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Enhancers and super-enhancers as master regulators in cancer

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Abstract

Gene expression regulation is one of the most fundamental cellular processes, enabling the activation of a gene to produce either the translatable protein-coding transcript (mRNA) or a functional non-coding RNA with gene regulatory functions, ultimately determining cell identity and function. Although gene expression regulation can occur at transcriptional, translational, and post-translational levels, transcription initiation is the first and the most important step in gene expression, facilitating the transfer of biological information from DNA to protein. Enhancers and super-enhancers are among the master regulators of tissue- and cell-specific transcription regulation involved in cell differentiation and tumor formation. Despite four decades passing since the first discovery of enhancers in eukaryotes and extensive efforts undertaken to identify enhancers on a genomic scale during the last decade, the discovery of enhancers still faces certain limitations and needs further investigation. The perturbation of enhancer function due to genetic or epigenetic changes is closely linked to a range of human disorders, including the development and progression of cancers. Thus, the detection of early cancer-related enhancer activity and the subsequent normalization of expression abnormalities using enhancer-targeting CRISPR epigenetic editing, as well as enhancer-targeting pharmaceuticals, are regarded as groundbreaking therapeutic tactics in preclinical stages.

Keywords: CRISPR; Epigenetic editing; Enhancer-promoter loop; Enhancer-targeting drugs; Non-coding transcript; Super-enhancers; Transcription

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1. Introduction

Enhancers are non-coding regions of DNA, ranging from 200 to 2,000 base pairs in length that can be bound by transcription factors (TFs) to modulate the transcription of cell-specific genes. Importantly, the action of enhancers on gene expression is not restricted by their position or distance from the target gene. Interestingly, enhancers can be located upstream, downstream, adjacent to promoters, or even up to one million base pairs away from the target gene.[1] Regardless of their distance, distal enhancers can form an enhancer-promoter loop complex to physically interact with the promoter of a target gene. Enhancers typically contain specific DNA elements recognized by tissue-specific TFs. Research has shown that enhancers recruit transcription complexes at the enhancer-promoter loop, including cell-specific TFs such as OCT4, SOX2, KLF4, and Nanog, RNA polymerase II (RNA pol II), co-activators, the mediator complex, enhancer

RNAs (eRNAs), and histone-modifying enzymes including methyltransferases, histone acetyltransferase EP300, and CBP. This cooperative binding initiates and promotes transcription (Figure 1A).[2-4] Although most enhancers are located in intergenic and intronic regions of the genome, some are located within exons.[5] Super-enhancers (SEs) are extensive genomic regions formed by clusters of enhancers. SEs exhibit a higher (several-fold) binding enrichment for transcriptional factors than typical enhancers, spanning more than 20 kb on average.[6] SEs have a greater impact on the transcription of specific genes in comparison to regular enhancers and have the ability to simultaneously activate a significant number of promoters. These SEs are typically found in close proximity to genes crucial for cell differentiation.[7,8] Scientific findings suggest that active enhancers are often marked by the co-occurrence of H3K4me1 and H3K27ac. However, certain enhancers can become active solely through H3K4me1 modification.[9,10] Genes linked to the H3K27ac enhancer mark exhibit higher expression levels compared to those associated with the H3K4me1 enhancer mark.[11] Further studies described CBP/EP300-mediated H3K27 acetylation as a marker of active enhancers, since repressing this modification reduces enhancer activity, indicating that H3K27ac is causative, not just correlative, to enhancer activity.[12] An illustration of this concept is the discovery that EP300 regulates enhancers in neuroblastoma (NB) by adding the H3K27ac mark to colorectal cancer-associated SEs. This process involves interaction with the recently identified TF TFAP2β in NB cells.[13] Moreover, EP300 has been shown to disrupt the activity of epigenetic modifiers known to regulate enhancers, such as histone deacetylases and non-coding RNA (ncRNAs), hence promoting pulmonary fibrosis.[14] Intriguingly, genome-wide RNA sequencing has

