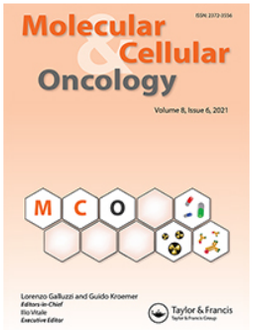

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AUTHOR'S VIEWS

CTCF puts a new twist on UV damage and repair in skin cancer

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ABSTRACT

Somatic mutations in skin cancers are highly enriched at binding sites for CCCTC-binding factor (CTCF). We have discovered that CTCF binding alters the DNA structure to render it more susceptible to UV damage. Elevated UV damage formation at CTCF binding sites, in conjunction with subsequent repair inhibition, promotes UV mutagenesis.

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Genome sequencing of tumors has revealed that somatic mutation rates vary widely across the genome, being particularly enriched at the DNA-binding sites of many transcription factors (TFs).[1] Mutation hotspots at transcription factor binding sites (TFBS) may in some cases reflect positive selection for a non-coding driver mutation, such as for recurrent mutations in the promoter of the telomerase reverse transcriptase (*TERT*) gene.[2,3] However, non-coding driver mutations at TFBS appear to be special cases,[3] as many recurrent TFBS mutations may instead be neutral 'passenger' mutations. These recurrent passenger mutations are not under selection due to a putative role in carcinogenesis, but instead arise from mutagenic processes associated with TF binding to DNA. For example, the binding of ETS (E26 transformation specific) TFs alters the DNA structure to render it more susceptible to damage by ultraviolet (UV) light,[1,3,4] resulting in very high rates of somatic mutations in skin cancers at ETS binding sites.

Somatic mutation rates in skin cancer are also highly elevated at the DNA binding sites of CCCTC-binding factor (CTCF), which functions as a key regulator of chromatin topology (in conjunction with the cohesin complex) and an insulator factor that controls enhancer function during transcriptional regulation.[5] CTCF binding sites (CBS) are mutation hotspots in many gastrointestinal cancers and skin cancers,[1,3,6,7] among others. Whether these mutation hotspots are associated with potential driver mutations or simply reflect elevated rates of neutral passenger mutations has not been fully elucidated. Previous bioinformatics analyses have suggested that CTCF binding may increase the rates of passenger mutations in skin cancers by inhibiting repair of UV-induced cyclobutane pyrimidine dimers (CPDs) at CBS, so that lesions persist to eventually form mutations.[7,8] However, whether CTCF can remain bound to a lesion-containing binding site to directly inhibit repair was unclear.

In our recent study, we used an *in vitro* system to show that purified CTCF protein can readily bind a CBS containing a site-specific CPD lesion, with only a slight (~2-fold) loss of affinity.[9] In contrast, the presence of a CPD lesion has a much greater impact on binding affinity for other TFs, with binding affinity decreasing ~10- to 60-fold.[10] Analysis of CTCF-DNA structures indicates that CTCF makes relatively few direct contacts with the pyrimidine-rich DNA strand of the CBS, which may explain why lesions on this strand do not significantly disrupt CTCF binding. A key question was whether CTCF bound to a CPD-containing site would inhibit its repair. Previous bioinformatics analysis had suggested that binding of the cohesin complex in conjunction with CTCF was required for repair inhibition.[7] However, our *in vitro* data indicated that CTCF binding inhibited repair of the CPD lesion by a model repair enzyme, even though cohesin was absent from this reaction. Moreover, bioinformatics analysis indicated that active CBS that had low cohesin occupancy (i.e. lacked a RAD21 binding site) also were associated with inhibited CPD repair activity and elevated mutation enrichment in melanoma.[9] Taken together, these findings indicate that CTCF is able to bind and directly inhibit the repair of CPD-containing binding sites, independent of cohesin.

A lingering mystery was why the mutation hotspot at CBS in skin cancers was largely confined to a pair of nucleotides (i.e., gCCccCTnnTGG, underline indicates mutation hotspot), while CTCF-mediated repair inhibition broadly extended throughout the CBS and adjacent DNA (Figure 1a). One possibility was that CTCF binding might specifically induce UV damage at sites in the binding motif that coincide with the mutation hotspot, similar to what was previously observed for ETS transcription factors.[1,3,4] We used our CPD-seq method[4] to map CPD lesions across the human genome in UV-irradiated human skin cells. This revealed that UV damage levels are significantly elevated in CBS at nucleotides corresponding to the mutation hotspot (Figure 1b), but are largely depleted at other positions in the motif.[9] Parallel experiments using purified CTCF protein and UV-irradiation of oligonucleotides containing a CBS confirmed that CTCF binding also

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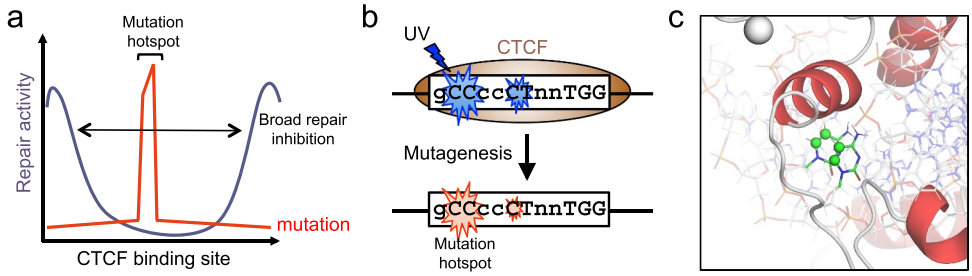

