pH 响应活体自组装生物材料及其在肿瘤诊疗中的应用进展

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摘要 自组装纳米材料在肿瘤部位具有被动靶向富集效应, 提高了药物的递送能力, 在生物医学领域具有广阔的发展前景. 然而, 本质上处于动态平衡的自组装纳米材料在体内传送时会与各种生物界面或生物大分子相互作用, 存在结构不稳定的问题, 目前还难以实现对其结构的精准控制, 这妨碍了它们的进一步临床应用. pH会影响氨基酸的电荷状态, 从而对多肽自组装过程中的静电相互作用造成影响, 因此改变pH是调控多肽自组装过程的常用方法之一. 在此, 我们基于活体自组装(*in vivo self-assembly*)的理念, 着重综述了基于pH响应型多肽的纳米生物材料原位制备方法并探讨了其在肿瘤诊断和治疗中的应用研究. 通过把用于肿瘤诊疗的功能分子与pH响应型多肽结合, 可以构建更复杂的具有生物功能的组装体系, 提高成像剂的诊断功能和化疗药物的治疗效果. 因此, 利用pH响应型多肽在活体中原位构建有序组装体的新策略为开发肿瘤诊疗的纳米材料铺平了道路, 为推向临床应用带来了新希望.

关键词 活体自组装, 生物纳米材料, pH 响应, 多肽, 诊断治疗

目前与肿瘤相关的研究集中在开发可装载药物或诊断试剂, 并将它们特异性地递送到肿瘤部位^[1]. 纳米材料是用于肿瘤诊断和治疗的理想材料, 有效提高了各种药物的稳定性, 实现了药物的可控释放, 提高了生物利用度. 其中纳米材料中的多肽分子可以实现各种各样的功能, 如特异性靶向、触发响应、细胞渗透和治疗功能, 可以将其巧妙地结合在纳米系统中, 以实现靶向传递、可控药物释放和协同治疗等^[2~4]. 纳米材料在肿瘤诊疗方面具有很大的应用价值, 在实体肿瘤部位具有渗透和滞留增强效应^[5,6](enhanced permeability and retention effect, EPR). 为增强被动靶向富集效果, 纳米材料需要避免网状内皮系统的摄取作用和体内非特

异性的识别作用, 延长血液循环半衰期^[7].

将小分子自组装成复杂结构使其具有高级功能, 这在生物体中是普遍存在的现象, 比如多肽链经过盘曲折叠形成蛋白质^[8]. 受到这一有趣过程的启发, 近年来研究人员设计和构建了在生理或病理条件下的自组装, 这可以与大自然的进化相媲美, 甚至超越大自然的进化^[9~13]. 研究人员构建的自组装药物递送体系利用了氧化还原反应和酯酶活性聚合物载体, 实现了肿瘤部位的可控药物释放^[14~16]. 然而, 这些刺激响应的聚合物在体内复杂生理环境传递过程中稳定性差, 而且一些聚合物存在生物相容性差的问题. 因此, 开发由生理环境触发的可控自组装分子是至关重要

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的,通过对自组装过程的精确控制来适应复杂的生物环境,从而构建生物相容性纳米材料.我们发展了活体原位自组装的新策略^[17~28],即利用肿瘤酸性微环境触发材料进行自组装形成高度有序且具有特定诊疗功能的纳米材料,具有的聚集诱导滞留(aggregation/assembly induced retention, AIR)效应大大提高了材料的成像性能和治疗效果^[29].目前基于活体自组装策略发展了各种具有聚集诱导滞留效应的纳米材料.其中一类是无机纳米材料^[30,31],例如, Xing课题组^[32]报道的表面修饰功能基团的上转换纳米材料通过生物正交反应在肿瘤部位交联,实现药物的高效富集.文献报道较多的还有聚合物材料, Wang课题组^[33]采用一种含有硫醇基团的低聚对苯撑乙烯与紫杉醇连接,通过肿瘤部位高浓度活性氧化硫醇基团使分子交联形成聚合物,与游离的紫杉醇相比半抑制浓度降低了124倍.但是目前发展的热点是多肽材料,由于多肽分子合成简单、易修饰且生物相容性好,为药物递送提供了一个有发展前景的平台^[34].本课题组发展了一系列多肽生物材料,通过活体原位自组装的策略在原位构筑多肽纳米材料,其具有的AIR效应大大提高了材料的诊疗效果.

