

幽门螺杆菌耐药机制的研究进展

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摘 要

幽门螺杆菌(*Helicobacter pylori*, Hp)是一种微需氧的螺旋状革兰阴性杆菌, 能引起多种胃肠道疾病。根除Hp可显著降低消化性溃疡的复发率和胃癌的发病风险, 但由于抗生素的不合理应用等问题, Hp的耐药率日渐升高, 给临床根除治疗带来极大困难。因此, 本文就Hp耐药机制的研究现状和进展作一综述。

关键词

幽门螺杆菌, 抗生素, 耐药机制

Progress on Drug Resistance Mechanism of *Helicobacter pylori*

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Abstract

Helicobacter pylori (*H. pylori*) is a microaerobic, spiral-shaped, and Gram-negative bacterium that causes multiple gastrointestinal diseases. *H. pylori* eradication can significantly reduce peptic ulcer occurrence and recurrence, and the risk of gastric cancer. However, due to the unreasonable application of antibiotics and other problems, *H. pylori* antibiotic resistance rate is increasing, which brings great difficulties to clinical *H. pylori* eradication. Therefore, this article reviews the

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current status and progress in study of drug resistance mechanisms of *H. pylori*.

Keywords

Helicobacter pylori, Antibiotic, Resistance Mechanism

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1. 引言

幽门螺杆菌(*Helicobacter pylori*, Hp)是一种生存于人体胃部的具有传染性的革兰氏阴性杆菌。迄今为止,全球约有 44 亿 Hp 感染患者,占世界人口的 50%左右,甚至在发展中国家 Hp 发病率已高达 80% [1]。Hp 作为 I 类致癌原与消化性溃疡、胃癌等消化系统疾病以及缺铁性贫血、特发性血小板减少性紫癜等胃肠外疾病密切相关[2] [3], 国内外指南均推荐 Hp 阳性患者接受根除治疗[4] [5]。目前, 抗生素治疗一直是临床上根除 Hp 的主要方法, 常用的抗生素主要有克拉霉素、甲硝唑、阿莫西林、左氧氟沙星、呋喃唑酮、四环素等。但由于抗生素的不合理应用等问题, Hp 的耐药率正逐渐升高, 特别是甲硝唑、克拉霉素和左氧氟沙星耐药率均处于较高水平, 而阿莫西林耐药率虽低, 但也呈逐年上升趋势[6] [7]。在我国大陆地区针对抗 Hp 耐药性分析发现甲硝唑、克拉霉素、左氧氟沙星与阿莫西林在 2019~2020 年达到或接近最高值, 耐药率分别为 85.2%、33.0%、41.6%与 17.5% [6]。因此, Hp 的抗生素耐药性问题亟待解决, 本文通过研究国内外相关文献, 对 Hp 耐药机制的研究进展进行梳理。

2. 基因突变与抗菌药物

2.1. 导致 Hp 对克拉霉素耐药的突变

克拉霉素通过与细菌 50S 亚基结合, 干扰细菌蛋白质的合成而发挥抗菌作用。事实上, 早在 1996 年就有关于 Hp 对克拉霉素耐药的研究, 该研究[8]提出 Hp 对克拉霉素产生耐药性是其核糖体 50S 中亚基 23SrRNA 的肽基转移酶环(V 结构域)发生点突变的结果。所有 Hp 耐药菌株均有 A2146G (以前称为 A2142G)和 A2147G (以前称为 A2143G), 这些突变影响核糖体与药物相互作用的亲和力, 从而不能抑制细菌蛋白质的合成[9]。但除了上述两个突变外, 还有研究发现了一些新的突变, 如 A2115G、A2144T、G2141A 和 T2182C, 这些新突变和经典突变之间的差异可能在于克拉霉素最低抑菌浓度(minimum inhibitory concentration, MIC)不同[10] [11]。此外, 编码细菌核糖体蛋白 L22 的 hp1314 (rpl22)基因和编码翻译起始因子 IF-2 的 hp1048 (infB)基因的突变也与克拉霉素耐药有关, 并与 23SrRNA 突变显示协同作用[12] [13]。另外, 通过基因组学分析, 有研究发现 hp1181 和 hp1184 基因突变可能是 Hp 对克拉霉素耐药产生的最早、最持久的反应, 这项研究也可能有助于 Hp 耐药的早期诊断、预防和治疗[14]。

