基于组氨酸的肿瘤靶向药物递送系统研究进展

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摘要: 组氨酸作为一种碱性氨基酸, pK_a接近肿瘤弱酸微环境pH, 其带电性和溶解性具有pH敏感性, 在中性环境中, 组氨酸不带电, 表现为疏水特性, 而在肿瘤酸性环境中, 组氨酸可质子化带正电, 同时转变为亲水特性, 因此, 组氨酸被广泛应用于靶向肿瘤弱酸性微环境的药物递送系统设计。本文全面综述了近年来基于组氨酸构建肿瘤靶向药物递送系统的研究进展, 系统总结了利用组氨酸促进细胞摄取和调控药物释放的设计思路, 并指出了相关研究工作的共性问题和未来发展方向。

关键词: 组氨酸; 细胞穿膜肽; 肿瘤微环境; 药物递送系统; 药物治疗

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Research progress of histidine-based tumor-targeting drug delivery systems

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Abstract: As a basic amino acid, histidine has a pK_a close to the acidity of the tumor microenvironment, thus the charge and solubility of histidine are able to vary as the pH changes. Under a neutral environment, histidine is not charged and exhibits hydrophobic properties, while it can be protonated and becomes hydrophilic when exposed to mildly acidic pH, such as tumor microenvironment. Therefore, histidine is widely used in the design of drug delivery systems to target the mildly acidic pH of tumor microenvironment. This article reviews the recent progresses of histidine-based tumor-targeting drug delivery systems, and summarizes the principles on promoting internalization and tuning drug release by taking advantage of histidine. Finally, we point out the common issues on histidine application and illustrate its future prospects.

Key words: histidine; cell-penetrating peptide; tumor microenvironment; drug delivery system; therapy

组氨酸 (histidine, His) 又名 α-氨基-β-咪唑基丙酸, 是负责生物系统缓冲能力的主要氨基酸, 由德国物理学家艾布瑞契•科赛尔于1986年首次从鲑精蛋白中分离出来[1]。组氨酸的pK_a约为6.5, 是组成蛋白质的20种氨基酸中pK_a值唯一接近7.0的特殊氨基酸, 其结构中含有咪唑基团[2], 咪唑基团是一种很强的亲核基团, 能够快速提供与接受质子 (图1)[3], 在中性环境中, 组氨酸不带电, 表现为疏水特性, 而当pH < pK_a时, 组氨酸质子化带正电, 同时转变为亲水性分子。

由于增殖迅速、供氧不足等原因, 肿瘤细胞主要通过无氧酵解进行能量代谢, 造成大量乳酸积累, 导致肿瘤组织细胞外环境呈弱酸性, pH值在6.5～7.2之间,

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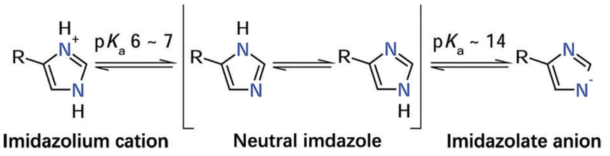

Figure 1　Protonation equilibria and tautomeric forms of the imidazole side chain of histidine. (Adapted from Ref. 3 with permission. Copyright © 2014 RSC)

低于人体正常组织及血液的pH值 (pH = 7.4), 而肿瘤细胞溶酶体pH进一步降低, 约为4.0～6.0, 因此酸性微环境是抗肿瘤药物靶向递送的一个有效靶点[4]。而组氨酸的$\mathrm{p}K_\mathrm{a}$值接近肿瘤组织的微酸环境, 可在肿瘤部位发生质子化, 实现电性及溶解性的转变, 因此被广泛应用于肿瘤靶向递送领域。

一方面, 组氨酸在肿瘤部位迅速发生质子化由中性转变为正电性的特点, 常被用于细胞穿膜肽 (cell-penetrating peptides, CPPs) 的构建和修饰, 促进细胞对药物的选择性高效摄取; 另一方面, 在肿瘤环境中, 组氨酸可由疏水特性转变为亲水特性, 利用此特点, 组氨酸常被用于构建pH响应型智能纳米递送载体, 赋予制剂多重功能, 如促进药物快速释放和增强制剂渗透能力等。此外, 组氨酸还具有质子海绵效应[5], 可促进药物迅速从溶酶体中逃逸到达胞质, 显著提高药物生物利用度。本文将主要介绍组氨酸在穿膜肽及pH响应型智能纳米药物递送系统的应用。

1　组氨酸在细胞穿膜肽中的应用

为提高药物的溶解度及其在血液循环中的稳定性, 制剂需要具有较强的亲水性及负电性, 但是细胞膜的亲脂性及负电性会导致药物的摄取量较低, 严重影响药物的抗肿瘤治疗效果。CPPs是一类由不多于30个氨基酸残基组成的小分子多肽, 具有很强的穿膜能力, 能够携带比其相对分子质量大100倍的外源性大分子进入细胞, 显著改善细胞对药物的摄取效果, 增强药物的抗肿瘤活性[6]。常用的穿膜肽主要由精氨酸、赖氨酸及一些疏水性的其他氨基酸残基组成。近年来, 组氨酸由于其自身的特殊性质, 被广泛应用于CPPs构建及修饰, 以携带抗肿瘤药物高效入胞。

1.1　组氨酸发挥细胞穿膜作用　组氨酸修饰的药物或递送载体由血液循环进入肿瘤部位后, 咪唑基团可响应弱酸微环境发生质子化, 呈现正电性, 表现出较强的穿膜作用, 可携带制剂高效入胞。基于此, Mo等[7]合成了一种由六氢苯甲酸-组氨酸-谷氨酸-疏水碳十八长链构成的脂质结构化合物 (1,5-dioctadecyl-*L*-glutamyl-2-histidyl-hexahydrobenzoic acid, $\mathrm{HHG2C}_{18}$), 该脂质的亲水端包括组氨酸、谷氨酸和一个pH响应基团六氢苯甲酸, 两个硬脂烷基长链作为疏水端, 结构类似于天然磷脂, 用于脂质体的构建。当到达肿瘤环境时, 组氨酸发生质子化, 电荷翻转, 且六氢苯甲酸基团水解, 致脂质体表面呈现更强的正电性, 利于脂质体的穿膜入胞 (图2)。Hung等[8]将*N*-乙酰组氨酸偶联维生素E聚乙二醇琥珀酸酯 (*N*-acetyl histidine-*D*-*α*-tocopheryl polyethylene glycol succinate, NAcHis-TPGS) 修饰到聚乙丙交酯 [poly(lactic-*co*-glycolic acid), PLGA] 纳米粒表面, 并包载多柔比星 (doxorubicin, DOX) 及光敏剂吲哚菁绿 (indocyanine green, ICG), 在血液循环中该纳米粒呈负电特性, 到达肿瘤细胞外, 组氨酸发生质子化呈正电性, 迅速被肿瘤细胞摄取 (图3)。