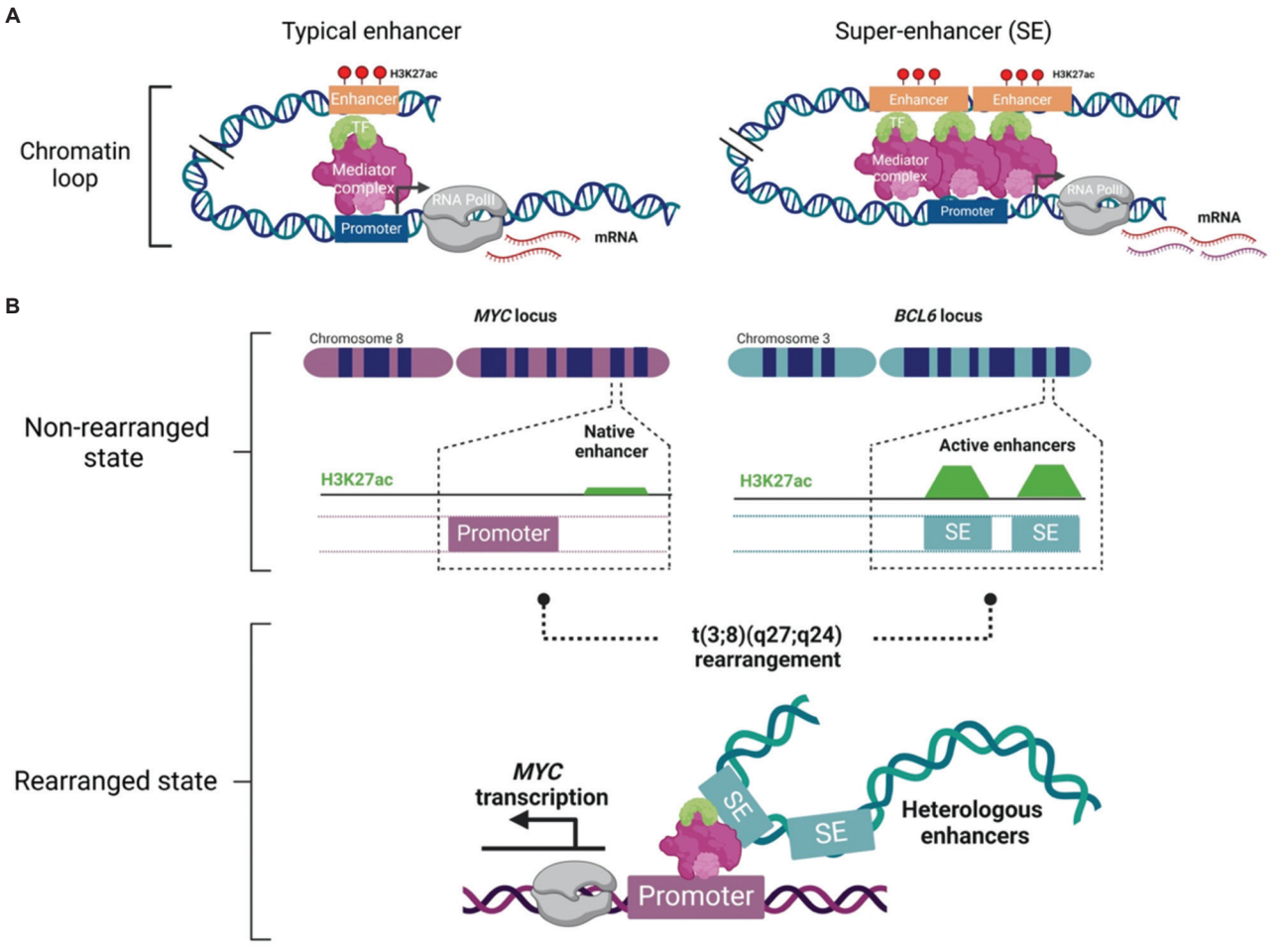


Figure 1. Enhancer hijacking and *MYC*-activating rearrangement. Heterologous genomic rearrangements linking the *BCL6* and *MYC* loci can activate *MYC* promoter through *BCL6* enhancers in B cell lymphomas. (A) Enhancers/super-enhancers elements. (B) Compared to a non-rearranged state, the genomic rearrangement t(3;8)(q27;q24) results in enhanced activation of *MYC* due to the interaction with BCL6 active distal enhancers, which are enriched with the active H3K27ac chromatin immunoprecipitation-seq mark. This rearrangement, which activates *MYC*, is linked to germline polymorphisms that modify the risk of developing lymphoma. Figure created using BioRENDER.com.

discovered that a significant portion of enhancers and SEs can be transcribed to give rise to eRNAs/SE RNAs that can further facilitate enhancer-promoter interactions, RNA pol II elongation, and can even act as decoys for repressive cofactors.[15-18]

2. Oncogenic enhancer and SEs activation by genomic rearrangements and variations

Chromosomal rearrangements, such as deletions, inversions, duplications, or translocations, can misplace active enhancers within the genome, causing abnormal gene expression. Such chromosomal rearrangements can lead to the activation of oncogenes or the silencing of tumor suppressor genes, commonly associated with cancer development.[19-22] Recent findings suggest that genomic rearrangements can lead to the repositioning of distal enhancers to the promoters of oncogenes specific to certain tumor types. These enhancers, which were not originally intended for these oncogenes, contribute to the initiation and progression of tumorigenesis by activating oncogenic signals. This phenomenon, known as enhancer hijacking, is a crucial cancer-driver mechanism.

