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(54)发明名称

猴腺病毒(大猩猩)或腺病毒载体及其使用方法

(57)摘要

本发明提供了一种腺病毒或腺病毒载体,其特征为包含一种或多种特定的核酸序列或一种或多种特定的氨基酸序列,或其部分,所述腺病毒或腺病毒载体涉及例如腺病毒pIX蛋白、DNA聚合酶蛋白、五邻体蛋白、六邻体蛋白和/或纤维蛋白。

1. 一种腺病毒或腺病毒载体,其包括一种或多种选自下组的核酸序列:
 - (a) 与SEQ ID NO:6具有至少98.7%同一性的核酸序列,
 - (b) 与SEQ ID NO:7具有至少98.9%同一性的核酸序列,
 - (c) 与SEQ ID NO:8具有至少99.4%同一性的核酸序列,
 - (d) 与SEQ ID NO:9具有至少99.1%同一性的核酸序列,
 - (e) 与SEQ ID NO:10具有至少81.25%同一性的核酸序列,
 - (f) 与SEQ ID NO:11具有至少90.83%同一性的核酸序列,以及
 - (g) 与SEQ ID NO:12具有至少82.5%同一性的核酸序列。
2. 一种腺病毒或腺病毒载体,其包括一种或多种选自下组的核酸序列:
 - (a) 包括SEQ ID NO:6的至少162个连续的核苷酸的核酸序列,
 - (b) 包括SEQ ID NO:7的至少162个连续的核苷酸的核酸序列,
 - (c) 包括SEQ ID NO:8的至少121个连续的核苷酸的核酸序列,
 - (d) 包括SEQ ID NO:9的至少531个连续的核苷酸的核酸序列,
 - (e) 包括SEQ ID NO:10的至少156个连续的核苷酸的核酸序列,
 - (f) 包括SEQ ID NO:11的至少192个连续的核苷酸的核酸序列,以及
 - (g) 包括SEQ ID NO:12的至少84个连续的核苷酸的核酸序列。
3. 一种腺病毒或腺病毒载体,其包括一种或多种选自下组的氨基酸序列:
 - (a) 与SEQ ID NO:14具有至少85%同一性的氨基酸序列,
 - (b) 与SEQ ID NO:15具有至少80%同一性的氨基酸序列,以及
 - (c) 与SEQ ID NO:16具有至少80%同一性的氨基酸序列。
4. 一种腺病毒或腺病毒载体,其包括一种或多种选自下组的核酸序列:
 - (a) 编码与SEQ ID NO:13具有至少99.7%同一性的氨基酸序列的核酸序列,
 - (b) 编码与SEQ ID NO:14具有至少85%同一性的氨基酸序列的核酸序列,
 - (c) 编码与SEQ ID NO:15具有至少80%同一性的氨基酸序列的核酸序列,以及
 - (d) 编码与SEQ ID NO:16具有至少80%同一性的氨基酸序列的核酸序列。
5. 一种腺病毒或腺病毒载体,其包括一种或多种选自下组的氨基酸序列:
 - (a) 与SEQ ID NO:18具有至少99%同一性的氨基酸序列,
 - (b) 与SEQ ID NO:20具有至少81.4%同一性的氨基酸序列,
 - (c) 与SEQ ID NO:21具有至少91.3%同一性的氨基酸序列,以及
 - (d) 与SEQ ID NO:22具有至少83.4%同一性的氨基酸序列。
6. 一种腺病毒或腺病毒载体,其包括一种或多种选自下组的核酸序列:
 - (a) 编码与SEQ ID NO:18具有至少99%同一性的氨基酸序列的核酸序列,
 - (b) 编码与SEQ ID NO:19具有至少99.75%同一性的氨基酸序列的核酸序列,
 - (c) 编码与SEQ ID NO:20具有至少81.4%同一性的氨基酸序列的核酸序列,
 - (d) 编码与SEQ ID NO:21具有至少91.3%同一性的氨基酸序列的核酸序列,
 - (e) 编码与SEQ ID NO:22具有至少83.4%同一性的氨基酸序列的核酸序列。
7. 一种腺病毒或腺病毒载体,其包括一种或多种选自下组的氨基酸序列:
 - (a) 包括SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列,
 - (b) 包括SEQ ID NO:20的至少114个连续的氨基酸残基的氨基酸序列,

(c) 包括SEQ ID NO:21的至少231个连续的氨基酸残基的氨基酸序列,以及

(d) 包括SEQ ID NO:22的至少30个连续的氨基酸残基的氨基酸序列。

8. 一种腺病毒或腺病毒载体,其包括一种或多种选自下组的核酸序列:

(a) 编码包括SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列的核酸序列,

(b) 编码包括SEQ ID NO:19的至少428个连续的氨基酸残基的氨基酸序列的核酸序列,

(c) 编码包括SEQ ID NO:20的至少114个连续的氨基酸残基的氨基酸序列的核酸序列,

(d) 编码包括SEQ ID NO:21的至少231个连续的氨基酸残基的氨基酸序列的核酸序列,

以及

(e) 编码包括SEQ ID NO:22的至少30个连续的氨基酸残基的氨基酸序列的核酸序列。

9. 根据权利要求1-8任意一项所述的腺病毒或腺病毒载体,其进一步包括转基因。

10. 一种组合物,所述组合物包括权利要求1-9任意一项所述的腺病毒或腺病毒载体以及药学上可接受的载体。

猴腺病毒(大猩猩)或腺病毒载体及其使用方法

[0001] 相关申请的交叉引用

[0002] 本申请是申请号为201280059668.2、申请日为2012年10月5日、发明名称为“猴腺病毒(大猩猩)或腺病毒载体及其使用方法”的中国发明专利申请的分案申请,原申请为国际申请号为PCT/US2012/058978的国家阶段申请,该国际申请要求2011年10月5日提交的申请号为61/543,652的美国临时专利申请的优先权,其通过引用并入本申请。

[0003] 通过引用并入的电子提交材料

[0004] 与本申请一并提交的可机读核苷酸/氨基酸序列表标识如下:2012年10月5日创建的文件名为“711249Sequence Listing_ST25.TXT”的719,903字节的ASCII(文本)文件,其通过引用整体并入本申请。

[0005] 发明背景

[0006] 以生物学上有意义的形式和量在体内递送蛋白已阻碍药物和疫苗的发展数十年。已证实成功代替了传统蛋白递送方法的一项措施是递送在体内产生蛋白的外源性核酸序列。基因转移载体理想地能进入多种细胞类型,能够接纳较大的核酸序列,是安全的并且能够生产出治疗患者所需的量。病毒载体是具有这些有利性质的基因转移载体(参见例如Thomas等,Nature Review Genetics,4:346-358(2003))。而且,尽管多种病毒载体被工程化改造以感染广泛的细胞类型,但是还能够对病毒载体进行修饰,使其靶向特定的细胞类型,这能够增强这种载体的治疗效力(参见例如Kay等,Nature Medicine,7(1):33-40(2001))。

[0007] 在用于治疗目的将外源性蛋白递送至哺乳动物细胞方面,使用病毒载体已经取得了一些成功,包括例如逆转录病毒(参见例如Cavazzana-Calvo等,Science,288(5466):669-672(2000))、慢病毒(参见例如Cartier等,Science,326:818-823(2009))、腺相关病毒(AAV)(参见例如Mease等,Journal of Rheumatology,27(4):692-703(2010))、单纯疱疹病毒(HSV)(参见例如Goins等,Gene Ther.,16(4):558-569(2009))、牛痘病毒(参见例如Mayrhofer等,J.Virol.,83(10):5192-5203(2009))和腺病毒(参见例如Lasaro和Ertl,Molecular Therapy,17(8):1333-1339(2009))。

[0008] 尽管具有有利的性质,但是病毒基因转移载体的广泛使用仍受到一些因素的阻碍。在这方面,某些细胞不会容易地顺从通过目前可用的病毒载体进行的基因递送。例如,淋巴细胞在摄取腺病毒方面存在障碍(Silver等,Virology,165:377-387(1988)),和Horvath等,J.Virology,62(1):341-345(1988))。此外,整合至宿主细胞基因组的病毒载体(例如逆转录病毒载体)可能会导致在癌基因中插入突变(参见例如Cavazzana-Calvo等,如上所示,和Hacein-Bey-Abina等,N.Engl.J.Med.,348:255-256(2003))。

[0009] 病毒载体用于基因转移的用途还受到病毒载体免疫原性的阻碍。大多数美国人已经接触过多种野生形式的目前正作为基因转移载体开发的病毒(例如腺病毒)。其结果是,很多美国人口已经预先产生了对基于某些病毒的基因转移载体的免疫力。这种载体在血液中会被迅速清除,从而减弱了载体递送生物学上有意义的量的基因产物的有效性。而且,某些病毒载体的免疫原性妨碍了有效的重复给药,当在疫苗应用中使用病毒载体时,这可以

有利于“激发”针对病原体的免疫系统,从而导致病毒载体的剂量中仅有一小部分有效地将载荷的物质递送至宿主细胞。

[0010] 因此,仍需要能够用于在体内有效地将基因递送至哺乳动物细胞的改良的病毒载体。本发明提供了这种病毒载体。

[0011] 发明概述

[0012] 本发明提供了一种腺病毒或腺病毒载体。所述腺病毒或腺病毒载体包括一种或多种选自下组的核酸序列:(a)与SEQ ID NO:1具有至少96%一致性的核酸序列,(b)与SEQ ID NO:2具有至少99%一致性的核酸序列,(c)与SEQ ID NO:3具有至少80%一致性的核酸序列,(d)与SEQ ID NO:4具有至少80%一致性的核酸序列和(e)与SEQ ID NO:5具有至少85.4%一致性的核酸序列。

[0013] 本发明提供了一种包括一种或多种选自下组的核酸序列的腺病毒或腺病毒载体:(a)与SEQ ID NO:6具有至少98.7%一致性的核酸序列,(b)与SEQ ID NO:7具有至少98.9%一致性的核酸序列,(c)与SEQ ID NO:8具有至少99.4%一致性的核酸序列,(d)与SEQ ID NO:9具有至少99.1%一致性的核酸序列,(e)与SEQ ID NO:10具有至少81.25%一致性的核酸序列,(f)与SEQ ID NO:11具有至少90.83%一致性的核酸序列和(g)与SEQ ID NO:12具有至少82.5%一致性的核酸序列。

[0014] 本发明提供了一种包括一种或多种选自下组的核酸序列的腺病毒或腺病毒载体:(a)包括SEQ ID NO:6的至少162个连续的核苷酸的核酸序列,(b)包括SEQ ID NO:7的至少162个连续的核苷酸的核酸序列,(c)包括SEQ ID NO:8的至少121个连续的核苷酸的核酸序列,(d)包括SEQ ID NO:9的至少531个连续的核苷酸的核酸序列,(e)包括SEQ ID NO:10的至少156个连续的核苷酸的核酸序列,(f)包括SEQ ID NO:11的至少192个连续的核苷酸的核酸序列和(g)包括SEQ ID NO:12的至少84个连续的核苷酸的核酸序列。

[0015] 本发明提供了一种包括一种或多种选自下组的氨基酸序列的腺病毒或腺病毒载体:(a)与SEQ ID NO:14具有至少85%一致性的氨基酸序列,(b)与SEQ ID NO:15具有至少80%一致性的氨基酸序列和(c)与SEQ ID NO:16具有至少80%一致性的氨基酸序列。

[0016] 本发明提供了一种包括一种或多种选自下组的核酸序列的腺病毒或腺病毒载体:(a)编码与SEQ ID NO:13具有至少99.7%一致性的氨基酸序列的核酸序列,(b)编码与SEQ ID NO:14具有至少85%一致性的氨基酸序列的核酸序列,(c)编码与SEQ ID NO:15具有至少80%一致性的氨基酸序列的核酸序列和(d)编码与SEQ ID NO:16具有至少80%一致性的氨基酸序列的核酸序列。

[0017] 本发明提供了一种包括一种或多种选自下组的氨基酸序列的腺病毒或腺病毒载体:(a)与SEQ ID NO:18具有至少99%一致性的氨基酸序列,(b)与SEQ ID NO:20具有至少81.4%一致性的氨基酸序列,(c)与SEQ ID NO:21具有至少91.3%一致性的氨基酸序列和(d)与SEQ ID NO:22具有至少83.4%一致性的氨基酸序列。

[0018] 本发明提供了一种包括一种或多种选自下组的核酸序列的腺病毒或腺病毒载体:(a)编码与SEQ ID NO:18具有至少99%一致性的氨基酸序列的核酸序列,(b)编码与SEQ ID NO:19具有至少99.75%一致性的氨基酸序列的核酸序列,(c)编码与SEQ ID NO:20具有至少81.4%一致性的氨基酸序列的核酸序列,(d)编码与SEQ ID NO:21具有至少91.3%一致性的氨基酸序列的核酸序列,(e)编码与SEQ ID NO:22具有至少83.4%一致性的氨基酸序

列的核酸序列。

[0019] 本发明提供了一种包括一种或多种选自下组的氨基酸序列的腺病毒或腺病毒载体：(a) 包括SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列，(b) 包括SEQ ID NO:20的至少114个连续的氨基酸残基的氨基酸序列，(c) 包括SEQ ID NO:21的至少231个连续的氨基酸残基的氨基酸序列和(d) 包括SEQ ID NO:22的至少30个连续的氨基酸残基的氨基酸序列。

