Frequent $PVT1$ rearrangement and novel chimeric genes $PVT1$-$NBEA$ and $PVT1$-$WWOX$ occur in multiple myeloma with 8q24 abnormality

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**Running title:** Recurrent $PVT1$ rearrangement in multiple myeloma

**Keywords:** multiple myeloma, translocation, 8q24 abnormalities, $PVT1$, $MYC$

**Disclosure of potential conflicts of interest:** No potential conflicts of interest were disclosed.

**Financial support:** This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare of Japan, by a Grant-in-aid for Scientific Research (B) and (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by the National Cancer Center Research and Development Fund.
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Word count: 4020 words

Total number of figures and tables: Four figures, one table, six supplementary figures and one supplementary table
Abstract

Chromosome 8q24 rearrangements are occasionally found in multiple myeloma (MM) and are associated with tumor progression. 8q24 rearrangements were detected by fluorescence in situ hybridization (FISH) in 12 of 54 MM patients (22.2%) and in 8 of 11 MM cell lines (72.7%). The breakpoints of 8q24 in 10 MM patients and in all MM cell lines were assigned to a 360 kb segment, which was divided into four regions: approximately 120 kb centromeric to MYC (5’ side of MYC), the region centromerically adjacent to PVT1 (approximately 170 kb region, including MYC, of 5’ side of PVT1), the PVT1 region, and the telomeric region to PVT1. PVT1 rearrangements were most common and found in 7 of 12 patients (58.3%) and 5 of 8 cell lines (62.5%) with 8q24 abnormalities. A combination of spectral karyotyping (SKY), FISH, and oligonucleotide array identified several partner loci of PVT1 rearrangements, such as 4p16, 4q13, 13q13, 14q32 and 16q23-24. Two novel chimeric genes were identified: PVT1-NBEA in the AMU-MM1 cell line harboring t(8;13)(q24;q13) and PVT1-WWOX in RPMI8226 cell line harboring der(16)t(16;22)ins(16;8)(q23;q24). The PVT1-NBEA chimera in which PVT1 exon 1 was fused to NBEA exon 2 and the PVT1-WWOX in which PVT1 exon 1 was fused to WWOX exon 9 were associated with the expression of abnormal NBEA and WWOX lacking their N-terminus respectively. These findings suggest that PVT1 rearrangements may represent a novel molecular paradigm underlying the pathology of 8q24 rearrangement-positive MM.

Precis: Not only MYC but also PVT1 has a pivotal role in the pathophysiology of multiple myeloma and possibly other tumors with 8q24 abnormalities where Myc resides.
Introduction

Genetic abnormalities play a crucial role in the pathogenesis of various malignancies, including multiple myeloma (MM). The primary cytogenetic abnormalities associated with disease development are either nonrandom chromosomal gains known as hyperdiploid which is characterized by trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19 and 21, or structural rearrangements involving the immunoglobulin heavy chain gene (IGH) located at 14q32.33 (IGH translocation) (1, 2). Secondary cytogenetic abnormalities implicated in disease progression include 8q24 rearrangements, gain of the long arm of chromosome 1 (1q+), and loss of the short arm of chromosome 17 (17p-) (1, 3).

