评述

肝素/硫酸肝素在恶性疟原虫入侵红细胞过程中的作用机制

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摘要 疟疾是疟原虫通过雌性按蚊为媒介传播的寄生虫病, 是当今世界公共卫生的突出问题。寄生于人体的疟原虫主要包括恶性疟原虫、间日疟原虫、三日疟原虫、卵形疟原虫和诺氏疟原虫5种, 其中, 恶性疟原虫的致病性最为强烈, 是导致全球疟疾发病和死亡的重要病原体。硫酸肝素是广泛分布于脊椎动物细胞表面的无支链多糖, 为疟原虫入侵宿主红细胞的一个重要受体, 在疟原虫入侵过程中发挥着重要的作用。疟原虫入侵宿主红细胞是一个快速而复杂的过程, 恶性疟原虫入侵相关蛋白质在疟原虫入侵过程中具有重要的作用, 主要参与黏附宿主红细胞、形成移动连接复合体、形成和修饰纳虫空泡等过程。黏附是恶性疟原虫入侵宿主红细胞的第一步, 入侵相关蛋白质与宿主红细胞表面硫酸肝素受体结合, 继而完成后续的入侵。综合国内外文献, 本文围绕肝素/硫酸肝素在治疗恶性疟疾中的应用、与恶性疟原虫入侵相关蛋白质的结合以及恶性疟原虫入侵相关蛋白质在裂殖子入侵宿主红细胞过程中的作用机制等研究方面进行综述, 为恶性疟原虫入侵过程中相关蛋白质与肝素结合的分子机制提供理论依据, 同时也为新型抗疟药物和疫苗的研制以及治疗提供参考。

关键词 恶性疟原虫, 肝素/硫酸肝素, 治疗, 肝素结合蛋白质, 入侵机制

疟疾是一个全球性的公共卫生问题, 尤其是在非洲地区(2.13亿, 占93%)。据报告, 2018年全球疟疾死亡人数估计为405000^[1-3]。疟原虫是一种专性细胞内寄生虫, 寄生于人体的疟原虫包括恶性疟原虫、间日疟原虫、三日疟原虫、卵形疟原虫和诺氏疟原虫, 其中恶性疟原虫的感染最为广泛^[4]。恶性疟原虫在生长发育阶段需要人和按蚊两个宿主, 在人体内先后寄生于肝细胞和红细胞内, 进行裂体增殖, 在红细胞内, 除进行

裂体增殖外, 部分裂殖子形成配子体, 开始有性生殖的初期发育。在按蚊体内, 完成配子生殖, 继而进行孢子增殖^[5,6]。恶性疟原虫裂殖子入侵宿主红细胞是一个复杂的过程, 整个过程涉及细胞的黏附、重新定位、移动连接的形成、纳虫空泡的形成和红细胞膜的闭合等, 其中入侵的必要条件是疟原虫与宿主红细胞表面受体黏附^[7]。随着人们对疟原虫入侵机制的不断深入研究, 发现肝素类分子是裂殖子入侵宿主红细胞的主要受体,

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且肝素类分子能够破坏玫瑰花环的形成和抑制裂殖子对宿主红细胞的入侵^[8,9]。本文对肝素/硫酸肝素在治疗恶性疟疾中的应用、肝素结合类蛋白质及恶性疟原虫入侵相关蛋白质在裂殖子入侵宿主红细胞过程中的作用机制的研究做进一步阐述, 将为寻找抑制疟原虫在宿主体内繁殖的药物靶标和疫苗候选抗原奠定基础。

1 肝素/硫酸肝素

早期的临床研究发现, 微血栓和血脑屏障(blood brain barrier, BBB)是脑型疟(cerebral malaria, CM)发病机制的关键组成部分, 在严重的脑型疟患者切片中发现染虫红细胞广泛分布于血管内皮, 有些病例则有血栓的形成或染虫红细胞的高度聚集, 在电子显微镜下可观察到染虫红细胞的小疣与血管内皮细胞紧密连接^[10-12]。肝素因具有抗凝作用, 过去曾用作疟疾的辅助治疗药物, 但却常引起严重的副作用(如出血)^[13]。先前的研究表明, 其他具有安全性的硫酸化多糖能够阻止恶性疟原虫黏附于宿主红细胞、抑制裂殖子的入侵并破坏玫瑰花环^[14,15]。后来Chen团队^[16]首次发现, 氨基葡聚糖家族中的硫酸肝素是恶性疟原虫入侵宿主红细胞的主要受体。氨基葡聚糖(glycosaminoglycans, GAGs)是广泛分布于脊椎动物细胞表面的无支链多糖, 可分为肝素/硫酸肝素(heparan sulfate, HS)、软骨素/硫酸软骨素(chondroitin sulfuric acid, CSA)及透明质酸等, 在细胞黏附、控制生长、细胞信号传导和抗炎镇痛等方面具有重要功能^[17,18]。肝素/HS结构相似, 均包含1→4-连接的 α -D-氨基葡萄糖(GlcN)和 β -D-葡萄糖醛酸(GlcA)或 α -L-艾杜糖醛酸(IdoA), 而肝素和HS的硫酸化水平以及 β -D-葡萄糖醛酸或 α -L-艾杜糖醛酸的比例不同, 其中肝素的硫酸化程度要比硫酸肝素高^[19]; 其次, 二者分布部位不同, 肝素存在于肥大细胞颗粒中, 而HS则普遍存在于细胞表面和细胞外基质中^[20-23]。早期的研究发现, 能与肝素结合的多肽都包含一段或多段一致性序列, 即[-X-B-B-X-B-X-]和[-X-B-B-B-X-X-B-X-], 其中B是赖氨酸、精氨酸和组氨酸等碱性氨基酸基本残基, X是亲水氨基酸残基^[24]。

