

诱骗寡聚核苷酸技术在纤维化疾病中的运用及其递送策略的选择

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Decoy oligonucleotide technology in fibrosis: Application and delivery strategy

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Abstract

Fibrosis is a pathological condition caused by a variety of etiologies, which is characterized by an increase in the fibrous connective tissue and a reduction in the parenchymal cells of several organs and can result in structural damage and functional impairment of organs. With the development of molecular biology and cellular biology technology in recent years, gene therapy methods for fibrosis are drawing attention, including antisense oligonucleotides, RNA interference, Decoy oligonucleotide (ODN) technology and so on. Among them, Decoy ODN technology can block the target gene expression by capturing specific transcription factors, having the potential to interfere with the expression of the fibrosis related genes. This paper will review the application of Decoy ODN technology in fibrosis as well as the delivery strategy *in vivo*.

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Key Words: Decoy oligonucleotides technology; Transcription factor; Delivery strategy; Fibrosis

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背景资料

分子生物学技术已经广泛应用于纤维化疾病的基础研究, 其中包括反义寡核苷酸、RNA干扰、诱骗寡聚核苷酸技术等。近年来, 诱骗寡聚核苷酸技术在纤维化疾病的基础研究和临床应用方面得到了快速发展。

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随着分子生物学技术的快速发展, 目前针对转录因子的诱骗寡聚核苷酸(Decoy oligodeoxynucleotide, Decoy ODN)的形态结构、体内投递方法及不同的Decoy ODN之间的嵌合等方面的探索是该领域内的研究热点。

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摘要

纤维化(fibrosis)是由多种病因所致的纤维结缔组织增多, 实质细胞减少为特征的器官结构破坏和功能减退的病理变化, 可发生于多种器官。近年来, 包括反义寡核苷酸、RNA干扰、诱骗寡聚核苷酸(Decoy oligodeoxynucleotide, Decoy ODN)技术等多种分子生物学技术应用于纤维化疾病的基础研究和临床防治, 其中, Decoy ODN技术通过诱骗策略, 能够快速靶向性阻断特异性转录因子与其靶基因启动子的结合, 从而干扰其表达, 具有干预纤维化基因表达的潜能, 在纤维化疾病的基础研究和临床应用方面得到了快速发展。本文将对Decoy ODN技术应用于纤维化疾病及其形态结构和在体内的投递策略作一评述。

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关键词: 诱骗寡聚核苷酸技术; 转录因子; 递送策略; 纤维化

核心提示: 本文对诱骗寡聚核苷酸(Decoy oligodeoxynucleotide, Decoy ODN)技术的作用原理及针对转录因子核因子-κB(nuclear factor-κB)和转录因子特异性蛋白1(specificity protein 1)的Decoy ODN在纤维化疾病中的应用, 在肝纤维化疾病的应用展望及Decoy ODN的形态及递送策略的选择进行了详细的描述。

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0 引言

纤维化(fibrosis)以细胞外基质的过度沉积, 伴随着实质细胞的减少为特征, 涉及皮肤瘢痕、腹膜后纤维化、胰腺和胃肠道纤维化、肾小球硬化、肾间质纤维化、骨髓纤维化、动脉硬化、肺纤维化、肝纤维化等多种疾病。在多种致病因素包括创伤、感染、炎症、血液循环障碍及免疫反应等的作用下, 由炎症因子和细胞因子介导肌成纤维细

胞(myofibroblast, MF)分泌大量的细胞外基质(extracellular matrix, ECM), 同时, 起降解ECM作用的金属蛋白酶(matrix metalloproteinases, MMPs)的功能受到抑制, 引起ECM的分泌与降解失衡, 过度沉积于器官组织内导致纤维化^[1,2]。纤维化的发病机制极其复杂, 涉及多条信号转导通路, 如转化生长因子-β(transforming growth factor-β, TGF-β)、结缔组织生长因子(connective tissue growth factor, CTGF)和血小板衍生生长因子(platelet-derived growth factor, PDGF)^[3,4]等炎症因子相关的信号转导通路, 但是, 由于这些信号的多效性和各条信号转导通路之间的串话, 使得抗纤维化的分子治疗存在诸多障碍^[5]。针对特异性的细胞核转录因子(transcription factor, TF)结合纤维化相关基因启动子的特定DNA基序(motif), 人工合成这些特定的DNA序列, 反向诱捕转录因子, 即诱骗寡聚核苷酸(Decoy oligonucleotides, Decoy ODN)技术。对这些短片段的DNA进行修饰和加工, 采用不同的投递策略进行体内递送, 从而干扰纤维化的病理进程, 是备受关注的基因疗法^[6]。肝纤维化是脏器纤维化的典型例证, 我们实验室从事肝纤维化的基础和临床研究已经十余年, 因此, 本文就Decoy ODN技术在纤维化疾病中的运用, 特别是在肝纤维化疾病中的应用前景及Decoy ODN在体内的递送策略作一阐述。