2.2. 导致 Hp 对甲硝唑和左氧氟沙星耐药的突变

甲硝唑通过还原反应产生亲电物质, 能有效破坏细菌蛋白质和核酸从而杀死细菌, 达到抗 Hp 的目的[15]。许多研究表明 rdxA (编码对氧不敏感的 NADPH 硝基还原酶)、frxA (编码 NADPH 黄素氧化还原酶)和 fdxB (编码铁氧还原蛋白类似物)基因的突变与不同水平的甲硝唑耐药有关, 但是单一的 fdxB 基因突变只导致 Hp 对甲硝唑的低水平耐药甚至不耐药, 而 rdxA 和 frxA 突变则与高水平的甲硝唑耐药有关[16]

[17] [18]。另有研究提出在 *rdxA* 突变中似乎只有 R16H/C 和 M21A 位点与甲硝唑耐药相关[19]。但是 Hp 对甲硝唑产生耐药作用似乎不能用单一机制来解释, 有研究[13]发现在 MIC 值最高的菌株中除全长 *rdxA* 错义突变外, 还存在 *dppA* 基因和 *dapF* 基因的突变, 但这项研究的样本数量可能比较有限, 未来仍需加大实验样本, 明确上述突变位点与甲硝唑耐药的相关性。

左氧氟沙星作为根除 Hp 感染的常用治疗药物之一, 是一种氟喹诺酮类抗生素, 以细菌的脱氧核糖核酸(DNA)为靶, 妨碍 DNA 旋转酶(拓扑异构酶 II)和拓扑异构酶 IV, 进一步造成细菌 DNA 的不可逆损害, 达到抗菌效果[20]。其中 DNA 旋转酶, 由 2 个 *gyrA* 和 2 个 *gyrB* 亚基组成, 是维持 DNA 螺旋结构所必需的酶[20]。Hp 对左氧氟沙星耐药主要是与 *gyrA* 基因内喹诺酮类药物耐药决定区的 Asn-87 和(或)Asp-91 点突变有关, 同样 *gyrB* 基因突变也可以在耐药中发挥作用, 但这些突变通常与 *gyrA* 基因突变共存[19] [21]。此外, 近期也有研究发现并证实 *gyrA* 基因内新的 Gly-85 点突变与氟喹诺酮类耐药相关[22]。在此研究中分析了 2006 株耐药菌株, 其中, 15 株在 *gyrA* 中表现出 Asn-87 突变(51.7%), 12 株在 Asp-91 (41.4%)出现突变, 并且发现了之前从未报道过的 Gly-85 点突变[22]。

2.3. 导致 Hp 对阿莫西林耐药的突变

阿莫西林作为治疗 Hp 的四联方案中常用抗生素之一, 同其他 β -内酰胺类抗生素一样, 能抑制胞壁粘肽合成酶, 即青霉素结合蛋白(PBPs), 从而阻碍细胞壁粘肽合成, 使细菌胞壁缺损, 菌体膨胀裂解[23]。不少学者认为 PBPs 突变是引起阿莫西林耐药的根本原因, 编码 PBPs 的 *pbp1A* 的基因突变可能是 Hp 对阿莫西林耐药的主要原因[24] [25]。同时, 有研究[26]分析表明, 除了 *pbp1A* 的基因突变外, 其他分子机制还可能导致 Hp 对阿莫西林耐药, 例如其他蛋白质 PBP2、PBP3 和 PBP4 的变化等。

