tRF的主要功能及研究方法

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摘要 非编码RNA(ncRNA)是指不编码蛋白质但具有重要功能的RNA。转运RNA的衍生片段(tRF)是一类新兴的ncRNA, 越来越多的研究发现它在许多方面发挥着重要作用, 而不是简单的降解产物。比如, tRF可以控制基因表达水平, 调节转录和翻译; 且tRF与细胞的自我更新、增殖、分化密切相关。因此, tRF可能会导致多种疾病, 如癌症、神经退行性疾病、免疫系统紊乱等。该文则主要介绍tRF的分类、主要生物学功能以及分子学生物和生物信息学研究方法。

关键词 tRF; 分类; 生物学功能; 生物化学方法; 生物信息学; 数据库

Function and Research Methods of tRF in Cancer

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Abstract ncRNA (noncoding RNA) is a kind of RNA that does not encode protein but has important function. tRF is a new type of ncRNA. More and more studies have found that it plays important roles in many aspects, rather than a simple degradation product. For example, tRF can control gene expression, regulate transcription and translation, and it is closely related to cell self-renewal, proliferation and differentiation. Therefore, tRF may be involved in a variety of diseases, such as cancer, neurodegenerative diseases, immune system disorders and so on. This paper mainly introduces the classification, main biological functions and research methods of biomolecules and bioinformatics of tRF.

Keywords tRF; classification; biological function; biochemical method; bioinformatics; database

1 tRF简介及功能

1.1 tRF概念及分类

tRNA衍生片段(tRNA fragments, tRF)由前体tRNA和成熟的tRNA产生, 是长度为14~30 nt的小片段非编码RNA[1]。根据起源和原始位置tRF可分为5个主要亚型: tRF-1、tRF-3、tRF-5、i-tRF以及tRF-2[2]。tRF-1来源于前体tRNA 3′非编码区(non-coding region), 由内切酶RNaseZ或ELAC2剪切而成[3]; tRF-3由成熟的tRNA 3′末端产生, 被核糖核酸内切酶(Dicer)、血管生成素(ANG)、TψC环外切酶等从T环上剪切而形成, 通常含有CCA末端序列[4]; tRF-5由成熟的tRNA 5′端产生, 在D环或者D环与反密码子环之间的茎区被Dicer剪切而成[5]; i-tRF主要来源于成熟tRNA所有内部区域, 但不包含5′端和3′端[6]; tRF-2是由tRNA的反密码子环裂解而成的[7]。

1.2 tRF主要生物学功能

tRF大小与miRNA(microRNA)相似, 但比miRNA稳定性更好, 丰度更高。tRF的组织特异性、

收稿日期: 2022-08-25 接受日期: 2022-11-21

国家自然科学基金(批准号: 81660238)、云南省基础研究计划(批准号: 202101AT070238)、云省医学学科后备人才项目(批准号: H2019001)和云南省临床医学中心开放项目(批准号: 2020LCZXKF-XY13)资助的课题

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Received: August 25, 2022 Accepted: November 21, 2022

This work was supported by the National Natural Science Foundation of China (Grant No.81660238), the Fundamental Research Program of Yunnan Province (Grant No.202101AT070238), the Yunnan Medical Discipline Reserve Talent Project (Grant No.H2019001), and the Yunnan Provincial Clinical Medical Center Open Project (Grant No.2020LCZXKF-XY13)

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疾病相关性以及表达时序性, 使其在肿瘤、代谢性疾病和神经系统疾病等重大疾病中都具有重要的调控功能[8]。tRF通过各种机制发挥生物作用, 可与蛋白质或RNA相互作用, 调节癌症相关mRNA的稳定性, 进而调控肿瘤细胞的生长、凋亡、侵袭和转移[9], 本文主要从tRF调节基因表达(转录、翻译、表观遗传修饰)、细胞功能这两个方面进行介绍, 且整理了tRF在不同疾病中的相关机制研究(表1)。

