·综述·

幽门螺杆菌耐药的分子机制研究现状

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摘要:近年抗生素耐药率普遍升高,耐药成为根除幽门螺杆菌治疗失败的主要原因。克拉霉素耐药的主要原因为23S rRNA基因点突变。甲硝唑低水平耐药主要与硝基还原酶基因rdxA和frxA基因的失活有关,高水平耐药是多基因突变的累积效果所致。阿莫西林耐药与pbp1A基因多位点突变相关。porD和oorD两基因的突变可能与幽门螺杆菌对呋喃唑酮产生低水平耐药相关。

关键词:幽门螺杆菌;耐药性;突变

目前已明确幽门螺杆菌(*Hp*)是慢性活动性胃炎的直接病因,与消化性溃疡、黏膜相关性淋巴样组织淋巴瘤密切相关,是胃癌发生的危险因子。根除*Hp*治疗是临床治疗慢性胃病的重要策略,根除*Hp*不仅能加速消化性溃疡的愈合,而且能显著降低溃疡复发率,也可以减少早期胃癌术后的复发。目前,阿莫西林、甲硝唑和克拉霉素是临床上根除*Hp*治疗最常选用的抗生素,但随着抗生素的广泛应用,*Hp*的耐药率逐年上升[1]。国内不同地区、不同民族人群胃内*Hp*检出率在30%~80%之间,有很大差别。因此,*Hp*耐药机制的研究在临床诊断和治疗中意义重大。

1 克拉霉素耐药的分子机制

克拉霉素是新一代的大环内酯类抗生素,其通过抑制细菌蛋白质的合成,达到杀菌目的。近年来*Hp*对克拉霉素的耐药率呈上升趋势,有文献报道,日本地区*Hp*对克拉霉素耐药率约13%~18%,欧洲约1.7%~23.4%,北美地区约12.2%~25%[2]。

目前对克拉霉素耐药机制的研究主要集中在对*Hp* 23S rRNA基因的分析。目前为止已证实23SrRNA基因主要有3种不同的点突变与*Hp*对克拉霉素耐药有关:A2143G、A2142G、A2142C,其中最常见为A2143G,其次为A2142G,而A2142C则较少见。已有多项研究证实,A2142G和A2143G两种突变可以致*Hp*对克拉霉素高水平耐药,其中A2142G/C的平均最低抑菌浓度(MIC)较A2143G高,A2142G和A2143G的联合突变较单一突变MIC更高[2]。但并非所有A2142G突变MIC均较A2143G高,在Versalovic等的研究中,26.3%的A2142G并非高水平耐药,也有研究发现2株A2142G的MIC较A2143G低,说明A2142G突变并非高水平耐药所必需[3]。

其他已发现的较少见突变包括A2115G-G2141A联合突变、T2717C、A2142T、T2182C、T2244C、G2223A、T2288C、C2195T-A2143G联合突变、T2221C-A2143G联合突变、G1939A、T1942C和C2147G三个位点联合突变[4]。Hulten于一株耐药株中发现A2115G-G2141A联合突变,而无A2143G、A2142G突变,证明A2115G-G2141A联合突变与*Hp*耐药相关[5],其他位点与耐药的关系还有待于进一步研究。Rimbara等[4]将日本1995到2004年间检测的*Hp*临床分离株进行META分析,发现A2142G或者A2143G突变出现于所有*Hp*耐克拉霉素菌株,敏感株则未检测出,T2182C、G2223A、T2288C、C2244T突变在敏感株和耐药株中均有较高的检出率,这4种突变与*Hp*耐克拉霉素无关。Matsuoka等[6]也证实T2182C、T2244C突变敏感株和耐药株均存在,与耐药无关。

由此可见,A2142G、A2143G、A2142C、A2115G-G2141A基因突变与*Hp*克拉霉素耐药相关,因前三者较常见,分子生物学方法检测克拉霉素耐药多立足于这三个基因突变位点,值得一提的是,有些学者检测克拉霉素耐药选择位点2182[7-8],前面已提到Rimbara和Matsuoka已证实2182位点突变在敏感株和耐药株中均有较高的检出率,与耐药无关,故选择2182位点作为耐药检测位点显然是不合适的。

