端粒酶的非端粒功能研究进展

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摘要 端粒酶是一种具有逆转录酶活性的核糖核蛋白复合体, 主要功能是进行染色体末端端粒DNA的复制, 维持端粒长度。然而, 新的研究发现, 端粒酶逆转录酶(telomerase reverse transcriptase, TERT)亚基还参与了肿瘤的发生、基因表达调节和线粒体调控等非端粒的功能。端粒酶的非端粒功能的研究有助于全面阐明端粒酶的生物学效应与作用机制, 并且对肿瘤等疾病的治疗具有重要的指导意义。

关键词 端粒酶; 端粒酶逆转录酶; 非端粒功能

Research Progress of Non-telomeric Functions of Telomerase

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Abstract Telomerase is a ribonucleoprotein enzyme complex completes telomere DNA replication of chromosomes. However, additional non-telomeric roles emerge for the telomerase protein TERT (telomerase reverse transcriptase) that can impact tumourigenesis, gene expression regulation and mitochondrial functionality. Researches on the telomere-independent functions of telomerase will contribute to an overall and deep elucidation of the biological behavior of telomerase and related mechanisms, with important significance on the therapy of tumor and other related diseases.

Keywords telomerase; telomerase reverse transcriptase; non-telomeric functions

端粒(telomere)是染色体末端一种特殊结构, 是真核细胞维持染色体稳定性的重要组分, 细胞每分裂一次, 端粒会缩短50~100 bp[1], 当端粒长度缩短到某一阈值时, 过短的端粒无法保护基因组维持正常的稳定性, 最终造成细胞的老化、凋亡或恶变[2-4]。端粒和端粒酶的发现揭示了一个重要的生物学问题, 即线性染色体末端复制是如何进行的[5]。2009年, 诺贝尔生理学或医学奖授予了美国加利福尼亚旧金山大学的Elizabeth H. Blackburn、美国巴尔的摩约翰·霍普金斯医学院的Carol Greider和美国哈佛医学院的Jack W. Szostak, 以表彰他们对端粒和端粒酶保护染色体机制做出的贡献。随着人们对遗传物质DNA结构的解析以及染色体末端复制问题的提出, 到20世纪80年代, Elizabeth H. Blackburn

收稿日期: 2016-01-15 接受日期: 2016-03-28

国家自然科学基金(批准号: 31270737)、高等学校学科创新引智计划资助项目(批准号: B13007)、长江学者和创新团队发展计划资助项目(批准号: IRT13047)和北京市自然科学基金(批准号: 6112016)资助的课题

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Received: January 15, 2016 Accepted: March 28, 2016

This work was supported by the National Natural Science Foundation of China (Grant No.31270737), Institutions of Higher Learning Discipline Innovation (Grant No.B13007), Changjiang Scholars and Innovative Team Development Plan Projects (Grant No.IRT13047) and the Beijing Natural Science Foundation of China (Grant No.6112016)

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网络出版时间: 2016-05-30 10:59:27 URL: http://www.cnki.net/kcms/detail/31.2035.Q.20160530.1059.006.html

和Jack W. Szostak合作鉴定了染色体末端的特殊结构——端粒[6]。随后, Elizabeth H. Blackburn和Carol W. Greider又发现了合成端粒DNA的端粒酶[7-8]。

