Implications of enhancer transcription and eRNAs in cancer

Santanu Adhikary\textsuperscript{1,2}, Siddhartha Roy\textsuperscript{2}, Jessica Chacon\textsuperscript{7}, Shrikanth S. Gadad\textsuperscript{3,4,5,7,8} and Chandrima Das\textsuperscript{1,6,8}

\textsuperscript{1}Biophysics and Structural Genomics Division, Saha Institute of Nuclear Physics, 1/AF Bidhannagar, Kolkata-700064
\textsuperscript{2}Structural Biology and Bioinformatics Division, CSIR-Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Kolkata-700032
\textsuperscript{3}Center of Emphasis in Cancer, Department of Molecular and Translational Medicine, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center El Paso, Texas-79905, United States
\textsuperscript{4}Graduate School of Biomedical Sciences, Texas Tech University Health Sciences Center El Paso, Texas-79905, United States
\textsuperscript{5}Cecil H. and Ida Green Center for Reproductive Biology Sciences, Department of Obstetrics and Gynaecology, University of Texas Southwestern Medical Center, Dallas, TX 75390, United States
\textsuperscript{6}Homi Bhaba National Institute, Mumbai, India
\textsuperscript{7}Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center El Paso, Texas-79905, United States

\textsuperscript{8}Lead Contacts / Address correspondence to:
Shrikanth S. Gadad, Ph.D.
Center of Emphasis in Cancer
Department of Molecular and Translational Medicine
Paul L. Foster School of Medicine
Texas Tech University Health Sciences Center El Paso
5001 El Paso Drive
El Paso, Texas 79905, USA
Phone: 915.215.6431
Fax: 915.783.5222
E-mail: shrkanth.gadad@ttuhsc.edu

Chandrima Das, Ph.D.
Biophysics and Structural Genomics Division
Saha Institute of Nuclear Physics
Sector-I, Block- AF, Salt Lake
Kolkata-700064, West Bengal, India
Phone: 033-2337-0221 Ext- 4623
Fax: +91-33-2337-4637
Email: chandrima.das@saha.ac.in

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Abbreviations

eRNA: enhancer RNA
TNBC: Triple-Negative Breast Cancer
NSCLC: Non-Small Cell Lung Cancer
TF: Transcription Factors
ESR1: Estrogen Receptor 1
ERα: Estrogen Receptor α
AR: Androgen Receptor
AML: Acute Myeloid Leukemia
CML: Chronic Myeloid Leukemia
ALL: Acute Lymphoid Leukemia
EMT: Epithelial to Mesenchymal Transition
CSC: Cancer Stem Cells
TME: Tumor Microenvironment
TAM: Tumor-Associated Macrophages
EGFR: Epidermal Growth Factor Receptor
IGF1R: Insulin Growth Factor 1 Receptor
TKI: Tyrosine Kinase Inhibitor
HDACi: Histone Deacetylase inhibitor
LncRNA: Long noncoding RNA
CRPC: Castration-Resistant Prostate Cancer
E-P: Enhancer-Promoter
GRO-seq: Global Nuclear Run-On Sequencing
PRO-seq: Precision Nuclear Run-On Sequencing
TT-seq: Transient Transcriptome Sequencing
PRC2: Polycomb Repressive Complex 2
HAT: Histone Acetyl Transferase
MEF: Mouse Embryonic Fibroblast
BET: Bromodomains and Extraterminal
NELF: Negative Elongation Factor
PTEFb: Positive Elongation Factor b
CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
CRISPRi: CRISPR interference
Cas9: CRISPR associated protein 9
dCas9: Dead Cas9
Abstract