3. 外排泵与生物膜

3.1. 外排泵的表达

外排泵是由一系列转运体组成, 革兰氏阴性菌表达大量外排泵, 可以将具有不同结构(包括抗生素)的分子转运出细胞, 这种外排降低了细胞中抗生素的浓度, 并使细菌能够在更高的抗生素浓度下存活[27]。因此, 外排泵的过表达可导致 Hp 的临床相关耐药性。研究发现, 外排泵介导了 Hp 对阿莫西林、甲硝唑和克拉霉素的耐药性[28] [29] [30]。研究[28]指出, 影响药物外排的转运蛋白 HP0939、HP0497 和 HP0471 同时参与了 Hp 多药耐药和生物膜形成。敲除转运蛋白 HP0939、HP0497 和 HP0471 后不仅增加了 Hp 对多种抗生素的敏感性, 并在体外抑制了完整生物膜的形成[28]。另外, 在具有生物膜的细菌中, 外排基因的表达也显著增加[31] [32]。因此, 外排泵与生物膜可以通过协同作用以增加耐药性。

3.2. 生物膜

Hp 具有在体内和体外形成生物膜的能力, 生物膜在体外可增加 Hp 对克拉霉素、阿莫西林和甲硝唑的耐药[32]。细胞外聚合物(EPS)在 Hp 生物膜耐药性中起重要作用。EPS 位于生物膜的最外层, 可以为生物膜内的细胞提供保护, 避免了人体免疫细胞与细菌的直接相互作用并减少了抗生素的渗透[33]。近年来, 外膜蛋白在致病性和耐药性中的作用也是主要研究热点。Hathroubi 等人发现, 生物膜形成导致的耐药性部分取决于外膜蛋白的变化, 并且通过增加细胞外蛋白酶 K 的水平可以降低克拉霉素耐药性[34]。

4. 毒力因子分泌

近年来, 一些研究[35] [36]提出毒力因子与 Hp 耐药性之间是有关联的, 但该结论目前尚存在争议。其中有研究[35]提出甲硝唑耐药性与细胞毒素相关基因 A (*cagA*)和空泡细胞毒素基因 A (*vacA*)的基因型

有关。在耐药菌株中, *vacA s1m1/cagA+*型最常见, 其次是 *vacA s1m1/cagA-*型[35]。另有研究[36]指出 *cagA+*菌株会显著增加 Hp 对甲硝唑耐药的风险。此外, *vacA s1m1* 不仅显著增加了对甲硝唑的耐药性, 也增加了对阿莫西林和左氧氟沙星的耐药性, 而 *vacA s1m2* 则降低了对克拉霉素和甲硝唑的耐药性[36]。但是同时有研究提出毒力因子与耐药性或易感性之间没有显著关联[37]。因此, Hp 毒力因子和耐药性之间的关系及作用机制仍需进一步研究阐明。

5. 结论

近年来, Hp 对多种抗菌药物的耐药性迅速增加, 尤其对克拉霉素、甲硝唑、左氧氟沙星和阿莫西林的耐药率更是逐年递增。Hp 可以迅速适应变化的环境, 并通过多种耐药机制(基因突变、外排泵、生物膜形成、毒力因子)抑制抗生素的活性。因此, 全面了解 Hp 的耐药机制, 有助于临床医师实施个体化的治疗策略, 优化根除疗法, 尽量选择敏感抗生素的根除方案, 减少耐药菌株的形成与播散, 避免抗生素的滥用, 从而提高 Hp 根除率。

