmosphere for 15 days. Colonies with at least 50 cells were counted using a microscope at 10× magnification and colony formation efficiency in soft agarose was quantified.

2.4. Tumor xenograft model

6-Week-old Balb/c nude mice were provided by Experimental Animal Center of Guangdong Province (Foshan, China). All experimental procedures involving animals were performed in accordance with the Experimental Animal Center Guide for the Care and Use of Laboratory Animals, and the Institutional Ethical Guidelines for Experiments with Animals. Malignantly transformed cells (BEAS-2B-T), or the 45th passage of untransformed control cells (BEAS-2B-N) were propagated and 1×10^6 cells were injected subcutaneously into the bilateral inguino-abdominal flanks of 10 nude mice; BEAS-2B-T cells were injected on the right and the BEAS-2B-N cells group on the left side. After 25 days, the mice were killed and the xenograft tissues were harvested and fixed with 10% formalin solution for histological analysis.

BEAS-2B-T cells transfected with shRNA1 or negative control (shRNA NC) were propagated and 5×10^6 cells were injected subcutaneously into seven nude mice. shRNA1-transfected cells were injected on the right and shRNA NC-transfected cells on the left side. Untransfected BEAS-2B-T cells (NT) were injected subcutaneously on the right side of another seven nude mice. The mice remained in a pathogen-free environment and tumor growth was monitored by measuring tumors externally in two dimensions with a slide caliper. Tumor volume was determined from the equation: $V = 0.5\ (LW^2)$, where L and W are, respectively the length and width of the tumor (Anumanthan et al., 2005; Jiang et al., 2011). The tumor diameter was measured every 5 days. After 25 days, the mice were killed and the xenograft tissues were harvested.

2.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. To detect the expression of lncRNA-DQ786227, first strand cDNA was generated by SYBR PrimesScript RT-PCR kit (TaKaRa Biotechnology, Kyoto, Japan) using 500 ng total RNA. We performed qRT-PCR to detect expression of lncRNA-DQ786227 with the SYBR Premix Ex Taq (TaKaRa) using the Applied Biosystems 7500 real-time PCR system (Foster City, CA, USA). 18S rRNA was used as an internal control. The qRT-PCR conditions for lncRNA-DQ786227 were as follows: polymerase activation 30 s at 95 °C, 40 cycles at 95 °C for 5 s, 60 °C for 30 s, and 72 °C for 20 s. PCR products were identified by melting curve analysis. The data were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). All primers were synthesized by Invitrogen. The primer sets used in lncRNA-DQ786227 qRT-PCR were as follows:

18s rRNA-F: 5′-GTAACCCGTTGAACCCCATT-3′,
18s rRNA-R: 5′-CCATCCAATCGGTAGTAGCG-3′,
LncRNA-DQ786227-F: 5′-ACTGCCTCACAACGTTTGTGCCT-3′,
LncRNA-DQ786227-R: 5′-CCAGCGTGAGCTCCAGTGCTTT-3′.

2.6. RNAi and shRNA

To investigate the biological function of lncRNA-DQ786227, BEAS-2B-T cells were transfected with 40 nM each of siRNA targeting lncRNA-DQ786227 or negative control (siRNA NC) (GenePharma) using Lipofectamine 2000 transfection reagent (Invitrogen), according to the manufacturer's instructions. The sequences of carboxyfluorescein (FAM) siRNA primers were as follows: siRNA1, 5′-CUCCAUUGUUUCUCCUUAATT-3′ and 5′-UUAAGGAGAAACAAUGGAGTT-3′; siRNA2, 5′-GCAUCUAGCAUGCUCAAUATT-3′ and 5′-UAUUGAGCAUGCUAGAUGCTT-3′; siRNA3, 5′-GCUCCUGCGAAUAGCUAAATT-3′ and 5′-UUUAGCUAUUCGCAGGAGCTT-3′; siRNA4, 5′-GAGGGUAACAAUACUUACUTT-3′ and 5′-AGUAAGUAUUGUUACCCUCTT-3′; siRNA NC, 5′-UUCUCCGAAGUGUCACGUTT-3′ and 5′-ACGUGACACGUUCGGAGAATT-3′. At 24, 48 and 72 h post transfection, the expression of lncRNA-DQ786227 was determined by qRT-PCR. Next, we used the most efficient sequence, siRNA1, to generate cell lines stably expressing the shRNA1. Recombinant lentiviruses containing shRNA1 or control (shRNA NC) were purchased from GenePharma.

2.7. Analysis of cell viability, apoptosis and cell cycle after RNAi

For the cell viability assay, BEAS-2B-T cells were plated in 96-well plates (3×10^3 cells per well) and cultured for 24 h and then transfected with siRNA targeting lncRNA-DQ786227 or siRNA NC. After 48 h incubation, the absorbance determined at 450 nm after transfection with carboxyfluorescein-labeled siRNA was done using a Synergy 2 microplate reader (BioTek, Winooski, VT, USA) to determine transfection rate. The transfection efficiency was >90%. Each cell type was analyzed in triplicate. Cell viability was evaluated with a Cell Counting Kit-8 (CCK-8) (Dojindo, Tokyo, Japan). CCK-8, being nonradioactive allows sensitive and convenient colorimetric assays for the determination of the number of the viable cells in cell proliferation and cytotoxicity assays (Yang et al., 2008). The cell viability (% of control) was expressed as the percentage of $(OD_{test} - OD_{blank})/(OD_{control} - OD_{blank})$, where OD_{test} was the OD of the transfected cells, $OD_{control}$ was the optical density of BEAS-2B-T cells, and OD_{blank} was the OD of the wells without BEAS-2B-T cells. For the apoptosis and cell cycle assay, BEAS-2B-T cells were plated in six-well plates (5×10^4 cells per well), cultured for 24 h, and then transfected with siRNA targeting lncRNA-DQ786227 or siRNA NC. For the apoptosis assay, cells were harvested using 0.25% trypsin and washed twice with ice-cold PBS after 48 h incubation, and then resuspended in 500 μl binding buffer (10 mM HEPES-NaOH [pH 7.4], 140 mM NaCl, 2.5 mM $CaCl_2$). Cells were stained with 5 μl annexin V–fluoresein-5-isothiocyanate (FITC) and 5 μl propidium iodide (PI) for 15 min in the dark at room temperature, and then analyzed by flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA). Results were calculated as the percentage of apoptotic cells among the total number of cells counted. For the cell cycle assay, upon 24 h of serum-starvation, and 48 h after transfection, cells were harvested and fixed in 70% ice-cold ethanol at 4 °C overnight, then washed twice with 0.1% (vol/vol) Triton X-100 in PBS, treated with RNAse A (200 mg/l) at 37 °C for 30 min, stained with 20 mg/l PI, and then immediately analyzed by flow cytometry with FACScan (Becton Dickinson). Data were gated using the FlowJo FACS analysis software (Tree Star, Ashland, OR, USA).

Download English Version:

https://daneshyari.com/en/article/2599143

Download Persian Version:

https://daneshyari.com/article/2599143

Daneshyari.com