Despite extensive progress in developing anti-cancer therapies, therapy resistance remains a major challenge that promotes disease relapse. The changes that lead to therapy resistance can be intrinsically present or may be initiated during treatment. Genetic and epigenetic heterogeneity in tumors make it more challenging to deal with therapy resistance. Recent advances in genome-wide analyses have revealed that the deregulation of distal gene regulatory elements, such as enhancers, appears in several pathophysiological conditions, including cancer. Beyond the conventional function of enhancers in recruiting transcription factors to gene promoters, enhancer elements are also transcribed into noncoding RNAs known as enhancer RNAs (eRNA). Accumulating evidence suggests that uncontrolled enhancer activity with aberrant eRNA expression promotes oncogenesis. Interestingly, tissue-specific, transcribed eRNAs from active enhancers can serve as potential therapeutic targets or biomarkers in several cancer types. This review provides a comprehensive overview of the mechanisms of enhancer transcription and eRNAs as well as their potential roles in cancer and drug resistance.
1. Introduction

Cancer is one of the leading causes of death worldwide; it is a heterogeneous disease controlled by genetic and epigenetic alterations and deregulated transcription (1,2). Advances in DNA-based technologies have led to the development of precision-based therapeutic strategies, including surgery, targeted therapy, radiation therapy, chemotherapy, hormonal therapy, and immunotherapy (3–5). Yet, most cancers evolve and become refractory to therapy, resulting in death because the continuous selection of fit cells leads to resistance against therapeutic strategies (6–8).

In the past two decades, with the completion of the Human Genome Project and advances in high throughput sequencing, it has become quite feasible to identify regulatory and functional elements and determine their function, especially abnormal biological processes such as cancer. Sequencing has provided a blueprint for gene expression and has led to new study areas comprising the assessment of multiple action mechanisms involved in gene regulation, providing researchers with therapeutic and diagnostic targets for many heritable diseases. Transcription is a critical regulatory step controlled by promoters, which are nearby cis-regulatory elements, and other distal regulatory elements, such as enhancers and silencing elements (9). Understanding the intricate details of transcriptional regulation at enhancers in cancer is the key to developing next-generation therapeutic strategies to treat this ever-evolving disease.

Enhancers and enhancer transcription

Enhancers are short clusters of regulatory DNA elements, spanning 500–2000bp, that are transcribed and contain transcription factor (TF) recognition sequences. They are present either near to or distant from the target gene promoters and regulate expression across space and time (10–12). The first enhancer was described as a distal cis-regulatory DNA element of the gene activation of the simian virus 40 (SV40) (13). Active enhancers have been found in undifferentiated and pluripotent embryonic stem cells, which drive gene expression to maintain pluripotency (14). However, those involved in lineage commitment remain inactive (15). During cellular differentiation, enhancers become active and control lineage specification (14,15). Emerging studies have shown that most oncogenes are governed by actively transcribing enhancers and/or clusters of enhancers (sometimes termed super-enhancers), previously known as locus control regions (16–18). The binding of TFs to enhancers leads to the recruitment of chromatin-modifying machinery and chromatin remodeling complexes. Enhancers are typically marked by histone modifications, such as histone H3 lysine 27 acetylation (H3K27ac) and H3 lysine 4 mono-methylation (H3K4me1).
(Figure 1A), which result in enhancer transcription to generate enhancer RNAs (eRNAs) via RNA polymerase II (RNA Pol II) (Figure 1A) (19–25). The discovery of eRNAs has revealed an untapped wealth of biological information. We have just begun to understand their potential in designing strategies to combat many diseases, including cancer.

