Trends in Genetics

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REVIEWS

Review

Chromatin Interactions and Regulatory Elements in Cancer: From Bench to Bedside

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Chromatin interactions regulate gene expression by bringing distal regulatory elements, such as super-enhancers, to promoters in close spatial proximity. It has been recognized that in cancer, chromatin interactions can be dysregulated, leading to aberrant oncogene expression. Chromatin interactions may potentially serve as biomarkers, or be modulated via CRISPR therapy and small molecule inhibitors against transcription. However, these methods face challenges that must be resolved and raise questions for further research. Understanding chromatin interactions is essential for safety aspects of anticancer therapies, such as the mechanism of action of epigenetic regulators and transcription factors in cancer, and potential off-target effects arising from targeting super-enhancers and promoters. In this review article, we discuss how chromatin interactions and regulatory elements may become dysregulated in cancer, potential methods to target them for clinical therapy, and outline outstanding questions that require addressing before epigenetic therapies can translate to the clinic safely and effectively.

Highlights

In cancer, mutations in noncoding distal regulatory elements and aberrant chromatin remodeling can alter the chromatin interaction landscape, resulting in the dysregulation of gene expression.

Chromatin interactions and regulatory elements can potentially serve as diagnostic and predictive biomarkers for epigenetically driven cancers.

Chromatin interaction analysis can guide the selection of regulatory elements for genetic and epigenetic editing, as well as identify potential off-target effects.

General inhibitors of transcription preferentially target cancer addiction to oncogenes driven by super-enhancers, but their effects on chromatin interactions in off-target normal cells remain to be elucidated.

The mechanisms of chromatin interaction formation need to be better understood to translate epigenetic therapies to the clinic safely and effectively.

Regulatory Elements Regulate Gene Expression through Chromatin Interactions

In the 3D genome, **chromatin interactions** (see Glossary) bring two or more linearly distant regions of chromatin into close spatial proximity, allowing distal regulatory elements, such as **enhancers** and **silencers**, to recruit transcription factors to proximal gene **promoters**, thereby modulating transcription. The chromatin interaction landscape is unique across different cell types and plays an important role in regulating cell-type-specific **epigenetic** control of gene expression [1,2]. The development of chromatin interaction analysis techniques such as fluorescence *in situ* hybridization (FISH) and chromosome conformation capture (3C) has enabled rapid progress in this field in recent years (see Figure I in Box 1).

Enhancers are primarily defined by their ability to regulate gene expression across long genomic distances, by working coordinately with promoters, which directs transcription of the proximal gene. Enhancer elements, open chromatin regions marked by mono-methylation of histone H3 at lysine 4 (H3K4me1) and acetylation of histone H3 at lysine 27 (H3K27ac) [3], recruit transcription factors, chromatin remodelers, and transcriptional machinery to distant target gene promoters via chromatin interactions, activating transcription of specific genes. Unlike promoters, which are located proximal to the transcription start site, enhancers are orientation independent and can be located upstream or downstream of their target genes. Enhancers are also frequently bi-directionally transcribed [4,5], producing noncoding enhancer RNAs (eRNAs). eRNAs frequently remain tethered to the template DNA after transcription and mediate transcription of interacting genes by recruiting transcription factors and chromatin remodelers through RNA–protein interactions, or by acting as decoys to inhibit transcription repressors [6,7].

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Recent studies have defined a category of enhancers known as super-enhancers: clusters of enhancers with exceptionally high enrichment of transcriptional activators [8–10]. Super-enhancers are particularly bound by master transcription factors and regulate expression of genes key to cell identity [10]. Super-enhancers have been shown to be associated with more chromatin interactions than typical enhancers, even after normalizing for enhancer size, and these chromatin interactions loop super-enhancers to multiple distant target genes [11].

Enhancer–promoter interactions generally work within the confines of **topologically associated domains** (TADs), regions segregated away from each other by chromatin interactions between **insulator** elements [12]. TADs form a higher-order chromatin organization that is crucial for preventing ectopic enhancer–promoter interactions and gene misexpression [13].

TAD boundaries are enriched for structural proteins such as the CCCTC-binding factor (CTCF) and the cohesin complex [12]. CTCF brings TAD boundaries together by forming a homodimer in an orientation-dependent manner, requiring convergent binding motifs at the two contact points [14]. In the 'loop extrusion' model, the ring-shaped cohesin complex binds and passes DNA through its lumen to form a loop, until it reaches the CTCF homodimer in the convergent orientation [15]. Recent papers have shown that the Yin Yang 1 (YY1) protein may perform similar functions as CTCF in enhancer–promoter chromatin interactions [16,17].

Box 1. Chromatin Interaction Analysis Techniques

Chromatin interactions can be identified through imaging techniques such as fluorescence *in situ* hybridization (FISH) and genetic approaches such as the chromosome conformation capture techniques (C techniques).

In FISH, DNA probes bound by different fluorescent molecules are designed to target the genomic loci of interest, allowing for the measurement of 3D distances between them via microscopic imaging [95].

