HHS Public Access

Author manuscript

Bioessays. Author manuscript; available in PMC 2017 October 01.

Published in final edited form as:

Bioessays. 2016 October ; 38(10): 1003–1015. doi:10.1002/bies.201600106.

Enhancer deregulation in cancer and other diseases

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Summary

Mutations in enhancer-associated chromatin-modifying components and genomic alterations in non-coding regions of the genome occur frequently in cancer and other diseases pointing to the importance of enhancer fidelity to ensure proper tissue homeostasis. In this review, I will use specific examples to discuss how mutations in chromatin-modifying factors might affect enhancer activity of disease-relevant genes. I will then consider direct evidence from single nucleotide polymorphisms, small insertions or deletions but also larger genomic rearrangements such as duplications, deletions, translocations and inversions of specific enhancers to demonstrate how they have the ability to impact enhancer activity of disease genes including oncogenes and tumor suppressor genes. Considering that the scientific community only fairly recently has begun to focus its attention on "enhancer malfunction" in disease, I propose that multiple new enhancer-regulated and disease-relevant processes will be uncovered in the near future that will constitute the mechanistic basis for novel therapeutic avenues.

Keywords

cancer; enhancer deregulation; enhancer mutations and genomic alterations; Kabuki syndrome; MLL3/KMT2C; MLL4/KMT2D; UTX/KDM6A

Introduction

History of enhancers

The term "enhancer" was initially coined in the early eighties based on studies of a viral DNA element from Simian virus 40 (SV40) when it was demonstrated that this particular DNA sequence had the ability to convey increased activity towards a *T-antigen* or β-*globin* reporter in mammalian cells [1]. Based on these seminal studies the hallmarks of an enhancer were defined as having the capacity to work with different promoters, independent of genomic location and distance from the transcriptional start site and regardless of orientation. This standard enhancer definition paved the way for functional studies in the metazoan system and, for the most part with certain restrictions, is still the prevalent working model within the enhancer field today. Shortly thereafter other virus enhancers with

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The author has declared no conflict of interest.

similar properties albeit at times with higher tissue or host specificity were also described [2].

Subsequent studies on the mouse immunoglobulin heavy chain locus uncovered the first eukaryotic enhancer –the *E*μ enhancer- thus confirming the existence of operatively similar genomic elements in metazoans [3]. Additional insight into the mechanisms of enhancer function particularly as it pertains to their importance in organismal development was provided by genetic studies in the fruit fly *Drosophila melanogaster*. These studies demonstrated that important developmental genes can often be regulated by several enhancers in time and space, that enhancers can function in a combinatorial and modular manner and have the ability to act over very large distances (summarized in [4]).

Enhancers are bound by transcription factors and chromatin-modifying co-activators/co-repressors

Both viral and metazoan enhancers are bound by activating and/or repressing transcription factors on specific sites which are often characterized by factor-specific DNA binding motifs [5] (summarized in [6, 7]) (Fig. 1A). Additionally, transcription factors often form a platform for the recruitment of co-activators and co-repressors (Fig. 1A). While transcription factors often appear to be “master regulators” with profound effects on enhancer activation or repression, co-activators and co-repressors in many instances occupy a modulatory role in this process. Additionally, they often constitute proteins with chromatin-modifying capabilities which generally fall under the categories (1) DNA or histone-modifying enzymes, (2) chromatin-associated factors (“readers” of DNA or histone modifications) and (3) chromatin-remodeling proteins (summarized in [8]) (Box 1). Additionally, the enzymatic activities and chromatin-altering abilities of these co-activators and co-repressors also directly affect the DNA methylation, histone modification and DNA accessibility patterns within and around enhancers and thus sets them apart from other regulatory elements within the genome (Box 1). Thus in summary, the combinatorial binding of transcription factors along with these chromatin-modifying components to enhancers results in so called “enhancer signatures” which can serve as a readout to define enhancers in a tissue-specific manner and on a global scale.

Mediators of enhancer-promoter communication

Early on, factors with the potential to mediate between enhancer- and promoter-bound factors were discovered and were shown to play a major role in enhancer-promoter communication (Fig. 1A, Box 1). In order to effectively explain enhancer-promoter communication particularly over large distances a looping mechanism was proposed by which enhancers are brought into close proximity to their cognate promoters by these mediating factors (summarized in [9, 10]). More recently, enhancer-promoter looping has been experimentally validated by chromosome confirmation capture (3C) technology-based approaches confirming this initial model [11–13]. Additionally, work on the β-*globin* locus has confirmed that looping between the β-*globin* enhancer region (the β-*globin* locus control region) and the β-*globin* promoter causally underlies induction of β-*globin* transcription [14, 15].

Mutations in chromatin-modifying enhancer-associated factors as well as germline and somatic variants in enhancers occur frequently in cancer

Over the past few years whole genome sequencing (WGS) and genome wide association (GWAS) studies across many different cancer types, including solid tumors and different forms of leukemia, have made it increasingly clear that many of the abovementioned chromatin-modifying and remodeling proteins play a central role in cancer pathogenesis and other diseases [16, 17] (summarized in [18–22]). At the same time similar WGS and GWAS studies have also revealed that the majority of germline and somatic variants in cancer occur in non-coding regions of the genome including enhancers and thus point to the importance of enhancer misregulation in tumorigenesis [23–27] (summarized in [28]).

In this review I focus on the most recent advances in the field that connect enhancer "malfunction" to various diseases including cancer. I will discuss several possible mechanisms that result in enhancer misregulation of disease-relevant genes. In particular, I will first consider mutations in enhancer-bound chromatin-modifying proteins and will then move on to describe how genomic alterations such as single nucleotide polymorphisms (SNPs), small insertions or deletions but also larger genomic rearrangements such as duplications, deletions, translocations and inversions can impact enhancer activity of disease genes including oncogenes and tumor suppressor genes.

