OXFORD

Briefings in Functional Genomics, 2025, **24**, elaf007
https://doi.org/10.1093/bfgp/elaf007
Review Paper

Enhancer RNA in cancer: identification, expression, resources, relationship with immunity, drugs, and prognosis

Ruijie Zhang, Zhengxin Chen, Tianyi Li, Dehua Feng, Xinying Liu, Xuefeng Wang, Huirui Han, Lei Yu, Xia Li, Bing Li*, Limei Wang*, Jin Li*

College of Biomedical Informatics and Engineering, Kidney Disease Research Institute at the second affiliated hospital, Hainan Engineering Research Center for Health Big Data, Hainan Medical University, 3 Xueyuan Rd, Longhua District, Haikou, Hainan 571199, China

*Corresponding authors. Bing Li, Limei Wang, and Jin Li, College of Biomedical Informatics and Engineering, Kidney Disease Research Institute at the second affiliated hospital, Hainan Engineering Research Center for Health Big Data, Hainan Medical University, 3 Xueyuan Rd, Longhua District, Haikou, Hainan 571199, China. E-mail: binglijpn2003@aliyun.com; lijin@muhn.edu.cn; wanglm@muhn.edu.cn.

Abstract

Enhancer RNA (eRNA), a type of non-coding RNA transcribed from enhancer regions, serves as a class of critical regulatory elements in gene expression. In cancer biology, eRNAs exhibit profound roles in tumorigenesis, metastasis, and therapeutic response modulation. In this review, we outline eRNA identification methods utilizing enhancer region prediction, histone H3 lysine 4 monomethyl chromatin signatures, and nucleosome positioning analysis. We quantitate eRNA expression through RNA-seq, single-cell transcriptomics, and epigenomic integration approaches. Functionally, eRNAs regulate gene expression, protein function modulation, and chromatin modification. Key databases detailing eRNA annotations and interactions are highlighted. Furthermore, we analyze the connection of eRNA with immune cells and its potential in immunotherapy. Emerging evidence demonstrates eRNA's critical involvement in immune cell crosstalk and tumor microenvironment reprogramming. Notably, eRNA signatures show promise as predictive biomarkers for immunotherapy response and chemoresistance monitoring in multiple malignancies. This review underscores eRNA's transformative potential in precision oncology, advocating for integrated multiomics approaches to fully realize their clinical applicability.

Keywords: enhancer RNA; cancer; biomarkers; personalized therapy

Introduction

Enhancers are chromosome sequences located around genes, especially upstream of transcription start sites, and act as binding sites for transcription factors (TFs) that regulate gene expression [1, 2]. The transcripts that come from the transcription of enhancers are called enhancer RNA (eRNA) [3]. The eRNA-associated region represents the active region of the enhancer [4]. eRNAs can promote the binding of essential TFs to enhancers and promote the formation and stabilization of enhancer–promoter loops [5, 6].

eRNAs have been demonstrated to possess a range of functions in the context of diverse types of cancer. For example, eRNA ADCY10P1 acts as a tumor suppressor in ovarian cancer (OC) [7]. eRNA NET1e is a promising target for therapy in breast invasive carcinoma [8]. eRNA P2RY2e exerted a carcinogenic role in bladder cancer (BLCA) [9]. The expression of eRNAs can be affected by other factors. Copy number variation activates eRNA expression in lung adenocarcinoma [10] and gastric cancer (GC) [11]. eRNAs have many applications in prognosis. For example, high expression of eRNA SPRY4-AS1 was correlated with shorter overall survival in hepatocellular carcinoma (HCC) [12]. Increased expression of eRNA LINC02257 was associated with a worse prognosis in colorectal cancer [13]. eRNAs have a significant correlation with immune responses. eRNA AC005515.1 can be used as a predictor of the immune response to esophageal cancer (ESCA) [14]. The process of subtyping can be performed by combining eRNA expression, thereby facilitating more accurate immune treatment [15].

As eRNA research expands, many scientists have explored and summarized various aspects related to eRNAs. Wang *et al.* explored the roles of eRNAs in regulating cancer signaling pathways and discussed the potential of eRNA-targeted therapy for human cancers [16]. Vittorio Sartorelli *et al.* explored the important role of eRNAs in epigenome [17]. Wang *et al.*'s study indicates the enormous therapeutic potency of eRNA across human diseases [18]. Ankit Patel *et al.* focus areas and strategies for future in-depth research on eRNA in brain health and disease [19].

According to a consensus statement published in 2024 [20], researchers distinguish between eRNAs and enhancer-lncRNAs. They are both RNAs that are transcribed from enhancer regions, but they differ greatly in length, structure, function, and research methodology. eRNAs are typically shorter and serve primarily

Received: January 9, 2025. **Revised:** March 14, 2025. **Accepted:** April 7, 2025

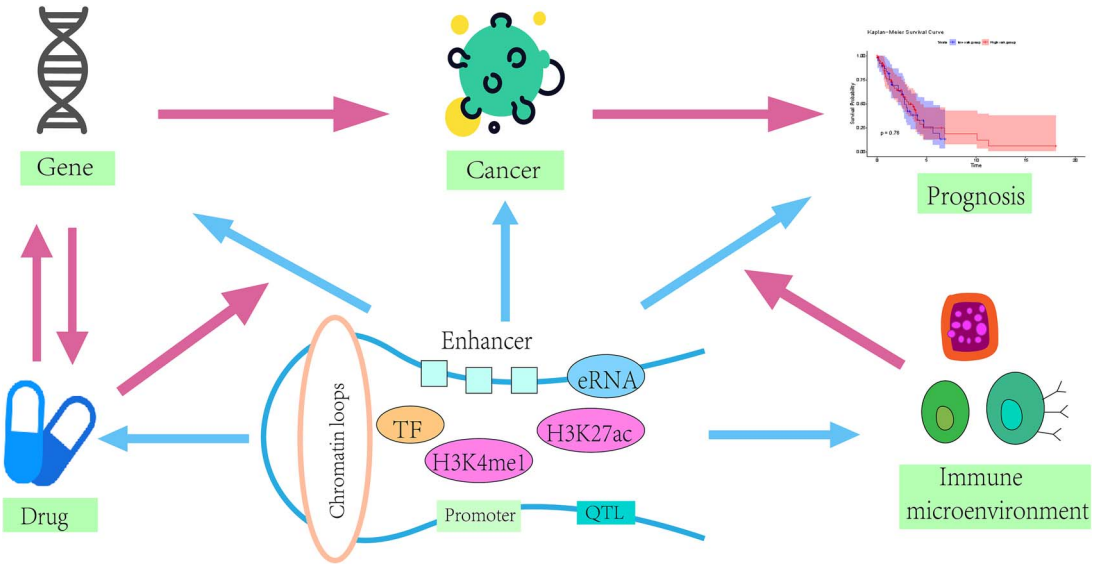

Figure 1. Schematic diagram of eRNA in cancer.

as markers of enhancer activity, while enhancer-lncRNAs are typically longer and more structurally complex, with multiple regulatory functions.

In this review, we summarize some bioinformatical concepts and applications for eRNAs. We provide a comprehensive understanding of eRNA identification, eRNA expression, eRNA regulation, database resources, drug response, immune response, and cancer prognosis (Figure 1).

eRNA identification

We provided a comprehensive overview of the methodologies employed for the identification of eRNAs. These eRNAs can be successfully identified through the utilization of specific markers, including enhancer regions, the histone H3 lysine 4 monomethyl (H3K4me1) marker, and nucleosome regions.

Enhancer region-based method

A significant proportion of researchers have identified eRNA based on the enhancer region.

The initial stage of the process is to gather data on enhancers from a number of databases. Wu *et al.* [21] retrieved enhancers from the FANTOM5 [22]. Zhang *et al.* [8] retrieved data from three databases: ENCODE [23], FANTOM5 [22], and Roadmap Epigenomics [8, 24]. Subsequently, the enhancers present in at least two databases were identified and designated as "annotated enhancers" [25]. The second step is to remove any areas that are not relevant. Wu *et al.* proceeded to remove all known gene annotation regions and introns [21]. Zhang *et al.* excluded regions that were found to overlap with known coding genes and lncRNA regions [8]. Guo *et al.* excluded any regions that were found to overlap with known coding genes [25]. The remaining region is subsequently defined as the eRNA region [8, 21, 25].

