



(12) 发明专利

(10) 授权公告号 CN 102458418 B

(45) 授权公告日 2015. 09. 16

(21) 申请号 201080028245. 5

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(22) 申请日 2010. 06. 07

(51) Int. Cl.

(30) 优先权数据

A61K 31/7088(2006. 01)

61/184, 937 2009. 06. 08 US

C07H 21/02(2006. 01)

61/235, 381 2009. 08. 20 US

(56) 对比文件

(85) PCT国际申请进入国家阶段日

US 20080269156A1 , 2008. 10. 30,

2011. 12. 23

审查员 楼杜鹃

(86) PCT国际申请的申请数据

PCT/US2010/037565 2010. 06. 07

(87) PCT国际申请的公布数据

W02010/144336 EN 2010. 12. 16

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(54) 发明名称

一种寡核苷酸化合物的制药用途

(57) 摘要

本发明涉及用于治疗慢性肾病 (CKD) 的方法, 包括用于预防或者延迟 CKD 发作的方法和预防 CKD 的恶化和发展的方法。在特定的实施方案中, 本发明提供用于治疗处于正在发展的 CKD 风险之中的受试者的方法, 该方法包括向受试者施用组合物, 该组合物包含 :a) 治疗有效量的至少一种抑制与肾病有关的人类靶基因表达的寡核苷酸化合物 ; 和 b) 药学上可接受的赋形剂或载体、或其混合物, 从而在受试者中降低 CKD 风险。

1. 一种寡核苷酸化合物在制备用于在处于源自暴露于复发性肾脏损害的慢性肾病发展的风险的受试者中预防或减弱慢性肾病发展的药物中的用途,所述寡核苷酸化合物调低与肾损伤有关的靶基因的表达,

其中所述寡核苷酸化合物是化学修饰的 siRNA,

其中所述靶基因是其序列以 SEQ ID NO:67-73 中任一种阐述的 p53 基因,

其中在各肾脏损害 24 小时之内向所述受试者施用所述寡核苷酸化合物,从而在所述受试者中减弱所述慢性肾病的发展。

2. 根据权利要求 1 所述的用途,其中在各肾脏损害 4 小时内向所述受试者施用所述寡核苷酸化合物。

3. 根据权利要求 1 所述的用途,其中在各肾脏损害 0.5 小时内向所述受试者施用所述寡核苷酸化合物。

4. 根据权利要求 1 所述的用途,其中在各肾脏损害后 0-4 小时向所述受试者施用所述寡核苷酸化合物。

5. 根据权利要求 1 所述的用途,其中各肾脏损害导致急性肾损伤。

6. 根据权利要求 1 所述的用途,其中各肾脏损害与下列一种或多种情况有关:包括心血管手术在内的手术、暴露于肌红蛋白尿、局部贫血/再灌注性损伤;败血症;尿路梗阻;暴露于包括肾毒放射对比成像剂、抗生素或化疗剂在内的肾毒素;蛋白尿;带有间质损伤的肾脏氨生成上升;高血脂;有磷酸钙累积的高磷血症。

7. 根据权利要求 6 所述的用途,其中各肾脏损害和所述局部贫血/再灌注性损伤、或暴露于所述肾毒素、或两者有关。

8. 根据权利要求 6 所述的用途,其中各肾脏损害和在所述心血管手术或心肺手术期间或之后的发生的所述局部贫血/再灌注性损伤有关。

9. 根据权利要求 6 所述的用途,其中各肾脏损害和所述肌红蛋白尿有关。

10. 根据权利要求 6 所述的用途,其中各肾脏损害和所述肾毒素有关。

11. 根据权利要求 1 所述的用途,其中和所述肾损伤有关的靶基因是表达被所述肾脏损害上调的人类基因。

12. 根据权利要求 1 所述的用途,其中所述 siRNA 具有一般的双链结构:

5' (N)_x - Z 3' (反义链)

3' Z' - (N')_y - z" 5' (正义链)

其中 N 和 N' 均是核糖核苷酸,所述核糖核苷酸可以是未修饰的或修饰的、或非常规部分;

其中 (N)_x 和 (N')_y 均是寡核苷酸,其中每一个连续的 N 或 N' 通过共价键连接到下一个 N 或 N' ;

其中 Z 和 Z' 可以存在或不存在,但如果存在的话独立地是共价地连接到其存在的链的 3' 末端的 1-5 个连续的核苷酸或非-核苷酸部分;

其中所述 z" 可以存在或不存在,但如果存在的话是共价地连接到 (N')_y 的 5' 末端的封端部分;

x 和 y 各自独立地是 18 到 40 的整数;

其中 (N')_y 的序列和 (N)_x 的序列是基本上互补的;并且其中所述 (N)_x 包含与 SEQ ID

NO:67-73 中任一种阐述的 mRNA 中存在的 18 到 40 个连续核糖核苷酸基本上互补的反义序列。

13. 根据权利要求 12 所述的用途,其中所述 siRNA 是 I5siRNA 化合物。

一种寡核苷酸化合物的制药用途

[0001] 相关专利

[0002] 本申请要求 2009 年 6 月 8 日提交的美国临时专利申请 No. 61/184937 和 2009 年 8 月 20 日提交的 61/235381 的优先权；两者全部通过引用的方式并入本文。

[0003] 本申请引用了很多专利和出版物。为了更充分地描述本发明所涉及领域的情况，这些文件的公开内容全部通过引用的方式并入本申请。

技术领域

[0004] 本发明涉及用于治疗慢性肾病 (CKD) 的方法，包括用于预防或者延迟 CKD 发作的方法和预防 CKD 的恶化和发展的方法。在特定的实施方案中，本发明提供用于治疗处于正在发展的 CKD 风险之中的受试者的方法，该方法包括向受试者施用组合物，该组合物包含：
a) 治疗有效量的至少一种抑制与肾病有关的人类靶基因表达的寡核苷酸化合物；和 b) 药学上可接受的赋形剂或载体、或其混合物，从而在受试者中降低 CKD 风险。

背景技术

[0005] 慢性肾病

[0006] 慢性肾病 (CKD) 是世界范围内的公共健康问题，并被认为是和上升的心血管疾病和晚期肾疾病 (ESRD) 的风险有关的普遍病症。

[0007] 国家肾基金 (NKF) 肾脏病预后质量指导 (K/DOQI) 将慢性肾病定义为持续三个或更多月的肾损坏或下降的肾小球滤过率 (GFR)。其他 CKD 的标志也是已知的并用于诊断。通常，带有不可逆的硬化和肾单位减少的肾质量功能丧失导致渐进性 GFR 下降并最终导致 ESRD。

[0008] 最近，K/DOQI 出版了 CKD 阶段分类的指标，如下所述：

[0009] 阶段 1：GFR 正常或降低的肾损坏 ($> 90\text{mL}/\text{min}/1.73\text{m}^2$)

[0010] 阶段 2：GFR 轻微降低 ($60\text{--}89\text{mL}/\text{min}/1.73\text{m}^2$)

[0011] 阶段 3：GFR 中度降低 ($30\text{--}59\text{mL}/\text{min}/1.73\text{m}^2$)

[0012] 阶段 4：GFR 严重降低 ($15\text{--}29\text{mL}/\text{min}/1.73\text{m}^2$)

[0013] 阶段 5：肾衰竭 ($\text{GFR} < 15\text{mL}/\text{min}/1.73\text{m}^2$ 或透析)

[0014] 在 CKD 阶段 1 和 2，GFR 不能单独确定诊断。还依赖其他肾损坏的指标，包括在血液或尿液组成成分中的异常或在影像测试中的异常。

[0015] CKD 病理生理学

[0016] 每个肾脏有大约 1 百万肾单位，每个单位都对总 GFR 有贡献。不考虑肾脏损伤病原学，利用剩余健康肾单位的超滤和代偿性肥大，处于渐进性肾单位功能丧失的肾能够维持 GFR。当肾储备耗尽时，这种肾单位适应性为继续正常的血浆溶质清除留出了余地，因此仅在总 GFR 降低到 50% 后才开始出现尿素和肌酸酐在血浆中的水平显著上升。当 GFR 下降 50% 时，血浆肌酸酐值将翻倍。因此，病人体内的血浆肌酸酐从基准值 $0.6\text{mg}/\text{dL}$ 翻倍到 $1.2\text{mg}/\text{dL}$ 意味着损失了 50% 的有功能的肾单位量。

[0017] 虽然对所述的原因有益处,但是剩余肾单位超滤和肥大是引起渐进性肾功能障碍的主要原因。这种情况发生的原因在于上升的肾小球毛细血管压力,其能破坏毛细血管并最初导致病灶和部分肾小球硬化症,并最终导致肾小球硬化症。这个假设是基于对 5/6 肾切除大鼠的研究,这些大鼠中发展处的病变类似于在人类中观察到的 CKD。

[0018] 引起慢性肾病最普遍的原因是糖尿病和高血压。其他因素包括来自肾毒素(包含放射性对比剂)的急性损害或灌注降低(局部贫血);败血症;蛋白尿;带有间质损伤的肾脏氨生成上升;高血脂;有磷酸钙累积的高磷血症;氧化亚氮和烟气水平降低。

[0019] 在美国,这对健康体系来说是花费高、预后差的 CKD 的发病率和流行率都在上升。肾病是美国的第九致死原因。高死亡率导致联邦公共卫生署署长为美国公民要求健康国民 2010 计划为 CKD 保留一定的章节。这些章节的目的在于阐明目标并提供降低美国慢性肾病发病率、疾病率、死亡率和医疗成本的战略。多项指标可以评估慢性肾病的负担,所有指标都强调需要改善临床检查、治疗和监护和经济效益。降低肾衰竭将需要更多的公共卫生努力,包括有效地预防策略和慢性肾病的早期检查和治疗。

[0020] 自从 1989 年以来,国际间晚期肾病的发病率(ESRD)也在稳步增长。美国的 ESRD 发病率最高,其次是日本。日本有最高的每百万人口流行率,其次是美国。

[0021] 和血液透析有关的死亡率是惊人的,并且说明进入血液透析阶段的病人的生命预期明显缩短。在同一个年龄层,当与非透析的病人和没有肾病的个体相比,做透析的 ESRD 病人的死亡率显著升高。在 60 岁,健康人预期再活 20 年,然而 60 岁开始做血液透析的病人只能期望 4 年。(Aurora and Verelli, May 21, 2009. Chronic Renal Failure: Treatment & Medication. Emedicine. <http://emedicine.medscape.com/article/239798-治疗>).

[0022] 转让给本发明的受让人之一的国际专利公开 No. WO 2006/035434、WO 2008/104978、WO 2008/106102 和 WO 2009/001359 涉及治疗急性肾病(包括心脏手术后的急性肾衰竭)的方法。

[0023] 用于治疗 CKD 和减弱 CKD 发展的方法和组合物应该具有很好的治疗价值。

[0024] 发明概述

[0025] 根据一个方面,本发明提供在受试者中治疗或预防肾损坏的方法,该受试者处于和暴露于肾脏损害复发有关的慢性肾病 CKD 的风险之中,该方法包括向受试者施用治疗有效剂量的抑制和肾损害有关的靶基因的化合物,其中在肾脏损害的 24 小时之内将寡核苷酸化合物施用于受试者。在一些实施方案中,该化合物是寡核苷酸化合物。在一些实施方案中,寡核苷酸化合物抑制靶基因的表达。

[0026] 根据另一个方面,本发明提供了在受试者中减弱慢性肾病(CKD)发展的方法,该受试者处于由暴露于肾脏损害复发而导致的 CKD 发展的风险之中,该方法包括向受试者施用治疗有效剂量的寡核苷酸化合物(其下调与肾损伤有关的靶基因的表达),其中在每次肾脏损害 24 小时之内,将寡核苷酸化合物施用于受试者。在一些实施方案中,肾损害导致急包括急性肾损伤(AKI)的性肾脏损害。

[0027] 在各种实施方案中,处于慢性肾病(CKD)或 CKD 发展风险的受试者,该受试者患有的一种或多种 I 型或 II 型糖尿病、高血压(hypertension)、高胆固醇、心脏病、肝病、淀粉状蛋白病、镰状细胞病、系统性红斑狼疮、肾小球肾炎、多囊性肾病、动脉粥样硬化、血管疾病(例如动脉炎、血管炎、或纤维发育异常),或该受试者即将接受放射照相检查(即

放射性对比剂的施用),或该受试者使用肾毒药物,包括但不限于,镇痛剂例如醋氨酚(Tylenol®)和非-类固醇抗炎症药物(NSAID)(例如布洛芬(Motrin®,Advil®)),当长时间有规律地服用时这些药物能引起止痛药肾病变。

[0028] 在另一个方面本发明涉及减弱肾损坏(由在患有慢性肾病(CKD)的受试者中的肾脏损害而导致)严重性的方法,该方法包括向受试者施用治疗有效剂量的寡核苷酸化合物(其抑制与肾局部贫血有关的靶基因表达),从而减弱肾损坏。在一些实施方案中肾损坏是AKI。

[0029] 在其它的实施方案中,提供了预防由在患有CKD的受试者中的急性肾损伤或损害导致的CKD发展的方法,该方法包括向受试者施用治疗有效剂量的寡核苷酸化合物(其抑制与肾局部贫血有关的靶基因表达),从而预防CKD发展。

[0030] 在一些本发明方法的实施方案中,肾脏损害选自手术(包括心血管手术)、暴露于放射性对比剂、肌红蛋白尿、局部贫血/再灌注性损伤、尿路梗阻接触肾毒素,该毒素包括含放射性对比剂的对比试剂;灌注降低;蛋白尿;带有间质损伤的肾脏氨生成上升;高血脂;有磷酸钙累积的高磷血症。在一些实施方案中,肾脏损害选自局部贫血/再灌注、败血症和暴露于放射性对比剂。

[0031] 在某些实施方案中,在心血管手术或心肺手术期间或之后发生局部贫血/再灌注性损伤。肌红蛋白尿由作为导致直接近端小管细胞(PTC)损伤和肾脏血管收缩内源肾毒素肌红蛋白导致。复发或多种肾脏损害是指2、3、4、5或更多同一种或多种肾脏损害。

[0032] 在一些实施方案中将寡核苷酸施用于受试者在72小时之内(即之前、同时地或之后),在48小时之内、在24小时之内、在16小时之内、在8小时之内、在肾脏损害4小时之内、之前或之后,优选地和在约肾脏损害72小时之前到约肾脏损害后8小时之间。在一些实施方案中,在肾脏损害后0到4小时,施用寡核苷酸。在一些实施方案中,在肾脏损害前后将寡核苷酸施用于受试者。前后是指肾脏损害1小时之内、在50、45、40、35、30、25、20、15、10、5、4、3、2分钟之内或肾脏损害后1分钟。

[0033] 在各种实施方案中寡核苷酸化合物选自包含下列物质的组未修饰的或化学修饰的siRNA、shRNA、适体、反义分子、miRNA和核酶。在最优选的实施方案中,抑制剂化学修饰的siRNA。

[0034] 在一些实施方案中,靶基因是人类基因,其表达在肾脏损害后被调高。一些实施方案中,与肾损伤、肾损坏、肾局部贫血有关的靶基因选自具有在下面表1中阐述的mRNA序列的基因。靶基因非限制性示例包含p53、肿瘤蛋白p53结合蛋白2(TP53BP2);富含亮氨酸重复基因和含(LRDD)的死亡结构域;细胞色素b-245、 α 多肽(CYBA、p22phox);活化转录因子3(ATF3);半胱天冬酶2、细胞凋亡-相关的半胱氨酸肽酶(CASP2);NADPH氧化酶3(NOX3);harakiri、BCL2相互作用蛋白(HRK、BID3);补体成分1、q亚成分结合蛋白(C1QBP);BCL2/腺病毒E1B 19kDa相互作用蛋白3(BNIP3);分裂素激活蛋白激酶8(MAPK8、JNK1);分裂素激活蛋白激酶14(MAPK14、p38);ras-相关的C3肉毒杆菌毒素底物1(rho族、小GTP结合蛋白RAC1);糖原合成酶激酶3 β (GSK3B);嘌呤能受体P2X、配体门控离子通道、7(P2RX7);瞬时感受器电位通道、亚族M、成员2(TRPM2);聚(ADP-核糖)多糖水解酶(PARG);CD38分子(CD38);STEAP族成员4(STEAP4);骨形成蛋白2(BMP2);间隙连接蛋白、 α 1、43kDa(connexin43, GJA1);TYRO蛋白酪氨酸激酶结合蛋白(TYROBP);结缔组

织生长因子 (CTGF) ;分泌磷蛋白 1(骨桥蛋白、SPP1) ;ras 同源基因族、成员 A(RHOA) ;双氧化酶 1(DUOX1)、NOX1、NOX2(gp91phox、CYBB)、NOX4、NOX5、DUOX2 和相关的蛋白、NOXO1、NOXO2(p47phox、NCF1)NOXA1、NOXA2(p67phox、NCF2) 和 p40phox(NCF4)、ASPP1、CTDS、CAPNS1、REDD1、REDD2、HTRA2、KEAP1、SHC1、ZNHT1、LGALS3、HI95、TGFb-1、ACE、MCP-1、CDK、MIF、ECE-1、ET-1、TSA、Smad2、Smad3、ALK5、STAT3、PTGDS、TLR2。在一些实施方案中靶基因选自 p53 核 CASP2。不受理论束缚,任何一种或多种上述的基因可因肾脏损害而被上调,并且在肾脏中抑制一种或多种这些基因的上调可以保护肾脏细胞免受损坏(包括局部缺血损伤)。

[0035] 在另一个方面,本发明提供了预防在受试者中慢性肾病 (CKD) 发展的方法,该受试者处于正在发展的由暴露于多种肾脏损害而导致的 CKD 的风险之中,该方法包括向受试者施用治疗有效剂量的寡核苷酸化合物(其抑制和肾局部贫血有关的基因地表达),其中在肾脏损害 16 小时之内将寡核苷酸化合物施用于受试者。

[0036] 在又一个方面,本发明提供了预防在受试者中发生慢性肾病 (CKD) 的方法,该受试者可以处于正在发展的由暴露于多种肾脏损害而导致的 CKD 的风险之中(或受试者倾向于这种 CKD),该方法包括向受试者施用治疗有效剂量的寡核苷酸化合物(其抑制和肾局部贫血有关的基因地表达),其中在肾脏损害 16 小时之内将寡核苷酸化合物施用于受试者。方法包括用于局部或系统递送的持续的递送和可控的递送,该递送包括 siRNA 递送使用例如递送媒介物(包含泵、缓慢的或持续的释放的组合物或含有 siRNA 贮存的植入物)。递送媒介物包含自然的和合成的物质或自然的和合成的物质的联合物。

[0037] 也提供了用于治疗或预防慢性肾病 (CKD) 的试剂盒。在一些实施方案中,本发明提供了用于治疗或预防与暴露于放射性对比剂有关的慢性肾病 (CKD) 的试剂盒。在一些实施方案中,试剂盒包含含有治疗有效剂量的寡核苷酸化合物(抑制和肾损害有关的基因的表达)的组件,该组件的量有效于预防放射性对比剂导致的肾损坏,且该放射性对比剂的量可以有效完成一次放射照相检查。在某些实施方案中,包含作为单独个体制剂寡核苷酸化合物和作为单独个体制剂放射性对比剂。在一些实施方案中,寡核苷酸化合物和放射性对比剂被合并为一种组合物。在一些实施方案中,寡核苷酸化合物制剂和放射性对比剂制剂以不同的形式提供(例如一种制剂是液体而另一种制剂是冻干制剂)。在一些实施方案中,试剂盒进一步包含使用说明书。

[0038] 在各种实施方案中,本发明提供了使用双链寡核糖核苷酸化合物的方法,该化合物能抑制与 AKI 和 CKD 发展有关的靶基因的表达。在各种实施方案中,靶基因是和局部贫血/再灌注性损伤 (IRI) 有关的基因。在某些优选的实施方案中,靶基因选自列于如下文阐述的表 1 的基因。在特定的实施方案中,双链寡核糖核苷酸化合物是化学修饰的 siRNA。

[0039] 在一些实施方案中,根据以下结构对 siRNA 化合物进行化学修饰:

[0040] 5' (N)_x-Z 3' (反义链)

[0041] 3' Z'-(N')_y-z 5' (正义链)

[0042] 其中每个 N 和 N' 是核糖核苷酸,该核糖核苷酸可以是未修饰的或修饰的,或非常规部分;

[0043] 其中每一个 (N)_x 和 (N')_y 是寡核苷酸,在其中每一个连续的 N 或 N' 通过共价键连接于下一个 N 或 N' ;

[0044] 其中 Z 和 Z' 可以存在或不存在,但如果存在的话是独立地共价地连接于它所在链的 3' 末端的 1-5 个连续的核苷酸;

[0045] 其中 z'' 可以存在或不存在,但如果存在的话是共价地连接到 (N')_y 的 5' 末端的封端部分;

[0046] x 和 y 各独立地是 18 到 40 的整数;

[0047] 其中 (N')_y 的序列和 (N)_x 的序列是充分互补的;其中 (N)_x 包含与约 18 到约 40 个连续的核糖核苷酸充分互补的反义序列,这些核糖核苷酸存在于如表 1 所示的 mRNA 中,该表 1 阐述了任一 SEQ ID NO:1-115。

[0048] 在一些实施方案中,连接每个连续的 N 或 N' 的共价键是磷酸二酯键。在各种实施方案中所有的共价键都是磷酸二酯键。

[0049] 在各种实施方案中,化合物包含核糖核苷酸,其中 x = y 并且每个 x 和 y 是 19、20、21、22 或 23。在一些实施方案中 x = y = 23。在其它的实施方案中 x = y = 19。

[0050] 在一些实施方案中化合物是钝末端的,例如其中 Z 和 Z' 都不存在。在可选地实施方案中,化合物包含至少 1 个 3' 垂悬,其中至少 Z 和 Z' 两者之一是存在的。Z 和 Z' 可以独立地包含一种或多种共价地连接的已修饰的或非-修饰的核苷酸,例如反向 dT 或 dA; dT、LNA, 镜像核苷酸等等。在一些实施方案中,每个 Z 和 Z' 是独立地选自 dT 和 dTdT。

[0051] 在一些实施方案中,每个 (N)_x 和 (N')_y 包含未修饰的核苷酸。

[0052] 在一些实施方案中,在一种或多种核糖核苷酸的糖残基中,N 或 N' 包含修饰结构。在其它的实施方案中,化合物包含至少一个在糖残基修饰的核糖核苷酸。在一些实施方案中,化合物含糖残基的 2' 位的修饰结构。在一些实施方案中,在 2' 位的修饰结构包含氨基、氟、烷氧基或烷基部分。在某些实施方案中,2' 修饰结构包含甲基氧部分(也通称 2'-O-甲基; 2'-O-Me; 2'-O-CH₃)。在一些实施方案中,在每个 (N)_x 和 (N')_y 中核糖核苷酸在修饰的核糖核苷酸和未修饰的核糖核苷酸之间交替,每个已修饰的核糖核苷酸是被修饰过的,由此在它的糖上具有 2'-O-甲基,并且位于 (N)_x 中间位置的核糖核苷酸是未修饰过的且位于 (N')_y 中间位置的核糖核苷酸是已修饰的。在一些实施方案中,优选的化合物是 15,其靶基因是 p53。

[0053] 在一些实施方案中,在反义链和正义链的一条或两条中,siRNA 化合物包含已修饰的交替的核糖核苷酸。在其它的实施方案中,仅在反义链 (N)_x 中,化合物包含已修饰的交替的核糖核苷酸。在某些实施方案中,反义链的中间核糖核苷酸是未修饰的;例如核糖核苷酸在 19- 节链的第 10 位或在 23- 节链的第 12 位。

[0054] 在其他的实施方案中,化合物在交替的位置中包含已修饰的核糖核苷酸,其中每个在 (N)_x 5' 和 3' 末端的 N 的糖残基是被修饰过的,和每个在 (N')_y 5' 和 3' 末端的 N' 的糖残基是未被修饰过的。在一些实施方案中,(N)_x 和 (N')_y 的 3' 和 5' 末端都未被磷酸化。在其它的实施方案中,(N)_x 和 (N')_y 之一或两者的 3' 末端被磷酸化。

[0055] 在一些实施方案中,(N)_x 包含已修饰的和未修饰的核糖核苷酸,每个已修饰的核糖核苷酸在它的糖上具有 2'-O-甲基,其中在 (N)_x 的 3' 末端的 N 是已修饰的核糖核苷酸,(N)_x 包含至少 5 个在 3' 末端起始的交替的已修饰的核糖核苷酸和至少总共 9 个已修饰的核糖核苷酸,而且每个剩余的 N 是未修饰的核糖核苷酸。在一些实施方案中,(N)_x 包含在第 2、4、6、8、11、13、15、17 和 19 位的 2'-O-Me 修饰的核糖核苷酸。在其它的实施方案中,(N)_x

包含在第 1、3、5、7、9、11、13、15、17 和 19 位的 2' O Me 修饰的核糖核苷酸。

[0056] 在一些实施方案中,非常规部分选自镜像核苷酸 (mirror nucleotide) 和通过 2'-5' 核苷酸间磷酸键连接到邻近核苷酸的核苷酸。在一些实施方案中,镜像核苷酸选自 L-核糖核苷酸 (L-RNA) 和 L-脱氧核糖核苷酸 (L-DNA)。在一些实施方案中 (N')_y 包含至少 1 个非常规部分。

[0057] 在一个上述结构的实施方案中,化合物在 (N')_y 的一个或两个末端包含至少一个镜像核苷酸。在各种实施方案中,化合物包含 2 个连续的镜像核苷酸,一个在 (N')_y3' 倒数第二位且另一个在 (N')_y3' 末端。在一个优选的实施方案中, $x = y = 19$ 而且在 (N')_y 第 18 位包含 L-DNA。

[0058] 在一些实施方案中, $x = y = 19$ 和 (N')_y, 包含在第 1-17 和 19 位的未修饰的核糖核苷酸和 1 个在 3' 倒数第二位 (第 18 位) 的 L-DNA。在其它的实施方案中, $x = y = 19$ 并且 (N')_y 包含在第 1-16 和 19 位的未修饰的核糖核苷酸和 2 个在 3' 倒数第二位的连续的 L-DNA (第 17 和 18 位)。

[0059] 在另外一个上述结构的实施方案中, (N')_y 进一步包含一种或多种核苷酸 (在 1 个或 2 个末端含有内-糖桥联)。

[0060] 附图简述

[0061] 图 1 提供了在 ESRD 发展过程中的建议的 AKI 和 CKD 之间互相加强的恶性循环 (来自: B. Molitoris (2008) "Contrast nephropathy: are short-term outcome measures adequate for quantification of long-term renal risk", Nat Clin Pract Nephrol. 20084 :594-5.)。

[0062] 图 2 提供了如下文实施例 2 所描述的研究设计大纲。

[0063] 图 3 显示了在反复性局部缺血损伤之后, p53 siRNA 在肾功能上的作用。

[0064] 图 4 显示了 siP53 保护 GFR 和减少蛋白尿。

[0065] 图 5 在 5 个月的局部缺血损伤的循环之后, siP53 (12mg/kg) 在组织学上的作用。

[0066] 图 6 提供了为了通过肾切除术和以双月间隔诱导多发性 AKI 建立 CKD 模型的研究设计。通过使大鼠暴露于全肾切除术和多发性 AKI (7-8 月期间 3-4 次) 诱导 CKD, 并用高盐 (Na, 钠) 食物喂养。

[0067] 图 7 显示了以高或低 Na 食物喂养 7-8 月后, 处理前的肾功能参数。血清肌酐、GFR 和尿液蛋白在仅用 QM5 siRNA 或载体处理的大鼠中是相似的。高 Na 食物导致更快速的 CKD 发展, GFR (而非蛋白尿) 下降。

[0068] 图 8 显示了在 CKD 诱导的动物中, QM5 (siP53) 用于预防 AKI 的效果。

[0069] 图 9A-9H 显示了在 CKD 研究 (如图 6 概括的和如实施例 2.2 所述) 最结束时的组织病理学评估。图 9A-9C 涉及急性损伤参数: 肾小管坏死 (9A)、肾小管膨胀 (9B) 和管型 (9C)。图 9D-9H 涉及慢性损伤参数: 肾小球损坏 (9D), 间质细胞渗透 (9E); 间质纤维化 (9F) 肾小管萎缩或膨胀 (9G) 和血管病 (9H)。

[0070] 图 10 显示了在结束时的组织病理学评估结果, 并记录了在处理过和未处理过的组中的平均急性和慢性损伤评分。

[0071] 发明详述

[0072] 本发明基本上涉及在受试者中减弱慢性肾病发展或预防 CKD 发展恶化, 该受试者

处于其风险之中。该方法通常使用能调低急性肾损伤和特别是局部缺血再灌注性损伤有关的各种靶基因的表达的化合物。该方法使用化学修饰的小干扰 RNA 寡核苷酸 (siRNA), 其具有结构和修饰结构, 当与未修饰的化合物做比较时, 修饰结构能提高活性、提高稳定性和或减少毒性、降低靶向作用或降低先天免疫应答。

[0073] 下面的表 1 为靶基因非限制性示例, 用 NCBI 登陆码标记了基因识别号 (gi)。该表为相应地 mRNA, 阐述了各个 mRNA 序列、gi 号 (基因识别号) 和序列识别号 (SEQ ID NO)。

[0074] 表 1 : 非限制性靶基因列表

[0075]

基因	全名和人类基因 ID
REDD1	DDIT4, DNA-损坏-可诱导的转录物 4 gi 56676369 ref NM_019058 (SEQ ID NO: 1)
REDD2	DNA-损坏-可诱导的转录物 4-样 gi 34222182 ref NM_145244 (SEQ ID NO: 2)
TP53BP2 (ASPP2)	肿瘤蛋白 p53 结合蛋白、2 gi 112799848 ref NM_001031685.2 (SEQ ID NO: 3) gi 112799845 ref NM_005426.2 (SEQ ID NO: 4):
LRDD	富含亮氨酸重复基因和死亡结构域, 其包含: gi 61742781 ref NM_018494.3 (SEQ ID NO: 5)

[0076]

	gi 61742783 ref NM_145886.2 (SEQ ID NO: 6) gi 61742785 ref NM_145887.2 (SEQ ID NO: 7)
CYBA	细胞色素 b-245、 α 多肽 gi 68509913 ref NM_000101.2 (SEQ ID NO: 8)
ATF3	活化转录因子 3 gi 95102484 ref NM_001030287.2 v.3 (SEQ ID NO: 9) gi 71902534 ref NM_001674.2 v. 1 (SEQ ID NO: 10) gi 95102482 ref NM_001040619.1 (SEQ ID NO: 11)
CASP2	半胱天冬酶 2、细胞凋亡-相关的半胱氨酸肽酶 gi 39995058 ref NM_032982.2 (SEQ ID NO: 12) gi 39995060 ref NM_032983.2 (SEQ ID NO: 13)
NOX3	NADPH 氧化酶 3 Gi 11136625 ref NM_015718.1 (SEQ ID NO: 14)
HRK	harakiri gi 4504492 ref NM_003806.1 (SEQ ID NO: 15)
C1QBP	补体成分 1、q 亚成分结合蛋白 gi 28872801 ref NM_001212.3 (SEQ ID NO: 16)
BNIP3	BCL2/腺病毒 E1B 19kDa 相互作用蛋白 3 Gi 7669480 ref NM_004052.2 (SEQ ID NO: 17)
MAPK8	分裂素激活蛋白激酶 8 gi 20986493 ref NM_002750.2 v.2 (SEQ ID NO: 18) gi 20986522 ref NM_139049.1 v. 1 (SEQ ID NO: 19) gi 20986518 ref NM_139046.1 v.3 (SEQ ID NO: 20) gi 20986520 ref NM_139047.1 v.4 (SEQ ID NO: 21)
MAPK14	分裂素激活蛋白激酶 14 gi 20986511 ref NM_139012.1 v.2 (SEQ ID NO: 22) gi 20986515 ref NM_139014.1 v. 4 (SEQ ID NO: 23) gi 4503068 ref NM_001315.1 v.1 (SEQ ID NO: 24) gi 20986513 ref NM_139013.1 v.3 (SEQ ID NO: 25)
Rac1	ras-相关的 C3 肉毒杆菌毒素底物 1(rho 族,小 GTP 结合蛋白) gi 156071511 ref NM_018890.3(SEQ ID NO: 26) gi 156071503 ref NM_006908.4(SEQ ID NO: 27)
GSK3B	糖原合成酶激酶 3 β gi 21361339 ref NM_002093.2(SEQ ID NO: 28) gi 225903436 ref NM_001146156.1 (SEQ ID NO: 29)
P2RX7	嘌呤能受体 P2X、配体门控离子通道,7 gi 34335273 ref NM_002562.4 (SEQ ID NO: 30)

[0077]

TRPM2	瞬时感受器电位通道、亚族 M、成员 2 gi 67906812 ref NM_003307.3 v. L (SEQ ID NO: 31)
PARG	聚(ADP-核糖)多糖水解酶 gi 70610135 ref NM_003631.2 (SEQ ID NO: 32)
CD38	CD38 分子 Gi 38454325 ref NM_001775.2 (SEQ ID NO: 33)
STEAP4	STEAP 族成员 4 Gi 13375867 ref NM_024636.1 (SEQ ID NO: 34)
BMP2	骨形成蛋白 2 gi 80861484 ref NM_001200.2(SEQ ID NO: 35)
GJA1	间隙连接蛋白、 α 1、43kDa gi 4755136 ref NM_000165.2(SEQ ID NO: 36)
TYROBP	TYRO 蛋白酪氨酸激酶结合蛋白 gi 291045273 ref NM_001173515.1 变体 4 (SEQ ID NO: 37) gi 291045270 ref NM_198125.2 变体 2 (SEQ ID NO: 38) gi 291045269 ref NM_003332.3 变体 1 (SEQ ID NO: 39) gi 291045271 ref NM_001173514.1 变体 3 (SEQ ID NO: 40)
CTGF	结缔组织生长因子 gi 4503122 ref NM_001901.1(SEQ ID NO: 41)
SPP1	分泌磷蛋白 1 gi 91206461 ref NM_001040058.1(SEQ ID NO: 42) gi 38146097 ref NM_000582.2 (SEQ ID NO: 43) gi 91598938 ref NM_001040060.1(SEQ ID NO: 44)
RHOA	ras 同源基因族成员 A gi 50593005 ref NM_001664.2(SEQ ID NO: 45)
DUOX1	双氧化酶 1 gi 28872749 ref NM_017434.3 (SEQ ID NO: 46) gi 28872750 ref NM_175940.1 (SEQ ID NO: 47)
NOX4	NADPH 氧化酶 4 gi 219842344 ref NM_016931.3 v. 1 (SEQ ID NO: 48) gi 219842345 ref NM_001143836.1 v. 1 (SEQ ID NO: 49) gi 219842347 ref NM_001143837.1 v. 1 (SEQ ID NO: 50)
NOX1	NADPH 氧化酶 1 (gi: 21614529、NM_007052 亚型 1L; SEQ ID NO: 51) (gi: 7669509、NM_013955 亚型 1Lv; SEQ ID NO: 52)
NOX2 (gp91pho、	NADPH 氧化酶 2 (gi: 6996020, NM_000397; SEQ ID NO: 53)

[0078]

