Ubiquitous Expression of MAKORIN-2 in Normal and Malignant Hematopoietic Cells and Its Growth Promoting Activity

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Abstract

Makorin-2 (MKRN2) is a highly conserved protein and yet its functions are largely unknown. We investigated the expression levels of MKRN2 and RAF1 in normal and malignant hematopoietic cells, and leukemia cell lines. We also attempted to delineate the role of MKRN2 in umbilical cord blood CD34⁺ stem/progenitor cells and K562 cell line by over-expression and inhibition of MKRN2 through lentivirus transduction and shRNA nucleofection, respectively. Our results provided the first evidence on the ubiquitous expression of MKRN2 in normal hematopoietic cells, embryonic stem cell lines, primary leukemia and leukemic cell lines of myeloid, lymphoid, erythroid and megakaryocytic lineages. The expression levels of MKRN2 were generally higher in primary leukemia samples compared with those in age-matched normal BM cells. In all leukemia subtypes, there was no significant correlation between expression levels of MKRN2 and RAF1. sh-MKRN2-silenced CD34⁺ cells had a significantly lower proliferation capacity and decreased levels of the early stem/progenitor subpopulation (CFU-GEMM) compared with control cultures. Over-expression of MKRN2 in K562 cells increased cell proliferation. Our results indicated possible roles of MKRN2 in normal and malignant hematopoiesis.

Introduction

Makorin-2 (HSPC070; MKRN2) belongs to the MKRN gene family of which the ribonucleoproteins are characterized by a variety of zinc-finger motifs [1,2]. MKRN2 holds four C3H zinc fingers and a signature C3HC4 RING zinc finger domain. MKRN2 is a highly conserved gene [1] yet its function remains largely unknown. Previous studies reported that mkrn2 in Xenopus laevis acted upstream of glucogen synthease kinase-3β in the phosphatidylinositol 3-kinase/Akt pathway. The third C3H zinc finger and the RING motif are required for the anti-neurogenesis activity [3,4]. MKRN2 was first identified in human CD34⁺ stem/progenitor cells, as well as in some leukemic cell lines [2,5,6]. In chromosome 3p25, MKRN2 is located next to the proto-oncogene RAF1. Interestingly, they share a sequence of 105 bp in the 3' UTR in a reversed transcription orientation [2]. This antisense sequence-overlapping of MKRN2 with RAF1 suggested that these two genes may regulate each other and be involved in normal hematopoietic and leukemic development. In this study, we investigated the expression levels of MKRN2 and RAF1 in normal and malignant hematopoietic cells, and leukemia cell lines. We also attempted to explore the role of MKRN2 in umbilical cord blood CD34⁺ stem/progenitor cells and K562 cell line by over-expression and inhibition of MKRN2 through lentivirus transduction and shRNA nucleofection, respectively. Our results demonstrated ubiquitous mRNA expression of MKRN2 and RAF1 in normal hematopoietic cells, embryonic stem cell lines, primary leukemia and leukemic cell lines. We also showed MKRN2 functions on promoting cell proliferation of primary CD34⁺ progenitor cells and K562 cells, indicating its possible involvement in normal and malignant hematopoiesis.

Materials and Methods

Ethics statement

Written informed consents were obtained for collection of all human samples. For minors/children enrolled in the study, written consents were obtained from their parents on their behalf. This study was approved by the Ethics Committee for Clinical Research of The Chinese University of Hong Kong. All necessary permits were obtained for the described study, which complied with all relevant regulations.

Patients and samples

Primary leukemic cells (over 70% blast cells) were obtained from the bone marrow of children (age ≤19 years) who were newly diagnosed with chronic myeloid leukemia (CML), acute lymphoid (ALL) or acute myeloid (AML) leukemia at the Prince of Wales
Hospital, The Chinese University of Hong Kong. Age-matched normal subjects were siblings of patients who donated bone marrow for transplantation.