参考文献

- [1] Jiang, X., Xu, Z., Zhang, T., *et al.* (2021) Whole-Genome-Based *Helicobacter pylori* Geographic Surveillance: A Visualized and Expandable Webtool. *Frontiers in Microbiology*, **12**, Article ID: 687259. <https://doi.org/10.3389/fmicb.2021.687259>
- [2] Cho, J., Prashar, A., Jones, N.L., *et al.* (2021) *Helicobacter pylori* Infection. *Gastroenterology Clinics of North America*, **50**, 261-282. <https://doi.org/10.1016/j.gtc.2021.02.001>
- [3] Santos, M.L.C., De Brito, B.B., Da Silva, F.A.F., *et al.* (2020) *Helicobacter pylori* Infection: Beyond Gastric Manifestations. *World Journal of Gastroenterology*, **26**, 4076-4093. <https://doi.org/10.3748/wjg.v26.i28.4076>
- [4] 苑旭晔, 陈宏楨, 郭金波, 等. 《第六次全国幽门螺杆菌感染处理共识报告(非根除治疗部分)》解读[J]. 河北医科大学学报, 2023, 44(3): 249-251.
- [5] Suzuki, S., Kusano, C., Horii, T., *et al.* (2022) The Ideal *Helicobacter pylori* Treatment for the Present and the Future. *Digestion*, **103**, 62-68. <https://doi.org/10.1159/000519413>
- [6] 赵霞, 徐薇薇, 刘益萌, 等. 中国大陆地区幽门螺杆菌对常用抗生素耐药性的临床分析[J]. 基础医学与临床, 2022, 42(7): 1077-1082.
- [7] Megraud, F., Bruyndonckx, R., Coenen, S., *et al.* (2021) *Helicobacter pylori* Resistance to Antibiotics in Europe in 2018 and Its Relationship to Antibiotic Consumption in the Community. *Gut*, **70**, 1815-1822. <https://doi.org/10.1136/gutjnl-2021-324032>
- [8] Versalovic, J., Shorridge, D., Kibler, K., *et al.* (1996) Mutations in 23S rRNA Are Associated with Clarithromycin Resistance in *Helicobacter pylori*. *Antimicrobial Agents and Chemotherapy*, **40**, 477-480. <https://doi.org/10.1128/AAC.40.2.477>
- [9] Gong, E.J., Ahn, J.Y., Kim, J.M., *et al.* (2020) Genotypic and Phenotypic Resistance to Clarithromycin in *Helicobacter pylori* Strains. *Journal of Clinical Medicine*, **9**, Article No. 1930. <https://doi.org/10.3390/jcm9061930>
- [10] Kocazeybek, B., Sakli, M.K., Yuksel, P., *et al.* (2019) Comparison of New and Classical Point Mutations Associated with Clarithromycin Resistance in *Helicobacter pylori* Strains Isolated from Dyspeptic Patients and Their Effects on Phenotypic Clarithromycin Resistance. *Journal of Medical Microbiology*, **68**, 566-573. <https://doi.org/10.1099/jmm.0.000944>
- [11] Albasha, A.M., Elnosh, M.M., Osman, E.H., *et al.* (2021) *Helicobacter pylori* 23S rRNA gene A2142G, A2143G, T2182C, and C2195T Mutations Associated with Clarithromycin Resistance Detected in Sudanese Patients. *BMC Microbiology*, **21**, Article No. 38. <https://doi.org/10.1186/s12866-021-02096-3>
- [12] Binh, T.T., Shiota, S., Suzuki, R., *et al.* (2014) Discovery of Novel Mutations for Clarithromycin Resistance in *Helicobacter pylori* by Using Next-Generation Sequencing. *The Journal of Antimicrobial Chemotherapy*, **69**, 1796-1803. <https://doi.org/10.1093/jac/dku050>
- [13] Miftahussurur, M., Shrestha, P.K., Subsomwong, P., *et al.* (2016) Emerging *Helicobacter pylori* Levofloxacin Resistance and Novel Genetic Mutation in Nepal. *BMC Microbiology*, **16**, Article No. 256. <https://doi.org/10.1186/s12866-016-0873-6>
- [14] Li, X.H., Huang, Y.Y., Lu, L.M., *et al.* (2021) Early Genetic Diagnosis of Clarithromycin Resistance in *Helicobacter*