H3K4me1 signature-based method

Shino Murakami *et al.* proposed the PreSTIGE algorithm to identify eRNA regions [26]. In PreSTIGE, mRNA genes with elevated tissue-specific expression were identified, and they were likely targets of tissue-specific enhancers. Then, the H3K4me1 domains in the vicinity were identified from ChIP-seq data and were considered enhancers. The overlap between the enhancer region and lncRNA region was defined as eRNAs [26, 27].

Combining super-enhancers and nucleosomes

eRNA regions are subject to regulation by evolutionarily conserved, well-positioned nucleosomes and are frequently dysregulated in cancer. The identification of eRNA can be achieved through the utilization of nucleosome regions. Chen *et al.* identified eRNA from the super-enhancer region. First, super-enhancer regions were characterized using polymeric RNA-seq data from a large cohort. Subsequently, the nucleosome regions were identified through the use of MNase-seq. In conclusion, super-enhancer regions that overlap with nucleosome regions are defined as eRNAs [28].

Although enhancer region-based approaches exhibit operational simplicity and high reproducibility through the identification of overlapping regions while eliminating confounding effects from gene annotations, these methods inherently depend on predefined genomic annotations and demonstrate limited capacity to identify novel or tissue-specific regulatory elements. H3K4me1 signature-based strategies integrate tissue-specific transcriptional profiles with H3K4me1 chromatin marks to identify active enhancers; however, this approach may fail to capture non-H3K4me1-associated enhancer elements due to its reliance on specific histone modification signals. By contrast, the integrative framework combining super-enhancer clusters with nucleosome positioning data significantly enhances predictive reliability through cross-tissue validation guided by multiomics evidence, thereby establishing a more comprehensive landscape of enhancer activity patterns.

eRNA expression

We presented a detailed account of the methodologies employed for the quantification of eRNA expression. The estimation of eRNA expression can be achieved through the utilization of various techniques, including RNA-seq, single-cell technology, and epigenomic features.

eRNA expression on based on RNA-seq

eRNA expression quantification workflow initiated with raw RNA-seq data processing, including quality control, removal of non-target RNA contaminants, and splice-aware alignment to the human reference genome (hg38) using STAR [29]. eRNA-expressing regions were annotated, followed by read quantification and normalization to Reads Per Kilobase of transcript per Million mapped reads (RPKM)/Transcripts Per Million (TPM) metrics to mitigate technical biases arising from transcript length, sequencing depth, and library size variations, thereby estimating eRNA expression [29].

eRNA expression estimation based on single-cell technology

Random displacement amplification sequencing (RamDA-seq) is the first full-length total RNA-sequencing method for single cells [30]. In RamDA-seq studies, eRNA expression estimation at the single-cell level relies on non-poly(A) RNA capture technology and integrated multiomics analysis. This technology breaks the traditional poly(A)-dependent limitations by directly capturing short-stranded non-coding RNAs, including eRNAs, through not-so-random primers (NSRs) and Reverse transcription with random displacement amplification (RT-RamDA). In Tetsutaro Hayash *et al.*'s research, experiments were optimized for trace RNAs from single-cell lyses, genomic DNA removal, to full-length cDNA amplification, and combined with bioinformatics screening. They identified 1515 active enhancers in single mouse embryonic stem cells, of which 75% were consistent with the bulk data and 25% were single-cell-specific enhancers [30].

eRNA expression estimation based on epigenomic features

Epigenomic features can be used to estimate eRNA expression. Luo *et al.* employed DNase hypersensitive sites as indicators of chromatin status [31]. They observed that enhancers with the capacity to produce eRNAs are typically situated within open chromatin regions. The integration of DNase microarray peak data facilitated the implementation of a more rigorous methodology for the identification of candidate regions. Following the delineation of candidate regions, eRNAs were identified through the utilization of Random Forest and XGBoost algorithms, with DNA methylation levels [32] and H3K27ac levels serving as the defining features [31].

Zhu *et al.* developed a logistic regression model to predict eRNA transcriptional status [33]. In the model, chromatin modifications were employed as predictors, with Global nuclear Run-On sequencing (GRO-seq) levels serving as the response variables. The results demonstrated that the combination of four modifications (H3K27ac + H3K27me3 + H3K79me1 + H3K9ac) yielded the most optimal eRNA expression prediction [31, 33].

Kim *et al.* [34] detected in ~25% of enhancers (3000/12,000) by integrating H3K4me1/CBP ChIP-seq technology (~12,000 neuronal activity-dependent enhancers identified) in combination with total RNA sequencing (without polyA screening) and strand-specific library building (sequencing depth > 50 million reads) RNAPII-mediated bidirectional transcriptional signaling, systematically revealing the presence of non-polyadenylated eRNAs in over 2000 intergenic enhancer regions (average length < 2 kb, two-fold upregulation of expression upon stimulation) [34].

RNA-seq-based methodologies enable systematic analysis through quantification of enhancer-associated transcripts using normalized metrics (e.g. RPKM/TPM), yet these approaches require stringent experimental parameters, including deep sequencing depth and strand-specific library preparation protocols. Notably, such techniques exhibit limited sensitivity for non-polyadenylated eRNAs due to their transcriptional characteristics. Single-cell approaches, while enabling high-resolution transcriptomic profiling, present technical challenges including complex experimental workflows and inherent limitations in detecting low-abundance eRNAs (TPM < 1). Epistasis-based feature identification strategies offer cost-effective solutions but demonstrate critical dependencies on the quality of epigenetic interaction data and necessitate experimental validation.

eRNA regulation

In cancer, eRNA can regulate various elements, such as genes, proteins, and other molecular factors.

eRNA-gene regulation

eRNA binding to cohesion and mediator complexes stabilizes chromatin loop architecture through physical interactions, enhancing target gene transcriptional activation [35]. For example, antisense eRNAs within the procalcitonin gene cluster orchestrate long-range chromatin interactions *via* R-loop-mediated chromatin looping, thereby modulating neurodevelopmental gene expression programs essential for neuronal differentiation [36]. eRNAs can serve as molecular scaffolds to directly recruit TFs (e.g. androgen receptor) and RNA polymerase II (RNAPII) to the enhancer region, promoting transcription initiation and elongation [37, 38]. Estrogen receptor (ER)-dependent enhancer engagement in breast cancer coordinates eRNA biogenesis through R-loop formation, thereby activating adjacent oncogenic gene expression programs *via* chromatin remodeling and transcriptional co-activation [39, 40].

eRNA–protein regulation

eRNA binding to the CBP HAT domain allosterically activates histone acetylation, enhancing downstream gene transcriptional output through chromatin relaxation [41]. eRNA interacts with specific proteins to perform regulatory functions. In the context of acute myeloid leukemia (AML), S100-eRNA has been observed to typically promote an inhibitory inflammatory microenvironment [42].

eRNA-QTL regulation

Zhang *et al.* performed eRNA-quantitative trait loci (QTL) discovery by concatenating eRNA transcriptomic data, whole-genome single nucleotide polymorphism (SNP) genotypes, and clinical/environmental covariates into Matrix eQTL's linear regression architecture [43]. SNPs with a false discovery rate < 0.05 were defined as significant eRNA-QTL. Cis-eRNA-QTLs (acting close together) directly regulate local enhancer activity; trans-eRNA-QTLs (acting far apart) affect distal genes through cross-chromosomal interactions. Chen *et al.* identified 626 eRNAs associated with susceptibility to multiple cancers through eRNA-based transcriptome-wide association studies [44]. Following this, experiments validated the effects of two newly identified susceptibility eRNAs (CCND1e and SNAPC1e) on prostate cancer (PCa) cell proliferation, demonstrating the important role of eRNA-QTL in cancer risk [44].