In the C techniques, formaldehyde is used to crosslink DNA, resulting in a frozen snapshot of chromatin conformation within the nucleus. The pairs of interacting chromatin are then digested using restriction enzymes, followed by intermolecular ligation, ultimately yielding a library of DNA molecules containing chimeric sequences from pairs of interacting chromatin.

The chromosome conformation capture (3C) technique quantifies chromatin interactions between two genomic loci (one to one) (Figure IA) [96]. This is done through PCR amplification of the chimeric DNA library using primers designed at each locus.

Circularized chromosome conformation capture (4C) identifies all interactions with a specific locus (one to many) (Figure IB) [97]. Inverse PCR primers are designed at the locus of interest, to enrich for chimeric DNA molecules containing the locus. The interacting chromatin can then be identified using next-generation sequencing.

Carbon copy chromosome conformation capture (5C) technique identifies interacting pairs within a large genomic region (many to many) (Figure IC) [98]. Unlike 3C, 5C uses primers designed at all restriction enzyme cut sites within the region of interest to assay all pairwise combinations of interactions. Microarrays or next-generation sequencing techniques are used to read out the different interaction combinations.

Hi-C technique leverages on next-generation sequencing to obtain a genome-wide view of all interactions within the cell (all to all) (Figure ID) [99].

Chromatin interaction analysis by paired-end tag sequencing (ChIA-PET) identifies genome-wide chromatin interactions that are associated to a specific protein of interest, by performing an additional chromatin immunoprecipitation step to enrich for protein-bound sequences (many to many) [100].

Glossary

Chromatin interaction: the physical interaction between two or more genetic loci in the genome.
CRISPR: clustered regularly interspaced short palindromic repeats. CRISPR-associated proteins (CRISPR-Cas) can bind to DNA sequences determined by single-guide RNAs (sgRNAs) through complementary base pairing.
Enhancer: distal regulatory region that interacts with gene promoters via long-range chromatin interactions to recruit transcription factors and chromatin remodelers to activate transcription.
Epigenetics: a category of potentially heritable features that regulates gene expression without involving changes to the DNA sequences. These features include chromatin interactions, histone modifications (such as acetylation and methylation of lysine residues), DNA methylation, nucleosome positioning, and RNA signaling.
Genome-wide association studies (GWASs): studies that identify genetic variants, such as SNPs, that occur at higher frequencies in a particular disease compared to the normal population.
Insulator: genomic regions binding proteins that function as barriers to constrain enhancer–promoter interactions.
Long noncoding RNA: RNA transcripts longer than 200 bases that do not have protein coding function.
Phase separation (in biology): the localized concentration and interaction between certain cellular components form droplets of higher density that separate from the liquid environment surrounding it, akin to how oil separates from water.
Promoter: proximal regulatory regions upstream of gene transcription start sites that contain binding sites for RNA polymerases and other transcription factors.
Silencer: a distal regulatory region, bound by repressive transcription factors, that represses gene transcription via long-range chromatin interactions to promoters.
Topologically associated domain (TAD): compartmentalized DNA regions formed via insulator chromatin interactions. TADs show

higher intra-region gene interactions and reduced chromatin interactions between two different regions.

