INTRODUCTION

Cell identity and cell-type-specific gene expression are regulated by cis-regulatory elements such as promoters, enhancers, and insulators in non-coding regions. These regulatory elements form cell-type-specific 3D chromatin structures through direct interactions within the nucleus. Aberrations in regulatory elements and the disruption of chromatin structure can result in the development of diseases, including cancer.

Promoters and enhancers are epigenetically regulated by TFs that recruit chromatin regulators to these regions. The human genome encodes a relatively large number of TFs; only a small number of master TFs function to define cell identity by regulating the lineage-specific expression of various genes. The master TFs cooperatively bind to
enhancer regions with co-activators that include transcription complexes and histone-modifying enzymes. Through this cooperative binding, they regulate epigenetic states and chromatin interaction (Figure 1A). Enhancer regions are marked by histone modifications of H3K27ac and H3K4me1.1,2 When bound by master TFs, clusters of enhancers known as SEs regulate gene expression that plays a prominent role in cell identity or specialized cellular function.3,4 SEs span larger regions compared with other typical enhancers and are highly occupied by TFs and their cofactors. In cancer cells, genomic or epigenomic aberrations lead to oncogenic enhancer formation or activation, resulting in the upregulation of the neighboring oncogenes.

Enhancers function to increase transcriptional output by directly interacting with their target promoter regions. A recent genome-wide analysis of chromatin interactions revealed transcription regulatory networks between promoters and enhancers (Figure 1B).5,7 Enhancer-promoter interactions were significantly enriched for cell-type-specific genes. Many transcription cofactors, including the mediator complex, cohesin, and the cohesin loader, function to mediate loop structure between promoters and enhancers.8 The typical loop structure between promoters and enhancers is restricted within an insulated domain structure known as a TAD that possesses an average size of approximately 1 Mb (Figure 1C).9-11 Chromatin interactions are more frequent within TADs than outside TADs. TADs are formed by binding of insulation proteins CCCTC-binding factor (CTCF) and cohesins.10,12,13 It is now established that TADs have the ability to restrict long-range enhancer-promoter interactions. Therefore, both genomic and epigenomic disruptions of TAD boundaries allow loop formation between proto-oncogenes and enhancers that are partitioned naturally.

2 | ONCOGENIC ENHANCER FORMATION BY GENOMIC CHANGES AT NON-CODING REGIONS

The typical phenotype of cancers includes defects in DNA repair mechanisms and cell cycle regulation. As a result of these defects, the cancer genome is frequently mutated. Mutations in coding genes contribute greatly to tumorigenesis by promoting oncogene activation and repressing tumor suppressor gene expression. Additionally, genomic changes, such as insertions, mutations, and amplifications,
at non-coding regions cause oncogenic enhancer formation, ultimately leading to neighboring oncogene activation (Table 1 and Figure 2).

Large numbers of small somatic mutations, including single base alterations, insertions, or deletions, are found in the non-coding regions in cancer. However, identifying driver mutations in non-coding regions is difficult because whole-genome sequence information in cancers is not sufficient. In addition to this, predicting the function of the non-coding mutation is difficult. To date, few mutations have been identified in non-coding enhancers, and these mutations may function in various cancers.

