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CTCF and BORIS in genome regulation and cancer

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CTCF plays a vital role in chromatin structure and function. CTCF is ubiquitously expressed and plays diverse roles in gene regulation, imprinting, insulation, intra/interchromosomal interactions, nuclear compartmentalisation, and alternative splicing. *CTCF* has a single paralogue, the testes-specific *CTCF*-like gene (*CTCFL*)/*BORIS*. *CTCF* and *BORIS* can be deregulated in cancer. The tumour suppressor gene *CTCF* can be mutated or deleted in cancer, or CTCF DNA binding can be altered by epigenetic changes. BORIS is aberrantly expressed frequently in cancer, leading some to propose a pro-tumourigenic role for BORIS. However, BORIS can inhibit cell proliferation, and is mutated in cancer similarly to CTCF suggesting BORIS activation in cancer may be due to global genetic or epigenetic changes typical of malignant transformation.

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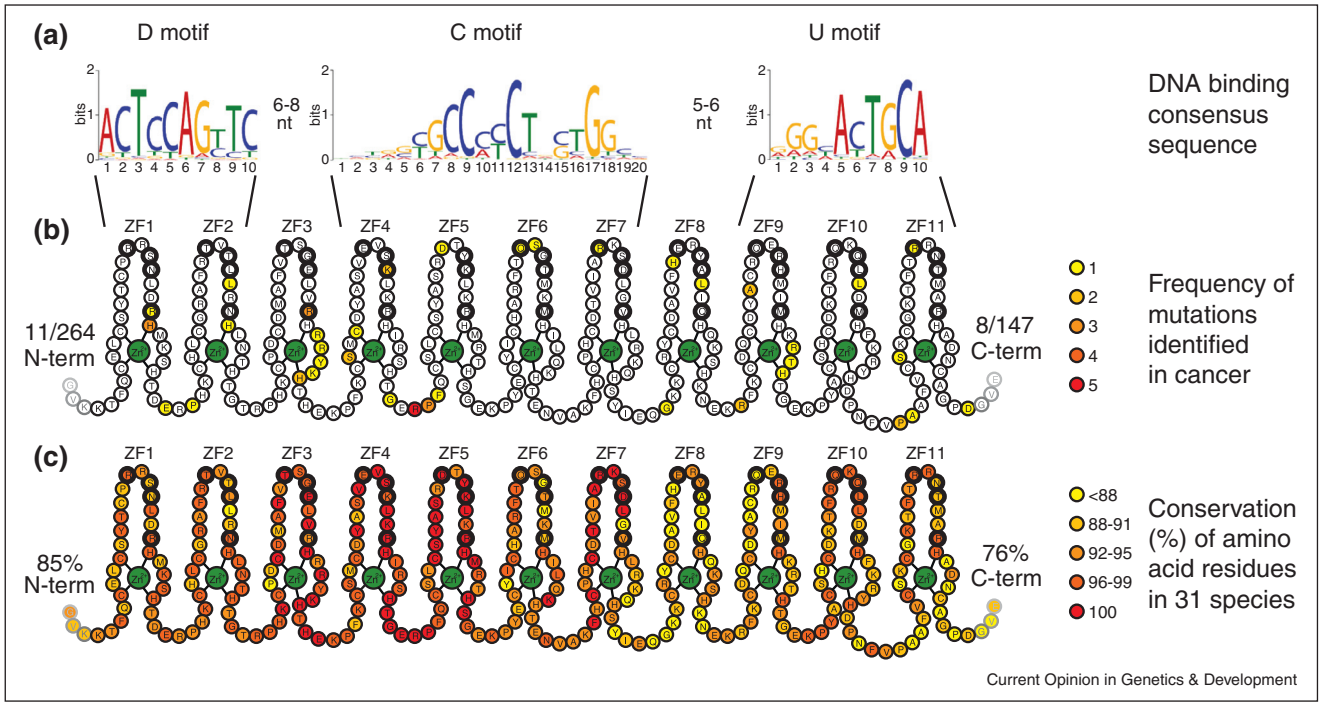
CTCF and BORIS in the genomic landscape