Indirect evidence of enhancer malfunction in disease

Epigenetic alteration of the "enhancer landscape" in cancer

Based on already known "enhancer signatures" (see Introduction) several studies have started to explore the hypothesis that changes in the "enhancer landscape" including DNA methylation and histone modification patterns might correlate with tumorigenesis. Evidence for DNA methylation changes on enhancers in cancer comes from several studies in breast, cervical, lung and prostate cancer [29–32]. Gains and losses in H3K4 monomethylation (H3K4me1) on many enhancers have been reported in colon cancer [33] and similar findings were reported for changes in the DNA accessibility pattern on cis-regulatory elements in various cancers [34]. Interestingly, two recent studies also suggest that changes in the "enhancer landscape" can underlie therapy resistance of cancer cells. Endocrine therapy-resistant breast cancer cells for example rely on the NOTCH signaling pathway and are characterized by gains and losses in H3 lysine 4 dimethylation (H3K4me2) on many enhancers with a concomitant change in chromatin accessibility [35]. Furthermore, in NOTCH1-dependent T cell acute lymphoblastic leukemia (T-ALL) with a resistance to γ-secretase inhibitors increased chromatin compaction and reduced H3 lysine 27 acetylation (H3K27ac), an active enhancer mark, can be observed on various enhancers [36].

Enhancer-associated factors are often mutated in cancer and other diseases

In addition to changes of the "enhancer landscape" in cancer which could be an indirect effect of various processes, enhancer-associated chromatin-modifying proteins (Box 1) and factors that mediate enhancer-promoter interaction (Box 1) are often mutated and/or misexpressed in many different cancer types (Table 1) but also play a role in other diseases (Table 2). Based on the enhancer-associated nature of these factors, it is therefore very likely

that their misregulation will directly affect enhancer activity. Plausible scenarios that could explain how mutations in enhancer-associated factors drive tumorigenesis or certain diseases include inappropriate activation of oncogenic enhancers, deactivation of enhancers of tumor suppressor genes and ectopic activation or deactivation of enhancers of disease-relevant genes (other than cancer genes). Below I will discuss these possibilities in more detail based on a "case study" of the enhancer-associated histone H3 lysine 27 demethylase UTX/KDM6A and the histone H3 lysine 4 (H3K4) methyltransferases MLL3/KMT2C and MLL4/KMT2D and their roles in cancer and a genetic disease called Kabuki syndrome (Fig. 1B, 2).

UTX and MLL3/MLL4 function as histone H3 lysine 27 demethylases and lysine 4 monomethyltransferases on enhancers

MLL3 and MLL4 are both mammalian homologs of Set1 which constitutes the sole H3K4 methyltransferase in yeast, implements all three H3K4 methylation states -H3 lysine 4 mono-, di- and trimethylation (H3K4me1, -me2 and –me3)- and exists in a large protein complex termed COMPASS (Complex of Proteins Associated with Set1) (summarized in [37, 38]). In the fruit fly *Drosophila* three Set1-related proteins exist: Set1, Trithorax (Trx) and Trithorax-related (Trr). The mammalian genome contains six yeast Set1-related proteins: SET1A/SET1B (homologous to *Drosophila* Set1), MLL1/MLL2 (homologous to *Drosohila* Trx) and MLL3/MLL4 (homologous to *Drosophila* Trr) (Fig. 1B). All metazoan complexes share identical core subunits (Fig. 1B, green) but also contain complex-specific subunits (Fig. 1B, blue) that are conserved only within the Set1, Trx and Trr branches. For example Utx (*Drosophila*)/UTX (mammals) exists as a complex-specific subunit only in the *Drosophila* Trr and mammalian MLL3 or MLL4 complexes. Trr in *Drosophila* and MLL3/MLL4 in a redundant fashion in mammals constitute major H3K4 monomethyltransferases on enhancers while Utx/UTX acts as an H3K27 demethylase removing H3K27 trimethylation (H3K27me3), an inhibitory histone modification, from enhancers [39–41]. This suggests a model in which prior removal of H3K27me3 via Utx/UTX is required on inactive/"poised" enhancers before they can transition to an activated state via addition of H3K4me1 through Trr/MLL3/MLL4 (see also Box 1).

UTX, MLL3 and MLL4 are frequently mutated across a broad spectrum of cancers

WGS studies across many different cancers have revealed that *UTX*, *MLL3* and *MLL4* are frequently mutated within many different forms of solid tumors but also certain leukemias such as non-Hodgkin lymphoma (Fig. 2, Table 1). Interestingly, this is not equally the case for other subunits of the MLL3/MLL4 complexes. The functional requirement for core subunits within H3K4 methyltransferase complexes other than MLL3/MLL4 might render the effects of their mutation too deleterious in cancer cells to administer a competitive advantage. However, this would not explain why other complex-specific subunits are not mutated at comparable frequencies suggesting that UTX, MLL3 and MLL4 might actually have tumor suppressive/oncogenic roles outside the canonical MLL3/MLL4 complexes. Matters are additionally complicated by the fact that depending on the context *UTX, MLL3 and MLL4* can act as tumor suppressors or oncogenes. For example, *Drosophila* studies have shown that *Utx* and *trr* display tumor suppressive properties in the eye [42, 43]. In contrast, *trr* acts as an oncogene in collaboration with the Hippo signaling pathway in another context [44, 45]. Similar findings have been reported in the mammalian system. In most cases *UTX*,