### TABLE 1 Oncogene activation mechanism by enhancer aberrations in various cancers

| Genomic/epigenomic aberrations       | Cancer type                          | Activated oncogenes                      | References | Figure |
|--------------------------------------|--------------------------------------|------------------------------------------|------------|--------|
| **Genomic enhancer formation**      |                                      |                                          |            |        |
| Mutation                             | Lymphoblastoid B-cell neuroblastoma  | PAX5                                     | 18         | Figure 2A |
| Insertion                            | T-cell acute lymphoblastic leukemia   | TAL1                                     | 14         | Figure 2A |
| Amplification                        | Neuroblastoma                        | MYCN                                     | 22         | Figure 2A |
| **Enhancer rewiring by genomic/epigenomic aberrations** |                                      |                                          |            |        |
| Translocation of an enhancer         | T-cell acute lymphoblastic leukemia   | TLX1, TLX3, TAL1, TAL2, NOTCH1, MYC       | 23         | Figure 3A |
| Insertion                            | B-cell lymphomas                     | MYC, BCL6                                | 24         |        |
| Adenoid cystic carcinoma             | MYB                                   |                                          | 25         |        |
| Medulloblastomas                     | GFI1, GFI1B                           |                                          | 26         |        |
| Gastric adenocarcinoma               | CCNE1, IGF2                           |                                          | 27         |        |
| Inversion including an enhancer      | Acute myeloid leukemia                | EV1                                      | 28         | Figure 3B |
| Deletion of a TAD boundary           | Sarcoma                               | IRS4                                     | 32         |        |
| Inversion including a TAD boundary   | Colorectal cancer                     |                                          | 32         |        |
| Mutation at a TAD boundary           | T-cell acute lymphoblastic leukemia   | TA1, LMO2                                | 33         | Figure 3C |
| DNA methylation at a TAD boundary    | IDH-mutated gliomas                   | PDGFR                                    | 34         |        |
| SDH-deficient gastrointestinal stromal tumors |                                      | KIT, PDGFR                              | 35         |        |
| **Epigenomic enhancer activation**  |                                      |                                          |            |        |
| Mutations in coding regions of        | Prostate cancer                       |                                          | 36,37      | Figure 4A |
| transcription factors                | Pancreatic cancer                     |                                          | 38         |        |
| MLL fusion                           | Acute myeloid leukemia                | MLL-AF6, MLL-AF9                         | 55         | Figure 4B |
| Oncovirus integration                | HPV-associated cervical cancer        | Viral oncogenes (E6, E7)                 | 59         | Figure 5A |
| Oncovirus episomal formation         | EBV-associated gastric cancer         |                                          | 60         | Figure 5B |

### Figure 2 Models of oncogenic enhancer formation by genomic changes.

A. New enhancer formation by point mutation or small insertions. Newly formed enhancer region is activated by tissue-specific transcription factors. Asterisk indicates point mutations or small indels. B. New enhancer formation by focal amplifications.
in a tumor type-specific manner. In lymphoblastoid B-cell lines, SEs are generated by a mutation cluster located 330 kb upstream from the PAX TSS (Table 1 and Figure 2A). Another example of mutations in enhancers is in neuroblastoma cells, a GATA3-dependent enhancer is generated at the LMO1 oncogene locus. In T-cell acute lymphoblastic leukemia, binding sites for the transcription factor MYB are
produced by small insertions in the intergenic non-coding region upstream of the TAL1 oncogene, allowing enhancer formation to upregulate TAL1 expression. MYB recruits the acetyltransferase complex, CBP and p300, and TAL1 to produce oncogenic enhancers and to activate key oncogenes in leukemogenesis.

Somatic copy-number changes are also widespread in tumor cells. Analysis of somatic copy-number changes from 3131 cancer patient samples from 26 different tumor types revealed that extensive copy-number changes were observed in 25% of the cancer genome. In addition, focal amplifications or small deletions were observed in 10% of the cancer genome. Many copy-number variations are observed in key oncogenes, however some are observed in the non-coding genome. Genomic amplification in non-coding regions can also result in oncogenic enhancer activation (Table 1, Figure 2B). Somatic copy-number analyses and epigenetic analyses of 12 cancer cell types revealed that SEs generated by focal amplification could aberrantly activate KLF5, USP12, PARD6B, or MYC. Another study also revealed that the 350-2000 kb genomic region, including the MYCN oncogene, was amplified aberrantly in neuroblastoma, resulting in the activation of MYCN expression.

