BPB Reports

Report

Identifying Long Non-Coding RNA as Potential Indicators of Bacterial Stress in Human Cells

Rina Abe,[a,b] Yuka Yagi,[a,b] and Hidenori Tani[b,*]

[a]*Department of Clinical Pharmacy, Yokohama University of Pharmacy, 601 Matano, Totsuka, Yokohama, 245-0066, Japan;*
[b]*Department of Health Pharmacy, Yokohama University of Pharmacy, 601 Matano, Totsuka, Yokohama, 245-0066, Japan*
Received November 10, 2023; Accepted November 20, 2023

Long non-coding RNAs (lncRNAs) are transcripts exceeding 200 nucleotides in length that do not code for proteins. However, they play pivotal roles in various biological processes. The mechanisms by which bacterial infections induce lncRNA expression remain elusive. Our past study, we identified a unique class of lncRNAs with short half-lives, less than 4 h in human HeLa cells. These short-lived lncRNAs include to many regulatory functions, such as HOTAIR, NEAT1, or GAS5. Due to their potential influence on human biology, these short-lived lncRNAs might serve as important markers to gauge the stress from bacterial infections. In this study, we identified three lncRNAs, named MIR22HG, GABPB1-AS1, and IDI2-AS1. Their expression significantly decreased after exposure to lipopolysaccharide, simulating bacterial infection in human A549 cells. Our findings suggest that short-lived lncRNAs react to bacterial infections, with their expression levels dropping notably. We propose that these lncRNAs could act as potential indicators of cellular responses to bacteria.

Key words non-coding RNA, lncRNA, LPS, short half-life

INTRODUCTION

Numerous long non-coding RNAs (lncRNAs), including intergenic, intronic, and cis-antisense types, that are non-protein-coding and exceed 200 nucleotides in length, have been discovered in mammals.[1)] They are transcribed based on tissue type and developmental phase.[2,3)] These lncRNAs have diverse structural and regulatory functions in several cellular activities, encompassing chromatin alteration, imprinting, transcription, translation, and epigenetic control.[4,5)]

In 2012, two research groups revealed that the stabilities of lncRNAs vary greatly, similar to mRNAs in mouse neuro-2a neuroblastoma cells[6)] and human HeLa cells.[7)] Using the 5′-bromouridine immunoprecipitation chase-deep sequencing (BRIC-seq), which is genome-wide method for determining RNA stability, renowned regulatory lncRNAs like HOTAIR,[8)] NEAT1,[9)] and GAS5[10)] were found to have brief half-lives, whereas housekeeping-like lncRNAs have extended half-lives.[7)] These findings imply the lifespans of lncRNAs may relate to their roles, with short-lived lncRNAs possibly having more regulatory purposes. Thus, undiscovered short-lived lncRNAs might influence human biological activities, serving as chemical stress indicators in cells.[11)]

Evidence derived from next-generation sequencing (NGS) underscores the significance of lncRNAs in a variety of disease pathologies, including infections, inflammation, and stress.[12–14)] In numerous disease processes, lncRNAs exhibit altered regulation. A deeper comprehension of lncRNA functionalities can offer molecular insights into inflammation, stress, and immune control, with a particular focus on the interplay between host and pathogen. Such insights could pave the way for potential therapeutic strategies against infectious ailments.[15)] Infection is primarily characterized by oxidative stress, endoplasmic reticulum stress, unfolded protein response, immune responses, and inflammation.

Our research aimed to find new short-lived lncRNAs in human cells that alter their expression due to bacterial infection simulation using lipopolysaccharide (LPS). We used A549 cells that are adenocarcinomic human alveolar basal epithelial cells. A549 cells are useful *in vitro* model of bacterial invading research, is easy to handle, and provides a reproducible human model system. We identified three lncRNAs, MIR22HG, GABPB1-AS1, and IDI2-AS1, in A549 cells that decreased expression in response to LPS.

MATERIALS AND METHODS

Chemicals Lipopolysaccharide (LPS) was purchased from Wako (Japan). LPS was dissolved in water and diluted in culture medium at a final concentration of 0.1% vol/vol.

Cell Culture A549 cells were grown in Dulbecco's modified Eagle's medium (DMEM; Wako, Japan) supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified incubator under an atmosphere of 5% CO_2.

