基于RVG肽的中枢神经系统靶向递送策略进展

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摘要 血脑屏障(blood-brain barrier, BBB)严格调控物质进出中枢神经系统(central nervous system, CNS), 是CNS治疗的重要障碍。源自狂犬病病毒糖蛋白(rabies virus glycoprotein, RVG)的多肽具有高度嗜神经性, 能够特异性结合CNS中的烟碱型乙酰胆碱受体(nicotinic acetylcholine receptor, nAChR), 可通过受体介导的转胞吞(receptor-mediated transcytosis, RMT)机制穿过BBB将缀合的核酸、蛋白质分子递送至特异性组织、细胞中。RVG肽还能结合CNS药物递送载体外泌体、纳米颗粒, 赋予其CNS靶向特性。该文就RVG肽来源、靶向机制及其结合外泌体、纳米颗粒进行CNS靶向递送进行综述, 这为CNS治疗提供了一种安全、无创的递送策略。

关键词 RVG肽; CNS靶向递送; 外泌体; 纳米颗粒

Advances in Targeted Delivery Strategies for Central Nervous System Based on RVG Peptide

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Abstract The BBB (blood-brain barrier) strictly regulates the substances flow of CNS (central nervous system), which is an important obstacle to CNS treatment. The polypeptide derived from the RVG (rabies virus glycoprotein) is highly neurotropic and can specifically bind to the nAChR (nicotinic acetylcholine receptors) in CNS, and then penetrate BBB through the RMT (receptor-mediated transcytosis) mechanism. The conjugated nucleic acids or proteins of RVG peptide are delivered to specific brain tissues or cells. RVG peptide can also combine with exosomes or nanoparticles, which are CNS drug delivery carriers, giving them CNS targeting properties. This article reviews the source and targeting mechanism of RVG peptide and its combination with exosomes and nanoparticles for CNS-targeted delivery, which provides a safe and non-invasive delivery strategy for CNS therapy.

Keywords RVG peptide; CNS-targeted delivery; exosomes; nanoparticles

血脑屏障(blood-brain barrier, BBB)由连续、无窗的中枢神经系统(central nervous system, CNS)内皮细胞在神经血管单位诱导下形成, 严格调控正常神经元所需的微环境[1]。BBB阻止100%的大分子和98%以上的小分子药物进入CNS[2]; 穿过BBB进行CNS治疗面临药物选择性差、BBB破坏、低BBB渗透或快速消除等挑战[3]。外泌体、纳米颗粒被发现能够克服BBB进行CNS递送, 然而其均具有靶向性