CTCF (CCCTC-binding factor) is a ubiquitous multivalent 11-zinc finger (ZF) protein that can bind tens of thousands of sites across the human genome depending on cell type [1,2••,3••,4]. CTCF was initially identified as a transcriptional regulator [5]. It is now recognised as having diverse roles as an insulator, regulator of imprinted loci, in X chromosome inactivation (XCI) and governing higher-order chromatin architecture. Further functional complexity is added by CTCF's ability to homodimerise with other bound CTCF proteins to influence higher-order chromatin structure and heterodimerise with an increasingly larger set of protein partners [6,7]. *BORIS* ('Brother' of the Regulator of Imprinted Sites) is the sole paralogue of *CTCF*. BORIS binds a subset of DNA targets identical to that of CTCF [8••], but its role in the genomic landscape has not been as thoroughly examined.

Genome-wide analysis of CTCF occupancy and the development of techniques to map CTCF-mediated chromosomal interactions have provided further mechanistic insights into CTCF's role in gene regulation. Approximately 60% of CTCF target sites are situated in intergenic regions and sites upstream of transcription start sites (TSSs) [9], suggesting that CTCF can co-ordinate long range gene expression. CTCF occupancy genome-wide is frequently juxtaposed with transcriptional activity: genes proximal to low-occupancy sites exhibit higher expression and are cell-specific; while high-occupancy sites more often define co-ordinately expressed loci and are associated with repressive histone marks [10]. Comprehensive mapping of human cell lines (38 in total) showed the overlap of CTCF binding sites between different cell types can be as little as 26%, with up to 6% of CTCF binding sites being unique to a single cell-line [3••]. Such results indicate that CTCF binding is differentially regulated. The majority of CTCF binding sites have been shown to be cell type-specific [11,12] with differential methylation being associated with 41% of the variable occupancy seen [12]. Furthermore, ubiquitous CTCF binding sites exhibit stronger CTCF binding, higher evolutionary conservation, and increased intergenic localisation compared to cell-type specific binding sites [3••]. Comparing 56 human cell lines, constitutive CTCF binding sites (bound in 90% of cell lines) were located in open chromatin and associated with cohesin binding [11]. These data are consistent with a conserved role for CTCF in partitioning the genome into discrete topological domains.

CTCF's multiple functions are related to its ability to engage different, yet specific, DNA targets using combinations of its 11 ZFs. DNA binding studies originally indicated that only ZFs 4–7 were required for strongest binding to a core 12 bp consensus sequence found in cognate CTCF target sites [13]. Further refinement by chromatin immunoprecipitation combined with tiling arrays (ChIP-chip) identified a 15–20 bp consensus [4]. Subsequent systematic mutation of all individual CTCF ZFs revealed ZFs 4–7 bound to ~80% of CTCF target sites containing the core (C) 15–20 bp motif; and flanking ZFs 1–3 and 8–11 bound additional downstream (D) and upstream (U) consensus motifs respectively [2••] (Figure 1a). The conserved U motif, identified by CTCF ChIP-Seq from six mammalian species [14], is bound by ZFs 9–11 and separated from the C motif by 5–6 nucleotides. The D motif is bound by ZFs 1-2 separated from the C motif by a 6–8 nucleotide (nt) spacer (Figure 1a). CTCF can bind to target sites containing combinations of C, UC, CD and UCD motifs [2••]. The UC motif,

Figure 1



Conserved CTCF zinc finger domain residues are frequently mutated in cancer. **(a)** CTCF DNA binding consensus motifs D (downstream), C (core) and U (upstream) identified by Nakahashi *et al.* [2••]. The D motif is bound by ZFs 1-2, C motif is bound by ZFs 4-7 and the U motif is bound by ZFs 9-11. Spacer sequences between DNA motifs are indicated in nucleotides (nt). **(b)** Location and frequency of known missense mutations in CTCF in diverse cancers (COSMIC and cBio databases) [15•,57–60,75]. Frequencies of missense mutation within the N and C-termini are shown as number of mutations/amino acid number. Amino acids in bold circle directly bind to DNA. **(c)** Percentage of conservation of CTCF amino acid residues across 31 species. Overall conservation in the N-termini and C-termini is indicated.

particularly when it contains a 6 nt spacer (U_6C), displayed the highest CTCF occupancy and the presence of the D motif reduced CTCF binding even in the presence of the U motif. ZF 3 was found to be critical for binding in the absence of the U motif, and ZFs 1–2 and 8–11 all help to stabilise CTCF occupancy when D and U motifs are absent. CTCF was more weakly bound to sites lacking a C motif based on ChIP-Seq peak intensity. These data prompted the proposal of a 'saddle' model of CTCF binding where engagement of the core C motif or 'seat' through binding of ZFs 4–7 was stabilised by binding of peripheral ZFs to flanking U and D motifs, acting as the 'stirrups' [2••].

