Identification of novel fusion genes with 28S ribosomal DNA in hematologic malignancies

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Abstract. Fusion genes are frequently observed in hematologic malignancies and soft tissue sarcomas, and are usually associated with chromosome abnormalities. Many of these fusion genes create in-frame fusion transcripts that result in the production of fusion proteins, and some of which aid tumorigenesis. These fusion proteins are often associated with disease phenotype and clinical outcome, and act as markers for minimal residual disease and indicators of therapeutic targets. Here, we identified the 28S ribosomal DNA (*RN28S1*) gene as a novel fusion partner of the B-cell leukemia/lymphoma 11B gene (*BCL11B*), the immunoglobulin κ variable 3-20 gene (*IGKV3-20*) and the component of oligomeric Golgi complex 1 gene (*COG1*) in hematologic malignancies. The *RN28S1-BCL11B* fusion transcript was identified in a case with mixed-lineage (T/myeloid) acute leukemia having t(6;14)(q25;q32) by cDNA bubble PCR using *BCL11B* primers; however, the gene fused to *BCL11B* on 14q32 was not on 6q25. *IGKV3-20-RN28S1* and *COG1-RN28S1* fusion transcripts were identified in the Burkitt lymphoma cell line HBL-5, and the multiple myeloma cell line KMS-18. *RN28S1* would not translate, and the breakpoints in partner genes of *RN28S1* were within the coding exons, suggesting that disruption of fusion partners by fusion to *RN28S1* is the possible mechanism of tumorigenesis. Although further analysis is needed to elucidate the mechanism(s) through which these *RN28S1*-related fusions play roles in tumorigenesis, our findings provide important insights into the role of rDNA function in human genomic architecture and tumorigenesis.

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Introduction

Fusion genes are frequently observed in hematologic malignancies and soft tissue sarcomas (1). These fusion genes are usually associated with chromosome abnormalities such as translocations, inversions, and deletions, but have also been identified in cryptic chromosome abnormalities. Fusion genes have also been identified in various solid tumors, including *ETS*-family fusion genes in prostate cancer (2), *ETV6-NTRK3* in secretory breast cancer (3), and *ALK* fusion genes in lung cancer (4). Many of these fusion genes create in-frame fusion transcripts that result in the production of fusion proteins, and some of which aid tumorigenesis. These fusion proteins are often associated with disease phenotype and clinical outcome, and act as markers for minimal residual disease and indicators of therapeutic targets. However, several fusion genes that do not create in-frame fusion transcripts have also been identified. Oncogenic rearrangements of immunoglobulin (*IG*) or T-cell receptor (*TCR*) genes are well-known fusion genes, and some of these create fusion transcripts, such as *IGH-BACH2* by t(6;14)(q15;q32) in B-cell lymphoma/leukemia (5), *IGH-MMSET* by t(4;14)(p16.3;q32.3) (6) and *Cα-IRTA1* by t(1;14)(q21;q32) (7) in multiple myeloma, and *BCL11B-TRDC* by inv(14)(q11.2q32.31) in T-cell acute lymphoblastic leukemia (8). Furthermore, chromosome abnormalities led to fusion transcripts in the non-coding gene *PVT1* such as *PVT1-NBEA* and *PVT1-WWOX* in multiple myeloma (9), *PVT1-CHD7* in small-cell lung carcinoma (10), and *PVT1-MYC* and *PVT1-NDRG1* in medulloblastoma (11). The role of *PVT1*-fusions is uncertain, but they may represent another type of fusion transcript in cancer cells and possibly in normal cells.

In the present study, we unexpectedly identified additional fusion genes involving 28S ribosomal DNA (*RN28S1*) in hematologic malignancies.