1 pH响应型自组装

多肽自组装是自发的热力学过程,需要多种分子间非共价相互作用的协同参与,进而决定热力学

稳定的自组装体的最小能量状态.这些非共价相互作用包括在多肽和蛋白质形成稳定结构过程中关键的氢键相互作用、能诱导分子方向性生长的 π - π 相互作用、离子对形成后的静电相互作用、在界面系统中起重要作用的疏水相互作用和作用范围很广的范德华相互作用^[35].同时,自组装过程可以由各种动力学参数精确合理地调控,比如pH、温度、离子、浓度和溶剂.在某个动力学参数的调控下,自组装体会暂时稳定在亚稳态,如果没有动力学参数的继续干预,最终会发展成为热力学稳定态(图1).因此,自组装过程中动力学和热力学驱动间的竞争关系提供了从热力学控制转变为动力学控制的可能,通过动力学控制可以设计响应型的自组装材料.虽然自组装体的结构可以在明确的化学条件下控制,但复杂的生理环境会导致自组装过程的物理化学参数受到影响,研究动力学因素的调节作用和机制有助于我们利用肿瘤部位pH的改变实现对自组装体结构的控制.

肿瘤细胞在进行生理活动过程时会持续与周围微环境发生相互作用,进而造成生理状态与正常组织存在差异,表现为弱酸和蛋白表达异常等,这为肿瘤诊疗药物的靶向递送提供了多个靶点^[36].正常细胞主要靠线粒体氧化磷酸化来产生能量,但是大多数肿瘤细胞则依赖于有氧糖酵解,这会增加额外代谢酸的生成^[37].同时代谢酸不能及时得到清除,这会导致肿瘤部位细胞外微环境中的pH(extracellular pH, pH_e)降低到6.5~7.0^[38],细胞内溶酶体的pH降低

图1 热力学和动力学共同控制的自组装过程示意图. 热力学控制因素包括氢键相互作用、 π - π 相互作用、静电相互作用、疏水相互作用和范德华相互作用. 动力学控制因素包括pH、温度、离子、浓度和溶剂

Figure 1 Self-assembly process controlled by thermodynamics and kinetics. The thermodynamic control factors include hydrogen bonding, π - π stacking, electrostatic interaction, hydrophobic interaction, and van der Waals interactions. The kinetic control factors include pH, temperature, ions, concentrations, and solvents

到4.0~5.0^[39],与正常组织微环境中的pH 7.4形成差异(图2),进而使弱酸环境成为肿瘤的特异性靶点之一。我们从小分子多肽的设计出发,利用肿瘤部位pH的改变原位构建了pH响应型活体自组装材料,用这种方法来实现精准可控的诊疗功能,同时通过延长滞留时间提高了材料的生物利用度,进而将多肽与其他功能性生物分子结合,可以构建更复杂的具有生物功能的组装系统用于肿瘤诊断和治疗。

2 pH响应型多肽材料

多肽分子的序列、电性、亲疏水性和二级结构会对纳米材料的结构造成影响,因此把多肽分子作为纳米材料的构建块是一种广泛使用的分子设计策略^[40]。此外,多肽分子具有生物相容性好、易于化学修饰和环境敏感性等特点,被广泛用于构建药物靶向递送和控制释放的功能性纳米材料,有望成为最广泛的纳米药物之一。多肽分子在纳米材料中可以担任不同功能的模块,包括靶向模块、响应型模块、疏水模块和治疗模块等。此外,多肽分子具有的环境响应性引起了人们对特定生理微环境的关注,利用肿瘤部位的pH与正常组织的差异可以设计出对肿瘤酸性微环境响应的多肽,进而将多肽与不同的功能分子偶联,可以构建出具有不同结构和功能的活体自组装材料。多肽分子的化学性质和生物功能与其结构密切相关,引入的自组装策略可以改变其结构,进而提高其化学稳定性和生物效应。因此,在特定条