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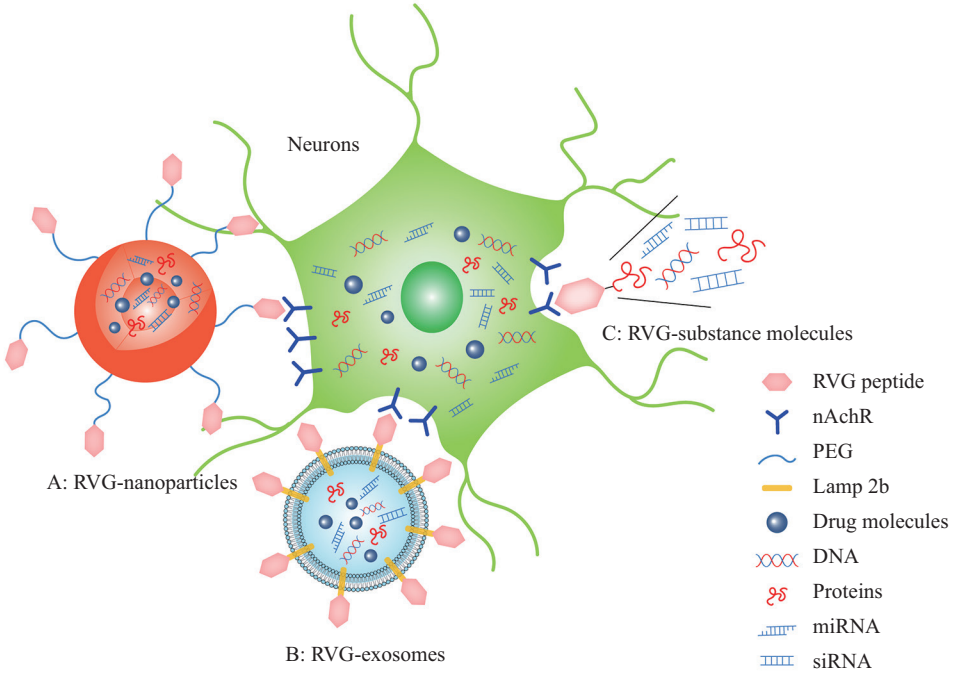
低的不足。基于BBB特性，多种转运蛋白、受体蛋白被用于CNS靶向递送[3]。RVG肽具有高度嗜神经性，能够通过受体介导的转胞吞(receptor-mediated transcytosis, RMT)机制将缀合的分子递送至皮质、海马及神经元、小胶质等组织、细胞中；RVG肽还可结合外泌体、纳米颗粒，赋予其CNS靶向能力；偶联RVG肽的外泌体、纳米颗粒能够装载基因及药物分子进行CNS靶向递送，基因及药物分子发挥后续疾病治疗、肿瘤成像、科学研究等作用。因此，RVG肽为CNS靶向递送提供了一种新的方法。

1 RVG肽来源

狂犬病病毒糖蛋白(rabies virus glycoprotein, RVG)是狂犬病病毒膜上插入的一个65 kDa的三聚体I型糖蛋白[4]，成熟RVG由505个氨基酸组成，包括细胞质结构域、跨膜结构域和细胞外结构域三部分[5]。RVG具有高度嗜神经性，能够识别特异性受体，在低pH下诱导病毒与靶细胞融合，介导病毒在CNS中的逆向轴突传播，但不参与病毒基因组的转录、复制[6-7]。RVG肽是从RVG受体识别关键序列189—214中衍生的由29个氨基酸(YTIWMPENPRP-GTPCDIFTNSRGKRASNGGGG)组成的短肽，具有低免疫原性，能够特异性识别CNS受体靶向递送基因及药物分子[8]。

2 RVG肽CNS靶向机制

研究发现，RVG肽能够特异性识别并结合CNS中普遍存在的烟碱型乙酰胆碱受体(nicotinic acetylcholine receptor, nAChR)，并被α-银环蛇毒素竞争性抑制[9]。nAChR是一种五聚体配体门控离子通道，广泛分布于脑微血管内皮细胞、丘脑、纹状体、海马、皮质等多种CNS结构中[6-10]。RVG肽与nAChR结合后，通过RMT机制介导物质或载体穿过BBB(图1)[11]。RMT是BBB高度特异性运输系统的一部分，负责CNS内必需的肽或蛋白质等大分子物质的转运[12]；RMT通过配体–受体相互作用介导BBB内皮细胞以内吞方式摄取腔膜侧大分子，经细胞内转运后以胞吐方式将分子释放到基底膜侧，受体通过再循环回到腔膜侧[13-14]。靶向RMT已被成功用于跨BBB递送生物治疗性物质；与参与BBB穿透的被动扩散途径相比，RVG肽与nAChR介导的物质转运对BBB具有高度特异性，可有效提高药物分子的治疗效率[15-16]。



A、B: RVG肽分别结合纳米颗粒、外泌体，通过nAChR介导的RMT途径靶向神经元递送核酸、蛋白质及药物分子等；C: RVG肽靶向神经元递送物质分子。

A,B: nanoparticles and exosomes modified with RVG peptide respectively target neurons to deliver nucleic acids, proteins or drug molecules through the nAChR-mediated RMT pathway; C: RVG peptide targets neurons to deliver substance molecules.

