DOI: 10.11844/cjcb.2013.06.0280

细胞分裂周期蛋白6: 新的抗肿瘤靶点

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摘要 真核细胞中细胞分裂周期蛋白6(cell division cycle 6, Cdc6)是起始细胞DNA复制的必需蛋白, 其主要功能是参与组装完成“复制前复合体(pre-replication complexes, pre-RC)”。近期的研究发现, Cdc6在调控细胞有丝分裂方面也具有重要作用。Cdc6介导基因组异常情况下ATR监测点信号的活化, 从而阻止有丝分裂的提前进入。Cdc6还可抑制有丝分裂期CDK1的活性, 促进有丝分裂滑脱。因此, Cdc6必然与细胞的恶性增殖及恶性转化密切相关, 抑制Cdc6将会产生有效的抗肿瘤作用。该文总结了近年来的研究成果, 阐述了Cdc6在肿瘤发生发展中的作用, 重点讨论了Cdc6在调控有丝分裂中的作用, 以及抑制Cdc6在抗肿瘤中的作用。

关键词 Cdc6; DNA复制; ATR监测点; 有丝分裂滑脱

Cell Cycle Division 6: New Anticancer Target

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Abstract Cell division cycle 6 (Cdc6) is an essential regulator of DNA replication in eukaryotic cells. Its best-characterized function is the assembly of pre-replication complexes (pre-RC). Recent studies have shown that Cdc6 also participates in the regulation of mitosis. Cdc6 can inhibit pre-mature mitotic entry under replication stress by mediating the ATR checkpoint activation. In addition, Cdc6 can promote mitotic slippage by inhibiting mitotic CDK1 activity. Therefore, as a tumor-highly-related protein, Cdc6 promotes cell proliferation and malignant transformation. Cdc6 function disturbance could elicit potent anticancer effects. In this review, we summarized the relations of Cdc6 function with tumor malignant progress, emphasizing on the mitosis regulation of Cdc6 and the possible anticancer activity by Cdc6 targeting.

Key words Cdc6; DNA replication; ATR checkpoint; mitotic slippage

Cdc6最初作为一个在酿酒酵母中起始S期所必需的基因被发现。随后的研究表明, Cdc6在真核生物中高度保守, 人的*cdc6*基因位于17q21.3[1]。Cdc6的主要作用是参与构成“复制前复合体(pre-replication complexes, pre-RC)”。真核细胞DNA复制需要在DNA起始位点(origin)依次结合组装Orc、Cdc6、Cdt1和Mcm2-7, 这些蛋白一起被称为pre-RC。pre-RC组装完成后, 细胞便具有了起始DNA复制的能力。Cdc6是组成pre-RC的重要分子, 如果Cdc6功能被抑制, 细胞将不能完成pre-RC的组装, DNA复制也将受到抑制[2]。近年来的研究表明, Cdc6并不只是一个单纯的DNA复制起始蛋白。除了起始DNA复制以外, Cdc6还参与对S-M监测点及有丝分裂的调控, 通过多条途径影响细胞分裂增殖, 并参与细胞的恶性转化。本文将总结相关领域的研究结果, 归纳

收稿日期: 2012-12-06 接受日期: 2013-03-06
国家自然科学基金(批准号: 30901822、81272482)资助的课题
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Received: December 6, 2012 Accepted: March 6, 2013
This work was supported by the National Natural Science Foundation of China (Grant No.30901822, 81272482)
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网络出版时间: 2013-05-16 15:31
URL: http://www.cnki.net/kcms/detail/31.2035.Q.20130516.1531.002.html

Cdc6蛋白的功能, 阐述Cdc6蛋白与肿瘤发生发展的关系以及在肿瘤治疗中的作用。

1 Cdc6起始DNA复制

Cdc6首先被发现可调控DNA复制。利用酵母“条件性敲除(KO)”及温度敏感突变株研究发现, 清除Cdc6后DNA复制受到抑制, 细胞停滞在G_1/S, 不能进入S期[3-4]。随后多项研究证实, Cdc6是组成pre-RC的重要蛋白, 其作用主要是介导Mcm蛋白的染色质组装[5-6]。人Cdc6也被证实是DNA复制的必需蛋白。血清饥饿的成纤维细胞中, Cdc6的mRNA及蛋白表达均受到抑制; 加入血清后细胞细胞重新进入增殖周期, 此时Cdc6的表达相应增高[1]。在G_1期细胞中显微注射Cdc6抗体, 可阻止细胞进入S期[7-8]。与酵母细胞一样, 在人细胞中Cdc6也是介导Mcm蛋白的染色质组装。Cook等[2]的研究证实, Cdc6可通过其ATPase结构域介导Mcm蛋白组装到染色质, 且此过程不需要CDK活性。