8q24 rearrangements have been identified by conventional cytogenetic analysis in 3.5–5.0% of MM patients (4, 5) and by fluorescence in situ hybridization (FISH) and spectral karyotyping (SKY) in 9.5–20% (6-9). 8q24 rearrangements are frequently associated with advanced disease in MM patients and MM cell lines (10, 11). Ig chromosomal translocations, such as t(8;14)(q24;q32) and t(8;22)(q24;q11), occur in approximately 25% of MMs with 8q24 rearrangements, while non-Ig chromosomal loci, including 1p13, 1p21-22, 6p21, 6q12-15, 13q14 and 16q22, in which no candidate genes have been delineated so far, have also been identified as translocation partners (7, 8, 12, 13). MYC has long been a possible candidate target gene for 8q24 rearrangements; however, many of the breakpoints within 8q24 have been assigned to various regions that encompassed more than 2 Mb centromeric or telomeric to MYC (9, 11). In contrast to the typical Burkitt lymphoma (BL) translocation t(8;14) with breakpoints within the MYC gene (14), rearrangements of plasmacytoma variant translocation 1/Moloney leukemia virus integration-1 locus (PVT1), which is located 57 kb 3’ of MYC, have been identified in variant BL translocations t(8;22) and t(2;8). In the latter translocations, fusion of the constant region of the IG γ or κ chain gene to PVT1 was detected, resulting in a lack of protein production (15).
In this study, 8q24 rearrangements were analyzed in MM patients and cell lines by FISH and SKY combined with oligonucleotide arrays. Results showed frequent PVT1 rearrangements with several partners and novel PVT1-NBEA and PVT1-WWOX chimeric genes.

Materials and Methods

Patients and cell lines

The use of clinical samples was approved by the Institutional Review Board of Kyoto Prefectural University of Medicine. Informed consent was obtained from all patients. Primary samples were obtained from the bone marrow of 53 patients and the lymph node of one patient between April 2005 and January 2011. Eleven MM cell lines, AMU-MM1, KMS-12-BM, KMS-18, KMS-20, KMS-28-PE, KMS-34, AMO1, IM9, LP-1, NCI-H929 and RPMI8226, were also analyzed. AMU-MM1 was established at Aichi Medical University from the tumor cells of the cerebrospinal fluid of a 72-year-old Japanese female patient with IgA-κ MM (16).

FISH analysis

FISH was performed as described previously (17). To assess 8q24 rearrangement patterns and identify the genes involved, three sets of probes were used. The first set of probes was the 8q24 probe-LSI MYC Dual Color, Break Apart Rearrangement Probe. It is consisting of the SpectrumOrange-labeled 5' LSI MYC probe, which begins 119 kb upstream of the 5' end of MYC and extends 266 kb toward the centromere, and the SpectrumGreen-labeled 3' LSI MYC probe, which starts approximately 1.5 Mb 3' of MYC and extends 407 kb toward the telomere (Abbott Japan, Tokyo, Japan). The second set of probes was designed to hybridize to adjacent both sides on the PVT1 gene, defined as PVT1-adjacent (PVT1-A) probe. PVT1-A probe set was comprised of two specific bacterial artificial chromosome (BAC) clones CTD-3066D1, a fragment...
approximately 170 kb in length adjacent to the 5’ end of PVT1, and RP11-628C14, a fragment approximately 190 kb in length adjacent to the 3’ end of PVT1. The third set of probes was the PVT1-spanning (PVT1-S) probe consisting of two BAC clones; CTD-22267H2, a fragment approximately 120 kb in length covering 5’ regions of PVT1, and RP11-164J24, a fragment approximately 190 kb in length covering 3’ regions of PVT1 (Fig. 1A). For interphase analysis, signals were evaluated in a minimum of 100 nuclei with a hybridization efficiency greater than 90%.

**SKY analysis and SKY combined with FISH analysis (SKY-FISH)**

SKY analysis was performed as described previously (18). For SKY-FISH analysis, SKY and FISH probe mixtures simultaneously hybridized to chromosomes for two days at 37°C. Ten to 20 metaphase spreads were analyzed and karyotypes were defined according to ISCN 2009 (19). For complex abnormalities with rearranged 8q24 locus, such as translocations or insertions, which cannot be detected by either FISH or SKY, a SKY-FISH procedure was used to detect chromosomal locations involving MYC and PVT1.