CYBB)	
NOX5	NADPH 氧化酶 5 (gi: 20127623, NM_024505; SEQ ID NO: 54)
DUOX2	双氧化酶 2 (gi: 132566531, NM_014080; SEQ ID NO: 55)
NOXO1	NADPH 氧化酶组织原 1 (gi: 34222190, 变异体 a, NM_144603, SEQ ID NO: 56) (gi: 41281810, 变异体 b, NM_172167; SEQ ID NO: 57) (gi: 41281827, 变异体 c, NM_172168; SEQ ID NO: 58)
NCF1 (p47phox, NOXO2)	NADPH 氧化酶组织原 2 (gi: 115298671, NM_000265; SEQ ID NO: 59)
NOXA1	NADPH 氧化酶激活物 1 (gi: 41393186, NM_006647; SEQ ID NO: 60)
NCF2 (p67phox, NOXA2)	NADPH 氧化酶激活物 2 (gi: 67189969, NM_000433; SEQ ID NO: 61) (gi 189083741 ref NM_001127651.1 v.2 (SEQ ID NO: 62)
ASPP1	蛋白磷酸酶 1, 调节(抑制剂)亚单位 13B (PPP1R13B) gi 121114286 ref NM_015316.2 (SEQ ID NO: 63)
CTSD	组织蛋白酶 D gi 23110949 ref NM_001909.3 (SEQ ID NO: 64)
CAPNS1	钙蛋白酶小亚单位 1 gi 51599152 ref NM_001749.2 变异体 1 (SEQ ID NO: 65) gi 51599150 ref NM_001003962.1 变异体 2 (SEQ ID NO: 66)
p53 (TP53)	肿瘤蛋白 p53 gi 187830767 ref NM_000546.4 变异体 1 (SEQ ID NO: 67) gi 187830776 ref NM_001126112.1 变异体 2 (SEQ ID NO: 68) gi 187830854 ref NM_001126114.1 变异体 3 (SEQ ID NO: 69) gi 187830822 ref NM_001126113.1 变异体 4 (SEQ ID NO: 70) gi 187830893 ref NM_001126115.1 变异体 5 (SEQ ID NO: 71) gi 187830900 ref NM_001126116.1 变异体 6 (SEQ ID NO: 72) gi 187830908 ref NM_001126117.1 变异体 7 (SEQ ID NO: 73)
HTRA2	Htra 丝氨酸蛋白酶 2 var 1 gi: 73747817, NM_013247 (SEQ ID NO: 74) var 2 gi: 73747818, NM_145074 (SEQ ID NO: 75)
KEAP1	Kelch-样 ECH-相关的蛋白 1 var 1 gi: 45269144 NM_203500 (SEQ ID NO: 76)

[0079]

	var 2 gi: 45269143 NM_012289 (SEQ ID NO: 77)
SHC1	Src 同源 2 结构域, 其包含转化的 prot. 1 gi 194239661 ref NM_183001.4 (SEQ ID NO: 78) gi 194239660 ref NM_003029.4 (SEQ ID NO: 79) gi 194239663 ref NM_001130040.1 (SEQ ID NO: 80) gi 194239667 ref NM_001130041.1 (SEQ ID NO: 81)
ZNHIT1	Zn 指 HIT 一型 gi: 37594439; NM_006349 (SEQ ID NO: 82)
LGALS3	植物凝血素半乳糖苷-结合可溶 3 var 1 gi: 115430222 NM_002306 (SEQ ID NO: 83) var 2 gi: 115430224 NR_003225 (SEQ ID NO: 84) var 3 gi 294345474 ref NM_001177388.1 (SEQ ID NO: 85)
HI95	Sestrin2 gi: 32454742 NM_031459 (SEQ ID NO: 86)
TGFB-1	转化生长因子 β -1 gi: 63025221 NM_000660 (SEQ ID NO: 87)
ACE	血管紧张素-转换酶 转录物变异体 2: gi 23238213 ref NM_152830.1 (SEQ ID NO: 88) 转录物变异体 1, gi 23238217 ref NM_000789.2 (SEQ ID NO: 89)
CCL2	智人趋化因子 (C-C 基序) 配体 2 (CCL2), mRNA gi 56119169 ref NM_002982.3 (SEQ ID NO: 90)
CDK1 (CDC2)	智人细胞分裂周期 2, G1 to S 和 G2 to M (CDC2), gi 195927038 ref NM_001786.3 v.1 (SEQ ID NO: 91) gi 195927039 ref NM_033379.3 v.2 (SEQ ID NO: 92) gi 195927040 ref NM_001130829.1 v.3 (SEQ ID NO: 93)
MIF	巨噬细胞抑制性因子 gi: 4505184 NM_002415 (SEQ ID NO: 94)
ECE-1	内皮素转换酶 gi 164519130 ref NM_001397.2 变异体 1, (SEQ ID NO: 95) gi 164519139 ref NM_001113349.1 (SEQ ID NO: 96) gi 164519135 ref NM_001113347.1 (SEQ ID NO: 97) gi 164519137 ref NM_001113348.1 (SEQ ID NO: 98)
ET-1 (EDN1)	智人内皮素 1, mRNA gi 154800436 ref NM_001955.3 (SEQ ID NO: 99)
TSA (LY6E)	(胸腺共享抗原 1) 智人淋巴细胞抗原 6 复合体, 基因座 E (LY6E), mRNA 变异体 1, gi 187827163 ref NM_002346.2 (SEQ ID NO: 100)

[0080]

	变异体 2, gi 187761330 ref NM_001127213.1 (SEQ ID NO: 101)
Smad2	智人 SMAD 族成员 2 (SMAD2), mRNA 变异体 1, gi 118572580 ref NM_005901.4 (SEQ ID NO: 102) 变异体 2, gi 118572581 ref NM_001003652.2 (SEQ ID NO: 103) gi 209693425 ref NM_001135937.1 var 3 (SEQ ID NO: 104)
Smad3	智人 SMAD 族成员 3 (SMAD3), mRNA 变异体 1, gi 52352808 ref NM_005902.3 (SEQ ID NO: 105) gi 223029439 ref NM_001145102.1 v.2 (SEQ ID NO: 106) gi 223029441 ref NM_001145103.1 v.3 (SEQ ID NO: 107) gi 223029443 ref NM_001145104.1 v.4 (SEQ ID NO: 108)
TGFBR1	智人转化生长因子、 β 受体 1、mRNA(ALK5、活化素-受体例如激酶) 转录物变异体 1, gi 66346739 ref NM_004612.2 (SEQ ID NO: 109) 转录变异体 2, gi 195963411 ref NM_001130916.1 (SEQ ID NO: 110)
STAT3	转录 3 的智人信号传导物和激活物(急性-阶段应答因子)(STAT3),mRNA 转录物变异体 1, gi 47080104 ref NM_139276.2 (SEQ ID NO: 111) 转录物变异体 2 gi 47080105 ref NM_003150.3 (SEQ ID NO: 112) 转录物变异体 3 gi 47458819 ref NM_213662.1 (SEQ ID NO: 113)
PTGDS	智人前列腺素 D2 合成酶 21kDa(脑)(PTGDS), mRNA gi 38505192 ref NM_000954.5 (SEQ ID NO: 114)
TLR2	智人 toll-样受体 2 (TLR2), mRNA gi 68160956 ref NM_003264.3 (SEQ ID NO: 115)

[0081] 定义

[0082] 为了方便起见,本文描述了某些使用于说明书、实施例和权利要求中的术语。

[0083] 应该注意,除非有特别清楚的说明,如本文所用单数形式“a”、“an”和“the”包括复数形式。

[0084] 当本发明的各方面或实施方案就马库什组或其它替换物组描述时,本领域熟练的技术人员应该意识到本发明也是由此描述该组任一种体成员或成员分组。

[0085] “抑制剂”是化合物,其使基因的表达或该基因产物的活性降低(部分地或全部地)或调低到足够的程度,以达到所需的生物或生理效应。术语“抑制剂”如本文所用是指一种或多种寡核苷酸抑制剂,其包含 siRNA、shRNA、合成 shRNA ;miRNA、反义 RNA 和 DNA 和核酶。

[0086] “siRNA 抑制剂”是化合物,其使基因的表达或该基因产物的活性降低(部分地或全部地)或调低到足够的程度,以达到所需的生物或生理效应。如本文所用,术语“siRNA 抑制剂”是指一种或多种 siRNA、shRNA、合成 shRNA ;miRNA。抑制也可以指下调或 RNAi 沉默。

[0087] 如本文所用,术语“抑制”或“下调”是指,使基因的表达或该基因产物的活性降低到足够的程度以达到所需的生物或生理效应。抑制或调低可以是完全的或部分的。

[0088] 如本文所用,术语靶基因的“抑制”或“下调”,是指降低基因的表达(转录或翻译)或基因的多肽活性,该基因选自任一 SEQ ID NO:1-115 或 SNP(单核苷酸多态性)或其另外的变异体组成的组。靶基因的 mRNA gi 号如表 1 所阐述。靶 mRNA 序列的多核苷酸序列是指本文所阐述的 mRNA 序列,或任一其同源序列,该序列与任一本文所述的 mRNA 优选地具有至少 70%一致性、更优选地 80%一致性、还更优选地 90%或 95%一致性。因此,本发明包含多核苷酸,其已暴露于如本文所述的突变、改变或修饰。术语“mRNA 多核苷酸序列”和“mRNA”可交换地使用。

[0089] 如本文所用,可交换地使用的术语“多核苷酸”和“核酸”是指包含脱氧核糖核酸(DNA)和核糖核酸(RNA)的核苷酸序列。术语也应该被理解为包括由核苷酸类似物产生的 RNA 或 DNA 的等价物、类似物。在本申请始终,所述 mRNA 序列代表相应的基因。

[0090] “寡核苷酸”或“寡聚物”是指从约 2 到约 50 个核苷酸的脱氧核糖核苷酸或核糖核苷酸序列。每种 DNA 或 RNA 核苷酸可以是独立地天然的或合成的,和或已修饰的或未修饰的。修饰包括发生在糖部分、碱基部分和/或在寡核苷酸中的核苷酸间的连接上的改变。本发明的化合物包括分子,其含有脱氧核糖核苷酸、核糖核苷酸、已修饰的脱氧核糖核苷酸、已修饰的核糖核苷酸及其联合物。

[0091] “核苷酸”是指包含脱氧核糖核苷酸和核糖核苷酸,可以是天然的或合成的,和/或已修饰的或未修饰的。修饰包括发生在糖部分、碱基部分和/或在寡核苷酸中的核苷酸间的连接上的改变。如本文所用,术语“核糖核苷酸”包含自然的和合成的、未修饰的和已修饰的核糖核苷酸。修饰包括发生在糖部分、碱基部分和/或在寡核苷酸中的核苷酸间的连接上的改变。

[0092] 核苷酸/寡核苷酸的类似物或修饰优选地用于本发明,所提供的所述类似物或修饰不会显著地不利地影响核苷酸/寡核苷酸的功能。在一些实施方案中,化学修饰导致提高活性或稳定性或降低脱-靶向作用、或诱导先天免疫应答。可接受的修饰包括糖部分修饰、碱基部分修饰、在核苷酸间键合和其联合物中的修饰。

[0093] 核苷酸可选自自然地存在的或合成修饰的碱基。自然地存在的碱基包含腺嘌呤、鸟嘌呤、胞嘧啶、胸腺嘧啶和尿嘧啶。已修饰的核苷酸碱基包含肌苷、黄嘌呤、次黄嘌呤、2-氨基腺嘌呤、6-甲基、2-丙基和其它烷基腺嘌呤、5-卤基尿嘧啶、5-卤基胞嘧啶、6-氮杂胞嘧啶和 6-氮杂胸腺嘧啶、伪尿嘧啶、4-硫代尿嘧啶、8-卤基腺嘌呤、8-氨基腺嘌呤、8-硫醇腺嘌呤、8-硫醇烷基腺嘌呤、8-羟基腺嘌呤和其它 8-取代的腺嘌呤、8-卤基鸟嘌呤、8-氨基鸟嘌呤、8-硫醇鸟嘌呤、8-硫代烷基鸟嘌呤、8-羟基鸟嘌呤和其它取代的鸟嘌呤、其它氮杂和去氮腺嘌呤、其它氮杂和去氮鸟嘌呤、5-三氟甲基尿嘧啶和 5-三氟胞嘧啶。

[0094] 另外,可以制备包含核苷酸类似物的化合物,其中一种或多种核苷酸的结构被根本地改变并且更适合作为治疗或实验试剂。核苷酸类似物的一个示例是缩氨酸核酸(PNA),其中 DNA(或 RNA)中的脱氧核糖(或核糖)磷酸基骨架被聚酰胺骨架(和在缩氨酸中所发现的类似)取代。已经发现 PNA 类似物能抵抗酶促降解并且扩大存活范围至体内和体外。

[0095] 对于糖残基的可以的修饰结构是多样的,并且包含 2'-O 烷基、锁核酸(LNA)、乙二醇核酸(GNA)、苏糖核酸(TNA)、阿拉伯糖苷、阿卓糖醇(ANA)和其他,包含吗啉代和环己基的 6-元糖。

[0096] LNA 化合物见诸于国际专利公开 No. WO 00/47599、WO 99/14226 和 WO 98/39352。

包含 LNA 核苷酸的 siRNA 化合物的示例见诸于 Elmen et al., (NAR 2005. 33(1) :439-447) 和国际专利公开 No. WO 2004/083430。

[0097] 骨架修饰结构,例如乙基(导致二氧磷基-乙基三酯);丙基(导致二氧磷基-丙基三酯);和丁基(导致二氧磷基-丁基三酯)也是可以的。其他骨架修饰结构包含聚合体骨架、环状骨架、无环骨架、硫代磷酸基-D-核糖骨架、酰胺基、磷酰基乙酸基衍生物。某些结构包含具有一个或多个 2'-5' 核苷酸 间键合(桥联或骨架)的 siRNA 化合物。

[0098] 由本发明分子表示的其他修饰结构包含核苷修饰结构,例如人工核酸、缩氨酸核酸(PNA)、吗啉代和锁核酸(LNA)、乙二醇核酸(GNA)、苏糖核酸(TNA)、阿拉伯糖苷和镜像核苷(例如 β -L-脱氧核苷而不是 β -D-脱氧核苷)。进一步说,所述分子可以还在糖部分包含修饰结构,例如 2'-烷基、2'-氟、2'-脱氧-2'-氟、2'-O-烯丙基、2'-胺和 2'-烷氧基。其他糖修饰结构如本文所述。

[0099] 另外,本发明抑制性核酸分子可包含一种或多种缝隙和/或一种或多种缺口和/或一种或多种错配。不希望受理论束缚,缝隙、缺口和错配有利于核酸/siRNA 部分去稳定化,因此内源细胞结构,例如 DICER、DROSHA 或 RISC 可以更容易地将它加工成抑制性成分。

[0100] 就本发明来说,在核酸中的缝隙是指在一条链中缺乏一个或多个内在核苷酸,同时在核酸中的缺口是指在一条链中缺乏在邻近的核苷酸之间的核苷酸间键合。任一本发明的分子可含有一种或多种缝隙和/或一种或多种缺口。

[0101] siRNA 和 RNA 干扰

[0102] RNA 干扰(RNAi)涉及双链(ds)RNA-依赖基因的特定后转录沉默现象。起初,试图研究这一现象并实验地操纵哺乳动物细胞,但是能活性的、非特异性的抗病毒防御机制(被针对长 dsRNA 分子的应答所激活)挫败了这种努力(Gil et al. Apoptosis, 2000. 5 : 107-114)。后来发现在哺乳动物细胞中,21 种核苷酸 RNA 的合成双链能调节基因特异性 RNAi,同时不刺激基因抗病毒防御机制(参见 Elbashir et al. Nature 2001, 411 :494-498 and Caplen et al. PNAS USA 2001, 98 :9742-9747)。因此,在试图理解基因功能的研究中,短双链的小干扰 RNA(siRNA) 成为很有用的工具。这样的 RNA 干扰(RNAi) 是指,在哺乳动物中小干扰 RNA(siRNA) 或微 RNA 调节的序列-特异性后转录基因沉默受的过程(Fire et al, Nature 1998. 391, 806) (miRNA ;Ambros, Nature 2004 431 :7006, 350-55 ;和 Bartel, Cell. 2004. 116(2) :281-97)。在植物中,相应的过程通常是指特异性后转录基因沉默或 RNA 沉默,并且优选地不包括真菌。

[0103] siRNA 是双链 RNA 分子,其能抑制(部分地或全部地)或下调基因或者内源的、对应细胞、外源基因(例如病毒核酸)的基因/mRNA 的表达。RNA 干扰的机制在下文详述。

[0104] 很多研究表明 siRNA 治疗在哺乳动物和人类体内是有效的。Bitko 等人指出,当以鼻内施用的特定 siRNA 分子在治疗小鼠中是有效的,该 siRNA 分子能直接对抗呼吸道合胞病毒(RSV) 壳包核酸 N 基因(Bitko et al., Nat. Med. 2005, 11(1) :50-55)。最近,siRNA 被成功地用于灵长类中的抑制(Tolentino et al., Retina 2004. 24(1) :132-138)。使用 siRNA 作为疗法的综述,参见例如 Barik(J. Mol. Med. 2005. 83 :764-773) 或 Dykxhoorn et al (2006. Gene Ther. 13 :541-552)。

[0105] siRNA 结构

[0106] 对应已知基因的 siRNA 的选择和合成被广泛地报道;(参见例如 Ui-Tei et al.,

J Biomed Biotech. 2006 ;2006 :65052 ;Chalk et al., BBRC. 2004, 319(1) :264-74 ;Sioud & Leirdal, Met. Mol Biol. ;2004, 252 :457-69 ;Levenkova et al., Bioinform. 2004, 20(3) :430-2 ;Ui-Tei et al., NAR. 2004, 32(3) :936-48).

[0107] 例如,已修饰的 siRNA 的制备和用途参见,例如 Braasch et al., Biochem. 2003, 42(26) :7967-75 ;Chiu et al., RNA, 2003, 9(9) :1034-48 ;PCT 公开 WO 2004/015107(atugen AG) 和 WO 02/44321(Tuschl et al)。美国专利 No. 5,898,031 和 6,107,094,教导了化学修饰的寡聚物。美国专利公开 No. 2005/0080246 和 2005/0042647 分别地涉及具有改变的基元的寡聚化合物和具有化学修饰核苷间键合的 dsRNA 化合物。

[0108] 其它修饰结构也被公开了。5' - 磷酸基部分的修饰显示能提高在果蝇胚胎中的 siRNA 活性 (Boutla, et al., Curr. Biol. 2001, 11 :1776-1780), 并且这对在人类 HeLa 细胞中的 siRNA 功能也是需要的 (Schwarz et al., Mol. Cell, 2002, 10 :537-48). Amarzguioui et al., (NAR, 2003, 31(2) :589-95) 说明了依赖 2' -O- 甲基修饰结构定位 siRNA 活性。Holen et al (NAR. 2003, 31(9) :2401-07) 报告了相对天然型,具有少量 2' -O- 甲基修饰的核苷的 siRNA 具有良好的活性,但是随着 2' -O- 甲基修饰的核苷数量增加活性降低。Chiu 和 Rana (RNA. 2003, 9 :1034-48) 教导了相对于未修饰的 siRNA,在正义或反义链(全部修饰的链)中引入 2' -O- 甲基修饰的核苷,将严重降低 siRNA 活性。有报告称,在反义链的 5' - 末端 2' -O- 甲基基团的取代将严重限制活性,然而在反义链的 3' - 末端和在正义链的两个末端的这种取代却是耐受的 (Czauderna et al., NAR. 2003, 31(11) :2705-16 ;WO 2004/015107)。本发明的分子的优点是非毒性的并且可以作为药物组合物制备,用于治疗多种疾病。

[0109] 国际专利公开 No. WO 2008/050329(转让给本发明)涉及 siRNA 化合物、包含其的组合物和用其治疗与凋亡前基因表达相关的疾病和疾患的方法,并且将该专利全部地并入本文。US Ser. No. 11/655610 涉及通过抑制一般的或 p53 特定的凋亡前基因,而治疗听觉受损的方法。

[0110] 寡核苷酸

[0111] 本发明提供了使用包含双链寡核苷酸(例如 siRNA)的寡核苷酸抑制剂的方法,该抑制剂可以调低所需基因的表达。siRNA 是双链寡核糖核苷酸,其中正义链来源于所需基因的 mRNA 序列,并且反义链与正义链充分互补。通常,在不损失 siRNA 活性的前提下,一些靶向 mRNA 序列的偏差是允许的(参见例如 Czauderna et al., NAR. 2003, 31(11) :2705-2716)。不受理论束缚,本发明 siRNA 在以后转录水平抑制基因表达的同时,可以会或不会破坏 mRNA ;siRNA 可以靶向 mRNA 以完成特定裂解和降解和 / 或可抑制靶向信息的翻译。

[0112] 在各种实施方案中,siRNA 包含含有第一链和第二链的 RNA 双链,因此第一链包含核糖核苷酸序列,其至少部分地与靶向核酸(转录自靶基因的 mRNA)的约 18 到约 40 个连续的核苷酸互补,并且第二链包含的核糖核苷酸序列至少部分地与第一链互补,并且其中所述第一链和 / 或所述第二链包含一种或多种化学修饰的核糖核苷酸和 / 或非常规部分。

[0113] 在一个实施方案中,siRNA 化合物包含至少一种在糖部分上包含 2' 修饰结构的核糖核苷酸(2' 糖修饰结构)。在某些实施方案中,化合物包含 2'-O- 烷基或 2'- 氟或 2'-O- 烯丙基或任一其它 2' 修饰结构,可选地在不同的位置。其它稳定的修饰结构也是可以的(例

如末端修饰结构)。在一些实施方案中,优选的 2'-O-烷基是 2'-O-甲基(甲基氧、2'-OMe)糖修饰结构。

[0114] 在一些实施方案中,寡核苷酸的骨架是被修饰过的,并且包含磷酸基-D-核糖实体,而且也包含硫代磷酸基-D-核糖实体、三酯、硫代酯、2'-5'桥联骨架(也可以表示为 5'-2')、PACE 等。

[0115] 如本文所用,术语“非配对核苷酸类似物”是指核苷酸类似物其包含非-碱基配对部分包括但不限于:6-脱氨基腺苷(水粉草素)、4-Me-吡啶、3-硝基吡咯、5-硝基吡啶、Ds、Pa、N3-Me-riboU、N3-Me-riboT、N3-Me-dC、N3-Me-dT、N1-Me-dG、N1-Me-dA、N3-乙基-dC、N3-Me-dC。在一些实施方案中,非-碱基配对核苷酸类似物是核糖核苷酸。在其它的实施方案中它是脱氧核糖核苷酸。另外,可以制备多核苷酸的类似物,其中一种或多种核苷酸的结构被根本地改变,并且更适合作为治疗或实验试剂。核苷酸类似物的一个示例是缩氨酸核酸(PNA)其中DNA(或RNA)中的脱氧核糖(或核糖)磷酸基骨架被聚酰胺骨架(和在缩氨酸中所发现的类似)取代。已经发现PNA类似物能抵抗酶促降解并且在体内和体外提高了稳定性。其它有用的修饰结构包含聚合体骨架、环状骨架、无环骨架、硫代磷酸基-D-核糖骨架、三酯骨架、硫代脂骨架、2'-5'桥联骨架、人工核酸、吗啉代核酸、乙二醇核酸(GNA)、苏糖核酸(TNA)、阿拉伯糖苷、和镜像核苷(例如 β -L-脱氧核糖核苷而不是 β -D-脱氧核糖核苷)。一种或多种反向核苷酸,例如反向胸苷或反向腺嘌呤能用来合成本发明的化合物(参见,例如Takei, et al., 2002, JBC 277(26):23800-06)。

[0116] 其它的修饰结构包含末端修饰结构在寡核苷酸地 5' 和 / 或 3' 部分,而且也通称封端部分。这样的末端修饰结构选自核苷酸、已修饰的核苷酸、脂质、缩氨酸、糖和反向脱碱基部分。

[0117] 在本发明中有时提到的“脱碱基核苷酸”或“脱碱基核苷酸类似物”,更恰当地说是指伪-核苷酸或非常规部分。核苷酸是核酸单体单元,包含核糖或脱氧核糖、磷酸基、和碱基(在DNA中的腺嘌呤、鸟嘌呤、胸腺嘧啶、或胞嘧啶;在RNA中的腺嘌呤、鸟嘌呤、尿嘧啶或胞嘧啶)。已修饰的核苷酸包含在糖、磷酸基和 / 或碱基中的一种或多种的修饰结构。脱碱基伪-核苷酸没有碱基,并且严格地说这样不是核苷酸。

[0118] 术语“非常规部分”如本文所用是指脱碱基核糖部分、脱碱基脱氧核糖部分、脱氧核糖核苷酸、已修饰的脱氧核糖核苷酸、镜像核苷酸、非-碱基配对核苷酸类似物和通过 2'-5'核苷酸间磷酸键连接到邻近核苷酸的核苷酸;桥联核酸(包括LNA和亚乙基桥联核酸)。在一些本发明的实施方案中,优选的非常规部分是脱碱基核糖部分、脱碱基脱氧核糖部分、脱氧核糖核苷酸、镜像核苷酸和通过 2'-5'核苷酸间磷酸键连接到邻近核苷酸的核苷酸。

[0119] 脱碱基脱氧核糖部分包含例如脱碱基脱氧核糖-3'-磷酸基;1,2-双脱氧-D-呋喃核糖-3-磷酸基;1,4-无水-2-脱氧-D-核糖醇-3-磷酸基。反向脱碱基脱氧核糖部分包含反向脱碱基脱氧核糖;3',5'反向脱氧脱碱基 5'-磷酸基。

[0120] 相对于自然地存在的或通常使用的核苷酸,“镜像”核苷酸是有相反手性的核苷酸,即自然地存在的(D-核苷酸)的镜像(L-核苷酸),就镜像核糖核苷酸来说也是指L-RNA,和“镜像异构体”。核苷酸,可以是核糖核苷酸或脱氧核糖核苷酸,并且可以进一步包含至少1个糖、碱基和 / 或骨架修饰结构。参见美国专利 No. 6, 586, 238 和美国专利

No. 6, 602, 858, 公布了包含至少一种 L-核苷酸取代的核酸催化剂。镜像核苷酸包含例如 L-DNA (L-脱氧核糖腺苷酸-3'-磷酸基(镜像 dA); L-脱氧核糖胞啶-3'-磷酸基(镜像 dC); L-脱氧核糖鸟苷-3'-磷酸基(镜像 dG); L-脱氧核糖胸苷-3'-磷酸基(镜像 dT)) 和 L-RNA (L-核糖腺苷酸-3'-磷酸基(镜像 rA); L-核糖胞啶-3'-磷酸基(镜像 rC); L-核糖鸟苷-3'-磷酸基(镜像 rG); L-核糖尿嘧啶-3'-磷酸基(镜像 dU))。术语“封端部分”如本文所用包含脱碱基核糖部分、脱碱基脱氧核糖部分、包含 2' O 烷基修饰结构的脱碱基核糖和脱碱基脱氧核糖部分的修饰结构;反向脱碱基核糖和脱碱基脱氧核糖部分和其修饰结构;C6-亚氨基-Pi;包含 L-DNA 和 L-RNA 镜像核苷酸;5'-O-Me 核苷酸;和包含 4', 5'-亚甲基核苷酸的核苷酸类似物;1-(β-D-赤呋喃糖基)核苷酸;4'-硫核苷酸、碳环核苷酸;5'-氨基-烷基磷酸基;1,3-二氨基酸-2-丙基磷酸基、3-氨丙基磷酸基;6-氨基己基磷酸基;12-氨基十二烷基磷酸基;羟丙基磷酸基;1,5-脱水己糖醇核苷酸;α-核苷酸;苏-戊糖基核苷酸;无环 3', 4'-seco nucleotide;3,4-二羟基丁基核苷酸;3,5-二羟基戊基核苷酸、'-5'-反向脱碱基部分;1,4-丁二醇磷酸基;5'-氨基;和桥联的或非桥联的甲基磷酸基和 5'-巯基部分。

[0121] 某些优选的封端部分是脱碱基核糖或脱碱基脱氧核糖部分;反向脱碱基核糖或脱碱基脱氧核糖部分;C6-氨基-Pi;包含 L-DNA 和 L-RNA 镜像核苷酸。

[0122] 进一步的末端修饰结构是生物素基团。这样的生物素基团可优选地连接于第一和/或第二链的最 5' 端或最 3' 端之一或者两端的核苷酸。在更优选的实施方案中,生物素基团链接于多肽或蛋白。本发明的范围包含多肽或蛋白链接到任一其它上述的末端修饰结构。

[0123] 根据本发明,如本文公布的各种末端修饰结构优选地位于核酸的核苷酸的核糖部分。更特定地,如果这样修饰的核苷酸是末端核苷酸,末端修饰结构可连接于或取代任一核糖部分的 OH-基团(包括但不限于 2' OH, 3' OH 和 5' OH)。反向脱碱基或脱碱基是核苷酸,不具有核碱基部分的脱氧核糖核苷酸或核糖核苷酸(例如参见 Sternberger, et al., (2002). Antisense nucleic acid drugs Dev, 12, 131-43)。

[0124] 已修饰的脱氧核糖核苷酸包含,例如可以用作为在 5' 末位(第 1 位)的核苷酸的 5' OMe DNA (5-甲基-脱氧核糖鸟苷-3'-磷酸基);PACE (脱氧核糖腺嘌呤 3' 磷酰基乙酸基、脱氧核糖胞啶 3' 磷酰基乙酸基、脱氧核糖鸟苷 3' 磷酰基乙酸基、脱氧核糖胸苷 3' 磷酰基乙酸基)。

[0125] 桥联核酸包含 LNA (2' -O, 4' -C-亚甲基桥联核酸腺苷 3' 单磷酸基、2' -O, 4' -C-亚甲基桥联核酸 5-甲基-胞啶 3' 单磷酸基、2' -O, 4' -C-亚甲基桥联核酸鸟苷 3' 单磷酸基、5-甲基-尿苷(或胸苷) 3' 单磷酸基);和 ENA (2' -O, 4' -C-亚乙基桥联核酸腺苷 3' 单磷酸基、2' -O, 4' -C-亚乙基桥联核酸 5-甲基-胞啶 3' 单磷酸基、2' -O, 4' -C-亚乙基桥联核酸鸟苷 3' 单磷酸基、5-甲基-尿苷(或胸苷) 3' 单磷酸基)。

[0126] 在某些实施方案中,在所述第一链和靶向核酸之间的互补性是完美的。在一些实施方案中,链是充分互补的,即在所述第一链和靶向核酸之间有 1, 3 或最多 3 个错配。充分互补的是指对另一个序列有超过 84% 的互补性。例如在包含 19 碱基对的双链区域中,1 个错配导致 94.7% 互补性、2 个错配导致约 89.5% 互补性和 3 个错配导致约 84.2% 互补性,这使得双链区域是充分互补的。相应地显著地一致性是指对另一个序列有超过 84% 的互补

性。

[0127] 在一些实施方案中,化合物的第一链和第二链通过环状结构连接,该结构包含非-核酸聚合体例如尤其是聚乙二醇。可选地,该环状结构包含核酸(包含已修饰的和未-修饰的核糖核苷酸以及已修饰的和未-修饰的脱氧核糖核苷酸)。

[0128] 在进一步的实施方案中,siRNA 第一链的 5' - 末端连接于第二链的 3' - 末端,或第一链的 3' - 末端连接于第二链的 5' - 末端,所述连接是通过核酸接头完成的,该接头通常具有长度在 2-100 之间的核碱基,优选地约 2 到约 30 个核碱基。

[0129] 在优选的实施方案中,本发明的方法使用具有改变的核糖核苷酸的寡核苷酸化合物,其中核糖核苷酸在至少化合物的反义和正义链之一中被修饰过,对于 19 节链和 23 节链寡聚物,在反义链的 5' 和 3' 末端的核糖核苷酸的糖残基是已修饰的,并且在正义链的 5' 和 3' 末端的核糖核苷酸的糖残基是未修饰的。对于 21 节链寡聚物,在正义链的 5' 和 3' 末端的核糖核苷酸的糖残基是已修饰的,在反义链的 5' 和 3' 末端的核糖核苷酸的糖残基是未修饰的,或在 3' 末端可以有可选的其他的修饰结构。如上面所提到的,优选的情况是反义链的中间核苷酸是未被修饰的。

[0130] 根据一个本发明的优选的实施方案,寡核苷酸/siRNA 的反义链和正义链在 3'-末端而不在 5'-末端磷酸化。根据另一个优选的本发明的实施方案,反义链和正义链是非-磷酸化的。根据还另一个优选的本发明的实施方案,在正义链中的最 5' 端核糖核苷酸是已修饰的,以消除在体内 5' - 磷酸化的任何可能性。

[0131] 可以制备具有任一本文公开的修饰结构/结构的任一 siRNA 序列。同时含有序列和结构的化合物可以用于治疗本文公布的病症。

[0132] 结构基序

[0133] 在一些本发明的实施方案中,根据任一在结构 (A)-(P) 中阐述的修饰结构或作为串联 siRNA 或 RNA_{tar},寡核苷酸抑制剂是化学修饰的 siRNA。

[0134] 在一个方面,本发明提供了如结构 (A) 阐述的化合物:

[0135] (A) 5' (N)_x-Z3' (反义链)

[0136] 3' Z'-(N')_y5' (正义链)

[0137] 其中每个 N 和 N' 是核苷酸选自未修饰的核糖核苷酸,已修饰的核糖核苷酸,未修饰的脱氧核糖核苷酸和已修饰的脱氧核糖核苷酸;

[0138] 其中每一个 (N)_x和 (N')_y是寡核苷酸,在其中每一个连续的 N 或 N' 通过共价键连接于下一个 N 或 N' ;

[0139] 其中 x 和 y 各是 18 到 40 的整数;

[0140] 其中每个 Z 和 Z' 可以存在或不存在,但如果存在的话是共价地连接于它所在链的 3' 末端的 1-5 个连续的核苷酸;

[0141] 其中 (N')_y序列是与 (N)_x 充分互补的序列;并且其中 (N)_x的序列包含与约 18 到约 40 个连续的核糖核苷酸充分互补的反义序列,这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0142] 在某些实施方案中,本发明提供了具有结构 (B) 的化合物

[0143] (B) 5' (N)_x-Z 3' (反义链)

[0144] 3' Z'-(N')_y 5' (正义链)

[0145] 其中每一个 $(N)_x$ 和 $(N')_y$ 是寡聚物,在该寡聚物中连续的 N 或 N' 是通过共价键连接于下一个 N 或 N' 的未修饰的核糖核苷酸或已修饰的核糖核苷酸;

[0146] 其中每个 Z 和 Z' 可以存在或不存在,但如果存在的话是共价地连接于它所在链的 3' 末端的 1-5 个连续的核苷酸;

[0147] 其中每个 x 和 $y = 19, 21$ 或 23 , 并且 $(N)_x$ 和 $(N')_y$ 是完全互补的。

[0148] 其中交替的核糖核苷酸在每个 $(N)_x$ 和 $(N')_y$ 中是已修饰的,这导致了在核糖核苷酸的糖残基上的 2' -O- 甲基修饰结构;

[0149] 其中 $(N')_y$ 序列是与 $(N)_x$ 充分互补的序列;并且其中 $(N)_x$ 的序列包含与约 18 到约 40 个连续的核糖核苷酸充分互补的反义序列,这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0150] 在一些实施方案中,在 3' 和 5' 末端每个 $(N)_x$ 和 $(N')_y$ 是独立地磷酸化或非 - 磷酸化。