MLL3 and *MLL4* appear to have tumor suppressive properties [46–48] but have also been implicated in oncogenesis [49–52]. Furthermore, it has not been fully elucidated to date whether haploinsufficiency of *UTX*, *MLL3* and *MLL4* is sufficient to drive tumorigenesis or whether homozygous mutations are required to achieve the same effect. For example, in homozygous *TP53* mutant acute myeloid leukemia (AML) *MLL3* was validated as a haploinsufficient tumor suppressor [48] whereas most studies to date have mainly investigated the effects of homozygous *UTX*, *MLL3* and *MLL4* deletions or siRNA/shRNA-mediated knock-down in various tumor contexts and thus not specifically addressed heterozygous versus homozygous mutations. An interesting connection between transcription-associated DNA damage and enhancer malfunction might be made by a recent study which suggests that MLL4 functions as a guardian of genome stability by regulating RNA polymerase II fidelity over the bodies of actively transcribed genes [53]. While this study only focused on the effects of *MLL4* deletion over actively transcribed genes, it is very likely that due to the enhancer-associated nature of MLL4 similar effects could also occur on transcribed enhancers resulting in accumulation of enhancer mutations over time. Thus, MLL4 (and possibly UTX and MLL3) might potentially be involved in enhancer regulation by directly affecting enhancer activity and indirectly through co-transcriptional DNA damage-inducing mechanisms which might alter transcription factor recruitment on specific enhancers of tumor suppressor and/or oncogenes. This hypothesis however needs to be further tested in the future.

UTX and MLL4 in Kabuki syndrome

MLL4 is also very frequently mutated (>56%) in the genetic disease Kabuki syndrome which is characterized by craniofacial anomalies but also other clinical features (Table 2) [54, 55]. Mutations in *UTX* have also been described albeit with lower frequency (Table 2) [55, 56]. All identified mutations are heterozygous dominant thus suggesting haploinsufficiency for *MLL4* and *UTX* in Kabuki syndrome. To date it has not been investigated whether Kabuki syndrome mutations result in changes of enhancer activity but based on the role of UTX and MLL4 in regulating enhancer activity this is a very plausible scenario. However, the lack of a clear association between Kabuki syndrome and an increased cancer risk seems to imply that *MLL4* haploinsufficiency is an unlikely event in most cancers with *MLL4* mutations.

In summary, this “case study” on *UTX*, *MLL3* and *MLL4* in cancer and Kabuki syndrome provides us with valuable insight how mutations in enhancer-associated chromatin-modifying factors might result in disease. Firstly, it implies that based on the tissue- and context-dependent expression of transcription factors mutations in enhancer-associated chromatin-modifiers might either have tumor suppressive or oncogenic potential and secondly that their role of haploinsufficiency needs to be more rigorously tested in the future.

Direct evidence of enhancer malfunction in disease

Mutations and genomic alterations of enhancers associated with diseases other than cancer

Specific enhancer mutations or genomic alterations of enhancers have been associated with or directly implicated in campomelic dysplasia, celiac disease, cleft palate, coronary heart disease, Crohn's disease, Hirschsprung's disease, multiple sclerosis, preaxial polydactyly, rheumatoid arthritis, systemic lupus, ulcerative colitis, Van Buchem disease and X-linked deafness which supports the idea that enhancer misregulation in these cases might confer disease susceptibility [23, 57–61] (summarized in [62]). For example, aniridia which is characterized by an absence of the iris and generally caused by heterozygous null mutations in the *PAX6* coding sequence can also result from genomic rearrangements downstream of the *PAX6* locus. It was shown that these rearrangements are responsible for the inactivation of enhancer elements that have the ability to drive eye-specific expression of *PAX6* [63, 64]. Furthermore, X-linked deafness can be caused by deletion of an enhancer element located nearly one megabase (Mb) upstream of *POU3F4* [58, 65]. Similarly, in Van Buchem disease, a bone sclerosing dysplasia, a non-coding region ~35 kilobases (kb) downstream of *SOST* is homozygously deleted [66]. Additionally, in a skeletal malformation syndrome called campomelic dysplasia genomic translocations interrupt putative cis-regulatory elements upstream of *SOX9* [67]. Even single nucleotide changes in enhancers have been implicated in disease. Point mutations in a long-range limb bud-specific enhancer located ~1 Mb upstream of the *sonic hedgehog* gene (*SHH*) in the intron of a neighboring gene results in overactivation of *SHH* and preaxial polydactyly [61, 68]. Furthermore, a SNP within an *IRF6* enhancer results in disruption of an AP-2α transcription factor binding site and is associated with cleft lip [69]. In summary, these studies provide direct evidence that enhancer mutations or genomic alterations of enhancers are directly causative of disease.

Select examples of enhancer mutations and genomic alterations of enhancers in cancer

Similar mechanisms including different types of genomic enhancer alterations as described for some genetic disorders above are also operative in cancer. Below I will discuss select examples of individual misregulated enhancers in different cancer types in more detail. This certainly does not constitute an exhaustive list but will provide a representative cross section of the most recently reported mechanisms that can result in altered enhancer activity of oncogenes and tumor suppressor genes. I will attempt to cover a broad spectrum of genomic alterations including SNPs, small insertions or deletions and larger genomic rearrangements including duplications, deletions, translocations and inversions.

Point mutations in enhancers resulting in increased/decreased affinity of transcription factor binding

Multiple genome-wide association studies have revealed several SNPs located in a gene desert upstream of *MYC* and have associated individual SNPs with an increased risk to develop certain cancer types (summarized in [70]). The investigation of individual cancer risk loci in the context of specific cancers such as colorectal cancer, prostate cancer and breast cancer showed an increased enrichment of known enhancer marks such as histone H3K4me1. Some of these SNP-bearing regions were also demonstrated to drive reporter

gene expression, and showed differential binding of transcription factors such as TCF7L2 and an ability to form 3D interactions with the *MYC* promoter (Fig. 3) [71–75]. Remarkably, studies in a mouse model containing a deletion in a non-coding region 500 kb upstream of *MYC* that usually harbors a SNP associated with many cancers show that these mice are resistant to the development of intestinal tumors [76]. This suggests that the gene desert upstream of *MYC* contains enhancer elements with the ability to tissue-specifically regulate *MYC* expression (Fig. 3).