近年来, 有研究表明聚组氨酸自身是一种非典型CPP, 在不同pH条件下均具有穿膜作用。Iwasaki等[9]设计了不同长度的聚组氨酸多肽, 对其穿膜能力进行考察。结果表明, 由16个组氨酸聚合而成的多聚组氨酸 (HHHHHHHHHHHHHHHH, H_{16}) 在多种哺乳动物细胞中的摄取远远优于经典的穿膜肽八聚精氨酸 (RRRRRRRR, R_8), 并且对多种细胞无显著毒性, 其还可以将荧光染料和蛋白质等分子递送到细胞中。

之后, Hayashi等[10]将硬脂酰化的H_{16} (stearyl-H_{16} peptide, STR-H_{16}) 插入磷脂双分子层中对脂质体进行修饰, 考察其携带纳米药物穿膜的效果。研究表明, 当H_{16}在脂质膜材中占比超过10%时, STR-H_{16}显著增加了HT1080细胞对脂质体的摄取, 并在20%左右达到最佳摄取量, 这意味着聚组氨酸多肽是一种有巨大潜力的CPP, 可用于肿瘤药物的高效递送。

1.2　组氨酸调节细胞穿膜肽活性　虽然CPPs可介导药物高效入胞, 但是由于缺乏选择性, 限制了其进一步的应用。组氨酸可响应pH实现电荷的转变, 可以利用此特性调节CPPs的活性, 改善CPPs的特异性。

Zhang等[11]用组氨酸代替穿膜肽TK [AGYLLGKINLKKLAKL(Aib)KKIL-NH_2] 中所有的赖氨酸, 构建了一种新的多肽TH [AGYLLGHINLHHLAHL(Aib)HHIL-NH_2]。与TK相比, TH中的组氨酸残基能够响应肿瘤细胞外pH, 呈正电特性, 因此TH的穿膜效果显著强于TK。另外, 与TK相比, TH在pH 6.0条件下被HeLa细胞的摄取量显著高于pH 7.4环境, 即TH只在低pH环境下发挥穿膜功能, 因此该多肽的肿瘤靶向性显著改善, 对正常组织的毒性明显降低。通过将TH与抗癌药喜树碱 (camptothecin, CPT) 连接后, 得到的大分子TH-CPT可以pH依赖方式杀伤肿瘤细胞, 进一步证实了TH在肿瘤治疗中的显著优势 (图4)。随后, Zhang等[12]用TH修饰脂质体构建出一种pH响应脂质体 (TH-modified liposome, TH-Lip), 该脂质体在肿瘤

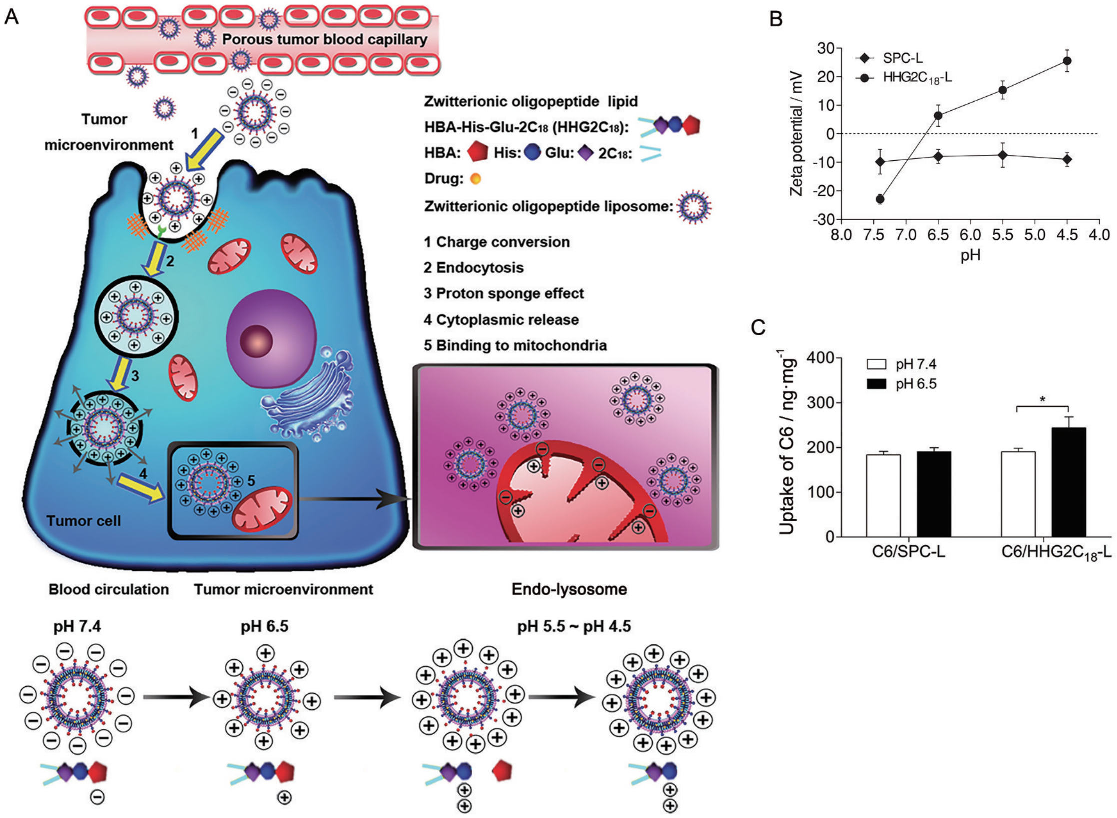

Figure 2　Multistage pH-responsive liposomes for mitochondrial-targeted anticancer drug delivery. A: Schematic design of the smart liposomes (HHG2C$_{18}$-L); B: Zeta potential of HHG2C$_{18}$-L and SPC-L at different pH values; C: Cellular uptake of C6/HHG2C$_{18}$-L and C6/SPC-L on A498 cells at pH 7.4 and pH 6.5. Uptake of C6 is the ratio between the amount of C6 in the cells (ng) and the amount of cell proteins (mg). $n = 3$, $\bar{x} \pm s$. $^{*}P < 0.05$. (Adapted from Ref. 7 with permission. Copyright © 2012 Wiley). SPC-L: Liposome consists of soy phosphatidylcholine and cholesterol; HHG2C$_{18}$-L: Liposome consists of soy phosphatidylcholine, cholesterol and HHG2C$_{18}$; C6: Coumarin 6