- pylori*. *World Journal of Gastroenterology*, **27**, 3595-3608. <https://doi.org/10.3748/wjg.v27.i24.3595>
- [15] 杜乐, 喻世静, 殷智鑫, 等. 硝基咪唑类药物的研究进展[J]. 化学世界, 2020, 61(2): 92-98.
- [16] Kwon, D.H., El-Zaatari, F.A., Kato, M., *et al.* (2000) Analysis of *rdxA* and Involvement of Additional Genes Encoding NAD(P)H Flavin Oxidoreductase (*FrxA*) and Ferredoxin-Like Protein (*FdxB*) in Metronidazole Resistance of *Helicobacter pylori*. *Antimicrobial Agents and Chemotherapy*, **44**, 2133-2142. <https://doi.org/10.1128/AAC.44.8.2133-2142.2000>
- [17] Yang, Y.J., Wu, J.J., Sheu, B.S., *et al.* (2004) The *rdxA* Gene Plays a More Major Role than *frxA* Gene Mutation in High-Level Metronidazole Resistance of *Helicobacter pylori* in Taiwan. *Helicobacter*, **9**, 400-407. <https://doi.org/10.1111/j.1083-4389.2004.00270.x>
- [18] Lee, S.M., Kim, N., Kwon, Y.H., *et al.* (2018) *rdxA*, *frxA*, and Efflux Pump in Metronidazole-Resistant *Helicobacter pylori*: Their Relation to Clinical Outcomes. *Journal of Gastroenterology and Hepatology*, **33**, 681-688. <https://doi.org/10.1111/jgh.13906>
- [19] Zhang, S., Wang, X., Wise, M.J., *et al.* (2020) Mutations of *Helicobacter pylori* *RdxA* Are Mainly Related to the Phylogenetic Origin of the Strain and Not to Metronidazole Resistance. *The Journal of Antimicrobial Chemotherapy*, **75**, 3152-3155. <https://doi.org/10.1093/jac/dkaa302>
- [20] 温国琴. 抗生素耐药性现状及研究进展[J]. 中国处方药, 2022, 20(8): 186-190.
- [21] Ziver-Sarp, T., Yuksel-Mayda, P., Saribas, S., *et al.* (2021) Point Mutations at *gyrA* and *gyrB* Genes of Levofloxacin Resistant *Helicobacter pylori* Strains and Dual Resistance with Clarithromycin. *Clinical Laboratory*, **67**. <https://doi.org/10.7754/Clin.Lab.2021.210843>
- [22] Rhie, S.Y., Park, J.Y., Shin, T.S., *et al.* (2020) Discovery of a Novel Mutation in DNA Gyrase and Changes in the Fluoroquinolone Resistance of *Helicobacter pylori* over a 14-Year Period: A Single Center Study in Korea. *Antibiotics (Basel, Switzerland)*, **9**, Article No. 287. <https://doi.org/10.3390/antibiotics9060287>
- [23] Nauta, K.M., Ho, T.D. and Ellermeier, C.D. (2021) The Penicillin-Binding Protein PbpP Is a Sensor of β -Lactams and Is Required for Activation of the Extracytoplasmic Function σ Factor $\sigma(P)$ in *Bacillus thuringiensis*. *mBio*, **12**, e00179-21. <https://doi.org/10.1128/mBio.00179-21>
- [24] Kwon, Y.H., Kim, J.Y., Kim, N., *et al.* (2017) Specific Mutations of Penicillin-Binding Protein 1A in 77 Clinically Acquired Amoxicillin-Resistant *Helicobacter pylori* Strains in Comparison with 77 Amoxicillin-Susceptible Strains. *Helicobacter*, **22**, e12437. <https://doi.org/10.1111/hel.12437>
- [25] Zerbetto De Palma, G., Mendiondo, N., Wonaga, A., *et al.* (2017) Occurrence of Mutations in the Antimicrobial Target Genes Related to Levofloxacin, Clarithromycin, and Amoxicillin Resistance in *Helicobacter pylori* Isolates from Buenos Aires City. *Microbial Drug Resistance (Larchmont, NY)*, **23**, 351-358. <https://doi.org/10.1089/mdr.2015.0361>
- [26] Tran, T.T., Nguyen, A.T., Quach, D.T., *et al.* (2022) Emergence of Amoxicillin Resistance and Identification of Novel Mutations of the *pbp1A* Gene in *Helicobacter pylori* in Vietnam. *BMC Microbiology*, **22**, Article No. 41. <https://doi.org/10.1186/s12866-022-02463-8>
- [27] Zgurskaya, H.I., Walker, J.K., Parks, J.M., *et al.* (2021) Multidrug Efflux Pumps and the Two-Faced Janus of Substrates and Inhibitors. *Accounts of Chemical Research*, **54**, 930-939. <https://doi.org/10.1021/acs.accounts.0c00843>
- [28] Cai, Y., Wang, C., Chen, Z., *et al.* (2020) Transporters HP0939, HP0497, and HP0471 Participate in Intrinsic Multidrug Resistance and Biofilm Formation in *Helicobacter pylori* by Enhancing Drug Efflux. *Helicobacter*, **25**, e12715. <https://doi.org/10.1111/hel.12715>
- [29] Rimbara, E., Mori, S., Kim, H., *et al.* (2018) Mutations in Genes Encoding Penicillin-Binding Proteins and Efflux Pumps Play a Role in β -Lactam Resistance in *Helicobacter cinaedi*. *Antimicrobial Agents and Chemotherapy*, **62**, e02036-17. <https://doi.org/10.1128/AAC.02036-17>
- [30] Geng, X., Li, W., Chen, Z., *et al.* (2017) The Bifunctional Enzyme SpoT Is Involved in the Clarithromycin Tolerance of *Helicobacter pylori* by Upregulating the Transporters HP0939, HP1017, HP0497, and HP0471. *Antimicrobial Agents and Chemotherapy*, **61**, e02011-16. <https://doi.org/10.1128/AAC.02011-16>
- [31] Ge, X., Cai, Y., Chen, Z., *et al.* (2018) Bifunctional Enzyme SpoT Is Involved in Biofilm Formation of *Helicobacter pylori* with Multidrug Resistance by Upregulating Efflux Pump Hp1174 (*gluP*). *Antimicrobial Agents and Chemotherapy*, **62**, e00957-18. <https://doi.org/10.1128/AAC.00957-18>
- [32] Yonezawa, H., Osaki, T., Hojo, F., *et al.* (2019) Effect of *Helicobacter pylori* Biofilm Formation on Susceptibility to Amoxicillin, Metronidazole and Clarithromycin. *Microbial Pathogenesis*, **132**, 100-108. <https://doi.org/10.1016/j.micpath.2019.04.030>
- [33] Penesyan, A., Paulsen, I.T., Gillings, M.R., *et al.* (2020) Secondary Effects of Antibiotics on Microbial Biofilms. *Frontiers in Microbiology*, **11**, Article No. 2109. <https://doi.org/10.3389/fmicb.2020.02109>
- [34] Hathroubi, S., Zerebinski, J., Clarke, A., *et al.* (2020) *Helicobacter pylori* Biofilm Confers Antibiotic Tolerance in Part