1 Decoy ODN技术作用原理

在转录起始阶段, 真核生物的基因表达主要由转录因子负责识别启动子的特异结合序列(transcription factor binding site, TFBS), 然后, 再结合RNA聚合酶, 形成转录起始复合物, 启动转录。随着特异性转录因子对靶基因的调控研究不断深入, 通过干预各种特异性转录因子的活性, 实现对靶基因表达的调控, Decoy ODN技术用于疾病的防治备受关注^[7]。Decoy ODN技术又称转录因子诱骗技术, Decoy ODN是含有TFBS的双链寡聚核苷酸片段, 长度一般在10-30 bp之间。他能与特定基因启动子上的TFBS竞争性的结合转录因子, 其结果是转录因子不能有效的结合内源性靶基因启动子的TFBS, 在转录水平促进或者抑制下游基因的表达, 该技术作为一种特

殊的基因治疗方法, 已经在动物实验中被人们接受^[8,9].

2 Decoy ODN在纤维化疾病模型中的应用

生物体内存在大量的特异性转录因子, 目前, 以他们的TFBS设计Decoy ODN, 用于疾病的干预性实验治疗也屡见报道, 但是, 用于纤维化疾病防治方面的报道不多, 其中, 核因子- κ B (nuclear factor- κ B, NF- κ B) Decoy ODN用于纤维化的实验研究最多, 其次, 转录因子特异性蛋白1 (specificity protein 1, SP1) Decoy ODN, Smad Decoy ODN和AP1 Decoy ODN也有报道.

2.1 NF- κ B的Decoy ODN在纤维化疾病中的应用 NF- κ B是一个转录因子蛋白家族, 包括5个亚单位: Rel、p65、RelB、p50和p52. Rel、p65和RelB的N端含有Rel同源区 (Rel homology domain, RHD)及核定位区域 (nuclear-localization sequence, NLS); C端含有反式激活结构域(transactivation domain, TD). 在生物体内通常是以二聚体的形式存在, 最常见的是p65与p50组成的异二聚体. NF- κ B的抑制单位I κ B通过其C末端的锚定蛋白重复序列覆盖NLS阻止NF- κ B向细胞核内转移. 外界信号刺激细胞后, I κ B磷酸化, 使NF- κ B暴露NLS. 游离的NF- κ B迅速进入细胞核, 诱导相关基因转录^[10,11]. NF- κ B调节细胞的多种生命活动, 如细胞增殖、细胞凋亡、炎症反应^[12]等.

1990年, Bielinska等^[13]首先报道了含有NF- κ B结合序列的双链DNA片段, 即NF- κ B Decoy ODN, 能竞争性的与内源性DNA结合NF- κ B. 2002年, Griesenbach等^[14]尝试用NF- κ B Decoy ODN治疗纤维化疾病, 他们采用脂质体包裹硫代磷酸化修饰的NF- κ B Decoy ODN, 通过呼吸道吸入的方式治疗博来霉素引起的小鼠肺纤维化, 但是, 其结果没有支持NF- κ B Decoy ODN具有降低白介素-6(interleukin-6, IL-6)表达的作用. 之后, 有大量报道证明此技术能干扰NF- κ B的活性^[15], 逐渐用于纤维化疾病的研究中, 并在NF- κ B Decoy ODN的投递方式上有新突破. 动脉粥样硬化是一个慢性炎症和纤维化的过程, 通过注射脂多糖引起的小鼠动脉粥样硬化模型中, Kim等^[16]、Lee等^[17]采用尾静