2 肝素/硫酸肝素在治疗恶性疟疾中的应用

疟原虫为了避免其所寄生的红细胞被脾脏所清

除, 需要“滞留”在血管内。被感染的红细胞可以与其他染虫红细胞或未染虫红细胞黏附形成玫瑰花环, 这可能会导致局部氧气输送受阻, 从而导致宿主死亡^[25], 然而恶性疟原虫玫瑰花环能被低浓度的肝素破坏^[26]。恶性疟原虫表面蛋白1(*Plasmodium falciparum* erythrocyte membrane protein 1, PfEMP1)由var基因编码, 是第一个暴露在红细胞表面的抗原, 介导恶性疟原虫感染的红细胞与血管内皮细胞及未感染的红细胞发生黏附, 但其具体功能尚不清楚^[27,28]。Chen等人^[29]通过单细胞操作技术, 将单个疟原虫虫体所表达的抗原变异基因进行了全部扩增和克隆, 在PfEMP1序列中发现了GAG结合基序簇, 通过比较重组蛋白质DBL-1-GST(含有糖胺聚糖结合基序)和ATS-GST(不含糖胺聚糖结合基序)与肝素结合的能力, 发现含有GAG结合基序的蛋白质能与肝素基质结合且具有浓度依赖性, 而CSA同样作为GAGs家族的一员, 却未能抑制其与肝素结合; 进一步将DBL-1-GST加到虫体培养液中, 发现玫瑰花环的生成遭到破坏, 这表明PfEMP1与红细胞结合的受体是硫酸肝素。2006, Vogt等人^[30]用高碘酸盐处理肝素, 生成了不具有抗凝活性的氨基葡聚糖(dGAG), 但在其他方面仍与肝素/HS相似。在体外实验中发现, dGAG具有与肝素相同的特性, 能够抑制恶性疟原虫的入侵和玫瑰花环的形成, 且在新型动物模型中发现dGAG能够在大鼠和猕猴体内抑制玫瑰花环的形成、自身凝集和细胞黏附。由于不相容性, 大多数注射的人染虫红细胞均被大鼠和猕猴的肝脏、脾脏和肾脏吸收。令人惊奇的是, 人染虫红细胞能够在大鼠和猕猴的肺静脉血管中与其他染虫红细胞或大鼠和猕猴的健康红细胞发生由硫酸肝素介导的细胞黏附, 这两种动物有望成为用于筛选抗疟疾药物和疫苗的新型动物模型。目前已有成功使用肝素治疗严重疟疾的报道, 但由于肝素具有抗凝血作用, 因此出血并发症的发生率很高^[13]。Wahlgren团队^[31-33]开发了一种低抗凝肝素衍生物, 可以逆转某些恶性疟原虫分离株的玫瑰花环和细胞黏附, 还可以阻止裂殖子的入侵。以上研究表明, 红细胞使用硫酸肝素分子作为玫瑰花环受体, 且肝素/硫酸肝素能够抑制玫瑰花环的形成和裂殖子的入侵。研究疟原虫硫酸肝素受体有助于探究疟原虫的发病机制, 为该病的治疗提供新思路。