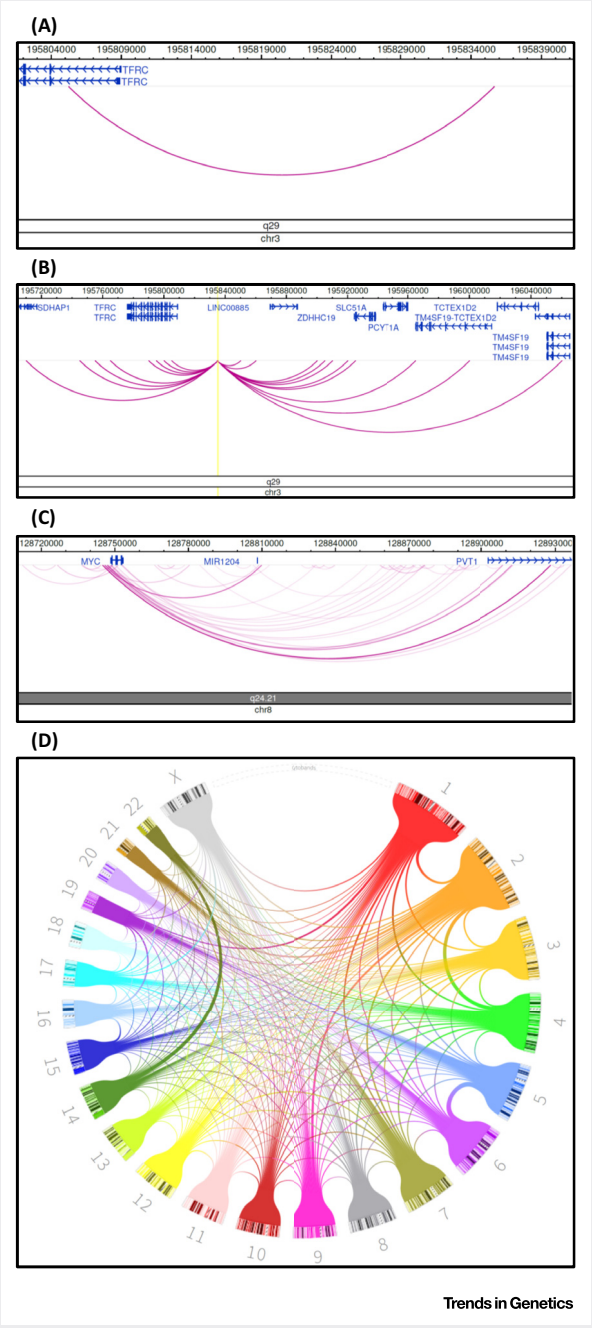

Figure I. C Techniques Allow for the Identification and Quantification of Chromatin Interactions. Visualization of the different C techniques was generated using the WashU Epigenome Browser with simulated data (http://epigenomegateway.wustl.edu/browser/) [101] and the Rondo analysis tool (http://rondo.ws) using K562 Hi-C from Rao *et al.* [102]. (A) Chromosome conformation capture (3C) is used to quantify interactions from one locus to another (one to one). (B) Circularized chromosome conformation capture (4C) identifies all interacting partners of the gene locus of interest (one to many). (C) Carbon copy chromosome conformation capture (5C) identifies all chromatin interactions within a genomic region (<1 megabase) (many to many). (D) Hi-C captures all chromatin interactions within the genome (all to all). Abbreviation: chr, chromosome.

An additional class of genetic elements is the silencers. Silencer elements have been defined as distal binding sites for repressive transcription factors, such as the Polycomb Group proteins [18] and the neuron-restrictive silencer factor [19]. However, unlike enhancers, not much is known about silencers, such as their general genetic and epigenetic signatures and modes of action.

Recent studies have put forth a **phase-separation** model of transcription regulatory control by chromatin interactions [20], whereby the clustering of transcriptional machinery at enhancer–promoter interactions forms phases of different densities that act as membrane-less compartments (Figure 1A). Transcription co-activators with intrinsically disordered regions, such as BRD4, RNA polymerase II, and the Mediator complex, have been shown to form phase-separated condensates at super-enhancers and interacting promoters *in vitro* and *in vivo* [21–24], sequestering transcriptional machinery locally to drive transcription. RNA has also been shown to bind proteins and participate in phase separation [25–27].

Chromatin Interactions and Regulatory Elements in Cancer

Chromatin interactions play a major role in regulating gene expression; hence, alterations in the chromatin interaction landscape can result in major pathological changes [13]. Unsurprisingly, cancer-associated alterations in the chromatin interaction landscape and their participants can contribute to the dysregulation of gene expression [28] and form distinct cancer-specific signatures [29,30].

Enhancers are commonly mutated in cancer, throwing gene regulation into disarray. For example, at the chromosome 8q24 region, genome-wide association studies (GWASs) identified risk loci for multiple cancer types that coincide with enhancers [31]. These enhancers form chromatin interactions with the *MYC* oncogene and likely contribute to oncogenesis through these interactions [31]. In addition, oncogenes can become dysregulated through the translocation of enhancers to the vicinity [32] (Figure 2A).

Aberrant transcription factor binding can also alter chromatin interactions. In T cell acute lymphoblastic leukemia, overexpression of c-MAF increases its binding at the *TAL1* gene promoter and mediates an inter-chromosomal enhancer–promoter interaction that upregulates *TAL1* expression (Figure 2B) [33]. Transcription factor binding can also be gained from point mutations, mediating new chromatin interactions [34] (Figure 2C).

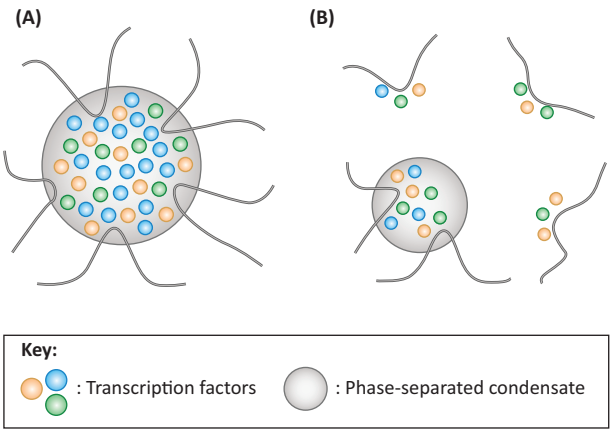

Figure 1. Phase Separation at Enhancer–Promoter Interactions. (A) Enhancers and promoters recruit and concentrate various transcription factors and co-activators such as Mediator, BRD4, and RNA polymerase II, creating localized regions of high density. These regions form phase-separated condensates (in gray) that act as membrane-less compartments, accumulating transcriptional machinery (colored circles) to facilitate transcription of participating genes. (B) Inhibition of transcription factors may reduce the size and density of phase-separated condensates, dysregulating the enhancer–promoter interactions within these condensates.

