多配基组合修饰提升抗肿瘤纳米药物功效的研究进展

张小雨[1], 吴送姑[1], 徐 晖[2], 龚俊波[1], 邢金峰[1], 魏振平[1*]

(1. 天津大学化工学院, 天津 300350; 2. 沈阳药科大学药学院, 辽宁 沈阳 110016)

摘要: 抗肿瘤纳米药物从给药位置进入体内后, 还需跨越一系列生理病理障碍, 到达目标作用位置才能有效发挥抗肿瘤疗效。配基修饰策略是提升纳米药物体内递送效率的经典方法, 但具有单一性和阶段性特点的单配基修饰策略与具有多变性和全过程性特点的体内递送进程之间的矛盾, 决定了仅使用单一配基修饰的纳米药物不能满足目标药效需求。所以凭借纳米药物表面积优势使用多配基组合修饰策略是推进新一代智能纳米药物的关键, 本文在对应体内递送进程总结分类了常用功能配基的基础上, 重点讨论了多配基组合修饰抗肿瘤纳米药物的优势及研究进展, 并依据配基组合方式将多配基组合修饰分为协同型及互补型, 这对于保障抗肿瘤纳米药物顺利克服多重生理病理障碍实现精准递送具有重要意义。

关键词: 纳米药物; 协同型组合修饰; 互补型组合修饰; 肿瘤递送

中图分类号: R943 **文献标识码**: A **文章编号**: 0513-4870(2024)07-1942-10

Research progress on the antitumor efficacy improvement for nanomedicine by combinatorial modification with multiligand

ZHANG Xiao-yu[1], WU Song-gu[1], XU Hui[2], GONG Jun-bo[1], XING Jin-feng[1], WEI Zhen-ping[1*]

(*1. School of Chemical Engineering, Tianjin University, Tianjin 300350, China; 2. School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China*)

Abstract: After entering the body from the drug delivery site, antitumor nanomedicines need to cross a series of physiopathological barriers to reach the target site of action to effectively exert antitumor therapeutic effects. The ligand modification strategy is a classic method to enhance the efficiency of nanomedicine delivery *in vivo*, but the contradiction between the single ligand modification strategy, which is characterized by unity and stage, and the *in vivo* delivery process, which is characterized by versatility and whole-process characteristics, determines that nanomedicines modified by a single ligand alone cannot satisfy the target efficacy requirements. Therefore, the use of multiligand combinatorial modification strategies by virtue of nanomedicine surface area advantages is key to advancing the next generation of smart nanomedicines. In this paper, on the basis of summarizing and classifying the commonly used functional ligands for *in vivo* delivery, the advantages and research progress of multiligand combination modification of antitumor nanomedicines are discussed with special focus, and the multiligand combination modification is classified as synergistic and complementary according to the combination of the ligands, which is of great significance to ensure that antitumor nanomedicines can overcome the multiple physiopathological barriers to achieve precise delivery.

Key words: nanomedicine; synergistic combinatorial modification; complementary combinatorial modification; tumor delivery

收稿日期: 2023-11-19; 修回日期: 2024-04-27.

基金项目: 国家自然科学基金资助项目 (22078234, 22178254).

*通讯作者 Tel: 13752416756, E-mail: zpwei2000@sina.com

DOI: 10.16438/j.0513-4870.2023-1306

肿瘤是指在致瘤因子的作用下局部组织细胞在基因水平上失去对生长的正常调控导致细胞异常增殖所形成的一种新生物，据报道2020年在中国与癌症相关的死亡人数相比2005年增加了21.6%，达到了239.78万，可见肿瘤治疗仍然面临着巨大的挑战[1]。化疗作为肿瘤治疗的主要方法，是通过使用细胞毒药物来达到抑制肿瘤的目的。其中纳米载药系统凭借独有的纳米尺寸能有效负载并递送抗肿瘤药物，成为了肿瘤化疗领域的研究热点[2,3]，其系统剂型特点与需要跨越的生理病理障碍之间高度复杂的相互关系决定了药物在体内的抗肿瘤疗效。

配基修饰策略是常用的纳米药物剂型优化手段，但传统单配基修饰策略只选择一种配基对纳米药物进行优化修饰，结果制备出的纳米药物机制单一，不能应对复杂的体内递送[4]。Li等[5]欲使用转铁蛋白 (transferrin, Tf) 单配基修饰策略来增强胡椒碱 (piperine, PIP) 纳米颗粒的抗肿瘤作用，但体内实验表明使用Tf修饰后的纳米颗粒在治疗荷瘤小鼠后得到的最终肿瘤体积，与使用Tf修饰策略前的纳米颗粒在治疗荷瘤小鼠后得到的最终肿瘤体积相似，分别为353.42和363.20 mm^3。这种配基修饰前后抗肿瘤作用没有明显改善的根本原因是，仅用Tf修饰的纳米颗粒只能特异性亲和肿瘤部位中的转铁蛋白受体 (transferrin receptor, TfR)，具有单一性和阶段性特点，导致修饰后的纳米药物依然不能有效面对具有多变性和全过程性特点的体内递送进程。多配基组合修饰策略是解决目前单配基修饰纳米药物由于不能应对实际体内递送，而导致出现药效低、毒性大、稳定性差的有效途径，所以本文首先依据纳米药物体内递送进程总结分析了常用配基的功能机制，进一步重点讨论了使用多配基组合修饰手段提升纳米药物递送效率的一种新策略。

1 纳米药物体内递送进程

尽管纳米药物能够有效杀死培养皿中的肿瘤细胞，但一旦应用于体内就很难达到预想效果，这是因为在实体中纳米药物从给药位置到目标作用部位的递送是一项具有多重阶段且富含变化的逐步过程 (图1)，纳米药物首先需在主要包括单核吞噬细胞系统 (mononuclear phagocyte system, MPS) 的其他部位和肿瘤部位之间进行选择分布，其次需渗透到肿瘤内部，然后进入肿瘤细胞内、进一步到达细胞质和最终定位在亚细胞作用部位才能有效发挥药效。相对应纳米药物从给药位置进入体内后需经过多重阻碍才能完成这一递送过程，其中主要包括MPS清除障碍[6]、肿瘤定位障碍[7]、肿瘤低渗透性障碍[8]、细胞膜障碍[9]、溶酶体降解障碍[10,11]和亚细胞定位障碍[12]，每经过一个障碍都会导致到达目标部位的纳米药物总量减少，出现药效减小毒性加大的现象。有效的纳米药物设计应该考虑所有这些阶段和障碍，以最高效方式将药物输送到目标作用部位，从而达到药效大而毒性小的设计初衷。

2 对应体内递送进程的常用配基功能机制

纳米药物递送系统的设计目的是将药物从给药位置递送到目标作用部位，这段旅程主要经过分布、渗透、内化、到达细胞质和细胞内定位5个阶段，所以纳米药物在理想状态下应具有非肿瘤排斥、肿瘤亲和、肿瘤渗透、细胞膜穿透、溶酶体逃逸和亚细胞靶向功能才能实现最大程度的精准用药。