[0020] 本发明提供了一种包括一种或多种选自下组的核酸序列的腺病毒或腺病毒载体：(a) 编码包括SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列的核酸序列，(b) 编码包括SEQ ID NO:19的至少428个连续的氨基酸残基的氨基酸序列的核酸序列，(c) 编码包括SEQ ID NO:20的至少114个连续的氨基酸残基的氨基酸序列的核酸序列，(d) 编码包括SEQ ID NO:21的至少231个连续的氨基酸残基的氨基酸序列的核酸序列和(e) 编码包括SEQ ID NO:22的至少30个连续的氨基酸残基的氨基酸序列的核酸序列。

[0021] 发明详述

[0022] 腺病毒通常与人类的良性病变相关，并且已对从多个种属(包括人)中分离得到的腺病毒基因组进行了广泛的研究。腺病毒是中型的(90–100nm)，含有约36kb双链DNA的无包膜二十四面体病毒。腺病毒的衣壳介导病毒感染细胞早期阶段的关键相互作用，并且在腺病毒生命周期结束时用于包装腺病毒基因组。所述衣壳含有252个壳粒，其包括240个六邻体、12个五邻体基底蛋白和12个纤维(Ginsberg等, *Virology*, 28:782–83 (1966))。所述六邻体包含三个相同的蛋白，即多肽II (Roberts等, *Science*, 232:1148–51 (1986))。所述五邻体基底包含五个相同的蛋白以及所述纤维包含三个相同的蛋白。蛋白IIIa、VI和IX存在于腺病毒的外壳中并且其被认为具有稳定病毒衣壳的作用(Stewart等, *Cell*, 67:145–54 (1991) 和Stewart等, *EMBO J.*, 12 (7):2589–99 (1993))。除了pIX以外，衣壳蛋白的表达依赖于腺病毒聚合酶蛋白。因此，仅当聚合酶蛋白基因存在和表达时，腺病毒颗粒的主要成分才能由基因组表达。

[0023] 腺病毒的若干特性使其成为治疗应用(即“基因治疗”)中用于将遗传物质转移至细胞或者疫苗应用中作为抗原递送系统使用的理想载体。例如，腺病毒能够以较高滴度(例如约 10^{13} 颗粒单位(pu))生产，并且能够将遗传物质转移至非复制和复制细胞。可以对腺病毒基因组进行操作使其携带大量的外源性DNA(高达约8kb)，并且腺病毒衣壳能够增强更长的序列的转移(Curiel等, *Hum. Gene Ther.*, 3:147–154 (1992))。此外，腺病毒通常不会整合至宿主细胞的染色体，而是保持线性游离体形式，从而极大降低了重组的腺病毒干扰正常细胞功能的可能性。

[0024] 本发明至少部分地基于此前未经鉴定或分离的腺病毒的发现和分离。本申请所述的腺病毒分离自大猩猩。在东部大猩猩(*Gorilla beringei*)和西部大猩猩(*Gorilla gorilla*)两个种属中有四个广泛认可的大猩猩亚种。西部大猩猩种属包括亚种西部低地大猩猩(*Gorilla gorilla gorilla*)和克罗斯河大猩猩(*Gorilla gorilla diehli*)。东部大猩猩种属包括亚种山地大猩猩(*Gorilla beringei beringei*)和东部低地大猩猩(*Gorilla beringei graueri*) (参见例如Wilson和Reeder, eds., *Mammalian Species of the World*, 3rd ed., Johns Hopkins University Press, Baltimore, Maryland (2005))。本发明的腺病毒分离自东部低地大猩猩(*Gorilla beringei graueri*)。

[0025] 已对若干这种腺病毒的基因组进行了分析,并且已确定所述腺病毒可以具有核酸序列例如SEQ ID NO:23、SEQ ID NO:24、SEQ ID NO:25、SEQ ID NO:26、SEQ ID NO:27、SEQ ID NO:28、SEQ ID NO:29、SEQ ID NO:30、SEQ ID NO:31、SEQ ID NO:32、SEQ ID NO:33、SEQ ID NO:34、SEQ ID NO:35或SEQ ID NO:36,其均包括多个子序列以唯一地定义所述腺病毒,即核酸序列SEQ ID NO:1-12和氨基酸序列SEQ ID NO:13-22。SEQ ID NO:6-12分别编码氨基酸序列SEQ ID NO:17-22。SEQ ID NO:1-5分别是核酸序列SEQ ID NO:6和9-12的子集。SEQ ID NO:13-16分别是氨基酸序列SEQ ID NO:19-22的子集。

[0026] 可以按照此前已知的将腺病毒用作腺病毒载体例如基因递送载体的相同方式对腺病毒进行修饰。

[0027] 术语“腺病毒”,如本申请所使用的,指保留了参与腺病毒生命周期的能力且未通过例如破裂(例如超声)、变性(例如使用加热或溶剂)或者交联键(例如通过福尔马林形成交联)被物理灭活的腺病毒。所述“腺病毒生命周期”包括(1)病毒结合和进入细胞,(2)腺病毒基因组转录和腺病毒蛋白翻译,(3)腺病毒基因组复制和(4)病毒粒子组装(参见例如Fields Virology,5th ed.,Knipe等(eds.),Lippincott Williams&Wilkins,Philadelphia,PA(2006))。

[0028] 术语“腺病毒载体”,如本申请所使用的,指其中的腺病毒基因组已被操作以容纳相对于腺病毒基因组是非天然的核酸序列的腺病毒。典型地,通过将一个或多个突变(例如缺失、插入或取代)引入腺病毒的腺病毒基因组以容纳非天然核酸序列(例如用于基因转移的核酸序列)插入至腺病毒的方法产生腺病毒载体。

[0029] 腺病毒和腺病毒载体可以是具有复制能力的、有条件地具有复制能力的或复制缺陷的。具有复制能力的腺病毒或腺病毒载体能够在典型的宿主细胞(即典型地能够被腺病毒感染)中复制。具有复制能力的腺病毒或腺病毒载体与野生型腺病毒相比在腺病毒基因组中具有一个或多个突变(例如一个或多个缺失、插入和/或取代),但该突变不会抑制其在宿主细胞中的病毒复制的腺病毒。例如,所述腺病毒或腺病毒载体能够部分或完全缺失称为E3区的腺病毒早期区,其不是腺病毒或腺病毒基因组的繁殖所必须的。

[0030] 有条件地复制的腺病毒或腺病毒载体是被设计成在预先确定的条件下复制的腺病毒或腺病毒载体。例如,可以将复制必需的基因功能例如由腺病毒早期区编码的基因功能可操作地与可诱导的、可阻遏的或组织特异性转录控制序列例如启动子连接。在这种实施方式中,复制需要存在或不存在与转录控制序列相互作用的特异性因素。在美国专利号5,998,205中进一步描述了有条件地复制的腺病毒载体。

[0031] 复制缺陷的腺病毒或腺病毒载体是需要补偿一个或多个复制所需的腺病毒基因组的功能或区域的腺病毒或腺病毒载体,作为结果,例如一个或多个复制必需基因功能或区域的缺乏,使得腺病毒或腺病毒载体在典型的宿主细胞中不能复制,特别是在被腺病毒或腺病毒载体所感染的人体中的那些宿主细胞。

[0032] 在基因功能或基因组区域中的缺陷,如本申请所使用的,被定义为腺病毒基因组足量的遗传物质被破坏(例如缺失)以消除或损害所述基因的功能(例如基因产物的功能降低至少约2倍、5倍、10倍、20倍、30倍或50倍),其核酸序列全部或部分被破坏(例如缺失)。整个基因区的缺失通常不需要破坏复制必需基因功能。但是,出于在腺病毒基因组中为一个或多个转基因的提供足够空间的目的,除去一个或多个基因区的大部分可能是可取的。尽

管缺失遗传物质是优选地,但是通过增加或取代使遗传物质突变也适用于破坏基因功能。复制必需基因功能是腺病毒复制(例如繁殖)所需的基因功能并且其由例如腺病毒早期区(例如E1、E2和E4区)、晚期区(例如L1、L2、L3、L4和L5区)、参与病毒包装的基因(例如IVa2基因)和病毒相关RNA(例如VA-RNA-1和/或VA-RNA-2)所编码。无论腺病毒或腺病毒载体是具有复制能力的还是复制缺陷的,所述腺病毒或腺病毒载体仍保留至少一部分腺病毒基因组。所述腺病毒或腺病毒载体可以包含腺病毒基因组的任意一部分,包括蛋白编码和非蛋白编码区。理想地,所述腺病毒或腺病毒载体包含编码腺病毒蛋白的至少一条核酸序列。所述腺病毒或腺病毒载体可以包含编码任意适宜地腺病毒蛋白的核酸序列,其中腺病毒蛋白例如由任意一个早期区基因(即E1A、E1B、E2A、E2B、E3和/或E4区)编码的蛋白,或者由任意一个晚期区基因编码的蛋白,所述基因编码病毒结构蛋白(即L1、L2、L3、L4和L5区)。

[0033] 理想地,所述腺病毒或腺病毒载体包含一个或多个编码pIX蛋白、DNA聚合酶蛋白、五邻体蛋白、六邻体蛋白和/或纤维蛋白的核酸序列。所述腺病毒或腺病毒载体可以包含编码腺病毒蛋白的全长氨基酸序列的全长核酸序列。或者,所述腺病毒或腺病毒载体可以包含编码腺病毒蛋白全长氨基酸序列一部分的全长核酸序列的一部分。

[0034] 核酸序列的“一部分”包含至少10个核苷酸(例如约10个至约5000个核苷酸)。优选地,核酸序列的“一部分”包含10个或更多个(例如15个或更多、20个或更多、25个或更多、30个或更多、35个或更多、40个或更多、45个或更多、50个或更多或者100个或更多)核苷酸,但是少于5,000个(例如4900个或更少、4000个或更少、3000个或更少、2000个或更少、1000个或更少、800个或更少、500个或更少、300个或更少或者100个或更少)核苷酸。优选地,核酸序列的一部分是约10个至约3500个核苷酸(例如约10、20、30、50、100、300、500、700、1000、1500、2000、2500或3000个核苷酸),约10个至约1000个核苷酸(例如约25、55、125、325、525、725或925个核苷酸),或约10个至约500个核苷酸(例如约15、30、40、50、60、70、80、90、150、175、250、275、350、375、450、475、480、490、495或499个核苷酸),或者任意两个前述值所定义的范围。更优选地,核酸序列的“一部分”包含不超过约3200个核苷酸(例如约10个至约3200个核苷酸、约10个至约3000个核苷酸、或约30个至约500个核苷酸,或者任意两个前述值所定义的范围)。

[0035] 氨基酸序列的“一部分”包含至少3个氨基酸(例如约3个至约1,200个氨基酸)。优选地,氨基酸序列的“一部分”包含3个或更多个(例如5个或更多、10个或更多、15个或更多、20个或更多、25个或更多、30个或更多、40个或更多或者50个或更多)氨基酸,但是少于1,200个(例如1,000个或更少、800个或更少、700个或更少、600个或更少、500个或更少、400个或更少、300个或更少、200个或更少或者100个或更少)氨基酸。优选地,氨基酸序列的一部分是约3个至约500个氨基酸(例如约10、100、200、300、400或500个氨基酸),约3个至约300个氨基酸(例如约20、50、75、95、150、175或200个氨基酸),或约3个至约100个氨基酸(例如约15、25、35、40、45、60、65、70、80、85、90、95或99个氨基酸),或者任意两个前述值所定义的范围。更优选地,氨基酸序列的“一部分”包含不超过约500个氨基酸(例如约3个至约400个氨基酸、约10个至约250个氨基酸、约50个至约100个氨基酸,或者任意两个前述值所定义的范围)。

[0036] 腺病毒pIX蛋白存在于腺病毒衣壳中,已发现其增强六邻体九聚物之间的相互作用,并且是包装全长基因组所必需的(参见例如Boulangier等,J.Gen.Virol.,44:783-800

(1979); Horwitz M.S., "Adenoviridae and their replication" in *Virology*, 2nd ed., B.N.Fields等, (eds.), Raven Press, Ltd., New York, pp.1679-1721 (1990), Ghosh-Choudhury等, *EMBO J.*, 6:1733-1739 (1987) 和 van Oostrum等, *J.Virol.*, 56:439-448 (1985)。除了其对腺病毒结构的贡献以外,还发现pIX显示出了转录性质,如刺激腺病毒主要晚期启动子(MLP)活性(参见例如Lutz等, *J.Virol.*, 71(7):5102-5109 (1997))。编码全部或部分腺病毒pIX蛋白的核酸序列包括例如SEQ ID NO:6、SEQ ID NO:7、SEQ ID NO:8。SEQ ID NO:1是SEQ ID NO:6的子集。包含全长pIX蛋白或其部分的氨基酸序列包括例如SEQ ID NO:17和SEQ ID NO:18。

[0037] 腺病毒DNA聚合酶蛋白是病毒DNA在体外和体内复制所必需的。所述聚合酶与末端蛋白(TP)前体的复合物共纯化,所述末端蛋白与腺病毒DNA的5'端共价连接(Field等, *J.Biol.Chem.*, 259:9487-9495 (1984))。腺病毒DNA聚合酶和pTP均由E2区编码。所述聚合酶蛋白是除pIX以外的全部结构蛋白的表达所需的。没有聚合酶蛋白的基因序列,就不产生聚合酶蛋白。其结果是,病毒基因组不复制,主要的晚期启动子不活化和衣壳蛋白不表达。编码全部或部分腺病毒DNA聚合酶蛋白的核酸序列包括例如SEQ ID NO:9。SEQ ID NO:2是SEQ ID NO:9的子集。包括全长腺病毒DNA聚合酶或其部分的氨基酸序列包括例如SEQ ID NO:19和SEQ ID NO:13。

