增强子RNA的功能特性及其在人类疾病中的重要调控作用

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摘要 增强子是基因组上一段可以被转录调控蛋白识别并结合的区域, 作为顺式调控元件和启动子共同参与基因转录过程。增强子在激活状态下, 打开局部染色质并暴露DNA基序以吸引转录因子, 从而进一步招募RNA聚合酶产生一类非编码RNA, 即增强子RNA(enhancer RNA, eRNA)。eRNA可促进增强子与启动子特异性染色质远程互作参与基因转录调节, 或与转录因子等调控蛋白结合促进基因转录, 具有多样的功能和调控机制, 从而在细胞的发育和分化、疾病发生发展等众多生物过程中起重要作用。该文就eRNA特性、功能、鉴定、数据库资源, 以及eRNA在人类神经系统疾病、癌症、免疫代谢类疾病、心血管疾病等中的功能作用研究进展作一系统综述, 探讨eRNA的未来研究方向, 及在疾病中作为潜在治疗靶点的可能及目前存在的挑战。

关键词 增强子RNA(eRNA); 数据库; 调控机制; 疾病

Functional Properties of Enhancer RNA and Its Important Regulatory Roles in Human Diseases

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Abstract Enhancer is a region of the genome that can recognize and bind to transcriptional regulatory proteins, and is involved in the gene transcription process as a *cis*-regulatory element together with promoter. Enhancers, in their activated state, open local chromatin and expose DNA mores to attract transcription factors, thereby further recruiting RNA polymerase to produce a class of non-coding RNA—eRNA (enhancer RNA). eRNA can promote the remote interaction between enhancer and promoter-specific chromatin to participate in gene transcription regulation, or combine with regulatory proteins such as transcription factors to promote gene transcription. eRNA has a variety of functions and regulatory mechanisms, and thus plays important roles in many biological processes such as cell development and differentiation and disease development. In this paper, the characteristics, function, identification and database resources of eRNA, as well as the functional role of eRNA in human neurological diseases, cancers, immune metabolism diseases and cardiovascular diseases are systematically reviewed, and the future research direction of eRNA, as well as the possibility as potential therapeutic targets in diseases and the existing challenges are discussed.

Keywords eRNA (enhancer RNA); database; regulatory mechanism; disease

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转录作为基因表达调控的关键步骤, 调控过程涉及许多顺式调控DNA元件和反式作用蛋白。顺式调节元件包括基因近端的启动子以及其他远端调节元件(如增强子和沉默元件等)。其中, 增强子是与辅因子和转录因子(transcription factor, TF)结合的DNA元件, 位于基因组非编码区域, 具有特征性的染色质结构, 并以三维染色质重塑、增强子–启动子环等多种方式参与驱动靶基因的转录[1]。

增强子在激活状态下, 打开局部染色质并暴露DNA基序以吸引TF, 从而进一步招募RNA聚合酶(RNA Pol II), 使增强子转录并表达非编码RNA, 这类RNA被称为增强子RNA(ehancer RNA, eRNA)[2]。随着生物学及测序技术的发展, 研究发现eRNA在人类细胞和组织内大量存在, 且具有多种生物学功能, 以各种机制调控近距离和远距离靶基因的表达[3]。例如eRNA通过吸收黏连蛋白并随后调节基因表达来稳定增强子–启动子(enhancer-promoter, E-P)环结构[4]; 通过连接辅因子CP300/p300和RNAP II直接诱导E-P环形成[5]; 通过与TF[例如阴阳蛋白1(Yin Yang 1, YY1)]结合正向调节增强子转录并稳定基因表达等[6]。eRNA在人类不同组织和细胞发育中具有不同的表达模式, 与多种细胞类型中响应各种刺激的基因的表达调节有关[7-8]。在癌症中, eRNA调节肿瘤细胞发育和分化相关的基因, 是癌症潜在的治疗靶点[9]。此外, 关于癌症的全基因组关联分析(genome wide association study, GWAS)也发现增强子和超级增强子往往是突变的热点区域[10], 数量性状位点(quantitative trait locus, QTL)分析提示增强子表达可解释精神分裂症(6.8±1.5)%的遗传力[11], 进一步说明eRNA在人类疾病复杂调控机制中发挥重要作用。