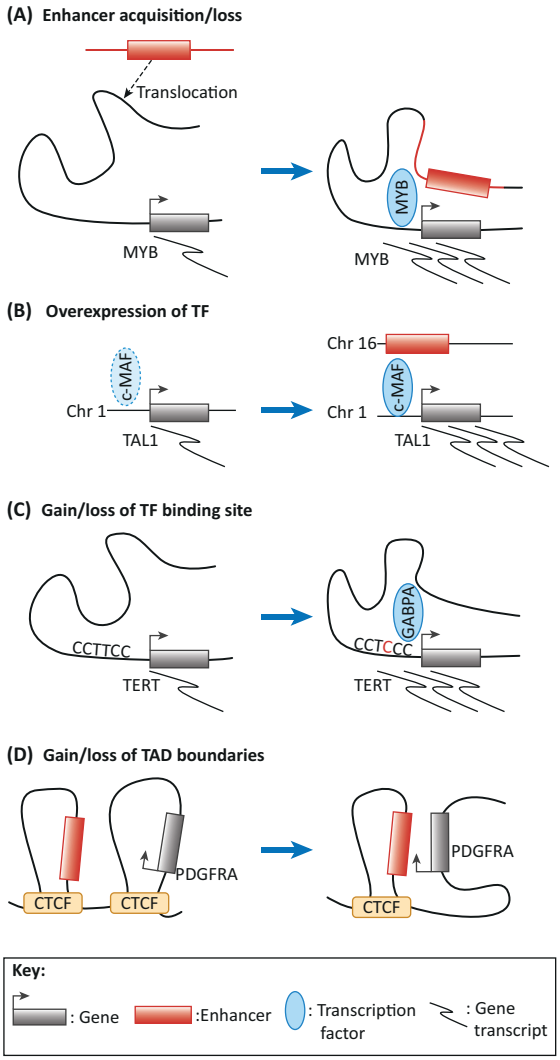

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Figure 2. Acquisition of New Chromatin Interactions in Cancer Can Lead to Aberrant Gene Expression. (A) Translocation of a NFIB super-enhancer to the vicinity of the MYB oncogene in adenoid cystic carcinoma forms a chromatin interaction, leading to overexpression of MYB and the establishment of a positive feedback loop. (B) Overexpression of c-MAF in T cell acute lymphoblastic leukemia upregulates TAL1 expression by mediating an interchromosomal chromatin interaction between TAL1 on chromosome (Chr) 1 and the TIL16 enhancer element on Chr 16. (C) In multiple cancers, an SNP in the promoter region of the TERT gene enables binding of GABPA, which mediates the formation of long-range chromatin interactions and upregulates TERT transcription. (D) Loss of CTCF binding in IDH-mutant gliomas compromises topologically associated domain (TAD) insulation and allows for aberrant chromatin interactions between an enhancer and the PDFGRA gene. Abbreviations: TF, transcription factor; Chr, chromosome; TAD, topologically associated domain.

Alterations in higher-order chromatin organization can contribute to cancer as well. Breast cancer cells were observed to have abnormal genomic compartmentalization compared to non-malignant cells, with increased open chromatin regions correlated to upregulation of oncogenes [35,36]. TAD boundaries are also frequently disrupted in cancer, thereby rewiring chromatin interactions and contributing to oncogenesis [37]. This disruption can be due to the

loss of CTCF binding, either through mutation or DNA hyper-methylation of CTCF binding sites [38,39] (Figure 2D), leading to aberrant chromatin interactions across TADs [40,41].

Overexpression of **long noncoding RNAs (lncRNAs)** can also facilitate cancer-associated chromatin looping. Cancer-specific expression of the *CCAT1-L* lncRNA was shown to facilitate chromatin interactions at the *MYC* locus to upregulate the *MYC* oncogene [42].

GWASs of multiple types of cancer show that most risk loci harboring cancer-associated mutations are noncoding in nature [43]. These noncoding risk loci frequently fall within distal regulatory elements that modulate gene expression via chromatin interactions. To target these regions for cancer biomarker and therapy discovery, it is thus important to understand their associated chromatin interactions. Chromatin interactions are also important for us to understand the mechanisms of action and safety of epigenetic drugs, drugs based on transcription factors, as well as genetic and epigenetic editing.

Given the importance of chromatin interactions in cancer, in this review article, we discuss the potential for translating our understanding of genomic elements and associated chromatin interactions into the clinic and the work that needs to be done to make this a reality.

Chromatin Interactions Guide Cancer Biomarker Discovery

Understanding chromatin interactions may be important for the identification of diagnostic and predictive biomarkers for epigenetically driven cancers, where dysregulation of oncogenes arise from epigenetic alterations in addition to or instead of coding mutations. Aberrant DNA methylation, chromatin remodeling, and transcription factor binding can result in the formation or disruption of cancer-associated chromatin interactions that facilitate overexpression of driver oncogenes or silencing of tumor suppressors [44].

