## SUMMARY

It is shown herein that mutants of fibroblast growth factor (FGF) 1 having reduced or eliminated mitogenic activity can be used to reduce blood glucose in a mammal. Based on these observations, methods for reducing blood glucose in a mammal, for example to treat a metabolic disease, are disclosed. Such FGF1 mutants can have an N-terminal truncation, point mutations, or combinations thereof, for example to reduce the mitogenic activity of the native FGF1 protein. Such FGF1 mutants can be used alone or in combination with other agents, such as other glucose reducing agents, such as thiazolidinedione. In one aspect, the present invention refers to an isolated mutated mature fibroblast growth factor (FGF) 1 protein comprising at least 90% sequence identity to the amino acid sequence shown in SEQ ID NO: 234 wherein the isolated mutated mature FGF1 protein comprises a Y94V mutation, and wherein the amino acid numbering is based on SEQ ID NO: 5.

In some examples, the FGF1 mutant is part of a chimeric protein, such as one that includes at least 10, at least 20, at least 30, at least 40, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, or at least 50 contiguous amino acids from a C-terminal end of FGF19 or FGF21.

In some examples, the FGF1 mutant is part of a chimeric protein, such as one that includes at least 10, at least 20, at least 30, at least 35, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 120, at least 150, at least 180, or at least 200 amino acids (such as 20-500, 20 to 250, 30 to 200, 35 to 180, 37 to 90, or 37 to 180 amino acids) of a protein that selectively binds to beta-Klotho (β-Klotho), such as SEQ ID NO: 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145 or 146.

In some examples, the FGF1 mutant is part of a chimeric protein, such as one that includes at least 10, at least 20, at least 30, at least 35, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 120, at least 150, at least 180, or at least 200 amino acids (such as 20-500, 20 to 250, 30 to 200, 35 to 180, 37 to 90, or 37 to 180 amino acids) of a protein that selectively binds to FGFR1c, such as SEQ ID NO: 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, or multimers thereof (*e.g.*, dimers, timers), such as SEQ ID NO: 190.

In some examples, the FGF1 mutant is part of a chimeric protein, such as one that includes at least 10, at least 20, at least 30, at least 35, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 120, at least 150, at least 180, or at least 200 amino acids (such as 20-500, 20 to 250, 30 to 200, 35 to 180, 37 to 90, or 37 to 180 amino acids) of a protein that selectively binds to β-Klotho, and that includes at least 10, at least 20, at least 30, at least 35, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 120, at least 150, at least 180, or at least 200 amino acids (such as 20-500, 20 to 250, 30

to 200, 35 to 180, 37 to 90, or 37 to 180 amino acids) of a protein that selectively binds to FGFR1c, such as SEQ ID NO: 168, 169, 170 or 171.

In some examples, chimeric proteins include a linker between the FGF1 mutant and the FGF19, FGF21, FGFR1c-binding, or β-Klotho-binding sequence. In some examples, use of the disclosed methods result in one or more of: reduction in triglycerides, decrease in insulin resistance, reduction of hyperinsulinemia, increase in glucose tolerance, or reduction of hyperglycemia in a mammal.

Disclosed herein are mutated FGF1 proteins, which can include deletion of an N-terminal portion of FGF1, point mutations (such as amino acid substitutions, deletions, additions, or combinations thereof), or combinations of N-terminal deletions and point mutations, and methods of their use to lower glucose, for example to treat a metabolic disease. In some examples, such mutations reduce the mitogenicity of mature FGF1 (*e.g.*, SEQ ID NO: 5), such as a reduction of at least 20%, at least 50%, at least 75% or at least 90%. In some examples, the mutant FGF1 protein is a truncated version of the mature protein (*e.g.*, SEQ ID NO: 5), which can include for example deletion of at least 5, at least 6, at least 10, at least 11, at least 12, at least 13, or at least 20 consecutive N-terminal amino acids. In some examples, one or more of the deleted N-terminal amino acids are replaced with corresponding amino acids from FGF21 (or any FGF having low affinity for FGFR4, including FGF3, FGF5, FGF7, FGF9 and FGF10), such as at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, or at least 15, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 corresponding amino acids from FGF21 (*e.g.*, see SEQ ID NOS: 21, 219, 221, 222 and 223). In some examples, the mutant FGF1 protein is a mutated version of the mature protein (*e.g.*, SEQ ID NO: 5), such as one containing at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9 or at least 10 amino acid substitutions (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 or 41 substitutions), such as one or more of those shown in Table 1. In some examples, the mutant FGF1 protein includes both an N-terminal truncation and point mutations. In some examples, the mutant FGF1 protein includes at least 120 consecutive amino acids from amino acids 5-141 of FGF1 (*e.g.*, of SEQ ID NO: 2 or 4), (which in some examples can include 1-20 point mutations, such as substitutions, deletions, or additions).

