Brief Report

Stability of MALAT-1, a nuclear long non-coding RNA in mammalian cells, varies in various cancer cells

Hidenori Tani, Yo Nakamura, Kenichi Ijiri, Nobuyoshi Akimitsu*

Radioisotope Center, The University of Tokyo, Tokyo, Japan.

ABSTRACT: Recent large-scale transcriptome analyses have revealed a large number of transcripts with low protein-coding potential, known as non-coding RNAs (ncRNAs). Many studies revealed that several long ncRNAs are involved in the regulation of genome organization and gene expression, or in the structural components of functional domains in the nucleus. As regulation of mRNA decay in the cytoplasm is crucial for controlling the abundance of cellular transcripts and the levels of protein expression, so regulation of long non-coding RNA decay in the nucleus is considered to be important for biological function. Although enzymatic pathways involved in cytoplasmic mRNA decay have been studied extensively, far less is known about those in nuclear long ncRNA decay. Here, we have investigated decay of metastasis associated lung adenocarcinoma transcript 1 (MALAT-1), which is a long (~ 8 kb) ncRNA that is misregulated in many human cancers and was shown to be retained specifically in the nucleus in nuclear speckles, as a model of nuclear long ncRNA in mammalian cells. We have found that the half-life of MALAT-1 ranges from ~ 9 h to > 12 h in various cancer cells. Moreover, Xrn2, PM/Scl-75, PARN, and Mtr4, known nuclear RNases or RNA helicases, did not affect MALAT-1 degradation or single knockdown of these components did not change the MALAT-1 decay rate.

Keywords: Non-coding RNA, metastasis associated lung adenocarcinoma transcript 1 (MALAT-1), RNA degradation, cancer, nuclear speckle

1. Introduction

Most of the eukaryotic genome is transcribed, yielding a complex network of transcripts that include tens of thousands of long noncoding RNAs (ncRNAs) with little or no protein coding capacity. Recent studies revealed that several long ncRNAs in the nucleus are required for epigenetic silencing of multiple genes *in cis* within large chromosomal domains such as Xist, Air, and Kcnq1ot1 (*1-3*), or are essential structural/organizational components of paraspeckles such as MENε/β (*4-6*). As regulation of mRNA decay in the cytoplasm is important for controlling the abundance of cellular transcripts and the levels of protein expression, so regulation of long ncRNA decay in the nucleus is considered to be important for their biological function. Although the enzymatic pathways involved in cytoplasmic mRNA decay have been studied extensively (*7-9*), far less is known about those in nuclear long ncRNA decay.

Metastasis associated lung adenocarcinoma transcript 1 (MALAT-1), also known as NEAT2, is a long (~ 8 kb) ncRNA (*10*) which was shown to be retained specifically in the nucleus in nuclear speckles (*11*). It has domains that are thought to be involved in the assembly, modification, and/or storage of the pre-mRNA processing machinery. MALAT-1 was originally identified as a transcript showing significant expression in individuals exhibiting high risk for metastasis of non-small cell lung tumors (*10*), and subsequently showed broad expression in normal human and mouse tissues and is overexpressed in many human carcinomas, including those of the breast, pancreas, lung, colon, prostate, and liver (*10-12*). This implies that MALAT-1 misregulation may play a role in the development of numerous cancers. The nascent MALAT-1 transcript is processed to yield two ncRNAs that localize to different subcellular compartments (*13*). Recently, several studies revealed that MALAT-1 is associated with cell invasion (*14,15*).

In this study, we have investigated the decay of MALAT-1 as a model of nuclear long ncRNA in mammalian cells. We have found that the half-life of MALAT-1 ranges from ~ 9 h to > 12 h in various cancer cells. Moreover, we investigated whether nuclear nuclease components such as Xrn2, PM/Scl-75, PARN, and Mtr4 affect the decay rate of MALAT-1.