配基修饰策略作为提高纳米药物递送效率的经典优化手段，深入了解各种配基功能机制是科学应用的

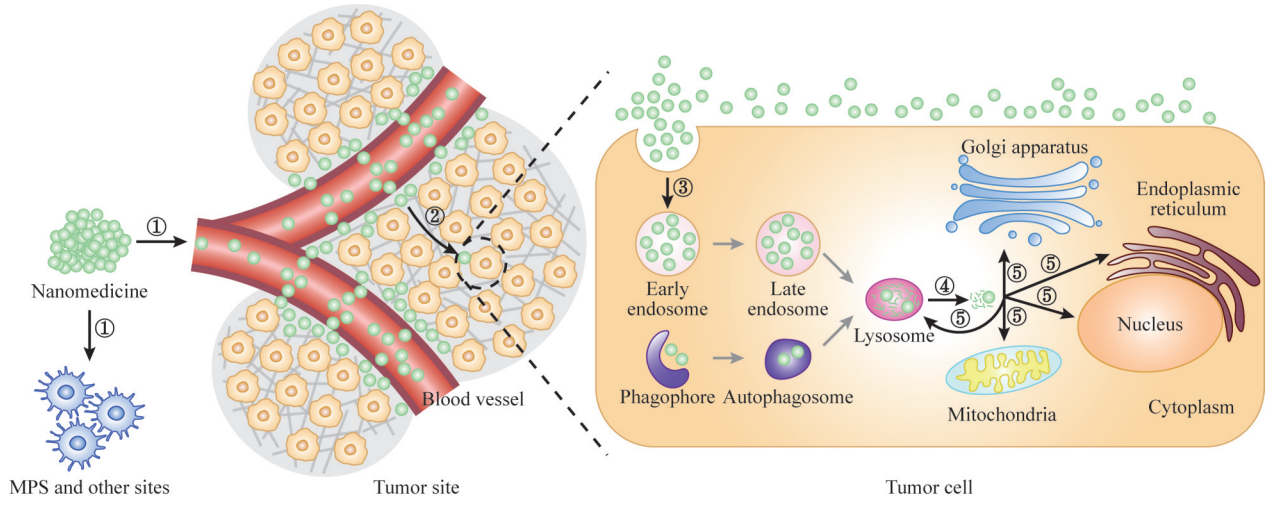

Figure 1 The stepwise delivery process of nanomedicine from the site of administration to the target site of action. ① Selective distribution at other sites including mainly MPS and tumor sites; ② Penetration inside the tumor; ③ Entry into the tumor cell; ④ Reaching the cytoplasm; ⑤ Localization of subcellular action sites. MPS: Mononuclear phagocyte system

前提, 依据纳米药物体内递送进程, 配基可以分为非肿瘤排斥型配基、肿瘤亲和型配基、肿瘤渗透型配基、细胞膜穿透型配基、溶酶体逃逸型配基和亚细胞靶向型配基6类 (图2), 分别对应了纳米药物从给药部位到作用部位的5个阶段和6个障碍 (表1)[13-32]。

2.1 非肿瘤排斥型配基

导致抗肿瘤药物在肿瘤中低分布的一个主要障碍是肿瘤部位之外的MPS对纳米药物的竞争。MPS是先天免疫系统的一部分, 由树突状细胞、单核细胞和巨噬细胞组成, 能够吞噬纳米药物等外来异物[33]。在血液循环中的纳米药物首先容易被调理素快速吸附, 进而就会被MPS快速识别清除[34]。大多数不受保护的纳米药物, 无论它们的组成材料是什么, 都可以从血液循环中被MPS迅速移除[35]。

为了使纳米药物留在血液中直到到达肿瘤部位, 它们需要最大限度地避开MPS的吞噬捕获。在纳米药物表面修饰隐形配基就可以有效躲避MPS的识别清除, 从而产生长期循环的纳米药物, 达到一种非肿瘤部位排斥的效果。在所有非肿瘤排斥型配基中最有效和最常见的就是聚乙二醇 (polyethylene glycol, PEG), PEG是一种柔性亲水聚合物, 可以利用空间斥力阻碍血浆蛋白质的吸附而躲避MPS的清除[36]。一项生物分布研究表明PEG修饰纳米颗粒在静脉注射后的所有时间点 (60 min、360 min和24 h), 在MPS主要器官肝脏中的荧光强度都低于未修饰纳米颗粒, 相反PEG修饰纳米颗粒在肿瘤组织中却能实现有效聚集[13]。说明非肿瘤排斥型配基的修饰能够赋予纳米药物躲避MPS清除的能力, 从而反向提高肿瘤部位对纳米药物的竞争力。

