周翊峰教授，博导，东吴学者，江苏省杰出青年基金获得者，2021年度国家杰出青年科学基金获得者，中国抗癌协会肿瘤病因学会专业委员会常务委员，现就职于苏州大学苏州医学院。2005年毕业于中国协和医科大学/中国医学科学院，获得博士学位。2005年至2008年期间在美国华盛顿大学从事博士后研究。2008年9月，作为引进人才到苏州大学工作，并被聘为教授。以通讯作者的身份在国际学术期刊，其中包括*Gastroenterology*(封面推荐)、*EMBO J*、*J Exp Med*、*Cancer Res*、*Mol Cancer*、*Oncogene*、*Int J Cancer*、*Breast Cancer Res*、*Carcinogenesis*、*Hum Mutat*、*J Biol Chem*等上发表文章30余篇；文章总引用6 000余次，单篇通讯作者最高引用450余次；以第一完成人分别获得2017年度教育部高等学校科学研究优秀成果(自然科学)二等奖和2018年度江苏省科学技术三等奖；担任*Cancer Med*、*Gene*杂志学术编辑，*Nature*、*Gastroenterology*、*Gut*、*Oncogene*、*Carcinogenesis*、*Cancer Letter*、*Theranostics*等杂志的特约审稿人，国家自然科学基金二审专家。

闪亮的“暗物质”——可编码的非编码RNA

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摘要　非编码RNA(non-coding RNA, ncRNA)是指不编码蛋白质的RNA, 这些ncRNA被认为是基因组中的“暗物质”。然而, 大量的新发现修正了这一看法: 微小RNA(microRNA, miRNA)、环状RNA(circular RNA, circRNA)和长链非编码RNA(long non-coding RNA, LncRNA)都具有小开放阅读框(small open reading frame, sORF), 能够编码功能性微肽或蛋白质。该综述根据不同功能对ncRNA编码肽的研究进行总结, 阐述这些ncRNA编码肽的研究方法与功能机制, 以使这些闪亮的“暗物质”——ncRNA编码肽能够在不同领域得到学者的关注。

关键词　非编码RNA; ncRNA编码肽; 肿瘤; 微小RNA; 环状RNA; 长链非编码RNA

The Shiny “Dark Matters” — Encodable Non-Coding RNA

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Abstract　NcRNAs (non-coding RNAs), which do not encode proteins, have been considered as the ‘dark matter’ of the genome. However, a large number of new discoveries have revised this view: miRNAs (microRNAs), circRNAs (circular RNAs) and LncRNAs (long non-coding RNAs) all have sORFs (small open reading frames), which can encode functional micropeptides or proteins. This review summarizes the researched ncRNA-encoded

收稿日期: 2022-03-01　　接受日期: 2022-03-07

国家杰出青年科学基金(批准号: 82125027)资助的课题

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Received: March 1, 2022　　Accepted: March 7, 2022

This work was supported by the National Science Fund for Distinguished Young Scholars of China (Grant No.82125027)

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peptides according to their different functions, and expounds on the research methods and functional mechanisms of these ncRNA-encoded peptides, so that these shiny “dark matters”—ncRNA-encoded peptides can attach the attention of scholars in different research fields.

Keywords non-coding RNA; ncRNA-encoded peptides; tumor; microRNA; circRNA; LncRNA

普遍观点认为蛋白质是遗传信息的最终产物，并是遗传信息发挥功能的主要体现方式，而实际上仅有不到2%的基因编码蛋白质[1-2]，其余不编码的转录本被统称为非编码RNA(non-coding RNA, ncRNA)，根据长度等特征其可被分为小核仁RNA(small nucleolar RNA, snoRNA)、微小RNA(microRNA, miRNA)、环状RNA(circular RNA, circRNA)和长链ncRNA(long ncRNA, LncRNA)[3]。随着研究的深入，这些起初被认为是基因组中“暗物质”的ncRNA的功能被揭示了出来，它们以不同的方式参与生理和发育过程相关基因的表达调控[4]。不仅如此，LncRNA、miRNA和circRNA上含有小开放阅读框(small open reading frame, sORF)并能编码具有功能的微肽或蛋白质[5-6]，越来越多“闪亮的暗物质”被发现和鉴定。其中，肿瘤相关的LncRNA编码肽受到了研究人员的重视，认为其能够成为临床应用的新型治疗靶标和生物标志物。本文对ncRNA编码性的研究方法进行了梳理与评价，总结了功能已被阐明的ncRNA编码肽，并着重关注肿瘤相关的ncRNA编码肽，以期对癌症的早期发现、准确诊断、精准分类、精准治疗、预后判定等方面的应用提供借鉴。