2 Cdc6参与调控细胞有丝分裂

研究发现, 抑制Cdc6不但可有效地抑制DNA复制[9-12], 还可以导致细胞出现凋亡改变[11,13-15]。仅从pre-RC组装的角度不能完全解释抑制Cdc6后凋亡的出现。另外, 细胞进入S后, 依然有Cdc6结合于染色质上[16], 在M期还有部分Cdc6结合于纺锤体上[17]。而且, 在S期和M期细胞中Cdc6通过不同的途径被降解[18]。这些结果都提示, 在DNA复制起始之后Cdc6仍发挥作用; 除了在G_1期参与DNA复制之外, Cdc6还参与S、M期的细胞事件。

2.1 Cdc6抑制细胞进入有丝分裂

最初人们发现, 在酵母中清除G_1期细胞Cdc6会抑制DNA复制, 但随后这些细胞不会停滞于M期, 而是在染色体未复制的情况下依然分裂, 细胞出现减数分裂[4]。而Cdc6-K114E突变株(Cdc6的Walker A基序的突变, 不能起始DNA复制)却不能进入有丝分裂, 细胞阻滞于M期[19]。这说明Cdc6在除起始DNA复制以外, 还参与调控细胞进入M期, 而且是由Cdc6的不同蛋白位点发挥作用。随后的研究表明, 过表达不被泛素酶降解的Cdc6[20], 或在G_2期细胞中外源性高表达野生型Cdc6, 会阻止细胞进入有丝分裂[3]。这种Cdc6介导的M期阻滞依赖于wee1/mik1(抑制有丝分裂)激酶, 并受到Cdc25/MIH(诱导有丝分裂)磷酸酶的影响, 提示Cdc6可通过wee1/mik1间接抑制M期p34Cdc2/Cdc28激酶[3]。在哺乳动物包括人的细胞中也有类似报道。在S期细胞中清除Cdc6会使DNA复制减慢, 但此时Chk1不能被有效激活, 导致仍在进行DNA复制的细胞提前进入有丝分裂[9]。而Cdc6的过量表达可阻止细胞从G_2期到M期的过渡, 此过程中有Chk1的激活以及对CDK1的抑制[21]。

随后多项研究证实, Cdc6通过促进ATR信号阻止细胞进入有丝分裂。ATR在细胞中主要负责应对DNA复制压力。当DNA复制受阻时会产生单链DNA, 此时ATR与ATR结合蛋白(ATR-interacting protein, ATRIP)形成复合体, 共同结合于受损DNA处, 进一步激活Chk1, 抑制细胞进入有丝分裂[22-23]。在酵母细胞中, 羟基脲作用后, Cdc6与Rad3(酵母中的ATR同源蛋白)、Rad26(酵母中的ATRIP同源蛋白)共同结合于染色质上, 清除Cdc6蛋白会抑制Rad3和Rad26与染色质的结合, 细胞不能实现周期阻滞。进一步的研究证实, Rad26蛋白可通过直接与Cdc6蛋白结合, 从而将Rad3招募到染色质[24]。在人细胞中的研究也发现, Cdc6蛋白可与ATR-ATRIP结合; Cdc6 siRNA会抑制ATR通路的活化[25]。