eRNAs were first identified in neurons and macrophages (26,27). eRNAs are bidirectionally transcribed from enhancers (24,26) via RNA Pol II and are usually noncoding (10,22,26,28–32). In contrast to mRNAs, the majority of these eRNAs are unspliced and have non-polyadenylated tails (11). Furthermore, eRNAs are more unstable, more abundant, and shorter, and they are retained in the nucleus, making them vulnerable to exosome-mediated decay (11,33–35). The lack of polyadenylation (polyA) is one of the primary causes of their reduced stability (11,33,34,36). Mechanistically, the proximity of the polyA signal to the transcription start site (TSS) prevents the assembly of polyadenylation machinery at RNA Pol II’s C-terminal domain (CTD), which results in the recruitment of exosomes (37). It is evident from recent studies that eRNAs have secondary structures similar to other ncRNAs and undergo post-transcriptional modifications, such as cytosine methylation via Nsun7, that stabilize the eRNA (38). eRNAs bind to TFs and are densely located at the target gene’s TSS to induce enhancer–promoter (E–P) interactions (39) (Figure 1B and 1C). These characteristics make them poorly detectable through conventional polyA+ RNA-seq (10,11,22,24,26).

The identification and quantification of eRNAs in specific target cell lines and tissues are feasible via techniques such as ribosomal RNA (rRNA)-depleted total RNA-seq or cap analysis of gene expression (CAGE) (11,40,41). Other studies have identified eRNAs using Global Run-On Sequencing (GRO-seq), Precision Nuclear Run-On Sequencing (PRO-seq), or Transient Transcriptome Sequencing (TT-seq) (42–48). The CAGE by the FANTOM consortium revealed 43,011 active enhancers that transcribe eRNA (49). The genomic regions marked with H3K27ac and H3K4me1 reveal the active sites of eRNA transcription (50). BruUV-seq, which involves the transcription blocking of DNA lesions using UV light coupled with bromouridine labeling and nascent RNA-seq, is also an efficient method to detect eRNAs (51). Other techniques, such as fluorescence in situ hybridization, use fluorescein-tagged RNA probes to detect eRNAs and their steady-state levels (52). These methods reveal an unprecedented view of the active transcription landscape, enabling the discovery of eRNAs.
This review will discuss the relevance of enhancer transcription and eRNAs in cancer, including therapy-resistant cancers. Lastly, we will propose plausible new therapeutic strategies to combat resistant cancers.

2. The molecular features and actions of eRNAs

E RNAs are the primary products of active enhancers, which promote gene transcription and facilitate the interaction of TFs (Figure 1B) and coactivators via an E–P loop (Figure 1C) and prevent Pol II release from pausing by interacting with NELF (Figure 1D) (39,42,53–56). Recent studies have suggested that the deletion or disruption of DNA-encoding ncRNAs may not reveal their molecular functions (57,58); hence, ncRNAs such as eRNAs should be targeted using classical approaches such as shRNA- or siRNA-mediated silencing. Possible precautions should be taken to ensure minimal or no interference in TF or RNA Pol II functions (34,39,42,59,60). Generally, non-polyadenylated eRNAs act in cis; however, recent reports have indicated the existence of trans-acting, stable polyadenylated eRNAs (10,42,53,59).

2.1. eRNAs as positive regulators of gene transcription

E RNAs alter the epigenetic landscape and chromatin architecture at the enhancer site (61); for example, the SERPINB2 eRNA upregulates its mRNA transcription (62). Conversely, the number of transcripts generated from enhancers located in the intragenic regions can inversely correlate with the host’s gene expression levels (63). Nonetheless, recent reports have suggested that eRNAs have an enormous impact on transcription regulation, with eRNAs inducing neighboring mRNA transcription (11,26,40,42,61,64). RNA Pol II is not ubiquitously bound to the enhancers; however, its recruitment and removal are crucial for the initiation and termination of eRNA, which regulate the length of transcripts (65). Knockdown studies have revealed that the downregulation of eRNAs leads to fewer target mRNAs (62,66). The specificity of eRNAs to their target mRNA synthesis is contextually dependent on the chromatin landscape, TFs, and E–P interactions (67).