图1 基于RVG肽的CNS靶向递送机制

Fig.1 Mechanism of CNS-targeted delivery based on RVG peptide

3 RVG肽CNS靶向递送

靶向给药的主要目标是减少药物的非特异性摄取, 以及促进药物在靶组织、细胞的累积。基于RVG肽的CNS特异性、BBB穿透性、低免疫原性以及无创性等特点, RVG肽被用于结合核酸、蛋白质等治疗分子进行CNS靶向递送。相比于大分子蛋白载体, 肽具有较小的分子量、易于合成、低细胞毒性及可降解等优势[17]。

3.1 递送核酸分子

RVG肽不能直接结合核酸, KUMAR等[18]在RVG肽羧基末端加入壬基精氨酸残基合成嵌合肽RVG-9R, 使RVG-9R通过静电相互作用结合siRNA。其在体外靶向表达nAChR的神经细胞并转导siRNA, 引起有效的基因沉默。静脉注射结合抗病毒siRNA的RVG-9R对致死性病毒性脑炎小鼠提供了有力的保护, 且经反复给药后未诱导炎性因子或抗肽抗体产生。KIM等[19]发现, RVG-9R能够将siRNA靶向递送至表达nAChR的CNS巨噬细胞/小胶质细胞, 抑制肿瘤坏死因子-α(tumor necrosis factor-α, TNF-α)介导的神经炎症和神经元凋亡。ROHN等[20]研究表明, RVG肽可转染cdk4 siRNA, 使得Neuro2A细胞中cdk4的表达水平减少75%; 立体定向注射可将cdk4 siRNA特异性递送到海马神经元, 在注射部位400 μm处敲低cdk4表达。因此RVG肽被证明提供了一种高效、安全的方法, 用于穿过BBB靶向CNS递送siRNA及潜在治疗分子。

VILLA-CEDILLO等[21]为提高基因导入神经细胞的效率, 在RVG肽中引入Asn194Lys突变。突变型RVG肽、亲核肽及编码绿色荧光蛋白的质粒形成的复合物能够通过能量依赖的胞吞作用将DNA分子递送到神经元细胞中。Asn194Lys突变增加了基因向神经母细胞瘤和星形胶质细胞的传递。这种新的RVG多肽的亲核能力使其有希望作为基因输送载体进入脑细胞。

3.2 递送蛋白质

XIANG等[22]通过基因工程构建质粒转染细胞将RVG肽与β-半乳糖苷酶(beta-galactosidase, β-Gal)融合, 在静脉注射或胃内给药后, RVG肽均能将生物活性蛋白穿过BBB递送到海马区神经元。这可能为用于神经退行性疾病, 如阿尔茨海默病(Alzheimer's disease, AD)等的蛋白质疗法提供新的策略。ZOU等[23]将RVG肽与基因编辑蛋白Cre重组酶结合, 经尾静脉注射将RVG-Cre全身性递送到两个Cre报告小鼠系中, 证明RVG-Cre可以靶向大脑皮层并介导体细胞中有效的基因组编辑。这种通过RVG肽将基因编辑酶蛋白直接导入小鼠大脑的方法比基于质粒或病毒的方法要安全得多, 有望在各种CNS疾病治疗中进一步应用。

4 RVG肽结合外泌体

外泌体(exosomes, Ex)是一种用于细胞间通讯、可由多种细胞分泌的膜结合纳米囊泡, 直径约30~150 nm, 是核酸和蛋白质的天然载体[24]; 因其具有天然跨越BBB的特性, 故而成为CNS理想的药物递送工具[25]。Ex具有良好的生物相容性、循环稳定性、生物屏障通透性、低免疫原性和低毒性等优势[26]。然而, 天然Ex存在特异性CNS靶向能力低, 易积聚于肝肾组织进而导致清除不足[27]。溶酶体相关膜蛋白2b(lysosome-associated membrane protein 2b, Lamp2b)是Ex膜表面大量表达的非特异性单跨膜蛋白, 常被用作嵌合表达蛋白载体来制备连接RVG肽的工程靶向Ex(即RVG-Ex)[28]。RVG肽可与不同来源的Ex结合, 介导其CNS靶向递送, 增强受体细胞摄取, 减少药物脱靶效应[29-30]。