1 ncRNA编码肽的研究方法

翻译组学与蛋白组学方法常用于探寻可编码的ncRNA。翻译组学中多核糖体分析(polysome profiling)、全长翻译RNA测序(full length translating RNA sequencing, RNC-seq)、核糖体印迹测序(ribosome profiling, Ribo-seq)常用于检测样本中翻译中的RNA，蛋白组学分析则通过质谱技术鉴定样本中的蛋白质种类以找到相应的ncRNA编码肽。原则上，这些技术能鉴定出可编码的ncRNA。但在研究早期，由于研究体系的不完善，学界对“基因组中98%的“非编码”是否可以编码功能肽/蛋白”有着长期的争论。2013年，GUTTMAN等[7]提出Ribo-seq检测出的翻译中长链基因间ncRNA(large intergenic ncRNA, lincRNA)虽然被核糖体结合，但lincRNA的终止密码子处缺乏编码RNA中所具有的核糖体释放行为，因此推定绝大多数lincRNA不编码蛋白质。次年，两项研究通过质谱(mass spectrometry, MS)技术公布了人类蛋白质组草图[8-9]，声称发现千余个LncRNA所编码的“新蛋白质”。然而人类蛋白质组组织对此提出质疑：在更加严格的标准下进行质控分析，大部分“新蛋白质”不再出现在结果中，这表示原本的分析不够规范。

近十年中，ncRNA编码肽的研究与日俱增[10]，ncRNA编码肽的研究流程逐步成熟(图1)，ncRNA编码性的争论因此尘埃落定。2019年，LU等[11]利用RNC-seq和鸟枪蛋白质组学，检测到了308种LncRNA编码肽，其中207种得到多反应监测和/或平行反应监测的验证，最终经免疫印迹鉴定出了其中10种新蛋白。2020年，FLOWER等[12]对lincRNA编码肽研究设计了一种大同小异的工作流程。首先，通过高分辨率的液相色谱–串联质谱(liquid chromatography-tandem mass spectrometry, LC-MS/MS)对小鼠肾脏内髓质(inner medulla, IM)的匀浆样本进行测序，并结合RNA-seq构建lincRNA编码肽数据库对样本中的肽段进行匹配；其次，通过多数据综合的质量控制标准和生物信息学方法进一步评估由LC-MS/MS鉴定的肽。另外不同以上的流程，基于核糖体蛋白S6(ribosomal protein S6, RPS6)是识别ORF的重要因子，能够介导多聚A尾mRNA的翻译起始[13]，PANG等[14]通过使用针对RPS6的抗体对样本进行了RNA免疫沉淀，接着对得到的RNA进行高通量测序和编码潜力分析，最后对具有编码能力的LncRNA进行鉴定与功能研究。此外，FESENKO等[15]还总结了一些植物中的LncRNA编码肽的研究方法与流程。值得注意的是，由于circRNA的特殊性(缺乏末端5′帽和3′多腺苷酸尾的环状结构)，在研究中需要鉴定circRNA ORF翻译所依赖的结构，如内部核糖体进入位点(internal ribosome entry site, IRES)元件，N6-甲基腺嘌呤(N6-methyladenosine, m^6A)修饰[5]。

虽然研究ncRNA编码性研究体系已较完整，但目前仍无法大规模地鉴定编码性ncRNA。翻译组学和MS分析出的多数ncRNA编码肽未能通过免疫印迹鉴定，产生这一现象的因素众多，例如：Ribo-seq准