In some examples, the FGF1 mutants disclosed herein are used to generate a chimeric protein, such as an FGF1/FGF21, FGF1/FGF19, FGF1/β-Klotho-binding protein, FGF1/FGFR1c-binding protein or FGF1/β-Klotho-binding protein/FGFR1c-binding protein. For example, the C-terminal end or the N-terminal end of the disclosed FGF1 mutants can be

joined directly or indirectly to the N-terminal end of a C-terminal fragment of FGF21 or FGF19, such as SEQ ID NO: 86 or 100, respectively. Similarly, the C-terminal end of the disclosed FGF1 mutants can be joined directly or indirectly to the N-terminal end of a β-Klotho binding domain (such as SEQ ID NO: 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146 or β-Klotho binding portion of SEQ ID NO: 168, 169, 170 or 171), or the N-terminal end of the disclosed FGF1 mutants can be joined directly or indirectly to the C-terminal end of a β-Klotho-binding domain. In addition, the C-terminal end of the disclosed FGF1 mutants can be joined directly or indirectly to the N-terminal end of a FGFR1c-binding domain (such as SEQ ID NO: 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167 or 190), or the N-terminal end of the disclosed FGF1 mutants can be joined directly or indirectly to the C-terminal end of a FGFR1c-binding domain. In some examples, the C-terminal end of the disclosed FGF1 mutants can be joined directly or indirectly to an FGFR1c-binding domain (such as any of SEQ ID NOS: 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 190 or FGFR1c-binding portion of 168, 169, 170 or 171) and a β-Klotho-binding domain, the N-terminal end of the disclosed FGF1 mutants can be joined directly or indirectly to the C-terminal end of a FGFR1c-binding domain and a β-Klotho-binding domain, or both (such as SEQ ID NO: 168, 169, 170 or 171). Such chimeric proteins can be used to reduce blood glucose in a mammal, for example to treat a metabolic disease.

Specific exemplary FGF1 mutant proteins are shown in SEQ ID NOS: 6, 7, 8, 9, 10, 11, 12, 13, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 113, 114, 115, 116, 117, 118, 119, 120, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237 and 238, which can be used to generate any of the chimeras disclosed herein. Specific exemplary FGF1/FGF21 chimeras are shown in SEQ ID NOS: 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 219, 221, 222, and 223. Specific exemplary FGF1/FGF19 chimeras are shown in SEQ ID NOS: 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 220, and 224. Specific exemplary FGF1/β-Klotho-binding chimeras are shown in FIGS. 23-25 (and in SEQ ID NOS: 173, 174, 175, 177, 178, 179, 181, 182, 183, 185, 186, and 187). Specific exemplary FGF1/FGFR1c-binding chimeras are shown in FIGS. 23J and 24I (and in SEQ ID NOS: 188 and 189). Specific exemplary β-Klotho-binding/FGFR1c-binding chimeras that can be linked

directly or indirectly to an N- or C-terminal end of a FGF1 mutant protein are shown in SEQ ID NOS: 168, 169, 170 and 171.

Also disclosed are FGFR1c-binding protein dimers and multimers (such as trimers) and their use to treat metabolic disease. An example is shown in FIG. 25E (also see SEQ ID NO: 190).

Also disclosed are isolated nucleic acid molecules encoding the disclosed mutant FGF1 proteins (which includes chimeras), and the FGFR1c binding proteins. Vectors and cells that include such nucleic acid molecules are also disclosed. In another aspect, the present invention refers to an isolated nucleic acid encoding the isolated protein as disclosed herein. In yet another aspect, the present invention refers to a nucleic acid vector comprising the isolated nucleic acid as disclosed herein. In a further aspect, the present invention refers to a host cell comprising the nucleic acid vector as disclosed herein.