2.2. E–P interactions

Historically, active enhancers have been shown to regulate distal gene promoters via chromatin looping, and recent GRO-seq-based studies have indicated that eRNAs are actively transcribed from these enhancers, yet their biological function remains elusive (47,68). In the initial study, researchers performed loss of function and chromosomal conformation capture assays, which showed an eRNA–E–P interaction in the presence of estrogen in breast cancer cells (42). It was found that eRNAs regulate the interaction between active enhancers and
proximal promoters through association with the cohesion complex via the RAD21 and SMC3 subunits (Figure 1D). Later, it was affirmed that the eRNA–RAD21 interaction is required for the stability of the E–P loop, as RNase H1-mediated digestion prevents RAD21 recruitment to the chromatin (69). Furthermore, such chromatin looping and eRNA interactions were found to regulate gene expression at the β-globin gene locus (70). Similarly, using 4C-seq and ChIP-seq assays, it was found that Evf2 eRNA, transcribed from the ultra-conserved enhancer, modulates cohesion positioning in interneurons, thereby regulating enhancer interactions in long-range cis (> 30Mb) and trans (71). Additionally, in muscle, DRR eRNA modulates Cohesin recruitment in distal promoters, facilitating a long-range E–P interaction and regulating muscle differentiation (72).

eRNAs have also been shown to interact with heterogeneous nuclear ribonucleoprotein U (hnRNPU) and the mediator complex to regulate the E–P interaction (66,73). Heparinase (HPSE) eRNA regulates the transcription of the HPSE oncogene through an interaction with hnRNPU and p300, which is mediated by EGR1. This interaction facilitates the increased recruitment of p300 to the super-enhancer, may promote chromatin looping, and activates the promoter (66). eRNAs have also been found to associate with the mediator coactivator complex at E–P looping sites. The loss of eRNA removes the mediator complex from the target promoters, and the looping is disrupted (53,74). For instance, eRNA interacts with mediator complex subunit 1 (MED1), facilitating the E–P interaction via chromatin looping in prostate cancer cells (53). In T-cell acute lymphoblastic leukemia cells, ARIEL eRNA connects to mediator complex MED12 (75), and in colon cancer cells, CTCF (the CCCTC binding factor) eRNAs facilitate the E–P interaction via chromatin looping (76). Likewise, PRC2, which trimethylates H3K27, interacts with the mediator complex, assisting in poised enhancers’ looping by binding to RNA (77,78). However, the PRC2-dependent mechanism of the eRNA-driven transition of poised to active enhancers requires further investigation.

Furthermore, the integrator, which is a multi-subunit complex associated with the CTD of RNA Pol II, regulates eRNA processing, which, in turn, modulates the eRNA-dependent E–P interaction (35,79). Contrastingly, several studies have shown that the E–P interaction is maintained even after the inhibition of eRNA production or enhancer transcription (54,80). Collectively, the exact mechanism of the eRNA-mediated E–P interaction remains to be explored and may be distinct for different eRNAs.

2.3. Interactions with DNA, R-loop formation, and the RNA exosome
It is evident from recent studies that eRNAs hybridize with templated DNA, leaving non-templated DNA single-stranded, which is known as the R-loop (81). These R-loops are associated with DNA damage and genomic instability and are implicated in cancer (82,83).

Studies have suggested that R-loops are associated with an RNA processing complex known as the RNA exosome (34). RNA exosomes have nine core subunits that bind to RNA, while the two non-core subunits are endowed with ribonuclease activity (84). The catalytic subunit DIS3 regulates CTCF binding element eRNAs (cbeRNAs; cbeRNAs and DIS3-sensitive RNAs have a substantial overlap), which has been shown to play a crucial role in the maintenance of immunoglobulin heavy chain integrity and somatic hypermutation in B cells (85). The genetic depletion of exosome component 3 (Exosc3) and Exosc10 of the RNA exosome complex leads to the upregulation of eRNAs that results in R-loop formation and genomic instability at the enhancers of mouse embryonic stem cells (mESCs) and B cells (34). Significantly, tumor aneuploidy correlates with global enhancer activation and eRNA transcription, indicating genomic instability, DNA damage, and cancer progression (86). Further studies are required to elucidate the relationship between eRNA transcription and R-loop formation at the enhancers of cancer cells.

