Oncogenic Ras influences the expression of multiple lncRNAs

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Abstract Recent ultrahigh-density tiling array and large-scale transcriptome analysis have revealed that large numbers of long non-coding RNAs (lncRNAs) are transcribed in mammals. Several lncRNAs have been implicated in transcriptional regulation, organization of nuclear structure, and post-transcriptional processing. However, the regulation of expression of lncRNAs is less well understood. Here, we show that the exogenous and endogenous expression of an oncogenic form of small GTPase Ras (called oncogenic Ras) decrease the expression of lncRNA *ANRIL* (antisense non-coding RNA in the *INK4* locus), which is involved in the regulation of cellular senescence. We also show that

forced expression of oncogenic Ras increases the expression of lncRNA *PANDA* (p21 associated ncRNA DNA damage activated), which is involved in the regulation of apoptosis. Microarray analysis demonstrated that expression of multiple lncRNAs fluctuated by forced expression of oncogenic Ras. These findings indicate that oncogenic Ras regulates the expression of a large number of lncRNAs including functional lncRNAs, such as *ANRIL* and *PANDA*.

Keywords Long non-coding RNA · Oncogenic Ras · *ANRIL* · *PANDA*

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Introduction

Long non-coding RNAs (lncRNAs) range in size from 200 nucleotides to over 10 kb and are spliced and polyadenylated post-transcriptionally. Although mass scale transcriptome analysis has revealed the existence of large numbers of lncRNAs in mammals (Carninci et al. 2005), the function of most lncRNAs remain unclear. Recent studies revealed that several lncRNAs are involved in biological processes including development, cellular senescence, apoptosis, and cancer (Batista and Chang 2013; Fatica and Bozzoni 2014; Kitagawa et al. 2013, 2012). Previously, we and another group have reported the role of the lncRNA *ANRIL* (antisense non-coding RNA in the *INK4* locus) in the regulation of *INK4* locus (Kotake et al. 2011;

Yap et al. 2010), which encodes two cyclin-dependent kinase inhibitors, $p15^{INK4B}$ and $p16^{INK4A}$ and a positive regulator of p53, *ARF*. The *INK4* locus is frequently mutated or its expression is silenced in human cancers (Ruas and Peters 1998; Sharpless 2005). *ANRIL* (3.8 kb transcript) is expressed in the opposite direction from the $p16^{INK4A}$ -*ARF*- $p15^{INK4B}$ gene cluster. *ANRIL* binds to polycomb repression complex 1/2, that stably silence gene expression and is required for the recruitment and repression of $p15^{INK4B}$ and $p16^{INK4A}$ transcription, resulting in the prevention of cellular senescence. *PANDA* (p21 associated ncRNA DNA damage activated; 1.5 kb transcript) is a p21 promoter-derived lncRNA induced by DNA damage in a p53-dependent manner (Hung et al. 2011). *PANDA* directly binds to the transcription factor NF-YA and inhibits its association with promoters of pro-apoptotic genes, such as *FAS*, *NOXA*, and *PUMA*. Depletion of *PANDA* sensitizes cells to apoptosis by DNA damage, suggesting that *PANDA* functions by blocking apoptosis to maintain the survival of cells that have sustained DNA damage.

Recent studies have revealed that several lncRNAs, such as *MALAT1* and *HOTAIR*, are also associated with cancer progression (Gupta et al. 2010; Tano et al. 2010). Although the biological importance of lncRNAs has gradually been demonstrated, the regulation of lncRNAs expression is poorly understood. In this study, we analyzed the expression of lncRNAs induced by oncogenic Ras signaling.

Materials and methods

Cell culture and retroviral transduction

WI38 and TIG-3 cells are normal human diploid fetal lung fibroblasts. WI38 cells were obtained from American Type Culture Collection (Frederick, MD, USA) and TIG-3 cells were obtained from Health Science Research Resource Bank (Osaka, Japan). Human colorectal cancer HCT116 cells were obtained from American Type Culture Collection. HKe3 cells were established from HCT116 cells with a disruption in the oncogenic K-Ras (Shirasawa et al. 1993). All cells were cultured in DMEM (Invitrogen, Carlsbad, CA, USA) containing 10 % fetal bovine serum (GIBCO, Grand Island, NY, USA). WI38 and TIG-3 cells were infected with retroviruses expressing H-Ras^{G12V} (kindly

provided by Dr. CJ. Der, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA). The pMX-puro retrovirus vector was kindly provided by Dr. T Kitamura (The University of Tokyo, Tokyo, Japan). Retrovirus production and transduction were performed as previously described (Kotake et al. 2007).