2.2 肿瘤亲和型配基

理想的纳米药物应该对肿瘤部位有特异亲和性, 从而能最大限度地聚集在肿瘤部位发挥疗效。纳米药

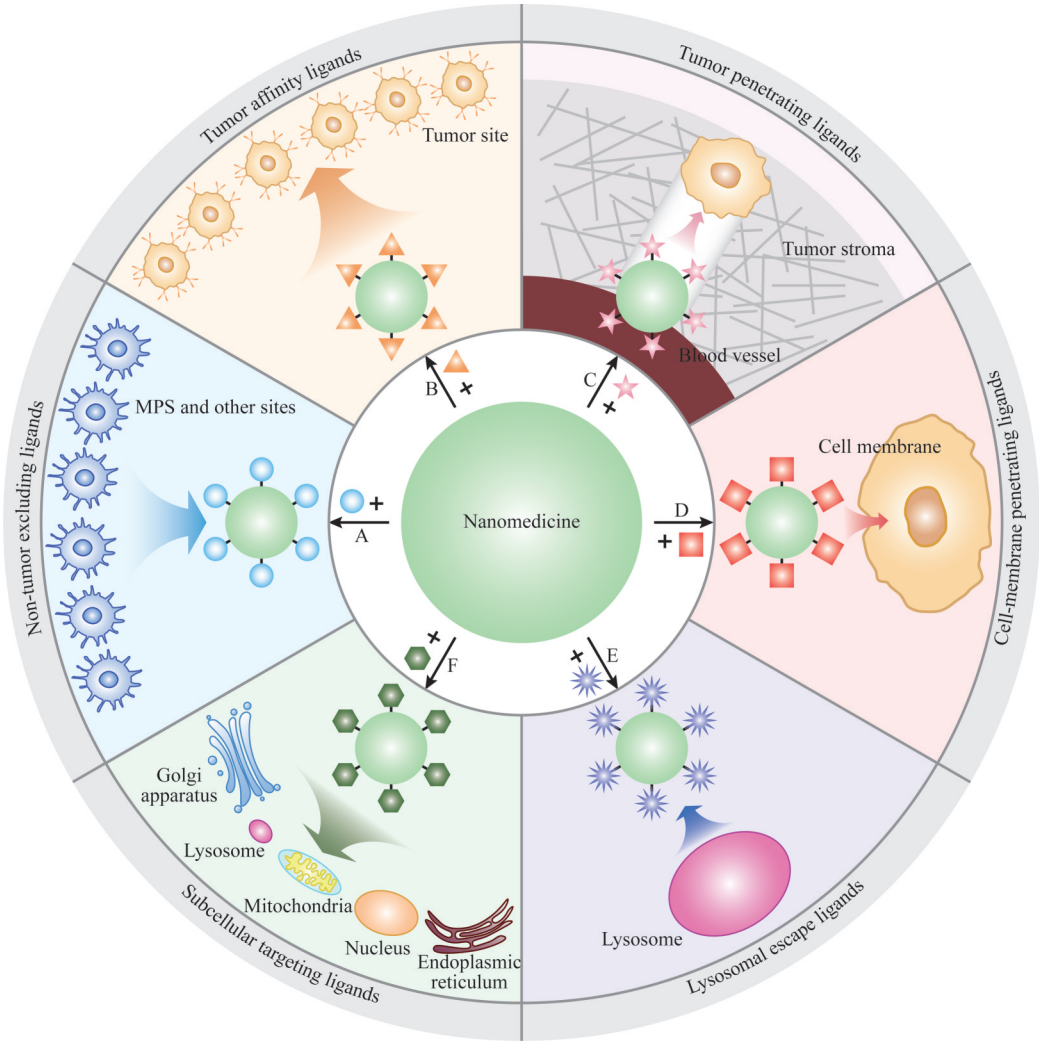

Figure 2 Classification of ligands according to the *in vivo* delivery process of nanomedicine. A: Non-tumor excluding ligands; B: Tumor affinity ligands; C: Tumor penetrating ligands; D: Cell-membrane penetrating ligands; E: Lysosomal escape ligands; F: Subcellular targeting ligands

Table 1 Summary of commonly used ligands corresponding to nanomedicine delivery processes and barriers *in vivo*[13-32]. PEG: Polyethylene glycol; FA: Folic acid; HA: Hyaluronic acid; Tf: Transferrin; RGD: Arg-Gly-Asp; GSH: Glutathione; TAT: RKKRRQRRR; PEI: Polyethyleneimine; LA: Lauric acid; NLS: Nuclear localization signal; TPP: Triphenylphosphine; CS: Chondroitin sulfate

Delivery process	Delivery barrier	Type of ligands	Commonly used ligand	Ref.
Distribution	MPS clearance	Non-tumor excluding ligands	PEG	[13]
	Tumor localization	Tumor affinity ligands	Biotin	[14]
			FA	[15]
			HA	[16]
			Tf	[17]
			Trastuzumab	[18]
			RGD	[19]
			AS1411 aptamer	[20]
Penetration	Tumor hypo-permeability	Tumor penetrating ligands	GSH	[21]
			Chitosan	[22]
			Collagenase	[23]
Internalization	Cell membrane	Cell-membrane penetrating ligands	TAT	[24]
			Melittin	[25]
Reaching the cytoplasm	Lysosomal degradation	Lysosomal escape ligands	PEI	[26]
			LA	[27]
Subcellular localization	Subcellular localization	Subcellular targeting ligands	NLS	[28]
			TPP	[29]
			Morpholine	[30]
			Pardaxin	[31]
			CS	[32]

物可以利用肿瘤表面过度表达的特征受体作为肿瘤亲和位点, 并利用受体配体结合原理, 将能识别肿瘤特征受体的相应配体如小分子[14,15]、聚糖[16]、蛋白质[17]、抗体[18]、多肽[19]和核酸适配体[20]修饰于纳米药物上, 就能够顺利获得对肿瘤部位的特异性亲和力, 从而增加在肿瘤部位的蓄积量。Deng等[17]统计了一种被肿瘤亲和型配基Tf修饰前后的纳米颗粒在肿瘤与全身之间的信号比, 数据表明Tf修饰后的纳米颗粒在肿瘤与全身之间的信号比能达到7%, 然而无Tf修饰时的纳米颗粒的信号比与游离染料相似, 在所有时间点都没有在肿瘤部位检测到明显的信号, 此研究表明了肿瘤亲和型配基对纳米药物体内分布结果的重要性。

2.3 肿瘤渗透型配基

抗肿瘤纳米药物需渗出血管, 穿透肿瘤组织接触肿瘤细胞才能发挥药效, 但由于高通透性和滞留 (enhanced permeability and retention, EPR) 效应存在异质性, 肿瘤环境中存在血管紊乱、间质压力高和细胞外基质 (extracellular matrix, ECM) 致密等问题及脑肿瘤治疗中存在的血脑屏障 (blood brain barrier, BBB), 都会导致纳米药物出现无法进入肿瘤组织或肿瘤细胞完成药物输送的现象[37,38]。特别是在脑肿瘤治疗中, 由于脑部存在一道由脑部毛细血管内皮细胞、基底膜、周细胞和星形胶质细胞等构成的BBB, 有效分隔了血液和脑实质, 进一步阻碍了纳米药物接触目标脑肿瘤细胞[38]。纳米药物如何跨出血管并有效地进入穿透肿瘤实质仍然是目前肿瘤治疗的主要障碍。

如果一种配基能利用主动转胞吞作用, 从细胞的一侧被运送到另一侧, 那么纳米药物使用此配基修饰后就能主动地渗入和渗透实体瘤[39]。谷胱甘肽 (glutathione, GSH) 就是一种可利用主动转胞吞作用的肿瘤渗透型配基, 它首先在肿瘤环境中被γ-谷氨酰转肽酶水解生成阳离子, 再利用带负电的细胞膜来触发主动转胞吞作用, 一种脂质体在使用GSH修饰策略后在激光共聚焦显微镜下显示能快速渗出血管并穿透整个肿瘤球体, 相反不使用GSH修饰的脂质体滞留在血管里很难渗出, 并且渗出的脂质体也只分布在肿瘤的周边而不是内部, 这项研究展示了肿瘤渗透型配基对于纳米药物渗透的重要性[21]。另外利用转胞吞作用也能使纳米药物有效渗透BBB, Qian等[22]为了提高负载卡莫司汀 (carmustine, BCNU) 的纳米颗粒对脑胶质瘤的治疗效果, 将一种肿瘤渗透型配基 (壳聚糖) 修饰在纳米颗粒表面, 纳米颗粒在被壳聚糖修饰后, 颗粒表面由负电转为正电, 进而能顺利引发带负电的脑部毛细血管内皮细胞的转胞吞作用, 最后分布结果显示, 无论是在正常脑组织还是脑部的肿瘤组织中, 纳米颗粒的蓄积量都显著高于游离药物, 成功揭示了壳聚糖作为肿瘤渗透型配基治疗脑胶质瘤的前景。

另外胶原作为ECM的主要成分是阻碍纳米药物在肿瘤中顺利转运的主要原因[40], 利用能特异性降解胶原的胶原酶来修饰纳米药物, 就可以有效提高纳米药物的肿瘤渗透力。Cui等[23]用体外肿瘤细胞球模型评估了胶原酶修饰前后纳米颗粒的肿瘤穿透能力, 结

果显示无胶原酶修饰时的纳米颗粒仅能穿透球体的0.1%, 而胶原酶修饰后的纳米颗粒却能穿透球体的27%, 相比无胶原酶修饰组穿透力提高了269倍, 这种渗透改善可以归因于胶原酶能降解ECM中存在的胶原, 从而促进了纳米颗粒在肿瘤内的深度分布。

