WO 2007/071789A1 and WO2012/123419A1 describe technology allowing for targeted downregulation of proteins of interest, utilising *de novo* designed peptide-based molecules (referred to therein as 'interferors') comprising at least one β-aggregating sequence which is directed to and can interact with a corresponding β-aggregation prone region (APR) in a protein of interest. Such APRs can be determined in protein sequences using publically available algorithms and computer programs, such as TANGO (Fernandez-Escamilla et al. Nat Biotechnol. 2004, vol. 22, 1302-6, http://tango.embl.de/) or Zyggregator (Pawar et al. J Mol Biol. 2005, vol. 350, 379-92; Tartaglia and Vendruscolo, Chem Soc Rev. 2008, vol. 37, 1395-401).

It was proposed in WO 2007/071789A1 and WO2012/123419A1 that upon contact between a protein of interest comprising an APR in its amino acid sequence and an interferor molecule comprising a β-aggregating sequence corresponding to said APR, a specific β-sheet interaction and co-aggregation occurs between the interferor and the protein of interest, leading to reduced solubility of the protein of interest and its sequestration into aggregates or inclusion bodies, and consequently an effective down-regulation or knock-down of the biological function of said protein of interest.

SUMMARY

The present disclosure is at least in part based on the unexpected finding that certain missense mutations at G12 or G13 of human RAS proteins alter the profile of β-aggregation prone regions (APRs) in the human RAS proteins, which can be successfully exploited to design novel molecules targeting specifically such mutant RAS proteins.

In particular, human RAS proteins are predicted to contain 5 APR regions of at least 5 amino acids (see Table 6). The most N-terminal APR (TEYKLVVVGAG, SEQ ID NO: 1) is C-terminally delineated by G12 (underlined) in the wild-type proteins. However, certain G12 missense mutations, such as particularly G12V, G12C, G12A, or G12S enlarge this APR such that the APRs in the respective RAS mutants include not only the mutated residue at position 12 but additionally one or more subsequent residues. Further, certain G13 missense mutations, such as particularly G13V, G13C, or G13S, enlarge this APR such that the APRs in the respective RAS mutants include not only the glycine at position 12 but additionally the mutated residue at position 13 and optionally one or more subsequent residues.

Hence, this APR is predicted to span positions 2-15 and display the sequence TEYKLVVVGAVGVG (SEQ ID NO: 2) in the G12V RAS mutant; to span positions 2-14 and display the sequence TEYKLVVVGACGV (SEQ ID NO: 3) in the G12C RAS mutant; to span positions 2-14 and display the sequence TEYKLVVVGAAGV (SEQ ID NO: 4) in the G12A RAS

mutant; and to span positions 2-13 and display the sequence TEYKLVVVGASG (SEQ ID NO: 5) in the G12S RAS mutant. Additionally, at least some mutations at G12 of human RAS, such as in particular the G12V mutation, also significantly increase the predicted aggregation propensity of the corresponding APR. For example, the TANGO algorithm predicts an aggregation score of about 20% for the wild-type APR as set forth in SEQ ID NO: 1, but almost 41% for the APR in the G12V RAS mutant as set forth in SEQ ID NO: 2. Further, this APR is predicted to span positions 2-14 and display the sequence TEYKLVVVGAGCV (SEQ ID NO: 81) in the G13C RAS mutant; to span positions 2-15 and display the sequence TEYKLVVVGAGVVG (SEQ ID NO: 82) in the G13V RAS mutant; and to span positions 2-13 and display the sequence TEYKLVVVGAGS (SEQ ID NO: 83) in the G13S RAS mutant. Additionally, at least some mutations at G13 of human RAS, such as in particular the G13V mutation, also significantly increase the predicted aggregation propensity of the corresponding APR. For example, the TANGO algorithm predicts an aggregation score of about 20% for the wild-type APR as set forth in SEQ ID NO: 1, but almost 41% for the APR in the G13V RAS mutant as set forth in SEQ ID NO: 82.