[0151] 在某些实施方案中,其中每个 x 和 $y = 19$ 或 23 ,在 $(N)_x$ 的 5' 和 3' 末端的每个 N 是已修饰的;而且在 $(N')_y$ 的 5' 和 3' 末端的每个 N' 是未修饰的。

[0152] 在某些实施方案中,其中每个 x 和 $y = 21$,在 $(N)_x$ 的 5' 和 3' 末端的每个 N 是未修饰的;而且在 $(N')_y$ 的 5' 和 3' 末端的每个 N' 是已修饰的。

[0153] 在特定的实施方案中,当 x 和 $y = 19$ 时, siRNA 是已修饰的,使得 2' -O- 甲基 (2'-OMe) 基团存在于反义链 $(N)_x$ 的第 1、第 3、第 5、第 7、第 9、第 11、第 13、第 15、第 17 和第 19 位核苷酸,而且由此同样的修饰结构,即 2' -OMe 基团,存在于正义链 $(N')_y$ 的第 2、第 4、第 6、第 8、第 10、第 12、第 14、第 16 和第 18 核苷酸。在各种实施方案中,这些特定的 siRNA 化合物在两个末端被钝末端化。

[0154] 在一些实施方案中,本发明提供了具有结构 (C) 的化合物:

[0155] (C) 5' $(N)_x$ -Z3' 反义链

[0156] 3' Z' - $(N')_y$ 5' 正义链

[0157] 其中每个 N 和 N' 是独立地选自未修饰的核糖核苷酸、已修饰的核糖核苷酸、未修饰的脱氧核糖核苷酸和已修饰的脱氧核糖核苷酸的核苷酸;

[0158] 其中每一个 $(N)_x$ 和 $(N')_y$ 是寡聚物,在该寡聚物中通过共价键每一个连续的核苷酸连接于下一个核苷酸,并且 x 和 y 各是 18 到 40 的整数;

[0159] 其中在 $(N)_x$ 中核苷酸是未修饰的或 $(N)_x$ 包含交替的已修饰的核糖核苷酸和未修饰的核糖核苷酸;每个已修饰的核糖核苷酸是被修饰过的,由此在它的糖上具有 2' -O- 甲基并且位于 $(N)_x$ 中间位置的核糖核苷酸是已修饰的或未修饰的,优选地是未修饰的;

[0160] 其中 $(N')_y$ 包含未修饰的核糖核苷酸,并在末端或倒数第二位进一步包含 1 个已修饰的核苷酸,其中已修饰的核苷酸选自由下列物质组成的组:镜像核苷酸、二环核苷酸、2' -糖修饰的核苷酸、阿卓糖醇核苷酸,或通过核苷酸间键合(选自 2' -5' 磷酸二酯键、P- 烷氧基连接或 PACE 连接)连接到邻近核苷酸的核苷酸。

[0161] 其中如果在 $(N')_y$ 中不止 1 个核苷酸是已修饰的,已修饰的核苷酸可以是连续的;

[0162] 其中每个 Z 和 Z' 可以存在或不存在,但如果存在的话是共价地连接于任一其所连接的寡聚物的 3' 末端的 1-5 个脱氧核糖核苷酸;

[0163] 其中 $(N')_y$ 的序列包含与 $(N)_x$ 充分互补的序列；其中 $(N)_x$ 包含与约 18 到约 40 个连续的核糖核苷酸充分互补的反义序列，这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0164] 在特定的实施方案中， $x = y = 19$ 并且自 (N) 中每个已修饰的核糖核苷酸是修饰过的，使得在它的糖上具有 2'-O- 甲基，而且位于 $(N)_x$ 中间的核糖核苷酸是未修饰的。相应地，在化合物中，其中 $x = 19$ ， $(N)_x$ 包含在第 1、3、5、7、9、11、13、15、17 和 19 位 2'-O- 甲基糖修饰的核糖核苷酸。在其它的实施方案中， $(N)_x$ 包含在第 2、4、6、8、11、13、15、17 和 19 位的 2'-O Me 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 5 位。在其它的实施方案中， $(N)_x$ 包含在第 2、4、8、11、13、15、17 和 19 位的 2'-O Me 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 6 位。在其它的实施方案中， $(N)_x$ 包含在第 2、4、6、8、11、13、17 和 19 位的 2'-O Me 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 15 位。在其它的实施方案中， $(N)_x$ 包含在第 2、4、6、8、11、13、15、17 和 19 位的 2'-O Me 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 14 位。在其它的实施方案中， $(N)_x$ 包含在第 1、2、3、7、9、11、13、15、17 和 19 位的 2'-O Me 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 5 位。在其它的实施方案中， $(N)_x$ 包含在第 1、2、3、5、7、9、11、13、15、17 和 19 位的 2'-O Me 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 6 位。在其它的实施方案中， $(N)_x$ 包含在第 1、2、3、5、7、9、11、13、17 和 19 位的 2'-O Me 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 15 位。在其它的实施方案中， $(N)_x$ 包含在第 1、2、3、5、7、9、11、13、15、17 和 19 位的 2'-OMe 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 5 位。在其它的实施方案中， $(N)_x$ 包含在第 1、2、4、6、7、9、11、13、15、17 和 19 位的 2'-OMe 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 5 位。在其它的实施方案中， $(N)_x$ 包含在第 2、4、6、8、11、13、14、16、17 和 19 位的 2'-O Me 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 15 位。在其它的实施方案中， $(N)_x$ 包含在第 1、2、3、5、7、9、11、13、14、16、17 和 19 位的 2'-OMe 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 15 位。在其它的实施方案中， $(N)_x$ 包含在第 2、4、6、8、11、13、15、17 和 19 位的 2'-OMe 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 7 位。在其它的实施方案中， $(N)_x$ 包含在第 2、4、6、11、13、15、17 和 19 位的 2'-OMe 修饰的核糖核苷酸，并且可以进一步包含至少 1 个脱碱基或反向脱碱基非常规部分，例如在第 8 位。

[0165] 在另外其他的实施方案中，相对于任一基因 $(N)_x$ 包含至少 1 个核苷酸错配。在某些优选的实施方案中，在第 5、6 或 14 位上 $(N)_x$ 包含 1 个单核苷酸错配。在一个结构 (C) 的实施方案中，在 $(N')_y$ 5' 和 3' 之一或 2 个的末端，至少 2 个核苷酸通过 2'-5' 磷酸二酯键连接。在某些优选的实施方案中， $x = y = 19$ 或 $x = y = 23$ ；在 $(N)_x$ 中核苷酸在已修饰

的核糖核苷酸和未修饰的核糖核苷酸之间交替,每个已修饰的核糖核苷酸是被修饰过的,由此在它的糖上具有 2'-O-甲基,并且位于 (N)_x 中间的核糖核苷酸是未修饰的;而且通过 2 个 2'-5' 磷酸二酯键在 (N')_y3' 末端的 3 个核苷酸连接在一起(如本文所述的结构 I)。在其它优选的实施方案中, $x = y = 19$ 或 $x = y = 23$;在 (N)_x 中核苷酸在已修饰的核糖核苷酸和未修饰的核糖核苷酸之间交替,每个已修饰的核糖核苷酸是被修饰过的,由此在它的糖上具有 2'-O-甲基,并且位于 (N)_x 中间的核糖核苷酸是未修饰的;并且 4 个在 (N')_y5' 末端的连续的核苷酸通过 3 个 2'-5' 磷酸二酯键连接起来。在进一步的实施方案中,用 2'-O-甲基修饰另外 1 个位于 (N)_y 的之间位置的核苷酸的糖部分。在另一个优选的实施方案中,在 (N)_x 中,核苷酸在 2'-O-甲基修饰的核糖核苷酸和未修饰的核糖核苷酸之间交替,4 个在 (N')_y5' 末端的连续的核苷酸通过 3 个 2'-5' 磷酸二酯键连接起来,而且 5' 末端核苷酸或者在 5' 末端的 2 个或 3 个连续的核苷酸包含 3'-O-甲基修饰结构。

[0166] 在某些优选的结构 C 的实施方案中, $x = y = 19$,而且在 (N')_y 中,核苷酸至少在一个位置上包含镜像核苷酸、脱氧核糖核苷酸,和通过 2'-5' 核苷酸间键与邻近的核苷酸连接的核苷酸;

[0167] 在某些优选的结构 C 的实施方案中, $x = y = 19$ 而且 (N')_y 包含镜像核苷酸。在各种实施方案中,镜像核苷酸是 L-DNA 核苷酸。在某些实施方案中,L-DNA 是 L-脱氧核糖胞嘧啶。在一些实施方案中,(N')_y 在第 18 位包含 L-DNA。在其它的实施方案中,(N')_y 在第 17 和 18 位包含 L-DNA。在某些实施方案中,(N')_y 包含在第 2 位以及在第 17 和 18 位中的一位或两位取代的 L-DNA。在某些实施方案中,(N')_y 进一步包含 5' 末端封端核苷酸,例如作为垂悬的 5'-O-甲基 DNA 或脱碱基或反向脱碱基部分。

[0168] 在另外其他的实施方案中,(N')_y 在第 15 位包含 DNA 以及在第 17 和 18 位中的一位或两位包含 L-DNA。在这种结构中,第 2 位可以进一步包含 L-DNA 或脱碱基非常规部分。

[0169] 设想的其它结构 C 的实施方案,其中 $x = y = 21$ 或其中 $x = y = 23$;在这些实施方案中,如上所述 (N')_y 的修饰结构是在 21 节链的第 17、18、19、20 位和 23 节链的第 19、20、21、22 位上,而不是在第 15、16、17、18 位;相似地,在第 17 和 18 位中的一位或两位上的修饰结构是在 21 节链的第 19 和 20 位中的一位或两位上以及在 23 节链的第 21 和 22 位中的一位或两位上。根据 21 和 23 节链,所有的在 19 节链中的修饰结构做类似的调整。

[0170] 根据各种结构 (C) 的实施方案,在 (N')_y 中,在 3' 末端的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸通过 2'-5' 核苷酸间键合连接。在一个优选的实施方案中,4 个在 (N')_y3' 末端的连续的核苷酸通过 3 个 2'-5' 磷酸二酯键连接起来,其中一种或多种形成 2'-5' 磷酸二酯键的 2'-5' 核苷酸进一步包含 3'-O-甲基糖修饰结构。优选地,(N')_y 的 3' 末端核苷酸包含 2'-O-甲基糖修饰结构。在某些优选的结构 C 的实施方案中, $x = y = 19$,并且在 (N')_y 中,2 个或多个在第 15、16、17、18 和 19 位的连续的核苷酸包含通过 2'-5' 核苷酸间键与邻近的核苷酸连接的核苷酸。在各种实施方案中,形成 2'-5' 核苷酸间键的核苷酸包含 3' 脱氧核糖核苷酸或 3' 甲基氧核苷酸。在一些实施方案中,在 (N')_y 第 17 和 18 位的核苷酸通过 2'-5' 核苷酸间键连接在一起。在其它的实施方案中,在 (N')_y 第 16、17、18、16-17、17-18 或 16-18 位的核苷酸通过 2'-5' 核苷酸间键连接在一起。

[0171] 在某些实施方案中,(N')_y 包含在第 2 位的 L-DNA 和在第 16-17、17-18 或 16-18

位的 2'-5' 核苷酸间键。在某些实施方案中, (N')_y 包含在第 16-17、17-18 或 16-18 位的 2'-5' 核苷酸间键和 5' 末端封端核苷酸。

[0172] 根据各种结构 (C) 的实施方案, 在 (N')_y 中, 在每个末端的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核苷酸或在每个 5' 和 3' 末端的 2-8 个已修饰的核苷酸独立地是镜像核苷酸。在一些实施方案中, 镜像核苷酸是 L-核糖核苷酸。在其它的实施方案中, 镜像核苷酸是 L-脱氧核糖核苷酸。镜像核苷酸可在糖或碱基部分或在核苷酸间键合上, 被进一步修饰的。

[0173] 在一个优选的结构 (C) 的实施方案中, 3' 末端的核苷酸或者在 (N')_y 的 3' 末端的 2 个或 3 个连续的核苷酸是 L-脱氧核糖核苷酸。

[0174] 在其它的结构 (C) 的实施方案中, 在 (N')_y 地末端之一的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸或在每个 5' 和 3' 末端的 2-8 个已修饰的核苷酸独立地是 2' 糖修饰的核苷酸。在一些实施方案中, 2' 糖修饰结构包含氨基、氟、烷氧基或烷基部分。在某些实施方案中, 2' 糖修饰结构包含甲基氧部分 (2'-OMe)。在一系列优选的实施方案中, 在 (N')_y 5' 末端的 3、4 或 5 个连续的核苷酸包含 2'-OMe 修饰结构。在另一个优选的实施方案中, 在 (N')_y 3' 末端的 3 个连续的核苷酸包含 2'-O-甲基修饰结构。

[0175] 在一些结构 (C) 的实施方案中, 在 (N')_y 中, 在 5' 和 3' 末端之一的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸或在每个 5' 和 3' 末端的 2-8 个已修饰的核苷酸独立地是二环核苷酸。在各种实施方案中, 二环核苷酸是锁核酸 (LNA)。2'-O, 4'-C-亚乙基-桥联核酸 (ENA) 是 1 种 LNA (参见下文)。

[0176] 在各种实施方案中, (N')_y 5' 末端或在 3' 和 5' 两末端包含已修饰的核苷酸在。

[0177] 在一些结构 (C) 的实施方案中, 在 (N')_y 5' 和 3' 之一或 2 个末端中的至少 2 个核苷酸通过 P-乙氧基骨架修饰结构相连。在某些优选的实施方案中, $x = y = 19$ 或 $x = y = 23$; 在 (N)_x 中核苷酸在已修饰的核糖核苷酸和未修饰的核糖核苷酸之间交替, 每个已修饰的核糖核苷酸是被修饰过的, 由此在它的糖上具有 2'-O-甲基, 并且位于 (N)_x 中间位置的核糖核苷酸是未修饰的; 而且在 (N')_y 3' 末端或 5' 末端的 4 个连续的核苷酸通过 3 个 P-乙氧基骨架修饰结构连接。在另一个优选的实施方案中, 在 (N')_y 3' 末端或 5' 末端的 3 个连续的核苷酸通过 2 个 P-乙氧基骨架修饰结构连接。

[0178] 在一些结构 (C) 的实施方案中, 在 (N')_y 中, 在每个 5' 和 3' 末端的 2、3、4、5、6、7 或 8 个连续的核糖核苷酸独立地是镜像核苷酸、通过 2'-5' 磷酸二酯键连接核苷酸、2' 糖修饰的核苷酸或二环核苷酸。在一个实施方案中, 在 (N')_y 的 5' 和 3' 末端的修饰结构是相同的。在一个优选的实施方案中, 4 个在 (N')_y 5' 末端的连续的核苷酸通过 3 个 2'-5' 磷酸二酯键连接起来, 而且在 (N')_y 3' 末端的 3 个连续的核苷酸通过 2 个 2'-5' 磷酸二酯键连接在一起。在另外一个实施方案中, 在 (N')_y 5' 末端的修饰结构与在 (N')_y 3' 末端的修饰结构是不同的。在一个特定的实施方案中, 在 (N')_y 5' 末端的已修饰的核苷酸是镜像核苷酸, 而且在 (N')_y 3' 末端的已修饰的核苷酸通过 2'-5' 磷酸二酯键连接。在另外一个特定的实施方案中, 在 (N')_y 5' 末端的 3 个连续的核苷酸是 LNA 核苷酸, 而且通过 2 个 2'-5' 磷酸二酯键在 (N')_y 3' 末端的 3 个连续的核苷酸连接在一起。在 (N)_x 中核苷酸在已修饰的核糖核苷酸和未修饰的核糖核苷酸之间交替, 每个已修饰的核糖核苷酸是被修饰过的, 由此在它的糖上具有 2'-O-甲基, 并且位于 (N)_x 中间的核糖核苷酸是未修饰的,

或在 $(N)_x$ 中的核糖核苷酸是未修饰过的。

[0179] 在另外一个结构 (C) 的实施方案中, 本发明提供了化合物其中 $x = y = 19$ 或 $x = y = 23$; 在 $(N)_x$ 中核苷酸在已修饰的核糖核苷酸和未修饰的核糖核苷酸之间交替, 每个已修饰的核糖核苷酸是被修饰过的, 由此在它的糖上具有 2'-O- 甲基, 而且位于 $(N)_x$ 中间的核糖核苷酸是未修饰的; 通过 2 个 2'-5' 磷酸二酯键在 $(N')_y$ 3' 末端的 3 个核苷酸连接在一起在 $(N')_y$ 5' 末端的 3 个核苷酸是 LNA, 例如 ENA。

[0180] 在另外一个结构 (C) 的实施方案中, 在 $(N')_y$ 5' 末端的 5 个连续的核苷酸包含 2'-O- 甲基糖修饰结构, 而且在 $(N')_y$ 3' 末端的连续的核苷酸是 L-DNA。

[0181] 在又一个实施方案中, 本发明提供了化合物其中 $x = y = 19$ 或 $x = y = 23$; $(N)_x$ 包含未修饰的核糖核苷酸; 通过 2 个 2'-5' 磷酸二酯键在 $(N')_y$ 3' 末端的 3 个连续的核苷酸连接在一起, 而且在 $(N')_y$ 5' 末端的 3 个连续的核苷酸是 LNA, 例如 ENA。

[0182] 根据其它结构 (C) 的实施方案, 在 $(N')_y$ 中, 5' 或 3' 末端的核苷酸, 或在 5' 和 3' 末端的之一的 2、3、4、5 或 6 连续的核苷酸或在每个 5' 和 3' 末端的 1-4 个已修饰的核苷酸独立地是磷酸羧酸酯或磷基羧酸酯核苷酸 (PACE 核苷酸)。在一些实施方案中, PACE 核苷酸是脱氧核糖核苷酸。在一些优选的实施方案中, 在 $(N')_y$ 中, 在每个 5' 和 3' 末端的 1 或 2 连续的核苷酸是 PACE 核苷酸。

[0183] 在一些实施方案中, 本发明提供了具有结构 (D) 的化合物:

[0184] (D) 5' $(N)_x$ -Z3' 反义链

[0185] 3' Z'-(N')_y5' 正义链

[0186] 其中每个 N 和 N' 是选自未修饰的核糖核苷酸、已修饰的核糖核苷酸、未修饰的脱氧核糖核苷酸或已修饰的脱氧核糖核苷酸的核苷酸;

[0187] 其中每一个 $(N)_x$ 和 $(N')_y$ 是寡聚物, 在该寡聚物中通过共价键每一个连续的核苷酸连接于下一个核苷酸, 并且 x 和 y 各是 18 到 40 的整数;

[0188] 其中 $(N)_x$ 包含未修饰的核糖核苷酸, 并在 3' 最末端或倒数第二位进一步包含 1 个已修饰的核苷酸, 其中已修饰的核苷酸选自由下列物质组成的组: 二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸或通过核苷酸间键合 (选自 2'-5' 磷酸二酯键、P- 烷氧基连接或 PACE 连接) 连接到邻近核苷酸的核苷酸。

[0189] 其中 $(N')_y$ 包含未修饰的核糖核苷酸, 并在 5' 末端或倒数第二位进一步包含 1 个已修饰的核苷酸, 其中已修饰的核苷酸选自由下列物质组成的组: 二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸或通过核苷酸间键合 (选自 2'-5' 磷酸二酯键、P- 烷氧基连接或 PACE 连接) 连接到邻近核苷酸的核苷酸。

[0190] 其中在每个 $(N)_x$ 和 $(N')_y$ 中已修饰的和未修饰的核苷酸不是交替的;

[0191] 其中每个 Z 和 Z' 可以存在或不存在, 但如果存在的话是共价地连接于任一其所连接的寡聚物的 3' 末端的 1-5 个脱氧核糖核苷酸;

[0192] 其中 $(N')_y$ 序列是与 $(N)_x$ 充分互补的序列; 其中 $(N)_x$ 包含与约 18 到约 40 个连续的核糖核苷酸充分互补的反义序列, 这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0193] 在一个结构 (D) 的实施方案中, $x = y = 19$ 或 $x = y = 23$; $(N)_x$ 包含未修饰的核糖核苷酸, 在其中在 3' 末端的 2 个连续的核苷酸通过 1 个 2'-5' 核苷酸间键合连接; 而且

(N')_y 包含未修饰的核糖核苷酸,在其中在 5' 末端的 2 个连续的核苷酸通过 1 个 2' -5' 核苷酸间键合连接。

[0194] 在一些实施方案中, $x = y = 19$ 或 $x = y = 23$; (N)_x 包含未修饰的核糖核苷酸,在其中在 3' 末端的 3 个连续的核苷酸通过 2 个 2' -5' 磷酸二酯键连接在一起;而且 (N')_y 包含未修饰的核糖核苷酸,在其中在 5' 末端的 4 个连续的核苷酸通过 3 个 2' -5' 磷酸二酯键连接在一起(如本文所述的结构 II)。

[0195] 根据各种结构 (D) 的实施方案,在 (N)_x3' 末端最末位或倒数第二位起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸和在 (N')_y5' 末端最末位或倒数第二位起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸通过 2' -5' 核苷酸间键合连接。

[0196] 根据一个优选的结构 (D) 的实施方案,4 个在 (N')_y 5' 末端的连续的核苷酸通过 3 个 2' -5' 磷酸二酯键连接起来,而且通过 2 个 2' -5' 磷酸二酯键在 (N')_x 3' 末端的 3 个连续的核苷酸连接在一起。在 (N')_y 5' 末端的 3 个核苷酸和在 (N')_x 3' 末端的 2 个核苷酸也可包含 3' -O- 甲基修饰结构。

[0197] 根据各种结构 (D) 的实施方案,在 (N)_x3' 末端最末位或倒数第二位起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核苷酸以及在 (N')_y5' 末端最末位或倒数第二位起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸独立地是镜像核苷酸。在一些实施方案中,镜像核糖核苷酸是 L- 核糖核苷酸。在其它的实施方案中,镜像核苷酸是 L- 脱氧核糖核苷酸。

[0198] 在其它的结构 (D) 的实施方案中,在 (N)_x3' 末端最末位或倒数第二位起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸以及在 (N')_y5' 末端最末位或倒数第二位起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸,独立地是 2' 糖修饰的核苷酸。在一些实施方案中,2' 糖修饰结构包含氨基、氟、烷氧基或烷基部分。在某些实施方案中,2' 糖修饰结构包含甲基氧部分 (2' -OMe)。

[0199] 在一个优选的结构 (D) 的实施方案中,在 (N')_y 5' 末端的 5 个连续的核苷酸包含 2' OMe 糖修饰结构,而且在 (N')_x3' 末端的 5 个连续的核苷酸包含 2' OMe 糖修饰结构。在另一个优选的结构 (D) 的实施方案中,在 (N')_y 5' 末端的 10 个连续的核苷酸包含 2' OMe 糖修饰结构而且在 (N')_x3' 末端的 5 个连续的核苷酸包含 2' OMe 糖修饰结构。在另一个优选的结构 (D) 的实施方案中,在 (N')_y 5' 末端的 13 个连续的核苷酸包含 2' OMe 糖修饰结构,而且在 (N')_x3' 末端的 5 个连续的核苷酸包含 2' -O- 甲基修饰结构。

[0200] 在一些结构 (D) 的实施方案中,在 (N')_y 中,在 (N)_x3' 末端最末位或倒数第二位起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸,以及在 (N')_y5' 末端最末位或倒数第二位起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸,独立地是二环核苷酸。在各种实施方案中,二环核苷酸是锁核酸 (LNA),例如 2' -O, 4' -C- 亚乙基 - 桥联核酸 (ENA)。

[0201] 在各种结构 (D) 的实施方案中, (N')_y 包含已修饰的核苷酸,其选自二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸或通过核苷酸间键合(选自 2' -5' 磷酸二酯键、P- 烷氧基连接或 PACE 连接)连接到邻近核苷酸的核苷酸。

[0202] 在各种结构 (D) 的实施方案中, (N)_x 包含已修饰的核苷酸,其选自二环核苷酸、2'

糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸或通过核苷酸间键合（选自 2'-5' 磷酸二酯键、P- 烷氧基连接或 PACE 连接）连接到邻近核苷酸的核苷酸。

[0203] 在实施方案中，其中相同链的每一个 3' 和 5' 末端包含已修饰的核苷酸，在 5' 和 3' 末端的修饰结构是相同的。在另外一个实施方案中，在 5' 末端的修饰结构与在同一链 3' 末端的修饰结构是不同的。在一个特定的实施方案中，在 5' 末端的已修饰的核苷酸是镜像核苷酸，而且在同一链 3' 末端的已修饰的核苷酸通过 2'-5' 磷酸二酯键连接。

[0204] 在一个特定的结构 (D) 的实施方案中，在 (N')_y 5' 末端的 5 个连续的核苷酸包含 2' OMe 糖修饰结构，而且在 (N')_y 3' 末端的 2 个连续的核苷酸是 L-DNA。另外，化合物可进一步包含 5 个连续的 2' OMe 糖修饰的核苷酸在 (N')_x 3' 末端。

[0205] 在各种结构 (D) 的实施方案中，在 (N)_x 中的已修饰的核苷酸与在 (N')_y 中的已修饰的核苷酸是不同的。例如，在 (N)_x 中的已修饰的核苷酸是 2' 糖修饰的核苷酸，而在 (N)_y 中的已修饰的核苷酸是通过 2'-5' 核苷酸间键合连接的核苷酸。在另一个示例中，在 (N)_x 中的已修饰的核苷酸是镜像核苷酸，而在 (N)_y 中的已修饰的核苷酸是通过 2'-5' 核苷酸间键合连接的核苷酸。在另一个示例中，在 (N)_x 中的已修饰的核苷酸是通过 2'-5' 核苷酸间键合连接的核苷酸，而在 (N')_y 中已修饰的核苷酸是镜像核苷酸。

[0206] 在其他的实施方案中，本发明提供了具有结构 (E) 的化合物：

[0207] (E) 5' (N)_x-Z 3' 反义链

[0208] 3' Z'-(N')_y 5' 正义链

[0209] 其中每个 N 和 N' 是选自未修饰的核糖核苷酸、已修饰的核糖核苷酸、未修饰的脱氧核糖核苷酸或已修饰的脱氧核糖核苷酸的核苷酸；

[0210] 其中每一个 (N)_x 和 (N')_y 是寡聚物，在该寡聚物中通过共价键每一个连续的核苷酸连接于下一个核苷酸，并且 x 和 y 各是 18 到 40 的整数；

[0211] 其中 (N)_x 包含未修饰的核糖核苷酸，并在 5' 末端或倒数第二位进一步包含 1 个已修饰的核苷酸，其中已修饰的核苷酸选自由下列物质组成的组：二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸，或通过核苷酸间键合（选自 2'-5' 磷酸二酯键、P- 烷氧基连接或 PACE 连接）连接到邻近核苷酸的核苷酸。

[0212] 其中 (N')_y 包含未修饰的核糖核苷酸，并在 3' 最末端或倒数第二位进一步包含 1 个已修饰的核苷酸，其中已修饰的核苷酸选自由下列物质组成的组：二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸，或通过核苷酸间键合（选自 2'-5' 磷酸二酯键、P- 烷氧基连接或 PACE 连接）连接到邻近核苷酸的核苷酸。

[0213] 其中在每个 (N)_x 和 (N')_y 中已修饰的和未修饰的核苷酸不是交替的；

[0214] 其中每个 Z 和 Z' 可以存在或不存在，但如果存在的话是共价地连接于任一其所连接的寡聚物的 3' 末端的 1-5 个脱氧核糖核苷酸；

[0215] 其中 (N')_y 序列是与 (N)_x 充分互补的序列；并且其中 (N)_x 的序列包含与约 18 到约 40 个连续的核糖核苷酸充分互补的反义序列，这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0216] 在某些优选的实施方案中，在 (N)_x 5' 末端最末位的核苷酸是未修饰的。

[0217] 根据各种结构 (E) 的实施方案，在 (N)_x 5' 末端的最末位或倒数第二位（优选地在 5' 倒数第二位）起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸，和在

(N')y3' 末端的最末位或倒数第二位起 始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸通过 2' -5' 核苷酸间键合连接。

[0218] 根据各种结构 (E) 的实施方案,在 (N)x5' 末端的最末位或倒数第二位 (优选地在 5' 倒数第二位) 起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核苷酸,和在 (N')y3' 末端的最末位或倒数第二位起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核苷酸独立地是镜像核苷酸。在一些实施方案中,镜像核苷酸是 L- 核糖核苷酸。在其它的实施方案中,镜像核苷酸是 L- 脱氧核糖核苷酸。

[0219] 在其它结构 (E) 的实施方案中,在 (N)x5' 末端的最末位或倒数第二位 (优选地在 5' 倒数第二位) 起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸,和在 (N')y3' 末端的最末位或倒数第二位起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸独立地是 2' 糖修饰的核苷酸。在一些实施方案中,2' 糖修饰结构包含氨基、氟、烷氧基或烷基部分。在某些实施方案中,2' 糖修饰结构包含甲基氧部分 (2' -OMe)。

[0220] 在一些结构 (E) 的实施方案中,在 (N')y 中,在 (N)x5' 末端的最末位或倒数第二位 (优选地在 5' 倒数第二位) 起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸,和在 (N')y3' 末端的最末位或倒数第二位起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸独立地是二环核苷酸。在各种实施方案中二环核苷酸是锁核酸 (LNA),例如 2' -0,4' -C- 亚乙基 - 桥联核酸 (ENA)。

[0221] 在各种结构 (E) 的实施方案中,(N')y 包含的已修饰的核苷酸选自二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸、通过 P- 烷氧基骨架修饰结构连接于邻近核苷酸的核苷酸,或通过核苷酸间键合 (选自 2' -5' 磷酸二酯键、P- 烷氧基连接或在 3' 末端或在 3' 和 5' 末端之一的 PACE 连接) 连接到邻近核苷酸的核苷酸。

[0222] 在各种结构 (E) 的实施方案中,(N)x 包含的已修饰的核苷酸选自二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸,或通过核苷酸间键合 (选自 2' -5' 磷酸二酯键、P- 烷氧基连接或在 5' 末端或在 3' 和 5' 末端之一的 PACE 连接) 连接到邻近核苷酸的核苷酸。

[0223] 在一个实施方案中,其中同一链的 3' 和 5' 两个末端包含已修饰的核苷酸,在 5' 和 3' 末端的修饰结构是相同的。在另外一个实施方案中,在 5' 末端的修饰结构与在同一链 3' 末端的修饰结构是不同的。在一个特定的实施方案中,在 5' 末端的已修饰的核苷酸是镜像核苷酸,而且在同一链 3' 末端的已修饰的核苷酸通过 2' -5' 磷酸二酯键连接。

[0224] 在各种结构 (E) 的实施方案中,在 (N)x 中的已修饰的核苷酸与在 (N')y 中的已修饰的核苷酸是不同的。例如,在 (N)x 中的已修饰的核苷酸是 2' 糖修饰的核苷酸,而在 (N)y 中的已修饰的核苷酸是通过 2' -5' 核苷酸间键合连接的核苷酸。在另一个示例中,在 (N)x 中的已修饰的核苷酸是镜像核苷酸,而在 (N)y 中的已修饰的核苷酸是通过 2' -5' 核苷酸间键合连接的核苷酸。在另一个示例中,在 (N)x 中的已修饰的核苷酸是通过 2' -5' 核苷酸间键合连接的核苷酸,而在 (N')y 中已修饰的核苷酸是镜像核苷酸。

[0225] 在其他的实施方案中,本发明提供了具有结构 (F) 的化合物:

[0226] (F) 5' (N)x-Z3' 反义链

[0227] 3' Z' -(N')y5' 正义链

[0228] 其中每个 N 和 N' 是选自未修饰的核糖核苷酸、已修饰的核糖核苷酸、未修饰的脱

氧核糖核苷酸或修饰的脱氧核糖核苷酸的核苷酸；

[0229] 其中每一个 $(N)_x$ 和 $(N')_y$ 是寡聚物,在该寡聚物中通过共价键每一个连续的核苷酸连接于下一个核苷酸,并且 x 和 y 各是 18 到 40 的整数；

[0230] 其中每个 $(N)_x$ 和 $(N')_y$ 包含未修饰的核糖核苷酸,在其中每个 $(N)_x$ 和 $(N')_y$ 在 3' 最末端或倒数第二位独立地包含 1 个已修饰的核苷酸,其中已修饰的核苷酸选自由下列物质组成的组:二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、通过 P- 烷氧基骨架修饰结构连接于邻近核苷酸的核苷酸或通过 2' -5' 磷酸二酯键连接与邻近的核苷酸的核苷酸；

[0231] 其中在每个 $(N)_x$ 和 $(N')_y$ 中已修饰的和未修饰的核苷酸不是交替的；

[0232] 其中每个 Z 和 Z' 可以存在或不存在,但如果存在的话是共价地连接于任一其所连接的寡聚物的 3' 末端 1-5 个脱氧核糖核苷酸；

[0233] 其中 $(N')_y$ 序列是与 $(N)_x$ 充分互补的序列；并且其中 $(N)_x$ 的序列包含与约 18 到约 40 个连续的核糖核苷酸充分互补的反义序列,这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0234] 在一些结构 (F) 的实施方案中, $x = y = 19$ 或 $x = y = 23$; $(N')_y$ 包含未修饰的核糖核苷酸,其中在 3' 末端的 2 个连续的核苷酸包含 2 个连续的镜像脱氧核糖核苷酸；而且 $(N)_x$ 包含未修饰的核糖核苷酸,其中在 3' 末端的 1 个核苷酸包含镜像脱氧核糖核苷酸 (如所述的结构 III)。

[0235] 根据各种结构 (F) 的实施方案,在 $(N)_x$ 和 $(N')_y$ 的 3' 末端的最末或倒数第二位独立地起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸通过 2' -5' 核苷酸间键合连接。

[0236] 根据一个优选的结构 (F) 实施方案,通过 2 个 2' -5' 磷酸二酯键在 $(N')_y$ 3' 末端的 3 个连续的核苷酸连接在一起,而且通过 2 个 2' -5' 磷酸二酯键在 $(N')_x$ 3' 末端的 3 个连续的核苷酸连接在一起。

[0237] 根据各种结构 (F) 的实施方案,在 $(N)_x$ 和 $(N')_y$ 的 3' 末端的最末或倒数第二位独立地起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核苷酸独立地是镜像核苷酸。在一些实施方案中镜像核苷酸是 L- 核糖核苷酸。在其它的实施方案中,镜像核苷酸是 L- 脱氧核糖核苷酸。

[0238] 在其它的结构 (F) 的实施方案中,在 $(N)_x$ 和 $(N')_y$ 的 3' 末端的最末或倒数第二位独立地起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸独立地是 2' 糖修饰的核苷酸。在一些实施方案中,2' 糖修饰结构包含氨基、氟、烷氧基或烷基部分。在某些实施方案中,2' 糖修饰结构包含甲基氧部分 (2' -OMe)。

[0239] 在一些结构 (F) 的实施方案中,在 $(N)_x$ 和 $(N')_y$ 的 3' 末端的最末或倒数第二位独立地起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸,独立地是二环核苷酸。在各种实施方案中二环核苷酸是锁核酸 (LNA) 例如 2' -0,4' -C- 亚乙基 - 桥联核酸 (ENA)。

[0240] 在各种结构 (F) 的实施方案中, $(N')_y$ 包含已修饰的核苷酸,其选自二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸,或通过核苷酸间键合 (选自 2' -5' 磷酸二酯键、P- 烷氧基连接或在 3' 末端或在 3' 和 5' 两者末端的 PACE 连接) 连接到邻近核苷酸的核苷酸。

[0241] 在各种结构 (F) 的实施方案中, (N)_x 包含的已修饰的核苷酸选自二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸, 或通过核苷酸间键合 (选自 2' -5' 磷酸二酯键、P- 烷氧基连接或在 3' 末端或在 3' 和 5' 末端 之一的 PACE 连接) 连接到邻近核苷酸的核苷酸。

[0242] 在一个实施方案中, 其中每个同一链的 3' 和 5' 末端包含已修饰的核苷酸, 在 5' 和 3' 末端的修饰结构是相同的。在另外一个实施方案中, 在 5' 末端的修饰结构与在同一链 3' 末端的修饰结构是不同的。在一个特定的实施方案中, 在 5' 末端的已修饰的核苷酸是镜像核苷酸, 而且在同一链 3' 末端的已修饰的核苷酸通过 2' -5' 磷酸二酯键连接。

[0243] 在各种结构 (F) 的实施方案中, 在 (N)_x 中的已修饰的核苷酸与在 (N')_y 中的已修饰的核苷酸是不同的。例如, 在 (N)_x 中的已修饰的核苷酸是 2' 糖修饰的核苷酸, 而在 (N)_y 中的已修饰的核苷酸是通过 2' -5' 核苷酸间键合连接的核苷酸。在另一个示例中, 在 (N)_x 中的已修饰的核苷酸是镜像核苷酸, 而在 (N)_y 中的已修饰的核苷酸是通过 2' -5' 核苷酸间键合连接的核苷酸。在另一个示例中, 在 (N)_x 中的已修饰的核苷酸是通过 2' -5' 核苷酸间键合连接的核苷酸, 而在 (N')_y 中已修饰的核苷酸是镜像核苷酸。

[0244] 在其他的实施方案中, 本发明提供了具有结构 (G) 的化合物:

[0245] (G) 5' (N)_x-Z3' 反义链

[0246] 3' Z' -(N')_y5' 正义链

[0247] 其中每个 N 和 N' 是选自未修饰的核糖核苷酸、已修饰的核糖核苷酸、未修饰的脱氧核糖核苷酸或修饰的脱氧核糖核苷酸的核苷酸;

[0248] 其中每一个 (N)_x 和 (N')_y 是寡聚物, 在该寡聚物中通过共价键每一个连续的核苷酸连接于下一个核苷酸, 并且 x 和 y 各是 18 到 40 的整数;

[0249] 其中每个 (N)_x 和 (N')_y 包含未修饰的核糖核苷酸, 其中每个 (N)_x 和 (N')_y 在 5' 末端或倒数第二位独立地包含 1 个已修饰的核苷酸, 其中已修饰的核苷酸选自由下列物质组成的组: 二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、通过 P- 烷氧基骨架修饰结构连接于邻近核苷酸的核苷酸或通过 2' -5' 磷酸二酯键连接与邻近的核苷酸的核苷酸;

[0250] 其中 (N)_x 的已修饰的核苷酸优选地是在 5' 末端倒数第二位;

[0251] 其中在每个 (N)_x 和 (N')_y 中已修饰的和未修饰的核苷酸不是交替的;

[0252] 其中每个 Z 和 Z' 可以存在或不存在, 但如果存在的话是共价地连接于任一其所连接的寡聚物的 3' 末端的 1-5 个脱氧核糖核苷酸;

[0253] 其中 (N')_y 序列是与 (N)_x 充分互补的序列; 并且其中 (N)_x 的序列包含 与约 18 到约 40 个连续的核糖核苷酸充分互补的反义序列, 这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0254] 在一些结构 (G) 的实施方案中, x = y = 19 或 x = y = 23.