In prostate cancer a risk-associated SNP on chromosome 6 is located in a non-coding region in the vicinity of the *RFX6* gene and contains a binding site for the homeodomain-containing transcription factor HOXB13. The region of the risk-associated SNP displays increased binding of HOXB13 compared to control tissue resulting in allele-specific upregulation of *RFX6*. Prostate cancers bearing this SNP essentially require RFX6 for proper proliferation, migration and invasion. Additionally, increased RFX6 levels appear to be a more general hallmark of prostate cancers as they are indicative of poor clinical outcome [77].

The identification of a highly risk-associated SNP located within the first intron of the *LMO1* gene in neuroblastoma affirms that SNPs can also result in reduced transcription factor binding to enhancers. This particular SNP located within a super-enhancer highly enriched for H3K27ac alters a consensus GATA binding motif to a TATA motif resulting in loss of GATA3 binding on the TATA allele and decreased *LMO1* expression [78].

Deletions within enhancers

Deletions in non-coding regions can also promote tumorigenesis, if affecting enhancers of tumor suppressor genes. Some indirect evidence for this was provided by studies on a deletion within a non-coding region on chromosome 15. This region is characterized by enrichment for H3K4me1, increased DNAse hypersensitivity and recruitment of the transcription factor c-JUN as evidenced by the presence of multiple activating protein 1 (AP-1) binding motifs and long range interactions with distant genes [79]. However, the exact mechanism by which this deletion might potentially affect tumor progression remains unresolved.

Small insertions resulting in de novo creation of transcription factor binding sites

Super-enhancers which are also known as locus control regions or stretch enhancers are defined as regions that contain clusters of individual enhancers and are prominently bound by many different transcription factors, chromatin-modifying proteins or enhancer-promoter mediating factors (see Introduction) [80–83]. They regulate genes that play important roles in cell identity and within disease-relevant cell types often show increased enrichment for mutations within regions that have been associated with certain diseases such as Alzheimer's disease, type 1 diabetes and systemic lupus erythematosus [81]. Super-enhancers are also often enriched at/around important oncogenes including *MYC* in various cancers such as diffuse large B cell lymphoma, multiple myeloma, glioblastoma multiforme and small-cell lung cancer compared to normal tissue [81, 84, 85].

One mechanism of this "*de novo*" creation of super-enhancers has recently been described by Look and colleagues in a subset of T-ALL. In these cases heterozygous insertions upstream of the *TAL1* oncogene result in a new binding site for the transcription factor MYB thus creating a super-enhancer which drives allele-specific *TAL1* expression [86].

Focal amplification of enhancers

An increase in oncogenic enhancer activity can also be achieved by focal amplification, and copy number gains of non-coding regions have also been described for super-enhancers across various tumor types [87]. In particular, the *MYC* locus appears to constitute a hotspot for focal amplifications which are often confined to non-coding regions excluding the *MYC* gene itself, thus pointing to a prominent role of tissue-specific *MYC* enhancers in tumorigenesis (Fig. 3).

For example, a focal amplification located 500 kb upstream of *MYC* was described in approximately 5% of chronic lymphocytic leukemia (CLL) cases [88] and also occurs in the cervical HeLa cancer cell line where it was caused by integration of the human papillomavirus and is thought to have been the tumor-initiating event (Fig. 3) [89]. More direct evidence that focal amplifications containing enhancers are directly involved in enhanced/aberrant oncogene activation comes from studies on enhancers located downstream of *MYC* (Fig. 3) [87, 90, 91].

Two non-coding amplifications were detected 450 kb and 800 kb downstream of *MYC* in approximately 2% of lung adenocarcinoma and 4% of uterine corpus endometrial carcinoma, respectively [87]. Both amplified regions in lung adenocarcinoma and endometrial carcinoma encompass a cluster of enhancers with high enrichment for H3K27ac and form stable chromatin interactions with the *MYC* promoter (Fig. 3). Enrichment for H3K27ac on the amplified region in lung adenocarcinoma and enhancer-promoter interaction are tissue-specific as they cannot be observed in an endometrial carcinoma cell line and vice versa [87]. A small sequence of 150 base pairs (bp) within the amplified lung adenocarcinoma region is responsible for the majority of enhancer activity, contains binding motifs for several important transcription factors and depends on the recruitment of NFE2L2 and CEBPβ. Furthermore, repression or deletion of this enhancer element via CRISPR/Cas9 in two lung adenocarcinoma cell lines resulted in a reduction of *MYC* expression and a reduced ability of anchorage-independent and clonogenic growth [87].

A long-range *MYC* enhancer situated 1.47 Mb downstream of the *MYC* promoter is recurrently duplicated in 5% of T-ALL and was demonstrated to depend on the NOTCH signaling pathway and to be essentially required for proper thymocyte development and leukemogenesis (Fig. 3) [91].

Another enhancer region located 1.7 Mb downstream of *MYC* contains a cluster of five enhancers and is amplified in 3–5% of AML cases (Fig. 3). For example, in mouse RN2 AML cells the analogous enhancer region is occupied by the SWI/SNF chromatin remodeling complex, the enhancer mark H3K27ac and the histone lysine acetyl reader Brd4. Work in mouse and human leukemia cell lines confirmed that enhancer activity and recruitment of Brg1/BRG1, the catalytic subunit of SWI/SNF, are restricted to the

hematopoietic lineage. Brg1/BRG1 is also required for chromatin looping of these enhancers to the *Myc/MYC* promoter and for appropriate recruitment of hematopoietic transcription factors [90].

