A novel *RET* rearrangement (*ACBD5/RET*) by pericentric inversion, inv(10)(p12.1;q11.2), in papillary thyroid cancer from an atomic bomb survivor exposed to high-dose radiation

KIYOHIRO HAMATANI[1], HIDETAKA EGUCHI[3], KAZUAKI KOYAMA[1], MAYUMI MUKAI[1,2], KEI NAKACHI[1] and YOICHIRO KUSUNOKI[1]

Departments of [1]Radiobiology/Molecular Epidemiology and [2]Genetics, Radiation Effects Research Foundation, Hiroshima, Japan; [3]Division of Translational Research, Research Center for Genomic Medicine, Saitama Medical University, Saitama, Japan

Received May 30, 2014; Accepted August 6, 2014

DOI: 10.3892/or.2014.3449

Abstract. During analysis of *RET/PTC* rearrangements in papillary thyroid cancer (PTC) among atomic bomb survivors, a cDNA fragment of a novel type of *RET* rearrangement was identified in a PTC patient exposed to a high radiation dose using the improved 5' RACE method. This gene resulted from the fusion of the 3' portion of *RET* containing tyrosine kinase domain to the 5' portion of the acyl-coenzyme A binding domain containing 5 (*ACBD5*) gene, by pericentric inversion inv(10)(p12.1;q11.2); expression of the fusion gene was confirmed by RT-PCR. *ACBD5* gene is ubiquitously expressed in various human normal tissues including thyroid. Full-length cDNA of the *ACBD5-RET* gene was constructed and then examined for tumorigenicity. Enhanced phosphorylation of ERK proteins in the MAPK pathway was observed in NIH3T3 cells transfected with expression vector encoding the full-length *ACBD5/RET* cDNA, while this was not observed in the cells transfected with empty expression vector. Stable NIH3T3 transfectants with *ACBD5-RET* cDNA induced tumor formation after their injection into nude mice. These findings suggest that the *ACBD5-RET* rearrangement is causatively involved in the development of PTC.

Introduction

The *RET* proto-oncogene encodes a tyrosine kinase (TK) receptor protein that is expressed normally in neural crest-derived cells (1-3). In papillary thyroid cancer (PTC), the *RET* proto-oncogene fuses to sequences of the 5' portion of partner genes at its TK domain via chromosomal inversion or translocation, resulting in rearranged and constitutively activated chimeric oncogenes called *RET/PTC* (4-7). To date, at least 15 different types of rearranged *RET/PTC* have been found, with 12 different partner genes (8-10). The majority of rearranged *RET* oncogenes found in PTC are *RET/PTC1* and *RET/PTC*3, which are formed by paracentric inversions of chromosome 10. Most rare types of these *RET* rearrangements have been isolated from radiation-associated PTCs, such as those arising post-Chernobyl (10-17). All the partner genes of rearranged *RET/PTC* thus far identified are widely expressed in various human tissue including thyroid. The chimeric products of *RET/PTC* are constitutively expressed and form homo-dimers mediated by the coiled-coil domains of the partner genes in PTC, resulting in a ligand-independent activation of TK of the RET.

During molecular analyses of ~100 PTC cases from atomic bomb (A-bomb) survivors, rearrangements of genes with kinase domains, such as *RET*, *NTRK1* and *ALK*, have frequently been detected in survivors exposed to high radiation doses (18,19). Among these, *RET/PTC1* was the most common rearranged gene in the PTC cases examined in A-bomb survivors exposed to a radiation dose of >500 mGy. An improved rapid amplification of cDNA ends (RACE) method was established to identify unknown *RET* rearrangement with RNA extracted from archival formalin-fixed, paraffin-embedded (FFPE) thyroid cancer tissue specimens. Using this method, a PTC A-bomb survivor exposed to a high radiation dose was found to possess *RET/PTC*8 (20), which had been identified in thyroid cancer from post-Chernobyl children (14). In addition, a novel type of *RET* rearrangement, i.e., acyl-coenzyme A binding domain containing 5 (*ACBD5*)-*RET*, was found in a PTC from another high-dose A-bomb survivor. In the present study, we reported the identification and molecular characteristics of the *ACBD-RET* rearrangement that was formed by fusion of the tyrosine kinase domain of *RET* to the 5' portion of *ACBD5* gene located in the short arm of chromosome 10, via pericentric inversion inv(10)(p12.1;q11.2).