弱酸性环境中可迅速改变其带电行为, 使脂质体带正电荷, 显著促进脂质体在肿瘤内的渗透能力及入胞能力。研究结果表明, C26细胞及HepG2细胞对TH-Lip的摄取量显著高于聚乙二醇化的脂质体 (pH 6.3), 证明了TH能够促进纳米制剂的跨膜转运。

基于组氨酸的$\mathrm{p}K_{\mathrm{a}}$特殊性, 研究人员还将其用于构建可逆活化细胞穿膜肽 (reversibly activatable cell-penetrating peptides, RACPPs)[13], 以提高CPPs的特异性。Zaro等[14]设计了一种pH响应组氨酸−谷氨酸 (histidine-glutamic acid, HE) 重复序列, 并将其与一种CPP融合, 得到重组多肽 (histidine-glutamic acid-cell-penetrating peptide, HE-CPP)。研究发现, 与pH 7.4相比, HE-CPP被HeLa细胞摄取量显著低于pH 6.8, 证实了HE重复序列的pH敏感性。这是因为在正常生理条件下, 组氨酸 ($\mathrm{p}K_{\mathrm{a}} = 6.5$) 不带电荷, 带负电的谷氨酸 ($\mathrm{p}K_{\mathrm{a}} = 4$) 与带正电的赖氨酸 ($\mathrm{p}K_{\mathrm{a}} = 10$) 或精氨酸 ($\mathrm{p}K_{\mathrm{a}} = 12$) 通过静电相互作用屏蔽CPP上的正电荷。当融合蛋白HE-CPP处于弱酸环境时, 组氨酸发生质子化带正电, 谷氨酸与CPP序列中阳离子氨基酸之间的静电作用被解除, 从而使CPP的细胞穿膜活性被重新激活。更重要的是, HE-CPP作为一段氨基酸序列, 能够响应pH变化发生可逆的净电荷变化和构象变化, 因此被称为RACPP。

而在上述体系中, CPP既可以选自两亲性CPP, 又可选自阳离子CPP; 此外, HE-CPP不仅可以用于生物大分子的靶向递送, 还可以用于纳米药物的递送, 充分体现了HE-CPP序列构建的灵活性及其应用的广泛性 (表1)[13,15-20]。

2　pH响应型智能纳米药物递送系统

纳米药物递送系统因具有多种优势, 如提高药物溶解度和减少药物不良反应等, 被广泛研究用于抗肿瘤药物递送, 但是传统的纳米制剂难以克服体内存在的多重屏障, 实现药物的高效递送, 如大粒径的纳米制剂难以渗透进入肿瘤深部, 亲水性及负电性特征限制了制剂快速入胞, 稳定的结构难以实现药物的快速释放等。为解决这些问题, 智能响应型纳米递送系统应运而生, 可通过响应肿瘤内外环境的刺激而呈现不同的功能特性以达到理想的疗效[21]。其中, 肿瘤酸性环

