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The cysteine-rich domain of TET2 binds preferentially to mono- and dimethylated histone H3K36

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Missense mutations in Ten-eleven translocation 2 (TET2) gene are frequently found in leukaemia patients. Although mutations span the entire coding region, they tend to cluster in the C-terminal enzymatic domain and a cysteine-rich (CR) domain of unknown function. Herein, we found the CR domain binds chromatin preferentially at the histone H3 tail by recognising H3 lysine 36 mono- and dimethylation (H3K36me1/2). Importantly, missense mutations in the CR domain perturbed TET2 recruitment to the target locus and its enzymatic activities. Our findings identify a novel H3K36me recognition domain and uncover a critical link between histone modification and DNA hydroxylilation in leukaemogenesis.

Keywords: epigenetics; TET2; leukaemia; histone H3K36 methylation; histones < chromosomes.
CR domain was prepared and incubated with biotinylated histone H3 peptides methylated at various lysine residues by microscale thermophoresis. CRTET2 showed preferential binding to histone H3K36me1/2 (see online supplementary material for Table). We then simulated the binding mechanisms between H3 peptides and TET2 using CABS-dock (7). Interestingly, H3 (31–40) peptides appeared to interact with the DNA recognition pocket of CRTET2 (see online supplementary material for a colour version of Fig. S2), which is frequently mutated in leukaemia patients (1). W1291 was predicted to lie close to H3K36 (Fig. 1F). Because the W1291R mutation is often found in leukaemia patients (1), we next tested the importance of the sequence surrounding H3 in histone binding to WT and W1291R mutant proteins. Histone H321-44 K36me1 (688.4 ± 160.9 nM) and K36me2 peptides (734.5 ± 158.8 nM) showed similar binding affinity (Fig. 1H), whereas binding was impaired for unmodified H321-44 (20,980 ± 7,884 nM) peptides. On the other hand, the W1291R mutant displayed a significantly reduced binding affinity to histone H321-44 K36me1 (11,050 ± 3,316 nM) and K36me2 peptides (27,530 ± 5,688 nM); Fig. 1H), suggesting that W1291 is an important residue for recognition of H3K36 methylation. Surprisingly, the W1291R mutant bound preferentially to histone H321-44 K36me0 peptide (945.5 ± 230.6 nM), indicating that CR mutations can affect the recognition of K36 methylation. Collectively, these results showed that CRTET2 binds preferentially methylated H3K36 and suggest that mutations in the CR domain of leukaemia patients may compromise its recognition ability in vitro.

Next, we investigated whether H3K36 methylation coincides with TET2 localization in vivo. Because the full-length TET2 protein bound preferentially to dimethylated histone H3K36 in vitro (data not shown), we examined the subnuclear distribution of HA-tagged WT and mutant TET2 in HeLa cells after
anti-H3K36me2 antibody staining by immunofluorescence and confocal microscopy. TET2 WT and the DSBH mutant were predominantly localized to the nucleus (Fig. 2A and D, HA, left panel), and line profile analysis revealed that both were colocalized with H3K36me2 (right panel). CRTET2 mutants were also localized to the nucleus (Fig. 2B and C, HA, left panel), but neither colocalized well with the H3K36me2 signal (right panel). Next, we validated TET2 recruitment to chromatin by ChIP-qPCR. The results showed that the TET2 R1214W mutant was unable to recruit either TET2-bound (E) or TET2-unbound (F) loci. HEK293T cells were transfected with the indicated expression plasmids and analysed by ChIP-qPCR. *P<0.05; ***P<0.0001 (student t-test). (G) TET2 mutations in the CR domain alter enzymatic activity in cells. Dot-blot analysis of 5-hmC levels in HEK293T cells over-expressing wild-type or mutant TET2. Oligonucleotides containing 5-hmC or DNA from HEK293T cells transfected with TET2 containing mutations in the HxD motif (TET2-HxDmut) were used for positive and negative controls, respectively. (H–K). Immunocytochemical detection of 5-hmC (green) and HA-TET2 (red) in HEK293T cells transiently transfected with wild-type or mutant TET2. Arrows indicate signature nuclear dot patterns and euchromatin regions.