件下原位构建自组装体是一种很有前景的策略,可以广泛应用到肿瘤诊断和药物递送等领域。

pH会影响氨基酸和取代官能团的电荷状态,从而对多肽自组装过程中的静电相互作用造成影响,因此改变pH是控制多肽自组装和解组装的常用方法之一。发展pH响应型纳米材料是一种独特的策略,不需要明确肿瘤位置就能触发响应,比使用热疗^[41]和超声波^[42]等外部刺激更具优势。许多对酸性微环境响应的多肽都是酸性氨基酸(天冬氨酸和谷氨酸)去质子化和碱性氨基酸(赖氨酸、精氨酸和组氨酸)质子化的结果^[43](图3)。多组氨酸就具有对酸性肿瘤微环境的响应能力,组氨酸上咪唑基团的酸度系数(acidity coefficient, pK_a)约为6.5,在酸性条件下会被质子化,使多肽更亲水^[44],Zhang课题组^[45]利用此性质设计了一个在肿瘤部位对pH响应的多肽分子 $H_7K(R)_2$ 。由这种酸性环境中的亲水多肽分子与疏水信号分子相结合,可以构建活体自组装纳米材料用于体内成像和药物递送。另一种类型的pH响应型多肽分子由酸性和碱性氨基酸共同组成,通过合理的序列设计使等电点位于6~7之间,可以对肿瘤酸性微环境做出响应。其中一种等电点约为6.4的多肽分子 E_4K_4 ^[46],在生理条件下带有负电荷,在酸性肿瘤微环境中则不带电荷甚至带正电荷,使静电相互作用受到影响^[47],进而影响自组装过程。

此外,大自然中存在随pH变化而构象发生变化的现象,比如流感病毒蛋白血凝素在体内pH条件下,

图2 肿瘤酸性微环境示意图

Figure 2 Schematic diagram of tumor acidic microenvironment

图3 氨基酸及pH响应型多肽分子在肿瘤酸性微环境中的转变图示. 在肿瘤酸性微环境中, 酸性氨基酸和碱性氨基酸会分别发生去质子化和质子化, pH响应型多肽分子 $H_7K(R_2)_2$ 、 E_4K_4 和pHLIP分别发生亲疏水性、电性和二级结构的改变

Figure 3 Schematic illustrations of the transformation of amino acids and peptides in acidic tumor microenvironment. In acidic tumor microenvironment, the acidic amino acids and basic amino acids respectively deprotonate and protonate. pH-responsive peptide $H_7K(R_2)_2$, E_4K_4 and pHLIP respectively show the change of hydrophobicity, electrical property and secondary structure

通过构象重组将三股盘绕线圈结构转化为疏水性环螺旋结构^[48]. 受此现象启发, Szoka课题组^[49]开发了一种含有4个EALA重复单元的pH响应多肽GALA, 在pH为7时呈现无规卷曲二级结构, 而在pH为5时呈现 α -螺旋二级结构. Reshetnyak课题组^[50]报道了一种具有pH响应性和肿瘤靶向性的多肽pHLIP, 在正常生理条件下呈无规卷曲结构, 而在肿瘤部位酸性微环境中会转变为 α -螺旋二级结构. 近年来越来越多的研究证明, 纳米粒子的形态对其循环时间、渗透能力和细胞摄取难易程度有重要的影响^[51]. 因此, 设计出形态可控的纳米粒子是获得新生物效应的一种策略, 可以增强肿瘤治疗的效果. 肿瘤微环境响应型多肽的触发因素除pH外, 还可以是过表达的酶, 比如基质金属蛋白酶(matrix metalloproteinases, MMPs)^[52-54]. 以酶为触发因素的响应具有特异性和条件温和等优点, 酶促自组装过程为原位形成组装体提供了另一种可能, 在生物医学领域具有很大的应用潜力. 因此, 在活体中原位形成有序组装体的新策略为开发用于肿瘤诊疗的纳米生物材料铺平了道路, 具有广泛的发展前景.

3 pH响应型活体自组装材料在肿瘤诊疗中的应用

肿瘤微环境的弱酸性pH是设计特异性环境响应

型成像分子的良好触发因素, 据此设计的智能成像纳米探针有望实现对肿瘤组织的准确检测和特异性成像^[55]. 最近的一些报道显示, 通过靶向肿瘤的特定微环境, 研究人员已经开发出了可用于成像的纳米材料^[56-58]. 本课题组^[59]报道了一种在活体中原位形成自组装体的超分子系统, 包括pH响应载体模块PbAE和自组装模块BPs. 在pH为7.4时, BPs分子在疏水作用下分散在PbAE胶束的疏水区域中, 进而通过胞吞作用进入活细胞的溶酶体中. 在溶酶体的酸性环境中, BPs分子被释放并在原位自组装, 进而荧光被激活. 这种将单体整合到pH响应的载体模块中, 进而在生物体内实现原位自组装的策略为未来开发高灵敏分子探针开辟了新途径.