Regulation between eRNA and chromatin structure and modification

eRNA promotes the modification of histones H3K27ac and H3K4me1 by binding to acetyltransferases such as CBP/p300, thereby increasing chromatin openness and enhancer activity [36]. Inhibition of eRNA reduces H3K4me3 levels in the promoter region of target genes and inhibits transcription [36, 38]. m6A-modified eRNAs regulate chromatin high-level structure by recruiting the YTHDC1 protein, promoting the formation of transcriptional condensate in the nucleus, and enhancing the enrichment efficiency of transcriptional activators such as BRD4 [38]. Knockdown of eRNA leads to enhancer–promoter loop dissociation and reduces the efficiency of gene expression; e.g. eRNA deletion in the KLK3 locus significantly inhibits PCa cell proliferation [36, 37].

eRNA resources

We summarized databases that provide information on eRNAs (Table 1), categorized by function and research content, as follows.

eRNA and cancers

eRic database

The eRic Database is a database of eRNA expression data based on The Cancer Genome Atlas (TCGA) samples [8].

In the eRNA expression profiles module, gene expression profiles for 30 cancer types can be searched. In the eRNA clinical characterization module, eRNA data and clinical relevance indicators can be searched. In the eRNA target gene module, eRNA-related target genes can be searched. In the eRNA drug response module, information on drugs and eRNAs based on the GDSC and CTRPv2 databases can be searched.

TceA database

The TceA database is developed to leverage aggregated RNA-seq data from TCGA and Genotype-Tissue Expression (GTEx) for the exploration of eRNA expression within super-enhancers [28].

In the eRNA site discovery module, users can explore eRNA sites from the tissue-specific super-strong enhancer regions defined. In the sample module, the database includes, 9300 TCGA tumor samples and 700 paired normal samples. In the comprehensive analysis module, there are statistical associations between eRNAs and CpGs, eRNA–gene pairs, non-coding amplified foci of somatic copy number alterations (SCNAs), deletion amplified foci of SCNAs, TF occupancy, eQTLs, and somatic mutation.

eRNA expression databases

HeRA database

HeRA is a database storing eRNAs related to those in normal human tissues [45].

In the eRNA expression module, eRNA expression profiles of 52 human normal tissues can be retrieved. In the trait-related module, the database collects the differences in eRNA expression based on gender, race, age group, height, weight, and BMI. In the regulatory factor-related module, it collects eRNA expression in different human tissues associated with different TFs. In the target gene-related module, it collects eRNA-related target genes and their locations in different human tissues, as well as the correlation between them.

Animal-eRNAdb database

Animal-eRNAdb is an animal eRNA database constructed from the RNA-seq data of 5085 samples from 10 species [46].

In the eRNA expression module, 185,177 eRNAs can be retrieved. In the trait-related module, 100,723 trait-related eRNAs can be retrieved. In the regulator-related module, 157,500 eRNAs related to 12,232 TFs can be retrieved. In the eRNA-related target gene module, 135,501 eRNAs related to 151,673 target genes can be retrieved. In the sequence similarity module, users can browse eRNAs with sequence similarities in multiple species.

eRNAbase database

The eRNAbase database records a large number of available resources for human and mouse eRNAs and provides comprehensive annotation and analysis of eRNAs [47].

In the Browse module, the samples include genome proportion, peak distribution, chromosome distribution, and eRNA-related information. In the online analysis module, there are three submodules: eRNA-mediated pathway regulation analysis, eRNA-based variant interpretation analysis, and eRNA-mediated TF target gene analysis. In the Genome Browser module, human and mouse eRNA regulatory information is visualized with customizable tracks of interest.

eRNA QTLs

The eaQTLdb database

eaQTLdb is a database to explore enhancer activity QTL (eaQTL), i.e. SNPs, that affect eRNA expression based on multiomics data from TCGA [48].

In the Expression Landscape of eaQTL module, basic information on eRNAs and their neighboring genes, targets, and SNPs can be found. In the signature eaQTL correlation module, basic information about the eRNAs and their neighboring genes, targets, and SNPs is provided. In the survival eaQTL module, the basic information on survival-related eRNAs and their neighboring genes, targets, and SNPs can be retrieved. In the immune eaQTL module, the basic information on eRNAs and their neighboring genes, targets, SNPs, and immune cell types is available. In the module for eaQTL associated with disease/trait GWAS signal co-localization, information on eRNAs and their neighboring genes, predicted and captured targets, basic information on SNPs, trait/disease type, and PP4 *a posteriori* probability can be obtained.

CancereRNAQTL database

The CancereRNAQTL database systematically explores the effects of SNPs on eRNA expression in tumor tissues based on data from TCGA [49].

In the eRNAQTL module, basic information on cancer types, eRNAs and their neighboring genes, SNPs and the expression of eRNAs in different genotypes is available. In the survival-eRNAQTL module, basic information about cancer types, eRNAs and their neighboring genes, SNPs, and survival time in different genotypes can be obtained. In the GWAS-eRNAQTL module, the basic information of cancer types, eRNAs and their neighboring genes, SNPs, and the corresponding disease phenotypes can be obtained.

Drug response and immune infiltration

GPIeR is a multidimensional data portal based on TCGA large-scale genomics data for exploring genetic variation, drug response, and immune infiltration of eRNAs [43].

Table 1. Overview of eRNA-Related Databases

Database	Functional description	Module
eRic	The database under consideration is one of eRNA expression data, with a foundation in TCGA samples	(1) Expression landscape (2) Clinical relevance (3) Target genes (4) Drug response
TCeA	The database explores the expression of eRNA within super-enhancers, utilising RNA-seq data from the TCGA and GTEx databases	(1) Annotation and samples (2) eRNA quantification (3) Integrated analysis
HeRA	The database investigates the process of storing eRNA databases associated with normal human tissues	(1) Expression (2) Traits (3) Regulators (4) Target genes
Animal-eRNAdb	The database is composed of 5085 samples from 10 different species mouse samples	(1) Expression (2) Trait (3) Regulator (4) Target genes (5) Sequence
eRNAbase	The database documents a substantial resource of human and mouse eRNAs, accompanied by comprehensive annotation and analysis	(1) Browse (2) Search (3) Analysis (4) Genome browser
eaQTLdb	The database explores a database of eaQTL affecting eRNA expression	(1) eaQTL (2) Hallmark-eaQTL (3) Survival-eaQTL (4) Immune-eaQTL (5) GWAS-eaQTL
CancereRNAQTL	The database describes a systematic exploration of SNP databases that influence eRNA expression in tumor tissues	(1) eRNAQTL (2) Survival-eRNAQTL (3) GWAS-eRNAQTL
GPIeR	The database proposes a multidimensional data portal, based on large-scale TCGA genomic data, for the exploration of genetic variation, drug response, and immunity of eRNAs	(1) Cis eRNA-QTL (2) Trans eRNA-QTL (3) Survival eRNA-QTL (4) Drug response (5) Immune infiltration
eRNA-IDO	The database is a comprehensive resource for the identification of human eRNA, the exploration of its interactions, and the acquisition of its functional annotations	(1) eRNA-ID (2) eRNA-Anno
M2ED2	The database has been compiled to provide a comprehensive description of the eRNA landscape during the early stages of mouse embryonic development	(1) Expression (2) Regulators (3) Targets

In the cis module, the basic information of eRNAs, SNPs, and the correlation between eRNAs can be found. In the trans module, the basic information of eRNAs, SNPs, and the correlation between eRNAs can be found. In the Survival eRNA-QTL module, the basic information on eRNAs, SNPs, and the KM curve can be obtained. In the drug response module, the basic information on drugs, eRNAs, and the correlation between eRNAs and drug responses can be obtained. In the immune infiltration module, the basic information on immune cells, eRNAs, and the correlation between eRNA expression and the abundance of immune cells can be obtained.

Integrated databases

eRNA-IDO database

The eRNA-IDO database is a one-stop platform for human eRNA identification, interactome discovery, and functional annotation [50].

In the eRNA-ID module, users can input transcripts assembled from scratch and filter them according to chromatin coordinates and coding sites to obtain eRNAs. In the eRNA-Anno module, eRNA functions can be predicted by discovering eRNA-connected protein-coding genes in the eRNA-centric regulatory network.