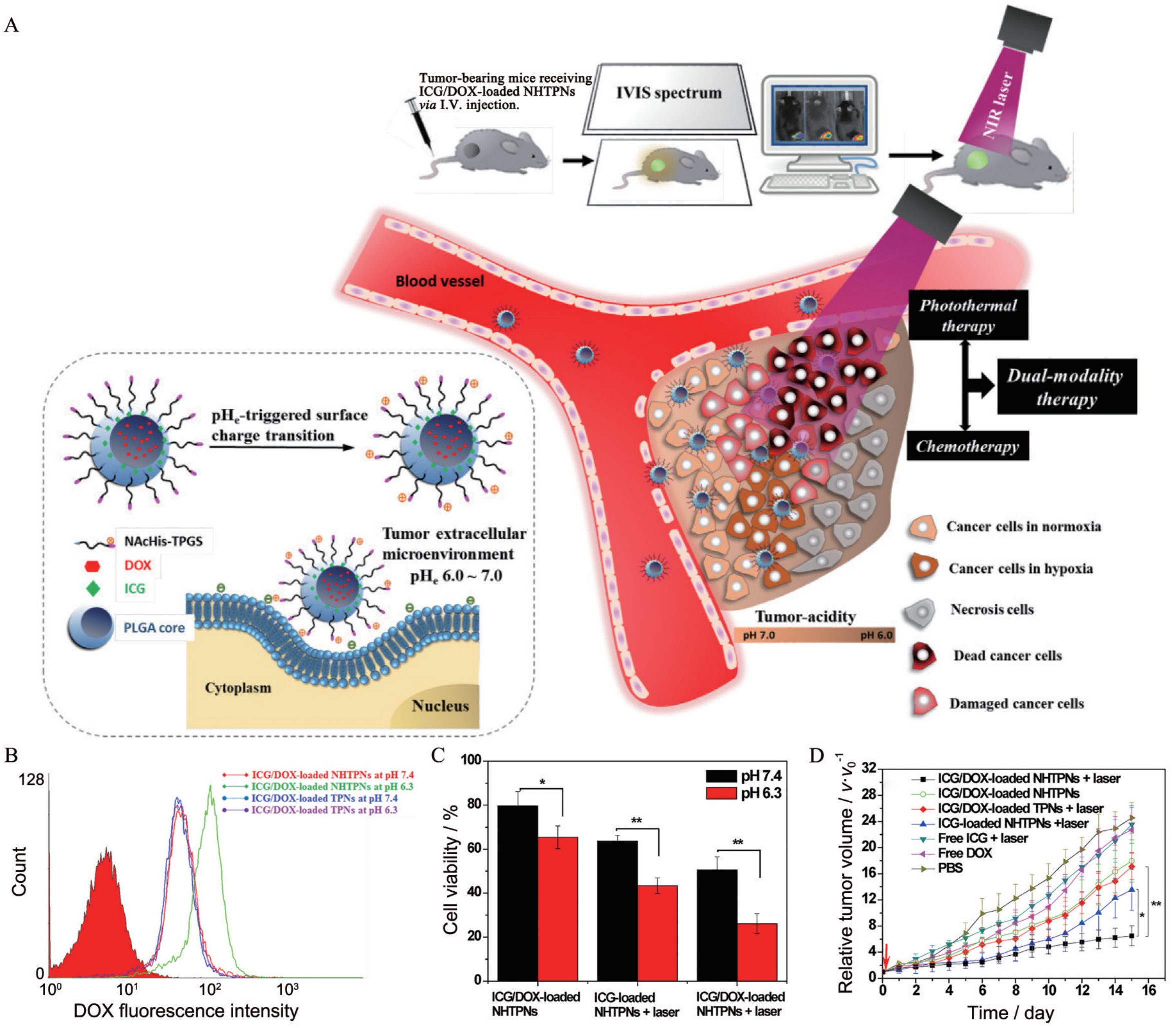

Figure 3　Active tumor permeation and uptake of surface charge-switchable theranostic nanoparticles for imaging-guided photothermal/chemo combinatorial therapy. A: Illustration of active tumor penetration and uptake of dual drug-loaded nanoparticles with pH_e-triggered surface charge transition for the imaging-guided photo-thermal/chemo combinatorial therapy; B: Flow cytometric histograms TRAMP-C1 cells treated with ICG/DOX-loaded TPNs and NHTPNs at pH 7.4 and 6.3 for 2 h; C: Cell viability of TRAMP-C1 cells incubated respectively with ICG-loaded NHTPNs and ICG/DOX-loaded NHTPNs; D: Tumor growth inhibition profiles of the mice bearing TRAMP-C1 tumor injected with various formulations, followed by NIR laser irradiation (5 min, 1.0 $W \cdot cm^2$) 6 h post-injection or without any laser treatment. $n = 6$, $\bar{x} \pm s$. $^{*}P < 0.05$, $^{**}P < 0.01$. (Adapted from Ref. 8 with permission. Copyright © 2016 Ivyspring). pH_e: Tumor extracellular pH; TPNs: TPGS/PLGA nanoparticles; NHTPNs: NAcHis-TPGS/PLGA nanoparticles; PBS: Phosphate buffered saline; NIR: Near-infrared

境是一个重要的靶点, 而组氨酸 pK_a 接近肿瘤酸性微环境 pH, 在生理条件下呈疏水性且不带电, 到达肿瘤微环境后迅速发生质子化转变正电性且亲水性增加。研究人员通常利用这种电性变化及“疏水-亲水”转变特性赋予胶束不稳定性以促进药物快速释放; 此外, 组氨酸质子化可以使药物载体呈正电性, 有利于增强纳米制剂的细胞摄取及渗透能力; 更重要的是, 纳米制剂在进入溶酶体后, 组氨酸可随着 pH 的不断下降进一步质子化, 发挥质子海绵效应, 促进药物的溶酶体逃逸。

2.1　组氨酸促进纳米制剂中药物快速释放　自 1984 年 Bader 等首次提出聚合物胶束的概念以来, 被广泛研究用于递送抗肿瘤药物[22]。尽管聚合物胶束具有良好的稳定性, 但在肿瘤部位药物通过扩散作用自胶束中释放的速度却较缓慢, 因此肿瘤细胞内游离药物浓度低、疗效差, 且具有诱导肿瘤细胞产生耐药性的风险。聚组氨酸 pK_a 接近肿瘤酸性微环境 pH, 在生理条件下呈疏水性, 到达肿瘤微环境后迅速发生质子化转变为亲水性高分子。研究人员将聚组氨酸的这种转变特性用于 pH 响应型聚合物胶束的构建, 其亲水疏水转变导致聚合物失去两亲性, 胶束解聚而释药, 提高了胶束中药物释放的可控性及其在肿瘤部位的药物蓄积量。

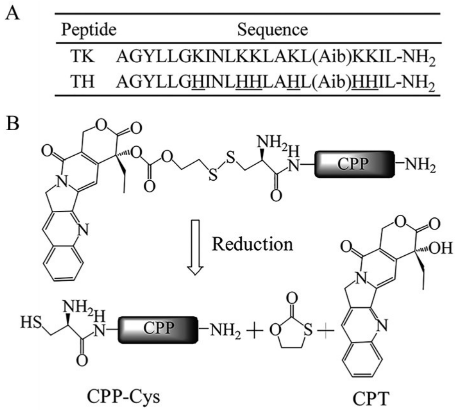

Figure 4　Sequences of peptides and structure of cell-penetrating peptides-camptothecin (CPP-CPT) conjugations. A: Sequences of TK and TH; B: Disulfide mediated release of CPT from CPPs. (Adapted from Ref. 11 with permission. Copyright © 2011 American Chemical Society). CPP-Cys: Cysteine-modified cell-penetrating peptides

2003年, Lee等[23]首次将聚组氨酸引入聚合物胶束中, 采用开环聚合的方式制备聚组氨酸-聚乙二醇嵌段共聚物 [poly(*L*-histidine)-poly(ethylene glycol) diblock copolymer, polyHis-*b*-PEG], 其在pH 8.0条件下可自组装为球形胶束, 在弱酸性条件下放置24 h后由于胶束解体释放出聚合物单体, 导致粒径呈双峰分布, 表明胶束结构已被破坏。进一步研究发现该两亲性聚合物对pH的变化过于敏感, 在pH 7.2时胶束已明显解体。因此, 该胶束尽管有利于药物在肿瘤部位的快速释放, 但在体内稳定性差, 对正常组织具有较大的不良反应。

于是, 研究人员采用与另外一种聚合物构建混合胶束的方式改善其在生理条件下的稳定性。Yin等[24]利用聚乳酸-聚乙二醇共聚物 [poly(*L*-lactic acid)-*b*-poly(ethylene glycol), PEG-PLLA] 与polyHis-*b*-PEG构建混合胶束, 通过调节二者摩尔比, 可显著改变体系的pH敏感性, 随着PEG-PLLA比例的逐步增加, 引起粒径发生显著变化所需pH值也逐渐下降。Wu等[25]构建出一种由二硬脂酰基磷脂酰乙醇胺-聚乙二醇 [1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(polyethylene glycol), DSPE-PEG] 及polyHis-*b*-PEG组成的混合胶束, 研究发现, 当pH从7.4下降至6.5左右时, 胶束粒径开始显著增加; 体外释放实验也表明, 胶束在pH 6.0及5.0条件下的释放速度及程度远远高于pH 7.4。

除了将聚组氨酸用于控制聚合物胶束中药物的可控释放, 其还被应用于无机纳米递送系统, Mu等[26]将polyHis-*b*-PEG修饰到介孔硅上, 在血液循环中, 聚组氨酸保持去质子化状态, 防止药物从介孔硅泄漏, 到达肿瘤部位后, 聚组氨酸发生质子化, 快速释放所负载的药物索拉非尼, 有效发挥抗肿瘤功效。