**Address correspondence to:*
Dr. Nobuyoshi Akimitsu, Radioisotope Center, The University of Tokyo, 2-11-16 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan.
e-mail: akimitsu@ric.u-tokyo.ac.jp

2. Materials and Methods

2.1. *Cell culture*

H1299 (human lung cancer cells), H1975 (human lung cancer cells), and HT1080 (human fibrosarcoma cells) cells were cultured at 37°C in the presence of 5% CO_2 in RPMI-1640 medium (Wako) supplemented with 10% fetal bovine serum and penicillin/streptomycin. A549 (human lung cancer cells) and HeLa Tet-off (TO) (human cervical cells) cells were cultured at 37°C in the presence of 5% CO_2 in Dulbecco's Modified Eagles Medium (D-MEM) supplemented with 10% fetal bovine serum and penicillin/streptomycin.

2.2. *siRNA treatment*

siRNAs were purchased from SIGMA, Gene Pharms, or NIPPON EGT. The sequences of the siRNAs for Xrn2, PM/Scl-75, PARN, and Mtr4 are 5'-AAGAGUACAGA UGAUCAUGUU-3', 5'-AACAUCGAGAGAUUUGUA CUA-3', 5'-CCGCAACAAUAGUUUUACAGC-3', and 5'-GCCUAUGCACUUCAAAUGATT-3', respectively. These siRNAs were transfected into each HeLa TO cell using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. As controls, cells were transfected with control siRNA (SIGMA). Quantitative real-time reverse transcriptase PCR was used to determine whether RNA interference achieved significant depletion of each target sequence.

2.3. *RNA isolation and Northern blot analysis*

Total RNA was isolated according to the method of Chomczynski and Sacchi (*16*) at different times after the addition of 20 μg/mL of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB). Total RNA (4 μg) was resolved using 1% formaldehyde agarose gel electrophoresis under denaturing conditions and was transferred to a nylon membrane. RNA blots were stained with methylene blue to check for equal loading and transfer. Hybridization was performed using the ULTRAhyb (Ambion) with random primed [α-^{32}P]dCTP-labeled cDNA probes corresponding to MALAT-1 and 7SK. Hybridization signals were visualized and quantified with a FLA-9000 (FUJIFILM).

2.4. *Quantitative real-time reverse transcriptase PCR*

Total RNA was reverse transcribed into cDNA using the QuantiTect Reverse Transcription Kit (Qiagen). cDNA was amplified using the following primers: Xrn2 forward (5'-CTTTTCCAGCAGCAAAGGTT-3') and reverse (5'-TGGAAGGCTGCATTCTGG-3'), PM/Scl-75 forward (5'-TCAGGATCTCATTTGGAA CAGA-3') and reverse (5'-CACAGGAAACCTGTCC AAGAA-3'), PARN forward (5'-GAAGGAAAAAG GCCAAGAAATTA-3') and reverse (5'-GGCTGTTC TTCGAGATGCTT-3'), Mtr4 forward (5'-GTTGAAG GGTGTACACATGAGG-3') and reverse (5'-TCCAA CTCGTGGTTTAAGTGG-3'), and GAPDH forward (5'-GCACCGTCAAGGCTGAGAAC-3') and reverse (5'-TGGTGAAGACGCCAGTGGA-3'). GAPDH was used for normalization. The SYBR® Premix Ex Taq™ II (Perfect Real Time) (TaKaRa) was used according to the manufacturer's instructions. Quantitative real-time reverse transcriptase PCR analysis was performed using a Thermal Cycler Dice Real Time System (TaKaRa).

