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胶质瘤中的表观遗传学:一场基因革命

祁 婧[1],闵 彬[2],涂艳阳[1]
([1]空军军医大学唐都医院实验外科,陕西 西安 710038;[2]空军工程大学门诊部,陕西 西安 710051)

Epigenetics in glioma: a genetic revolution

QI Jing[1], *MIN Bin*[2], *TU Yan-Yang*[1]
[1]Department of Experimental Surgery, Tangdu Hospital, Air Force Military Medical University, Xi'an 710038, China; [2]Outpatient Department of Air Force Engineering University, Xi'an 710051, China

【**Abstract**】The ever-changing epigenetic phenomenon and its role in the process of genome and tumor progression are one of the emerging areas of cancer research. In humans, the most common primary brain tumor gliomas, especially the most malignant glioblastoma, abnormal DNA methylation, histone methylation and mRNA expression will fundamentally subvert our heterogeneous understanding of tumor. In this paper, the value of glioma epigenetics in the diagnosis and prognosis of glioma is discussed, which provides a theoretical basis for understanding the molecular mechanism of glioma development and finding new and more effective drug targets.

【**Keywords**】glioma; epigenetics; methylation; target

【**摘 要**】一直以来异常的表观遗传学现象以及其在基因组和肿瘤进程、预后中的角色都是癌症研究的新兴领域之一. 在人类最常见的原发性脑瘤胶质瘤中,特别是高度恶性的胶质母细胞瘤,异常的 DNA 甲基化、组蛋白甲基化以及 mRNA 表达都会从根本上颠覆我们对于异质性肿瘤的认识。本文针对胶质瘤表观遗传学在胶质瘤诊断和预后中的价值进行探讨,为理解胶质瘤发生发展的分子机制以及寻找新的更有效的药物靶点提供理论基础。

【**关键词**】胶质瘤;表观遗传学;甲基化;靶点
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0 引言

神经胶质瘤是渗透性的脑瘤,最常见的是星形细胞瘤和少突神经胶质瘤,被分为低级别(WHO 等级Ⅰ和Ⅱ)和高级别肿瘤(WHO 等级Ⅲ和Ⅳ)。胶质母细胞瘤(Ⅳ级星形细胞肿瘤)是最致命且最具有破坏性的神经胶质瘤。尽管经过几十年的研究,胶质母细胞瘤和高级别神经胶质瘤的预后仍然很差。这强调了阐明肿瘤发病机制的重要性。近年来在胶质瘤分子遗传学方面取得了许多新进展,并已应用于胶质瘤的分型中,其中许多基因和分子改变可导致细胞代谢的显著变化。

传统分子靶向治疗方法主要集中在诸如点突变、基因缺失和重排等基因的结构变化,这些改变参与了胶质瘤的发生及演进,对其诊断、治疗及预后判断也具有重要的作用。例如,胶质瘤中出现的各种基因改变(如 EGFR 扩增、PTEN 损失、PDGFRA 扩增)可导致受体酪氨酸激酶信号增强和 PI3K/AKT 通路的失调,从而刺激葡萄糖摄取和有氧糖酵解。在Ⅱ级和Ⅲ级星形细胞瘤、少突神经胶质瘤和胶质母细胞瘤中70%都存在 NADP+依赖性酶异柠檬酸脱氢酶 1(socitrate dehydrogenase 1, IDH1)的突变。IDH1 催化氧化胞质内异柠檬酸脱羧生成 α-酮戊二酸(α-KG)。IDH1 突变改变了细胞代谢,其代谢产物 2-羟戊二酸(2-HG)的积累会在肿瘤的发生发展中发挥作用.