Correspondence to: Dr Kiyohiro Hamatani, Department of Radiobiology/Molecular Epidemiology, Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami-ku, Hiroshima-shi, Hiroshima 732-0815, Japan
E-mail: hamatani@rerf.or.jp

Key words: RET rearrangements, pericentric inversion, atomic bomb survivors

Materials and methods

Tissue specimens. FFPE PTC specimens from A-bomb survivors, preserved at room temperature for 20-50 years, were collected and analyzed with approval of the Human Investigation Committee, and the Ethics Committee for Genome Research at the Radiation Effects Research Foundation (RERF). A novel *RET* rearrangement (*ACBD5-RET*) was discovered in PTC specimen from one A-bomb survivor whose DS02 dose (21) was 1.8 Gy (weighted thyroid dose).

RNA extraction. RNA was isolated from dissected tissue using the High Pure RNA Paraffin kit according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany) with some modifications, as described in a previous study (22).

cDNA synthesis and improved switching mechanism at 5' end of RNA transcript (SMART) RACE. cDNA synthesis and improved SMART RACE with RNA from archival FFPE PTC tissues was carried out as described in a previous study (20).

Cloning and sequencing of cDNA fragments of ACBD5-RET gene. Target candidate cDNA fragments derived from the second SMART RACE-PCR products were eluted from 8% acrylamide gel and cloned into *Hinc*II digested plasmid vector. Plasmid DNA containing an insert of >70 bp was sequenced using DNA sequencer CEQ8000 (Beckman Coulter Inc., Fullerton, CA, USA), since the total length of the SMART adaptor and the 5' portion of exon 12 of *RET* was 55 bp.

RT-PCR detection of ACBD5-RET. RT-PCR was carried out as previously described (20). RT-PCR products were cloned in a cloning vector and confirmed as actual *ACBD5-RET* products by sequencing.

Determination of ACBD5 gene expression levels in normal tissue by real-time PCR. Human normal tissue RNAs were purchased from BioChain Institute, Inc. (USA). Total RNA (2 μg) was reverse transcribed with 100 units of ReverTra Ace (Toyobo Co., Japan) as described in a previous study (20). Quantitative PCR amplifications were carried out using ABsolute™ qPCR SYBR-Green Mixes according to the manufacturer's instructions (Thermo Fisher Scientific Inc., USA). Amplification conditions optimized for the iCycler instrument (Bio-Rad, Hercules, CA, USA) resulted in a single PCR product, which was judged by using melting curve and electrophoretic analysis. 18SrRNA was used as a reference. The assays were run in duplicate using the following primer set: ACBD5-RT56F, 5'-GCCATGATTGCATATGTTGAAG-3' and ACBD5-RT56R, 5'-AACTCCTGCCACTCTTTTTGTC-3' for ACBD5 gene; and 18SrRNART-F12, 5'-GTAGTCGCCGTGCCTACCAT-3' and 18SrRNART-R12, 5'-GTTTCTCAGGCTCCCTCTCC-3' for 18SrRNA.

Expression of variant 1 and 2 of ACBD5 gene in normal tissue by RT-PCR. RT-PCR of variant 1 and 2 of *ACBD5* gene was carried out as described elsewhere (20). Briefly, the primer sets used were: ACBD5ex2F2, 5'-TCTTCTGCTAGACATGCTCTTCCT-3' and ACBD5ex3R1, 5'-CCCAAGAGCCTGCATGAAAC-3' for variant 1; and ACBD5ex1F2, 5'-GACAGGCCTTGTCCGGCAATA-3' and ACBD5ex3R1, 5'-CCCAAGAGCCTGCATGAAAC-3' for variant 2. The cDNA derived from 2 ng of total RNA was used as a template for RT-PCR. PCR conditions consisted of initial denaturation (95˚C for 2 min), followed by 36 cycles (denaturation at 95˚C for 30 sec, annealing at 60˚C for variant 2 or 62˚C for variant 1, for 30 sec, extension at 72˚C for 30 sec), and final extension at 72˚C for 3 min.