纳米材料的形态转变可以通过pH的改变来进行精确调节, 进而对其循环、渗透、积累、保留等行为产生影响, 最终实现肿瘤诊断和治疗的精准化, 目前研究人员已经成功制备了形态转变的纳米材料^[51]. Hu课题组^[60]提出了一种由多肽分子STP和芳香分子TPE组成的pH响应型活体自组装纳米形变材料, 在到达肿瘤部位酸性微环境后, 多肽偶联物自组装体的形态从纳米颗粒转变为纳米纤维, 激活了体内靶向成像, 提高了药物的递送速度和治疗效果. 同时, 响应型多肽分子可以结合其他功能分子构建活体自组装纳米材料, 使材料具有更高的选择性、稳定性和

治疗效果. 本课题组^[61]设计了对pH敏感的分子BP-KLVFF-His₆-PEG(图4), 其中BP为疏水模块, KLVFF为组装模块, His₆为pH响应模块, PEG为亲水模块. 在正常生理环境中分子为纳米颗粒, 到达肿瘤部位酸性环境(pH 6.5)后亲疏水平衡被破坏, 在KLVFF片段氢键作用的驱动下, 分子自组装形成纳米纤维. 这种方法可以显著提升分子的滞留时间, 通过结合功能分子尼罗红(成像剂)和阿霉素(化疗药物), 可以用于高效的诊断治疗. 96 h后, 与对照组相比, 肿瘤部位的荧光强度和药物浓度分别提高了7.5和2倍, 可用于长期且高特异性的肿瘤成像和高效的化疗.

对肿瘤部位酸性微环境响应的活体自组装材料也被用于提高药物递送效率, 进而提高治疗效果. 同时, 为了使纳米材料对肿瘤微环境具有更精确和快速的响应, 对pH和酶双重响应的材料设计也引起了研究人员的关注. 本课题组^[62]将pH响应的治疗多肽HLAH和末端结合有PEG的MMP-2响应多肽SPEG连接在聚合物上, 设计出了对pH和酶双重响应的活体自组装纳米材料P-S-H(图5). P-S-H在生理环境中自组装形成HLAH在内的球形组装体, 到达肿瘤部位后,

P-S-H纳米粒子会受到过度表达的MMP-2和酸性pH的刺激, 造成SPEG剪切后PEG壳的脱落和HLAH的质子化, 重新自组装形成了HLAH在表面的球形组装体. 重组装的分子可以在肿瘤部位有效积累, 增强了抗肿瘤能力, 且治疗剂量无明显毒性, 这种原位重组装策略为活体自组装材料的设计提供了新思路.

4 总结与展望

纳米技术的出现和发展为设计新一代智能材料提供了一个新方法, 通过灵活选择构建块可以设计出不同结构的纳米材料, 然而要想真正应用到临床还需解决许多问题. 活体自组装提供了一种利用外界环境变化控制纳米材料行为的新方法, 自组装纳米材料在pH的触发下可以呈现较强的荧光信号, 从而可以对实体肿瘤进行高效、特异性的原位诊断, 对临床肿瘤诊断具有重要意义. 同时, 基于pH响应型多肽构建的自组装生物材料可以实现药物靶向递送的功能, 从而提高肿瘤治疗效果.

尽管目前有一些文献报道了基于活体自组装的生物医学应用, 但分子设计和自组装行为的认识仍处

图4 pH触发从自组装纳米颗粒(nanoparticles, NPs)到纳米纤维(nanofibers, NFs)的形态学转化原理及活体内肿瘤部位原位组装示意图. (a) 由BP, KLVFF, His₆和PEG组成的分子结构. pH响应机制是His₆在pH为6.5时被质子化, 导致其亲水性显著增加. (b) 在肿瘤酸性的溶液环境中单体在pH为7.4时自组装成NPs, 在pH为6.5时转换为 β -折叠结构的NFs. 小分子可以通过疏水相互作用被NFs捕获. 在体内: NPs被静脉注射并通过被动靶向机制在肿瘤中积累. 在酸性肿瘤微环境中NPs转化为NFs, 并形成了巢状结构包裹肿瘤, 随后注射的诊疗分子会在巢状结构处聚集. Reprinted with permission from Ref. [61]. Copyright (2017) Wiley