[0255] 根据各种结构 (G) 的实施方案, 在 (N)_x 和 (N')_y 的 5' 末端的最末或倒数第二位独立地起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸通过 2' -5' 核苷酸间键合连接。对于 (N)_x 修饰的核苷酸优选地在 5' 末端倒数第二位开始。

[0256] 根据各种结构 (G) 的实施方案, 在 (N)_x 和 (N')_y 的 5' 末端的最末或倒数第二位独立地起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核苷酸独立地是镜像核苷酸。在一些实施方案中镜像核苷酸是 L- 核糖核苷酸。在其它的实施方案中, 镜像核苷酸是 L- 脱

氧核糖核苷酸。对于 (N)_x 修饰的核苷酸优选地在 5' 末端倒数第二位开始。

[0257] 在其它的结构 (G) 的实施方案中, 在 (N)_x 和 (N')_y 的 5' 末端的最末或倒数第二位独立地起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸独立地是 2' 糖修饰的核苷酸。在一些实施方案中, 2' 糖修饰结构包含氨基、氟、烷氧基或烷基部分。在某些实施方案中, 2' 糖修饰结构包含甲基氧部分 (2' -OMe)。在一些优选的实施方案中, 连续的已修饰的核苷酸优选地在 (N)_x 5' 末端的倒数第二位开始。

[0258] 在一个优选的结构 (G) 的实施方案中, 在 (N')_y 5' 末端的 5 个连续的核糖核苷酸包含 2' OMe 糖修饰结构, 而且在 (N')_x 5' 倒数第二位的 1 个核糖核苷酸包含 2' OMe 糖修饰结构。在另一个优选的结构 (G) 的实施方案中, 在 (N')_y 5' 末端的 5 个连续的核糖核苷酸包含 2' OMe 糖修饰结构, 而且在 (N')_x 5' 末端的 2 个连续的核糖核苷酸包含 2' OMe 糖修饰结构。

[0259] 在一些结构 (G) 的实施方案中, 在 (N)_x 和 (N')_y 的 5' 末端的最末或倒数第二位独立地起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸是二环核苷酸。在各种实施方案中, 二环核苷酸是锁核酸 (LNA), 例如 2' -O, 4' -C- 亚乙基 - 桥联核酸 (ENA)。在一些优选的实施方案中, 连续的已修饰的核苷酸优选地在 (N)_x 5' 末端的倒数第二位开始。

[0260] 在各种结构 (G) 的实施方案中, (N')_y 包含已修饰的核苷酸, 其选自二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸, 或通过核苷酸间键合 (选自 2' -5' 磷酸二酯键、P- 烷氧基连接或在 5' 末端或在 3' 和 5' 末端之一的 PACE 连接) 连接到邻近核苷酸的核苷酸。

[0261] 在各种结构 (G) 的实施方案中, (N)_x 包含的已修饰的核苷酸选自二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸, 或通过核苷酸间键合 (选自 2' -5' 磷酸二酯键、P- 烷氧基连接或在 5' 末端或在 3' 和 5' 末端之一的 PACE 连接) 连接到邻近核苷酸的核苷酸。

[0262] 在一个实施方案中, 其中每个同一链的 3' 和 5' 末端包含已修饰的核苷酸, 在 5' 和 3' 末端的修饰结构是相同的。在另外一个实施方案中, 在 5' 末端的修饰结构与在同一链 3' 末端的修饰结构是不同的。在一个特定的实施方案中, 在 5' 末端的已修饰的核苷酸是镜像核苷酸, 而且在同一链 3' 末端的已修饰的核苷酸通过 2' -5' 磷酸二酯键连接。在各种结构 (G) 的实施方案中, 在 (N)_x 中的已修饰的核苷酸与在 (N')_y 中的已修饰的核苷酸是不同的。例如, 在 (N)_x 中的已修饰的核苷酸是 2' 糖修饰的核苷酸, 而在 (N)_y 中的已修饰的核苷酸是通过 2' -5' 核苷酸间键合连接的核苷酸。在另一个示例中, 在 (N)_x 中的已修饰的核苷酸是镜像核苷酸, 而在 (N)_y 中的已修饰的核苷酸是通过 2' -5' 核苷酸间键合连接的核苷酸。在另一个示例中, 在 (N)_x 中的已修饰的核苷酸是通过 2' -5' 核苷酸间键合连接的核苷酸, 而在 (N')_y 中已修饰的核苷酸是镜像核苷酸。

[0263] 在其他的实施方案中, 本发明提供了具有结构 (H) 的化合物:

[0264] (H) 5' (N)_x-Z3' 反义链

[0265] 3' Z' -(N')_y5' 正义链

[0266] 其中每个 N 和 N' 是选自未修饰的核糖核苷酸、已修饰的核糖核苷酸、未修饰的脱氧核糖核苷酸或已修饰的脱氧核糖核苷酸的核苷酸;

[0267] 其中每一个 $(N)_x$ 和 $(N')_y$ 是寡聚物,在该寡聚物中通过共价键每一个连续的核苷酸连接于下一个核苷酸,并且 x 和 y 各是 18 到 40 的整数;

[0268] 其中 $(N)_x$ 包含未修饰的核糖核苷酸,并在 3' 最末端或倒数第二位或 5' 末端或倒数第二位进一步包含 1 个已修饰的核苷酸,其中已修饰的核苷酸选自由下列物质组成的组:二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸,或通过核苷酸间键合(选自 2' -5' 磷酸二酯键、P- 烷氧基连接或 PACE 连接)连接到邻近核苷酸的核苷酸。

[0269] 其中 $(N')_y$ 包含未修饰的核糖核苷酸,并在内部位置进一步包含 1 个已修饰的核苷酸,其中已修饰的核苷酸选自由下列物质组成的组:二环核苷酸、2' 糖修饰的核苷酸、镜像核苷酸、阿卓糖醇核苷酸,或通过核苷酸间键合(选自 2' -5' 磷酸二酯键、P- 烷氧基连接或 PACE 连接)连接到邻近核苷酸的核苷酸。

[0270] 其中在每个 $(N)_x$ 和 $(N')_y$ 中已修饰的和未修饰的核苷酸不是交替的;

[0271] 其中每个 Z 和 Z' 可以存在或不存在,但如果存在的话是共价地连接于任一其所连接的寡聚物的 3' 末端的 1-5 个脱氧核糖核苷酸;

[0272] 其中 $(N')_y$ 序列是与 $(N)_x$ 充分互补的序列;并且其中 $(N)_x$ 的序列包含与约 18 到约 40 个连续的核糖核苷酸充分互补的反义序列,这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0273] 在一个结构 (H) 的实施方案中,在 $(N)_x$ 的 3' 最末端或 3' 末端倒数第二位或 5' 末端或两个末端独立地起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸独立地是 2' 糖修饰的核苷酸、二环核苷酸、镜像核苷酸、阿卓糖醇核苷酸或通过 2' -5' 磷酸二酯键连接与邻近的核苷酸的核苷酸,而且在 $(N')_y$ 中的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的内部核糖核苷酸,独立地是 2' 糖修饰的核苷酸、二环核苷酸、镜像核苷酸、阿卓糖醇核苷酸或通过 2' -5' 磷酸二酯键连接与邻近的核苷酸的核苷酸。在一些实施方案中,2' 糖修饰结构包含氨基、氟、烷氧基或烷基部分。在某些实施方案中,2' 糖修饰结构包含甲基氧部分 (2' -OMe)。

[0274] 在另外一个结构 (H) 的实施方案中,在 $(N')_y$ 的 3' 最末端或 3' 末端倒数第二位或 5' 末端的独立地起始的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核糖核苷酸,或者在 $(N')_y$ 的每个 5' 和 3' 末端的 2-8 个连续的核苷酸,独立地是 2' 糖修饰的核苷酸、二环核苷酸、镜像核苷酸、阿卓糖醇核苷酸或通过 2' -5' 磷酸二酯键连接与邻近的核苷酸的核苷酸,并且在 $(N)_x$ 中的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的内部核糖核苷酸独立地是 2' 糖修饰的核苷酸、二环核苷酸、镜像核苷酸、阿卓糖醇核苷酸或通过 2' -5' 磷酸二酯键连接与邻近的核苷酸的核苷酸。

[0275] 在一个实施方案中,其中每个同一链的 3' 和 5' 末端包含已修饰的核苷酸,在 5' 和 3' 末端的修饰结构是相同的。在另外一个实施方案中,在 5' 末端的修饰结构与在同一链 3' 末端的修饰结构是不同的。在一个特定的实施方案中,在 5' 末端的已修饰的核苷酸是镜像核苷酸,而且在同一链 3' 末端的已修饰的核苷酸通过 2' -5' 磷酸二酯键连接。

[0276] 在各种结构 (H) 的实施方案中,在 $(N)_x$ 中已修饰的核苷酸与在 $(N')_y$ 中已修饰的核苷酸是不同的。例如,在 $(N)_x$ 中的已修饰的核苷酸是 2' 糖修饰的核苷酸,而在 $(N)_y$ 中的已修饰的核苷酸是通过 2' -5' 核苷酸间键合连接的核苷酸。在另一个示例中,在 $(N)_x$ 中的已修饰的核苷酸是镜像核苷酸,而在 $(N)_y$ 中的已修饰的核苷酸是通过 2' -5' 核苷酸

间键合连接的核苷酸。在另一个示例中,在 $(N)_x$ 中的已修饰的核苷酸是通过 2'-5' 核苷酸间键合连接的核苷酸,而且在 $(N')_y$ 中的已修饰的核苷酸是镜像核苷酸。

[0277] 在一个优选的结构 (H) 的实施方案中, $x = y = 19$; 在 $(N')_y$ 第 9-11 位的 3 个连续的核糖核苷酸包含 2' OMe 糖修饰结构, 而且在 $(N')_x$ 3' 末位的 5 个连续的核糖核苷酸包含 2' OMe 糖修饰结构。

[0278] 对于所有上述的结构 (A)-(H), 在各种实施方案中 $x = y$ 并且每个 x 和 y 是 19、20、21、22 或 23。在某些实施方案中, $x = y = 19$ 。在另外其他的实施方案中, $x = y = 23$ 。在其他的实施方案中, 化合物在交替的位置包含已修饰的核糖核苷酸, 其中每个在 $(N)_x$ 5' 和 3' 末端的 N 的糖残基是被修饰过的而且中间核糖核苷酸是未修饰的, 例如核糖核苷酸在 19- 节链的第 10 位, 在 21- 节链的第 11 位以及在 23- 节链的第 12 位。

[0279] 在一些实施方案中, 其中 $x = y = 21$ 或 $x = y = 23$, 在反义链的中间核苷酸优选地是未修饰的前提下, 为 21 和 23 节链, 调整 19 节链中的修饰结构的位置。

[0280] 在一些实施方案中, $(N)_x$ 和 $(N')_y$ 的 3' 和 5' 末端都未被磷酸化。在其它的实施方案中, $(N)_x$ 和 $(N')_y$ 之一或两者的 3' 末端被磷酸化。在又一个实施方案中, $(N)_x$ 和 $(N')_y$ 之一或两者的 3' 末端被以不可裂解的磷酸基基团磷酸化。在又一个实施方案中, 用可裂解的或不可裂解的磷酸基基团, 使 $(N)_x$ 和 $(N')_y$ 的之一或两者的 2' 最末端位置磷酸化。也可将这些特定 siRNA 化合物的末端钝化和非-磷酸化; 然而, 比较性实验显示, 与非-磷酸化化合物相比, 在 1 个或 2 个 3'-末端的磷酸化的 siRNA 化合物在体内 具有相似活性。

[0281] 在某些实施方案中, 对于所有上文提到的结构, 化合物是钝末端的, 例如其中 Z 和 Z' 都不存在。在可选地实施方案中, 化合物包含至少 1 个 3' 垂悬, 其中至少 Z 和 Z' 两者之一是存在的。Z 和 Z' 独立地包含一种或多种共价地连接的已修饰的或非-修饰的核苷酸, 例如反向 dT 或 dA; dT、LNA、镜像核苷酸等。在一些实施方案中, 每个 Z 和 Z' 是独立地选自 dT 和 dTdT。Z 和 / 或 Z' 存在的 siRNA 和 Z 和 / 或 Z' 不存在的 siRNA 具有相似的活性和稳定性。

[0282] 在某些实施方案中, 对于所有上文提到的结构, 化合物包含一种或多种膦酰基羧酸酯和 / 或磷基羧酸酯核苷酸 (PACE 核苷酸)。在一些实施方案中 PACE 核苷酸是脱氧核糖核苷酸而且磷基羧酸酯核苷酸是磷基醋酸核苷酸。PACE 核苷酸和类似物的示例见诸于美国专利 No. 6, 693, 187 和 7, 067, 641, 两者都通过引用的方式并入本文。

[0283] 在某些实施方案中, 对于所有上文提到的结构, 化合物包含一种或多种锁核酸 (LNA) (也被定义为桥联核酸或二环核苷酸)。优选的锁核酸是 2' -O, 4' -C- 亚乙基核苷 (ENA) 或 2' -O, 4' -C- 亚甲基核苷。其它 LNA 和 ENA 核苷酸的示例见诸于 WO 98/39352, WO 00/47599 和 WO 99/14226, 都通过引用的方式并入本文。

[0284] 在某些实施方案中, 对于所有上文提到的结构, 化合物包含一种或多种阿卓糖醇单体 (核苷酸), 也被定义为 1,5 无水 -2- 脱氧 -D-altrito- 己糖醇 (参见例如, Allart, et al., 1998. nucleotide & ribonucleotide 17:1523-1526; Herdewijn et al., 1999. nucleotide & ribonucleotide 18:1371-1376; Fisher et al., 2007, NAR 35(4): 1064-1074; 都通过引用的方式并入本文)。

[0285] 本发明明确地排除每个 N 和 / 或 N' 是脱氧核糖核苷酸 (D-A、D-C、D-G、D-T) 的化

合物。在某些实施方案中, $(N)_x$ 和 $(N')_y$ 可独立地包含 1、2、3、4、5、6、7、8、9 或更多个脱氧核糖核苷酸。在某些实施方案中, 本发明提供了化合物, 其中每个 N 是未修饰的核糖核苷酸而且 3' 末端核苷酸或在 $(N')_y$ 3' 末端的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核苷酸是脱氧核糖核苷酸。在另外其他的实施方案中, 每个 N 是未修饰的核糖核苷酸, 而且 5' 末端核苷酸或在 $(N')_y$ 5' 末端的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核苷酸是脱氧核糖核苷酸。在进一步的实施方案中, 5' 末端核苷酸或在 5' 末端的 2、3、4、5、6、7、8、或 9 个连续的核苷酸, 以及在 $(N)_x$ 3' 末端的 1、2、3、4、5 或 6 个连续的核苷酸是脱氧核糖核苷酸, 并且每个 N' 是未修饰的核糖核苷酸。在更进一步实施方案中, $(N)_x$ 包含未修饰的核糖核苷酸, 和独立地在每个 5' 和 3' 末端的 1 或 2、3 或 4 个连续的脱氧核糖核苷酸, 和在内部位置的 1 或 2、3、4、5 或 6 连续的脱氧核糖核苷酸; 而且每个 N' 是未修饰的核糖核苷酸。在某些实施方案中, 3' 末端核苷酸或在 $(N')_y$ 3' 末端的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核苷酸, 以及末端 5' 核苷酸或 $(N)_x$ 5' 末端的 2、3、4、5、6、7、8、9、10、11、12、13 或 14 个连续的核苷酸是脱氧核糖核苷酸。本发明排除每个 N 和 / 或 N' 是脱氧核糖核苷酸的化合物。在一些实施方案中, 5' 末端的 N 的核苷酸或 2 或 3 个连续的 N 和 1、2、或 3 个 N' 是脱氧核糖核苷酸。有活性的 DNA/RNA siRNA 嵌合体的某些示例见诸于美国专利公开 2005/0004064, 和 Ui-Tei, 2008 (NAR 36(7):2136-2151) 全部通过引用的方式并入本文。

[0286] 除非另有说明, 在本文所述结构的优选的实施方案中, 在每个连续的 N 或 N' 之间的共价键是磷酸二酯键。

[0287] 另外一个本发明提供的新分子是包含连续的核苷酸的寡核苷酸, 其中这种核苷酸的第一片段编码第一抑制性 RNA 分子, 这种核苷酸的第二片段编码第二抑制性 RNA 分子, 而且这种核苷酸的第三片段编码第三抑制性 RNA 分子。每个第一、第二和第三片段可包含双链 RNA 的 1 条链, 而且第一、第二和第三片段可通过接头连接到一起。进一步说, 寡核苷酸可包含通过一种或多种接头结合的 3 个双链片段。

[0288] 由此, 本发明提供的 1 个分子是包含连续的核苷酸的寡核苷酸, 其编码 3 种抑制性 RNA 分子; 所述寡核苷酸可具有三链结构, 以致 3 个双链臂通过一种或多种接头 (例如任一如上文所述的接头) 连接到一起。这种分子形成“星”-样结构, 并且在本文中也可以被定义为 RNAsstar。这样的结构见诸于 PCT 专利公开 WO 2007/091269 (转让给本发明的受让人), 并且全部通过引用的方式并入本文。

[0289] 共价键是指将 1 个核苷酸单体连接于邻近的核苷酸单体的核苷酸间键合。共价键包含例如磷酸二酯键、二氧硫代磷酸酯键、P- 烷氧基键、P- 羰基键等。RNA 和 DNA 的常规核苷间键合是 3' 到 5' 磷酸二酯连接。在某些优选的实施方案中, 共价键是磷酸二酯键。共价键包含非含磷核苷间键合, 例如尤其公布于 WO2004/041924 的那些例子。除非另有说明, 在优选的本文所述结构实施方案中, 在每个连续的 N 或 N' 之间的共价键是磷酸二酯键。

[0290] 对于所有上述结构, 在一些实施方案中, $(N)_x$ 寡核苷酸序列与 $(N')_y$ 寡核苷酸序列是完全互补的。在其它的实施方案中, $(N)_x$ 和 $(N')_y$ 是充分互补的。在某些实施方案中, $(N)_x$ 与靶向序列是完全互补的。在其它的实施方案中, $(N)_x$ 与靶向序列是充分互补的。

[0291] 在一些实施方案中, $(N)_x$ 和 $(N')_y$ 的 3' 和 5' 末端都未被磷酸化。在其它的实施方案中, $(N)_x$ 和 $(N')_y$ 之一或两者的 3' 末端被磷酸化 (3' Pi)。在又一个实施方案中, (N)

x 和 (N')_y 之一或两者的 3' 末端被不可裂解的磷酸基基团磷酸化。在又一个实施方案中, (N)_x 和 (N')_y 的之一或两者的 2' 末端末位, 被可裂解的或不可裂解的磷酸基基团磷酸化。进一步说, 本发明抑制性核酸分子可包含一种或多种缝隙和 / 或一种或多种缺口和 / 或一种或多种错配。不希望受理论束缚, 缝隙、缺口和错配有利于部分地使核酸 / siRNA 去稳定化, 因此内源细胞结构, 例如 DICER、DROSHA 或 RISC 可以更容易地将它加工成抑制性成分。

[0292] 就本发明来说, 在核酸中的缝隙是指在一条链中缺乏一个或多个内在核苷酸, 同时在核酸中的缺口是指在一条链中缺乏在邻近的核苷酸之间的核苷酸间键合。任一本发明的分子可含有一种或多种缝隙和 / 或一种或多种缺口。在一个方面本发明提供了具有结构 (I) 的化合物:

[0293] (I) 5' (N)_x-Z 3' (反义链)

[0294] 3' Z'-(N')_y-z" 5' (正义链)

[0295] 其中每个 N 和 N' 是核糖核苷酸, 该核糖核苷酸可以是未修饰的或已修饰的, 或非常规部分;

[0296] 其中每一个 (N)_x 和 (N')_y 是寡核苷酸, 在其中每一个连续的 N 或 N' 通过共价键连接于下一个 N 或 N';

[0297] 其中 Z 和 Z' 可以存在或不存在, 但如果存在的话, 独立地是共价地连接于它所在链的 3' 末端的 1-5 个连续的核苷酸或非-核苷酸部分;

[0298] 其中 z" 可以存在或不存在, 但如果存在的话是共价地连接到 (N')_y 的 5' 末端的封端部分;

[0299] 其中 x = 18 到 27;

[0300] 其中 y = 18 到 27;

[0301] 其中 (N)_x 包含已修饰的和未修饰的核糖核苷酸, 每个已修饰的核糖核苷酸在它的糖上具有 2'-O-甲基, 其中在 (N)_x 的 3' 末端的 N 是已修饰的核糖核苷酸, (N)_x 包含至少 5 个在 3' 末端起始的交替的已修饰的核糖核苷酸, 和至少总共 9 个已修饰的核糖核苷酸, 而且每个剩余的 N 是未修饰的核糖核苷酸;

[0302] 其中在 (N')_y 中, 至少 1 种非常规部分是存在的, 其中非常规部分可以是脱碱基核糖部分、脱碱基脱氧核糖部分、已修饰的或未修饰的脱氧核糖核苷酸、镜像核苷酸, 和通过 2'-5' 核苷酸间磷酸键连接到邻近核苷酸的核苷酸; 和

[0303] 其中 (N')_y 的序列是与 (N)_x 有互补性的序列; 并且其中 (N)_x 的序列包含与约 18 到约 27 个连续的核糖核苷酸有互补性的反义序列, 这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0304] 在一些实施方案中 x = y = 19。在其它的实施方案中 x = y = 23。在一些实施方案中, 至少 1 个非常规部分存在于 (N')_y 的第 15、16、17 或 18 位。在一些实施方案中, 非常规部分选自镜像核苷酸、脱碱基核糖部分和脱碱基脱氧核糖部分。在一些优选的实施方案中, 非常规部分是镜像核苷酸, 优选地是 L-DNA 部分。在一些实施方案中 L-DNA 部分存在于第 17 位、第 18 位或第 17 和 18 位。

[0305] 在其它的实施方案中, 非常规部分是脱碱基部分。在各种实施方案中, (N')_y 包含至少 5 个脱碱基核糖部分或脱碱基脱氧核糖部分。

[0306] 在另外其他的实施方案中, (N')_y 包含至少 5 个脱碱基核糖部分或脱碱基脱氧核

糖部分和至少 1 个 N' 是 LNA。

[0307] 在一些实施方案中, (N)_x 包含 9 个交替的已修饰的核糖核苷酸。在其它的结构 (I) 的实施方案中, (N)_x 包含 9 个交替的已修饰的核糖核苷酸 (进一步在第 2 位包含 2' O 修饰的核苷酸)。在一些实施方案中, (N)_x 在第 1、3、5、7、9、11、13、15、17、19 位的奇数位置包含 2' O Me 修饰的核糖核苷酸。在其它的实施方案中, (N)_x 进一步在第 2 和 18 位中的一位或两位包含 2' O Me 修饰的核糖核苷酸。在另外其他的实施方案中, (N)_x 在第 2、4、6、8、11、13、15、17、19 位包含 2' O Me 修饰的核糖核苷酸。

[0308] 在各种实施方案中, z'' 是存在的并选自脱碱基核糖部分、脱氧核糖部分; 反向脱碱基核糖部分、脱氧核糖部分; C6-氨基 -Pi; 镜像核苷酸。

[0309] 在另一个方面本发明提供了具有如下阐述的结构 (J) 的化合物:

[0310] (J) 5' (N)_x-Z 3' (反义链)

[0311] 3' Z' -(N')_y-z'' 5' (正义链)

[0312] 其中每个 N 和 N' 是核糖核苷酸, 该核糖核苷酸可以是未修饰的或已修饰的, 或非常规部分;

[0313] 其中每一个 (N)_x 和 (N')_y 是寡核苷酸, 在其中每一个连续的 N 或 N' 通过共价键连接于下一个 N 或 N' ;

[0314] 其中 Z 和 Z' 可以存在或不存在, 但如果存在的话是独立地 1-5 个连续的核苷酸共价地连接于它所在链的 3' 末端;

[0315] 其中 z'' 可以存在也可以不存在, 但如果存在的话是共价地连接到 (N')_y 的 5' 末端的封端部分;

[0316] 其中 x = 18 到 27 ;

[0317] 其中 y = 18 到 27 ;

[0318] 其中 (N)_x 包含已修饰的或未修饰的核糖核苷酸, 可选地和至少 1 个非常规部分;

[0319] 其中在 (N')_y 中, 至少 1 种非常规部分是存在的, 其中非常规部分可是脱碱基核糖部分、脱碱基脱氧核糖部分、已修饰的或未修饰的脱氧核糖核苷酸、镜像核苷酸、非 - 碱基配对核苷酸类似物或通过 2' -5' 核苷酸间磷酸键连接到邻近核苷酸的核苷酸; 而且

[0320] 其中 (N')_y 的序列是与 (N)_x 有互补性的序列; 并且其中 (N)_x 的序列包含与约 18 到约 27 个连续的核糖核苷酸有互补性的反义序列, 这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0321] 在一些实施方案中 x = y = 19。在其它的实施方案中 x = y = 23。在一些优选的实施方案中, (N)_x 包含已修饰的和未修饰的核糖核苷酸, 和至少一种非常规部分。

[0322] 在一些实施方案中, 在 (N)_x 中, 在 3' 末端的 N 是已修饰的核糖核苷酸, 而且 (N)_x 包含至少 8 个已修饰的核糖核苷酸。在其它的实施方案中, 至少 8 个已修饰的核糖核苷酸中的至少在 3' 末端起始的 5 个是交替的。在一些实施方案中, (N)_x 在第 5、6、7、8、9、10、11、12、13、14 或 15 位中的一个位置包含脱碱基部分。

[0323] 在一些实施方案中, 至少 1 个非常规部分存在于 (N')_y 的第 15、16、17 或 18 位。在一些实施方案中, 非常规部分选自镜像核苷酸、脱碱基核糖部分和脱碱基脱氧核糖部分。在一些优选的实施方案中, 非常规部分是镜像核苷酸, 优选地是 L-DNA 部分。在一些实施方案中, L-DNA 部分存在于第 17 位、第 18 位或第 17 和 18 位。在其它的实施方案中, 至少 1 个

在 $(N')_y$ 中的非常规部分是脱碱基核糖部分或脱碱基脱氧核糖部分。

[0324] 在又一个方面本发明提供了具有如下阐述的结构 (K) 的化合物：

[0325] $(K) 5' (N)_x - Z 3'$ (反义链)

[0326] $3' Z' - (N')_y - z'' 5'$ (正义链)

[0327] 其中每个 N 和 N' 是核糖核苷酸, 该核糖核苷酸可以是未修饰的或已修饰的, 或非常规部分；

[0328] 其中每一个 $(N)_x$ 和 $(N')_y$ 是寡核苷酸, 在其中每一个连续的 N 或 N' 通过共价键连接于下一个 N 或 N'；

[0329] 其中 Z 和 Z' 可以存在或不存在, 但如果存在的话是独立地共价地连接于它所在链的 3' 末端的 1-5 个连续的核苷酸；

[0330] 其中 z'' 可以存在也可以不存在, 但如果存在的话是共价地连接到 $(N')_y$ 的 5' 末端的封端部分；

[0331] 其中 $x = 18$ 到 27；

[0332] 其中 $y = 18$ 到 27；

[0333] 其中 $(N)_x$ 共同包含已修饰的或未修饰的核糖核苷酸和非常规部分, 任一已修饰的核糖核苷酸在糖上具有 2' -O- 甲基；

[0334] 其中 $(N')_y$ 包含已修饰的或未修饰的核糖核苷酸, 可选地和非常规部分, 任一已修饰的核糖核苷酸在糖上具有 2' -OME；

[0335] 其中 $(N')_y$ 的序列是与 $(N)_x$ 有互补性的序列；并且其中 $(N)_x$ 的序列包含与约 18 到约 27 个连续的核糖核苷酸有互补性的反义序列, 这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0336] 在一些实施方案中 $x = y = 19$ 。在其它的实施方案中 $x = y = 23$ 。在一些优选的实施方案中, 至少 1 个非常规部分存在于 $(N)_x$ 并且是脱碱基核糖部分或脱碱基脱氧核糖部分。在其它的实施方案中, 至少 1 个非常规部分存在于 $(N)_x$ 并且是非 - 碱基配对核苷酸类似物。在各种实施方案中, $(N')_y$ 包含未修饰的核糖核苷酸。在一些实施方案中, $(N)_x$ 包含至少 5 个脱碱基核糖部分或脱碱基脱氧核糖部分或其联合物。在某些实施方案中, $(N)_x$ 和 / 或 $(N')_y$ 包含已修饰的核糖核苷酸, 其与在 $(N')_y$ 和 / 或 $(N)_x$ 中的相应的已修饰的或未修饰的核糖核苷酸, 不能碱基配对。

[0337] 在各种实施方案中本发明提供了如结构 (L) 所阐释的 siRNA：

[0338] $(L) 5' (N)_x - Z 3'$ (反义链)

[0339] $3' Z' - (N')_y 5'$ (正义链)

[0340] 其中每个 N 和 N' 是核苷酸选自未修饰的核糖核苷酸、已修饰的核糖核苷酸、未修饰的脱氧核糖核苷酸和已修饰的脱氧核糖核苷酸；

[0341] 其中每一个 $(N)_x$ 和 $(N')_y$ 是寡核苷酸, 在其中每一个连续的 N 或 N' 通过共价键连接于下一个 N 或 N'；

[0342] 其中 Z 和 Z' 是不存在的；

[0343] 其中 $x = y = 19$ ；

[0344] 其中在 $(N')_y$ 中, 至少在第 15、16、17、18 和 19 位中的一位的核苷酸包含核苷酸, 其选自脱碱基非常规部分、镜像核苷酸、脱氧核糖核苷酸, 和通过 2' -5' 核苷酸间键与邻近

的核苷酸连接的核苷酸；

[0345] 其中 $(N)_x$ 包含交替的 2'OMe 糖修饰的核糖核苷酸和未修饰的核糖核苷酸，从而在 $(N)_x$ 的中间位置具有 2' OMe 糖修饰的核糖核苷酸；而且

[0346] 其中 $(N')_y$ 的序列是与 $(N)_x$ 有互补性的序列；并且其中 $(N)_x$ 的序列包含与约 18 到约 40 连续的核糖核苷酸互补的反义链，这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0347] 在一些结构 (L) 的实施方案中，在 $(N')_y$ 中在第 17 和 18 位中的一位或两位的核苷酸包含已修饰的核苷酸，其选自脱碱基非常规部分、镜像核苷酸和通过 2'-5' 核苷酸间键与邻近的核苷酸连接的核苷酸。在一些实施方案中镜像核苷酸选自 L-DNA 和 L-RNA。在各种实施方案中镜像核苷酸是 L-DNA。

[0348] 在各种实施方案中， $(N')_y$ 在第 15 位包含已修饰的核苷酸，其中已修饰的核苷酸选自镜像核苷酸和脱氧核糖核苷酸。

[0349] 在某些实施方案中， $(N')_y$ 在第 2 位进一步包含已修饰的核苷酸或伪核苷酸，其中伪核苷酸可是脱碱基非常规部分和已修饰的核苷酸可选地是镜像核苷酸。

[0350] 在各种实施方案中，反义链 $(N)_x$ 在奇数位置 (5' 到 3'；在第 1、3、5、7、9、11、13、15、17、19 位) 包含 2' O-Me 修饰的核糖核苷酸。在一些实施方案中， $(N)_x$ 在第 2 和 18 位中的一位或两位，进一步包含 2' O-Me 修饰的核糖核苷酸。在其它的实施方案中， $(N)_x$ 在第 2、4、6、8、11、13、15、17、19 位包含 2' O Me 修饰的核糖核苷酸。