1.2.1 调节基因表达 (1) tRF对转录机制的调节。tRF通过不同的机制参与转录调节, 主要通过与AGO蛋白家族结合、与mRNA的竞争性结合使基因沉默。

有研究人员将现有实验数据进行大规模元分析, 发现tRF负载到AGO1(Argonaute 1)蛋白上来调节基因表达[29]。KUSCU等[30]研究发现, tRNA过度表达产生的tRF-3与含有RNA诱导沉默复合体(RNA-induced silencing complex, RISC)的Argonaute-GW182相关联, 以促进翻译和抑制目标mRNA降解。一些研究人员通过Ago-免疫沉淀(Ago-immunoprecipitation, Ago-IP)结合生信分析发现, tRF-5存在于多个Ago-IP中, 而tRF-3与Ago2-IP的相互作用最强[31]。Ago2具有独特的核糖核酸内切酶活性, 该酶活性对特异性miRNA的生物合成和mRNA切割至关重要[15]。DAUGAARD等[31]推测, Ago2-tRF-3的相互作用可能会通过直接剪切靶基因来抑制反义报告基因。

tRF对基因的沉默还有其他作用机制, 比如通过目标蛋白与mRNA的竞争性结合。在肝细胞癌细胞系Huh7中tRF_U3_1可直接与La/SSB蛋白(干燥综合征抗原B, 也称LARP3和La自身抗原)结合, 并抑制La/SSB依赖性病毒基因表达[23]。 已有研究表明, IGF2BP1能够与不稳定编码区(the coding region instability determinant, CRD)结合来防止*MYC*(MYC proto-oncogene) mRNA裂解[32]。KRISHNA等[32]发现, 在维甲酸(retinoic acid, RA)诱导的分化状态下, 特异的5′tRF将IGF2BP1从*MYC* mRNA-蛋白质复合物中分离, 并且5′-tRF会优先与mRNA结合蛋白IGF2BP1相互作用。这种相互作用影响了转录稳定性, 从而抑制了*MYC*的翻译[32]。

(2) tRF对翻译进程的调节。tRF通过与生物体内的某些RNA和蛋白质结合来影响其翻译过程, 这种相互作用可以抑制或促进翻译的进程。

有研究表明, tRF会优先抑制翻译机制的核心组成部分, 例如核糖体蛋白、翻译的启动因子及延长因子, 从而控制整个mRNA的翻译[33]。LUO等[33]发现在果蝇中, tRF通过保守的反义序列配对整个mRNA的目标位点来抑制靶mRNA。GEBETSBERGER等[34]研究发现, 嗜盐古菌(*Haloferax volcanii*)在特定的应力条件下产生的Val-tRF能够与核糖体小亚基结合, 抑制嗜盐古菌的翻译及肽键的形成。LALANDE等[35]证明拟南芥中的tRF与多核糖体作用来抑制蛋白质合成。HUTVAGNER小组[36]提供相关数据显示: 来源于$tRNA^{Gln}$的tRF-5的3′端含有“GG”二核苷酸基序, 它能够与哺乳动物多合成酶复合物(multisynthetase complex, MSC)相互作用, 从而抑制翻译。

除此之外, 许多tRF还具有增强翻译的作用。在乳腺癌(breast cancer, BC)中过度表达的RNA结合蛋白核仁素(nucleolin, NCL)通过结合*p53* mRNA的5′→3′UTR碱基配对区域来抑制*p53*翻译, tRF3E与NCL竞争性相互作用, 使*p53* mRNA释放并促进其翻译, 从而调节癌细胞生长[18]。KIM等[22]研究证明, 在哺乳动物中来源于LeuCAG-tRNA的tRF-3能够结合至少两种核糖体蛋白mRNA(RPS28和RPS15), 以增强翻译作用, 并通过保持核糖体蛋白S28水平增加核糖体数量。FRICKER小组[37]确定了布鲁氏锥虫(*Trypanosoma brucei*)的翻译激活功能, 在tRNA衍生的小RNA(tRF&tiRNA, tsRNA)存在时核糖体上高丰度微管蛋白的mRNA水平提高了1.3至3.8倍, 这表明了tsRNA可增强翻译起始作用。

(3) tRF对表观遗传修饰的调节。几项研究表明, tRF可通过影响不同的表观遗传过程来调节基因表达。

为进一步证明精子来源的内源性tRF的稳定性和活性可能依赖于转录后修饰, 研究人员发现高脂肪饮食(high-fat diet, HFD)喂养小鼠衍生的sncRNA中m5C和m2G碱基修饰水平显著提高, 这表明RNA修饰可能会影响精子衍生tRFs的生物发生和功能, 从而促进新陈代谢表型在后代的传播[38]。一项对人类精子的研究表明, 健康精子细胞中tsRNA水平是对饮食干预快速进行反应的, 糖敏感tsRNA的变化与精子运动的同步变化呈正相关, 而与肥胖呈负相关[39]。这种对人类精子中tsRNA饮食干预的快速反应与模式生物中发现的父系代际代谢反应相协调[39]。

