

环状RNA在头颈部肿瘤中的作用

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摘要 环状RNA(circular RNA, circRNA)是一类闭合环状非编码RNA, 它们大量稳定存在于各种生物细胞中并具有组织特异性表达等特征。近年来研究表明, 一些circRNA分子具有微RNA海绵、RNA结合蛋白海绵和转录调节等作用, 在生物应用领域潜力巨大。头颈部肿瘤患者呼吸、吞咽和发音均可受到严重影响, 患者“有苦难言”、生活质量不高。为提高对此类肿瘤的认识, 该文就近年来circRNA的研究新进展及其在头颈部肿瘤中的作用作一综述, 旨在为进一步研究其在头颈部肿瘤中的作用提供新的思路和依据。

关键词 环状RNA; 微RNA; 头颈部肿瘤; 基因表达

Roles of Circular RNA in Head and Neck Cancers

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Abstract Circular RNA (circRNA) is closed continuous loops noncoding RNA. They are stably expressed in a variety of bio-logical cells, and have tissue specificity and other characteristics. Recently, the studies on circRNAs have shown that many circRNAs play roles as microRNA sponges, RNA-binding protein sponges, transcriptional regulators and so on. These roles give circRNAs a great potential in biological applications. Patients with head and neck cancers usually have serious troubles in respiration, swallowing and pronunciation. They suffer from pain of “hard to express” and have poor quality of life. As a result, to improve our understanding of this kind of cancers, this paper briefly reviewed the research progress of circRNA and their roles in head and neck cancers in recent years. The purpose is to bring some new view-points and bases for further study on circRNAs in head and neck cancers.

Keywords circular RNA; microRNA; head and neck cancer; gene expression

头颈部肿瘤(head and neck cancer, HNC)包括源自鼻腔、口腔、咽、喉、鼻窦等头颈部组织中的肿瘤, 其中约95%为鳞状细胞癌^[1]。据报道, 头颈部鳞状细胞癌(head and neck squamous cell carcinoma, HNSCC)在全球癌症常见死亡原因中排名第6, 而吸烟和酗酒是其重要的诱因^[2]。在过去的30年里, 虽然

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新的手术方法、化疗药物和先进的放射疗法已被用于HNSCC的治疗,但HNSCC患者总生存期一直没有得到改善^[3]。不仅如此,治疗引起的副作用给患者身体和心理上带来了极大的痛苦。肿瘤细胞区域和/或远处转移是造成HNSCC患者死亡的主要原因^[4],造成这种结果的根本原因是早期诊断的准确性不高及疗效不佳^[5]。因此,研究头颈部肿瘤发生的分子机制,探索新的、安全的、有效的诊疗方法具有重要意义。

环状RNA(circular RNA, circRNA)作为非编码RNA(non-coding RNA, ncRNA)的新成员,凭借其自身独特的环状结构,在真核细胞中稳定表达,且其表达水平具有细胞和发育阶段特异性^[6-8]。目前,功能研究表明, circRNA可以靶向结合内源性微RNA(microRNA, miRNA),抑制miRNA的生物学活性,继而发挥miRNA海绵的作用,亦称为竞争性内源RNA(competing endogenous RNA, ceRNA)作用^[8-9]。小脑退化相关蛋白1反义转录物(cerebellar degeneration-related protein 1 transcript, Cdr1as/ciRS-7)和 cirSry(sex-determining region Y)作为miRNA的分子海绵可算作其中最为经典的2个例子^[9-10]。其中, ciRS-7拥有超过60个miR-7的结合位点,远远超过之前发现的任何一种线性分子海绵^[10-12]。研究发现, circRNA可能参与了多种疾病的发生、发展过程,有望成为新一代疾病诊断的生物标志物^[13-17]。本文主

要介绍circRNA的形成和生物学功能,并讨论其在头颈部肿瘤中的作用。

1 circRNA的形成

长期以来,真核生物mRNA前体(pre-mRNA)都被认为在内含子剪切及外显子拼接后产生线性mRNA,随后翻译产生蛋白质。近年来研究发现,蛋白质编码基因也可以通过可变剪接产生多种多样的转录产物, circRNA就是来源于一种特殊的反向可变剪接(back splicing),其中一些基因的circRNA表达水平比相应的线性还高得多^[11,18]。

circRNA有3种存在形式:外显子环状RNA(exonic circRNA)、内含子环状RNA(circular intronic RNA, ciRNA)和外显子-内含子环状RNA(exon-intron circRNAs, EICIcRNAs)。关于外显子环化的形成, Jeck等^[19]提出了两种模型(图1)。(1)套索驱动环化(lariat-driven circularization):与外显子反向剪接有关,一个外显子的3'剪接供体(splice donor)与另一个外显子的5'剪接受体(splice acceptor)共价结合,接着切除内含子,然后形成circRNA(MODEL-1);(2)内含子配对驱动环化(intron-pairing-driven circularization):主要驱动力为内含子与内含子之间的互补配对,首先将Pre-mRNA从链状变为环状结构,再进一步剪切环中的内含子,最终形成 circRNA(MODEL-2)。在这2种模型的外

