

胆管癌的表现遗传学进展

唐思敏, 邓雪婷, 李全朋, 葛贤秀, 缪林*

南京医科大学第二附属医院消化医学中心, 南京医科大学消化内镜研究所, 江苏 南京 210011

[摘要] 胆管癌是起源于胆管上皮的恶性肿瘤, 预后较差。目前对胆管癌发生发展的具体分子机制并不是很清楚, 既往研究较多关注胆管癌遗传学层面的基因突变, 随着研究进展, 越来越多的研究发现, 胆管癌发生发展过程的复杂性并不仅仅是由基因组DNA改变导致的。DNA甲基化、组蛋白修饰、染色质重塑、非编码RNA的调控及基因印记等表现遗传学调控方式, 在胆管癌的发生发展中起着重要作用, 并受到了较多关注。文章就目前胆管癌的表现遗传学进展作一综述, 旨在揭示表现遗传学调控在胆管癌发生发展中的作用。

[关键词] 胆管癌; 表现遗传学; DNA甲基化; 组蛋白修饰; miRNA调控

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Advances in epigenetics of cholangiocarcinoma

Tang Simin, Deng Xueting, Li Quanpeng, Ge Xianxiu, Miao Lin*

Medical Center for Digestive Diseases, Department of Gastrointestinal Endoscopy, the Second Affiliated Hospital of NMU, Nanjing 210011, China

[Abstract] Cholangiocarcinoma is a malignant tumor originating from the bile duct epithelium with a poor prognosis. At present, the specific molecular mechanism of cholangiocarcinoma is not clear, and the previous studies have focused on gene mutations in the histological level of cholangiocarcinoma. However, more and more studies have found that the development of cholangiocarcinoma is not only caused by the changes of DNA. Epigenetic regulations, such as DNA methylation, histone modification, chromatin remodeling, non-coding RNA and genetic imprinting regulation also play an important role on development of cholangiocarcinoma, which receive more attention now. We summarize the current epigenetic progress of cholangiocarcinoma, and aims to reveal the role of epigenetic regulation in the development and progression of cholangiocarcinoma.

[Key words] cholangiocarcinoma; epigenetics; DNA methylation; histone modification; miRNA regulation

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胆管癌是继肝癌之后第二常见的肝胆系统肿瘤^[1]。胆管癌早期诊断困难, 发现时大多处于晚期。目前, 手术切除是胆管癌唯一有效的治疗方法, 但仅适用于少部分早期患者, 且术后复发率高, 5年生存率20%~30%。由于胆管癌具有较强侵袭性, 且缺乏有效的非手术治疗手段, 因此胆管癌患者预后较差^[2-3]。

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*通信作者(Corresponding author), E-mail: miaofrest@163.com

表现遗传学是指DNA序列不发生改变但基因或者蛋白表达发生变化, 主要调控机制包括: DNA甲基化、组蛋白修饰、染色质重塑、非编码RNA调控及基因印记等。Waddington^[4]在1939年首先提出了表现遗传改变的现象。胆管癌的早期研究大多关注遗传学层面, 近年来随着研究深入, 表现遗传学与胆管癌发病机制的关系越来越受到人们重视^[1]。本文就胆管癌在表现遗传学的研究进展作一综述。

1 流行病学现状及危险因素

近年来, 胆管癌在全球范围内的发病率显著增

高,泰国和亚洲其他一些地区是胆管癌的高发区,而西方国家胆管癌发生率则较低^[5]。胆管癌的发生与多种危险因素相关,目前公认的因素包括:肝吸虫病、原发性硬化性胆管炎、胆道囊肿、胆石症,这些疾病均存在慢性炎症及胆汁淤积^[6]。此外,肥胖、HBV感染、HCV感染、糖尿病等也是胆管癌发生发展的危险因素^[7-9]。

2 表观遗传学机制

2.1 DNA 甲基化

DNA 甲基化是最早发现的表观遗传修饰途径之一,也是目前发现的唯一DNA自然修饰方式。DNA甲基化有较多方面的生理和病理学意义,正常的DNA甲基化可以维持机体的正常生理功能,异常的DNA甲基化则有可能引起疾病甚至是恶性肿瘤的发生^[10]。