Peripheral blood samples were collected from normal adult volunteers. Mononuclear cells (MNC) were enriched by Ficoll-Hypaque density gradients (Amersham, Piscataway, NJ, USA). Human umbilical cord blood (CB) MNC and enriched CD34+ cells were obtained from full-term deliveries as described previously [7].

Human leukemic cell lines and culture condition

Leukemic cell lines of B-cell lymphoid (RS411, 697, REH, Raji, IM9), T-cell lymphoid (HSB2, CEM119, Jurkat, Molt 3, SupT1), myeloid (KG1a, Kasumi-1, HL60, K562), natural killer (NK-92) lineages, and myeloma NCI-H929 line were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). These cell lines were cultured in Iscove modified Dulbecco medium (IMDM; Invitrogen, Carlsbad, CA, USA) or RPMI 1640 medium (Invitrogen) containing 10% fetal bovine serum (FBS; Invitrogen) [20% for Kasumi-1 cells], 1 x Penicillin-Streptomycin (Invitrogen) according to the manufacturer’s instruction. Megakaryoblastic cell lines (MEG01, MO7e, CHRF288) were obtained and maintained as previous described [8]. The human embryonic stem cell (ESC) lines H9 (P48-53) and H14 (P44-68) were products of WiCell (Madison, WI, USA) and maintained as previously described [9].

Over-expression of MKRN2 in CD34+ and K562 cells by lentivirus transduction

Full length morakin cDNA was subcloned into lentiviral vector (pLEF1xIG-MKRN2) (Fig A in File S1). The empty vector (pLEF1xEG) was used as a control. The VSV-G pseudotyped lentivirus was produced by cotransfecting 293T cells with the transfer vector and three packaging vectors [10]. CD34+ or K562 cells were infected by the lentivirus at the multiplicity of infection (MOI) of 30. K562 cells were selected as the study model because K562 blasts are multipotential, hematopoietic malignant cells that could spontaneously differentiate into recognizable progenitors of the erythroid, granulocytic and monocytic lineages. In addition, we observed that MKRN2 and RAF1 were consistently and highly expressed in K562 and in primary myelocytic leukemia cells. Cells were first transduced for 16 hr, followed by 12 hr recovery in IMDM containing 10% FBS (Invitrogen) and 10% fetal bovine serum (FBS; Invitrogen) (20% for Kasumi-1 cells). 1 x Penicillin-Streptomycin (Invitrogen) according to the manufacturer’s instruction. Megakaryoblastic cell lines (MEG01, MO7e, CHRF288) were obtained and maintained as previous described [8]. The human embryonic stem cell (ESC) lines H9 (P48-53) and H14 (P44-68) were products of WiCell (Madison, WI, USA) and maintained as previously described [9].

Silencing of MKRN2 by nucleofection using shRNA

A set of 29 mer shRNA constructs targeting MKRN2 (pGFP-V-RRS-MKRN2, 4 unique sh cassettes in retroviral GFP vector) was introduced to inhibit the expression of MKRN2 in primary CD34+ cells and K562 cells. pGFP-V-RRS (retroviral GFP vector) and pGFP-V-RRS-NE (non-effective 29-mer sh GFP cassette retroviral GFP vector) were used as control experiments. All Hush constructs were purchased from OriGene Technologies (Rockville, MD, USA). Briefly, 200 ng of each of the shRNA plasmids were used for nucleofection. Enriched CB CD34+ cells (2 x 10^7/mL) and K562 cells (1 x 10^7/mL) were transfected using the Human CD34+ Cell Nucleofection Kit and K562 Nucleofection Kit (Amaxa Biosystems, Koeln, Germany), respectively. After nucleofection, cells were allowed to grow for 48 hr prior to measurement of readout parameters. The stable suppression of MKRN2 in K562 cells was maintained using Puromycin treatment (1 μg/mL; Invitrogen).

Cell viability

Transduced cells from each treatment were plated in duplicate wells (12-well plates, Corning) with the appropriate culture conditions (starting at 2 x 10^4 cells/mL and split at a ratio of 1:3 on day 6). Cells were counted daily by a hemacytometer under light-microscope, with trypan-blue staining (0.4%; Bio-Rad, Hercules, CA, USA) to exclude dead cells.