[0351] 其他设想的结构 (L) 的实施方案，其中 $x = y = 21$ 或其中 $x = y = 23$ ；在这些实施方案中，上文所述的 $(N')_y$ 的修饰结构不是在第 17 和 18 位，而是在 21- 节链寡核苷酸的第 19 和 20 位以及在 23- 节链寡核苷酸的第 21 和 22 位上；相似地，在第 15、16、17、18 或 19 位的修饰结构，是在 21- 节链寡核苷酸的第 17、18、19、20 或 21 位以及在 23- 节链寡核苷酸的第 19、20、21、22 或 23 位上。在反义链上的 2' O Me 修饰结构也做相似地调整。在一些实施方案中， $(N)_x$ 在奇数位置包含 2' O Me 修饰的核糖核苷酸，该在奇数位置 (5' 到 3') 是指：21- 节链寡核苷酸 [在第 11 位的核苷酸是未修饰的] 的第 1、3、5、7、9、12、14、16、18、20 位，以及 23- 节链寡核苷酸 [在第 12 位的核苷酸是未修饰的] 的第 1、3、5、7、9、11、13、15、17、19、21、23 位。在其它的实施方案中， $(N)_x$ 在 21- 节链寡核苷酸 [在第 11 位的核苷酸是未修饰的] 的第 2、4、6、8、10、12、14、16、18、20 位包含 2' OMe 修饰的核糖核苷酸，以及在 23- 节链寡核苷酸 [在第 12 位的核苷酸是未修饰的] 的第 2、4、6、8、10、13、15、17、19、21、23 位包含 2' OMe 修饰的核糖核苷酸。

[0352] 在一些实施方案中， $(N')_y$ 进一步包含 5' 末端封端核苷酸。在各种实施方案中末端封端部分选自脱碱基非常规部分、反向脱碱基非常规部分、L-DNA 核苷酸和 C6- 亚胺磷酸基 (在末端的 C6 氨基接头和磷酸基)。

[0353] 在其它的实施方案中，本发明提供了具有如下阐述的结构 (M) 的化合物：

[0354] $(M) 5' (N)_x - Z 3' (反义链)$

[0355] $3' Z' - (N')_y 5' (正义链)$

[0356] 其中每个 N 和 N' 选自伪 - 核苷酸和核苷酸；

[0357] 其中每个核苷酸选自未修饰的核糖核苷酸、已修饰的核糖核苷酸、未修饰的脱氧核糖核苷酸和已修饰的脱氧核糖核苷酸；

[0358] 其中每一个 $(N)_x$ 和 $(N')_y$ 是寡核苷酸, 在其中每一个连续的 N 或 N' 通过共价键连接于下一个 N 或 N' ;

[0359] 其中 Z 和 Z' 是不存在的 ;

[0360] 其中 $x = 18$ 到 27 ;

[0361] 其中 $y = 18$ 到 27 ;

[0362] 其中 $(N')_y$ 的序列是与 $(N)_x$ 有互补性的序列 ; 并且其中 $(N)_x$ 的序列包含与约 18 到约 27 个连续的核糖核苷酸有互补性的反义序列, 这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0363] 在其它的实施方案中本发明提供了具有如下所述的结构 (N) 的双链化合物 :

[0364] $(N)5' (N)_x - Z 3'$ (反义链)

[0365] $3' Z' - (N')_y 5'$ (正义链)

[0366] 其中每个 N 和 N' 是核苷酸选自未修饰的核糖核苷酸、已修饰的核糖核苷酸、未修饰的脱氧核糖核苷酸和已修饰的脱氧核糖核苷酸 ;

[0367] 其中每一个 $(N)_x$ 和 $(N')_y$ 是寡核苷酸, 在其中每一个连续的 N 或 N' 通过共价键连接于下一个 N 或 N' ;

[0368] 其中 Z 和 Z' 是不存在的 ;

[0369] 其中 x 和 y 各是 18 到 40 的整数 ;

[0370] 其中 $(N')_y$ 的序列是与 $(N)_x$ 有互补性的序列 ; 并且其中 $(N)_x$ 的序列包含与约 18 到约 40 个连续的核糖核苷酸有互补性的反义序列, 这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 的反义序列中 ;

[0371] 其中 $(N)_x$, $(N')_y$ 或 $(N)_x$ 和 $(N')_y$ 包含非碱基 - 配对已修饰的核苷酸, 以致在双链化合物中 $(N)_x$ 和 $(N')_y$ 形成了不到 15 个碱基对。

[0372] 在其它的实施方案中, 本发明提供了具有如下阐述的结构 (O) 的化合物 :

[0373] $(O)5' (N)_x - Z 3'$ (反义链)

[0374] $3' Z' - (N')_y 5'$ (正义链)

[0375] 其中每个 N 是核苷酸选自未修饰的核糖核苷酸、已修饰的核糖核苷酸、未修饰的脱氧核糖核苷酸和已修饰的脱氧核糖核苷酸 ;

[0376] 其中每个 N' 是选自 6 元糖核苷酸、7 元糖核苷酸、吗啉代部分、缩氨酸核酸和其联合物的核苷酸类似物 ;

[0377] 其中每一个 $(N)_x$ 和 $(N')_y$ 是寡核苷酸, 在其中每一个连续的 N 或 N' 通过共价键连接于下一个 N 或 N' ;

[0378] 其中 Z 和 Z' 是不存在的 ;

[0379] 其中 x 和 y 各是 18 到 40 的整数 ;

[0380] 其中 $(N')_y$ 的序列是与 $(N)_x$ 有互补性的序列 ; 并且其中 $(N)_x$ 的序列包含与约 18 到约 40 个连续的核糖核苷酸有互补性的反义序列, 这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0381] 在其它的实施方案中本发明提供了具有如下阐述的结构 (P) 的化合物 :

[0382] $(P)5' (N)_x - Z 3'$ (反义链)

[0383] $3' Z' - (N')_y 5'$ (正义链)

[0384] 其中每个 N 和 N' 是核苷酸选,其自未修饰的核糖核苷酸、已修饰的核糖核苷酸、未修饰的脱氧核糖核苷酸和已修饰的脱氧核糖核苷酸;

[0385] 其中每一个 (N)_x 和 (N')_y 是寡核苷酸,在其中每一个连续的 N 或 N' 通过共价键连接于下一个 N 或 N' ;

[0386] 其中 Z 和 Z' 是不存在的;

[0387] 其中 x 和 y 各是 18 到 40 的整数;

[0388] 其中在 (N)_x 或 (N')_y 的内部位置的 N 或 N' 之一,或在 (N)_x 或 (N')_y 末位的一个或多个 N 或 N' 包含脱碱基部分或 2' 修饰的核苷酸;

[0389] 其中 (N')_y 的序列是与 (N)_x 有互补性的序列;并且其中 (N)_x 的序列包含与约 18 到约 40 个连续的核糖核苷酸有互补性的反义序列,这些核糖核苷酸存在于和急性肾损伤有关的靶基因的 mRNA 中。

[0390] 在各种实施方案中, (N')_y 在第 15 位包含已修饰的核苷酸其中,该已修饰的核苷酸选自镜像核苷酸和脱氧核糖核苷酸。

[0391] 在某些实施方案中, (N')_y 在第 2 位进一步包含已修饰的核苷酸,其中已修饰的核苷酸选自镜像核苷酸和脱碱基非常规部分。

[0392] 在各种实施方案中,反义链 (N)_x 在奇数位置 (5' 到 3';第 1、3、5、7、9、11、13、15、17、19 位) 包含 2' O-Me 修饰的核糖核苷酸。在一些实施方案中, (N)_x 在第 2 和 18 位之一或两位上,进一步包含 2' O-Me 修饰的核糖核苷酸。在其它的实施方案中, (N)_x 在第 2、4、6、8、11、13、15、17、19 位包含 2' O Me 修饰的核糖核苷酸。

[0393] 另外一个本发明提供的新分子是包含连续的核苷酸的寡核苷酸,其中这种核苷酸的第一片段编码第一抑制性 RNA 分子,这种核苷酸的第二片段编码第二抑制性 RNA 分子,而且这种核苷酸的第三片段编码第三抑制性 RNA 分子。每个第一、第二和第三片段可包含双链 RNA 的 1 条链,而且第一、第二和第三片段可通过接头连接到一起。进一步说,寡核苷酸可包含通过一种或多种接头结合的 3 个双链片段。

[0394] 由此,本发明提供的 1 个分子是包含连续的核苷酸的寡核苷酸,其编码 3 种抑制性 RNA 分子;所述寡核苷酸可具有三链结构,以致 3 个双链臂通过一种或多种接头(例如任一如上文所述的接头)连接到一起。这种分子形成“星”-样结构,并且在本文中也可以被定义为 RNA_{tar},并且诸于 PCT 专利公开 WO 2007/091269(转让给本发明的受让人)。

[0395] 所述三链寡核苷酸可是具有通用结构的寡核糖核苷酸:

[0396] 5' 寡聚物 1(正义)接头 A 寡聚物 2(正义)3'

[0397] 3' 寡聚物 1(反义)接头 B 寡聚物 3(正义)5'

[0398] 3' 寡聚物 3(反义)接头 C 寡聚物 2(反义)5'

[0399] 或

[0400] 5' 寡聚物 1(正义)接头 A 寡聚物 2(反义)3'

[0401] 3' 寡聚物 1(反义)接头 B 寡聚物 3(正义)5'

[0402] 3' 寡聚物 3(反义)接头 C 寡聚物 2(正义)5'

[0403] 或

[0404] 5' 寡聚物 1(正义)接头 A 寡聚物 3(反义)3'

[0405] 3' 寡聚物 1(反义)接头 B 寡聚物 2(正义)5'

[0406] 5' 寡聚物 3(正义) 接头 C 寡聚物 2(反义) 3'

[0407] 其中一种或多种接头 A、接头 B 或接头 C 是存在的 ; 只要链的极性和分子的通用结构还继续存在, 任一 2 个或多个寡核苷酸和接头 A-C 的一种或多种的联合体是可以的。进一步说, 如果接头 A-C 的 2 个或多个是存在的, 它们可以相同或不同。

[0408] 因此, 形成了三臂结构, 其中每个臂包含正义链和互补的反义链 (即反义寡聚物 1 碱基与正义寡聚物 1 配对等)。三臂结构可以是三链化的, 从而每个臂具有碱基配对。

[0409] 进一步说, 上述三链结构在一条或多条链中, 可以具有缝隙却没有接头。这样的具有 1 个缝隙的分子技术上是四链化的而不是三链化的 ; 插入额外的缝隙或缺口将导致分子具有额外的链。本发明的发明人得到的初步结果显示, 在抑制某些靶基因时, 所述有缝隙的分子比相似的无缝隙的分子更具有活性。这也是有缺口分子的情况。

[0410] 根据一个本发明的优选的实施方案, siRNA 的反义链和正义链仅在 3' - 末端而不在 5' - 末端磷酸化。根据另一个优选的本发明的实施方案, 反义链和正义链是非 - 磷酸化的。根据还另一个优选的本发明的实施方案, 在正义链的最 5' 端核糖核苷酸是已修饰的, 以排除任何在体内 5' - 磷酸化的可能性。

[0411] 本发明进一步提供了在细胞中能表达任一上述的寡核糖核苷酸的载体, 表达之后可以产生了适当的修饰结构。在优选的实施方案中, 细胞是哺乳动物细胞, 优选地是人类细胞。

[0412] 治疗方法

[0413] 在一个实施方案中, 本发明涉及治疗受试者的方法, 该受试者需要治疗以减弱 CKD (与一种或多种表 1 靶基因的表达有关) 发展, 该方法包括包含向受试者使用一定量的寡核苷酸抑制剂, 其可以降低、下调或抑制一种或多种那些基因的表达或上调。

[0414] 很多病症可以引起肾脏的永久损坏和 / 或影响肾功能并导致 CKD。引起成人 CKD 的最常见原因如下 :

[0415] a) 糖尿病, 糖尿病肾病 (DN) 是常见糖尿病的并发症 ;

[0416] b) 高血压, 未治疗或治疗不善的高血压是引起 CKD 的主因 ;

[0417] c) 肾脏老化, 表现出和年龄有关的肾功能衰退 ;

[0418] d) 急性或慢性肾局部贫血 (这是我们用于大鼠的模型, 也是你们用于人类的模型) ;

[0419] e) 败血症。

[0420] 其它能导致 CKD 的比较不普遍的病症包含 : 肾小球疾病、例如肾小球肾炎 (在肾脏中的肾小球炎症) ; 肾脏动脉狭窄 (窄化)、溶血性尿毒综合症、多囊性肾病、排尿障碍、药物和毒素 - 导致的肾损坏、和复发性肾感染。

[0421] 在一些实施方案中, 本发明涉及治疗受试者的方法, 该受试者需要治疗慢性肾病 (CKD) (与一种或多种上文表 1 靶基因的表达有关), 该方法包括向受试者使用一定量的寡核苷酸抑制剂, 其可以在受试者的肾中预防一种或多种那些基因的上调或超表达。在某些实施方案中, 一种或多种靶基因的上调或超表达是对肾脏损害或损伤的应答。在一些实施方案中, 肾脏损害是包含急性肾损伤 (AKI) 的急性肾脏损害。在各种本发明的实施方案中, 治疗包括预防或者延迟 CKD 发作, 以及预防 CKD 的恶化和发展。

[0422] 在优选的实施方案中, 接受治疗的受试者是热血动物, 和尤其是包含非 - 人类灵

长类和人类的哺乳动物。

[0423] 本发明的方法包括向受试者施用一种或多种治疗有效剂量的抑制性化合物,其可以调低表 1 靶基因的表达;特别是和 siRNA,从而治疗受试者。

[0424] 在各种实施方案中,抑制剂选自包含下列物质的组:siRNA、shRNA、适体、反义分子、miRNA 和核酶。在最近的优选的实施方案中,抑制剂是 siRNA。在优选的实施方案中,siRNA 是化学合成的、已修饰的化合物。

[0425] 术语“治疗”是指关于治病的治疗和预防疾病的或预防性方法,其目的是预防或者延迟 CKD 发作,减弱、预防或减慢上面所列的 CKD 或 CKD 的发展或严重性。需要治疗的对象包括那些正在患有疾病或病症、有 CKD 倾向,和需要预防 CKD 的对象;例如在暴露于反复性肾脏损害的受试者中,该肾脏损害包含因肾毒药物引起的肾脏损害或局部贫血-再灌注性损伤(IRI),肾毒药物例如但不仅限于抗生素(例如氨基苄类)、化疗的药物(例如顺氯氨铂)、免疫抑制剂药物(例如 Cyclosporin A, Tacrolimus(也称为 FK-506 或 Fujimycin))和放射性对比剂。根据多种本发明的实施方案,在暴露于肾脏损害之前、期间和之后,优选地在损害之后施用寡核苷酸抑制剂。在一些实施方案中,寡核苷酸抑制剂是 siRNA 化合物。在各种实施方案中,在肾脏损害后约 4 小时,向受试者施用 siRNA。如果治疗是以预防为目的,那么本发明涉及延缓 CKD 发作或防止 CKD 发展的方法。在一些实施方案中,CKD 的发展是因为对包含反复性急性肾损伤(AKI)的反复性肾脏损害产生的应答。

[0426] 急性肾衰竭(ARF),也通称急性肾损伤(AKI),是迅速地肾脏功能的损失,其原因是肾损坏而导致在血液中滞留含氮的(尿素和肌酸酐)和非-含氮的废物。根据肾功能障碍严重性和持续性,这种累积也伴随着代谢障碍,例如代谢酸中毒(血液酸化)和高钾血症(升高的血清钾水平)、体内液体平衡的改变,和在其它器官系统上的影响。它的特征在于少尿症或无尿症(尿液产生的降低或中止)。

[0427] 肾小球滤过率“GFR”是指经过肾小球过滤液体的流率。GFR 评估是最常用的肾脏功能测试方式。在一些实施方案中,相对于未受治疗的受试者,在受治疗的受试者中的 GFR 提高了约 5%、10%、20%、30%、40%或更多,GFR 可以用来评估减弱发展 CKD 或预防 CKD 恶化的方法。

[0428] 肌酐清除率(CCr, mL/min/1.73m²)是在单位时间内已清除肌酸酐的血浆的体积,它可以用来测量真实 GFR 的近似值。

[0429] SCr(mg/dL)涉及血清肌酸酐水平。在一些实施方案中,相对于未受治疗的受试者,在受治疗的受试者中的 SCr 降低了约 5%、10%、20%、30%、40%或更多,SCr 可以用来评估减弱发展 CKD 或预防 CKD 恶化的方法。

[0430] 在一些实施方案中,本发明的方法涉及治疗 CKD 的方法,该 CKD 是由反复性急性肾损伤(AKI)损害导致的,尤其是因外科术后病人局部贫血引起的急性肾衰竭、因化疗,例如施用顺氯氨铂引起的急性肾衰竭、败血症-相关的急性肾衰竭、肾毒素导致的 AKI(包含放射性对比剂导致的 AKI)。造影剂导致的 AKI(CIAKI)(也通称造影剂肾病)是指,例如在暴露于心血管造影术和特定冠状动脉心血管造影术的病人中静脉施用含碘造影剂导致的 AKI。在另外一个实施方案中,本发明的方法是指在暴露于主心脏手术或血管手术的高-危病人中预防 CKD。病人处于正发展的急性肾衰竭(在某些情况下向 CKD 发展)的风险之中。可以使用多种计分方法来鉴定这些病人,例如由 US Academic Hospitals(QMMI)

和 Veterans' Administration(CICSS) 研发的 Cleveland 临床算法。

[0431] 在另一个优选的实施方案中,本发明的方法涉及治疗或预防在受试者中的 CKD,通过肾毒素处理诱导该受试者,所用肾毒素包含利尿药、 β -阻断剂、利尿剂、血管扩张剂、ACE 抑制剂、免疫抑制剂(例如环孢素)、氨基苷抗生素(例如庆大霉素)、抗真菌(例如两性霉素乙)、化疗试剂(例如顺铂)、造影剂、抗体(例如免疫球蛋白)、甘露醇、非甾体抗炎药(例如阿司匹林、布洛芬、双氯芬酸)、环磷酰胺、甲氨蝶呤、阿昔洛韦、聚乙二醇、 β -内酰胺抗生素、万古霉素、利福平、环丙沙星、磺胺类药物、雷尼替丁、西咪替丁、速尿、噻嗪类、苯妥英、青霉素、锂盐、氟化物、去甲金霉素、磷、马兜铃酸、抗氧化剂、钙通道阻滞剂、血管活性物质、生长因子、抗炎剂以及更多。

[0432] 在多数就医的 ARF 病人中,ARF 是由急性肾小管坏死(ATN)引起,而 ATN 是由局部缺血、败血性和/或肾毒损害导致的。血量减少性、心脏性和败血性休克和施用引起血管收缩的药物或肾血管损伤都可以引起肾脏灌注不足。肾毒素包含外源毒素,例如放射性对比剂、氨基苷类和顺铂和顺铂-样化合物,也包含内源毒素,例如肌红蛋白。不希望受理论束缚,最近的研究支持以下理论:就 ARF 而言,在肾脏组织中的细胞凋亡在大多数人类中是显著地。凋亡性细胞死亡的主要位置是末端肾单位。在局部缺血损伤的初始阶段,肌动蛋白细胞骨架的完整性丧失导致上皮扁平化(同时刷毛缘丧失、病灶细胞接触减少)和随后细胞从基底的脱离。有人认为细胞凋亡性小管细胞死亡比坏死性细胞死亡更能预报功能改变(Komarov et al., Science 1999,10;285(5434):1733-7);Supavekin et al., Kidney Int. 2003,63(5):1714-24)。

[0433] 如本文所用,"造影剂"是指用于提高内部身体结构在影像中的可见度的化合物,例如 X-光影像或扫描影像(例如,CAT(Computerized Axial Tomography)扫描、MRI(Magnetic Resonance Imaging)扫描)。术语造影剂在本文中也是指放射性对比剂。造影剂用于多种诊断(例如栓塞;心导管插入术)和治疗过程。造影剂的使用仍是造影剂导致的肾病(CIN)主要风险因素。已患有肾衰竭和糖尿病的病人尤其高风险。此外,CIN 是与显著住院的和长期的疾病率和死亡率有关的。

[0434] 其他的机制也可促使 AKI 的发展:局部贫血、血管收缩、与选定的内源物质(例如在由挤压损伤和严重钝伤引起的横纹肌溶解中的肌红蛋白)有关的毒性损伤、放射性对比剂(用于包含 CT 心血管造影术、心脏动脉造影术的放射学检查的含碘的和 IV 造影剂)、磷酸盐(某些肾病由使用磷酸钠的为结肠镜检查而做的肠道准备工作引起)、肾毒药物(例如,NSAID、氨基苷抗生素、庆大霉素、青霉素,两性霉素 B)、微循环改变、如观察到的败血症和其它炎症状态、溶血、诊断心导管术,特别是在高龄或糖尿病病人中的股动脉造影、经由皮肤的冠状动脉介入(PCI)、冠状动脉旁路术(CABG)、败血症、胸腹部主动脉手术、主动脉动脉瘤修复例如 forinfra-肾脏主动脉腹部手术或胸或胸腹部主动脉手术。

[0435] 冠状动脉疾病(CAD)、心脏衰竭、糖尿病、血管并发症(例如动脉粥样硬化疾病和肾脏静脉血栓形成)、HIV-感染的病人、性别、高龄(>60)、已经存在的慢性肾病或潜在的肾脏机能不做、血容量不足、肝炎协同感染、肝病、肝肾综合症、癌症病人、患有严重水和电解质代谢障碍的病人、患有血液和非血液系统恶性肿瘤的病人、肝硬化、COPD、严重烧伤、心包炎和胰腺炎等已存在的病症可预测 AKI 病人的严重性和长期预后。

[0436] 对肾脏的内在损坏:

[0437] 毒素或药物（例如一些 NSAID、氨基苄类抗生素、含碘造影剂、锂、磷酸盐，某些肾病由使用磷酸钠的为结肠镜检查而做的肠道准备工作引起）

[0438] 横纹肌溶解（肌肉组织的破坏）- 由此导致的在血液中的肌红蛋白释放影响肾脏；它可以是由损伤（尤其是挤压损伤和严重钝伤）、抑制素、刺激物和其它药物引起的。

[0439] 溶血作用（血红细胞的破坏）- 血红蛋白损坏小管；其由多种病症（例如镰刀-细胞疾病和红斑狼疮）引起。

[0440] 多发性骨髓瘤，归因于高钙血或“管型肾病”（多发性骨髓瘤通过不同机制也能引起慢性肾衰竭）

[0441] 急性肾小球肾炎其可归因于多种起因，例如抗肾小球基底膜疾病 / Goodpasture's 综合症、Wegener's 肉芽肿病或含系统性红斑狼疮的急性狼疮性肾。

[0442] 后-肾脏（在尿路中的梗阻原因）归因于干扰膀胱正常排空的药物（例如抗胆碱能类）、良性前列腺肥大或前列腺癌、肾结石、腹部恶性肿瘤（例如卵巢癌、结肠直肠癌）、阻塞的尿道管、能引起晶尿症的药物和能导致肌红蛋白尿和膀胱炎的药物。

[0443] 总之，目前没有令人满意的用于预防和 / 或治疗由复发性肾急性损害导致的 CKD 的治疗方式，由此为了这个目的需要研发新的化合物。

[0444] 药物组合物

[0445] 本发明的化合物可以作为化学原料来施用，优选地以药物组合物呈现。相应地，本发明提供了施用包含一种或多种本发明寡核苷酸化合物的药物组合物方法；和药学上可接受的载体。这种组合物可包含 2 种或多种不同 siRNA 化合物的混合物。

[0446] 在一些实施方案中，药物组合物包含至少一种共价地或非-共价地结合于一种或多种本发明 siRNA 化合物的本发明 siRNA 化合物，它的量可以有效地抑制本发明的靶基因；和药学上可接受的载体。通过内源细胞复合体可在细胞内产生该化合物，从而制备本发明一种或多种寡核糖核苷酸。

[0447] 本发明进一步提供了包含药学上可接受的载体和一种或多种本发明化合物的药物组合物，它的量可以有效抑制在细胞中本发明人类靶基因的表达，包含序列 (N)_x 的化合物与靶向核酸序列是充分互补的。

[0448] “具有互补性”或“充分互补的”是指对另一个序列有超过 84% 的互补性。例如在包含 19 碱基对的双链区域中，1 个错配导致 94.7% 互补性、2 个错配导致约 89.5% 互补性和 3 个错配导致约 84.2% 互补性，这使得双链区域是充分互补的。相应地显著地一致性是指对另一个序列有超过 84% 的互补性。

[0449] 另外，与包含使本发明靶基因的 mRNA 转录物和一种或多种本发明化合物接触的对照相比，本发明提供了抑制本发明靶基因表达的方法，抑制率至少 40%，优选地是 50%、60% 或 70%，更优选地是 75%、80% 或 90%。

[0450] 在一个实施方案中，寡核糖核苷酸抑制一种或多种本发明靶基因，由此这种抑制选自包含抑制基因功能、抑制多肽和抑制 mRNA 表达的组。

[0451] 在一个实施方案中，化合物抑制靶向多肽，由此该抑制选自下面的组：抑制功能（可以通过酶法检测或用内源基因 / 尤其是多肽的已知交互子（interactor）的结合法检测来检验）、抑制蛋白（可以通过 Western 印迹法，尤其是 ELISA 或免疫沉淀法来检验）和抑制 mRNA 表达（可以通过 Northern 印迹法、量化 RT-PCR、尤其是原位杂交或微阵列杂交来检

验)。

[0452] 另外,本发明提供了治疗或预防在受试者中肾损坏的方法,该受试者处于和一种或多种本发明靶基因的激活或上调或超表达有关的 CKD 的风险之中,该方法包括向受试者施用治疗有效剂量的本发明的化合物,从而在受试者中治疗或预防肾损坏。

[0453] 在其他的实施方案中,本发明提供了治疗受试者的方法,该受试者处于 CKD(伴随于或有关于或起因于一种或多种本发明靶基因的水平升高)发展的风险之中,该方法包括向受试者施用治疗有效剂量的本发明的化合物,从而降低在受试者中 CKD 发展的风险。

[0454] 递送

[0455] 将用于本发明方法的 SiRNA 化合物作为化合物本身(即作为无修饰的 siRNA)或作为药学上可接受的盐施用,也可以单独地或作为有效成分结合一种或多种药学上可接受的载体、溶剂、稀释剂、赋形剂、助剂和媒介物施用。在一些实施方案中,通过直接施用以载体或稀释剂制备的裸分子,将用于本发明方法的 siRNA 分子输送到靶向组织。

[0456] 术语“裸 siRNA”是指 siRNA 分子不含任一递送媒介物,包含病毒序列、病毒微粒、脂质体制剂、脂转染物或沉淀剂等媒介物可以协助、提升或促进进入细胞。例如,在 PBS 中的 siRNA 是“裸 siRNA”。

[0457] 然而,在一些实施方案中,本发明的 siRNA 分子可以在脂质体制剂和脂转染制剂等中输送,并且通过该领域熟练技术人员熟知的方法来制备。这样的方法见诸于,例如,在美国专利 No. 5,593,972;5,589,466,和 5,580,859,(以引用的方式并入本文)中。

[0458] 已经研发出尤其旨在提高和改善 siRNA 递送进入哺乳动物细胞的递送系统(参见例如 Shen et al., FEBS Let. 2003,539:111-115;Xia et al., Nat. Biotech. 2002, 20:1006-1010;Reich et al., Mol. Vision 2003,9:210-216;Sorensen et al., J. Mol. Biol. 2003. 327:761-766;Lewis et al., Nat. Gen. 2002,32:107-108 和 Simeoni et al., NAR 2003,31,11:2717-2724)。最近,siRNA 已经全部成功地用于抑制在灵长类中的基因表达(参见例如 Tolentino et al., Retina 24(4):660)。

[0459] 药学上可接受的载体、溶剂、稀释剂、赋形剂、助剂和媒介物,也包含植入物载体(通常是指惰性的、非毒性的固体或液体填充物)、稀释剂或胶囊材料(不与本发明有效成分反应),以及脂质体和微球体等等。用于本发明的递送系统的示例包含美国专利 No. 5,225,182;5,169,383;5,167,616;4,959,217;4,925,678;4,487,603;4,486,194;4,447,233;4,447,224;4,439,196 和 4,475,196。其它类似的植入物、递送系统和组件对本领域的熟练技术人员是熟知的。依照良好医疗实践,考量个体受试者的临床病症、所需治疗的疾病、施用的部位和方法、施用安排、病人年龄、性别、体重和执业医师所知的其它因素,来施用本发明的 SiRNA 或药物组合物和确定剂量。

[0460] 本文所用的“治疗有效剂量”由那些该领域已知的因素来确定。该剂量必须有效达到改善之目的,所谓改善包括但不限于提高存活率或更快恢复,或改善或消除症状和其它选自该领域熟练的技术人员做的恰当检测的指标。

[0461] 通常,在每次肾脏损害 24 之内施用单剂或多剂(例如或 2 剂或 3 剂或更多剂)的方案中,用于人类的化合物的有效剂量在 1ng/kg 到约 20-100mg/kg 体重每天之间,优选地是约 0.01mg 到约 2-10mg/kg 体重每天。通过任一常规施用方式,可施用用于本发明方法的 SiRNA 化合物。可以口服、皮下或胃肠外(包含静脉、动脉、肌肉、腹腔内)施用该化合物,以

及鼻内和稍内施用,也可以使用灌注技术。化合物植入物也是可用的。为注射制备液体形式,该术语包含皮下、透皮、静脉、肌肉、稍内和其它胃肠外施用途径。液体组合物包含水性溶液,含有和不含有有机共-溶剂、水性或油悬浮液、含可食用油乳化液,以及相似的药物媒介物。在特定的实施方案中,该施用包含静脉施用。

[0462] 能以每日一次、每日四次、每日三次、每日两次、QD,或以医学上恰当的任一间隔和持续时间,施用包含本文公布的核酸分子的药物组合物。然而,也可以以剂量单位施用组合物,该剂量单位包含 2、3、4、5、6 或更多个亚-剂量(以在整天内的合理间隔施用)。

[0463] 在一些实施方案中,将剂量单位复合为数天内使用的单剂,例如,使用常规持续释放的制剂,该制剂可以在数天之内提供持续的和一致的 dsRNA 释放。持续的释放制剂在该技术领域是熟知的。在某些实施方案中,本发明的方法包括以持续的或可控的递送为目的,向受试者施用一种或多种 siRNA 化合物。本发明的方法主要依赖肠胃外施用途径,更特定地使用植入积存或积存注射,其可以延长生物试剂释放进入循环系统。用于肠胃外递送系统的设备包括非-可注射的和可注射的设备。非-可注射的设备包括植入物,例如 siRNA 积存植入物,或相似设备。已知的积存植入物包括但不限于,合成的和自然的包含固体可生物降解的和非-可生物降解的聚合体材料的物质,该聚合体材料包含泡沫、胶、基质等(含有一种或多种葡聚糖、纤维蛋白、透明质酸、壳聚糖等),而且泵和微泵系统在该领域也是已知的。可注射的设备包括推注(化合物在注射后释放和消散在),和贮藏性或积存注射,其可以在注射部位提供储藏库或积存,使得生物试剂得到超时的持续的释放。

[0464] 本发明也提供了制备用于本发明方法的药物组合物的方法,其包含:

[0465] 提供一种或多种双链的本发明化合物;和

[0466] 使所述化合物与药学上可接受的载体混合。

[0467] 本发明也提供了制备用于本发明方法的药物组合物的方法,其包含使一种或多种本发明的 siRNA 化合物与药学上可接受的载体混合。

[0468] 在优选的实施方案中,将用于制备药物组合物(用于本发明的方法)的药学上有效剂量的 siRNA 化合物与载体混合。在特定的实施方案中,用于本发明方法的 siRNA 化合物共轭地连接于类固醇、维生素或脂质或其他合适的分子(例如胆固醇)。

[0469] 下文用实施例来详细阐述本发明,但这不能被理解为是限制性的。

[0470] 本文任一资料的引用不是旨在承认这些资料是相关的先进技术,或认为这些材料涉及任何本申请或权利要求的专利性。关于任一资料的内容和日期的任何申明,都是基于本申请人在提交时可以得到的信息,并不意味着承认任何这些申明的正确性。

实施例

[0471] 在分子生物学中的常见方法

[0472] 在业内是已知的并且不做特别说明的标准分子生物学技术基本上依据以下文献, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York(1989)、和 Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland(1989) and as in Perbal, *A Practical Guide to Molecular Cloning*, John Wiley & Sons, New York(1988)、和 Watson et al., *Recombinant DNA*, Scientific American Books, New York 和 Birren

et al(eds)Genome Analysis:A Laboratory Manual Series, Vols.1-4 Cold Spring Harbor Laboratory Press, New York(1998),以及如在美国专利 4,666,828 ;4,683,202 ;4,801,531 ;5,192,659 和 5,272,057 中阐释的方法,并且通过引用的方式并入本文。聚合酶链式反应 (PCR) 的操作是根据 PCR 标准 :A Guide To Methods And Applications,Academic Press,San Diego,CA(1990)。与 Flow Cytometry 结合的原位 (在细胞中)PCR 能用于检测包含特定 DNA 和 mRNA 序列的细胞 (Testoni et al.,1996,Blood 87 :3822.),完成 RT-PCR 的方法在该技术领域是熟知的。

[0473] 实施例 1 :针对靶向基因的有效 siRNA 化合物序列的产生和 siRNA 制备

[0474] 使用独有算法和已知靶基因序列,产生很多潜在 siRNA 序列。除了算法以外,通过 19- 节链序列的 5' 和 / 或 3' 延长可以产生一些 23- 节链寡聚物序列。这种方法产生的序列与相应的 mRNA 序列是完全互补的。

[0475] 序列表 :已经以序列表文件 (标题为“209-PCT1_ST25.txt”创建于 2010-06-07,459kb) 电子提交了本申请序列表 (SEQ ID NO :1-SEQ ID NO :119)。申请人通过引用序列表将其并入本说明书。

[0476] 实施例 2 :在体外测试 siRNA 化合物

[0477] 1. 总体情况

[0478] 约 $1.5-2 \times 10^5$ 将测试细胞 (用于靶向人类基因的 siRNA 的 HEPG2 或 PC3 细胞) 接种到 6 孔板的每一个孔 (70-80% 汇合)。

[0479] 24h 后,使用最终浓度为 500pM、5nM、20nM 或 40nM 的 Lipofectamine™ 2000 试剂 (Invitrogene),以 siRNA 寡聚物转染细胞。在 CO₂ 孵育器中,以 37℃ 孵育细胞 72h。

[0480] 将使用以 PTEN-Cy3 标记的 siRNA 寡聚物的细胞转染作为阳性对照。将使用以 GFP 标记的 siRNA 寡聚物的细胞转染作为阴性对照。

[0481] 转染后约 72h,获得细胞并从细胞提取 RNA。通过荧光显微检查测试转染效率。

[0482] 使用 qPCR 分析在细胞中表达内源基因的靶基因,测定使用特定 siRNA 对基因表达抑制百分率。

[0483] 本发明化合物对靶基因的抑制性活性,或结合的本发明多种化合物对靶基因的抑制性活性可以用来测定其它化合物与靶向多肽的互相作用,例如,如果其它化合物为抑制靶基因与本发明寡核苷酸竞争,或如果其它化合物促进所述抑制。通过多种方式测试所述抑制或激活,尤其地例如靶向多肽活性产物检测,或在放射性或荧光竞争测定法中,结合化合物从靶向多肽的取代。