Finally, we investigated whether mutations in the CR domain of TET2 also affect its cellular catalytic activity in vivo. All patient-derived CR mutations tested displayed low or no catalytic activity in cells (Fig. 2G, Lanes 3–8). Some mutants also showed altered expression levels (see online supplementary material for a colour version of Fig. S4A, HA), but the enzyme activity was low regardless of the expression level (see online supplementary material for a colour version of this Fig. S4B and C). We further confirmed the subnuclear distribution of HA-tagged WT and mutant TET2 in HEK293T cells by immunofluorescence microscopy to determine the role of CR_{TET2} in nuclear localization and enzyme activity of TET2. HA-tagged TET2 (HA-TET2) showed clear nuclear localization that coincided with the level of the 5hmC signal (Fig. 2H). However, CR_{TET2} mutants were diffusely localized to the nucleus, and some mutants formed abnormal foci (Fig. 2I–K and see online supplementary material for a colour version of Fig. S5). Consistent with the results in Fig. 2G, CR_{TET2} mutants exhibited a reduced hmC signal (Fig. 2I–K and see online supplementary material for a colour version of Fig. S5) and some also formed foci (arrows in Fig.

Fig. 2 TET2 CR domain mutations disrupt the recognition of H3K36 methylation, its cellular localization, and enzyme activity in vivo. (A–D) Wild-type TET2 (A) and the DSBH domain mutant (H1881R) (D) but not CR domain mutants (B and C) localize to H3K36me2 loci. HeLa cells were transfected with the indicated expression plasmids and analysed by immunostaining using confocal fluorescence microscopy. Line profile analysis of colocalization between TET2 proteins and H3K36me2 is shown in the panel on the right. The white bar indicates 10 μm. (E and F) CR mutations disrupt the correct localization of TET2-bound (E) and TET2-unbound (F) loci. HEK293T cells were transfected with the indicated expression plasmids and analysed by ChIP-qPCR. *P<0.05; ***P<0.0001 (student t-test). (G) TET2 mutations in the CR domain alter enzymatic activity in cells. Dot-blot analysis of 5-hmC levels in HEK293T cells over-expressing wild-type or mutant TET2. Oligonucleotides containing 5-hmC or DNA from HEK293T cells transfected with TET2 containing mutations in the HxD motif (TET2-HxDmut) were used for positive and negative controls, respectively.

(T-K). Immunocytochemical detection of 5-hmC (green) and HA-TET2 (red) in HEK293T cells transiently transfected with wild-type or mutant TET2. Arrows indicate signature nuclear dot patterns and euchromatin regions.
4l–K and see online supplementary material for a colour version of Fig. S5A–E), suggesting that colocalization with HA-TET2 did not occur. These results suggest that CR₇E₂T₆ mutations abolish both enzymatic activity and cellular localization. Together, our findings suggest that leukaemia-derived mutations of CR₇E₂T₆ impair catalytic activity in cells.

In this report, we demonstrated that CR₇E₂T₆ binds preferentially to mono- and dimethylated H3K36, and leukaemia-related mutations disrupt binding to the target genome (see online supplementary material for a colour version of Fig. S6). Notably, the binding of the W1291R mutant to non-TET2 loci is the first report that mutations in CR₇E₂T₆ can change its genomic localization, potentially opening the prospect that genomic mislocalization of CR₇E₂T₆ mutants to non-target loci could serve as a biomarker for leukaemia. Loss of TET also contributes to cancer development in many cancers through abnormal regulation of DNA methylation (9), especially in leukaemias (1). Given that abnormal DNA methylation occurs in cancer and TET2 mutations are found in elderly people not showing signs of leukaemia, our findings indicate that CR₇E₂T₆ mutations likely cause mis-localization of mutated TET2 proteins, resulting in abnormal DNA methylation and gene expression during the early stages of leukaemogenesis.

In conclusion, our results reveal an important relationship between TET2 and H3K36 methylation. The discovery of their direct interaction via the CR domain sheds new light on the connection between DNA hydroxylation and histone methylation, and its impact on chromatin and DNA biology, the pathogenic mechanisms of TET2-mutated leukaemia, and the epigenetic mechanisms in which they participate.

Supplementary Data
Supplementary Data are available at JB online.

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Conflict of Interest
None declared.

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