For instance, enhancers specific to the lymphoma subtype within the *MYC* locus were shown to be silenced in lymphomas and associated with germline polymorphisms that alter the risk of developing lymphoma. Additionally, enhancers within the *BCL6* locus are subject to acetylation and possess the capability to undergo genomic duplication. Moreover, they can activate the *MYC* promoter, thereby functioning as an enhancer donor in a translocation phenomenon referred to as enhancer hijacking (Figure 1).[23]

In another study, Gröschel *et al.*[24] demonstrated that chromosomal 3q rearrangements result in the relocation of a distal *GATA2* enhancer (located 110 kb away from the *GATA2* gene at 3q21) to the *EVI1* locus. This event leads to the ectopic activation of *EVI1* expression, which is a crucial oncogenic driver in acute myeloid leukemia (AML). Furthermore, the elimination of the ectopic *EVI1* enhancer using the CRISPR/Cas9 genome-editing system resulted in decreased cell proliferation and increased apoptosis in the MUTZ-3 cell line derived from myeloid leukemia. These effects were remarkably similar to those observed when EVI1 knockdown was achieved using small hairpin RNA in the same cells.

These findings propose that chromosomal rearrangements can lead to the repositioning of a single enhancer, consequently impacting the regulation of two distinct distal genes, ultimately contributing to cancer development. In addition to chromosomal rearrangements, genetic variations such as deletions, mutations, or epigenetic modulations, such as methylation at the enhancer site, can affect the interaction among transcription regulatory elements (enhancer, promoter, and TFs binding site). Such genetic variations or epigenetic modifications often result in loop formation between proto-oncogenes and enhancers, leading to the upregulation of neighboring oncogenes and tumorigenesis. For example, a single nucleotide polymorphism (SNP) within the 15q15.1 chronic lymphocytic leukemia risk locus can create SEs that are correlated with decreased proapoptotic *BMF* expression. This SNP also hinders the interaction between the TF RELA (p65) and SE, resulting in an enhancement of BCL2's antiapoptotic function, thereby facilitating tumor growth.[25]

In addition, chromosomes are folded and arranged into 3D genomic segments that are megabases in length and have the ability to self-interact. However, interactions with regions beyond the designated topologically associated domains (TADs) are infrequent. Interactions of enhancers with their target genes are constrained due to the TAD boundaries enriched with insulator proteins such as CTCF in mammalian cells.[26,27] TAD boundary disruption due to chromosomal rearrangements or mutations can result in enhancers interacting with genes outside of the original TAD, resulting in inappropriate enhancer-promoter interactions. Such interactions have been implicated in rare diseases such as adult-onset demyelinating leukodystrophy,[28] and human limb malformation.[29,30]

A few studies have investigated TAD boundary disruption in carcinogenesis.[31,32] Interestingly, mutations in the CTCF motif at the TAD boundary result in NOTCH1 misregulation associated with ovarian cancer, due to the aberrant activity of enhancers caused by the disruption of the TAD.[33] However, more studies are required to unravel TAD boundary disruptions and their connection to cancer initiation and development.

3. Influence of SEs on tumor microenvironment

Cancer cells exhibit modified patterns of SE regulations, which contribute to the activation of oncogenes and other genes associated with key cancer features.[34,35] Chromosomal translocations in malignant lymphomas can relocate SEs to immunoglobulin loci near *MYC*, resulting in elevated levels of *MYC* expression.[36] Mechanistically, *MYC* overexpression may be reciprocally related to hijacking histone deposition, which alters cancer genome organization, as observed in the U2OS osteosarcoma cell line. Immunofluorescence labeling demonstrated that MYC molecules formed punctate foci at active transcriptional SEs, which were abrogated on removal of the architectural protein CTCF.[37]

Hijacking histone variants and chaperones, which transport histones across the cell and deposit them in

chromatin, is altered in solid tumors. H2A.Z, a highly conserved histone variant with 60% identity with H2A, is related to transcriptional activation. In mammals, there are two paralogues of H2A.Z: H2A.Z.1 and H2A.Z.2. Their expression is typically upregulated in numerous tumor types. MYC, ERα, and AR TFs can drive the addition of H2A.Z.1 to genomic sites in hormone-regulated malignancies such as breast and prostate cancer.[38] Furthermore, SEs activate the histone chaperone HJURP, resulting in abnormally high HJURP expression in t(4;14)-positive multiple myeloma. Overexpression of HJURP enhances tumor cell proliferation and is linked to poor outcomes in t(4;14)-positive multiple myeloma patients.[39] Enhancer hijacking may potentially increase resistance to treatment, rendering SEs more vulnerable to epigenetic therapies than canonical enhancers.[40] This is because SEs arise when master TFs attach to each component enhancer, attracting unusually high densities of cofactors (mediators and coactivators) that are proposed to interact with enhancers.

