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(54) **Verfahren und Medikament zur Hemmung der Expression eines vorgegebenen Gens**

Method and medicament for inhibiting the expression of a defined gene

Méthode et médicament destinés à inhiber l'expression d'un gène donné

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(56) Entgegenhaltungen:
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WO-A-99/32619

- **UHLMANN E ET AL: "ANTISENSE OLIGONUCLEOTIDES: A NEW THERAPEUTIC PRINCIPLE" CHEMICAL REVIEWS,US,AMERICAN CHEMICAL SOCIETY. EASTON, Bd. 90, Nr. 4, 1. Juni 1990 (1990-06-01), Seiten 543-584, XP000141412 ISSN: 0009-2665**
- **MADHUR K. ET AL.: "Antisense RNA : function and fate of duplex RNA in cells of higher eukaryotes." MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Bd. 62, Dezember 1998 (1998-12), Seiten 1415-1434, XP000909741**

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71. Verwendung nach einem der Ansprüche 56 bis 70, wobei die chemische Verknüpfung mittels einer oder mehrerer Verbindungsgruppen gebildet ist, wobei die Verbindungsgruppen vorzugsweise Poly-(oxyphosphinicooxy-1,3-propandiol)- und/oder Polyethylenglycol-Ketten sind.

72. Verwendung nach einem der Ansprüche 56 bis 71, wobei die chemische Verknüpfung durch in den komplementären Bereichen II anstelle von Purinen benutzte Purinanaloga gebildet ist.

73. Verwendung nach einem der Ansprüche 56 bis 72, wobei die chemische Verknüpfung durch in den komplementären Bereichen II eingeführte Azabenzoleinheiten gebildet ist.

74. Verwendung nach einem der Ansprüche 56 bis 73, wobei die chemische Verknüpfung durch in den komplementären Bereichen II anstelle von Nukleotiden benutzte verzweigte Nukleotidanaloga gebildet ist.

75. Verwendung nach einem der Ansprüche 56 bis 74, wobei zur Herstellung der chemischen Verknüpfung mindestenes eine der folgenden Gruppen benutzt wird: Methylenblau; bifunktionelle Gruppen, vorzugsweise Bis-(2-chlorethyl)-amin; N-acetyl-N'-(p-glyoxyl-benzoyl)-cystamin; 4-Thiouracil; Psoralen.

76. Verwendung nach einem der Ansprüche 56 bis 75, wobei die chemische Verknüpfung durch an den Enden des doppelsträngigen Bereichs angebrachte Thiophosphoryl-Gruppen gebildet ist.

77. Verwendung nach einem der Ansprüche 56 bis 76, wobei mindestens eine 2'-Hydroxylgruppe der Nukleotide der dsRNA in dem komplementären Bereich II durch eine chemische Gruppe, vorzugsweise eine 2'-Amino- oder eine 2'-Methylgruppe, ersetzt ist.

78. Verwendung nach einem der Ansprüche 56 bis 77, wobei mindestens ein Nukleotid in mindestens einem Strang des komplementären Bereichs II ein "locked nucleotide" mit einem, vorzugsweise durch eine 2'-O, 4'-C-Methylenbrücke, chemisch modifizierten Zuckerring ist.

79. Verwendung nach einem der Ansprüche 56 bis 78, wobei die dsRNA an mindestens ein von einem Virus stammendes, davon abgeleitetes oder ein synthetisch hergestelltes virales Hüllprotein gebunden, damit assoziiert oder davon umgeben ist.

80. Verwendung nach einem der Ansprüche 56 bis 79, wobei die dsRNA zum primären oder prozessierten RNA-Transkript des Zielgens komplementär ist.

81. Verwendung nach einem der Ansprüche 56 bis 80, wobei die Zelle eine menschliche Zelle ist.

82. Verwendung nach einem der Ansprüche 56 bis 81, wobei mindestens zwei voneinander verschiedene dsRNAs verwendet werden, wobei ein Strang jeder dsRNA zumindest abschnittsweise komplementär zu jeweils einem von mindestens zwei verschiedenen Zielgenen ist.

83. Verwendung nach Anspruch 82, wobei eines der Zielgene das PKR-Gen ist.

84. Verwendung nach einem der Ansprüche 56 bis 83, wobei das Medikament in die Blutbahn oder das Interstitium des zu therapierenden Organismus injizierbar ist.

Claims

1. Method for inhibiting the expression of a given target gene in a mammalian cell in vitro, wherein an oligoribonucleotide having a double-stranded structure (dsRNA) comprising 15 to 49 base pairs is introduced into the mammalian cell, wherein one strand of the dsRNA has a region I with not more than 49 successive nucleotide pairs and which is at least in segments complementary to the target gene, and wherein a complementary region II within the double-stranded structure is formed by two separate RNA single strands.