[0038] 腺病毒六邻体蛋白是腺病毒衣壳中最大的和最丰富的蛋白。所述六邻体蛋白是病毒衣壳组装、确定衣壳的十二面体对称性(其反过来定义了衣壳体积和DNA包装尺寸的限度)和衣壳的完整性所必需的。此外,为了减少腺病毒载体的中和,六邻体是修饰的主要靶点(参见例如Gall等, *J.Virol.*, 72:10260-264 (1998) 和Rux等, *J.Virol.*, 77(17):9553-9566 (2003))。六邻体蛋白的主要结构特征在不同血清型的腺病毒之间是相同的,但是六邻体蛋白的尺寸和免疫学性质在不同血清型之间不同(Jornvall等, *J.Biol.Chem.*, 256(12):6181-6186 (1981))。对15种腺病毒六邻体蛋白进行比较的结果显示六邻体的主要抗原和血清型特异性区域出现在环1和2(即分别为LI或11和LII或12),在其中存在七个在不同的腺病毒血清型之间长度和序列不同的离散的高变区(HVR1至HVR7)(Crawford-Miksza等, *J.Virol.*, 70(3):1836-1844 (1996))。编码全部或部分腺病毒六邻体蛋白的核酸序列包括例如SEQ ID NO:11和SEQ ID NO:4。包含全长腺病毒六邻体蛋白或其部分的氨基酸序列包括例如SEQ ID NO:21和SEQ ID NO:15。

[0039] 腺病毒纤维蛋白是具有三个结构域的腺病毒多肽IV的同源三聚体:尾、轴和隆突。(Devaux等, *J.Molec.Biol.*, 215:567-88 (1990), Yeh等, *Virus Res.*, 33:179-98 (1991))。所述纤维蛋白主要通过隆突和轴结构域介导病毒与细胞表面受体的结合(Henry等, *J.Virol.*, 68(8):5239-46 (1994))。用于三聚化的氨基酸序列位于隆突,其是所述纤维(尾)的氨基末端与五邻体基底结合所必需的(Novelli等, *Virology*, 185:365-76 (1991))。除了识别细胞受体并且与五邻体基底结合以外,所述纤维有助于血清型鉴定。来自于不同腺病毒血清型的纤维蛋白具有极大差异(参见例如Green等, *EMBO J.*, 2:1357-65 (1983), Chroboczek等, *Virology*, 186:280-85 (1992) 和Signas等, *J.Virol.*, 53:672-78 (1985))。因此,所述纤维蛋白具有对腺病毒的生命周期至关重要的多种功能。编码全部或部分腺病毒纤维蛋白的核酸序列包括例如SEQ ID NO:12和SEQ ID NO:5。包含全长腺病毒纤维蛋白或其部分的氨基酸序列包括例如SEQ ID NO:22和SEQ ID NO:16。

[0040] 腺病毒五邻体基底蛋白位于十二面体衣壳的顶部并且包含五个相同的单体。所述五邻体基底蛋白提供了用于将六邻体蛋白桥连于十二面体衣壳的多个小平面上的结构,并且提供了用于将纤维蛋白掺入衣壳的必要接口。所述五邻体基底的各单体均含有RGD三肽基序 (Neumann等, *Gene*, 69:153-157 (1988))。所述RGD三肽介导与 α v整合素的结合并且在五邻体基底的RGD序列具有点突变的腺病毒感染细胞的能力受到限制 (Bai等, *J. Virol.*, 67:5198-5205 (1993))。因此,五邻体基底蛋白是衣壳的结构和病毒-细胞相互作用的最大效能所必需的。编码全部或部分腺病毒五邻体基底蛋白的核酸序列包括例如SEQ ID NO:10和SEQ ID NO:3。包含全长腺病毒五邻体基底蛋白或其部分的氨基酸序列包括例如SEQ ID NO:20和SEQ ID NO:14。

[0041] 核酸或氨基酸序列的“一致性”,如本申请所述,可以通过将目的核酸或氨基酸序列与对照核酸或氨基酸序列比较确定。计数在对照序列中被改变和/或修饰 (例如通过点突变、插入或缺失) 以得到目的序列的核苷酸或氨基酸残基的数量。从目的序列的总长减去这种改变的总数,用所述差值除以目的序列的长度并以百分率表示。用于获得最佳比对和计算两条或多条序列之间一致性多种数学算法是公知的并且被纳入多种可用的软件程序中。这种程序的例子包括CLUSTAL-W、T-Coffee和ALIGN (用于核酸和氨基酸序列的比对)、BLAST程序 (例如BLAST 2.1、BL2SEQ和之后的版本) 以及FASTA程序 (例如FASTA3x、FASTM和SSEARCH) (用于序列比对和序列相似性检索)。序列比对算法也公开于例如Altschul等, *J. Molecular Biol.*, 215 (3):403-410 (1990), Beigert等, *Proc. Natl. Acad. Sci. USA*, 106 (10):3770-3775 (2009), Durbin等编著 *Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids*, Cambridge University Press, Cambridge, UK (2009), Soding, *Bioinformatics*, 21 (7):951-960 (2005), Altschul等, *Nucleic Acids Res.*, 25 (17):3389-3402 (1997) 和Gusfield, *Algorithms on Strings, Trees and Sequences*, Cambridge University Press, Cambridge UK (1997) 中。

[0042] 在一个实施方式中,腺病毒或腺病毒载体包括一种或多种下述核酸序列:(a) 与SEQ ID NO:1具有至少96%一致性的核酸序列 (例如至少96%或100%一致性), (b) 与SEQ ID NO:2具有至少99%一致性的核酸序列 (例如至少99.08%、至少99.16%、至少99.23%、至少99.31%、至少99.39%、至少99.47%、至少99.55%、至少99.62%、至少99.70%、至少99.78%、至少99.86%、至少99.93%或100%一致性), (c) 与SEQ ID NO:3具有至少80%一致性的核酸序列 (例如至少82%、至少84.22%、至少86.44%、至少88.67%、至少90.89%、至少93.11%、至少95.33%、至少97.56%、至少99.78%或100%一致性), (d) 与SEQ ID NO:4具有至少80%一致性的核酸序列 (例如至少80.58%、至少82.67%、至少84.75%、至少86.83%、至少88.92%、至少91.00%、至少93.08%、至少95.17%、至少97.25%、至少99.33%或100%一致性) 和 (e) 与SEQ ID NO:5具有至少85.4%一致性的核酸序列 (例如至少87.48%、至少89.57%、至少91.65%、至少93.73%、至少95.82%、至少97.90%、至少99.98%或100%一致性)。

[0043] 所述腺病毒或腺病毒载体可以单独包含一种、两种、三种、四种或全部五种前述序列或其任意组合。在这一方面,所述腺病毒或腺病毒载体可以包括任意两种前述序列的任意组合、任意三种前述序列的任意组合、任意四种前述序列的任意组合或者全部五种前述序列。例如,所述腺病毒或腺病毒载体可以包括与SEQ ID NO:1具有至少96%一致性的核酸

序列。所述腺病毒或腺病毒载体可以包括与SEQ ID NO:2具有至少99%一致性的核酸序列和与SEQ ID NO:3具有至少80%一致性的核酸序列。所述腺病毒或腺病毒载体可以包括与SEQ ID NO:1具有至少96%一致性的核酸序列,与SEQ ID NO:3具有至少80%一致性的核酸序列和与SEQ ID NO:5具有至少85.4%一致性的核酸序列。所述腺病毒或腺病毒载体可以包括(a) SEQ ID NO:1所示的核酸序列,(b) SEQ ID NO:2所示的核酸序列,(c) SEQ ID NO:3所示的核酸序列,(d) SEQ ID NO:4所示的核酸序列或(e) SEQ ID NO:5所示的核酸序列。所述腺病毒或腺病毒载体可以包括(a)与SEQ ID NO:1具有至少96%一致性的核酸序列,(b)与SEQ ID NO:2具有至少99%一致性的核酸序列,(c)与SEQ ID NO:3具有至少80%一致性的核酸序列,(d)与SEQ ID NO:4具有至少80%一致性的核酸序列和(e)与SEQ ID NO:5具有至少85.4%一致性的核酸序列。所述腺病毒或腺病毒载体可以包括(a) SEQ ID NO:1所示的核酸序列,(b) SEQ ID NO:2所示的核酸序列,(c) SEQ ID NO:3所示的核酸序列,(d) SEQ ID NO:4所示的核酸序列和(e) SEQ ID NO:5所示的核酸序列。

[0044] 在另一个实施方式中,所述腺病毒或腺病毒载体包括一种或多种下述核酸序列:(a)与SEQ ID NO:6具有至少98.7%一致性的核酸序列(例如至少98.95%、至少99.20%、至少99.45%、至少99.70%、至少99.95%或100%一致性),(b)与SEQ ID NO:7具有至少98.9%一致性的核酸序列(例如至少99.15%、至少99.40%、至少99.65%、至少99.90%或100%一致性),(c)与SEQ ID NO:8具有至少99.4%一致性的核酸序列(例如至少99.65%、至少99.90%或100%一致性),(d)与SEQ ID NO:9具有至少99.1%一致性的核酸序列(例如至少99.13%、至少99.16%、至少99.19%、至少99.23%、至少99.26%、至少99.29%、至少99.32%、至少99.35%、至少99.38%、至少99.42%、至少99.45%、至少99.48%、至少99.51%、至少99.54%、至少99.57%、至少99.61%、至少99.64%、至少99.67%、至少99.70%、至少99.73%、至少99.76%、至少99.79%、至少99.83%、至少99.86%、至少99.89%、至少99.92%、至少99.95%、至少99.98%或100%一致性),(e)与SEQ ID NO:10具有至少81.25%一致性的核酸序列(例如至少81.30%、至少81.36%、至少81.41%、至少81.46%、至少81.52%、至少81.57%、至少81.63%、至少81.68%、至少81.73%、至少81.79%、至少81.84%、至少81.89%、至少81.95%、至少82.00%、至少82.06%、至少82.11%、至少82.16%、至少82.22%、至少82.27%、至少82.32%、至少82.38%、至少82.43%、至少82.48%、至少82.54%、至少82.59%、至少82.65%、至少82.70%、至少82.75%、至少82.81%、至少82.86%、至少82.91%、至少82.97%、至少83.02%、至少83.08%、至少83.13%、至少83.18%、至少83.24%、至少83.29%、至少83.34%、至少83.40%、至少83.45%、至少83.50%、至少83.56%、至少83.61%、至少83.67%、至少83.72%、至少83.77%、至少83.83%、至少83.88%、至少83.93%、至少83.99%、至少84.04%、至少84.09%、至少84.15%、至少84.20%、至少84.26%、至少84.31%、至少84.36%、至少84.42%、至少84.47%、至少84.52%、至少84.58%、至少84.63%、至少84.69%、至少84.74%、至少84.79%、至少84.85%、至少84.90%、至少84.95%、至少85.01%、至少85.06%、至少85.11%、至少85.17%、至少85.22%、至少85.28%、至少85.33%、至少85.38%、至少85.44%、至少85.49%、至少85.54%、至少85.60%、至少85.65%、至少85.71%、至少85.76%、至少85.81%、至少85.87%、至少85.92%、至少85.97%、至少86.03%、至少86.08%、至少86.13%、至少86.19%、至少86.24%、至少

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[0045] 所述腺病毒或腺病毒载体可以单独包含一种、两种、三种、四种、五种、六种或全部七种前述序列或其任意组合。在这一方面,所述腺病毒或腺病毒载体可以包括任意两种前述序列的任意组合、任意三种前述序列的任意组合、任意四种前述序列的任意组合、任意五种前述序列的任意组合或者全部六种前述序列。例如,所述腺病毒或腺病毒载体可以包括与SEQ ID NO:6具有至少98.7%一致性的核酸序列。所述腺病毒或腺病毒载体可以包括与SEQ ID NO:10具有至少81.25%一致性的核酸序列和与SEQ ID NO:12具有至少82.50%一致性的核酸序列。所述腺病毒或腺病毒载体可以包括与SEQ ID NO:8具有至少99.4%一致性的核酸序列,与SEQ ID NO:9具有至少99.1%一致性的核酸序列,与SEQ ID NO:10具有至少81.25%一致性的核酸序列和与SEQ ID NO:12具有至少82.5%一致性的核酸序列。所述腺病毒或腺病毒载体可以包括(a) SEQ ID NO:6所示的核酸序列,(b) SEQ ID NO:9所示的核酸序列,(c) SEQ ID NO:10所示的核酸序列,(d) SEQ ID NO:11所示的核酸序列或(e) SEQ ID NO:12所示的核酸序列。所述腺病毒或腺病毒载体可以包括(a) 与SEQ ID NO:8具有至少99.4%一致性的核酸序列,(b) 与SEQ ID NO:9具有至少99.1%一致性的核酸序列,(c) 与SEQ ID NO:10具有至少81.25%一致性的核酸序列,(d) 与SEQ ID NO:11具有至少90.83%一致性的核酸序列和(e) 与SEQ ID NO:12具有至少82.5%一致性的核酸序列。所述腺病毒或腺病毒载体可以包括(a) SEQ ID NO:7所示的核酸序列,(b) SEQ ID NO:9所示的核酸序列,(c) SEQ ID NO:10所示的核酸序列,(d) SEQ ID NO:11所示的核酸序列和(e) SEQ ID NO:

