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# RN7SL1 may be translated under oncogenic conditions

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**RN7SL1 (RNA component of signal recognition particle 7SL1), a component of the signal recognition particle, is a non-coding RNA possessing a small ORF (smORF). However, whether it is translated into peptides is unknown. Here, we generated the RN7SL1-Green Fluorescent Protein (GFP) gene, in which the smORF of RN7SL1 was replaced by GFP, introduced it into 293T cells, and observed cells emitting GFP fluorescence. Furthermore, RNA-seq of GFP-positive cells revealed that they were in an oncogenic state, suggesting that RN7SL1 smORF may be translated under special conditions.**

RN7SL1 | ncRNA | smORF

In the cell, RNA is transcribed by three types of RNA polymerases: 1) RNA polymerase I, which transcribes ribosomal RNA (rRNA) precursors, except 5S rRNA; 2) RNA polymerase II, which transcribes messenger RNA (mRNA); and 3) RNA polymerase III, which transcribes non-coding RNA (ncRNA), such as 5S rRNA, U6 spliceosomal RNA, and transfer RNAs (tRNA) (1–3). Proteins are produced by translation of mRNAs with 5' cap and 3' poly (A) tail transcribed by RNA polymerase II (4). RN7SL1 (RNA component of signal recognition particle 7SL1), which is transcribed by RNA polymerase III, is a part of the signal recognition particle and contributes to the generation of transmembrane and secreted proteins (5, 6).

Some ncRNAs possess small open reading frames (smORFs) that encode small proteins of less than 100 amino acids (7, 8). *LINC01013*, a type of long intergenic ncRNA (lncRNA), contains smORF, and micropeptide translated from it was reported to activate myocardial fibroblasts (9). It was also reported that smORFs are also present in pri-miRNAs, which are precursors of microRNAs (miRNAs), and that smORFs regulate their expression levels in pri-miR-155 and pri-miR-497 (10). Although RN7SL1 theoretically possesses two smORFs encoding micropeptides of 7 and 16 amino acids from the 5' side, their function is unknown. Here, we aimed to investigate under what conditions RN7SL1 is translated and we will explore the function of RN7SL1 under those special conditions.

## Results

Since smORFs theoretically exist in RN7SL1, a plasmid vector expressing RN7SL1-GFP was constructed by replacing the one of them with AcGFP1 (Fig. 1A). The vector is the one used in previous reports and is transcribed at the RNA polymerase III promoter (11). 293T cells were electroporated or lipofected with the plasmid and GFP fluorescence was detected in some cells in both cases (Fig. 1B). GFP-positive cells were also detected in the analysis by FACS (Fig. 1C). These results suggest that cell overcrowding was stressful and created a situation in which GFP was expressed. To examine the expression of micropeptide, immunostaining was performed using micropeptide polyclonal antibody (Fig. 1D). Fluorescence was detected, suggesting the possibility of micropeptide expression. To see under what conditions the percentage of GFP-positive cells increases, we added 2-mercaptoethanol or UV irradiation as a cell stress inducer, both of which increased GFP-positive cells by about 1% (Fig. 1E). Forced expression of micropeptide reduced the percentage of GFP positive cells expressing GFP from the internal ribosome entry site (Fig. 1F). These results suggest that smORF micropeptides are expressed under stress conditions and repress translation. Because there may be hidden cell-side factors that induce expression of micropeptide from smORF in GFP-positive cells, we decided to investigate gene expression in GFP-positive cells. For this purpose, GFP-positive and GFP-negative cells were isolated by FACS. Total RNA was extracted from these cells, and RNA-seq was performed to confirm gene expression under each condition. Volcano plot revealed that the increase in vault RNA1-3 (vtRNA1-3) expression was more pronounced in GFP-positive cells (Fig. 1G). To characterize gene expression in GFP-positive cells, Gene Set Enrichment Analysis (GSEA)