Methods of using the disclosed mutant FGF1 proteins and FGFR1c binding protein multimers (or nucleic acid molecules encoding such) are disclosed, such as a mutated mature FGF1 protein having a deletion of at least six contiguous N-terminal amino acids, at least one point mutation, or combinations thereof, for example to reduce or eliminate mitogenic activity. In some examples the methods include administering a therapeutically effective amount of a disclosed mutant FGF1 protein or FGFR1c binding protein (or nucleic acid molecules encoding such) to reduce blood glucose in a mammal, such as a decrease of at least 5%. In some examples the methods include administering a therapeutically effective amount of a disclosed mutant FGF1 protein or FGFR1c binding protein multimer (or nucleic acid molecules encoding such) to treat a metabolic disease in a mammal. Exemplary metabolic diseases that can be treated with the disclosed methods include but are not limited to: diabetes (such as type 2 diabetes, non-type 2 diabetes, type 1 diabetes, latent autoimmune diabetes (LAD), or maturity onset diabetes of the young (MODY)), polycystic ovary syndrome (PCOS), metabolic syndrome (MetS), obesity, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), dyslipidemia (*e.g.*, hyperlipidemia), and cardiovascular diseases (*e.g.*, hypertension). In some examples, one or more of these diseases are treated simultaneously with the disclosed FGF21 mutants. Also disclosed are methods of reducing fed and fasting blood glucose, improving insulin sensitivity and glucose tolerance, reducing systemic chronic inflammation, ameliorating hepatic steatosis in a mammal, reducing food intake, or combinations thereof, by administering a therapeutically effective amount of a disclosed mutant FGF1 protein or FGFR1c binding protein multimer (or nucleic acid molecules encoding such).

The foregoing and other objects and features of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

We claim:

1. An isolated mutated mature fibroblast growth factor (FGF) 1 protein comprising at least 90% sequence identity to the amino acid sequence shown in SEQ ID NO: 234 wherein the isolated mutated mature FGF1 protein comprises a Y94V mutation, and wherein the amino acid numbering is based on SEQ ID NO: 5.

2. The isolated protein of claim 1, wherein the isolated mutated mature FGF1 protein further comprises a mutation at K12, and wherein the amino acid numbering is based on SEQ ID NO: 5.

3. The isolated protein of claim 2, wherein the mutation at K12 is K12V, and wherein the amino acid numbering is based on SEQ ID NO: 5.

4. The isolated protein of claim 1, wherein the isolated mutated mature FGF1 protein further comprises a mutation at C117, and wherein the amino acid numbering is based on SEQ ID NO: 5.

5. The isolated protein of claim 4, wherein the mutation at C117 is C117V, and wherein the amino acid numbering is based on SEQ ID NO: 5.

6. The isolated protein of claim 1, wherein the isolated mutated mature FGF1 protein further comprises a mutation at K12 and C117, and wherein the amino acid numbering is based on SEQ ID NO: 5.

7. The isolated protein of claim 6, wherein the mutation at K12 is K12V, the mutation at C117 is C117V, and amino acid numbering is based on SEQ ID NO: 5.

8. The isolated protein of claim 1, wherein the isolated mutated mature FGF1 protein comprises at least 95% sequence identity to the amino acid sequence shown in SEQ ID NO: 234, wherein the isolated mutated mature FGF1 protein comprises a Y94V mutation, and wherein the amino acid numbering is based on SEQ ID NO: 5.

9. The isolated protein of claim 1, wherein the isolated mutated mature FGF1 protein comprises at least 98% sequence identity to the amino acid sequence shown in SEQ ID NO: 234,

wherein the isolated mutated mature FGF1 protein comprises a Y94V mutation, and wherein the amino acid numbering is based on SEQ ID NO: 5.

10. The isolated protein of claim 1, wherein the isolated mutated mature FGF1 protein comprises at least 99% sequence identity to the amino acid sequence shown in SEQ ID NO: 234, wherein the isolated mutated mature FGF1 protein comprises a Y94V mutation, and wherein the amino acid numbering is based on SEQ ID NO: 5.

11. An isolated nucleic acid encoding the isolated protein of claim 1.

12. A nucleic acid vector comprising the isolated nucleic acid of claim 11.

13. A host cell comprising the nucleic acid vector of claim 12.

14. The isolated protein of claim 1, wherein the isolated mutated mature FGF1 protein comprises the amino acid sequence shown in SEQ ID NO: 234.

15. The isolated protein of claim 1, wherein the isolated mutated mature FGF1 protein consists of the amino acid sequence shown in SEQ ID NO: 234.