12所示的核酸序列。

[0046] 在另一个实施方式中,所述腺病毒或腺病毒载体包括一种或多种下述核酸序列:(a)包括SEQ ID NO:6的至少162个连续的核苷酸的核酸序列,(b)包括SEQ ID NO:7的至少162个连续的核苷酸的核酸序列,(c)包括SEQ ID NO:8的至少121个连续的核苷酸的核酸序列,(d)包括SEQ ID NO:9的至少531个连续的核苷酸的核酸序列,(e)包括SEQ ID NO:10的至少156个连续的核苷酸的核酸序列,(f)包括SEQ ID NO:11的至少192个连续的核苷酸的核酸序列或(g)包括SEQ ID NO:12的至少84个连续的核苷酸的核酸序列。所述腺病毒或腺病毒载体可以包括SEQ ID NO:6的至少162个(例如165个或更多、170个或更多、190个或更多、200个或更多、250个或更多或者300个或更多)连续的核苷酸,但不超过SEQ ID NO:6的399个(例如398个或更少、350个或更少或者275个或更少)连续的核苷酸的核酸序列。优选地,所述腺病毒或腺病毒载体包括包含SEQ ID NO:6的162至300个连续的核苷酸(例如163、175、200、250或275个连续的核苷酸)或者162至200个连续的核苷酸(例如164、166、167、168、169、171、172、173、174、176、177、178、179、180、181、182、183、184、185、186、187、188、189、191、192、193、194、195、196、197、198或199个连续的核苷酸)或者任意两个前述值所定义的范围的核酸序列。

[0047] 所述腺病毒或腺病毒载体可以包括SEQ ID NO:7的至少162个(例如165个或更多、170个或更多、190个或更多、200个或更多、250个或更多或者300个或更多)连续的核苷酸,但不超过SEQ ID NO:7的399个(例如398个或更少、350个或更少或者275个或更少)连续的核苷酸的核酸序列。优选地,所述腺病毒或腺病毒载体包括包含SEQ ID NO:7的162至300个连续的核苷酸(例如163、175、200、250或275个连续的核苷酸)或者162至200个连续的核苷酸(例如164、166、167、168、169、171、172、173、174、176、177、178、179、180、181、182、183、184、185、186、187、188、189、191、192、193、194、195、196、197、198或199个连续的核苷酸)或者任意两个前述值所定义的范围的核酸序列。

[0048] 所述腺病毒或腺病毒载体可以包括SEQ ID NO:8的至少121个(例如125个或更多、130个或更多、150个或更多、200个或更多、250个或更多或者300个或更多)连续的核苷酸,但不超过SEQ ID NO:8的399个(例如398个或更少、350个或更少或者275个或更少)连续的核苷酸的核酸序列。优选地,所述腺病毒或腺病毒载体包括包含SEQ ID NO:8的121至300个连续的核苷酸(例如125、150、175、200、250或275个连续的核苷酸),121至200个连续的核苷酸(例如130、140、145、160、165、170、180、185、190、195或199个连续的核苷酸)或121至150个连续的核苷酸(例如122、123、124、125、126、127、128、129、130、131、132、133、134、135、136、137、138、139、140、141、142、143、144、145、146、147、148或149个连续的核苷酸)或者任意两个前述值所定义的范围的核酸序列。

[0049] 所述腺病毒或腺病毒载体可以包括SEQ ID NO:9的至少531个(例如540个或更多、600个或更多、700个或更多、800个或更多、900个或更多或者1,000个或更多)连续的核苷酸,但不超过SEQ ID NO:9的3168个(例如3,100或更少、3,000个或更少、2,500个或更少、2,000个或更少或者1,500个或更少)连续的核苷酸的核酸序列。优选地,所述腺病毒或腺病毒载体包括包含SEQ ID NO:9的531至2,000个连续的核苷酸(例如550、600、700、1,000、1,200、1,500或1,700个连续的核苷酸),531至1,000个连续的核苷酸(例如535、575、600、650、675、725、750、800、850、900或950个连续的核苷酸)或531至800个连续的核苷酸(例如540、

545、550、560、565、570、580、585、590、595、615、625、630、640、660、665、670、680、685、690、695、705、715、730、740、755、760、765、770、775、780、785、790、795或799个连续的核苷酸)或者任意两个前述值所定义的范围的核酸序列。

[0050] 所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:10的至少156个(例如160个或更多、200个或更多、225个或更多、235个或更多、250个或更多、300个或更多、350个或更多、400个或更多、450个或更多或者500个或更多)连续的核苷酸,但不超过SEQ ID NO:10的1,863个(例如1,800个或更少、1,500个或更少、1,200个或更少、1,000个或者更少、850个或者更少、800个或者更少、750个或者更少或者700个或者更少)连续的核苷酸的核酸序列。优选地,所述腺病毒或腺病毒载体包括包含SEQ ID NO:10的156至1,500个连续的核苷酸(例如175、210、225、245、255、265、275、290、300、400、500、600、700、800、900、1,000或1,200个连续的核苷酸)、156至1,000个连续的核苷酸(例如165、180、185、195、205、230、240、260、270、295、350、450、550、650、750、850或950个连续的核苷酸)或者156至500个连续的核苷酸(例如199、230、235、290、305、310、315、325、340、345、360、365、370、375、380、385、390、395、405、425、430、440、455、460、465、470、475、480、485、490、495或499个连续的核苷酸)或者任意两个前述值所定义的范围的核酸序列。

[0051] 所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:11的至少192个(例如200个或更多、300个或更多、400个或更多、500个或更多或者600个或更多)连续的核苷酸,但不超过SEQ ID NO:11的2841个(2,800个或更少、2,500个或更少、2,000个或更少、1,800个或更少或者1,500个或者更少)连续的核苷酸的核酸序列。优选地,所述腺病毒或腺病毒载体包括包含SEQ ID NO:11的192至2,000个连续的核苷酸(例如200、250、300、350、400、450、500、550、600、650、700、800、900、1,000、1,200、1,500、1,700或1,900个连续的核苷酸)、192至1,000个连续的核苷酸(例如275、375、475、575、675、775、875或975个连续的核苷酸)或者192至500个连续的核苷酸(例如220、235、240、255、260、270、280、285、290、295、299、305、315、330、335、340、345、355、360、365、370、385、390、395、399、405、415、430、435、440、445、455、460、465、470、485、490、495、499个连续的核苷酸)或者任意两个前述值所定义的范围的核酸序列。

[0052] 所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:12的至少84个(例如90个或更多、100个或更多、200个或更多、300个或更多、500个或更多、700个或更多或者900个或更多)连续的核苷酸,但不超过SEQ ID NO:12的1,740个(1,700个或更少、1,500个或更少、1,200个或更少或者1,000个或者更少)连续的核苷酸的核酸序列。优选地,所述腺病毒或腺病毒载体包括包含SEQ ID NO:12的84至1,200个连续的核苷酸(例如95、100、200、400、600、800、1,000或1,200个连续的核苷酸)、84至1,000个连续的核苷酸(例如95、150、195、250、350、450、550、650、750、850或950个连续的核苷酸)或者84至500个连续的核苷酸(例如90、99、115、125、130、145、155、165、175、190、225、230、240、255、260、265、270、275、315、325、330、340、355、360、365、370、375、380、385、390、395、415、425、430、440、455、460、465、470、475、480、485、490、495或499个连续的核苷酸)或者任意两个前述值所定义的范围的核酸序列。

[0053] 所述腺病毒或腺病毒载体可以单独包含一种、两种、三种、四种、五种、六种或全部七种前述序列或其任意组合。在这一方面,所述腺病毒或腺病毒载体可以包括任意两种前

述序列的任意组合、任意三种前述序列的任意组合、任意四种前述序列的任意组合、任意五种前述序列的任意组合、任意六种前述序列的任意组合或者全部七种前述序列。例如,所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:6的至少162个连续的核苷酸的核酸序列。所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:8的至少121个连续的核苷酸的核酸序列,包含SEQ ID NO:10的至少156个连续的核苷酸的核酸序列和包含SEQ ID NO:12的至少84个连续的核苷酸的核酸序列。所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:7的至少162个连续的核苷酸的核酸序列,包含SEQ ID NO:9的至少531个连续的核苷酸的核酸序列和包含SEQ ID NO:11的至少192个连续的核苷酸的核酸序列。所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:8的至少121个连续的核苷酸的核酸序列和包含SEQ ID NO:9的至少531个连续的核苷酸的核酸序列。所述腺病毒或腺病毒载体可以包括(a)包含SEQ ID NO:6或SEQ ID NO:7至少162个连续的核苷酸的核酸序列,(b)包含SEQ ID NO:9至少531个连续的核苷酸的核酸序列,(c)包含SEQ ID NO:10至少156个连续的核苷酸的核酸序列,(d)包含SEQ ID NO:11至少192个连续的核苷酸的核酸序列和(e)包含SEQ ID NO:12至少84个连续的核苷酸的核酸序列。

[0054] 在另一个实施方式中,所述腺病毒或腺病毒载体包括一种或多种下述氨基酸序列:(a)与SEQ ID NO:14具有至少85%一致性的氨基酸序列(例如至少91.67%、至少98.33%或100%一致性),(b)与SEQ ID NO:15具有至少80%一致性的氨基酸序列(例如至少86.25%、至少92.50%、至少98.75%或100%一致性)和(c)与SEQ ID NO:16具有至少80%一致性的氨基酸序列(例如至少80.25%、至少86.50%、至少92.75%、至少99.00%或100%一致性)。

[0055] 所述腺病毒或腺病毒载体可以单独包含一种、两种或全部三种前述氨基酸序列或其任意组合。在这一方面,所述腺病毒或腺病毒载体可以包括前述序列之一、任意两种前述序列的任意组合或者全部三种前述序列。例如,所述腺病毒或腺病毒载体可以包括与SEQ ID NO:14具有至少85%一致性的氨基酸序列。所述腺病毒或腺病毒载体可以包括与SEQ ID NO:14具有至少85%一致性的氨基酸序列和与SEQ ID NO:15具有至少80%一致性的氨基酸序列。所述腺病毒或腺病毒载体可以包括(a)与SEQ ID NO:14具有至少85%一致性的氨基酸序列,(b)与SEQ ID NO:15具有至少80%一致性的氨基酸序列和(c)与SEQ ID NO:16具有至少80%一致性的氨基酸序列。所述腺病毒或腺病毒载体可以包括(a)SEQ ID NO:14所示的氨基酸序列,(b)SEQ ID NO:15所示的氨基酸序列或(c)SEQ ID NO:16所示的氨基酸序列。所述腺病毒或腺病毒载体可以包括(a)SEQ ID NO:14所示的氨基酸序列,(b)SEQ ID NO:15所示的氨基酸序列和(c)SEQ ID NO:16所示的氨基酸序列。

[0056] 在另一个实施方式中,所述腺病毒或腺病毒载体包括一种或多种下述氨基酸序列:(a)与SEQ ID NO:18具有至少99%一致性的氨基酸序列(例如至少99%或100%一致性),(b)与SEQ ID NO:20具有至少81.4%一致性的氨基酸序列(例如至少81.56%、至少81.72%、至少81.88%、至少82.04%、至少82.21%、至少82.37%、至少82.53%、至少82.69%、至少82.85%、至少83.01%、至少83.17%、至少83.33%、至少83.49%、至少83.65%、至少83.82%、至少83.98%、至少84.14%、至少84.30%、至少84.46%、至少84.62%、至少84.78%、至少84.94%、至少85.10%、至少85.26%、至少85.43%、至少85.59%、至少85.75%、至少85.91%、至少86.07%、至少86.23%、至少86.39%、至少

86.55%、至少86.71%、至少86.88%、至少87.04%、至少87.20%、至少87.36%、至少87.52%、至少87.68%、至少87.84%、至少88.00%、至少88.16%、至少88.32%、至少88.49%、至少88.65%、至少88.81%、至少88.97%、至少89.13%、至少89.29%、至少89.45%、至少89.61%、至少89.77%、至少89.93%、至少90.10%、至少90.26%、至少90.42%、至少90.58%、至少90.74%、至少90.90%、至少91.06%、至少91.22%、至少91.38%、至少91.54%、至少91.71%、至少91.87%、至少92.03%、至少92.19%、至少92.35%、至少92.51%、至少92.67%、至少92.83%、至少92.99%、至少93.16%、至少93.32%、至少93.48%、至少93.64%、至少93.80%、至少93.96%、至少94.12%、至少94.28%、至少94.44%、至少94.60%、至少94.77%、至少94.93%、至少95.09%、至少95.25%、至少95.41%、至少95.57%、至少95.73%、至少95.89%、至少96.05%、至少96.21%、至少96.38%、至少96.54%、至少96.70%、至少96.86%、至少97.02%、至少97.18%、至少97.34%、至少97.50%、至少97.66%、至少97.83%、至少97.99%、至少98.15%、至少98.31%、至少98.47%、至少98.63%、至少98.79%、至少98.95%、至少99.11%、至少99.27%、至少99.44%、至少99.60%、至少99.76%、至少99.92%或100%一致性), (c) 与SEQ ID NO:21具有至少91.3%一致性的氨基酸序列(例如至少91.41%、至少91.51%、至少91.62%、至少91.72%、至少91.83%、至少91.93%、至少92.04%、至少92.14%、至少92.25%、至少92.36%、至少92.46%、至少92.57%、至少92.67%、至少92.78%、至少92.88%、至少92.99%、至少93.10%、至少93.20%、至少93.31%、至少93.41%、至少93.52%、至少93.62%、至少93.73%、至少93.83%、至少93.94%、至少94.05%、至少94.15%、至少94.26%、至少94.36%、至少94.47%、至少94.57%、至少94.68%、至少94.78%、至少94.89%、至少95.00%、至少95.10%、至少95.21%、至少95.31%、至少95.42%、至少95.52%、至少95.63%、至少95.74%、至少95.84%、至少95.95%、至少96.05%、至少96.16%、至少96.26%、至少96.37%、至少96.47%、至少96.58%、至少96.69%、至少96.79%、至少96.90%、至少97.00%、至少97.11%、至少97.21%、至少97.32%、至少97.42%、至少97.53%、至少97.64%、至少97.74%、至少97.85%、至少97.95%、至少98.06%、至少98.16%、至少98.27%、至少98.37%、至少98.48%、至少98.59%、至少98.69%、至少98.80%、至少98.90%、至少99.01%、至少99.11%、至少99.22%、至少99.33%、至少99.43%、至少99.54%、至少99.64%、至少99.75%、至少99.85%、至少99.96%或100%一致性) 和 (d) 与SEQ ID NO:22具有至少83.4%一致性的氨基酸序列(例如至少83.57%、至少83.74%、至少83.92%、至少84.09%、至少84.26%、至少84.43%、至少84.61%、至少84.78%、至少84.95%、至少85.12%、至少85.30%、至少85.47%、至少85.64%、至少85.81%、至少85.99%、至少86.16%、至少86.33%、至少86.50%、至少86.68%、至少86.85%、至少87.02%、至少87.19%、至少87.37%、至少87.54%、至少87.71%、至少87.88%、至少88.06%、至少88.23%、至少88.40%、至少88.57%、至少88.74%、至少88.92%、至少89.09%、至少89.26%、至少89.43%、至少89.61%、至少89.78%、至少89.95%、至少90.12%、至少90.30%、至少90.47%、至少90.64%、至少90.81%、至少90.99%、至少91.16%、至少91.33%、至少91.50%、至少91.68%、至少91.85%、至少92.02%、至少92.19%、至少92.37%、至少92.54%、至少92.71%、至少92.88%、至少93.06%、至少93.23%、至少93.40%、至少