最近, BOSKOVIC等[40]研究发现, tRF-GG在产生各种非编码RNA snoRNA、scaRNA和snRNA中发

表1 tRF在不同疾病中的相关机制研究

Table 1 Research on the mechanism of tRF in different diseases

疾病 Disease	tRF名称 tRF name	上调/下调 Up/down	作用机制 Mechanism	影响癌症发生 Affect cancer	参考文献 References
NSCLC	tRF-Leu-CAG1, tRF-Leu-CAG2	Up	Directly target AURKA protein	Promote cell cycle progression and cell proliferation	[10]
Lung cancer	ts-101, ts-53, ts-46, ts-47	Down	Associate with PiwiL2 protein	Inhibition of lung cancer cell proliferation	[11]
LUAD	tsRNA-5001a	Up	Inhibit the expression of GADD45G and the anti-tumor function of GADD45G	Promote LUAD cell proliferation	[12]
NSCLC	tRF-007333	Up	Bind to HSPB-1 protein	Promote NSCLC cell proliferation	[13]
NSCLC	AS-tDR-007333	Up	Interaction with HSPB1 activates H3K4me1 and H3K27ac to enhance MED29 expression; stimulate the expression of ELK4 to enhance the activity of MED29 promoter	Promote NSCLC cell proliferation and migration	[14]
CRC	tRF/miR-1280	Down	Inhibition of Notch signaling pathways that support cancer stem-like cells (CSC) phenotypes	Inhibition of colorectal cancer growth and metastasis	[15]
CRC	5′-tRF-GlyGCC	Up	Regulated by AlkB homolog 3 (ALKBH3)	The level of 5′-tRF-GlyGCC in plasma is a promising diagnostic biomarker for CRC diagnosis	[16]
CRC	tRF-20-MEJ-B5Y13	Up	Regulated by Dicer1 and positively correlate with Dicer1 expression	Promote colon cancer cell invasion and migration	[17]
BC	tRF3E	Not expressed	Specifically interacts with nucleolin (NCL)	Inhibition of cancer cell growth	[18]
BC	ts-112	Up	Regulated by RUNX1 transcription factor	Enhance BC cell proliferation and promote normal mammary epithelial proliferation	[19]
BC	tRF-19-W4PU732S	Up	Inhibition of ribosomal protein-L27A (RPL27A) by directly targeting 3′UTR	Promote BC cell viability, invasion, migration, EMT and CSC phenotypes, and the suppression of apoptosis	[20]
HGSOC	tRF-03357	Up	Inhibit the expression of HMBOX1 gene	Promote cell proliferation, migration and invasion	[21]
HCC	LeuCAG 3′tsRNA	Up	Inhibition impairs ribosome biogenesis	Promote tumor growth	[22]
HCC	tRF_U3_1	Up	Associate and interaction with La/SSB protein	Suppress viral gene expression or replication	[23]
GC	tRF-Val-CAC-016	Down	Modulates the transduction of CACNA1d-mediated MAPK signaling pathways	Suppress the proliferation of gastric carcinoma	[24]
GC	tRF-Val	Up	Bind to EEF1A1 protein and transport it to the nucleus, promote its interaction with MDM2, and inhibit the downstream molecular pathway of p53	Promote proliferation and inhibit apoptosis	[25]
PC	tRF-Leu-AAG	Up	Combine with 3′UTR to regulate UPF1 expression	Promote cell proliferation, migration and invasion	[26]
Pan-Cancer	tRF-20-S998LO9D	Up	Not clear	Promote the proliferation of tumor cells *in vitro*	[27]
RSV	tRF5-Gly-CCC/ tRF5-Lys-CTT	Up	Exert the function of gene trans silencing at post-transcriptional level	Enhance virus replication	[28]

NSCLC: 非小细胞肺癌; LUAD: 肺腺癌; CRC: 结直肠癌; BC: 乳腺癌; HGSOC: 高级别卵巢浆液性癌; HCC: 肝细胞癌; GC:胃癌; PC: 胰腺癌; RSV: 呼吸道合胞病毒感染。

NSCLC: non-small cell lung cancer; LUAD: lung adenocarcinoma; CRC: colorectal cancer; BC: breast cancer; HGSOC: high-grade serous ovarian cancer; HCC: hepatocellular carcinoma; GC: gastric carcinoma; PC: pancreatic cancer; RSV: respiratory syncytial virus.