**Genome copy number analysis**

The DNA gain and loss assay based on high-density oligonucleotide microarrays (GeneChip Human Mapping 50K, 250K, or 6.0 SNP array, Affymetrix, Santa Clara, CA) was performed with genomic DNA extracted from cell lines and tumor specimens. Breakpoints in chromosomal translocations were identified by means of genome copy number analysis combined with SKY, and the SNP array data were analyzed to determine total copy numbers using the CNAG3.0 or 3.3 programs (20).
Reverse-transcription polymerase chain reaction (RT-PCR) and sequencing analysis

RT-PCR analysis was performed as described previously (21). The following primers were used:
P1S (forward primer in exon 1 of \textit{PVT1}, NR_003367.1), 5’-TTGCGGAAAGGATGTGCGGCG-3’, and N3A (reverse primer in exon 3 of \textit{NBEA}, NM_015678.3), 5’-GCTCCATATTCTGCTTGACA-3’, for the detection of any chimeric gene on der(8)t(8;13); N2S (forward primer in exon 2 of \textit{NBEA}), 5’-CATACAGGTCGGAGAGGTC-3’, and P2A (reverse primer in exon 2 of \textit{PVT1}), 5’-AGGGCTTCACCGGCTCAAT-3’, or P3A (reverse primer in exon 3 of \textit{PVT1}), 5’-GGGTCTTACATTCCATAGG-3’, for the detection of any chimeric gene on der(13)t(8;13); P1S and W9A (reverse primer in exon 9 of \textit{WWOX}, NM_016373.2), 5’-CAGGGAGATACGGAACCTAC-3’, for the detection of any chimeric gene on der(16)t(16;22)ins(16;8)(q23;q24). The nucleotide sequences of PCR products were determined with the fluorometric method (Dye Terminator Cycle Sequencing Kit, Applied Biosystems).

Real-time quantitative RT-PCR (RQ-PCR)

\textit{NBEA} and \textit{WWOX} mRNA levels were measured with specific primer probe sets from Assays-on-Demand\textsuperscript{TM} (Applied Biosystems, Branchburg, NJ) or SYBR Green method using the ABI Prism 7300 system (Applied Biosystems) according to the manufacturer’s instructions. Primers were Assays-on-Demand NBEA 2-3 (Hs00995629_m1), NBEA 58-59 (Hs00995655_m1), W8S (forward primer in exon 8 of \textit{WWOX}), 5’-GCAACATCCTCTTCTCAACGA -3’, W9A(2) (reverse primer in exon 9 of \textit{WWOX}), 5’-TGGGACAGCACGACAGTACA-3’, W9S (forward primer in exon 9 of \textit{WWOX}), 5’-TGACTGTGCTGTGCTTCCCA-3’ and W9A(3) (reverse primer in exon 9 of \textit{WWOX}), 5’-CCGGTCTTGGCATGACCTCCT-3’. These primer sets were used to distinguish abnormal chimeric \textit{NBEA} or \textit{WWOX} transcripts from normal transcripts: NBEA 2-3, spanning the breakpoint of 8q24 in t(8;13)(q24;q14), can detect only the normal \textit{NBEA} transcript,
While NBEA 58-59 can detect both normal and chimeric transcripts, similarly, combination of W8S and W9A(2) can detect only the normal WWOX transcript and W9S and W9A(3) can detect both normal and chimeric WWOX transcripts. The β-actin mRNA level was measured as an internal control. In addition to the 11 MM cell lines, normal peripheral lymphocytes, an Epstein-Barr virus-transformed B-cell line derived from normal healthy volunteers, the erythroleukemia cell line K562, and the Burkitt lymphoma cell line Daudi were analyzed. Each assay was done in triplicate.