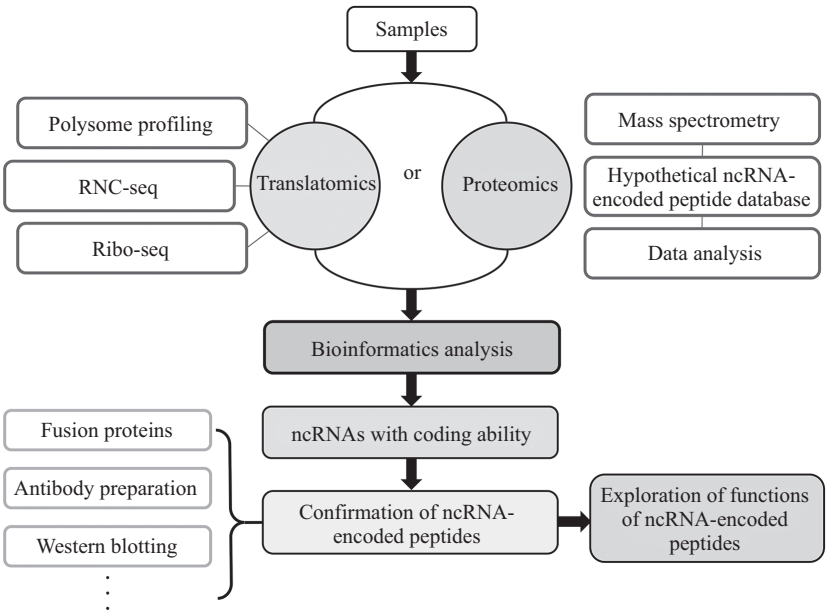


图1 鉴定ncRNA编码肽的工作流程

Fig.1 Workflow for identification of ncRNA-encoded peptides

表1 研究ncRNA编码性常用技术的局限性

Table 1 Limitations of common techniques for studying LncRNA with coding ability

技术 Techniques	局限性 Limitations
Polysome profiling[17]	Low RNA concentration in each fraction. Difficult to acquire enough amount mRNA for full-spectrum analysis. High concentration of sucrose inhibits some further enzymatic reactions.
Full length translating RNA analysis, RNC-seq[11]	Low sequencing accuracy. Cannot obtain information on ribosomal bound ORFs. Difficult to separate intact ribosome nascent-chain complex (RNC). The fragility of RNC leads to ribosome dissociation and mRNA breakage/degradation, which result in Biased analyses of RNC-mRNAs.
Ribosome profiling, Ribo-seq[18]	Ribosome protected fragments (RFPs) cannot represent translational activity. Repeatability was limited due to the influence of sample preparation. A considerable false positive rate. High sequencing and computational cost. Cannot detect and quantify the translating circRNA.
Mass spectrometry	Rely on protein databases to search.
Fusion protein	May affect the structure and function of original protein.
Western blot	Rely on high-quality antibodies. The smaller the molecular weight of the micropeptide, the more difficult it is to detect.

确度有限, MS技术对低丰度蛋白的灵敏性不足、未知序列的新蛋白无法进行搜库, 免疫印迹的技术局限等(表1)。也正因此, 需要研究人员的共同努力, 早日突破这些技术壁垒。

2 LncRNA编码肽与肿瘤

LncRNA是一类由RNA聚合酶II转录, 长度大于200个核苷酸的RNA转录本, 转录后常进行修饰, 例如聚腺苷酸化、加帽和剪接[19-22]。与mRNA相比, LncRNA具有更多的时空特异性和更低的物种间保守性[22-23]。具有细胞和组织特异性的LncRNA极可能具有在不同细胞和组织中执行特殊功能的属性。因此, 肿瘤特异表达的LncRNA一直以来在癌症诊断与治疗中备受关注, 顺理成章这些LncRNA编码

的功能性微肽也成为了研究的热点(表2和表3)。

2.1 肿瘤相关LncRNA编码肽的调控方式

LncRNA可以通过调控翻译影响肿瘤发展进程。LncRNA *ASH1L-AS1*编码的一个位于内质网的小肽(a peptide located in ER, APPLE), 通过直接结合poly(A)结合蛋白1[poly(A)-binding protein cytoplasmic 1, PABPC1]和真核翻译起始因子(eukaryotic translation initiation factor 4G, eIF4G)并增强两者的互作, 促进eIF4F起始复合体的组装和mRNA环化, 以增强细胞分化、增殖和凋亡关键通路中特异癌基因(如*KRAS*和*PIM1*等)的蛋白合成, 从而加速急性髓性白血病(acute myeloid leukemia, AML)恶性转化进程[25]。

LncRNA编码肽还通过调控mRNA剪接参与肿瘤的发生发展。结直肠癌(colorectal cancer, CRC)中高表达的LncRNA *LOC90024*编码微肽——剪接调节小蛋白(splicing regulatory small protein, SRSP) [26]。SRSP通过与富含丝氨酸和精氨酸的剪接调控因子3(serine- and arginine-rich splicing factor 3, SRSF3)相互作用, 促进SRSF3与*Sp4*的外显子3结合, 进而诱导包含外显子3的*Sp4*的转录以形成具有反式激活域的致癌的*L*-Sp4蛋白, 而缺乏反式激活域的“非癌性”*S*-Sp4的形成则受到抑制。同样地, 由LncRNA HOXB-AS3编码的保守多肽也调控了mRNA的剪接[27]。HOXB-AS3肽通过竞争性结合核内不均一性核糖核蛋白A1(heterogeneous nuclear ribonucleoproteins A1, hnRNP A1)的RGG基序中的精氨酸残基来阻断hnRNP A1与丙酮酸激酶(pyruvate kinase isozymes, *PKM*) mRNA的结合, 抑制*PKM* mRNA的剪接以及PKM2和miR-18a的形成, 从而通过抑制有氧糖酵解

表2 肿瘤相关功能性LncRNA编码肽的基本信息及功能(根据参考文献[24]修改)