Western blotting

For western blotting, cells were lysed with RIPA buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 % NP-40, 0.5 % DOC (sodium deoxycholate), 0.1 % SDS, 1 mM Na₃VO₄, 1 mM DTT, 1 mM PMSF) supplemented with protease inhibitors (10 mg/L antipain (PEPTIDE INSTITUTE. INC., Osaka, Japan), 10 mg/L leupeptin (PEPTIDE INSTITUTE. INC.), 10 mg/L pepstatin (PEPTIDE INSTITUTE. INC.), 10 mg/L trypsin inhibitor (Sigma), 10 mg/L E64 (PEPTIDE INSTITUTE. INC.), and 2.5 mg/L chymostatin (PEPTIDE INSTITUTE. INC.)). Western blotting was performed as previously described (Kotake et al. 2007). Antibodies to H-Ras (OP23; Calbiochem-Merck, Darmstadt, Germany) and α -tubulin (Sigma, St. Louis, MO, USA) were purchased commercially. Horseradish peroxidase-conjugated antibodies (Promega, Tokyo, Japan) were used as secondary antibodies and the chemiluminescence system from PerkinElmer (Tokyo, Japan) for detection.

Reverse transcription-polymerase chain reaction (RT-PCR)

For RT-PCR, total RNA was extracted by RNeasy Plus kit (Qiagen, Tokyo, Japan), and 1 μ g of total RNA was used for cDNA synthesis primed with oligo dT primers (Invitrogen). The specific PCR pairs for *PANDA*, *ANRIL*, and *GAPDH* were as follows: *PANDA*, 5'-AGACCCAGTGGCACCTGAC-3' and 5'-GGGCAGAACTTGGCATGATG-3'; *ANRIL*, 5'-TGCTCTATCCGCCAATCAGG-3' and 5'-GGGCCTCAGTGGCACATACC-3'; *GAPDH*, 5'-GCAAATTCATGGCACCGT-3' and 5'-TCGCCCCACTTGATTTTGG-3'.

DNA microarray

DNA microarray analysis was performed using SurePrint G3 Human GE 8x60K (Agilent Technologies, Santa Clara, CA, USA) according to the

manufacturer's protocol by Hokkaido System Science (Sapporo, Japan). Three chips per group were hybridized. Briefly, total RNA was extracted by RNeasy Mini kit (Qiagen), and 200 µg of total RNA was amplified and labeled with cyanine 3 CTP by the Low Input Quick Amp Labeling kit (Agilent Technologies). Labeled cRNA was purified by RNeasy mini spin column (Qiagen) and 600 ng of cyanine 3 labeled cRNA was hybridized to SurePrint G3 Human GE 8x60K (Agilent Technologies). The chips were analyzed by Agilent Technologies Microarray Scanner.

Results and discussion

The small GTPase Ras controls cell proliferation, survival, and differentiation by regulating downstream effector molecules, such as mitogen-activated protein kinases, phosphoinositide 3-kinase, and Ral guanine nucleotide-dissociation stimulator (Karnoub and Weinberg 2008; Schubert et al. 2007). Activating Ras mutations including G12V and G13D amino acid substitution lead to oncogenic transformation and occur in a wide range of human cancers. Previously

and here, we showed that *ANRIL* expression is decreased by forced expression of H-Ras^{G12V} in WI38 cells (Kotake et al. 2011) (Fig. 1a, b). Furthermore, we examined by using HCT116 and HKe3 cells whether endogenous expression of oncogenic Ras affect the expression of *ANRIL*. HCT116 cells have a heterozygous K-Ras mutation (G13D). HKe3 cells were generated by disrupting the K-Ras^{G13D} of HCT116 cells (Shirasawa et al. 1993). RT-PCR analysis demonstrated that *ANRIL* is expressed at a low level in HCT116 cells as compared to HKe3 cells (Fig. 1c). We also examined the effect of oncogenic Ras on the expression of *PANDA*. The stable cell lines expressing H-Ras^{G12V} in TIG-3 cells in which *PANDA* expression was detectable were established, and the expression of *PANDA* was determined. Western blotting results demonstrated the stable expression of H-Ras^{G12V} (Fig. 2a). *PANDA* expression was substantially increased by H-Ras^{G12V} transduction as assessed by RT-PCR analysis (Fig. 2b). These data indicate that oncogenic Ras regulates the expression of two lncRNAs, *ANRIL* and *PANDA*, which have a pivotal role in cell fate determination, such as cellular senescence and apoptosis.