1　端粒酶基本结构与功能

端粒和端粒酶的生物学研究已从模式生物嗜热四膜虫和酵母拓展到了人[9]、小鼠[10]、寄生虫[11]、植物[12-18]等高等生物中。端粒酶是真核细胞中具有逆转录酶活性的核糖核蛋白, 主要功能是阻止染色体终端端粒的缩短, 维持染色体的稳定和完整, 保证正常的细胞增殖。目前认为, 端粒酶主要由3个组分构成, 即端粒酶逆转录酶(telomerase reverse transcriptase, TERT)、端粒酶RNA(telomerase RNA, TER)和端粒酶相关蛋白(telomerase-associated protein, TEP)[19]。芽殖酿酒酵母的端粒酶在体内对端粒的维护至少包含5个基因, 分别为: *EST2*(the isoamyl acetate-hydrolyzing esterase gene 2)、*TLC1*(telomerase component 1)、*EST1*、*EST3*和*CDC13*(cell division cycle protein 13)[20-21]。其中, *EST2*编码102 kDa的端粒酶逆转录催化亚基[22], *TLC1*编码1 301 nt的端粒酶RNA亚基[23], EST2和TLC1构成了端粒酶的催化核心[24]。CDC13结合单链端粒DNA, 可能是端粒的一个组成元件[25-26]。EST1在体外可以绑定TLC1和端粒DNA富含鸟嘌呤的单链结构[27]。此外, CDC13和EST1相互作用[28], 为端粒招募端粒酶[29], 并且使不活跃的端粒酶变活跃[30]。EST3是稳定端粒酶全酶的组成成分[31], 能和EST2的N-端区域相互作用[32], 并且预测发现, EST3类似于哺乳动物的端粒蛋白(telomeres protect protein 1, TPP1)能够形成OB结构域(Oligonucleotide/oligosaccharide binding domains), 起到招募端粒酶的作用[33]。端粒酶是以端粒酶RNA为模板, 以端粒酶逆转录酶(TERT)为催化亚单位, 以端粒的3′末端为引物, 利用逆转录方式合成端粒重复序列, 来延长细胞分裂增殖时丢失的端粒结构。

端粒缩短对于干细胞的增殖有非常大的影响, 会损害再生组织的能力[34]。然而, 在癌细胞中, 端粒酶使得端粒维护和无限的细胞分裂得以实现[35-36]。端粒酶不仅可以作为癌症早期诊断依据和预后指标, 还能够成为癌症治疗的有效靶点, 因此, 对端粒酶结构、功能、活性及作用机理的研究具有很高的实际应用价值[37]。Bodnar等[38]对*TERT*基因的克隆证明了TERT在细胞衰老过程中端粒缩短以及端粒酶在细胞无限增殖中的作用, 发现TERT在肿瘤细胞中表达水平较高, 而在正常细胞中表达水平较低。最新的研究结果显示, *TERT*基因启动子突变与肿瘤发生密切相关; 多种数据均显示, *TERT*基因启动子突变是癌症发生、发展和转移等过程的普遍特征[39]。值得关注的是, 多种癌症*TERT*基因启动子突变并非随机发生, 而是主要集中在两个位点, –124C和–146C(基因编码起始位点定义为+1), 这些位点均突变为T, 发生突变膀胱癌细胞系的*TERT* mRNA含量平均增加18倍, 蛋白质含量和酶活性均增加2倍, 最终使细胞系的端粒长度平均增加1.8倍, 说明*TERT*基因的启动子突变可有效增加*TERT*基因转录、蛋白表达和端粒酶催化活性, 通过持续延长端粒长度而逃脱正常死亡命运, 最终达到“永生化”而癌变[40-42]。

然而, 越来越多的证据表明, 端粒酶除行使端粒DNA合成的典型功能外, 还具有许多其他作用, 尤其是端粒酶催化亚基参与了基因的表达调节、线粒体功能调控以及肿瘤的发生等, 这些功能被称为端粒酶的非端粒功能[43]。

