**Promiscuous Structural Variants Drive Myeloma Initiation and Progression**

P. Leif Bergsagel and W. Michael Kuehl

**Summary:** A comprehensive genomic analysis of structural variants in multiple myeloma in this issue highlights the key role of these events, involving primarily the immunoglobulin heavy chain locus in disease initiation and the MYC locus in disease progression. However, the current study reveals the large number of genomic hotspots, oncogenes, tumor suppressor genes, and recombination mechanisms that contribute to multiple myeloma heterogeneity.

See related article by Rustad et al., p. 258 (1).
it would be interesting to know whether the structures of MYC with t(11;14) translocations (9). The current article shows 8;11 breakpoints in two primary multiple myeloma tumors. We also note that others have used an IGH super-enhancer and tion products containing 3′ sequences flanking both breakpoints, resulted in transloca-

between der14 t(11;14) and chromosome 8, with duplicated chromosome 8 sequences including MYC; for (8) cell lines, there was a der14 t(11;14) presumptive primary translocation, with one of the 19 IGH breakpoints similar to canonical IGH translocations. This is an interesting but not totally convincing suggestion. However, if this is correct, then other mechanisms that increase MAP3K14 activity, for example, deletions of BIRC2 and BIRC3 or TRAF3 might also be initiating events.

Recurrent intrachromosomal copy-number abnormalities (CNA) represent an important oncogenic event in multiple myeloma, and they reported that 83% of CNAs are attributed to a specific structural variant. The authors conclude that at least one driver IGH is present in 47% of all chromothripsis events, 42% of chromoplexy events, and 28% of all templated insertions involving two or more chromosomes. As expected, chromothripsis was associated with copy-number gain and copy-number loss, chromoplexy with copy-number loss, and templated insertions with copy-number gains. Excluding structural events involving immunoglobulin loci, structural variants that affected copy number had the strongest average effect on expression, with an increase or decrease in expression for each gain or loss of a gene copy.

The authors identified 68 recurrent structural variant hotspots not involving immunoglobulin loci or known fragile sites. Hotspots were defined using a piecewise constant algorithm comparing local structural variant breakpoint density with an empirical background model. Gain-of-function hotspots (n = 49), defined by the presence of copy-number gains and translocation-type events, were associated with templated insertions and tandem duplication. These included well-defined multiple myeloma oncogenes (MYC, CCND1, NSD2/PGRF3, IRF4, and MAP3K14) and novel putative driver genes that are targets of therapy (TNFRSF17, SLAMF7, and MCL1). In addition, several gain-of-function hotspots lacking an oncogene were located near enhancers, many of which have been reported to be hijacked by MYC (FAM46C, FBXW7, FOXO3, TXNDC5, NSMCE2, and FCHSD2). They also identified 19 loss-of-function hotspots, defined by copy-number loss, typically resulting from single deletion of tumor suppressor genes (CDKN2C, SP3, SP140, RPL5, CDKN2A, CYLD, RB1, CDKN1B, MAX, TRAF3, TP53, and KDM6A).

The current study highlights the importance of whole-genome sequencing for the detection and classification of structural variants in multiple myeloma. As the authors note, future studies should use greater sequencing coverage to identify subclonal mutations and follow their course over time in patients that have not yet progressed to multiple myeloma, and in those in stable remission, at risk for relapse. When whole-genome sequencing structural variant analysis is combined with RNA-sequencing expression analysis, it becomes evident that structural variants are major

**Figure 1.** A, Model for possible resolutions of a double-strand break with duplicated sequences. A double-strand break (1), with generation of duplicated sequences “cded” on both sides of the breakpoint (2), can be repaired with generation of tandem duplicated sequence (3), insertion of an unrelated piece of DNA (4), and reciprocal translocation without (5) or with duplication “UVWX” on the partner chromosome (6). B, Insertion of an 11;14 fragment containing 3′ IGH enhancer (3′E) 11q13 breakpoint, but not CCND1 gene, in the KMS12 multiple myeloma cell line. C, Insertion of an 11;14 fragment containing the 3′ IGH enhancer 11q13 breakpoint, but not CCND1 gene, and also a chromosome 8 fragment containing the MYC gene between duplicated sequences on chromosome 4. For B and C, chromosome 8 is blue, chromosome 14 is green, chromosome 11 is orange, and chromosome 4 is red with duplicated sequences underlined and a possible super-enhancer (SE) indicated.

an 11;14 sequence containing the 3′ IGH super-enhancer, but not the CCND1 gene. For the KMS12 (Fig. 1B) and MM.M1 (8) cell lines, there was a der14 t(11;14) presumptive primary translocation and insertion of an 11;14 fragment between duplicated chromosome 8 sequences including MYC; for each cell line, precisely the same unique chr11:14 breakpoint was found on the der14 t(11;14) chromosome and the chromosome 8 with the inserted fragment. The MOLP8 cell line was more complex with an 11;14 fragment from the der14 t(11;14) and a chromosome 8 fragment containing MYC inserted between duplicated chromosome 4 sequences (Fig. 1C). For the Karpas620 (8) cell line, a translocation between der14 t(11;14) and chromosome 8, with duplicated sequences flanking both breakpoints, resulted in translocation products containing 3′ IGH super-enhancer and MYC sequences on both translocation products. We also note that others have used an IGH capture procedure that identified 8;11 breakpoints in two primary multiple myeloma tumors with t(11;14) translocations (9). The current article shows that templated sequences often are associated with MYC or CCND1 sequences, but not NSD2 sequences. Therefore, it would be interesting to know whether the structures of templated sequences associated with the CCND1 locus in the current article are similar to the four multiple myeloma cell lines described above (8).
contributors to dysregulated gene expression in multiple myeloma and a critical piece of the puzzle to understand disease pathogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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