However, chromatin interaction analysis of clinical samples has always been stymied by high cell number requirement and long processing time. In one study, the authors surmounted these obstacles by first narrowing down probable chromatin interaction sites around five melanoma-associated genes using a pattern-recognition algorithm that accounts for various genetic and epigenetic features, before testing them with a modified 3C-PCR assay (Figure 3A) [45]. A panel of 15 cancer-specific chromatin interactions was shortlisted as a potential diagnostic biomarker for melanoma, and 3C-PCR analysis of this panel correctly distinguished between cancer and normal clinical blood samples 80% of the time [45,46].

The position of genes relative to the center of the nucleus (radial position) is relatively conserved. Chromosomes occupy specific territories within the nucleus, but gene-poor and transcriptionally silent regions tend to situate near the nuclear periphery, whereas active genes can loop out of their chromosome territories to cluster more centrally [47]. In cancer, dysregulation of cancer-associated chromatin interactions can lead to a concomitant radial repositioning of certain genes, independent of their expression [48]. This change in radial positioning can be captured using high-throughput FISH imaging [49], and diagnostic FISH biomarkers for prostate and breast cancer have been proposed [50,51].

Chromatin interactions and regulatory elements can also serve as predictive biomarkers to guide for drug selection. In one study, acute myeloid leukemia (AML) patients were clustered based on their super-enhancer profile, and a novel subset of AML patients was identified based on the presence of a retinoic acid receptor alpha (RARA)-associated super-enhancer formed by increased H3K27ac histone modifications [52]. This super-enhancer biomarker was shown to

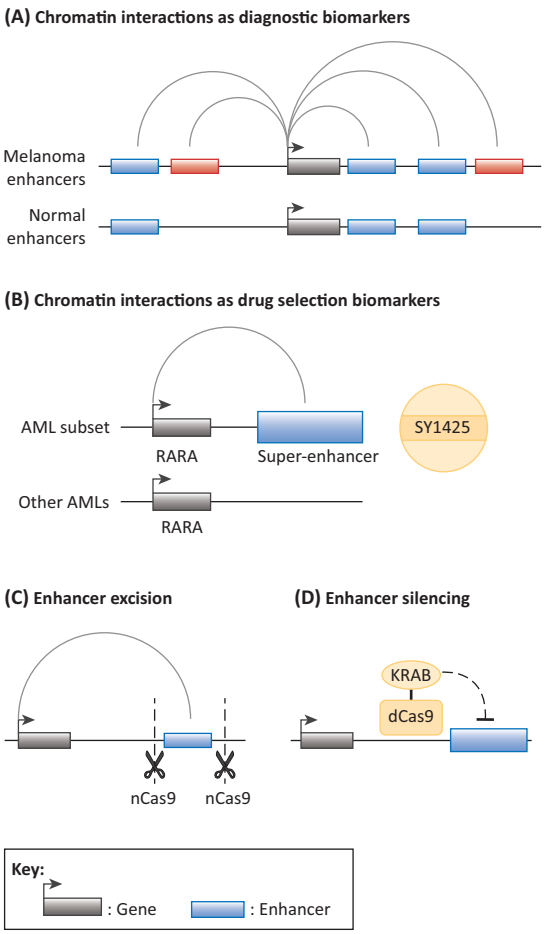

Figure 3. Chromatin Interactions Can Influence Cancer Therapy. (A) Chromatin interactions (shown as loops) can serve as diagnostic biomarkers for cancers such as melanoma, before discernible changes in gene and protein expression. Enhancers are shown as blue boxes. (B) Chromatin interactions can influence drug selection for chemotherapy, by identifying novel subtypes of cancer. In this example, a novel subset of acute myeloid leukemia (AML) patients with cancer-associated RARA super-enhancer are more sensitive to SY1425 retinoic acid receptor alpha (RARA) agonist treatment. (C) Excision of cancer-associated enhancers can be achieved by targeting Cas9 nickase (nCas9) to both ends, reducing expression of oncogenes associated via chromatin interactions. (D) Enhancers can alternatively be silenced using nuclease-deactivated Cas9 (dCas9) fused with transcriptional repressive domains such as Krüppel associated box (KRAB).

predict for increased tumor sensitivity toward RARA-targeting compounds, and a biomarker-directed Phase II clinical trial is ongoing (Figure 3B) [53]. Another study also shortlisted potential drug targets for ependymoma based on association with tumor- and subset-specific super-enhancers [54]. Since super-enhancers activate target genes via chromatin interactions, the chromatin interactions acquired by these super-enhancers can also be ideal candidates for predictive biomarkers.