93.57%、至少93.74%、至少93.92%、至少94.09%、至少94.26%、至少94.43%、至少94.61%、至少94.78%、至少94.95%、至少95.12%、至少95.30%、至少95.47%、至少95.64%、至少95.81%、至少95.99%、至少96.16%、96.33%、至少96.50%、至少96.68%、至少96.85%、至少97.02%、至少97.19%、至少97.37%、至少97.54%、至少97.71%、至少97.88%、至少98.06%、至少98.23%、至少98.40%、至少98.57%、至少98.74%、至少98.92%、至少99.09%、至少99.26%、至少99.43%、至少99.61%、至少99.78%、至少99.95%或100%一致性)。

[0057] 所述腺病毒或腺病毒载体可以单独包含一种、两种、三种或全部四种前述氨基酸序列或其任意组合。在这一方面,所述腺病毒或腺病毒载体可以包括任意两种前述序列的任意组合、任意三种前述序列的任意组合或者全部四种前述序列。例如,所述腺病毒或腺病毒载体可以包括与SEQ ID NO:18具有至少99%一致性的氨基酸序列。所述腺病毒或腺病毒载体可以包括与SEQ ID NO:18具有至少99%一致性的氨基酸序列和与SEQ ID NO:21具有至少91.3%一致性的氨基酸序列。所述腺病毒或腺病毒载体可以包括与SEQ ID NO:18具有至少99%一致性的氨基酸序列,与SEQ ID NO:20具有至少81.4%一致性的氨基酸序列和与SEQ ID NO:21具有至少91.3%一致性的氨基酸序列。所述腺病毒或腺病毒载体可以包括(a) SEQ ID NO:18所示的氨基酸序列,(b) SEQ ID NO:20所示的氨基酸序列,(c) SEQ ID NO:21所示的氨基酸序列或(d) SEQ ID NO:22所示的氨基酸序列。所述腺病毒或腺病毒载体可以包括(a)与SEQ ID NO:18具有至少99%一致性的氨基酸序列,(b)与SEQ ID NO:20具有至少81.4%一致性的氨基酸序列,(c)与SEQ ID NO:21具有至少91.3%一致性的氨基酸序列和(d)与SEQ ID NO:22具有至少83.4%一致性的氨基酸序列。所述腺病毒或腺病毒载体可以包括(a) SEQ ID NO:18所示的氨基酸序列,(b) SEQ ID NO:20所示的氨基酸序列,(c) SEQ ID NO:21所示的氨基酸序列和(d) SEQ ID NO:22所示的氨基酸序列。

[0058] 在另一个实施方式中,所述腺病毒或腺病毒载体包括一种或多种下述氨基酸序列:(a)包含SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列,(b)包含SEQ ID NO:20的至少114个连续的氨基酸残基的氨基酸序列,(c)包含SEQ ID NO:21的至少231个连续的氨基酸残基的氨基酸序列和(d)包含SEQ ID NO:22的至少30个连续的氨基酸残基的氨基酸序列。

[0059] 所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:18的至少89个(例如90个或更多、100个或更多或者110个或更多)连续的氨基酸残基,但不超过SEQ ID NO:18的133个(例如130个或更少、125个或更少、120个或更少或者115个或更少)连续的氨基酸残基的氨基酸序列。优选地,所述腺病毒或腺病毒载体包括包含SEQ ID NO:18的89至130个(例如90、100、110、115、120或125个连续的氨基酸残基)连续的氨基酸残基,SEQ ID NO:18的89至115个(例如95、110或112个连续的氨基酸残基)连续的氨基酸残基,或SEQ ID NO:18的89至100个(例如89、90、91、92、93、94、95、96、97、98或99个连续的氨基酸残基)连续的氨基酸残基或者任意两个前述值所定义的范围的氨基酸序列。

[0060] 所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:20的至少114个(例如125个或更多、150个或更多、175个或更多、200个或更多、250个或更多、250个或更多、275个或更多、300个或更多或者400个或更多)连续的氨基酸残基,但不超过SEQ ID NO:20的621个(例如620个或更少、600个或更少、550个或更少或者450个或更少)连续的氨基酸残基的氨基酸序

列。优选地,所述腺病毒或腺病毒载体包括包含SEQ ID NO:20的114至600个连续的氨基酸残基(例如155、180、200、250、275、300、400或500个连续的氨基酸残基),SEQ ID NO:20的114至500个连续的氨基酸残基(例如130、160、190、220、250、280、310、340、370、375、400、425、450或475个连续的氨基酸残基)或者SEQ ID NO:20的114至300个连续的氨基酸残基(例如165、175、185、195、199、15、225、235、245、255、265、285、295、299、315、325、335、345、355、360、365、380、385、390、395或399个连续的氨基酸残基)或者任意两个前述值所定义的范围的氨基酸序列。

[0061] 所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:21的至少231个(例如250个或更多、300个或更多、400个或更多或者500个或更多)连续的氨基酸残基,但不超过SEQ ID NO:21的947个(例如940个或更少、900个或更少、800个或更少、700个或更少或者600个或更少)连续的氨基酸残基的氨基酸序列。优选地,所述腺病毒或腺病毒载体包括包含SEQ ID NO:21的231至800个连续的氨基酸残基(例如250、275、290、325、350、375、390、400、500、600或700个连续的氨基酸残基),SEQ ID NO:21的231至600个连续的氨基酸残基(例如235、260、285、300、335、360、375、385、395、425、445、450、465、475、525、545、550、565或575个连续的氨基酸残基),或SEQ ID NO:21的231至400个连续的氨基酸残基(例如245、255、265、285、295、299、315、345、355、365、385、389、395或399个连续的氨基酸残基)或者任意两个前述值所定义的范围的氨基酸序列。

[0062] 所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:22的至少30个(例如50个或更多、75个或更多、100个或更多、200个或更多或者300个或更多)连续的氨基酸残基,但不超过SEQ ID NO:22的580个(例如575个或更少、550个或更少、500个或更少、450个或更少或者400个或更少)连续的氨基酸残基的氨基酸序列。优选地,所述腺病毒或腺病毒载体包括包含SEQ ID NO:22的30至500个连续的氨基酸残基(例如35、55、85、105、135、155、175、195、200、205、235、250、275、295、300、305、335、350、375、395、400、405、435、450、475、495或499个连续的氨基酸残基),SEQ ID NO:22的30至300个连续的氨基酸残基(例如40、60、70、90、125、140、160、170、180、190、200、210、220、230、240、250、260、270、280或290个连续的氨基酸残基),或SEQ ID NO:22的30至100个连续的氨基酸残基(例如33、34、39、42、43、44、49、52、58、59、62、68、69、72、78、79、81、84、87、88、91、92、93、94、97、98或99个连续的氨基酸残基)或者任意两个前述值所定义的范围的氨基酸序列。

[0063] 所述腺病毒或腺病毒载体可以单独包含一种、两种、三种或全部四种前述氨基酸序列或其任意组合。在这一方面,所述腺病毒或腺病毒载体可以包括任意两种前述序列的任意组合、任意三种前述序列的任意组合、或者全部四种前述序列。例如,所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列。所述腺病毒或腺病毒载体可以包括包含SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列以及SEQ ID NO:20的至少114个连续的氨基酸残基的氨基酸序列。所述腺病毒或腺病毒载体可以包括(a)包含SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列,(b)包含SEQ ID NO:21的至少231个连续的氨基酸残基的氨基酸序列和(c)包含SEQ ID NO:22的至少30个连续的氨基酸残基的氨基酸序列。所述腺病毒或腺病毒载体可以包括(a)包含SEQ ID NO:20的至少114个连续的氨基酸残基的氨基酸序列,(b)包含SEQ ID NO:21的至少231个连续的氨基酸残基的氨基酸序列和(c)包含SEQ ID NO:22的至少30个连续的氨基酸残基

的氨基酸序列。所述腺病毒或腺病毒载体可以包括(a)包含SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列,(b)包含SEQ ID NO:20的至少114个连续的氨基酸残基的氨基酸序列,(c)包含SEQ ID NO:21的至少231个连续的氨基酸残基的氨基酸序列和(d)包含SEQ ID NO:22的至少30个连续的氨基酸残基的氨基酸序列。在其他实施方式中,所述腺病毒或腺病毒载体包括编码一种或多种任意前述氨基酸序列例如如本申请所述的SEQ ID NO:13-22的任意氨基酸序列或其任意变体和/或部分的一种或多种核酸序列。例如,所述腺病毒或腺病毒载体可以包括编码与SEQ ID NO:19具有至少99.75%一致性(例如至少99.84%、至少99.94%或100%一致性)的氨基酸序列的核酸序列,或者编码与SEQ ID NO:13具有至少99.7%一致性(例如至少99.93%或100%一致性)的氨基酸序列的核酸序列。

[0064] 所述腺病毒或腺病毒载体可以包括例如SEQ ID NO:23、SEQ ID NO:24、SEQ ID NO:25、SEQ ID NO:26、SEQ ID NO:27、SEQ ID NO:28、SEQ ID NO:29、SEQ ID NO:30、SEQ ID NO:31、SEQ ID NO:32、SEQ ID NO:33、SEQ ID NO:34、SEQ ID NO:35或SEQ ID NO:36所示的核酸序列。

[0065] 如本申请所讨论的,所述腺病毒或腺病毒载体可以是具有复制能力的、有条件地具有复制能力的或复制缺陷的。优选地,所述腺病毒或腺病毒载体是复制缺陷的,这样所述复制缺陷的腺病毒或腺病毒载体需要对用于繁殖(例如以形成腺病毒载体颗粒)的腺病毒基因组一个或多个区域至少一个复制必需基因功能进行补偿。

[0066] 可以采用任何适宜的方式对复制缺陷的腺病毒或腺病毒载体进行修饰以导致其用于繁殖的腺病毒基因组一个或多个区域的一个或多个复制必需基因功能缺陷。在腺病毒基因组一个或多个区域的一个或多个复制必需基因功能缺陷进行补偿是指使用外源性方法提供缺陷的复制必需基因的功能。这种补偿可以通过任意适宜的方式实现,例如使用补偿细胞和/或编码被破坏的复制必需基因功能的外源性DNA(例如辅助腺病毒)。

[0067] 腺病毒或腺病毒载体可以仅在腺病毒基因组早期区(即E1-E4区),仅在腺病毒基因组晚期区(即L1-L5区),在腺病毒基因组早期和晚期区或者整个腺病毒基因(即高容量腺病毒载体(HC-Ad))的一个或多个复制必需基因功能产生缺陷。参见Morsy等,Proc.Natl.Acad.Sci.USA,95:965-976(1998);Chen等,Proc.Natl.Acad.Sci.USA,94:1645-1650(1997);和Kochanek等,Hum.Gene Ther.,10:2451-2459(1999)。复制缺陷腺病毒载体的例子已在美国专利5,837,511;5,851,806;5,994,106;6,127,175;6,482,616;和7,195,896以及国际专利申请公开WO 1994/028152、WO 1995/002697、WO 1995/016772、WO 1995/034671、WO 1996/022378、WO 1997/012986、WO 1997/021826和WO 2003/022311中披露。

[0068] T腺病毒基因组的早期区包括E1、E2、E3和E4区。E1区包含E1A和E1B亚区,并且在E1区中复制必需基因功能的一个或多个缺陷可以包括在E1A或/和E1B亚区中复制必需基因功能的一个或多个缺陷,从而需要对用于腺病毒或腺病毒载体繁殖(例如以形成腺病毒载体颗粒)的腺病毒基因组E1A亚区和/或E1B亚区进行补偿。E2区包含E2A和E2B亚区,并且在E2区中复制必需基因功能的一个或多个缺陷可以包括在E2A或/和E2B亚区中复制必需基因功能的一个或多个缺陷,从而需要对用于腺病毒或腺病毒载体繁殖(例如以形成腺病毒载体颗粒)的腺病毒基因组E2A亚区和/或E2B亚区进行补偿。

