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Activation of MIPEP Using CRISPR-Cas9 for Mitochondrial Stability and Telomere Preservation: A Novel In Silico Approach to Age-related Type-II Diabetes Treatment and Ageing

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Abstract

This research primarily focuses on ageing, disintegrated function, and age-related diseases such as T2D. Telomere shortening, mitochondrial dysfunction, and oxidative stress drive ageing, resulting in cellular and metabolic decay. Researchers have identified the downregulation of the MIPEP gene as a factor involved in these processes, which is relevant to mitochondrial health and cellular metabolism. The research unveils the modulation of MIPEP using the computational CRISPR-Cas9 technique as a preclinical solution for ageing-related impairments. Upregulation of MIPEP improves mitochondrial quality, decreases oxidative stress, and preserves pancreatic β-cells important for insulin synthesis and glucose control. Exercising the mitochondria may thereby slow the shortening of telomeres, another ageing biomarker associated with cellular senescence and metabolic dysfunction due to MIPEP activation. Furthermore, the role of MIPEP activation for important signalling pathways like Insulin Signaling, arginine metabolism, AMPK and Akt signalling pathways involved in energy regulation and β-cell survival is presented in the research. This research may present a unique approach to addressing T2D and other geriatric diseases given that they focus on the primary processes of ageing: mitochondrial malfunctioning and telomere shortening. The results highlighted the importance of mitochondrial quality of life in ageing and offered a platform for developing targeted medicine for ageing and ageing diseases.

Keywords: Telomere shortening, mitochondrial dysfunction, oxidative stress, MIPEP gene, CRISPR-Cas9, upregulation, pancreatic β-cells, AMPK and Akt signaling.

Introduction

MIPEP plays a part in processing and maintaining mitochondrial mRNA. Because of this, it is directly linked to processes necessary for ageing and controlling age-related diseases like type 2 diabetes. Mitochondria, known as the "powerhouse of the cell" is important in generating energy, regulating metabolism, and cellular signalling processes that preserve cell structure (Munir et al., 2025). MIPEP has a functional role in the final steps of maturation of proteins implicated from the cytoplasm to the mitochondria compartment. Bioenergetic proteins in mitochondria are made up of proteins that are connected to the ETC and some of the enzyme systems in mitochondria. In this case, MIPEP keeps proteostasis stable on mitochondria, which stops the membrane potential from

dropping and misfolded proteins from sticking together, both of which would lead to oxidative stress. A problem with MIPEP's expression or regulation, or maybe the pathways it works in, has been linked to several metabolic disorders, such as getting older and having age-related type 2 diabetes, which is marked by less insulin release and glucose metabolism. In addition, given the fact that MIPEP deficiency is unveiled, mitochondrial dysfunction deteriorates with increasing ROS and a shortening in telomere length, which also contributes to ageing and reduced cellular viability over time. MIPEP's role becomes more crucial when it comes to pancreatic cells, as these cells heavily rely on mitochondrial function for insulin release. Defective β-cell mitochondria reduce the responsiveness of these cells to glucose oscillations and cause diabetes development due to low insulin secretion (Davidson et al., 2020). The protection of MIPEP against oxidative stress in turn relieves stress-induced apoptosis in β-cells, which provides the basis of its therapeutic target activation. The beneficial effects of activating the MIPEP are also associated with the enhancement of the signals of insulin. In this way, MIPEP helps control the amount of glucose in the body by activating Akt (Diaz-Vegas et al., 2024). This keeps the cells safe from damage. In addition, the reduction of the expression levels of reactive oxygen species (ROS) by MIPEP prevents oxidative β-cell damage and maintains mitochondrial structural integrity, which is critical to their function. This is especially important in the orientation of pancreatic β-cells, in which functional mitochondria are crucial for insulin secretion. Due to the impact in the viewpoint of stabilization of mitochondria in the cells and so stopping cellular damage, MIPEP is recognized to be a worthy target in the context of anti-ageing methodologies and treatment of Type 2 diabetes (Eldomery et al., 2016). Due to its impact on the stabilization of mitochondria in the cells and stopping cellular damage, MIPEP is recognized as a worthy target in the context of anti-ageing methodologies and the treatment of type 2 diabetes. It looks at how stable mitochondria are and how that affects the ageing process and the start of related diseases, with type 2 diabetes being the most well-known example (Li et al., 2024). The new CRISPR-Cas9 system is used in this experiment to get the gene expression of MIPEP, which codes for mitochondrial intermediate peptidase, to work the way it's supposed to. Because manufactured proteins are what make the oxidative phosphorylation pathway work, MIPEP plays a big role in keeping the structure and function of mitochondria (Li et al., 2018). Upregulation of MIPEP is likely to offset the injurious effects of mitochondrial dysfunction including oxidative stress and reduced energy generation. These issues are relevant to the advancement of Type 2 diabetes because β-cell health is necessary for focused insulin release and glucose regulation (Liu et al., 2021). The development approach used in this research makes it possible to design and test gRNA sequences specific to the MIPEP promoter region. This strategy is considered to be aimed at increasing the mechanism of expression of MIPEP and, consequently, addressing the causes of instabilities of mitochondria and general ageing of the cells. The effectiveness of this approach is based on the presented opportunity to open a therapeutic channel not only for diabetes but also for diverse age-related diseases (Prel et al., 2021). Consequently, the Seri/activation of the MIPEP sensor has the potential to transform pro-ageing targets into anti-ageing targets, thereby advancing the field of precision medicine in metabolic and age-related diseases, which are primarily focused on bioenergetic stability, antioxidant defence, and telomere integrity. Using the CRISPR-Cas9 system to specifically activate MIPEP is a new way to get past molecular and cellular barriers that are related to getting older and having T2DM. MIPEP, a mitochondrial intermediate peptidase, remains a pivotal protein to maintain and regulate mitochondrial as well as cellular stability. Mitochondria that have impaired function have been established to have direct relations with ageing as well as the development of age-related illnesses, such as diabetes (Rodrigues, 2019). These organelles are not only involved in energy creating energy-creating processes but also in oxidative stress reactions, cell death, and metabolic signalling. When these processes don't work right, it leads to more cellular dysfunction, shorter telomeres, and fewer ways for tissues to heal, especially in pancreatic

β-cells that are needed to make insulin. MIPEP expression can then be increased through bioengineering with CRISPR-Cas9 to modify the promoter region of the gene, thus releasing the therapeutic potential of the protein. Probably, the activation of MIPEP could directly impact unfavorable processes common to both ageing and diabetes, such as mitochondrial dysfunction and elevated levels of oxidative stress (Saxena et al., 2013). In addition, this strategy might affect the main metabolic signalling pathways, including AMPK and Akt, which control how glucose is used and how insulin works. Here, we shift the focus of CRISPR-Cas9 treatment from the conventional functioning of cell pathways to a more targeted, mechanism-correcting intervention. It offers an opportunity not only to combat the indication but also such causes of ageing as metabolic disorders. This concept aligns with the objectives of precision medicine, which focuses on the molecular and genetic factors of various diseases, to manage ageing and type 2 diabetes.

Material and method

Sequence Retrieval

We obtained the MIPEP gene sequence from Ensembl (http://asia.ensembl.org/index.html), a well-known database that contains a variety of genome-related information. Ensembl operates confirmed annotations and data for all types of organisms to ensure that top-caliber genetic data is employed. We searched for the supplementary data by locating the MIPEP gene using its identifier, which we derived from the chromosome number and the gene sequence. We selected this sequence to incorporate the regulatory segments of the MIPEP promoter region, which are essential for designing gRNAs to activate the MIPEP gene using CRISPR Cas9. We selected this sequence to incorporate the regulatory segments of the MIPEP promoter region, which are essential for designing gRNAs to activate the MIPEP gene using CRISPR Cas9. Special attention was paid to formally fixing sequence ID and version checks to confirm that the presented sequence matched the current genomic configuration provided under Ensembl. (accessed on 26 October 2024).

Overlap of the Sequence at the Local Level

One of the computer programs used in this research BLAST, is used to align the MIPEP gene sequence to the reference genome. Often, we retrieve the MIPEP nucleotide sequence from Ensembl and align it to a target reference genome to identify homologous areas and confirm the sequence using Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi). It is used for its capacity to align the input sequences to the database and look out for areas of local similarity. This alignment aids in identifying the conserved motif in the MIPEP promoter region, essential for designing guide RNA (gRNA) that targets those regions for CRISPR-Cas9 activation. Apart from confirming the presence and coordinative conserved sequence, the alignment process enables the identification of some of the functional domains and regulatory regions related to the MIPEP gene. Apart from confirming the presence and coordination of conserved sequences, the alignment process enables the identification of some of the functional domains and regulatory regions related to the MIPEP gene. These reasons are very important for the next steps because they determine the best ways to target the genome accurately, make sure there is little off-targeting, and make sure the MIPEP cassette is highly activated. (accessed on 27 October 2024).

