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文献综述 REVIEW

端粒长度测量方法的研究进展

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Progress in telomere length measurement methods

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Abstract

Research indicate that telomere is relative to certain diseases such as cancer. Telomerase activity, replicative history and genetic stability of the cells are all reflected in telomere length. Since telomerase is known to be activated and have its length changed by certain cancers, assessments of telomere length are important for disease investigation and analysis. We here describe several methods for detecting telomere length.

Key Words: Telomere; Measurement

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摘要

近年来研究表明端粒的长短同癌症的发生有密切联系, 各国学者报道了大量与端粒相关的研究. 端粒长短测量法则成为研究端粒的重要手段. 本文总结了多种端粒测量方法, 对相关疾病研究有一定指导意义.

关键词: 端粒; 测量

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■背景资料
近年有关端粒研究端粒长短的变化与疾病的发生密切相关, 在科学研究中经常通过测量端粒长度来研究某种疾病.

0 引言

端粒长度的变化是癌症发生的重要指针, 监测端粒长度变化则是研究癌症发生的重要手段. 近年来报道了多种端粒长度测量方法, 从简单易行的DNA印迹法到单个染色体水平上的端粒测量方法都可以测量端粒的长度. 根据不同的试验材料及试验目的, 应该有针对性地选择端粒测量方法.

1 端粒及其作用

端粒-线性DNA分子末端特殊的核蛋白结构决定染色体末端长度, 使DNA免受不恰当的修复以及防止端-端融合和核酸外切酶的降解[1-2]. 端粒通常为富含TG简单重复序列(TTAGGG)[3], 但是不同真核生物的端粒重复序列不同. 除了端粒酶可以保护端粒不被降解以外, Rad50蛋白也有维持端粒长度的作用[4]. 研究表明, 端粒和端粒相关序列与某些疾病发生相关[5]. 端粒还能够抑制临近基因的转录[1]. 因此研究端粒、端粒相关序列在疾病发生特别是癌症发生中有重大意义.

2 端粒长度的测量方法

2.1 DNA印迹法(Southern blot, SB) SB已广泛应用于分析DNA和端粒结构[6]. 用限制性核酸内切酶*Hin*f Ⅰ或*Rsa* Ⅰ消化DNA, 然后琼脂糖电泳分离不同大小的片段, 转移到硝酸纤维或尼龙膜上. 用^{32}P同位素或生物素、碱性磷酸酯酶标记的端粒特异探针与其杂交(CCCATT)n. 末端限制酶切片段(TRF)通过光密度计定量测量[7]. 选用*Hin*f Ⅰ或*Rsa* Ⅰ是因为这两种酶能频繁的切断DNA, 使亚端粒区域变得尽可能小. 用于SB分析

■应用要点
本文总结了多种测量端粒长度的方法; 在何种条件下应用哪种方法较合适, 对疾病研究有一定参考意义.

的DNA应该是没有被打碎和纯度较高的, 但是因为DNA的高分子量、高黏度使得这点较难保证. TRF法得到的数据除代表所有染色体端粒的长度外, 还包括部分亚端粒区长度. 因此TRF法不能提供端粒的实际长度[7].

2.2 杂交保护分析法(hybridization protection assay, HPA) HPA需要制备基因组DNA、细胞或组织溶胞产物同吖啶酯(AE)标记端粒的探针进行杂交, 检测发光强度, 确定端粒在Alu序列中比例. 研究表明, Alu序列中比例为0.01的端粒大约与2 kb的TRF法测量的端粒长度对应. HPA不需要完整的或没有修剪的DNA, 但是, 推荐使用下限为10 ng修剪过的DNA. 纯化的细胞DNA或至少含有1000个细胞的组织溶胞产物都能用于测量. 虽然HPA不包括亚端粒序列, 但是HPA法没有给出详细的细胞或染色体水平的端粒信息, 也不能直接测定端粒长度[8].