然而, 目前关于eRNA的研究并不多, 大部分eRNA的功能和作用仍然不明确, 因此, eRNA的表达和功能研究是一个重要而迫切的问题。本文就eRNA的结构功能特点、数据资源及其在疾病尤其是神经系统和癌症中的功能作用的最新研究进展作一综述, 并讨论eRNA作为潜在生物标志物和疾病治疗靶点的可能性以及未来研究的一些方向。

1 eRNA的特性和功能

1.1 eRNA由活性增强子转录

目前, DNA元件百科全书(the ENCyclopedia of DNA Elements, ENCODE)[12], 哺乳动物基因组联盟(the ENCyclopedia of DNA Elements, FANTOM)[9]和表观组学路线图计划(Roadmap Epigenomics Mapping Consortium)[13]项目已经在不同的细胞和组织中检测到数以万计的增强子。早在2010年, KIM等[14]研究发现增强子可以双向转录出一类新的非编码RNA——eRNA。与其他lncRNA或mRNA相比, eRNA具有几个独特的特征: eRNA由高度组蛋白甲基化修饰的增强子区域双向转录产生, 因此具有高水平的H3K4me1和H3K4me2修饰[14-16]; 与mRNA相比, 大多数eRNA较短(约为500 bp)且非多聚腺苷酸化、不稳定、多存在于细胞核中[17-18]; 少数由活性增强子特异性转录产生的eRNA则较长(近5 Kb), 且发生多聚腺苷酸化。非多聚腺苷酸化的短eRNA主要发挥顺式调控作用, 而存在于细胞核中的更稳定的聚腺苷酸化eRNA则可在反式调控中发挥作用。

1.2 eRNA的调节机制

eRNA作为调节性非编码RNA, 调控机制很多, 其中被广泛认可的一种作用机制是建立和/或稳定E-P染色质环。研究发现, eRNA可通过招募黏连蛋白提高E-P环的动态稳定来促进mRNA转录[19-21]。同样, 染色质相互作用研究证明, 与蛋白质编码基因启动子成环的增强子具有更高的eRNA表达, 可见eRNA对E-P环的形成至关重要。此外, eRNA还可形成局部R环促进增强子与启动子之间的特异性染色质远程互作, 从而改变三维基因组中的染色质高级结构, 实现对靶基因的调节[22]。例如小鼠模型中HS5-1增强子区域产生的eRNA PEARL(Pcdh eRNA associated with R-loop formation)通过形成局部RNA-DNA双链体(R环), 来调节靶基因原钙黏蛋白(protocadherin, Pcdh)的表达, 以促进远端增强子和靶启动子之间的长距离染色质相互作用[22]。

eRNA的另一个重要调节机制与功能活性增强子染色质修饰状态相关, 并且eRNA参与转录修饰过程[23]。p300/CBP作为转录共激活剂, 参与细胞周期进展以及细胞的生长、分化和发展, 是一类非常重要的转录共激活因子, 与多种肿瘤疾病密切相关。在活性增强子处, CBP被募集, eRNA可通过结合CBP的组蛋白乙酰化转移酶(histone acetyltransferase, HAT)结构域促进CBP的乙酰化, 从而促进靶基因转录[24]。在表观遗传上, eRNA相比于pre-mRNA或某些mRNA具有更高的m6A水平[25]; 这些携带m6A修饰的eRNA可通过招募

YTHDC1(YTH N^6-methyladenosine RNA binding protein C1)蛋白，在增强子附近形成转录凝聚体，从而正向调节增强子和靶基因的转录激活，而eRNA和其他染色质相关RNA上m6A的缺失也会导致开放染色质的积累[25]。