2　TERT与基因的表达调节及肿瘤发生

转录调控是生物系统的基本生命过程之一, 越来越多的证据表明, TERT可以直接调控基因表达。端粒酶通过调控细胞分裂和组织中基因组的稳定性, 来影响细胞衰老和肿瘤发生[44]。Smith等[45]发现, 在人类乳腺上皮细胞中异位表达TERT, 导致5个生长促进因子的上调及7个生长抑制基因的下调。通过比较人成纤维BJ细胞和被TERT转染的BJ细胞的表达谱, 检测到172个差异表达基因, 并且认为上皮调节蛋白(epiregulin)是一个强有力的生长因子, 在hTERT-BJ细胞表达水平较高, 抑制上皮调节蛋白表达, 即发生hTERT-BJ细胞的衰老程序[46]。也有证据表明, hTERT在转录水平调节Mac-2BP(Mac2 binding protein)、VEGF(vcular eothelial gowth factor)和细胞周期蛋白D1(cyclin D1)的表达[47-48]。此外, 牛肾上腺皮质细胞异源过表达hTERT实验发现, 有284个基因与细胞周期调控、新陈代谢、分化和凋亡

有关[49]。小鼠表皮细胞TERT过表达激发了休眠干细胞的活性, 参与了干细胞的分化[50]。进一步研究证实, TERT在小鼠皮肤细胞的适度表达后, TERT通过Wnt途径参与转录过程的调节, 在干细胞的保护、增殖以及细胞分化中起到了重要作用[51]。

然而, 端粒酶参与基因表达的具体机制还未明确, 目前主要有两种假设。一种假设认为, 端粒酶可能参与表观遗传修饰或调控染色质结构, 间接影响基因的表达。研究发现, 在正常人成纤维细胞中, hTERT的过表达可调控DNA 5′甲基转移酶活性[52], 而hTERT表达被抑制则影响了染色质重塑[53]。另一种可能的机制是, 端粒酶与转录因子或染色质修饰因子相互作用调节相关基因转录。已发现hTERT通过调节NF-κB(nuclear factor-κB)和Wnt/β-catenin信号通路来影响靶基因的转录[54]。Park等[55]研究表明, TERT在β-catenin转录调节复合物中起辅助因子作用, 来调节Wnt信号通路。此外还发现, TERT同响应Wnt信号的基因启动子结合[56]。

TERT已经被证实参与了不依赖于端粒酶RNA(TER)的Wnt-β-catenin信号通路, 通过调节Wnt靶基因的表达, 在肿瘤的发生中起重要作用[57]。Shibani等[58]发现, TERT采用端粒帽化方式来稳定染色体末端, 而不是通过端粒延长来增加细胞的寿命。同样, Li等[59]的研究表明, 用端粒酶RNA特异siRNA对肿瘤细胞进行干扰处理, 可以引发快速且特异的肿瘤增殖减慢效应, 但不引起端粒的脱帽和DNA损伤反应, 而且大部分端粒没有缩短, 说明端粒酶发挥着非依赖端粒长度的作用。他们还发现, siRNA干扰导致细胞基因组表达的改变, 其中涉及细胞周期蛋白G2(cyclin G2)和Cdc27(cell division cycle 27)的表达下调, 说明肿瘤增殖受到抑制, 这可能与端粒酶活性下降导致细胞周期蛋白表达下调相关。此外, 研究发现, 端粒酶RNA(TER)除了在延长端粒结构时作为模板的典型作用外, 还有感应DNA损伤、增加RNA稳定性的功能[60]。

Okamoto等[61]研究发现, TERT通过对癌症干细胞(cancer stem cell, CSC)的维护, 影响肿瘤的发生。他们认为, TERT和转录调节因子BRG1(brahma-related gene 1)以及在干细胞和癌细胞过表达的GTP偶联蛋白(核干细胞因子)形成了一个复合物, 这个复合物对于维护CSC表型、调节相关的转录过程非常关键。最新的一篇综述也介绍了在肿瘤发生过程中, 端粒酶参与CSC的调控, 起着非依赖端粒功能的作用[62]。

Hoffmeyer等[63]发现, 胚胎干细胞和癌细胞都显示出TERT与Wnt途径是相关联的, 与野生型的小鼠相比, β-catenin缺失小鼠的胚胎干细胞端粒短且端粒酶活性不高; 而被β-catenin激活的胚胎干细胞端粒长, 端粒酶活性高。在人类癌细胞的实验中也存在上述类似的现象[64]。以上表明, TERT能够通过Wnt通路在转录过程中起到一定作用。