Having performed extensive experiments, the present inventors now demonstrate that these differences between the N-terminal most APRs predicted in the G12 or G13 mutant RAS proteins and wild-type RAS allow to design molecules comprising β-aggregating sequence(s) which specifically target the APRs found in the G12 or G13 mutant RAS proteins, and which can downregulate the G12 or G13 mutant RAS proteins but substantially not wild-type RAS.

Accordingly, there is provided a non-naturally occurring molecule configured to form an intermolecular beta-sheet with a G12 or G13 mutant human RAS protein (or put otherwise, a human RAS protein mutated at G12 or G13, or yet differently, a human RAS protein in which the glycine at position 12 or the glycine at position 13 is mutated) and substantially not with wild-type human RAS protein. The capacity of such molecule to specifically target the G12 or G13 mutant RAS protein for the intermolecular β-sheet formation may manifest in particular as the molecule's ability to downregulate, decrease solubility of and/or induce aggregation or inclusion body formation of the G12 or G13 mutant RAS protein but substantially not of wild-type RAS, such as for example in an appropriate *in vitro*, cell culture or *in vivo* setup.

In one aspect, there is provided a non-naturally occurring molecule configured to form an intermolecular beta-sheet with a human RAS protein mutated at position 12 or 13 and substantially not with wild-type human RAS protein.

In another aspect, there is provided a nucleic acid encoding the molecule as defined herein, wherein the molecule is a polypeptide.

Further, there is provided any molecule as taught herein for use in medicine.

In another aspect, there is provided a molecule as defined herein or a nucleic acid as defined herein for use in medicine.

Further, there is provided any molecule as taught herein for use in a method of treating a disease caused by or associated with a G12 or G13 mutation in human RAS protein.

In another aspect, there is provided a molecule as defined herein or a nucleic acid as defined herein for use in treating a disease caused by or associated with a mutation at position 12 or 13 in human RAS protein.

In another aspect, there is provided use of a molecule as defined herein or a nucleic acid as defined herein in the manufacture of a medicament for the treatment of a disease caused by or associated with a mutation at position 12 or 13 in human RAS protein.

There is provided a method for treating a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any molecule as taught herein.

In another aspect, there is provided a pharmaceutical composition comprising a molecule as defined herein; or a nucleic acid as defined herein.

Further, there is provided a pharmaceutical composition comprising any molecule as taught herein.

It shall be appreciated that to the extent that the present molecules allow to target non-human mutant RAS molecules, (such as RAS molecules from other eukaryotes, particularly from yeast, fungi or animals, more particularly from animals, even more particularly warm-blooded animals, and still more particularly mammals, such as domesticated animals, farm animals, sport animals, or pets), for example owing to an acceptable degree of sequence identity between the targeted APR in human RAS and a corresponding APR in a non-human RAS, the molecules may also be used in non-human animals similarly as described herein for humans. Hence, medical interventions and pharmaceutical compositions as contemplated herein may also subsume veterinary treatments and compositions for veterinary use. By the same token, the present molecules may lend themselves for a variety of *in vitro*, *in cellulo* or *in vivo* applications (e.g., diagnostics, imaging, use in cell or non-human animal models, research tool use, etc.) not only in human cells or tissues, but also in non-human cells or tissues and in non-human animals. Hence, also provided is an *in vitro* method for downregulating the amount or biological activity of a mutant RAS in a cell (e.g., a human or non-human cell) expressing, e.g., endogenously or exogenously expressing, the mutant RAS, the method comprising contacting the cell with a RAS targeting pept-in as taught herein or with a nucleic acid molecule encoding the same (an alternative available for polypeptide peptins). Also provided is thus a method for downregulating the amount or biological activity of a mutant RAS in a non-human organism expressing, e.g., endogenously or exogenously expressing, the mutant RAS, the method comprising administering to the organism a RAS targeting pept-in as taught herein or a nucleic acid molecule encoding the same.