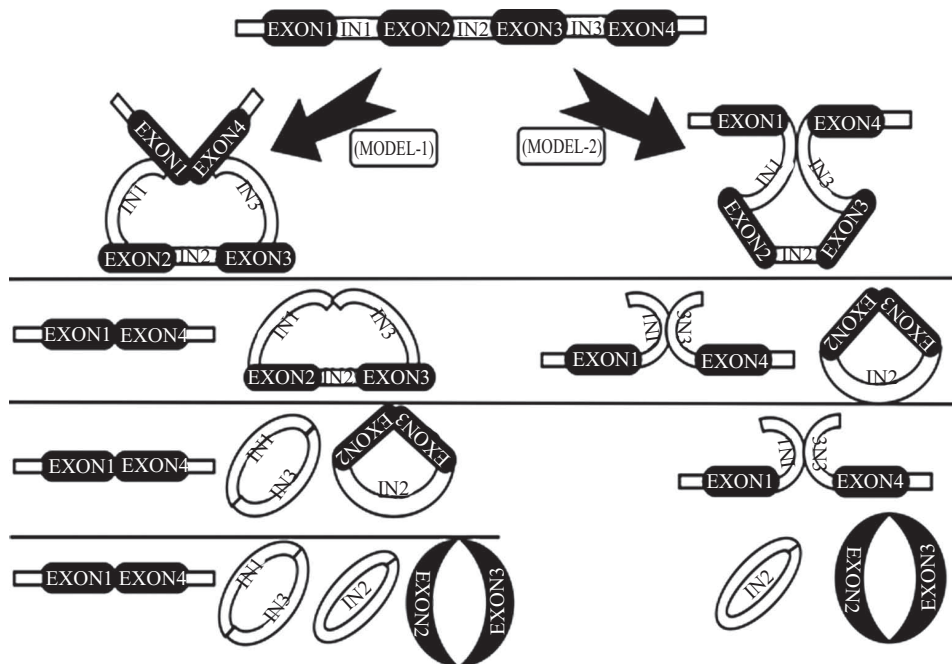


图1 外显子环状RNA的形成过程(根据参考文献[19]修改)

Fig.1 The formation of exonic circRNA (modified from reference [19])

显子侧翼区域都发现有重复且互补的ALU长内含子(long flanking introns)序列。Zhang等^[20]采用RNaseR酶对去rRNA的样品进行处理富集circRNA, 在人源胚胎干细胞H9中检测出近万条circRNA, 并且利用高通量测序的方法, 证明circRNA可由外显子侧翼内含子互补配对驱动外显子环化, 为上述Jeck提出的内含子配对驱动环化模型提供了事实依据。此前, Zhang等^[21]发现了内含子在传统剪切过程中可形成RNA套索结构, 这种套索结构因其无3'末端, 能大量稳定地存在于细胞内, 又称为内含子circRNA(ciRNA)。Li等^[22]在宫颈癌HeLa细胞的细胞核中发现一类EIciRNA, 这类circRNA的内含子在成环过程中并没有被剪切, 而是保留在外显子之间。这一研究更加丰富了circRNA的形成原理, 体现了基因转录、转录后水平的多样性和复杂性(图2)。

除了上述几种由Pre-mRNA可变剪接产生的三种circRNA外, Lu等^[23]发现一类由tRNA前体(pre-tRNA)剪接产生的新型circRNA, 称为tRNA内含子环状RNA(tRNA intronic circular, tricRNA)。研究发现, tricRNA的生物合成需要保守的tRNA序列及加工酶, 而且其表达具有时间和组织特异性。不仅建立的tricRNA表达系统为circRNA在人类或动物体内的表达提供了一个新方法, 而且内源性的tricRNA自身可能也扮演着重要角色。已有研究报道, tRNA缺陷可以引起神经退化性疾病, 那么tricRNA可能作为一种治疗的生物标志物, 修复tRNA的缺陷。

2 circRNA的功能

2.1 circRNA的海绵作用

2.1.1 circRNA的miRNA海绵作用 miRNA可与mRNA的非翻译区特异性结合, 发挥其在转录后水

平调节基因表达的能力。然而, 在真核生物细胞中存在一类ceRNA具有miRNA应答元件(miRNA response element, MRE), 可以与miRNA结合并使其丧失对靶基因表达的抑制作用, 即miRNA海绵作用^[24]。近年来, 研究发现, 外显子circRNA存在于细胞质中, 其中一部分发现拥有大量MRE^[9-10,25]。

Hansen等^[9]首先发现来自鼠的性别决定区Y(sex-determining region Y, SRY)的circSry具有miRNA海绵功能, circSry拥有16个miR-138结合位点。不仅如此, 在人和小鼠脑细胞胞质中发现的Cdr1as/ciRS-7拥有超过70个miR-7选择性的保守结合位点。作为反义转录物, Cdr1as/ciRS-7不仅可以调节正义链的表达, 而且可以与miR-7结合, 调节其靶基因的表达。实验证明, 过表达Cdr1as/ciRS-7或circSry, miRNA靶基因的表达水平增加, 而降低这些circRNA则得到相反的结果^[10]。体内实验进一步表明, 用人类或小鼠的Cdr1as/ciRS-7注射斑马鱼胚胎能减少其中脑体积的大小, 类似于miR-7沉默的表型, 且注射miR-7前体后情况有所好转^[10]。紧接着, Geng等^[26]和Yu等^[27]分别在心肌细胞和肝癌细胞中也发现了Cdr1as/miR-7轴的存在。除了上述经典的2个circRNA, 在疾病研究中也发现了大量具有miRNA海绵作用的circRNA。Wang等^[28]发现一种与心脏相关的circRNA(HRCR)可作为内源性miR-233的分子海绵抑制心肌肥厚和心力衰竭。在骨关节炎的软骨细胞中, Liu等^[29]发现一种CircRNA-CER可以与MMP13竞争miR-136的结合位点参与软骨细胞外基质降解过程, 可作为骨关节炎治疗靶点。不仅如此, circRNA、长链非编码RNA(long non-coding RNA, lncRNA)、miRNA三者之间的共同作用也有相关研究报道。在铅神经毒性小鼠模型的海马和大脑