2.3 荧光原位杂交(FISH)[9] FISH直接将寡核苷酸探针标记在端粒序列上[10-12]. 标准的FISH包括制作分裂中期的染色体以及DNA变性, 与异硫氰酸荧光素(FITC)或Cy3标记的寡核苷酸探针杂交, 用DAPI或PI复染, 最后用荧光显微镜检测信号[13]. FISH方法被广泛地应用于特定基因的细胞遗传学研究. FISH自身的特点使其适合于测定端粒长度, 如小的端粒探针可增加进入细胞的渗入度, 而且单链探针不需要退火.

几种改良的FISH: 用FISH方法进行的端粒定量被称作Q-FISH[14-15](Quantitative-FISH). Lansdorp *et al*[10]用核酸肽(PNA)寡核苷酸探针(PNA-FISH)代替寡核苷酸探针改良了Q-FISH法. 因为带电的脱氧核糖磷酸盐主链被肽链连接的不带电N-(2-氨基乙基)-甘氨酸主链替代[16], PNA探针的复式结构远比DNA/DNA或DNA/RNA的稳定. 结合FISH和免疫染色(TELI-FISH)的方法可以测量用甲醛固定, 石蜡包埋的人组织样本的端粒, 同时还可以鉴别细胞种类. 在TELI-FISH中只需用很少的细胞, 这种方法可以忽略不同细胞种类而直接比较正常和癌变细胞的端粒长度[17]. Perner *et al*[18]发明了端粒/着丝粒-FISH(T/C-FISH)测量每个单独的染色体臂的端粒长度的方法, 2号染色体着丝粒作内参. 荧光强度的比率经过计算同TRF法有很好的相关性. Yan *et al*在染色质/DNA纤维制备中应用了Fiber-FISH法[19-20]. 端粒的特异PNA Green探针和1q亚端粒的特异Red探针在染色质纤维上同时杂交, 已知的1q亚端粒探针大小为100 kb. 因为解压缩的染色质的可变伸缩性使得纤维杂交的时候显色长度变得可变. 然而, 目的序列同亚端粒探针有相似的伸缩程度. 因此, 用已知大小的亚端粒探针作对照对判断DNA纤维解压缩的程度起很重要的作用. 在检测淋巴母细胞系1q时, 端粒和亚端粒排列在纤维上的信号范围分别在0.9 μm-2.9 μm和13.8-29 μm之间. 这意味着根据纤维的不同伸展程度杂交到纤维上的探针可见长度为3.4-7.2 kb/μm(平均5.5 kb/μm) .

2.4 流式细胞计量-荧光原位杂交(Flow-FISH) Flow-FISH包括6个基本步骤: 细胞分离, DNA变性并与PNA探针杂交, 洗去多余探针, 复染后用流式细胞计量术采集和分析[21]. 来自不同细胞系和临床样本的骨髓、血液淋巴结、经过检测的扁桃体的端粒长度值同TRF法有很好的相关性[22]. 同Q-FISH相反, Flow-FISH可以分析周期中和非周期中的细胞端粒, 整个操作只需1 d[21-23]. 此外, 不同的细胞亚群能够同时处理, 这对于分析较难纯化的细胞是很有用的[24]. Flow-FISH法测定端粒长度在研究有核血细胞在正常个体和病态个体的血液病时有很高的实用性[25-26]. Flow-FISH同时分析端粒长度和细胞表型的技术难度很高, 因为只有很少的带有合适发射光谱的荧光染料能承受DNA变性和PNA杂交的条件. Baerlocher *et al*[27]运用Hydra 96孔微型分注器测量端粒长度[28], 他可以定量差异很小(0.5 kb)的端粒片段, 而且只用很少的细胞(1000个). 当同时测量复杂样品时, 这种自动化操作尽显优势.