转录/翻译水平上的基因表达的调控机制是癌症研究中最新兴的领域。最近的研究[8-9]表明恶性转化是由遗传变异和表观遗传学改变的复杂相互作用所致的,从而影响各种细胞生物学过程的变化,包括细胞增殖和侵袭、DNA 修复、凋亡、血管生成和细胞周期调控,最终导致肿瘤形成。

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作者简介:祁 婧。硕士。E-mail:qj15034774786@163.com
通讯作者:涂艳阳。博士,副教授,副主任医师。E-mail:tu.fmmu@gmail.com

表观遗传学的现象很多,已知的有 DNA 甲基化、基因组印记、母体效应、基因沉默、核仁显性、休眠转座子激活以及 RNA 编辑等。DNA 甲基化是恶性胶质瘤中研究最广泛的表观遗传现象。组蛋白是核小体形成过程中所必需的蛋白质。每一个核小体由约 147 个 DNA 碱基对缠绕在组蛋白八聚体上,组蛋白八聚体由 H2A、H2B、H3 和 H4 组成。组蛋白氨基酸的尾巴可以接受各种翻译后修饰如乙酰化、甲基化、磷酸化、泛素化和精氨酸(R)和赖氨酸(K)残基类泛素化。组蛋白赖氨酸残基的乙酰化作用通常激活转录,而甲基化(H3K9、H3K27、H4K20、H3K4 甲基化)可以激活或抑制转录相关因子。

1 DNA 甲基化

研究最多的表观遗传修饰是胞嘧啶甲基化,在哺乳动物细胞中,DNA 甲基化主要发生在胞嘧啶残基上,其次是鸟嘌呤。DNA 甲基化是由 DNMT(DNA 甲基转移酶)家族的催化转移 S-腺苷甲硫氨酸的甲基到 DNA 上。到目前为止,五个 DNMT 家族成员已被确定,分别为 DNMT1、DNMT2、DNMT3a、DNMT3b 和 DNMT3L。DNA 甲基化与转录活性相关。众所周知,在人类的癌症中 DNA 甲基化谱在肿瘤发生和进展中扮演着重要角色[15-16]。

人神经胶质瘤表现出甲基化模式的肿瘤典型变化[17-20]。低甲基化主要发生在 DNA 重复区域,并可通过激活致癌基因和增加基因组不稳定性来促进肿瘤生长。高甲基化主要发生在基因启动子 CpG 岛上,参与肿瘤形成和进展的过程,这些基因大都与肿瘤抑制[21-22]、DNA 修复[23]、细胞周期调控[24]、凋亡[25-26]、侵袭[27-28]和迁移[29]相关(表 1)。有趣的是,胶质瘤分级不同甲基化模式也不同,在胶质瘤 WHO 的Ⅱ级,Ⅲ级和Ⅳ级之间基因的甲基化的状态也呈现出明显差异[30]。

在癌症基因组图谱(the cancer genome atlas, TCGA)项目的框架内,Noushmehr 等[6]研究甲基化谱分析确定了 GBM 肿瘤表型,其特征在于大量基因位点的协同高甲基化,被称为 G-CIMP。G-CIMP 与延长生存期以及基因表达谱(突变表达模式)有关[31]。此外,发现 IDH 突变导致酶活性的变化,α-KG 产生减少,产生代谢物 2-羟基戊二酸(2-HG),其竞争性地抑制调节 DNA 和组蛋白甲基化的酶的活性(α-KG 依赖性双加氧酶),包括组蛋白脱甲基酶[32]和 TET5mC 羟化酶家族[33-35]。TET 蛋白能通过转化 5-甲基胞嘧啶(5mC)至 5-羟甲基胞嘧啶(5hmC)改变 DNA 甲基化状态。5hmC 的生物学功能尚未得到确凿的阐明。与正常脑相比,在人类胶质瘤中,5hmC 显著减少,并且已经有研究显示 5hmC 水平与细胞增殖之间呈现反比关系[36-37]。这些发现揭示了基因调节的另一种水平,并证明与胶质瘤发生中遗传和表观遗传密切相关[38-39]。