[0069] E3区不包括任何复制必需基因功能,这样缺失部分或整个E3区均不需要对用于腺

病毒或腺病毒载体繁殖(例如以形成腺病毒载体颗粒)的E3区任何基因功能进行补偿。在本发明背景下,将E3区定义为以编码与来自人腺病毒5E3区的12.5K蛋白(NCBI参照序列AP_000218)具有较高同源性的蛋白的开放阅读框架起始并且以编码与来自人腺病毒5E3区的14.7K蛋白(NCBI参照序列AP_000224.1)具有较高同源性的蛋白的开放阅读框架结束的区域。E3区可以全部或部分缺失,或者全部或部分保留。可以定制缺失的尺寸以保留基因组与最佳包装尺寸高度匹配的腺病毒或腺病毒载体。在腺病毒或腺病毒基因组中更大的缺失将容纳更大外源性核酸序列的插入。在本发明的一个实施方式中,保留存在于E3区中的L4多聚腺苷酸化信号序列。

[0070] E4区包含多个开放阅读框架(ORE)。具有除ORF6和在一些情况下ORF3以外全部开放阅读框架缺失的E4区的腺病毒或腺病毒载体不需要对用于腺病毒或腺病毒载体繁殖(例如以形成腺病毒载体颗粒)的E4区任意基因的功能进行补偿。而相反的是,具有ORF6和在一些情况下ORF3破坏或缺失的E4区的腺病毒或腺病毒载体(例如E4区具有基于ORF6和/或ORF3的复制必需基因功能的缺陷),同时具有或不具有E4区或天然E4启动子的任意其他开放阅读框架的破坏或缺失、多聚腺苷酸化序列和/或右侧反向末端重复

[0071] (ITR),需要对用于腺病毒或腺病毒载体繁殖(例如以形成病毒载体颗粒)的E4区进行补偿(特别是,E4区的ORF6和/或ORF3)。腺病毒基因组的晚期区包括L1、L2、L3、L4和L5区。腺病毒或腺病毒载体还可能在主要晚期启动子(MLP)中具有突变,如在国际专利申请公开WO 2000/000628中讨论的,如有需要其可以使腺病毒或腺病毒载体产生复制缺陷。

[0072] 含有所需的一个或多个复制必需基因功能缺陷的腺病毒基因组的一个或多个区域是腺病毒基因组的一个或多个早期区即E1、E2和/或E4区,其任选地缺失部分或全部E3区。与野生型腺病毒相比,复制缺陷的腺病毒或腺病毒载体还可以在腺病毒基因组中具有一个或多个突变(例如一个或多个缺失、插入和/或取代),所述突变不会抑制病毒在宿主细胞中的复制。因此,除了一个或多个复制必需基因功能的缺陷以外,所述腺病毒或腺病毒载体还可以在非复制必需的其他方面存在缺陷。例如,所述腺病毒或腺病毒载体可以具有部分或整个称为E3区的腺病毒早期区的缺失,其不是腺病毒或腺病毒基因组的繁殖所必需的。

[0073] 在一个实施方式中,所述腺病毒或腺病毒载体是复制缺陷的并且至多需要对腺病毒基因组用于繁殖(例如以形成腺病毒载体颗粒)的E1区或E4区进行补偿。因此,所述复制缺陷的腺病毒或腺病毒基因组需要对用于繁殖(例如以形成腺病毒载体颗粒)的腺病毒基因组E1A亚区和/或E1B区(表示为E1缺陷的腺病毒载体)或腺病毒基因组E4区(表示为E4缺陷的腺病毒载体)至少一个复制必需基因功能进行补偿。所述腺病毒或腺病毒载体可以是在腺病毒基因组E1区的至少一个复制必需基因功能(理想的情况是全部复制必需基因功能)和腺病毒基因组非必需E3区的至少一个基因功能是缺陷的(表示为E1/E3缺陷的腺病毒载体)。所述腺病毒或腺病毒载体可以是在腺病毒基因组E4区的至少一个复制必需基因功能(理想的情况是全部复制必需基因功能)和腺病毒基因组非必需E3区的至少一个基因功能是缺陷的(表示为E3/E4缺陷的腺病毒载体)。

[0074] 在一个实施方式中,所述腺病毒或腺病毒载体是复制缺陷的并且至多需要对腺病毒基因组用于繁殖(例如以形成腺病毒载体颗粒)的E2区优选E2A亚区进行补偿。因此,所述复制缺陷的腺病毒或腺病毒基因组需要用于繁殖(例如以形成腺病毒载体颗粒)的腺病毒

基因组E2A亚区至少一个复制必需基因功能(表示为E2A缺陷的腺病毒载体)进行补偿。所述腺病毒或腺病毒载体可以是在腺病毒基因组E2A区的至少一个复制必需基因功能(理想的情况是全部复制必需基因功能)和腺病毒基因组非必需E3区的至少一个基因功能是缺陷的(表示为E2A/E3缺陷的腺病毒载体)。

[0075] 在一个实施方式中,所述腺病毒或腺病毒载体是复制缺陷的并且至多需要对腺病毒基因组用于繁殖(例如以形成腺病毒载体颗粒)的E1区和E4区进行补偿。因此,所述复制缺陷的腺病毒或腺病毒基因组需要对用于繁殖(例如以形成腺病毒载体颗粒)的腺病毒基因组E1区和E4区二者至少一个复制必需基因功能(表示为E1/E4缺陷的腺病毒载体)进行补偿。所述腺病毒或腺病毒载体可以是在腺病毒基因组E1区的至少一个复制必需基因功能(理想的情况是全部复制必需基因功能),腺病毒基因组E4区的至少一个复制必需基因功能和腺病毒基因组非必需E3区的至少一个基因功能是缺陷的(表示为E1/E3/E4缺陷的腺病毒载体)。所述腺病毒或腺病毒载体优选地需要对至多用于繁殖的腺病毒基因组E1区进行补偿,并且不要求对用于繁殖的腺病毒基因组任意其他缺陷进行补偿。更优选地,所述腺病毒或腺病毒载体需要至多是对用于繁殖的腺病毒基因组E1区和E4区进行补偿,并且不要求对用于繁殖的腺病毒基因组任意其他缺陷进行补偿。

[0076] 当在腺病毒基因组中的多个复制必需基因功能存在缺陷时(例如E1/E4缺陷的腺病毒载体),所述腺病毒或腺病毒载体可以包括间隔序列以使得病毒在补偿细胞系中以类似于单一复制必需基因功能缺陷的腺病毒或腺病毒载体(例如E1缺陷的腺病毒载体)的方式生长。所述间隔序列可以含有任意核苷酸序列所需长度的序列,如具有至少约15个碱基对的序列(例如在约15个核苷酸至约12,000个核苷酸之间),长度为优选地约100个核苷酸至约10,000个核苷酸,更优选地约500个核苷酸至约8,000个核苷酸,甚至更优选地约1,500个核苷酸至约6,000个核苷酸,以及最优选地约2,000至约3,000个核苷酸,或者任意两个前述值所定义的范围。所述间隔序列对于腺病毒基因组而言可以是编码的或非编码的和天然的或非天然的,但是不能恢复缺陷区的复制必需功能。所述间隔还可以含有表达盒。更优选地,所述间隔包含多聚腺苷酸化序列和/或相对于腺病毒或腺病毒载体而言是非天然的基因。在例如美国专利5,851,806和国际专利申请公开W0 1997/021826中进一步描述了间隔在腺病毒载体中的用途。

[0077] 通过除去全部或部分腺病毒基因组,例如腺病毒基因组的E1、E3和E4区,所得到的腺病毒或腺病毒载体能够接纳外源性核酸序列插入的同时,保留包装进入腺病毒衣壳的能力。可以将外源性核酸序列插入腺病毒基因组中的任意位置,只要在该位置的插入能够允许形成腺病毒或腺病毒载体颗粒即可。所述外源性核酸序列优选的位置是腺病毒基因组中的E1区、E3区或E4区。

[0078] 可以在补偿细胞系中生产本发明的复制缺陷的腺病毒或腺病毒载体,其以适当水平提供了复制缺陷的腺病毒或腺病毒载体中不存在但是病毒繁殖需要的基因功能,以产生较高滴度的病毒载体储备。这种补偿细胞系是公知的并且包括,但不限于293细胞(参见例如Graham等,J.Gen.Virol.,36:59-72(1977))、PER.C6细胞(参见例如国际专利申请公开W0 1997/000326以及美国专利5,994,128和6,033,908)和293-ORF6细胞(参见例如国际专利申请公开W0 95/34671和Brough等,J.Virol.,71:9206-9213(1997))。生产本发明的复制缺陷的腺病毒或腺病毒载体的其他适宜补偿细胞系包括已产生的用于繁殖编码其表达抑制病

毒在宿主细胞中生长的转基因的腺病毒载体的补偿细胞(参见例如美国专利申请号2008/0233650)。其他适宜的补偿细胞参见例如美国专利6,677,156和6,682,929以及国际专利申请公开号WO 2003/020879。在一些情况下,细胞基因组不需要包含核酸序列,其基因产物补偿复制缺陷的腺病毒载体的全部缺陷。在复制缺陷的腺病毒载体中缺乏的一个或多个复制必需基因功能可以由辅助病毒提供,例如提供了复制缺陷的腺病毒或腺病毒载体所需的反式的一个或多个必需基因功能的腺病毒载体。或者,本发明的腺病毒或腺病毒载体可以包含非天然存在的复制必需基因,其补偿本发明的复制缺陷的腺病毒或腺病毒载体缺乏的一个或多个复制必需基因功能。例如,可以对E1/E4-缺陷的腺病毒载体进行工程化改造以使其含有获得或来源于不同腺病毒(例如与本发明的腺病毒或腺病毒载体具有不同血清型的腺病毒或者与本发明的腺病毒或腺病毒载体不同种属的腺病毒)的编码E4ORF 6的核酸序列。

[0079] 所述腺病毒或腺病毒载体可以进一步包含转基因。术语“转基因”在本申请中被定义为可操作地与适宜的调控元件(例如启动子)连接的非天然存在的核酸序列,这样非天然存在的核酸序列能够被表达以产生蛋白(例如肽或多肽)。所述调控元件(例如启动子)对腺病毒或腺病毒载体来说可以是天然的或非天然的。

[0080] “非天然的”核酸序列是任意核酸序列(例如DNA、RNA或cDNA序列),其是在天然存在的位置上的腺病毒的非天然存在的核酸序列。因此,所述非天然存在的核酸序列可以是天然存在于腺病毒中,但位于腺病毒基因组中的非天然位置和/或可操作地与非天然的启动子连接。术语“非天然存在的核酸序列”、“异源性核酸序列”和“外源性核酸序列”是同义词并且在本发明的上下文中可以互换使用。所述非天然存在的核酸序列优选地是DNA并且优选地编码蛋白(即编码一个或多个蛋白的一个或多个核酸序列)。

[0081] 所述非天然存在的核酸序列能够用于编码治疗性蛋白,所述治疗性蛋白能够用于预防或治疗哺乳动物的疾病。适宜的治疗性蛋白的例子包括细胞因子、毒素、肿瘤抑制蛋白、生长因子、激素、受体、丝裂原、免疫球蛋白、神经肽、神经递质和酶。或者,所述非天然存在的核酸序列可以编码病原体(例如细菌或病毒)抗原,以及所述腺病毒或腺病毒载体可以用作疫苗。

[0082] 本发明提供了一种包含本申请所述的腺病毒或腺病毒载体及其载体(例如药学上可接受的载体)的组合物。所述组合物理想地是生理上可接受的(例如药学上可接受的)组合物,其包含载体,优选地生理上(例如药学上)可接受的载体和腺病毒或腺病毒载体。在本发明的背景下可以使用任意适宜的载体,并且这种载体是本领域公知的。载体的选择将部分地由所述组合物的特定用途(例如给予动物)和给予所述组合物的特定方法决定。理想地,在复制缺陷的腺病毒载体的背景下,所述药物组合物优选地不含具有复制能力的腺病毒。所述药物组合物任选可以是无菌的。

[0083] 适宜的组合物包括水性或非水性等渗无菌溶液,其可以含有抗氧化剂、缓冲剂和抗菌剂,以及水性和非水性无菌混悬剂,其可以包括助悬剂、增溶剂、增稠剂、稳定剂和防腐剂。所述组合物可以存在于单位剂量或多剂量的密封容器中,如安瓿和小瓶,并且可以贮存在冷冻干燥(冻干)条件下,仅需要在使用前加入无菌液体载体例如水。即用溶液和混悬液可以由无菌粉剂、颗粒剂和片剂制备。优选地,所述载体是缓冲盐溶液。更优选地,将所述腺病毒或腺病毒载体配制成组合物的一部分以保护腺病毒或腺病毒载体在给药前不被损坏。

例如,可以将所述组合物制剂以降低腺病毒或腺病毒载体在用于制备、贮存或给予所述腺病毒或腺病毒载体的装置上(例如玻璃器皿、注射器或针头)的损失。可以将所述组合物制剂以降低腺病毒或腺病毒载体的光敏性和/或温度敏感性。出于此目的,所述组合物优选地包括药学上可接受的液体载体,例如上文所述的那些,和选自下组的稳定剂:聚山梨醇酯80、L-精氨酸、聚乙烯吡咯烷酮、海藻糖及其组合。使用这种组合物将延长腺病毒或腺病毒载体的储存期限,并且便于其给药。含有腺病毒或腺病毒载体的组合物的制剂进一步参见例如美国专利6,225,289、美国专利6,514,943和国际专利申请公开WO 2000/034444。