Figure 4 Principle of pH-triggered morphological transformation from self-assembled nanoparticles to nanofibers and the schematic illustration of *in situ* assembly of tumor sites *in vivo*. (a) The molecular structures of designed with building blocks BP, KLVFF, His₆, and PEG motifs. The pH-responsive mechanism was that peptide His₆ was protonated at pH 6.5, leading to a remarkable increase of hydrophilicity. (b) In solution: monomers self-assembled into NPs at pH 7.4 and converted to β -sheet NFs at pH 6.5. Small molecules were captured by NFs through hydrophobic interactions. *In vivo*, the NPs were intravenously injected and accumulated in tumor via passive targeting mechanisms. NPs transformed into NFs to construct the nest-like host of tumor in acidic tumor microenvironment. The tumor nest-like host can gather following intravenously injected theranostic molecules^[61]

图5 肿瘤微环境下P-S-H纳米药物重组过程示意图. P-S-H纳米颗粒到达肿瘤部位后, 过表达的MMP2和酸性pH诱导可重构肽H原位活化, 表面带正电荷的纳米药物发生重组, 有效地诱导了细胞凋亡. Reprinted with permission from Ref. [62]. Copyright (2017) American Chemical Society

Figure 5 Schematic illustrations of the reorganization process of P-S-H nanodrugs in the tumor microenvironment. After the P-S-H nanoparticles arrived at the tumor site, the overexpressed MMP2 and acidic pH induced the *in situ* activation of the reconfigurable peptide H and the reassembly of nanodrugs with positive charges on the surfaces, which induced cell apoptosis effectively^[62]

于初级阶段, 有待进一步探索. 这个领域的研究才刚刚起步, 这种活体自组装策略需要发展更细致的结构设计方法. 根据疾病诊断和治疗的需要, 应该更精确地调控纳米材料在特定病理条件下的结构, 包括大小、表面电荷和形态, 以满足延长滞留时间等需要.

同时, 为了充分研究部分活体自组装纳米材料具有的形态转变性能, 应大力开发纳米材料的体内结构表征技术. 相信通过材料科学、生物和化学等领域研究人员的合作, 基础理论研究会不断发展, 活体自组装必将极大地推动生物医学以及材料学的长足进步.

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Summary for “pH 响应活体自组装生物材料及其在肿瘤诊疗中的应用进展”

pH-responsive *in vivo* self-assembled biomaterials and their application for tumor diagnosis and therapy

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The passive targeting of self-assembled nanomaterials at tumor sites can improve drug delivery capability, which has great prospects in biomedical field. However, dynamically balanced self-assembled nanomaterials will interact with a variety of biological interfaces or biological macromolecules *in vivo*, and there are problems with structural instability, and it is difficult to achieve accurate control of their structure, which hampers their further clinical application.

pH can affect the electrical charge of the amino acids, which affects the electrostatic interaction of polypeptides in the process of self-assembly, so changing the pH is one of the ways to regulate the process of self-assembly. In this way, we propose *in vivo* self-assembly strategy based on the pH-response peptide for tumor diagnosis and therapy. After reaching the tumor sites, the exogenous molecules structure will change under the stimulus of the acidic tumor microenvironment, and then form a highly ordered nanostructure, which can prolong the retention time of the material and improve the utilization effect. Starting from the design of small molecular peptides, polypeptides are combined with other functional biomolecules, and pH change at tumor sites is used to construct pH-responsive *in vivo* self-assembled materials. In this way, accurate and controllable diagnosis and therapy can be realized.

The acidic tumor microenvironment is one of the most prominent characteristics of tumor microenvironment and is a good trigger for designing microenvironmental responsive molecules. Our group designed a pH-sensitive molecule BP-KLVFF-His₆-PEG, the hydrophilic and hydrophobic balance was destroyed after reaching the acidic environment at the tumor site (pH 6.5). Driven by the hydrogen bonds of the KLVFF fragment, the molecule was self-assembled to form nanofibers. Combined with the imaging agent Nero red and doxorubicin, it can be used for long-term tumor imaging and enhancement to therapeutic efficacy. By combining functional molecules of tumor diagnosis and therapy with pH-responsive polypeptides, a more complex assembly system with biological functions can be constructed to improve the diagnostic function of imaging agents and the therapeutic effect of chemotherapy drugs. In this way, the new strategy of using pH-responsive polypeptides to build ordered assembly *in vivo* is paving the way for the development of nanomaterials for tumor diagnosis and therapy, which brings new hope for clinical application.

***in vivo* self-assembly, nanobiomaterials, pH-responsive, peptides, diagnosis and therapy**

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