3 恶性疟原虫入侵相关蛋白质与肝素/硫酸肝素结合的研究

氨基葡聚糖途径是恶性疟原虫入侵宿主红细胞的主要途径, 研究发现, 硫酸化的氨基葡聚糖可以作为恶性疟原虫接触和黏附宿主红细胞的主要受体^[34]. Zhang等人^[16]通过受体亲和层析再结合高通量蛋白质质谱分析方法以及一系列实验, 发现了恶性疟原虫与肝素相互作用的多种蛋白质分子, 该研究结果不仅说明肝素样分子是疟原虫入侵宿主红细胞的重要受体, 还揭示了肝素抑制虫体入侵宿主红细胞的作用机制, 也为肝素样分子作为抗疟药物提供了理论依据. Glushakova等人^[35]通过外源肝素竞争与恶性疟原虫结合, 发现外源肝素可降低恶性疟原虫对宿主红细胞的入侵, 且肝素浓度越高, 恶性疟原虫的入侵率越低, 与此同时发现肝素能够抑制恶性疟原虫从红细胞内的逸出; 肝素与其靶标结合的快速动力学以及结合位点的FITC-肝素高强度的信号表明, 肝素对大量寄生虫蛋白具有高结合亲和力, 其中之一很可能是裂殖子表面蛋白1(merozoite surface protein 1, MSP1). 与此类似的是, Boyle等人^[36]证明肝素样分子主要在裂殖子入侵宿主红细胞的早期发挥抑制作用, MSP1-42和MSP1-33可以结合肝素, 提示其使用肝素样分子作为入侵的受体. Kobayashi等人^[37]发现, 恶性疟原虫与红细胞表面黏附, 之后进行再定位, 而在肝素存在的情况下, 恶性疟原虫无法进行再定位, 且发现Duffy样结合蛋白(Duffy binding-like, DBL)和网织红细胞结合蛋白(reticulocyte binding-like, RBL/PfRh)家族中的蛋白质能与肝素结合, 并且其中一些以高亲和力结合, 这一发现为开发新型抗疟药和了解HS在裂殖子入侵宿主红细胞过程中发挥的作用提供了有用的信息. 恶性疟原虫必须识别宿主红细胞表面上的受体才能进行入侵, Kobayashi等人^[38,39]使用改良的逆转录病毒介导的表达克隆方法筛选出恶性疟原虫受体且与入侵相关的蛋白质BAEBL(erythrocyte binding antigens-140, EBA-140), 流式细胞术检测结果表明, 肝素能够竞争性抑制BAEBL与红细胞表面的硫酸肝素受体结合, 但是该发现并不足以说明肝素对入侵的完全抑制作用, 因为BAEBL并不是入侵的必需配体^[40]. 顶膜抗原1(apical membrane antigen 1, AMA1)和棒状体颈蛋白(rhoptry neck protein, RONs)参与形成移动连接, 但研究发现该种复合

物并不是肝素破坏的真正靶标, 而一项惊人的发现是肝素能与PfRH2, PfRH4和PfRH5结合, 它们都是唾液酸介导的非依赖性途径的所有配体^[37]. 恶性疟原虫入侵相关蛋白质可以与肝素/硫酸肝素受体结合, 作为细胞黏附的第一步, 暗示肝素结合相关蛋白质可以作为疟疾疫苗研制的新突破点.

4 恶性疟原虫入侵相关蛋白质在裂殖子入侵宿主红细胞过程中的作用机制

恶性疟原虫是一种专性细胞内寄生原虫, 不能在宿主细胞外进行复制增殖, 因此, 裂殖子入侵宿主红细胞是恶性疟原虫增殖和扩散的必要条件. 多年前通过开创性的视频显微镜和电子显微镜研究将其入侵宿主红细胞的过程可视化^[41,42], 发现恶性疟原虫裂殖子入侵宿主红细胞主要包括四个阶段: 黏附、再定位、形成紧密连接复合体和入侵, 其中黏附是裂殖子完成入侵的必要条件^[5,7], 且许多实验结果表明, 肌动球蛋白在裂殖子入侵宿主红细胞过程中提供马达动力支撑^[43-46].

裂殖子的入侵过程非常迅速, 涉及一系列的力学步骤^[47,48]. 裂殖子的入侵始于最初对红细胞的低亲和力附着, 是由糖基磷脂酰肌醇(glycosylphosphatidylinositol, GPI)锚定裂殖子表面蛋白介导的, 裂殖子表面蛋白质(MSPs)等与其结合促进恶性疟原虫与宿主红细胞黏附^[49,50]; 硫酸化的肝素可以作为裂殖子与宿主红细胞发生黏附的受体^[37], 具有糖胺聚糖结合基序的裂殖子表面蛋白质(如PF3D7_1104400, PF3D7_0811600和PF3D7_1361800等)能和红细胞发生黏附且具有肝素结合特性, 以完成裂殖子入侵的第一步^[51-53]; 恶性疟原虫裂殖子暴露于血液中, 低浓度钾离子会给虫体提供天然信号, 触发细胞内钙离子的升高, 进而触发如175 kD红细胞结合抗原(EBA175)微线体蛋白和AMA1等分泌到裂殖子表面^[54-57]. 随后, 释放的微线体蛋白EBA175与它的受体糖蛋白A(glycophorin A, glyA)结合, 恢复了基础胞质钙水平并触发了棒状体蛋白(如CLAG3.1和PfRH2b)的释放^[58,59]. 随后裂殖子调整其黏附部位, 使棒状体和微线体的顶端与红细胞表面发生顶端黏附, 进行再定位, 恶性疟原虫网织红细胞结合蛋白(Rh/RBL)家族和红细胞结合样蛋白(erythrocyte binding-like, EBL)在与宿主细胞的识别和入

