·综述·

NOTCH1突变与慢性淋巴细胞白血病Richter转化的关系研究进展

陈秀丽 王诗芬 许贞书

The relationship between NOTCH1 mutation and the Richter transformation in chronic lymphocytic leukemia

Chen Xiuli, Wang Shifen, Xu Zhenshu

Corresponding author:Xu Zhenshu, Fujian Institute of Hematology, Fujian Provincial Key Laboratory of Hematology, Fujian Medical University Union Hospital, Fuzhou 350001, China. Email: zhenshuxu@yahoo.com

慢性淋巴细胞白血病(CLL)是老年常见的惰性B淋巴细胞肿瘤。随着步入老年化社会,我国初诊CLL患者逐年增多,成为主要的老年血液肿瘤之一。以利妥昔单抗为基础的免疫化学治疗(如FCR方案)是治疗CLL的首选方案,治疗总反应率(ORR)达70%。但对于合并TP53突变、17p-等细胞遗传学异常的CLL患者,FCR方案治疗的ORR仅30%[1]。有些患者对治疗反应差,病情容易进展而转化成弥漫大B细胞淋巴瘤(DLBCL)等侵袭性淋巴瘤(即Richter转化)。发生Richter转化的患者预后差,传统FCR方案治疗后的中位生存时间<12个月,即使采用R-CHOP方案(DLBCL的一线治疗方案)治疗,中位生存时间也只有21个月,明显低于原发DLBCL[2]。FDA已经批准新药ibrutinib、idelalisib和venetoclax应用于临床,给CLL患者带来更多治疗选择。复发难治的CLL患者采用新药治疗,虽然ORR可达65%~85%,但是部分患者仍然出现疾病进展发生Richter转化,或因无法耐受新药的严重出血、心房纤颤等不良反应而终止治疗[3-5]。因此,Richter转化可能还存在其他潜在分子机制,CLL新的预后标志和靶向治疗方法成为研究热点。

一、CLL细胞Richter转化的分子机制

CLL虽然是惰性淋巴肿瘤,但约10.7%的患者发生Richter转化,转化为DLBCL占多数(90%),少部分(10%)转化为霍奇金淋巴瘤[6-8]。我们随访了210例初治CLL,发现有10%的患者出现Richter转化,均为DLBCL[9]。研究表明,IGHV4-39、IGHD6-13、IGHJ5基因偏向性使用频率增加是CLL发生Richter转化的高危因素,特别是独特型BCR以及IGHV4-39基因偏向性使用频率增加是转化的独立预后因素[10]。其他因素还有CD38、ZAP-70、CD49d的高表达和端粒的缩短等,以及del(11q22.3)、del(17p13)、del(15q21.3)、del(9p21)、add(2p25.3)等细胞遗传学改变[7,11-13]。此外,BCL-2、CD38、LRP4的基因多态性也与转化有关[12,14]。虽然转化的DLBCL和原发DLBCL在形态学、免疫表型方面相似,但前者具有特别之处。首先,CLL细胞Richter转化有不同模式。多数是线性模式转化,即转化克隆直接来自父辈的CLL克隆;少数是分支模式转化,转化的克隆和原来的CLL克隆来自共同的前体细胞[12]。其次,CLL容易出现Richter转化相关的染色体拷贝数改变事件。最常见的是17p的缺失,直接导致TP53失活,后者促使其他基因改变,包括体细胞突变和染色体重排。比如,发生转化的患者易检测到19p21和CDKN2A/B的双等位缺失,而且CDKN2A的缺失和TP53失活、MYC活化常常并存,这种现象和分支转化模式密切相关[12]。其他和转化相关的细胞遗传学改变有12三体、del(7q31.31-36.6)、del(8p)、del(14q23.2-q32.33),以及11、13、18、8q24/MYC的扩增[15-16]。我们曾报道了1例伴12三体染色体核型异常的CLL,患者在治疗期间很快出现疾病进展,转化为DLBCL[17]。再次,CLL的许多重现性基因突变也和Richter转化密切相关,包括TP53(肿瘤抑制基因)、NOTCH1和MYC(细胞增殖相关)、CDKN2A/B(细胞周期调控)等,转化时90%的重现性突变是发生在上述基因[12]。但有研究表明,原发DLBCL却很少见NOTCH1和TP53的基因突变,提示NOTCH1基因的突变是Richter转化的较特异的分子事件[18-20]。