此外，eRNA还可与转录调节因子相互作用以控制基因的表达，如eRNA可与转录因子(如YY1和BRD4)相互作用，增加转录因子在增强子上的富集程度，从而调节靶基因转录，发挥生物学作用[6,26-27]。综上，目前研究发现eRNA主要是通过促进E-P环或局部R环形成等调控机制间接干预转录过程；也可以通过改变染色质修饰状态、与转录因子相互作用等调节靶基因。除此之外，eRNA还可通过与NELF或P-TEFb相互作用致使RNA聚合酶II(RNAP II)暂停释放从而使转录过程进入生产性延伸阶段，直接干预转录过程[28]。然而，由于eRNA在不同状态下的表达和结构等异质性，其功能机制仍需进一步探索。

1.3 eRNA的鉴定

由于大多数eRNA 3′端无polyA，容易被核酸内切酶识别降解，只能存在于细胞核内，因此eRNA无法在稳态RNA测序方法中被广泛捕获[17-18]；此外，由于eRNA丰度比基因转录本低，往往需要更高覆盖率的测序技术来准确捕获eRNA，因此，传统的依赖稳态RNA的测序技术如RNA-seq能够检测的eRNA有限，量化生物样本中的增强子活性以及对eRNA的鉴定仍然具有挑战性。

随着高通量测序技术的发展，目前一些对于新生RNA更加敏感的测序技术逐渐发展并用于eRNA的鉴定。如全球核连续测序(global nuclear run-on sequencing, GRO-seq)[29]、精确核连续测序(precision nuclear run-on sequencing, PRO-seq)[30]及其衍生物5′GRO-seq和PRO-cap，以及瞬时转录组测序(transient transcriptome sequencing, TT-seq)[31]、加帽端测序(cap analysis gene expression, CAGE)[32,35]、5′末端测序(Exo-seq)[28]等，这些测序方法在检测新生的低丰度RNA上具有高灵敏度。如RAHNAMOUN等[29]利用GRO-seq在SW480细胞中鉴定出增强子区域的双向转录产物eRNA；KRISTJÁNSDÓTTIR等[30]通过PRO-seq量化淋巴母细胞系(lymphoblastoid cell line, LCL)增强子转录起始的变化，检测到数十万个eRNA转录位点；SCHWALB等[31]利用TT-seq检测人K562细胞系中增强子的瞬时表达情况。基于测序技术方法所获得的eRNA可以采用实验方法(如3′-RACE、5′-RACE、RNA原位杂交、qRT-PCR或Northern blot等)进行验证。此外，siRNA、shRNA和CRISPR-Cas13a等实验方法可以进一步用于研究eRNA在疾病转录调控中的潜在功能作用[3,33,36]。

在单细胞水平上，2017年SHIBAYAMA等[38]提出一种通过原位杂交后进行酪胺信号放大(tyramide signal amplification, TSA)的单分子荧光原位杂交(single molecule fluorescence *in situ* hybridization, smFISH)成像方案，可在单细胞水平上检测短而低表达的转录本，并且可以同时在转录延伸位点可视化eRNA和蛋白质编码转录本。在测序方法上，HAYASHI等[37]开发了一种单细胞全长总RNA测序方法，随机置换扩增测序(random displacement amplification sequencing, RamDA-seq)，这种测序方法对非polyA RNA具有较高的敏感性。HAYASHI等[37]对小鼠胚胎干细胞分化样本进行RamDA-seq后检测到单细胞水平eRNA及其细胞特异性活性，该方法有助于识别稀有细胞中的eRNA和研究eRNA在单细胞中的时间和空间表达模式，且可帮助理解eRNA的调控机制。