Ex vivo expansion of transfected CD34+ cells

Enriched CD34+ cells at 2 x 10^5/mL were expanded in IMDM containing 10% FBS (StemCell Technology, Vancouver, Canada), 0.1% BSA, thrombopoietin (TPO; 50 ng/mL), stem cell factor (SCF; 50 ng/mL) and Flt-3 ligand (FL; 80 ng/mL). All cytokines were products of Peprotech (Rocky Hill, NJ, USA). After 8 days, multilineage stem/progenitor cells in the expansion culture were quantified by further culture for 14 days in cytokine-enriched methylcellulose medium (StemCell Technology). The number of colony forming units (CFU) of the erythroid (BFU-E, CFU-E), myeloid (CFU-GM) and early CFU (CFU-GEMM) lineages was counted under a microscope.

MTT assay

Effects of over-expression and inhibition of MKRN2 on proliferation of K562 cells were assessed by the methabenzthiazuron (MTT) method. Cells (5 x 10^4 per well) were seeded in duplicates onto a 24-well plate (Corning, NY, USA) and incubated with 100 μL MTT (5 mg/mL; Invitrogen) for 30 min at 37°C. The insoluble violet formazan crystals and cells were collected by centrifugation at 18,300 x g for 10 min and dissolved in 100 μL dimethylsulphoxide (DMSO, Invitrogen). Absorbance was read at 570 nm. Duplicate measurements were determined in 3 independent experiments and expressed as percentage of the control.

Reverse transcription and qPCR

Total RNA was extracted from cell cultures (1 x 10^7/samples), peripheral blood MNC or bone marrow samples using Trizol reagent (Invitrogen). cDNA was synthesized from 1 μg of total RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). qPCR analysis was carried out using human specific Taqman Gene Expression Assays (Applied Biosystems). These primer and probe sets (MKRN2, Hs00274055_m1 and RAF1, Hs0024119_m1) have been recommended for specific gene expression experiments because they detect the maximum number of transcripts for target genes. Results were expressed as relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Statistical analysis

The significance of growth or inhibitory effects exerted by over-expression or shRNA suppression of MKRN2 in CD34+ cells and K562 cell line were determined by the paired-samples t test. The differences in MKRN2 and RAF1 mRNA expression levels between normal and malignant hematopoietic cells, and leukemic cell lines were determined by independent samples t test. Correlations of MKRN2 and RAF-1 in primary leukemic samples and normal bone marrow samples were analyzed by the Pearson Correlation test. All analyses were performed using SPSS for Windows 17 software (SPSS, Chicago, II, USA). A P value of
0.05 was considered significant. Results are expressed as mean ± standard error of the mean (SEM).

**Results**

mRNA expression of MKRN2 in normal and malignant hematopoietic cells

By qPCR analysis, we observed ubiquitous expressions of MKRN2 and RAF1 in primary hematopoietic cells including adult MNC, neutrophils, cord blood MNC, enriched CD34+ cells, and human embryonic stem cell lines H9 and H14 (n = 2–6) (Fig 1). The expression levels are represented as relative to GAPDH (Y-axis, Fig 1–3). We also demonstrated expressions of MKRN2 and RAF1 in leukemia cell lines of B-ALL, T-ALL, AML, CML, NK and MK lineages (n = 2–3) (Fig 2). In primary leukemia samples obtained from BM of patients, we showed positive expressions of MKRN2 and RAF1 in B-ALL Philadelphia chromosome (BCR/ABL or Ph) positive and negative, T-ALL, AML and CML samples (n = 5–22) (Fig 3). The expression levels of MKRN2 were generally higher in leukemia samples (P<0.05 in Ph−B-ALL, Ph+B-ALL, T-ALL and AML samples) compared with those in age-matched normal BM cells (n = 9), whilst RAF1 was higher in Ph−B-ALL and

![Figure 1. Expression of MKRN2 and RAF1 in primary hematopoietic cells and embryonic stem cell lines.](image1)

![Figure 2. Expression of MKRN2 and RAF1 in leukemic cell lines.](image2)
AML samples ($P<0.05$). In all leukemia subtypes, there was no significant correlation between expression levels of MKRN2 and RAF1 (Fig B in File S1). In CML samples, there was no notable difference between $BCR/ABL$ major ($n = 8$) and $BCR/ABL$ minor samples ($n = 3$) in terms of MKRN2 or RAF1 mRNA expression (Fig C in File S1).