Materials and methods

Clinical sample and cell lines. Leukemic cells from a 15-year-old boy with mixed-lineage (T/myeloid) acute leukemia having t(6;14)(q25;q32) were analyzed after obtaining informed consent from the patient's parents. In

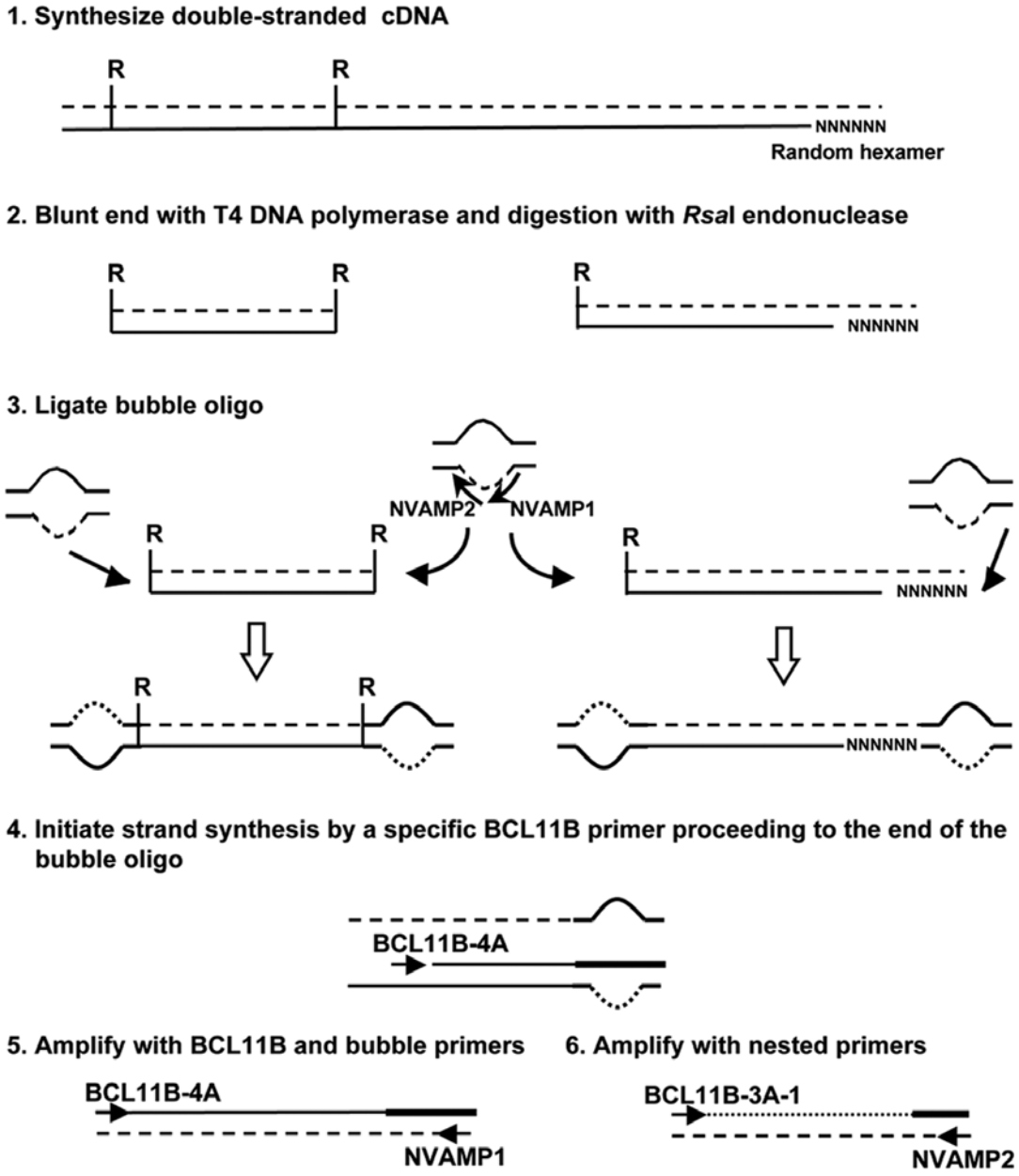

Figure 1. Outline of cDNA bubble PCR method used for the detection of *RN28S1-BCL11B* fusion transcript.

addition, 14 B-cell non-Hodgkin's lymphoma, 11 multiple myeloma, 4 B-cell precursor acute lymphoblastic leukemia cell lines, and 3 EB virus-transformed B-cell lines from normal healthy volunteers were analyzed; the cell lines were as described previously (5). The Institutional Review Board of Kyoto Prefectural University of Medicine approved this study.

cDNA bubble PCR. cDNA bubble PCR was used to detect the fusion partner of *BCL11B*, as described previously (Fig. 1) (12). Total RNA was used to generate double-stranded cDNA. Primers used were: NVAMP-1 and BCL11B-4A for first-round PCR, and NVAMP-2 and BCL11B-3A-1 for second-round PCR (Fig. 1 and Table I).