4.1 结合树突状细胞源性Ex

ALVAREZ-ERVITI等[31]首次构建了Lamp2b N-端融合RVG肽的质粒, 质粒转染树突状细胞(dendritic cells, DC)获取低免疫原性RVG-Ex, 用电穿孔法使RVG-Ex负载siRNA, 装载siRNA的RVG-Ex通过RVG的CNS靶向性将GAPDH siRNA递送至小鼠神经元、小胶质细胞及少突胶质细胞中引发特定的基因敲除。相比单纯的RVG肽, RVG-Ex可更有效地将AD治疗靶基因BACE1 siRNA递送至CNS中, 强烈敲除BACE1 mRNA(60%)和蛋白质(62%)表达。EL-ANDALOUSSI等[32]研究表明, DC源性RVG-Ex可将siRNA靶向递送至中脑、皮质和纹状体中, 敲除60%的RNA和蛋白质表达, 且在重复给药后未发现毒性或免疫原性。该体系还可将α-突触核蛋白(alpha-synuclein, a-Syn) siRNA、shRNA微环(shRNA-MCs)递送至黑质多巴胺能神经元, 显著降低中脑、纹状体和皮质中a-Syn的病理性聚集, 减少多巴胺能神经元丢失, 改善帕金森病(Parkinson's disease, PD)症状[33-34]; 将miR-124递送至CNS中可减轻可卡因介导的小胶质细胞激活引发的神经炎症[35]。这表明RVG-Ex递送策略能够向CNS递送基因物质用于

表1 基于RVG-Ex的CNS靶向递送

Table 1 CNS-targeted delivery based on RVG-Ex

外泌体来源 Sources of Ex	递送物质 Delivery substances	靶向部位 Target sites	应用 Applications	参考文献 References
Dendritic cells	BACE1 siRNA	Neurons, microglia, oligodendrocytes	AD	[31]
	siRNA	Midbrain, cortex, striatum	Gene silencing	[32]
	a-Syn siRNA	Dopaminergic neurones of the substantia nigra	PD	[33]
	shRNA-MCs			[34]
	miR-124	Microglia	Cocaine	[35]
Stem cells	miR-124	Ischemic region	Stroke	[36]
	miR-193b-3p	Intracerebral hemorrhage area	SAH	[37]
	MSCs-Ex	Cortex, hippocampus	AD	[38]
HEK293T	HMGB1siRNA,NGF	Ischemic region	Stroke	[39-40]
	DNA aptamer	Neurons	PD	[41]
	CD10dm	Hippocampus	AD	[42]
	MOR siRNA	Cortex	Morphine addiction	[43]

AD、PD等疾病诊疗。

4.2 结合干细胞源性Ex

YANG等[36]将表达RVG肽的Lamp2b质粒转染骨髓间充质干细胞(BMSCs)获取BMSCs源性RVG-Ex, 将负载的miR-124有效递送到卒中后梗死部位, 通过miR-124发挥促进皮质前体细胞再生, 保护卒中引起的缺血性损伤作用。LAI等[37]用BMSCs源性RVG-Ex向动脉瘤性蛛网膜下腔出血(subarachnoid hemorrhage, SAH)区递送miR-193b-3p, 来抑制HDAC3的表达和活性, 上调NF-κB p65的乙酰化可减轻炎症反应和神经行为缺损。CUI等[38]将RVG肽修饰的间充质干细胞(mesenchymal stem cells, MSCs)源性Ex靶向递送至转基因APP/PS1小鼠皮层和海马, 通过Ex中继承自干细胞的相关基因物质来降低β-淀粉样蛋白(amyloid-β, Aβ)水平, 显著改善AD小鼠学习记忆能力。

