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Identification of a common microdeletion cluster in 7q21.3 subband among patients with myeloid leukemia and myelodysplastic syndrome

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ABSTRACT

Monosomy 7 and interstitial deletions in the long arm of chromosome 7 (−7/7q−) is a common nonrandom chromosomal abnormality found frequently in myeloid disorders including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and juvenile myelomonocytic leukemia (JMML). Using a short probe-based microarray comparative genomic hybridization (mCGH) technology, we identified a common microdeletion cluster in 7q21.3 subband, which is adjacent to 'hot deletion region' thus far identified by conventional methods. This common microdeletion cluster contains three poorly characterized genes; *Samd9*, *Samd9L*, and a putative gene *LOC253012*, which we named *Miki*. Gene copy number assessment of three genes by real-time PCR revealed heterozygous deletion of these three genes in adult patients with AML and MDS at high frequency, in addition to JMML patients. Miki locates to mitotic spindles and centrosomes and downregulation of Miki by RNA interference induced abnormalities in mitosis and nuclear morphology, similar to myelodysplasia. In addition, a recent report indicated Samd9 as a tumor suppressor. These findings indicate the usefulness of the short probe-based CGH to detect microdeletions. The three genes located to 7q21.3 would be candidates for myeloid tumor-suppressor genes on 7q.

Introduction

Monosomy 7 and interstitial deletions in 7q (−7/7q−) are a common nonrandom chromosomal abnormality found frequently in myeloid disorders. In 1964, prior to chromosome band identification, monosomy 7 was first reported in three patients with refractory anemia as monosomy of a C-group chromosome [1]. Since that time, −7/7q− have been identified in 10–20% of a wide range of myeloid malignancies including MDS, AML, and JMML [2].

Enormous efforts have been made to identify genes responsible for −7/7q−. In the absence of definitive familial cases, the basic strategy for gene hunting began with identifying patients that carried 7q−. Detailed maps of regions deleted from individual patients were then generated from the results of loss of heterogeneity assays or fluorescence *in situ* hybridization. Unfortunately, the cumulative results from thousands of patients were confounded by the fact that the boundaries of commonly deleted regions derived by separate research groups showed a poor degree of overlap [3]. Currently, it is generally accepted that two or more genes near bands 7q22 and/or 7q34 are involved in myeloid tumors.

Microarray-based comparative genomic hybridization (mCGH) technology allows efficient detection of microdeletions (<100 kb) that affect one or a few genes, enabling to search for small 7q deletions that are not visible cytogenetically in marrow cells of MDS/AML patients. Initially, bacterial artificial chromosome (BAC)-based mCGH systems were developed, but this system had limited potential to detect microdeletions because of the long probe size (>100 kb). Thereafter, SNP-array hybridization turned out to be a powerful method for detecting not only single nucleotide polymorphism, but also microdeletions [4]. However, because SNPs tend to cluster within introns and intergenic spaces, SNP-array may bias against the detection of microdeletions in critical genes.

Here, we describe the application of a modified BAC-based mCGH system that uses short (<10 kb) genomic DNA fragments without any repetitive sequences as probes to improve the detection of small deletions and reduce background hybridization. Because repeat-free fragments generally overlap exon-containing regions, this type of probe not only yields a high signal/noise ratio, but also can be useful in determining the copy number of a corresponding gene. Using this system for identification of responsible gene(s) for −7/7q−, we report the isolation of a common microde-

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letion among JMML patients that contains three poorly characterized genes.

Materials and methods

Short probe-based mCGH. This system was similar to that described by others [5]. Briefly, total 292 repeat-free segments (2.7–9.5 kb) were identified using BlastN at the NCBI server (235 probes in 7q21.2–7q31.1, 15 in 4q12, 27 in 20q, and 15 in 21q). Each of these fragments was PCR amplified from human placenta DNA (Clontech, Mountain View, CA) and cloned into the pCR-XL-TOPO vector (Invitrogen, Carlsbad, CA). The primer sets used to amplify probes #14–#16 are listed in Table 1. Sequences of other primer sets are available upon request. Five micrograms of each target DNA, PCR-amplified fragments using universal primers in the vector, was printed on poly-L-lysine coated glass slides (Matsunami Glass, Osaka, Japan) using a spotter (SPBIO, Hitachi Software, Tokyo, Japan). Bone marrow samples were obtained after informed consent and approval from the Institutional Review Board at Hiroshima University. Test samples and reference placenta DNA (2.5 μg) were random-prime labeled with CY3- and CY5-dCTP (GE healthcare), respectively, and then hybridized to the slide. Scanning of microarrays was performed using G2505A scanner (Agilent Technologies, Santa Clara, CA) and signals were analyzed with ArrayVision (GE healthcare).

Cell culture and gene transfer. EOL-1 and MUTZ-3 cells ([6] and references in it) were cultured in RPMI1640 medium with 10% (FBS). 293 and HeLa(tc) [7] cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS). siRNA oligonucleotides (100 nM, otherwise indicated, Table 2) were transfected using Oligofectamine (Invitrogen). C-terminal FLAG-tagged Mikiα or β protein, or MikiΔN-FLAG(C) protein (lacking N-terminal hydrophobic 30 aa of Mikiβ) was expressed using the pcDNA3 expression vector (Invitrogen).