2. Method according to claim 1, wherein the dsRNA has 15 to 21 base pairs.

3. Method according to claim 1, wherein the dsRNA has 21 to 49 base pairs.

4. Method according to claim 1, wherein the dsRNA has 21 base pairs.

5. Method according to one of the preceding claims, wherein the dsRNA is enclosed within micellar structures, preferably within liposomes.

6. Method according to one of the preceding claims, wherein the target gene is expressed in eukaryotic cells.

7. Method according to one of the preceding claims, wherein the target gene is selected from: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

8. Method according to one of the preceding claims, wherein the target gene is part of a virus or viroid.

9. Method according to claim 8, wherein the virus is a virus or viroid which is pathogenic for humans.

10. Method according to claim 8, wherein the virus or viroid is a virus or viroid which is pathogenic for animals.

11. Method according to one of the preceding claims, wherein the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

12. Method according to one of the preceding claims, wherein the cohesion of the complementary region II, which is caused by the nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

13. Method according to one of the preceding claims, wherein the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

14. Method according to one of the preceding claims, wherein the chemical linkage is generated at at least one, preferably both, ends of the complementary region II.

15. Method according to one of the preceding claims, wherein the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

16. Method according to one of the preceding claims, wherein the chemical linkage is formed by purine analogs used in the complementary regions II in place of purines.

17. Method according to one of the preceding claims, wherein the chemical linkage is formed by azabenzene units introduced into the complementary regions II.

18. Method according to one of the preceding claims, wherein the chemical linkage is formed by branched nucleotide analogs used in the complementary regions II in place of nucleotides.

19. Method according to one of the preceding claims, wherein at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxyl-benzoyl)cystamine; 4-thiouracil; psoralene.

20. Method according to one of the preceding claims, wherein the chemical linkage is formed by thiophosphoryl groups attached at the ends of the double-stranded region.

21. Method according to one of the preceding claims, wherein at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the complementary region II is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

22. Method according to one of the preceding claims, wherein at least one nucleotide in at least one strand of the complementary region II is a "locked nucleotide" with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

23. Method according to one of the preceding claims, wherein the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthet-

ically.

24. Method according to one of the preceding claims, wherein the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

25. Method according to one of the preceding claims, wherein the cell is a human cell.

26. Method according to one of the preceding claims, wherein at least two dsRNAs which differ from each other are introduced into the cell, wherein at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

27. Method according to one of the preceding claims, wherein one of the target genes is the PKR gene.

28. Medicament comprising at least one oligoribonucleotide having a double-stranded structure (dsRNA) comprising 15 to 49 base pairs for inhibiting the expression of a given target gene in mammalian cells, wherein one strand of the dsRNA has a region I with not more than 49 successive nucleotide pairs and which is at least in segments complementary to the target gene, and wherein a complementary region II within the double-stranded structure is formed by two separate RNA single strands.

29. Medicament according to claim 28, wherein the dsRNA has 15 to 21 base pairs.

30. Medicament according to claim 28, wherein the dsRNA has 21 to 49 base pairs.

31. Medicament according to claim 28, wherein the dsRNA has 21 base pairs.

32. Medicament according to one of claims 28 to 31, wherein the dsRNA is enclosed within micellar structures, preferably within liposomes.

33. Medicament according to one of claims 28 to 32, wherein the target gene can be expressed in eukaryotic cells.

34. Medicament according to one of claims 28 to 33, wherein the target gene is selected from: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

35. Medicament according to one of claims 28 to 34, wherein the target gene can be expressed in pathogenic organisms, preferably in plasmodia.

36. Medicament according to one of claims 28 to 35, wherein the target gene is part of a virus or viroid.

37. Medicament according to claim 36, wherein the virus is a virus or viroid which is pathogenic for humans.

38. Medicament according to claim 36, wherein the virus or viroid is a virus or viroid which is pathogenic for animals.

39. Medicament according to one of claims 28 to 38, wherein the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

40. Medicament according to one of claims 28 to 39, wherein the cohesion of the complementary region II, which is caused by the nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

41. Medicament according to one of claims 28 to 40, wherein the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

42. Medicament according to one of claims 28 to 41, wherein the chemical linkage is generated at at least one, preferably both, ends of the complementary region II.

43. Medicament according to one of claims 28 to 42, wherein the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

44. Medicament according to one of claims 28 to 43, wherein the chemical linkage is formed by purine analogs used in the complementary regions II in place of purines.

45. Medicament according to one of claims 28 to 44, wherein the chemical linkage is formed by azabenzene units inserted into the complementary regions II.

46. Medicament according to one of claims 28 to 45, wherein the chemical linkage is formed by branched nucleotide analogs used in the complementary regions II in place of nucleotides.

47. Medicament according to one of claims 28 to 46, wherein at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxyl-benzoyl)cystamine; 4-thiouracil; psoralene.

48. Medicament according to one of claims 28 to 47, wherein the chemical linkage is formed by thiophosphoryl groups provided at the ends of the double-stranded region.