BORIS shows high conservation of the 11 ZF DNA binding domain (>70% homology) with CTCF, but with divergent N-terminal and C-terminal regions (<18% homology) [15•]. Consequently, BORIS and CTCF ZF domains are predicted to bind similar DNA targets, however other proteins recruited to those sites *via* binding of the divergent N-termini and C-termini regions may be different and thus lead to different functional outcomes. While CTCF expression is ubiquitous, BORIS expression is limited to specific cells of the testes [16,17]. Because of the restriction of BORIS to spermatogonia and preleptotene spermatocytes, BORIS has been proposed to act as a male germ cell gene regulator. This is consistent with *BORIS* null (*Ctcfl*$^{-/-}$) male mice which show partially penetrant subfertility [8••] due to a meiosis defect [16].

BORIS binds to a core consensus sequence very similar to the C motif bound by CTCF and 64% of BORIS binding sites overlap with CTCF binding sites; but these represent only about 10% of all CTCF binding sites. Increasing BORIS expression reduced CTCF occupancy at BORIS binding sites, indicating BORIS can compete with CTCF for DNA binding — but only at specific target sites. BORIS appears to act locally by binding proximal to TSSs and associating with transcriptionally active genes coincident with H3K4me3 and PolII Ser5 phosphorylation marks, whilst CTCF acts more distally to TSSs [8••]. A more thorough characterisation of the role of BORIS in genome organisation will be made possible with the availability of high-quality antibodies suitable for ChIP and chromatin interaction applications.

Diverse roles for CTCF in chromatin structure and genome organisation

CTCF has many diverse roles in regulating chromatin function and nuclear structure, however BORIS appears

to share relatively little function. CTCF can mediate both intrachromosomal and interchromosomal interactions [3••,18,19] (Figure 2a,b) and can exert a positive or negative influence on gene transcription depending on the genomic context [20–26] (Figure 2c). Chromatin loops <200 kb are more likely to contain active chromatin features, while loops >200 kb demonstrate repressive domain characteristics [18]. CTCF interacts with binding partners cohesin and mediator complexes to shape three-dimensional genomic architecture [27]. The length of the chromatin loops varies by interaction partner. CTCF alone or in concert with cohesin was biased towards loop distributions larger than 1 Mb, while the CTCF-cohesin-mediator complex mediated loops typically <300 kb in size [27].

CTCF also modulates other aspects of chromatin structure. CTCF binding serves as an anchor to phase up to 10 nucleosomes either side of a target site [28] (Figure 2d). This nucleosome phasing is particularly evident at ubiquitous CTCF binding sites [3••]. Conversely, BORIS binding shows no association with nucleosome phasing [8••]. CTCF binding to DNA is also associated with DNAse I hypersensitivity regions, elevation of all histone modifications except H3K27me3 and methylation-poor overlapping CpG dinucleotides in a cell type-specific manner [3••]. Reduced CTCF DNA binding negatively correlates with the degree of DNA methylation at CpGs within the CTCF binding sites [12]. It is not clear from such data, whether CTCF is a cause or effect of this phenomenon. Pre-existing DNA methylation can prevent CTCF binding *in vitro* [29,30]. Conversely, CTCF can actively inhibit DNA methylation at CTCF binding sites. At unmethylated CTCF binding sites, CTCF has been shown to interact with self-PARylated PARP1 (poly[-ADP-ribose] polymerase 1) which can prevent DNA methylation *via* inhibition of DNMT1 (DNA methyltransferase 1) activity. PAR depletion by poly[ADP-ribose] glycohydrolase (PARG) overexpression reduced CTCF and PARP1, but not DNMT1 DNA binding [31•] (Figure 2e).