脉注射环状的NF- κ B Decoy ODN, 不仅降低了动脉粥样硬化相关分子的表达, 如血管黏附分子(vascular adhesion molecule, VCAM)和细胞间黏附分子(intercellular adhesion molecule, ICAM), 而且纤维化相关炎症介质TGF- β 也有所下降. Tomita等^[18]用日本血凝素病毒脂质体载体(hemagglutinating virus of Japan with liposome-based non-viral vectors, HVJ-liposome)携带NF- κ B Decoy ODN治疗大鼠系膜增生性肾小球肾炎、炎症肾炎及肾纤维化, 治疗组的IL-1及TNF- α 的表达量明显降低. 在肺纤维化研究方面, De Stefano等^[19]利用生物裂解聚酯(poly d, l-lactide-coglycolide, PLGA)技术包装NF- κ B Decoy ODN, 用于治疗脂多糖引起的大鼠肺纤维化, 取得了良好的效果. 他们用PLGA技术包装的大型多孔颗粒(large porous particles, LPPs)用作NF- κ B Decoy ODN的载体, 采用吸入方式给药, 对脂多糖引起的支气管肺泡嗜中性粒细胞浸润的抑制长达72 h, 而用单纯裸露的NF- κ B Decoy ODN只能持续6 h.

应用NF- κ B Decoy ODN的治疗策略, 针对各种刺激引起的囊性纤维化(cystic fibrosis, CF)机制及治疗的研究更为深入. CF是以产生和释放细胞因子和趋化因子为特征的炎症过程, 其中, IL-8是最重要的, NF- κ B Decoy ODN能有效的阻断NF- κ B对IL-8基因启动子的调控作用, 引起IL-8表达下调^[20]. 最近, 在分子生物学和基因治疗领域, 肽核酸(peptide nucleic acids, PNA)备受关注, 他是一类DNA类似物, 以氨基酸取代糖磷酸主链, 与核酸分子比较, 具有较高的水溶性、稳定性和碱基特异性, 容易被细胞吸收. 在绿脓假单胞菌引起的囊包性纤维化体外模型中, 为了进一步提高NF- κ B Decoy ODN下调IL-8表达的效果, Gambari等^[11]选用PNA作为投递工具. 他们运用PNA-DNA-PNA嵌合物包裹NF- κ B Decoy ODN对CF的进程进行干预, 结果显示: PNA-DNA-PNA嵌合物更易与脂质体融合; 对核酸酶有更强的耐受性及自身的稳定性; 能更好地下调IL-8的表达.

2.2 SP1的Decoy ODN在纤维化疾病中的应用 转录因子特异性蛋白1 (specificity protein 1, SP1)属于Kruppel样转录因子家族, 其共同结构特征是包含3个锌指结构锚定区域, 可特

■ 相关报道

不同转录因子的Decoy ODN之间的组合在多种纤维化疾病如肺纤维化、肾纤维化等疾病模型研究中已经有所报道, 但其稳定性和靶向性有待进一步提高.

■创新盘点

本文详细总结了转录因子的Decoy ODN的多种形态结构及体内投递策略在纤维化疾病模型中的应用以及各自的优缺点, 为感兴趣的研究者提供一些新的思路。

异性结合含有重复GC序列(GC盒)的DNA片段。因为能结合GC盒, 该转录因子超家族不但可以调节包括管家基因在内的所有基因的表达, 而且还可以调控组织特异性基因的转录^[21]。运用SP1 Decoy ODN同样可以下调CF过程中IL-6和IL-8的表达^[22], 从而减缓纤维化进程。而在人类皮肤的增生性瘢痕中, SP1 Decoy ODN可以显著减少病变部位胶原蛋白的沉积^[23], 而胶原蛋白是各种纤维化疾病的主要病理沉积物。在单侧输尿管结扎引起的大鼠肾纤维化模型中, Chae等^[24]通过脂质体投递SP1 Decoy ODN能通过阻断TGF- β 信号减少各种细胞外基质的产生, 在肺纤维化疾病模型中具有同样的效果^[25,26]。在体外培养的大鼠肝星状细胞系中, SP1 Decoy ODN具有抑制大部分肝纤维化相关基因表达的作用, 包括TGF- β 、PDGF、胶原蛋白^[27]等。