表 1 人类胶质瘤中主要的表观遗传改变

DNA 甲基化	
基因	功能
GATA4、NDRG2	肿瘤抑制
MGMT	DNA 修复
p14ARF	调节细胞周期
TMS1/ASC、WWOX	细胞凋亡
SOCS3、PCDH-gamma-A11	侵袭
Sox2	迁移
组蛋白修饰	
组蛋白脱乙酰基酶	(HDAC1、HDAC2、HDAC3、HDAC9)
组蛋白去甲基化酶	(JMJD1A、JMJD1B)
组蛋白甲基转移酶	(SET7、SETD7、MLL、MLL3、MLL4)
Micro-RNA	
下调 miRNAs	
miR-34a	c-Met、Notch
miR-146a	Notch
miR-7	EGFR
miR-128	Bmi-1
miR-195	E2F3、CCND3
上调 miRNAs	
miR-21	RECK、TIMP3
miR-26a	pTEN、RB1
miR-10b	Cell-cycle inhibitor
miR-30e	IjBa
miR-221/222	p27Kip1、PTPl、PUMA

在过去十年中,关于 DNA 甲基化过程的研究发现了许多肿瘤重要的生物标志物。O6-甲基鸟嘌呤-DNA 甲基转移酶(methylguanine methyl transferase, MGMT)是一种 DNA 修复酶,其去除鸟嘌呤 O6 位置的烷基加合物,从而保护正常细胞免受致癌物质的侵害,相反的是,MGMT 也可以来保护接受化疗的肿瘤细胞。MGMT 表达可以通过启动子甲基化进行表观遗传沉默,这种情况出现在 35%~45%的恶性胶质瘤和 80%的 WHO Ⅱ级胶质瘤中[40-41]。启动子甲基化状态已被确定为恶性胶质瘤患者进行烷基化剂化疗中治疗效果明显且独立的预测因子。通常与未甲基化的 MGMT 启动子相比,存在 MGMT 启动子甲基化形式的患者用替莫唑胺治疗效果更加显著[42-43]。这说明 MGMT 启动子甲基化状态已被确定为神经肿瘤

学的重要临床标志物。然而不是所有 MGMT 启动子甲基化的患者在替莫唑胺治疗后均有显著的治疗效果。在这些患者中,已经发现 MGMT 启动子甲基化和 MGMT 的 mRNA 表达不一致,不论 MGMT 启动子甲基化或是未甲基化,在 25%的胶质母细胞瘤中检测到 MGMT 的 mRNA 表达异常;携带低转录活性的 MGMT 患者具有更好的治疗效果,这一结果与 MGMT 启动子甲基化结果正好相反,这种不一致的基本机制尚不清楚。我们假设,MGMT 低表达水平与未甲基化的启动子组合的情况可能由转录物不稳定和/或转录抑制因子如 miRNA 调节或组蛋白修饰引起[44]。

2 组蛋白修饰

染色质是细胞核中 DNA 和组蛋白的缩合形式。在真核生物中,染色质由 147 个碱基对的 DNA 组成,紧紧缠绕在两个拷贝的四个核心组蛋白 H2A,H2B,H3 和 H4 八聚体周围的。

所得到的核小体是染色质的基础重复单元[45]。由于每个核心组蛋白具有从核小体突出的氨基末端“尾”,组蛋白,特别是其尾巴可能存在一定数量的翻译后修饰。组蛋白修饰包括乙酰化、甲基化和磷酸化,但也存在研究较少的修饰,如泛素化、ADP 核糖基化、脱氨基和脯氨酸异构化[46]。这些组蛋白修饰中的每一种都能够影响染色质结构,从而导致 DNA 修复以及基因转录的改变。组蛋白修饰可以广泛地分为主动标记和被动标记。特别是组蛋白乙酰化和甲基化在致癌机制中发挥显著作用[15,47]。

