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INVITED REVIEW ARTICLE

Spatial genome architecture and the emergence of malignancy

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Abstract

Human chromosomes are large spatially and hierarchically structured entities, the integrity of which needs to be preserved throughout the lifespan of the cell and in conjunction with cell cycle progression. Preservation of chromosomal structure is important for proper deployment of cell type-specific gene expression programs. Thus, aberrations in the integrity and structure of chromosomes will predictably lead to disease, including cancer. Here, we provide an updated standpoint with respect to chromatin misfolding and the emergence of various cancer types. We discuss recent studies implicating the disruption of topologically associating domains, switching between active and inactive compartments, rewiring of promoter–enhancer interactions in malignancy as well as the effects of single nucleotide polymorphisms in non-coding regions involved in long-range regulatory interactions. In light of these findings, we argue that chromosome conformation studies may now also be useful for patient diagnosis and drug target discovery.

Introduction

Despite the century-long interest scientists have had in the organization of eukaryotic nuclei, it was not until relatively recently that the spatial architecture of chromosomes could be studied on the molecular level. Historically, it was Carl Rabl and Theodori Boveri who first hypothesized over the existence of chromosomal territories (1,2). This concept was proven years later (3–5) and developed further with the discovery of 'sub-chromosomal foci' that divided the territories into either early- or late-replicating sub-compartments (6). The era of genomics that ensued allowed for the interrogation of nuclear architecture at increasingly higher resolution and throughput. This way, large lamina-associated chromatin domains (LADs) (7) and dynamic euchromatin-interacting LADs (eLADs) (8) were mapped and found to be insulated from active chromatin at positions bound by the CTCF protein. Then, the introduction and development of chromosome conformation capture (3C) technologies (9; see Box 1) revealed chromatin compartmentalization into Mbp-sized domains. 'Open' and generally active transcribed euchromatin was predominantly allocated to so-called A-compartments, while 'closed' and more densely packed heterochromatin to B-compartments (10). At the sub-Mbp scale, the existence of self-interacting topologically associating domains (TADs) was revealed (11). Neighbouring TADs are insulated from one another by 'boundaries' that are typically demarcated by CTCF and/or active transcription units (i.e. housekeeping promoters or tRNA genes). Chromatin segments in a given TAD interact more often among themselves than with segments in other TADs (12,13). Interestingly, TAD boundaries often mark transition regions between A- and B-compartments, LADs and non-LADs, as well as early- and late-replicating domains (12,14). Finally, at the sub-TAD level, kbp-resolution Hi-C studies discovered thousands of chromatin loops. These are anchored at convergently oriented CTCF-bound sites that also bind cohesin, and have a median size of 185 kbp (13,15,16). As a result of these and other observations, a model for the formation of such 'structural' loops was proposed: chromatin is extruded via the cohesin ring by consuming ATP

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(17) and extrusion stops once cohesin encounters the two CTCF anchors (18,19). However, another widespread type of looping, albeit far more dynamic than that of 'structural' CTCF loops, involves contacts among gene promoters and enhancers (13). These interactions are crucial for regulating the magnitude and timing of gene transcription and are often nested inside CTCF loops (20). The interplay between CTCF and loops underlies the contribution of 3D chromatin conformation in gene expression (13,16,21). Although CTCF is often present at one or the other anchor of such enhancer/promoter loops, RNA polymerases and transcription factors—such as Myc (22), AP-1 (23) or YY1 (24)—seem to define their emergence, and even small changes in contact probability can induce large transcriptional differences (25). In light of this functional significance of genome topology, it is no surprise that its alterations are now understood to be involved in human disease manifestation (26–30), including cancer.