2 eRNA数据库资源

尽管eRNA的表达量低，且多为不带polyA，然而目前常规RNA-seq数据仍然是研究eRNA丰富且方便的信息资源。例如，HAN等[39-40]利用转录组RNA-seq数据进行增强子比对和注释，将鉴定到的增强子中心上下游3 Kb定义为eRNA，并进行进一步的eRNA表达定量、生物信息学分析和功能实验验证。此外，有多项研究通过整合现有TCGA、GTEX大规模人类癌症和正常组织RNA-seq数据集来表征人类基因组eRNA表达情况，HeRA[40]和eRic[39]数据库通过集成分析多个数据集(如ENCODE、FANTOM eRNA注释数据，以及GTEx、TCGA的RNA-seq数据)，提供了正常人体组织和肿瘤组织中eRNA的表达图谱和调控网络。HeRA数据库提供了GTEx中54种人类正常组织中eRNA表达谱、性状相关eRNA、eRNA调节因子和eRNA靶基因信息。eRic数据库主要整合了大规模癌症患者样本和癌细胞系的多组学和药物基因组学数据。TCeA数据库[41]利用TCGA和GTEx中的RNA-seq数据探索了超级增强子中的eRNA表达模式，提供了超级增强子中超30万

表1 eRNA数据库信息

Table 1 eRNA database information

数据库 Database	数据来源 Source	模块 Module	URL	参考文献 References
HeRA	GTEx (normal tissue)	(1) eRNAs expression profile; (2) trait-related eRNAs; (3) regulators of eRNAs; (4) eRNAs target genes	https://hanlab.uth.edu/HeRA/	[40]
eRic	TCGA (tumor tissue)	(1) eRNAs expression profile; (2) clinical relevance eRNAs; (3) eRNAs target genes; (4) drug-related eRNAs	https://hanlab.uth.edu/eRic/	[39]
TCeA	TCGA/GTEx /CCLE (normal/tumor)	(1) super enhancer region; (2) eRNAs expression profile; (3) super-enhancer eRNA loci and genes associated with responses to immunotherapy; (4) clinical outcome, CNV, and CpG methylation related eRNA; (5) 3D eRNA-locus/promoter interactions	https://bioinformatics.mdanderson.org/public-software/tcea	[41]
GPIeR	TCGA (tumor tissue)	(1) *cis*-eRNA-QTLs; (2) *trans*-eRNA-QTLs; (3) eRNA-QTLs associated with patient overall survival; (4) eRNA and drug response; (5) eRNA and different type of immune cell abundance	https://hanlaboratory.com/GPIeR/	[42]
Animal-eRNAdb	RNA-seq from 10 species	(1) eRNA expression; (2) trait-related eRNA; (3) eRNA regulator; (4) eRNA target gene; (5) sequence similarity	http://gong_lab.hzau.edu.cn/Animal-eRNAdb/	[43]
eRNAbase	Human/mouse	(1) genetic annotation; (2) eRNA-mediated pathway; (3) eRNA-based genetic variation; (4) eRNA-mediated TF-target gene	http://bio.liclab.net/eRNAbase/index.php	[44]

个精确eRNA位点的高分辨率图谱。GPIeR数据库[42]使用来自TCGA的大规模组学数据分析了遗传变异对RNA表达和疾病的影响, 鉴定了约100万个eRNA数量性状位点(eRNA-QTL)。GPIeR数据库可用于探索eRNA相关的遗传变异、药物反应和免疫浸润, 促进eRNA在癌症中的功能和临床研究。Animal-eRNAdb数据库[43]提供了10个物种上千样本中eRNA表达及物种间eRNA序列相似性等信息。eRNAbase数据库[44]从GEO/SRA和ENCODE数据库中收集了14种eRNA相关实验类型数据, 对人类和小鼠eRNA区域进行了详细的表观遗传注释, 提供了eRNA介导的通路调控分析、基于eRNA的变异解释分析和eRNA介导的TF-靶基因分析等信息。eRNA相关数据库的主要信息如表1所示。

