LYMPHOID NEOPLASIA

The E3 ubiquitin ligase *UBR5* is recurrently mutated in mantle cell lymphoma

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Key Points

- *UBR5* is recurrently mutated in mantle cell lymphoma with a C-terminal cluster of deleterious mutations.

We have recently reported the application of RNAseq to mantle cell lymphoma (MCL) transcriptomes revealing recurrent mutations in *NOTCH1*. Here we describe the targeted resequencing of 18 genes mutated in this discovery cohort using a larger cohort of MCL tumors. In addition to frequent mutations in *ATM*, *CCND1*, *TP53*, and *NOTCH1*, mutations were also observed recurrently in *MEF2B*, *TRAF2*, and *TET2*. Interestingly, the third most frequently mutated gene was *UBR5*, a gene encoding a 2799aa protein, with multiple functions, including E3 ligase activity based on a conserved cysteine residue at the C-terminus. Nonsynonymous mutations were detected in 18% (18/102) of tumors, with 61% of the mutations resulting in frameshifts in, or around, exon 58, predicted to result in the loss of this conserved cysteine residue. The recurrence and clustering of deleterious mutations implicate *UBR5* mutations as a critical pathogenic event in a subgroup of MCL. (*Blood*. 2013;121(16):3161-3164)

Introduction

Mantle cell lymphoma (MCL) accounts for 7% of non-Hodgkin lymphomas and represents a particularly challenging disease with patient outcomes inferior to most other lymphoma subtypes.[1,2] MCL is characterized on the molecular level by the hallmark t(11;14)(q13;q32) translocation that results in overexpression of cyclin D1, and by frequent additional cytogenetic alterations.[3] The complete mutational landscape has not been described to date. Using RNAseq as a discovery tool, we recently described recurrent *NOTCH1* and *CCND1* mutations.[4] Herein we describe the targeted resequencing, in a larger cohort of patients, of mutated genes identified by RNAseq. We report frequent mutations in *UBR5*, a gene encoding an E3 ubiquitin-protein ligase that has not been implicated, thus far, in lymphomagenesis.[5]

Materials and methods

Sample selection and targeted capture sequencing

RNAseq was performed on the RNA from the tumors of 18 patients and 2 MCL cell lines (Mino and Jeko-1), as reported by Kridel et al.[4] We selected 92 diagnostic MCL samples (supplemental Table 1), including 8 samples from patients that were part of the RNAseq discovery set, for targeted resequencing. A capture protocol was used to select specific target regions from a constructed multiplexed library of genomic DNA utilizing complementary DNA (cDNA) clones and PCR amplicons as baits (supplemental Table 2).

For the description of mutational recurrence and patterns, all 102 clinical cases were included. The study was approved by the University of British Columbia–British Columbia Cancer Agency Research Ethics Board and was conducted in accordance with the Declaration of Helsinki.

Variant calling and validation

Short read sequences were aligned using Burrows-Wheeler Aligner.[6] To identify mutations, the Genome Analysis Toolkit v3 was used in adherence to the recommended best practices.[7] Mutation calls have been validated using Sanger sequencing of genomic and/or cDNA, including constitutional DNA for 37 cases. cDNA sequence flanking exon 58 of UBR5 was analyzed in all 102 cases (supplemental Figure 1).

Results and discussion

As previously reported,[4] we performed RNAseq on the MCL tumors from 18 patients and 2 MCL cell lines. In addition to the novel mutations reported for *NOTCH1* and *CCND1* and known somatic variants in *ATM* and *TP53*, mutations were also discovered in other genes involved in cell cycle, proliferation, and DNA damage response (eg, *TRAF2*, *CDKN2A*, *CHEK2*). To determine the incidence and spectrum of mutations in these genes, we performed targeted resequencing of 92 MCL tumors focusing on 18 genes (supplemental Table 2), based on preliminary results from the discovery cohort and proposed gene function. The total capture space was 335 512 bp of genomic DNA including exons, adjacent intronic sequence and untranslated regions (supplemental Table 2). Massively parallel genomic sequencing was performed achieving