以上结果说明, 在S期阻滞细胞Cdc6位于ATR信号上游, 可发挥类似受体的功能, 促进ATR-ATRIP与染色质的结合, 活化ATR-Chk1监测点, 抑制细胞进入有丝分裂[26]。这一作用可保证细胞在DNA未复制完全时不进入有丝分裂, 避免发生异常有丝分裂。但也有研究发现Cdc6位于ATR信号下游, 受到ATR的调节。在羟基脲诱导的S期细胞中, 通过RNAi抑制ATR, 可以降低染色质结合的Cdc6蛋白水平, 而且ATR可以直接磷酸化Cdc6[27]。在缺氧状态下, ATR可通过抑制Cdc25a进一步下调CDK2, 促进Cdc6的降解。此时细胞新生DNA减少, 说明DNA复制起始受到抑制[28]。这些结果提示, Cdc6与ATR参与组成了一个相互影响的复杂网络, 共同调控细胞在应激状态下的反应。

2.2 Cdc6可促进细胞退出有丝分裂

退出有丝分裂需要M期CDK1的失活。在酵母中细胞通过Sic1(CDK抑制蛋白)或cyclinB/Clb2的降解来失活CDK1。研究显示, Cdc6蛋白具有与Sic1类似的功能, 可通过抑制CDK1-Clb2促进有丝分裂退出, 在有丝分裂后期参与CDK的失活, 促进细胞退出有丝分裂[29-30]。哺乳动物细胞中, Cdc6也被证实参

与调控有丝分裂的退出。研究表明, 有丝分裂后期(anaphase)中plk1(polo-like kinase1)与Cdc6共同定位于纺锤体。plk1可介导Cdc6磷酸化(T37)。野生型Cdc6可与CDK1结合而Cdc6-TV(Cdc6的T37V突变)不能结合CDK1。野生型Cdc6与Cdc6-TV相比, 细胞CDK1活性降低, Separase活性增强。这些结果说明, plk1可磷酸化Cdc6, 磷酸化后的Cdc6与Cdk1结合并抑制Cdk1活性, 进一步释放Separase, 导致细胞进入有丝分裂后期[31-32]。

有丝分裂期是细胞最"脆弱"的时期, 退出有丝分裂是一种细胞逃避死亡的方式。众所周知, 细胞发生异常有丝分裂后, 纺锤体组装检测点(spindle assembly checkpoint, SAC)活化, 使细胞阻滞于M期(主要是有丝分裂中期), 并启动死亡机制清除细胞。但是, 细胞异常有丝分裂后的归宿并不一定就是死亡。最近一些研究发现, 细胞出现异常有丝分裂时仍可通过"有丝分裂滑脱(mitotic slippage)"的方式来逃避死亡: 细胞在SAC活化的情况下, 实现染色体分离, 退出有丝分裂而进入间期(interphase)。有研究证实, 通过RNAi抑制APC/cdc20阻止细胞退出有丝分裂可有效地杀伤肿瘤细胞[33]。有丝分裂滑脱的实质就是有丝分裂的提前退出, 而M期CDK1失活是细胞退出有丝分裂的必要条件。对于已发生异常有丝分裂的细胞来说, 死亡和滑脱可被看作是两个相互竞争的细胞归宿[34], 竞争的焦点就是M期(有丝分裂中期)阻滞时间的长短[35], 而对M期CDK1的调节在这一竞争的关键[36-37]。因此, Cdc6通过失活CDK1促进有丝分裂退出, 一方面可为随后pre-RC的组装创造条件[30], 另一方面在细胞处于异常有丝分裂时, 可促进有丝分裂滑脱, 有助于细胞逃避死亡。Cdc6通过抑制进入和促进退出有丝分裂, 可避免细胞停留在有丝分裂期, 以促进细胞在应激状态下的存活。

3 Cdc6与肿瘤

3.1 Cdc6 在肿瘤细胞中高表达

Cdc6在肿瘤组织中, 包括宫颈癌[38-39]、肺癌[40]、口腔鳞状上皮癌[41]、前列腺癌[42]等组织中呈现高表达, 且其表达水平与组织的恶性进程相关。Williams等[43]用免疫组化与免疫荧光方法, 检测了正常与病变宫颈切片中Cdc6蛋白的表达, 发现Cdc6在非正常组织切片中染色明显增强, 其特异性与敏感性超过传统的肿瘤标记物Ki67和PCNA。Karakaidos等[40]检测了75例NSCLC病例癌组织与周边正常组织中*cdc6* mRNA与蛋白水平的差异, 发现在癌组织中*cdc6*的mRNA水平比正常组织高出5倍, Western Blot结果证实, 其蛋白水平也相应地偏高。