Open Reading Frame (ORF) screening

ORF screening is a process of searching for the strands of DNA that may code for a protein. In this step localized within each sequence, using tools like the NCBI ORF Finder, regions between start and stop codons, which are likely to represent real ORFs, are identified. In this step, localized within each sequence using tools like the NCBI ORF Finder, regions between start and stop codons, which are likely to represent real ORFs(https://www.ncbi.nlm.nih.gov/orffinder), are identified. The NCBI ORF Finder searches an input sequence for all consistent open reading

frames and, for each frame, reveals its length together with the position of the initiation and termination codons. Determining the translated segments is crucial as it identifies the structurally functional parts of a gene, such as the previously mentioned MIPEP. For other tasks later on, like finding the guide RNA for CRISPR Cas9 systems, correct ORF prediction is useful because it needs to match specific target areas with important coding sequences. It also confirms the accuracy of sequences being assessed based on their coding capacity, making up a standard process in a range of computational and experimental genomic approaches. The outcomes are again compared with database gene predictions to validate the outlines of the predicted ORFs and their functionality based on the biological role of the gene. (accessed on 29 October 2024).

Formation of gRNA

The CRISPR gRNA design against the MIPEP promoter region was done using the CHOPCHOP design tool. The CRISPR-gRNA design against the MIPEP promoter region was done using the CHOPCHOP design tool (https://chopchop.cbu.uib.no/). The tool also allows the user to accurately position the target sequences by comparing the genomic location of the region where CRISPR may bind. Notably, the core promoter region of MIPEP was scanned for protospacer adjacent motif (PAM) requirements for the function of the CRISPR-Cas9 system. Notably, the core promoter region of MIPEP was scanned for protospacer-adjacent motif (PAM) requirements for the function of the CRISPR-Cas9 system. They identified general criteria such as specificity and on-target efficiency and minimized off-target effects used to make the right choices of gRNA. These factors are considered by CHOPCHOP through the filtering of candidate gRNA sequences by their alignment with the genome for assessment of possible off-target hybridization, while sequences with the highest on-target efficiency and minimal chance of undesired interactions are desired. These factors are considered by CHOPCHOP through the filtering of candidate gRNA sequences based on their alignment with the genome for an assessment of possible off-target hybridization, while sequences with the highest on-target efficiency and minimal chance of undesired interactions are desired. In addition, the gRNA candidates were checked for GC content because it affects the stability and the efficiency of the interaction. We efficiently designed the gRNAs for high efficiency and specificity and selected only those with these qualities to upregulate the MIPEP gene. This approach combines computational accuracy with experimental practicality, making it possible to fashion a successful strategy for the activation of MIPEP. (accessed on 1 November 2024).

Verification of gRNA

To obtain highly specific and efficient gRNA sequences, the CRISPR-specific analysis platform available from Integrated DNA Technologies (IDT) was used. IDT's online design tool (https://www.idtdna.com/site/order/designtool/index/CRISPR_SEQUENCE), the mentioned resource provides a reliable base for designing gRNA sequences to the genetic sequence of the MIPEP gene. This tool assists researchers in identifying potential off-target effects and fine-tuning gRNA for precise targeting. On this platform, the gRNA sequences specific to the promoter regions of MIPEP were tested for specificity to the genome to reduce off-target effects. Furthermore, the platform offers an efficiency rating so that it is possible to identify potential candidates shown to have on-target activity. This step is very important to confine the CRISPR-Cas9 so that it targets the desired gene MIPEP, and very little or no other gene in other areas of the genome is activated, which makes the work much more reliable and precise. (accessed on 2 November 2024).

Assembling of gRNA expression vector

To make the guide RNA (gRNA) expression vector. Which is needed to turn on the MIPEP gene with CRISPR-Cas9, the first step is to clone the designed gRNA into the right expression vector,

which is pSpCas9(BB) using the benchling tool (https://www.benchling.com/). CRISPR-Cas9 studies widely utilize this vector due to its ability to generate two expression systems, Cas9 protein and guide RNA, in mammalian cells. According to the present method, the gRNA is designed to bind to the promoter region of the MIPEP gene to enhance its expression. The cloning procedure starts with the formulation of the gRNA sequence, which is the opposite of the MIPEP promoter area. This sequence is then electroporated into pSpCas9(BB) using restriction enzyme digestion and ligation techniques. These restriction enzymes are designed to cut the vector at specific areas, thereby facilitating the installation of the gRNA sequence. An attempt is made to obtain positive clones with colony PCR or restriction digestion analysis. Once we achieve this, we use a vector containing the correct gRNA sequence for the transfection process into target cells. This step confirms the reinforcement of the CRISPR-Cas9 system for the activation of the MIPEP gene, potentially enhancing mitochondrial dysfunction and aiding in the fight against age-related diseases such as Type II diabetes. (accessed on 10 November 2024).

Calculation of the GC Content

Figuring out how much GC is in the sequences of guide RNAs (gRNAs) is an important part of making the CRISPR-Cas9 system stable and effective. The GC content refers to the percentage of nucleotides in the sequence that are either guanine (G) or cytosine (C), and this determines the stability of the gRNA. The GC content is higher than in the secondary structure due to higher sensitivity to hydrogen in G-C pairs than in A-T pairs. However, a high percentage of GC can result in secondary structures, which may interfere with the gRNA binding with the target DNA owing to the poor efficiency of the CRISPR system. However, a high percentage of GC can result in secondary structures, which may interfere with the gRNA's ability to bind with the target DNA due to the poor efficiency of the CRISPR system. Therefore, the GC content should not be excessively low or high, as this could lead to the emergence of unwanted secondary structures, nor should it be excessively high, as a simple gRNA may not be sufficiently stabilized at the required site for the CRISPR-Cas9 complex to reach the target site. Biocomputational tools (https://www.endmemo.com/bio/gc.php) predict and optimize the GC content of gRNAs for target gene activation or editing in this process. (accessed on 11 November 2024).

Calculation of Thermodynamic Ensemble Prediction

We used the RNA folding tool from RNA World of Vienna at the University of Vienna, Austria (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) to predict the thermodynamic stability of the designed gRNA-DNA complex, which also dictates the efficiency of the CRISPR-Cas9. This tool predicts the minimum free energy (MFE) and thermodynamic ensemble of RNA secondary structures that reflects the stability and efficiency of gRNA to bind its target DNA. This tool predicts the minimum free energy (MFE) and thermodynamic ensemble of RNA secondary structures that reflect the stability and efficiency of gRNA in binding to its target DNA. The intuitive nature of the predicted secondary structures and base pair probabilities gives us a better idea of the folding patterns that might change how gRNA binds to target DNA and how well and specifically the CRISPR-Cas9 system works. Furthermore, the accuracy of the thermodynamic ensemble predictions is confirmed by calculating the Gibbs free energy (ΔG) for gRNA-DNA interactions, which reflects the stability of the complexes. The theoretically calculated lower ΔG values reflect the higher stability of complexes that correlate with the increased efficiency of CRISPR-Cas9 editing. The visualization of RNA structures obtained from this analysis helps in the determination of areas susceptible to instability and misfolding within the gRNA structure to enable further refinement of gRNA design for improved utility. In this way, the study integrates predictive insights to guarantee that the gRNAs possess a highly efficient structure for gene

targeting, which is essential for precise MIPEP activation when required for therapeutic purposes. (accessed on 15 November 2024).

Secondary Structure Prediction

In addition, the secondary structure of the gRNA was predicted to guarantee the highest possible efficacy of the CRISPR-Cas9 system. This process was done using an enhanced online RNA structure prediction tool (https://rna.urmc.rochester.edu/RNAstructureWeb/) procured from Mathew's laboratory. The RNA structure web interface incorporates our tool, which rates RNA sequences to determine their thermodynamically favourable secondary structures. Based on these folding patterns, the tool determines the possible structural motifs in the target RNA sequence and energy levels that could affect the binding of the gRNA to the target DNA. This is a very important step in the design of gRNAs, which will reduce any possibility of secondary structure interfering with the targeting and efficiency of the gRNAs. The predicted structures also permit the determination of early folding problems, such as hairpin loops or self-complementary sequences that may affect the overall efficiency of CRISPR-Cas9. Maintaining a thermodynamically favourable conformer towards the formation of the second structure is key in the design of a highly effective and stable genome editing mechanism. Maintaining a thermodynamically favourable conformer for the formation of the second structure is key to the design of a highly effective and stable genome editing mechanism. (accessed on 18 November 2024).