Results

Frequent involvement of PVT1 locus in 8q24 rearrangements

8q24 rearrangements were detected using three FISH probe sets in 12 of the patients (22.2%) and 8 of the cell lines (72.7%). The breakpoints of 8q24 were assigned to a 360 kb segment containing the MYC and PVT1 genes in 10 MM patients and in all MM cell lines, and 11 breakpoint types were identified (Fig. 1, Supplementary Fig. S1, and Table S1). Breakpoint regions at 8q24 could be divided into four regions: approximately 120 kb centromeric to MYC (5′ side of MYC; region A), the region centromerically adjacent to PVT1 (approximately 170 kb region, including MYC, of 5′ side of PVT1; region B), the PVT1 region (region C), and the telomeric region to PVT1 (3′ side of PVT1; region D) (Fig. 1). In MM patients, region C was the most frequent (Fig. 1B), with breakpoints detected within the PVT1 gene in five patients (41.7%) (Pt-1 to 4, and 7). Region B was identified in four patients (33.3%) (Pt-7 to 10), and both regions A and C in two patients (16.7%) (Pt-5 and 6). The remaining two patients (16.7%) (Pt-11 and 12) showed breakpoints outside of these three regions (region D). Among the eight cell lines with 8q24 rearrangements, breakpoints were identified in the PVT1 (region C) in three cell lines (37.5%) (AMU-MM1, LP-1, and KMS-34), in region B in one cell line (12.5%) (AMO1), and in both regions A and C in two cell lines (25.0%) (RPMI8226 and KMS-18). The breakpoints observed in KMS-28-PE and
NCI-H929 were either centromeric or telomeric to MYC and PVT1. SNP array analysis validated the interphase FISH data by showing copy number gains at the regions of interest in KMS-18, RPMI8226 and KMS-28-PE (Supplementary Fig. S2). In summary, breakpoints were assigned to the PVT1 gene in 7 of 54 patients (13.0%) and 5 of 11 cell lines (45.5%), and to the MYC gene in four patients (7.4%) and one cell line (9.1%).

**Partners of 8q24 rearrangement detected by SKY-FISH and genome copy number analysis**

Metaphase analysis of two patients (Pt-1 and 8) and eight MM cell lines by SKY, SKY-FISH and FISH identified various translocation/insertion partner loci for 8q24 rearrangements (Fig. 2 and Supplementary Fig. S3). To detect the candidate genes within partner loci of 8q24 rearrangement, the boundaries of copy number gains and losses in the regions of interest were mapped using SNP arrays (Table 1 and Supplementary Fig. S4). Chromosomal breakpoints, partner genes and 8q24 rearrangement patterns identified five partner loci of PVT1 translocations or insertions, 4p16, 4q13, 13q13, 14q32 and 16q23-24, in five of the eight cell lines and one of the two primary MM cells (Table 1).

**Identification of the PVT1-NBEA and PVT1-WWOX chimeric genes in MM cell lines**

In AMU-MM1 cell line, SKY analysis identified the unbalanced chromosomal translocation t(8;13)(q24;q13), which resulted in two der(8) and one der(13) (Fig. 3A). SNP array analysis clearly demonstrated that the copy number change at 8q24 occurred within the region including PVT1 exon 1 and intron 1 (between the physical positions of 128871130 and 128909458, Fig. 3B). At 13q13, the copy number change occurred within intron 2 of NBEA (between the physical positions of 34477756 and 34734115, Fig. 3B). Based on these results, RT-PCR analysis was performed to detect chimeric products using primers P1S and N3A, and N2S and P3A. Direct
sequencing of the PCR products generated using primers P1S and N3A, and N2S and P3A revealed the fusion of 5'-'PVT1' exon 1 with NBEA exon 3-3', and that of 5'-'NBEA' exon 2 with PVT1' exon 3-3' (Fig. 3C, D and E). The primers N2S and P2A did not yield any PCR product, suggesting the splicing out of PVT1' exon 2 (Fig. 3C and D). In RPMI8226 cell line, SKY-FISH analysis identified der(16)t(16;22)ins(16;8)(q23;q24) (Fig. 3F). SNP array analysis clearly demonstrated that the copy number change at 8q24 occurred within intron 1 of PVT1 (between the physical position of 128891891 and 128902659, Fig. 3G). At 16q23, the copy number change occurred within intron 8 of WWOX (between the physical position of 77399684 and 77401485, Fig. 3G). Based on these results, RT-PCR analysis was performed to detect chimeric products using primers P1S and W9A (Fig. 3H) and direct sequencing of this product revealed the fusion of 5'-'PVT1' exon 1 with WWOX exon 9-3' (Fig. 3I and J). However, these chimeric transcripts were not detected in seven patients with PVT1 rearrangements.