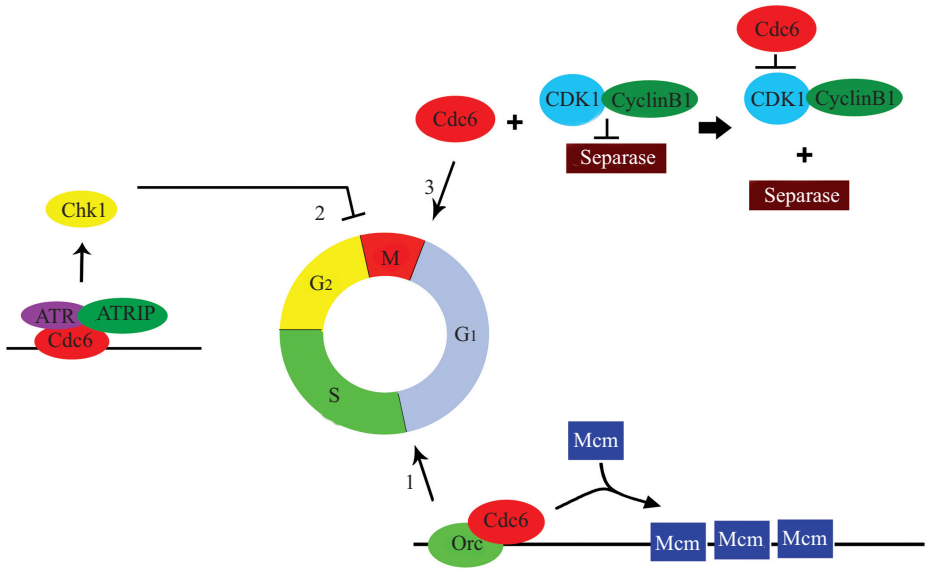
3.2 Cdc6与肿瘤细胞恶性转化、转移

Cdc6与肿瘤发生发展密切相关, 参与细胞的恶性转化。有文章报道, Cdc6会抑制INK4/ARF基因座位(编码三种肿瘤抑制因子p15、ARF和p16)的转录, 下调三种肿瘤抑制因子的表达。此外, Cdc6与Ras共同作用可使细胞获得永生化和恶性转化能力。作者提出Cdc6蛋白具有癌基因的功能, 可促进细胞的恶性转化[44]。雄激素是促进前列腺癌恶性转化的重要因子, 研究发现雄激素会导致Cdc6的高表达[45], 进一步研究证实AR可作为转录因子与*cdc6*基因启动子结合促进*cdc6*的转录[46]。

Cdc6的高表达可促进肿瘤转移。临床病例的检测发现, 肝癌出现淋巴结转移的病人其肿瘤组织中Cdc6表达明显升高[47]。对于Cdc6促进转移的机制目前研究较少, Wu等[42]的研究证实, *cdc6*是抑癌基因PTEN(phosphatase and tensin homolog)的靶基因, PTEN可抑制Cdc6的表达, 而PTEN在大多数转移性肿瘤(如前列腺癌)中是缺失的。作者认为, PTEN的缺失将导致Cdc6的上调, 从而导致远处转移灶克隆增殖能力增强。另有研究发现, Cdc6通过与钙黏蛋白E(CDH1)启动子区E盒结合, 抑制钙黏蛋白的转录, 而钙黏蛋白的缺失将促进转移和侵袭[48]。

3.3 Cdc6与肿瘤细胞代谢

肿瘤细胞存在明显区别于正常细胞的代谢特征。肿瘤细胞因其增殖迅速往往处于缺血缺氧的环境下, 此时肿瘤细胞可通过调整代谢途径以尽可能的获得能量, 如有氧糖酵解(Warburg effect)。最近有研究发现, 在缺血缺氧环境下, 细胞还会通过下调Cdc6进而抑制DNA复制, 从而暂时停止分裂增殖以避免死亡。Martin等[28]的研究发现, 体外缺氧(<0.1%)培养环境下8 h, 肿瘤细胞Cdc6即显著下调, DNA复制受到抑制, 而稳定转染Cdc6可恢复细胞DNA复制。甲硫氨酸依赖也是肿瘤细胞的一个特性。肿瘤细胞的生长依赖绝对的甲硫氨酸, 在缺乏甲硫氨酸并代之以前体物半胱氨酸(Met^-Hcy^+)的培养基中不能生长, 而正常细胞可以在Met^-Hcy^+培养基中生长。Booher等[49]的研究发现, 在Met^-Hcy^+培养基中的乳