Reverse Transcription-Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) Total RNA was extracted from cells with TRIzol Reagent (Thermo Fisher Scientific, USA), in accordance with the manufacturer's instructions. The isolated RNA was reverse transcribed into cDNA using PrimeScript RT Master Mix (Perfect Real Time) (TaKaRa, Japan). The resulting cDNA was amplified using the primer sets listed in Table 1, and the levels were normalized relative to glyc-

*To whom correspondence should be addressed. e-mail: hidenori.tani@yok.hamayaku.ac.jp

Table 1. Primer Pairs for RT-qPCR

Name	Sence sequence (5'-3')	Antisence sequence (5'-3')
GAPDH	GCACCGTCAAGGCTGAGAAC	TGGTGAAGACGCCAGTGGA
ACTB	CCAACCGCGAGAAGATGA	CCAGAGGCGTACAGGGATAG
MIR22HG	CGGACGCAGTGATTTGCT	GCTTTAGCTGGGTCAGGACA
GABPB1-AS1	AGGGAAAGAAAATATGCCATTTCTA	ATCATTCCGCCGCTTTCT
SLC12A2-DT	GCAATTATCACGGGAAACCTAT	ATCATTCCGCCGCTTTCT
LINC00473	TATGCGCGTCAGCATACTTT	TCTCCCAAAGCACAACGAG
FAM222A-AS1	CAACATGGAAATGGAGACCA	CTTCCGGGATCCCAGTGT
CYTOR	CGTGCCTGTCTTCAGATCTTC	TCATCTCCCAGTTATTCAAGGAG
IDI2-AS1	GTGTTAAACAAGACAACGCTGAA	AAGAGCGCTGGAAAAACCTT
SNHG15	GCAACTCCTTTGCAAGATGC	CTCAAGGAGGGACCTCAGC
OIP5-AS1	GATTTCTGCTCACTGCAGTCTCT	CCTAGCTACTTGGGAGGCTGA
LITATS1	GGGGGAAGTTGTGTAACCTCT	CTCCAAGGGGCTTCGTTC

Table 2. The 10 Short-Lived LncRNAs That Were Investigate in This Study

Name	Accession No.	Length (nt)	$t_{1/2}$*
MIR22HG	NR_028502	2699	2.4
GABPB1-AS1	NR_024490	4139	3.4
SLC12A2-DT	NR_015360	2977	3.5
LINC00473	NR_026861	1123	2.4
FAM222A-AS1	NR_026661	1178	2.4
CYTOR	NR_024204	828	2.4
IDI2-AS1	NR_024628	1107	3.7
SNHG15	NR_003697	837	2.6
OIP5-AS1	NR_026757	5320	3.4
LITATS1	NR_038842	997	3.0

*These values are taken from a previous report[7)]

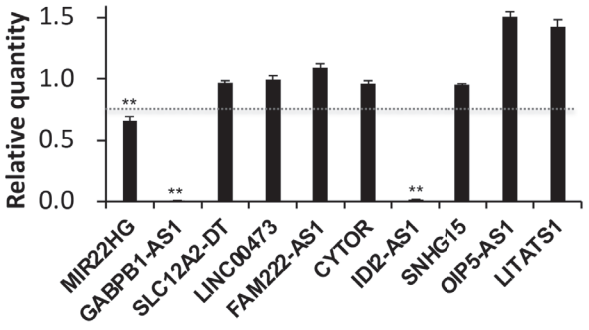

Fig. 1. Alterations in LncRNA Expression Levels in Response to LPS

A549 cells were treated with 1 ug/mL LPS for 24 h. Expression levels of the indicated RNAs were determined by RT-qPCR. GAPDH and ACTB were used for normalization. Values represent the mean ± SD obtained from three independent experiments (**P < 0.01, Student's t test). P values of MIR22HG, GABPB1-AS1, or IDI2-AS1 were 0.0078, 0.0021, or 0.0025, respectively. Y-axis indicated that the expression levels of treated-cells was divided by the those of untreated-cells. Thus, zero indicated the RNA not detectable and one indicated the there was no change in expression levels comparing the untreated-cells. Gray dotted lines indicated cut-off values.

eraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin (ACTB) mRNAs. Relative RNA quantities were calculated as treated values normalized relative to untreated values. For significance testing, the Student's t-test was used. THUNDERBIRD SYBR qPCR mix (Toyobo, Japan) was used in accordance with the manufacturer's instructions. RT-qPCR analysis was performed using a Quantstudio 7 Flex (Thermo Fisher Scientific).

RESULTS AND DISCUSSION

We initially chose ten lncRNAs that have brief half-lives ($t_{1/2} \leq 4$ h) in Hela cells[7)]: (1) they exceed 200 nucleotides in length; (2) they are either highly or moderately expressed with an RPKM greater than 1 according to RNA-Seq data in HeLa cells; (3) they can be found on the NCBI database; (4) they do not intersect with known protein-coding gene regions; and (5) they do not include widely recognized lncRNAs (as shown in Table 2). A549 cells were treated with lipopolysaccharide (LPS; bacterial infection simulation) at a concentration of 1 μg/mL for 24 h, and alterations in the expression levels of the 10 selected lncRNAs were examined. These levels were normalized relative to GAPDH and ACTB mRNAs. Differences in expression could be measured easily for lncRNAs that showed marked and rapid downregulation on exposure to LPS. Therefore, we focused on lncRNAs that were downregulated in response to bacterial infection simulation as potential stress indicators. Significant decreases of 0.6-, 0.01-, and 0.01-fold in the expression levels of MIR22 host gene (MIR22HG), GABPB1 antisense RNA 1 (GABPB1-AS1), and IDI2 antisense RNA 1 (IDI2-AS1) were observed following treatment with 1 μg/mL (Fig. 1).