Figure 1. CTCF modulates UV damage fixation and inhibits repair to promote recurrent mutations at CTCF-binding sites (CBS) in skin cancers. (a) Inhibition of repair activity by CCCTC-binding factor (CTCF) occurs broadly throughout CTCF binding sites (CBS), while mutation enrichment in skin cancers is primarily localized to a specific hotspot in the CBS. Adapted from previous bioinformatics analysis[9]. (b) CTCF binding modulates UV (ultraviolet light) damage formation to promote mutations at a hotspot in the CBS motif. Upper case letters in motif indicate highly conserved positions of CBS motif; lower case letters indicate less conserved positions; 'n' is any nucleotide. Adapted from [9]. (c) CTCF-bound DNA structures demonstrate a contacted CC dinucleotide (green) that comprises the mutation hotspot. The C5 and C6 atoms involved in CPD formation are highlighted as spheres. CTCF is rendered in cartoon.

induces CPDs at the location of the mutation hotspot *in vitro*. These experiments indicate that CTCF binding directly modulates UV damage formation, which, coupled with repair inhibition, likely causes the observed mutation hotspot at CBS in skin cancers.

Finally, we wondered how CTCF binding modulates UV damage formation. Analysis of published structures revealed that CTCF binding induces a DNA structure more susceptible to UV damage (Figure 1c). Specifically, the C5-C6 double bonds of adjacent pyrimidines comprising the mutation hotspot adopt smaller distance and torsion angles that are poised for CPD formation.[9] In contrast, CTCF binding induces unfavorable geometries at a number of other locations in the CBS, thereby explaining why CPD formation is suppressed at these locations. This analysis was confirmed by molecular dynamics (MD) simulations of a DNA fragment containing a CBS in the presence or absence of bound CTCF.

In summary, these findings reveal the molecular etiology of passenger mutation hotspots at CBS. It will be important in future studies to determine whether the mechanism of UV damage induction and repair inhibition are responsible for all CBS mutation hotspots in skin cancers, or if selected binding sites may also be potential driver mutations under positive selection. It will also be important to determine whether related mechanisms (i.e., alterations in DNA damage formation and/or repair inhibition) are responsible for CBS mutation hotspots in gastrointestinal cancers.

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Disclosure statement

No potential conflict of interest were disclosed.

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References

1. Gonzalez-Perez A, Sabarinathan R, Lopez-Bigas N. Local determinants of the mutational landscape of the human genome. Cell. 2019;177:101–114. doi:10.1016/j.cell.2019.02.051.
2. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013;339:957–959. doi:10.1126/science.1229259.
3. Elliott K, Larsson E. Non-coding driver mutations in human cancer. Nat Rev Cancer. 2021;21:500–509. doi:10.1038/s41568-021-00371-z.
4. Mao P, Brown AJ, Esaki S, Lockwood S, Poon GMK, Smerdon MJ, Roberts SA, Wyrick JJ. ETS transcription factors induce a unique UV damage signature that drives recurrent mutagenesis in melanoma. Nat Commun. 2018;9:2626. doi:10.1038/s41467-018-05064-0.
5. Merkenschlager M, Nora EP. CTCF and cohesin in genome folding and transcriptional gene regulation. Annu Rev Genomics Hum Genet. 2016;17:17–43. doi:10.1146/annurev-genom-083115-022339.
6. Katainen R, Dave K, Pitkanen E, Palin K, Kivioja T, Valimaki N, Gylfe AE, Ristolainen H, Hanninen UA, Cajuso T, et al. CTCF/cohesin-binding sites are frequently mutated in cancer. Nat Genet. 2015;47:818–821.
7. Poulos RC, Thoms JA, Guan YF, Unnikrishnan A, Pimanda JE, Wong JW. Functional mutations form at CTCF-cohesin binding sites in melanoma due to uneven nucleotide excision repair across the Motif. Cell Rep. 2016;17:2865–2872. doi:10.1016/j.celrep.2016.11.055.
8. Sabarinathan R, Mularoni L, Deu-Pons J, Gonzalez-Perez A, Lopez-Bigas N. Nucleotide excision repair is impaired by binding of transcription factors to DNA. Nature. 2016;532:264–267. doi:10.1038/nature17661.
9. Sivapragasam S, Stark B, Albrecht AV, Bohm KA, Mao P, Emehiser RG, Roberts SA, Hrdlicka PJ, Poon GMK, Wyrick JJ. CTCF binding modulates UV damage formation to promote mutation hot spots in melanoma. Embo J. 2021;40:e107795. doi:10.15252/embj.2021107795.
10. Tommasi S, Swiderski PM, Tu Y, Kaplan BE, Pfeifer GP. Inhibition of transcription factor binding by ultraviolet-induced pyrimidine dimers. Biochemistry. 1996;35:15693–15703. doi:10.1021/bi962117z.