3. eRNAs in cancer

eRNAs have emerged as excellent markers of active enhancers and genes (Table 1). Aberrant changes in eRNA levels are associated with deregulated enhancer transcription and gene expression in cancer (32,41,86,87). For example, in breast cancer, the estrogen-dependent binding of estrogen receptor alpha (ERα) to oncogenic enhancers leads to the production of eRNAs (80,88). Similarly, in prostate cancer, androgen receptor (AR)-induced KLK3 eRNA, a regulator of KLK3 oncogene expression eRNA, acts as a scaffold for the AR-protein complex (53). Conversely, studies have indicated that tumor suppressors, such as TP53, induce eRNAs, which play a critical role in cell-cycle regulation in several cancer cells (60). In acute myeloid leukemia (AML), chromosomal rearrangements such as inv(3)/t(3;3) leads to aberrant transcriptional activity at GATA2 gene distal enhancer, which elevates EVII expression. Mechanistically, inv(3)/t(3;3) loops with the EVII promoter to control EVII expression (89). Additionally, in pancreatic cancer, the enhancer landscape’s reprogramming, which leads to genome-wide alterations in enhancer activity, promotes oncogenesis with the worst prognosis (90–92). These observations have revealed the role of enhancer transcription in regulating oncogenesis (93), and may generate eRNAs, which can be potential therapeutic
targets, although a direct connection between pancreatic cancer and eRNA expression is yet to be established.

3.1. eRNAs as tumor suppressors

eRNAs function as tumor suppressors by regulating tumor suppressor gene expression (32), such as tumor suppressor p53. The transcription of several eRNAs upon Nutlin-3a treatment in breast cancer cells is stimulated by p53 (60). Intriguingly, the loss of these eRNAs perturbs p53-dependent gene transcription (60). In another instance, LED lncRNA (the lncRNA activator of enhancer domains) regulates enhancer activity and eRNA transcription to control the expression of the p53-dependent genes CDKN1A, p21, PRKAG2, TOBI, and SUFU (94). Interestingly, LED expression is regulated by DNA hypermethylation; 5-azacytidine treatment promotes LED expression, which, in turn, facilitates p53’s tumor-suppressive role. Therefore, eRNAs are crucial for maintaining tumor-suppressive functions.

3.2. eRNAs as positive regulators of oncogenes

eRNAs are master regulators of oncogenes and show potential oncogenic functions in various human cancers (32,93). In ER+ breast cancers, 17β-estradiol (E2) treatment leads to ERα-bound enhancers’ activation, inducing eRNA transcription and E2-dependent gene regulation (42). E2 treatment activates eRNA transcription and promotes the expression of genes, including GREB1, NRIP1, P2RY2, SMAD7, PGR, KCNK5, SIAH2, CA12, TFF1, and FOXC1 (42). Interestingly, the interaction between NRIP1 and TFF1 loci is lost upon the depletion of NRIP1e, indicating eRNA-mediated E–P looping. Mutation in the enhancer element of the ACTRT1 gene, which encodes actin-related protein T1 (ARP-T1), leads to aberrant eRNA transcription (95). Several reports have suggested that mutations or misregulation in enhancer regions lead to aberrant eRNA production (95) and deregulated signaling pathways (47,61,95–97). Therefore, the ectopic expression of ACTRT1 reduces cell proliferation and activates the Hedgehog signaling pathway aberrantly (95).

In prostate cancer, AR binding to target the PSA gene enhancer produces PSA eRNA (PSAe) and activates its expression in castration-resistant prostate cancer (CRPC) cells (98). PSAe regulates androgen-dependent gene expression both in cis and trans and promotes tumor progression (98). PSAe is structurally similar to the HIV-1 TAR-L motif and interacts with P-TEFb and cyclin T1 to regulate transcription. However, during trans-activation, PSAe modulates AR-dependent gene expression via a feed forward loop.