[0084] 还可以将所述组合物制剂以增强转导效率。此外,本领域的普通技术人员将理解所述腺病毒或腺病毒载体可以存在于与其他治疗剂或生物活性剂的组合物中。例如,可以将控制炎症的因子例如布洛芬或类固醇作为组合物的一部分以减轻与体内给予腺病毒或腺病毒载体相关的肿胀和炎症。如果腺病毒或腺病毒载体用于向宿主递送编码抗原的核酸序列,则可以给予免疫系统刺激剂或佐剂例如白介素、脂多糖或双链RNA以增强或改善针对所述抗原的任意免疫应答。可以存在抗生素即杀菌剂和杀真菌剂以治疗现有感染和/或降低未来感染的风险,如与基因转移程序相关的感染。

[0085] 本发明还包含以下实施方式:

[0086] 实施方式1.一种腺病毒或腺病毒载体,其包括一种或多种选自下组的核酸序列:

[0087] (a) 与SEQ ID NO:1具有至少96%同一性的核酸序列,

[0088] (b) 与SEQ ID NO:2具有至少99%同一性的核酸序列,

[0089] (c) 与SEQ ID NO:3具有至少80%同一性的核酸序列,

[0090] (d) 与SEQ ID NO:4具有至少80%同一性的核酸序列,以及

[0091] (e) 与SEQ ID NO:5具有至少85.4%同一性的核酸序列。

[0092] 实施方式2.根据实施方式1所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:1具有至少96%同一性的核酸序列。

[0093] 实施方式3.根据实施方式2所述的腺病毒或腺病毒载体,其包括SEQ ID NO:1所示的核酸序列。

[0094] 实施方式4.根据实施方式1-3任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:2具有至少99%同一性的核酸序列。

[0095] 实施方式5.根据实施方式4所述的腺病毒或腺病毒载体,其包括SEQ ID NO:2所示的核酸序列。

[0096] 实施方式6.根据实施方式1-5任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:3具有至少80%同一性的核酸序列。

[0097] 实施方式7.根据实施方式6任意一项所述的腺病毒或腺病毒载体,其包括SEQ ID NO:3所示的核酸序列。

[0098] 实施方式8.根据实施方式1-7任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:4具有至少80%同一性的核酸序列。

[0099] 实施方式9.根据实施方式8所述的腺病毒或腺病毒载体,其包括SEQ ID NO:4所示的核酸序列。

[0100] 实施方式10.根据实施方式1-9任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:5具有至少85.4%同一性的核酸序列。

- [0101] 实施方式11.根据实施方式10所述的腺病毒或腺病毒载体,其包括SEQ ID NO:5所示的核酸序列。
- [0102] 实施方式12.一种腺病毒或腺病毒载体,其包括一种或多种选自下组的核酸序列:
- [0103] (a) 与SEQ ID NO:6具有至少98.7%同一性的核酸序列,
- [0104] (b) 与SEQ ID NO:7具有至少98.9%同一性的核酸序列,
- [0105] (c) 与SEQ ID NO:8具有至少99.4%同一性的核酸序列,
- [0106] (d) 与SEQ ID NO:9具有至少99.1%同一性的核酸序列,
- [0107] (e) 与SEQ ID NO:10具有至少81.25%同一性的核酸序列,
- [0108] (f) 与SEQ ID NO:11具有至少90.83%同一性的核酸序列,以及
- [0109] (g) 与SEQ ID NO:12具有至少82.5%同一性的核酸序列。
- [0110] 实施方式13.根据实施方式12所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:6具有至少98.7%同一性的核酸序列。
- [0111] 实施方式14.根据实施方式13所述的腺病毒或腺病毒载体,其包括SEQ ID NO:6所示核酸序列。
- [0112] 实施方式15.根据实施方式12-14任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:7具有至少98.9%同一性的核酸序列。
- [0113] 实施方式16.根据实施方式15所述的腺病毒或腺病毒载体,其包括SEQ ID NO:7所示的核酸序列。
- [0114] 实施方式17.根据实施方式12-16任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:8具有至少99.4%同一性的核酸序列。
- [0115] 实施方式18.根据实施方式17所述的腺病毒或腺病毒载体,其包括SEQ ID NO:8所示的核酸序列。
- [0116] 实施方式19.根据实施方式12-18任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:9具有至少99.1%同一性的核酸序列。
- [0117] 实施方式20.根据实施方式19所述的腺病毒或腺病毒载体,其包括SEQ ID NO:9所示的核酸序列。
- [0118] 实施方式21.根据实施方式12-20任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:10具有至少81.25%同一性的核酸序列。
- [0119] 实施方式22.根据实施方式21所述的腺病毒或腺病毒载体,其包括SEQ ID NO:10所述的核酸序列。
- [0120] 实施方式23.根据实施方式12-22任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:11具有至少90.83%同一性的核酸序列。
- [0121] 实施方式24.根据实施方式23所述的腺病毒或腺病毒载体,其包括SEQ ID NO:11所示的核酸序列。
- [0122] 实施方式25.根据实施方式12-24任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:12具有至少82.5%同一性的核酸序列。
- [0123] 实施方式26.根据实施方式25所述的腺病毒或腺病毒载体,其包括SEQ ID NO:12所示的核酸序列。
- [0124] 实施方式27.一种腺病毒或腺病毒载体,其包括一种或多种选自下组的核酸序列:

- [0125] (a) 包括SEQ ID NO:6的至少162个连续的核苷酸的核酸序列，
- [0126] (b) 包括SEQ ID NO:7的至少162个连续的核苷酸的核酸序列，
- [0127] (c) 包括SEQ ID NO:8的至少121个连续的核苷酸的核酸序列，
- [0128] (d) 包括SEQ ID NO:9的至少531个连续的核苷酸的核酸序列，
- [0129] (e) 包括SEQ ID NO:10的至少156个连续的核苷酸的核酸序列，
- [0130] (f) 包括SEQ ID NO:11的至少192个连续的核苷酸的核酸序列，以及
- [0131] (g) 包括SEQ ID NO:12的至少84个连续的核苷酸的核酸序列。
- [0132] 实施方式28. 根据实施方式27所述的腺病毒或腺病毒载体，其包括包含SEQ ID NO:6的至少162个连续的核苷酸的核酸序列。
- [0133] 实施方式29. 根据实施方式28所述的腺病毒或腺病毒载体，其包括SEQ ID NO:6所示的核酸序列。
- [0134] 实施方式30. 根据实施方式27-29任意一项所述的腺病毒或腺病毒载体，其包括包含SEQ ID NO:7的至少162个连续的核苷酸的核酸序列。
- [0135] 实施方式31. 根据实施方式30所述的腺病毒或腺病毒载体，其包括SEQ ID NO:7所示的核酸序列。
- [0136] 实施方式32. 根据实施方式27-31任意一项所述的腺病毒或腺病毒载体，其包括包含SEQ ID NO:8的至少121个连续的核苷酸的核酸序列。
- [0137] 实施方式33. 根据实施方式32所述的腺病毒或腺病毒载体，其包括SEQ ID NO:8所示的核酸序列。
- [0138] 实施方式34. 根据实施方式27-33任意一项所述的腺病毒或腺病毒载体，其包括包含SEQ ID NO:9的至少531个连续的核苷酸的核酸序列。
- [0139] 实施方式35. 根据实施方式34所述的腺病毒或腺病毒载体，其包括SEQ ID NO:9所示的核酸序列。
- [0140] 实施方式36. 根据实施方式27-35任意一项所述的腺病毒或腺病毒载体，其包括包含SEQ ID NO:10的至少156个连续的核苷酸的核酸序列。
- [0141] 实施方式37. 根据实施方式36所述的腺病毒或腺病毒载体，其包括SEQ ID NO:10所示的核酸序列。
- [0142] 实施方式38. 根据实施方式27-37任意一项所述的腺病毒或腺病毒载体，其包括包含SEQ ID NO:11的至少192个连续的核苷酸的核酸序列。
- [0143] 实施方式39. 根据实施方式38所述的腺病毒或腺病毒载体，其包括SEQ ID NO:11所示的核酸序列。
- [0144] 实施方式40. 根据实施方式27-39任意一项所述的腺病毒或腺病毒载体，其包括包含SEQ ID NO:12的至少84个连续的核苷酸的核酸序列。
- [0145] 实施方式41. 根据实施方式40所述的腺病毒或腺病毒载体，其包括SEQ ID NO:12所示的核酸序列。
- [0146] 实施方式42. 一种腺病毒或腺病毒载体，其包括一种或多种选自下组的氨基酸序列：
- [0147] (a) 与SEQ ID NO:14具有至少85%同一性的氨基酸序列，
- [0148] (b) 与SEQ ID NO:15具有至少80%同一性的氨基酸序列，以及

- [0149] (c) 与SEQ ID NO:16具有至少80%同一性的氨基酸序列。
- [0150] 实施方式43. 根据实施方式42所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:14具有至少85%同一性的氨基酸序列。
- [0151] 实施方式44. 根据实施方式43所述的腺病毒或腺病毒载体,其包括SEQ ID NO:14所示的核酸序列。
- [0152] 实施方式45. 根据实施方式42-44任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:15具有至少80%同一性的氨基酸序列。
- [0153] 实施方式46. 根据实施方式45所述的腺病毒或腺病毒载体,其包括SEQ ID NO:15所示的氨基酸序列。
- [0154] 实施方式47. 根据实施方式42-46任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:16具有至少80%同一性的氨基酸序列。
- [0155] 实施方式48. 根据实施方式47所述的腺病毒或腺病毒载体,其包括SEQ ID NO:16所示的氨基酸序列。
- [0156] 实施方式49. 一种腺病毒或腺病毒载体,其包括一种或多种选自下组的核酸序列:
- [0157] (a) 编码与SEQ ID NO:13具有至少99.7%同一性的氨基酸序列的核酸序列,
- [0158] (b) 编码与SEQ ID NO:14具有至少85%同一性的氨基酸序列的核酸序列,
- [0159] (c) 编码与SEQ ID NO:15具有至少80%同一性的氨基酸序列的核酸序列,以及
- [0160] (d) 编码与SEQ ID NO:16具有至少80%同一性的氨基酸序列的核酸序列。
- [0161] 实施方式50. 根据实施方式49所述的腺病毒或腺病毒载体,其包括编码与SEQ ID NO:13具有至少99.7%同一性的氨基酸序列的核酸序列。
- [0162] 实施方式51. 根据实施方式50所示的腺病毒或腺病毒载体,其包括编码SEQ ID NO:13所示的氨基酸序列的核酸序列。
- [0163] 实施方式52. 根据实施方式49-51任意一项所述的腺病毒或腺病毒载体,其包括编码与SEQ ID NO:14具有至少85%同一性的氨基酸序列的核酸序列。
- [0164] 实施方式53. 根据实施方式52所示的腺病毒或腺病毒载体,其包括编码SEQ ID NO:14所示的氨基酸序列的核酸序列。
- [0165] 实施方式54. 根据实施方式49-53任意一项所述的腺病毒或腺病毒载体,其包括编码与SEQ ID NO:15具有至少80%同一性的氨基酸序列的核酸序列。
- [0166] 实施方式55. 根据实施方式54所示的腺病毒或腺病毒载体,其包括编码SEQ ID NO:15所示的氨基酸序列的核酸序列。
- [0167] 实施方式56. 根据实施方式49-55任意一项所述的腺病毒或腺病毒载体,其包括编码与SEQ ID NO:16具有至少80%同一性的氨基酸序列的核酸序列。
- [0168] 实施方式57. 根据实施方式56所示的腺病毒或腺病毒载体,其包括编码SEQ ID NO:16所示的氨基酸序列的核酸序列。
- [0169] 实施方式58. 一种腺病毒或腺病毒载体,其包括一种或多种选自下组的氨基酸序列:
- [0170] (a) 与SEQ ID NO:18具有至少99%同一性的氨基酸序列,
- [0171] (b) 与SEQ ID NO:20具有至少81.4%同一性的氨基酸序列,
- [0172] (c) 与SEQ ID NO:21具有至少91.3%同一性的氨基酸序列,以及