These and further examples of the disclosure are described in the following sections and in the appended claims. The subject-matter of the appended claims is hereby specifically incorporated in this specification.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 illustrates a screen of RAS-targeting molecules ('pept-ins') according to certain embodiments of the present invention on NCI-H441 tumor cell line cultures. (**A**) Single-dose (25µM) screen of RAS-targeting pept-ins on adherently growing (2D) NCI-H441 cells. Viability was assessed after 4 days of exposure to the test compounds and normalized to the vehicle condition (30mM Urea). (**B**) Single-dose (25µM) screen of RAS-targeting pept-ins on NCI-H441 cells growing as suspension spheroid cultures (3D). Viability was assessed after 5 days of exposure to the test compounds and normalized to the vehicle condition (30mM Urea). NT: Not tested. Error bars represent the SD.

Fig. 2 illustrates dose-response and IC_{50} determination of RAS-targeting molecules ('pept-ins') according to certain embodiments of the present invention and a negative control. Pept-ins were

CLAIMS

1. A non-naturally occurring molecule configured to form an intermolecular beta-sheet with a human RAS protein mutated at position 12 or 13 and substantially not with wild-type human RAS protein.

2. The molecule according to claim 1, wherein the mutant human RAS protein is a G12V, G12C, G12A, or G12S mutant human RAS protein, preferably a G12V mutant human RAS protein, or is a G13V, G13C, or G13S mutant human RAS protein, preferably a G13V mutant human RAS protein.

3. The molecule according to claim 1 or 2, wherein the RAS protein is KRAS, NRAS or HRAS protein, preferably KRAS protein.

4. The molecule according to any one of claims 1 to 3, wherein the intermolecular beta-sheet involves the amino acid at position 12 or 13 of the mutant human RAS protein.

5. The molecule according to claim 4, wherein the intermolecular beta-sheet involves:

a) a portion of or the whole of the amino acid sequence TEYKLVVVGAVGVG (SEQ ID NO: 2) in the G12V mutant human RAS protein; or

b) a portion of or the whole of the amino acid sequence TEYKLVVVGACGVG (SEQ ID NO: 6) or preferably TEYKLVVVGACGV (SEQ ID NO: 3) in the G12C mutant human RAS protein; or

c) a portion of or the whole of the amino acid sequence TEYKLVVVGAAGVG (SEQ ID NO: 7) or preferably TEYKLVVVGAAGV (SEQ ID NO: 4) in the G12A mutant human RAS protein; or

d) a portion of or the whole of the amino acid sequence TEYKLVVVGASGVG (SEQ ID NO: 8) or preferably TEYKLVVVGASGV (SEQ ID NO: 9) or more preferably TEYKLVVVGASG (SEQ ID NO: 5) in the G12S mutant human RAS protein; or

e) a portion of or the whole of the amino acid sequence TEYKLVVVGAGVVG (SEQ ID NO: 82) in the G13V mutant human RAS protein; or

f) a portion of or the whole of the amino acid sequence TEYKLVVVGAGCVG (SEQ ID NO: 84) or preferably TEYKLVVVGAGCV (SEQ ID NO: 81) in the G13C mutant human RAS protein; or

g) a portion of or the whole of the amino acid sequence TEYKLVVVGAGSVG (SEQ ID NO: 85) or preferably TEYKLVVVGAGSV (SEQ ID NO: 86) or more preferably TEYKLVVVGAGS (SEQ ID NO: 83) in the G13S mutant human RAS protein.

6. The molecule according to any one of claims 1 to 5, wherein the molecule is able to decrease the solubility or to induce the aggregation or inclusion body formation of the human RAS protein mutated at position 12 or 13.

7. The molecule according to any one of claims 1 to 6, wherein the molecule comprises an amino acid stretch which participates in the intermolecular beta-sheet.