2.4 细胞膜穿透型配基

细胞膜是一种以磷脂为主的半透膜, 具有吸收营养物质、排出代谢废物和维持细胞内环境等重要功能[41]。然而在抗肿瘤药物面前, 细胞膜对物质进出的严格控制是药物进入细胞内的障碍, 抗肿瘤纳米药物通常必须克服这一障碍才能到达目标作用部位。

细胞穿透肽 (cell-penetrating peptide, CPP) 是一类对细胞膜具有强力穿透作用的短肽, 修饰在纳米药物表面能有效增加纳米药物的入胞率[42,43]。有研究表明一种用经典穿透肽TAT (RKKRRQRRR) 修饰的金纳米颗粒在孵育肿瘤细胞24 h后, 胞内的金含量是未修饰纳米颗粒的3.1倍[24]。同时随着对细胞膜穿透研究的不断深入, 多种新型CPP被陆续开发, 例如一种来源于蜜蜂毒液依靠穿孔作用实现膜穿透功能的蜂毒肽 (GIGAVLKVLTTGLPALISWIKRKRQQ) [44,45], 在修饰纳米颗粒后, 能使细胞中的乳酸脱氢酶 (lactate dehydrogenase, LDH) 大量释放, 8 h后释放率可达60%以上[46]。并且使用蜂毒肽修饰策略的纳米颗粒能显著提高所负载多柔比星 (doxorubicin, DOX) 在肿瘤细胞中的药物浓度, 此细胞内化效果的提升主要依靠于蜂毒肽的膜穿孔作用[25]。

2.5 溶酶体逃逸型配基

通过内吞途径进入细胞的纳米药物首先被包裹在早期内体中, 然后早期内体逐渐成熟成晚期内体与含有很多消化酶的溶酶体融合, 纳米药物被引导进行降解消化后才被允许进入细胞质[47]。另外进入了细胞质的药物又可能诱导细胞发生自噬, 产生自噬小体来重新捕获纳米药物, 进一步将纳米药物递送到溶酶体进行降解[11,48]。溶酶体是内吞和自噬途径共同的降解终点, 对于以溶酶体为靶点的纳米药物, 接触溶酶体提供了直接接近其目标的途径, 然而对于大多数纳米药物来说, 导致纳米药物被困在溶酶体中被降解是不可取的, 有效逃逸溶酶体降解是纳米药物入胞后发挥药效的关键[49]。

作为最具代表性和使用频率最高的溶酶体逃逸型配基, 聚乙烯亚胺 (polyethyleneimine, PEI) 是一种含有丰富氨基的阳离子聚合物, 能有效驱使纳米药物在内体阶段发生逃逸, 进而有效避免溶酶体降解[50]。PEI能通过两种机制驱动内体逃逸功能, 第一种是与内体膜作用使内体膜变得多孔, 从而使货物泄漏到细胞质中, 第二种机制是质子海绵效应, 通过捕获大量质子, 使离子和水内流, 增大内体内渗透压造成膜的胀破来实现内体逃逸[51,52]。一种使用PEI修饰策略的丝素纳米粒在共聚焦显微镜下显示其与内体几乎无重合, 表明PEI修饰的纳米颗粒不在内体中聚集停留, 具有良好的内体逃逸能力[26]。

另外在纳米药物表面修饰氯喹 (chloroquine, CQ) 和月桂酸 (lauric acid, LA) 等针对自噬途径的溶酶体逃逸型配基, 逐渐成为一种促使纳米药物进入细胞质避免溶酶体降解的有效策略[27,53]。Jiang等[27]研究发现一种能够诱导细胞内部发生自噬的蛋白质纳米胶囊在使用LA修饰后, 最终能有效存在于细胞质中而不是溶酶体中, 推测LA可通过自噬途径保护纳米药物不被溶酶体降解。

2.6 亚细胞靶向型配基

普通纳米药物在到达细胞质之后, 与细胞内结构是随机作用的关系, 但根据药物作用机制, 药物在目标亚细胞位置聚集才能最大限度地发挥药效。根据药物作用机制, 设计出能靶向目标亚细胞位置的纳米递送系统将能有效确保疗效, 将药物治疗推进到更精确的阶段[54]。

2.6.1 细胞核靶向型配基 细胞核作为细胞的心脏, 遗传物质在这里储存、复制和转录, 是靶点中最敏感和最脆弱的位置[55]。在跨越一系列生理病理障碍后, 细胞核通常是DOX、顺铂 (cisplatin, DDP) 和羟基喜树碱 (hydroxycamptothecin, HCPT) 等抗肿瘤药物的最终目标[56]。核定位信号 (nuclear localization signal, NLS) 作为最常用的细胞核靶向型配基, 能够高效介导纳米药物向细胞核转运的过程[57-59]。Maity等[28]指出用低、中、高含量的乱序重排肽修饰的纳米量子点在细胞核及其附近积累量很低, 平均分别占细胞内量子点总数的17.3%、21.1%和25.5%, 然而用同样含量的NLS多肽修饰的量子点却具有明显的核靶向性, 在细胞核及其附近的积累量平均分别占细胞内量子点总数的30.4%、43.3%和49.0%, 纳米量子点积累量的显著提升依赖于NLS多肽的核定位作用, 此结果对核靶向配基的研究开发提供了重要理论依据。

2.6.2 线粒体靶向型配基 线粒体作为参与细胞代谢、信号传递和能量产生的重要细胞器, 已被证明是包括二甲双胍等许多抗肿瘤药物治疗的靶点[60,61]。对于以线粒体为治疗靶点的抗肿瘤纳米药物, 能否有效聚集在线粒体是治疗效果的关键, 其中三苯基膦 (triphenylphosphine, TPP) 作为一种亲脂性阳离子配基由于可以穿过疏水带负电的线粒体膜, 常用作线粒体靶向型配基推动纳米药物在线粒体的靶向聚集[62]。Tan

等[29]为提高负载DOX和吴茱萸碱胶束的抗肿瘤疗效, 采用TPP修饰策略, 结果显示胶束在被TPP修饰后明显增加了和线粒体的重叠率, 同时体外细胞毒性显示未修饰胶束的半抑制浓度 (half maximal inhibitory concentration, IC_{50}) 是TPP修饰胶束IC_{50}的1.52倍, 此项结果表明纳米药物在TPP的作用下能有效聚集在线粒体, 从而最终有效抑制了肿瘤细胞。

2.6.3 溶酶体靶向型配基 溶酶体不仅是细胞中的"垃圾处理系统", 还参与了细胞程序性死亡、代谢和免疫调节等其他细胞过程, 是肿瘤治疗中一个有吸引力的靶点[63,64]。其中姜黄素 (curcumin, Cur) 就能以溶酶体为药效靶点来影响肿瘤的增殖、侵袭、转移、耐药和免疫功能[64]。吗啉可以凭借自身碱性基团来靶向溶酶体的酸性环境, 是常用的溶酶体靶向型配基之一[65]。一种用于递送Cur的脂质体在使用吗啉修饰后, 在细胞内与溶酶体的重叠系数从0.598提高到了0.836, 使脂质体对溶酶体的靶向力提高了39.8%, 溶酶体靶向型配基的修饰让纳米药物在细胞内不再是随机分布, 而是能靶向聚集在溶酶体部位[30]。