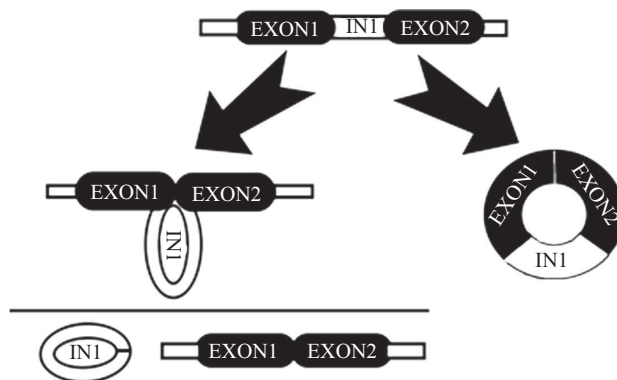


图2 内含子环状RNA(ciRNA)和外显子-内含子环状RNA(EIciRNAs)的形成过程(根据参考文献[22]修改)

Fig.2 The formation of ciRNA and EIciRNAs (modified from reference [22])

皮层中,促凋亡长链非编码RNA(lncRNA)和凋亡相关circRNA(circRar1)表达上调,可通过共同的靶标miR-671促进神经元凋亡^[30]。Lan等^[31]通过芯片和生物信息学数据分析发现,人类椎间盘中RNA丰度组成分别为:mRNA(14 635, 37.93%)、miRNA(2 059, 5.34%)、lncRNA(18 995, 49.23%)、circRNA(2 894, 7.5%),同一miRNA可与不同的circRNA相互作用。对RNA全貌的认识有利于揭示疾病发生中的RNA相互作用机制。

然而,又有研究表明,circRNA的miRNA海绵作用只是其中某些circRNA独有的功能,而不是circRNA的普遍特性^[8]。有人挑选了7 112个人的circRNA和635只小鼠的circRNA为实验对象,发现其中大多数人circRNA只在少数在细胞中表达,而且细胞特异性不强、丰度较低,小鼠circRNA中与人同源的circRNA表达量也并未高于相邻的线性外显子^[8]。

2.1.2 circRNA的RNA结合蛋白海绵作用 有些circRNA分子还可以与RNA结合蛋白(RNA-binding protein, RBP)相结合,作为蛋白质海绵。如Cdr1as/ciRS-7和circSry可与miRNA效应因子阿格蛋白(argonaute, AGO)相结合^[10,32],从而被切割或者抑制翻译,最终被降解。Guo等^[8]应用采光可激活的核苷增强交联和免疫共沉淀(photo-activatable ribonucleoside cross-linking and immunoprecipitation, PAR-CLIP)对20种RBP进行分析,发现circRNA外显子RBP的结合密度比其相邻的外显子略高。然而,You等^[33]对38种RBP的序列进行生物信息学分析发现,circRNA外显子中RBP结合密度比编码序列或mRNA的3'-非翻译区(3'-untranslated region, 3'-UTR)低。这两种结论未必是相反的,可能因为circRNA无蛋白质编码功能,与其结合的RBP不容易被转移,造成circRNA中RBP的结合密度比生物信息学预测的要高。Ashwal-Fluss等^[34]发现,果蝇和人MBL/MBNL1(muscleblind)基因的pre-mRNA可通过剪接形成circRNA(circMbL),在circMbL侧翼内含子区域含有大量MBL蛋白质的结合位点。实验证明,circMbL与线性MbL的生物合成之间存在竞争关系,circMbL可以通过与MBL蛋白质特异性结合来调控MBL的表达水平,而且这种调控强度取决于所含MBL结合位点的多少。Lesca等^[35]发现,circANRIL与pescadillo核糖体生物合成因子1(pescadillo homologue 1, PES1)结合可以抑制核糖体RNA的成

熟、诱导核仁应激和p53的活化,从而诱导细胞凋亡和抑制细胞增殖,可能在动脉粥样硬化中起关键作用。

近期,circRNA与蛋白质的关系又有了新的突破。Tim等^[36]发现,circRNA可与蛋白结合形成circRNA蛋白质复合物(circRNA-protein complexes, circRNPs)。这些circRNPs大小不一,可通过甘油密度梯度离心在细胞质裂解液中分离得到。实验以RNA结合蛋白胰岛素样生长因子2结合蛋白3(insulin-like growth factor 2 binding protein 3, IMP3)为主,验证了可与IMP3形成circRNP的几种circRNA,并将其归为同一个亚类,而且发现,由特异性蛋白质定义的circRNA亚类可能具有共同的生物学功能和合成途径。

2.2 circRNA的转录调节作用

circRNA无3'末端,可以逃脱分支酶的降解作用而在细胞中累积。Zhang等^[21]在细胞核中发现大量ciRNA,只有少量的MRE,无法充当miRNA海绵,但它们却有一定调控转录效率的作用。实验发现^[21],将ci-ankrd52、ci-mcm5和ci-sirt7沉默后,其亲本基因的mRNA表达量减少,其中ci-ankrd52被证明具有调控RNA聚合酶II转录活性的功能,在此过程中ciRNA可能作为顺式作用元件发挥作用。Li等^[22]发现,EIciRNA可在基因启动子区域与U1核小核糖核蛋白(small nuclear ribonucleoprotein, SnRNP)及RNA聚合酶II相互作用增强基因转录水平。

2.3 circRNA的翻译作用

1995年,Chen等^[37]在体外构建了环状mRNA,可特异地依赖内部核糖体进入位点(internal ribosome entry site, IRES)结合核糖体,从而启动蛋白质翻译,表明有些没有5'帽和3'多聚(A)尾的mRNA可依靠IRES起始翻译。有些circRNA也可依赖此途径开始翻译。近年,Guo等^[8]在人骨肉瘤细胞U2OS中发现,circRNA具有低效率翻译功能。前不久,Abe等^[38]首次提出体外合成的circRNA,在特异性内部启动元素缺失的情况下,也可在人类活体细胞中通过滚环扩增机制进行翻译,而且比同型线性RNA翻译速率快2个数量级。