M2ED2 database

The M2ED2 database is a database describing the eRNA landscape during early mouse embryonic development. The database has three modules: eRNA expression module, eRNA regulation module, and eRNA target gene module [51].

In the eRNA expression module, the eRNA landscapes of multiple developmental stages of the early mouse embryo can be queried, and in the eRNA regulation module, the eRNA TFs of multiple developmental stages of the early mouse embryo can be queried. In the eRNA target module, target genes regulated

by eRNAs at multiple developmental stages in the early mouse embryo can be queried.

Some databases (e.g. TCGA and GTEx) integrate RNA-seq data from different platforms, batches, and experimental conditions; this heterogeneity may lead to bias in the quantification of eRNA expression. Some databases rely on computational predictions (e.g. eRNA–target gene co-expression) and lack experimental support from CRISPR or RNA interference studies [44]. It is suggested that future databases could include the missing data mentioned above. It is recommended that a gold standard for establishing eRNA functional annotation should be developed through systematic large-scale knockdown of different eRNAs combined with transcriptome sequencing or phenotypic analyses (e.g. cell proliferation and apoptosis), which would help screen out key eRNAs that promote tumorigenesis or possess therapeutic potential.

eRNA and immunity

eRNAs are inextricably linked to immune cells, making them promising targets for immunotherapy, particularly when they interact with immune checkpoints. Moreover, the expression levels of eRNAs offer a valuable means of classifying patients into distinct immune subtypes, thereby facilitating the development of more targeted and efficacious immunotherapy strategies.

Immune microenvironment

Specific eRNAs are strongly linked to the expression and infiltration of immune cells, indicating their pivotal role in modulating the immune response. Moreover, the expression profile of eRNAs in cancer patients is found to be correlated with that of the tumor immune microenvironment. Consequently, eRNAs may become a key factor in improving immunotherapeutic strategies. A negative correlation between ZFHX4-AS1 expression and B cell [52] expression, as well as a positive correlation with T cell CD8+ [53], neutrophil, macrophage, and myeloid dendritic cell expression. The aforementioned results indicate that ZFHX4-AS1 may exert a significant influence on the tumor immune microenvironment [54]. A notable positive correlation was observed between AC003092.1 and immunosuppressive cells, including MDSCs, macrophages, and regulatory T cells. Conversely, a negative correlation was evident between AC003092.1 and NK cells [55] and neutrophils [56]. Twenty-eight immune cells and pathways were found to be significantly associated with differentially expressed eRNAs in patients with spinal cord injury (SCI) [57]. The expression of the eRNA LINC02257 was found to be significantly correlated with the infiltration of various immune cells and different T cells [13].eRNAs associated with the prognosis of AML were focused on immune-related pathways and suggested that AML leukocytes shaped the pattern of the immune microenvironment through eRNAs and related genes and that eRNAs promoted tumor growth by enhancing the suppressive immune microenvironment in the AML environment to promote tumor growth [42]. The eRNA AC003092.1 was found to be significantly enriched in immune-associated GO terms [58]. Patients were subsequently stratified into two subgroups, designated AC003092.1_H and AC003092.1_L, based on the expression of AC003092.1. The results of the single-sample gene-set enrichment analysis [59] indicated that AC003092.1_H was enriched for immune cell types, functions, and pathways. Furthermore, it exhibited a higher presence of immune and stromal cells in comparison with AC003092.1_L [60].

Immunotherapy

Immunotherapy, as an important tool in cancer treatment [61], is influenced by a variety of factors. eRNAs interact with immune checkpoints [62] and become potential targets for immunotherapy [8, 11]. A substantial body of evidence from extensive research has demonstrated a correlation between the expression levels of specific eRNAs and the response rates observed in immuno-therapy treatments [63]. Furthermore, eRNA-based risk scores and immunity scores predict the efficacy of immunotherapy, thus providing a valuable tool for clinical decision-making [43]. The objective remission rate of immunotherapy was significantly predicted by three eRNAs across 25 distinct cancer types [64]. Patients with low eRNA-based risk scores exhibited superior outcomes with immune checkpoint inhibitor (ICI) treatment [65]. Patients in the high eRNA-derived risk-scoring group exhibited enhanced outcomes with immunotherapy in GC [11]. A two-subtype classification was devised for colorectal cancer patients based on immunoscore, comprising C1 (high-risk score) and C2 (low-risk score). The C1 subtype was found to demonstrate a robust immune response. The eRNA locus [28] and associated genes that are implicated in the immunotherapeutic response thus offer a novel direction for immunotherapy research. Patients with high expression of eRNA AC003092.1 demonstrated enhanced efficacy in response to ICIs [60].

Classification of immune subtypes

Patients could be classified into distinct immune subtypes based on eRNA expression levels. These subtypes were found to be closely associated with treatment response. Jiang *et al.* distinguished AML into two clinically significant immune subtypes based on the different expression of immune-related eRNAs [42]. Hu *et al.* conducted consensus cluster analysis [66] based on 349 TCGA GC samples, thereby obtaining eRNA clusters A and B [11]. According to the stromal score, GC patients in the eRNA cluster B were clustered into high- and low-stromal score groups. In accordance with the state of immune infiltration and matrix score. Patients in cluster A were classified as belonging to the "medium-immune score subtype" (Immune_M). In cluster B, the high-score group was designated as the "medium immunity score subtype" (Immune_M), whereas the low-score group was classified as the "low immunity score subtype" (Immune_L). The abundance of tumor-infiltrating immune cells in these three subtypes was then evaluated. There were significant differences in epithelial–mesenchymal transition, Tumor Mutation Burden, and mRNA expression-based stemness index scores among the three subtypes [11]. Bu *et al.* performed non-negative matrix factorization analysis [67] based on eRNA expression to classify HCC patients into three subtypes: clusters 1, 2, and 3 (C1, C2, and C3, respectively). The pathway enrichment results demonstrated notable discrepancies in the extent of immune cell infiltration between these subtypes. In particular, genes within cluster C1 were found to be enriched in pathways associated with metastasis, immune infiltration, and tumorigenesis. This suggests that cluster C1 is linked to immune infiltration [68].

eRNA and drugs

eRNAs have the potential to serve as therapeutic targets, allowing for the stratification of patients based on their eRNA expression profiles. This stratification enables the tailored application of specific drugs to distinct patient groups, facilitating a more precise and personalized approach to drug therapy.

Table 2. Prognosis Biomarkers for cancers

Types of cancer	Biomarkers
OSCC	IRS2e [76]
ESCA	AC007255.1 [77]
OC	ZFHX4-AS1 [54]
	FOXP4-AS1 [77]
UVM	LINC00689 [78]、ELFN1-AS1 [78]
CCRCC	AC003092.1 [56]
GBM	CYP1B1-AS1 [79]
UCEC	IGFBP7-AS1 [80]
PCa	GAS1RR [81]、MAPK15、ZNF467、and MC1R [85]
Ga	ADCYAP1R1、BMP2、BMPR1A、CD4、DDX17、ELN、FGF13、MAPT、PDIA2、PSMB8、PTPN6、SEMA6C 、and SSTR5 [82]
CM	AC009495.2、LINC02446、LINC00189、RSRP1、CUTALP、CMAHP、and MOSMO [83]
OS	CD8A、CDK6、FAAH、and SAMD4A [84]
	[85]
THCA	FAAHP1、TP73-AS1、WDFY3-AS2
	LINC01184、AL365259.1、TMEM184A、
	AC007255.1、IQANK1、and AC084375.1 [65]

Targeted drugs

Several eRNAs (or eRNA targets) could be targeted by drugs. Huang *et al.* identified 19 potential compounds targeting SCI-associated eRNAs in CMap. TSA and 15-delta prostaglandin J2 were considered to be the best compounds for SCI [57]. In HCC, patients can be classified into three immune subtypes based on eRNA expression. In the immune-rich subtype, patients are more likely to benefit from sorafenib [69] and cabozantinib [68, 70].