2.6.4 内质网靶向型配基 内质网作为一种动态多功能细胞器, 参与了脂质的生物合成、蛋白质的折叠和翻译后修饰、生物信号的转导及钙的稳态储存和动态调动, 与肿瘤的发生发展密切相关[66,67]。相比于细胞核和线粒体, 内质网的靶向研究较少, 其主要原因在于内质网中可利用进行靶向的特征较少[68]。Qin等[31]为内质网靶向提供了一项新实例, 其设计的一种Pardaxin肽 (HGFFALIPKIISSPLFKTLLSAVGGSAVGSALSSGGQE) 修饰的阳离子脂质体相比于无Pardaxin肽修饰的阳离子脂质体, 在细胞内与内质网的极高共定位现象证实了脂质体使用Pardaxin肽修饰后能特异性地增加在内质网中的积聚, 揭示了开发高效内质网靶向型配基的必要性。

2.6.5 高尔基体靶向型配基 高尔基体作为参与细胞分泌功能的主要细胞器, 与肿瘤发生发展之间存在密切联系, 是抗肿瘤策略中的潜力靶点[69]。比如Khine等[70]介绍了布雷菲德菌素A等一系列针对高尔基体的抗肿瘤天然产物, 并讨论了利用这些途径开发新化疗药物的可能性。以高尔基体为作用靶点的抗肿瘤药物需要高尔基体靶向型配基的研究开发, 其中半胱氨酸和硫酸软骨素 (chondroitin sulfate, CS) 是常用的高尔基体靶向型配基[71]。一种共载吡柔比星与长春瑞宾的胶束被CS修饰后, 在激光共聚焦显微镜下显示了与高尔基体的极高共定位, 此共定位源于CS对高尔基体的亲和靶向作用[32]。

3 多配基组合修饰抗肿瘤纳米药物

纳米药物递送系统应以最高效方式将药物从给药位置递送到目标作用部位, 但由于体内环境的复杂性, 仅用单配基修饰的纳米药物机制单一, 不能较好地应对体内复杂的生理病理障碍, 而导致实际应用过程中出现纳米药物使用单配基修饰后, 抗肿瘤效果并没有明显提升的现象。Fu等[72]制备的一种紫杉醇 (paclitaxel, PTX) 脂质体在使用PEG修饰策略后, 在体内的肿瘤抑制率 (14.7%) 依然很低, 此低肿瘤抑制率进一步证明了单配基修饰策略在实际应用时的低效率和不稳定性。利用纳米药物的极大表面积优势, 完全可以使用多配基组合修饰来有效避免单配基修饰的局限性。如表2[72-80]根据药物作用机制将非肿瘤排斥型配基、肿瘤亲和型配基、肿瘤渗透型配基、细胞膜穿透型配基、溶酶体逃逸型配基和亚细胞靶向型配基6类功能配基组合搭配制备多配基修饰纳米药物是智能纳米药物的发展前景。

3.1 协同型多配基组合修饰

依据纳米药物体内递送进程归纳的同一类功能配基中不止含有一种配基, 而是含有多种从不同机制出发但针对纳米药物同一体内阶段和障碍的配基。将这样属于同一类的多种配基进行组合搭配后修饰纳米药

Table 2 Examples of combinatorial multiligand modified nanomedicine. CPP: Cell-penetrating peptide

Type of combinatorial modifications	Ligand	Type of ligands	Nanocarrier	Ref.
Synergistic	Fructose and biotin	Tumor affinity ligands and tumor affinity ligands	Liposome	[73]
	FA and methionine	Tumor affinity ligands and tumor affinity ligands	Micelle	[74]
	FA, biotin and HA	Tumor affinity ligands, tumor affinity ligands and tumor affinity ligands	Micelle	[75]
Complementary	PEG and FA	Non-tumor excluding ligands and tumor affinity ligands	Nanoparticle	[76]
	PEG and TAT	Non-tumor excluding ligands and cell-membrane penetrating ligands	Liposome	[72]
	Anti-CD133 antibody and polyarginine	Tumor affinity ligands and cell-membrane penetrating ligands	Nanoparticle	[77]
	HA and TPP	Tumor affinity ligands and subcellular targeting ligands	Micelle	[78]
	PEG, *D*-mannose and *L*-fucose	Non-tumor excluding ligands, tumor affinity ligands and tumor affinity ligands	Liposome	[79]
	PEG, Tf and CPP	Non-tumor excluding ligands, tumor affinity ligands and cell-membrane penetrating ligands	Liposome	[80]

物, 就能够从不同机制出发, 有效地解决单配基修饰纳米药物由于无法应对体内复杂变化而导致药效不稳定和效率低下的问题。其中多种肿瘤亲和型配基之间的组合搭配是协同型多配基修饰策略中最广泛使用的组合类型, 这种组合类型能够有效避免由于体内肿瘤受体动态变化及受体饱和等问题导致单配基修饰纳米药物出现疗效不佳的状况。

将针对肿瘤表面不同特征受体 (果糖转运蛋白和多维生素转运体) 的两种肿瘤亲和型配基 (果糖和生物素) 共修饰到脂质体后, 肿瘤中脂质体的积聚含量得到有效增强, 分别是无配基修饰脂质体、果糖单配基修饰脂质体和生物素单配基修饰脂质体肿瘤积聚量的2.76、1.60 和 1.96 倍[73]。这种使用两种肿瘤亲和型配基组合修饰后的纳米药物, 相比于单配基修饰时对肿瘤部位的亲和效果得到明显增强的实验现象, 反映出了协同型组合修饰能在单一修饰的基础上显著提高配基所对应体内阶段的递送效率。Chen 等[74]同样使用协同型组合修饰策略, 将两种肿瘤亲和型配基叶酸 (folic acid, FA) 和蛋氨酸同时修饰在负载 PTX 的胶束上, 旨在提高体内抗肿瘤效果, 结果显示使用双配基修饰胶束治疗后的肿瘤体积 (600 mm^3) 比使用 FA 单配基修饰胶束治疗后的肿瘤体积 (800 mm^3) 减少了25%, 进一步证实了协同型多配基组合修饰策略能更有效的应对体内复杂变化, 从而成功地达到预期抗肿瘤效果。

3.2 互补型多配基组合修饰

纳米药物从给药位置到目标作用位置, 所历经每个阶段的递送效率共同决定了最终的抗肿瘤疗效。从针对体内多种递送屏障的多类配基中, 选取一类以上的配基来修饰纳米药物是一种更为全面的修饰手段, 这种互补型修饰策略从递送的多个屏障出发, 能促进多个阶段的递送效率, 最终共同提高纳米药物的抗肿瘤疗效。