3 circRNA与头颈部肿瘤

3.1 头颈部肿瘤中充当miRNA海绵作用

研究发现,circRNA在肿瘤发生、发展中发挥

表1 与circRNA相互作用的miRNA及其在头颈部肿瘤中的作用
Table 1 The miRNAs related to circRNAs and their roles in head and neck tumors

环状RNA circRNA	微RNA miRNA	肿瘤位置 Tumour location	miRNA的作用 Role of miRNA	参考文献 References
ciRS-7, cir-ITCH	miR-7	Laryngeal	Antioncogene	[40]
		Nasopharyngeal	Antioncogene	[41-43]
		Tongue	Antioncogene	[44]
cir-Sry	miR-138	Laryngeal	Antioncogene	[49]
		Nasopharyngeal	Antioncogene	[54]
		Tongue	Antioncogene	[51-52]
		Oral	Antioncogene	[53]
cir-ITCH	miR-17	Nasopharyngeal	Oncogene	[56-57]
		Oral	Antioncogene	[58]
	miR-214	Nasopharyngeal	Oncogene	[59-60]
		Tongue	Oncogene	[61]

重要作用, 有潜力成为肿瘤诊断和治疗的生物标志物。circRNA具有miRNA海绵的作用, 而这些miRNA在头颈部肿瘤中的作用可为circRNA在头颈部肿瘤中的研究提供思路(表1)。

3.1.1 ciRS-7/miR-7 Hansen等^[32]在脑组织中发现大量Cdr1as/ciRS-7, 并且拥有超过70个miR-7的结合位点。在神经母细胞瘤和星形细胞瘤中下调miR-7的表达时, miR-7对癌基因的抑制作用下降, 致使肿瘤生长增速。Cdr1as/ciRS-7可被miR-617降解, 从而瞬间释放大量miR-7, 提示Cdr1as/ciRS-7可能具有储存miR-7的功能, 将miR-7带到特定部位发挥作用^[12]。在原发性肝细胞肝癌中, Cdr1as可通过靶向抑制miR-7充当癌基因^[27]。已知Cdr1as/ciRS-7在肿瘤中的作用是通过抑制miR-7的表达实现的。为了明确Cdr1as/ciRS-7在肿瘤中的生物学作用, 可从miR-7在各类肿瘤中复杂的调控作用着手。研究表明, miR-7可下调肿瘤相关信号通路中的致癌因子的表达, 如表皮生长因子受体(epidermal growth factor receptor, EGFR)、胰岛素受体底物1/2(insulin receptor substrate 1/2, IRS-1/IRS-2)、p21蛋白活化激酶-1(p21-activating kinases 1, Pak1)、黏着斑激酶(focal adhesion kinase, FAK)、磷脂酰肌醇3激酶(phosphatidylinositol 3-kinase, PI3K)/蛋白激酶B(protein kinase B, PKB, 又称为Akt)、蛋白酶体激活因子28γ(proteasome activator 28 gamma, PA28γ)等^[39]。

miR-7在头颈部肿瘤中主要作为抑癌基因发挥作用。如在喉癌细胞系Hep-2中, miR-7可通过抑制PI3K/Akt信号通路从而抑制肿瘤的增殖和迁移^[40]。

在鼻咽癌中, miR-7可影响鼻咽癌细胞株CNE-1和CNE-2对放射治疗的敏感性, 其表达量越高细胞对射线越敏感^[41]。进一步研究发现, 由于放射敏感性不同, 鼻咽癌细胞受X线辐射后miR-7表达变化的方向也不同, 放射敏感性高的细胞miR-7表达下调, 敏感性低的细胞与之相反; 而且miR-7的表达调整幅度与X线辐射的剂量有关, 低剂量照射, miR-7的调整幅度较大^[42]。在鼻咽癌5-8F细胞中, miR-7亦可抑制EGFR/PI3K/Akt信号通路, 导致细胞增殖和克隆形成显著受到抑制^[43]。在舌鳞癌细胞中, 过表达miR-7导致胰岛素样生长因子1受体(insulin-like growth factor 1 receptor, IGF1R)的表达在mRNA和蛋白水平显著减少, 使用荧光素酶报告基因实验得出miR-7可与IGF1R mRNA的3'-UTR相结合, 通过抑制IGF1R的表达, 从而减弱由胰岛素样生长因子1(insulin-like growth factor 1, IGF1)诱导的Akt激活, 导致细胞增殖减慢和细胞周期的阻滞, 促进细胞的凋亡^[44]。

3.1.2 circSry/miR-138 circSry拥有16个miR-138结合位点^[9], miR-138在多种肿瘤中也常常发挥抑癌基因的作用, 如肝细胞肝癌^[45]、黑色素瘤^[46]、宫颈癌^[47]、骨肉瘤^[48]等。

miR-138在喉癌组织中表达水平显著降低, 与此同时, E盒结合锌指蛋白2(zinc finger E-box binding homeobox 2, ZEB2)在喉癌组织中的表达水平升高。通过实验证明, ZEB2作为miR-138的靶基因, 其翻译受miR-138抑制, 从而影响喉癌细胞的侵袭^[49]。Liu等^[50]发现, miR-138在鼻咽癌标本和鼻咽癌细胞株中表达均下调, 过表达miR-138可显著抑制细胞增