Table 2 Basic information and functions of tumor-associated functional peptides encoded by LncRNAs (modified from reference [24])

微肽 Micropeptide	基因名 Symbol	长度/aa Length /aa	癌症 Cancer	功能 Function
APPLE[25]	*ASH1L-AS*1	90	AML	Enhance protein synthesis of specific oncogenes in key pathways of cell differentiation, proliferation, and apoptosis
ASAP[28]	*LINC00467*	94	CRC	Promote colorectal cancer cell proliferation
ASPRS[40]	*LINC00908*	60	TNBC	Inhibit tumor angiogenesis and restricts tumor cell migration
CIP2A-BP[37]	*LINC00665*	52	TNBC	Inhibit tumor invasion and metastasis
CRNDEP[45]	*CRNDE*	84	OC	May be involved in the cell proliferation
HESRG[42]	*ESRG*	105	Germinoma & EC	A novel, sensitive and specific biomarker for intracranial germinoma and EC
HBVPTPAP[38]	*HBVPTPAP*	145	HCC	Raise the mitochondrial membrane potential and induce the apoptosis
HOXB-AS3[27]	*HOXB-AS3*	53	CC	Inhibiting cell proliferation, invasion, and metastasis
KRASIM[46]	*NCBP2-AS2*	99	HCC	Inhibit carcinogenic signaling in hepatocellular carcinoma cells
PACMP [43]	*CTD-2256P15.2*	44	BC	Regulate cancer progression and drug resistance by modulating DDR
RBRP[47]	*LINC00266*	71	CRC	Promote cell proliferation and metastasis
SMIM30[14]	*LINC00998*	59	HCC	Promote cell proliferation and migration
SRSP[26]	*LOC90024*	130	CRC	Induce “cancerous” Sp4 splicing variant formation, promote CRC tumorigenesis and progression
UBAP1-AST6[11]	*UBAP1-AST6*	Not mentioned	LC	Promote the cell proliferation
YY1BM[39]	*LINC00278*	21	ESCC	Promote apoptosis and downregulate the survival rate of ESCC cells

aa: 氨基酸; AML: 急性髓性白血病; CRC: 结直肠癌; TNBC: 三阴性乳腺癌; OC: 卵巢癌; EC: 胚胎癌; HCC: 肝细胞癌; CC: 结肠癌; BC: 乳腺癌; LC: 肺癌; ESCC: 食管鳞状细胞癌; DDR: DNA损伤应答。

aa: amino acid; AML: acute myelocytic leukemia; CRC: colorectal cancer; TNBC: triple-negative breast cancer; OC: ovarian cancer; EC: embryonal carcinoma; HCC: hepatocellular carcinoma; CC: colon cancer; BC: breast cancer; LC: lung cancer; ESCC: esophageal cell squamous carcinoma; DDR: DNA damage response.

表3 其他功能性LncRNA编码肽的基本信息及功能

Table 3 Basic information and functions of other functional peptides encoded by LncRNAs