In other malignancies, such as CpG island methylator phenotype colon cancer, cMYC oncogene expression is driven by super-enhancer-derived colon cancer–associated transcript
1 (CCAT1) (99). Furthermore, the interrogation of the cancer genome atlas has shown a positive correlation between CCAT1 and cMYC expression in several tumor types (99), indicating a functional relationship between them. Inhibition of BRD4 by JQ1 or its knockdown leads to reduced cMYC expression, which arrests tumor growth (99). CCATIL, a longer form of CCAT1, mediates a long-distance interaction between the CCAT1-producing enhancer and the cMYC promoter (76).

In the past few years, genome-wide studies have shown aberrant enhancer activity in the deregulation of many signaling pathways in cancer (12,100,101). For example, in breast cancer cells, the hormone-driven YAP/TEAD of the Hippo pathway modulates eRNA transcription (102); other pathways, such as inflammatory signaling in p53-mutated cancers, drive eRNA transcription to regulate tumor progression (103). Specifically, inflammatory genes such as CCL2 are activated by mutant p53 and NFκB enhancers (103,104). Collectively, these studies have suggested that eRNAs play a role in promoting tumor initiation and maintenance.

4. Therapy resistance, enhancers, and eRNAs

Resistant cancer is a well-defined phenomenon that occurs when cancer cells become tolerant of an administered drug dose. Resistance to therapy occurs due to various factors, including genetic and/or epigenetic alterations, the increased efflux of drugs, and several other molecular processes (Figure 2A). These alterations collude to develop a patient’s resistance to classical or novel chemotherapeutic drugs and immunotherapy-based approaches, leading to the disease’s relapse (3,5–8). Traditionally, many chemotherapeutic drugs induce DNA damage in cancer cells; however, off-target effects could potentially lead to cytotoxicity in normal cells. After repeated exposure, the drug’s efficacy recedes, leading to a relapse of the disease in most patients. For instance, 40–55% of triple-negative breast cancer patients develop resistance to chemotherapy and radiation therapy (105,106). Similarly, 50–70% of ovarian cancers relapse within a year post-surgery and -chemotherapy (107). Approximately 40% of non-small-cell lung cancers (NSCLC) relapse due to acquired resistance, resulting in death (108). Furthermore, 20% of lymphoblastic leukemia and pediatric cancers develop resistance and relapse (109). Hence, continuous efforts are being made to understand and identify the mechanisms of resistance in cancer.

In the past decade, the deregulation of enhancer transcription, affecting ncRNA expression (eRNAs, lncRNAs, etc.), has been implicated in several cancers and its therapies
(Figure 2A) (93,110–114); however, the role of eRNAs in drug resistance is not well known. Though the role of enhancers in therapy-resistant cancers is emerging, such as, in combination with cisplatin, BET inhibitors sensitize cisplatin-resistant ovarian cancer to cisplatin by inhibiting the ALDH1 super-enhancer (115). Enhancer overactivation is also implicated in hormone-refractory breast cancers; a recent report has suggested that TFs such as GATA3 and AP1 reprogram the enhancer landscape to promote enhancer overactivation, leading to phenotypic plasticity in endocrine-resistant breast cancer (116).

Given the role of enhancers in therapy-resistant cancers and eRNAs being shown as functional units of enhancer activity, the eRNAs involvement in resistant cancers can’t be ruled out. Neuroepithelial cell transforming gene 1 eRNA (NET1e) is abundantly expressed in breast cancer, and, notably, its overexpression results in resistance to the drugs BEZ235 and Obatoclax in MCF7 cells (21). In enzalutamide CRPC cells, AR-eRNAs are differentially expressed (117); the study has shown that prolonged treatment with enzalutamide leads to resistance and enhances the expression of AR-eRNAs and oncogenic mRNAs such as FTO, LUZP2, MARC1, and NCAM2, thereby promoting more resistant phenotypes (117).