Over-expression of MKRN2 in cord blood CD34$^+$ cells

Lentiviral transduction of MKRN2 resulted in $1.14 \pm 0.09$ fold change of MKRN2 mRNA and $0.93 \pm 0.27$ fold change of RAF1 in total CD34$^+$ cells, relative to their respective levels in control cells containing the empty vector ($n = 3$). There were trends of increased cell expansion (Fig 4) and the number of multilineage stem/progenitor cells (CFU-GEMM) were also decreased in the pGFP-V-RS-MKRN2 nucleofected cell expansion culture (Fig 7).

ShRNA-silencing of MKRN2 in cord blood CD34$^+$ cells

ShRNA inhibition of MKRN2 resulted in reduction of MKRN2 expression ($0.66 \pm 0.12$ fold vs. pGFP-V-RS; and $0.79 \pm 0.07$ fold vs. pGFP-V-RS-NE) [Fig 6]. RAF1 expression in MKRN2-silenced cells was $1.28 \pm 0.31$ fold compared with that in control pGFP-V-RS cells. CD34$^+$ cells expressing pGFP-V-RS-MKRN2 had significantly lower proliferation capacity as shown in day 7 culture ($P = 0.005$) and a trend of reduced expansion at day 2 and day 8 cultures ($n = 3$), when compared with either non-effective sh cassette (pGFP-V-RS-NE) or empty vector (pGFP-V-RS) control cultures. The early stem/progenitor cells (CFU-GEMM) were also decreased in the pGFP-V-RS-MKRN2 nucleofected cell expansion culture (Fig 7).

Over-expression of MKRN2 in K562 cells

Lentiviral transduction of MKRN2 in K562 cells resulted in $1.54 \pm 0.21$ fold change of MKRN2 mRNA expression compared with pLEF1α-IG control cells ($n = 3$). RAF1 expression in MKRN2-tranduced cells was $1.06 \pm 0.03$ fold of control cells. By flow cytometric analysis, $83 \pm 15.3\%$ (range $52–98\%$) of pLEF1α-IG and $90 \pm 4.79\%$ ($80.1–95.2\%$) of pLEF1α-IG-MKRN2 transduced K562 cells expressed GFP ($n = 3$) (Fig D in File S1). MTT assay of pLEF1α-IG-MKRN2 transduced cells showed a significantly increased proliferation in culture, compared with that of the pLEF1-IG control cells ($P = 0.05$) (Fig 8).

ShRNA-silencing of MKRN2 in K562 cells

At 2 days post-nucleofection, Sh-MKRN2-silenced K562 had $0.86 \pm 0.02$ fold change of MKRN2 expression and $1.39 \pm 0.27$ fold change of RAF1 expression ($n = 3$). GFP positive cells ranged 62.2–85.3% of the total cell population. However, shRNA silencing of MKRN2 did not reduce cell proliferation of K562 cells in culture (MTT assay).

Discussion

Our data provided the first evidence on the ubiquitous expression of MKRN2 in multi-lineage normal hematopoietic and leukemic cells, as well as its function on promoting CD34$^+$ and K562 cell proliferation. In spite of the known conservation of the MKRN2 gene through evolution, little has been reported on its role in any organism other than the anti-neurogenic activity in Xenopus laevis [3,4]. Using shRNA silencing, we demonstrated the activity of MKRN2 on promotion of CD34$^+$ cell expansion to early progenitor cells, indicating its role on normal hematopoiesis. However, over-expression of MKRN2 in CD34$^+$ cells did not
significantly affect cell expansion and lineage development, possibly because endogenous levels of MKRN2 protein were sufficient for its cell promoting functions. Upregulated expressions of MKRN2 in primary leukemia cells prompted us to further investigate the effects of forced expression and silencing of MKRN2 in the leukemic cell line K562. Again, we observed the stimulating activity of over-expressing MKRN2 on K562 proliferation. In contrast to CD34+ cells, sh-silencing of MKRN2 in K562 did not affect cell proliferation, indicating possible differences between the regulatory mechanism of MKRN2 in CD34+ cells and leukemic cell line K562. Further evaluation of MKRN2 gene manipulation on cell cycle regulation might reveal its specific mechanism on hematopoietic cell proliferation.