4.3 结合人胚胎肾细胞源性Ex

KIM等[39]使用人胚胎肾细胞系(HEK293T)制备RVG-Ex, 用电穿孔法装载HMGB1 siRNA, 经静脉递送至CNS用于缺血性卒中治疗。相比未修饰Ex, RVG-Ex更有效地降低了CNS中HMGB1、TNF-α的表达水平, 抑制了细胞凋亡, 缩小了梗死面积。该体系还可将神经生长因子(nerve growth factor, NGF)靶向递送至缺血皮层来重塑小胶质细胞极化, 调节神经炎症反应[40]。HEK293T源性RVG-Ex还可向CNS递送特异性识别a-Syn的DNA适配体减少脑内a-Syn病理性聚集来治疗PD[41]; 递送CD10突变体(CD10dm)调节脑内炎症反应用于AD治疗[42]。LIU等[43]将表达RVG肽的质粒和阿片类受体mu(MOR) siRNA共转染HEK293T细胞, 使MOR siRNA与Ex的AGO2蛋白结合后靶向递送至脑皮质中, 通过下调MOR的表达水平来介导吗啡复发的治疗。这些研究表明, RVG肽可成功介导Ex靶向CNS递送核苷酸、蛋白质等物质用于AD、PD、卒中等CNS疾病治疗(表1)。

5 RVG肽结合纳米颗粒

纳米颗粒(nanoparticles, NPs)包括聚合物纳米颗粒、树枝状聚合物、脂质纳米颗粒及无机纳米颗粒等多种类型[44], 可以将用于PD、AD、脑肿瘤等疾病的基因治疗物质、蛋白质、荧光探针等多种物质递送到CNS中。NPs在多功能化、携带药物有效载荷的能力、控制药物释放和改变药物的药物动力学方面具有优势[45]。NPs可以缀合RVG肽通过RMT机制穿过BBB靶向CNS递送物质分子(表2)。异双官能团聚乙二醇(NHS-PEG-MAL)可作为连接头, 其中MAL基团与RVG肽末端的半胱氨酸残基反应, NHS基团与NPs胺基结合, 完成RVG-NPs制备; PEG还能增强聚合物纳米颗粒的稳定性、生物相容性和体内靶向性[46-48]。

5.1 结合阳离子聚合物

引入二硫键的阳离子聚合物聚乙烯亚胺(SS-PEI)具有低免疫原性、生物可降解等优势, 能够通过静电相互作用结合miR-124a、pDNA, 经RVG肽修饰后可穿透BBB将其特异性递送至CNS中表达nAChR的神经元胞质中介导基因治疗[49]。RVG肽

表2 基于RVG-NPs的CNS靶向递送

Table 2 CNS-targeted delivery based on RVG-NPs

纳米颗粒类型 Types of NPs	递送载体 Delivery carriers	递送物质 Delivery substances	靶向部位 Target sites	应用 Applications	参考文献 References
Cationic polymers	SSPEI	miR-124a, pDNA	Neurons	Delivery of nucleic acids	[46,49,51]
	PMT	BACE1 siRNA	Hippocampus, cortex	AD	[50]
	Pluronic Nps	β-Gal	Brain	Delivery of proteins	[47]
Polymer Nps	PIC micelles	siRNA	Neurons	Gene delivery carriers	[48]
	PEG	Bacitracin A	Neurons	Pneumococcal meningitis	[51]
	PLGA	Deferoxamine, miR-124,	Substantia nigra,striatum	PD	[52-54]
	GNPs	DMC	Neuroblastoma	Tumor imaging, treatment	[55]
Dendrimers	PAM-ABP	Fluorescent probe, cocktail	Stem cells	Gene delivery system	[56]
Lipid NPs	SLNS/NLC	DNA	Neurons	AD	[57-58]
		BACE1 siRNA, quercetin	Neuronal mitochondria	AD	[59-60]
Inorganic Nps	Go-pSiNPs	Resveratrol, Genistein	Damaged brain tissue	Gene silencing	[61]
	Gd2O3-Nps	siRNA	Neuroblastoma	Tumor imaging	[62]
		Gd2O3			