3 \hspace{1cm} ENHANCER REWIRING BY CHROMOSOMAL REARRANGEMENTS

Genomic rearrangements such as translocations or inversions that create master TF binding sites alter the position of enhancer regions from the genomic regions in normal cells to proto-oncogene neighboring regions, therefore leading to oncogene activation (Table 1, Figure 3). This phenomenon is known as “enhancer hijacking.” The phenomenon has been reported in various cancers, including T-cell acute lymphoblastic leukemia, B-cell lymphomas, adenoid cystic carcinoma cells, medulloblastoma, gastric cancer, and acute myeloid leukemia.

For example, in T-cell acute lymphoblastic leukemia, chromosomal translocations often result in movement of different proto-oncogenes, including TLX1, TLX3, TAL1, TAL2, NOTCH1, and MYC, near to highly activated enhancer regions that regulate T-cell receptors in T-cells (Table 1, Figure 3A). In B-cell lymphomas, the novel mechanisms underlying aberrant activation of oncogenes MYC and BCL6 by the de novo enhancer have been identified. Translocation or duplication of MEF2B-bound enhancers that are usually located at BCL6-locus have been identified. Another example is enhancer repositioning, as a result of chromosomal translocation in adenoid cystic carcinoma cells. These enhancers move to the neighboring regions of the MYB gene, therefore leading to high MYB expression. In medulloblastomas, mutually exclusive translocations that activate the GFI1 or GFI1B gene have been identified. The GFI1B gene translocates to neighboring regions of the enhancer around the DDX31 gene. Similar enhancer translocations between chromosomes 1 and 21 have been also observed at the GFI1 locus on chromosome 1. In gastric adenocarcinoma, the super-enhancer at chromosome 2 translocates to the neighboring regions of CCNE1 at chromosome 19, leading to aberrant activation of CCNE1, which is associated with CDK4/6 inhibitor treatment. Another translocation of the IGF2 enhancer has been also observed in gastric adenocarcinoma. These IGF2 translocations are lineage specific and also observed in colorectal cancer, in addition to gastric adenocarcinoma.

Inversions in the cancer genome also lead to enhancer rewiring (Table 1, Figure 3B). The enhancer that regulates the tumor suppressor GATA2 moves to the neighboring regions of proto-oncogene EV1 by inversion of a 9-kb fragment in acute myeloid leukemia cells, therefore leading to downregulation of tumor suppressors and activation of oncogenes.
More recently, chromatin structural analysis, chromosome conformation capture, and its derivative genome-wide methods, were performed in various types of cells including cancers and then analyzed comprehensively in the context of gene expression. The chromosome conformation capture technique can detect chromatin interactions by quantitating chimeric reads between interacting regions that are produced by restriction enzyme digestion and proximity ligation and Hi-C, a genome-wide derivative method using next-generation sequencing.29,30 These analyses revealed the presence of cancer-related gene overexpression associated with TAD structural aberrations caused by genomic or epigenetic aberrations in TAD boundary regions (Table 1, Figure 3C).31

Integrated analysis of pan-cancer copy-number alteration and TAD structure detected by human fibroblast Hi-C revealed TAD structural aberrations and concomitant enhancer rewiring caused by copy-number changes.32 In sarcoma and squamous cancer cells, the formation of strongly activated enhancers as a result of a TAD boundary deletion led to the upregulation of the IRS4 gene.32 In another example, de novo loop formation between IGF2 and lineage-specific enhancers as a result of tandem duplications intersecting the TAD boundary was identified in colorectal cancer cells.32 Furthermore, binding sites for CTCF and cohesin were often mutated in several cancer cell types such as colorectal cancer, esophageal carcinoma, and liver carcinoma.15,32 In T-cell acute lymphoblastic leukemia patients, deletions of the CTCF-binding TAD boundary and simultaneous TAL1 activation was observed, and this enhancer rewiring was confirmed by CRISPR/Cas9-mediated deletion using a normal cell line. Disruption of the CTCF-binding TAD boundary generated de novo loops from enhancers that naturally insulated and activated TAL1 and LMO2, ultimately resulting in T-cell transformation.33