49. Medicament according to one of claims 28 to 48, wherein at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the complementary region II is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

50. Medicament according to one of claims 28 to 49, wherein at least one nucleotide in at least one strand of the complementary region II is a "locked nucleotide" with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

51. Medicament according to one of claims 28 to 50, wherein the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically.

52. Medicament according to one of claims 28 to 51, wherein the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

53. Medicament according to one of claims 28 to 52, wherein the cell is a human cell.

54. Medicament according to one of claims 28 to 53, wherein at least two dsRNAs which differ from each other are contained in the medicament, wherein at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

55. Medicament according to claim 54, wherein one of the target genes is the PKR gene.

56. Use of an oligoribonucleotide having a double-stranded structure (dsRNA) comprising 15 to 49 base pairs for the preparation of a medicament, wherein one strand of the dsRNA has a region I with not more than 49 successive nucleotide pairs and which is at least in segments complementary to a given target gene in mamalian cells, and wherein a complementary region II within the double-stranded structure is formed by two separate RNA single strands.

57. Use according to claim 56, wherein the dsRNA has 15 to 21 base pairs.

58. Use according to claim 56, wherein the dsRNA has 21 to 49 base pairs.

59. Use according to claim 56, wherein the dsRNA has 21 base pairs.

60. Use according to one of claims 56 to 59, wherein the dsRNA is enclosed within micellar structures, preferably within liposomes.

61. Use according to one of claims 56 to 60, wherein the target gene can be expressed in eukaryotic cells.

62. Use according to one of claims 56 to 61, wherein the target gene is selected from: oncogene, cytokin gene, Id-protein gene, development gene, prion gene.

63. Use according to one of claims 56 to 62, wherein the target gene can be expressed in pathogenic organisms, preferably in plasmodia.

64. Use according to one of claims 56 to 63, wherein the target gene is part of a virus or viroid.

65. Use according to claim 64, wherein the virus is a virus or viroid which is pathogenic for humans.

66. Use according to claim 64, wherein the virus or viroid is a virus or viroid which is pathogenic for animals.

67. Use according to one of claims 56 to 66, wherein the ends of the dsRNA are modified in order to counteract degradation in the cell or dissociation into the single strands.

68. Use according to one of claims 56 to 67, wherein the cohesion of the complementary region II, which is caused by the nucleotide pairs, is increased by at least one, preferably two, further chemical linkage(s).

69. Use according to one of claims 56 to 68, wherein the chemical linkage is formed by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably van-der-Waals or stacking interactions, or by metal-ion coordination.

70. Use according to one of claims 56 to 69, wherein the chemical linkage is generated at at least one, preferably both, ends of the complementary region II.

71. Use according to one of claims 56 to 70, wherein the chemical linkage is formed by means of one or more compound groups, the compound groups preferably being poly(oxyphosphinicooxy-1,3-propanediol) and/or polyethylene glycol chains.

72. Use according to one of claims 56 to 71, wherein the chemical linkage is formed by purine analogs used in the complementary regions II in place of purines.

73. Use according to one of claims 56 to 72, wherein the chemical linkage is formed by azabenzene units introduced into the complementary regions II.

74. Use according to one of claims 56 to 73, wherein the chemical linkage is formed by branched nucleotide analogs used in the complementary regions II in place of nucleotides.

75. Use according to one of claims 56 to 74, wherein at least one of the following groups is used for generating the chemical linkage: methylene blue; bifunctional groups, preferably bis(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; psoralene.

76. Use according to one of claims 56 to 75, wherein the chemical linkage is formed by thiophosphoryl groups attached to the ends of the double-stranded region.

77. Use according to one of claims 56 to 76, wherein at least one 2'-hydroxyl group of the nucleotides of the dsRNA in the complementary region II is replaced by a chemical group, preferably a 2'-amino or a 2'-methyl group.

78. Use according to one of claims 56 to 77, wherein at least one nucleotide in at least one strand of the complementary region II is a "locked nucleotide" with a sugar ring which is chemically modified, preferably by a 2'-O, 4'-C-methylene bridge.

79. Use according to one of claims 56 to 78, wherein the dsRNA is bound to, associated with or surrounded by, at least one viral coat protein which originates from a virus, is derived therefrom or has been prepared synthetically.

80. Use according to one of claims 56 to 79, wherein the dsRNA is complementary to the primary or processed RNA transcript of the target gene.

81. Use according to one of claims 56 to 80, wherein the cell is a human cell.

82. Use according to one of claims 56 to 81, wherein at least two dsRNAs which differ from each other are used,

wherein at least segments of one strand of each dsRNA are complementary to in each case one of at least two different target genes.

83. Use according to claim 82, wherein one of the target genes is the PKR gene.

84. Use according to one of claims 56 to 83, wherein the medicament is injectable into the bloodstream or into the interstitium of the organism to undergo therapy.