修饰的聚(甘露醇–共-PEI)基因转运体(PMT)通过与BBB表达的nAChR结合, 将BACE1 siRNA递送至小鼠海马和皮层用于AD治疗[50]。共结合RVG肽和阳离子聚合物壳聚糖的基于pluronic的NPs具有结构稳定、易装载蛋白质、无细胞毒性等优点, 可实现β-Gal等蛋白质在脑内的有效递送, 被用作CNS靶向成像和蛋白质递送载体[47]。

5.2 结合聚合物NPs

聚合物NPs包括聚乙二醇(PEG)、聚(乳酸–共–乙醇酸)(PLGA)和聚乳酸(PLA)等。RVG肽标记的由聚乙二醇化聚天冬氨酸酰肼(PAHy)衍生物组成的新型聚离子复合物(PIC)胶束负载的siRNA更容易被Neuro2A细胞内化, 具有更强的CNS靶向能力, 能有效抑制基因表达[48]。由RVG肽、P-糖蛋白抑制剂(P85)共修饰的杆菌肽A聚乙二醇化纳米载体通过RMT途径、ATP耗竭和膜微黏度降低的协同作用穿过BBB在脑实质蓄积, 抑制药物敏感和耐药肺炎链球菌在脑内的生长, 来治疗肺炎球菌脑膜炎[51]。

YOU等[52]研究表明连接RVG肽的PLGA可向CNS靶向递送去铁胺, 降低黑质纹状体内的铁含量和氧化应激水平, 减轻多巴胺能神经元损伤, 逆转神经行为缺陷, 延缓PD进展; 该体系还可递送miR-124降低促炎因子, 升高神经保护因子水平, 并靶向NF-κb通路, 降低MEKK3和P-p65表达, 改善PD的神经炎症[53]; 递送天然自噬诱导剂4,4'-二甲氧基查耳酮(DMC)至神经元和小胶质细胞, 减轻炎症反应, 改善PD小鼠运动缺陷[54]。

LEE等[63]发现, 聚(D,L-丙交酯–乙交酯)(PLG)纳米颗粒包裹碳酸钙可形成产气聚合物纳米颗粒(GNPs), 在酸性条件下释放二氧化碳来增强超声信号。RVG肽修饰, 可将GNPs靶向积聚在神经母细胞瘤中, 用于肿瘤超声成像。该体系还可携带荧光探针检测神经母细胞瘤荷瘤小鼠中的肿瘤部位, 包裹治疗性基因鸡尾酒(siMyc、siBcl-2和siVEGF)可显著抑制肿瘤生长[55]。

5.3 结合树枝状聚合物

干细胞对非病毒基因转染试剂的容受性很差, BELOOR等[56]将RVG偶联到氧化还原敏感型树枝状可降解精氨酸接枝聚合物(PAM-ABP)上, 可在不改变母体DNA释放特性和低毒性的前提下, 与质粒DNA形成纳米颗粒, 向人类胚胎干细胞和MSCs转染非病毒基因。RVG-PAM-ABP是一种具有生物还原性、生物相容性、无毒的, 用于向表达nAChR的干细胞进行基因输送的新型合成系统。