2 甲硝唑耐药的分子机制

甲硝唑耐药率是几种抗*Hp*药物中最高的,一些发展中国家达90%,西欧国家也达5%~50%[9]。甲硝唑耐药可能与rdxA、frxA、fdxB基因突变有关。

rdxA和frxA基因在甲硝唑耐药中所起的作用

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存在着争议。大部分学者认为，rdxA 基因失活突变是 *Hp* 甲硝唑耐药的基因基础。Moore 等[9]认为，frxA 基因的框移突变在敏感株和耐药株中出现的频率相同，因此与 *Hp* 甲硝唑耐药无关，只有 rdxA 基因与耐药相关。Jeong 等[10]认为，rdxA 的功能对于 *Hp* 对甲硝唑的敏感性起主要作用，临床分离株对甲硝唑的耐药基本上都有 rdxA 的突变，在 rdxA 基因突变的基础上，frxA 基因的失活突变可以增加 *Hp* 细菌的 MIC，frxA 基因的失活可以使甲硝唑的杀菌作用减缓，增加已有 rdxA 突变的 *Hp* 耐药水平，但在 rdxA 基因有活性的情况下不能使 *Hp* 产生耐药。rdxA 基因和 frxA 联合突变的耐药水平比 rdxA 单独突变的耐药性要高。Yang 等[11]证实 rdxA 基因突变对 *Hp* 甲硝唑耐药起主要作用，耐药株中 89% 存在 rdxA 基因突变，而敏感株中 rdxA 基因无突变而存在 frxA 基因突变，高水平耐药株中 86% 存在 rdxA 基因突变，只有 43% 存在 frxA 基因突变，表明 rdxA 基因突变在甲硝唑高水平耐药中所起的作用较 frxA 基因突变重要。

虽然 rdxA 基因的突变失活与甲硝唑耐药密切相关，很多研究显示，rdxA 基因治疗前后敏感株和耐药株的序列分析并无显著差别，说明 rdxA 基因非耐药必需基因，可能存在其他的机制。Marais 等[12]证明甲硝唑耐药可以只由 frxA 突变引起，也可以没有 rdxA 或 frxA 突变，存在其他耐药机制。目前的观点为，甲硝唑低水平的耐药(MTZ 32μg/ml)是由于 rdxA 和 frxA 基因失活所致，高水平耐药很可能是多基因突变的累积效果。Albert 等[13]发明了一种以芯片杂交为基础的比较基因组测序法(CGS)，用 CGS 来研究基因突变与 *Hp* 对甲硝唑耐药的关系，发现从敏感到高水平的耐药是从 rdxA 基因突变开始，接着是 frxA 基因突变，高水平耐药是由于多重基因突变叠加所致。

3 阿莫西林耐药的分子机制

阿莫西林又称羟氨苄西林是 *Hp* 根除治疗中常用的 β-内酰胺类抗生素，很多地区耐药率低于 1%[14]。PBP1 对阿莫西林亲和力下降可能是耐药的主要原因。近来研究涉及 PBP1 氨基酸序列和 pbp1 基因突变的具体位点之间关系。Paul 等[15]发现以下突变与阿莫西林耐药有关：pbp1(S414R、Y484C、T541I 和 P600T)。Gerrits 等[16]证明 Ser414Arg 在阿莫西林 Hardenberg strain 耐药株中起主要作用后进一步证实，S402G、E406A、S417T、S414R、T555S 和 N561Y 位点突变为耐药主要原因。Rimbara 等[14]发现 9 个突变位点仅发生于耐药株和低度敏感株，其中 Asn562→Tyr 突变出现于所有阿莫西林耐药株中，为阿莫西林耐药株共同而特异的突变。Kwon 等[17]通过序列比对分析发现耐药株 pbp-1A 基因发生 51 个碱基突变，将其转入敏感株使其 MIC 升至 8 g/ml，而其他报道 pbp-1A 有 1 到 4 个位点突变时 MIC 为 2 g/ml。

高水平耐药是多基因多位点共同作用的结果，pbp1 基因突变并不能造成阿莫西林高水平耐药，阿莫西林高水平耐药存在 pbp1 突变以外的其他机制。Co 等[18]实验证明，pbp1-T438M、HopB、Hop 基因突变均参与阿莫西林耐药。Rimbara 等[19]实验证明 pbp1，pbp2，和 pbp3 突变均与阿莫西林耐药相关，在 pbp1 突变的基础上 pbp2 和 pbp3 的突变更进一步提高了 MIC。

4 呋喃唑酮耐药的分子机制

呋喃唑酮近年来被越来越多地用于 *Hp* 感染的一线治疗，同样导致耐药出现。呋喃唑酮与甲硝唑作用机制类似，Kown[20]推测 *Hp* 对呋喃唑酮的低水平耐药可能与 por 和 oor 两基因突变有关。Masaoka 等[21]发现呋喃唑酮耐药株 porD 基因有三个位点突变(G353A，A356G，和 C357T)，以 C357T 为常见，oorD 突变位点为 A041G，A122G，C349A(G)，首先在国内外用数据支持了 Kown 等的推测。

5 展望

Hp 是胃肠疾病的重要病原体，清除 *Hp* 对胃肠疾病的转归有重要作用。耐药基因突变检测若能成为 *Hp* 检查的常规检测，将会为临床与 *Hp* 相关性疾病的诊治提供依据。虽然目前国内外对 *Hp* 耐药性的研究取得了一定进展，但仍存在着耐药机制的不同观点，基于 *Hp* 耐药基因突变检测的分子生物学检测方法存在分子基础上的不足，因此今后的研究方向重点将是分子机制与耐药性之间的关系。

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(下转第 124 页)

(上接第 104 页)

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(上接第121页)

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