DNA 甲基化过程主要由DNA甲基转移酶(DNA methyltransferase, DNMT)催化^[11]。在人类, DNMT包括DNMT1、DNMT3a和DNMT3b 3种类型。早前文献报道,SOX17基因启动子区高甲基化抑制Wnt/ β 连环蛋白通路信号转导与胆管癌发生发展相关^[12]。最近有文献再次报道胆管癌患者SOX17启动子甲基化,并且SOX17表达与甲基化水平呈负相关。研究证实,胆管癌患者SOX17启动子区甲基化抑制基因表达现象与DNMT相关:与正常胆管细胞相比,胆管癌细胞系中DNMT1和DNMT3b表达升高,SOX17表达降低;SOX17表达水平降低,与胆管癌患者术后不良预后相关。降低DNMT1表达水平后,SOX17表达明显上升^[13]。

此外,由于DNA甲基化常发生在肿瘤早期阶段,所以甲基化相关生物标志物的发现可能有助于胆管癌的早期诊断。有文献报道,在对39例胆管癌组织及54例对照组织研究发现,生物标记物CNRI1、MAL、SPG20、FBN1、INA、SNCA、SEPT9、VIM及TMEFF2发生甲基化的敏感性及其特异性均较高,特异性为100%,其中FBN1、INA、SNCA、SEPT9、VIM敏感性在50%以上。研究者又对35例胆汁细胞刷片进行qMSP分析,发现13种启动子甲基化基因,其中CDO1、CNRI1、SEPT9和VIM甲基化率在50%~91%之间,32例胆管癌样本中有29例(91%)发生这4个基因的甲基化。此外,研究者分析得出这4个生物标志物组的敏感性达85%,特异性达98%,表明它们可能适用于恶性胆管癌的诊断^[14]。除此之外,Uhm等^[15]也指出,SFRP1、ID4和DLC-1基因启动子

区异常甲基化所致的转录失活可促进胆管癌的发生。

2.2 组蛋白水平修饰

除了常见的DNA甲基化修饰,蛋白质修饰及空间构象改变也可实现对基因的表达调控,目前基于蛋白质水平的表观遗传学研究主要是组蛋白修饰。组蛋白不仅是一种染色质结构蛋白,而且可作为基因表达的活性调节因子参与翻译后化学修饰。组蛋白修饰是指通过对特殊蛋白修饰或改变组蛋白构象,实现对基因表达的调控,包括组蛋白甲基化、乙酰化、磷酸化等,可影响染色质结构并产生染色质相关亲和力蛋白,调节基因表达^[16]。目前组蛋白水平对胆管癌的研究主要集中在乙酰化。

组蛋白乙酰化修饰主要由组蛋白乙酰转移酶(histone acetyltransferase, HAT)及组蛋白脱乙酰酶(histone deacetylase, HDAC)共同协调完成,其中组蛋白脱乙酰酶在细胞周期及肿瘤发生发展过程中起重要作用,可参与染色质重塑、基因表达、细胞周期分化和发育等过程^[17-18]。目前在人类中发现了18种HDAC,研究发现HDAC2、HDAC3和HDAC8在胆管癌组织中高表达。其中HDAC2和HDAC3的高表达与胆管癌患者的淋巴结转移、TNM分期及肿瘤分化相关,可促进肿瘤进展并导致临床不良预后。这些结果表明,HDAC2和HDAC3可作为胆管癌预后重要的生物标志物,并且组蛋白乙酰化调节可能成为治疗胆管癌的新策略^[19]。也有文献报道,HDAC1表达上调能显著促进肝内胆管癌的恶性进展。HDAC1可能通过激活缺氧诱导因子-1 α (hypoxia inducible factor-1, HIF-1 α)调控肿瘤干细胞生成,进而在肿瘤血管生成、肿瘤生长和转移中起重要作用^[18]。随着对HDAC的研究深入,组蛋白脱乙酰酶抑制剂(histone deacetylase inhibitor, HDACI)的研究也越来越多,HDACI曲古抑菌素A(trichostatin A, TSA)和辛二酰苯胺异羟肟酸(suberoylanilide hydroxamic acid, SAHA)被证实在胆管癌细胞系中可以抑制细胞增殖、促进细胞凋亡、诱导细胞G2/M周期停滞并抑制上皮间质转化^[19]。其中,SAHA已经被批准用于治疗T细胞皮肤淋巴瘤^[20]。随着相关研究的深入,未来HDACI也将可能被用于胆管癌的治疗。