- [0173] (d) 与SEQ ID NO:22具有至少83.4%同一性的氨基酸序列。
- [0174] 实施方式59. 根据实施方式58所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:18具有至少99%同一性的氨基酸序列。
- [0175] 实施方式60. 根据实施方式59所述的腺病毒或腺病毒载体,其包括SEQ ID NO:18所示的氨基酸序列。
- [0176] 实施方式61. 根据实施方式58-60任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:20具有至少81.4%同一性的氨基酸序列。
- [0177] 实施方式62. 根据实施方式61所述的腺病毒或腺病毒载体,其包括SEQ ID NO:20所示的氨基酸序列。
- [0178] 实施方式63. 根据实施方式58-62任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:21具有至少91.3%同一性的氨基酸序列。
- [0179] 实施方式64. 根据实施方式63所述的腺病毒或腺病毒载体,其包括SEQ ID NO:21所示的氨基酸序列。
- [0180] 实施方式65. 根据实施方式58-64任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:22具有至少83.4%同一性的氨基酸序列。
- [0181] 实施方式66. 根据实施方式65所述的腺病毒或腺病毒载体,其包括SEQ ID NO:22所示的氨基酸序列。
- [0182] 实施方式67. 一种腺病毒或腺病毒载体,其包括一种或多种选自下组的核酸序列:
- [0183] (a) 编码与SEQ ID NO:18具有至少99%同一性的氨基酸序列的核酸序列,
- [0184] (b) 编码与SEQ ID NO:19具有至少99.75%同一性的氨基酸序列的核酸序列,
- [0185] (c) 编码与SEQ ID NO:20具有至少81.4%同一性的氨基酸序列的核酸序列,
- [0186] (d) 编码与SEQ ID NO:21具有至少91.3%同一性的氨基酸序列的核酸序列,
- [0187] (e) 编码与SEQ ID NO:22具有至少83.4%同一性的氨基酸序列的核酸序列。
- [0188] 实施方式68. 根据实施方式67所述的腺病毒或腺病毒载体,其包括编码与SEQ ID NO:18具有至少99%同一性的氨基酸序列的核酸序列。
- [0189] 实施方式69. 根据实施方式68所述的腺病毒或腺病毒载体,其包括编码SEQ ID NO:18所示的氨基酸序列的核酸序列。
- [0190] 实施方式70. 根据实施方式67-69任意一项所述的腺病毒或腺病毒载体,其包括与SEQ ID NO:19具有至少99.75%同一性的氨基酸序列的核酸序列。
- [0191] 实施方式71. 根据实施方式70所述的腺病毒或腺病毒载体,其包括编码SEQ ID NO:19所示的氨基酸序列的核酸序列。
- [0192] 实施方式72. 根据实施方式67-71任意一项所述的腺病毒或腺病毒载体,其包括编码与SEQ ID NO:20具有至少81.4%同一性的氨基酸序列的核酸序列。
- [0193] 实施方式73. 根据实施方式72所述的腺病毒或腺病毒载体,其包括编码SEQ ID NO:20所示的氨基酸序列的核酸序列。
- [0194] 实施方式74. 根据实施方式67-73任意一项所述的腺病毒或腺病毒载体,其包括编码与SEQ ID NO:21具有至少91.3%同一性的氨基酸序列的核酸序列。
- [0195] 实施方式75. 根据实施方式74所述的腺病毒或腺病毒载体,其包括编码SEQ ID NO:21所示的氨基酸序列的核酸序列。

[0196] 实施方式76.根据实施方式67-75任意一项所述的腺病毒或腺病毒载体,其包括编码与SEQ ID NO:22具有至少83.4%同一性的氨基酸序列的核酸序列。

[0197] 实施方式77.根据实施方式76所述的腺病毒或腺病毒载体,其包括编码SEQ ID NO:22所示的氨基酸序列的核酸序列。

[0198] 实施方式78.一种腺病毒或腺病毒载体,其包括一种或多种选自下组的氨基酸序列:

[0199] (a) 包括SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列,

[0200] (b) 包括SEQ ID NO:20的至少114个连续的氨基酸残基的氨基酸序列,

[0201] (c) 包括SEQ ID NO:21的至少231个连续的氨基酸残基的氨基酸序列,以及

[0202] (d) 包括SEQ ID NO:22的至少30个连续的氨基酸残基的氨基酸序列。

[0203] 实施方式79.根据实施方式78所述的腺病毒或腺病毒载体,其包括包含SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列。

[0204] 实施方式80.根据实施方式79所述的腺病毒或腺病毒载体,其包括SEQ ID NO:18的氨基酸序列。

[0205] 实施方式81.根据实施方式78-80任意一项所述的腺病毒或腺病毒载体,其包括包含SEQ ID NO:20的至少114个连续的氨基酸残基的氨基酸序列。

[0206] 实施方式82.根据实施方式81所述的腺病毒或腺病毒载体,其包括SEQ ID NO:20的氨基酸序列。

[0207] 实施方式83.根据实施方式78-82任意一项所述的腺病毒或腺病毒载体,其包括包含SEQ ID NO:21的至少231个连续的氨基酸残基的氨基酸序列。

[0208] 实施方式84.根据实施方式83所述的腺病毒或腺病毒载体,其包括SEQ ID NO:21的氨基酸序列。

[0209] 实施方式85.根据实施方式78-84任意一项所述的腺病毒或腺病毒载体,其包括包含SEQ ID NO:22的至少30个连续的氨基酸残基的氨基酸序列。

[0210] 实施方式86.根据实施方式85所述的腺病毒或腺病毒载体,其包括SEQ ID NO:22的氨基酸序列。

[0211] 实施方式87.一种腺病毒或腺病毒载体,其包括一种或多种选自下组的核酸序列:

[0212] (a) 编码包括SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列的核酸序列,

[0213] (b) 编码包括SEQ ID NO:19的至少428个连续的氨基酸残基的氨基酸序列的核酸序列,

[0214] (c) 编码包括SEQ ID NO:20的至少114个连续的氨基酸残基的氨基酸序列的核酸序列,

[0215] (d) 编码包括SEQ ID NO:21的至少231个连续的氨基酸残基的氨基酸序列的核酸序列,以及

[0216] (e) 编码包括SEQ ID NO:22的至少30个连续的氨基酸残基的氨基酸序列的核酸序列。实施方式88.根据实施方式87所述的腺病毒或腺病毒载体,其包括编码包含SEQ ID NO:18的至少89个连续的氨基酸残基的氨基酸序列的核酸序列。

[0217] 实施方式89.根据实施方式88所述的腺病毒或腺病毒载体,其包括编码SEQ ID

N0:18所示的氨基酸序列的核酸序列。

[0218] 实施方式90.根据实施方式87-89任意一项所述的腺病毒或腺病毒载体,其包括编码包含SEQ ID N0:19的至少428个连续的氨基酸残基的氨基酸序列的核酸序列。

[0219] 实施方式91.根据实施方式90所述的腺病毒或腺病毒载体,其包括编码SEQ ID N0:19所示的氨基酸序列的核酸序列。

[0220] 实施方式92.根据实施方式87-91任意一项所述的腺病毒或腺病毒载体,其包括编码包含SEQ ID N0:20的至少114个连续的氨基酸残基的氨基酸序列的核酸序列。

[0221] 实施方式93.根据实施方式92所述的腺病毒或腺病毒载体,其包括编码SEQ ID N0:20所示的氨基酸序列的核酸序列。

[0222] 实施方式94.根据实施方式87-93任意一项所述的腺病毒或腺病毒载体,其包括编码包含SEQ ID N0:21的至少231个连续的氨基酸残基的氨基酸序列的核酸序列。

[0223] 实施方式95.根据实施方式94所述的腺病毒或腺病毒载体,其包括编码SEQ ID N0:21所示的氨基酸序列的核酸序列。

[0224] 实施方式96.根据实施方式87-95任意一项所述的腺病毒或腺病毒载体,其包括编码包含SEQ ID N0:22的至少30个连续的氨基酸残基的氨基酸序列的核酸序列。

[0225] 实施方式97.根据实施方式96所述的腺病毒或腺病毒载体,其包括编码SEQ ID N0:22所示的氨基酸序列的核酸序列。

[0226] 实施方式98.根据实施方式1-97任意一项所述的腺病毒或腺病毒载体,其中所述腺病毒或腺病毒载体需要对用于增殖的腺病毒基因组的一个或多个早期区的缺陷进行补偿,但不需要对用于增殖的腺病毒基因组的任何其他缺陷进行补偿。

[0227] 实施方式99.根据实施方式98所述的腺病毒或腺病毒载体,其中所述一个或多个早期区选自下组:腺病毒基因组的E1区、E2区和E4区。

[0228] 实施方式100.根据实施方式99所述的腺病毒或腺病毒载体,其中所述腺病毒或腺病毒载体需要对用于增殖的腺病毒基因组的E1区的缺陷进行补偿,但不需要对用于增殖的腺病毒基因组的任何其他缺陷进行补偿。

[0229] 实施方式101.根据实施方式99所述的腺病毒或腺病毒载体,其中所述腺病毒或腺病毒载体需要对用于增殖的腺病毒基因组的E1A区或E1B区的缺陷进行补偿,但不需要对用于增殖的腺病毒基因组的任何其他缺陷进行补偿。

[0230] 实施方式102.根据实施方式99所述的腺病毒或腺病毒载体,其中所述腺病毒或腺病毒载体至多需要对用于增殖的腺病毒基因组的E4区的缺陷进行补偿,但不需要对用于增殖的腺病毒基因组的任何其他缺陷进行补偿。

[0231] 实施方式103.根据实施方式99所述的腺病毒或腺病毒载体,其中所述腺病毒或腺病毒载体需要对用于增殖的腺病毒基因组的E1区的缺陷和腺病毒基因组的E4区的缺陷进行补偿,但不需要对用于增殖的腺病毒基因组的任何其他缺陷进行补偿。

[0232] 实施方式104.根据实施方式1-103任意一项所述的腺病毒或腺病毒载体,其进一步包括转基因。

[0233] 实施方式105.一种组合物,所述组合物包括实施方式1-104任意一项所述的腺病毒或腺病毒载体以及药学上可接受的载体。

[0234] 下述实施例进一步说明本发明,当然,不应将其解释为以任何方式限定其范围。

[0235] 实施例1

[0236] 本实施例显示了编码呼吸道合胞病毒 (RSV) F蛋白的腺病毒载体在棉鼠中的免疫原性。通过基因工程对具有SEQ ID NO:28所示的核酸序列的大猩猩腺病毒进行修饰以(1)通过缺失E1区使其复制缺陷,和(2)表达人呼吸道合胞病毒 (RSV) 融合(F)糖蛋白。由于RSV在细胞质中复制,因此通过除去RNA加工信号(例如RNA剪接位点)对编码F蛋白的基因进行修饰使其在细胞核中表达,并且对密码子进行优化使其在哺乳动物细胞中表达。通过体外感染HEK-293细胞,以及通过使用感染细胞蛋白提取物和商品化抗-RSV多克隆抗体(Pab7133P, Maine Biotechnology, Portland, Maine)的Western印迹检测对来自腺病毒载体的F蛋白的表达进行验证。

[0237] 在胫骨肌单次注射给予棉鼠 (*Sigmodon hispidus*) 10^7 颗粒单位 (pu) 的表达RSV F糖蛋白的E1缺失的腺病毒载体。然后,在28天后使用活的人RSV (鼻内给予 10^6 颗粒形成单位 (pfu)) 攻击所述动物。在攻击后的第5天,检测动物肺部RSV的病毒载量。使用表达F蛋白的腺病毒载体免疫动物的肺部不具有可检测的RSV (检测限为70pfu/克肺组织)。本实施例的结果表明本发明的编码RSV F蛋白的腺病毒载体在体内具有免疫原性并且其在棉鼠中针对RSV感染能够产生完全的保护作用。

[0238] 在本申请中引用的所有参考文献,包括出版物、专利申请、和专利,都以参考的形式并入本申请,相当于每篇参考文献都单独地和特别地被指出以参考的形式并入,并且在在本申请中以全文列出。

[0239] 在本发明描述的上下文中(特别是在随后的权利要求的上下文中),术语“一”和“一”以及“所述”以及相似指示词的使用应理解为包括单数和复数,除非在本申请中另有说明或者与上下文明显矛盾。术语“包含”“具有”“包括”以及“含有”都应理解为开放性术语(即,意思是“包括,但不限于”),除非另有注明。本申请中对范围的描述仅仅旨在作为一种对每个落在该范围内的单独数值的简略写法,且每个单独的数值都被包括在说明书中,就像单独地写在本申请中一样。所有在本申请中描述的方法都可以按照任何适当的顺序进行,除非在本申请中另有指明或者除非与上下文明显矛盾。在本申请中任何和所有的实施例,或示例性语言(例如,“例如”)的使用,都仅旨在更好地阐明本发明,而不是在本发明的范围上加以限制,除非权利要求另有要求。在说明书中的语言都不应被理解为指明任何未在权利要求中包括的方案是为本发明所必需。

[0240] 本申请中描述了本发明的优选实施方式,包括发明人所知道的实施本发明的最佳方式。通过阅读前面的说明,那些优选实施方式的变通方式对本领域普通技术人员而言是很明显的。发明人预期,熟练的技术人员可以根据需要应用这样的变通方式,且发明人预期,本发明可以以本申请中特定描述以外的方式实施。因此,本发明包括随后附具的权利要求中所述主题的所有适用法律准许的改变和等同实施。而且,本发明包括以上所述元件的所有可能的变通方式的任意组合,除非在本申请中另有说明或者与上下文明显矛盾。

序列表

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<211> 2841

<212> DNA

<213> 东部低地大猩猩

<400> 11

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<211> 1740

<212> DNA

<213> 东部低地大猩猩

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<210> 13

<211> 429

<212> PRT

<213> 东部低地大猩猩

<400> 13

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Pro	Asn	Glu	Asp	Gln	Leu	Asp	Pro	Leu	Pro	Pro	Phe	Cys	Ser	Arg	Lys
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<213> 东部低地大猩猩

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His	Glu	Cys	Ser	Ala	Arg	Arg	Arg	Asp	Phe	Tyr	Phe	His	His	Ile	Asn
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770	775	780	

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835 840 845
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His Arg Leu Met Glu Ser Arg Gly Lys Lys Arg Ile Lys Lys His Gly
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Cys Pro Ser Cys Gly Ala Thr Ser Lys Gly Lys Leu Arg Ala Lys Gly
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<212> PRT

<213> 东部低地大猩猩

<400> 20

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Abstract

The invention provides an adenovirus or adenoviral vector characterized by comprising one or more particular nucleic acid sequences or one or more particular amino acid sequences, or portions thereof, pertaining to, for example, an adenoviral pIX protein, DNA polymerase protein, penton protein, hexon protein, and/or fiber protein.