Due to the common sequence between MKRN2 and RAF1 in the antisense orientation, we suspected existence of a mutual regulatory mechanism between the two genes [11,12]. RAF1, a protein closely associated with the RAP1, RAS, ERK and AKT pathways, plays multiple roles in hematopoietic cells [13]. It is required for growth factor-induced proliferation of normal hematopoietic and leukemic cells [14]. RAF1 is also implicated in drug resistance of BCR/ABL expressing leukemic cells [15]. In normal and leukemic cells, however, we only observed ubiquitous expressions of MKRN2 and RAF1. They did not exhibit any convincingly significant correlation in their expression patterns. It is anticipated that a larger sample size of each leukemia subtype would be required to accurately address the relationship between MKRN2 and RAF1, as well as between specific translocations such as BCR-ABL.

To our knowledge, there have been very few reports on the involvement of MKRN2 in malignancy, except some microarray

Figure 5. Colony forming capacity of ex vivo expanded CD34+ cells over-expressing MKRN2. CD34+ cells were transduced with MKRN2 cDNA subcloned into lentiviral vector (pLEF1α-IG-MKRN2) and expanded for 8 days, and subjected to CFU culture for 14 days (n = 3). There was no difference between MKRN2-transduced cells and control cells containing the empty vector (pLEF1α-IG).

doi:10.1371/journal.pone.0092706.g005

Figure 6. ShRNA-silencing of MKRN2 in cord blood CD34+ cells. MKRN2 expression was down-regulated in CD34+ cells by nucleofection of shRNA. CD34+ cells expressing pGFP-V-RS-MKRN2 had lower expansion capacity in day 7 culture (P = 0.005; n = 4) and a trend of reduced expansion at days 2 and 8, compared with cells transfected with non-effective sh-pGFP-V-RS-NE or empty vector (pGFP-V-RS).

doi:10.1371/journal.pone.0092706.g006
screening data on papillary thyroid cancer [16]. MKRN1, the most studied member of the MKRN family has been shown to participate in a variety of mechanisms such as RNA-II-dependent transcription [17], Oct-4 signaling in mouse embryonic stem cells [18], telomere length homeostasis in cancer cell lines [19,20], polycystic kidney [21], ubiquitinase activity [22], and p14ARF-associated cellular senescence and gastric tumorigenesis [23]. Based on the ubiquitous expression and proliferative promoting activity of MKRN2 in the various developmental windows of hematopoiesis, we suggest that MKRN2 may play a house-keeping role on normal hematopoiesis. Our study has provided evidence that MKRN2 might also be involved in the proliferation of human leukemic cells. Further knowledge on MKRN2 interaction with known proto-oncogenes and involvement in leukemogenesis may lead to development of alternative treatment for the malignancy.

Supporting Information

File S1

Figure A: MKRN2 construct for lentiviral transduction.

Figure B: Correlation of MKRN2 and RAF1 Expression in Leukemia Samples. Expression levels of MKRN2 and RAF1 mRNA, relative to GAPDH, in bone marrow cells collected from leukemic patients (Ph-B-ALL, n = 8; Ph+B-ALL, n = 7; T-ALL, n = 5; AML, n = 22 and CML, n = 11) and age-matched normal bone marrow donors (n = 9) were measured by qPCR and analyzed by Pearson correlation test. A positive correlation (P = 0.042) was observed in Ph+B-ALL samples. However, the correlation became insignificant when the one sample with extremely high expressions of both MKRN2 and RAF1 was excluded from analysis. Ph = Philadelphia chromosome or BCR/ABL translocation.