KEGG PATHWAY and STRING for MIPEP Protein Interactions

The 'Pathway database' of KEGG (https://www.genome.jp/kegg/pathway.html) is a comprehensive resource and visualization that offers information on complex networks, including metabolic pathways, signal transduction, and disease states, among others. Each pathway is assigned a minimum five-digit number, with additional codes such as map, ko, etc, or rn, as well as a brief three- or four-letter organism code. This work is made better by the STRING tool (https://string-db.org/), which puts together known and inferred PPIs from other databases, literature, and prediction algorithms. STRING observes a more complex interactive network of the MIPEP gene, highlighting its significance in mitochondrial biology, the processing of mitochondrial precursor proteins, and cellular homeostasis. Altogether, these utilities offer a strong basis for in-depth aboriginal investigation of the molecular roots of health and illness. (accessed on 19 November 2024).

Results

Retrieval of Sequence :

The MIPEP gene sequence was successfully retrieved from the Ensembl database as shown in Table 1. The sequence ID-ENST00000382172.4 is verified to ensure that the obtained data reflects the newest genomic pattern on record in the Ensembl database.

Table 1: The sequential retrieval and validation method for the MIPEP gene is as follows: The Ensembl database was used to obtain the MIPEP gene sequence through its gene identification number based on chromosome number 13 and the MIPEP gene sequence. The regulatory parts of the MIPEP promoter region were incorporated for CRISPR-Cas9 guide RNA design. The sequence was then validated using sequence ID-ENST00000382172.4 to match with extant genomic data as a final sign of concordance.

MIPEP SEQUENCE	GGAAACGCGGAGCGCGCGCTCCCAGCGAAAGCAGCAGGGCAGGGATCTG CGTTGGAGGAAGGGACTGCTCTGGTGCTAGAATGCTGTGCGTCGGAAGGC TGGGCGGCTTGGGAGCCAGAGCAGCAGCTCTGCCGCCCCGCCGGGCGGG CCGGGGAAGCCTCGAAGCCGGGATCCGGGCCCGAAGGGTCAGCACCAGC TGGTCTCCCGTGGGCGCCGCCTTCAATGTCAAGCCCCAGGGCAGCCGCTT GGACCTGTTCGGCGAGCGCCGGGGTCTTTTTGGAGTTCCTGAGCTGAGTG CCCCAGAAGGATTTCATATTGCACAAGAAAAAGCCTTGAGAAAGACAGAA TTGCTTGTGGACCGTGCATGTTCCACCCCACCTGGGCCCCAGACCGTGCT GATCTTCGATGAGCTCTCGGATTCCTTATGCAGAGTGGCCGACTTGGCTGA TTTTGTGAAAATCGCTCACCCTGAGCCAGCATTCAGAGAAGCTGCGGAAG AAGCTTGTAGAAGTATTGGCACCATGGTAGAGAAGTTGAACACAAATGTG GATTTATATCAAAGTTTGCAAAAATTACTAGCTGATAAAAAACTTGTGGATT CCCTTGATCCAGAAACAAGGCGAGTGGCTGAACTGTTTATGTTTGATTTTG AAATTAGTGGAATCCATCTAGACAAAGAAAAGCGTAAAAGAGCAGTGGAC CTCAATGTTAAAATCTTGGATTTGAGTAGTACATTTCTTATGGGAACCAATT TTCCCAACAAGATTGAGAAGCATCTCTTACCAGAACACATTCGTCGTAACT TTACATCTGCTGGGGATCATATCATAATTGATGGTCTCCACGCAGAATCACC AGATGACTTGGTGCGAGAAGCTGCTTATAAAATTTTTCTTTATCCCAATGCT GGTCAATTGAAATGTTTAGAAGAATTGCTCAGCAGCAGAGATCTTCTGGCA AAGTTGGTGGGGTATTCCACGTTTTCTCACAGGGCTCTCCAAGGAACGATA GCTAAAAATCCAGAGACTGTCATGCAGTTCCTTGAAAAACTATCTGACAA ACTTTCTGAAAGAACTCTGAAAGATTTTGAGATGATACGAGGGATGAAAA TGAAACTGAATCCTCAAAATTCCGAAGTAATGCCCTGGGACCCCCCTTACT ACAGTGGTGTGATTCGTGCAGAAAGGTATAATATTGAGC

Overlap of the Sequence at the Local Level using BLASTn :

By running a BLAST on the obtained MIPEP gene sequence and comparing it with the reference genome, the following observations were made. First, the analyzed MIPEP nucleotide sequence possessed high local identity with the reference genome, especially in the promoter region that is crucial for the regulation of CRISPR-Cas9 activation as shown in Figure 1 and Figure 2. Furthermore, functional domains and the regulatory regions of the MIPEP gene were observed to be a potential factor for gene activation and expression as shown in Figure 3. The alignment also emphasized locations where very high sequence conservation is observed, indicating functional constraint. Such findings are pivotal for identifying the correct location of the MIPEP gene and abating interactivity with other genes as well as for enhancing the efficiency of activation mediated by CRISPR-Cas9.

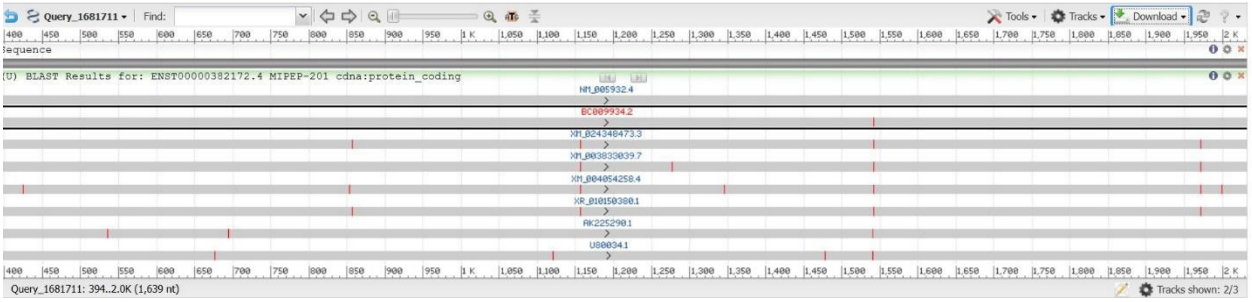


Figure 1: The MIPEP gene was aligned to the reference genome using the BLASTn sequence alignment tool. The above figure zooms in the portion at the local level by showing the local alignment of the MIPEP gene sequence and the reference genome where conserved motifs in the promoter region are shown.

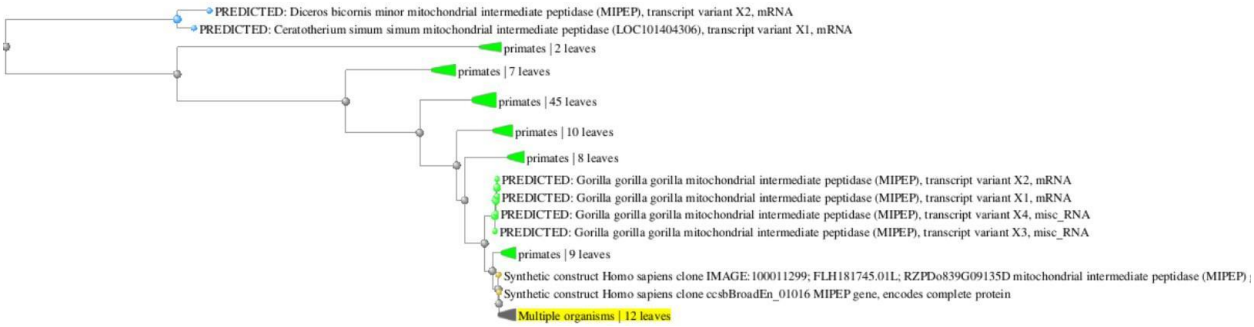


Figure 2: The above figure shows that the MIPEP nucleotide sequence possessed a high local identity with these sequences and this shows the distance tree of the results.

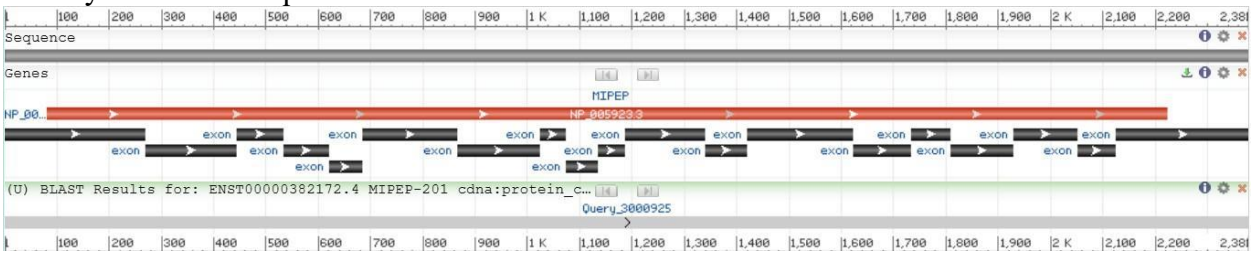


Figure 3: This shows that the Functional domains and regulatory regions are described, to be used as the basis for designing guide RNA for the activation of MIPEP. This alignment validates the existence and synchronization of such sequences that facilitate precise genome focus and optimal MIPEP activation.