Structural genomic rearrangements/enhancer hijacking

In three recent studies "enhancer hijacking" was described as one means of ectopic oncogene activation [92–94]. "Enhancer hijacking" describes a process by which an enhancer, through genomic rearrangements, is removed from its natural genomic context and brought into proximity of another gene to activate it ectopically.

The first description of this phenomenon dates back more than thirty years describing a translocation between chromosome 8 and 14 in Burkitt's lymphoma presumably as a faulty result of class switch recombination thus bringing an enhancer of the *immunoglobulin H* (*IgH*) gene into close proximity of the *MYC* gene resulting in *MYC* overexpression [95].

A similar phenomenon was described in follicular lymphoma for the *BCL2* locus. Here, a translocation between chromosome 14 and 18 places the *IgH* enhancer downstream of the antiapoptotic *BCL2* gene. This leads to overexpression of *BCL2* and suppression of apoptosis [96, 97].

For example, in glioblastoma a translocation from chromosome 10 to chromosome 5 brings a putative super-enhancer into close proximity to the *telomerase reverse transcriptase* (*TERT*) promoter. The functional consequence of this event has not been investigated. However, TERT as the catalytic subunit of telomerase is known to be overexpressed in cancer. Furthermore, recurrent mutations in the *TERT* promoter which create new binding motifs for transcription factors and thus result in *TERT* overexpression have been reported in several tumor types (summarized in [98]).

In AML, cases with a particular inversion or translocation on chromosome 3 are characterized by the repositioning of a distal *GATA2* enhancer close to the *EVI1* (also known as *PRDM3* or *MECOM*) locus thus resulting in inactivation of the rearranged *GATA2* allele while simultaneously activating ectopic expression of the oncogene *EVI1* [93].

Furthermore, within two particular subgroups of pediatric medulloblastoma somatic structural variants including tandem duplications, deletions or inversions can place potent enhancers located within the *DDX31* locus proximal to *GFI1B*. These enhancers which generally function to presumably activate *DDX31* or one of its neighboring genes now in a different context start to drive expression of the oncogene *GFI1B*. Similarly, interchromosomal translocations and tandem duplications were also found to drive ectopic expression of the *GFI1B* paralog *GFI1* [92].

Further support that "enhancer hijacking" might be a more generally employed mechanism by which oncogenes are activated in cancers was provided by the finding that binding sites of the insulator protein CTCF and the enhancer-promoter mediating cohesin complex are frequently mutated in colorectal cancer (Box 1) [99]. CTCF and cohesin are also involved in the creation of loops that isolate subgroups of genes within so-called insulated neighborhoods, thus creating boundaries to prevent ectopic activation via enhancers from

other genes that lie outside these loops [100]. Indeed, in T-ALL some deletions involve CTCF/cohesin boundaries that flank insulated neighborhoods which contain proto-oncogenes. Specifically, deletion of a CTCF/cohesin boundary element via CRISPR/Cas9 in human embryonic kidney (HEK-293T) cells and primary human T cells demonstrated ectopic activation of the oncogene *TAL1*. Additional data supports a model in which an active enhancer element that is located outside the insulated *TAL1*-containing neighborhood and is usually prevented from interaction with the *TAL1* locus is now able to drive *TAL1* expression. A similar phenomenon in HEK-293T cells for the proto-oncogene *LMO2* was reported with a larger deletion containing several CTCF sites, and data mining of esophageal and liver carcinoma samples confirms an increased enrichment for mutations in CTCF boundary sites [101].

Conclusions and prospects

The amassment of genome-wide sequencing data via GWAS and WGS studies across many different cancer types and various genetic diseases over the past few years has revealed the importance of maintaining the integrity of non-coding regulatory elements, a fact that was previously largely underappreciated. It has become increasingly clear that tumorigenesis or disease state is often associated with epigenetic changes of the “enhancer landscape” and that mutations in or misregulation of enhancer-associated chromatin modifiers can have profound effects on enhancer activity holding the potential to cause inappropriate activation of oncogenic enhancers, inactivation of tumor-suppressive enhancers or inappropriate activation/inactivation of disease-relevant genes. As discussed here, this notion is strongly supported by multiple concrete studies on disease-relevant enhancers or enhancers of oncogenes and tumor suppressor genes. I predict that the next few years will see a further surge in the discovery of new disease-relevant enhancers. Despite a huge amount of disease-relevant data from GWAS and WGS studies our mechanistic understanding of individual enhancer-mediated processes in cancer and other diseases is strongly lacking behind and should lie at the forefront of future research investigations. Efforts are currently being made to target misregulated enhancers in cancer. For example, BROMO domain inhibitors are now tested in clinical cancer trials. They target the histone acetyl-binding BROMO domain of the enhancer-associated and -activating factor BRD4, thus preventing its recruitment (Box 1). BRD4 has a strong preference to bind to enhancers of oncogenic and lineage-specific genes also known as super enhancers (see above) making it a promising target [84, 85]. Time will tell whether this will prove a successful strategy to target individual tumors as BROMO-domain inhibitors apparently indiscriminately target most super enhancers. Thus, combination therapeutic approaches including BROMO domain inhibitors and the development of more enhancer-specific therapies should be a major focus of future clinical treatment regimens.

Acknowledgments

We thank all the members of the Herz laboratory for helpful discussions. We apologize to all whose work could not be discussed or referenced due to space limitations. This work was supported by a grant from the National Institutes of Health 4R00CA181506-02 to Hans-Martin Herz.