2.3 Decoy ODN组合在纤维化疾病中的应用
纤维化的发病机制极其复杂, 通过单因素的干预, 虽然可以下调部分细胞因子和纤维化相关基因的表达, 但从整体上抑制纤维化的病理进程还有巨大的差距, 因此, 将不同的Decoy ODN进行组合, 通过寻找其协同作用, 可能成为抑制纤维化病理进程的重要策略。NF- κ B Decoy ODN和SP1 Decoy ODN已经被用于肾间质性纤维变性^[28]和动脉粥样硬化^[17]。其中, 在肾间质性纤维变性中, 这种组合能减少间质体积和巨噬细胞浸润, 下调纤维化相关基因的表达和调节上皮间质转换(epithelial-mesenchymal transition, EMT)相关基因的表达, 从而抑制肾纤维化^[28]。将SP1 Decoy ODN和Smad Decoy ODN合成在同一个DNA片段上, 用于治疗单侧输尿管结扎引起的肾间质性纤维变性中, 该Decoy ODN较单一的SP1 Decoy ODN或者Smad Decoy ODN的干预效果更好^[29]。Yuan等^[30]筛选出能同时有效结合转录因子Smad和AP1的Decoy ODN片段AFODN4, 并证实同时抑制Smad和AP1是发展新的抗纤维化试剂的有效策略。

3 Decoy ODN技术在肝纤维化疾病中的应用展望

肝纤维化是以TGF- β 等炎症因子参与的损伤-炎症-修复的病理过程^[31], 肝星状细胞(hepatic stellate cell, HSC)扮演着重要角色。正常情况

下, HSC处于静止状态, 主要功能是储存维生素A(vitamin A, VA)和甘油三酯等脂质成分^[32]。在炎症因子的刺激下, 静止期的HSC活化并分化为肌成纤维细胞(myofibroblast, MF), 表现为致纤维化基因的表达、白细胞因子的释放、具有收缩性和趋化性等特征^[33], 结果是ECM的分泌与降解失衡。

NF- κ B在肝脏中既能引起细胞凋亡也能促进细胞增殖, 但是, 其作用的细胞类型需要进一步阐明^[34]。当体外培养的大鼠HSC自然活化时, p50和p65的表达是上升的, 同时伴随着 α -平滑肌肌动蛋白(α -smooth muscle actin, α -SMA)的增加^[35]。研究^[36]表明, 在非酒精性脂肪肝炎及酒精性脂肪性肝病中, p65的表达量显著增加, 其中, 在脂肪性肝炎引起的肝纤维化中表达更高。因此, NF- κ B Decoy ODN可能具有潜在的治疗肝纤维化疾病的价值。EMT是肝纤维化发展的中间环节, 最终导致ECM的产生及降解失衡, 波形蛋白(vimentin)是EMT的标志蛋白, E-钙联蛋白(E-cadherin)具有抑制EMT的作用。在TGF- β 诱导小鼠肝细胞AML12的EMT模型中和四氯化碳所致的小鼠肝纤维化模型中, Kim等^[37]采用闭合环状NF- κ B Decoy ODN进行干预性治疗, 导致了波形蛋白表达的下调和E-cadherin表达的恢复, 因此, NF- κ B Decoy ODN能通过干预EMT, 进而阻断肝纤维化进程。也有研究^[38]认为, NF- κ B Decoy ODN并不能抑制HSC的增殖和分化, 而是通过抑制巨噬细胞的功能, 减轻炎症反应, 进而减轻肝纤维化。E-cadherin是转录因子Snail直接作用的靶基因, Snail主要以其锌指区域与E-cadherin启动子区E盒主链上的CAGGTG序列结合而发挥下调作用^[39]。Snail可以间接上调MMP-1、MMP-2和MMP-7的表达, 抑制ECM的合成, 加速ECM的降解, 增强细胞的侵袭能力^[40]。但是, 在肝纤维化疾病的治疗中, 针对Snail的Decoy ODN策略未见报导。

SP1是调节炎症-修复过程的转录因子, 其调节范围较广, 如TGF- β 、多种致纤维化细胞因子和ECM基因的表达。研究^[27]显示, 在HSC中, SP1 Decoy ODN能够下调细胞周期蛋白cyclin D1及p27的表达, 也能下调TGF- β 、PDGF-BB、 α -SMA、Collagen I α 1和TIMP-1等纤维化基因的表达, 具有抑制HSC活化的