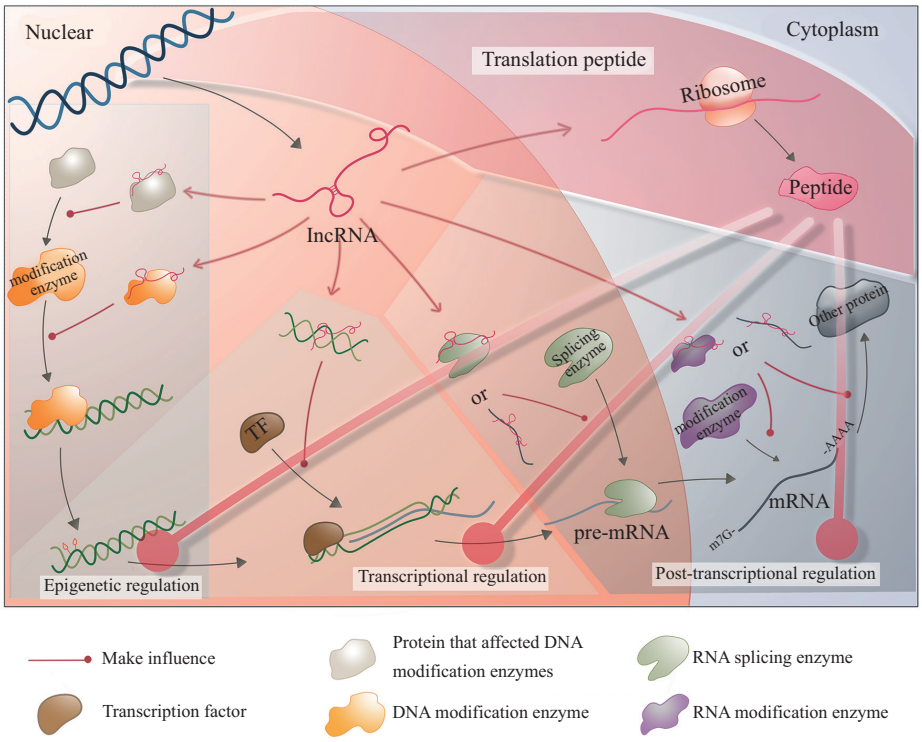
物种 Species	微肽 Micropeptide	基因名 Symbol	功能 Function
Human	SPAR[48-49]	*LINC00961*	Inhibit mTORC1[48] Promote muscle development [49]
Human	Mtln[50]	*MTLN* (LINC00116)	Supports mitochondrial supercomplexes and respiratory efficiency
Mouse	MOXI[51]	*Mtln* (1500011K16Rik*)*	Enhances fatty acid beta-oxidation
Human	NoBody[52-53]	*LINC01420*	Decrease P-bodies and mRNA.
Human	P155[54]	*MIR155HG*	Suppress autoimmune inflammation.
Human	STORM[55]	*LINC00689*	Inhibit protein secretion.
Mouse	MLN[56]	*Mrln*	Inhibit SERCA (sarcoendoplasmic reticulum calcium transport ATPase)
Mouse	DWORF[56]	*Strit1*	Activate SERCA.
Mouse	EPRp[57]	*EPR*	Promote epithelial tight junction.
Mouse	Kastor & Polluks[58]	*Gm9999*	Regulate sperm development
Mouse	Aw112010-encoded Peptide[59]	*AW112010*	Cause immune response.
Fruit fly	Scl[60]	*SclB*	Regulate calcium transport and hence influence regular muscle contraction
Fruit fly	Pgc[61]	pgc	Repressing CTD Ser 2 phosphorylation
Chicken	Six1 ORF2[62]	*SIX1*	Promote cell proliferation and involved in muscle growth.
Zebrafish	Toddler[63]	*apela*	Promote gastrulation movements
Soybean	ENOD40[64]	*ENOD40B*	Interact with sucrose synthase and control sucrose use in nitrogen-fixing nodules
Bacillus subtilis	MciZ[65]	*mciZ*	Prevent inappropriate Z-ring formation during sporulation
Bacillus subtilis	Sda[66]	*sda*	Inhibit the sporulation of strains utilizing either KinA or KinB as the sole sporulation-specific histidine kinase

来抑制CRC细胞的增殖、侵袭和转移。

LncRNA编码肽能够以不同的方式调控肿瘤细胞的新陈代谢。与HOXB-AS3肽不同, 由*LINC00467*编码的ATP合酶相关肽(ATP synthase-associated peptides, ASAP)促进了CRC的增殖[28]。ASAP通过与ATP合酶的亚基α和γ相互作用增强了ATP合酶的构建, 提高了ATP合酶活性和线粒体耗氧率, 在CRC细胞中敲除*ASAP*能够抑制细胞增殖和移植瘤的生长。综上所述, 如同LncRNA能够以多种方式(表观遗传、转录及转录后)对靶基因进行调节, LncRNA编码的多肽也以不同的方式调控细胞的生命活动(图2)。揭示在同一细胞生命活动中具有不同调控方式的微肽, 能够有条理地揭示细胞内复杂的调控网络的隐秘地带, 也可以为靶向癌症治疗提供新的理论基础。

2.2 肿瘤相关LncRNA编码肽的功能

LncRNA编码肽是肿瘤发生发展的关键信号通路的调控因子, 影响了肿瘤细胞的增殖、迁移、侵袭与转移。在肝细胞癌(hepatocellular carcinoma, HCC)组织中, 由高表达的*LINC00998*编码的促癌微肽——SMIM30通过与蛋白激酶SRC/YES1的氨基末端结构域相互作用, 介导其膜锚定和磷酸化, 进而激活下游MAPK信号通路, 促进HCC细胞的增殖和迁移[14]。而在三阴性乳腺癌(triple-negative breast cancer, TNBC)中我们鉴定了一个由*LINC00665*编码的抑癌微肽[37], 该微肽与蛋白磷酸酶2A癌性抑制因子(cancerous inhibitor of protein phosphatase 2A, CIP2A)结合, 因此我们将其命名为CIP2A结合肽(CIP2A binding peptide, CIP2A-BP)。CIP2A-BP通过取代蛋白磷酸酶2(protein phosphatase 2, PP2A)的B56γ亚基的结合位点, 释放了PP2A活性, 抑制PI3K/AKT/NF-λB通路的激活, 进一步抑制*MMP2*、*MMP9*和*SNAIL*的表达, 从而抑制三阴性乳腺癌细胞的侵袭和转移。同时, CIP2A-BP的翻译受到转化生长因子β(transforming growth factor-β, TGF-β)的调控, TGF-β激活Smad信号通路后促进翻译抑制蛋白4E-BP1的表达, 从而抑制LINC00665的翻译, 这进一步解释了TGF-β的促癌作用。这提示, LncRNA编码肽不仅从LncRNA的转录, 还从翻译上受到了调控, 紧