Reverse transcription (RT)-PCR. RT-PCR analysis was performed as described previously (5). Primers used for the detection of *BCL11B-RN28S1* fusion transcripts were BCL11B-3A-2 and RN28S1-GA1 for first-round PCR, and BCL11B-3A-1 and RN28S1-GA2 for second-round PCR; those for *IGKV3-20-RN28S1* were IGKV3-20-2F and

Table I. Primers used for PCR.

Primer	Sequence 5'-3'
BCL11B-3A-1	ACGCAGAGGTGAAGTGATCAC
BCL11B-3A-2	GACAACTGACACTGGCATCC
BCL11B-4A	ACCACGCGCTGTTGAAGGG
RN28S1-GA1	CCTTAGCGGATTCCGACTTCCAT
RN28S1-GA2	GTCCTGCTGTCTATATCAACCAACAC
IGKV3-20-2F	GGCTCCTCATCTATGGTGCATC
COG1-11F	AACAGCAACCTTCATCGCCTG

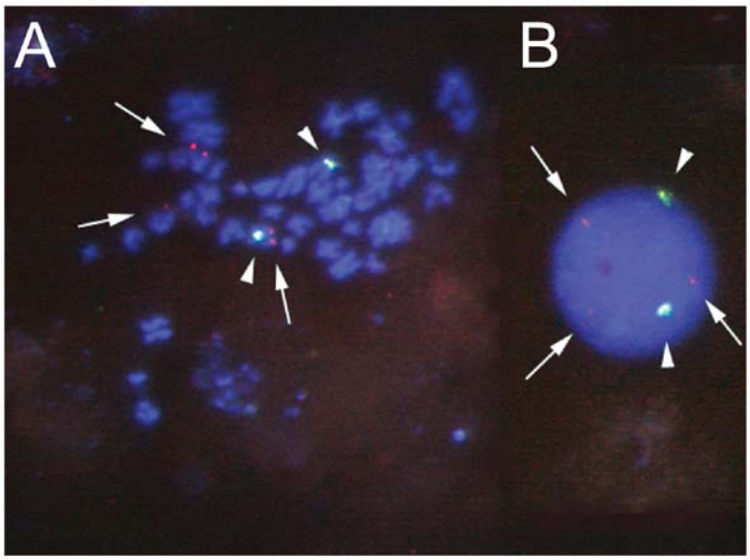

Figure 2. Fluorescence *in situ* hybridization analysis of the leukemic metaphase (A) and interphase (B). Split signals of BAC clone, RP11-431B1 (red, arrows), that encompassed *BCL11B* gene on 14q32, were detected. Arrowheads indicate CEP6 probe (centromere of chromosome 6, green).