殖和细胞克隆形成,而且可抑制在体肿瘤的生长。miR-138的靶标可能为细胞周期蛋白D1(cyclin D1, CCND1),其mRNA水平与miR-138的表达呈负相关。miR-138还与舌鳞癌细胞的转移潜能相关,miR-138可抑制舌鳞癌细胞迁移和侵袭^[51]。不仅如此,研究发现,miR-138还可与G蛋白 α 抑制活性多肽2(G protein alpha inhibiting activity polypeptide 2, *GNAI2*)的mRNA 3'-UTR区相结合,抑制*GNAI2*的表达,导致细胞增殖减少、周期阻滞和促进细胞凋亡^[52]。miR-138在口腔鳞状细胞癌组织和细胞系中显著下调,miR-138可与Yes相关蛋白1(Yes-associated protein 1, *YAP1*)mRNA的3'-UTR相结合,抑制细胞增殖,可作为治疗口腔鳞癌的一个潜在的治疗靶标^[53]。

3.1.3 cir-ITCH/miR-7、miR-17和miR-214 最近的一项研究报道,cir-ITCH作为抑癌基因,在食管鳞状细胞癌组织中的表达水平显著下降,具有miR-7、miR-17和miR-214海绵功能,导致ITCH水平增加,从而促进泛素介导的蓬乱蛋白2(dishevelled segment polarity protein 2, *DVL2*)降解、抑制癌基因*c-Myc*(v-myc avian myelocytomatosis viral oncogene homolog)的表达,抑制Wnt/ β -catenin信号通路^[54]。cir-ITCH不仅在食管癌中发挥抑癌作用,Li等^[55]在肺癌中也得出了相似结论。

在头颈部肿瘤中的相关研究证明,miR-17具有致癌作用。王雯珺等^[56]通过研究鼻咽癌发现,与非癌志愿者相比,miR-17在患者血清中的表达量较高。Zeng等^[57]研究机制时发现,miR-17-5p可特异性结合细胞周期蛋白依赖性激酶抑制因子1A(cyclin dependent kinase inhibitor 1A, *CDKN1A*)3'-UTR区的2个位点,过表达miR-17-5p可抑制*CDKN1A*的表达,从而促进鼻咽癌细胞系CNE2的增殖。Chang等^[58]研究发现,miR-17-92基因簇在晚期迁徙的口腔鳞癌细胞株中显著下调,并具有调控口腔鳞癌细胞迁移的能力。进一步研究表明,miR-17-92基因簇抑制口腔鳞癌的迁移能力是通过抑制下游的一个主要目标整合蛋白 β 8(integrin β 8, *ITGB8*)来实现的。miR-17-92基因簇,尤其是miR-17和miR-20a是调节口腔鳞癌细胞株迁移关键因子,且与患者淋巴结转移及临床预后具有显著相关性。

miR-214在头颈肿瘤中也作为癌基因发挥作用。miR-214在鼻咽癌细胞和组织中高表达,这不仅促进鼻咽癌细胞体外增殖和侵袭,也加速了肿

瘤的形成和肺转移。miR-214还与鼻咽癌的转移相关。miR-214可以激活Akt信号通路抑制乳运铁蛋白(lactotransferrin, LTF)的表达,miR-214可能是造成LTF在鼻咽癌组织中表达量下调的原因^[59]。将鼻咽癌细胞中的miR-214沉默可促进细胞凋亡和抑制细胞增殖,而且研究发现,与细胞凋亡相关的B细胞淋巴瘤/白血病-2基因介导因子(Bcl-2-interacting mediator, BIM)为miR-214的直接目标,低表达BIM的鼻咽癌患者预后较差^[60]。在舌鳞癌Tca8113细胞株中斑螋素可下调miR-214,从而增加p53蛋白质的表达,抑制Bcl-2/Bax蛋白质信号通路。然而,miR-214过表达降低了斑螋素在舌鳞癌Tca8113细胞株中的抑癌作用,抑制p53的表达并增加了的Bcl-2/Bax的信号转导途径。结果显示,miR-214在舌鳞癌细胞中起促癌作用^[61]。

3.2 转录调节作用

circRNA可以看作是选择性剪接形成的异构体,它们可能在剪接水平调节亲本基因表达。如ci-ankrd52、ci-mcm5和ci-sirt7可以提高其亲本基因的表达水平^[21]。这些circRNA的亲本基因中有些与肿瘤的发生、发展密切相关,发挥抑癌或癌基因的作用。其中,微小染色体维持蛋白5(minichromosome maintenance complex component 5, *MCM5*)的表达水平在口腔癌前病变阶段就处于升高状态,*MCM5*的高表达和口腔鳞状细胞癌的进展和预后不良密切相关^[62]。去乙酰基酶SIRT7(Sirtuin 7)在头颈部肿瘤病人的循环血液中表达上调^[63],在肿瘤组织中表达下调,尤其在肿瘤晚期病人的癌组织中下调幅度更大^[64],可能成为头颈部肿瘤诊断和预后的生物学标志物。

3.3 作为喉癌的生物标志物

近期,Xuan等^[65]通过基因芯片分析了circRNA在4对喉鳞状细胞癌组织和癌旁组织中的表达差异,其中hsa_circ_100855上调倍数最大,hsa_circ_104912下调倍数最大,并对52对组织标本进行了验证。验证结果表明,与癌旁组织相比,hsa_circ_100855在喉癌组织中表达上调,与肿瘤分期、分型和是否有淋巴结转移等密切相关。相反,hsa_circ_104912在喉癌组织中下调,且与患者肿瘤分期、有否淋巴结转移及低分化晚期阶段具有临床相关性。总之,这些circRNA可作为新的、稳定的喉癌生物标志物。

4 结语与展望

近年来研究发现, circRNA具有miRNA海绵、RBP海绵、转录调控因子和翻译等功能, 而且能大量稳定存在于唾液^[66]、血液^[67]和外体(exosome)^[68]中, 有潜力成为疾病诊断和治疗的新型靶向分子。circRNA可能通过与miRNA相互作用或转录调节等手段调控其他可编码RNA的表达, 以达到促癌或抑癌的作用。然而, 这仅仅是个开始, 上述生物学功能可能只是少数circRNA所具有的, 也可能只是circRNA众多功能中的一小部分, circRNA的神秘面纱正等待被揭开。与circRNA相关的miRNA、靶基因及蛋白质在头颈部肿瘤中已有大量研究, 但circRNA究竟如何发挥作用以及其中具体的分子机制尚未完全明确。随着circRNA研究的深入, 可将其与相关的生物分子串联起来, 编织出转录后水平调控基因表达的巨型网络。