LncRNA广泛参与到靶基因的表观遗传调节、转录调节及转录后调节中。在表观遗传调节中, LncRNA不仅能够直接作用于DNA修饰酶[29], 还能够作用于影响DNA修饰酶稳定性的蛋白[30], 从而直接或间接地对DNA修饰进行调控。在转录调节中, LncRNA一方面直接结合靶DNA 形成三螺旋结构调节其转录[31-32], 另一方面通过与转录相关蛋白相互作用[33-34]影响DNA的转录。在转录后调节中, LncRNA能够与靶mRNA或pre-mRNA互补杂交, 影响mRNA的剪辑、编辑和稳定性[35]。此外, LncRNA还能够通过结合RNA剪接因子或RNA修饰酶发挥转录后调节功能[36]。同样地, LncRNA 编码肽也能够以这样不同的方式发挥功能, 具体的例子在上文中已提及。

LncRNAs are widely involved in the epigenetic, transcriptional, and post-transcriptional regulation of target genes. In epigenetic regulation, LncRNAs can not only directly act on DNA modification enzymes[29], but also act on proteins that affect the stability of DNA modifying enzymes[30], so as to regulate DNA modification directly or indirectly. In transcriptional regulation, on the one hand, LncRNAs directly bind to target DNA to form a triple helix structure[31-32] to regulate DNA transcription. On the other hand, it affects DNA transcription by interacting with transcription related proteins[33-34]. In post-transcriptional regulation, LncRNA targets complementary RNA or pre-mRNA, affecting mRNA editing, editing and stability[35]. In addition, LncRNAs can also play a post transcriptional regulatory function by binding to RNA splicing factors or RNA modification enzymes[35]. Similarly, LncRNA-encoded peptides are able to function in such different ways. These specific examples are mentioned above.

图2　LncRNA及其编码肽的常见功能方式(根据参考文献[16]修改)

Fig.2　Common functional modes of LncRNAs and their encoded peptides (modified from reference [16])

密参与到偌大的信号调控网络中。

LncRNA编码肽以不同路径参与调控肿瘤细胞的凋亡过程。LncRNA *HBVPTPAP*编码的微肽与配对免疫球蛋白样2型受体α(paired immunoglobin like type 2 receptor alpha, PILRA)胞内结构域的相互作用, 激活下游JAK/STAT信号通路, 进而提高线粒体膜电位, 促进HCC细胞凋亡[38]。由*LINC00278*编码的阴阳1(Yin Yang 1, YY1)结合微肽(YY1-binding micropeptide, YY1BM)则以不同的方式诱导食管鳞状细胞癌(esophageal cell squamous carcinoma, ESCC)细胞凋亡[39]。YY1BM通过阻断YY1和雄激素受体(AR)之间的结合, 抑制真核细胞延伸因子2激酶(eukaryotic elongation factor 2 kinase, *eEF2K*)基因的转录, 增强真核翻译延伸因子2(eukaryotic translation elongation factor 2, eEF)的活性, 从而促进ESCC细胞的凋亡。而在营养剥夺条件下, 低表达的YY1BM导致了eEF2K的高表达, 逆转了营养缺乏诱导的ESCC细胞凋亡, 使得癌细胞具有更强的生存能力。

LncRNA编码肽还参与调控肿瘤的血管生成。众所周知, 实体瘤的生长依赖于血管生成, 而血管内皮细胞生长因子(vascular endothelial growth factor, VEGF)/血管内皮细胞生长因子受体(VEGF receptor, VEGFR)信号通路是肿瘤血管生成的关键调控网络。由*LINC00908*编码的微肽能够结合信号转导与转录激活因子3(signal transducer and activator of transcription 3, STAT3), 因而被命名为STAT3小调节肽(a

small regulatory peptide of STAT3, ASRPS)。ASRPS通过结合STAT3 C-端的卷曲螺旋域(coiled-coil domain, CCD), 抑制STAT3在Tyr705位点的磷酸化, 从而抑制VEGF的转录和表达, 减少肿瘤血管生成, 限制肿瘤细胞的增殖迁移[40]。在TNBC中*LINC00908*表达下调与TNBC患者预后不良相关。