Construction of ACBD5-RET. Total RNA from the PTC cell line TPC1 was used to amplify *RET/PTC1* using a sense primer (H4PRTF1, TTAAGCTTCTGCTGCTCCTCCTCCTTTCCA) located in the 5' flanking region of *H4* gene and an antisense primer (RET-3' end R3, AATCTAGATCAGGGGTAGTGGCTGCTCAGTA) located in the 3' untranslated region of *RET* gene. After digestion of amplified fragments with *Hin*dIII and *Xba*I, a fragment of ~2,400 bp was inserted into *Hin*dIII and *Xba*I-digested pBluescript II SK(-). To replace the partner of *RET/PTC1* with 5' region of *ACBD5* gene, a fragment of ~1,200 bp covering the region from exon 5 to exon 12 of the *ACBD5* gene was amplified with total RNA from human normal thymus using sense primer ACBD5F2, TTAAGCTTAATGGGATGCTTGGAGTTCACTG, located in the joint region of exons 4 and 5 of *ACBD5* gene, and antisense primer ACBD5-RETR1, CTTTGGATCCTCCTGTGAGGTGGGCTGAGGAGCA, which corresponds to regions of 3' end of exon 12 of *ACBD5* and of 5' end of exon 12 of *RET*. Following digestion with *Hin*dIII and *Bam*HI, a fragment of ~1,200 bp was inserted into *Hin*dIII and *Bam*HI-digested pBluescript II SK(-) containing *Bam*HI-*Xba*I fragment of *RET* kinase domain. To complete the construction of *ACBD5-RET*, a fragment of ~500 bp containing the region from initiation codon to exon 5 of *ACBD5* gene was amplified with total RNA from human normal thymus using sense primer ACBD5F1, ACAACAAGCTTTCATGCAGGCTCTTGGGAAAGC, and antisense primer ACBD5R2, CGGTTTTGGCGTTCGGAGTAGA. After digestion of the amplified fragments with *Hin*dIII and *Nde*I, whose recognition site is located in exon 5 of *ACBD5* gene, the digested fragments were ligated to *Hin*dIII- and *Nde*I-digested pBluescript II SK(-) containing regions of exons 4-12 of *ACBD5* and exons 12-20 of *RET*. Underlined primers indicate the restriction enzyme sites that were artificially created to make the construct of *ACBD5-RET*.

DNA transfection. NIH3T3 cells ($5x10^4$) were transfected with 10 μg of empty vector, *ACBD5-RET* or *RET*/PTC3 expression vectors (pcDNA3.1) (Invitrogen, Carlsbad, CA, USA) using FuGENE6 Transfection Reagent (Roche Diagnostics GmbH). After 2 days, cells were further incubated in DMEM containing 10% calf serum and 500 μg/ml of G418. G418-resistant colonies were screened for transfected cDNA expression by RT-PCR. Selected clones were then analyzed for phosphorylation of ERK protein by western blotting.

Western blot analysis. After scraping out the cells from 100 mm dishes, $1x10^6$ stable transfectants were lysed in 100 μl of RIPA lysis buffer (Merck Millipore Co., Germany). After adding 2X SDS buffer to cell lysate, and heating at 95˚C, an aliquot (~2 μg of proteins) was separated by 8% SDS-polyacrylamide