用非肿瘤排斥型配基 PEG 和肿瘤亲和型配基 FA 共同修饰的负载表柔比星的纳米颗粒, 与仅用 PEG 单配基修饰的纳米颗粒相比, 给药后荷瘤小鼠中位存活时间延长了 9 天, 出现这一结果是因为 PEG 和 FA 能分别从避免 MPS 系统吞噬和主动亲和肿瘤部位两个方向出发, 共同促进纳米药物在肿瘤部位的聚集, 使药效增强而毒性下降, 最终体现在荷瘤小鼠存活时间的延长[76]。Fu 等[72]为了解决 PTX 脂质体在使用 PEG 修饰策略后依然对肿瘤抑制效果不佳 (肿瘤抑制率为 14.7%) 的局限性, 开发了一种 PEG 和 TAT 组合修饰的 PTX 脂质体, 目的从分布和细胞内化两个阶段进行双点位促进, 最终实现更强的肿瘤抑制效果, 结果显示双配基修饰脂质体的肿瘤抑制率确实大幅度提高, 达到了 69.4%, 是 PEG 单配基修饰脂质体肿瘤抑制率的 4.72 倍, 可见互补型多配基组合修饰策略能使纳米药物体内递送更加稳定和高效。另外使用肿瘤亲和型配基透明质酸 (hyaluronic acid, HA) 和线粒体靶向型配基 TPP 组合修饰的纳米胶束在体内的肿瘤抑制率能达到 81.7%, 是 TPP 单配基修饰胶束肿瘤抑制率的 2.12 倍[78]。这种优越的肿瘤抑制效果是由于纳米药物在 HA 的作用下能首先聚集在肿瘤部位, 然后在 TPP 的作用下能进一步靶向聚集在目标亚细胞作用位置, 提高了分布和亚细胞定位两个阶段的递送效率, 从而实现了药效的明显提升。这些研究都说明了根据纳米药物体内递送进程, 选择针对不同体内阶段和障碍的多种配基互补对纳米药物进行修饰, 能够有效避免单配基修饰策略在实际应用过程中出现药效低、毒性大、稳定性差的局限性, 从而实现稳定高效的药物递送。

4 总结与展望

体内递送具有的多变性和全程性与单配基修饰策略具有的单一性和阶段性之间的矛盾, 决定了传统单配基修饰策略不能满足目标疗效。其中多配基组合修饰策略因更符合实际体内递送过程, 能使纳米药物在实际应用中稳定实现高药效而低毒性的治疗目的, 有更加广阔的应用前景。本文首先依据纳米药物体内递送进程总结分类了常用功能配基, 又依据配基组合方式将多配基组合修饰分为协同型及互补型, 提出这种配基功能分类及多配基组合形式分类的意义在于, 在未来设计多配基组合修饰抗肿瘤纳米药物时, 可以根据纳米药物在体内转运过程中所遇到的特定生理病理屏障, 有针对性地选择最适配基进行特定组合, 使修饰后的纳米药物在给药后能有效克服相应屏障, 顺利递送至目标部位发挥疗效。

本文提出的协同型及互补型多配基组合修饰提升抗肿瘤纳米药物功效的研究工作在面临实际应用转化时, 可以围绕以下 3 个方面进行展开: ① 基于纳米载体原剂型特点及包载药物的作用机制, 可以首先考察未经修饰时的纳米药物在给药后的实际体内命运, 并据此推测其到达目标部位并发挥疗效所急需克服的生理病理屏障; ② 基于纳米药物在体内递送过程中急需克服的屏障, 有针对性地选择对应此屏障发挥功能的修饰配基; ③ 最后还应考察所选定修饰配基之间的数量比及质量比对药物靶向递送效率的影响, 用理论指导设计才能制备出理想的多配基组合修饰纳米药物递送系统, 必要时可与成像系统进行结合以期进一步优化配基修饰方案。相信随着药剂学、肿瘤学、生物学及相关学科的发展, 多配基组合修饰抗肿瘤纳米药物策略

将会更加完善, 能引领肿瘤化疗进入一个全新的智能时代。

作者贡献: 张小雨主要负责论文撰写、图形绘制、格式修改及论文投稿; 吴送姑、徐晖、龚俊波、邢金峰和魏振平主要负责论文的构思、修改和审核。

利益冲突: 本论文中不涉及任何利益冲突。

References

[1] Qi JL, Li ML, Wang LJ, et al. National and subnational trends in cancer burden in China, 2005-20: an analysis of national mortality surveillance data [J]. Lancet Public Health, 2023, 8: e943-e955.

[2] Kaur N, Popli P, Tiwary N, et al. Small molecules as cancer targeting ligands: shifting the paradigm [J]. J Control Release, 2023, 355: 417-433.

[3] Zhang P, Ye GH, Xie GF, et al. Research progress of nanomaterial drug delivery in tumor targeted therapy [J]. Front Bioeng Biotechnol, 2023, 11: 1240529.

[4] Khan N, Ruchika, Dhritlahre RK, et al. Recent advances in dual-ligand targeted nanocarriers for cancer therapy [J]. Drug Discov Today, 2022, 27: 2288-2299.

[5] Li S, Guo JS, Tian ZH, et al. Piperine-loaded glycyrrhizic acid- and PLGA-based nanoparticles modified with transferrin for antitumor piperine-loaded glycyrrhizic acid- and PLGA-based nanoparticles [J]. AAPS PharmSciTech, 2021, 22: 239.

[6] Zhou YM, Dai ZF. New strategies in the design of nanomedicines to oppose uptake by the mononuclear phagocyte system and enhance cancer therapeutic efficacy [J]. Chem Asian J, 2018, 13: 3333-3340.

[7] Vyas D, Patel M, Wairkar S. Strategies for active tumor targeting-an update [J]. Eur J Pharmacol, 2022, 915: 174512.

[8] Hu J, Yuan XW, Wang F, et al. The progress and perspective of strategies to improve tumor penetration of nanomedicines [J]. Chin Chem Lett, 2021, 32: 1341-1347.

[9] Shin MC, Zhang J, Min KA, et al. Cell-penetrating peptides: achievements and challenges in application for cancer treatment [J]. J Biomed Mater Res A, 2014, 102: 575-587.

[10] Shete HK, Prabhu RH, Patravale VB. Endosomal escape: a bottleneck in intracellular delivery [J]. J Nanosci Nanotechnol, 2014, 14: 460-474.

[11] Mei L, Zhang XD, Feng SS. Autophagy inhibition strategy for advanced nanomedicine [J]. Nanomedicine (Lond), 2014, 9: 377-380.

[12] D'Souza GG, Weissig V. Subcellular targeting: a new frontier for drug-loaded pharmaceutical nanocarriers and the concept of the magic bullet [J]. Expert Opin Drug Deliv, 2009, 6: 1135-1148.

[13] Sasaki D, Kusamori K, Nishikawa M. Delivery of corn-derived nanoparticles with anticancer activity to tumor tissues by modification with polyethylene glycol for cancer therapy [J]. Pharm Res, 2023, 40: 917-926.

[14] Zhu L, Song ZM, Feng SJ, et al. Biotin-modified oligochitosan-F127 micelles for honokiol's encapsulation [J]. J Nanopart Res, 2021, 23: 116.

[15] Sun YT, Zhao YR, Teng SS, et al. Folic acid receptor-targeted human serum albumin nanoparticle formulation of cabazitaxel for tumor therapy [J]. Int J Nanomedicine, 2018, 14: 135-148.