2.3 肿瘤相关LncRNA编码肽的应用

在肿瘤组织中特异性表达的LncRNA被认为是潜在的有效生物标志物[41]。同样地, 肿瘤组织中特异性表达的LncRNA编码肽作为生物标志物为癌症的诊断提供新的视角。例如, 由LncRNA *ESRG*编码的微肽被鉴定为生殖细胞瘤和胚胎癌的特异性生物标志物[42], 该肽在生殖细胞瘤和胚胎癌中特异性高表达, 而在其他颅内生殖细胞肿瘤(germ cell tumor, GCT)和非GCT组织中未被检测到。不仅如此, 最新的研究还揭示了参与调节肿瘤耐药性的LncRNA编码肽。GUO等[43]发现了由LncRNA *CTD-2256P15.2*编码的微肽以双重机制调控DNA损伤应答(DNA damage response, DDR)过程, 影响肿瘤的发展和耐药性。LncRNA *CTD-2256P15.2*在表柔比星(epirubicin, EPI)耐药性乳腺肿瘤中高度表达, 其编码的微肽一方面通过与羧基末端连接蛋白反应蛋白(CtBP-interacting protein, CtIP)竞争结合泛素连接酶Cullin3的底物衔接分子——Kelch样蛋白15(Kelch-like protein 15, KLHL15)抑制CtIP的泛素化和降解, 从而促进DNA同源重组修复; 另一方面直接结合多聚ADP核糖化(poly ADP-ribose, PAR)链, 通过其携带的正电荷中和PAR链的负电荷, 促进多聚ADP核糖聚合酶1(poly ADP-ribose polymerase 1, PARP1)催化的PAR。因此该微肽命名为PAR扩增和维持CtIP稳定微肽(PAR-amplifying and CtIP-maintaining micropeptide, PACMP)。单独靶向PCAMP能显著抑制肿瘤细胞生长, 还能促进肿瘤细胞对放疗、化疗(喜树碱、表阿霉素)、靶向治疗(PARPi、ATR以及CDK4/6抑制剂)等多种药物的敏感性, 明显改善疗效。

几十年来, 人们一直认为靶向癌症标志中心的特定蛋白质可以代表能够显着降低癌症死亡率的重大治疗进展[44]。然而, 越来越多的证据表明, ncRNA表达水平的改变在癌细胞生物学中起着相关作用, 与癌症患者的不良临床结果有关, 并且可能成为新型和更有效的癌症疗法的宝贵靶标。鉴于微肽的小分子性和细胞膜渗透性、溶解性, 微肽可以很容易地在细胞区间穿梭, 并通过细胞外囊泡参与细胞间的细胞间通讯, 相对于大蛋白, 微肽是更为理想的药物。同时, 与miRNA不同, 微肽不会潜在地结合和激活Toll样受体, 从而诱导细胞因子风暴对肾脏和肝脏功能较差的转移性癌症患者产生严重的副作用。除了胰岛素这一经典的例子, 卡非佐米、硼替佐米、地加瑞克等也能够佐证多肽类药物的优势及有效性。因此, 使用天然的抑癌性微肽治疗癌症是一种更安全的值得发展的方法, 可能具有振奋人心的癌症治疗潜力。以上提到的微肽ASRPS、CIP2A-BP以及YY1BM均在相应的动物模型中验证了它们的肿瘤抑制作用[37,39-40]。由于微肽高特异性、高效价和低毒性的特点, 其作为治疗剂具有极大的吸引力, 我们希望这些能作为癌症药物的ncRNA编码的天然微肽被不断地鉴定出来。

3 circRNA编码肽与肿瘤

circRNA是具有共价闭合结构的一类ncRNA, 具有与线性RNA相似的转录过程[67-69], 但缺乏末端5′帽和3′多腺苷酸尾结构[70]。circRNA具有重要的生理功能, 例如, 与RNA聚合酶II和小核核糖核蛋白(small nuclear ribonucleoproteins, snRNPs)相互作用来调节mRNA的转录和剪接[71], 以及作为miRNA海绵与靶miRNA结合并影响肿瘤发生和转移[72-73]。与LncRNA一样, circRNA也在多种癌症中表现出异常表达, 具有高度的组织特异性[74-77]。不同的是, circRNA比线性RNA更能耐受核酸外切酶降解, 有望成为肿瘤诊断的生物标志物, 并为癌症治疗提供新靶点[78]。circRNA中也存在高度保守的ORF, 并以独立于5′帽结构的方式编码微肽: circRNA ORF通过IRES元件招募核糖体进行翻译[79]; 或者通过包含m^6A位点的短序列结合m^6A修饰结合蛋白YTHDF与翻译起始因子eIF4G2相互作用启动circRNA的翻译[80]。此外, 环状结构造成了circRNA翻译的滚动循环[81-84], 进而产生超过100 aa的蛋白质[85]。