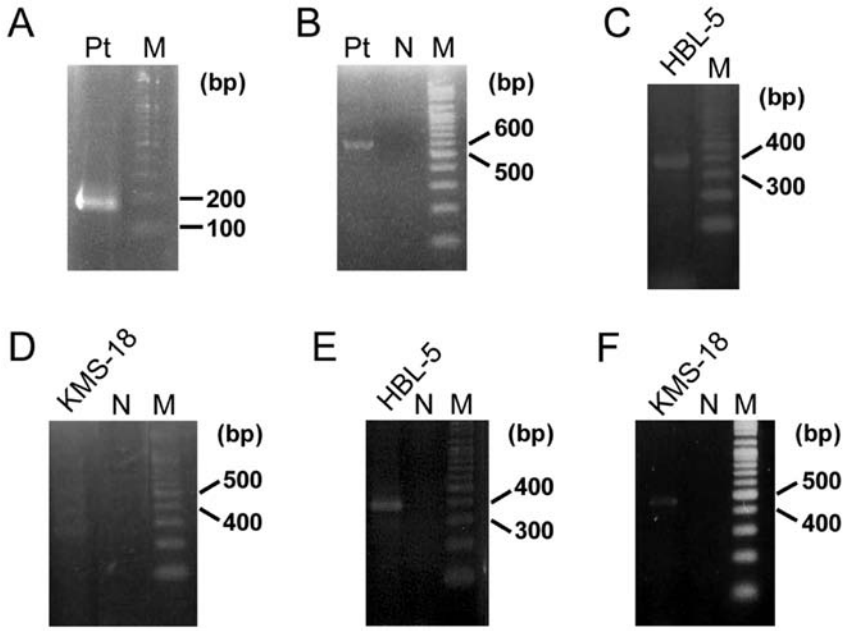

Figure 3. Identification of *BCL11B-RN28S1*, *IGKV3-20-RN28S1* and *COG1-RN28S1* fusion transcripts. (A) Bubble PCR products from a mixed-lineage acute leukemia patient sample. (B) Confirmation of *BCL11B-RN28S1* fusion transcript by nested RT-PCR. Detection of *IGKV3-20-RN28S1* fusion transcript (C) and multiple amplicons including *COG1-RN28S1* fusion transcript (D) by nested RT-PCR for the detection of *BCL11B-RN28S1*. RT-PCR using specific primers confirmed *IGKV3-20-RN28S1* (E) and *COG1-RN28S1* (F) fusion transcripts. Lanes are: Pt, mixed-lineage acute leukemia patient; N, negative control (water); M, size marker.

RN28S1-GA1; and those for *COG1-RN28S1* were COG1-11F and RN28S1-GA1 (Table I).

Nucleotide sequencing. Nucleotide sequences of PCR products and, if necessary, subcloned PCR products were analyzed as previously described (5).

Results

Identification of RN28S1-BCL11B fusion transcript by cDNA bubble PCR method. Leukemic cells from a patient with mixed-lineage acute leukemia having t(6;14)(q25;q32) were analyzed using fluorescence *in situ* hybridization analysis, and rearrangement of the B-cell leukemia/lymphoma 11B (*BCL11B*) gene was suggested (Fig. 2). Thus, we performed cDNA bubble PCR to identify the gene on chromosome 6q25 that was fused to the *BCL11B* gene on 14q32. Sequence analysis of multiple products amplified by second-round PCR of bubble PCR detected a product that contained a 34-bp sequence of *BCL11B* exon 3 fused to an unknown 96-bp sequence (Fig. 3A). A BLAST search revealed that the unknown sequence was 28S ribosomal DNA (*RN28S1*)