2.5 寡核苷酸引物原位DNA合成法(PRINS)[29-31] 寡核苷酸引物(CCCTAA)7与分裂中期或分裂间期的同源染色体退火、杂交, 加入热稳定DNA聚合酶和荧光标记的核苷酸后引物开始延伸, 最终通过荧光显微镜分析荧光信号[32]. 许多位点的引物同时扩增保证了充足的荧光信号得以检测, 但是不均匀的引物退火可能导致端粒长度测量不充分. 一些用于增加标记和退火功效的改良, 双脱氧核苷酸的参与可以通过减少染色体被随机打断的非相关DNA的扩增而使更多特异端粒序列被标记[33-34]. 最近, Yan *et al*[35]用双链PRINS法, 即(TTAGGG)7和(CCCTAA)7两个引物标记端粒DNA的正链和反链. 两个方向的标记使产物的信号得以加强, 标记功效增加到78%至95%.

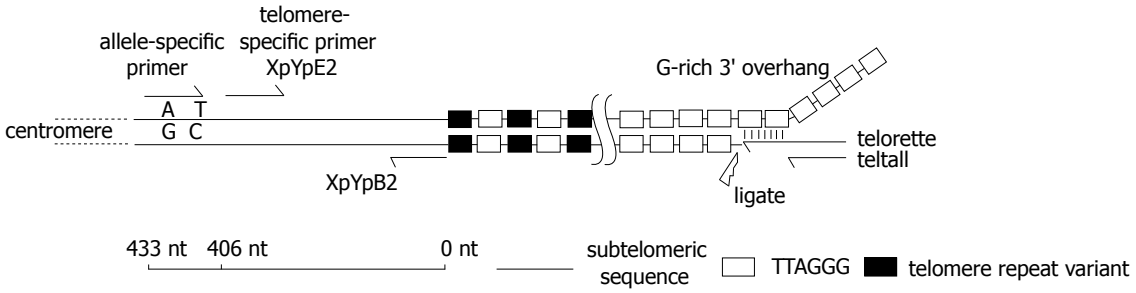

图 1 STELA原理示意图.

2.6 定量PCR[36] 以前认为用寡核苷酸引物TTAGGG和CCCTAA[37]进行的PCR反应不可能用于测量端粒, 因为引物自身之间可能发生杂交[38-39]. 但是如果引物被加以修饰, 6个重复碱基中包括两个错配碱基和4个连续配对碱基, 这样两个引物便不会互相杂交. 在Q-PCR中端粒(T)重复拷贝的比例同单个拷贝的基因(S)的比例是确定的. T/S的比例同端粒平均长度成正比关系, 端粒长度可根据T/S率测定[38].

2.7 单个端粒长度分析(STELA) STELA是在单个染色体水平上基于PCR的端粒测量方法[40]. 第1步是“telorette”(在与端粒G悬突不配对的20个核苷酸后有7个同源的TTAGGG接头)的退火. 第2步是将“telorette”连接到富C链5末端. 此后用识别telorette尾巴的teltail引物同亚端粒上游引物进行PCR扩增[40-41]. 在研究这个区域的多态现象时, 上游引物可以是特异的等位基因(图1). STELA最先被用于分析人类X染色体短臂[20]. 最近对秀丽隐杆线虫的端粒和沃纳综合征的成纤维细胞的端粒的研究表明, 在单个染色体水平上STELA的分析有很高的可靠性[42]. STELA的PCR扩增具有高度的端粒特异性, 用STELA测量人类成纤维细胞的XpYp端粒长度始终比TRF的平均值小, 且这两者成线性关系, 说明STELA没有包括亚端粒长度[40].

3 结论

测量端粒长度在研究癌症等疾病发生时有重大意义. 端粒结构的特殊性和复杂性对分析带来很大不便, 特别是高度重复序列和各种空间结构使得测量的精度有所降低. 近年来对端粒的研究越来越深入, 方法越来越完善. 总的来说, Southern blot是应用最广泛的方法, 但是针对不同的目的在测量端粒长度时还应有所选择.

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■同行评价
本文内容全面, 层次清楚, 有一定的参考意义.

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