挥作用, 其中tRF-GG对U7 snRNA的调节通过支持组蛋白的充足供应来调节异染色质介导的MERVL元件转录抑制。tRF已被证明可强烈抑制内源性转座因子(transposable elements, TE), TE的转录通常受到组蛋白修饰和DNA甲基化等表观遗传标记的抑制[41]。在没有这些抑制的情况下, 3′-tRF显著性高表达, 因此研究者提出tRF介导的反转子沉默机制的两种可能: 短链18 nt 3′-tRF通过靶向逆转录酶引物结合位点来干扰逆转录; 22 nt 3′-tRF通过与AGO2关联来诱导病毒RNA的转录后沉默[41]。

1.2.2 *调节细胞功能* tRF通过调节基因的表达来促进细胞增殖和细胞周期进展。

在正常细胞中, tRF可作为内源性凋亡信号, 直接或间接地导致细胞凋亡; 当细胞受到压力时, tRF增加使凋亡过程紊乱、恶性细胞增殖[36]。有研究表明tRF-3027与RISC的重要组成部分AGO结合, 可阻止复制蛋白A1(replication protein A1, RPA1)的表达从而抑制细胞增殖[42]。COSENTINO等[43]发现, $tRNA^{Gln}$的低甲基化会形成5′-$tRFs^{Gln}$碎片, 该片段介导的TRMtRNA甲基转移酶(tRNA methyltransferase 10A, T10A)缺乏会导致胰腺β细胞死亡。

tRNA衍生的小RNA被发现在癌症中经常失调, 它们的失调可以干扰不同水平的基因表达在癌症中发挥关键作用[44]。tRF-Leu-CAG在非小细胞肺癌(non-small cell lung cancer, NSCLC)细胞系和肿瘤组织中高表达, 增强了NSCLC中极光激酶A(aurora kinase A, AURKA)的活性, 从而促进了NSCLC细胞的增殖, 同时也促进了G_0/G_1细胞周期进展[10]。tRF/miR-1280是来自$tRNA^{Leu}$和pre-miRNA的17 bp长的片段, 可将Notch配体*JAG2*基因作为直接结合靶点, 再通过抑制支持癌症干细胞样细胞(cancer stem-like cell, CSC)表型的Notch信号通路来抑制结肠直肠癌细胞的增殖和转移[15]。BALATTIE等[11]发现ts-46、ts-47和ts-53的表达导致H1299和A549细胞系集落形成明显减少。ZHOU等[45]通过改进miR-Catch的方法和双荧光素酶报告基因检测证实tRF5-Glu直接与乳腺癌乳腺癌抗雌激素药物耐药性基因3(the breast cancer anti-estrogen resistance 3, *BCAR3*) mRNA的3′UTR位点结合来下调其表达, 从而抑制卵巢癌细胞的增殖。

1.3 tRF与miRNA、tiRNA的异同

已有研究表明, 许多短链非编码RNA(small non-coding RNA, sncRNA), 如miRNA、tiRNA等与tRF在长度、生物发生和功能等不同方面上存在相似性。miRNA的生物发生十分复杂, 遵循从细胞核到细胞质中的一系列裂解过程, 这与tRF的形成完全不同。通常, 成熟的miRNA加载到AGO蛋白与GW182(glycine-tryptophan)及相关蛋白一起构成的RISC上[46-47]。miRNA通过位于5′端的核苷酸2~7的“种子”序列识别靶mRNA的3′UTR的互补序列, 并引导RISC到该区域抑制翻译[48-49]。当miRNA与靶点完全互补时, 靶mRNA可被直接切割和降解; 不完全互补时, miRNA以非完美配对的方式影响mRNA稳定性来调控转录翻译[50]。

tRF的功能与miRNA类似, 可通过与靶mRNA的3′UTR、AGO蛋白及其他蛋白结合形成RISC, 抑制mRNA表达[51-53]。例如, 在胃癌中异常表达的tRF-3017A通过与AGO蛋白结合形成RISC来调节肿瘤抑制基因*NELL2*的表达[54]。WANG等[55]发现, tRF-24-V29K9UV3IU可以发挥miRNA样功能, 与AGO2结合, 并通过与*GPR78* mRNA的3′UTR结合, 直接沉默*GPR78*的表达。与tRF相比, miRNA通常依赖于AGO蛋白和其他miRNA结合蛋白(miRNA binding protein, miRBP)行使功能, 其中AGO可以帮助miRNA调控靶基因并保护其不被降解[56], 但tRF能够直接进行生物功能调节, 这表明tRF比miRNA更稳定和丰富, 且具有更复杂的调节潜力从而发挥更重要的作用[1]。

tRF与tiRNA的生物合成过程非常相似, 主要的区别是两者具有不同的长度和切割位点。在应激条件下由ANG从tRNA反密码子环剪切产生的30~50 nt的tsRNA被称为tiRNA(tRNA-derived stress-induced RNAs)[42,57]。结构上tRF具有类似于miRNA的5′磷酸和3′羟基[51], tiRNA不具有5′磷酸而是5′羟基[58]。