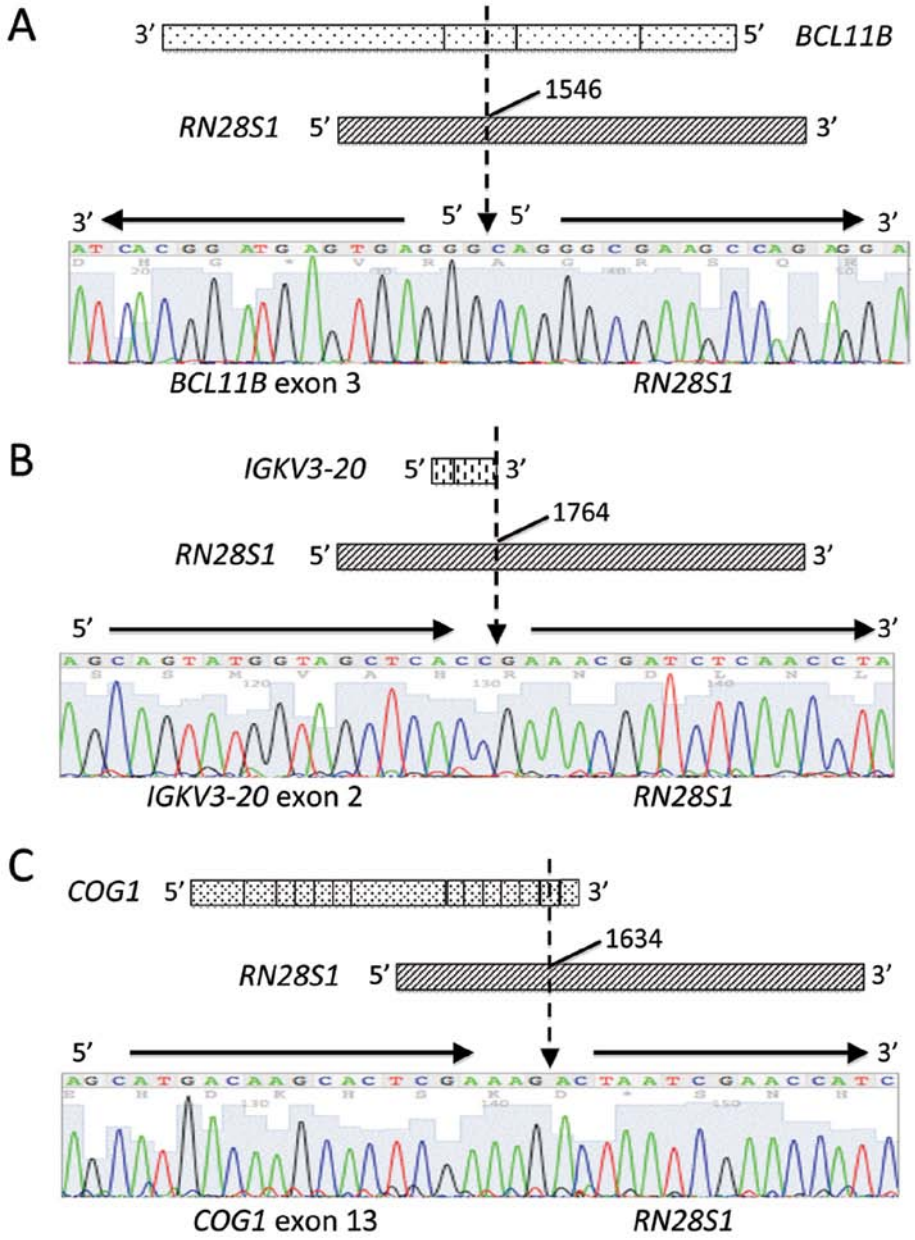

Figure 4. Structure of three fusion transcripts of *RN28S1*. (A) *BCL11B-RN28S1*. (B) *IGKV3-20-RN28S1*. (C) *COG1-RN28S1*. Dashed arrows indicate fusion points. Horizontal arrows indicate transcriptional orientation.

(Fig. 4A). This fusion transcript was confirmed by RT-PCR (Fig. 3B). The transcriptional directions of the contributing genes in the fusion transcript were opposed, and the fusion point of *BCL11B* was within the exon (Fig. 4A). No sequences from chromosome 6q25 were identified.

Accidental detection of IGK-RN28S1 and COG1-RN28S1 fusion transcripts in B-cell malignancy cell lines. *RN28S1* is one of three ribosomal DNAs encoding the 18S, 5.8S and 28S rRNAs, which exist in nucleolar organizer regions on the five acrocentric chromosomes (13p, 14p, 15p, 21p and 22p). Therefore, in this case, the *RN28S1-BCL11B* fusion transcript was considered not to be generated by the chromosome translocation of t(6;14)(q25;q32). Thus, we could infer that a mechanism other than chromosome abnormalities was involved in the creation of the *RN28S1-BCL11B* fusion transcript. To analyze whether the *RN28S1-BCL11B* fusion transcripts were expressed in other hematologic malignancy cell lines, RT-PCR using *RN28S1* and *BCL11B* primers was performed. PCR products were detected in several cell lines. In the Burkitt lymphoma cell line HBL-5, a 367-bp PCR product (Fig. 3C), contained 119-bp of immunoglobulin κ variable 3-20 (*IGKV3-20*) exon 2 fused to an *RN28S1* sequence (Fig. 4B). The multiple myeloma cell line KMS-18, amplified multiple PCR products (Fig. 3D) including a 441-bp product that resulted from a 62-bp sequence of the component of oligomeric Golgi complex 1 (*COG1*) gene fused to an *RN28S1* sequence (Fig. 4C). These fusions both occurred within the exons of *IGKV3-20* and *COG1*, as in the *RN28S1-BCL11B* fusion, and they were confirmed by RT-PCR using each specific primer (Fig. 3E and F).