Personalized drugs

There is a significant positive correlation between eRNA-based risk scores and sensitivity to anticancer drugs [71]. Yang *et al.* identified two distinct expression groups based on eRNA expression levels and subsequently evaluated drug response sensitivity [72]. Cisplatin exhibited heightened sensitivity in cohorts with elevated CDK6-AS1 expression levels relative to those with lower expression levels. Conversely, paclitaxel demonstrated enhanced sensitivity in the cohort with lower expression levels [72].

Hu *et al.* categorized GC into three subgroups: Immune_H, Immune_M, and Immune_L based on eRNA expression. They applied a predictive model for the analysis of chemotherapy responses [11]. Patients in the Immune_L group exhibited lower estimated IC50s for acitretin, bicalutamide, cisplatin, and dasatinib, suggesting that these agents were most effective in this group, enabling precise treatment for these patients [11]. Immunological drugs associated with eRNAs have advantages in patient treatment. Expression of eRNA ID2AS1 is positively correlated with response to ICI therapy in BLCA patients [73, 74]. Patients with high expression of AC003092.1 are more likely to benefit from ICIs [60].

Prognosis biomarkers

eRNAs display both tissue-specific and individual-specific expression patterns [75] and have a potential prognostic value in various types of cancers.

Single eRNA biomarker

The expression of eRNAs was found to be closely associated with cancer prognosis. There were notable correlations between the expression levels of particular eRNAs and cancer prognosis, including survival.

A multitude of eRNA biomarkers have been identified across a range of cancer types (Table 2), allowing for the stratification of patients into high-risk and low-risk groups based on eRNA expression levels. Subsequent prognostic analyses have been conducted for these risk groups, including eRNA IRS2 in oral squamous cell carcinoma (OSCC) [76], ESCA [86], OC [54, 77], uveal melanoma (UVM) [78], clear cell renal cell carcinoma (CCRCC) [56], glioblastoma (GBM) [79], uterine corpus endometrial carcinoma (UCEC) [80], and PCa [81].

Multiple eRNA biomarkers

Studies have found that not only can a single eRNA biomarker be used as a prognostic indicator, but risk scores derived from multiple eRNA biomarkers can also be used to predict patient prognosis. The researchers used Lasso–Cox regression model and developed prognostic risk scores based on the expression of these eRNAs to categorize patients into high- and low-risk groups [65, 83, 87].

eRNA-related prognostic models have been used in a variety of cancers, including glioma (Ga) [82], CCRCC [56], head and neck squamous cell carcinoma [88], cutaneous melanoma (CM) [83], osteosarcoma (OS) [84], PCa [85], and thyroid cancer (THCA) [65]. The AUC for these markers ranges from 0.651 to 0.93.

These biomarkers play important roles in biological processes, such as regulatory actions and associated molecular pathways. Xiong *et al.* revealed the role of eRNA GAS1RR as an independent prognostic marker in PCa [81]. By analyzing the TCGA and UCSC Xena databases, six eRNA–target gene pairs associated with recurrence-free survival in BCR were screened, of which GAS1RR was highly positively correlated with the tumor suppressor gene GAS1 ($r = 0.86$). Low expression of GAS1RR was significantly associated with high Gleason score, advanced pathological stage, high PSA value, and increased risk of BCR ($P < .05$). Multivariate Cox regression showed that GAS1RR was an independent prognostic factor for PCa (HR = 0.188, $P = .028$). Functional enrichment analysis revealed that GAS1RR was involved in tumor progression by regulating cell adhesion, calcium signaling, and MAPK pathways. Immune infiltration analysis revealed a positive correlation with immune cell infiltration, such as B cells and CD4+/CD8+ T cells

($P < 1e\text{-}19$). RT-qPCR verified the low expression and strong correlation of GAS1RR and GAS1 in 18 pairs of PCa tissues ($r = 0.85$ for tumor tissues). The study suggests that GAS1RR may inhibit PCa progression by regulating GAS1 and the tumor microenvironment, providing a new direction for prognostic assessment and targeted therapy [81].

Tian *et al.* screened 27 immune target genes highly associated with eRNAs, and functional enrichment analysis of the 27 target genes yielded 8 KEGG pathways and several GO terms [82]. KEGG pathways are involved in the regulation of immune function, neural function, and cellular signaling. They include PD-L1 expression and the PD-1 checkpoint pathway in cancer, neuroactive ligand–receptor interaction, and the Janus kinase–Signal transducer and activator of transcription (JAK–STAT) signaling pathway. The GO terminology suggests that IRG is rich in various signaling pathways and "axonogenesis". Filtering by Lasso–Cox analysis returned 13 IRGs that were significantly associated with survival outcomes. They were used to construct a comprehensive prognostic tool for Ga's. The distribution of risk scores points to low- and high-risk groups describing all Ga patients. High-risk patients exhibited heavier immune and stromal cell infiltration but fewer tumor cells in the TME [82].

Discussion

In the preceding decade, eRNA has made significant contributions to the field of cancer research. It is evident that eRNAs fulfill a multifaceted role within the context of cancer biology. First, evidence has demonstrated that eRNAs promote the release of negative elongation factors from promoters [89]. A mechanism that contributes to the expression of cancer-associated target genes and thus may drive tumor development. In addition, eRNAs have been demonstrated to play a pivotal role in the gene expression regulatory network by influencing the formation of chromatin loops and the activity of TFs [3]. Furthermore, the interaction of eRNAs with the immune–tumor environment has been demonstrated to impact their function [90], which may involve immune responses and inflammatory processes in the tumor microenvironment, with significant implications for cancer therapy [91].

The absence of a uniform methodology for the identification of eRNA necessitates the standardization of subsequent research in this field, with the aim of enhancing the accuracy of identification. The expression of eRNAs can be determined through the use of RNA-seq, single-cell technology, and epigenomic features. The application of deep learning can facilitate the identification of tissue-specific eRNAs [92]. eRNAs play a central role in cancer initiation and progression by driving oncogene expression and remodeling the tumor microenvironment through mechanisms such as regulating chromatin structure, recruiting TFs, mediating histone modifications, and interacting with eRNA-QTLs. A substantial number of eRNA-related resources are equipped with sophisticated data storage and analysis capabilities, and it would be beneficial for researchers to utilize these web resources. The present study was conducted with the objective of elucidating the relationship between eRNAs and immunity. To this end, an in-depth examination of eRNAs from the perspective of the immuno-oncological microenvironment, immunotherapy, and subtype classification was undertaken.

eRNA is intimately associated with drug sensitivity, which serves as a crucial point of reference for the development of personalized medication. Emerging preclinical studies suggest that transcription inhibitors can modulate eRNA for therapy. BET inhibitors, including JQ1, exert inhibitory effects on oncogenes such as MYC through disruption of super-enhancers that harbor eRNA activity [93, 94]. CDK9 inhibitors indirectly suppress eRNA synthesis by blocking RNAPII-mediated transcription elongation [95, 96]. However, currently, no therapeutic agents targeting eRNA have been clinically approved, and to our knowledge, none are in late-stage clinical trials. This gap highlights the infancy of eRNA research in drug development.

eRNAs are of significant value as prognostic markers in the context of cancer. However, the majority of current research employs Lasso–Cox regression analysis to identify prognostically relevant eRNAs, while machine learning methods can also be applied for this purpose.

At present, most eRNA research remains at the level of high-throughput sequencing [97]. Furthermore, the application of single-cell technology [98] to eRNA research represents a significant avenue for future investigation. This will facilitate a deeper comprehension of eRNA regulation of cancer gene expression and eRNA influence on cancer development.

Key Points

- We provided the most comprehensive summary of eRNA-related databases to date, an invaluable resource for understanding eRNA expression, drug response, immune response, and prognostic indicators in cancer.
- We highlighted the strong link between eRNAs and immune cells, positioning eRNAs as potential targets for immunotherapy and as crucial elements in the development of immunotherapeutic strategies.
- We highlighted the potential of eRNAs as therapeutic targets and their ability to stratify patients for personalized drug therapy based on eRNA expression profiles.
- We advocate for future research directions, including the application of deep learning and single-cell technologies. Ultimately, this review aims to inform the development of personalized cancer therapies by elucidating eRNAs' potential as diagnostic markers, therapeutic targets, and prognostic indicators.