3D genome architecture and malignancy

For many years, cancer was thought to be a genetic disease, driven by mutations in the coding genome that promote tumour formation (42). It was not until quite recently that the contribution of epigenetics to carcinogenesis became widely acknowledged (42–45). Still, how aberrations in spatial chromosome architecture underlie cancer emergence and tumour evolution remains largely unexplored. It had been confined to the 'macroscopic' identification of chromosomal translocations and aneuploidy (46,47), but an increasing body of evidence now suggests that carcinogenesis indeed involves alterations at all levels of 3D genome organization (summarized in Fig. 1).

Alterations in A/B-compartments and chromosomal territories

As regards Mbp-sized alterations, major disruptions of chromosomal territories were documented in breast cancer with concomitant changes in compartmentalization and promotion of cancer-related transcriptional changers. Genes embedded in the regions that switched compartments belonged to the oncogenic WNT pathway (48). In glioblastoma, compartment switching represents a key force for gene expression determination, as exemplified by the *RGS6* locus. *RGS6* expression is 400x higher in patient-derived cells, where it is now found in compartment A, despite similar epigenetic marking of the locus in patient and control lines (49). In a model of colon mucosa carrying a duplication of chr7 (an early event in colorectal cancer development), compartment switching leads to the silencing of the *Kcnh5* gene encoding a potassium voltage-gated channel implicated in cell cycle regulation and proliferation (50,51). Similar switching events have been documented in blood malignancies and correlate with gene expression changes contributing to tumorigenesis. For example, in multiple myeloma, ~20% of genomic regions altered are involved in A/B-compartment switching and encompass activated cell adhesion pathway genes and repressed apoptotic and hematopoietic lineage genes (52). Similarly, in lymphoma, switching underlies the differential regulation of genes involved in V(D)J recombination and in the negative regulation of T-cell apoptotic pathways, both of which support carcinogenesis (53). However, unlike in the above cases, compartment switching in leukemias only applies to ~10% of the affected chromosomal regions, and the corresponding gene expression changes are less pronounced when leukemic subtypes are compared with normal cells (21). Nevertheless, these changes correlate well with epigenetic changes in both A and B compartments. Hence, the effects on chromatin accessibility and the promotion of transcriptional changes underlying leukemogenic transformation (54). Altogether, these and other examples demonstrate how Mbp-scale alterations in chromatin conformation can be a major contributor to tumorigenesis by repositioning loci in a different chromatin context, thereby inducing or suppressing gene expression.

Box 1. 3C-based technologies for studying chromatin conformation.

The idea of mapping the physical communication between *cis*-regulatory elements has long intrigued scientists interested in how the nucleus is organized. In 1995, Cullen and colleagues were the first to show the formation of an actual loop between the prolactin gene promoter and a nearby regulatory fragment that correlated with the gene's activation (31). In 2002, the chromosome conformation capture (3C) method was introduced and quickly became a popular tool for studying chromatin topology. For 3C, cells are crosslinked, chromatin is digested using a restriction enzyme and cohesive ends that lie in spatial proximity are ligated *in situ*. These ligation hybrids would initially be detected in a 'one-to-one' manner using polymerase chain reaction, where the detection frequency was used as a readout of contact frequency of the fragments targeted (32). To increase throughput, chromosome conformation capture-on-chip (4C) was developed. 4C allows for the detection of essentially all contacts involving a given preselected 'viewpoint' (or 'bait') and was quickly adapted to next-generation sequencing readouts (33,34). However, it was not until 2009 that a 3C variant of genome-wide coverage, namely Hi-C, was introduced (10). Hi-C allowed mapping contacts in an 'all-to-all' fashion, and although the first maps were only of 1 Mbp resolution (10,35), kbp-resolution Hi-C maps were soon generated thanks to advances in sequencing technology (13). Around the same time as higher resolution Hi-C, more 'focused' 3C-based technologies emerged. These included an enrichment step, either by using oligonucleotides against preselected genomic targets (36–39) or by adding an immunoprecipitation step to isolate interactions forming via a specific factor or histone mark (40,41). Such enrichment steps are becoming increasingly popular, because they offer improved cost-to-resolution balance compared with Hi-C. Finally, the emergence of these and other 3C variants is also being followed by the development of methods that are orthogonal to it, like methods that do away with cell fixation (i3C), with ligation (GAM, SPRITE) or with both (DamC, TALE-iD)—or even methods that study interactions in single nuclei (ScHi-C, Dip-C) or single molecules (ChIA-Drop; for a review of these methods see 9).