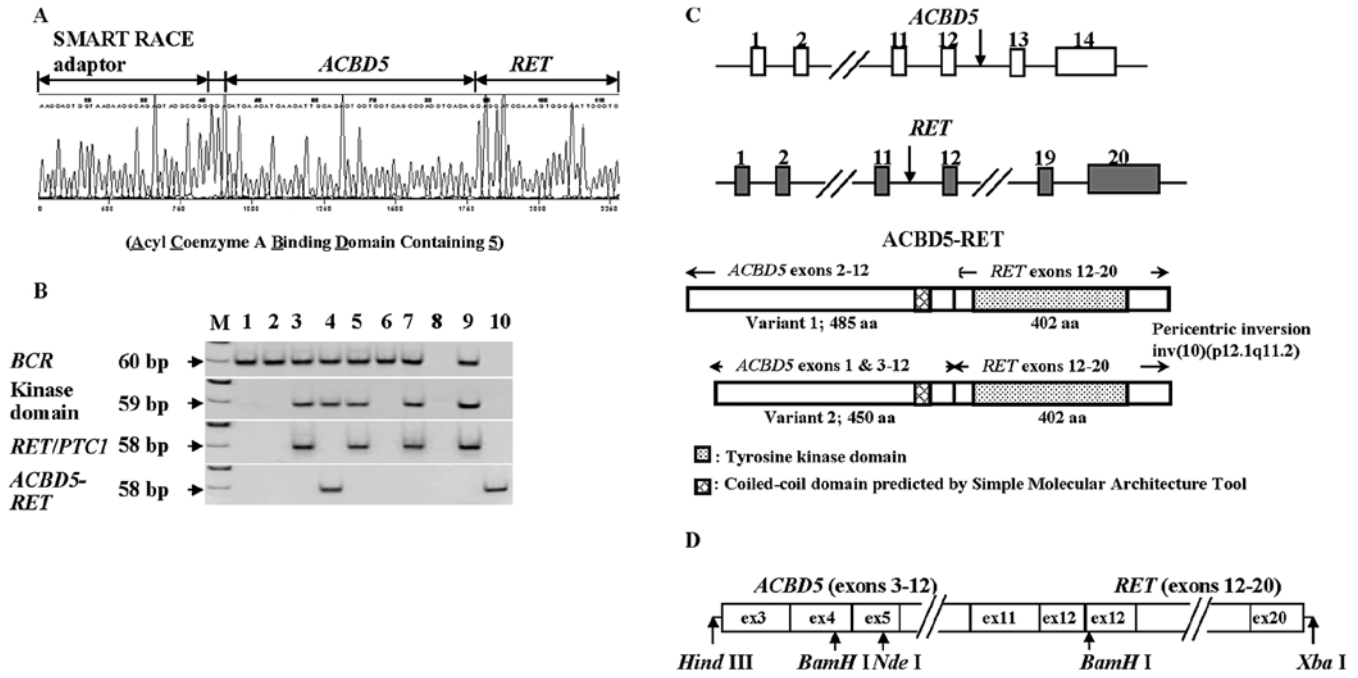

Figure 1. Identification of *ACBD5-RET* fusion gene in PTC of one A-bomb survivor exposed to 1.8 Gy. (A) Sequence of *ACBD5-RET* fragments isolated by SMART RACE method. (B) RT-PCR analysis of expression of *ACBD5-RET* along with *RET/PTC1* fusion genes in PTC of the A-bomb survivors. Lanes 1-7 show RT-PCR products from RNA in PTC specimens of A-bomb survivors. Lanes 1, 2 and 6 show no detectable expression of *RET* tyrosine kinase domain; lanes 3, 5 and 7, *RET/PTC1*; lane 4, *ACBD5-RET*; lane 8, H_2O for negative control; lane 9, cell line TPC1 harboring *RET/PTC1*; lane 10, 77 base-synthesized nucleotides plus genomic DNA for positive control of *ACBD5-RET*. Lane M indicates pUC19-*Msp*I digest used as a DNA size marker. (C) Predicted *ACBD5-RET* fusion gene isolated from radiation-exposed PTC. Both variants 1 and 2 of *ACBD5* gene contain coiled-coil domain in exon 12. (D) Construct of *ACBD5-RET* for transfection assay. This construct consists of exons 3-12 of *ACBD5* gene and exons 12-20 of *RET* gene. PTC, papillary thyroid cancer.

gel and then transferred to a PVDF membrane. Specific bands were detected by incubating with primary antibodies and then with horseradish peroxidase-conjugated anti-rabbit antibody (Cell Signaling Technology Co., USA), and finally by treatment with enhanced chemiluminescence reagent (Amersham Biosciences). For analysis of RET protein, Ret (C31B4) rabbit monoclonal antibody (Cell Signaling Technology Co.) was used as the primary antibody. Phospho-p44/42 MAP kinase antibody and p44/42 MAP kinase antibody (Cell Signaling Technology Co.) were used as primary antibodies for analysis of phosphorylation of ERK1/2.

Transformation assay. Stable transfectants were suspended at a cell concentration of $2x10^6$/ml in culture media and injected subcutaneously into the flank of BALB/c nu/nu female mice. Three clones of stable transfectants with *ACBD5-RET* and one clone of stable transfectants with *RET/PTC3* and G418-resistant NIH3T3 cells with empty vector were injected into one flank of the mice at different cell densities ($1x10^6$, $5x10^5$ and $2.5x10^5$ cells). Animal experiments in this study were conducted on the basis of approval from the RERF's Experimental Animal Care Committee.

Results and Discussion

Identification and expression of ACBD5-RET gene in PTC of one A-bomb survivor exposed to high-dose radiation. During analysis of *RET/PTC* rearrangements in PTC specimens among A-bomb survivors, a cDNA fragment of a new type of *RET*/PTC, with a size of 102 bp, was isolated from one A-bomb survivor exposed to 1.8 Gy using the improved RACE method (20) in PTC. Sequence analysis revealed that the isolated fragments contained a portion of exon 12 of the *ACBD5* gene that was fused in frame with exon 12 of the *RET* gene (Fig. 1A). The fragment obtained from the 5' SMART RACE method contained an unknown insert of 3 bp (GGA) between the adaptor for RACE and ACBD5; RT-PCR-amplified fragments covering the corresponding region did not include any 3 bp-insert (GGA), and were coincident with sequences of the reported *ACBD5* (data not shown). The 3 bp-insert was presumably derived from the template-independent addition of a few nucleotides by terminal transferase activity of reverse transcriptase in the process of SMART RACE.