作用. Park等^[41]采用环状的DNA片段(R-SP1 Decoy ODN)治疗四氯化碳所致的小鼠肝纤维化, 能够抑制肝巨噬细胞的作用, 明显下调炎症因子TNF- α 、IL-1的表达, 炎症因子IL-6、VEGF和MCP-1的表达也有下降; 能够明显抑制纤维化相关基因纤维连接蛋白(fibronectin)、MMP-13和TIMP-1的表达, 也能抑制TGF- β 、 α -SMA和胶原蛋白的表达, 因此, R-SP1 Decoy ODN具有抑制肝纤维化的作用, 其应用前景值得进一步研究.

4 Decoy ODN的结构形态及其递送

Decoy ODN片段在体内的靶向递送是影响其临床应用的重要环节, 通过对Decoy ODN进行改造和修饰以及采用先进的运载工具进行递送, 是提高体内递送效率的两个重要策略.

4.1 不同形态的Decoy ODN在疾病模型中的应用 不加修饰的双链Decoy ODN片段在体内很容易降解, Miyake等^[42]用硫代磷酸化的NF- κ B Decoy ODN用于干预兔的血管重构的研究, 结果显示他能抑制血管平滑肌细胞的增殖和迁移, 但是, 对血管内皮细胞没有作用. 之后又有将NF- κ B和SP1的特异结合位点通过几个脱氧核苷酸连接起来, 两端在用多聚腺苷酸进行封闭形成闭合环状的DNA序列, 这种闭合环状的双链Decoy ODN形似哑铃, 又称哑铃状Decoy ODN, 具有抗核酸酶的作用^[28]. 在治疗大鼠单侧输尿管结扎引起的肾纤维化实验研究中, 哑铃状Decoy ODN比两端开放Decoy ODN的疗效持久^[43]. 相比之下, PNA-DNA-PNA嵌合物能更加容易穿过细胞膜, 对核酸酶有更强的耐受性及自身的稳定性^[11,20].

4.2 Decoy ODN递送策略的选择 对于外源性的Decoy ODN, 仅仅从形态结构对其进行改造, 生物体内的核酸酶依然会将其分解. 针对这一问题, 国内外学者探索出了几种Decoy ODN的递送方式. 在治疗大鼠的肾纤维化中, Kim等^[28]用Mirus脂质体递送NF- κ B和SP1的嵌合Decoy ODN, 7 d后还能检测到带荧光标签的Decoy ODN. Son等^[44]用日本血凝素病毒载体(HVJ)携带NF- κ B Decoy ODN治疗小鼠肝纤维化, 通过小鼠脾脏注射, 在小鼠肝脏的巨噬细胞和部分纤维化的肝脏组织中, 能观察到荧光标记的Decoy ODN; 在小鼠肾脏纤维化治疗中, 也用这种病毒载体递送过Decoy

ODN^[20]. Bezzerri等^[45]、De Stefano等^[46]用从绿脓杆菌提取的脂多糖刺激人类支气管上皮细胞, 分泌大量IL-6和IL-8, 然后用PLGA技术包装的大型多孔颗粒LPPs用作NF- κ B Decoy ODN的载体进行细胞水平投递, 颗粒直径为30 μ m左右, 这种投递系统能保护其内部携带的药物不易被降解, 结果显示经过包装的实验组相对于裸露的Decoy ODN实验组无论IL-6还是IL-8都有所下降; 将其用于肺纤维化的治疗, 通过吸入方式给药, 抑制支气管肺泡嗜中性粒细胞浸润可达到72 h, 而未包装的只能持续6 h^[47]. 此项投递技术最大的优点是能保证携带的药物如Decoy ODN能缓慢释放出来, 延长作用时间^[48]. 近年来, 纳米技术开始用于药物递送^[49], Wardwell等^[50]用N端三甲壳聚糖(polysialic acid-N-trimethyl chitosan, PSA-TMC)纳米颗粒包裹NF- κ B Decoy ODN用于治疗CF, 与脂质体转染NF- κ B Decoy ODN作比较, 投递24 h后, 纳米颗粒递送组较脂质体转染组明显地下调IL-8的表达; 48 h后, 纳米颗粒组的IL-6表达量明显低于脂质体转染组. 这几种投递方式各自有他们的优缺点, 其中, 病毒和脂质体作为载体相比之下能较好的保护Decoy ODN短时间内不被降解, 但是, 作为治疗存在安全性的隐患; PLGA包装的大型多孔颗粒和纳米材料能使携带的Decoy ODN达到一个缓慢释放的效果; 特别是智能型纳米材料的应用将为Decoy ODN的体内递送提供新思路.