8. The molecule according to claim 7, wherein the amino acid stretch comprises at least 6 contiguous amino acids of the amino acid sequence:

a) TEYKLVVVGAVGVG (SEQ ID NO: 2); or

b) TEYKLVVVGACGVG (SEQ ID NO: 6) or preferably TEYKLVVVGACGV (SEQ ID NO: 3); or

c) TEYKLVVVGAAGVG (SEQ ID NO: 7) or preferably TEYKLVVVGAAGV (SEQ ID NO: 4); or

d) TEYKLVVVGASGVG (SEQ ID NO: 8) or preferably TEYKLVVVGASGV (SEQ ID NO: 9) or more preferably TEYKLVVVGASG (SEQ ID NO: 5); or

e) TEYKLVVVGAGVVG (SEQ ID NO: 82); or

f) TEYKLVVVGAGCVG (SEQ ID NO: 84) or preferably TEYKLVVVGAGCV (SEQ ID NO: 81); or

g) TEYKLVVVGAGSVG (SEQ ID NO: 85) or preferably TEYKLVVVGAGSV (SEQ ID NO: 86) or more preferably TEYKLVVVGAGS (SEQ ID NO: 83);

including the amino acid at position 11 of the respective sequences a) to d), or the amino acid at position 12 of the respective sequences e) to g).

9. The molecule according to claim 7 or 8, wherein the molecule comprises the amino acid stretch VVVGAV (SEQ ID NO: 10), LVVVGAV (SEQ ID NO: 11), VVVGAVG (SEQ ID NO: 12), VVVGAVGVG (SEQ ID NO: 13), VVVGAGV (SEQ ID NO: 99), LVVVGAGV (SEQ ID NO: 100), VVVGAGVV (SEQ ID NO: 101) or VVVGAGVVG (SEQ ID NO: 102).

10. The molecule according to any one of claims 7 to 9, wherein the amino acid stretch comprises one or more D-amino acids and/or analogues of one or more of its amino acids.

11. The molecule according to any one of claims 7 to 10, wherein the molecule comprises two or more, preferably two, said amino acid stretches, which are identical or different.

12. The molecule according to any one of claims 7 to 11, wherein the amino acid stretch or stretches are each independently flanked, on each end independently, by one or more amino acids that display low beta-sheet forming potential or a propensity to disrupt beta-sheets.

13. The molecule according to any one of claims 7 to 12, wherein the molecule comprises, consists essentially of or consists of the structure:

a) NGK1-P1-CGK1,

b) NGK1-P1-CGK1-Z1-NGK2-P2-CGK2,

c) NGK1-P1-CGK1-Z1-NGK2-P2-CGK2-Z2-NGK3-P3-CGK3, or

d) NGK1-P1-CGK1-Z1-NGK2-P2-CGK2-Z2-NGK3-P3-CGK3-Z3-NGK4-P4-CGK4,

wherein:

P1 to P4 each independently denote an amino acid stretch as defined in any one of claims 7 to 10,

NGK1 to NGK4 and CGK1 to CGK4 each independently denote 1 to 4 contiguous amino acids that display low beta-sheet forming potential or a propensity to disrupt beta-sheets, such as 1 to 4 contiguous amino acids selected from the group consisting of R, K, D, E, P, N, S, H, G, Q, and A, D-isomers and/or analogues thereof, and combinations thereof, preferably 1 to 4 contiguous amino acids selected from the group consisting of R, K, D, E, P, N, S, H, G, and Q, D-isomers and/or analogues thereof, and combinations thereof, more preferably 1 to 4 contiguous amino acids selected from the group consisting of R, K, D, E, and P, D-isomers and/or analogues thereof, and combinations thereof, and

Z1 to Z3 each independently denote a direct bond or preferably a linker;

optionally wherein:

NGK1 to NGK4 and CGK1 to CGK4 is each independently 1 to 2 contiguous amino acids selected from the group consisting of R, K, A, and D, D-isomers and/or analogues thereof, and combinations thereof, preferably NGK1 to NGK4 and CGK1 to CGK4 is each independently 1 to 2 contiguous amino acids selected from the group consisting of R, K, and D, D-isomers and/or analogues thereof, and combinations thereof, such as wherein NGK1 to NGK4 and CGK1 to CGK4 is each independently K, R, D, A or KK, preferably each independently K, R, D or KK; and/or

each linker is independently selected from a stretch of between 1 and 10 units, preferably between 1 and 5 units, wherein a unit is each independently an amino acid or poly(ethylene glycol) (PEG), such as wherein each linker is independently GS, PP or GSGS (SEQ ID NO: 14), preferably GS, or D-isomers and/or analogues thereof.