5.4 结合脂质NPs

脂质NPs可分为固体脂质NPs(SLN)和纳米结构脂质载体(NLC)[64]。偶联RVG肽、包被壳聚糖的SLNS将BACE1 siRNA经鼻内给药方式递送至CNS, 用于AD的治疗[57]。RVG肽功能化的SLNS和NLC可装载槲皮素靶向BBB并诱导神经元保护, 减弱AD中的Aβ聚集[58]。共修饰RVG肽和靶向线粒体的三苯基膦阳离子的红细胞膜或巨噬细胞膜包裹的NLC成功将负载的抗氧化剂白藜芦醇、金雀异黄素靶向递送至神经元线粒体内, 降低线粒体氧化应激反应, 减轻AD症状, 表明了靶向修饰的纳米系统在AD中的应用前景[59-60]。

5.5 结合无机型NPs

JOO等[61]研究表明, 偶联RVG肽的多孔硅–氧化石墨烯核壳纳米载体能够保护高效负载的siRNA免受核酶降解, 将其靶向递送到损伤脑组织中, 并延迟siRNA释放, 介导基因沉默。JIN等[62]构建了RVG肽和IRDye800标记的牛血清白蛋白包被的三角形氧化钆纳米颗粒, 作为神经母细胞瘤术前诊断的靶向MRI试剂和指导术中精确切除肿瘤的荧光显像剂, 使得肿瘤小鼠术后42天的存活率从0%提高到80%。这表明RVG肽介导的CNS靶向纳米颗粒在疾病诊断和治疗方面具有巨大潜力。

6 RVG肽与CNS递送策略

目前, 穿过BBB进行CNS递送的非侵入性策略主要涉及RMT、Ex及NPs等。RMT通过特定配体与BBB受体的结合触发物质摄入囊泡, 经多阶段完成转胞吞, 是广为研究的BBB递送过程。理想的受体应在BBB中高表达, 在外周低表达, 基于RMT的CNS递送安全有效性还需临床试验验证。Ex作为CNS递送载体广受关注, 然而, 关于Ex的来源、功能、副作用等还未完全掌握, Ex穿过BBB的具体机制尚不清楚, 且未经靶向修饰的Ex只有少部分能够进入脑内。基于NPs的CNS递送策略显示出极大的临床价值, 但其穿过BBB能力有限, 对NPs表面进行多功能化修饰可以增强其CNS应用。此外, 嗜神经病毒载体可经鞘内注射等侵入性方法向CNS递送基因物质, 然其存在自身免疫的风险[3,65]。RVG肽属于nAChR特异性配体, 能够以RMT进行CNS靶向递送, 然其更多地被作为靶向配体修饰Ex、NPs, 弥补其CNS靶向性缺乏、脑内累积量低的不足。

7 结语与展望

BBB与CNS疾病密切相关, 仍然是CNS药物干预的主要障碍。RVG肽作为CNS中nAChR特异性配体可直接介导核酸、蛋白质分子通过RMT途径穿过BBB进入CNS, 也可结合Ex、NPs介导高效的CNS靶向递送。相较于RVG肽, 其他传统的转铁蛋白受体、低密度脂蛋白受体等RMT靶点在多种组织中表达, 并不适用于CNS特异性递送[11]。基于RVG肽的CNS靶向递送策略广泛应用于PD、AD、卒中、脑肿瘤等疾病中, 使得核酸、蛋白质、小分子药物等可以跨过BBB的阻碍发挥疗效, 成为一种有吸引力的CNS靶向递送新方法。

关于RVG肽还有一些观点需要探究。LIU等[66]研究表明, RVG肽修饰的聚酰胺树状大分子(PAMAM)可能通过γ-氨基丁酸受体介导DNA的CNS靶向递送。FU等[67]提出来自RVG的由39个氨基酸组成的肽(RDP)可与γ-氨基丁酸受体结合, 介导蛋白质的神经元递送。而XIN等[68]从RVG肽中重新筛选了由15个氨基酸组成的肽, 这种新筛选的RVG肽能够介导脂质体通过nAChR向神经胶质瘤递送紫杉醇。这表明了RVG肽及其CNS靶向的复杂性, 还需进一步探索RVG序列及其他潜在靶点。总之, 基于RVG肽的CNS靶向递送策略具有广阔的应用前景。

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