二、NOTCH1突变参与CLL细胞Richter转化

NOTCH1基因位于9q34,编码的NOTCH1蛋白表达于细胞膜表面,是一种依赖配体激活的转录因子,对淋巴细胞分化与凋亡发挥重要影响[21-22]。研究表明,NOTCH1功能失调与T-ALL、CLL等淋巴肿瘤细胞的增殖、分化、凋亡过程密切相关[23-24]。随着二代测序技术的普及,NOTCH1突变与CLL Richter转化的关系越来越受到重视,成为近几年血液肿瘤领域的研究热点。首先,Richter转化时NOTCH1突变较频繁。国内Xia等[25]和国外Shedden等[26]采用二代测序结合拷贝数分析,发现CLL初诊时NOTCH1突变的发生频率为10%~20%。如果出现17p-、12三体等细胞遗传学改变时,NOTCH1突变频率会明显提高,特别是发生Richter转化或化疗耐药时,这种突变发生频率可达30%[27]。我们采用巢式PCR结合测序分析Richter转化患者的基因,发现

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作者单位:350001 福州,福建省血液病研究所,福建血液病学重点实验室,福建医科大学附属协和医院

通信作者:许贞书,Email:zhenshuxu@yahoo.com

NOTCH1的PEST区域容易发生突变，该突变表现为2 bp的框移缺失（△CT7544-7545, P2515fs）[17]。Lopez等[27]也证实，这种突变位于NOTCH1基因外显子34，可以引起NOTCH1分子的PEST区的C端截短。其次，Richter转化和NOTCH1突变之间存在密切联系。有学者将NOTCH1突变作为CLL预后不良的重要指标之一[20, 28-29]，因为诸多研究证明NOTCH1突变和细胞转化之间存在着因果关系。如NOTCH1突变可以促进活性成分ICN的释放和稳定，更强地抑制CLL细胞的凋亡[28]。我们的研究表明Richter转化是突变产生的ICN蛋白直接活化NF-κB通路的结果[30]，Baldoni等[31]的研究也支持这一观点。此外，突变型ICN也可以通过抑制蛋白酶系统依赖的降解，或与共抑制分子竞争性结合CSL等多种方式持续激活下游HES-1、DTX等癌基因而影响细胞功能[32-33]。然而，以上研究都局限于分析NOTCH1突变对下游信号通路或癌基因的影响。该分子突变是否还通过其他方式影响细胞功能呢？

三、NOTCH1突变通过调节趋化因子影响细胞转化

最近有学者发现NOTCH1突变可以通过调节DNMT3A影响抑癌基因DUSP22启动子的甲基化，从而激活趋化因子CCL19，促进CLL细胞归巢[34]。这项研究在阐明NOTCH1调控CLL细胞分泌细胞因子的功能方面做了有意义的探索，虽然仍有许多问题还没有答案。我们在近期研究中发现，NOTCH1突变患者的趋化因子CCL17的基因和蛋白表达明显高于未突变患者；通过构建NOTCH1突变质粒并进行转录组测序，我们发现突变型ICN可以上调许多基因，特别是与细胞趋化功能相关的基因，包括CCL17、CCL22，和临床标本检测结果相互印证；另外，我们还发现与野生型ICN相比，突变型ICN募集抑制性转录辅因子HDAC1/MTA2的能力明显减弱（待发表）。总之，野生型ICN可以在部分靶基因（如CCL17）上募集转录抑制复合物HDAC1/MTA2，使该靶基因低表达；但突变型ICN因缺失了PEST结构域，不能再募集HDAC1/MTA2，从而该靶基因得以恢复高表达。CCL17表达上调后，增强对Th细胞的趋化功能，后者持续刺激CLL细胞归巢、增殖、抗凋亡，导致细胞转化。

综上所述，CLL细胞发生Richter转化的原因和机制很复杂，深刻理解其中的机制有助于寻找CLL新的预后标志和靶向治疗方法。

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