Conflict of interest: The authors declare no conflict of interest.

Funding

This work was supported by the Natural Science Foundation of Hainan Province [Nos. 824RC514, 821QN0894, 621MS041] and National Natural Science Foundation of China [No. 32260155].

References

1. Bulger M, Groudine M. Enhancers: the abundance and function of regulatory sequences beyond promoters. *Dev Biol* 2010;**339**:250–7. https://doi.org/10.1016/j.ydbio.2009.11.035.
2. Wang L, Wei C, Wang Y., *et al.* Identification of the enhancer RNAs related to tumorgenesis of pituitary neuroendocrine tumors. *Front Endocrinol (Lausanne)* 2023;**14**:1149997. https://doi.org/10.3389/fendo.2023.1149997.
3. Chen Q, Zeng Y, Kang J., *et al.* Enhancer RNAs in transcriptional regulation: recent insights. *Front Cell Dev Biol* 2023;**11**:1205540. https://doi.org/10.3389/fcell.2023.1205540.
4. Ahmed I, Yang SH, Ogden S., *et al.* eRNA profiling uncovers the enhancer landscape of oesophageal adenocarcinoma and reveals new deregulated pathways. *Elife* 2023;**12**:e80840. https://doi.org/10.7554/eLife.80840.

5. Lai F, Orom UA, Cesaroni M., *et al.* Activating RNAs associate with mediator to enhance chromatin architecture and transcription. *Nature* 2013;**494**:497–501. https://doi.org/10.1038/nature11884.
6. Plank JL, Dean A. Enhancer function: mechanistic and genome-wide insights come together. *Mol Cell* 2014;**55**:5–14. https://doi.org/10.1016/j.molcel.2014.06.015.
7. Mo J, Zhang L, Li H., *et al.* The enhancer RNA ADCY10P1 is associated with the progression of ovarian cancer. *J Ovarian Res* 2022;**15**:61. https://doi.org/10.1186/s13048-022-00987-1.
8. Zhang Z, Lee JH, Ruan H., *et al.* Transcriptional landscape and clinical utility of enhancer RNAs for eRNA-targeted therapy in cancer. *Nat Commun* 2019;**10**:4562. https://doi.org/10.1038/s41467-019-12543-5.
9. Ding M, Zhan H, Liao X., *et al.* Enhancer RNA - P2RY2e induced by estrogen promotes malignant behaviors of bladder cancer. *Int J Biol Sci* 2018;**14**:1268–76. https://doi.org/10.7150/ijbs.27151.
10. Qin N, Ma Z, Wang C., *et al.* Comprehensive characterization of functional eRNAs in lung adenocarcinoma reveals novel regulators and a prognosis-related molecular subtype. *Theranostics* 2020;**10**:11264–77. https://doi.org/10.7150/thno.47039.
11. Hu X, Wu L, Yao Y., *et al.* The integrated landscape of eRNA in gastric cancer reveals distinct immune subtypes with prognostic and therapeutic relevance. *iScience* 2022;**25**:105075. https://doi.org/10.1016/j.isci.2022.105075.
12. Ye M, Wang S, Qie JB., *et al.* SPRY4-AS1, a novel enhancer RNA, is a potential novel prognostic biomarker and therapeutic target for hepatocellular carcinoma. *Front Oncol* 2021;**11**:765484. https://doi.org/10.3389/fonc.2021.765484.
13. Xiao J, Liu Y, Yi J., *et al.* LINC02257, an enhancer RNA of prognostic value in colon adenocarcinoma, correlates with multi-omics immunotherapy-related analysis in 33 cancers. *Front Mol Biosci* 2021;**8**:646786. https://doi.org/10.3389/fmolb.2021.646786.
14. Jia L, Chen J, Zhao J., *et al.* Identification of enhancer RNA AC005515.1 as a novel biomarker for prognosis in esophageal cancer and predictors of immunotherapy response. *Transl Cancer Res* 2023;**12**:3266–83. https://doi.org/10.21037/tcr-23-777.
15. Bu X, Liu S, Zhang Z., *et al.* Comprehensive profiling of enhancer RNA in stage II/III colorectal cancer defines two prognostic subtypes with implications for immunotherapy. *Clin Transl Oncol* 2024;**26**:891–904. https://doi.org/10.1007/s12094-023-03319-x.
16. Wang R, Tang Q. Current advances on the important roles of enhancer RNAs in molecular pathways of cancer. *Int J Mol Sci* 2021;**22**:5640. https://doi.org/10.3390/ijms22115640.
17. Sartorelli V, Lauberth SM. Enhancer RNAs are an important regulatory layer of the epigenome. *Nat Struct Mol Biol* 2020;**27**:521–8. https://doi.org/10.1038/s41594-020-0446-0.
18. Wang Y, Zhang C, Wang Y., *et al.* Enhancer RNA (eRNA) in human diseases. *Int J Mol Sci* 2022;**23**:11582. https://doi.org/10.3390/ijms231911582.
19. Patel A, Dharap A. An emerging role for enhancer RNAs in brain disorders. *Neuromolecular Med* 2024;**26**:7. https://doi.org/10.1007/s12017-024-08776-3.
20. Mattick JS, Amaral PP, Carninci P., *et al.* Long non-coding RNAs: definitions, functions, challenges and recommendations. *Nat Rev Mol Cell Biol* 2023;**24**:430–47. https://doi.org/10.1038/s41580-022-00566-8.
21. Wu Y, Yang Y, Gu H., *et al.* Multi-omics analysis reveals the functional transcription and potential translation of enhancers. *Int J Cancer* 2020;**147**:2210–24. https://doi.org/10.1002/ijc.33132.
22. Andersson R, Gebhard C, Miguel-Escalada I., *et al.* An atlas of active enhancers across human cell types and tissues. *Nature* 2014;**507**:455–61. https://doi.org/10.1038/nature12787.
23. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012;**489**:57–74. https://doi.org/10.1038/nature11247.
24. Bernstein BE, Stamatoyannopoulos JA, Costello JF., *et al.* The NIH roadmap Epigenomics mapping consortium. *Nat Biotechnol* 2010;**28**:1045–8. https://doi.org/10.1038/nbt1010-1045.
25. Guo G, Wang H, Tong X., *et al.* Transcriptional landscape of enhancer RNAs in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. *J Inflamm Res* 2022;**15**:775–91. https://doi.org/10.2147/JIR.S331188.
26. Murakami S, Gadad SS, Kraus WL. A PreSTIGEous use of LncRNAs to predict enhancers. *Cell Cycle* 2015;**14**:1619–20. https://doi.org/10.1080/15384101.2015.1032650.
27. Vučićević D, Corradin O, Ntini E., *et al.* Long ncRNA expression associates with tissue-specific enhancers. *Cell Cycle* 2015;**14**:253–60. https://doi.org/10.4161/15384101.2014.977641.
28. Chen H, Liang H. A high-resolution map of human enhancer RNA loci characterizes super-enhancer activities in cancer. *Cancer Cell* 2020;**38**:701–715.e5. https://doi.org/10.1016/j.ccell.2020.08.020.
29. Withanage MHH, Liang H, Zeng E. RNA-Seq experiment and data analysis. *Methods Mol Biol* 2022;**2418**:405–24. https://doi.org/10.1007/978-1-0716-1920-9_22.
30. Hayashi T, Ozaki H, Sasagawa Y., *et al.* Single-cell full-length total RNA sequencing uncovers dynamics of recursive splicing and enhancer RNAs. *Nat Commun* 2018;**9**:619. https://doi.org/10.1038/s41467-018-02866-0.
31. Luo X, Li Q, Tang Y., *et al.* Predicting active enhancers with DNA methylation and histone modification. *BMC Bioinformatics* 2023;**24**:414. https://doi.org/10.1186/s12859-023-05547-y.
32. von Känel T, Huber AR. DNA methylation analysis. *Swiss Med Wkly* 2013;**143**:w13799. https://doi.org/10.4414/smw.2013.13799.
33. Zhu Y, Sun L, Chen Z., *et al.* Predicting enhancer transcription and activity from chromatin modifications. *Nucleic Acids Res* 2013;**41**:10032–43. https://doi.org/10.1093/nar/gkt826.
34. Kim TK, Hemberg M, Gray JM., *et al.* Widespread transcription at neuronal activity-regulated enhancers. *Nature* 2010;**465**:182–7. https://doi.org/10.1038/nature09033.
35. Ne II, Heward JA, Roux B., *et al.* Long non-coding RNAs and enhancer RNAs regulate the lipopolysaccharide-induced inflammatory response in human monocytes. *Nat Commun* 2014;**5**:3979.
36. Zhou Y, Xu S, Zhang M., *et al.* Systematic functional characterization of antisense eRNA of protocadherin α composite enhancer. *Genes Dev* 2021;**35**:1383–94. https://doi.org/10.1101/gad.348621.121.
37. Bose DA, Donahue G, Reinberg D., *et al.* RNA binding to CBP stimulates histone acetylation and transcription. *Cell* 2017;**168**:135–149.e22. https://doi.org/10.1016/j.cell.2016.12.020.
38. Lee JH, Wang R, Xiong F., *et al.* Enhancer RNA m6A methylation facilitates transcriptional condensate formation and gene activation. *Mol Cell* 2021;**81**:3368–3385.e9. https://doi.org/10.1016/j.molcel.2021.07.024.
39. Hsieh CL, Fei T, Chen Y., *et al.* Enhancer RNAs participate in androgen receptor-driven looping that selectively enhances gene activation. *Proc Natl Acad Sci U S A* 2014;**111**:7319–24. https://doi.org/10.1073/pnas.1324151111.
40. Schaukowitch K, Joo JY, Liu X., *et al.* Enhancer RNA facilitates NELF release from immediate early genes. *Mol Cell* 2014;**56**:29–42. https://doi.org/10.1016/j.molcel.2014.08.023.
41. Bose DA, Berger SL. eRNA binding produces tailored CBP activity profiles to regulate gene expression. *RNA Biol* 2017;**14**:1655–9. https://doi.org/10.1080/15476286.2017.1353862.