In addition to mutations in TAD boundaries, epigenetic change has also led to TAD disruption in gliomas and in a subset of gastrointestinal stromal tumors.34,35 In IDH-mutated gliomas that showed a CpG island methylator phenotype, methylation induction at the binding sites of CTCF reduced CTCF binding.36 3C analysis detected the disruption of TAD structure and de novo loop formation between enhancers and the neighboring oncogene PDGFRα. This structural change led to activation of PDGFRα.34 In SDH-deficient gastrointestinal stromal tumors that also showed global DNA hypermethylation, Hi-C analysis revealed TAD structure reorganization by CTCF loss as a result of DNA methylation, ultimately leading to Kit and PDGFRα upregulation.35 Additionally, a TAD boundary that naturally insulated a core enhancer from the FGF4 oncogene was also disrupted, ultimately leading to strong FGF4 activation.

A DNA element from Simian virus 40 activated the T antigen or a β-globin reporter gene in mammalian cells independently of its distance from the TSS.54 Virus DNA sequences that possessed enhancer function have also been discovered in other viruses.57 These viral enhancers are activated by certain TFs that bind to specific sites within...
enhancers. Human papillomaviruses (HPVs) are another example that functions as an enhancer through integration. HPV is known as an oncovirus that is associated with cervical cancers and with ano- genital and oropharyngeal cancers. During their normal viral life cycle, HPVs are maintained as extrachromosomal circular-DNA, known as the episome, within the host cell nucleus. However, in HPV-associated cancers, HPV genomes are integrated into host cellular chromatin. Tandemly integrated HPV16 could result in the formation of an SE that drives transcription of the viral oncogenes (Table 1, Figure 5A).

Recently, we identified a novel mechanism for epigenetically activating silenced enhancers in heterochromatin regions induced by oncovirus. Unlike HPV, EBV is associated with various cancers such as Burkitt lymphoma, nasopharyngeal cancer, and gastric cancer. EBV encodes a sequence-specific DNA-binding protein, EBNA1, that connects the viral episome to the host metaphase chromosome during mitosis. We identified specific chromatin structural changes that occurred during inactive to active structural shifts in EBV-positive gastric cancers. We discovered that regions that displayed these features also showed chromatin interactions with the EBV genome. The EBV genome targets and reprograms latent host enhancers even within H3K9me3-positive heterochromatin regions. This aberrant activation of enhancers contributes to tumorigenesis through chromatin rewiring, allowing enhancers to interact with and activate neighboring proto-oncogenes. This phenomenon is termed "enhancer infestation" and is another mechanism that induces epigenetic activation of enhancers without genetic alteration (Table 1, Figure 5B). This finding is the first to show that direct interaction between exogenous virus DNA and host chromatin affected the host chromatin structure and epigenetic states. This discovery provides researchers with new insight that virus DNA may affect host chromatin epigenetically and allows for a more detailed investigation of virus-associated cancer.

7 | CONCLUSION

Aberrant enhancer activation is a critical process that drives oncogene activation in cancer. Intense enhancer activities in cancer cells drive uncontrolled proliferation, and enhancers can be targeted for cancer therapy. Although transcription is a general property present within all cells, cancer cells are more dependent on increased transcription levels from enhancers. A further understanding of transcriptional dysregulation by aberrant enhancers in cancer is required to aid in the development of clinically useful epigenetic inhibitors of various components associated with enhancer regions.

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DISCLOSURE

The authors have no conflict of interest.