参考文献 (References)

- Kang H, Kiess A, Chung CH. Emerging biomarkers in head and neck cancer in the era of genomics. *Nat Rev Clin Oncol* 2015; 12(1): 11-26.
- Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; 55(2): 74-108.
- Rudolph E, Dyckhoff G, Becher H, Dietz A, Ramroth H. Effects of tumour stage, comorbidity and therapy on survival of laryngeal cancer patients: A systematic review and a meta-analysis. *Eur Arch Otorhinolaryngol* 2011; 268(2): 165-79.
- Roepman P, Wessels LF, Kettelarij N, Kemmeren P, Miles AJ, Lijnzaad P, *et al.* An expression profile for diagnosis of lymph node metastases from primary head and neck squamous cell carcinomas. *Nat Genet* 2005; 37(2): 182-6.
- Mao L, Hong WK, Papadimitrakopoulou VA. Focus on head and neck cancer. *Cancer Cell* 2004; 5: 311-6.
- Qu S, Yang X, Li X, Wang J, Gao Y, Shang R, *et al.* Circular RNA: A new star of noncoding RNAs. *Cancer Lett* 2015; 365(2): 141-8.
- Salzman J, Chen RE, Olsen MN, Wang PL, Brown PO. Cell-type specific features of circular RNA expression. *PLoS Genet* 2013; 9(9): e1003777.
- Guo JU, Agarwal V, Guo H, Bartel DP. Expanded identification and characterization of mammalian circular RNAs. *Genome Biol* 2014; 15(7): 409.
- Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, *et al.* Natural RNA circles function as efficient microRNA sponges. *Nature* 2013; 495(7441): 384-8.
- Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, *et al.* Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 2013; 495(7441): 333-8.
- Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS One* 2012; 7(2): e30733.
- Hansen TB, Kjems J, Damgaard CK. Circular RNA and miR-7 in cancer. *Cancer Res* 2013; 73(18): 5609-12.
- Li JQ, Yang J, Zhou P, Le YP, Zhou CW, Wang SM, *et al.* Circular RNAs in cancer: Novel insights into origins, properties, functions and implications. *Am J Cancer Res* 2015; 5(2): 472-80.
- Li PF, Chen SC, Chen HL, Mo XY, Li TW, Shao YF, *et al.* Using circular RNA as a novel type of biomarker in the screening of gastric cancer. *Clin Chim Acta* 2015; 444: 132-6.
- Wang F, Nazarali AJ, Ji S. Circular RNAs as potential biomarkers for cancer diagnosis and therapy. *Am J Cancer Res* 2016; 6(6): 1167-76.
- Xuan L, Qu L, Zhou H, Wang P, Yu H, Wu T, *et al.* Circular RNA: A novel biomarker for progressive laryngeal cancer. *Am J Transl Res* 2016; 8(2): 932-9.
- Qin M, Liu G, Huo X, Tao X, Sun X, Ge Z, *et al.* Hsa_circ_0001649: A circular RNA and potential novel biomarker for hepatocellular carcinoma. *Cancer Biomark* 2016; 16(1): 161-9.
- Wilusz JE. Repetitive elements regulate circular RNA biogenesis. *Mob Genet Elements* 2015; 5(3): 1-7.
- Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, *et al.* Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA* 2012; 19(2): 141-57.
- Zhang XO, Wang HB, Zhang Y, Lu X, Chen LL, Yang L. Complementary sequence-mediated exon circularization. *Cell* 2014; 159(1): 134-47.
- Zhang Y, Zhang XO, Chen T, Xiang JF, Yin QF, Xing YH, *et al.* Circular intronic long noncoding RNAs. *Mol Cell* 2013; 51(6): 792-806.
- Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, *et al.* Exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol* 2015; 22(3): 256-64.
- Lu Z, Filonov GS, Noto JJ, Schmidt CA, Hatkevich TL, Wen Y, *et al.* Metazoan tRNA introns generate stable circular RNAs *in vivo*. *RNA* 2015; 21(9): 1554-65.
- Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: Competitive inhibitors of small RNAs in mammalian cells. *Nat Methods* 2007; 4(9): 721-6.
- Chen I, Chen CY, Chuang TJ. Biogenesis, identification, and function of exonic circular RNAs. *Wiley Interdiscip Rev RNA* 2015; 6(5): 563-79.
- Geng HH, Li R, Su YM, Xiao J, Pan M, Cai XX, *et al.* The circular RNA Cdr1as promotes myocardial infarction by mediating the regulation of miR-7a on its target genes expression. *PLoS One* 2016; 11(3): e0151753.
- Yu L, Gong XJ, Sun L, Zhou QY, Lu BL, Zhu LY. The circular RNA Cdr1as act as an oncogene in hepatocellular carcinoma through targeting miR-7 expression. *PLoS One* 2016; 11(7): e0158347.
- Wang K, Long B, Liu F, Wang JX, Liu CY, Zhao B, *et al.* A circular RNA protects the heart from pathological hypertrophy and heart failure by targeting miR-223. *Eur Heart J* 2016; 37(33): 2602-11.
- Liu Q, Zhang X, Hu X, Dai L, Fu X, Zhang J, *et al.* Circular RNA related to the chondrocyte ECM regulates MMP13 expression by functioning as a miR-136 'Sponge' in human cartilage degradation. *Sci Rep* 2016; 2(6): 22572.