eRNA transcription from active enhancers is implicated in both drug-resistant solid tumors and therapy-resistant leukemias. In daunorubicin-resistant AML, in response to stress, enhancers are activated to induce ABCB1 expression and promote resistance (118). Daunorubicin treatment to fresh blast cells from human AML samples leads to the activation of ABCB1 expression via the binding of ATF4-like TFs to stress-responsive enhancers. It has been reported that U0126 and ISRIB inhibit ABCB1 expression, thereby sensitizing cancer cells to drug treatment; however, the mechanism of inhibition is still unknown (118). These inhibitors may also affect enhancer activity and eRNA transcription by suppressing ABCB1 expression. Thus, by uncovering eRNA mechanisms in therapy-resistant cancers, researchers can identify potential therapeutic targets in resistant cancers.

**eRNAs as emerging targets for anti-cancer therapy**

Emerging studies have shown aberrant enhancer activity and eRNA expression in tumor samples when compared to normal tissues (21,86). Enhancer overactivation is a unique phenomenon in oncogenesis, and targeting eRNAs could be a potential new anti-cancer therapeutic strategy (21,119–121). The tissue-specific expression of eRNAs across different cancer types may make them tissue-specific therapeutic targets. These eRNAs can be targeted by shRNAs, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Cas9, CRISPR interference (CRISPRi)-dCas9, antisense oligos (ASOs or locked nucleic acids
[LNAs]), or enhancer activity suppression using BET inhibitors, which can control target gene expression and tumor growth (Figure 2B) (21,53,66,122–124). *NET1e* is overexpressed in breast cancer cell lines, such as MCF7, with low or no expression seen in normal breast cell lines, such as MCF10A, or other cancer cell lines, such as HeLa or HepG2 (21). Targeting *NET1e* with LNAs inhibits cell proliferation (21), and this eRNA could serve as a potential drug target; however, it needs to be tested in preclinical models. In another study, the *HPSE* eRNA has been shown to have an oncogenic function in vivo (66); a detailed mechanism of action will reveal its therapeutic potential.

Interestingly, regarding other potential targets, a strong correlation between enhancer 9 expression (chr9:5580709-5581016) and programmed death-ligand 1 (PD-L1) has been found in a plethora of cancers (86). PD-L1 reportedly plays a significant role in cancer immunotherapy; therefore, using the expression of eRNA as a biomarker can help in designing immunotherapy-based approaches since the deletion of this enhancer impairs PD-L1 expression (86). These studies have suggested that eRNAs significantly contribute to the development of novel therapeutic targets that are highly specific and unique due to their tight regulation and tissue specificity.

5. Conclusions and future perspectives

Tumor heterogeneity poses a considerable challenge in understanding tumor progression and creates obstacles in identifying the best therapeutic strategy; failure to determine a potential strategy could lead to drug resistance. Identifying the driver oncogene, which promotes drug resistance at any stage of tumorigenesis, is possible with high throughput genomics, proteomics, and metabolomics analyses. These technologies have helped the scientific community to advance the design of combinatorial or personalized therapy.

Therapy with a single drug often fails to affect heterogeneous polyclonal cells and increases the possibility of relapse. Therefore, combinatorial treatment shows promising results and minimizes the likelihood of a gain of resistance. However, predicting therapeutic outcomes can be difficult, as resistant tumors are genetically different across patients. As tumor cells often develop alternative mechanisms for survival, finding an alternative therapeutic strategy will not be the final step.

