One Strike and You're Out: CTCF is a Haploinsufficient Tumor Suppressor

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Hemizygous deletions at chromosomal location 16q22.1 frequently occur in many human cancers, including more than 50% of all breast cancers. One of the genes in this locus, *CTCF*, is critical for chromatin organization and acts as a master regulator of the genome. While the prevailing opinion in the field is that deletion of both copies of a tumor suppressor is necessary for oncogenesis, Drs. Chris Kemp and Gala Filippova (Human Biology Division), together with Brady Bernard and Ilya Shmulevich (Institute for Systems Biology) as well as Matt Teater and Ari Melnick (Cornell University), demonstrate that loss of a single copy of *Ctcf* triggers epigenetic changes and markedly increases cancer development and progression in mice and humans.

CTCF is a well-conserved zinc finger DNA-binding protein that binds several different target sequences across the genome to establish chromatin boundaries and facilitate chromatin-chromatin interactions. This protein also mediates multiple epigenetic phenomena including genomic imprinting, X chromosome inactivation, and regulation of spreading DNA methylation (Filippova, 2008). Human *CTCF*, a gene that was originally cloned at the Fred Hutchinson Cancer Research Center, was mapped to 16q22.1 more than 15 years ago (Filippova, 1998); however, a causal relationship between the partial loss of *CTCF* and oncogenesis was yet to be established.

To investigate the possibility that hemizygous deletion of *Ctcf* drives tumor progression, the researchers developed a *Ctcf* knockout mouse line. $Ctcf^{-/-}$ was embryonic lethal, confirming that CTCF is critical for development (Moore, 2012); however, $Ctcf^{+/-}$ mice were essentially normal. Interestingly, $Ctcf^{+/-}$ mice developed twice as many cancers as their wildtype littermates, and were three-times more likely to develop multiple tumors. These tumors also occurred in a variety of tissue types and were phenotypically more aggressive than those forming in wildtype mice. Similar patterns of tumor development were observed when $Ctcf^{+/-}$ mice were exposed to various oncogenic treatments, such as irradiation or chemical mutagenesis.

All $Ctcf^{+/-}$ mouse tumors that were examined retained a single wildtype Ctcf allele with no mutations or deletions in the coding region, confirming that CTCF is truly haploinsufficient for tumor suppression. To investigate the mechanism underlying this haploinsufficiency, the researchers

defined the methylation state of wildtype and mutant genomes. *Ctcf*$^{+/-}$ mice had alterations in CpG methylation that tended towards hypermethylation at multiple distinct sites across the genome relative to wildtype controls. These changes preceded the development of cancer, suggesting that the epigenetic changes may set the stage for tumor development. The authors next analyzed human tumors in the Cancer Genome Atlas (TCGA) and found that 57% of human breast tumors and 24% of uterine endometrial cancers contained hemizygous *CTCF* deletions. *CTCF* was also the 16th and 4th most mutated gene in these cancers, respectively. Similar to the mouse tumors, *CTCF*$^{+/-}$ human tumors also contained distinct CpG methylation differences across the genome, suggesting that the oncogenic mechanism may be conserved across species.

This study by Kemp, *et al*. clearly demonstrates that the epigenetic regulator CTCF is a potent tumor suppressor. The loss of a single allele of *Ctcf*, and the accompanying epigenetic instability, was sufficient to drive aggressive tumor formation in a variety of tissues. Data from TCGA analysis suggested that CTCF copy number variation may occur preferentially in some cancer subtypes. Going forward, "we should determine if tumors with loss of CTCF, with their distinct epigenetic profile, have different responses to therapy," said Dr. Gala Filippova.

Kemp CJ, Moore JM, Moser R, Bernard B, Teater M, Smith LE, Rabaia NA, Gurley KE, Guinney J, Busch SE, Shaknovich R, Lobanenkov VV, Liggitt D, Shmulevich I, Melnick A, Filippova GN. 2014. CTCF Haploinsufficiency Destabilizes DNA Methylation and Predisposes to Cancer. *Cell Rep.* 1020-9. doi: 10.1016/j.celrep.2014.04.004.

See also: Filippova GN, Lindblom A, Meincke LJ, Klenova EM, Neiman PE, Collins SJ, Doggett NA, Lobanenkov VV. 1998. A widely expressed transcription factor with multiple DNA sequence specificity, CTCF, is localized at chromosome segment 16q22.1 within one of the smallest regions of overlap for common deletions in breast and prostate cancers. *Genes Chromosomes Cancer.* 22(1):26-36.

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Moore JM, Rabaia NA, Smith LE, Fagerlie S, Gurley K, Loukinov D, Disteche CM, Collins SJ, Kemp CJ, Lobanenkov VV, Filippova GN. 2012. Loss of maternal CTCF is associated with peri-implantation lethality of Ctcf null embryos. *PLoS One.* 7(4):e34915. doi: 10.1371/journal.pone.0034915.

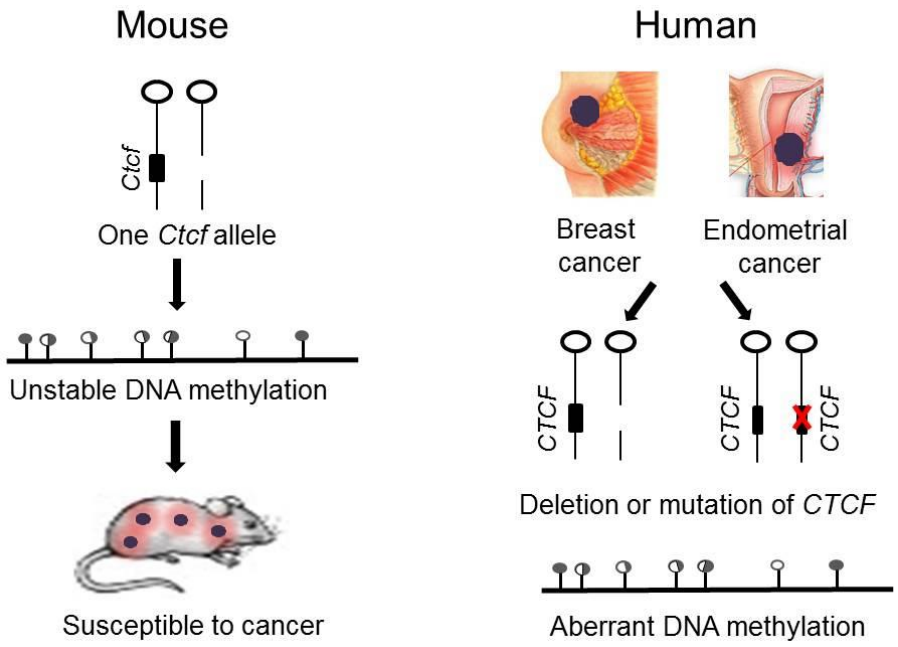

Image adapted from Kemp, et al., 2014.

Deletion of a single Ctcf allele in mice (left) led to alterations in DNA methylation and increased oncogenesis. Human tumors of the breast and uterus (right) had frequent loss of a single allele of CTCF and similar changes in genomic methylation patterns.