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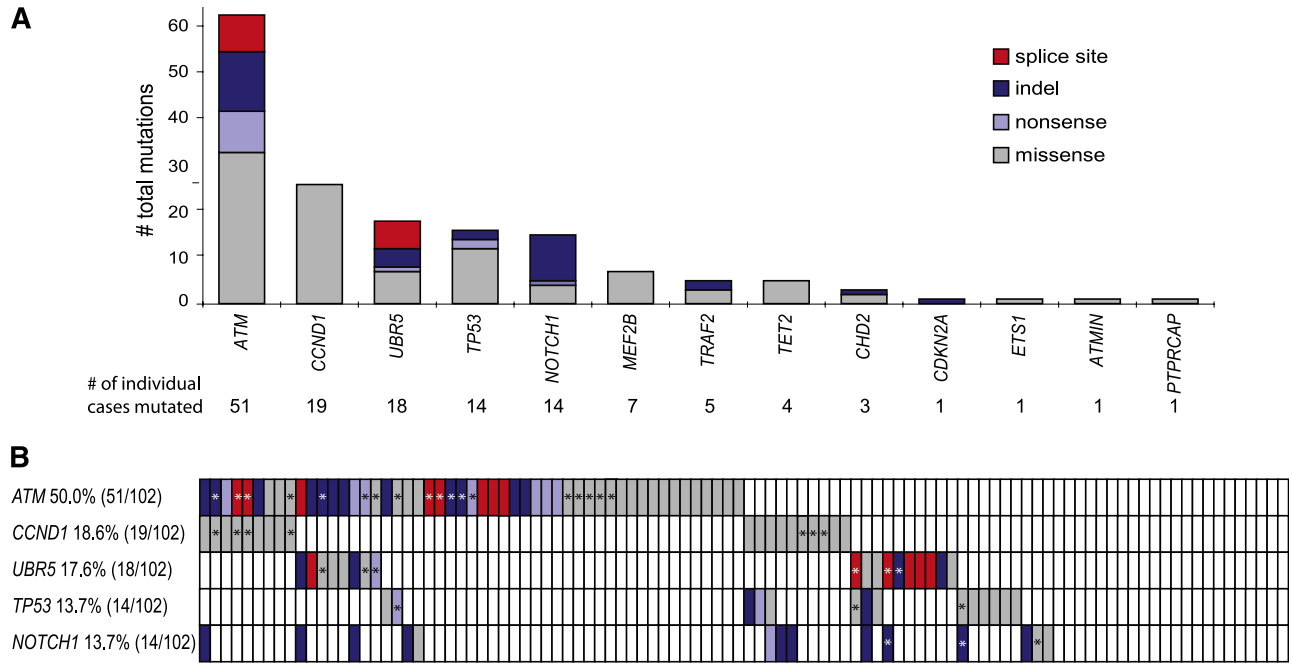

Figure 1. Mutations discovered by massive parallel sequencing of 102 MCL cases. (A) Total numbers of nonsynonymous mutations confirmed in 13 target genes analyzed. Next to the well-known and previously described mutations in *ATM, TP53,* and *NOTCH1*, *UBR5* has the highest percentage of deleterious mutations including 7 splice-site affecting mutations. (B) The schematic represents the status of the 5 most recurrently mutated genes (rows) in MCL in 102 patients (columns) analyzed (red: splice site mutation; dark blue: indel; light blue: nonsense mutation; gray: missense mutation; and white: wild type. Asterisks highlight mutations validated as being somatic by Sanger sequencing.

a mean coverage depth of 50× per sample, with 80% of targets covered at a depth of >16× (supplemental Figure 2).

Combining the results from the 92 cases that were part of the targeted capture experiment and 10 additional cases from our RNAseq discovery set, we analyzed sequence alterations in 102 clinical MCL tumors. In 13 genes, we identified a total of 162 nonsilent variants including 32 indels, 102 missense, 13 nonsense, and 15 splice mutations (Figure 1A and supplemental Table 3). Constitutional DNA was available for 37 of the 102 cases and we were able to confirm that all 51 mutations present in these cases were somatic. The mutational frequencies of *ATM, TP53, CCND1,* and *NOTCH1* were consistent with previously published reports.[4,8-12] *ATM* was mutated in 50% (51/102) of MCL cases with a total of 63 nonsilent mutations observed: 9 nonsense mutations, 13 indels, 8 frameshift-inducing splice-site mutations, and 33 missense mutations. In *CCND1*, 26 nonsynonymous mutations were identified in 19 of 102 cases (19%), all clustering in exon 1 (supplemental Table 3). In addition to those missense mutations, we found 60 synonymous mutations spanning a region of less than 600 bp including the 5′ UTR, exon 1, and an adjacent 150 bp sequence of intron 1 (supplemental Table 4). The proximity of *CCND1* to the *IGH* enhancer locus as a result of the translocation t(11;14), the clustering of variants within 2 kb of the transcriptional start site, and a high ratio of transitions in ratio to transversions (52:34) all suggest that these mutations arose through somatic hypermutation.[13] In addition to confirming recurrent somatic mutations in known targets, mutations were observed in genes not previously reported to be mutated in MCL. *MEF2B*, *TRAF2*, and *TET2* were found to be mutated in 7%, 5%, and 4% of cases, respectively (supplemental Table 3). No mutations were seen in *CHEK1*, *CHEK2*, *CD14*, *CUL1*, and *RB1* in clinical specimens.