- 30 Nan A, Chen L, Zhang N, Liu Z, Yang T, Wang Z, *et al.* A novel regulatory network among LncRpa, CircRar1, MiR-671 and apoptotic genes promotes lead-induced neuronal cell apoptosis. *Arch Toxicol* 2016; doi: 10.1007/s00204-016-1837-1.
- 31 Lan PH, Liu ZH, Pei YJ, Wu ZG, Yu Y, Yang YF, *et al.* Landscape of RNAs in human lumbar disc degeneration. *Oncotarget* 2016; 7(39): 63166-76.
- 32 Hansen TB, Wiklund ED, Bramsen JB, Villadsen SB, Statham AL, Clark SJ, *et al.* miRNA-dependent gene silencing in-volving Ago2-mediated cleavage of a circular antisense RNA. *EMBO J* 2011; 30(21): 4414-22.
- 33 You X, Vlatkovic I, Babic A, Will T, Epstein I, Tushev G, *et al.* Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. *Nat Neurosci* 2015; 18(4): 603-10.
- 34 Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, *et al.* CircRNA biogenesis competes with pre-mRNA splicing. *Mol Cell* 2014; 56(1): 55-66.
- 35 Lesca MH, Anika S, Kristina S, Garwin P, Nils AK, Wolfgang W, *et al.* Circular non-coding RNA ANRIL modulates ribosomal RNA maturation and atherosclerosis in humans. *Nat Commun* 2016; 7: 12429.
- 36 Schneider T, Hung LH, Schreiner S, Starke S, Eckhof H, Rossbach O, *et al.* CircRNA-protein complexes: IMP3 protein component defines subfamily of circRNPs. *Sci Rep* 2016; 6: 31313.
- 37 Chen CY, Sarnow P. Initiation of protein synthesis by the eukaryotic translational apparatus on circular RNAs. *Science* 1995; 268(5209): 415-7.
- 38 Abe N, Matsumoto K, Nishihara M, Nakano Y, Shibata A, Maruyama H, *et al.* Rolling circle translation of circular RNA in living human cells. *Sci Rep* 2015; 5: 16435.
- 39 Zhao J, Tao Y, Zhou Y, Qin N, Chen C, Tian D, *et al.* MicroRNA-7: A promising new target in cancer therapy. *Cancer Cell Int* 2015; 15: 103.
- 40 徐瑶, 欧阳学农, 陈曦, 赵忠全, 綦晓艳. miR-7抑制喉鳞癌Hep-2细胞增殖及迁移的研究. *临床肿瘤学杂志(Xu Yao, OuYang Xuenong, Chen Xi, Zhao Zhongquan, Qi Xiaoyan. Inhibition effect of miR-7 on proliferation and metastasis of Hep-2 laryngeal carcinoma cells. Chinese Clinical Oncology)* 2015; 20(3): 193-7.
- 41 王旭丹, 杨惠玲, 郭禹标, 梁志慧, 赵睿颖, 夏云飞, 等. 不同辐射抗拒鼻咽癌细胞微小RNA差异表达的研究. *中国病理生理杂志(Wang Xudan, Yang Huiling, Guo Yubiao, Liang Zhihui, Zhao Ruiying, Xia Yunfei, et al. Discrepancy of microRNA in different radioresistant nasopharyngeal carcinoma cells. Chinese Journal of Pathophysiology)* 2007; 23(6): 1045-8.
- 42 陈智贤, 孙爱民, 陈勇, 刘英, 詹军芳, 陈龙华, 等. 放射敏感性、X线辐射剂量对鼻咽癌细胞miR-7表达的影响. *南方医科大学学报(Chen Zhixian, Sun Aiming, Chen Yong, Liu Ying, Zhan Junfang, Chen Longhua, et al. Effects of radiosensitivity and X-ray dose on miR-7 expression in nasopharyngeal carcinoma. Journal of Southern Medical University)* 2010; 30(8): 1810-2,1816.
- 43 孙栋勋, 黄栋栋, 金巧智, 陈武兵, 蔡志毅. miRNA-7通过EGFR/PI3K/Akt通路抑制鼻咽癌5-8F细胞增殖. *中国病理生理杂志(Sun Dongxun, Huang Dongdong, Jin Qiaozhi, Chen Wubing, Cai Zhiyi. MiRNA-7 inhibits proliferation of human nasopharyngeal 5-8F carcinoma cells via EGFR/PI3K/Akt pathway. Chinese Journal of Pathophysiology)* 2014; 30(10): 1807-12.
- 44 Jiang L, Liu X, Chen Z, Jin Y, Heidbreder CE, Kolokythas A, *et al.* MicroRNA-7 targets IGF1R (insulin-like growth factor 1 receptor) in tongue squamous cell carcinoma cells. *Biochem J* 2010; 432(1): 199-205.
- 45 Liu Y, Zhang W, Liu K, Liu S, Ji B, Wang Y. MiR-138 suppresses cell proliferation and invasion by inhibiting SOX9 in hepatocellular carcinoma. *Am J Transl Res* 2016; 8(5): 2159-68.
- 46 Chen Y, Cao KE, Wang S, Chen J, He B, He GU, *et al.* MicroRNA-138 suppresses proliferation, invasion and glycolysis in malignant melanoma cells by targeting HIF-1 α . *Exp Ther Med* 2016; 11(6): 2513-8.
- 47 Li B, Yang XX, Wang D, Ji HK. MicroRNA-138 inhibits proliferation of cervical cancer cells by targeting c-Met. *Eur Rev Med Pharmacol Sci* 2016; 20(6): 1109-14.
- 48 Zhu Z, Tang J, Wang J, Duan G, Zhou L, Zhou X. MiR-138 acts as a tumor suppressor by targeting EZH2 and enhances Cisplatin-induced apoptosis in osteosarcoma cells. *PLoS One* 2016; 11(3): e0150026.
- 49 Gao S, Wang J, Xie J, Zhang T, Dong P. Role of miR-138 in the regulation of larynx carcinoma cell metastases. *Tumour Biol* 2015; doi: 10.1007/s13277-015-4244-y.
- 50 Liu X, Lv XB, Wang XP, Sang Y, Xu S, Hu K, *et al.* MiR-138 suppressed nasopharyngeal carcinoma growth and tumorigenesis by targeting the CCND1 oncogene. *Cell Cycle* 2012; 11(13): 2495-506.
- 51 Jiang L, Liu X, Kolokythas A, Yu J, Wang A, Heidbreder CE, *et al.* Downregulation of the Rho GTPase signaling pathway is involved in the microRNA-138-mediated inhibition of cell migration and invasion in tongue squamous cell carcinoma. *Int J Cancer* 2010; 127(3): 505-12.
- 52 Jiang L, Dai Y, Liu X, Wang C, Wang A, Chen Z, *et al.* Identification and experimental validation of G protein alpha inhibiting activity polypeptide 2 (GNAI2) as a microRNA-138 target in tongue squamous cell carcinoma. *Hum Genet* 2011; 129(2): 189-97.
- 53 Xu R, Zeng G, Gao J, Ren Y, Zhang Z, Zhang Q, *et al.* MiR-138 suppresses the proliferation of oral squamous cell carcinoma cells by targeting Yes-associated protein 1. *Oncol Rep* 2015; 34(4): 2171-8.
- 54 Li F, Zhang L, Li W, Deng J, Zheng J, An M, *et al.* Circular RNA ITCH has inhibitory effect on ESCC by sup-pressing the Wnt/beta-catenin pathway. *Oncotarget* 2015; 6(8): 6001-13.
- 55 Wan L, Zhang L, Fan K, Cheng ZX, Sun QC, Wang JJ. Circular RNA-ITCH suppresses lung cancer proliferation via inhibiting the Wnt/ β -Catenin pathway. *Biomed Res Int* 2016; 2016: 1579490.
- 56 王雯珺, 郭敏章, 列璞怡, 伍思培. miRNA-17-5p靶向调节CDKN1A对鼻咽癌细胞增殖的影响. *中国临床研究(Wang Wenjun, Guo Minzhang, Lie Puyi, Wu Sipei. Effect of miRNA-17-5p on the proliferation of nasopharyngeal carcinoma cells through targeting CDKN1A. Chinese Journal of Clinical Research)* 2016; 29(2): 149-52.
- 57 Zeng X, Xiang J, Wu M, Xiong W, Tang H, Deng M, *et al.*

