CLAIMS

What is claimed is:

1. Small hairpin RNA (shRNA) molecule having the structure, in 5' to 3' orientation,

X_1-L-X_2,

wherein

X_1 and X_2 are each nucleotide sequences of 8 to 30 nucleotides in length having sufficient complementarity to one another to form a double-stranded stem structure;

L is a nucleotide sequence forming a loop region;

the first nucleotide positioned at the 5′ terminal end of X_1 is designated as n1 and is di- or triphosphorylated and the last nucleotide at the 3' terminal end of X_2 is designated nx, wherein x is an integer of 25 to 65;

the shRNA molecule comprises a nucleotide insertion in X_1 in position n7 or higher or in X_2 at position nx-6 or lower that remains unpaired in the double-stranded stem structure and creates a kink.

2. The shRNA molecule of claim 1, wherein

(1) L is a nucleotide sequence of 1 to 10 nucleotides in length, preferably 2 to 4 nucleotides in length; and/or

(2) the nucleotide at the 5' terminal end is triphosphorylated; and/or

(3) the shRNA molecule is blunt-ended; and/or

(4) X_1 and X_2 are with the exception of the nucleotide insertion creating the kink, fully complementary to one another; and/or

(5) the nucleotide insertion is a nucleotide insertion of 1-2 nucleotides, preferably of a single nucleotide; and/or

(6) the nucleotide insertion is a nucleotide insertion in X_1.

3. The shRNA molecule of claim 1 or 2, wherein X_1 and X_2 are each nucleotide sequences of 10 to 25 nucleotides in length, preferably of 10, 11, 20, 21, 30 or 31 or 10-20 nucleotides in length, more preferably of 10 or 11 nucleotides in length.

4. The shRNA molecule of any one of claims 1 to 3, wherein X_1 and X_2 are, not counting the nucleotide insertion creating the kink, of the same length and are 10, 20 or 30 nucleotides in length, preferably 10.

5. The shRNA molecule of any one of claims 1 to 4, wherein the nucleotide insertion is in a position selected from the positions ranging from n7 to the penultimate position of X_1 or from the positions ranging from the second position of X_2 to nx-6.

6. The shRNA molecule of claim 5, wherein the nucleotide insertion is in a position selected from the positions ranging from n9 to the position 2 or 3 nucleotides upstream of the first nucleotide of the loop region or from the position 2 or 3 nucleotides downstream of the last nucleotide of the loop region to nx-8.

7. The shRNA molecule of claim 6, wherein the nucleotide insertion is in the X_1 sequence at position n9.

8. The shRNA molecule of claim 1, wherein the nucleotide insertion is G or A.

9. The shRNA molecule of any one of claims 1 to 8, wherein the loop region L comprises or consists of the sequence UUCG.

10. The shRNA molecule of any one of claims 1 to 9, wherein
(1) X_1 comprises or consists of a nucleotide sequence selected from the group consisting of:
rrrnnyyy**r**yy (SEQ ID NO:1);
sswwwwss**r**ss (SEQ ID NO:2);
ggannnnn**n**nn (SEQ ID NO:3);
ggannnnn**n**cc (SEQ ID NO:4);
ggannunc**n**cc (SEQ ID NO:5);
ggawwusc**n**cc (SEQ ID NO:6);
ggauuucc**r**cc (SEQ ID NO: 7);
ggauuucc**a**cc (SEQ ID NO: 8); or
ggauuucc**g**cc (SEQ ID NO: 9),
wherein r is g or a, y is u or c, w is a or u, s is g or c and n is a, g, u or c, and wherein the X_1 sequence preferably comprises the inserted unpaired nucleotide, this unpaired nucleotide preferably being the nucleotide at position 9; and/or
(2) X_2 comprises or consists of a nucleotide sequence selected from the group consisting of:
rrrrrnnyyy (SEQ ID NO:10);
sssswwwwss (SEQ ID NO:11);
nnnnnnnucc (SEQ ID NO:12);
ggnnnnnucc (SEQ ID NO:13);
gggnannucc (SEQ ID NO:14);
gggwawwucc (SEQ ID NO:15);
ggggaaaucc (SEQ ID NO: 16),
wherein r is g or a, y is u or c, w is a or u, s is g or c and n is a, g, u or c; and/or