Comparison of the sequence of BCL11B primer with those of *IGKV3-20* and *COG1* found similarities between them, with

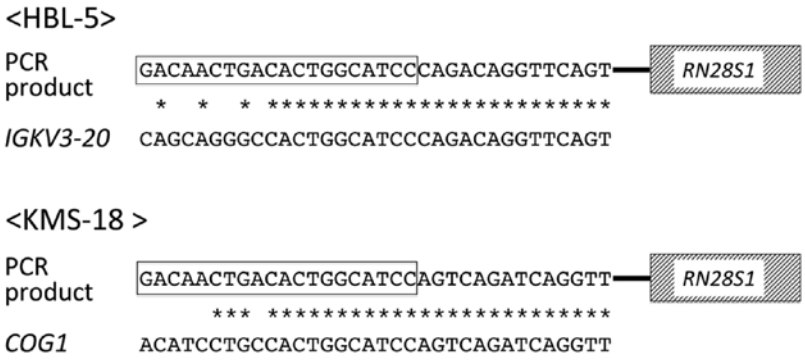

Figure 5. Comparison of nucleotide sequences of *BCL11B* primer and those of *IGKV3-20* and *COG1*. Primer BCL11B-3A-1 is boxed. *Identity.

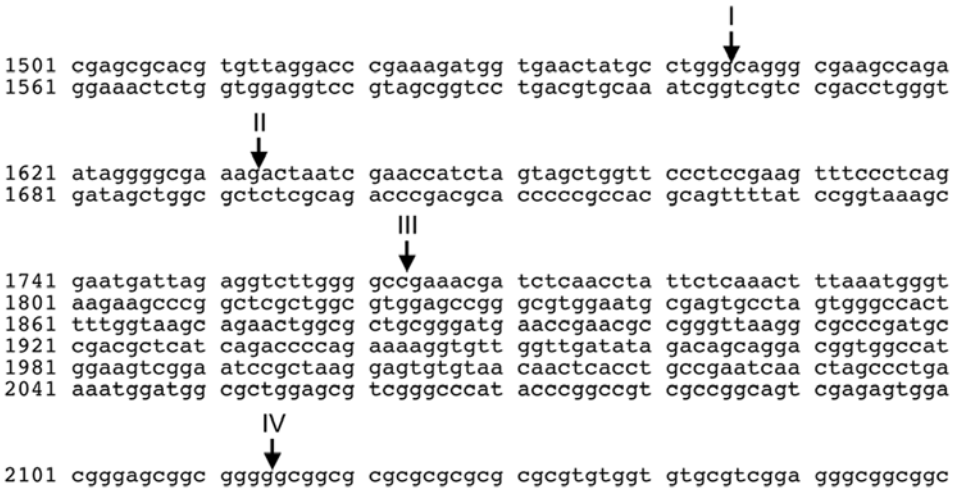

Figure 6. Breakpoint cluster region within *RN28S1*. Nucleotides are numbered (left side of the sequence) according to GenBank accession NR_003287. Vertical arrows indicate the breakpoints in cases with *BCL11B-RN28S1* (I), *COG1-RN28S1* (II), *IGKV3-20-RN28S1* (III) and *BCL-6-RN28S1* (IV). Three MYC binding sites are at positions 1344-1349, 1507-1521 (relates to the coanonical pathway) and 3655-3661 (relate to non-canonical pathway).

six mismatches in 20 nucleotides, suggesting that annealing of the primer to the similar sequences of *IGK* and *COG1* made it possible to amplify these fusion genes by chance (Fig. 5). Other products amplified by RT-PCR for the detection of *RN28S1-BCL11B* were either the normal sequence of *RN28S1* or non-specific sequences.

Discussion

In the present study, we identified three novel fusion transcripts involving *RN28S1*. Only one *RN28S1*-related fusion gene, *RN28S1-BCL6*, has been previously reported, and this was in a case of gastric lymphoma (13). *RN28S1* is a gene that would not translate to protein, and the breakpoints in partner genes of *RN28S1* were within the coding exons. Notably, *BCL11B* and *RN28S1* were fused with opposite transcription directions. These findings suggest that fusion genes involving *RN28S1* do not produce fusion proteins, but that disruption of the normal functions of fusion partners contribute to tumorigenesis in these cases.