Identification of Open Reading Frame (ORF) :

The results of ORF screening are shown in Figure 4 as several candidates' regions in the DNA sequences that may code functional proteins. Several ORFs were positioned through the NCBI ORF Finder pinpointing statistical significance for start codon stop codons, which specified the lengths and position of each of the ORFs identified. These results are important in identifying the translated segments that yield structural and functional proteins including those of the MIPEP gene. The identified ORFs were also tested with database-based gene prediction to confirm the coding capacity of the identified sequences. It also aided the discovery of target regions that would be relevant for other subsequent tasks such as the guide RNA design for gene editing with the CRISPR-Cas9 technique.

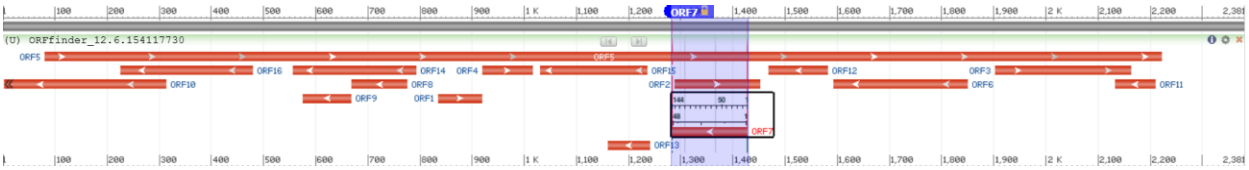


Figure 4: This figure demonstrates the NCBI ORF Finder for performing ORF screening of a MIPEP DNA sequence. The position of initiation and termination codons are labelled for each ORF and its size is shown. Additional evidence for the accuracy of the ORF predictions was obtained by comparing them with gene annotations in the databases. The predicted ORFs are extremely valuable for other applications such as CRISPR-Cas9 targeting and gene function studies.

Construction of gRNA:

The mCHOPCHOP tool was used to design the multiple gRNA sequences that target the MIPEP promoter region and these were highly efficient and specific as shown in Figure 5 and Table 2. To ensure better positioning of the gRNAs, the tool could locate and identify the genomic position of target sequences as shown in Figure 6 and Figure 7 and scan for the primers needed for CRISPR-Cas9 operation. Promoter sequences of MIPEP were analyzed for factors like identity and off-target effects to identify the best sites to bind. We selected gRNAs screened by CHOPCHOP for the candidate sequences, indicating high specificity and efficiency for off-target hybridization. The chosen gRNAs were characterized by good potential for upregulating the MIPEP gene. The target site prediction in computational analysis was complemented by the possibility of its experimental application. These candidates' sequences underwent further scrutiny depending on factors such as the off-target efficiency, specificity and extent of off-target effects. This design strategy offers a credible procedure for the activation of the MIPEP gene using the CRISPR-Cas9 system. The first rank sequence, TTCCCTCCCAAGTTGACTAGGGG, was found on chromosome 13 at position 23889577. This sequence has 50 % GC content and can form self-complementary structures twice. The left primer is from position 23,889,652 to 23,889,674 on chromosome 13 and has the following sequence AAGTACCTGCCGTCTCTCAAAG and the Tm of 59.9 °C. The right primer for the corresponding right region is situated within position 23,889,474-23,889,496 with the sequence GTTGTCGTCCAGAGAGCAAGAG as shown in Table 3. In the expected sequence of the control, only two off-target matches were found as shown in Table 4. The final product size is 200 base pairs. The second rank sequence, CGGAAGCGGGGCCGTCACGTGGG, was found on chromosome 13 at position 23889421. This sequence has 75 % GC content. The left primer is from position 23889540-23889562 on chromosome 13 and has the following sequence TTTCTTACTCTGGACACAGGGG and the Tm of 60.5°C. The right primer for the corresponding right region is situated within position 23889316-23889337 with the sequence AGCATTCTAGCACCAGAGCAG as shown in Table 5. The expected sequence has only one off-target match found as shown in Table 6. The final product size is 246 base pairs.

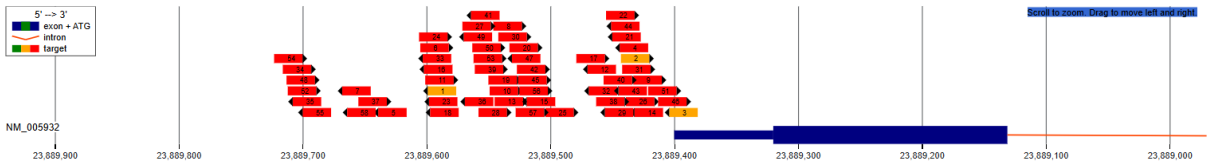


Figure 5: Structural representation of the sequence of the MIPEP gene.

Table 2: Target sequence and location of the target gRNA of the MIPEP gene.

Rank	Target sequence	Genomic location	Strand	GC content (%)	Self-complementarity	MM0	MM1	MM2	MM3	Efficiency
1	TTCCCTCCCAAGTTGACTAGGGG	chr13:23889577	+	50	1	0	0	0	2	60.50
2	CGGAAGCGGGGCCGTCACGTGGG	chr13:23889421	-	75	1	0	1	0	0	65.43

Rank: **1**
Target sequence: **TTCCCTCCCAAGTTGACTAGGGG**

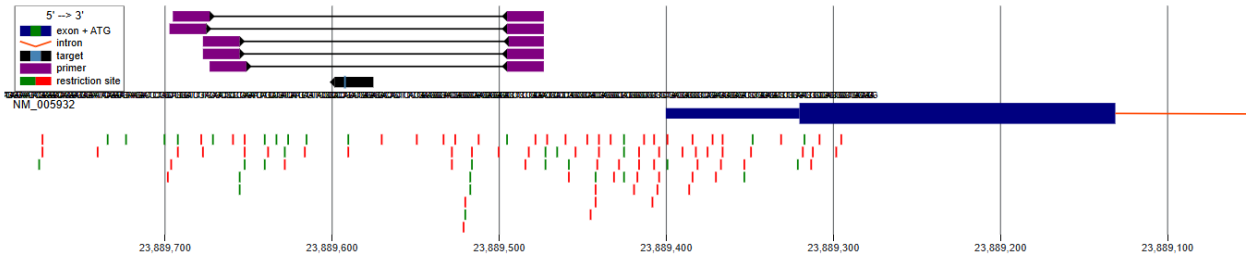


Figure 6: Graphical depiction of the gRNA of the gene for rank 1.

Table 3: The primer specification of the gRNA of the gene for rank 1.

Pair	Left primer coordinates	Left primer	Left primer Tm	Left primer off-targets	Right primer coordinates	Right primer	Right primer Tm	Right primer off-targets	Pair off-targets	Product size
1	chr13:23889652-23889674	AAGTACCTGCCGTCTCTCAAAG	59.9	0	chr13:23889474-23889496	GTTGTCGTCCAGAGAGCAAGAG	61.5	0	0	200
2	chr13:23889656-23889678	ACTCAAGTACCTGCCGTCTCTC	59.9	0	chr13:23889474-23889496	GTTGTCGTCCAGAGAGCAAGAG	61.5	0	0	204
3	chr13:23889656-23889678	ACTCAAGTACCTGCCGTCTCTC	59.9	0	chr13:23889474-23889495	GTTGTCGTCCAGAGAGCAAGA	60.6	0	0	204
4	chr13:23889676-23889698	ATGGTGGGCATTTCTTGTTTAC	60.1	1	chr13:23889474-23889496	GTTGTCGTCCAGAGAGCAAGAG	61.5	0	0	224
5	chr13:23889674-23889696	GGTGGGCATTTCTTGTTTACTC	59.9	1	chr13:23889474-23889496	GTTGTCGTCCAGAGAGCAAGAG	61.5	0	0	222

Table 4: The Off-target results of the gRNA of the gene for rank 1.

Off-targets		
Location	**Number of mismatches**	**Sequence (including mismatches)**
chr2:37139569	3	CCTC**a**AGTCAACTT**ca**GAGGGAA
chr3:140048934	3	TTCCCTCCC**g**A**t**TTGAC**c**AGCGG

Rank2

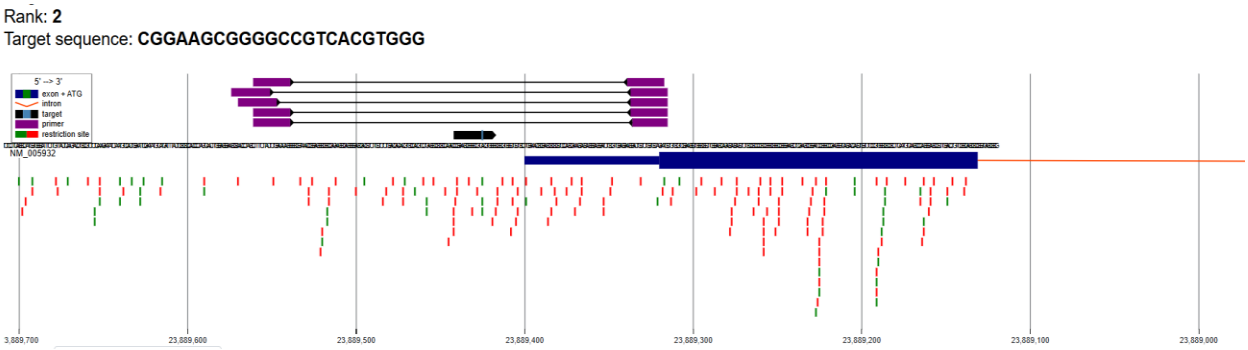


Figure 7: Graphical depiction of the gRNA of the gene for rank 2.