In this regard, studies that focus on understanding the mechanisms that drive enhancer function and the role of eRNAs in drug resistance have emerged as a potential avenue for investigating the identification of potential targets. However, there is still much to study...
before we can completely understand and exploit enhancer transcription’s therapeutic potential in cancer (11,21,32,40,86,93,112). Given the instability and dynamic nature of enhancer transcripts, the primary challenge is to validate their functional outcomes in vivo to explore their clinical utility. The proteome associated with eRNAs must also be investigated and can be identified via Chromatin Isolation by RNA Purification (ChIRP), RNA Antisense Purification (RAP), or Identification of Direct RNA Interacting Proteins (iDRiP) (125–127), which will potentially help to inhibit critical interactions by targeting protein partners. However, structural analyses would help in designing small-molecule modulators that can target eRNA–protein interactions with specificity.

Another potential avenue is to identify the role that RNA modifications play, including 5-cytosine methylation (m5C), N6-adenosine methylation (m6A), hydroxy-methyl cytosine (5hmC), and methyl-1 adenosine (m1A), which have been reported in several regulatory RNAs (128–131). The existence of such modifications in the context of eRNAs has not yet been explored. Additionally, the role of eRNAs in higher-order chromatin organization, as mediated by RNA–DNA, RNA–RNA, or RNA–protein interactions, should be investigated in future studies (132,133). Addressing these outstanding questions will not only broaden our knowledge of the enhanceosome but will significantly help in uncovering new eRNA-based cancer therapies, specifically for therapy-resistant cancers.

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Table 1: A list of the eRNAs implicated in oncogenesis

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>eRNAs</th>
<th>Transcriptional direction</th>
<th>References</th>
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<tr>
<td>Breast cancer</td>
<td>NRIPe, TFF1e, PGRe, KCNK5e, FOXC1e, GREB1e, CA12e, SMAD7e, P2RY2e, SIAH2e, NET1e, P21e, PRKAG2e, SUFUe, TOB1e</td>
<td>Bidirectional</td>
<td>(21,42,94)</td>
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<td>Castration-resistant prostate cancer</td>
<td>PSAe, ARHGEF26e, SLC16A7e, KLK15e, TLE1e, HTR3Ae, NCAM2e, FTOe, MARC1e, LUZP2e</td>
<td>Bidirectional</td>
<td>(98,117)</td>
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<td>Colorectal cancer</td>
<td>CCAT1e</td>
<td>Bidirectional</td>
<td>(99)</td>
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<tr>
<td>Basal cell carcinoma</td>
<td>ACTRT1e</td>
<td>Bidirectional</td>
<td>(95)</td>
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<tr>
<td>AML</td>
<td>MYC (E1–E5)</td>
<td>Not determined</td>
<td>(113)</td>
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<tr>
<td>ALL</td>
<td>ARIELe, LUNAR1e</td>
<td>Unidirectional</td>
<td>(75,114,122)</td>
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Figure Legends:

**Figure 1: The mechanism of eRNA action.** A. Increased histone modifications H3K27ac and H3K4me1 promote eRNA transcription from the enhancer of target genes. B. eRNAs interact with TFs and target them to bind to their cognate site. C. Enhancer–promoter interaction. eRNA interacts with the RAD21B and SMC3 subunits of the cohesion complex to mediate the E–P interaction via the mediator complex, promoting chromatin looping. D. Interaction with the transcriptional machinery. eRNAs remove NELF and recruit P-TEFb to release the pause of RNA Pol II, driving processive transcription and nascent mRNA transcription.

**Figure 2: Mechanism of drug resistance, eRNAs-dependent therapeutic strategy for cancer treatment.** A. The epigenetic mechanism of drug resistance. B. A schematic model of silencing eRNA production by CRISPR interference (CRISPRi), small-molecule-based targeted drugs, and inhibiting eRNA–protein interactions, thus suppressing the function of eRNA to deactivate its target oncogenes.
Figure 1

A. eRNA regulating transcription factor binding

B. eRNA facilitating enhancer-promoter interaction

C. Regulating RNA Pol II pausing
Implications of enhancer transcription and eRNAs in cancer

Santanu Adhikary, Siddhartha Roy, Jessica Chacon, et al.

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