2.2　组氨酸促进制剂摄取及渗透　基于增强渗透滞留 (enhanced permeability and retention effect, EPR) 效应的优势, 许多抗肿瘤纳米制剂被开发出来, 如Abraxane、Onivyde、Marqibo和Nanotherm已被FDA批准上市[27]。尽管与常规化疗药物相比, 纳米制剂在临床前研究中表现出显著改善的疗效, 但肿瘤组织细胞外基质密度高、脉管系统和淋巴循环受损, 导致肿瘤组织间质压力高, 这些因素形成了阻碍纳米药物渗透到肿瘤深处的生物屏障, 导致纳米药物在临床试验中疗效欠佳[28]。研究表明中性或带微负电荷的纳米粒有利于长时间的血液循环, 而带正电荷的纳米粒可改善在肿瘤组织的渗透能力, 但是正电荷纳米粒进入血液循环后迅速被吞噬系统清除, 且具有较高的细胞毒性[29]。因此, 研究人员通过表面电荷转换策略来改善纳米药物的血液稳定性及渗透能力。组氨酸在生理pH环境中呈微弱的负电性, 而在肿瘤微环境中, 由于质子化呈现正电特性, 因此利于其修饰的纳米粒渗透到肿瘤深处。

Li等[30]设计了一种阳离子脂质体, 用于拓扑异构酶依托泊苷以及siRNA的共递送, 聚乙二醇化的组氨酸接枝壳聚糖硫辛酸 (PEGylation histidine-grafted chitosanlipoic acid, PHCL) 能够屏蔽阳离子脂质体的正电性, 提高制剂在血液循环中的稳定性, 且降低全身

Table 1　Examples of HE-CPP-based drug delivery systems. $(HE)_{10}$MAP: HEHEHEHEHEHEHEHEHEHEKLALKLALKALKAALKLAY; GST: Glutathione-*S*-transferase; $(HE)_{10}G_5R_6$: HEHEHEHEHEHEHEHEHEHEGGGGGRRRRRR; $(HE)_{15}G_5$: HEHEHEHEHEHEHEHEHEHEHEHEHEHEHEGGGGG; $(HE)_5$: HEHEHEHEHE; $(RG)_5$: RGRGRGRGRG; mPEG: Methoxy-poly(ethylene glycol); PLA: Polylactide; CPSO: Cholesterol and polyoxyethylene sorbitol oleate

HE-CPP type	Carrier	Cargo	Tumor model	Ref.
$(HE)_{10}$MAP	Fusion protein	GST	MDA-MB-231 human breast cancer xenograft	[15]
$(HE)_{10}G_5R_6$	Fusion protein	GST	HeLa human cervical cancer	[16]
$(HE)_{10}G_5R_6$	Liposomes	Artemisinin	4T1 mouse mammary cancer	[17]
$(HE)_{10}G_5R_6$	mPEG-PLA micelles	Paclitaxel	4T1 mouse mammary cancer	[13]
$(HE)_{15}G_5$	Fusion protein	Arginine deiminase	MDA-MB-231 human breast cancer xenograft	[18]
$(HE)_5$&$(RG)_5$	mPEG-PLA micelles	Docetaxel	MCF-7 human breast cancer	[19]
$(HE)_5$&$(RG)_5$	CPSO micelles	Paclitaxel	U87 human glioma xenograft; G422 mouse glioblastoma	[20]

毒性，在肿瘤酸性pH条件下，PHCL中组氨酸发生质子化，导致该囊泡表面电荷发生翻转，促进该脂质体在肿瘤组织的渗透 (图5)。Swetha等[31]将pH敏感组件组氨酸偶联到星形PLGA末端，并包载两种疏水性药物，在肿瘤组织中组氨酸质子化，呈正电荷状态，促进纳米粒快速渗透到肿瘤深处发挥作用。

近年来研究人员还发现纳米粒的形状对其在肿瘤内的扩散及入胞有显著影响，盘状及蠕虫状纳米粒比常用的球状纳米粒更易于渗透[32]。组氨酸可用于形态可变纳米粒的构建，在生理条件下纳米粒呈球形结构，质子化后纳米粒形态转变为其他更易渗透到肿瘤深部的形状。