- Circulating miR-17, miR-20a, miR-29c, and miR-223 combined as non-invasive biomarkers in nasopharyngeal carcinoma. *PLoS One* 2012; 7(10): e46367.
- 58 Chang CC, Yang YJ, Li YJ, Chen ST, Lin BR, Wu TS, *et al.* MicroRNA-17/20a functions to inhibit cell migration and can be used a prognostic marker in oral squamous cell carcinoma. *Oral Oncol* 2013; 49(9): 923-31.
- 59 Deng M, Ye Q, Qin Z, Zheng Y, He W, Tang H, *et al.* MiR-214 promotes tumorigenesis by targeting lactotransferrin in nasopharyngeal carcinoma. *Tumour Biol* 2013; 34(3): 1793-800.
- 60 Zhang ZC, Li YY, Wang HY, Fu S, Wang XP, Zeng MS, *et al.* Knockdown of miR-214 promotes apoptosis and inhibits cell proliferation in nasopharyngeal carcinoma. *PLoS One* 2014; 9(1): e86149.
- 61 Tian X, Zeng G, Li X, Wu Z, Wang L. Cantharidin inhibits cell proliferation and promotes apoptosis in tongue squamous cell carcinoma through suppression of miR-214 and regulation of p53 and Bcl-2/Bax. *Oncol Rep* 2015; 33(6): 3061-8.
- 62 Yu SY, Wang YP, Chang JY, Shen WR, Chen HM, Chiang CP. Increased expression of MCM5 is significantly associated with aggressive progression and poor prognosis of oral squamous cell carcinoma. *J Oral Pathol Med* 2014; 43(5): 344-9.
- 63 Lu CT, Hsu CM, Lin PM, Lai CC, Lin HC, Yang CH, *et al.* The potential of SIRT6 and SIRT7 as circulating markers for head and neck squamous cell carcinoma. *Anticancer Res* 2014; 34(12): 7137-43.
- 64 Lai CC, Lin PM, Lin SF, Hsu CH, Lin HC, Hu ML, *et al.* Altered expression of SIRT gene family in head and neck squamous cell carcinoma. *Tumour Biol* 2013; 34(3): 1847-54.
- 65 Xuan L, Qu L, Zhou H, Wang P, Yu H, Wu T, *et al.* Circular RNA: A novel biomarker for pro-gressive laryngeal cancer. *Am J Transl Res* 2016; 8(2): 932-9.
- 66 Bahn JH, Zhang Q, Li F, Chan TM, Lin X, Kim Y, *et al.* The landscape of microRNA, Piwi-interacting RNA, and circular RNA in human saliva. *Clin Chem* 2015; 61(1): 221-30.
- 67 Memczak S, Papavasileiou P, Peters O, Rajewsky N. Identification and characterization of circular RNAs as a new class of putative biomarkers in human blood. *PLoS One* 2015; 10(10): e0141214.
- 68 Li Y, Zheng Q, Bao C, Li S, Guo W, Zhao J, *et al.* Circular RNA is enriched and stable in exosomes: A promising biomarker for cancer diagnosis. *Cell Res* 2015; 25(8): 981-4.