Abbreviations

AML	acute myeloid leukemia
GWAS	genome-wide association studies
SNP	single nucleotide polymorphism
T-ALL	T cell acute lymphoblastic leukemia
WGS	whole genome sequencing studies

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Box 1

Enhancer-associated chromatin modifying components, “enhancer signatures” and mediators of enhancer-promoter communication

To date many chromatin-modifying proteins have been identified to associate with enhancers and contain the ability to alter the DNA methylation, histone modification and DNA accessibility patterns within and around enhancers (see Introduction). For example some silent enhancers are marked by DNA methylation which is implemented by the DNA cytosine-5-methyltransferases DNMT1, DNMT3A and DNMT3B [29–32, 102] while on the other hand the family of TET enzymes including TET1, TET2 and TET3 is recruited to many active enhancers and catalyzes the oxidation of 5-methylcytosine to 5-hydroxy-metylcytosine [19, 103–106]. Many histone-modifying enzymes including histone lysine methyltransferases and demethylases also play a prominent role on enhancers (summarized in [8]) and particularly the histone modifications H3 lysine 4 monomethylation (H3K4me1), H3 lysine 9 acetylation (H3K9ac) and H3 lysine 27 acetylation (H3K27ac) are prominently enriched on many enhancers. While H3K4me1, H3K9ac and H3K27ac are usually strongly enriched on active enhancers some H3K4me1 and the repressive mark histone H3 lysine 27 trimethylation (H3K27me3) can be found on certain incactive and/or “poised” enhancers while H3K27 dimethylation (H3K27me2) occupies a protective role on non-cell-type-specific enhancers [107–111]. *Drosophila* Trithorax-related (Trr) and its mammalian homologs MLL3 and MLL4 in a redundant fashion constitute major H3K4 monomethyltransferases on enhancers (Fig. 1B) while the H3K4 demethylase LSD1/KDM1A can be responsible for enhancer “decommissioning” by removing H3K4me1 from enhancers [39–41, 112]. Additionally, a complex consisting of the chromatin “reader” RACK7 and the H3K4 trimethyl-specific demethylase SMCX/JARID1C/KDM5C prevents enhancer overactivation by keeping already active enhancers in a balanced state [113, 114]. All H3K27 methylation (including mono-, di- and trimethylation) is carried out by EZH1 and EZH2 the catalytic subunits of polycomb repressive complex 2 (PRC2). Interestingly, PRC2’s suppressive function on enhancer activity is antagonized by UTX an H3K27 demethylase which – either with MLL3 or MLL4 and additional components – forms a large macromolecular complex (Fig. 1B) [38]. Thus, the MLL3/MLL4 complexes with their ability to demethylate H3K27 and monomethylate H3K4 unite two enzymatic activities that are important for the transition from inactive/”poised” enhancers to active enhancers [39–41]. The GCN5L2 and PCAF containing histone acetyltransferase (HAT) complex ATAC catalyzes the deposition of H3K9ac [115–117] while the CBP and EP300 HATs implement the majority of H3K27ac on enhancers [117–119]. All these unique “enhancer signatures” implemented by DNA and histone modifying enzymes hold the potential to specifically recruit chromatin-associated factors. Among these are H3K4me1-binders such as TIP60 (also a HAT) [120], BROMO domain-containing proteins which recognize acetylated histones including BRD4 [81], but also components of ATP-dependent chromatin remodeling complexes such as CHD7 and BRG1 which interact with H3K4me1 and acetylated histones, respectively and CHD8, SMARCB1/BAF47, BAF155 and BAF170 [90, 121–125]. Some chromatin remodelers such as the NuRD complex can also be required to

keep enhancers in a repressed state and thus prevent inappropriate enhancer activation [126].

The cohesin complex is well known for its function in sister chromatid cohesion during meiosis and mitosis and postreplicative DNA damage repair but also functions in regulating enhancer activity. Members of the cohesin complex and their loading factors were first described as effectors of enhancer-promoter communication in *Drosophila* studies before this role was also confirmed in the mammalian system [127–130]. Another large complex, Mediator, assists in bringing together enhancer-associated transcription factors with the general transcription machinery including RNA polymerase II on promoters and the cohesin complex in this context is thought to further stabilize these long-range enhancer-promoter interactions (Fig. 1A) [129, 131] (summarized in [10, 132]). Cohesin also interacts with the insulator protein CTCF albeit not on enhancers. Instead, cohesin is involved in linking CTCF-bound loci called insulator elements with each other. These insulator elements are important in restricting enhancer activity to a given “neighborhood” often involving only a smaller subset of genes and thus preventing ectopic activation of other genes that otherwise might come under the influence of a given enhancer [10, 100, 131].