Several studies have noted an association of ribosomal DNA (RNA) with tumorigenesis. rRNA transcription and ribosome biogenesis are controlled by several cancer-related genes through the PI3 kinase/mTOR, MYC or RAS/ERK pathways (14). Proto-oncoprotein MYC controls ribosome biogenesis through the regulation of transcription by all three RNA polymerases (15). Another cancer-related gene, nucleophosmin (*NPM1*), which is frequently mutated in acute myeloid leukemia with a normal karyotype and creates fusion genes with *ALK* in anaplastic large cell lymphoma with t(2;5)(p23;q35), is necessary for MYC-mediated rRNA synthesis (16). *RN28S1* has three MYC-binding sites (17), and the breakpoints within *RN28S1* in our cases were to the 5' side of the MYC-binding sites (Fig. 6), suggesting that the *RN28S1*-fusion transcripts we detected are associated with tumorigenesis through the dysregulation of MYC-mediated rRNA synthesis. In other correlations of rDNA with tumorigenesis, a high frequency of rDNA rearrangements was noted in lung and colon cancers (18) and overexpression of rDNA was seen in prostatic cancer (19). The breakpoints of *RN28S1* in our three cases were within an ~600 bp region (Fig. 6); these may be related to recombinational hot spots in repetitive sequences.

The three genes fused to *RN28S1* that we found in our study are related to tumorigenesis in certain types of malignancies. *IGK* is one of the immunoglobulin light chain genes that is frequently rearranged by chromosome translocations, such as t(2;8)(p11;q24) in B-cell malignancies (20). Rearrangement of the *BCL11B* gene locus is observed in T-cell

malignancies, and three fusion transcripts involving *BCL11B* have been identified: *TLX3-BCL11B* fusion gene by cryptic t(5;14)(q35;q32.2) in T-ALL (21), *BCL11B-TRDC* by inv(14)(q11.2q32.31) in T-ALL (8) and *HELIOS-BCL11B* by t(2;14)(q34;q32) in adult T-cell leukemia (22). *COG1* is a component of the conserved oligomeric Golgi (COG) complex, Golgi transport complex, that is involved in glycosylation reactions and vesicular transport (23). Although the *COG1* gene has not been firmly associated with tumorigenesis to date, one *COG* family gene, *COG5*, was found fused to *HMGA2* in uterine leiomyoma (24), suggesting a possible link with tumorigenesis. Although the fusion genes we identified in this study were not related to chromosome translocations, each gene is involved in tumorigenesis in some way, suggesting that *RN28S1*-related fusion genes play some roles in tumorigenesis.

Molecular analysis of ribosomal DNA is challenging due to its repetitive nature. In this study, we attempted to confirm the three fusions at the genomic level; however, genomic PCR was unsuccessful. A possible explanation for the failure of amplification of genomic junctions is the quantitative imbalance of genomic DNAs between partner genes and *RN28S1* due to the ~400 copies of ribosomal DNA in a diploid human genome. Whole-transcriptome analysis by next-generation sequencing is usually a powerful tool for the detection of fusion transcripts. However, detection of *RN28S1* fusion transcripts using this method is difficult, because ribosomal RNAs, which comprises >95% of total RNA, is usually removed from total RNA prior to sequencing. While further analysis is needed to clarify the role of rDNA in tumorigenesis, our findings provide an important insight into the role of rDNA function in human genomic architecture and tumorigenesis.