[0484] 实施例 3 :siP53 化合物

[0485] QM5 是化学修饰的靶向大鼠和小鼠 p53 的 siRNA 化合物,并被公开于国际专利公开 WO 2006/035434 (转让给本发明的受让人之一)。QM5 化合物具有 2 条独立的链,分别是正义 (SEN ;乘客) 和反义 (AS ;向导) 链,每条链包含在两条链上交替的未修饰的核糖核苷酸 (大写字母) 和 2'- 甲基氧 (2'-O-Me ;2'-O-CH₃) 糖修饰的核糖核苷酸 (小写字母),这两条链形成如下文所示的特殊形式 :

[0486] 正义 (乘客) 序列 5' GaAgAaAaUuUcCgCaAaA 3' (SEQ ID NO :116)

[0487] 反义 (向导) 序列 3' cUuCuUuUaAaGgCgUuUu 5' (SEQ ID NO :117)

[0488] I5 化合物是 19- 节链钝化末端的双链核酸,它靶向人类 p53 基因 (在在应激反

应细胞凋亡途径中发挥重要作用)。化合物具有两条独立的链,分别是正义 (SEN) 和反义 (AS) 链,每条链包含在两条链上交错的未修饰的核糖核苷酸 (大写字母) 和 2'-甲基氧 (2'-O-Me) 糖修饰的核糖核苷酸 (小写字母),这两条链形成如下文所示的特殊形式:

[0489] 正义 (乘客) 序列 5' GaGaAuAuUuCaCcCuUcA 3' (SEQ ID NO:118)

[0490] 反义 (向导) 序列 3' cUcUuAuAaAgUgGgAaGu 5' (SEQ ID NO:119)

[0491] 为在人类受试者 (处于和暴露于肾脏损害复发有关的慢性肾病 CKD 的风险之中) 中治疗或预防肾损坏,在每次肾脏损害 24 小时之内,将治疗有效剂量的 I5 施用于受试者,从而治疗 CKD。I5 是 WO 2006/035434 (转让给本发明的受让人之一) 的主题。

[0492] 实施例 4:CKD 模型系统

[0493] 执行下列动物模型以支持本发明的方法。

[0494] (1) 预防受试者中的 CKD 发展,该受试者在处于由多发性肾脏损害引起的 CKD 的风险之中 (由实施例 4-1 支持);

[0495] (2) 预防 CKD 发展加速 / 发展,该 CKD 发展是由在有 CKD 背景的受试者中的 AKI 急性发作导致的 (由实施例 4-2 支持);和

[0496] (3) 减弱 AKI (有 CKD 背景的) 的严重性 (由实施例 4-2 支持)。

[0497] 实施例 4-1 双侧肾动脉止血钳 CKD 模型

[0498] 这种动物模型可用来评估用于预防 CKD 或减弱 CKD 发展的测试化合物,该 CKD 由反复性 AKI/ARF 损害导致。

[0499] 反复性 AKI/ARF 损害经常导致慢性肾病 (CKD) 恶化,CKD 发展或 CKD 发展 (参见图 1)。ARF 是临床综合症,其特征是在数天之内发生肾脏功迅速恶化。不受理论束缚,急性肾损伤可归因于肾局部贫血-再灌注性损伤,例如在正暴露于大手术 (例如大心脏手术) 的病人中的肾局部贫血-再灌注性损伤。ARF 的主要特征是肾小球滤过率 (GFR) 急剧下降,导致在血液中含氮的废物 (尿素、肌酸酐) 滞留。最近的研究支持以下理论:就 ARF 而言,在肾脏组织中的细胞凋亡在大多数人类中是显著地。凋亡细胞死亡的主要位置是末端肾单位。在局部缺血损伤的初始阶段,肌动蛋白细胞骨架的完整性丧失导致上皮扁平化 (同时刷毛缘丧失、病灶细胞接触减少) 和随后细胞从基底的脱离。

[0500] 如下所述并如图 2 所示,CKD 大鼠模型包含反复性 (5 次) 局部贫血-再灌注-导致的 ARF:

[0501] 夹住双侧肾动脉止血钳 45 分钟并随后释放止血钳,给予 24 小时再灌注之后,便可导致局部贫血-再灌注性损伤。在钳后 4 小时,将 PBS 或 QM5 (大鼠 siP53) (12mg/kg) 静脉注射进入个体实验动物。通过在手术之前 (基准) 和 24 小时、2 天和 7 天之后检测血清肌酸酐 (SCr) 水平,监测 ARF 发展。再重复做四个循环 (30-天间隔) 的处理 (I/R 损伤、QM5、SCr 检测),前后总共五个循环。第 5 个循环后,测试 7 天 24 小时肌酸酐清除 (CrCl) 代谢笼和尿液蛋白。代谢笼 2 天后外科摘除右肾 (第 5 个循环后第 10 天),并且针对 CKD 在组织学分析该肾脏。在右肾切除术 3 周后摘除左肾,并利用活体双-光子显微检查在体内研究该肾 (针对 Cy3-siRNA 摄取和滞留)。结果

[0502] 年龄一致的未处理的大鼠具有更均一的 Cy3 标记的 siRNA 的摄入和分布。在初次注射 24 小时后,在细胞中 siRNA 水平的下降是明显的。而且肾小管内腔基本上更舒张,只能观察到少量萎陷的内腔。

[0503] 生理盐水处理的 CKD 大鼠具有更不均一的 siRNA 的分布（不均衡）和摄入。可观察到小管薄上皮和内腔显著扩张。在肾小管细胞中确实有摄入，但比具有更正常形态的肾小管细胞周边摄入的水平低。在 24 小时，在这些大鼠中的残余荧光比在年龄一致的未处理大鼠中的更多，也就是说标记的 siRNA 的降解表现地比较低。

[0504] QM5 处理的 CKD 大鼠所显示的摄入和分布的特征是处于龄一致的未处理的组对照和生理盐水处理的 CKD 组对照之间。就个别领域来说，总摄入是更均一的。囊性小管任然偶尔出现。QM5 有助于 Cy3- 标记的 siRNA 均一地递送到肾小管上皮，这种效果在大鼠 #4 中更明显。24 小时 siRNA 新陈代谢也处于年龄一致的未处理的大鼠和生理盐水处理的局部缺血大鼠之间。在生理条件下，静脉注射后，在年龄一致的未处理的大鼠中 Cy3- 标记的 siRNA 迅速地从肾小球滤过，并被近端小管细胞（PTC）选择性地摄取。利用临界分析，量化在近端小管细胞中的总细胞和胞质累积，在 120 分钟使出现最大值并在下一个四小时内迅速衰退。siRNA 生物活性与荧光半排出期密切相关。

[0505] 图 3 显示了反复性局部缺血损伤后，p53siRNA 对于肾功能的作用。在用 PBS 或 siP53(QM5) (12mg/kg)（在每次局部贫血 AKI 后 4 小时静脉施用）处理的大鼠中，在每次局部贫血（AKI）循环之前，以及在每次局部贫血循环后的第 1、2 和 7 天的血清肌酸酐水平。数据表示平均数 +SD (n = 10/ 组)。

[0506] 图 4 显示了 siP53 保护 GFR 和减少蛋白尿。PBS 治疗的动物的测量结果以阴影柱显示，同时 QM5 治疗的动物的测量结果以实心柱显示。

[0507] 下文中的表 2 显示了，在 5 个月的局部缺血损伤循环之后，siP53 对肾功能的作用：siP53 保护肾小球滤过率和减少蛋白尿。在最后 1 个（第 5 个）AKI 循环后 7 天内，通过 24 小时尿液收集和尾部血液收集，测定肾小球滤过率（GFR）和蛋白尿（Uprot）。组：PBS- 在每次局部缺血损伤后 4 小时，对大鼠静脉施用 PBS；QM5- 在每次局部缺血损伤后 4 小时静脉施用 siP53 (12mg/kg)。数据代表平均数 +SD (n = 10/ 组)

[0508] 表 2：

[0509]

组织病理学评分参数	损伤评分,平均/组(n=10)±SD		
	PBS	QM5	p-值
肾小球损坏	0.1+0.3	0.1+0.3	1
间质细胞渗透	1.2+0.4	0.5+0.5	0.008
间质纤维化	1.3+0.5	0.9+0.6	0.12
肾小管状态	1.4+0.5	0.8+0.6	0.04
血管病	0.1+0.3	0	0.37
总体慢性损伤评分	4.1+0.7	2.3+1.2	0.02
肾小管坏死	0.1+0.3	0	0.37
肾小管膨胀	1.4+0.5	0.8+0.6	0.04
管型	1.3+1.3	0.5+0.5	0.16
总体急性损伤评分	2.8+1.8	1.3+0.9	0.05
总体病理学评分 慢性+急性	6.9+3.3	3.6+1.8	0.02

[0510] 图5显示了在第5个AKI循环后10天所得到的右肾组织病理学评分。在最后(第5次)一次局部缺血损伤后,对大鼠(每月局部缺血损伤后4小以PBS或siP53处理时)进行右肾切除术。由受广泛认可的病理学者对得到的右肾切片进行摸索性的分析。至少分析每只大鼠的2个典型的肾切片。分析急性(肾小管坏死、肾小管膨胀、管型)和慢性(肾小球损坏、间质细胞渗透、间质纤维化、肾小管状态和血管病)损坏参数。总体急性和总体慢性损伤评分是所有各自的慢性或急性损坏参数的和。总体病理学评分是每只大鼠的急性和慢性损伤评分的和。根据下面的评分系统,对病理学上的改变进行评级:

[0511] 级别0-无病理学上的改变;级别1-特征在于包含1到10%的范围(轻微和病灶);级别2-特征在于包含10到25%的范围(中等和多病灶);级别3-特征在于包含25到75%的范围(弥漫但没有正常结构损坏);级别4-特征在于包含多于75%的范围(弥漫并且有正常肾结构的显著损坏)。数据代表平均数+SD(n=10/组)。

[0512] 实施例4-2全肾切除术和高盐食物

[0513] 这种动物模型用于评估用于减低/减弱有CKD背景的AKI/ARF的测试化合物,从而为预防CKD(由在CKD病人中的复发性AKI/ARF损害引起)的恶化或发展提供模型,以及为在遭受CKD痛苦的病人中减弱AKI严重性(他暴露于可以引起AKI的过程或事件)提供模型。

[0514] 图6显示了用于建立CKD的研究设计。总地来说,在3或4个反复性AKI月循环(直到SCr和GFR值处于CKD水平)之前,对SD大鼠进行右肾切除术。第一个AKI循环包含用左蒂钳钳住45分钟,而下面的所有AKI包含钳住30分钟。整个周期始终,用高盐食物喂养大鼠。3或4个循环后,得到升高的肾功能参数:血清肌酐酞(SCr)、GFR和尿液蛋白。

[0515] 当血清肌酐酞(SCr)水平是高于0.8mg/ml且肾小球滤过率(GFR)是少于0.60ml/min/100gr时,可以认为该动物患有中等到严重的CKD。另外,3只大鼠被切除全肾并且以常规食物喂养相同的如上面的总时间段(7-8月)。在该时间段末期,他们的SCr、GFR和Uprot是升高的。结果如下文表3所示:

[0516] 表 3

[0517]

	SCr, mg/ml	GFR, ml/min/100g BW	Uprot, mg/24h
健康大鼠 (历史和公开的数据)	0.2-0.3	0.8-0.9*	
在3个AKI循环 (N=3) 后uninephr大鼠, HS饮食	1.13± 0.05	0.16 ± 0.03	221 ± 21
在4个AKI循环 (N=4) 后uninephr大鼠, HS饮食	0.98± 0.17	0.19 ± 0.03	646 ± 160

[0518] *Zaladek-Gil et al(1999),Braz J Med Biol Res,32 :107-113 ;Chamberlain et al, (2007)Exp Physiol,92 :251-262

[0519] 图 7 显示了在分组得动物中的肾功能参数 (在 AKI/ARF 损害和 siP53 治疗之前)。经过测试 SCr、GFR 和尿液蛋白 (Uprot) 水平,结果显示,相比全肾切除的接受正常食物的动物,全肾切除的接受高盐食物的动物表现出更严重的 CKD。在最后一次 AKI 损害后 4 小时,通过静脉注射,对 CKD 和对照动物施用 siP53 或 siGFP(12mg/kg) 或媒介物。图 8 显示了在患有 CKD 的动物中,siP53(QM5) 对于预防 AKI 损害的效果。

[0520] 图 9A-9H 显示了急性损伤 (9A-9C) 和慢性损伤 (9D-9H) 的组织病理学参数。组织病理学的评分系统如下所示:0:无;1:轻微和病灶;2:中等和多病灶;3:弥漫但没有正常结构损坏;4:弥漫且有显著的正常结构损坏。在 siP53 处理的动物中,所有急性参数肾小管坏死、肾小管膨胀和尿管型得到改善。在处理的动物中,慢性损伤参数肾小球损坏、间质细胞渗透、间质纤维化和肾小管萎缩得到降低。

[0521] 在处理的和未处理的动物中,急性和慢性损伤的平均组织病理学评分如图 10 所示。

[0522] 虽然已经图解和描述了某些本发明的实施方案,但是明显的是本发明不限于这些本文所述的实施方案。在不偏离所附权利要求所述的本发明的范围的条件下,本领域熟练的技术人员意识到修饰、改变、变化、代替和等同形式。

[0001]

序列表

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作为美国代表的退伍军人事务部（华盛顿，DC）
- <120> 治疗慢性肾病的方法
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[0021]

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[0022]

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<213> 人

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[0023]

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[0024]

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[0025]

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[0026]

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[0027]

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[0028]

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[0029]

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[0030]

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[0031]

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[0032]

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gagccuugcu gccaaugccu gccuuugggu cgacauagua uggaaguauu ugagagagag	3840
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<211> 2044

<212> RNA

<213> 人

<400> 14

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uuugaaugag ggucucucca ccuuuuuagu acucucaugg cugggaauaa auuuuuauuc	180
guuuauugac acguucucac gguaugaaga ggaggagucu uucauuaca cacaguuau	240
uuuggguuca acacuggcuu gggcacgagc auccgcacug ugccugaauu uuaacugcau	300
gcuaauucua auaccuguca gucgaaaccu uauuucuuuc auaagaggaa caaguauuug	360

[0033]

cugcagagga ccguggagga ggcaauuaga caaaaaccuc agauuucaca aacuggucgc	420
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cuaccacugg agccaguccg aggaggccca gggacuucug gccgcacuuu ccaagcuggg	540
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ugaauugcua aggacaauag caggcgucac cggucuggug aucucucugg cuuuagucuu	660
gaucaugacc ucgucaacug aguucaucag acaggccucc uaugaguugu ucugguacac	720
acaccauguu uucaucguu uuuuucucag ccuggccauc caugggacgg gucggauugu	780
ucgaggccaa acccaagaca gucucucucu gcacaacauc accuucugua gagaccgcu	840
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ggcuuggaaa uggauuuuag gccucuggu cuuguauuca ugugaaagaa uauuagguu	960
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ucuucacaua gcuuuacacu gggacgaaaa uacugacgug auuacaggcu uaaagcagaa	1620
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[0034]

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uggugaauaa uauuaacua uugugaacag uacacuauac cauacuuccu uagcuuauaa	1980
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ucaa	2044

<210> 15

<211> 716

<212> RNA

<213> 人

<400> 15

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augugcccgu gccccugca ccgcggccgc ggccccccgg ccgugugcgc cugcagcgcg	180
ggucgccugg ggcugcgcuc guccgccgcg cagcucaccg ccgcccggu caagcgcua	240
ggcgacgagc ugcaccagcg caccaugugg cggcgccgcg cgcggagccg gagggcgccg	300
gcgcccggcg cgcuccccac cuacuggccu uggcugugcg cggccgcgca gguggcggcg	360
cuggcggccu ggcugcucgg caggcggaaac uuguaggaac gcggggcuuc uugguggggc	420
cggagccgag acccagccgg agcgagcaac agguugguga aaaccugug uccuuggaga	480
aagcugguuc ccguuuucca gagggggagc ccagagcuug aaaggccgcg guuggcacuu	540
cgagaaggaa guggagagua aagacagcgc cuggagcgau cguagaaaca cagaauaggga	600
cuggggaagc ccuuuggaaa uccagcugca gaaacagaca ccccaaugcu auuuacauac	660
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[0035]

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uccgucgccg	gccuccgcgc	ugccgcgccc gccucgccuu uccggcagcu ccugcagccg 180
gcaccccggc	ugugcaccgc	gcccucgggg cugcucagcg ugcgcgcagg uuccgagcgg 240
cggccggggcc	uccugcggcc	ucgcggaccg ugcgccugug gcuguggcug cggcucgcug 300
cacaccgacg	gagacaaagc	uuuuguugau uuccugagug augaaauuaa ggaggaaaga 360
aaaauucaga	agcauaaaac	ccucccuaag augucuggag guugggagcu ggaacugaau 420
gggacagaag	cgaaauuagu	gcggaaaguu gccggggaaa aaauacggu cacuuucaac 480
auuaacaaca	gcaucccacc	aacauuugau ggugaggagg aaccucgca agggcagaag 540
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caagaagacg	aggcugagag	ugacaucuuc ucaucaggg aaguuaagcu ucaguccacu 720
ggcgagucug	aauggaagga	uacuaauuau acacucaaca cagauuccuu ggacugggcc 780
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caggcuuugg	ccagugaaca	aauccuacuc ugaagcuaga caugugcuuu gaaaugauua 1020
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[0036]

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ggcgccaugu cgcagaacgg agcgcccgagg augcaggagg agagccugca gggcuccugg	180
guagaacugc acuuacagca uaaugggaac gggggcagcg uuccagccuc gguuuuauu	240
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uccaagagcu cucacuguga cagcccaccu cgcucgcaga caccacaaga uaccaacagg	360
gcuucugaaa cagauacca uagcauugga gaaaaaaca gcucacaguc ugaggaagau	420
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gauuggucaa gucggccgga aaauuuucc cccaaggagu uccuuuuuaa acaccgaag	540
cgcacggcca ccucagcau gaggaacacg agcgucauga agaaaggggg cauauucucu	600
gcagaauuuc ugaaaguuuu ccuuccaucu cugcugcucu cucauuugcu ggccaucgga	660
uuggggaucu auauuggaag gcgucugaca accuccacca gcaccuuuug augaagaacu	720
ggagucugac uugguucguu aguggauuac uucugagcuu gcaacauagc ucacugaaga	780
gcuguuagau ccuggggugg ccacgucacu uguguuuuuu uguucuguaa augcugcguu	840
ccuaauuuag uaaaauaaaa gaauagacac uaaaaucaug uugaucuaa auuacaccua	900
ugggaucaau aagcauguca gacugauuaa ugucuacugu gaaaauuugg uaguauuuu	960
ucauuuguaa uuagauauaa auaucugaau auaaaauuuu uuaauuacu agucaugaug	1020

[0037]

uguguuguau uuuaaaaauu aucugcaacc uuaauucagc ugaaguacuu uauuuucaa	1080
aagaaugaau aacauugaua auaaaaucgc uacuuuaagg gguuugucca aaauaaauau	1140
uguggccuua uauaucacac uauuguagaa aguauuuuuu aaauuaaagc gaugcagguu	1200
gucuacuaaa gaaagauuau auauaacuau gcuaauuguu cauaaucaac agaaaccaag	1260
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<210> 18

<211> 1417

<212> RNA

<213> 人

<400> 18

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gagcucaagg aauaguagc gcagcuuagc augccauucu ugaaagaaau guugcaauca	180
agaagcuagc ccgaccauuu cagaaucaag cucaugccaa gcgggcuac agagagcuag	240
uucuuuagaa auguguuaau cacaaaaaua uauuuggccu uuugaauuuu uucacaccac	300
agaaaucccu agaagaauuu caagauguuu acauagucuu ggagcucaug gaugcaaac	360
uuugccaagu gauucagaug gagcuagauc augaaagaau guccuaccuu cucuaucaag	420
ugcugugugg aaucagcac cuucauucug cuggaauuuu ucaucgggac uuaagccca	480
guaauauagu aguaaaucuu gauugcacuu ugaagaauuu ugacuucggu cuggccagga	540

[0038]

cugcaggaac gaguuuuuug augacgccuu auguagugac ucgcuaacuac agagcacccg	600
aggucauccu uggcaugggc uacaaggaaa acguggauuu auggucugug gggugcauuu	660
ugggagaaau gguuugccac aaaauccucu uuccaggaag ggacuauuu gaucagugga	720
auaaaguuuu ugaacagcuu ggaacaccu guccugaaau caugaagaaa cugcaaccaa	780
caguaaggac uuacguugaa aacagaccua aaauugcugg auauagcuuu gagaaacucu	840
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caaagauccc ugacaagcag uuagaugaaa gggaacacac aaugaagag uggaaagaau	1080
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<210> 19

<211> 1412

<212> RNA

<213> 人

<400> 19

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gagcucaagg aauguauugc gcagcuuauug augccauucu ugaaagaaau guugcaauca	180
agaagcuuag ccgaccauuu cagaauacaga cucaugccaa gcgggcccua agagagcuag	240

[0039]

uucuuugaa auguguuaau cacaaaaaua uaauuggccu uuugaauguu uucacaccac	300
agaaaucccu agaagaauuu caagauguuu acauagucan ggagcucaug gaugcaaauc	360
uuugccaagu gauucagaug gagcuagauc augaaagaau guccuaccuu cucuaucaga	420
ugcugugugg aaucagcac cuucauucug cuggaauuu ucaucgggac uuaaagccca	480
guauauagu aguaaaau cuuugcacuu ugaagaau cuacuucgu cuggccagga	540
cugcaggaac gaguuuuug augacgccu auguagugac ucgcuaucac agagcaccgc	600
aggucauccu uggaucgggc uacaaggaaa acguggauuu auggucugug gggugcauuu	660
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auaauguuu ugaacagcuu ggaacaccan guccugaauu caugaagaaa cugcaaccaa	780
caguaaggac uuacguuga aacagaccua aaauugcugg auauagcuu gagaacucu	840
ucccugaugu cccuuucca gcugacucag aacacaaca acuuaaagcc agucaggcaa	900
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gucuagaagc agcagcugg ccucugggc gcuguagaug acuaucuggg ccaucggggg	1320
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<210> 20

<211> 1417

[0040]

<212> RNA

<213> 人

<400> 20

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agaagcuaag ccgaccuuuu cagaaucaaga cucaugccaa gcgggccuac agagagcuag	240
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ugcugugugg aaucacagac cuucauucug cuggaauuuu ucaucgggac uuaaagccca	480
guauauauagu aguaaaauuc gauugcacuu ugaagaauuc ugacuucggu cuggccagga	540
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caaagaucuu ugacaagcag uuagaugaaa gggaacacac aaugaagagc uggaaagaau	1080
ugauauauaa ggaaguuuug gacuuggagg agagaaccaa gaauaggagu auacgggggc	1140
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[0041]

gucgucuguc aaugaugugu cuucaauguc aacagaucg acuuuggccu cugauacaga	1260
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<212> RNA	
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agaagcuaag ccgaccuuu cagaaucaga cucaugccaa gcgggccuac agagagcuag	240
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[0042]

gggauuuuguu auccaaaaug cugguaauag augcaucuaa aaggaucucu guagaugaag	960
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cugucaauga ugugucuca augucaacag auccgacuuu ggccucugau acagacagca	1260
gucuagaagc agcagcuggg ccucugggcu gcuguagaug acuacuuggg ccaucggggg	1320
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<210> 22

<211> 4353

<212> RNA

<213> 人

<400> 22

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gaaccgcgac cacuggagcc uuagcgggag cagcagcugg aacgggagua cugcgacgca	180
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gugcgggugc aggcgggggc cccacagggc caccuucuug cccggcggu gccgcuggaa	480
aaugucucag gagaggccca cguucuaccg gcaggagcug aacaagaca ucugggaggu	540

[0043]

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guccaucauu caugcgaaaa gaaccuacag agaacugcgg uuacuuaaac auaugaaaca	720
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[0044]

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[0045]

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<210> 23

<211> 4274

<212> RNA

[0046]

<213> 人

<400> 23

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[0047]

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[0048]

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[0049]

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<210> 24

<211> 4353

<212> RNA

<213> 人

<400> 24

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[0050]

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[0051]

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[0052]

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<211> 1431

<212> RNA

<213> 人

<400> 25

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[0053]

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[0055]

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[0056]

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[0057]

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[0058]

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[0059]

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[0060]

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[0061]

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[0062]

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[0063]

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<213> 人

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[0064]

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[0076]

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<210> 32

<211> 4276

<212> RNA

<213> 人

<400> 32

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gaaaguugg auaguaaaga aaacaacaau acaagaauag aauccaugau gaguucugua	600
caaaaagaua acuuuuacca acuaaauga gaaaauuag aaaauuuuc ucagcuaagu	660

[0077]

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caagacucag aagcagauga ggagacaagu ccagguuuug augaacaaga agaugguagu	1260
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[0078]

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[0079]

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<210> 33

<211> 1494

<212> RNA

<213> 人

<400> 33

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[0080]

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[0081]

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<212> RNA	
<213> 人	
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[0082]

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[0083]

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[0084]

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<211> 3150

<212> RNA

<213> 人

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[0085]

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[0086]

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[0087]

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aacugauggu gucaaugugg acaugcacuu gaagcagauu gagauaaaga aguucaagua	660
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caguauccuc uucaagucua ucuuugaggu ggccuucuuug cugauccagu gguacaucau	780
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[0088]

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[0089]

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uuguuuгaca uucauguua aacuacgguc auguucagcu ucuuugcaug uaauguagac	3060
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<211> 572

<212> RNA

<213> 人

<400> 37

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[0090]

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ggccggcugg ucccucgggg gcgaggggcu gcggaggcga cccggaaaca gcguaucacu	300
gagaccgagu cgcuuuauca ggagcuccag ggucagaggu cggaugucua cagcgaccuc	360
aacacacaga ggccguuuu caaaugagcc cgaaucauga cagucagcaa caugauaccu	420
ggauccagcc auuccugaag cccaccucg accucauucc aacuccuacc gcgauacaga	480
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<210> 38

<211> 605

<212> RNA

<213> 人

<400> 38

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ccucuccugc uggcuguaag uggucuccu ccuguccagg cccaggcca gagcgauugc	180
aguugcucua cggugagccc gggcgugcug gcagggaucg ugaugggaga ccuggugcug	240
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ugcaccucau uccaacuccu accgcgauac agaccacag agugccaucc cugagagacc	540
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[0091]

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aaaaaaaa	608
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<212> RNA	
<213> 人	
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[0092]

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acugagaccg agucgccuua ucaggagcuc caggguacaga ggucggangu cuacagcgac	360
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agaccacag agugccaucc cugagagacc agaccgcucc ccaauacucu ccuaaaauaa	540
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<211> 2358

<212> RNA

<213> 人

<400> 41

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acggcugcgg cucugccgc gucugcgcca agcagcuggg cgagcugugc accgagcgcg	420
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[0093]

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[0094]

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<211> 1641

<212> RNA

<213> 人

<400> 42

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aacgacucug augauguaga ugacacugau gauucucacc agucugauga gucucaccau	540
ucugaugaau cugaugaacu ggucacugau uuucccacgg accugccagc aaccgaagu	600
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[0095]

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<211> 1616

<212> RNA

<213> 人

<400> 43

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uugcagccuu cucagccaaa cgcgcaccaa ggaaaacuca cuaccaugag aaugcagug 180

[0096]

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[0097]

uaagaauuug guggugucaa uugcuuauuu guuuucccac gguuguccag caauuaauaa	1560
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aguucugagg aaaagcagaa ugcugugucc ucugaagaaa ccaaugacuu uaaacaagag	300
acccuuccaa guaaguccaa cgaaagccau gaccacauagg augauaugga ugaugaagau	360
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[0098]

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<212> RNA

<213> 人

<400> 45

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[0099]

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[0100]

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[0101]

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[0102]

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[0103]

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[0104]

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[0105]

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[0106]

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[0107]

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[0108]

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<210> 48

<211> 4390

[0109]

<212> RNA

<213> 人

<400> 48

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[0110]

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[0111]

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[0112]

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<212> RNA

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<400> 49

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caucuguuu uaaccucaac ugcagccuu uccuuuuacc caugugccga acacucugg	480
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[0113]

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[0114]

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[0115]

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<212> RNA

<213> 人

[0116]

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[0117]

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[0118]

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[0119]

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<210> 51

<211> 2529

<212> RNA

<213> 人

<400> 51

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auguuccauu ccugaaggac cucuccagaa uccggauugc ugaauuucc cuguugcua	180
gaagggcucc aaaccaccuc uugacaauug gaaacugggu gguuaccac ugguuuucag	240
uuuuguuucu gguuguuugg uuagggcuga auguuuuccu guuuguggau gccuuccuga	300
aaauagagaa ggccgacaaa uacuacuaca caagaaaaau ccuuggguca acauuggccu	360
gugcccagac gucugcucuc ugcuuagaau uuaacagcac gcugauccug cuuccugugu	420

[0120]

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cagcuauuca caucauugca caccuguuuu acuuugacug cuauagcaga agccgacagg	600
ccacagaugg cucccuugcc uccauucucu ccagccuau ucaugaugag aaaaaggggg	660
guucuuugcu aaaucccauc caguuccgaa acacgacagu ggaguaugug acauucacca	720
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[0121]

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uaaucugcau uuugucucu uguaucuca guaauuuacu uggucucguc agguuugagc	1980
agucacuuua ggauaagaau gugccucuca agccuugacu ccugguauu cuuuuuuuga	2040
uugcauucua cuucguuacu ugagcuucag caacuuaaga acucugaag uucuuaaagu	2100
ucugaaguuc uuaaagccca uggauccuuu cucagaaaaa uaacuguaaa ucuuucugga	2160
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aucccaggug auuuuaucaa uuccaguguu accaucuccu gaguuuuggu uuguaaucuu	2280
uugucccucc cccccccaca gaagauuucu aaguaggug acuuuuuuaa uaaaauuua	2340
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ccccauccc auauaacacc aacaguguac auguuuacug ucacuuuuga uauggucuaa	2460
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<211> 2382

<212> RNA

<213> 人

<400> 52

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auguuccauu ccugaaggac cucuccagaa uccggauugc ugaauuucc cuguugccua	180
gaagggcucc aaaccaccuc uugacaauug gaaacugggu gguuaaccac ugguuuucag	240

[0122]

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gugcccagac	gucugcucuc	ugcuugaauu	uuacagcac	gcugauccug	cuuccugugu	420
gucgcaauu	gcuguccuuc	cugaggggca	ccugcucuu	uugcagccgc	acacugagaa	480
agcaauugga	ucacaaccuc	accuuccaca	agcugguggc	cuauaugauc	ugccuacaua	540
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ccacagaugg	cucccuugcc	uccauucucu	ccagccuau	ucaugaugag	aaaaaggggg	660
guucuuggcu	aaaucccauc	cagucccgaa	acacgacagu	ggaguaugug	acauucacca	720
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ugaacaagcg	uggcuucagc	auggaagugg	ggcaguauau	cuuuguuaau	ugccccucaa	1200
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[0123]

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auaaacauaa auauuaaaca aaauuaccga gaaccccauc cccauuaaac accaacagug	2280
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<211> 4353

<212> RNA

<213> 人

<400> 53

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guugaacguc uuccucuuug ucugguauua cggguuuuau gauauuccac cuaaguucuu	180
uuacacaaga aaacuucuuu ggucagcacu ggcacuggcc agggccccug cagccugccu	240
gaauuucaac ugcaugcuga uucucuugcc agucugucga aaucugcugu ccuuccucag	300

[0124]

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ucauaaaaug guggcaugga ugauugcacu ucacucugcg auucacacca uugcacauu	420
auuuaaugug gaauuggugug ugaaugcccg agucauaau ucugauccuu auucaguagc	480
acucucugaa cuuggagaca ggcaaaauga aaguuaucuc aauuuugcuc gaaagagaau	540
aaagaaccuu gaaggaggcc uguaccuggc ugugaccugc uuggcaggca ucacuggagu	600
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gcuggagagc cagaucagg aaaggaacaa ugccggcuuc cucagcuaca acaucuaccu	1500
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[0125]

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guuugauagu gaaaguuac auuuugugg agcucuaug uuuugagagc acuuuacaa	1980
acauuuuuuc auuuuuuucc ucucaguaa gucaguggaa guuagggaaa agauucugg	2040
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gugcuuggca gagagcgau acucaagua guuuuguua augaaugaau gaauuagaa	2520
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gccuuuuga auuuugaacu uaggauuugu gacaaauug uaucugauu ggucuuugu	2880
uuuuuuuac acccaccuu uuuuuuccgu aaauacacac aaaaaugga ucgaucugu	2940

[0126]

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[0127]

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[0128]

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[0129]

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<210> 55

<211> 6428

<212> RNA

<213> 人

<400> 55

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uccaccugg agaccccgug uucgaccccg accagcggg ggacguggug cugcccuucc	660
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[0130]

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gcagcagccc ugguuuugcc aucaccauca uugcucucug cugccuuccc uuagugaguc	2040
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[0131]

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[0132]

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[0133]

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[0134]

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cuauuguaga gauguaccac aguuuguuuu uucucuguu gauggacguu uggguuguuu	6300
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<211> 1587

<212> RNA

<213> 人

<400> 56

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gcagccugga agccacagcc auggcaggcc ccgauaccc aguuucagug caaggggcag	300
ccugggugca gaucaagagg cuccaacgu uugccuucuc ugugcgcugg ucagacggca	360
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[0135]

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cugcaugaaa aaaaaaaaa aaaaaaaaa aaaaaaaaa aaaaaaaaa aaaaaaaaa	1560
aaaaaaaaa aaaaaaaaa aaaaaaa	1587

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<211> 1132

<212> RNA

<213> 人

<400> 57

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[0136]

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<210> 58

<211> 1147

<212> RNA

<213> 人

<400> 58

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[0137]

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<210> 59

<211> 1409

<212> RNA

<213> 人

<400> 59

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[0138]

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[0139]

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<210> 60	
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<212> RNA	
<213> 人	
<400> 60	
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[0140]

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<210> 61

<211> 2429

<212> RNA

<213> 人

<400> 61

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guuauuuuaa gggaggcugg gcccacuuu acucaucugg cccagaaagu gaacaccuug	180
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ucagugccgu ccaggacccc cacucccgga uuugcuucaa cauuggcugc auguacacua	420
uccugaagaa caugacugaa gcagagaagg ccuuuaccag aagcauuuac cgagacaagc	480

[0141]

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[0142]

uuuuuguuga agacugcgca acuacagauu uggaaagcac ucggagagaa gucuaggaug	1860
uuucacaaac uacaaagcug aagaaaauga agcccuauua cuuguuuugua agauuuagca	1920
cccuucugcu guauacugua cugagacauu acaguuuugga aguguuaacu auuuauuccc	1980
uguuaaaauu uaaccuacua gacaaugaug ugaguacca ggaugauuuc cuggggcaca	2040
gugggugagg agauggggac aggugaauagg aggaguuagg ggagaggaaa aguggaugga	2100
agugucugga aagggcacga gagagucuuc cagguacuga uccuguuuuc ugcucugagu	2160
gcuagcuagc cagcuguguu cacacuguaa acauucauca agcuguacau uuggugcacu	2220
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uccaggccca aggaguaagu acaaaauuuc cuguuucuga accauuacug uaaauggcuc	2340
uaaaggcuug aaguaaccuu auagguuacu cauaaggcau auacaaauaa acuuguuugu	2400
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<210> 62