Oncogenic Ras might also affect other lncRNAs. Therefore, we next examined the effect of oncogenic Ras on the expression of lncRNAs by global analysis. DNA microarray analysis showed that 3,373 of the protein coding genes (PCG) had at least a twofold change by H-Ras^{G12V} transduction (Fig. 3a; Table 1). These data support a previous study demonstrating oncogenic Ras regulates diverse pathways (Karnoub

Fig. 1 Effect of oncogenic Ras on *ANRIL* expression. WI38 cells were infected with control (Mock) or H-Ras^{G12V}-expressing retroviruses and selected by puromycin. H-Ras protein levels were determined by western blotting (a). The levels of *ANRIL* expression were determined in WI38/Mock and WI38/H-Ras^{G12V} cells by RT-PCR (b). The levels of *ANRIL* expression were determined in HKe3 and HCT116 cells by RT-PCR (c)

Fig. 2 Effect of oncogenic Ras on *PANDA* expression. TIG-3 cells were infected with control (Mock) or H-Ras^{G12V}-expressing retroviruses and selected by puromycin. H-Ras protein levels were determined by western blotting (a). The levels of *PANDA* expression were determined in TIG-3/Mock and TIG-3/H-Ras^{G12V} cells by RT-PCR (b)

Fig. 3 Change of protein coding genes (PCG) and lncRNAs expression by oncogenic Ras. Microarray data of PCG (**a**) and lncRNAs (**b**). The mean value was calculated from triplicate samples of a representative experiment. The results are expressed relative to the corresponding values for control (WI38/Mock) cells. The vertical axis of the graph indicates fold-

change against control cells (Log2). The horizontal axis of the graph indicates the sample (*left*: control cells, *right*: WI38/H-Ras^{G12V}). The graph shows data where fold-change in gene expression was greater than twofold. A *p* value of <0.05 was determined as significant

Table 1 Summary of microarray results for PCG and lncRNAs

	Total entity	Twofold increase entity	Twofold increase (%)	Twofold decrease entity	Twofold decrease (%)
PCG	26,608	1,513	5.7	1,860	7.0
lncRNA	7,419	243	3.3	168	2.3

and Weinberg 2008). Additionally, of the 7,419 transcribed regions of lncRNAs, the expression level of 243 (3.3 %) increased more than twofold and the expression level of 168 (2.3 %) decreased by <50 % following H-Ras^{G12V} transduction (Fig. 3b; Table 1 and Supplemental Table 1). These data indicate that oncogenic Ras regulates the expression of multiple lncRNAs as well as PCG.

In this paper, we showed that both exogenous and endogenous expression of oncogenic Ras decreased the expression of *ANRIL*, which represses the transcription of *INK4* locus. In mouse and human fibroblasts, the transcription of *INK4* locus is activated by oncogenic Ras, causing stable cell cycle arrest to protect cells from hyperproliferative stimulation (Brookes et al. 2002; Serrano et al. 1997). The reduction of *ANRIL* by oncogenic Ras might be required for the activation of *INK4* locus by oncogenic Ras, resulting in the induction of premature senescence. We also found that enforced expression of oncogenic Ras increased the expression of *PANDA*, which represses apoptosis. Mutational activation of *ras* can protect some cell strains from apoptosis

(Downward 1998). The induction of *PANDA* by oncogenic Ras might be required for the repression of apoptosis, resulting in the progression of carcinogenesis. Indeed, *PANDA* is selectively increased in metastatic ductal carcinomas but not in normal breast tissue (Hung et al. 2011). It will be an important issue to reveal the role of *PANDA* in carcinogenesis. However, the molecular mechanism by which oncogenic Ras regulates *ANRIL* and *PANDA* expression is unclear. Recent studies showed that E2F1 transcription factor directly binds to and activates *ANRIL* transcription (Sato et al. 2010; Wan et al. 2013). It was also revealed that several lncRNAs including *PANDA* are induced in a p53-dependent manner (Huarte et al. 2010; Hung et al. 2011). Thus, it will be important to identify the downstream effector molecules of oncogenic Ras, which regulate the expression of lncRNAs.

Although approximately 10,000 primate-specific lncRNAs have been identified (Necsulea et al. 2014), the biological functions of most lncRNAs remain unclear. This study demonstrated that the expression of many lncRNAs was altered by oncogenic Ras. Among these lncRNAs regulated by oncogenic Ras,

uncharacterized functional lncRNAs associated with malignant transformation may be present.