Interestingly, 18% (18/102) of the tumors had mutations in *UBR5*, a gene not previously described as being mutated in lymphoma, making it the third most frequently mutated gene in MCL. We initially found 1 *UBR5* nonsense mutation (W2725Stop) and 1 *UBR5* missense mutation (H1932P) in 18 clinical by RNAseq, both validated as being somatic. The capture sequencing approach further identified 2 1-base pair deletions, 5 splice-site, and 6 missense mutations in *UBR5*. The effect of splice-site mutations was investigated by sequencing corresponding cDNA samples, and all mutations were shown to cause defective splicing leading to frameshift and premature stop codons in the coding sequence. All deleterious (those predicted to produce a truncated protein) mutations in *UBR5* gene were clustered within less than 100 bp of genomic sequence including exon 58 (Figure 2A). To identify more complex mutations within this region potentially missed by large scale sequencing approaches, we sequenced the cDNA from all cases. This resulted in the discovery of 3 additional mutations: 1 splice-site mutation, 1 4-bp deletion (missed by previous transcriptome sequencing analysis), and 1 38-bp intron retention causing a frameshift within exon 58. In summary, we found 11 deleterious and 7 missense mutations in *UBR5* in 18 of the 102 cases analyzed. Six of 18 mutations (33%) involve splice sites inducing frameshifts, an event almost 3 times higher than comparable mutations in *ATM* (8/63). The distribution of frequently mutated genes in MCL across all clinical cases is displayed in Figure 1B, highlighting the mutual exclusivity of *CCND1* and *UBR5* in our cohort. We did not observe any statistically significant differences of patient and disease characteristics between patients harboring wild-type and mutated *UBR5* (supplemental Table 1).

The significant recurrence and clustering of deleterious mutations implicate *UBR5* mutations in the pathogenesis of MCL. The *UBR5* gene is located on chromosome 8q22.3 encoding a 309 kDa homologous to E6-AP carboxyl terminus (HECT)-domain E3 ubiquitin ligase.[14] HECT ligases reversibly bind ubiquitin via a conserved cysteine residue at amino acid position 2768 in the UBR5 protein, and directly transfer ubiquitin to the substrate. Several substrates