3 eRNA在人类疾病中的调控作用及临床应用

3.1 神经系统疾病相关eRNA

eRNA最早在神经元细胞中被发现, 可作用于即刻早期基因(immediate early genes, IEGs)(如*c-fos*[45]和*Arc*[14])。研究表明, eRNA在大脑中存在时间空间以及细胞特异性, 可协助神经元传递遗传信号, 在调节大脑神经元可塑性和记忆形成中也发挥着重要作用[9,14]。人类神经系统疾病具有11%到24%的高度遗传率[46], 并且早在2015年表观遗传学研究发现大脑特异性eRNA富集大量与发育障碍相关的遗传变异位点[2]。

近些年来, 分子生物学、动物模型以及表观遗传学研究逐步发现临床中eRNA位点变异与神经系

统疾病相关，且通过不同功能机制参与调节疾病相关易感基因转录表达。表2列举了目前已知与神经系统疾病尤其是神经退行性疾病等相关的eRNA。例如，α-突触核蛋白(alpha-synuclein, *SNCA*)基因作为公认的帕金森病易感基因，GWAS研究显示帕金森显著相关遗传变异多富集在*SNCA*表达的非编码远端增强子元件上[47]；eRNA AANCR通过暂停RNA聚合酶II延伸使转录终止，改变*APOE*表达并影响阿尔茨海默病(Alzheimer's disease, AD)的易感性[48]；R6/11纹状体中超级增强子的H3K27ac活性降低能够影响亨廷顿舞蹈症(Huntington's disease, HD)患者神经元中eRNA的产生，导致相关神经元基因的转录失调[50]。此外，研究也发现了部分与大脑发育及精神障碍类疾病相关的eRNA。例如，eRNA PEARL可参与HS5-1增强子区域R环形成，从而调节靶基因原钙黏连蛋白(protocadherin α, *Pcdhα*)的表达，在大脑发育中发挥重要作用[22]；实验证据表明敲降大鼠脑中杏仁核中央核Kdm6b或Arc eRNA表达均可引起大鼠焦虑[49]；另外，eRNA-QTL分析也发现基因变异可通过影响eRNA表达变化从而介导神经疾病或脑部相关性状发生发展[11]。这些研究虽然揭示了eRNA在不同神经系统疾病中的可能机制，但其在疾病诊断或预后治疗中的临床效用在很大程度上仍然未知。

3.2 癌症相关eRNA

在人类癌症中，致癌基因或致癌信号通路的激活可表现为增强子活化和eRNA产生[39]。 eRNA不仅通过调节肿瘤抑制基因表达作为肿瘤抑制因子起作用，也可作为致癌基因的主要调节因子，表现出潜在的致癌功能[39,55]，表2列举了目前研究已知的与人类癌症相关的eRNA。致癌基因诱导的eRNA可以直接促进肿瘤发生，例如，MYC-eRNA的激活可促进一系列疾病如白血病、乳腺癌、前列腺癌、直肠癌和食管癌等的发展[56]；TP53-eRNA可参与乳腺癌、前列腺癌等癌细胞系中p53依赖性细胞周期停滞通路[57]；β-雌二醇(E2)相关eRNA可以激活乳腺癌中E2依赖性基因的表达[21]；eRNA NET1e激活可促进乳腺癌细胞的生长[39]；乳腺癌潜在药物靶点eRNA WAKMAR2通过调节免疫相关基因调节肿瘤微环境[33]；KLK3-eRNA可以控制前列腺癌中雄激素受体相关基因的表达，阻断雄激素信号通路，可能靶向雄激素反应基因或作为预后生物标志物[58]；P2RY2e[59]在膀胱肿瘤组织细胞中表达上调，可促进细胞增殖、侵袭和迁移，降低雌激素对膀胱癌细胞的促癌能力[60]；HPSE-eRNA通过驱动染色质循环和调节hnRNPU/p300/EGR1/HPSE轴来促进癌症的进展[61]；eNEMAL上调可促进*NEAT1*基因长转录本表达响应乳腺癌细胞缺氧状态等[62]。