The *ACBD5-RET* chimera gene was detected only in PTC from this A-bomb survivor by RT-PCR with gene-specific primers (Fig. 1B). Since the tissue samples used were specimens that had been fixed with unbuffered formalin, embedded in paraffin and preserved for >40 years (resulting in vigorous degradation of RNA), we were unable to clone a full-length cDNA of the *ACBD5-RET* gene using either 5' RACE method or RT-PCR. However, the *ACBD5* gene is ubiquitously expressed in various normal human tissues including thyroid, with two major products, variants 1 and 2 (Fig. 2A and B). The former starts from exon 2, while the latter starts at exon 1 and skips exon 2 (Fig. 1C). When we compared the expression levels of variants 1 and 2 of the *ACBD5* gene in various human tissues, we found that expression levels of variant 2 of *ACBD5* gene were higher than those of variant 1 in normal thyroid

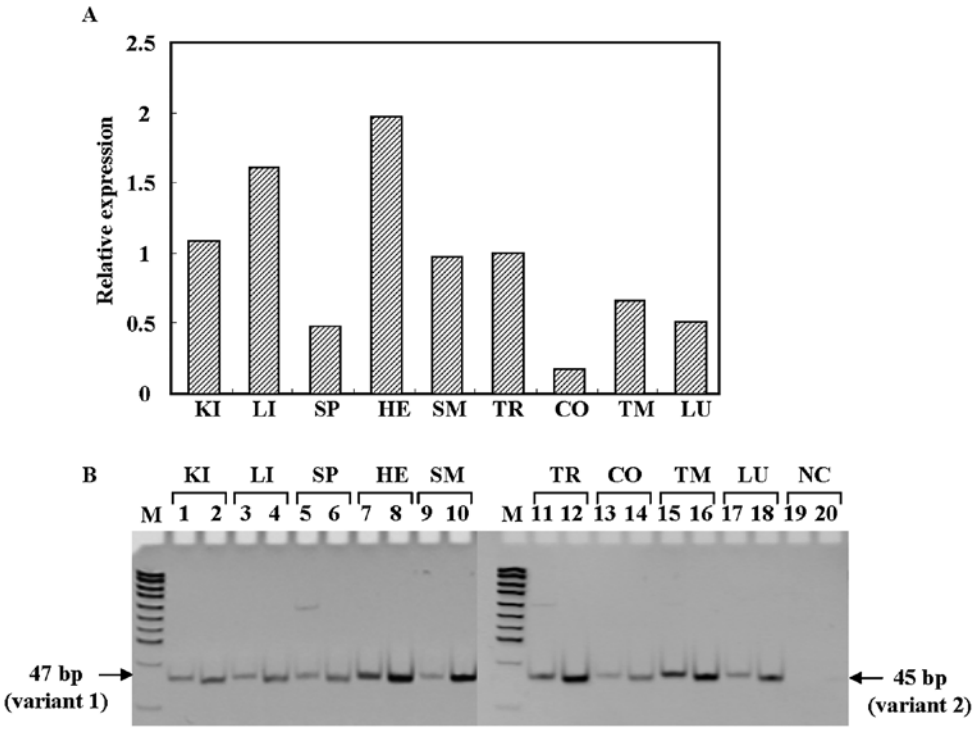

Figure 2. Expression of *ACBD5* mRNA (variants 1 and 2) in various human normal tissue. (A) Relative *ACBD5* mRNA expression levels determined by quantitative real-time RT-PCR. The tissue used is: KI, kidney; LI, liver; SP, spleen; HE, heart; SM, skeletal muscle; TR, thyroid; CO, colon; TM, thymus; LU, lung. NC shows H_2O for negative control, and M indicates pUC19-*Msp*I digest for DNA size marker. (B) Detection of the *ACBD5* transcripts by RT-PCR. Odd and even numbers indicate variants 1 and 2, respectively.