tRF和tiRNA的生物功能具有相似性, 如抑制翻译起始和延伸、作为表观遗传因子以及调节细胞增殖、转移、凋亡等[42,59-62]。例如tRF-19-W4PU732S通过抑制核糖体蛋白-L27A(ribosomal protein L27a, RPL27A)来抑制乳腺癌细胞凋亡[20]; 在结直肠癌中, 5′-tiRNA-His-GTG上调并抑制肿瘤抑制因子激酶2(large tumor suppressor kinase 2, LATS2)翻译, 使Hippo信号通路“关闭”, 最终诱导细胞凋亡[63]。与tRF不同的是没有直接研究表明, tiRNA能够调节核糖体的生物发生, 且两者在免疫调节方面的功能机

制尚不清楚[5]。tiRNA在调控方式上与tRF也有一定的差异, tiRNA通过与翻译起始复合物连接来抑制翻译起始[64]。例如含有TOG基序的5′tiRNA^{Ala}和5′tiRNA^{Cys}形成G-四联体(G-quadruplex, RG4), 该结构与eIF4G的HEAT1结构域(RNA结合区内含有的保守结构域)相互作用, 这种相互作用破坏了eIF4G/eIF4F在m^{7}GTP mRNA帽上的稳定性, 从而抑制翻译[65-67]。tRF和tiRNA介导的翻译抑制有AGO依赖性和非依赖性两种方式, AGO蛋白结合20~24 nt的小RNA, 因此tiRNA可能不会被结合[5,68]。JEHN等[69]发现, 在灵长类海马体中, 与典型的通过5′区域与目标的3′UTR结合的miRNA样调控方式不同, 5′tiRNA通过序列特异性过程使基因沉默, 目标3′UTR或CDS(coding sequence)与5′tiRNA的中间区域对齐结合。这些发现表明, AGO蛋白可能不是必不可少的tsRNA基因调控依赖性效应蛋白。

2 tRF研究方法

由于tRF特定的生物学功能对调控肿瘤细胞的疾病进程有着很大的影响, 所以研究tRFs在疾病发生发展过程中是否具有调节作用以及具体分子机制, 对寻找可靠的生物标记物以及新的药物作用靶点具有重要意义。现有的tRF分子机制研究手段, 主要包括分子生物学方法和生物信息学方法。本文列举了部分分子生物学实验手段及具体应用包括: 微阵列、RNA测序(RNA-seq)、实时定量逆转录聚合酶链式反应(qRT-PCR)、RNA免疫共沉淀(RNA immunoprecipitation, RIP)等, 并对常见的tRF生物信息学分析数据库进行介绍。

2.1 分子生物学方法

2.1.1 *微阵列和RNA测序* 微阵列和RNA-seq是高通量检测tRF表达的有效工具。

微阵列是基因芯片的一种, 原理是将已知序列带有荧光标记的基因探针附着在固相载体表面并以规则的网格形式排列, 加入待测的核酸序列与探针阵列进行杂交, 通过检测探针的荧光强度来获得基因的相对表达量。其优点是可以实现对阵列上数以千计的基因表达水平同时测量, 实现高通量检测, 且检测速度较快, 但由于miRNA样本量少, 序列短并且同家族序列极相似, 导致此方法灵敏度和选择性低、价格高昂、探针和靶分子杂交成双链的解链温度(*T*m)较低, 增加了错配的几率[70]。BALATTI等[11]对慢性淋巴细胞白血病(chronic lymphocytic leukemia, CLL)和肺癌的tsRNA表达概况进行分析发现tsRNA可能是癌症相关发展发病机制的重要影响因素。他们将患者的总RNA样本杂交到定制的tsRNA微阵列芯片上, 研究了有或不激活*MYC*癌基因的人类淋巴细胞中的tsRNA表达模式, 并发现了15个tsRNA的特征, 其中ts-47是MYC激活后下调最强的一个[11]。参考上述研究方法, FARINA小组[19]为确定乳腺癌中tsRNA的表达是否受转录因子RUNX1的调节, 使用经过验证的自定义tsRNA微阵列进行分析。结果表明RUNX1的缺失与四种tsRNA表达水平的变化一致; ts-19和ts-29与*RUNX1*(RUNX family transcription factor 1)基因敲除后的表达水平降低呈正相关, ts-46和ts-112分别与*RUNX1*基因敲除或过表达呈负相关[19]。

