Claim Amendments

1. An siRNA-mediated method to inhibit transforming growth factor beta (TGF-beta or TGF-β) expression via furin knockdown, the method comprising the steps of:

selecting a target cell; and

transfecting the target cell with an expression vector comprising a promoter and a nucleic acid insert operably linked to the promoter, wherein the insert encodes one or more short hairpin bifunctional RNAs (shRNA) capable of hybridizing to a region of a mRNA transcript encoding furin, wherein the transcript incorporates siRNA (cleavage dependent) and miRNA (cleavage-independent) motifs, thereby inhibiting furin expression via RNA interference.

2. The method of claim 1, wherein the region targeted by the shRNA is the 3' UTR region sequence of the furin mRNA transcript.

3. The method of claim 1, wherein the region targeted by the shRNA is the coding region of the furin mRNA transcript.

4. The method of claim 1, wherein the shRNA is defined further as comprising a picornaviral 2A ribosomal skip peptide intercalated between a first and a second nucleic acid insert in the expression vector.

5. The method of claim 1, wherein the promoter is a CMV mammalian promoter.

6. The method of claim 5, wherein the CMV mammalian promoter contains a CMV IE 5' UTR enhancer sequence and a CMV IE Intron A.

7. The method of claim 1, wherein the bi-functional shRNA furin is defined further as comprising two stem-loop structures with miR-30a backbone; the first stem-loop structure has complete complementary guiding strand and passenger strand, while the second stem-loop structure has three basepair (bp) mismatches at positions 9 to 11 of the passenger strand. ~~a first stem-loop structure comprising a complete complementary guide strand and a passenger strand and a second stem-loop structure comprising one or more basepair mismatches of the guide strand, wherein the bi-functional shRNA furin hybridized to a region of a mRNA transcript encoding furin, thereby inhibiting furin expression via RNA interference~~.

~~8. The method of claim 7, wherein the second stem-loop structure comprises three base pair mismatches.~~

~~9. The method of claim 7, wherein the basepair mismatches are at positions 9 to 11 of the guide strand.~~

Claims

1. An siRNA-mediated method to inhibit transforming growth factor beta (TGF-beta or TGF-β) expression via furin knockdown, the method comprising the steps of:

selecting a target cell; and

transfecting the target cell with an expression vector comprising a promoter and a nucleic acid insert operably linked to the promoter, wherein the insert encodes one or more short hairpin bifunctional RNAs (shRNA) capable of hybridizing to a region of a mRNA transcript encoding furin, wherein the transcript incorporates siRNA (cleavage dependent) and miRNA (cleavage-independent) motifs, thereby inhibiting furin expression via RNA interference.

2. The method of claim 1, wherein the region targeted by the shRNA is the 3' UTR region sequence of the furin mRNA transcript.

3. The method of claim 1, wherein the region targeted by the shRNA is the coding region of the furin mRNA transcript.

4. The method of claim 1, wherein the shRNA is defined further as comprising a picornaviral 2A ribosomal skip peptide intercalated between a first and a second nucleic acid insert in the expression vector.

5. The method of claim 1, wherein the promoter is a CMV mammalian promoter.

6. The method of claim 5, wherein the CMV mammalian promoter contains a CMV IE 5' UTR enhancer sequence and a CMV IE Intron A.

7. The method of claim 1, wherein the bi-functional shRNA furin is defined further as comprising a first stem-loop structure comprising a complete complementary guide strand and a passenger strand and a second stem-loop structure comprising one or more basepair mismatches of the guide strand, wherein the bi-functional shRNA furin hybridized to a region of a mRNA transcript encoding furin, thereby inhibiting furin expression via RNA interference.

8. The method of claim 7, wherein the second stem-loop structure comprises three base pair mismatches.

9. The method of claim 7, wherein the basepair mismatches are at positions 9 to 11 of the guide strand.

Claims

1. An siRNA-mediated method to inhibit transforming growth factor beta (TGF-beta or TGF-β) expression via furin knockdown, the method comprising the steps of:

selecting a target cell; and

transfecting the target cell with an expression vector comprising a promoter and a nucleic acid insert operably linked to the promoter, wherein the insert encodes one or more short hairpin bifunctional RNAs (shRNA) capable of hybridizing to a region of a mRNA transcript encoding furin, wherein the transcript incorporates siRNA (cleavage dependent) and miRNA (cleavage-independent) motifs, thereby inhibiting furin expression via RNA interference.

2. The method of claim 1, wherein the region targeted by the shRNA is the 3' UTR region sequence of the furin mRNA transcript.

3. The method of claim 1, wherein the region targeted by the shRNA is the coding region of the furin mRNA transcript.

4. The method of claim 1, wherein the shRNA is defined further as comprising a picornaviral 2A ribosomal skip peptide intercalated between a first and a second nucleic acid insert in the expression vector.

5. The method of claim 1, wherein the promoter is a CMV mammalian promoter.

6. The method of claim 5, wherein the CMV mammalian promoter contains a CMV IE 5' UTR enhancer sequence and a CMV IE Intron A.

7. The method of claim 1, wherein the bi-functional shRNA furin is defined further as comprising two stem-loop structures with miR-30a backbone; the first stem-loop structure has complete complementary guiding strand and passenger strand, while the second stem-loop structure has three basepair (bp) mismatches at positions 9 to 11 of the passenger strand.