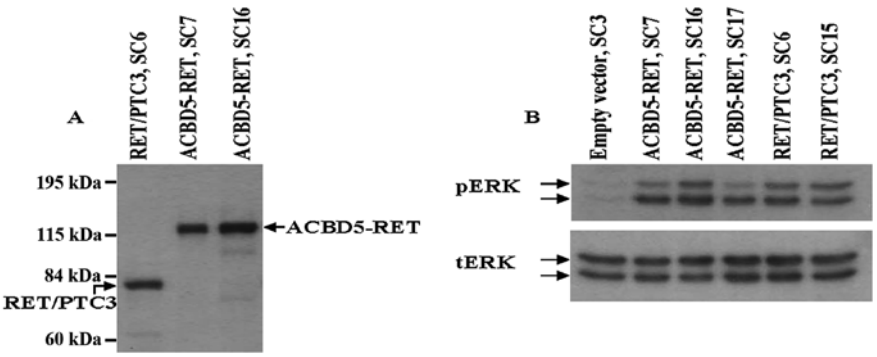

Figure 3. Activation of MAPK in stable transfectants with *ACBD5-RET*. (A) Expression of ACBD5-RET protein in the stable transfectants. Antibody used was specific for C-terminal of RET protein. (B) Western blot analysis of MAPK phosphorylation. Antibodies used were specific for phosphorylated ERK (pERK) and total ERK (tERK). tERK was used for quantity control of proteins. Empty vector-transfected NIH3T3 cells were used as a negative control, and *RET*/*PTC3*-transfected NIH3T3 cells were used as a positive control. SC7, SC16 and SC17 are subclones of NIH3T3 transfectants with *ACBD5-RET*; SC6 and SC15 are subclones of NIH3T3 transfectants with *RET*/*PTC3*; SC3 is a subclone of NIH3T3 transfectants with empty vector. PTC, papillary thyroid cancer.

(Fig. 2B). Assuming that the *ACBD5-RET* fusion gene utilized an initiation codon in exon 3 of the *ACBD5* gene, as is the case for variant 2 of *ACBD5*, we prepared a construct of the *ACBD5-RET* gene (Fig. 1D), as described in the Materials and methods section.

Activation of MAPK pathway in ACBD5-RET-introduced NIH3T3 stable transfectants. Since *RET*/*PTC* fusion gene products are involved in cell growth through phosphorylation of ERK protein, we evaluated the ability of the ACBD5-RET protein to phosphorylate ERK protein using stable transfectants of NIH3T3 cells constitutively expressing *ACBD5-RET*. Expression of ACBD5-RET or RET/PTC3 proteins in these stable transfectants is shown in Fig. 3A. Enhanced ERK protein phosphorylation was observed in three different transfectants: SC7, SC16 and SC17; and phosphorylation levels were comparable to those of two NIH3T3 stable transfectants with *RET*/*PTC3*, SC6 and SC15 (Fig. 3B). In RET/PTC fusion proteins, oligomerization is mediated by coiled-coil domains of the partner gene of *RET*/*PTC* genes, resulting in constitutive activation of tyrosine kinase through autophosphorylation of critical tyrosine residues in the *RET*/*PTC* genes (23). Enhanced phosphorylation of ERK in the stable transfectants with *ACBD5-RET* would result from the ligand-independent activation of tyrosine kinase by homodimerization of this chimera-protein through coiled-coil region located on exon 10

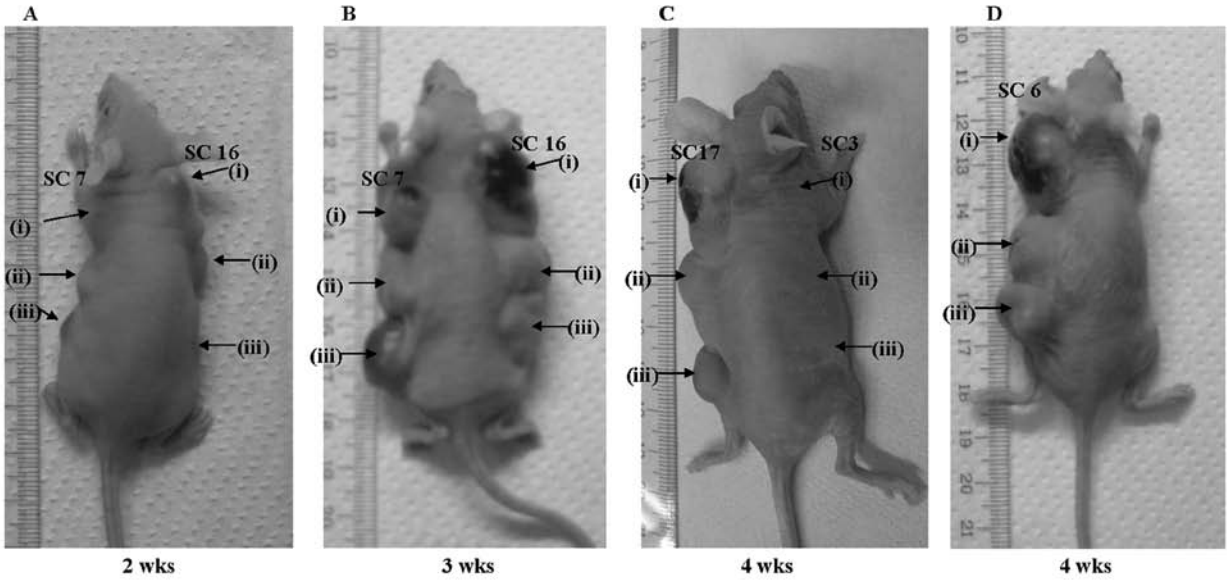

Figure 4. Transforming activity of *ACBD5-RET*. Mouse NIH3T3 fibroblasts were transfected with expression vector encoding *ACBD5-RET* cDNA. Stable NIH3T3 transfectants were injected into a nude mouse in amounts of $1x10^6$, $5x10^5$ and $2x10^5$ cells at positions of (i), (ii) and (iii), respectively. SC3, SC7, SC16, SC17 and SC6 are the same as the subclone numbers shown in Fig. 3.

of *ACBD5* gene, which was predicted by simple molecular architecture tool (EMBL).