RNA测序(RNA-seq)是使用高通量测序技术进行分析的过程, 不需要预先设计探针或序列抑制, 可检测新的转录物, 可检测多种RNA的表达水平且十分适用于尺寸小的非编码RNA[71-72]。RNA-seq的优势在于高通量性可有效地分析比较不同时间、不同组织样本以及疾病状态和药物干预等不同环境因素; 高分辨率性可识别单核苷酸变体、新的转录后修饰、新的可变剪接模式和未被识别的ncRNA[73-76]。但该方法也存在局限性和问题: 如测序要求比大多数mRNA短的序列并行处理, 需对RNA链或cDNA链进行片段化, 但该操作可能会使链产生偏差; 数据量大给数据存储和检索带来问题; 选择性剪接、反式剪接和对应于多个基因组位置的片段会影响后续的转录组分析[73]。KUSCU等[30]对总RNA进行质量控制和RNA-seq获得了3 000万个长度为50碱基的长配对末端测序片段, 通过数据分析及后续实验最终证明tRF-3009a在转录后引导AGO以不依赖Dicer的方式抑制靶基因的表达。KRISHNA等[32]为研究tsRNA富集现象, 在不同条件下培养的小鼠胚胎干细胞(mouse embryonic stem cells, mESCs)中提取RNA并进行小RNA测序, 得出数据经分析后发现与同源干细胞样状态相比在分化状态下50-tsRNA表达持续富集。

2.1.2 qRT-PCR qRT-PCR可分为三个基本类型: DNA结合染料法(SYBR Green I)、基于探针的化学法(TaqMan、Scorpion、分子信标、杂交探针)和淬灭染料引物法(Amplifluor、LUX荧光引物)。其中

DNA结合染料法(SYBR Green I)最为常见, 基本原理: 将带荧光的非特异性DNA染料结合到双链DNA上, 被结合的染料激发出强荧光, 以此来检测PCR过程中累积的扩增产物[77]。该方法的优点是可对任何双链DNA进行定量; 无需设计探针从而减少成本; 适合大量基因的分析; 操作简单。缺点是能结合所有DNA双链, 可能会产生大量引物二聚体引起背景干扰, 以及产生其他非特异性产物, 因此使用该方法需优化并进行后续分析以验证结果[77]。对于单一PCR产物的反应, 可通过精心设计引物来提高特异性。但tRF序列很短, 约为1条引物的长度, 因此常规引物设计不适用于tRF的逆转录。为解决此问题, 目前已有三种检测方法, 它们与miRNA检测方式相似。第一种加尾法: 利用聚腺苷酸聚合酶在3′端加上poly(A)尾巴, 然后再用5′端为poly(T)的通用引物进行逆转录; 或利用T4连接酶将所有分子末端加上共有序列, 再使用通用引物进行逆转录[70]。第二种茎环法: 利用茎–环状探针引物与3′端结合, 逆转录为互补的cDNA后进行扩增, 该方法特异性高, 可识别序列高度相似的同家族分子[70]。第三种加接头法: 使用Arraytar公司生产的rtStar™第一条cDNA合成试剂盒在去除修饰基团的总RNA 3′端与5′端添加接头, 该接头对tsRNA具有序列特异性, 在qRT-PCR过程中设计的引物包含了接头以及cDNA起始的对应互补序列, 进一步增加了特异性[78-80]。SCHORN等[41]在干细胞中发现两个内源性逆转录病毒(endogenous retroviruses, ERV)家族, IAP(intracisternal A particle)和MusD/ETn是主要的靶点, 在逆转录转座实验中被tRFs强烈抑制。为测量转染细胞中*MusD*(a coding-competent)和*ETn*(non-autonomous)的表达水平, 分别进行定量TaqMan qRT-PCR检测平均转录水平, 转染后2天和4天提取RNA, 此时可检测到*MusD*和*ETn*的表达, 并且可检测到逆转录病毒中间产物[41]。QIN等[81]在研究tsRNA在脊髓损伤(spinal cord injury, SCI)后的功能作用过程中, 通过生信分析筛选出tsRNA, 为观测其表达水平的变化趋势, 通过qRT-PCR进一步验证tsRNA及其潜在靶点mRNA, 得到的qRT-PCR数据显示与对照组相比, SCI组这些指标均显著上调。STEPHEN小组[82]利用RNA-seq鉴定出一种锥虫特异性小RNA, 在感染牛的血清中高水平存在。进一步进行qRT-PCR分析, 将根据特定序列定制的引物与探针混合使用进行了单步qRT-PCR反应, 结果表明可直接从血清中有效检测出小RNA, 无需预处理, 突显了sRNA在诊断人类疾病方面的潜力。