Table 5: The primer specification of the gRNA of the gene for rank 2.

Pair	Left primer coordinates	Left primer	Left primer Tm	Left primer off-targets	Right primer coordinates	Right primer	Right primer Tm	Right primer off-targets	Pair off-targets	Product size
1	chr13:23889540-23889562	TTTCTTACTCTGGACACAGGGG	60.5	0	chr13:23889316-23889337	AGCATTCTAGCACCAGAGCAG	59.8	0	0	246
2	chr13:23889540-23889562	TTTCTTACTCTGGACACAGGGG	60.5	0	chr13:23889316-23889338	AGCATTCTAGCACCAGAGCAGT	60.6	0	0	246
3	chr13:23889548-23889571	CCCTAGCCTTTTCTTACTCTGGA	60.2	0	chr13:23889316-23889338	AGCATTCTAGCACCAGAGCAGT	60.6	0	0	255

4	chr13:23889552-23889575	GCAACCCTAGCCTTTTCTTACTC	69.8	1	chr13:23889316-23889338	AGCATTCTAGCACCAGAGCAGT	60.6	0	0	259
5	chr13:23889540-23889562	TTTCTTACTCTGGACACAGGGG	60.5	1	chr13:23889318-23889340	CATTCTAGCACCAGAGCAGTCC	61.3	0	0	244

Table 6: The Off-target results of the gRNA of the gene for rank 2.

Off-targets		
Location	**Number of mismatches**	**Sequence (including mismatches)**
chr13:21298130	1	CGGA**g**GCGGGGCCGTCACGTGGG

Authentication of gRNA:

Using the online IDT tool, on-target and off-target scores for the each gRNA sequence were determined to achieve maximum efficiency. The on-target score which was an indication of efficiency of gRNA in targeting the MIPEP gene promoter was seen to be high concurring with high efficiency in gene activation. The above facts supported by a high on-target score also indicated that the gRNA is optimal for the targeting of the MIPEP gene and can efficiently bind to the target sequence. The off-target analysis also emphasized that high off-target activity was important because it meant it was possible to selectively activate a target gene in specific tissues such as pancreatic β-cells, where activation of other genes may be lethal. Thus, the identification of on-target, and off-target, gRNA sequences improved the CRISPR-Cas9 system, raising its capacity to activate the MIPEP gene for utilization in age-related diseases including diabetes as shown in Table 7 and Table 8.

Table 7: The Off-target and On-target results of the gRNA of the gene for rank 1.

Sequence	On-target score	Off-target score
TTCCCTCCCAAGTTGACTAG	64	66

Table 8: The Off-Target and On-Target results of the gRNA of the gene for rank 2.

Sequence	On-target score	Off-target score
CGGAAGCGGGGCCGTCACGT	61	80

Assembling of gRNA expression vector:

The pSpCas9(BB) guide RNA (gRNA) expression vector was confirmed by correctly inserting the gRNA in the designed sequence as shown in Figure 8. Here, the gRNA designed based on the MIPEP gene's promoter sequence was purposely used to activate the gene. The gRNA sequence

was then obtained by gene synthesis and then, the sequence was subsequently cloned in pSpCas9(BB) vector through restriction enzyme digestion and ligation. The ligation process was then done using the correct restriction enzymes to enable the gRNA to be well fixed at the corresponding sites on the vector.

In-silico PCR and restriction digestion analysis were performed to identify the positive clones and check whether the gRNA sequence has been integrated into the vector. These positive clones were then chosen and typed for transfection to the desired mammalian cells. The successful transfection and expression of the gRNA in target cells were then evaluated using the activation of the MIPEP gene, the possibility of improving mitochondrial function and combating age-related diseases including Type II diabetes.

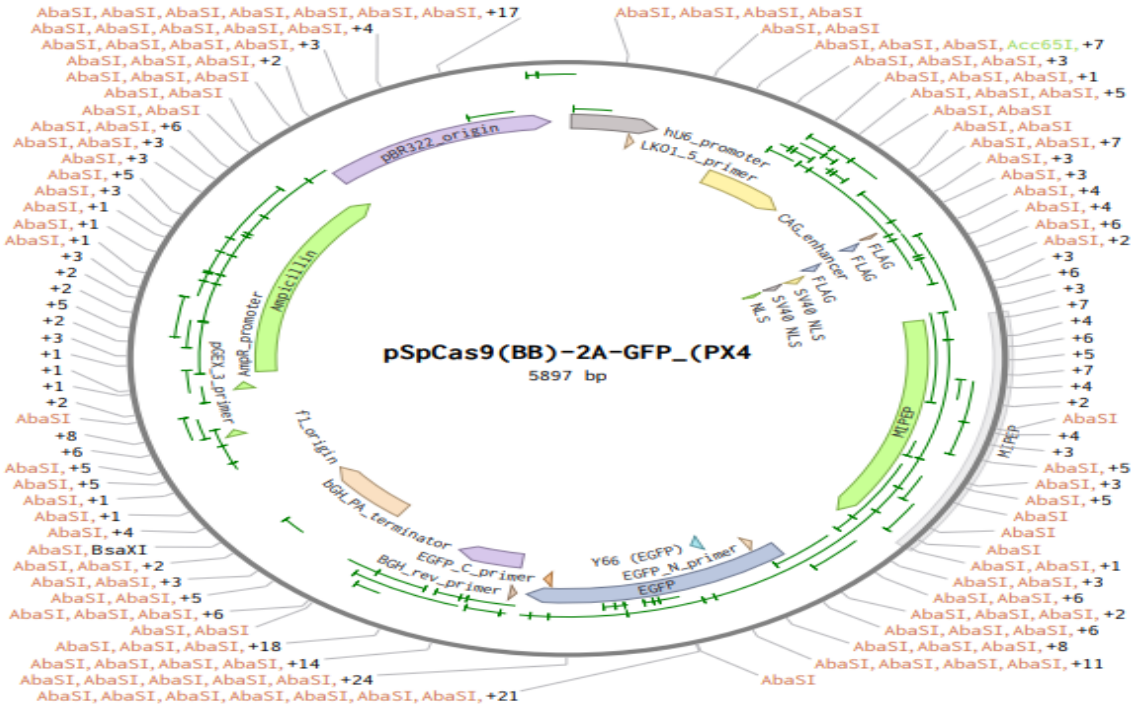


Figure 8: Preparation of the gRNA Expression Vector for MIPEP Gene Activation – This figure shows the cloning of the designed gRNA for the MIPEP gene into the vector named pSpCas9(BB). The vector is for CRISPR-Cas9-mediated activation of the MIPEP gene to improve mitochondrial biology. The gRNA is further designed to be complementary to the promoter region of MIPEP. Transfection competence was determined using colony PCR and the digested vectors were used to transfect target cells.. The results are designed to validate the CRISPR-Cas9 system for the possible treatment increase of mitochondrial activity in diseases associated with ageing including Type II diabetes.

Calculation of the GC Content:

The GC content of the multiple gRNA sequences necessary for both target loci was determined to evaluate the stability and efficiency of the CRISPR-Cas9 system as shown in Table 9 and Table 10. The GC content has core relevance with the secondary structures of the gRNA, with higher rates of GC providing more stable structures but the drawback of possible formation of secondary structures that cause hindrance for proper interaction of the gRNA with the target DNA. To this end, the results reveal that an optimal GC content was found for the respective gRNA enhancing its stability and avoiding the formation of objectionable secondary structures. Computational

strategies were employed to forecast and correct the GC content of the gRNAs on the premise that such sequences are ideal for target gene activation or editing.

Table 9: Forward and Reverse primer GC content for Rank 1.

Left primer coordinates	chr13:23889652-23889674	**Right primer coordinates**	chr13:23889474-23889496
Assembly 1 FWD	5' AAGTACCTGCCGTCTCTCAAAG 3'	**Assembly 1 REV**	**5' GTTGTCGTCCAGAGAGCAAGAG 3'**
Position	+/	**Position**	**-/**
Length	22	**Length**	**22**
GC content	**50%**	**GC content**	**50%**
Molecular Weight	6679.4	**Molecular Weight**	**6824.5**
Melting Temperature (T_M)	**59.9**	**Melting Temperature (T_M)**	**61.5**

Table 10: Forward and Reverse primer GC content for Rank 2.