1: Cdc6通过促进Mcm复合体组装到染色质, 起始DNA复制; 2: 复制压力时, Cdc6通过招募ATR/ATRIP到染色质参与S-M检测点, 抑制有丝分裂的提前进入; 3: Cdc6抑制有丝分裂期CDK1, 促进有丝分裂的退出。

1: Cdc6 promotes the Mcm complexes loading to chromatin to initiate DNA replication; 2: under replication stress, Cdc6 participates in S-M checkpoint signal by recruiting ATR/ATRIP to the chromatin, inhibits pre-mature mitosis entry; 3: Cdc6 inhibits mitotic CDK1 to promote mitosis exit.

图1 Cdc6 调控pre-RC组装与细胞存活

Fig.1 Cdc6 plays central roles controlling pre-RC assembly and cell survival

腺癌细胞被阻滞于G_1期, 此时Cdc6蛋白下调, pre-RC解聚。

3.4 Cdc6与肿瘤细胞凋亡

多种抗肿瘤药物及诱导细胞凋亡的因素可以抑制Cdc6。槲皮素作用于结肠癌细胞Caco-2后可下调*cdc6*基因的表达。阿多来新作用于K562、MDA以及MCF细胞后, 可下调Cdc6蛋白的表达[50]。鬼臼毒素、紫杉醇以及肿瘤坏死因子相关凋亡诱导配体作用于Hela、SK-HEP-1后, 可导致Caspase-3依赖的Cdc6蛋白的裂解[51]。紫外线照射可以下调肿瘤细胞的Cdc6蛋白水平[13]。这些结果说明, Cdc6与肿瘤细胞的凋亡密切相关, 而抑制Cdc6的确可以诱导细胞凋亡。在肝癌细胞HepG2、Hep3B、BEL-7402、Chang以及宫颈癌细胞Hela中, 以反义技术或RNAi的方法抑制*cdc6*, 会诱导肿瘤细胞的凋亡[11]。舌鳞状上皮癌细胞中, *cdc6* siRNA可抑制增殖并诱导凋亡[14-15]。

抑制Cdc6诱导细胞凋亡的机制目前尚未完全阐明。正常情况下, Cdc6进入核内完成pre-RC组装后会被CDK2磷酸化而转运出细胞核。有研究发现, Cdc6可被Caspase-3降解产生一个49 kDa片段(p49-tCdc6), 而这一片段可能因为缺乏出核序列而滞留在核内, 通过显性负性作用抑制DNA复制并诱导凋亡, 更有意义的是, 单独转染p49-tCdc6片段即可诱导细胞产生凋亡[51]。这似乎说明Cdc6处于凋亡信号的下游, 是Caspase-3的作用底物, Cdc6的降解是为了使细胞更快地完成凋亡。最近有研究发现, Cdc6本身具有抑制凋亡的作用, Cdc6可通过其ATPase结构域与凋亡蛋白酶激活因子Apaf-1形成稳定的复合物, 阻断凋亡体的形成[52]。

4 小结与展望

综上所述, Cdc6在细胞中的功能包括: 起始DNA复制、激活S-M监测点及促进有丝分裂滑脱(图1)。这三方面的作用都与肿瘤的发生发展密切相关: (1)起始DNA复制, 以满足肿瘤快速增殖的需要; (2)细胞DNA受损后, 活化S-M监测点, 抑制细胞进入有丝分裂, 避免产生异常有丝分裂; (3)细胞发生异常有丝分裂时, 促进有丝分裂退出, 导致有丝分裂滑脱, 逃避死亡。Cdc6的这三方面作用紧密关连: 促进肿瘤细胞在正常环境下快速增殖(第一个作用); 一旦出现损伤(如化疗药物作用后), 细胞停止周期进程并试图修复(第二个作用); 修复不成即通过滑脱逃避死亡(第三个作用)。

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