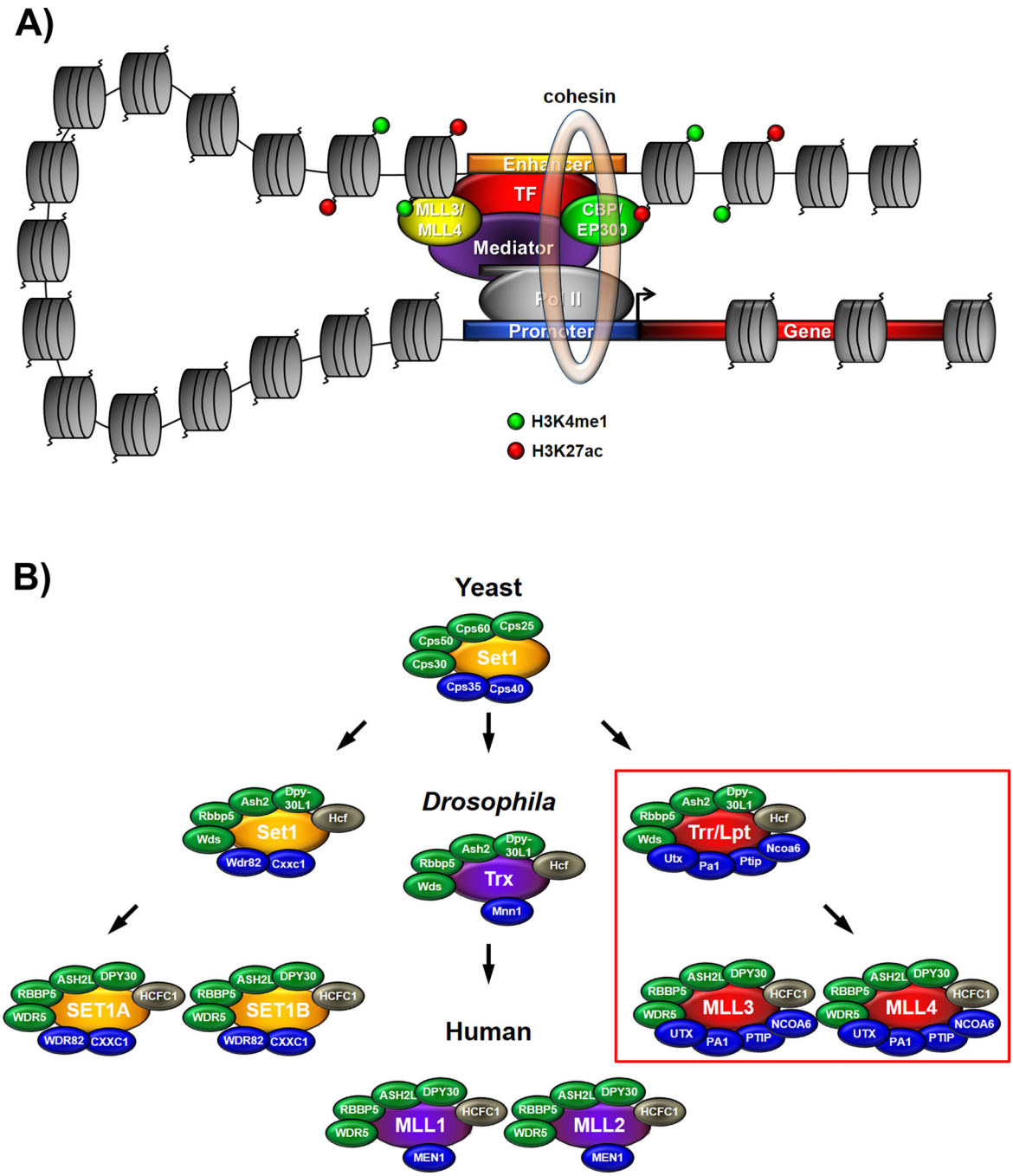

Figure 1.

A: Factors involved in enhancer-promoter interaction and communication. Tissue-specific transcription factors (TF, red) recruit co-activators to enhancers which often constitute chromatin-modifying proteins such as the histone H3 lysine 4 (H3K4) monometyltransferases MLL3/KMT2C or MLL4/KMT2D (yellow) and the histone H3 lysine 27 (H3K27) acetyltransferases CBP or EP300 (green). MLL3/MLL4 and CBP/EP300 implement H3K4 monomethylation (H3K4me1, green circles) and H3K27 acetylation (H3K27ac, red circles) on enhancers, respectively. Interactions of these enhancer-associated

factors with the general transcription machinery at the promoter including RNA Polymerase II (gray) are mediated by the Mediator complex (purple) and further stabilized by the ring-shaped cohesin complex (light brown transparent ring). **B:** The family of histone H3K4 methyltransferases in yeast, *Drosophila* and mammals. Core subunits which are commonly shared among all complexes are highlighted in green. Complex-specific subunits are only contained within one out of three branches and are highlighted in blue. UTX/KDM6A is an H3K27 demethylase and constitutes a complex-specific subunit within the Trithorax-related (Trr) branch (red box) while MLL3/KMT2C and MLL4/KMT2D form the catalytic core as H3K4 methyltransferases within the mammalian complexes of the Trr branch.

Figure 2.

Somatic mutations reported for *UTX/KDM6A* (A), *MLL3/KMT2C* (B) and *MLL4/KMT2D* (C) according to the Catalogue of Somatic Mutations in Cancer (COSMIC) based on the Pediatric Cancer (PeCan) Data Portal from St. Jude Children's Research Hospital.