However, not all cofactors are essential for SEs activation. In HCT116 cells, enhancers have been classified based on their cofactor dependencies, highlighting different mechanisms for activating their correlated SEs and, thus, transcription.[41] This framework of categorization permits us to comprehend how enhancers contribute to gene expression programs and regulatory specificity.[41] Furthermore, the amount of mediators is elevated compared to other regions, making it a useful indicator for identifying SEs. Therefore, the transcription-activated complexes recruited by SEs display about 10-fold their molecular density of conventional enhancers. These complexes require a stable structure to preserve their conformation in optimal conditions.[42] High-mobility group proteins, such as HMGA1, are necessary for preserving the enhancer substructures of coactivators such as mediator subunit 1 (MED1) and bromodomain-containing protein 4 (BRD4).[43] BRD4 functions as an epigenetic reader that targets and interacts with acetylated lysine residues on histone H3 and H4. When BRD4 binds to these residues, it recruits the mediator complex, RNA pol II, and the positive transcription elongation factor b, facilitating the process of transcription initiation and elongation.[44,45]

The high levels of RNA pol II and cofactors in SEs create a condensate by establishing multivalent interactions, resulting in the formation of liquid droplets. This phenomenon may be explained by a model based on the process of liquid-liquid phase separation. The model, proposed by Hnisz *et al.*,[46,47] suggests that the dense concentration of TFs, RNA pol II, cofactors, and eRNAs enables the formation of localized phase separation through weak multivalent interactions among molecules associated with SEs. This process would be more difficult to achieve with typical enhancers. It allows for the rapid formation of a highly concentrated and dynamic environment that promotes effective transcription.[48,49] Growing data from *in vitro* and *in vivo* studies strongly support the notion that phase separation may be employed to elucidate the characteristics of SEs, encompassing their function, development, and susceptibility. Nevertheless, this model attempts to elucidate the precise order of events involved in the development of long-distance chromatin connections or the generation of transcriptional condensates. Research has demonstrated that the levels and alterations of RNA molecules have a regulatory impact on the creation and dissolution of condensates.[47] Condensate production is facilitated by synergistic interactions among polyvalent molecules, such as RNA, DNA, and intrinsically disordered regions (IDRs) in proteins.[50]

Plenty of evidence indicates that SEs undergo sudden modifications in formation and dissolution. They arise by a single nucleation event and disassemble when chromatin factors or nucleation regions are removed. These features were observed in murine embryonic stem cells. The disruption of MED1 and BRD4 by 1,6-hexanediol leads to the formation of distinct structures at specific enhancer elements within the cell nuclei. This disruption also led to the excision of MED1 and BRD4 from the chromatin at enhancers, as well as the loss of RNA pol II.[51] RNA pol II selectively accessed the mediator condensates through the IDR located at the phosphorylated C-terminal domain of the large subunit. RNA-binding proteins located near the promoter[42] of downstream stemness genes, such as *TP63*, *MET*, and *FOSL1*, recruit RNA pol II to activate cancer stemness features in squamous cell carcinoma (Figure 2).[52] The administration of bromodomain and extra-terminal domain (BET) inhibitors effectively disrupted SEs, resulting in a strong inhibition of cancer stem cells (CSCs) self-renewal and the complete eradication of CSCs in a mouse model of human head-and-neck squamous cell carcinoma (HNSCC). Furthermore, the disruption of SEs also hinders the spread and migration of CSCs derived from human HNSCC to the lymph nodes.[52] Nevertheless, the use of anti-BRD4 agonists as a therapeutic option remains restricted due to their high toxicity and delivery limitations.[53] As a result, new methods combining genomic and computational frameworks have been developed to identify BRD4-enriched SEs and confirm their involvement in promoting the growth and movement of cancer cells through CRISPR knockouts.[40,54] Within this perspective, drug design can be accomplished through a physicochemical mechanism of action, which offers a new method to target cellular components that were previously considered difficult to drug, such as intrinsically disordered proteins.[47,55]