3 TERT与线粒体功能调控

近年来的研究表明, 无论是正常细胞还是癌症细胞, TERT不仅仅存在于细胞核中, 还有10%~20%的TERT位于线粒体中[65-66]。线粒体作为“细胞动力工厂”能够合成细胞所需的ATP, 同时还与细胞分化、细胞信息传递、细胞凋亡和氧化应激等生命活动密切相关, 线粒体还拥有独特的一套遗传物质——线粒体DNA(mitochondrial DNA, mtDNA), 与细胞核基因组协同作用调控细胞的生命活动。此外, 线粒体作为细胞内活性氧(reactive oxygen species, ROS)产生的主要来源地, 也是容易遭受活性氧攻击、发生氧化损伤的细胞器, 由于mtDNA没有组蛋白的保护以及有效修复系统, 因而受损伤的mtDNA容易导致肿瘤的发生与发展[67]。TERT与线粒体功能紧密联系的第一个证据来源于TERT被证实参与了细胞的内在凋亡途径。Zhang等[68]研究发现, 细胞凋亡过程中, 端粒酶对细胞的保护作用发生在线粒体凋亡的早期, 细胞色素c释放之前。早在2000年时, Seimiya等[69]就发现, 14-3-3信号蛋白能够与TERT结合, 该蛋白质的激活促进TERT定位在细胞核, 而当该蛋白质受到刺激表现出阴性时, TERT从细胞核中转出, 并且发现TERT线粒体定位信号在其N-端, 线粒体定位肽通过与线粒体膜上的出入转位酶结合来实现线粒体转位的功能[70]。研究表明, 生物体在氧化应激条件下, TERT可以从细胞核中转出, 进入线粒体[71]。目前, 端粒酶线粒体转位发生的机制并不是十分清楚, 猜测可能是通过ROS途径进行的, 即当细胞受到氧化刺激时, 引起线粒体损伤, 释放ROS作为信号分子, 传送信号激活Src激酶, 从而促进TERT的线粒体转

位[72]。研究发现, 酪氨酸707突变阻止了TERT蛋白激酶Src的磷酸化和TERT的核外转移[57]。Haendeler等[73]发现, 溴化乙锭刺激引起端粒酶线粒体转位后, TERT进入线粒体基质, 通过TOM与TIM共同作用结合到线粒体DNA的编码区ND1和ND2处, 帮助编码线粒体呼吸链的complex I组分, 从而有效地提高呼吸效率; 并且证实, shRNA沉默*TERT*基因后, 线粒体内活性氧升高。Kang等[74]发现, 在氧化胁迫条件下, *hTERT*基因表达量与线粒体膜电位的变化结果一致, 表明端粒酶线粒体转位对线粒体内外膜电位的维持有一定作用。在*hTERT*超表达的细胞中也表现出可以增加线粒体跨膜电位、产生较少ROS的现象[75]。对癌细胞的研究表明, 癌细胞中线粒体的端粒酶有助于保护细胞核DNA损伤和细胞凋亡[76-77]。

TERT一直被认为是依赖端粒酶RNA(TER)的DNA聚合酶, 在细胞中执行延长染色体末端的端粒结构的功能。Sharma等[78]在加入TER的兔网织红细胞系统(rabbit reticulocyte system, RRLs)中表达了TERT, 采用TRAP方法证实了TERT能够在体外合成端粒重复序列。随后, 分别在TERT-RRL中加入了TER活跃的HeLa细胞、TER不活跃的VA13细胞以及随机的引物, 通过PCR方法都检测到了cDNA; 原位杂交实验也证实, TERT能在没有TER的情况下合成cDNA, 从而表明TERT起到了不依赖TER的逆转录酶作用。在线粒体中, TERT的不依赖TER的反转录酶活性也已经被发现, Sharma等[78]证明了hTERT在线粒体中与mt-tRNAs的逆转录直接相关, 发现人端粒酶RNA(hTER)在线粒体中不存在, 认为mt-TERT能以mt-tRNAs作为模板, 起到了不依赖TER的逆转录酶的作用。新近研究发现, TERT在基因沉默过程中与线粒体中非编码RNA相结合, 作为RNA依赖的RNA聚合酶发挥着作用[79]。也有研究表明, *TERT*和*TER*基因敲除的小鼠端粒功能紊乱, 导致p53介导的线粒体生理和代谢功能受到影响, 最终使小鼠死亡[80]。