Alterations in TADs, TAD boundaries and enhancer–promoter looping

The identification of disruptions in TAD organization in developmental disorders (29) triggered the widespread application of 3C technologies when investigating disease mechanisms. To cite some recent discoveries in this area, duplication of an intra-TAD

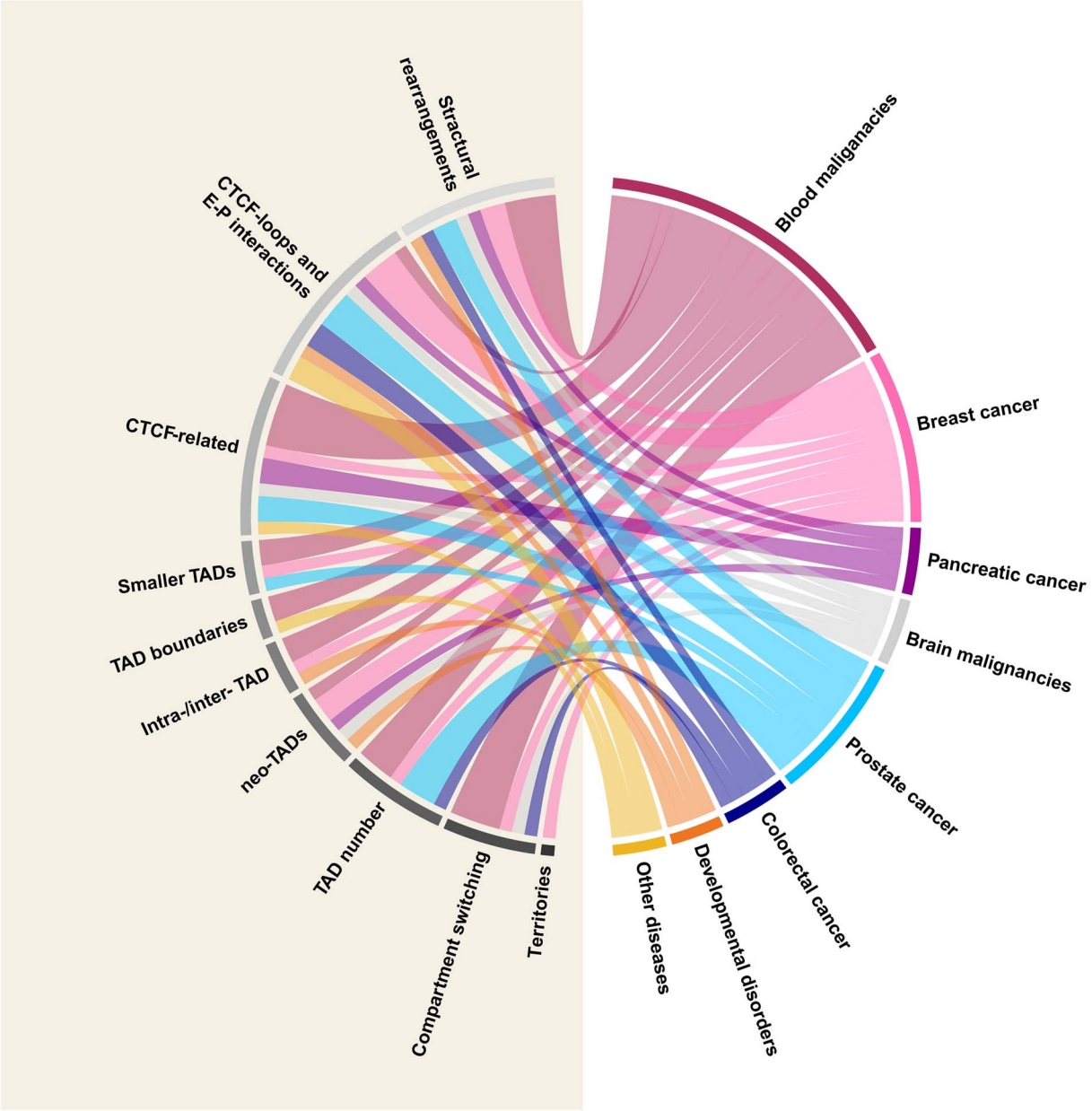

Figure 1. Genomic architecture alterations in cancer and disease. Different pathologies (colour-coded on the right half of the diagram) are connected to different 3D genome architecture alterations (grey-scale-coded on the left half of the diagram). Note that CTCF-related aberrations include mutations of both the *CTCF* gene and its binding sites, E-P signifies enhancer–promoter interactions and connector line width corresponds to the number of citations underlying it. CTCF loops and E-P interactions are affected in breast cancer (36,87,88); structural rearrangements and A/B-compartment switching often occur in blood malignancies (21,27,52–54,74,94); CTCF-related alterations have been seen in pancreatic cancer (69,81) and loss or gain of TADs has been documented in prostate cancer (59–61).

regulatory region alters gene expression and induces female-to-male sex reversal disorder of sex development (26,55); an inter-TAD duplication gives rise to a 'neoTAD' facilitating the overexpression of *Kcnj2* via ectopic interaction with a *Sox9* enhancer in Cooks syndrome (26); and in type-2 diabetes, TAD-confined chromatin hubs, which control islet identity and are composed of enhancer clusters and their target genes, display variation in enhancer content such that the expression of key genes and heritability of diabetes are affected (56). Along these lines, we now have evidence that TAD-level alterations are also implicated in various ways in tumorigenesis (57).