via a Protein-Dependent Mechanism. *Antibiotics (Basel, Switzerland)*, **9**, Article No. 355.

<https://doi.org/10.3390/antibiotics9060355>

- [35] Bachir, M., Allem, R., Tifrit, A., *et al.* (2018) Primary Antibiotic Resistance and Its Relationship with *cagA* and *vacA* Genes in *Helicobacter pylori* Isolates from Algerian Patients. *Brazilian Journal of Microbiology*, **49**, 544-551. <https://doi.org/10.1016/j.bjm.2017.11.003>
- [36] Karbalaee, M., Talebi Bezmin Abadi, A. and Keikha, M. (2022) Clinical Relevance of the *cagA* and *vacA* s1m1 Status and Antibiotic Resistance in *Helicobacter pylori*: A Systematic Review and Meta-Analysis. *BMC Infectious Diseases*, **22**, Article No. 573. <https://doi.org/10.1186/s12879-022-07546-5>
- [37] Oktem-Okullu, S., Cekic-Kipritci, Z., Kilic, E., *et al.* (2020) Analysis of Correlation between the Seven Important *Helicobacter pylori* (*H. pylori*) Virulence Factors and Drug Resistance in Patients with Gastritis. *Gastroenterology Research and Practice*, **2020**, Article ID: 3956838. <https://doi.org/10.1155/2020/3956838>