除上述这些癌症特异性表达的eRNA外，HAN等[39]利用癌细胞系百科全书(Cancer Cell Line Encyclopedia, CCLE)数据还发现癌细胞系中的eRNA表达水平与抗癌细胞药物敏感性之间存在较强关联。这些数据共同揭示了eRNA在肿瘤发生中的重要作用，为eRNA靶向治疗的临床实用性提供了更多的证据。

3.3 免疫代谢性疾病相关eRNA

除发病率高及常见的神经系统疾病和癌症外，eRNA在某些免疫相关炎症或者免疫代谢类疾病中的基因转录调节中也起着重要作用。同样早在2015年研究发现哺乳动物基因组功能注释数据库(function annotation of the mammalian genome, FANTOM)样本中增强子区域单核苷酸多态性 (single nucleotide polymorphism, SNP)可解释免疫类疾病15%的遗传力[71]。随着对人类转录组的全面分析，也逐渐发现单个及群体性的功能性eRNA在这些疾病中的作用机制。表2列举了目前研究已知的与人类免疫系统疾病相关的eRNA。例如，在小鼠模型中发现*Kdm6a*通过增加IFN-β特异性eRNA S-IRE1的转录而促进IFN-β的表达，从而参与免疫反应[58,72]；同样有研究在小鼠模型上发现生物钟核心基因脑和肌肉芳香烃受体核转运样蛋白1(brain and muscle arnt-like 1, *Bmal1*)可以通过调节巨噬细胞中eRNA转录以及增强子的表观遗传状态来控制响应炎症激活的基因表达时间[73]等。

除炎症伴随着的免疫系统疾病外，研究也发现了部分免疫代谢类疾病相关的eRNA。例如BENHAMMOU等[74]在肥胖手术患者肝脏测序数据及小鼠实验模型中发现具备eRNA功能的lncRNA-OLMALINC，可通过区域染色体DNA-DNA环相互作用促进肝脏中硬脂酰辅酶A去饱和酶表达来调节肝脏脂质代谢；G蛋白通路抑制因子2(G protein pathway suppressor 2, GPS2)和核受体辅阻遏物2(nuclear receptor corepressor 2, NCOR2/SMRT)的抗炎辅抑制因子复合物(GPS2复合物)在基因转录和代谢过程中具有重要调控作用，在肥胖和2型糖尿病患者的脂肪组织巨噬细胞和血液

表2 人类神经系统疾病、癌症、免疫代谢类疾病和心血管疾病相关eRNA及其功能

Table 2 eRNAs and their functions in human neurological diseases, cancers, immune metabolism diseases and cardiovascular diseases