Although generally conserved among cell types (13), TADs show a significant variation between cancer cells (58). For instance, breast (48), prostate (59–61) and myeloid tissue cancers (52) exhibit increased TAD numbers compared with normal tissue, whereas in B-cell lymphomas and colon mucosa, specific gains and losses have been recorded for both autosomal and sex chromosomes (50,53). The increase in TAD numbers typically occurs as a result of aberrant insulation; TADs seen in normal tissue are split in multiple smaller cancer-specific TADs via addition of new boundary positions (48,52,53,61). In addition to changes in TAD counts, intra-TAD alterations have also been

observed. In breast cancer for instance, the gain of proximal intra-TAD interactions occurs at the expense of inter-TAD ones (62). In T-ALL and in a leukemia differentiation model, *de novo* cancer-specific intra-TAD interactions correlate with altered NOTCH1 binding and CTCF occupancy at the interacting fragments and, thus, ultimately with altered enhancer activity (21,63). At the same time, T-ALL inter-TAD associations also increase as a result of loss of CTCF-mediated insulation that promotes TAD fusion (21).

Like in developmental disorders, neoTAD formation has now been reported for diffuse large B-cell lymphoma (64), neuroblastoma, pancreatic and breast cancer (27). NeoTADs result from Mbp-sized structural rearrangements (26,27) and here encompass cancer-promoting genes (27,64). Similarly, the formation of *de novo* eLADs that harbour genes with a key role in inducing epithelial-to-mesenchymal transition, characteristic of metastatic malignancies, has been reported (8). Altogether, these examples suggest that changes involving TAD boundaries, intra-TAD contacts or the emergence of eLADs can act to promote gene mis-regulation which is, in turn, exploited for cancer evolution.