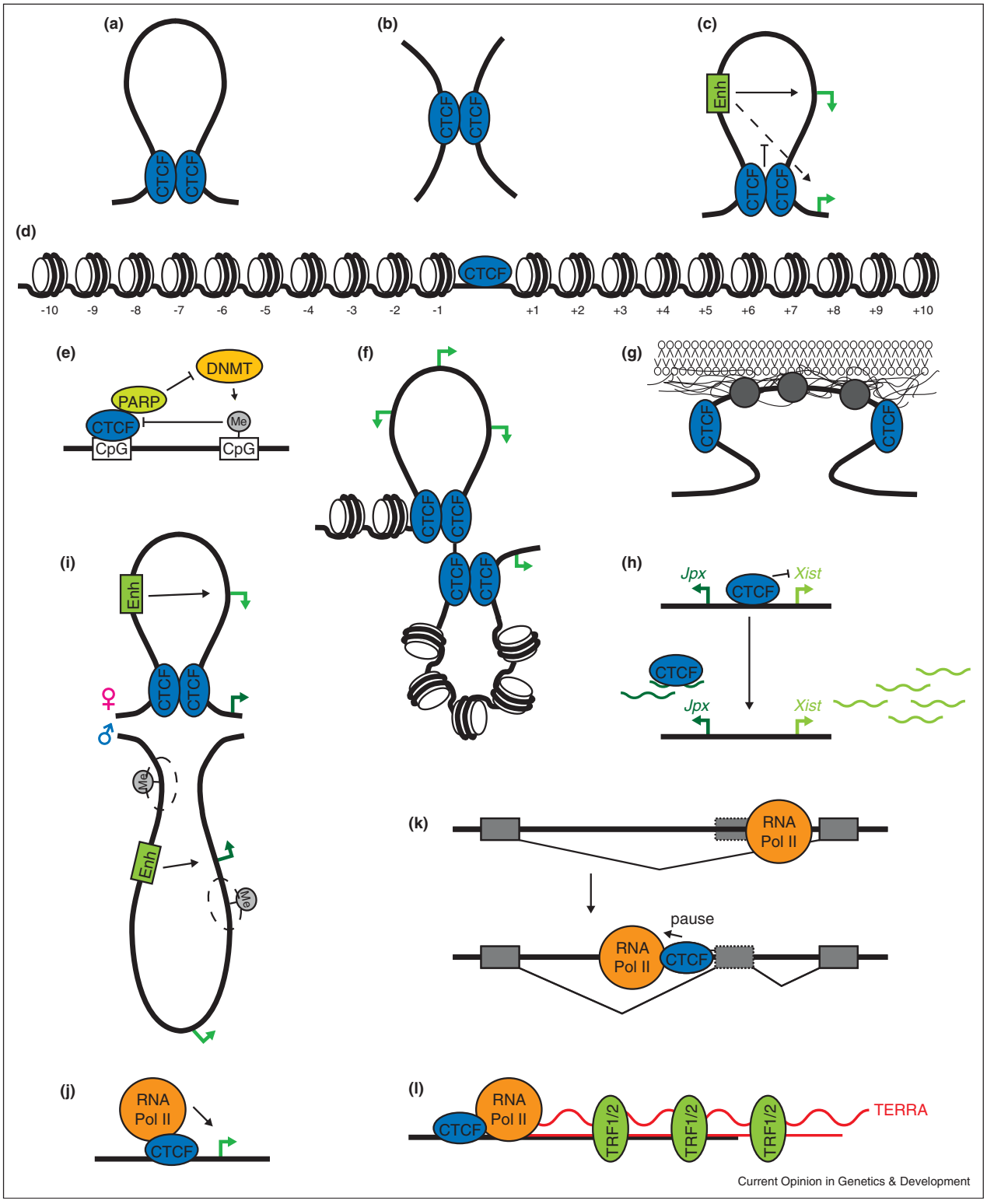
CTCF is the only known factor that shows insulator function in vertebrates [24,32–34]. Consistent with this function, CTCF binding is associated with chromatin boundaries [3••,35] by preventing the spread of repressive heterochromatin (Figure 2f). Also, by associating with genome-nuclear lamina interactions, CTCF demarcates the boundaries of lamina-associated domains [18,36] (Figure 2g). In females, X chromosome gene dosage is compensated for by XCI. CTCF plays a critical role in XCI, and conversely, escape from XCI [37,38]. However, 92% of CTCF binding sites are bound on both active and inactive X (Xi) chromosomes, while 8% of CTCF binding sites were Xi-specific and included boundaries flanking the X-inactivation centre. This indicates that CTCF binding does not necessarily dictate the state of chromatin on either side of the CTCF boundaries, and these can be differentially utilised to regulate chromatin structure and gene expression [37]. Before XCI, CTCF binding represses noncoding *Xist* mRNA expression, [39•]. At the onset of XCI, the neighbouring *Jpx* gene is up-regulated and the resultant RNA transcript binds CTCF and sequesters it away from one allele of *Xist*. By this mechanism, *Xist* expression is induced and XCI follows [39•] (Figure 2h). Intriguingly, this report was the first to convincingly demonstrate CTCF RNA binding activity.