2.3 非编码RNA的调控

非编码RNA是指不编码蛋白质的RNA,包括rRNA、tRNA、microRNA及lncRNA等。非编码RNA调控是指RNA通过某些机制实现对基因转录及转录后的调控。在胆管癌发生发展过程中,microRNA

(miRNA)参与表观遗传学调控的研究较多。miRNA是一类包含18~23个核苷酸的内源性非编码RNA,主要通过直接结合mRNA 3'非翻译区(UTR)在转录后调节基因表达^[21]。miRNA可参与细胞扩增、分化、转移、侵袭及凋亡等生物学进展^[22]。越来越多的研究发现,miRNA与表观遗传学存在密切关联,约50%编码miRNA的基因由DNA甲基化直接控制,另外,miRNA基因甲基化改变也与人类肿瘤发展相关^[23]。

有文献报道,miR-373在胆管癌患者中表达下调,甲基CpG结合域蛋白MBD2在胆管癌患者中表达上调。MBD2富集于miR-373启动子相关CpG岛,通过甲基化相关通路的调节使胆管癌患者miR-373表达下降。下调的miR-373与胆管癌的不良分型、较高的临床分期以及较低的生存率相关^[24-25]。Braconi等^[26]发现,在IL-6过表达的胆管癌细胞中,IL-6可通过抑制miR-148a和miR-152的表达,导致DNMT1水平上调、抑癌基因表达下调,从而促进胆管癌的发展。除此之外,还有很多在胆管癌中表达下调的miRNA,如miR-122、miR-145、miR-150、miR-638等^[27-28],他们也可参与胆管癌的发生发展。

除了低表达的miRNA,也存在许多高表达的miRNA与胆管癌的发生发展相关,如miR-21、miR-31、miR-191、miR-93、miR-425等^[27-29]。其中,miR-191可抑制肿瘤细胞凋亡,促进肿瘤细胞扩增、侵袭和转移,并诱导上皮间质转化的发生。miR-191通过抑制TET1的表达,减少TET1与p53基因CpG岛的结合,从而抑制抑癌基因p53的表达,促进肿瘤发生。此外,miR-191高表达与胆管癌患者的不良预后相关,是胆管癌发生的独立危险因素^[29]。除此之外,Wang等^[30]证实miRNA-21可调控胆管癌细胞扩增和肿瘤生长,高表达miRNA-21的胆管癌患者临床预后较差。研究者认为miRNA-21可能成为胆管癌诊断和预后的分子标志物及潜在治疗靶点。

2.4 染色质重塑

染色质重塑是表观遗传学又一重要的调节机制,是目前治疗多种肿瘤的主要治疗策略,是指在基因表达和重组过程中染色质结构发生一系列重要改变,包括染色质的包装状态、核小体中组蛋白及对应DNA分子的变化^[31]。共价组蛋白修饰复合物和ATP依赖性染色质重塑复合物共同参与染色质重塑的过程,其中ATP依赖性染色质重塑复合物分为4类:SWI/SNF、ISWI、CHD和INO80,而SWI/SNF复合体及其介导的核小体染色质重塑是近年来肿

瘤研究的焦点^[32]。随着研究的深入,染色质重塑与胆管癌之间的联系也逐渐被揭示。有报道指出,SWI/SNF复合体成员ARID1A、PBRM1在胆管癌中表达缺失,Sasaki等^[33]发现ARID1A在胆管癌组织中表达缺失,并且其失活突变可能参与胆道肿瘤发生的过程。另一项研究表明PBRM1突变以及由此导致的表达缺失是胆道癌变过程中的晚期事件,尽管其对浸润性肝内胆管癌的预后意义证据不足,但对染色质重塑基因在肿瘤发生过程中的基本机制提供了重要见解^[34]。

3 总结与展望

胆管癌的早期诊断对其防治具有重要意义,而表观遗传学层次的DNA甲基化、组蛋白修饰、非编码RNA的调控及染色质重塑等过程及相关分子标志物的改变,对胆管癌的早期诊断及预后评价等具有重要意义。此外,表观遗传学的修饰过程是可逆的,运用特定药物去调节表观遗传学的修饰,个体化靶向治疗,可能成为胆管癌治疗的新方向。

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