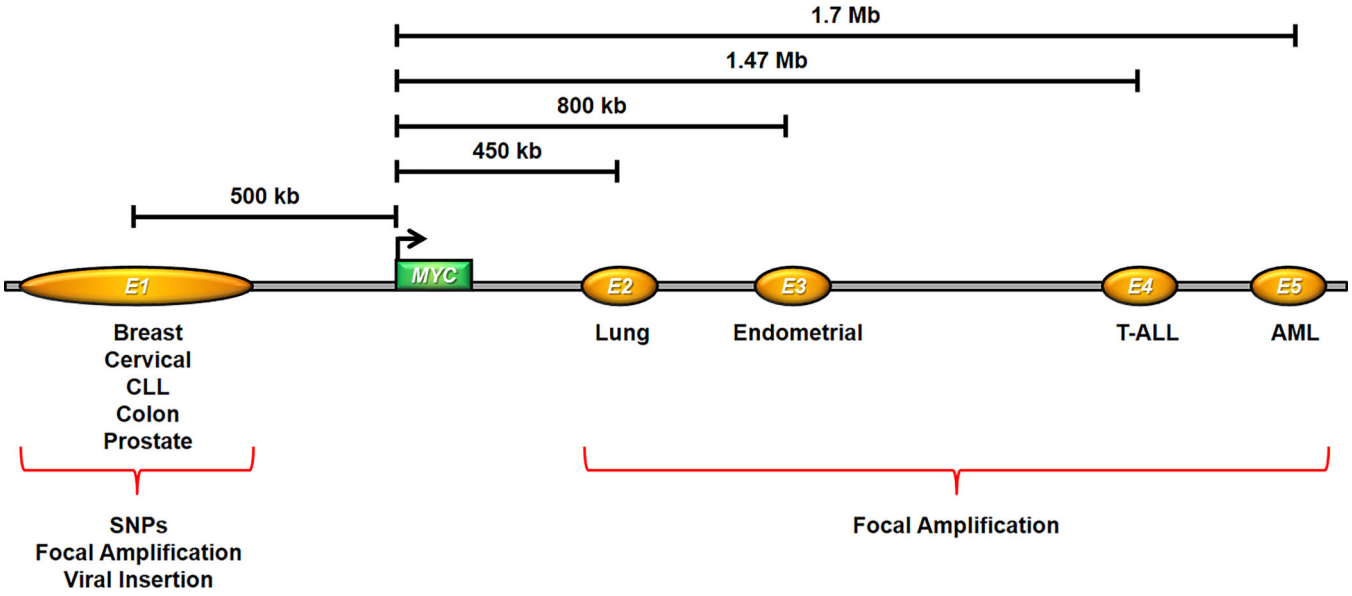

Figure 3.

Regions containing tissue-specific *Myc* enhancers. At least five regions (E1-5) that often contain several tissue-specific enhancer clusters have been reported for the *MYC* locus. The approximate distance from the center of each region to the *MYC* promoter is indicated by a black bar. E1 shows a high enrichment for SNPs in various cancers but is also affected by focal amplification or viral insertion. Regions E2-5 have been shown to be focally amplified in lung cancer (E2), endometrial cancer (E3), T-ALL (E4) and AML (E5).

Table 1

Mutations in mediators of enhancer-promoter interaction or chromatin-modifying proteins in cancer (with a frequency of >3%).

Gene	Molecular Function	Cancer Type	References
CTCF	Chromatin insulator	Endometrial, head and neck	[16, 17]
RAD21/SCC1	Cohesin complex	Acute myeloid leukemia	[16]
SMC1A	Cohesin complex	Acute myeloid leukemia, endometrial	[16, 17]
SMC3	Cohesin complex	Acute myeloid leukemia	[16]
STAG2/SCC3B	Cohesin complex	Acute myeloid leukemia, bladder, glioblastoma	[16, 17, 133]
ARID1A/BAF250	Chromatin remodeling (SWI/SNF complex)	Bladder, colorectal, endometrial, esophageal, gastric, kidney, liver, lung, ovarian	[16, 17, 133–137]
ARID1B/BAF250B	Chromatin remodeling (SWI/SNF complex)	Liver	[135]
ARID2/BAF200	Chromatin remodeling (SWI/SNF complex)	Liver, lung, melanoma	[16, 135]
PBRM1/BAF180	Chromatin remodeling (SWI/SNF complex)	Bladder, kidney	[17, 138]
SMARCA4	Chromatin remodeling (SWI/SNF complex)	Esophageal, medulloblastoma, lung	[16, 139, 140]
SMARCB1/BAF47/SNF5	Chromatin remodeling (SWI/SNF complex)	Rhabdoid tumor	[16, 141]
CHD4	Chromatin remodeling	Endometrial	[16]
CHD6	Chromatin remodeling	Bladder	[134]
CHD8	Chromatin remodeling	Glioblastoma	[16]
BCOR	Corepressor complex	Endometrial	[16]
NCOR1	Corepressor complex	Breast, head and neck, melanoma	[16]
DNMT3A	DNA methyltransferase	Acute myeloid leukemia	[16, 17]
TET2	DNA demethylase	Acute myeloid leukemia	[16, 17]
MLL1/KMT1A	H3K4 methyltransferase	Bladder, liver	[16, 134, 135]
MLL2/KMT1B	H3K4 methyltransferase	Bladder, endometrial, head and neck	[16, 17]
MLL3/KMT2C	H3K4 methyltransferase	Bladder, breast, colorectal, endometrial, gastric, head and neck, lung, liver, medulloblastoma	[16, 17, 134, 135, 137, 139, 142, 143]
MLL4/KMT2D	H3K4 methyltransferase	Non-Hodgkin lymphoma, bladder, breast, endometrial, head and neck, kidney, lung, medulloblastoma, squamous	[16, 17, 133, 139, 140, 144–146]

Gene	Molecular Function	Cancer Type	References
		cell carcinoma	
SMCX/JARID1C/ KDM5C	H3K4 demethylase	Kidney, lung	[16, 17, 145]
CBP	H3K27 acetyltransferase	Acute lymphoblastic leukemia, B cell lymphoma, bladder, non-Hodgkin lymphoma	[16, 134, 144, 146, 147]
EP300	H3K27 acetyltransferase	B cell lymphoma, bladder, endometrial, head and neck, lung	[16, 17, 133, 134, 146]
EZH2	H3K27 methyltransferase	Non-Hodgkin lymphoma, B-cell lymphoma	[144, 146]
UTX/KDM6A	H3K27 demethylase	Bladder, medulloblastoma, renal cell carcinoma	[16, 17, 133, 134, 140, 145]

Table 2

Mutations in enhancer-associated chromatin-modifying proteins in other diseases.

Gene	Molecular Function	Disease	References
MLL4/KMT2D	H3K4 methyltransferase	Kabuki Syndrome	[54, 55]
SMCX/JARID1C/KDM5C	H3K4 demethylase	X-linked mental retardation	[148]
CBP	H3K27 acetyltransferase	Rubinstein-Taybi Syndrome	[149, 150]
EP300	H3K27 acetyltransferase	Rubinstein-Taybi Syndrome	[149]
UTX/KDM6A	H3K27 demethylase	Kabuki Syndrome	[55, 56]