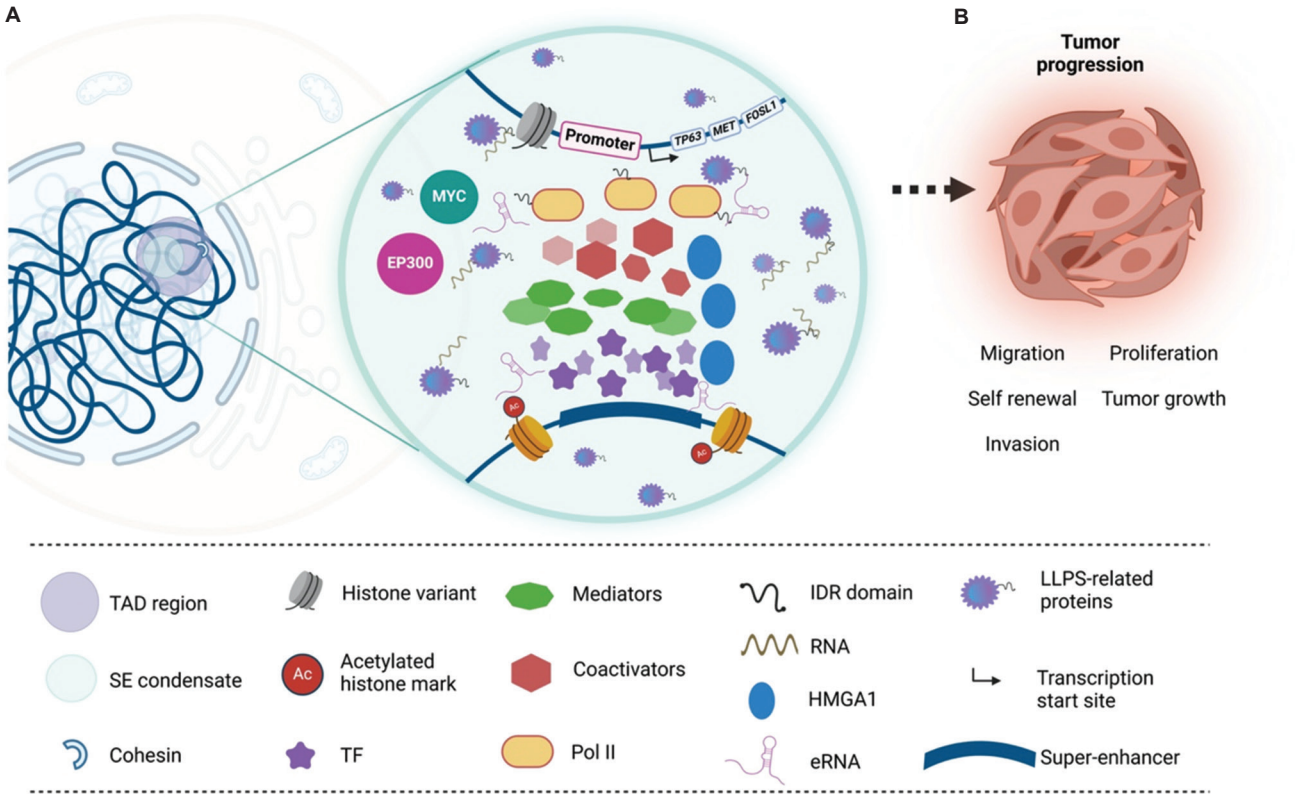


Figure 2. Oncogenic SEs assembled through LLPS processes. (A) At SE condensates, the transcription-activated complexes recruited by the SEs have about 10 times the molecular density of conventional enhancers. Such complexes require a stable structure to preserve their conformation under optimal conditions. The dense concentration of TFs, RNA polymerase II, cofactors (mediators and coactivators), and enhancer RNAs enables the formation of localized phase separation foci. SE condensate activation is facilitated by synergistic interactions among multivalent molecules, such as RNAs, DNA, and IDRs in proteins (LLPS-related proteins). RNA-binding proteins located near the promoter of downstream stemness genes, such as TP63, MET, and FOSL1, recruit Pol II to (B) activate cancer stemness features in squamous cell carcinoma, thereby creating a tumor microenvironment conducive to tumor progression. Figure created using BioRENDER.com.

Abbreviations: IDR: Intrinsically disordered regions; LLPS: Liquid-liquid phase separation; SE: Super-enhancer; TAD: Topologically associated domains; TF: Transcription factor.