Transforming activity of ACBD5-RET in NIH3T3 stable transfectants. To assess the tumorigenic activity of *ACBD5-RET*, we conducted a tumor formation assay in nude mice, using three clones (SC7, SC16 and SC17) from *ACBD5-RET*-introduced NIH3T3 stable transfectants. Administration of $1x10^6$ of each stable transfectant with *ACBD5-RET* resulted in tumor formation in nude mice as early as 10 days after injection. Detectable tumors formed in nude mice two weeks after injection of $5x10^5$ or $2.5x10^5$ cells (Fig. 4A). In all three clones, tumors grew to >15 mm 3-4 weeks after injection of each cell dilution (Fig. 4B and C). NIH3T3 stable transfectants, with *RET*/*PTC3* used as positive control, also formed detectable tumors 2-4 weeks after injection (Fig. 4D). By contrast, no tumors were detected even 4 weeks after injection of 0.25-$1x10^6$ of NIH3T3 cells with empty vectors (Fig. 4C). These results suggest that *ACBD5-RET* gene products may function as oncogene by constitutively activating MAP kinase through their homodimerization, as do products of other *RET*/*PTC* genes (23-25).

Acknowledgements

The Radiation Effects Research Foundation (RERF), Hiroshima and Nagasaki, Japan, is a public interest foundation funded by the Japanese Ministry of Health, Labour and Welware (MHLW), and the US Department of Energy (DOE). This study was funded in part through DOE award DE-HS0000031 to the National Academy Sciences, and in part by a Grant-in-Aid for Science Research from the Ministry of Education, Culture, Sports, Science and Technology, and a Grant-in Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan, as well as a Research Grant for the Princess Takamatsu Cancer Research Fund (08-24015). This publication was supported by RERF Research Protocols RP 5-02. The views of the authors do not necessarily reflect those of the two governments.

References

1. Pachinis V, Mankoo B and Costatini F: Expression of the *c-ret* proto-oncogene during mouse embryogenesis. Development 19: 1005-1017, 1993.
2. Avantaggiato V, Dathan NA, Griero M, Fabien N, Lazzaro D, Fusco A, Simeone A and Santoro M: Developmental expression of the *RET* protooncogene. Cell Growth Differ 5: 305-311, 1994.
3. Schuchardt A, D'Agati V, Larsson-Blomberg L, Costantini F and Pachnis V: Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. Nature 367: 380-383, 1994.
4. Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, Pierotti MA, Della Porta G, Fusco A and Vecchio G: PTC is a novel rearranged form of the *ret* proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell 60: 557-563, 1990.
5. Bongarzone I, Monzini N, Borrello MG, Carcano C, Ferraresi G, Arighi E, Mondellini P, Della Porta G and Pierotti MA: Molecular characterization of a thyroid tumor-specific transforming sequence formed by the fusion of ret tyrosine kinase and the regulatory subunit RI alpha of cyclic AMP-dependent protein kinase A. Mol Cell Biol 13: 358-366, 1993.
6. Bongarzone I, Butti MG, Coronelli S, Borrello MG, Santoro M, Mondellini P, Pilotti S, Fusco A, Della Porta G and Pierotti MA: Frequent activation of *ret* protooncogene by fusion with a new activating gene in papillary thyroid carcinomas. Cancer Res 54: 2979-2985, 1994.
7. Santoro M, Dathan N, Berlingieri MT, Bongarzone I, Paulin C, Grieco M, Pierotti MA, Vecchio G and Fusco A: Molecular characterization of *RET*/PTC3; a novel rearranged version of the *RET* proto-oncogene in a human thyroid papillary carcinoma. Oncogene 9: 509-516, 1994.
8. Tallini G and Asa SL: RET oncogene activation in papillary thyroid carcinoma. Adv Anat Pathol 8: 345-354, 2001.
9. Arighi E, Borrello MG and Sariola H: RET tyrosine signaling in development and cancer. Cytokine Growth Factor Rev 16: 441-467, 2005.
10. Ciampi R, Giordano TJ, Wikenheiser-Brokamp K, Koenig RJ and Nikiforov YE: *HOOK3-RET*: a novel type of *RET*/*PTC* rearrangement in papillary thyroid carcinoma. Endocr Relat Cancer 14: 445-452, 2007.