A pressing question is whether cancer-associated chromatin interactions can serve as diagnostic biomarkers for early detection of epigenetically driven cancers. It is not clear whether changes in chromatin interaction precede and cause corresponding changes in gene and

protein expression in epigenetically driven cancers, or vice versa. Global reorganization of chromatin interactions was observed in estrogen receptor activation [55], yet remains stable in glucocorticoid receptor activation [56], even though both processes involve widespread gene expression changes, highlighting the complex relationship between chromatin interactions and gene expression. Currently, the dynamics of chromatin interaction formation is still largely unexplored.

Modulating Regulatory Elements and Chromatin Interactions through Genetic and Epigenetic Engineering

The discovery of programmable DNA-binding domains (DBDs) that can precisely recognize specific DNA sequences has sparked a surge in research on genetic engineering. These include engineered proteins that recognize DNA sequences by structure, such as zinc fingers [57] and transcriptional activator-like effectors (TALEs) [58]. The discovery of clustered regularly interspaced short palindromic repeats (**CRISPR**)-associated systems (CRISPR-Cas) that recognize DNA sequences by complementary RNA-DNA base pairing with a guide RNA has greatly increased the ease of engineering DBDs [59].

DBDs with nuclease activity allow for precise cleavage of the target DNA sequence and optionally insert a replacement sequence through homology-directed repair (reviewed in [60]). This allows for modification of not only oncogene sequences but also dysfunctional cancer-specific distal regulatory elements. These oncogenes frequently play important physiological roles in normal cells. Since current DBD delivery methods cannot distinguish between cancer and normal cells *in vivo*, deletion of cancer-specific distal regulatory elements can minimize potential side effects by reducing gene expression instead of eliminating it altogether. Cancer cells frequently grow reliant on high expression of key oncogenes for survival and proliferation [61,62] and are therefore much more sensitive than normal cells to this reduction in oncogene expression.

Recently, it was shown that CRISPR/Cas9 excision of an entire 13-kb super-enhancer downstream of the *SOX2* gene can reduce SOX2 transcription by more than 90%, without affecting other genes along the same chromosome [63]. Myc-deficient mice embryos die from developmental defects, whereas mice with deletion of a *Myc* cancer-associated super-enhancer grew normally, highlighting the safety aspects of deleting distal regulatory elements instead of oncogenes themselves [64]. Targeted deletion of transcription factor binding sites within enhancers can also elicit a change in gene transcription. CRISPR/Cas9 deletion of a p300 binding site within a *GATA2* enhancer reduced *GATA2* transcription levels, without changing the expression of the neighboring *RPN1* gene [65].

Deletion of binding sites for chromatin architectural proteins, such as CTCF [66] and YY1 [16], can also disrupt physical interactions between enhancers and promoters, reducing transcription levels of the associated genes. However, deletion of individual CTCF binding sites appears to be redundant, possibly due to compensation from flanking binding sites and multiple chromatin contact points [67,68]. Further work has to be done to identify other structural factors that may be involved in maintaining chromatin interactions.

Gene editing techniques can also be adapted to modify epigenomic features for transcriptional regulation without modifying gene sequences. This is achieved by fusing nuclease-deactivated Cas9 protein (dCas9) with transcription-regulating domains. Transcriptional repression domains, such as KRAB (Krüppel associated box) [69], SID (mSin3 interacting domain) [70], and MeCP2 (methyl-CpG-binding protein 2) domains [71], close up chromatin and inhibit

translation initiation to silence the target gene. Transcriptional activation domains, such as VP16 (virus protein 16) and p300, increase chromatin accessibility for transcription factor binding to promote transcription. DNA methylation status can also be perturbed through the fusion of dCas9 to DNMT3a or Tet1 domains [72,73]. By itself, dCas9 binding can increase chromatin accessibility to facilitate gene transcription [74,75], or inhibit gene transcription by physically obstructing RNA polymerases along the gene body during transcription elongation [76].

Although there are advantages in editing regulatory elements instead of target genes themselves, there are also concerns about off-target effects. Enhancers can regulate genes through a complex network of regulatory relationships, facilitated by chromatin interactions [77]. Individual enhancers can skip over the nearest gene to regulate multiple distal genes via chromatin interactions [11]. For example, Ooi *et al*. [30] showed that CRISPR/Cas9 deletion of enhancer regions near *CLDN4* in gastric adenocarcinoma downregulated multiple distal interacting genes, without significant changes in the proximal genes. At the same time, enhancers can combine additively, synergistically, or repressively to regulate the same gene [78]. Moreover, recent findings have shown that super-enhancers are frequently associated with genes important in cancer and would likely be ideal targets for editing therapy [9,79]. Super-enhancers are associated with more chromatin interactions, and hence more target genes, compared to typical enhancers [11], thus making it even more important to understand the chromatin interactions associated with these super-enhancers.

It will be beneficial to perform chromatin interaction analysis at the gene of interest to identify all interacting enhancers, before narrowing down the list to cancer-specific enhancers with high regulatory activity (Figure 4A). Enhancer regulatory activity can be assayed using techniques such as self-transcribing active regulatory regions sequencing (STARR-seq) [80,81] in the 3′ untranslated region of a reporter gene such that the transcription level of the enhancer correlates with its regulatory strength. This should be followed by chromatin interaction analysis at the candidate enhancer, to identify potential off-target side effects (Figure 4B).