4. eRNAs as modulators of the epigenome in cancer

eRNAs are a subclass of ncRNAs, known as long non-coding RNAs. These molecules are mostly unspliced and bidirectionally transcribed from enhancer elements by RNA pol II.[56] Although ncRNAs play integral roles in tumor formation and development in various ways,[57-60] the exact biological functions of eRNAs are still under investigation. Interestingly, cap-analysis gene expression technology has estimated the presence of approximately 40,000–65,000 eRNAs in humans, indicating a significant abundance.[61,62] eRNAs lack polyadenylation (polyA) modification at their 3'-end and are retained in the nucleus and chromatin-enriched fractions.[17,61] Moreover, eRNAs have 90–100-fold less stability than mRNAs,[17] making them prone to degradation by exosomal complexes in the nucleus.[63] Enhancer transcription is considered the most common rapid transcriptional change occurring when cells undergo a state change, peaking as early as 15 min after the transition trigger in some time courses across multiple biological systems.[62] However, enhancer activity is no longer required once the target promoter has been activated, leading eRNA levels to frequently return to baseline. In some instances, enhancers are rapidly activated and then continuously expressed, suggesting that these generated eRNAs may have additional functional roles in promoting elongation.[64]

Multiple studies have demonstrated that eRNAs play a key role in transcriptional regulation, mainly during cellular differentiation.[65,66] For instance, the tumor suppressor TP53 has been shown to bind to regions with enhancer activity located distantly from any known TP53 target genes, known as TP53-bound enhancer regions (p53BERs). In addition, p53BERs generate eRNAs in a TP53-dependent manner, which are involved in the transcriptional enhancement of target genes interacting with the enhancer they are

produced from.[67-69] Furthermore, research indicates that eRNA presence can be indicative of enhancer activity,[70,71] and the levels of eRNA transcription can reflect the degree of enhancer or promoter activity.[72] Thus, eRNAs may serve as biological markers for active enhancer regions.[73-75] Moreover, studies show that eRNAs stabilize enhancer-promoter loops by attracting cohesin complexes, which are essential for the formation and stabilization of chromatin loop structure.[76]

Increasing lines of evidence gradually revealed the regulatory role of eRNAs in various diseases, including cancer.[77-80] For instance, Jiao *et al.*[81] identified a SE and its derived eRNA that facilitated the expression of heparanase (HPSE), an endo-β-D-glucuronidase essential for cancer invasion and metastasis. They demonstrated that HPSE eRNA was highly expressed and positively correlated with HPSE levels in cancer tissues, promoting tumorigenesis and aggressiveness of cancer cells both *in vitro* and *in vivo*. In addition, HPSE eRNA was shown to promote cancer progression by driving chromatin looping and regulating hnRNPU/p300/EGR1/HPSE axis. Consequently, HPSE eRNA serves as an important prognostic marker for cancer patients with poor outcomes. Qin *et al.*[82] applied genome-wide profiling of eRNAs in Chinese lung adenocarcinoma patients, integrating RNA-seq data analysis to present a comprehensive description of eRNAs in lung adenocarcinoma. They discovered that highly upregulated eRNAs identified upstream of *TERT* may contribute to lung cancer development by upregulating *TERT* expression. *TERT* is a well-known predisposition gene for lung cancer, encoding human telomere reverse transcriptase, which maintains telomere ends.[83-85] Intriguingly, they discovered that *FOXO6* expression was elevated in lung adenocarcinoma, attributed to the copy number amplification of *FOXO6* eRNA in lung adenocarcinoma patients.

Another study showed that CCAT1, an enhancer-templated RNA, forms a complex with TFs TP63 and SOX2, regulating *EGFR* expression by binding to the SEs of *EGFR*. This interaction activates both the MEK/ERK1/2 and PI3K/AKT signaling pathways in squamous cancer cells, promoting tumorigenesis.[86] Similarly, *NET1e*, an eRNA located about 90 kb downstream of the oncogene *NET1*, was highly expressed in breast cancer.[80] In addition, in the study, CRISPR activation of *NET1e* was found to accelerate cell growth in MCF7 breast cancer cell lines. Conversely, its knockdown by locked nucleic acids antisense RNA significantly reduced cell proliferation in the MCF7 breast cancer cell line, suggesting its therapeutic potential in clinical eRNA-targeted therapy. Therefore, eRNAs offer considerable therapeutic potential and warrant further intense investigations for their roles in cancer and other diseases.

5. Limitations and future perspectives of enhancer-targeted cancer therapy

After almost 40 years since the first discovery of enhancers in the Simian virus 40 genome,[87] the precise mechanisms by which enhancers exert their effect on gene activation remain elusive. The limitations arise from the intrinsic complexity of enhancers and our limited knowledge, which needs further advances in molecular techniques for elucidation. As discussed earlier, the locations of enhancer elements can be identified by genome-wide profiling of histone marks, with H3K4me1 and H3K27ac being the two major histone marks flanking active enhancers.[88] Recently, the application of molecular biology techniques such as chromatin immunoprecipitation (ChIP) followed by high-throughput sequencing has proven beneficial for genome-wide enhancer identification.[89,90] Nevertheless, the discovery of enhancers throughout the genome remains limited, and determining their target gene is even more challenging.