Cancer-promoting structural and copy number variants with respect to chromatin domains

Structural rearrangements are characteristic of most cancers (27,65,66) and originate from DNA double strand breaks (DSBs). Recent work combining Hi-C data and DSB induction has revealed that breaks are significantly enriched at CTCF loop anchors and TAD/domain boundaries marked by highly active gene promoters and topoisomerase II complexes (67–69). These 'hotspots' for structural rearrangements contribute to the generation of translocations within their topological neighborhood (70,71). In accordance, translocation partners, like in the case of the *MYC-IGH* fusion, present a higher-than-random contact probability in karyotypically normal cells, suggesting that spatial proximity precedes and facilitates the structural rearrangement itself (72,73).

In addition to large structural variants, copy number variations (CNVs) are also enriched at TAD boundaries (e.g. in myeloma; 52) and alter the expression of cancer-related genes both in *cis* and in *trans* via gene dosage changes (e.g. in leukemia; 74). Interestingly, TAD boundaries themselves were shown to be co-duplicated with enhancer clusters ('superenhancers'; 75) in a manner that correlates with leukemogenic transcriptional changes (74,76). In fact, enhancer amplification has been linked to oncogene activation and is considered as a driver mutation in DLBCL, follicular lymphoma (77) and colorectal carcinoma (78). On the other hand, the high occurrence of mutations in *CTCF* and cohesin complex genes (28,79) often leads to diminished CTCF/cohesin levels (e.g. in leukemia; 54,74) and to a concomitant loss of insulation at domain boundaries, which permits enhancer rewiring.

However, CTCF-relevant mutagenesis is not confined to the *CTCF* gene but also extends to its cognate binding motifs. CTCF binding sites at TAD boundaries or at 'superenhancers' are markedly enriched for cancer-related mutations (69,80). For example, a pancreatic cancer single nucleotide polymorphism at a TAD boundary weakens the expression of the *MFSD13A* tumor suppressor (81), and loss of insulation reported for T-ALL and glioma-specific TAD boundaries triggers oncogene activation due to altered CTCF occupancy (21,82). Given that CTCF binding is also sensitive to DNA methylation (28) and that cancer genomes are often aberrantly methylated (44), loss of insulation is also often attributed to hypermethylation (82,83).

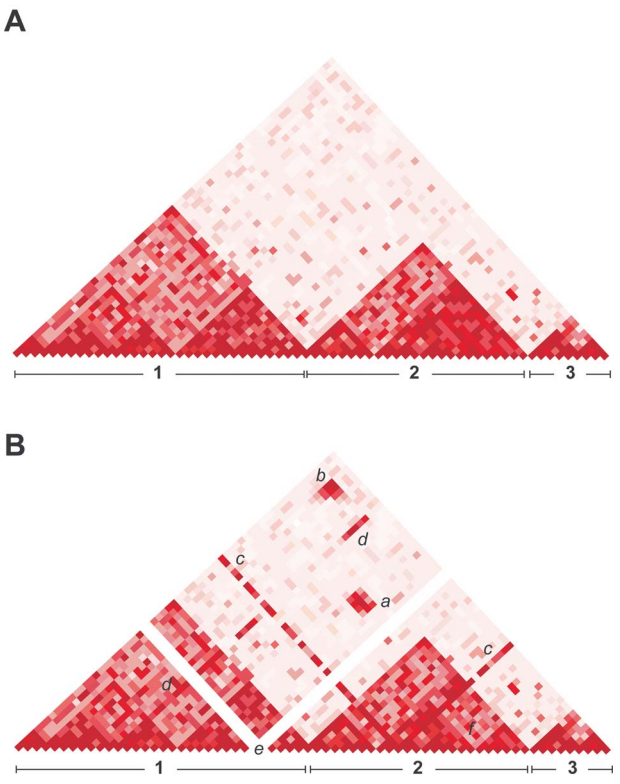

Figure 2. Conformation studies as a diagnostic tool. Illustration of a normal (**A**) or aberrant (**B**) Hi-C map involving data from three chromosomes. (A) Normal chromosomal territories (marked as 1, 2 and 3) with TADs and sub-TADs are depicted. (B) The same map as in panel A is shown, but with cancer-related alterations added. Namely, a translocation between chromosomes 1 and 2 (*a*); a balanced translocation between chromosomes 1 and 3 (*b*); a double minute signal (*c*); an amplification resulting in new interactions and stronger intra-TAD interactions (*d*); a deletion (*e*) and a TAD structure change (*f*). The depiction is based on data from (92).