The expression level of PVT1-NBEA and PVT1-WWOX transcripts was measured by RQ-PCR (Fig. 4). High expression of aberrant NBEA was detected in AMU-MM1 cell line using the NBEA 58-59 primer probe set. In contrast, no expression of normal NBEA was detected using the NBEA 2-3 primer probe set in AMU-MM1, while normal NBEA was detected in all other cell lines where it was expressed at similar levels. These results indicate that the 5'-'PVT1-NBEA-3' chimeric transcript is highly expressed in AMU-MM1 cells. Similarly, expression of WWOX exon 9 was higher than WWOX exon 8-9 in RPMI8226, suggesting that a part of expression of exon 9 was due to PVT1-WWOX chimeric transcript.

Discussion

The present study uncovered two aspects of molecular genetics in MM. The first finding is the frequent rearrangement of PVT1 gene in MM harboring 8q24 rearrangements. PVT1 is the human
homolog of the mouse Pvt1 oncogene, which was originally identified as a common retroviral integration site in murine leukemia virus (MLV)-induced T-lymphomas. PVTI/Pvt1 is also involved in variants t(2;8), t(8;22) or t(8;14) in human BLs and in variant t(6;15) in mouse plasmacytomas (10, 15, 22-25). Moreover, amplification of the PVTI gene has been observed in several cancers including lymphomas (26-30), and PVTI overexpression was found to contribute to the suppression of apoptosis (31). The PVTI locus is thought to encode several microRNAs important in oncogenesis (32). Hence, the current study suggests that PVTI is one of the target genes of rearrangement that may be responsible for driving MM, while other studies have shown breakpoints within the region centromeric to PVTI (9-11, 33).

The second finding is the novel chimeric genes, PVTI-NBEA and PVTI-WWOX, in the MM cell lines, AMU-MM1 cell line with t(8;13)(q24;q13) and RPMI8226 cell line with der(16)t(16;22)ins(16;8)(q23;q24), resulting in high expression of the abnormal chimeric transcript. In PVTI-NBEA, the breakpoint of PVTI was found within intron 1, and PVTI exon 1 was found to be fused to NBEA exon 3, resulting in the loss of the start codon in NBEA exon 2. In this setting, the second ATG in exon 3 of the NBEA gene may function as a start codon to produce a putative abnormal NBEA protein lacking 107 N-terminal amino acids (Fig. 3E), although the expression of PVTI-NBEA at the protein level remains to be investigated. Although knockdown of PVTI-NBEA using siRNA could not inhibit proliferation and induces cell death (Supplementary Fig. S5), it would be important to investigate the function of this NBEA chimera in MM tumorigenesis, since NBEA modulates signal transduction and vesicular trafficking in neurons and other cells and since gene abnormality and aberrant expression of NBEA have been associated with plasma cell dyscrasias (34-36). In addition, the association between the PVTI-NBEA fusion gene and the t(8;13) chromosomal abnormality, which has been reported in a small population of MMs, remains to be verified (7, 11, 37, 38). Chromosome 13 is often deleted in MM and this has been linked to
poorer prognosis. In such cases with loss of chromosome 13, *RB1* is thought to be a major target and driver. However, a group has reported *NBEA* to also be a target of recurrent interstitial deletions at 13q13 and proposed that *NBEA* might be a tumor suppressor gene in MM (36). *WWOX* is generally considered to be a candidate tumor suppressor gene, and known to have a proapoptotic effect by participating in the tumor necrosis factor (TNF) apoptotic pathway and via direct physical interaction with p53 and its homolog p73 (39). However, immunohistochemistry revealed that *WWOX* protein level were rather elevated in gastric and breast carcinoma (40). Therefore, *WWOX* seemed not to act as tumor suppressor gene simply. Interestingly, although both *NBEA* and *WWOX* are located at common fragile site, usually contributing to gene inactivation, FRA13A and FRA16D respectively, these genes highly express via fusion to *PVT1* (41, 42). It would be important to further elucidate the function of *NBEA* and *WWOX*. Several translocation/insertion partners of 8q24 rearrangements were identified in the remaining samples (Table 1). The genes located at translocation/insertion breakpoints, identified by copy number analysis, have been frequently associated with cancer (43-49). No chimeric genes could be cloned in other samples. In Pt-1, LP-1, and KMS-18, *PVT1* was translocated to the regions of *EPHA5*, *MMSET* or *ATG2B* with opposite direction of transcription, suggesting that these genes are not involved in the fusion with *PVT1*. In KMS-34, AMO1, Pt-8, KMS-28PE and NCI-H929, genome copy number changes were not identified within a single gene at 8q24 and/or partner loci (Supplementary Fig. S4). The translocation/insertion partner breakpoints identified in the present study warrant further molecular analysis of the candidate genes.