Next-generation sequencing technologies, such as mapping RNA interactome *in vivo* (MARIO), *in situ* mapping of RNA-genome interactome (iMARGI), multinucleic acid interaction mapping in single cells (MUSIC), CAGE, global RNA interactions with DNA by deep sequencing (GRID-seq), and global run-on sequencing (GRO-seq), open new horizons for understanding the interactions of genomic regions with RNA. Despite the broad spectrum of applications for RNA-seq technology, its utilization in the detection of eRNAs on a large scale has been limited primarily due to the poor stability of eRNAs and insensitivity of the RNA-seq technique. The MARIO technique involves cross-linking RNA molecules with their associated proteins before ligating them to a biotinylated RNAlinker, resulting in a chimeric RNA in the form of RNA1-Linker-RNA2. These linker-containing chimeric RNAs are then separated using streptavidin-coated magnetic beads and subjected to paired-end sequencing.[91] This technology allows for an equitable selection of interacting RNAs, enabling comprehensive mapping of an RNA-RNA interactome on a global scale.[91] This approach bypasses the necessity of having a specific antibody for a protein and eliminates the constraint of studying only one RNA-binding protein at a given time. In addition, this technique exclusively captures RNA molecules that are co-bound with a solitary protein molecule, preventing the capture of RNA molecules bound independently to multiple copies of a protein. This precautionary measure ensures the avoidance of reporting false interaction.[91-93]

The IMARGI method is employed for the identification of chromatin-associated RNAs (caRNAs) and the elucidation

of their specific genomic interaction sites. The IMARGI procedure initiates with *in situ* crosslinking and genome fragmentation, then converts each nearby RNA-DNA pair into an RNA-linker-DNA chimeric sequence.[94] Subsequently, the chimeric sequences are transformed into a sequencing library optimized for paired-end sequencing. To analyze paired-end sequencing data and unveil caRNA-DNA interactions, researchers can utilize the standardized bioinformatic software package known as iMARGI-Docker, available at https://sysbio.ucsd.edu/imargi_pipeline.[94]

The MUSIC GRID technique enables simultaneous profiling of multiple chromatin interactions, gene expression, and RNA-chromatin associations at the single-nucleus level. It represents an effective tool for investigating chromatin structure and gene expression at the cellular level within intricate tissues.[95] Of significant note, GRID-seq is capable of identifying both coding and ncRNAs that interact with tissue-specific promoters and enhancers, particularly SEs. Consequently, it enables the generation of a comprehensive map illustrating the connectivity between promoters and enhancers on a global scale.[96]

The FANTOM consortium utilized the CAGE technique to analyze extensive transcriptomes from various cell types, leading to the identification of 43,011 enhancer elements that were transcribed into eRNAs.[61]

The utilization of GRO-seq, a cutting-edge methodology, enables the identification of the precise genomic locations and orientations of all RNA polymerases actively involved in transcription. This powerful approach proves invaluable in monitoring the transcription of nascent enhancers. In addition, the distinctive transcription pattern exhibited by enhancers can be utilized to identify these regulatory elements, even in the absence of any information regarding the underlying TFs.[97] Consequently, GRO-seq proves to be a proficient approach for the identification, characterization, and comprehension of enhancer transcription regulation. The detection of enhancer transcription through GRO-seq analysis serves as a highly dependable method for identifying active enhancers. This approach can be effectively utilized to study and characterize enhancers and is considered the most reliable indicator of enhancer activity, surpassing the histone modifications commonly enriched at enhancers.[16,97,98]

A more integrative approach combining ChIP with high-throughput molecular biology techniques such as Hi-C, Hi-ChIP, ATAC-seq, and their single-cell sequencing alternatives would be more efficient in uncovering the mechanisms by which enhancers and SEs regulate transcription and oncogenesis.[99] Furthermore, experimental validation *in vitro*, *in vivo*, and *ex vivo* using enhancer reporter vectors would not only help in identifying enhancers and SEs and their target genes but also in assessing their pathological functions and cancer-driving potential.[100-103]

The application of enhancer-targeting drugs, such as BET inhibitors, is known to block the family member of BET proteins, which are preferentially located at active enhancers (H3K27ac). BET proteins have the ability to identify and attach to acetylated lysine residues. Inhibiting BET proteins shows significant potential for advancing cancer treatment strategies in the future.[104] For instance, in an *in vitro* investigation, the pharmacologic inhibitor GNE987 reduced NB cell growth and survival, promoted apoptosis, and caused cell cycle arrest by degrading BRD4. These observations were consistent with a reduction in xenograft tumor size. Chen *et al.*[105] also identified a new oncogenic gene, *FAM163A*, enriched with the H3K27Ac mark in GNE987-treated cells using RNA-seq and ChIP-seq data.