The degrees of change in expression of the three lncRNAs downregulated following treatment with LPS at various concentrations, i.e., MIR22HG, GABPB1-AS1, and IDI2-AS1, were examined (Fig. 2). The levels of MIR22HG, GABPB1-AS1, and IDI2-AS1 expression decreased with increasing LPS concentrations. The results for MIR22HG showed similar expression levels at all LPS concentrations. On the other hand, the results for GABPB1-AS1 and IDI2-AS1showed a dose-dependent response to LPS concentrations.

In this study, we identified three short-lived lncRNAs (MIR22HG, GABPB1-AS1, and IDI2-AS1) that respond to LPS, as bacterial infection simulation. These lncRNAs have the potential to be surrogate indicators of cell stresses. Recently, several lncRNAs with distinct regulatory roles in responses to cellular stresses have been identified, but our present knowledge of the stress transcriptome is limited. From our past studies, MIR22HG was upregulated in response to chemicals in HeLa cells and human-induced pluripotent stem cells (hiPSCs).[16,17)] GABPB1-AS1 was upregulated in response to chemicals in HepG2, HeLa cells, and hiPCSs.[16–18)] IDI2-AS1 was also upregulated in response to chemicals in HeLa cells. In the context of chemical stress, the upregulation of lncRNA is particularly intriguing, whereas in bacterial infection

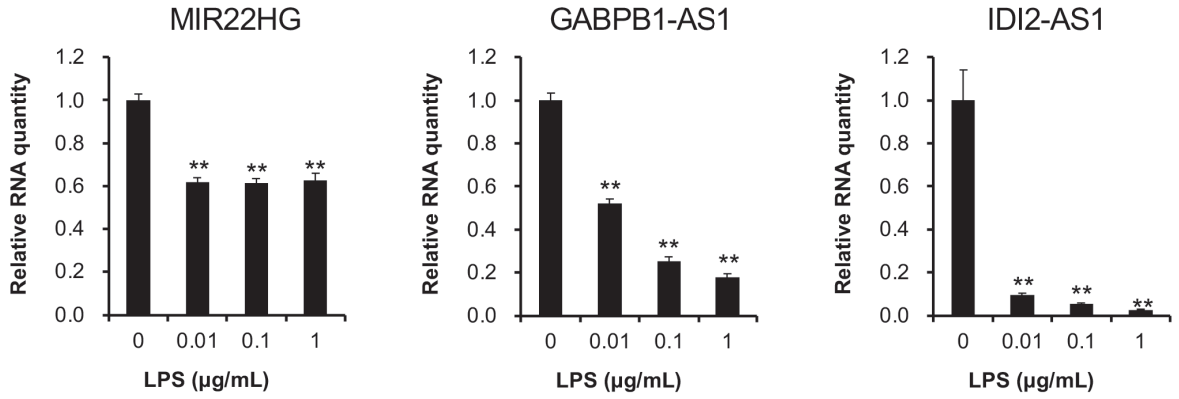

Fig. 2. Alterations in LncRNA Expression Levels in Response to Three Chemicals at Various Doses

A549 cells were treated with LPS for 24 h. Expression levels of the indicated RNAs were determined by RT-qPCR. GAPDH and ACTB were used for normalization. Values represent the mean ± SD obtained from three independent experiments (**P < 0.01, Student's t test). In MIR22HG, P values of 0.01, 0.1, or 1 (LPS conc.) were 0.0089, 0.0085, 0.0092. In GABPB1-AS1, P values of 0.01, 0.1, or 1 (LPS conc.) were 0.0056, 0.0037, 0.0033. In IDI2-AS1, P values of 0.01, 0.1, or 1 (LPS conc.) were 0.0044, 0.0021, 0.0017.

simulation, it is downregulated. Unlike the case of chemical stress in previous studies,[16–18] LPS stimulation induces an immune response in cells. The three lncRNAs (MIR22HG, GABPB1-AS1, and IDI2-AS1) were down-regulated upon LPS stimulation. These data suggest that the expressions of immune response genes might be suppressed by the lncRNAs under normal conditions. The switch in expression leads to the suppression of lncRNA expression, while the expression of immune response genes increases. These observations suggest that these lncRNAs may play a crucial role in the immune response. Moreover, recent studies showed that MIR22HG was identified as a tumor suppressor in several cancers.[19–21] These studies might be relation to our study. However, the functions of the GABPB1-AS1 and IDI2-AS1 remain unknown. We believe that this study will help to bridge the knowledge gap between digital genomic information and cellular function.

Conflict of interest The authors declare no conflict of interest.

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