The relationship between *MYC* and *PVT1* in terms of MM development and progression is difficult to elucidate. RQ-PCR analysis revealed high expression of *PVT1* and *MYC* in most MM cell lines regardless of *PVT1* or *MYC* rearrangement status (Supplementary Fig. S6). Results demonstrated that 8q24 rearrangements included various complex translocations with either deletion...
or insertion of a part of the 8q24 segment including MYC and the centromeric segment of PVT1. Additionally, gains of chromosome 8, including PVT1 and MYC, are frequently identified in MM cell lines (Fig 1B, Fig. S1B). These gains are likely to contribute to the amplification of the PVT1 and MYC genes (28). Beyond chromosomal abnormalities, the molecular mechanisms underlying MYC overexpression in MM, such as interaction with PVT1 or deregulation of microRNAs, warrant further research (32, 50).

In conclusion, PVT1 is frequently involved with various partner loci in MM with 8q24 abnormalities, and, PVT1-NBEA and PVT1-WWOX were identified as novel, highly expressed chimeric genes in which NBEA and WWOX are fused with PVT1 in MM cell lines harboring t(8;13)(q24;q13) and der(16)t(16;22)ins(16;8)(q23;q24), respectively. These findings suggest that PVT1 rearrangements may represent a novel molecular paradigm underlying the pathology of MM with 8q24 rearrangements.

Acknowledgments

The authors wish to thank Kayoko Kurita and Akari Kazami for their expert technical assistance.
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Figure Legends

Figure 1. Identification of breakpoints region at 8q24 by FISH analysis

(A) Location of FISH probes and mapping of putative breakpoints at 8q24. FISH probes are depicted as color bars. Vertical arrows indicate breakpoints and horizontal double-headed arrows indicate the possible range of breakpoints based on interphase FISH analysis. The combination of the FISH analysis with three sets of probes reveals four breakpoint regions and 11 breakpoint types as shown in Fig. 1B and Supplementary Fig. S1. (B) Mapping of breakpoints in MM patients and cell lines with 8q24 rearrangement. Dark gray boxes, the breakpoint regions; light gray boxes, duplicated regions. Pt, patient number. Breakpoint region A is the 120 kb length region of centromeric to MYC, B is the 170 kb length region centromerically adjacent to PVT1 including MYC, C is the PVT1 region and D is the region of telomeric to PVT1. *Frequency shows the case number for each of the breakpoint regions.