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References

- Batista PJ, Chang HY (2013) Long noncoding RNAs: cellular address codes in development and disease. *Cell* 152:1298–1307. doi:[10.1016/j.cell.2013.02.012](https://doi.org/10.1016/j.cell.2013.02.012)
- Brookes S, Rowe J, Ruas M, Llanos S, Clark PA, Lomax M, James MC, Vatcheva R, Bates S, Vousden KH, Parry D, Gruis N, Smit N, Bergman W, Peters G (2002) INK4a-deficient human diploid fibroblasts are resistant to RAS-induced senescence. *EMBO J* 21:2936–2945. doi:[10.1093/emboj/cdf289](https://doi.org/10.1093/emboj/cdf289)
- Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, Oyama R, Ravasi T, Lenhard B, Wells C, Kodzius R, Shimokawa K, Bajic VB, Brenner SE, Batalov S, Forrest AR, Zavolan M, Davis MJ, Wilming LG, Aidinis V, Allen JE, Ambesi-Impiomato A, Apweiler R, Aturaliya RN, Bailey TL, Bansal M, Baxter L, Beisel KW, Bersano T, Bono H, Chalk AM, Chiu KP, Choudhary V, Christoffels A, Clutterbuck DR, Crowe ML, Dalla E, Dalrymple BP, de Bono B, Della Gatta G, di Bernardo D, Down T, Engstrom P, Fagioli M, Faulkner G, Fletcher CF, Fukushima T, Furuno M, Futaki S, Gariboldi M, Georgii-Hemming P, Gingeras TR, Gojobori T, Green RE, Gustincich S, Harbers M, Hayashi Y, Hensch TK, Hirokawa N, Hill D, Huminicki L, Iacono M, Ikeo K, Iwama A, Ishikawa T, Jakt M, Kanapin A, Katoh M, Kawasawa Y, Kelso J, Kitamura H, Kitano H, Kollias G, Krishnan SP, Kruger A, Kummerfeld SK, Kurochkin IV, Lareau LF, Lazarevic D, Lipovich L, Liu J, Liuni S, McWilliam S, Madan Babu M, Madera M, Marchionni L, Matsuda H, Matsuzawa S, Miki H, Mignone F, Miyake S, Morris K, Mottagui-Tabar S, Mulder N, Nakano N, Nakauchi H, Ng P, Nilsson R, Nishiguchi S, Nishikawa S, Nori F, Ohara O, Okazaki Y, Orlando V, Pang KC, Pavan WJ, Pavesi G, Pesole G, Petrovsky N, Piazza S, Reed J, Reid JF, Ring BZ, Ringwald M, Rost B, Ruan Y, Salzberg SL, Sandelin A, Schneider C, Schönbach C, Sekiguchi K, Semple CA, Seno S, Sessa L, Sheng Y, Shibata Y, Shimada H, Shimada K, Silva D, Sinclair B, Sperling S, Stupka E, Sugiura K, Sultana R, Takenaka Y, Taki K, Tammoja K, Tan SL, Tang S, Taylor MS, Tegner J, Teichmann SA, Ueda HR, van Nimwegen E, Verardo R, Wei CL, Yagi K, Yamanishi H, Zabarovsky E, Zhu S, Zimmer A, Hide W, Bult C, Grimmond SM, Teasdale RD, Liu ET, Brusci V, Quackenbush J, Wahlestedt C, Mattick JS, Hume DA, Kai C, Sasaki D, Tomaru Y, Fukuda S, Kanamori-Katayama M, Suzuki M, Aoki J, Arakawa T, Iida J, Imamura K, Itoh M, Kato T, Kawaji H, Kawagashira N, Kawashima T, Kojima M, Kondo S, Konno H, Nakano K, Ninomiya N, Nishio T, Okada M, Plessy C, Shibata K, Shiraki T, Suzuki S, Tagami M, Waki K, Watahiki A, Okamura-Oho Y, Suzuki H, Kawai J, Hayashizaki Y, FANTOM Consortium, RIKEN Genome Exploration Research Group and Genome Science Group (Genome Network Project Core Group) (2005) The transcriptional landscape of the mammalian genome. *Science* 309:1559–1563. doi:[10.1126/science.1112014](https://doi.org/10.1126/science.1112014)
- Downward J (1998) Ras signalling and apoptosis. *Curr Opin Genet Dev* 8:49–54
- Fatica A, Bozzoni I (2014) Long non-coding RNAs: new players in cell differentiation and development. *Nat Rev Genet* 15:7–21. doi:[10.1038/nrg3606](https://doi.org/10.1038/nrg3606)
- Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY (2010) Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature* 464:1071–1076. doi:[10.1038/nature08975](https://doi.org/10.1038/nature08975)
- Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, Kenzelmann-Broz D, Khalil AM, Zuk O, Amit I, Rabani M, Attardi LD, Regev A, Lander ES, Jacks T, Rinn JL (2010) A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. *Cell* 142:409–419. doi:[10.1016/j.cell.2010.06.040](https://doi.org/10.1016/j.cell.2010.06.040)
- Hung T, Wang Y, Lin MF, Koegel AK, Kotake Y, Grant GD, Horlings HM, Shah N, Umbrecht C, Wang P, Wang Y, Kong B, Langerød A, Børresen-Dale AL, Kim SK, van de Vijver M, Sukumar S, Whitfield ML, Kellis M, Xiong Y, Wong DJ, Chang HY (2011) Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. *Nat Genet* 43:621–629. doi:[10.1038/ng.848](https://doi.org/10.1038/ng.848)
- Karnoub AE, Weinberg RA (2008) Ras oncogenes: split personalities. *Nat Rev Mol Cell Biol* 9:517–531. doi:[10.1038/nrm2438](https://doi.org/10.1038/nrm2438)
- Kitagawa M, Kotake Y, Ohhata T (2012) Long non-coding RNAs involved in cancer development and cell fate determination. *Curr Drug Targets* 13:1616–1621
- Kitagawa M, Kitagawa K, Kotake Y, Niida H, Ohhata T (2013) Cell cycle regulation by long non-coding RNAs. *Cell Mol Life Sci* 70:4785–4794. doi:[10.1007/s00018-013-1423-0](https://doi.org/10.1007/s00018-013-1423-0)
- Kotake Y, Cao R, Viatour P, Sage J, Zhang Y, Xiong Y (2007) pRB family proteins are required for H3K27 trimethylation and Polycomb repression complexes binding to and silencing p16INK4a tumor suppressor gene. *Genes Dev* 21:49–54. doi:[10.1101/gad.1499407](https://doi.org/10.1101/gad.1499407)
- Kotake Y, Nakagawa T, Kitagawa K, Suzuki S, Liu N, Kitagawa M, Xiong Y (2011) Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15(INK4B) tumor suppressor gene. *Oncogene* 30:1956–1962. doi:[10.1038/onc.2010.568](https://doi.org/10.1038/onc.2010.568)
- Necsulea A, Soumillon M, Warnefors M, Liechti A, Daish T, Zeller U, Baker JC, Grützner F, Kaessmann H (2014) The evolution of lncRNA repertoires and expression patterns in tetrapods. *Nature* 505:635–640. doi:[10.1038/nature12943](https://doi.org/10.1038/nature12943)
- Ruas M, Peters G (1998) The p16INK4a/CDKN2A tumor suppressor and its relatives. *Biochim Biophys Acta* 1378:F115–F177
- Sato K, Nakagawa H, Tajima A, Yoshida K, Inoue I (2010) ANRIL is implicated in the regulation of nucleus and