赖氨酸残基的乙酰化由组蛋白乙酰转移酶(HATs)和组蛋白脱乙酰酶(HDAC)的相反作用调节。乙酰化中和赖氨酸残基的正电荷,从而削弱 DNA 和组蛋白尾部之间的键。因此,组蛋白乙酰化与转录激活相关,而脱乙酰化通常与抑制转录有关。组蛋白甲基化主要发生在赖氨酸和精氨酸的侧链上,其影响转录机制的效应蛋白的活性。组蛋白甲基化可以激活(例如 H3K4me2、H3K4me3)或抑制(H3K9me2,H3K27me3)转录,这取决于各自的甲基化位点[48-50]。

组蛋白表达水平的改变也可能在胶质瘤发生中发挥作用。这些改变包括参与组蛋白修饰的基因的异常表达以及各基因的组蛋白修饰模式的变化(表 1)。组蛋白水平的畸变来源于调节基因突变,如 GBM(包括 HDAC2 和 HDAC9),组蛋白去甲基化酶(JMJD1A 和 JMJD1B),组蛋白甲基转移酶(SET7、SETD7、MLL、MLL3 和 MLL4)[5]。此外,HDAC 的表达水平的改变已被报道与肿瘤复发和进展相关(HDAC1、HDAC2 和 HDAC3)[51-52]。在几项研究中已经报道了组蛋白修饰调节单个基因。例如,抑制肿瘤抑制因子 RRP22 和细胞周期调节因子 p21 的表达以及促增殖转录因子 HOXA9 的增强表达与组蛋白修饰模式的改变相关[53-55]。但是,组蛋白修饰实际功能在胶质瘤中的作用及其作为生物标志物和/或治疗靶标的潜力仍有待充分阐明。

3 MicroRNAs

近来已发现非编码 RNA 在基因表达的表观遗传调控中起重要作用[56-57]。其中 miRNA 是约 22 个核苷酸(nt)长度的双链 RNA 分子,起源于人类基因组转录物前体。通过结合目标 mRNA 的 3’-UTR 内的特异性识别序列,抑制翻译或 mRNA 降解调节基因表达[58-59]。目标识别主要通过 miRNA 的 5’区域的 8 个核苷酸短序列的碱基配对进行介导[60]。虽然一些 miRNA 调节特定的目标,但是来自多个研究的证据表明某些关键的 miRNA 可以调节高达几百个靶基因,并且许多类型的 miRNA 协同调节其靶标[61-62]。研究人员使用计算预测方法和不同测序技术相结合等技术手段,已经确定了大量的调节分子[63-64]。目前发布的 mirbase 数据库,存在超过 140 种物种中含有的超过 17,000 个成熟 miRNA 序列[65]。最近的研究[66]表明大部分的转录组受到 miRNAs 调控。这些结论说明 miRNA 表达的调节异常与病理学特征及其预后相关联。

已经在许多类型的人类肿瘤中检测出 miRNA 的异常表达,包括神经胶质瘤[67-68]。然而,miRNA 不仅仅作为肿瘤抑制因子起作用,而且还依赖靶向 mRNA 的功能作为一种癌基因[69-71]。因此,改变的 miRNA 表达水平会对致癌过程产生重大影响。与正常细胞相比,miRNA 在恶性肿瘤中的差异表达的原因尚未完全阐明。然而,miRNA 的转录调控序列中的表观遗传修饰以及基因突变,基因组缺失或基因扩增等遗传改变可能影响 miRNA 成熟和/或与 mRNA 靶标相互作用[72-74]。

在 GBM 中,高通量分析已经确定了 miRNA 的差异表达[75-77]。因此,miRNA 被认为是 GBM 多重生物学特征的重要介质,包括细胞增殖、G1/S 细胞周期进程、细胞存活、细胞迁移和细胞侵袭[78]。尽管尚未阐明神经胶质瘤复杂网络中 miRNA 的确切功能,但越来越多的研究集中于 miRNA 在神经胶质瘤发生和进展过程中的不同功能(表 1)。例如,与正常脑相比,在神经胶质瘤中下调的 miRNA 已经被发现通过直接靶向致癌基因 c-Met、Notch[79-80]、Bmi-1[76]、表皮生长因子受体[81]、受体酪氨酸激酶[82]和细胞周期成分[83]