Left primer coordinates	**chr13:23889540-23889562**	**Right primer coordinates**	**chr13:23889316-23889337**
Assembly 1 FWD	5' TTTCTTACTCTGGACACAGGGG 3'	**Assembly 1 REV**	**5' AGCATTCTAGCACCAGAGCAG 3'**
Position	+/	**Position**	**-/**
Length	22	**Length**	**21**
GC content	**50%**	**GC content**	**52%**
Molecular Weight	6741.4	**Molecular Weight**	**6424.2**
Melting Temperature (T_M)	**60.5**	**Melting Temperature (T_M)**	**59.8**

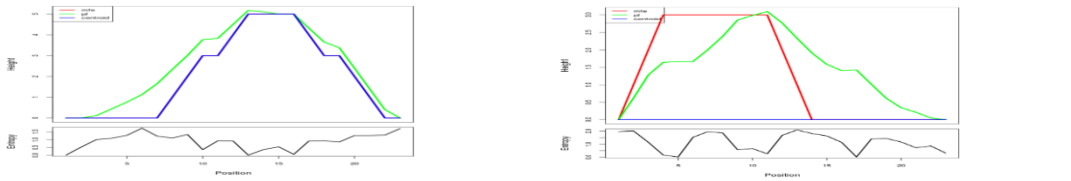
Calculation of Thermodynamic Ensemble Prediction:

The RNA folding tool from RNA World of Vienna was utilized to predict the thermodynamic stability of the designed gRNA-DNA complex concerning MFE and the thermodynamic RNA secondary structure ensemble as shown in Table 11. The expected secondary structures of gRNA were proved to be stable as indicated by the low. For the gRNA-DNA interactions, The Gibbs free energy change (ΔG) values were negative suggesting that the interaction was founded and stable. The lower ΔG values were also associated with interaction enthalpy points to a higher predicted stabilities of the gRNA-DNA complex suggesting higher CRISPR-Cas9 editome efficiency. The graphical visualization of the RNA structures went on to highlight the regions within the RNA that

could be unstable; information that could be applied to improving the design of the gRNA construct in future gene targeting schemes as shown in Figure 9. In total, the thermodynamic ensemble prediction supports the stability and efficiency of the gRNA design, which is essential for precise MIPEP activation for therapeutic purposes.

Table 11: The Free Energy of thermodynamics and the Frequency of the MFE prediction are depicted in this table.

Target No	Target RNA sequence	Free Energy of Thermodynamic	Frequency of the MFE	Ensemble Diversity
1	TTCCCTCCCAAGTTGACTAGGGG	**-5.15** kcal/mol.	**54.85** %	**5.21**
2	**CGGAAGCGGGGCCGTCACGTGGG**	**-2.88** kcal/mol	**28.12** %	**5.92**

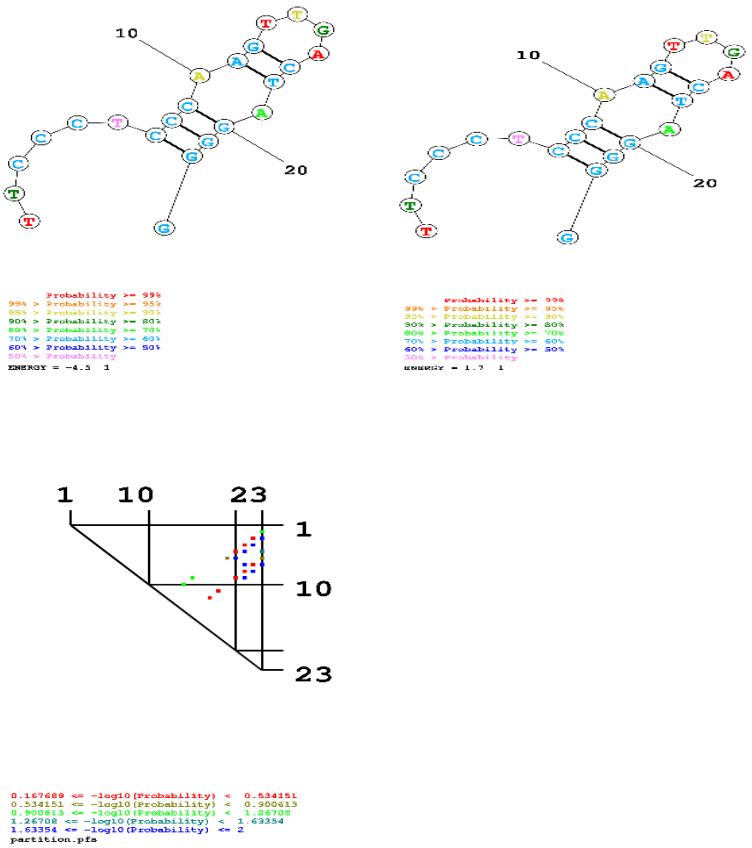


(A) (B)

Figure 9: A and B Figure depict the graphical thermodynamic free energy of gRNA-DNA complex stability. The following figure is the predicted RNA secondary structure of the designed gRNA, where the red and green colours represent the base pair probabilities of the structure stability. The MFE and ΔG define the stability of the gRNA-DNA complex; the lower ΔG numbers equal higher stability and better CRISPR-Cas9 activity. The regional overlain structures point areas demanding additional optimization to reduce misfolding and enhance GTP gRNA target DNA interaction for maximizing MIPEP activation.

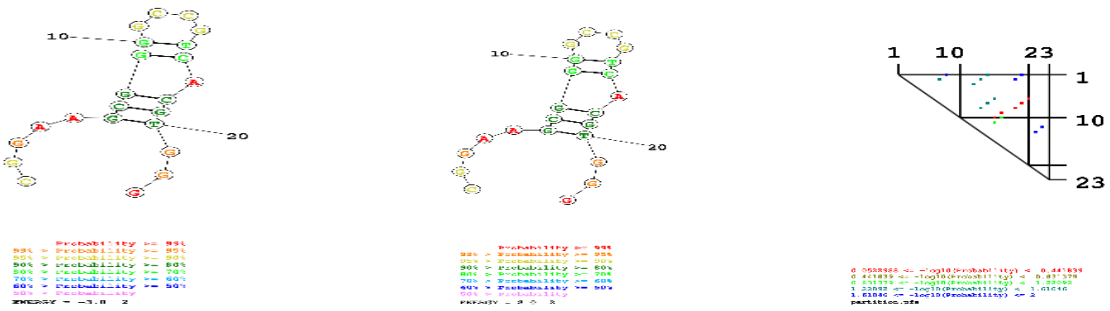
Secondary Structure Forecasting:

The secondary structure prediction of the guide RNA (gRNA) was performed using the enhanced RNA secondary structure prediction tool from Mathew's laboratory as shown in Figure 10 and Figure 11. The predicted structure plays an important role in improving the high efficacy of the CRISPR-Cas9 system because it enables the identification of any structural motifs that interfere with the gRNA binding ability to target DNA. The study showed that the designed gRNA does not have several adverse properties including hairpin loops or self-complementary features which would negatively affect its targeting ability. The thermodynamically stable folding motif allows the gRNA to adopt a structure that is least likely to cause structural interference to DNA targeting and cleavage by the highly conserved CRISPR-Cas9 effector complex.



(A) (B) (C)

Figure 10: (A) depicts the Fold secondary structure of the gRNA 1. (B) depicts the MaxExpect secondary structure of the gRNA 1 and (C) depicts the partition secondary structure of the gRNA 1.



(A) (B) (C)

Figure 11: (A) depicts the Fold secondary structure of the gRNA 2. (B) depicts the MaxExpect secondary structure of the gRNA 2 and (C) depicts the partition secondary structure of the gRNA 2.

KEGG PATHWAY and STRING for MIPEP Protein Interactions

The analysis of the molecular interactions and pathways of the MIPEP protein was carried out using molecular tools such as KEGG Pathway and STRING. The pathways showed in Figure 12

that MIPEP has a direct involvement in the process of mitochondrial precursor protein, oxidative phosphorylation and cellular energy metabolic proteins and the STRING analysis as shown in Figure 13 also corroborated with these data. Hence, the list of proteins that interact with MIPEP directly or indirectly is presented. It has been demonstrated that the MIPEP protein could bind to some other important mitochondrial proteins concerning the precursor protein import, mitochondrial biogenesis, and cellular metabolic processes. STRING also showed a focus on the relationships between proteins involved in stress and metabolism reflecting MIPEP's importance to health and diseases.