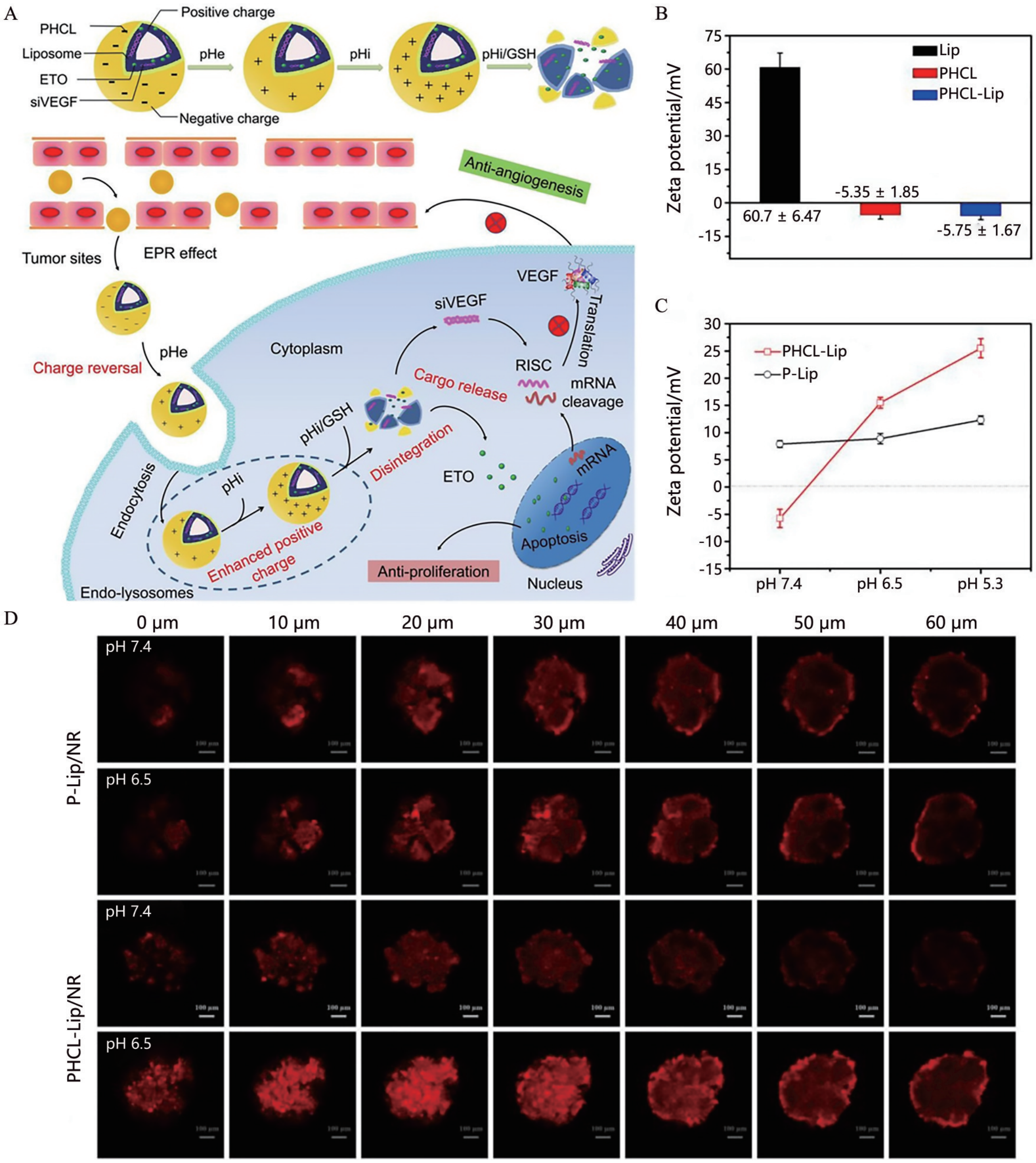

Figure 5　Co-delivery of VEGF siRNA and etoposide for enhanced anti-angiogenesis and anti-proliferation effect *via* multi-functional nanoparticles for orthotopic non-small cell lung cancer treatment. A: Schematic illustration of co-delivery of VEGF siRNA and etoposide; B: Zeta potential of the blank Lip, PHCL NP and PHCL-Lip; C: Zeta potential of PHCL-Lip and P-Lip at different pH; D: Scanned fluorescence distribution of the A549 spheroids after treated with PHCL-Lip/NR or P-Lip/NR at pH 7.4 or pH 6.5 conditions by CLSM. (Adapted from Ref. 30 with permission. Copyright © 2019 Ivyspring). ETO: Etoposide; siVEGF: Vascular endothelial growth factor small interference RNA; pH_i: Tumor intracellular pH; GSH: Glutathione; RISC: RNA-induced silencing complex; Lip: Blank cationic liposomes; PHCL: PEGylation histidine-grafted chitosanlipoic acid; PHCL-Lip: PHCL coated Lips; P-Lip: PEGylated Lips

Yang 等[33]设计了一种包含聚组氨酸的两亲性聚合物，在生理 pH 条件下，该两亲性聚合物自组装为粒径约为 40 nm 的球形纳米粒，可达到长循环和肿瘤靶向的效果；在肿瘤弱酸环境中，聚组氨酸质子化转变为亲水性分子，改变整个聚合物的亲疏水平衡，导致纳米粒形态转变为直径约为 7 nm 的纤维状结构，实现了较高的肿瘤渗透与滞留特性 (图 6)。

2.3　组氨酸促进基因递送效率　近年来，基因治疗因具有可持续发挥作用和彻底治愈肿瘤等优势，在抗肿瘤领域引起越来越多的关注，但是常用的基因递送载体如聚乙烯亚胺细胞毒性大和难以降解，并且所携带的基因难以从溶酶体逃逸，限制了基因治疗的进一步应用[34]。研究发现组氨酸的加入可显著改善基因治疗的效果，一方面，组氨酸咪唑环上的两个氢原子可以通过氢键与核酸分子相互作用，形成稳定的结构，保证核酸在血液循环中的稳定性；另一方面，组氨酸进入溶酶体后迅速中和酸性环境，导致质子主动运输，氯离子大量内流保持溶酶体电中性，这种流入导致大量水分子被动运输到溶酶体内，溶酶体发生膨胀和裂解，使核酸分子逸出到细胞质中 (质子海绵效应)[35]。

Gao 等[36]构建出一个 pH/还原双响应纳米载体用于递送多柔比星及 siRNA，其中聚乙二醇-聚乳酸-聚组氨酸三嵌段聚合物 [methoxy-poly(ethylene glycol)-polylactide-polyhistidine, mPEG-*b*-PLA-PHis] 通过二硫键与聚乙烯亚胺连接，该两亲性聚合物一方面可包载疏水药物多柔比星；另一方面聚乙烯亚胺可通过静电作用吸附 siRNA，肿瘤细胞内溶酶体的酸性使聚组氨酸质子化，有效增强了溶酶体逃逸能力，导致药物及 siRNA 快速释放 (图 7)。Dhanya 等[37]将组氨酸和精氨酸偶联的淀粉-聚乙烯亚胺修饰到金纳米粒上，用于 DNA 递送，显著提高了转染效率，且细胞相容性大大改善。

3　其他

近年来，金属离子与有机配体之间的配位作用所构建的递送体系如金属有机框架材料 (metal-organic frameworks, MOFs) 和金属配位聚合物 (metal coordination polymers, MCPs) 等，因其高负载能力，在药物递送领域受到越来越多的关注[38]。组氨酸标签 (histidine-tag, His-tag) 对多种金属离子具有较高的亲和力，基于此，Huang 等[39]制备出六聚组氨酸-锌离子配合物，该配合物不仅能够负载小分子荧光素 (载药量达 53%)，还能够负载分子质量高达 40 kDa 的葡聚糖 (载药量达 42%)。进一步研究发现，该配合物对于这两种分子质量相差较大的分子负载机制不完全相同，作者推测该配合物能够包载多种性质相差较大的药物分子，这有利于实现肿瘤的联合治疗。Tang 等[40]也利用组氨酸的这一性质，构建了金属配位凝胶。聚组氨酸主链上有多个咪唑基团，可诱导多价配位，改善水凝胶的稳定性。水凝胶还保留了组氨酸的 pH 响应能力，在 pH 7.5 时形成凝胶，当 pH 降低至 5.5 时呈现为溶胶状态，具备可注射性，该载体可用于药物的缓释递送。

Li 等[41]将次氮基三乙酸 (nitrilotriacetic acid, NTA) 与疏水长链偶联合成活性脂质，在镍离子和辅助脂类 (胆固醇及磷脂等) 作用下，制备一种新型非阳离子类脂纳米粒，用于组氨酸标记蛋白递送。研究表明，该类脂纳米粒能够将多种组氨酸标记蛋白，如绿色荧光蛋