Figure 2. Detection of partner loci of 8q24 rearrangements by metaphase analysis

(A) Metaphase FISH with PVT1-A probe (left), inverted DAPI staining (middle), and SKY-FISH (right) analysis of KMS-18. Red signals from the PVT1-A probe were detected on chromosome 14 by SKY-FISH (red arrows). Yellow arrows indicate fusion signals of red and green signals. Scale bar represents 10 μm. (B) Partial karyotype of 8q24 rearrangement-positive samples using SKY-FISH. Red and green signals derived from the 8q24 (KMS-28-PE) or PVT1-A probes (other cell lines and clinical samples) are shown on each chromosome. Partial karyotype demonstrates t(8;13)(q24;q13) in AMU-MM1, t(4;8)(p16;q24) in LP-1, t(8;16)(q24;q23-24) in KMS-34, t(8;14)(q24;q32) in KMS-18, der(16)t(16;22)ins(16;8)(q23;q24) in RPMI8226, t(8;14)(q24;q32) and t(8;15)(q24;?) in AMO1, t(8;14)(q24;q32) in KMS-28-PE, t(8;20)(q24;q11) in NCI-H929, t(4;8)(q22;q24) in Pt-1, and t(6;8)(p25;q24) and t(8;19)(q24;p13) in Pt-8.
In KMS-34, interphase FISH signal pattern was YYYRR (Supplementary Table S1), however one of red signals could not be detected in SKY-FISH analysis because of few metaphases in KMS-34.

Figure 3. Identification of PVT1-NBEA and PVT1-WWOX chimeric genes in MM cell lines

(A) SKY analysis of AMU-MM1 reveals a complex karyotype including t(8;13)(q24;q13) as 46,X,-X,+der(1;19)(q10;p10),der(2)t(2;17)(q37;q11.2),der(7)(qter→q11.2::p15→q11.2::?), t(8;13)(q24;q13),+der(8)t(8;13),del(12)(p11.2),-13,der(19)t(1;19)(q12;p13)×2,del(20)(p13). Arrow, breakpoint of der(8)t(8;13)(q24;q13); arrow head, breakpoint of der(13)t(8;13)(q24;q13). (B) Copy number changes at 8q24 (PVT1) and 13q13 (NBEA) detected by SNP array. Primers used to detect chimeric transcript. Y-axes indicate the linear scale corresponding to genome copy number of each chromosome. However, copy number data have never been corrected for the influence of tumor cell percentage or real copy number of some chromosomes. (C) Detection of PVT1-NBEA and NBEA-PVT1 chimeric transcripts by RT-PCR. (D) Sequencing of chimeric junction of PVT1-NBEA and NBEA-PVT1 chimeric transcripts. (E) Putative structure of abnormal NBEA fusion transcript. PVT1-NBEA lacks a start codon in NBEA exon 2; the second ATG in exon 3 might function as a start codon, resulting in an abnormal NBEA protein lacking its N-terminus. *ATG indicates start codon. (F) SKY-FISH analysis of RPMI8226 reveals a complex karyotype including der(16)t(16;22)ins(16;8)(q23;q24). Arrows indicate red signals of PVT1-A inserting to t(16;22). It is difficult to detect the 8q24 locus on der(16) using conventional cytogenetic technique. Scale bar represents 10 μm. (G) Copy number changes at 8q24 (PVT1) and 16q23 (WWOX) detected by SNP array. Primers used to detect chimeric transcript. Y-axes indicate genome copy number. (H) Detection of PVT1-WWOX chimeric transcript by RT-PCR. (I) Sequencing of chimeric junction of PVT1-WWOX chimeric transcript. (J) Putative structure of abnormal WWOX fusion transcript.
Figure 4. Expression of *NBEA* and *WWOX* in cell lines