One of the most thoroughly examined examples of CTCF's role in regulating gene imprinting involves the *IGF2/H19* locus. Differential CTCF binding due to allele-specific CpG methylation of a *cis*-regulatory element mediates differential gene expression from maternal and paternal alleles. CTCF binding to the maternal imprinting control region (ICR) of the *IGF2/H19* locus promotes *H19* expression. The ICR is methylated in the paternal allele allowing distal enhancers to induce IGF2 expression (reviewed in [40]) (Figure 2i). Similar CTCF-mediated mechanisms have also been identified at other imprinted loci including *CDKN1C* [41], *WT1* [42], *MEG3* [43] and *PLAGL1* [44].

CTCF has been shown to physically interact with RNA polymerase (Pol) II recruiting it to specific CTCF binding sites genome wide [45] (Figure 2j). This interaction between CTCF and RNA Pol II has been associated with transcriptional pausing [46] particularly when CTCF is bound proximal to TSSs [47]. Within the CD45 locus, CTCF binding upstream of exon 5 can cause RNA Pol II pausing, and subsequent inclusion of exon 5 in CD45 transcripts. CTCF binding to this site is inhibited by DNA methylation, providing a mechanistic link between epigenetic marks and alternative splicing [48•] (Figure 2k). CTCF also appears to facilitate RNA Pol II recruitment for the regulation of telomere length. Most human subtelomeres contain a CTCF/cohesin binding site within ~1–2 kb of the TTAGGG repeat tracts. Depletion of CTCF or cohesin led to decreased telomeric repeat-containing RNA (TERRA) transcription, telomere-induced DNA damage foci formation and destabilisation of terminal repeat binding factors (TRF1/2) binding [49•] (Figure 2l).

CTCF acts as a regulator of ribosomal RNA (rRNA) transcription in the nucleolus [50–54]. In this capacity, CTCF can facilitate the recruitment of upstream binding factor (UBF) to ribosomal DNA repeats and alters the local epigenetic state to activate rRNA expression [51,53,54]. This process is negatively regulated by methylation of CTCF binding sites in ribosomal DNA [54] and condensin binding [51]. Depletion of CTCF can alter normal nucleolar structure and number [52,54] and CTCF PARylation inhibits nucleolar transcription [50]. Interestingly, BORIS also binds to UBF, a mediator of

Figure 2



The varied functions of CTCF in conferring chromatin structure, genomic organisation and gene expression. These include **(a)** intrachromosomal looping; **(b)** interchromosomal looping; **(c)** gene regulation; **(d)** nucleosome phasing; **(e)** CTCF regulation of CpG methylation and CpG methylation regulation of CTCF binding; **(f)** insulator function and demarcation of chromatin domains; **(g)** demarcation of lamina associated domains; **(h)** RNA binding function in the regulation of X chromosome inactivation; **(i)** gene imprinting; **(j)** recruitment of RNA Pol II to gene promoters; **(k)** RNA Pol II pausing and alternative splicing; and **(l)** regulation of telomere length.

RNA Pol I transcription [54], and can bind to ribosomal DNA, but no functional role has been proposed [54].