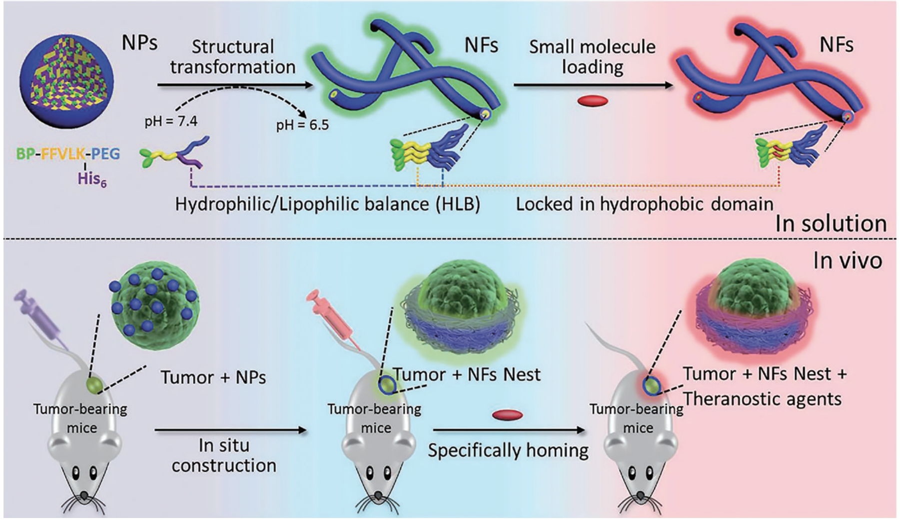

Figure 6　Principle of pH-triggered morphological transformation from self-assembled nanoparticles (NPs) to nanofibers (NFs). (Adapted from Ref. 33 with permission. Copyright © 2017 Wiley). BP: Bis-pyrene; FFVLK: Peptide Lys-Leu-Val-Phe-Phe; PEG: Polyethylene glycol; His6: Peptide His-His-His-His-His-His

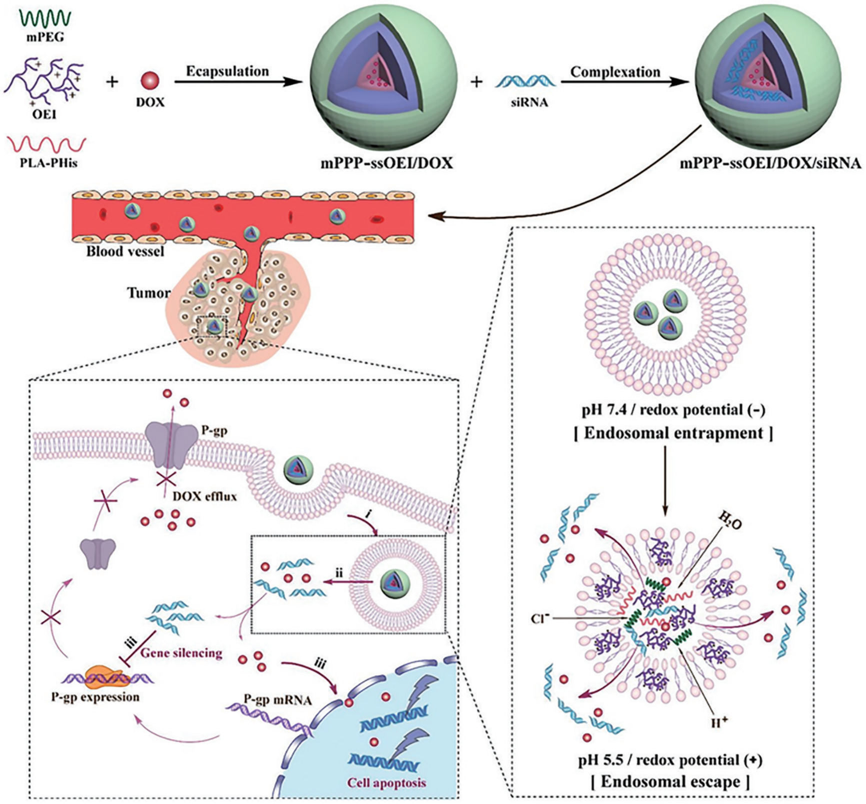

Figure 7　Schematic illustration of the pH/redox dual-responsive mPPP-ssOEI/DOX/siRNA codelivery polyplex with effective endo-lysosomal escape. (Adapted from Ref. 36 with permission. Copyright © 2019 American Chemical Society). OEI: Oligoethylenimine; PHis: Polyhistidine; mPPP-ssOEI: Methoxy-poly(ethylene glycol)-polylactide-polyhistidine-ss-oligoethylenimine; P-gp: P-Glycoprotein

白(green fluorescent protein, GFP)、绿色荧光蛋白及环化重组酶融合蛋白 [green fluorescent protein variant fused Cre recombinase, (-30)GFP-Cre] 导入哺乳动物细胞 (图8)。

组氨酸的这一性质还可用于蛋白纯化, 利用基因工程的方法将目的蛋白和His-tag进行融合, 而后通过一种固定化金属离子亲和色谱法 (immobilized metal-ion affinity chromatography, IMAC) 对目的蛋白进行纯化。Ma等[42]利用大肠杆菌构建一种肿瘤靶向肽, 并将组氨酸标签与其融合, 用IMAC从细菌粗提物中纯化目的蛋白, 纯化效果显著优于传统的纯化方法。

4　总结与展望

组氨酸作为一种具有pH响应能力的碱性氨基酸, 近年来已被广泛应用于肿瘤靶向递送领域, 一方面其可携带抗肿瘤药物分子高效入胞; 另一方面可用于构建pH响应型智能纳米药物递送系统, 赋予制剂多种功能特性 (促进纳米粒的深层渗透和细胞摄取, 以及调控药物释放), 在抗肿瘤领域发挥重要作用 (表2)[23-26,36,43-73]。

但是, 组氨酸作为药物递送系统的研究中, 仍存在一些亟待解决的问题, 如CPPs在血液中存在被血浆酶降解的风险、组氨酸构建的载体对肿瘤pH响应的灵敏度、在肿瘤部位的蓄积量等问题, 而解决这些问题的方