11. Fugazzola L, Pierotti MA, Vigano E, Pacini F, Vorontsova TV and Bongarzone I: Molecular and biochemical analysis of RET/PTC4, a novel oncogenic rearrangement between RET and ELE1 genes, in a post-Chernobyl papillary thyroid cancer. Oncogene 13: 1093-1097, 1996.
12. Klugbauer S, Demidchik EP, Lengfelder E and Rabes HM: Detection of a novel type of *RET* rearrangement (PTC5) in thyroid carcinomas after Chernobyl and analysis of the involved *RET*-fusion gene *RFG5*. Cancer Res 58: 198-203, 1998.
13. Klugbauer S and Rabes HM: The transcription coactivator *HTIF1* and a related protein are fused to the *RET* receptor tyrosine kinase in childhood papillary thyroid carcinomas. Oncogene 18: 4388-4393, 1999.
14. Klugbauer S, Jauch A, Lengfelder E, Demidchik E and Rabes HM: A novel type of *RET* rearrangement (PTC8) in childhood papillary thyroid carcinomas and characterization of the involved gene (*RFG8*). Cancer Res 60: 7028-7032, 2000.
15. Salassidis K, Bruch J, Zitzelsberger H, Lengfelder E, Kellerer AM and Bauchinger M: Translocation t(10;14)(q11.2:q22.1) fusing the *kinetin* to the *RET* gene creates a novel rearranged form (PTC8) of the *RET* proto-oncogene in radiation-induced childhood papillary thyroid carcinoma. Cancer Res 60: 2786-2789, 2000.
16. Corvi R, Berger N, Balczon R and Romeo G: *RET/PCM-1*: a novel fusion gene in papillary thyroid cancer. Oncogene 19: 4236-4242, 2000.
17. Saenko V, Rogounovitch T, Shimizu-Yoshida Y, Abrosimov A, Lushnikov E, Roumiantsev P, Matsumoto N, Nakashima M, Merimanov S, Ohtsuru A, Namba H, Tsyb A and Yamashita S: Novel tumorigenic rearrangement, Δ *rfp/ret*, in a papillary thyroid carcinoma from externally irradiated patient. Mutat Res 527: 81-90, 2003.
18. Hamatani K, Eguchi E, Ito R, Mukai M, Takahashi K, Taga M, Imai K, Cologne J, Soda M, Arihiro K, Fujihara M, Abe K, Hayashi T, Nakashima M, Sekine I, Yasui W, Hayashi Y and Nakachi K: *RET/PTC* rearrangements preferentially occurred in papillary thyroid cancer among atomic bomb survivors exposed to high radiation dose. Cancer Res 68: 7176-7182, 2008.
19. Hamatani K, Mukai M, Takahashi K, Hayashi Y, Nakachi K and Kusunoki Y: Rearranged anaplastic lymphoma kinase (*ALK*) gene in adult-onset papillary thyroid cancer amongst atomic bomb survivors. Thyroid 22: 1153-1159, 2012.
20. Hamatani K, Eguchi H, Taga M, Mukai M, Koyama K, Hayashi Y and Nakachi K: Improved method for analysis of RNA present in long-term preserved thyroid cancer tissue of atomic-bomb survivors. Thyroid 20: 43-49, 2010.
21. Young RW and Kerr GD (eds): Reassessment of the Atomic Bomb Radiation Dosimetry for Hiroshima and Nagasaki - Dosimetry System 2002. Vol 1. Radiation Effects Research Foundation, Hiroshima, Japan, 2005.
22. Hamatani K, Eguchi H, Takahashi K, Koyama K, Mukai M, Ito R, Taga M, Yasui W and Nakachi K: Improved RT-PCR amplification for molecular analyses with long-term preserved formalin-fixed, paraffin-embedded tissue specimens. J Histochem Cytochem 54: 773-780, 2006.
23. Tong Q, Xing S and Jhiang SM: Leucine zipper-mediated dimerization is essential for the *PTC1* oncogenic activity. J Biol Chem 272: 9043-9047, 1997.
24. Alberti L, Carniti C, Miranda C, Roccato E and Pierotti M: RET and NTRK1 proto-oncogenes in human diseases. J Cell Physiol 195: 168-186, 2003.
25. Kodama Y, Asai N, Kawai K, Jijiwa M, Murakumo Y, Ichihara M and Takahashi M: The *RET* proto-oncogene: a molecular therapeutic target in thyroid cancer. Cancer Sci 96: 143-148, 2005.