Loss of CTCF binding was shown to further occur by a larger mutation, namely the deletion of an entire CTCF binding site identified as a key prostate cancer risk-associated position and leading to context-relevant expression changes (84).

Analysis of such non-coding risk loci across multiple cancer types revealed that they represent *cis*-regulatory elements contacting promoters that change expression in malignancy (36,85–89). These long-range enhancer–promoter contacts almost invariably rely on CTCF/cohesin enrichment for their stabilization. Thus, they can work to sustain prostate cancer gene expression patterns (59,60) or multi-loop hubs involving cancer- and 'stemness'-related genes with high expression levels in glioblastoma (49). Altogether, examples like these describe a dose-dependent relationship between 'cancer-specific' chromatin interactions and gene expression changes.

Genome topology in diagnosis and treatment?

Most of the aforementioned observations were made possible by 3C-based technologies. However, in addition to explaining questions about cancer-specific alterations in chromatin conformation and gene expression at high resolution, Hi-C may also nowadays function as a complementary diagnostic tool to differentiate between cancer sub-types, identify rearrangements (especially balanced rearrangements and double minutes) and

map contacts in abnormal karyotypes (27,52,90–93) (Fig. 2). In fact, Hi-C can now be reliably applied to limiting the amounts of input cells (64) with costs equivalent to ~1/3 of those needed for sufficiently deep WGS diagnosis (92). This means that in a single experiment, one can now detect both conformational and sequence variants in patient samples with costs equivalent to that of WGS while also rendering patient-specific genome scaffolds *in silico* (27).

Finally, CD276 was recently identified as a potent drug target in glioblastoma by combining compartments, loop structure and expression data with gene ontology analyses. Elevated *CD276* levels correlate with poor prognosis and its pharmacological targeting appeared to limit glioblastoma stem cell self-renewal (49). Similarly, a two-TAD fusion in T-ALL allows for *de novo* interactions between the *MYC* promoter and a CDK7-sensitive enhancer cluster. Thus, treatment with CDK7 inhibitors could be used to decrease *MYC*-enhancer interactions and reduce its expression levels (21). Moreover, in a gastrointestinal stromal tumor subtype exhibiting widespread hypermethylation, reduced CTCF binding to its methylated cognate sites led to aberrant interactions of the *FGF3*, *FGF4* and *KIT* oncogenes with nearby enhancer clusters, and thus to their hyperactivation. Treating patient-derived xenografts with a drug combination that included KIT and FGF-receptor inhibitors resulted in a lasting growth attenuation of cancer cells *in vivo* (83). Though still not that many, these examples highlight the potential of genome conformation studies as tools directly relevant to clinical care and drug selection.

Outlook

The spatial conformation of chromosomes appears to play a crucial role in pathologies in general as well as in cancer specifically. Alterations in chromosomal architecture show how gene expression can be mis-regulated without the involvement of sequence mutations. In fact, new molecular pathways that can affect genome topology and gene regulation keep emerging, like the recent description of the contribution of transposable elements (TEs) in shaping chromatin (95), which can be conceptually linked to the aberrant activation of TEs during cancer evolution (96). Thus, such changes can serve as 'structural markers' for differential diagnosis. However, we are still far from having druggable targets involving spatial genome conformation. To achieve this, there is a need for systematic interrogation of cancer entities and pathologies that rely on aberrant chromatin folding. Only this way would it become possible to find new vulnerabilities and exploit 3D genome technology in personalized therapy (97).

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