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REFERENCES

1. Heintzman ND, Stuart RK, Hon G, et al. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet. 2007;39(3):311-318.
2. Creyghton MP, Cheng AW, Welstead GG, et al. Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc Natl Acad Sci U S A. 2010;107(50):21931-21936.
3. Hnisz D, Abraham BJ, Lee TI, et al. Super-enhancers in the control of cell identity and disease. Cell. 2013;155(4):934-947.
4. Whyte WA, Orlando DA, Hnisz D, et al. Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell. 2013;153(2):307-319.
5. Fullwood MJ, Liu MH, Pan YF, et al. An oestrogen-receptor-α-bound human chromatin interactome. Nature. 2009;462(7269):58-64.
6. Mumbach MR, Satpathy AT, Boyle EA, et al. Enhancer connectome reveals regulatory connectivity and cell type biases in the human genome. Nature. 2010;467(7314):58-64.
7. Li G, Ruan X, Auerbach RK, et al. Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. Cell. 2012;148(1-2):84-98.
8. Kagay MH, Newman JJ, Bilodeau S, et al. Mediator and cohesin connect gene expression and chromatin architecture. Nature. 2010;467(7314):430-435.
9. Dixon JR, Selvaraj S, Yue F, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature. 2012;485(7398):376-380.
10. Rao SSP, Huntley MH, Durand NC, et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell. 2014;159(7):1665-1680.
11. Sexton T, Yaffe E, Kenigsberg E, et al. Three-dimensional folding and functional organization principles of the Drosophila genome. Cell. 2012;148(3):458-472.
12. Nora EP, Goloborodko A, Valton AL, et al. Targeted degradation of CTCF decouples local insulation of chromosome domains from genomic compartmentalization. Cell. 2017;169(5):930-944.e22.
13. Zuin J, Dixon JR, Van Der Reijden MIJA, et al. Cohesin and CTCF differentially affect chromatin architecture and gene expression in human cells. Proc Natl Acad Sci U S A. 2014;111(3):996-1001.
14. Mansour MR, Abraham BJ, Anders L, et al. An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element. Science. 2014;346(6215):1373-1377.
15. Katainen R, Dave K, Pitkänen E, et al. CTCF/cohesin-binding sites are frequently mutated in cancer. Nat Genet. 2015;47(7):818-821.
16. Weinhold N, Jacobsen A, Schultz N, Sander C, Lee W. Genome-wide analysis of noncoding regulatory mutations in cancer. Nat Genet. 2014;46(11):1160-1165.
17. Melton C, Reuter JA, Spacek DV, Snyder M. Recurrent somatic mutations in regulatory regions of human cancer genomes. Nat Genet. 2015;47(7):710-716.
18. Puente XS, Beà S, Valdés- Mas R, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. Nature. 2015;526(7574):519-524.
19. Oldridge DA, Wood AC, Weichert-Leahy N, et al. Genetic predisposition to neuroblastoma mediated by a LMO1 super-enhancer polymorphism. Nature. 2015;528(7602):418-421.

20. Beroukhim R, Mermel CH, Porter D, et al. The landscape of somatic copy-number alteration across human cancers. Nature. 2010;463(7283):899-905.

21. Zhang X, Choi PS, Francis JM, et al. Identification of focally amplified lineage-specific super-enhancers in human epithelial cancers. Nat Genet. 2016;48(2):176-182.

22. Helmsauer K, Valieva ME, Ali S, et al. Enhancer hijacking determines extrachromosomal circular MYCN amplicon architecture in neuroblastoma. Nat Commun. 2020;11(1):1-12.

23. Cauwelier B, Dastugue N, Cools J, et al. Molecular cytogenetic study of 126 unselected T-ALL cases reveals high incidence of TCRβ locus rearrangements and putative new T-cell oncogenes. Leukemia. 2006;20(7):1238-1244.

24. Ryan RJH, Drier Y, Whittington H, et al. Detection of enhancer-associated rearrangements reveals mechanisms of oncogene dysregulation in B-cell lymphoma. Cancer Discov. 2015;5(10):1058-1071.