疾病类型 Disease type	疾病名称 Disease name	物种 Species	eRNA	靶基因/功能 Targer gene/function	参考文献 References
Neurological diseases	Anxiety	AIEMouse	Arc eRNA	Arc	[49]
	Neuron	Mouse, ESC cell	utNgn1	*Ngn1*/ESC differentiation	[51]
		Mouse/HEC-1-B cell	PEARL	Pcdhα/R-loop/act as a neuron	[22]
	Schizophrenia	Patients, HEK145 cell	enh3256	*GOLPH3L*	[52]
	Alzheimer's disease	GTEX	AANCR	*APOE*/R-loop	[48]
		Mouse	Bdnf-Enhg1, Bdnf-Enhg2	*Bdnf*	[53]
	Parkinson's disease	Human brain	KANSL1 eRNA	*KANSL1*	[54]
Cancers	Breast cancer	Patients	WAKMAR2	*IL27RA*, *RAC2*, *FABP7*, *IGLV1-51*, *IGHA1*, *IGHD*	[33]
		MCF7 cell	NET1e	*NET1*/cell proliferation	[39]
		MCF7 cell	eNEMAL	*MALAT1*	[62]
		MCF7 cell	PGRe, KCNK5e, TFF1e, FOXC1e, GREB1e, CA12e, P2RY2e, NRIPe,	Activated E2-dependent gene	[21]
		MCF-53/MCF7 cell	P21e, PRKAG2e, SUFUe, TOB1e/TP53-eRNA	p53-dependent gene	[55,64]
	Head and neck cancer	Patients	AP001056.1	*ICOSLG*	[64]
	Lung cancer	Patients	TBX5-AS1 eRNA	PI3K/AKT pathway	
	Carcinoma of urinary bladder	Patients	P2RY2e	Promote cell proliferation	[59-60]
		Patients	SMAD7e	SMAD7e knockdown leads to apoptosis	[66]
	Prostatic cancer	PSA cell	KLK3-eRNA	Involved in the androgen receptor circuit	[58]
		Patients/CPRC cell	PSAe	AR	[67]
	Squamous-cell carcinoma, intestinal cancer,	HCNSC/COAD cell	CCAT1	MEK/ERK1/2 pathway, PI3K/AKT pathway, *c-MYC* gene	[68-69]
	gastric carcinoma, prostatic cancer	SGC-7901/PC-3 cell	HPSE-eRNA	Drives chromatin loops and regulateshnrnpu /p300/EGR1/HPSE	[61]
	Ovarian cancer	TCGA	UCA1e	UCA1 overexpression via activation of HiPO-YAP pathway	[70]
	Leukemia	Patients	MYC-eRNA	*MYC*	[56]
Immune metabolism diseases	Inflammation	Mouse	S-IRE1	*IFN-β, IFNB1*	[58,72]
	Inflammation	Mouse	Unknown	Unknown	[73]
	Gastritis	Epithelial cell	BIRC3 eRNA	*CiAP2*/influence *Helicobacter pylori* infection/improve the anti-apoptosis ability of epithelial cells	[34]
	Obesity/type 2 diabetes	Mouse	CCL2 eRNA	Combined with CBP, Pol II was recruited to promote the E-P ring	[75]
	Obesity	Pregnant woman	IL6 eRNA, IL10RB eRNA	IL6/IL 10RB	[76]
	Obesity	Patients/mouse	OLMALINC	Regulates SCD/region chromosome DNA-DNA loop	[74]
Cardiovascular diseases	Myocardial fibrosis	Human/mouse	Wisper	Affect CF proliferation	[77]
	Dilated cardio myopathy/aortic stenosis	Human/mouse	CARMEN	*PRC2*	[78]
	Cardiac failure	Mouse myocardial cell	HERNA1	HERNA1-SMG1-SYT17	[79]
	Congenital heart defects	Mouse myocardial cell	Nkx2-5		[80]
	Progressive heart conduction disease	Mouse myocardial cell	RACER	The recruitment of Pol2 regulates the expression of TBX5-dependent gene *Ryr2*	[81]

单核细胞中*CCL2*(C-C motif chemokine ligand 2)是被GPS2复合物抑制的主要炎症靶基因之一。HUANG等[75]在小鼠实验模型中发现巨噬细胞特异性CCL2 eRNA可通过与CBP结合促使CBP乙酰化, 募集Pol II, 促进E-P相互作用来促进*CCL2*转录; 沉默CCL2 eRNA可有效降低*CCL2*的表达水平和巨噬细胞炎症水平, 还可逆转肥胖相关的胰岛素抵抗。这些动物实验模型研究表明了eRNA与体内免疫应答及代谢疾病之间的密切关系, 为免疫性疾病靶向治疗提供了新的视角。