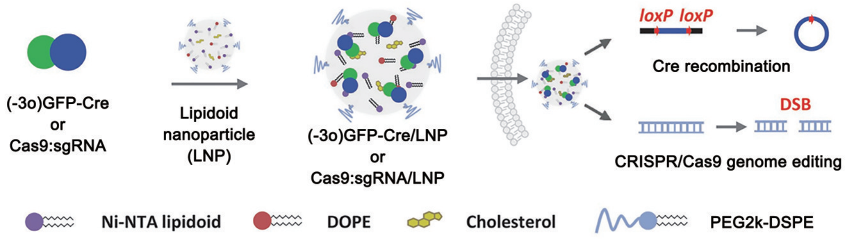

Figure 8　Schematic diagram of cargo protein loading and delivery by lipidoid nanoparticle formulations. (Adapted from Ref. 41 with permission. Copyright © 2018 Wiley). Cre: Cyclization recombination enzyme; Cas9: CRISPR-associated protein 9; sgRNA: Single-guide RNA; loxP: Locus of X (cross)-over in P1; DSB: Double strand breaks; DOPE: 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine

Table 2 Overview of drug delivery system employing histidine. PLLA: Poly(*L*-lactic acid); DSPE: 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine; RHMH18: Arg-Gly-Asp-human serum albumin-histidine18; MSN: Mesoporous silica nanoparticles; GA: Glycyrrhizic acid; DNP: Dinitrophenol; p(PEGA): PEG methyl ether acrylate; PLys: Poly(*L*-lysine); HA: Hyaluronic acid; Her2: Human epidermal growth factor receptor 2; TPGS: *D*-*α*-Tocopheryl polyethylene glycol succinate; 7pep: Transferrin receptor ligand; DTPA: Diethylenetriamine pentaacetic acid; VES: Vitamin E succinate; DexPHS: Dextran-*b*-poly(*L*-histidine); pHPMA: Poly *N*-(2-hydroxypropyl) methacrylamide; PIA: Poly(itaconic acid); FA: Folate acid; ALA: 5-Aminolevulinic acid; DPPE: 1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; IR820: New indocyanine green; SPION: Superparamagnetic iron oxide nanoparticle; PSD: Poly(sulfadimethoxine); R848: Resiquimod; GNR: Gold nanorod; ABT737: BH3 mimetic; ZnPc: Zinc phthalocyanine; UCNP: Upconversion nanoparticle; QDs: Quantum dots; Ab: Antibody; BHQ3: Black hole quencher 3; PBLG: Poly(*γ*-benzyl-*L*-glutamate)

Component	Carrier	Cargo	Ref.
PEG-PHis	Micelles	/	[23]
PHis-PEG/PLLA-PEG	Micelles	Doxorubicin	[24]
DSPE-PEG/PHis-PEG	Micelles	Paclitaxel	[25]
RHMH18	Nanoparticles	Paclitaxel	[43]
MSNs-PHis-PEG	Inorganic nanoparticles	Sorafenib	[26]
mPEG-PLA-PHis-ssOEI	Nanoparticles	Doxorubicin; siRNA	[36]
mPEG-PHis-PLLA	Micelles	Doxorubicin	[44]
mPEG-PLA-PHis	Micelles	Doxorubicin	[45]
PEG-Phis-PLLA	Micelles	Doxorubicin	[46]
PLA-*b*-PEG-*b*-PHis	Micelles	Doxorubicin	[47]
GA-PEG-PHis-PLGA	Micelles	Andrographolide	[48]
PHis-PLGA-PEG-PLGA-PHis	Micelles	Doxorubicin	[49]
PLGA-*b*-PHis-*b*-PEG-herceptin	Nanoparticles	Doxorubicin	[50]
mPEG-*b*-PLA-*b*-DNP-PHis	Micelles	Doxorubicin	[51]
p(PEGA)-*b*-PLys-*b*-PHis	Polymersomes	Doxorubicin	[52]
PHis-*b*-PEG/PLA-*b*-PEG-folate	Micelles	Doxorubicin	[53]
HA-PHis/Her2-TPGS	Micelles	Doxorubicin	[54]
PHis-PEG/7pep-DSPE-PEG	Micelles	Doxorubicin	[55]
HA-PHis/TPGS	Micelles	Doxorubicin	[56]
PLA-PEG-PLys-DTPA/PHis-PEG/Gd	Nanoparticles	Sorafenib; Gd	[57]
mPEG-PHis-VES/Biotin-PEG-VES	Micelles	Doxorubicin	[58]
DexPHS	Micelles	Doxorubicin	[59]
HA-PHis	Micelles	Doxorubicin	[60]
PHis-PLGA-TPGS	Micelles	Doxorubicin	[61]
pHPMA-PHis-PLeu	Micelles	Paclitaxel	[62]
PIA-PEG-FA-PHis	Micelles	Doxorubicin	[63]
ALA-PHis	Prodrug	ALA	[64]
DPPE-PHis-IR820	Nanovesicles	IR820; doxorubicin	[65]
SPION encapsulated PEG-PHis solid nanoparticles	Nanoparticles	Doxorubicin	[66]
mPEG-PHis-PSD /PLys/siRNA	Nanoparticles	siRNA	[67]
PLys-*g*-PHis	Nanoparticles	siRNA	[68]
HA-DOX/PHis/R848	Nanoparticles	R848; doxorubicin	[69]
GNRs/MSN/PHis/TPGS	Inorganic nanoparticles	Doxorubicin	[70]
ABT737@ZnPc-UCNPs	Up-converting nanoparticles	ZnPc; Bcl-2 inhibitor ABT737	[71]
QDs-Ab-BHQ3	Quantum dots	/	[72]
PLys-*b*-(PHis-*co*-PBLG)-b-PLys	Hydrogel	Gemcitabine	[73]

法往往需要再加入更多的高分子材料, 增加制剂的复杂性及不安全性, 因此可能阻碍临床转化的实现。在未来的研究中, 还需要重点解决以下几个方面的问题: ① 控制合成聚合物载体的多分散性, 实现更窄的分子质量分布, 以减小批间差异; ② 组氨酸的pH响应性可与其他具有刺激响应能力的载体联用, 开发多功能高分子载体用于肿瘤的治疗; ③ 重视肿瘤诊疗一体化系统的构建, 增加肿瘤可视性; ④ 开发适合大规模制备基于组氨酸的聚合物载体的方法, 且尽量减少有机溶剂的使用, 以确保载体的安全性。

此外, 作为人体内的一种氨基酸, 组氨酸对于人体是否还具有一些其他影响的研究目前还不够深入。对组氨酸进行深入研究, 将有望进一步优化肿瘤诊疗手段, 解决目前的诸多问题。

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利益冲突: 本文所有作者均声明不存在利益冲突。

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