(A) RQ-PCR analysis showing that the abnormal *NBEA* transcript is highly expressed in the AMU-MM1 cell line compared to other MM cell lines, normal lymphocytes, an EBV-transformed B-cell line, a leukemia cell line (K562) and a Burkitt lymphoma cell line (Daudi). Dotted and black bars indicate expression levels using the NBEA 2-3 and NBEA 58-59 primer sets, respectively. (B) RQ-PCR analysis showing that the abnormal *WWOX* transcript is relatively highly expressed in the RPMI8226 cell line compared to other cell lines. Dotted and black bars indicate expression levels using the WWOX 8-9 and WWOX 9 primer sets, respectively.
Table 1. Partners of 8q24 rearrangements and candidate genes

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<td>MYC</td>
<td>Translocation</td>
<td>14q32, No.15</td>
<td>IGH, No.15</td>
<td>TRAF3/TDRD9, TUBGCP5</td>
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<tr>
<td>Pt-8</td>
<td>MYC</td>
<td>Translocation</td>
<td>6p25, 19p13</td>
<td>IRF4, EFNA2/MUM1/GNG7</td>
<td>RNF8/FTSJD2, EFNA2/MUM1/GNG7</td>
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<tr>
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<td>Unknown</td>
<td>Translocation and gain</td>
<td>14q32</td>
<td>IGH</td>
<td>- a</td>
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<tr>
<td>NCI-H929</td>
<td>Unknown</td>
<td>Translocation</td>
<td>20q11</td>
<td>MAFB</td>
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</tbody>
</table>

NA, not available.

a No copy number change was found at chromosome 16 in KMS-34. The rearranged gene could not be identified, because there were too many genes within the region where copy number change occurred in KMS-28-PE (Supplementary Fig. S4).

b No gene has been reported as affected in MM.

c Duplication of both the MYC locus and centromeric part of PVT1 was detected in RPMI8226 and KMS-18 with PVT1 rearrangement.
**Figure 1.**

**A**

Chromosome 8

![Chromosome 8 Diagram](image)

BDP1 breakpoints:
- **PVT1-A**: CTD-3066D1 (~170kb - 266kb)
- **PVT1-S**: CTD-2267H2 (~120kb - 190kb)

**B**

<table>
<thead>
<tr>
<th>Patients and cell lines</th>
<th>Breakpoint types</th>
<th>Mapping of breakpoints and duplicated regions</th>
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</thead>
<tbody>
<tr>
<td>AMU-MM1</td>
<td>TP-1</td>
<td>MYC</td>
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<tr>
<td>Pt-3</td>
<td>TP-1</td>
<td>PVT1</td>
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<td>Pt-4</td>
<td>TP-1</td>
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<tr>
<td>LP-1</td>
<td>TP-2</td>
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<tr>
<td>Pt-1</td>
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<tr>
<td>Pt-2</td>
<td>TP-3</td>
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<td>Pt-7</td>
<td>TP-1 / TM</td>
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<tr>
<td>RPMI8226</td>
<td>DP-1</td>
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<tr>
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<td>Pt-5</td>
<td>DP-2</td>
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<td>DP-3</td>
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<td>TM</td>
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<td>Pt-9</td>
<td>TM</td>
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<td>Pt-10</td>
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<tr>
<td>Pt-11</td>
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Breakpoint regions:
- **A**
- **B**
- **C**
- **D**

<table>
<thead>
<tr>
<th>Frequency</th>
<th>A</th>
<th>B</th>
<th>C</th>
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Figure 2.

A

B

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<td>8 16 17</td>
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<td>1 4 8</td>
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<td>6 8 19</td>
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<table>
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<tr>
<td>8</td>
<td>7 8 14</td>
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<table>
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</tbody>
</table>
Recurrent PVT1 rearrangement in multiple myeloma

Figure 3.
Figure 4.

A

NBEA exon 2 - 3
NBEA exon 58 - 59

B

WWOX exon 8 - 9
WWOX exon 9

MM cell lines
Frequent PVT1 rearrangement and novel chimeric genes PVT1-NBEA and PVT1-WWOX occur in multiple myeloma with 8q24 abnormality


Cancer Res Published OnlineFirst August 6, 2012.

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Author Manuscript Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.