目前鉴定了胶质母细胞瘤(glioblastoma multiforme, GBM)中具有抑癌功能的三种circRNA编码肽。FBXW7-185aa由Circ-FBXW7上的IRES元件驱动编码, 通过阻止去泛素化酶USP28稳定促癌蛋白c-Myc来缩短c-Myc的半衰期, 因此FBXW7-185aa的上调能够抑制GBM肿瘤细胞增殖和细胞周期进程[85]。SHPRH-146aa由SNF2组蛋白连接子PHD环解旋酶(SNF2 his-

tone linker PHD RING helicase, *SHPRH*)基因的环状转录本circ-SHPRH编码, 它保护SHPRH免受蛋白酶体的降解, 是GBM中的肿瘤抑制因子[86]。同样地, 由*LINC-PINT*产生的circRNA编码的微肽通过直接与聚合酶相关因子复合物(polymerase associated factor complex, PAF1c)相互作用, 抑制多个癌基因的转录[87]。这些circRNA编码肽在GBM中的水平都较低, 过表达这些微肽则能抑制GBM细胞在体外和体内的增殖。这提示了这些具有抑癌功能的微肽作为肿瘤治疗药物具有一定的可行性。而在结肠癌组织中高度表达的*circPPP1R12A*编码了一种促癌的微肽——circPP1R12A-73aa[88], 在体外*circPPP1R12A*的沉默能够明显地抑制结肠癌细胞的增殖、迁移和侵袭, 这为结肠癌的治疗提供了新的方向与着眼点。

需要注意的是, LEGNINI等[89]发现在肌肉细胞中高表达的circ-ZNF609由*ZNF609*的第二个外显子的环化形成, 能够调节成肌细胞增殖, 并编码微肽[89]。在后续工作中, circ-ZNF609还被鉴定出在多种癌症中高表达并起促癌作用, 敲低circ-ZNF609可显著抑制癌细胞增殖、侵袭和转移[74-76]。但是, circ-ZNF609编码的微肽的功能一直以来未被发掘, 该微肽是否也促进了肿瘤的发展, 这些组织中微肽的编码是否具有特异性, 需要进一步的研究。

4 miRNA编码肽与肿瘤

miRNA长度约为22个碱基, 主要通过基于序列互补性靶向mRNA来控制基因表达, 从而参与发育和疾病的调节[90]。成熟的miRNA首先被转录为原代转录本(pri-miRNA), 随后经剪切产生pre-miRNA, 最后进一步剪切为成熟miRNA。目前发现的miRNA编码肽(miRNA encoded peptide, miPEP)较少, 均由pre-miRNA翻译。一些具有农艺应用的miPEP, 例如不同植物中与不定根的形成发育有关的miPE-P165a[6]、miPEP171b[6]以及vvi-miPEP171d1[91]最先被研究。此外, MONTIGNY等[92]在果蝇中也发现了发育相关的几种miPEP[92]。

肿瘤中miRNA的研究相对成熟, 多年来已经进行了大量涉及miRNA疗法的临床前研究。但由于其分子稳定性、潜在的毒性和脱靶效应等, 到目前为止只有少数miRNA疗法进入了临床开发[77]。值得注意的是, 前列腺癌细胞中, miR-200a和miR-200b的pri-miRNA分别编码两个肿瘤相关的miPEP——miPEP-200a和miPEP-200b, 这两个miPEP通过调控前列腺癌细胞从上皮到间充质的转变来抑制癌细胞的转移[93]。尽管例子有限, 但miPEP不可否认地参与到了肿瘤的发生发展中。几十年以来miPEP缓慢的研究进展提示了我们一些问题: 在ncRNA编码性翻译组学研究过程中, 具有核糖体结合的翻译中pri-mRNA是否被忽略？ miPEP是否偏向于在植物与昆虫中表达？问题的答案并不简单, 因为我们仍处于理解人类基因组"暗物质"功能的最初阶段。

5 结论与展望

参与肿瘤发生发展的ncRNA编码肽研究正在受到越来越多的研究者关注, 但尚有大量的ncRNA编码肽有待进一步发现和鉴定。ncRNA编码肽极有可能为肿瘤研究提供一个新的视角: 通过它揭示肿瘤发生发展的潜在机制; 同时, 因为ncRNA编码肽是一种内源性肽, 具有细胞毒性小、免疫原性低的优势, 将ncRNA编码肽用于肿瘤治疗也为新的肿瘤治疗方法提出一种新的尝试方向。

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