2.1.3 RNA免疫共沉淀 RNA免疫共沉淀(RNA immunoprecipitation, RIP)是用于研究RNA与蛋白结合情况和RNA上蛋白质结合位点的技术。该技术利用目标蛋白的抗体把相应的RNA-蛋白复合物沉淀下来, 再进行分离提纯获得特定RNA, 获得的RNA可通过末端标记和电泳对分子的大小进行鉴定, 或利用高通量测序对RNA序列进行分析。优点为步骤简单、灵敏性高, 可识别所有类别RNA, 包括非编码和反义RNA[78]。缺点为由于不是通过共价交联相互作用, 所需的严格洗涤条件可能会使与RNA结合蛋白(RNA-binding protein, RBP)亲和度较低的RNA无法回收; 动力学不稳定结合的RBP在剧烈的裂解条件下(如超声处理)可能会从其RNA靶标上解离并与其他RNA重新结合[83]。KIM等[22]确定tsRNA结合了至少两个核糖体蛋白质mRNA来增强翻译作用。通过免疫沉淀技术对tsRNA进行研究, 使用RNasin plus和蛋白酶抑制剂裂解细胞, 将RNA与制备的蛋白A/G偶联抗体特异性结合产生沉淀。再使用TRIzol提取出免疫沉淀中的RNA, 随后分离出免疫沉淀蛋白质[22]。KEAM等[36]在研究哺乳动物MSC与5′tRF、Gln19相互作用时也使用了RNA免疫共沉淀。首先从转染细胞中提取目的RNA, 再采用链霉亲和素包被的免疫磁珠进行免疫沉淀, 得到的沉淀分别用紫外、SDS两种方式进行洗脱[36]。KUSCU等[30]在研究tRF-3通过序列互补来抑制基因表达的过程中, 为证明tRNA过度表达产生的tRF-3可加载到Argonaute蛋白中, 进行了免疫沉淀实验。研究人员从tRNA过表达细胞中提取出Argonaute蛋白, 将其与Pan-Ago抗体结合, 再使用苯酚–氯仿从沉淀中提取RNA, 最后进行Northern blot印迹实验, 以表明被加载到该蛋白上的是tRF-3009a而不是tRF-3009b[30]。

2.2 生物信息学数据库

鉴定tRFs和阐明它们在细胞、疾病中的作用越来越受到关注, 为了促进研究和学术交流, 研究人员建立多个公开的tRF数据库(表2)。这些工具可以挖掘深度测序数据来识别和量化tRF。

线粒体和核tRNA数据(MINTbase)是由RI-GOUTSOS实验室PLIATSIKA等[84]建立的一个人类tRFs综合数据库, 提供了26 744个tRF的详细注释。由于MINTbase数据库只描述广泛表达的tRF的一般生

表2 常见tRF数据库
Table 2 Common tRF Databases

名称 Name	简介 Introduction	网址 Website	参考文献 References
DASHR v2.0	Integrate databases of all major categories of human sncRNA genes and RNA sequence maturation products	https://lisanwanglab.org/DASHRv2	[89]
BBcancer	Data on the expression of various RNA types (including tRFs) in 5 040 blood samples from normal people or 15 cancer patients were included	http://bbcancer.renlab.org/	[90]
GtRNAdb	The genomic tRNA database contains tRNAscan SE's prediction of tRNA genes with complete or near complete genome. The data can be retrieved using sequence or genetic characteristics	http://gtrnadb.ucsc.edu/	[91]
MINTbase	Mitochondrial and nuclear tRNA data (MINTbase) is a repository of tRNA fragments (tRFs), which can obtain information about tRF maximum abundance, its specific data and its parent tRNA modification	http://cm.jefferson.edu/MINTbase/	[84]
MINTmap	tRF is identified and quantified by mining deep sequencing data, and the original and standardized abundance of tRF were calculated	https://github.com/TJU-CMC-Org/MINTmap/	[92]
PtRFdb	The database specially developed for plant tRF analyzed 1 344 sequencing data sets and predicted different tRF types (i.e. tRF-5, tRF-3 and tRF-1)	http://www.nipgr.res.in/PtRFdb	[93]
RNA FRABASE	It can be used to search 3D fragments in 3D RNA structure, using the sequence and/or secondary structure given in dot bracket representation as input	http://rnafrabase.cs.put.poznan.pl	[94]
SPORTS1.0	Optimize tsRNA from sRNA-seq data and rely on nucleotide mismatch in sRNA to predict potential RNA modification domains	https://elkssl99cd-2175108d157588c04758296d1cfclib.link.nbu.edu.cn:8443/junchaoshi/sports1.0	[95]
tDRmapper	Tools for mapping, naming and quantifying tDRs, while annotating and quantifying mismatches and deletions, help to discover the biological function of tRF	https://github.com/sararselitsky/tDRmapper	[96]
tRF2Cancer	It is used to accurately identify tRFs from deep sequencing data, including the expression of tRFs in 10 991 samples of 32 cancers, and evaluate their expression in various cancers	http://rna.sysu.edu.cn/tRFfinder/	[97]
tRFdb	The first tRF database; retrieving tRF sequences may come from tRNA genome coordinates and names	http://genome.bioch.virginia.edu/ trfdb/	[98]
tRFexplorer	This system allows the study of the potential biological role of tRF in the absence of direct experimental evidence. The database contains 143 different tRNA derived ncRNAs, which are classified as tRNA derived fragments (9 tRF-5s, 45 tRF-3s), tRNA derived microRNAs and tRNA 5′ lead RNA	https://trfexplorer.cloud/	[99]
OncotRF	The use of a highly conservative filtering strategy ensures robust results of downstream analysis, including exploring tRF function and identifying diagnostic and prognostic biomarkers	http://bioinformatics.zju.edu.cn/Onco-tRF	[88]
tRFTar	The regulatory TGIs contains 25 281 tRF targets, and has the functions of customized search, co expression TGI filtering, genome browser, tRF function enrichment analysis based on TGI, etc.	http://www.rnanut.net/tRFTar/	[87]