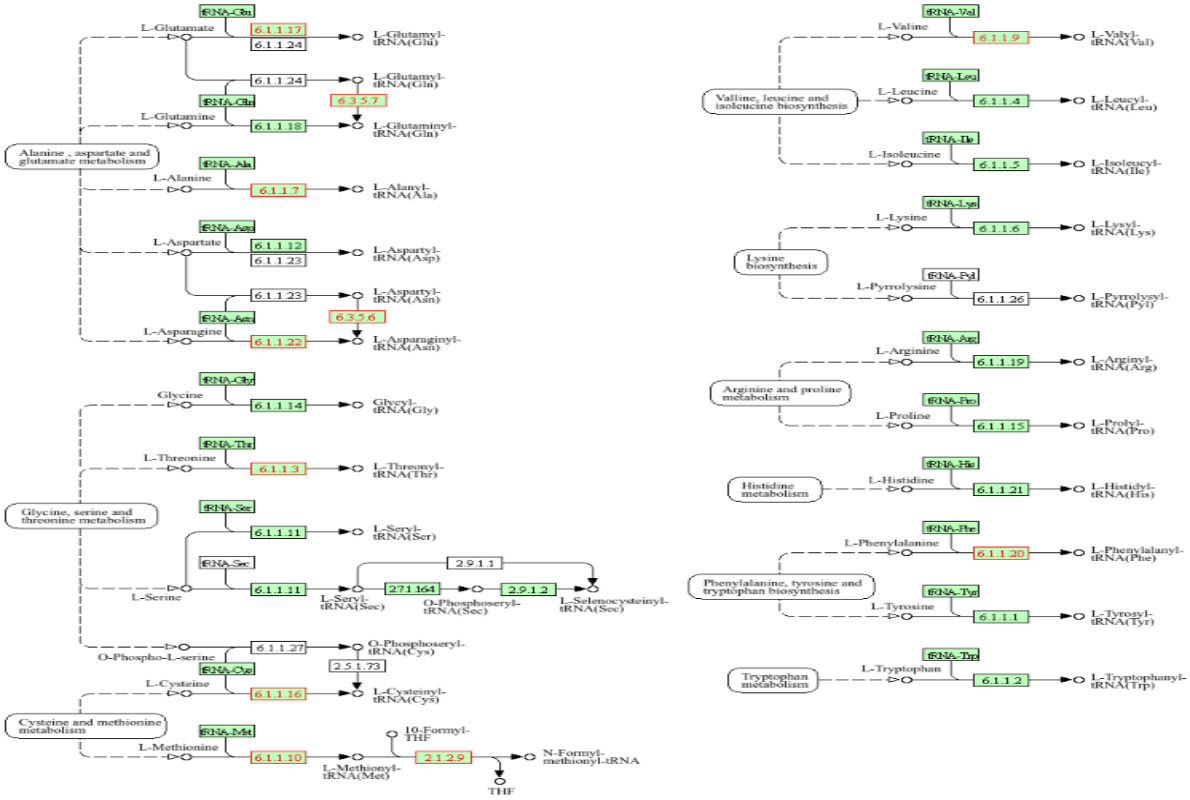


Figure 12: Increased expression of MIPEP can have tremendous benefits related to the treatment of type 2 diabetes through enhancement of mitochondrial bioactivity of those pathways. The AMPK pathway is among the key factors that are modulated through the activation of MIPEP. This pathway involved in energy homeostasis and glucose metabolism uses intermediate metabolites in the intermediary pathways such as glycine, serine, threonine metabolism and alanine, aspartate and glutamate metabolism. MIPEP affords enhanced mitochondrial efficiency and ample substrates coupled with optimal oxidative phosphorylation, which minimizes oxidative stress modifying glucose uptake; before this step, insulin sensitivity improves. Likewise, MIPEP activation sustains the integrity of mitochondria, thus promoting Akt signalling, which plays a crucial role in the cell survival process as well as insulin signalling. Valine, leucine, and isoleucine biosynthesis' L-leucine activate Akt mediated through mTOR. This, in turn, conserves the efficiency of these biosynthetic pathways, Contributor: MIPEP prevents apoptosis of pancreatic β-cells and normal glucose metabolism. Also, enhanced MIPEP can have the effect of increasing the insulin signalling pathway as well as positively regulating the path of glucose in enterocytes. Arginine and phenylalanine of arginine/ proline and phenylalanine/tyrosine/tryptophan metabolism are used for synthesizing nitric oxide and neurotransmitters, tying up metabolic health with vascular and neural functions. MIPEP supports the structural integrity of mitochondria, and guarantees the availability of ATP necessary for signaling, thus increasing glucose transport. Also, MIPEP's effects on decreasing the formation of ROS through pathways activated by Glu/Gln

guarantee the synthesis of a directly effective antioxidant – glutathione. Such moderation in the levels of oxidative stress helps to preserve the function of the β-cells necessary for the appropriate regulation of glucose levels and for the prevention of the cytotoxicity typical of T2DM. So, the upregulation of MIPEP can alleviate endothelial dysfunction and NO signalling through the metabolism of arginine. Nitric oxide (NO) is important in regulating blood flow and glucose supply to tissue. MIPEP maintains mitochondrial structure which helps to diminish oxidative impact and maintain NO synthesis and vascular function. This integrated action of MIPEP on these interacting pathways suggests that it may be a molecular target for the treatment of type 2 diabetes to increase insulin sensitivity, β-cell function and avoid the metabolic imbalance.

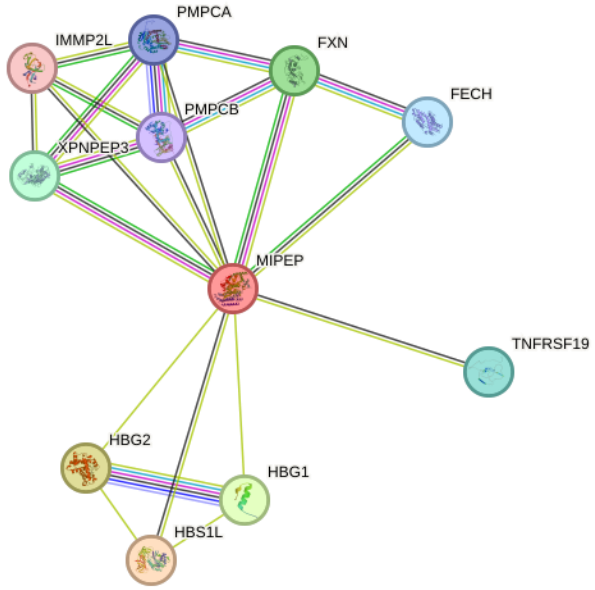


Figure 13: The MIPEP protein-protein interaction map suggests that MIPEP interacts with numerous proteins related to mitochondrial bioenergetics and cellular metabolism, as well as other age-related diseases such as T2D. It also suggests how MIPEP keeps the mitochondria in their optimal state through the proper dispatching and maturation of its retrieval proteins for efficient ATP manufacturing. This in turn decreases insulin resistance and facilitates cellular energy metabolism for the management of T2D. Since MIPEP is identified to be directly involved in primary metabolic control paths, such as the AMPK and Akt paths. Stimulation of the AMPK pathway by positively modulating the mitochondrial function and eliminating the oxidative burden promotes glucose transport and energy homeostasis. Conversely, the mitochondrial stabilizing effects of MIPEP showed positive modulation of the Akt pathway implicated in cellular survival and inflammation linked to proteins like FXN and TNFRSF19. The following pathways are important for glucose homeostasis by improving insulin signalling and β-cell survival. Moreover, MIPEP prevents oxidative stress through an effect on enzymes such as FECH related to the heme biosynthesis pathway. This decrease in ROS saves the mitochondria and avoids free radical-induced damage of the pancreatic β-cells which are vital for the synthesis of insulin. Also, by stabilizing the mitochondria, MIPEP specifically aids the β-cells to be less apoptotic and retain their insulin-secreting ability; meanwhile, HBG1 and HBG2 proteins look at broader oxygen transport and metabolic processes. Another major finding of the current study is that MIPEP is capable of reducing telomere shortening and it is known that this is associated with aging and cell stasis. They concluded that MIPEP activation ameliorates oxidative stress and mitochondrial

dysfunction and reduces metabolic decline typical of T2D and other age-related diseases. Last, MIPEP plays a part in arginine metabolism, which has an impact on nitric oxide (NO) synthesis – a molecule vital for vasculature health, glucose delivery, and metabolic homeostasis.