(3) the shRNA molecule comprises or consists of a nucleotide sequence selected from the group consisting of:
rrrnnyyy**r**yyuucgrrrrrnnyyy (SEQ ID NO:17);
sswwwwss**r**ssuucgsssswwwwss (SEQ ID NO:18);
ggannnnn**n**nnuucgnnnnnnnucc (SEQ ID NO:19);
ggannnnn**n**ccuucgggnnnnnucc (SEQ ID NO:20);
ggannunc**n**ccuucggggnannucc (SEQ ID NO:21);
ggawwusc**n**ccuucggggwawwucc (SEQ ID NO:22);
ggauuucc**n**ccuucgggggaaaucc (SEQ ID NO: 23);
ggauuucc**r**ccuucgggggaaaucc (SEQ ID NO:24);
ggauuuccaccuucgggggaaaucc (SEQ ID NO:25); or
ggauuuccgccuucgggggaaaucc (SEQ ID NO:26),
wherein r is g or a, y is u or c, w is a or u, s is g or c and n is a, g, u or c.

11. The shRNA molecule of any one of claims 1 to 10, wherein
(1) the shRNA molecule specifically binds to human retinoic acid-inducible gene 1 receptor (RIG-I); and/or
(2) the RNA is modified, optionally being a phosphorothioate RNA or being modified by 2'-fluoro groups.

12. Composition comprising at least one shRNA molecule according to any one of claims 1-11.

13. The composition of claim 12, wherein the composition comprises a plurality of shRNA molecules according to any one of claims 1-11.

14. The composition of claim 12 or 13, wherein the composition is
(1) a pharmaceutical composition, optionally an immunostimulatory composition, the immunostimulatory composition optionally being a vaccine composition further comprising a vaccine and wherein the shRNA molecule(s) are the adjuvant; or
(2) an antiviral composition, optionally further comprising an active antiviral agent; or
(3) an anticancer composition, optionally further comprising an active anticancer agent, preferably a chemotherapeutic or checkpoint inhibitor.

15. The composition of any one of claims 12 to 14, further comprising one or more excipients.

16. shRNA molecule of any one of claims 1 to 11 or composition of any one of claims 12 to 15 for stimulating the immune system or treating or preventing a viral infection or cancer in a subject in need thereof.

17. Use of an effective amount of the shRNA molecule according to any one of claims 1-11 or of the composition of any one of claims 12 to 14(1) or 15 in the manufacture of a medicament for stimulating the immune system in a subject in need thereof.

18. Use of an effective amount of the shRNA molecule according to any one of claims 1-11 or of the composition of any one of claims 12 to 15 in the manufacture of a medicament for preventing or treating a viral infection or cancer in a subject in need thereof .

19. The use according to claim 17 or 18, wherein the medicament is to be administered topically or systemically.

20. Method for modifying a small hairpin RNA (shRNA) molecule having the structure, in 5' to 3' orientation,

X_1-L-X_2,

wherein

X_1 and X_2 are each nucleotide sequences of 8 to 30 nucleotides in length having sufficient complementarity to one another to form a double-stranded stem structure;

L is a nucleotide sequence forming a loop region;

the first nucleotide positioned at the 5′ terminal end of X_1 is designated as n1 and the last nucleotide at the 3' terminal end of X_2 is designated nx, wherein x is an integer of 25 to 65;

the method comprising introducing a nucleotide insertion into X_1 in position n7 or higher or in X_2 at position nx-6 or lower that remains unpaired in the double-stranded stem structure to create a kink.