发挥抑癌作用。相反,在胶质瘤中具有高表达的miRNA可能被确定为致癌基因,例如miR-21通过靶向基质金属蛋白酶的调节剂,miR-26a靶向PTEN和miR-10b靶向细胞周期抑制剂[84-90]。

4 表观遗传网络

表观遗传调节途径通过相互作用形成复杂的调控网络:①miRNA本身的表达可以通过由组蛋白和/或DNA甲基化的共价修饰引起的染色质结构的变化来修饰[91-92]。②肿瘤可以相互利用miRNA来靶向表观遗传。例如,发现miR-29b在急性骨髓性白血病中靶向DNMT3a和3b,miR-449a控制前列腺癌细胞中的HDAC1。在人神经胶质瘤中,miR-185最近被证实作为DNMT1的调节因子,其过表达导致整体DNA低甲基化[93-95]。此外,已经发现miR-101靶向组蛋白甲基转移酶EZH2且在人类GBM中下调,从而促进肿瘤生长[96-97]。③组氨酸修饰酶可能被CpG高甲基化沉默。例如,NSD1基因编码参与染色质调节的组蛋白甲基转移酶,其沉默导致组蛋白残基H4K20和H3K36的甲基化减少,同时又导致致癌基因MEIS1的活化[98]。我们需要在全基因组范围进一步研究,以充分阐明神经胶质瘤中的表观遗传模式,这将为阐明胶质瘤等高度异质性肿瘤的表观遗传学网络的复杂模式提供理论依据[99]。

5 结论与展望

分子生物标志物的研究进展改变了目前在世界卫生组织分类框架内的诊断精确度,同时有利于揭示相同WHO级别但是不同预后和治疗反应的神经胶质瘤亚群之间的差异[2]。1p/19共缺失检测与化疗和/或放疗后的预后结果相关[100-101]。筛查IDH1/2突变有助于区分来自弥漫性星形细胞瘤的WHOⅠ级毛细胞星形细胞瘤和室管膜瘤(不含IDH突变)。IDH突变与间变性星形细胞瘤和胶质母细胞瘤的良好预后相关[102-103]。鉴于在实际病例中Ⅱ~Ⅳ级神经胶质瘤难以进行完全肿瘤切除,不完全切除术通常不能获得相应的预后效果,微创分子表征策略的发展受到越来越多的关注[104]。最近,组织病理学诊断与新颖的分子立体定向活检程序相结合已经在DNA和RNA水平上实现高度可重复和有效的结果;MGMT启动子甲基化,MGMT mRNA表达以及TP53突变状态和1p/19q-状态的信息可以从1 mm^3的立体定向组织样品中精确定义的位点收集[105-106]。

引入大规模的“二代”测序(next-generation sequencing, NGS)技术手段标志着基因组研究革命的开始[107]。目前,全基因组、转录组和甲基化测序在很小的肿瘤组织样本中也可以进行研究。NGS平台提供了对基因组和表观基因组更加全面的理论支持,并且基于NGS的数据的整合对于了解和鉴定神经胶质瘤的发生和发展的细胞内途径的致密网络是至关重要的[108]。随着NGS技术的成本下降和生物信息数据处理的改善,对于同一生物样品的转录组和遗传变异相结合的多个表观遗传修饰的基因组测序可能成为未来的临床研究方法。在这种情况下,通过微创方法(如分子立体定向程序)收集肿瘤样本将是特别有价值的。从这种方法获得的信息可以为每名患者创建个性化的治疗方案。将胶质瘤中研究发现的表观遗传学现象应用到胶质瘤的早期诊断,高危人群的监测,肿瘤风险评估,判断肿瘤复发情况,预测肿瘤疗效和预后,开发特异新靶点药物等方面具有很大的潜力,相信随着检测手段和实验方法的日臻完善,胶质瘤预防、诊断和治疗等领域必将取得喜人的成果。

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