Discussion

The activation of MIPEP using CRISPR-Cas9 technology can be regarded as a revolution in the study of the molecular aspect of ageing and Type 2 diabetes (CICATIELLO). It is possible to use computational tools in designing and selecting gRNA targeting the MIPEP promoter region to increase its expression without affecting other regions with high efficiency. This means that the described targeted activation may contribute to slowing down the ageing processes and impeding further development of Type 2 diabetes because in both cases, the main problem is mitochondrial disorder. MIPEP is singularly important in ensuring the stability of mitochondrial structure since it is involved in the import of proteins required for energy metabolism and antioxidant defence(Ji et al., 2022). As oxidation of fatty acids plays a central role in the generation of reactive oxygen species, the activation of MIPEP can protect mitochondrial function and prevent oxidative stress, a major causal effect of cellular ageing and insulin resistance (Kunová et al., 2022). The decrease of ROS not only rescues pancreatic β-cells from oxidative stress but also maintains their function in the secretion of insulin and glucose homeostasis (Kurylowicz, 2021). Additionally, the activation of MIPEP may influence telomeres by reducing the stress within the cellular area, which can lead to telomere shortening and impact cellular ageing and other age-related decline factors. From a diabetes perspective, it is postulated that initiating MIPEP will alter such signalling pathways as AMPK and Akt. Increasing the activity of AMPK is helpful because it helps control the amount of energy in the cell, makes the mitochondria work better, and takes in more glucose (Nikpay et al., 2020). All of these things make the cell more sensitive to insulin. Simultaneously, MIPEP's regulation of the Akt pathway can preserve cells and enhance insulin sensitivity, addressing the primary issues in the development of type 2 diabetes (Otani et al., 2023). Altogether, the described molecular and cellular mechanisms provide a perspective for the poly-pronged intervention on diabetes progression and age-related metabolic decline. This computational CRISPR-Cas9 approach underscores the potential of precision medicine in addressing polygenic and polyetiological disorders such as diabetes and ageing (Santovito & Weber, 2022). It means that advanced bioinformatics tools could be used to fine-tune mitochondrial pathways to find a basis for a treatment for metabolic diseases and ageing. Therefore, MIPEP activation not only supports the objectives of ageing and diabetes research but also provides possibilities for the utilization in other anti-ageing and anti-ageing disease-related diseases through the improvement of mitochondrial function (Souza et al., 2023). Therefore, MIPEP activation not only supports the objectives of ageing and diabetes research but also provides possibilities for its utilization in other anti-ageing and disease-related diseases through the improvement of mitochondrial function. Therefore, the activation of MIPEP pathways holds significant theoretical value in regulating metabolic health signalling pathways, such as the well-known AMP-Activated Protein Kinase (AMPK), Akt, and insulin signalling pathways. AMPK is an energy status reporting subunit in cells, and activation of this system is crucial in energy homeostasis. It is proposed that activation of MIPEP increases AMPK which promotes mitochondrial biogenesis as well as glucose uptake while decreasing oxidative stress. These effects acting in synergy improve insulin sensitivity, which is important in the management of type 2 diabetes(Sun et al., 2018). Based on the enhancement of AMPK activity, MIPEP may help maintain cellular adaptability that helps avoid the dysfunction in β-cells and conserve insulin production capability. The Akt pathway, also known as Protein Kinase B, serves as the primary signalling pathway for insulin and is another target of PTEN. It is thought that activating MIPEP increases Akt phosphorylation to protect pancreatic β-cells from oxidative stress and cell death. Akt signalling could enhance glucose metabolism and

protect β-cells stressed in the context of ageing and diabetes. These results suggested that MIPEP might help fix hyperglycemia and its effects because it controls Akt signalling and helps the body use glucose. Furthermore, the enhancement of mitochondrial function by MIPEP may have a direct impact on insulin-related signalling pathways. The optimal function of mitochondria is required for ATP synthesis, which is necessary for insulin granule release. Thus, MIPEP may maintain the proper function of mitochondria and guarantee the periphery tissues' sensitivity to insulin and appropriate insulin release to prevent systemic insulin resistance. Because MIPEP can lower ROS levels even more, it protects these pathways from oxidative stress, which is known to mess up insulin signal transduction. Through the integration of these related signalling pathways, activation of MIPEP provides a treatment strategy with multiple facets for enhancing insulin sensitivity and glucose control. These changes in AMPK, Akt, and insulin signalling suggest that it could be useful not only for preventing and controlling diabetes but also for improving other metabolic problems that come with getting older. Such a mechanistic perspective may help inform the precision medicine intervention approach for other age-related metabolic diseases. Although this work demonstrates an in silico method of targeting the MIPEP gene with CRISPR-Cas9, there are some issues to be considered for the potential application. The general use of in-silico studies, which rely on computer simulation and prediction, poses a significant constraint as it may not always accurately represent biological processes. Even though the designed gRNAs and components of CRISPR-Cas9 may look efficient in silico, their efficiency in the biological environment has its merits evaluated. That is why the laboratory evaluation of the candidates is crucial since the gene expression, cellular environment, and off-target effects might be quite different from in silico data. Similarly, the CRISPR-Cas9 technology has its pros and cons and potential ethical issues that are associated with it. Even though there are better efforts to reduce the unwanted effects of powerful CRISPR/Cas9 tools, these effects are still known to be a big problem because they can cause mutations that can mess up genes or regulatory regions that aren't the target. Such changes could have impacts on the cellular processes or could lead to side effects, especially for healing practices. Such changes could have impacts on cellular processes or lead to side effects, especially for healing practices (Zhang et al., 2024). Further, the long-term effects of gene activation through CRISPR-Cas9 are yet unknown in terms of overexpression or MIPEP dysregulation, which can cause some metabolic or cellular derangement. Moreover, the direct delivery of the CRISPR-Cas9 system with high efficiency and minimal toxicity to target cells, especially in vivo, remains a major challenge (Göransson et al., 2011). This remains an important consideration since most current delivery methods, including viral and non-viral vectors, differ concerning their efficiency, tissue specificity, and safety or toxicity (Hassan-Smith, 2014). Efficient adaptation of these delivery mechanisms is central to the effective use of this technology in therapy. Finally, translating this approach from proof-of-concept studies to clinical therapies is a problem of regulatory, ethical, and cost-related concerns that may impede the adoption and availability of the technologies. These observations emphasize the need for supplementing in silico studies with experimental confirmation and detailed safety assessment together with other in silico work to determine the applicability and efficiency of this approach in treating ageing and type 2 diabetes.

Conclusion

Interventions involving the gene, MIPEP, can be activated via CRISPR-Cas9 to treat aging as well as diabetes, according to the research. The use of MIPEP activation has begun to hold out the possibility of stabilizing mitochondria, which is vital for maintaining cellular balance and avoiding metabolic disorders. The research describes an understanding of the improvement of MIPEP expression that can increase mitochondrial bioenergetics, decrease oxidative stress, and prevent cell damage in pancreatic β-cells. These mechanisms go after deep aspects of ageing, such as mitochondrial breakdown and telomere loss, which are key factors in the development of ageing-

related diseases, such as type 2 diabetes. MIPEP activation may change the AMP-Activated Protein Kinase (AMPK) and Akt pathways to improve mitochondrial function, increase glucose uptake, and protect the β-cell. These effects in combination enhance insulin sensitivity and glucose homeostasis, address major factors in diabetes development, and lessen cellular ageing. Moreover, the in silico approaches used in the present work presents a solid basis for assessing the molecular mechanisms involved in MIPEP activation and suggest that this protein is involved in a variety of processes related to metabolic status and ageing. In summary, this research also provides evidence for the concept of MIPEP activation as an enticing new anti-ageing and anti-diabetic approach in addition to expanding knowledge of mitochondrial function and its application. Computational tools applied to CRISPR-Cas9 offer a new direction in precision medicine to counter the multifactorial aspects of age-related metabolic disorders. However, the research result suggests that MIPEP activation might be a therapeutic approach to treating the molecular mechanisms of ageing and age-related diseases, including diabetes type 2. The paper shows, using computational methods and through the lens of MIPEP, how it is involved in the regulation of key aspects of cellular ageing, including maintenance of mitochondrial homeostasis, control of oxidative stress, and prevention of telomere shortening. This approach not only expands our knowledge of the relationship between mitochondrial function and ageing but also highlights the need for the treatment of specific signalling pathways such as AMPK and Akt to increase metabolic health and insulin sensitivity. The prospects include conducting the utilization of these in silico predictions by performing additional in vitro and in vivo experiments. The prospects include conducting and utilizing these in silico predictions by performing additional in vitro and in vivo experiments. We could direct experimental work towards bolstering the hypothesis that MIPEP activation offers protection against disintegrated mitochondria, declining insulin-positive cells, and metabolic dysfunction. Additionally, it is fascinating to wonder about the potential of the pill regarding telomere activity analysis in human and animal models to have a better understanding of its functioning connected with ageing cellular processes. We are optimistic regarding the clinical opportunities in precision medicine; MIPEP activation may provide the basis for designing personalized treatments against age-related diseases. If treatments are tailored according to the state of one's mitochondria and genes, this work may open the door to new ways of influencing the ageing process and better control of metabolic disorders such as diabetes. Combining progressive gene-editing technologies including CRISPR-Cas9 improves the possibility of applying these discoveries into clinical treatments, promoting the improvement of age-related diseases.

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Conflict of interest Statement:
The Authors declare no conflicts of interest
Data Availability Statement:
All relevant data are within the paper.
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