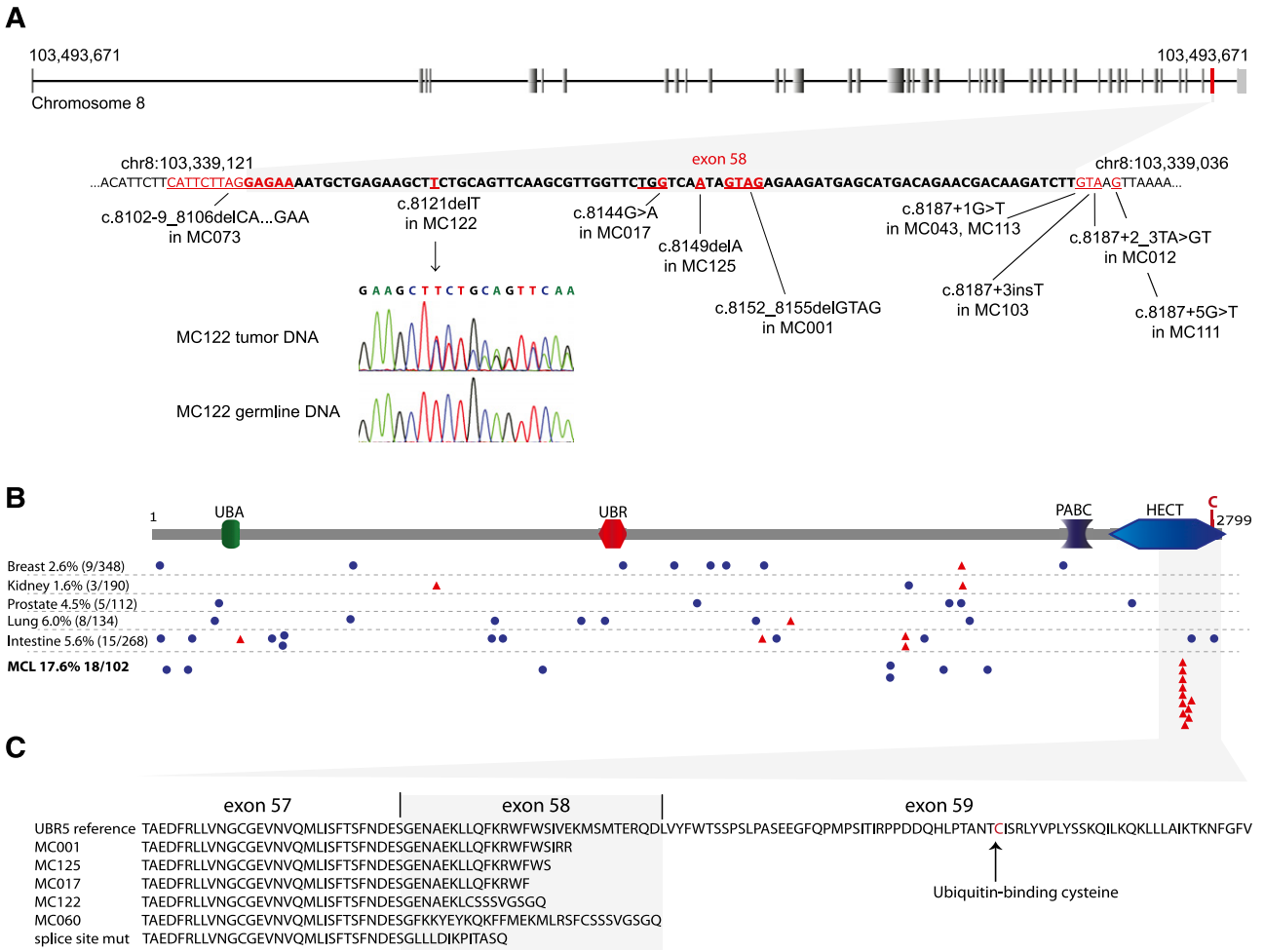

Figure 2. Details of *UBR5* mutations observed in MCL and mutations reported in other cancers. (A) Top panel: intron-exon structure of *UBR5* with Exon 58 highlighted in red. Bottom panel: details of deleterious mutations in the *UBR5* gene are shown. All mutations are clustering within the genomic region of exon 58, the second last exon of the gene. The DNA sequence of exon 58 is indicated in bold. The chromatogram is showing a heterozygote 1-base pair deletion in MC122, the corresponding germline DNA sequence confirms this mutation as somatic alteration. (B) UBR5 protein structure with its ubiquitin-associated domain (UBA), UBR box (a zinc finger-like domain), polyadenylate-binding protein C terminus domain (PABC), and a C-terminal HECT domain. Mutations reported in other cancers[23] are present in lower frequency and scattered throughout the protein. Blue circles: missense mutations; red triangles: deleterious mutations. All deleterious mutations found in MCL cluster within the C-terminal part of the HECT domain, upstream of the conserved cysteine residue at amino acid position 2768. (C) Protein alignment of truncated UBR5 mutant versions predicting a loss of ubiquitin-binding and transfer function.

including KATNA1 (katanin p60), TOPBP1, and PAIP2 have been shown to be ubiquitinated specifically by UBR5.[15-17] Accumulation of katanin p60 due to knockdown of *UBR5* leads to accumulation of tetraploid populations and polyploidy in affected cells.[16] In addition to E3 ligase function, multiple protein-protein interactions with UBR5 have been described, suggesting roles in DNA damage response,[18] cell-cycle control,[19,20] miRNA-mediated gene silencing,[21] and chromatin ubiquitination.[22] In contrast to *UBR5* mutations found in other cancers,[23] all deleterious mutations discovered in MCL cluster within the HECT domain, predicted to result in the loss of the conserved cysteine residue (Figure 2B-C). Functional studies are in progress to determine the specific role of these heterozygous truncating mutations in the pathogenesis of MCL.

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Authorship

Contribution: B.M. and R.K. designed and performed the research, analyzed and interpreted data, and wrote the paper; R.S.L. and S.R. analyzed sequencing data; K.T. designed research; D.W.S. designed experiments and wrote the paper; R.M., A.J.M., and M.A.M. oversaw data collection and analysis; J.M.C. curated the lymphoma database, reviewed the manuscript, and provided editorial input; and C.S. and R.D.G. participated in the design of the original project, reviewed the manuscript, and provided editorial input.

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