<211> 2256

<212> RNA

<213> 人

<400> 62

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aucagccucu ggaaugaagg ggugcuggca gcggacaaga aggacuggaa gggagcccug	180
gaugccuua gugccgucca ggacccccac ucccggauuu gcuucaacau uggcugcaug	240
uacacuaucc ugaagaacau gacugaagca gagaaggccu uuaccagaag cauaaccga	300
gacaagcacu uggcaguggc uuacuuccaa cgagggaugc ucuacuacca gacagagaaa	360
uaugauuugg cuaucaaaga ccuuaaagaa gccuugauuc agcuucgagg gaaccagcug	420

[0143]

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gccacgagca	ugaagucuga	gcccagacau	uccaaaaucg	acaaggcgau	ggaguguguc	600
uggaagcaga	agcuauauga	gccaguggug	auccugugg	gcaagcuguu	ucgaccaaau	660
gagagacaag	uggcucagcu	ggccaagaag	gauuaccuag	gcaaggcgac	ggucguggca	720
ucuguggugg	aucaagacag	uuucucuggg	uuugccccuc	ugcaaccaca	ggcagcugag	780
ccuccacca	gaccgaaaac	cccagagauc	uucagggcuc	uggaagggga	ggcucaccgu	840
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gucuuugucu	ugaagaaggg	caaugauaac	ugggccacgg	ucauguucaa	cgggcagaag	960
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ccccaggagg	aaagcucucc	gcaguccgac	auccagcuc	cuccuaguuc	caaagccccu	1080
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[0144]

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gggcacagug	ggugaggaga	uggggacagg	ugaauaggagg	aguuaaggga	gaggaaaagu	1920
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ucugagugcu	agcuagccag	cuguguucac	acuguaaaca	uucaucaagc	uguacauuug	2040
gugcacuuuu	cugugucaua	ccacaauaaa	aaaaaacua	gcaucuuaa	aaaacaagac	2100
acccaagucc	aggcccaagg	aguaaguaca	aaauuuccug	uuucugaacc	auuacuguaa	2160
uuggcucuua	aggcuugaag	uaaccuuua	gguuacucau	aaggcauua	caauuaaacu	2220
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<210> 63

<211> 4976

<212> RNA

<213> 人

<400> 63

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agcgagagcc	ccgcagcccg	ccgcagcugc	cgcucgccc	cggccgggcc	ggagagcacg	180
gcggcgggag	cgcggccuua	ggaggcggcc	ggagcggugg	gcacagcucg	gcgcggagcg	240
uccugucagg	cggcgcccg	gggcgucg	gacucucucc	gcgaugaugc	cgaugauuu	300
aacuguuuuc	uugagcaaca	augaacagau	uuuaacagaa	guuccuauaa	caccggaaac	360
aaccugucga	gauguuguag	aauuuugcaa	ggaaccugga	gaaggcagcu	gccauuuagc	420
ugaagugugg	aggggaaaug	aacguccau	accuuuugau	cauauaugu	acgaacaucu	480
ucagaaaugg	gguccacgga	gggaagaagu	gaaauuuuuc	cuucgacacg	aggacucucc	540
aacugagaac	agugaacaag	guggccguca	gacccaagag	caacgaacuc	agagaaaugu	600

[0145]

aauaaaugua ccuggagaaa aacguacuga aaaugggguu gggaaucac guguugaacu	660
uaccucuca gagcucaag auauggcagc uaggcaacag cagcagauug aaaaucagca	720
gcagauugug guugccaagg aacagcguuu acauuuucua aagcaacagg agcgccguca	780
gcagcagucu auuucugaaa augaaaagcu ucagaaauug aaagaacgag uugaagccca	840
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gaacggcaau cugucugcug aaauagaaag guucagugcc auguuccagg aaaagaagca	960
ggaaguacag acugcaauuu uaagggguuga ucagcuuagu cagcaauugg aagauuuaaa	1020
gaaaggaaaa cugaaugggu uccagucuuu caauggcaaa uugacgggac cagcggcggu	1080
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[0146]

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[0147]

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[0148]

gugaggccac cuuuguccca ggcccagccu caaggcaagg agggcgcuuc acugaggugu	4620
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uacccgaacu auguuggaaa uuuuuuuuuu cucauuaaaa uacaggcccu uaggcucuau	4740
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gcacuguagc ccaucaccuu ggagcacuga cuguacauag uguggugaag aaaagugaac	4860
gcccuguuag agcagcccgga ccacaggagc augggcgug ccagcccaga cgcugcugac	4920
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<210> 64

<211> 2205

<212> RNA

<213> 人

<400> 64

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ggcgcggcc gccaugcagc ccuccagccu ucugccgcuc gccucugcc ugcuggcugc	180
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cucguuugac auccacuauug gcucgggcag ccucuccggg uaccugagcc aggacacugu	600
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[0149]

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gggccccucu gccagcuug ggc_cagcug ggcucugcca ccuaccugu ucagugucc	1860
gggcccguug aggaugaggc cgc_uagaggc cugaggaua gcuggaagga gugagaggg	1920
acaaaacca ccuuguugga gccugcaggg uggugcuggg acugagccag ucccagggc	1980

[0150]

auguauuggc cuggaggugg gguugggauu gggggcuggu gccagccuuc cucugcagcu	2040
gaccucuguu guccuccccu ugggcggcug agagccccag cugacaugga aauacaguug	2100
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<213> 人	
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[0151]

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auucccacca ggcccugcac acaccacuc cguaaccucu cccuguaacc ugugccaage	1260
cuagcacuug ugaugccucc augccccgag ggcccucucu caguucuggg aggaugacuc	1320
cagucccugc acgcccuggc acaccuua cgguugcuac ccaggcggcc aaguccaga	1380
ccgugccaga cccaggugcc ccagugccuu ugucuauuu cugcuccag ccugccaggc	1440
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<210> 66

<211> 1489

<212> RNA

<213> 人

<400> 66

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agaacuccgg acgugugcg cgugagucgc agccauguuc cugguuaacu cguucuugaa	180
gggcggcggc ggcggcggcg ggggagggcg gggccugggu gggggccugg gaaaugugcu	240
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uggagggcgc ggugcgggug gaacggccau gcgcauccua ggcggaguca ucagegccau	360
cagegagcg gcugcgagu acaaccggga gccccgcc ccacgcacac auuacuccaa	420
cauugaggcc aacgagagug aggagguccg gcaguuccgg agacucuuug cccagcuggc	480

[0152]

uggagaugac auggagguca ggcgcacaga acucaugaac auucauaa agguugugac	540
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caacaucaaa agguggcagg ccuuauacaa acaguucgac acugacegau cagggaccu	720
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ucucucccag ggccgauccu gucugcaguc acaucuuugu ggggccugcu gaccacaag	1080
cuuuuguucu cucaguacuu guuaccagc uucucaacau ccagggecca auuugcccug	1140
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<210> 67

<211> 2586

<212> RNA

<213> 人

<400> 67

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aagucuagag ccaccgucca gggagcaggu agcugcuggg cuccggggac acuuugcguu 120

[0153]

cgggcuggga gcgugcuuuc cacgacggug acacgcuucc cuggauuggc agccagacug	180
ccuuccgggu cacugccaug gaggagccgc agucagaucc uagcgucgag cccccucuga	240
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cugcaccagc agcuccuaca ccggcgggccc cugcaccagc cccuccugg cccugucau	480
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acuacaugug uaacaguucc ugcaugggcg gcaugaaccg gaggccauc cucaccauca	960
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[0154]

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<210> 68

<211> 2583

[0155]

<212> RNA

<213> 人

<400> 68

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cgggcuggga gcgugcuuuc cacgacggug acacgcuucc cuggauuggc cagacugccu	180
uccgggucac ugccauggag gagccgcagu cagauccuag cgucgagccc ccucugaguc	240
aggaaacauu uucagaccua uggaaacuac uuccugaaaa caacguucug uccccuugc	300
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aagaccagcagg uccagaugaa gcucccagaa ugccagaggc ugccccccc guggccccug	420
caccagcagc uccuacaccg gcggccccug caccagcccc cuccuggccc cugucaucuu	480
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aacuggccaa gaccugcccu gugcagcugu ggguugauuc cacacccccg cccggcaccc	660
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gccccacca ugagcgucgc ucagauagcg augguucggc ccuuccucag caucuaucc	780
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[0156]

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uccauuugcu uugucccgagg gcuccacuga acaaguuggc cugcacuggu guuuuguugu	1560
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[0157]

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<212> RNA	
<213> 人	
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cgggcuggga gcgugcuuuc cacgaecggug acacgcuucc cuggauuggc agccagacug	180
ccuuccgggu cacugccaug gaggagccgc agucagauc uageguegag ccccucuga	240
gucaggaaac auuuucagac cuauaggaaac uacuuccuga aaacaacguu cuguccccu	300
ugccguccca agcaauggau gauuugaugc ugucuccgga cgauauugaa caaugguuca	360
cugaagacc agguccagau gaagcucca gaauccaga ggcugcucc cccguggccc	420
cugcaccagc agcuccuaca ccggcggccc cugcaccagc cccucuccg cccucugau	480
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auucugggac agccaagucu gugacuugca cguacuccc ugcccucaac aagauguuuu	600
gccaacuggc caagaccugc ccugucagc uguggguuga uuccacacc ccgccggca	660
cccgcguccg cgccauggcc auuacaagc agucacagca caugacggag guugugaggc	720
gcugccccc ccaugagcgc ugucagaua gcgauggucu ggcccuccu cagcaucua	780
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gugugguggu gcccuauagag ccgccugagg uuggcucuga cuguaccacc auccacuaca	900
acuacaugug uaacaguucc ugcaugggcg gcaugaaccg gaggeccauc cucaccauca	960
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[0158]

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cuccccagcc	aaagaagaaa	ccacuggaug	gagaauuuu	cacccuucag	gaccagacca	1200
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aaggauccc	aggcugggaa	ggagccagg	gggagcagg	cucacuccag	ccaccugaag	1440
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[0159]

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auggggucuc acaguguugc ccaggcuggu cucaaaccucc ugggcucagg cgauccaccu	2520
gucucagccu cccagagugc uggaauuaca auugugagcc accacgucca gcuggaaggg	2580
ucaacauuu uuacauucug caagcacauc ugcuuuuuca cccaccuccu cccuccuuc	2640
uccuuuuua uaucccauuu uuauaucgau cucuuuuuu acaauaaaac uuugcugcca	2700
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<210> 70

<211> 2646

<212> RNA

<213> 人

<400> 70

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ccuuccgggu cacugccaug gaggagccgc agucagaucc uagcgucgag ccccccuga	240
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ugccgucca agcaauggau gauuugaugc ugucuccgga cgauauugaa caaugguuca	360
cugaagacc agguccagau gaagcucca gaauccaga ggucgucucc cccgugccc	420
cugcaccage agcuccuaca ccggcgccc cugcaccage cccuccug cccugucau	480
cuucuguccc uucccagaaa accuaccagg gcagcuacgg uuuccgucug ggcuucugc	540
auucugggac agccaagucu gugacuugca cguacuccc ugcccucaac aagauguuuu	600
gccaacuggc caagaccugc ccugugcagc uguggguuga uuccacacc cgcgccgca	660

[0160]

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uccgagugga aggaaauuug cguguggagu auuuggauga cagaaacacu uuucgacaua	840
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acuacaugug uaacaguucc ugcaugggcg gcaugaaccg gaggcccac cucaccauca	960
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uuuucucuaa cuucaaggcc cauauugug aaugcuggc auuugcaccu accucacaga	1860
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uggggucua gacuugacc ccuugagggu gcuuguucc uucccuguu ggucgguggg	1980

[0161]

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<211> 2271

<212> RNA

<213> 人

<400> 71

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uugcugccgu cuuccaguug cuuuaucugu ucacuuguc ccugacuuuc aacucugucu	240
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ccugcccuu gcagcugugg guugauucca ccccccgcc eggcacccgc guccgcgcca	360
uggccaucua caagcaguca cagcacauga cggagguugu gaggcgcgc cccaccaug	420

[0162]

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augagccgcc ugagguuggc ucugacugua ccaccaucca cuacaacuac auguguaaca	600
guuccugcau gggcggcaug aaccggaggc ccauccucac caucaucaca cuggaagacu	660
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[0163]

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cuuuguugcc caggcuggag uggaguggcg ugaucuuugc uuacugcagc cuuugccucc	1920
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caauugugag ccaccacguc cagcuggaag gguaacauc uuuuacauuc ugcaagcaca	2160
ucugcauuuu caccaccacc uuccccuccu ucuccuuuu uauauccau uuuuauaucg	2220
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<210> 72

<211> 2404

<212> RNA

<213> 人

<400> 72

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[0164]

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cagccaaacc cugucugaca accucuuggu gaaccuuagu accuaaaagg aaucucacc	1860

[0165]

ccaueccaca ccugggagga uucaucucu uguauugau gaucuggauc caccaagacu	1920
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agacuggguc ucgcuuuguu gcccaggcug gaguggagug gcgugaucu ggcuuacugc	2040
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<213> 人	
<400> 73	
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uugcugccgu cuuccaguug cuuuaucugu ucacuuguc ccugacuuuc aacucugucu	240
ccuuccucu ccuacaguac uccccugccc ucaacaagau guuuugccaa cuggccaaga	300
ccugcccugu gcagcugugg guugauucca ccccccgcc cggcacccgc guccgcgcca	360
uggccaucua caagcaguca cagcacauga cggagguugu gaggcgcugc cccaccaug	420
agcgcugcuc agauagegau ggucuggccc cuccucagca ucuuauccga guggaaggaa	480
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[0166]

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guuccugeau gggcggcaug aaccggaggc ccauccucac caucaucaca cuggaagacu	660
ccagugguaa ucuacuggga cggaacagcu uugaggugcg uguuuugucc uguccuggga	720
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[0167]

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ccggcucgag	caguccugcc	ucagccuceg	gaguagcugg	gaccacaggu	ucaugccacc		2040
auggccagcc	aacuuuugca	uguuuuuguag	agaugggguc	ucacaguguu	gcccgaggcug		2100
gucucaaaacu	ccugggcuca	ggcgauccac	cugucucage	cucccagagu	gcugggauua		2160
caauugugag	ccaccacguc	cagcuggaag	ggucaacauc	uuuuacauuc	ugcaagcaca		2220
ucugcauuuu	cacecccacc	uuccccuccu	ucucccuuuu	uauaucceau	uuuuauaucg		2280
aucucuuaau	uuacaauaaa	acuuugcugc	caccugugug	ucugaggggu	g		2331

$\langle 210 \rangle$	74
$\langle 211 \rangle$	2550
$\langle 212 \rangle$	RNA
$\langle 213 \rangle$	人

<400> 74

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agcgcccggc	cgucgccgcc	gccgccauuu	ucgcgcccgg	ccgcaggggc	ucuugggaag	300
gcggagucuu	ugggcauccg	cccgggguga	ggggacccga	aguuccgagg	cgcgccggaa	360
gggcuagcgg	ucccagcaua	ccccgcggcc	ccuugggccg	ucucacaacu	cgcguccggc	420
ggagaccaca	auucccgga	uucguggggc	agggaggagu	cggccucccg	gaauccuggu	480
cccggegu	gc	gc	gc	gc	gc	540
ugcucgcguc	cugggugccg	ccucugagua	ggcggggcga	ggaggcagcc	aagcgcgagc	600

[0168]

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cccucgaca ccgggcuggu cugcggccug gugaugugau uuuggccauu ggggagcaga	1860
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[0169]

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ugaccuccua uuaaagaaaa ugagcugcug ccaucuuuug ugggcaguua gucaggugcu	2340
gcucuuugug gugugguggg cucuggucug uucugcucgg ugcugggccu gggagcaaag	2400
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<210> 75

<211> 2259

<212> RNA

<213> 人

<400> 75

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ggcgccgaag gccgagagca cgcggggauc ggucucuucc cgccgggucu cuuaccggug	180
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gggcuagcgg ucccagcaua ccccgcgccc ccuugggcgc ucucacaacu cgcguccggc	420
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[0170]

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[0171]

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uaaaauuaua ccuagcaaca uauuauagua aaaaauaggg ugggagggcu ggaucuuuuc	1920
ccccacaaa aggcuaagagg uaaagcugua uccccuaaa cuuaggggag auacuggagc	1980
ugaccauccu gaccuccuau uaaagaaaau gagcugcugc caucuuuugu ggcaguuag	2040
ucaggugcug cucuuugugg uguggugggc ucuggucugu ucugcucggu gcugggccug	2100
ggagcaaaga uucccaugcu ugguacaga uacugacagc ugccucuga aggaggguga	2160
aaacuucugc uugacaguuc cacauccaua gugcaugguc ugaugagugc gguugcugac	2220
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<211> 2606

<212> RNA

<213> 人

<400> 76

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gggucccccua cagccaaggu ccugagugc cagagguggu gguguugcuu aucuucugga	180
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auaccaagca ggccuuuggc aucaugaacg agcugcggu cagccagcag cugugugacg	420
ucacacugca ggucaaguac caggauccac cggccgccca guucauggcc cacaaggugg	480
ugcuggccuc auccagcccu gucuucaagg ccauguucac caacgggcug cgggagcagg	540
gcauggaggu gguguccauu gaggguaucc accccaaggu cauggagcgc cucauugaau	600

[0172]

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[0173]

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ccagggaugg gccuguacau agaagccacc ggauggcacu uccccaccgg auggacaguu	2520
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ucuuucaccc ugaaaaaaaa aaaaaa	2606

<210> 77

<211> 2577

<212> RNA

<213> 人

<400> 77

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ccagaggugg ugguguugcu uaucuucugg aacccaugc agccagauc caggccuagc	180
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aaccgcaccu ucagcuacac ccuggaggau cauaccaagc aggccuuugg caucaugaac	360
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[0174]

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cacccaagg ucauggagcg ccucauugaa uucgccuaca cggccuccau cuccaugggc	600
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[0175]

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cuuggaggcu augaugguca cacguuccug gacagugugg aguguuacga cccagauaca	1920
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<211> 3493

<212> RNA

<213> 人

<400> 78

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guccaaccag gcuaagggcc uggggauacc ccugccugg ccccuugcc caaacuggea	180
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[0176]

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[0177]

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[0178]

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<211> 3195

<212> RNA

<213> 人

<400> 79

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[0179]

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[0180]

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[0181]

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[0182]

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[0183]

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[0184]

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[0185]

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[0186]

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uggaggggaa acgcaaaacc ugcaguuccu gaguaccuuc uacaggcccg gccagccua	3000
ggccgggggu ggccacacca cagcaagccg gccccccuc uuuuggccuu guggauaagg	3060
gagaguugac cguuuucauc cuggccuccu uuugcuguuu ggauguuucc acgggucuca	3120
cuuauacca agggaaaacu cuucauuuuu guccguuuu cuucuaaaaa aaaaaaaaaa	3180
aaaaaaaaaa aa	3192

<210> 82

<211> 1208

<212> RNA

<213> 人

<400> 82

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ugggaguuca gcuuucugca gagggccacu aggaaccucg gauugcccac ggaagccagc	120
cacuuuuuuu gacaguccag cccaccuccu cuucugcccg gagaagcucc aggggcugcc	180
uuugugauca cagcaucuuc acaaggacca aaggaaaaua agauuuuug uaagaacacc	240
gugaccacau cuuuuuuug acccauuucg uggcucccac aagauuuaca ccuccacacu	300
gaggccggaa gugguuuugc ccuuuuuuu cauggcgaaa agcuuuuug ucuccaagga	360

[0187]

aacgccacgu aaugagucaa agcuguggcg cacgcgcaga aguacaagcu accggaagug	420
auggcgcccc uacuaaagcc uugggguuag uacgcgugcg cagcaguuuc uuccgacagu	480
uguguugugc caauggugga gaagaaaacu ucgguucgcu cccaggaccc cgggcagcgg	540
cgggugcugg accgggcugc cgggcagcgu cgcaucaacc ggcagcugga ggcccuggag	600
aaugacaacu uccaggauga ccccccacgcg ggacucccuc agcucggcaa gagacugccu	660
caguuugaug acgaugcgga cacuggaaag aaaaagaaga aaacccgagg ugaucuuuuu	720
aaacuucgcu uccgaaaaaa cuuucaggcc cuguuggagg agcagaacuu gaguguggcc	780
gagggcccuu acuaccugac ggccugugcg ggacccccau cgcggcccca gcgccccuuc	840
ugugcugucu guggcuuccc aucccccuaac accuguguca gcugcggugc ccgguacugc	900
acugugcgcu gucuggggac ccaccaggag accagguguc ugaaguggac ugugugagcc	960
ugggcuuucc cagagaggaa gggccgcugu gcacugcccc gccuucagaa agacagaaau	1020
ucaucaccca augcaggggg agcucuuccu ggaccaaggg aggagccgcu cauucaccca	1080
acaaaacugu gucuuauug ccaggaaaga ccagccucac uccugggaac ugucuggcag	1140
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aaaaaaaa	1208

<210> 83

<211> 1017

<212> RNA

<213> 人

<400> 83

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ccguccggag ccagccaacg agcggaaaau ggcagacaau uuuucgcucc augaugcguu	120
aucuggguu ggaaccccaa acccucaagg auggccuggc gcauggggga accagccugc	180
uggggcaggg ggcuaaccag gggcuuccua uccuggggcc uaccccgggc aggcaccccc	240

[0188]

aggggcuuau ccuggacagg caccuccagg cgccuaccu ggagcaccug gagcuuaucc	300
cggagcaccu gcaccuggag ucuaccagg gccaccagg ggccuuggg ccuaccuac	360
uucuggacag ccaagugcca ccggagccua ccugccacu ggcccuauug gcgccucug	420
ugggccacug auugugccu auaaccugcc uuugccuggg ggaguggugc cucgcaugcu	480
gauaacaauu cugggcacgg ugaagccaa ugcaaacaga auugcuuag auuuccaaag	540
agggaugau guugccuucc acuuuaacc acguucaau gagaacaaca ggagagucan	600
uguuugcaau acaaagcugg auauuaacug gggaaggga gaaagacagu cgguuuucc	660
auuugaaagu gggaaaccu ucaaaauaca aguacuggu gaaccugacc acuucaaggu	720
ugcagugaau gaugcucacu uguugcagua caucaucgg guuaaaaaac ucaugaaau	780
cagcaaacug ggaauuucug gugacauaga ccucaccagu gcuucauaua ccaugauaua	840
aucugaaagg ggcagauuaa aaaaaaaaaa agaaucaaa ccuacauau guaaagguu	900
cauguucacu gugagugaaa auuuuuacau ucaucaauu cccucuugua agucaucua	960
uuauuaaua uuacagugaa uuaccugucu cauauguca aaaaaaaaaa aaaaaa	1017

<210> 84

<211> 1973

<212> RNA

<213> 人

<400> 84

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guuagugggg acagaggga gauggguaag auucagagca caggcuagug ugacggaagu	180
uuuacuugu gaguuaua ggguuugga aucuagcugg auagcaucc ugcccuuga	240
agagauguuu uugugcgcc acacuacuga cuuaggcaua augccuagag auggaauaga	300

[0189]

acugcacaau gaacuagugg ugagguucag uuaauggaa auuggugaaa gcuuuuagga	360
uaaaaugaua aucuuuguuu cuuucaggaa aauggcagac aauiuuucgg uaaguguuuu	420
augccuguuu cuuucccuug aucagcucca caugguugag gguugggggu uuuguuuua	480
ccaugacuuu cccuuuucac ucuccacug cguggcuucc ccuggacuca uuuguccaau	540
gagggcuugc aagcuggagc cuuguuuuuc cagcagcaga uuugggaaga aagccaggca	600
gagcgaggcc ugaggacucac ucacaguaac ccuucacca aaagggccag ggcggaaggg	660
aguggacucu gccggcagga gcugagaaau ccucugagua gcgggaagug cgguacaguc	720
ugggcuuucu gauguuugug auuguuuuuc ucacggugau gaaaaaguau gugcuauaag	780
uagaggagcg cuaacuccug acuugagcua auuaugaaaa ugcagcccuc ccugaucuga	840
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cuuagaaacu caaacuauua auuaguggaa aaaguuuau gauaugcaug uaaugccuuu	960
gccauauucc ucuccuucuu agaucacaua uuccuauuuu ccugaaaauu cugcuuuuga	1020
gaaugcuuuc uguccguuaa uuguguaugu cuuucuuucc agcuccauga ugcguuaucu	1080
gggucuggaa acccaaacc ucaaggauug ccuggcgcua gggggaacca gccugcuggg	1140
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ccacugauug ugccuuaua ccugccuuug ccugggggag uggugccucg caugcugaua	1440
acaaucugg gcacggugaa gcccuaugca aacagaauug cuuagauuu ccaaagaggg	1500
aaugauguug ccuuccacuu uaaccacgc uucaaugaga acaacaggag agucauuguu	1560
ugcaauacaa agcuggauaa uaacugggga agggaagaaa gacagucggu uuuccauuu	1620

[0190]

gaaaguggga aaccuucaa aaacaagua cugguugaac cugaccacuu caagguugca	1680
gugaaugaug cucacuuguu gcaguacaau caucggguua aaaaacucua ugaaaucage	1740
aaacugggga uuucugguga cauagaccuc accagugcuu cauuuaccuu gauauaaucu	1800
gaaaggggca gauuaaaaaa aaaaaaagaa ucuaaaccuu acauguguaa agguuucaug	1860
uucacuguga gugaaaauuu uuacauucau cauuaucccu cuuguaaguc aucuacuuua	1920
uaauuuuac agugaauuac cugucucaau augucaaaaa aaaaaaaaaa aaa	1973
<210> 85	
<211> 744	
<212> RNA	
<213> 人	
<400> 85	
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aucuggguu ggaaacccaa acccucaagg auggccuggc gcauggggga accagccugc	180
uggggcaggg ggcuaaccag gggcuuccua uccuggggcc uaccccgggc aggcaccccc	240
aggggcuuuu ccuggacagg caccuccagg cgcuaaccu ggagcaccug gagcuuauc	300
cggagcaccu gcaccuggag ucuaccagg gccaccagc ggcccugggg ccuaccuac	360
uucuggacag ccaagugcca cggagccua ccugccacu ggcccuuag gcgccccugc	420
ugggccacug auugugccuu auaaccugcc uuugccuggg ggaguggugc cucgcaugcu	480
gauaacaauu cugggcacgg ugaagccaa ugcaaacaga auugcuuag auuuccaaag	540
agggaau gauugccuucc acuuuaacc acguucaau gagaacaaca ggagagucuu	600
uguuugcacu uacaugugua aagguuucau guucacugug agugaaaauu uuacauuca	660
ucaauauccc ucuuguaagu caucuacuuu auaauuuu cagugaauua ccugucuaa	720
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[0191]

<210>	86	
<211>	3503	
<212>	RNA	
<213>	人	
<400>	86	
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gcccuuccca	ggcccccgag gccguucgcc guucccgaaag cccgacuggg ggaagagucc	180
agcaccaaag	cggccguucu cggauuccgg agcguucugg agccccgaga gacgccccgg	240
gguucuagaa	gcuccccggc ggcgccaguu cccggcuuca uucgggcguc ccuccgaaac	300
ccacucgggu	gcacgggucg ucggcgagcc gcgaccgggu ccuggcgcgc accaugaucg	360
uggcggacuc	cgagugccgc gcagagcuca aggacuaccu gcgguucgcc ccgggcggcg	420
ucggcgacuc	gggccccgga gaggagcaga gggagagccg ggcucggcga ggcccucgag	480
ggcccagcgc	cuucauuccc guggaggagg uccuucggga gggggcugag agccucgagc	540
agcaccuggg	gcuggaggca cugauguccu cugggcgagu agacaaccug gcagugguga	600
ugggccugca	cccugacuac uuuaccagcu ucuggcgccu gcacuaccug cugcugcaca	660
cggauggucc	cuuggccagc uccuggcgcc acuacauugc caucauggcu gccgcccgcc	720
aucaguguuc	uuaccuggua ggcuccaca ugcccgaguu ucugcagacu gguggugacc	780
cugaguggcu	gcugggccuc caccgggccc ccgagaagcu gcgcaaacuc agcgagauga	840
acaaguugcu	ggcgcaucgg ccauggcuca ucaccaagga acacauccag gccuugcuga	900
agaccggcga	gcacacuugg ucccuggccg agcucauua ggcucugguc cugcucacce	960
acugccacuc	gcucuccucc uucguguuug gcuguggcau ccucccugag ggggaugcag	1020
auggcagccc	ugccccccag gcaccuacac ccccuaguga acagagcagc cccccaagca	1080

[0192]

gggacccguu gaacaacucu gggggcuuug agucugcccg cgacguggag gcgcugaugg	1140
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uggagagccg cuuugagcug gagaagucag agagccugcu ggugaccccc ucagcugaca	1260
uccuggagcc cucuccacac ccagacaugc ugugcuuugu ggaagaccu acuuucggau	1320
augaggacuu cacucggaga ggggcucagg cacccecuac cuuccgggcc caggauuua	1380
ccugggaaga ccauggcuauc ucgcugauc agcgccuuua ccugagggu gggcagcugc	1440
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guguggacac cuccgugcuc cgcagggcca ucuggaacua uauccacugc gucuuugca	1560
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gcaugcaagc cgcucugcug uacgccucc gugccaucac ccgcuaug accugacucc	1800
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cagaccuuu uguguccau gccaccuc cccacgcugc agugggcuug ugugugaug	1920
gcaguccga agccacacc uccuuuuucc ucacuggaau ggacaguuca uugcacugac	1980
ucugggauc cagcccugcu ccugggagcu ggaagagcac uuggagauc uaaggacca	2040
caccuuccu ccuucccug cccacagagg cagagggcac aggaagaag ccgggccaag	2100
cucggaaua augugccaca aguguugugg ccuuccugaa cugggaaguc ccuggcuggc	2160
ccccggggga gaggggcaa ugcuccggg acugacacuc caggcagcu ugccuucucu	2220
ccccuguc auccagauu cauaccucc uacuugccau ucacccauca augugaaagu	2280
caggguaca gcuggucugu guguccagu ccuaaaagc cuguucugu gggcagccug	2340
aggcuguugc ccgaauccu guucaguuu uugacuuccu uugccuuuu uccuuuuu	2400

[0193]

ccaugcuuaa uggugugagg cgucaggaga gaggccaagu acauaaaaa aaaaaaagca	2460
gauuauucucu agagaguuuug agccuuugcu ggucacauug ccuucugaag aggagggagu	2520
auuagauuau aaauccucuu uauuuugguc cuuuauvcuu gagguucaa ccuggagcca	2580
caguguguga gaggaggagg agaggagaa uucuguucuc ccagagcugc accugccucg	2640
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cagguuucuu guuuggaccc ugccccuggg ccauggccag gugaccaugg cuacauugcc	2820
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auaccgugc aggcgcggag aagcaaccgg caccuccuuc cggccugaaa gccucccug	3300
caagaaggug ugcaggagag aagaggcccc ggcaugggga ucuggguucu agagggaug	3360
ugaugacugu aaauuuacac ugggugggua gggaguggua uccaguguuc aagugcagaa	3420
aucuuuggcu uugcuaccag uuccauuga ugagaaauaa acguucgcug agguuuuguu	3480
ucauaaaaaa aaaaaaaaaa aaa	3503

<210> 87

<211> 2217

<212> RNA

<213> 人

[0194]

<400> 87	
ccccgccgcc gccgcccucc gcgccucggg ccaucuccu cccaccucc uccgcggagc	60
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cggcucugag ccgccgcgg ggccggccuc ggccgggagc ggaggaagga gucgccgagg	180
agcagccuga ggccccagag ucugagacga gccgccgccg ccccgccac ugcggggagg	240
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agacuuuucc guugccgcug ggagccggag gcgcggggac cucuuggcgc gacgcugccc	360
cgcgaggagg caggacuugg ggaccccaga ccgccuccu uugccgccgg ggacgcuuugc	420
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aggagacgga ucucucuccg accugccaca gaucccuau ucaagaccac ccaccuucg	660
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gaggucaccc gcgugcuauu gguggaaacc cacaacgaaa ucuauacaa guucaagcag	1260
aguacacaca gcuaauauau guucuucaac acaucagagc uccgagaagc gguaccugaa	1320

[0195]

cccguuuugc ucucccgggc agagcugcgu cugcugaggc ucaaguuaaa aguggagcag	1380
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cuggcaccca gcgacucgcc agagugguua ucuuuugaug ucaccggagu ugugcggcag	1500
ugguugagcc guggagggga aaauagaggc uuucgccuua gcgcccacug cuccugugac	1560
agcagggaua acacacugca aguggacauc aacggguuca cuaccggccg ccgaggugac	1620
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guguacuacg ugggcccga gccaaggug ggcagcugu ccaacaugau cgugcgcuuc	2040
ugcaagugca gcugaggucc cgccccgcc cgccccgcc cggcaggccc ggccccaccc	2100
cgccccgcc ccgugccuu gccaugggg gcuguauuuu aggacaccg ugccccagc	2160
ccaccugggg ccccauuuaa gauggagaga ggacugcgga aaaaaaaaaa aaaaaa	2217

<210> 88

<211> 2503

<212> RNA

<213> 人

<400> 88

cgcucuuaau ggccagggga cgguagcugc aggacucugc ucuccugcg ccaugggcca	60
ggguugggcu acugcaggac uucccagccu ccucuuccug cugcucugcu acgggcaccc	120
ucugcugguc cccagccagg aggcaucca acaggugaca guacccaug ggacaagcag	180

[0196]

ccaggcaaca accagcagcc agacaaccac ccaccaggcg acggcccacc agacaucage	240
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caucaccaca gagaccagca agauucugcu gcagaagaac augcaaauag ccaaccacac	420
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caagcggauc auaaagaagg uucaggaccu agaacgggca gcacugccug cccaggagcu	540
ggaggaguac aacaagaucc uguuggauau ggaaaccacc uacagcgugg ccacugugug	600
ccaccgaau ggcagcugcc ugcagcucga gccagaucug acgaauguga ugccacguc	660
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[0197]

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cuaccagucc aaggaggccg ggcagcgcgc ggcgaccgc augaagcugg gcuucaguag	1860
gccguggccg gaagccaugc agcugaucac gggccagccc acaugagcg ccucggccau	1920
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ccccagccca ccugcuccu ccugcccgcu ccugucccc cccccuccc aguccuccag	2400
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<210> 89

<211> 4195

<212> RNA

<213> 人

<400> 89

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gcugccgcug ccgcugcugu ugcugcugcc gccgcagccc gccuggcgcg uggaccccg 120

[0198]

gcugcagccc ggcaacuuuu cugcugacga ggccggggcg cageucuucg cgcagagcua	180
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caacaucacc gcggagaauug caaggcgcca ggaggaagca gcccugcuca gccaggaguu	300
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ggacccgcag cugcgcagga ucaucggagc ugugcgcacc cugggcucug ccaaccugcc	420
ccuggcuaag cggcagcagu acaacgccc ucuauagcaac augagcagga ucuacuccac	480
cgccaaagguc ugccucccca acaagacugc caccugcugg ucccuggacc cagaucucac	540
caacauccug gcuuccucgc gaagcuacgc caugcuccug uuugccuggg agggcuggca	600
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agccuacaag caggacggcu ucacagacac gggggccuac uggcgcuccu gguacaacuc	720
ccccaccuuc gaggacgauc uggaacaccu cuaccaacag cuagagcccc ucuaccugaa	780
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[0199]