5 结论

纤维化疾病是由多种病因导致的复杂的病理变化, 肝纤维化是脏器纤维化的典型例证. Decoy ODN技术作为一种新兴的基因诊疗方法, 通过竞争性地结合特异性转录因子, 从而干预肝纤维化相关基因的表达, 可能成为防治肝纤维化的新策略. 对Decoy ODN的形态结构和体内递送方式的研究将会为其临床应用提供安全及高效性的保障.

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应用要点

由于纤维化疾病的病因及病理过程极其复杂, 目前临床上的防治方法还存在诸多障碍. 诱骗寡聚核苷酸技术的快速有效及其靶向性阻断靶基因的表达, 在临床上具有巨大的防治潜能.

同行评价

纤维化是多种疾病共同的病理过程, 涉及多个炎症因子相关的信号通路, 诱骗寡聚核苷酸技术通过诱骗策略, 靶向阻断特异性转录因子与靶基因启动子的结合, 具有干预纤维化基因表达的潜能. 作者通过对有关文献的综述, 对纤维化的基础和临床研究具有一定的参考价值.

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•消息•

《世界华人消化杂志》外文字符标准

本刊讯 本刊论文出现的外文字符应注意大小写、正斜体与上下角标。静脉注射iv, 肌肉注射im, 腹腔注射ip, 皮下注射sc, 脑室注射icv, 动脉注射ia, 口服po, 灌胃ig。s(秒)不能写成S, kg不能写成Kg, mL不能写成ML, lcpm(应写为1/min)÷E%(仪器效率)÷60 = Bq, pH不能写PH或P^H, *H. pylori*不能写成HP, T_{1/2}不能写成tl/2或T, V_{max}不能Vmax, μ 不写为英文u。需排斜体的外文字, 用斜体表示。如生物学中拉丁学名的属名与种名, 包括亚属、亚种、变种。如幽门螺杆菌(*Helicobacter pylori*, *H. pylori*), *Ilex pubescens* Hook, et Arn. var. *glaber* Chang(命名者勿划横线); 常数*K*; 一些统计学符号(如样本数*n*, 均数mean, 标准差SD, *F*检验, *t*检验和概率*P*, 相关系数*r*); 化学名中标明取代位的元素、旋光性和构型符号(如*N*, *O*, *P*, *S*, *d*, *l*)如*n*-(normal, 正), *N*-(nitrogen, 氮), *o*-(ortho, 邻), *O*-(oxygen, 氧, 习惯不译), *d*-(dextro, 右旋), *p*-(para, 对), 例如*n*-butyl acetate(醋酸正丁酯), *N*-methylethanilide(*N*-甲基乙酰苯胺), *o*-cresol(邻甲酚), 3-*O*-methyl-adrenaline(3-*O*-甲基肾上腺素), *d*-amphetamine(右旋苯丙胺), *l*-dopa(左旋多巴), *p*-aminosalicylic acid(对氨基水杨酸)。拉丁字及缩写*in vitro*, *in vivo*, *in situ*; *Ibid*, *et al*, *po*, *vs*; 用外文字母代表的物理量, 如*m*(质量), *V*(体积), *F*(力), *p*(压力), *W*(功), *v*(速度), *Q*(热量), *E*(电场强度), *S*(面积), *t*(时间), *z*(酶活性, kat), *t*(摄氏温度, °C), *D*(吸收剂量, Gy), *A*(放射性活度, Bq), ρ (密度, 体积质量, g/L), *c*(浓度, mol/L), ϕ (体积分数, mL/L), *w*(质量分数, mg/g), *b*(质量摩尔浓度, mol/g), *l*(长度), *b*(宽度), *h*(高度), *d*(厚度), *R*(半径), *D*(直径), *T*_{max}, *C*_{max}, *V*_d, *T*_{1/2} *CI*等。基因符号通常用小写斜体, 如*ras*, *c-myc*; 基因产物用大写正体, 如P16蛋白。