侵中发挥着重要的作用^[43,60];成功入侵的关键是建立紧密的连接,这个连接点为裂殖子入侵运动提供了牵引力,使疟原虫能够将自身驱动到红细胞中,两种恶性疟原虫蛋白质已明确定位在紧密连接处,即顶膜抗原AMA1和棒状体颈蛋白RON4^[61];之后宿主红细胞膜下陷,将疟原虫包裹之中,形成纳虫空泡,当疟原虫完全被纳虫空泡包裹时,虫体与宿主红细胞连接处分离,虫体全部进入红细胞中;虫体的入侵导致红细胞发生脱水,因为钾离子和氯离子的流出,使正常的红细胞暂时经历棘状红细胞时期;裂殖子成功入侵到红细胞内5~11 min后,棘状突起逐渐消失,红细胞恢复呈正常形态^[5,62].

5 展望

恶性疟原虫裂殖子的红内期阶段是恶性疟原虫繁殖的重要阶段,裂殖子入侵宿主红细胞是一个快速而复杂的过程,主要包括细胞的黏附、重新定位、移动连接的形成、纳虫空泡的形成及修饰和红细胞膜

的闭合等.恶性疟原虫入侵相关蛋白质在恶性疟原虫入侵过程中发挥着重要的作用,但是其分子机制尚不清楚.研究发现,肝素/硫酸肝素广泛存在于几乎所有脊椎动物的细胞表面,且肝素/硫酸肝素能与恶性疟原虫入侵相关蛋白质发生相互作用.本实验室利用LC/LC-MS/MS方法首次确定恶性疟原虫与肝素特异性结合的811个蛋白质,通过研究发现筛选出的MSP1-42, RON3-48和PF3D7_1357800等十余种入侵相关蛋白质具有细胞黏附特性和抑制虫体入侵等功能且随着重组蛋白质或抗体浓度的增加,抑制虫体入侵活性逐渐增强,对裂殖子入侵相关蛋白质的单价或多价联合疫苗的研制和开发非常重要.因此,研究恶性疟原虫入侵相关的肝素结合蛋白质的功能,将进一步阐明恶性疟原虫入侵宿主红细胞的分子机制奠定基础,并将在药物靶标和疫苗候选抗原的筛选等方面取得更大的进步.与此同时,肝素/硫酸肝素作为恶性疟疾的辅助治疗药物,尚存在诸多问题,因此仍需对肝素/硫酸肝素衍生物进行进一步探索研究,其将为抗疟药物的研制开创新的局面.

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The mechanism of heparin/heparan sulfide in the process of *Plasmodium falciparum* invasion of red blood cells

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Malaria is a parasitic disease caused by *plasmodium*. *Plasmodium* is an obligate intracellular parasite that requires two hosts: human and *Anopheles*. The parasites of humans mainly include *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium Malariae*, *Plasmodium ovale*, and *Plasmodium Knosei*. *Plasmodium falciparum* has the strongest pathogenicity, and it is the main pathogen causing the morbidity and death of malaria worldwide. Heparan sulfate is an unbranched polysaccharide widely distributed on the surface of vertebrate cells. It is an important receptor for malaria to invade host red blood cells, and plays a key role in the process of malaria invasion. *Plasmodium* invasion of host red blood cells is a rapid and complex process. *Plasmodium* invasion-related proteins play an important role in the process of malaria parasite invasion. They are mainly involved in the processes of adhesion to host red blood cells, formation of mobile junction complexes, and formation and modification of parasitophorous vacuole membrane (PVM). Adhesion is the first step for *Plasmodium falciparum* to invade host red blood cells. Invasion-related proteins bind to heparan sulfide receptors on the surface of host red blood cells to complete subsequent invasion. Based on domestic and foreign literature, this article summarizes the research on heparin/heparan sulfate in the treatment of *Plasmodium falciparum*, the binding of *Plasmodium falciparum* invasion-related proteins and heparin/heparan sulfide, and the mechanism of *Plasmodium falciparum* invasion-related proteins in the process of merozoites invading host red blood cells. The molecular mechanism of the binding of related proteins to heparin in the process provides a theoretical basis, and also provides a reference for the development and treatment of new antimalarial drugs and vaccines.

***Plasmodium falciparum*, heparin/heparan sulfate, treatment, heparin binding protein, invasion mechanism**

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