[16] Wang XP, Song YR, Yu LCY, et al. Co-delivery of hesperetin and cisplatin *via* hyaluronic acid-modified liposome for targeted inhibition of aggression and metastasis of triple-negative breast cancer [J]. ACS Appl Mater Interfaces, 2023, 15: 34360-34377.

[17] Deng C, Liu YH, Zhou FZ, et al. Engineering of dendritic mesoporous silica nanoparticles for efficient delivery of water-insoluble paclitaxel in cancer therapy [J]. J Colloid Interface Sci, 2021, 593: 424-433.

[18] Niza E, Noblejas-López MDM, Bravo I, et al. Trastuzumab-targeted biodegradable nanoparticles for enhanced delivery of dasatinib in HER2+metastasic breast cancer [J]. Nanomaterials (Basel), 2019, 9: 1793.

[19] Fu S, Zhao YN, Sun J, et al. Integrin $\alpha v\beta 3$-targeted liposomal drug delivery system for enhanced lung cancer therapy [J]. Colloids Surf B Biointerfaces, 2021, 201: 111623.

[20] Jabbari A, Yaghoobi E, Azizollahi H, et al. Design and synthesis of a star-like polymeric micelle modified with AS1411 aptamer for targeted delivery of camptothecin for cancer therapy [J]. Int J Pharm, 2022, 611: 121346.

[21] Wang GW, Wu BH, Li QY, et al. Active transportation of liposome enhances tumor accumulation, penetration, and therapeutic efficacy [J]. Small, 2020, 16: e2004172.

[22] Qian LL, Zheng JJ, Wang K, et al. Cationic core-shell nanoparticles with carmustine contained within O6-benzylguanine shell for glioma therapy [J]. Biomaterials, 2013, 34: 8968-8978.

[23] Cui MJ, Naczynski DJ, Zevon M, et al. Multifunctional albumin nanoparticles as combination drug carriers for intra-tumoral chemotherapy [J]. Adv Healthc Mater, 2013, 2: 1236-1245.

[24] Song SL, Gui L, Feng QQ, et al. TAT-modified gold nanoparticles enhance the antitumor activity of PAD4 inhibitors [J]. Int J Nanomedicine, 2020, 15: 6659-6671.

[25] Li QQ, Shi ZQ, Ou MT, et al. pH-labile artificial natural killer cells for overcoming tumor drug resistance [J]. J Control Release, 2022, 352: 450-458.

[26] Norouzi P, Motasadizadeh H, Atyabi F, et al. Combination therapy of breast cancer by codelivery of doxorubicin and survivin siRNA using polyethylenimine modified silk fibroin nanoparticles [J]. ACS Biomater Sci Eng, 2021, 7: 1074-1087.

[27] Jiang LJ, Liang X, Liu G, et al. The mechanism of lauric acid-modified protein nanocapsules escape from intercellular trafficking vesicles and its implication for drug delivery [J]. Drug Deliv, 2018, 25: 985-994.

[28] Maity AR, Stepensky D. Nuclear and perinuclear targeting

efficiency of quantum dots depends on density of peptidic targeting residues on their surface [J]. J Control Release, 2017, 257: 32-39.

[29] Tan XY, Zhou YL, Shen L, et al. A mitochondria-targeted delivery system of doxorubicin and evodiamine for the treatment of metastatic breast cancer [J]. RSC Adv, 2019, 9: 37067-37078.

[30] Wang Y, Ding RH, Zhang Z, et al. Curcumin-loaded liposomes with the hepatic and lysosomal dual-targeted effects for therapy of hepatocellular carcinoma [J]. Int J Pharm, 2021, 602: 120628.

[31] Qin B, Yuan XL, Jiang MS, et al. Targeting DNA to the endoplasmic reticulum efficiently enhances gene delivery and therapy [J]. Nanoscale, 2020, 12: 18249-18262.

[32] Zhou HL, Li SH, Chen D, et al. Preliminary study on drug-loaded chondroitin sulfate-modified micelles targeting Golgi apparatus in tumor cells for the treatment of tumor metastasis [J]. J Sichuan Univ Med Sci Ed (四川大学学报(医学版)), 2022, 53: 619-625.

[33] Zalba S, Ten Hagen TLM, Burgui C, et al. Stealth nanoparticles in oncology: facing the PEG dilemma [J]. J Control Release, 2022, 351: 22-36.

[34] Mirkasymov AB, Zelepukin IV, Nikitin PI, et al. *In vivo* blockade of mononuclear phagocyte system with solid nanoparticles: efficiency and affecting factors [J]. J Control Release, 2021, 330: 111-118.

[35] Zheng HL, Song YZ, Deng YH. A review for phagocyte system: the executors of nanoparticles clearance [J]. J Shenyang Pharm Univ (沈阳药科大学学报), 2019, 36: 91-102.

[36] Wen PY, Ke WD, Dirisala A, et al. Stealth and pseudo-stealth nanocarriers [J]. Adv Drug Deliv Rev, 2023, 198: 114895.

[37] Munir MU. Nanomedicine penetration to tumor: challenges, and advanced strategies to tackle this issue [J]. Cancers (Basel), 2022, 14: 2904.

[38] Huang MY, Yang X, Xing JF, et al. Strategies for enhancing nanoscale brain targeting drug delivery [J]. Acta Pharm Sin (药学学报), 2019, 54: 629-637.

[39] Zhou Q, Dong CY, Fan WF, et al. Tumor extravasation and infiltration as barriers of nanomedicine for high efficacy: the current status and transcytosis strategy [J]. Biomaterials, 2020, 240: 119902.

[40] Sandha KK, Shukla MK, Gupta PN. Recent advances in strategies for extracellular matrix degradation and synthesis inhibition for improved therapy of solid tumors [J]. Curr Pharm Des, 2020, 26: 5456-5467.

[41] Zhang SY, Zhou ZR, Qian RC. Recent progress and perspectives on cell surface modification [J]. Chem Asian J, 2021, 16: 3250-3258.

[42] Khan MM, Filipczak N, Torchilin VP. Cell penetrating peptides: a versatile vector for co-delivery of drug and genes in cancer [J]. J Control Release, 2021, 330: 1220-1228.

[43] Peng ZR, Xia XH, Yan H. Advances in targeted drug delivery of cell-penetrating peptide-modified liposomes [J]. Chin J Cancer Biother (中国肿瘤生物治疗杂志), 2019, 26: 1275-1280.

[44] Wang AQ, Zheng Y, Zhu WX, et al. Melittin-based nano-delivery systems for cancer therapy [J]. Biomolecules, 2022, 12: 118.

[45] Rádis-Baptista G. Cell-penetrating peptides derived from animal venoms and toxins [J]. Toxins (Basel), 2021, 13: 147.

[46] Wang YQ, Qiu YZ, Chen SH, et al. Functionalized tumor cell membrane-camouflaged photo-activatable nanoparticle for spatio-temporal antitumor therapy [J]. Chem Eng J, 2023, 474: 145676.

[47] Ahmad A, Khan JM, Haque S. Strategies in the design of endosomolytic agents for facilitating endosomal escape in nanoparticles [J]. Biochimie, 2019, 160: 61-75.