3.4 心血管疾病相关eRNA

心血管疾病作为一种常见疾病, 也是造成人类死亡的重要疾病类型之一。心脏疾病相关lncRNA目前已得到较多研究, 而对于eRNA在心脏疾病中的研究仍不充分。表2列举了目前研究发现的心血管疾病相关eRNA。例如, MICHELETTI等[78]通过对小鼠心脏转录组数据分析发现了一种在心脏成纤维细胞(cardiac fibroblast, CF)中高表达的进化保守的*Wisp2*超级增强子相关RNA-Wisper, 其中CF可增殖分化为肌成纤维细胞引发心力衰竭等疾病[78], 在小鼠中进行功能丧失实验也发现沉默Wisper可显著减少CF增殖, 从而抑制心脏纤维化[78]; 与小鼠和人类心脏分化相关的超级增强子相关lncRNA-CARMEN[78], 位于心脏中胚层特异性基因调控网络的上游, 可与多梳蛋白抑制复合物2(Polycomb repressive complex 2, PRC2)的组成成分*SUZ12*和*EZH2*相互作用参与心脏病理过程; 并且在人类特发性扩张型心肌病(dilated cardiomyopathy, DCM)和主动脉瓣狭窄(aortic stenosis, AOS)患者中发现CARMEN亚型CARMEN3表达显著上调; 缺氧诱导因子1α(hypoxia-inducible factor-1α, HIF-1α)产生的eRNA(HERNA1)[79]在心室压力超负荷时表达显著上调, 实验发现抑制HERNA1可防止多能干细胞衍生的人心肌细胞的生长和功能障碍; 同样在小鼠心肌细胞测序数据分析中发现*Nkx2-5*基因转录起始位点上游的同一增强子区域可编码两个具有相反功能的eRNA(IRENE-SS、IRENE-div), 其中IRENE-SS可促进其靶基因*Nkx2-5*转录, IRENE-div作为转录抑制因子在心脏发育和心肌细胞稳态过程中发挥重要性[80]。

这些转录组数据分析结果也在体外人体心肌细胞实验中得到了验证, 由于人与小鼠之间相对较高的序列同一性和基因组位点的保守性, 这些证据也共同揭示了eRNA作为治疗人类心力衰竭等心血管疾病靶标的可能性。

4 结语和展望

通过高通量基因组学、蛋白质组学和代谢组学分析等大量研究手段发现eRNA具有多样生物功能, 在基因转录、神经细胞及心肌细胞等发育和分化多阶段发挥重要作用, 且参与神经系统疾病、癌症、免疫代谢性疾病和心血管疾病等多种疾病的发生发展; GWAS研究也发现许多与疾病相关的遗传变异富集在增强子元件中, 多角度证明eRNA在人类基因组中的重要性。但基于eRNA在细胞类型和状态下的特异性、不稳定性, 未来研究需要开发更加精准方便的单细胞eRNA测序方法。此外, 尽管目前大量的研究已经鉴定出一些与人类疾病相关的eRNA, 并建立了多个eRNA数据资源便于从多角度探索eRNA在正常细胞和癌细胞中的功能作用, 与上万哺乳动物增强子转录单位相比以及样本数量的增加, 这些eRNA数据资源仍然需要不断更新; 并且转录组数据和实验验证只阐明了人类疾病相关eRNA的部分功能机制, 需要更加详尽的机制来支持其治疗潜力; 由于复杂的调控机制及人体组织尤其是人脑样本取样复杂因素等, 进行体内验证eRNA功能以探索其临床效用仍具有很大挑战。

近些年来研究发现, eRNA包含与miRNA相似的序列和二级结构的特定区域, 因此在结构功能上, eRNA是否可发挥其他非编码RNA类似功能仍有广泛未知性, 并且与其他lncRNA和mRNA相比, eRNA在生物发生和转录调节中的独特机制亟待解决; 在转录调控机制中, 研究已经发现携带m6A修饰的eRNA可通过招募YTHDC1蛋白, 在增强子附近形成转录凝聚体, 从而正向调节增强子和靶基因的转录激活, 而eRNA在高阶染色质状态下, 是否也会通过RNA-DNA、RNA-RNA或RNA-蛋白质相互作用介导转录调控; 在疾病靶标研究中, 基于eRNA在肿瘤耐药性方面取得了部分研究进展, 其在调节癌细胞药物反应中的潜在作用也将是未来的重要研究方向。

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