Figure C: Expression of MKRN2 and RAF1 in CML patients with Major or Minor BCR/ABL. Expression levels of MKRN2 and RAF1 mRNA, relative to GAPDH, in bone marrow cells collected from CML BCR/ABL Major (n = 8) and Minor (n = 5) leukemic patients were measured by qPCR. There were no significant differences between the mRNA expression of either genes in the 2 subgroups of CML patients. Ph = Philadelphia chromosome or BCR/ABL translocation.

Figure D: Flow cytometric analysis of K562 transduction with MKRN2-GFP. Representative flow cytometric scatter plots of K562 cells lentiviral transduced with MKRN2-GFP. The empty vector GFP-IGV was used as a control. (A) Forward-scatter (x-axis) and side-scatter (y-axis) plot of K562 cells. R1 was gated for GFP expression analysis. (B) GFP expression (x-axis) and 7-AAD (y-axis, representing dead cells) of non-transduced cells. (C) K562 cells transduced with GFP-IGV control vector, showing 91.8% cells with GFP expression. (D) K562 cells transduced with MKRN2-GFP, showing 90.4% GFP-positive expression.

Author Contributions

Conceived and designed the experiments: KL, KYYC, HK, YCC, CKL, PCN. Performed the experiments: KY, KYYC, KTL, KST. Analyzed the data: KYL, KYYC, KTL. Contributed reagents/materials/analysis tools: KST, CKL, KYYC, PCN. Wrote the paper: KL, KYYC, KYL.

Figure 7. Colony forming capacity of ex vivo expanded CD34+ cells with silenced MKRN2. MKRN2 expression was down-regulated in CD34+ cells by nucleofection of shRNA. CD34+ cells expressing pGFP-V-RS-MKRN2 had a lower level of CFU-GEMM, compared with cells transfected with non-effective sh-pGFP-V-RS-NE or empty vector (pGFP-V-RS) (**P<0.01; n = 3). doi:10.1371/journal.pone.0092706.g007

Figure 8. Proliferation capacity of K562 cells over-expressing MKRN2. K562 cells were transduced with MKRN2 cDNA subcloned into lentiviral vector (pLEF1α-IG-MKRN2). MTT assay of pLEF1α-IG-MKRN2-transduced cells showed a significantly increased proliferation in culture, compared with pLEF1α-IG (empty vector) control cells (*P = 0.05; n = 3). doi:10.1371/journal.pone.0092706.g008

Figure 8. Proliferation capacity of K562 cells over-expressing MKRN2. K562 cells were transduced with MKRN2 cDNA subcloned into lentiviral vector (pLEF1α-IG-MKRN2). MTT assay of pLEF1α-IG-MKRN2-transduced cells showed a significantly increased proliferation in culture, compared with pLEF1α-IG (empty vector) control cells (*P = 0.05; n = 3). doi:10.1371/journal.pone.0092706.g008
References

1. Gray TA, Hernandez L, Carey AH, Schaldach MA, Smithwick MJ, et al. (2000) The ancient source of a distinct gene family encoding proteins featuring RING and C3H zinc-finger motifs with abundant expression in developing brain and nervous system. Genomics 66: 76–86.

2. Gray TA, Azuma K, Whitmore K, Min A, Abe S, et al. (2001) Phylogenetic conservation of the makorin-2 gene, encoding a multiple zinc-finger protein, antisense to the RAF1 proto-oncogene. Genomics 77: 119–126.

3. Cheung WK, Yang PH, Huang QH, Chen Z, Chen SJ, et al. (2008) Makorin-2 is a neurogenesis inhibitor downstream of phosphatidylinositol 3-kinase/Akt (PI3K/Akt) signal. J Biol Chem 283: 8486–8495.