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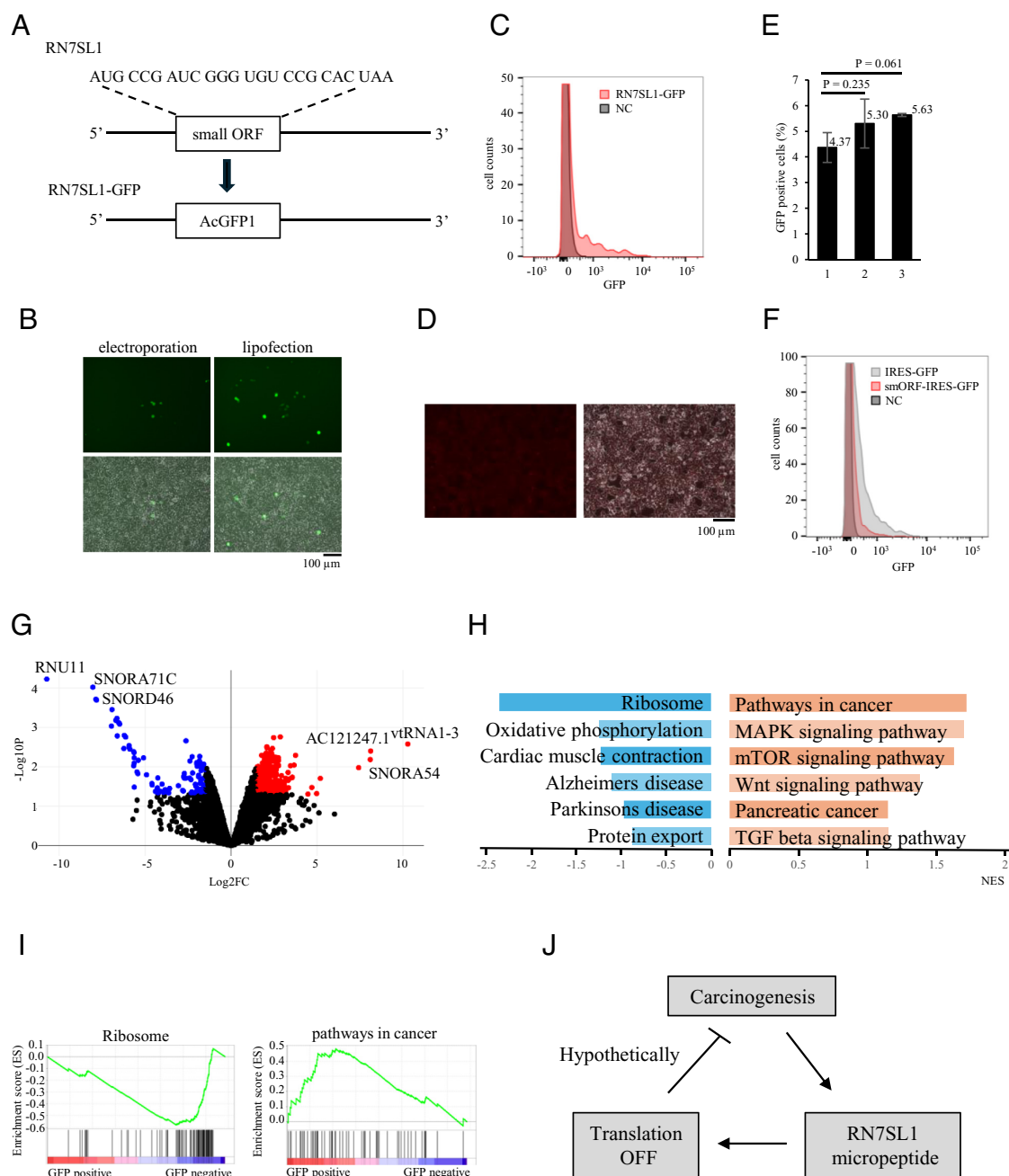
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was performed by using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. Activation of cancer-related pathways such as MAPK, Wnt, and TGF-beta signaling were observed in GFP-positive cells compared to GFP-negative cells (Fig. 1 H and I and Dataset S1). In contrast, only a few signals were inactivated in GFP-positive cells, and inactivation was observed in ribosomes and oxidative phosphorylation (Fig. 1 H and I and Dataset S1). These results suggest that GFP-positive cells are in an oncogenic state and that translation is suppressed in response. This process may be a suppressive control of carcinogenesis by global translational repression effect of RN7SL1 micropeptide (Fig. 1J).