Acknowledgements

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References

1. Taki T and Taniwaki M: Chromosomal translocations in cancer and their relevance for therapy. Curr Opin Oncol 18: 62-68, 2006.
2. Tomlins SA, Rhodes DR, Perner S, *et al*: Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310: 644-648, 2005.
3. Euhus DM, Timmons CF and Tomlinson GE: ETV6-NTRK3 - Trk-ing the primary event in human secretory breast cancer. Cancer Cell 2: 347-348, 2002.
4. Soda M, Choi YL, Enomoto M, *et al*: Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 448: 561-566, 2007.
5. Kobayashi S, Taki T, Chinen Y, *et al*: Identification of IGHCδ-BACH2 fusion transcripts resulting from cryptic chromosomal rearrangements of 14q32 with 6q15 in aggressive B-cell lymphoma/leukemia. Genes Chromosomes Cancer 50: 207-216, 2011.
6. Chesi M, Nardini E, Lim RS, Smith KD, Kuehl WM and Bergsagel PL: The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, MMSET, resulting in IgH/MMSET hybrid transcripts. Blood 92: 3025-3034, 1998.
7. Hatzivassiliou G, Miller I, Takizawa J, *et al*: IRTA1 and IRTA2, novel immunoglobulin superfamily receptors expressed in B cells and involved in chromosome 1q21 abnormalities in B cell malignancy. Immunity 14: 277-289, 2001.
8. Przybylski GK, Dik WA, Wanzeck J, *et al*: Disruption of the BCL11B gene through inv(14)(q11.2q32.31) results in the expression of BCL11B-TRDC fusion transcripts and is associated with the absence of wild-type BCL11B transcripts in T-ALL. Leukemia 19: 201-208, 2005.
9. Nagoshi H, Taki T, Hanamura I, *et al*: Frequent PVT1 rearrangement and novel chimeric genes PVT1-NBEA and PVT1-WWOX occur in multiple myeloma with 8q24 abnormality. Cancer Res 72: 4954-4962, 2012.
10. Pleasance ED, Stephens PJ, O'Meara S, *et al*: A small-cell lung cancer genome with complex signatures of tobacco exposure. Nature 463: 184-190, 2010.
11. Northcott PA, Shih DJ, Peacock J, *et al*: Subgroup-specific structural variation across 1,000 medulloblastoma genomes. Nature 488: 49-56, 2012.
12. Chinen Y, Taki T, Nishida K, *et al*: Identification of the novel AML1 fusion partner gene, LAF4, a fusion partner of MLL, in childhood T-cell acute lymphoblastic leukemia with t(2;21)(q11;q22) by bubble PCR method for cDNA. Oncogene 27: 2249-2256, 2008.
13. Chen YW, Hu XT, Liang AC, *et al*: High BCL6 expression predicts better prognosis, independent of BCL6 translocation status, translocation partner, or BCL6-deregulating mutations, in gastric lymphoma. Blood 108: 2373-2383, 2006.
14. Montanaro L, Treré D and Derenzini M: Changes in ribosome biogenesis may induce cancer by down-regulating the cell tumor suppressor potential. Biochim Biophys Acta 1825: 101-110, 2012.
15. Dai MS and Lu H: Crosstalk between c-Myc and ribosome in ribosomal biogenesis and cancer. J Cell Biochem 105: 670-677, 2008.
16. Li Z and Hann SR: Nucleophosmin is essential for c-Myc nucleolar localization and c-Myc-mediated rDNA transcription. Oncogene 32: 1988-1994, 2013.
17. Grandori C, Gomez-Roman N, Felton-Edkins ZA, *et al*: c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. Nat Cell Biol 7: 311-318, 2005.
18. Stults DM, Killen MW, Williamson EP, *et al*: Human rRNA gene clusters are recombinational hotspots in cancer. Cancer Res 69: 9096-10104, 2009.
19. Uemura M, Zheng Q, Koh CM, Nelson WG, Yegnasubramanian S and De Marzo AM: Overexpression of ribosomal RNA in prostate cancer is common but not linked to rDNA promoter hypomethylation. Oncogene 31: 1254-1263, 2012.
20. Croce CM and Nowell PC: Molecular basis of human B cell neoplasia. Blood 65: 1-7, 1985.
21. MacLeod RA, Nagel S, Kaufmann M, Janssen JW and Drexler HG: Activation of HOX11L2 by juxtaposition with 3'-BCL11B in an acute lymphoblastic leukemia cell line (HPB-ALL) with t(5;14)(q35;q32.2). Genes Chromosomes Cancer 37: 84-91, 2003.
22. Fujimoto R, Ozawa T, Itoyama T, Sadamori N, Kurosawa N and Isobe M: HELIOS-BCL11B fusion gene involvement in a t(2;14)(q34;q32) in an adult T-cell leukemia patient. Cancer Genet 205: 356-364, 2012.
23. Ungar D, Oka T, Brittle EE, *et al*: Characterization of a mammalian Golgi-localized protein complex, COG, that is required for normal Golgi morphology and function. J Cell Biol 157: 405-415, 2002.
24. Velagaleti GV, Tonk VS, Hakim NM, *et al*: Fusion of HMGA2 to COG5 in uterine leiomyoma. Cancer Genet Cytogenet 202: 11-16, 2010.