TERT已经在啮齿动物的神经细胞中被发现, 推测其可能在脑中起保护作用。新近, Alison等[81]研究不同程度(I-VI时期)阿尔茨海默症样本的TERT的原位表达, 并用一个体外病理模型分析TERT防止氧化损伤的功能。结果表明, 在阿尔茨海默症病人海马体线粒体以及在氧化刺激条件下培养的神经细胞中都有TERT定位, 体外实验表明, TERT降低了ROS的产生和神经细胞的氧化损伤。这表明, 在人脑神经细胞中TERT与线粒体共同作用, 对抵抗阿尔茨海默症可能起到一定作用。

总之, 大量的研究证据表明, 端粒酶尤其是端

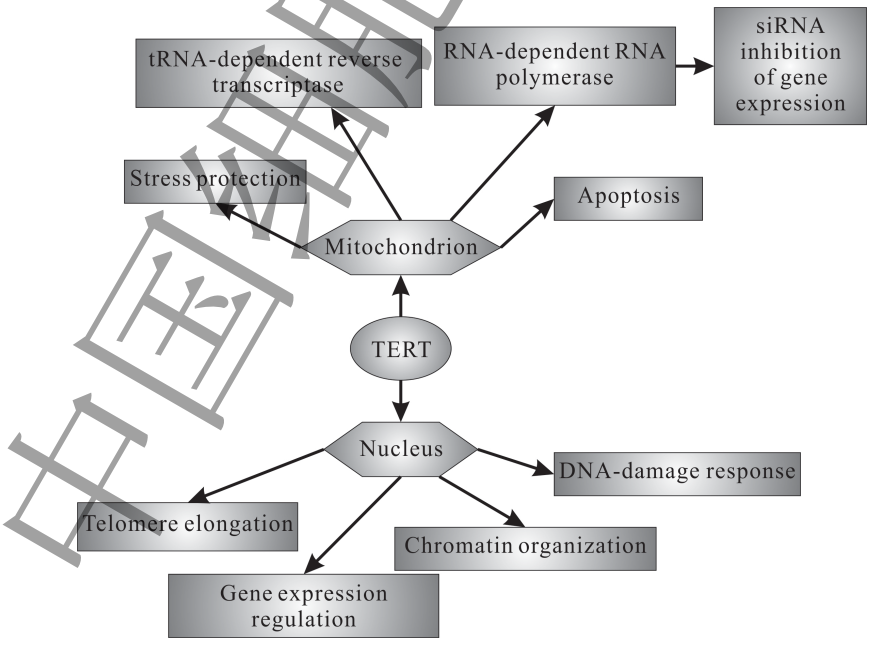

图1　端粒酶功能的多样性(根据参考文献[43]修改)

Fig.1　The diversity of telomerase function (modified from reference [43])

粒酶逆转录酶(TERT)亚基, 除了维持端粒长度和染色体稳定之外, 还具有许多非端粒的功能(图1)。对端粒酶非端粒功能的研究, 可以全方面了解端粒酶的功能, 这些新发现能够帮助确定在细胞老化过程中TERT及线粒体的关键角色。端粒酶对于癌症的发生和发展至关重要, 对端粒酶结构和功能的探索将会为解决细胞老化(衰老)和癌症治疗等世界性生物医学难题提供新的路径。

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