42. Jiang Z, Long J, Deng K., *et al.* eRNAs identify immune microenvironment patterns and provide a novel prognostic tool in acute myeloid Leukemia. *Front Mol Biosci* 2022;**9**:877117. https://doi.org/10.3389/fmolb.2022.877117.
43. Zhang Z, Luo M, Li Q., *et al.* Genetic, Pharmacogenomic, and immune landscapes of enhancer RNAs across human cancers. *Cancer Res* 2022;**82**:785–90. https://doi.org/10.1158/0008-5472.CAN-21-2058.
44. Chen W, Wang Z, Wang Y., *et al.* Enhancer RNA transcriptome-wide association study reveals a distinctive class of Pan-cancer susceptibility eRNAs. *Adv Sci (Weinh)* 2025;**12**:e2411974.
45. Zhang Z, Hong W, Ruan H., *et al.* HeRA: an atlas of enhancer RNAs across human tissues. *Nucleic Acids Res* 2021;**49**:D932–d938. https://doi.org/10.1093/nar/gkaa940.
46. Jin W, Jiang G, Yang Y., *et al.* Animal-eRNAdb: a comprehensive animal enhancer RNA database. *Nucleic Acids Res* 2022;**50**:D46–d53. https://doi.org/10.1093/nar/gkab832.
47. Song C, Zhang G, Mu X., *et al.* eRNAbase: a comprehensive database for decoding the regulatory eRNAs in human and mouse. *Nucleic Acids Res* 2024;**52**:D81–d91. https://doi.org/10.1093/nar/gkad925.
48. Yuan J, Tong Y, Liu X., *et al.* eaQTLdb: an atlas of enhancer activity quantitative trait loci across cancer types. *Int J Cancer* 2023;**153**:111–9. https://doi.org/10.1002/ijc.34481.
49. Cai YM, Lu ZQ, Li B., *et al.* Genome-wide enhancer RNA profiling adds molecular links between genetic variation and human cancers. *Mil Med Res* 2024;**11**:36. https://doi.org/10.1186/s40779-024-00539-2.
50. Zhang Y, Gong L, Ding R., *et al.* eRNA-IDO: a one-stop platform for identification, Interactome discovery, and functional annotation of enhancer RNAs. *Genomics Proteomics Bioinformatics* 2024;**22**:qzae059. https://doi.org/10.1093/gpbjnl/qzae059.
51. Yu H, Zhao J, Shen Y., *et al.* The dynamic landscape of enhancer-derived RNA during mouse early embryo development. *Cell Rep* 2024;**43**:114077. https://doi.org/10.1016/j.celrep.2024.114077.
52. Eibel H, Kraus H, Sic H., *et al.* B cell biology: an overview. *Curr Allergy Asthma Rep* 2014;**14**:434. https://doi.org/10.1007/s11882-014-0434-8.
53. van der Leun AM, Thommen DS, Schumacher TN. CD8(+) T cell states in human cancer: insights from single-cell analysis. *Nat Rev Cancer* 2020;**20**:218–32. https://doi.org/10.1038/s41568-019-0235-4.
54. Huang C, Cui H, Lang X., *et al.* An integrative analysis revealing ZFHX4-AS1 as a novel prognostic biomarker correlated with immune infiltrates in ovarian cancer. *J Immunol Res* 2022;**2022**:9912732.
55. Portale F, Di Mitri D. NK cells in cancer: mechanisms of dysfunction and therapeutic potential. *Int J Mol Sci* 2023;**24**:9521. https://doi.org/10.3390/ijms24119521.
56. Lv Y, Niu L, Li Q., *et al.* Identification of an immune-related eRNA prognostic signature for clear cell renal cell carcinoma. *Aging (Albany NY)* 2024;**16**:2232–48. https://doi.org/10.18632/aging.205479.
57. Huang R, Wang S, Zhu R., *et al.* Identification of key eRNAs for spinal cord injury by integrated multinomial bioinformatics analysis. *Front Cell Dev Biol* 2021;**9**:728242. https://doi.org/10.3389/fcell.2021.728242.
58. Ashburner M, Ball CA, Blake JA., *et al.* Gene ontology: tool for the unification of biology. The gene ontology consortium. *Nat Genet* 2000;**25**:25–9. https://doi.org/10.1038/75556.
59. Bindea G, Mlecnik B, Tosolini M., *et al.* Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity* 2013;**39**:782–95. https://doi.org/10.1016/j.immuni.2013.10.003.
60. Guo XY, Zhong S, Wang ZN., *et al.* Immunogenomic profiling demonstrate AC003092.1 as an immune-related eRNA in glioblastoma multiforme. *Front Genet* 2021;**12**:633812. https://doi.org/10.3389/fgene.2021.633812.
61. Kaur R, Bhardwaj A, Gupta S. Cancer treatment therapies: traditional to modern approaches to combat cancers. *Mol Biol Rep* 2023;**50**:9663–76. https://doi.org/10.1007/s11033-023-08809-3.
62. Wu M, Shen J. From super-enhancer non-coding RNA to immune checkpoint: frameworks to functions. *Front Oncol* 2019;**9**:1307. https://doi.org/10.3389/fonc.2019.01307.
63. Wu X, Zhao X, Zhou C., *et al.* Prognostic and onco-immunological value of immune-related eRNAs-driven genes in lung adenocarcinoma. *J Cancer Res Clin Oncol* 2024;**150**:188. https://doi.org/10.1007/s00432-024-05687-5.
64. Guo M, Lu Z, Xiong Y. Enhancer RNA-based modeling of adverse events and objective responses of cancer immunotherapy reveals associated key enhancers and target genes. *Front Oncol* 2022;**12**:1048127.
65. Wu P, Shi J, Wang Z., *et al.* Evaluate the immune-related eRNA models and signature score to predict the response to immunotherapy in thyroid carcinoma. *Cancer Cell Int* 2022;**22**:307. https://doi.org/10.1186/s12935-022-02722-8.
66. Wilkerson MD, Hayes DN. ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking. *Bioinformatics* 2010;**26**:1572–3. https://doi.org/10.1093/bioinformatics/btq170.
67. Nasser R, Eldar YC, Sharan R. Deep unfolding for non-negative matrix factorization with application to mutational signature analysis. *J Comput Biol* 2022;**29**:45–55. https://doi.org/10.1089/cmb.2021.0438.
68. Bu X, Liu S, Wen D., *et al.* Comprehensive characterization of enhancer RNA in hepatocellular carcinoma reveals three immune subtypes with implications for immunotherapy. *Mol Ther Oncolytics* 2022;**26**:226–44. https://doi.org/10.1016/j.omto.2022.07.001.
69. Abdelgalil AA, Alkahtani HM, Al-Jenoobi FI. Sorafenib. *Profiles Drug Subst Excip Relat Methodol* 2019;**44**:239–66. https://doi.org/10.1016/bs.podrm.2018.11.003.
70. Neuwelt AJ, Mathur S, Johnson AT., *et al.* Cabozantinib use in renal cell carcinoma. *Drugs Today (Barc)* 2017;**53**:299–307. https://doi.org/10.1358/dot.2017.53.5.2623107.
71. Wang L, Zhou S-Q, Zhou Y., *et al.* A two-eRNA-based signature can impact the immune status and predict the prognosis and drug sensitivity of lung adenocarcinoma. *J Immunol Res* 2022;**2022**:8069858.
72. Yang S, Zou X, Yang H., *et al.* Identification of enhancer RNA CDK6-AS1 as a potential novel prognostic biomarker in gastric cancer. *Front Genet* 2022;**13**:854211. https://doi.org/10.3389/fgene.2022.854211.
73. Zhang L, Ding D, Liu J., *et al.* Identification of prognostic and immunotherapy-related eRNA ID2-AS1 in bladder cancer. *Medicine (Baltimore)* 2022;**101**:e29759. https://doi.org/10.1097/MD.0000000000029759.
74. Naimi A, Mohammed RN, Raji A., *et al.* Tumor immunotherapies by immune checkpoint inhibitors (ICIs); the pros and cons. *Cell Commun Signal* 2022;**20**:44. https://doi.org/10.1186/s12964-022-00854-y.