14. The molecule according to claim 13, wherein the molecule comprises, consists essentially of or consists of a peptide of the amino acid sequence:

a) KVVVGAVKGSKVVVGAVK (SEQ ID NO: 15); or

b) KLVVVGAVKGSKLVVVGAVK (SEQ ID NO: 16); or

c) KVVVGAVGKGSKVVVGAVGK (SEQ ID NO: 17); or

d) KVVVGAVGVGKGSKVVVGAVGVGK (SEQ ID NO: 18); or

e) KVVVGAGVKGSKVVVGAGVK (SEQ ID NO: 128); or

f) KLVVVGAGVKGSKLVVVGAGVK (SEQ ID NO: 129); or

g) KVVVGAGVVKGSKVVVGAGVVK (SEQ ID NO: 130); or

h) KVVVGAGVVGKGSKVVVGAGVVGK (SEQ ID NO: 131);

optionally wherein the amino acid sequence comprises one or more D-amino acids and/or analogues of one or more of its amino acids, optionally wherein the N-terminal amino acid is acetylated and/or the C-terminal amino acid is amidated.

15. The molecule according to any one of claims 1 to 14, which comprises a detectable label, a moiety that allows for isolation of the molecule, a moiety increasing the stability or half-life of the molecule, a moiety increasing the solubility of the molecule, a moiety increasing the cellular uptake of the molecule, and/or a moiety effecting targeting of the molecule to cells.

16. A nucleic acid encoding the molecule according to any one of claims 1 to 15, wherein the molecule is a polypeptide.

17. The molecule according to any one of claims 1 to 15 or the nucleic acid according to claim 16 for use in medicine.

18. The molecule according to any one of claims 1 to 15 or the nucleic acid according to claim 16 for use in treating a disease caused by or associated with a mutation at position 12 or 13 in human RAS protein.

19. The molecule or nucleic acid for use according to claim 18, wherein the disease is a neoplastic disease, particularly cancer, optionally wherein the disease is pancreatic ductal adenocarcinoma, colorectal adenocarcinoma, multiple myeloma, lung adenocarcinoma, skin cutaneous melanoma,

uterine corpus endometrioid carcinoma, uterine carcinosarcoma, thyroid carcinoma, acute myeloid leukaemia, bladder urothelial carcinoma, gastric adenocarcinoma, cervical adenocarcinoma, head and neck squamous cell carcinoma, non-small cell lung cancer (NSCLC), or colorectal cancer.

20. Use of the molecule according to any one of claims 1 to 15 or the nucleic acid according to claim 16 in the manufacture of a medicament for the treatment of a disease caused by or associated with a mutation at position 12 or 13 in human RAS protein.

21. The use according to claim 20, wherein the disease is a neoplastic disease, particularly cancer, optionally wherein the disease is pancreatic ductal adenocarcinoma, colorectal adenocarcinoma, multiple myeloma, lung adenocarcinoma, skin cutaneous melanoma, uterine corpus endometrioid carcinoma, uterine carcinosarcoma, thyroid carcinoma, acute myeloid leukaemia, bladder urothelial carcinoma, gastric adenocarcinoma, cervical adenocarcinoma, head and neck squamous cell carcinoma, non-small cell lung cancer (NSCLC), or colorectal cancer.

22. A pharmaceutical composition comprising

the molecule according to any one of claims 1 to 15; or

the nucleic acid according to claim 16.