25. Drier Y, Cotton MJ, Williamson KE, et al. An oncogenic MYB feedback loop drives alternate cell fates in adenoid cystic carcinoma. Nat Genet. 2016;48(3):265-272.

26. Northcott PA, Lee C, Zichner T, et al. Enhancer hijacking accounted for two factors that bind promoter and enhancer sequences of the human gut microbiome. Science. 2009;3292:289-294.

27. Bradner JE, Hnisz D, Young RA. Transcriptional Addiction in Cancer. Cell. 2017;168(4):629-643.

28. Weischenfeldt J, Dubash T, Drainas AP, et al. Pan-cancer analysis of somatic copy-number alterations implicates IR54 and IGF2 in enhancer hijacking. Nat Genet. 2017;49(1):65-74.

29. Hnisz D, Weintraub AS, Day DS, et al. Activation of proto-oncogenes by disruption of chromosome neighborhoods. Science. 2016;351(6280):1454-1458.

30. Flavahan WA, Drier Y, Liu BB, et al. Insulator dysfunction and oncogene activation in IDH mutant gliomas. Nature. 2016;529(7602):110-114.

31. Flavahan WA, Drier Y, Johnstone SE, et al. Altered chromosomal topology drives oncogenic programs in SDH-deficient GISTs. Nature. 2019;575(7781):219-233.

32. Pomerantz MM, Li F, Takeda DY, et al. The androgen receptor cistrome is extensively reprogrammed in human prostate tumorigenesis. Nat Genet. 2015;47(11):1346-1351.

33. Gao S, Chen S, Han D, et al. Chromatin binding of FOXA1 is promoted by LSD1-mediated demethylation in prostate cancer. Nat Genet. 2020;52(10):1011-1017.

34. Roe JS, II HC, Somerville TDD, et al. Enhancer reprogramming promotes pancreatic cancer metastasis. Cell. 2017;170(5):875-888.e20.

35. Allen BL, Taatjes DJ. The Mediator complex: a central integrator of transcription. Nat Rev Mol Cell Biol. 2015;16(3):155-166.

36. Herz HM, Hu D, Shilatifard A. Enhancer malfunction in cancer. Mol Cell. 2014;53(6):859-866.

37. Ashktorab H, Schäffer AA, Daremipouran M, Smoot DT, Lee E, Brim H. Distinct genetic alterations in colorectal cancer. PLoS One. 2010;5(1).

38. Roe JS, Il HC, Somerville TDD, et al. Whole-genome analysis informs breast cancer response to aromatase inhibition. Nature. 2012;486(7403):353-360.

39. Fujimoto A, Totoki Y, Abe T, et al. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet. 2012;44(7):760-764.

40. Gui Y, Guo G, Huang Y, et al. Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. Nat Genet. 2011;43(9):875-878.

41. Jones DTW, Jäger N, Kool M, et al. Dissecting the genomic complexity underlying medulloblastoma. Nature. 2012;488(7406):100-105.

42. Ellis MJ, Ding L, Shen D, et al. Whole-genome analysis identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet. 2012;44(6):685-689.

43. Mäkinen N, Mehine M, Tolvanen J, et al. MED12, the mediator complex subunit 12 gene, is mutated at high frequency in uterine leiomyomas. Science. 2011;334(6053):252-255.

44. Meyer C, Kowarz E, Hofmann J, et al. New insights to the MLL reactivation pathway. Mol Cancer. 2012;11(1):1-12.

45. Okabe A, Hnisz D, Young RA. Transcriptional Addiction in Cancer. Cell. 2017;168(4):629-643.

46. Allen BL, Taatjes DJ. The Mediator complex: a central integrator of transcription. Nat Rev Mol Cell Biol. 2015;16(3):155-166.

47. Ashktorab H, Schäffer AA, Daremipouran M, Smoot DT, Lee E, Brim H. Distinct genetic alterations in colorectal cancer. PLoS One. 2010;5(1).