CTCF, BORIS and cancer

CTCF has been found to repress cancer cell growth and clonogenicity [15•,55] and has been found sporadically mutated in various cancer types including Wilms' tumour, leukaemia and uterine, breast and prostate cancers [15•,56–60]. The mutations identified included nonsense mutations [56,60], and missense DNA mutations in the 11 ZF domain [15•,56–58] that inhibit CTCF binding to specific target sites [57] (Figure 1b,c). Extensive characterisation of CTCF ZF mutations indicates that DNA binding is directly impacted particularly in ZFs responsible for core consensus binding (ZFs 4–7) [2••].

CTCF has been proposed as a possible target for 16q22.1 deletions that commonly occur in breast and prostate cancer [61]. Heterozygous deletion or mutation of *CTCF* has been reported in 8% of early T-cell precursor acute lymphoblastic leukaemia [62] and 20% of Down syndrome-related acute megakaryoblastic leukaemia [56]. Disruption of CTCF binding, and associated chromatin modification and structural changes at various loci in cancer cell lines, can lead to the deregulation of cancer associated genes [12,24–26,63–65]. This disruption of CTCF binding in colon cancer cells also disrupts a phenomenon called long range epigenetic silencing (LRES), which can be partially reversed by cell differentiation [66]. Therefore, changes in CTCF binding can influence both local and long range chromatin structure in cancer cells. Mutation of specific CTCF target sites within the *IGF2/H19* locus has been identified in patients with Beckwith–Wiedemann syndrome, an overgrowth disorder predisposing patients to paediatric cancer development [67,68]. These diverse observations indicate that the inactivation or disruption of normal CTCF DNA occupancy plays a role in cancer development.

BORIS expression is frequently induced in cancer leading some to conclude that BORIS may act in opposition to CTCF tumour suppressor function in cancer cells [17,69]. To date, findings on BORIS function have been inconsistent and a consensus has not been reached. Extensive characterisation of ectopic BORIS expression showed BORIS inhibits colony formation and cell proliferation in multiple cell types [15•]. In one unexplained finding, both ectopic BORIS expression and BORIS shRNA knockdown inhibited colony formation [70]. Two reports [71,72] showed that gross BORIS overexpression inhibited cell growth while low BORIS expression (using a doxycycline-inducible transient expression system) enhanced cell growth. Contrary to this result, Tiffen *et al.* [15•] did not observe a growth-enhancing effect of BORIS at any expression level (using a 'dox-off' lentiviral expression system) [15•]. The differences observed in these studies may be due to differences in the experimental models used and the differential function of BORIS in different cell types. However Tiffen *et al.* findings were reproducible in multiple cell types from different species as well as *in vitro* and *in vivo*. We favour the hypothesis that BORIS activation in cancer may be a consequence of global genetic and epigenetic dysregulation in cancer, and that BORIS may not specifically have pro-tumourigenic functions. Given that BORIS can function similarly to CTCF to inhibit cell growth [15•,70,71], aberrant BORIS expression in a cancer cell may actually be inhibitory of tumour growth. Consistent with this rationale, similar inactivating mutations (nonsense and missense) are seen in BORIS akin to those observed in CTCF in various cancer types [15•].

As a cancer testes antigen, the restriction of BORIS expression to cancer cells and spermatocytes could represent an immunotherapeutic avenue for the treatment of many cancers. Successful trials of a BORIS DNA-based vaccination or of dendritic cells loaded with BORIS antigen have already been performed in mice [73,74]. Irrespective of a pro-tumourigenic or tumour suppressor function, due to its tumour-specific expression, BORIS represents a viable target for cancer immunotherapy.

Conclusion

CTCF plays numerous critical roles in chromatin organisation and regulation of gene expression. Abrogation of these normal functions due to mutation or deletion in cancer likely contributes to cancer-associated epigenetic changes and gene deregulation. The *CTCF* paralogue *BORIS* is frequently deregulated in cancer. Some studies show potential oncogenic roles for BORIS, while others show directly conflicting functions, analogous to tumour suppressor CTCF. However, BORIS expression in cancer may simply reflect genetic and epigenetic dysregulation of tumour chromatin, representing an effect rather than a cause of oncogenic transformation. Further detailed investigation is required to determine the direct contributions of CTCF and BORIS to the molecular pathogenesis of cancer.

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