4. Mao M, Fu G, Wu JS, Zhang QH, Zhou J, et al. (1998) Identification of genes expressed in human CD34(+) hematopoietic stem/progenitor cells by expressed sequence tags and efficient full-length cDNA cloning. Proc Natl Acad Sci U S A 95: 8175–8180.

5. Zhang QH, Ye M, Wu XY, Ren SX, Zhao M, et al. (2000) Cloning and functional analysis of cDNAs with open reading frames for 300 previously undefined genes expressed in CD34(+) hematopoietic stem/progenitor cells. Genome Res 10: 1546–1560.

6. Lee KY, Fong BS, Tsang KS, Lau TK, Chu W, et al. (2011) The tetraspanin CD9 regulates migration, adhesion, and homing of human cord blood CD34(+) hematopoietic stem and progenitor cells. Blood 117: 1840–1850.

7. Li K, Yang M, Lam AC, Lau FW, Yuen PM (2000) Effects of flt-3 ligand in combination with TPO on the expansion of megakaryocytic progenitors. Cell Transplant 9: 125–131.

8. Lin MC, Yao H, Wang H, Zhang AG, et al. (2007) Lentivirus-mediated RNA interference targeting enhancer of zeste homolog 2 inhibits hepatocellular carcinoma growth through down-regulation of stathmin. Hepatology 46: 200–208.

9. Kristian GW, Armstrong BG, Battey JF (1990) N-myc mRNA forms an RNA-RNA duplex with endogenous antisense transcripts. Mol Cell Biol 10: 4180–4191.

10. Yelin R, Dahary D, Sorek R, Levanon EY, Goldstein O, et al. (2003) Widespread occurrence of antisense transcription in the human genome. Nat Biotechnol 21: 379–386.

11. Stork PJ, Dillon TJ (2005) Multiple roles of Rap1 in hematopoietic cells: complementary versus antagonistic functions. Blood 106: 2952–2961.

12. Muszynski KW, Russett PW, Heidecker G, Rapp U, Troppmair J, et al. (1995) Raf-1 protein is required for growth factor-induced proliferation of hematopoietic cells. J Exp Med 181: 2189–2199.

13. Demidenko ZN, An WG, Lee JT, Romanova LY, McCabrey JA, et al. (2005) Kinase-addiction and bi-phasic sensitivity-resistance of Bcr-Abl and Raf-1-expressing cells to imatinib and geldanamycin. Cancer Biol Ther 4: 484–490.

14. Jarzab B, Wiernich M, Fajarski D, Simek K, Jarzab M, et al. (2005) Gene expression profile of papillary thyroid cancer: sources of variability and diagnostic implications. Cancer Res 65: 1537–1597.

15. Omwancha J, Zhou XF, Chen SY, Baslan T, Fisher CJ, et al. (2006) Makorin RING finger protein 1 (MKRN1) has negative and positive effects on RNA polymerase II-dependent transcription. Endocrinology 29: 363–373.

16. Du Z, Cong H, Yao Y (2001) Identification of putative downstream genes of Oct-4 by suppression-subtractive hybridization. Biochem Biophys Res Commun 282: 701–706.

17. Kim JH, Park SM, Kang MR, Oh SY, Lee TH, et al. (2005) Ubiquitin ligase MKRN1 modulates telomere length homeostasis through a proteolysis of hTERT. Genes Dev 19: 776–781.

18. Salvatico J, Kim JH, Chung IK, Muller MT (2010) Differentiation linked regulation of telomerase activity by Makorin-1. Mol Cell Biochem 342: 241–250.

19. Yoshida N, Yano Y, Yoshiki A, Ueno M, Deguchi N, et al. (2003) Identification of a new target molecule for a cascade therapy of polycystic kidney. Hum Cell 16: 65–72.

20. Joazeiro CA, Weissman AM (2000) RING finger proteins: mediators of ubiquitin ligase activity. Mol Cell 10: 549–552.

21. Ko A, Shin JY, Seo J, Lee KD, Lee EW, et al. (2012) Acceleration of gastric tumorigenesis through MKRN1-mediated posttranslational regulation of p16ARF. J Natl Cancer Inst 104: 1660–1672.