The application of genome engineering tools to produce targeted mutations across different species has been described in various studies.[106-108] A pioneering study using CRISPR-Cas9 enhancer correction in treating sickle cell disease and β-thalassemia resulted in patients no longer needing transfusions and eliminated vaso-occlusive episodes.[109] These findings suggest that CRISPR genome/epigenome editing is not only a useful tool for generating and investigating chromosomal aberrations but also holds promise for correcting disease abnormalities, including cancer and age-related diseases.[110,111] For instance, it was shown that the application of CRISPR-Cas13a to knock down the SMAD7 enhancer, an estrogen-responsive eRNA, inhibited cell proliferation and migration while promoting cell apoptosis. This knockdown led to the suppression of cell invasion in bladder cancer in 5637 and T24 cells.[112] In another study, Mill *et al.*[113] discovered that using the CRISPR/Cas9 system to disrupt the SE region related to the *RUNX1* gene promoted apoptosis in acute leukemia cells (OCI-AML5), consequently modifying the survival rate of mice with AML. Additionally, Vincent *et al.*[114] demonstrated that epigenomic disruption of EGFR enhancers using CRISPRi (dCas9-KRAB) technology curtailed the invasive and proliferative competency of glioblastoma cells and enhanced their sensitivity to temozolomide treatment.

The landscape of drug delivery techniques in cancer treatment has undergone a remarkable revolution with the discovery of nanoparticles (NPs) and small-sized molecules. This breakthrough in nanotechnology has completely transformed conventional methods of delivering drugs to cancer tissues, presenting novel possibilities and renewed hope for effective cancer treatment. The distinctive characteristics of NPs, including decreased

toxicity, enhanced permeability, and precise targeting of cancer cells, offer a significant benefit in the treatment of cancer and aid in addressing the constraints and obstacles associated with traditional cancer treatment modalities[115] Huang *et al.*[116] made a significant discovery using ChIP-seq to identify top SE-associated genes, which they found to be promising oncogenes in pancreatic ductal adenocarcinoma (PDAC). These genes were shown to be highly susceptible to treatment with the cyclin-dependent kinase 7 inhibitor, THZ1, and BRD4 inhibitor, JQ1. In addition, it was shown that utilizing NPs containing a significant amount of JQ1, in combination with THZ1, could serve as a potentially effective therapeutic approach for treating PDAC by inhibiting SE-associated oncogenic transcription. While the study presents a novel approach for targeting SEs through the application of nanocarriers and opens new horizons for cancer treatment, the risks and hazards related to NPs still need to be addressed before approval for clinical applications.

6. Conclusion and perspectives

Enhancers play a crucial role in transcription regulation. The aberrant activation of enhancers and SEs due to chromosomal rearrangements and genetic/epigenetic variation drives oncogene activation, resulting in uncontrolled cell proliferation, resistance to apoptosis, and, consequently, tumor formation and progression. Enhancer-mediated regulation of genes is determined not only by their location but especially by their capability to physically bind to an appropriate promoter, which can even occur in a different chromosome topology. The epigenetic state of enhancers is crucial for their function, often used to identify their genomic locations. Active enhancers initiate the production of eRNAs and usually possess high levels of H3K4me1 and H3K27ac. However, more factors are required for robust enhancer identification.

The recent discovery of eRNAs adds another layer of complexity to the human transcriptome, encouraging intense research on the features and potential functions of this new class of ncRNAs. Evidence suggests that eRNAs may be powerful biological markers in cancer treatment and therapy. Abundant evidence shows that eRNAs are abnormally expressed in various cancers and that their expression is closely related to tumorigenesis. Investigations into these eRNAs reveal new oncogenic pathway activation in tumor cells and propose new potential targets for combination therapies. However, these findings on eRNAs and their association with cancer have been mainly established through genomic research, with limited supporting evidence from molecular assays, leaving the underlying molecular mechanisms involved unclear. Further molecular analyses are required to fully understand the complex molecular mechanisms of eRNAs in tumorigenesis. Moreover, a better understanding of enhancers and SEs structure, as well as reliable structural conformations of eRNAs and their interactions with target genes in three-dimensional space, is needed. Finally, CRISPR genome/epigenome-editing and enhancer-targeting drugs, such as BET inhibitors, present promising tools for correcting enhancers and SEs abnormalities in cancer therapy.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

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Ethics approval and consent to participate

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Consent for publication

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Availability of data

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