75. Panahi-Moghadam S, Hassani S, Farivar S., *et al.* Emerging role of enhancer RNAs as potential diagnostic and prognostic biomarkers in cancer. *Non-coding RNA* 2022;**8**:66. https://doi.org/10.3390/ncrna8050066.
76. Yan L, Wang J, Guo J., *et al.* Enhancer RNA IRS2e is essential for IRS2 expression and the oncogenic properties in oral squamous cell carcinoma. *Carcinogenesis* 2023;**44**:119–28. https://doi.org/10.1093/carcin/bgad010.
77. Hua T, Tian YJ, Wang RM., *et al.* FOXP4-AS1 is a favorable prognostic-related enhancer RNA in ovarian cancer. *Biosci Rep* 2021;**41**:BSR20204008. https://doi.org/10.1042/BSR20204008.
78. Zhao S, Jiang H, Liu J., *et al.* Comprehensive analysis of enhancer RNAs identifies LINC00689 and ELFN1-AS1 as novel prognostic biomarkers in uveal melanoma. *Dis Markers* 2022;**2022**:5994800.
79. Ye T, Li L-L, Peng X-M., *et al.* CYP1B1-AS1 is a novel biomarker in glioblastoma by comprehensive analysis. *Dis Markers* 2021;**2021**:8565943.
80. Liu J, Yin J, Wang Y., *et al.* A comprehensive prognostic and immune analysis of enhancer RNA identifies IGFBP7-AS1 as a novel prognostic biomarker in uterine corpus endometrial carcinoma. *Biol Proced Online* 2022;**24**:9. https://doi.org/10.1186/s12575-022-00172-0.
81. Xiong Z, Ge Y, Xiao J., *et al.* GAS1RR, an immune-related enhancer RNA, is related to biochemical recurrence-free survival in prostate cancer. *Exp Biol Med (Maywood)* 2023;**248**:1–13. https://doi.org/10.1177/15353702221131888.
82. Tian W, Chen K, Yan G., *et al.* A novel prognostic tool for glioma based on enhancer RNA-regulated immune genes. *Front Cell Dev Biol* 2021;**9**:798445.
83. Gan Y, Yang Y, Wu Y., *et al.* Comprehensive transcriptomic analysis of immune-related eRNAs associated with prognosis and immune microenvironment in melanoma. *Front Surg* 2022;**9**:917061. https://doi.org/10.3389/fsurg.2022.917061.
84. Yan P, Li Z, Xian S., *et al.* Construction of the prognostic enhancer RNA regulatory network in osteosarcoma. *Transl Oncol* 2022;**25**:101499. https://doi.org/10.1016/j.tranon.2022.101499.
85. Fan S, Wang Z, Zhao L., *et al.* A robust prognostic gene signature based on eRNAs-driven genes in prostate cancer. *Front Genet* 2021;**12**:676845. https://doi.org/10.3389/fgene.2021.676845.
86. Wang Q, Yu X, Yang N., *et al.* LncRNA AC007255.1, an immune-related prognostic enhancer RNA in esophageal cancer. *Peer J* 2021;**9**:e11698. https://doi.org/10.7717/peerj.11698.
87. Sahu D, Lin CC, Goel A. Transcriptomic profiling reveals an enhancer RNA signature for recurrence prediction in colorectal cancer. *Genes (Basel)* 2023;**14**:137. https://doi.org/10.3390/genes14010137.
88. Diao P, Huang R, Shi Y., *et al.* Development of a novel prognostic signature derived from enhancer RNA-regulated genes in head neck squamous cell carcinoma. *Head Neck* 2023;**45**:900–12. https://doi.org/10.1002/hed.27316.
89. Tan H, Liu T, Zhou T. Exploring the role of eRNA in regulating gene expression. *Math Biosci Eng* 2022;**19**:2095–119. https://doi.org/10.3934/mbe.2022098.
90. Wan L, Li W, Meng Y., *et al.* Inflammatory immune-associated eRNA: mechanisms, functions and therapeutic prospects. *Front Immunol* 2022;**13**:849451. https://doi.org/10.3389/fimmu.2022.849451.
91. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;**144**:646–74. https://doi.org/10.1016/j.cell.2011.02.013.
92. Zhang T, Li L, Sun H., *et al.* Deep ITEH: a deep learning framework for identifying tissue-specific eRNAs from the human genome. *Bioinformatics* 2023;**39**:btad375. https://doi.org/10.1093/bioinformatics/btad375.
93. Lovén J, Hoke HA, Lin CY., *et al.* Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell* 2013;**153**:320–34. https://doi.org/10.1016/j.cell.2013.03.036.
94. Whyte WA, Orlando DA, Hnisz D., *et al.* Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell* 2013;**153**:307–19. https://doi.org/10.1016/j.cell.2013.03.035.
95. Jonkers I, Lis JT. Getting up to speed with transcription elongation by RNA polymerase II. *Nat Rev Mol Cell Biol* 2015;**16**:167–77. https://doi.org/10.1038/nrm3953.
96. Kwiatkowski N, Zhang T, Rahl PB., *et al.* Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. *Nature* 2014;**511**:616–20. https://doi.org/10.1038/nature13393.
97. Lee JY. The principles and applications of high-throughput sequencing technologies. *Dev Reprod* 2023;**27**:9–24. https://doi.org/10.12717/DR.2023.27.1.9.
98. Jovic D, Liang X, Zeng H., *et al.* Single-cell RNA sequencing technologies and applications: a brief overview. *Clin Transl Med* 2022;**12**:e694. https://doi.org/10.1002/ctm2.694.

Briefings in Functional Genomics, 2025, **24**, elaf007
https://doi.org/10.1093/bfgp/elaf007
Review Paper