Translating Genetic and Epigenetic Editing to the Clinic

Gene editing technologies have advanced to clinical trials at various stages, primarily for *ex vivo* gene therapies. Zinc-finger, TALE, and CRISPR technologies have been used to generate chimeric antigen receptor T cells (CAR-T cells) for cancer treatment in multiple clinical trials, by engineering harvested patient T cells to recognize cancer-specific or cancer-associated antigens and replacing them back into the patient [82]. This has culminated in FDA approvals for CD19 CAR-T cell therapies against leukemia and lymphoma.

By contrast, *in vivo* editing therapies face more challenges. Unlike the controlled environments for *ex vivo* therapy, editing complexes or their coding sequences need to be delivered specifically to the target cells, without affecting other cells in the body. Viral vectors such as adeno-associated viruses (AAVs) can be engineered to target various glycans and coreceptors for cell-type specificity [83], and cell-type-specific promoters for inserted genes minimize effects on off-target cells. Nevertheless, *in vivo* insertion of therapeutic genes has recently entered clinical trials, with the first patient treated for mucopolysaccharidosis type II through the zinc-finger-mediated insertion of the IDS gene into hepatocytes (clinicaltrials.gov ID NCT03041324) [84].

More work needs to be done before epigenetic editing can be adopted in the clinic. For one, the fusion of various domains to dCas9 exceeds the capacity of a single AAV vector and impairs

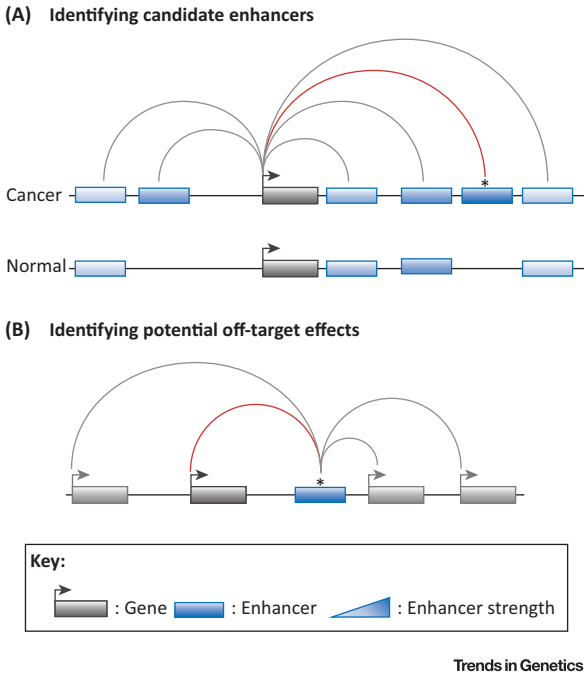

Figure 4. Chromatin Interactions Can Identify Ideal Enhancer Candidates for Genetic and Epigenetic Editing, as Well as Highlight Potential Off-Target Effects. (A) Chromatin conformation analyses at target genes identifies a list of potential enhancer candidates, which can be narrowed down based on cancer specificity and enhancer strength. (B) Chromatin conformation analyses at the shortlisted enhancer identifies all associated genes, which represent potential off-target side effects of targeting this enhancer.

delivery. Epigenetic editing also requires prolonged periods of activation, compared to the immediate action of gene excision and insertion, and may hence elicit immunogenic responses [85].

Small Molecule Inhibitors against Transcriptional Initiators

Transcription is important in cancer cells, and the discovery of super-enhancer dysregulation in cancer has suggested a mechanism for the specific targeting of pathways that are highly important in cancer maintenance. As such, small molecule inhibitors of transcription have been well studied in recent years, to target super-enhancer regulated oncogenes.

Super-enhancers are exceptionally enriched for BRD4, a BET bromodomain protein that mediates transcription activation [9]. These BRD4-enriched super-enhancers are associated with well-known oncogenes in multiple myeloma, small-cell lung cancer, and glioblastoma multiforme [9]. Inhibition of BRD4 using small molecule inhibitors, such as JQ1, especially depleted BRD4 at super-enhancers associated with oncogenes such as MYC [9]. As such, there are currently numerous ongoing clinical trials using BRD4 inhibitors to target various types of cancers [86].

Super-enhancers are also highly sensitive to inhibition of factors involved in the transcriptional machinery, because they require high densities of these factors to drive transcriptional activity [62]. Hence, cyclin-dependent kinases (CDKs) involved in transcription have emerged as potential drug targets to disrupt super-enhancers as well. Inhibitors of CDK7, such as THZ1, have been shown to preferentially downregulate genes associated with super-

enhancers in neuroblastoma [87], small-cell lung cancer [88], T cell acute lymphoblastic leukemia [89], triple-negative breast cancer [90], nasopharyngeal carcinoma [91], esophageal squamous cell carcinoma [92], and melanoma [93].