物学特征, 但该数据库不能在肿瘤发生、癌症进展和预后相关上对tRF进行特异性筛选, 因此多与含有癌症患者可用临床数据例如TCGA数据库结合使用, 以此来研究tRF在癌症中的作用。GU等[85]使用MINTbase v2.0从癌症基因组图谱(the cancer genome atlas, TCGA) 523名头颈部鳞状细胞癌(head and neck squamous cancer, HNSC)患者中获得tRF表达数据, 其与可用的TCGA临床数据相关, 共鉴定出23 413个丰度≥1.0每百万阅读数(reads-per-million, RPM)的成熟tRNA衍生片段, 以供进一步研究它们作为预后标志物的潜在用途。ZHOU等[86]基于MINTbase v2.0的注释和TCGA样本的临床信息, 在七种TCGA癌症类型中筛选癌症相关tRFs(cancer-associated tRFs, ca-tRFs)。随后通过整合来自tRFTar数据库的AGO

介导的tRF-靶基因相互作用的数据集, 定位了ca-tRF靶基因及靶基因模块[86]。通过tRFTar数据库分析146个交联免疫沉淀和高通量测序(high-throughput sequencing, CLIP-seq)数据集, 对潜在的AGO介导的tRF-靶基因相互作用(tRF-target gene interactions, TGIs)进行了系统计算筛选, 其中确定了12 102个tRF和5 688个目标基因之间的920 690个TGIs, 揭示了tRF和目标基因之间的广泛调节相互作用[87]。MA等[27]研究人员通过MINTbase检索tRF表达数据并使用R语言组织可视化了tRF-20-S998LO9D在泛癌和匹配对照组织中的表达差异, 然后使用OncotRF数据库检索tRF-20-S998LO9D在泛癌中的预后意义。OncotRF数据库能够识别差异表达的tRF, 预测其功能, 并根据使用者定义的群体评估tRF表达水平与临床结果的相关性, 并且还提供在线Kaplan-Meier绘图仪, 可根据使用者需求绘制生存曲线[88]。

3 展望

这些小片段的ncRNA以细胞和组织特异性的方式调节基因表达和蛋白质生物发生, 以及协调应力和其他信号, 以诱导其生物生成的精确度。但大多数tRF和其他RNA衍生片段的生物生成和生物功能仍不详尽清楚。了解不同组织中的tRNA片段生物学将使我们能够更准确地了解调节疾病进展的组织特异性。人们经不同疾病患者的基因组测序数据的可用性已经识别了tRNA衍生片段的特征, 这些片段已被证明具有致癌或肿瘤抑制活性。如今所需的是揭示tRNA衍生片段抑制或促进肿瘤生成的机制, 以及判断它们能否成为药物治疗靶点的最佳诊断工具, 以此为基础制定出有效的治疗策略, 使tRF疗法治疗不治之症成为可能。随着技术和实验方法的进步, 越来越多的tRNA衍生片段被研究人员发现, 下一代测序技术也在不断地发展, 不久的将来tRF的生物学功能或将被完全揭示。

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