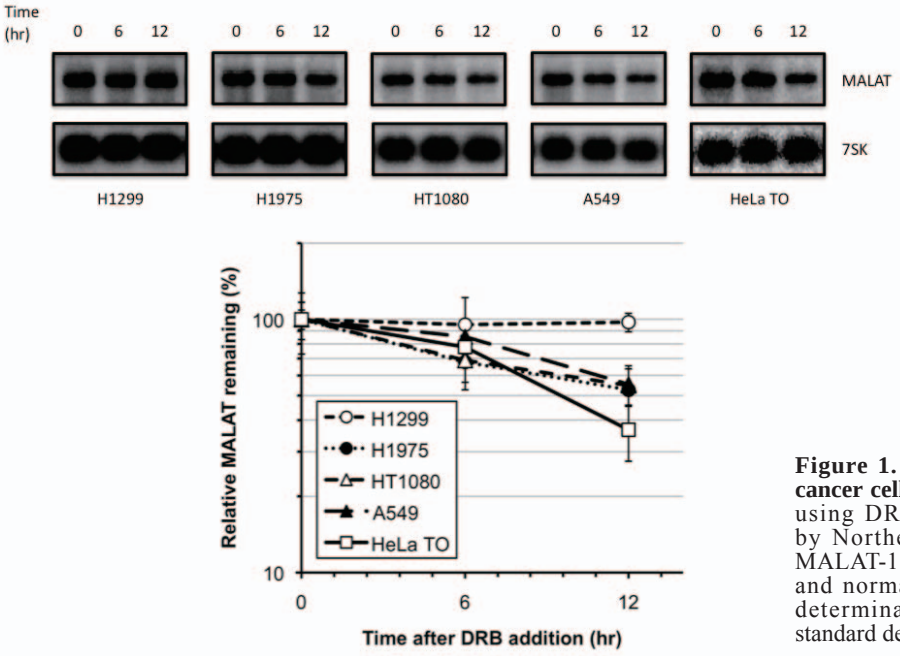

Figure 1. Decay of MALAT-1 in various cancer cells. MALAT-1 stability was assessed using DRB chase experiments, followed by Northern blot analysis. In the graph, MALAT-1 signal intensities were quantified and normalized to 7SK RNA in triplicate determinations. The error bars represent standard deviation.

3. Results and Discussion

3.1. *MALAT-1 stability in various cancer cells*

We first examined the decay of MALAT-1 in various cancer cells (H1299, H1975, A549, HT1080, and HeLa TO cells) by DRB chase experiments using 7SK RNA as a control (Figure 1). Since DRB specifically inhibits transcription elongation by RNA Polymerase II, 7SK RNA, which is transcribed by RNA Polymerase III, is stably transcribed. Half-lives of MALAT-1 in H1299, H1975, A549, HT1080, and HeLa TO cells were >