[48] Remaut K, Oorschot V, Braeckmans K, et al. Lysosomal capturing of cytoplasmic injected nanoparticles by autophagy: an additional barrier to non viral gene delivery [J]. J Control Release, 2014, 195: 29-36.

[49] Selby LI, Cortez-Jugo CM, Such GK, et al. Nanoescapology: progress toward understanding the endosomal escape of polymeric nanoparticles [J]. Wiley Interdiscip Rev Nanomed Nanobiotechnol, 2017, 9: e1452.

[50] Neuberg P, Kichler A. Recent developments in nucleic acid delivery with polyethylenimines [J]. Adv Genet, 2014, 88: 263-288.

[51] Chen ZH, Lv ZY, Sun YF, et al. Recent advancements in polyethyleneimine-based materials and their biomedical, biotechnology, and biomaterial applications [J]. J Mater Chem B, 2020, 8: 2951-2973.

[52] Guo X, Wei X, Chen Z, et al. Multifunctional nanoplatforms for subcellular delivery of drugs in cancer therapy [J]. Prog Mater Sci, 2020, 107: 100599.

[53] Wang JF, Zhou XF, Wang HF, et al. Autophagy-inhibiting polymer as an effective nonviral cancer gene therapy vector with inherent apoptosis-sensitizing ability [J]. Biomaterials, 2020, 255: 120156.

[54] Wang XW, Qiu YH, Wang MY, et al. Endocytosis and organelle targeting of nanomedicines in cancer therapy [J]. Int J Nanomedicine, 2020, 15: 9447-9467.

[55] Du WZ, Zhang LJ, Li XD, et al. Nuclear targeting subcellular-delivery nanosystems for precise cancer treatment [J]. Int J Pharm, 2022, 619: 121735.

[56] Goyal P, Malviya R. Advances in nuclei targeted delivery of nanoparticles for the management of cancer [J]. Biochim Biophys Acta Rev Cancer, 2023, 1878: 188881.

[57] Lu JN, Wu T, Zhang B, et al. Types of nuclear localization signals and mechanisms of protein import into the nucleus [J]. Cell Commun Signal, 2021, 19: 60.

[58] Nie YH, Fu G, Leng YX. Nuclear delivery of nanoparticle-based drug delivery systems by nuclear localization signals [J]. Cells, 2023, 12: 1637.

[59] Li RJ, Wu XX, Wang C. The anti-tumor nano-drug carrier

system modified by the nuclear localization signal peptide [J]. Chin J Biochem Mol Biol (中国生物化学与分子生物学报), 2022, 38: 35-41.

[60] Liu YE, Sun YH, Guo YD, et al. An overview: the diversified role of mitochondria in cancer metabolism [J]. Int J Biol Sci, 2023, 19: 897-915.

[61] Zheng BX, Bi CF, Hou WB, et al. Design and research progress of nano-drug delivery system based on mitochondria targeting [J]. Acta Pharm Sin (药学学报), 2022, 57: 681-694.

[62] Cheng XX, Feng D, Lv JY, et al. Application prospects of triphenylphosphine-based mitochondria-targeted cancer therapy [J]. Cancers (Basel), 2023, 15: 666.

[63] Tian XX, Shi AH, Yin H, et al. Nanomaterials respond to lysosomal function for tumor treatment [J]. Cells, 2022, 11: 3348.

[64] Shen Q, Pan X, Li Y, et al. Lysosomes, curcumin, and anti-tumor effects: how are they linked? [J]. Front Pharmacol, 2023, 14: 1220983.

[65] Zhang ZX, Dong CS, Yu GZ, et al. Smart and dual-targeted BSA nanomedicine with controllable release by high autolysosome levels [J]. Colloids Surf B Biointerfaces, 2019, 182: 110325.

[66] Shi YY, Wang SJ, Wu JL, et al. Pharmaceutical strategies for endoplasmic reticulum-targeting and their prospects of application [J]. J Control Release, 2021, 329: 337-352.

[67] Zhao Y, Yu QL. Advance in endoplasmic reticulum-targeting nanodrugs [J]. Chin J Biotechnol (生物工程学报), 2021, 37: 418-428.

[68] Chen LQ, Shen XR, Huang Y. Research progress of endoplasmic reticulum targeting drug delivery system for anti-tumor immunotherapy [J]. Acta Pharm Sin (药学学报), 2022, 57: 76-84.

[69] Dumitru AV, Stoica EE, Covache-Busuioc RA, et al. Unraveling the intricate link: deciphering the role of the Golgi apparatus in breast cancer progression [J]. Int J Mol Sci, 2023, 24: 14073.

[70] Khine MN, Sakurai K. Golgi-targeting anticancer natural products [J]. Cancers (Basel), 2023, 15: 2086.

[71] Li RS, Wen C, Huang CZ, et al. Functional molecules and nanomaterials for the Golgi apparatus-targeted imaging and therapy [J]. Trac-Trend Anal Chem, 2022, 156: 116714.

[72] Fu H, Shi KR, Hu GL, et al. Tumor-targeted paclitaxel delivery and enhanced penetration using TAT-decorated liposomes comprising redox-responsive poly (ethylene glycol) [J]. J Pharm Sci, 2015, 104: 1160-1173.

[73] Li R, Peng Y, Pu YC, et al. Fructose and biotin co-modified liposomes for dual-targeting breast cancer [J]. J Liposome Res, 2022, 32: 119-128.

[74] Chen HY, Chen YQ, Yang HB, et al. A dual-targeting nanocarrier based on modified chitosan micelles for tumor imaging and therapy [J]. Polym Chem, 2014, 5: 4734-4746.

[75] Liu MN, Wang BJ, Guo CJ, et al. Novel multifunctional triple folic acid, biotin and CD44 targeting pH-sensitive nano-actiniaes for breast cancer combinational therapy [J]. Drug Deliv, 2019, 26: 1002-1016.

[76] Ansari L, Derakhshi M, Bagheri E, et al. Folate conjugation improved uptake and targeting of porous hydroxyapatite nanoparticles containing epirubicin to cancer cells [J]. Pharm Dev Technol, 2020, 25: 601-609.

[77] Jin C, Bai L, Wang SQ. Multifunctional nanoparticles for targeting liver cancer stem cells and efficient endocytosis [J]. Chem Pap, 2023, 77: 1395-1403.

[78] Wang H, Zhang FK, Wen HY, et al. Tumor- and mitochondria-targeted nanoparticles eradicate drug resistant lung cancer through mitochondrial pathway of apoptosis [J]. J Nanobiotechnology, 2020, 18: 8.

[79] Li C, Lai CY, Qiu QJ, et al. Dual-ligand modification of PEGylated liposomes used for targeted doxorubicin delivery to enhance anticancer efficacy [J]. AAPS PharmSciTech, 2019, 20: 188.

[80] Lakkadwala S, Dos Santos Rodrigues B, Sun CW, et al. Biodistribution of TAT or QLPVM coupled to receptor targeted liposomes for delivery of anticancer therapeutics to brain *in vitro* and *in vivo* [J]. Nanomedicine, 2020, 23: 102112.