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ccuggaugcc cagccgcugc ucaaguacuu ccagccaguc acccaguggc ugcaggagca	1860
gaaccagcag aacggcgagg uccugggcug gcccgaguac caguggcacc cgcguugcc	1920
ugacaacuac ccggagggca uagaccuggu gacugaugag gcugaggcca gcaaguugu	1980
ggaggaauau gaccggacau cccagguggu guggaacgag uaugccgagg ccaacuggaa	2040
cuacaacacc acaucacca cagagaccag caagauucug cugcagaaga acaugcaau	2100
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ugccuacgug cgccgggccc ugcaaccguca cuacggggcc cagcacauca accuggaggg	2640
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[0200]

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cgggccccag uucggcuccg agguggagcu gagacacucc ugaggugacc cggcuggguc	3960
ggccugccc aaggccucc caccagagac ugggauggga acacuggugg gcagcugagg	4020
acacaccca caccagcc caccucguc cuccugccu guccugucc ccuuccuc	4080

[0201]

ccaguccucc agaccaccag ccgccccagc ccuucuccc agcacacggc ugccugacac	4140
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<211> 760	
<212> RNA	
<213> 人	
<400> 90	
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cuucauuccc caagggcucg cucagccaga ugcaaucaau gccccaguca ccugcuguua	180
uaacuucacc aauaggaaga ucucagugca gaggcucgcg agcuauagaa gaauccaccg	240
cagcaagugu cccaaagaag cugugaucuu caagaccuu guggccaagg agaucugugc	300
ugacccaag cagaaguggg uucaggauuc cauggaccac cuggacaagc aaacccaac	360
uccgaagacu ugaacacuca cuccacaacc caagaaucug cagcuaacuu auuuucccu	420
agcuuucccc agacaccug uuuuauuuua uuauauggaa uuuuguuugu ugaugugaaa	480
cauuaugccu uaaguaaugu uaaucuuau uuaaguuaau gauguuuuaa guuuaucuuu	540
caugguacua guguuuuua gauacagaga cuuggggaaa uugcuuuucc ucuugaacca	600
caguucuaacc ccugggaugu uuugaggguc uuugcaagaa ucauuauac aaagaauuuu	660
uuuuacauu ccaaugcauu gcuaaaauau uauuguggaa augaauuuu uguaacuauu	720
acaccaaua aauauuuuu uguacaaaaa aaaaaaaaa	760
<210> 91	
<211> 1916	
<212> RNA	
<213> 人	

[0202]

<400> 91	
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cccauugacu aacuauggaa gauuauacca aaauagagaa aauggagaa gguaccuau	180
gaguugugua uaaggguaa cacaaaacua caggucaagu gguagccaug aaaaaauca	240
gacuagaaag ugaagaggaa gggguuccua guacugcaau ucgggaaauu ucucuauuaa	300
aggaacuucg ucauccaaau auagucaguc uucaggauu gcuuauagcag gauuccaggu	360
uauaucucau cuuugaguuu cuuuccaugg aucugaagaa auacuuggau ucuauccuc	420
cuggucagua cauggauuc uacuuugua agaguuaauu auaccaaauc cuacagggga	480
uuguuuuug ucacucuaga agaguucuc acagagacuu aaaaccucaa aaucucuuga	540
uugaugacaa aggaacaauu aaacuggcug auuuuggccu ugccagagcu uuuggaauc	600
cuacagagu auauacacau gagguaguaa cacucuggua cagaucucca gaaguauugc	660
uggggucagc ucguuacua acuccaguug acuuuuggag uauagcacc auuuugcug	720
aacuagcaac uaagaaacca cuuuuccaug gggauucaga aauggaucaa cucuucagga	780
uuuucagagc uuugggcacu cccaauaau aguguggcc agaaguggaa uuuuacagg	840
acuauaagaa uacauuuccc aaauaggaa caggaagccu agcauccau gucaaaaacu	900
uggaugaaaa uggeuuggau uugcucucga aaauuuauu cuaugauca gccaaacgaa	960
uuucuggcaa aauggcacug aaucuccau auuuuauga uuuggacaau cagauuaaga	1020
agauguagcu uucugacaaa aaguuuccau auguuauuc aacagauagu uguguuuuu	1080
uuguuaacuc uugucuauuu uugucuaua uauuuuucu uguuaucaaa cuucagcugu	1140
acuucgucuu cuaauuucaa aaauuaacu uaaaaauga aaauuucuau augaauuuua	1200
auauaaucu guaaaugugu guaggucua cuguacaac uauuuguuac uauaauaaaa	1260
cuauaauuu gaugucagga aucaggaaaa aaauugagu ggcuaaauc aucucagucc	1320

[0203]

uuauaggcagu uuuaauuuucc uguaguugga acuacuaaaa uuuaaggaaaa ugcuaaguuc	1380
aaguuuucgua augcuuugaa guauuuuuau gcucugaaug uuuaaauguu cucaucaguu	1440
ucuugccaug uuguuaacua uacaaccugg cuaaagauga auauuuuucu acugguauuu	1500
uaauuuuuga ccuaaauguu uaagcauucg gaaugagaaa acuauacaga uuugagaaau	1560
gaugcuaaaau uuauaggagu uuucaguaac uuaaaaagcu acaugagag caugccaaaa	1620
uuugcuaagu cuuacaaaga ucaagggcug uccgcaacag ggaagaacag uuugaaaaau	1680
uuauagaacua ucuuauuuuu agguagguuu ugaaagcuuu uugucuaagu gaauucuuau	1740
gccuugguca gaguaauaac ugaaggaguu gcuuauucug gcuuucgagu cugaguuuaa	1800
aacuacacau uuugacauag uguuuauuag cagccaucua aaaaggcucu aauguauuu	1860
uaacuaaaaau uacuagcuuu gggaauuaaa cuguuuuaca aauaaaaaaa aaaaaa	1916

<210> 92

<211> 1636

<212> RNA

<213> 人

<400> 92

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gaaaaaaauc agacuagaaa gugaagagga agggguuccu aguacugcaa uucgggaaau	180
uucucuauua aaggaacuuc gucauccaaa uauagucagu cuucaggaug ugcuuangca	240
ggauuccagg uuauaucuca ucuuugaguu ucuuuccaug gaucugaaga aaacuugga	300
uucuaucucu ccuggucagu acauggauuc uucacuuguu aagguaguaa cacucuggua	360
cagaucucca gaaguauugc ugaggucagc ucguuacuca acuccaguug acuuuuggag	420
uauaggcacc auuuuugcug aacuagcaac uaagaaacca cuuuuccaug gggaauucaga	480

[0204]

aaauugaucaa cucuucagga uuuucagagc uuugggcacu cccaauaag aaguguggcc	540
agaaguggaa ucuuuacagg acuaaaagaa uacauuuccc aaauaggaaac caggaagccu	600
ageaucceau gucaaaaaacu uggaugaaaa ugguuggau uugcucucga aaauuuau	660
cuaugaucca gccaaacgaa uuucuggcaa aauggcacug aaucauceau auuuuaauga	720
uuuggacaau cagauuaaga agauguagcu uucugacaaa aaguuuccau auguuauauc	780
aacagauagu uguguuuuuu uuguuaacuc uugucuauuu uugucuuaa uauauuucuu	840
uguuaucaaa cuucagcugu acuuugcugu cuauuuucaa aaauauaacu uaaaaaugua	900
aaauuucua uauuuuuu auauaaauuc guaaaugugu guaggucua cuguacaac	960
uuuuuguuac uauauaaaa cuauauuuu gaugucagga aucaggaaaa aaauugagu	1020
ggcuuaaau aucucagucc uuauggcagu uuuaauuucc uguaguugga acuaacuaa	1080
uuuaggaaaa ugcuaaguuc aaguuuucua augcuuugaa guauuuuuu gcucugaug	1140
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auauuuuuc acugguauuu uauuuuuuga ccuaaauguu uaagcauucg gaugagaaa	1260
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uugucuagu gaauucuuu gccuugguca gaguaauaac ugaaggagu gcuuauucug	1500
gcuuucgagu cugaguuuu aacuacacau uuugacauag uguuuuuuag cagccauca	1560
aaaaggcucu aauguauuu uaacuaaaau uacuagcuuu gggaauuuu cuguuuuaca	1620
aaauaaaaaa aaaaaa	1636

<210> 93

<211> 1943

<212> RNA

[0205]

<213> 人

<400> 93

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gaaggccgga cuggaggauc uaccuuacc aaugacuaac uauugaagau uauaccaaaa	180
uagagaaaau uggagaaggu accuauagg uuguguauaa gguuagacac aaaacuacag	240
gucaaguggu agccaugaaa aaaaucagac uagaaaguga agaggaagg guuccuagua	300
cugcaauucg ggaaauuucu cuuuuaagg aacuucguca uccaaauuaa gucagucuuc	360
aggauugucu uaugcaggau uccagguuau aucucaucu uagauuuucu uccauggauc	420
ugaagaaaau cuuggauucu auccuccug gucaguacau ggauucuua cuuguuaaga	480
guuuuuuaa ccaaauccua caggggauug uguuuugua cucuagaaga guucuucaca	540
gagacuuuaa accucaaauu cuuuugaug augacaaagg aacaauuaa cuggcugauu	600
uuggccuugc cagagcuuuu ggaauaccua ucagaguua uacacaugag gcaauaacac	660
ucugguacag aucuccagaa guauugcugg ggucagcucg uuacucaacu ccaguugaca	720
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gaagccuagc auccauguc aaaaacuugg augaaaugg cuuggauuug cucucgaaaa	960
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uuauugauuu ggacaaucag auuaagaaga uguagcuuuc ugacaaaaag uuuccauug	1080
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auuucuuugu uaucaaacuu cagcuguacu ucgucuuua auuucaaaa uauaacuuua	1200
aaanguaaa auucuaauug aauuuuaaua uauucugua aaugugugua ggucucacug	1260

[0206]

uaacaacuaau uuguuacuaa aaauaaacua uaauauugau gucaggaauc agggaaaaau	1320
uugaguuggc uuaaaucauc ucaguccuaa uggcaguuuu auuuuccugu aguuggaacu	1380
acuaaaaauuu agggaaaugc uaaguucaag uuucguaaug cuuugaagua uuuuuaugcu	1440
cugaauuuuu aaauuuucuc aucaguuucu ugccauguug uuaacuaauac aaccuggcua	1500
aagaugaaua uuuuuuacu gguauuuuaa uuuuugaccu aaauguuuua gcauucggaa	1560
ugagaaaacu auacagauuu gagaaaugau gcuaaaauua uaggaguuuu caguaacuua	1620
aaaagcuaac augagagcau gccaaaauuu gcuaagucuu acaaagauca agggcugucc	1680
gcaacaggga agaacaguuu ugaaaauuua ugaacuaucu uauuuuuagg uagguuuuga	1740
aagcuuuuug ucuaagugaa uucuuaugcc uuggucagag uaauaacuga aggaguugcu	1800
uaucuuugcu uucgagucug aguuuuuuuac uacacauuuu gacauagugu uuauuagcag	1860
ccaucuaaaa aggcucuaau guauauuuua cuaaaauuac uagcuuuggg aaauaaacug	1920
uuuaacaaau aaaaaaaaaa aaa	1943

<210> 94

<211> 561

<212> RNA

<213> 人

<400> 94

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caacgugccc cgcgccuccg ugccggacgg guuccucucc gagcucaccc agcagcuggc	180
gcaggccacc ggcaagcccc ccaguuacau cgcggugcac guggucccgg accagcucau	240
ggccuucggc ggcuccagcg agccgugcgc gcucugcagc cugcacagca ucggcaagau	300
cggcggcgcg cagaaccgcu ccuacagcaa gcugcugugc ggccugcugg ccgagcgccu	360

[0207]

gcgcacagc cccgacaggg ucuacaucaa cuuuuacgac augaacgcgg ccaauguggg	420
cuggaacaac uccaccuucg ccuaagagcc gcagggaccc acgcugucug cgcuggcucc	480
acccgggaac ccgcgcacg cuguguucua ggcccgcga ccccaaccuu cuggugggga	540
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<210> 95	
<211> 5114	
<212> RNA	
<213> 人	
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cuggggauu cgacguaca gggggccacg cuggacgagg aggaccuggu ggacucguc	180
uccgagggcg acgcauacc caacggccug caggugaacu uccacagccc ccgaguggc	240
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cuggcggcag gacuggugc cugcuuggca gcacugggca uccaguacca gacaagauc	360
ccucugugu gccugagcg agcuugugc ucagugacca gucccaucuu gagcucaug	420
gacccacag uggaccccg ccaugacuuc uucagcuacg ccuguggggg cuggaucaag	480
gccaaaccag uccugaugg ccacucacg ugggggaccu ucagcaaccu cugggaacac	540
aaccaagcaa ucaucaagca ccucccgaa aacuccacgg ccagcgugag cgaggcagag	600
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gccaaaggca acuccagga caccugcag guggucaccg cccacuaccg caccuaccc	780
uuuuucucug ucuauugcag ugcgguucc aagaacucca acagcaacgu gaucaggug	840
gaccagucg gccugggcuu gccucgaga gacuauuacc ugaacaaaac ugaaaacgag	900

[0208]

aaggugcuga ceggauaucu gaacuacaug guccagcugg ggaagcugcu gggcggcggg	960
gacgaggagg ccauccggcc ccagauccag cagaucugg acuuugagac ggcacuggcc	1020
aacauacca ucccacagga gaagcgccgu gaugaggagc ucaucuacca caaagugacg	1080
gcagccgagc ugcagaccuu ggcacccgcc aucaacuggu ugccuuuucu caacaccauc	1140
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uuaaacuuug guggeauagg ugucgucgug ggccaugagc ugacuauugc uuuugaugau	1920
caaggacggg aguauagaaa ggacgggaac cuccggccau gguggaagaa cucauccgug	1980
gaggccuua agcgucagac cgagugcaug guagagcagu acagcaacua cagcgugaac	2040
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aaggcggccu aucgggcuua ccagaacugg gugaagaaga acggggcuga gcacucguc	2160
cccaccugg gccuaccaa uaaccagcuc ucuuuccugg gcuuugcaca ggucuggugc	2220

[0209]

uccguccgca caccugagag cuccacgaa ggccucauca ccgaucceca cagcccccuc	2280
cgcuuccggg ucaucggcuc ccucuccaau uccaaggagu ucucagaaca cuuccgcugc	2340
ccaccuggcuc cacccaugaa cccgccucac aagugcgaag ucugguaagg acgaagcgga	2400
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[0220]

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[0221]

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[0222]

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[0223]

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<211> 2117

<212> RNA

<213> 人

<400> 99

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[0224]

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[0225]

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uccuuuauau aaccggcuua ugaaagaggu uggauugaau uuugauguac uuauuuuuuu	1920
auagauuuuu auauucaaac aauuuauucc uuauuuuuac cauguuaaa auuuguuuug	1980
gcaggccaua uuggucuaug uauuuuuuuu auauguauuu cuaaaugaaa uugagaacau	2040
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<211> 1194

<212> RNA

<213> 人

<400> 100

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gguuugugac cuccaggcag gacggccauc cucuccagaa ugaagauuu cuugccagug	180
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[0226]

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<213> 人	
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[0227]

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<211> 10384

<212> RNA

<213> 人

<400> 102

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aaaaguggug ugagaaagca gugaaaaguc uggugaagaa gcuaaagaaa acaggacgau	420
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[0228]

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[0229]

aucuguuucc uauauuugaa uauuagaugg gaaaauuagu gucuagaaau acucucccau	1860
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ucccaaaggu uuauuaauaa caguaguagu uauguguaca gguaauguau caugauccag	1980
uaucaagua uugugcuguu uauauacauu uuuaguuuugc auagaugagg ugugugugug	2040
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ucugagcugu ucuuaacuga uccugguuuu gugauugaca auuuuucaug cucuguagug	3060
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[0230]

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[0231]

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<211> 6256

<212> RNA

<213> 人

<400> 105

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[0252]

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[0254]

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[0256]

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<211> 5997

<212> RNA

<213> 人

<400> 106

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[0257]

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[0258]

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[0259]

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[0260]

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[0261]

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<210> 107

<211> 5808

<212> RNA

<213> 人

<400> 107

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[0262]

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[0274]

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[0276]

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[0277]

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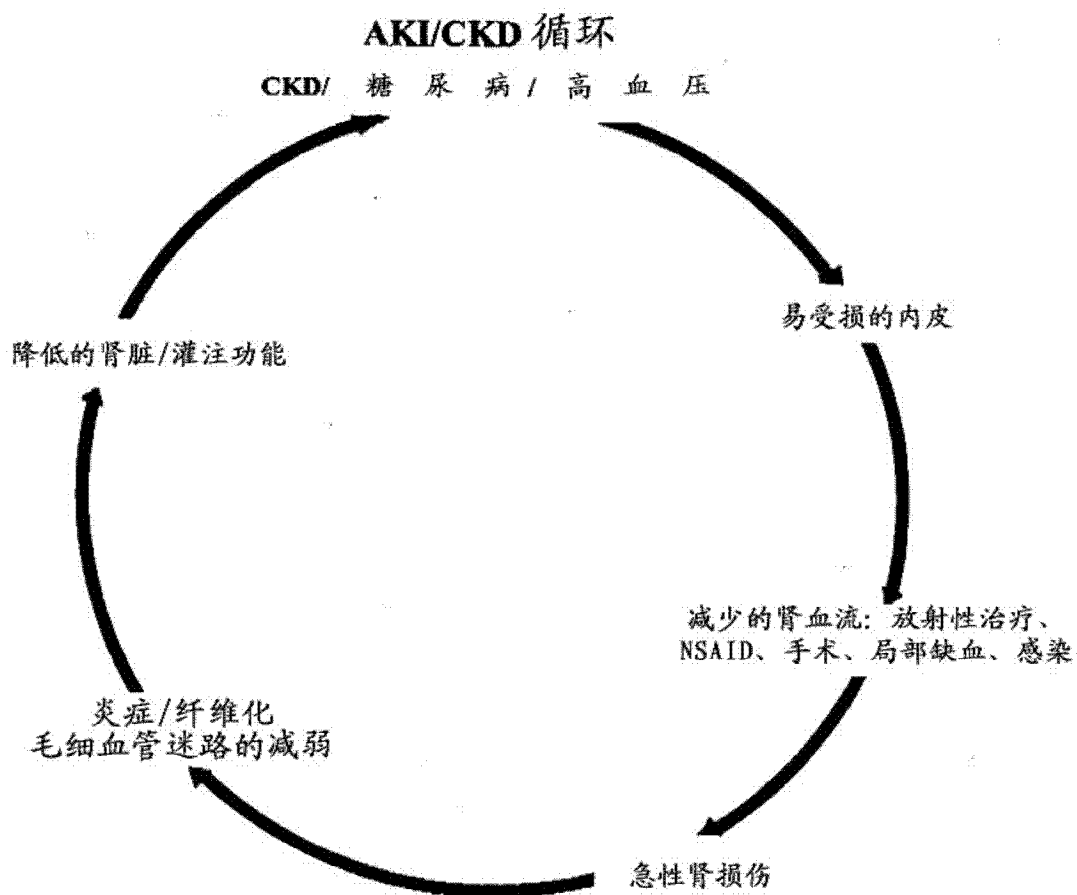


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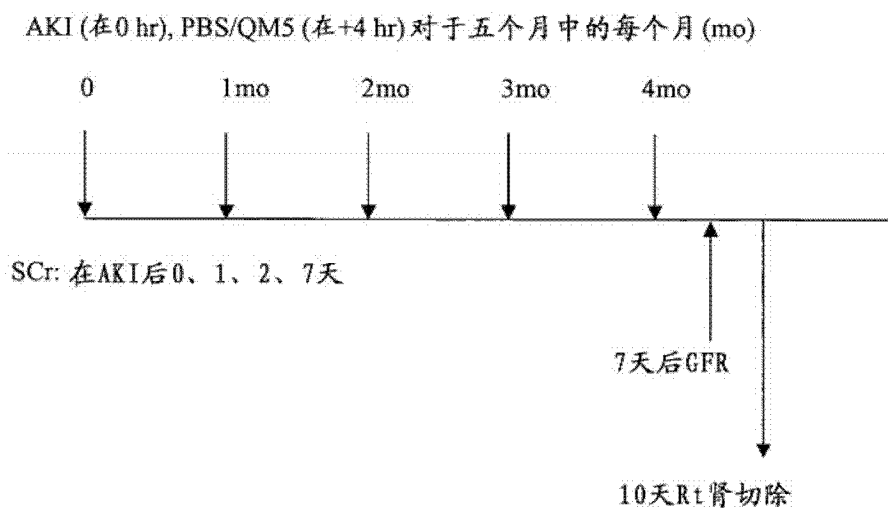


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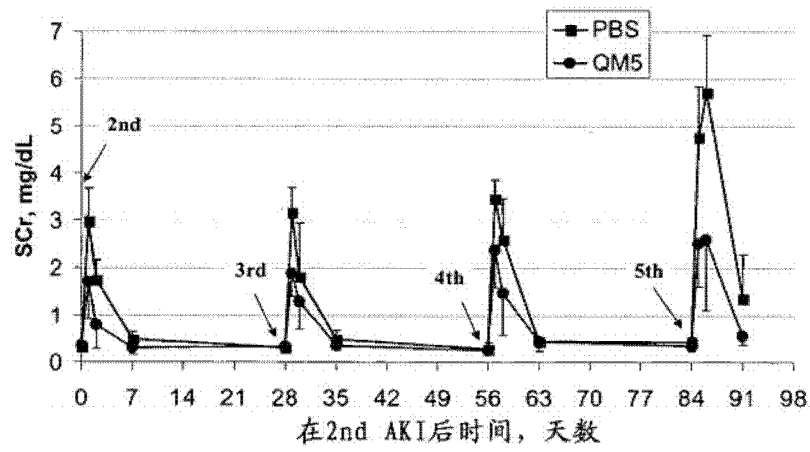


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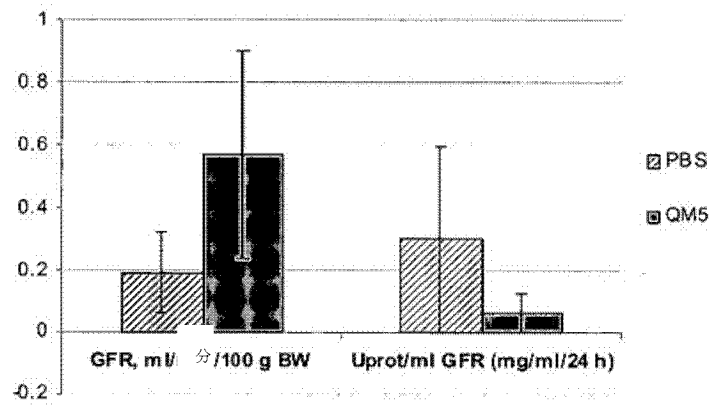


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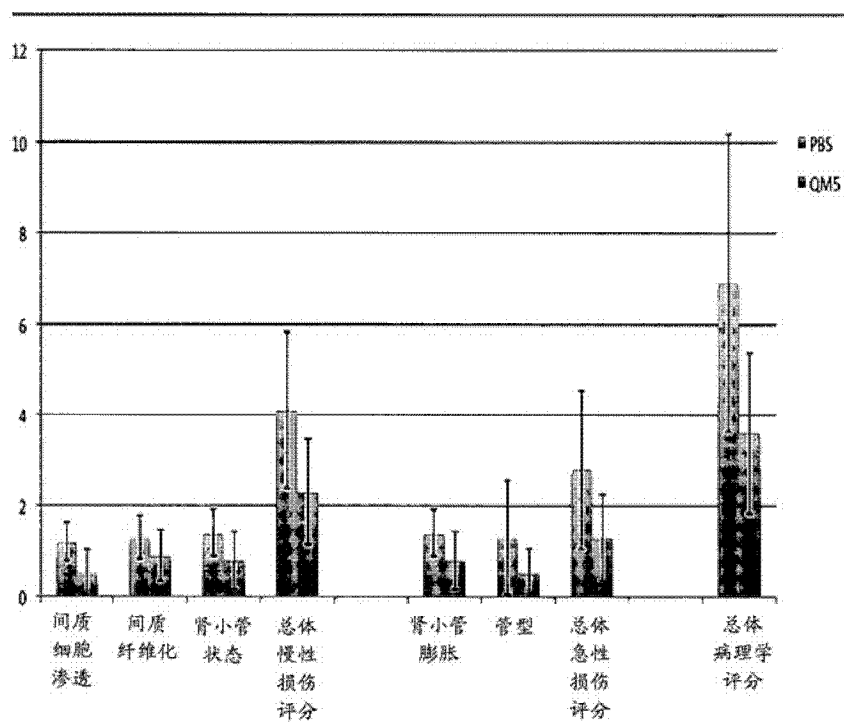


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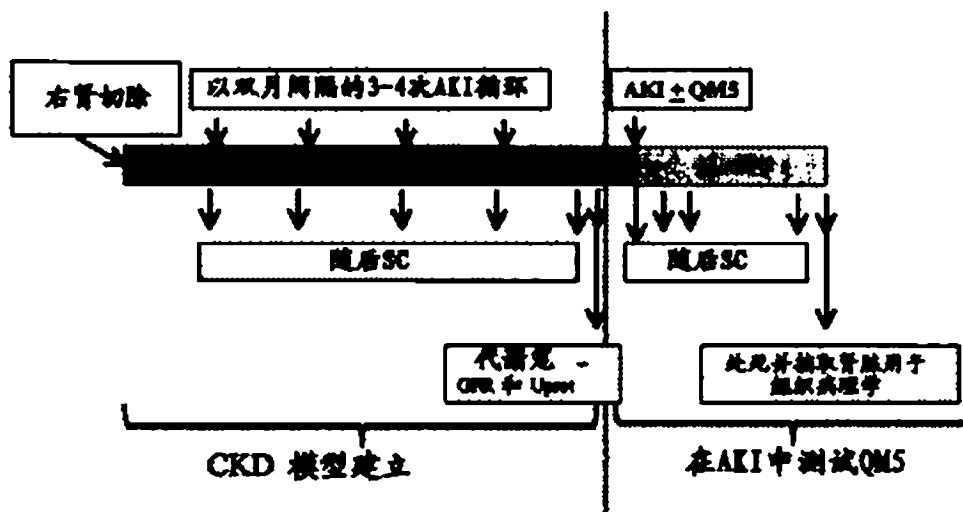


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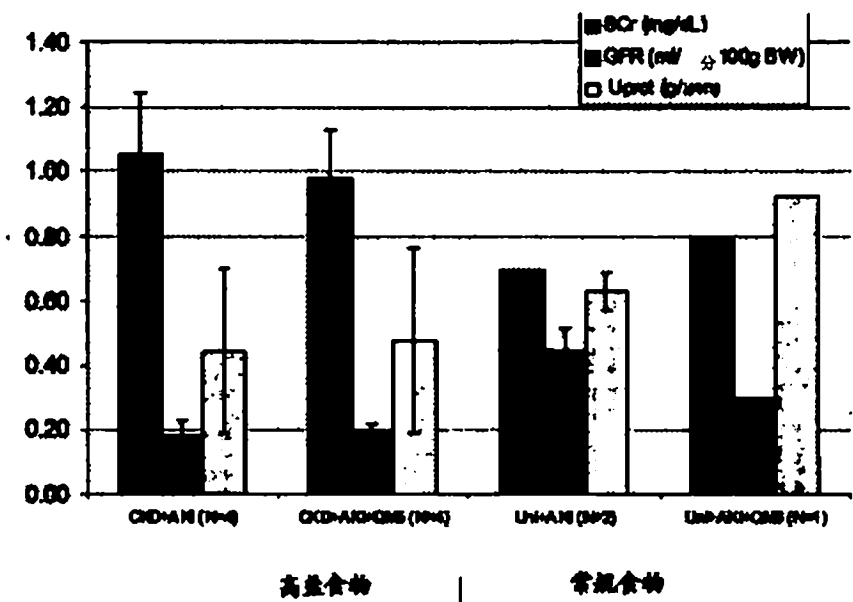


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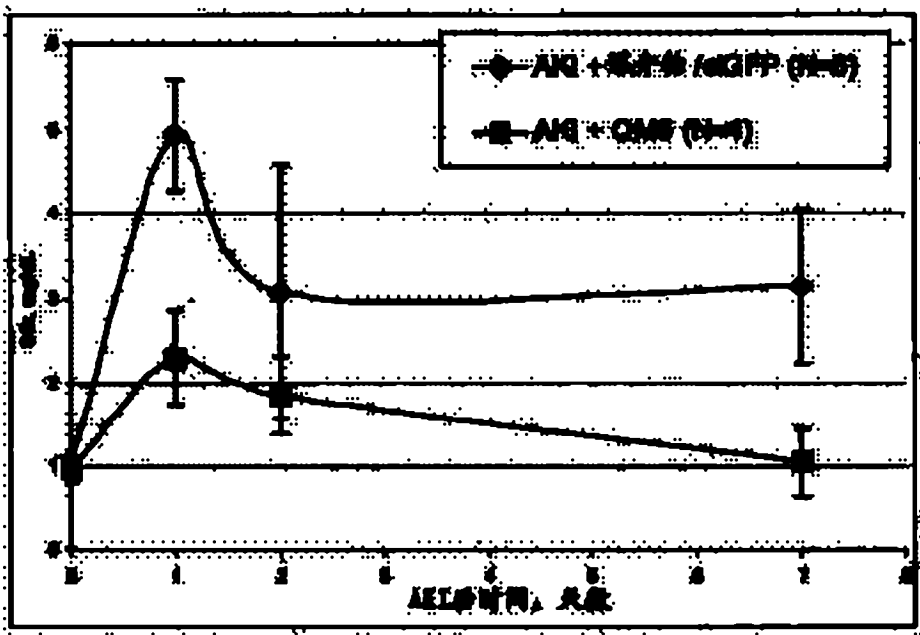


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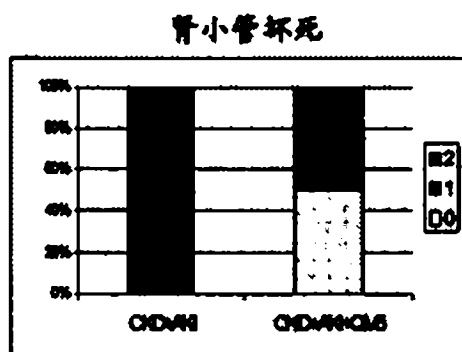


图 9A

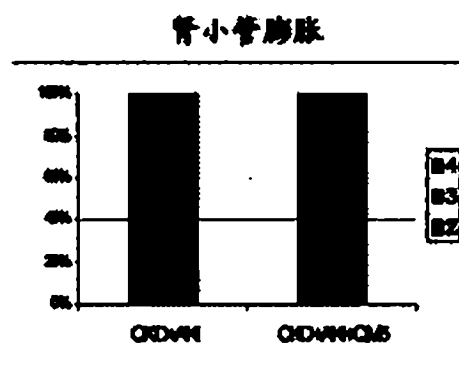


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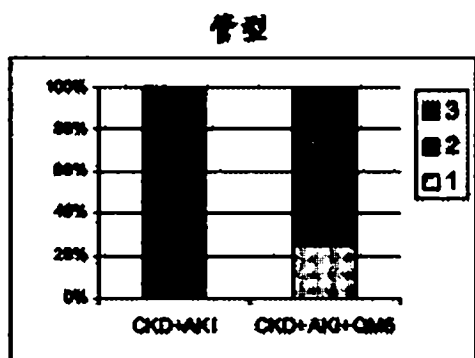


图 9C

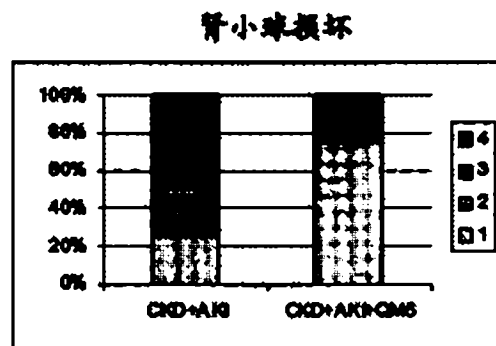


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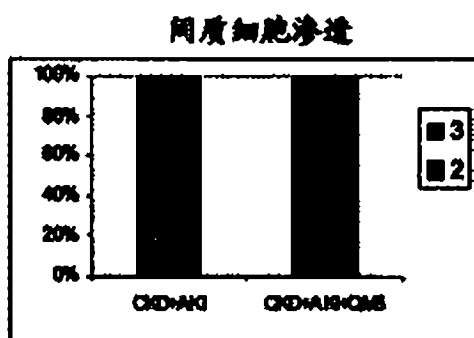


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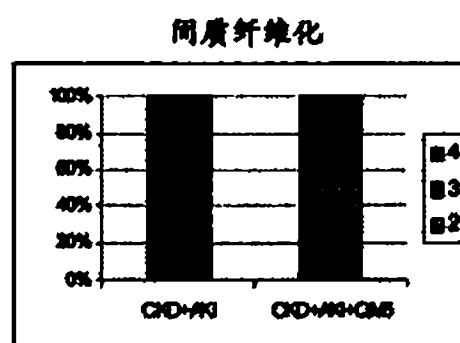


图 9F

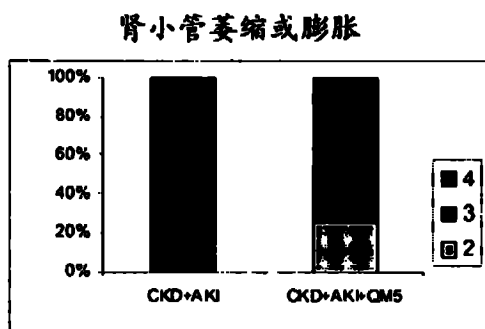


图 9G

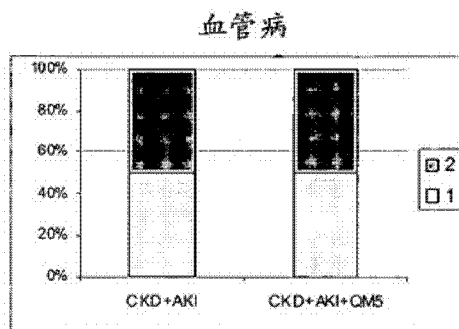


图 9H

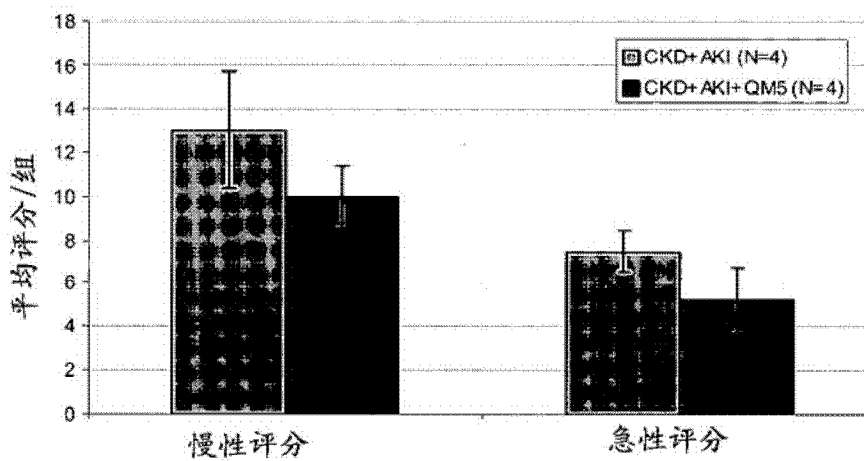


图 10