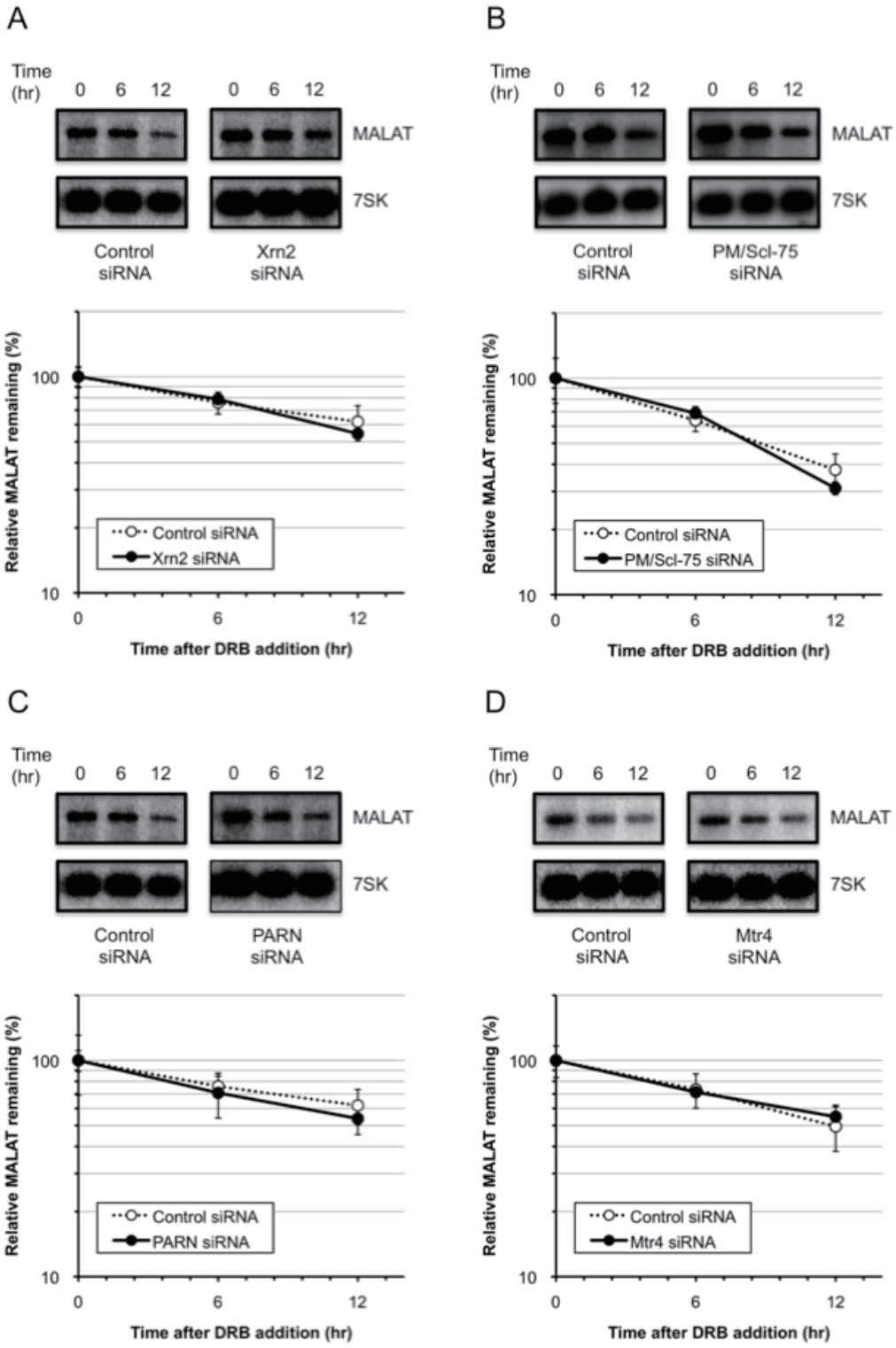

Figure 2. Decay of MALAT-1 after knockdown of several components by siRNA. HeLa TO cells were transfected with siRNA for **(A)** Xrn2, **(B)** Pm/Scl75, **(C)** PARN, or **(D)** Mtr4, and MALAT-1 stability was assessed using DRB chase experiments, followed by Northern blot analysis. In the graphs, MALAT-1 signal intensities were quantified and normalized to 7SK RNA in triplicate determinations. The error bars represent standard deviation.

12, ~ 12, ~ 12, ~ 12, and 9 h, respectively. MALAT-1 stabilities varied in various cancer cells. Interestingly, the half-life of MALAT-1 (~ 9 h) in HeLa TO cells is shorter than the median mRNA half-life (10 h) in human cells as described previously (*17*). This suggests that MALAT-1 may be actively degraded in HeLa TO cells. AU-rich elements (AREs) are present in the 3'-untranslated regions (3'-UTRs) of mRNA encoded in so-called early response genes, such as many proto-oncogenes, growth factors, and cytokines (*18*). AREs are recognized as the common RNA destabilizing element in mammalian cells. Interestingly, we found that MALAT-1 contains 21 AREs, and these are predominantly located in the 5'-untranslated regions (5'-UTRs). AREs encoded in MALAT-1 might be involved in fast degradation.

3.2. *Knockdown of Xrn2, PM/Scl-75, PARN, and Mtr4 in HeLa TO cells*

To investigate the contributing components to degradation of MALAT-1 in HeLa TO cells, we targeted Xrn2, PM/Scl-75/Rrp45, Poly(A)-specific ribonuclease (PARN), and Mtr4. Xrn2 is a nuclear 5'-to-3' exonuclease (*19*). PM/Scl-75 is a core component of the exosome, which is a complex of 3'-to-5' exonucleases (*20*). PARN is a deadenylation nuclease that was found largely in the nuclear fraction (*21*). Mtr4 is a putative RNA helicase, and is part of the recently identified TRAMP complex, which is required for the activation of the nuclear exosome *in vivo* by polyadenylation of target RNAs in yeast (*22*,*23*). Each siRNA for these components was introduced into HeLa TO cells, and the MALAT-1 decay rates in these cells were examined using Northern blot analysis. The siRNA of each target sequence was effective because knockdown of Xrn2, PM/Scl-75, PARN, or Mtr4 reduced each transcript level to 17, 13, 9, and 24%, respectively. However, the depletions of these components did not change the MALAT-1 decay rate (Figure 2). These results suggest that Xrn2, PM/Scl-75, PARN, Mtr4 did not affect MALAT-1 degradation or single knockdown of these components did not change the MALAT-1 decay rate.

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