- potential transcriptional target of E2F1. *Oncol Rep* 24:701–707
- Schubbert S, Shannon K, Bollag G (2007) Hyperactive Ras in developmental disorders and cancer. *Nat Rev Cancer* 7:295–308. doi:[10.1038/nrc2109](https://doi.org/10.1038/nrc2109)
- Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 88:593–602
- Sharpless NE (2005) INK4a/ARF: a multifunctional tumor suppressor locus. *Mutat Res* 576:22–38. doi:[10.1016/j.mrfmmm.2004.08.021](https://doi.org/10.1016/j.mrfmmm.2004.08.021)
- Shirasawa S, Furuse M, Yokoyama N, Sasazuki T (1993) Altered growth of human colon cancer cell lines disrupted at activated Ki-ras. *Science* 260:85–88
- Tano K, Mizuno R, Okada T, Rakwal R, Shibato J, Masuo Y, Ijiri K, Akimitsu N (2010) MALAT-1 enhances cell motility of lung adenocarcinoma cells by influencing the expression of motility-related genes. *FEBS Lett* 584:4575–4580. doi:[10.1016/j.febslet.2010.10.008](https://doi.org/10.1016/j.febslet.2010.10.008)
- Wan G, Mathur R, Hu X, Liu Y, Zhang X, Peng G, Lu X (2013) Long non-coding RNA ANRIL (CDKN2B-AS) is induced by the ATM-E2F1 signaling pathway. *Cell Signal* 25:1086–1095. doi:[10.1016/j.cellsig.2013.02.006](https://doi.org/10.1016/j.cellsig.2013.02.006)
- Yap KL, Li S, Muñoz-Cabello AM, Raguz S, Zeng L, Mujtaba S, Gil J, Walsh MJ, Zhou MM (2010) Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. *Mol Cell* 38:662–674. doi:[10.1016/j.molcel.2010.03.021](https://doi.org/10.1016/j.molcel.2010.03.021)