However, there are safety aspects to consider when using these transcription inhibitors. These drugs target transcription in general and may have long-term effects on normal cells. In the emerging model of phase separation in transcription, transcription factors such as BRD4 may contribute to the maintenance of phase-separated condensates and their associated enhancer–promoter interactions [24]. The use of transcription inhibitors may lead to dysregulation of the chromatin interaction landscape in off-target cells (Figure 1B), and more research needs to be done to elucidate such effects. In particular, no published studies have been done to date to examine the effects of CDK7 and BRD4 inhibitors on chromatin interactions or TADs.

Concluding Remarks and Future Perspectives

Chromatin interactions are known to be controlled by proteins such as CTCF and cohesin, as well as transcription factors. Since chromatin interactions are important in cancer, it would be desirable to target proteins that control chromatin interactions. However, targeting general factors such as CTCF and cohesin is likely to have deleterious side effects beyond that of affecting specific chromatin interactions. Therefore, an important question to address in the field is what are the determinants of specificity of chromatin interactions? How does a particular loop happen in one place, but not another? Are there specific cofactors involved in loop formation that may be targeted in cancer?

There is increasing evidence that lncRNAs participate in cancer-associated chromatin interactions, and many lncRNAs act in *cis* with the region where the lncRNAs are transcribed, which could provide locus specificity. A novel approach for the targeting of chromatin interactions is through the application of RNAi technology. A growing number of studies are applying RNAi technology to reduce cancer-specific oncogene expression, and Phase I clinical trials are being conducted [94]. However, the targeting of aberrant chromatin interactions in cancer via RNAi remains an area that is largely unexplored. These can be targeted via antisense oligonucleotides (ASOs) (Figure 5). ASOs can bind to lncRNAs through complementary base pairing, forming DNA–RNA complexes that can be recognized and degraded by the RNAse H enzyme, leading to the destabilization of cancer-associated enhancer–promoter interactions. Through

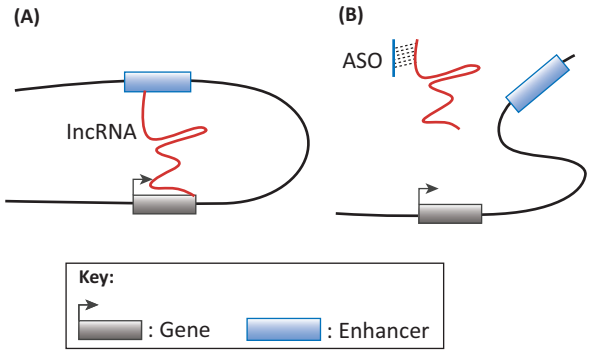

Figure 5. Antisense Oligonucleotides May Present as a Novel Method of Disrupting Cancer-Associated Chromatin Interactions. (A) Long noncoding RNAs (lncRNAs) facilitate chromosome looping of enhancers to promoters and recruit transcription factors. (B) Antisense oligonucleotides (ASO) bind to these lncRNAs via complementary base pairing and mark them for degradation, destabilizing the associated chromatin interactions.

these RNAi interventions, we may be able to reduce off-target effects that are common with conventional cancer treatments. As such, another key question is to define the roles of specific lncRNAs in loop formation that may be targeted in cancer.

Indeed, the mechanisms of chromatin interaction formation are still poorly understood and need to be better elucidated for us to develop strategies based on chromatin interactions in the clinic. While chromatin interactions disappear and re-form through the course of mitosis, it is unknown how this process occurs, and whether and how any cancer drugs that target mitosis may affect this process.

Moreover, many of the studies that have been published thus far have been done in cell lines and normal clinical samples. There is a pressing need for the field to analyze chromatin in more translational contexts such as cancer clinical samples and mouse models of cancer, to better elucidate the role of chromatin interactions in cancer.

Overall, the nascent field of chromatin interactions is starting to be increasingly recognized as playing roles in cancer, and more research needs to be done on understanding the basic mechanisms of chromatin interactions and the molecular players involved in chromatin interactions to translate our understanding of chromatin interactions into the clinic (see Outstanding Questions). At the same time, given the recent explosion of interest in CRISPR editing of enhancers and genes, it is critical to better understand how these methods affect chromatin interactions for our development of safe and effective treatments.

Outstanding Questions

What are the mechanisms by which chromatin interactions form?

What is the interplay between epigenetic factors and chromatin interactions?

What are the dynamics for the formation of chromatin interactions; which molecular events happen first?

How do specific enhancer–promoter chromatin interactions form? Are there specific cofactors and lncRNAs that are important in this process?

What happens to the associated chromatin interactions when an enhancer is excised or epigenetically modified? Does it cause a local rearrangement of chromatin interactions with wide-ranging effects?

What happens to the local chromatin interaction environment and phase separation at super-enhancers and promoters when epigenetic factors such as BRD4 and CDK7 are inhibited?

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