## Discussion

Increased amounts of RNA polymerase III transcripts have been reported in cancer (12). The activation of cancer-related pathways in GFP-positive cells in this study suggests that transcription by RNA polymerase III is involved in the translation of RN7SL1. It was reported that the smORF of MIR22HG RNA, an lncRNA, is translated in an influenza virus infection model (13). The activation of infection-related pathways was observed by GSEA under the conditions of electroporation in this study, suggesting that infection conditions by viruses or bacteria promote the translation of smORFs in lncRNAs. Four types of vtRNAs transcribed



**Fig. 1.** Validation and functional analysis of the translation of smORF in RN7SL1. (A) Design of artificially generated RN7SL1-GFP, in which the smORF of RN7SL1 was replaced by AcGFP1. (B) Fluorescence micrograph of 293T cells transfected with RN7SL1-GFP expression plasmid. (C) FACS analysis of GFP fluorescence. NC is cells without plasmid transfection. (D) Immunostaining with smORF micropeptide polyclonal antibody. RN7SL1 expression plasmid was transfected into 293T cells. (E) Cellular Stress Test Results. 1: RN7SL1-GFP, 2: RN7SL1-GFP and 55µM 2-mercaptoethanol, 3: RN7SL1-GFP and 1 min UV irradiation. (F) Co-expression of smORF micropeptide and GFP (G) Volcano plot of variable genes. (H) GSEA of GFP positive cells compared to GFP negative cells. Both electroporation and lipofection data were used for KEGG pathways analysis. (I) Enrichment plot of Ribosome and pathways in cancer. (J) Conceptual diagram of the function of the RN7SL1 micropeptide.

by RNA polymerase III have been found in humans: vtRNA1-1, 1-2, 1-3, and 2-1. vtRNA1-1 is involved in the regulation of apoptosis and autophagy, and vtRNA2-1 interferes with protein kinase R to apoptosis and cell proliferation, and vtRNA1-2 and vtRNA1-3 have been reported to have unknown functions (14). In the present study, vtRNA1-3 expression was markedly increased only in GFP-positive cells. Therefore, some abnormality in transcription by RNA polymerase III may occur in the carcinogenic state, which may cause the translation of RN7SL1. Since the production of abnormal peptides and proteins has been reported in cancer (15), it is presumed that they may be the cause. RN7SL1 is expressed in various cell lines and tissues, and the relatively high expression level of RN7SL1 in HCC1195 suggests the possibility of micropeptide expression (Datasets S2 and S3). The coding sequence of Ribosomal Protein S29 overlaps with the promoter region of RN7SL1, so genome-wide effects may also be involved in RN7SL1 translation, as several mutations are found in cancer (Dataset S4).

In this study, GFP was expressed with only one type of RNA polymerase III promoter (11). Therefore, we will confirm in the future whether there is a promoter dependence of protein expression from smORF observed in this study. The function of the micropeptide generated from the smORF of RN7SL1 will also be studied in the future.

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## Summary of the Methods

The p7SL-neo-TET (tetrahymena self-splicing intron) plasmid (addgene) was purchased and the artificial gene RN7SL1-GFP was incorporated at the Xba I, Aat II site. A Super Electroporator NEPA21 Type II (NEPA GENE) was used for the electroporation of 293T cells and Lipofectamine 3000 (Thermo Fisher Scientific) was used for the lipofection of 293T cells. Sorting was performed using Becton Dickinson fluorescence-activated cell sorting (BD FACS) AriaIIIu. Total RNA was extracted according to protocol using ISOGEN LS (Nippon Gene). Extracted RNA was submitted to the Genome Analysis Laboratory, Bioinformatics Center, Research Institute for Microbial Diseases, Osaka University, and RNA-seq was performed.

**Data, Materials, and Software Availability.** All study data are included in the article and/or supporting information. RNA-seq data is stored in the GEO database as GSE239474. (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE239474>) (16).

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