Other experimental procedures and reagents. Copy number assessment by qPCR was performed according to the procedure described [8]. qRT-PCR was performed as previously described [9] using primer sets (Table 1). Immunoprecipitation and immunoblot analyses were performed according to the standard procedures [10] using 2% gelatin as a blocking agent. Immunostaining and image analyses were performed as described [7,11]. Rabbit anti-Miki antibodies was raised against GST-Mikiα (377–462 aa) and affinity purified according to the standard procedures [10].

Table 2
Target sequences for RNAi.

Name	Sequence
siRNA#79	CGGUUGAUGAUCCUGUCAC
siRNA#80	GGAAGACAUUGGGAAUUAC
siRNA#81	AGGCAUUUAUGCAAAUUGAA
shMiki#1	AUGCAUCUCUGCUUAUCAACC
shMiki#2	GAAGGCAAUUACAUCGUGAAG
shMiki#3	UCAGGGAAAUGGAACUCUAUC
shMiki#4	AGAAGACAAUGGACUAUGUGU
shMiki#5	GAAACUCAUUUCACAGUUAUC
shMiki#6	UGACUUCGGAAUAUAUGAAUU

Results

Identification of three candidate myeloid tumor-suppressor genes in a common microdeletion cluster among JMML patients

Two hundred thirty-five probes in a region spanning 21.7 Mb within 7q21.2–7q31.1 and additional 57 control probes in 4q, 20q, and 21q were applied to a search for microdeletions using a short probe-based mCGH system (see Materials and methods). Test (leukemia) and reference DNA samples were labeled with CY3- and CY5-dCTP, respectively, and then hybridized to slides on which probes were printed.

We initially tested whether this system can detect copy number changes in a small region. Genomic DNA extracted from EOL-1 cells, which is known to harbor a deletion spanning 800 Kb between the *Rhe* (*FIP1L1*) gene and the *PDGFα* gene in 4q [12]. All eight probes (#239–#246) that locate within the deletion showed low fluorescence ratios (Fig. 1A, bracket), demonstrating the potential of this system to detect microdeletions. For detection of microdeletion in myeloid leukemia cells, we selected fresh bone marrow samples from adult AML/MDS patients or DNA from myeloid leukemia cell lines that did not show apparent 7q abnormalities. However, as shown, for an example, in Fig. 1B, gross regional copy number changes were still detected, and 'single copy events', which could include both real copy number changes in a small region and noise of the system, were frequently observed, recognized as general problems in detection of microdeletions in leukemia cells [13]. We then applied the microarray CGH system to samples from JMML patients, which is a subtype of MDS and is occasionally associated with monosomy 7 [2]. In contrast to adult MDS/AML pa-

Table 1
Primer sets.

	Forward	Reverse
Probe#14	5′-AACTTCTCCTGACTCCAGTCATAGCTCCTT-3′	5′-ATCCATAGACCTGACATGTGTATCATATCC-3′
Probe#15	5′-GTGGGAATCGTCTACTTCCTGCACTCAAGA-3′	5′-TGATTAAAGACTGGACCAAAGAGCATGTGA-3′
Probe#16	5′-TGCTCACTCAACCGAAATCAATATTGAGAT-3′	5′-ATGCTTTAGGCTCCTAAGCCTTCTTTCCTT-3′
Top2b	5′-CAACTTTTTGCTGGCATCTG-3′	5′-GCTGGAATGTCTGGAAAAGC-3′
Tel	5′-ACAAATCACCGGCCTTCTCCTGACCC-3′	5′-GGCTGGATGGCTTCGGGTGGGACTC-3′
Albumin	5′-AGCTATCCGTGGTCCTGAAC-3′	5′-TTCTCAGAAAGTGTGCATATATCTG-3′
c7orf16	5′-CAGGCCCAGCCTCGGTGAGC-3′	5′-GCACAACCCCGTGCCACCAG-3′
DDC	5′-CTCATGGCTCACGCGTCCAG-3′	5′-CAAGCCGACCTAGGTTGGTG-3′
Cdk6	5′-ACACTGCCTTGTTGGCAAAG-3′	5′-AGGTTTGCAGAATCGAGGCC-3′
Samd9–5′	5′-AACCCAGATATGGCTAATCC-3′	5′-CAGGTCTATGGATGGTTGCC-3′
Samd9–3′	5′-CGTTTACAAGGTCGAGCTGA-3′	5′-CCCAGGTAAAAAGACACCTT-3′
Samd9L	5′-CATTCCTGTGCTTCTCCTTG-3′	5′-GGATTCCGGGATCTCATGCA-3′
Miki-5′	5′-CCTGGTGAGGAACCCTGTCA-3′	5′-TCTCTGTGACTATCCTGGGA-3′
Miki-3′	5′-CAAGGCATTCGGTTTGTAAG-3′	5′-CTCTGGTGAAGCAGAATTCT-3′
CCDC132	5′-AGGATACCCTGGGTCGGCTC-3′	5′-TTCAGCCGCCGCGACTTACC-3′
Col1A2	5′-GCAGTAACCTTATGCCTAGC-3′	5′-GAGAGTCTGCCCTCCAAGTG-3′
Rint-1	5′-GCTGAGTATGTCTGTTGAAG-3′	5′-CCAAACTAGATACAGGTGCC-3′
Lep	5′-GTATCTCCAGGATTGAAGAG-3′	5′-CCCACTCTTTGCTGGGTGGA-3′
Miki(RT)	5′-AACTCTATCTGCCAGTCAGAAG-3′	5′-TTTAGCCATTGGTAAGCTAGCC-3′
HPRT(RT)	5′-CCTCATGGACTAATTATGGACAG-3′	5′-GCAGGTCAGCAAAGAATTTATAG-3′