To be or not to be: "Identification of the human Sphingomyelin phosphodiesterase 5"

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Abstract

Sphingolipids represent a major class of lipids that are important components of membranes in eukaryotic cells. There is little data regarding the human sphingomyelinase (SMPD5), which is believed to be associated with mitochondrial at mitochondria ER-associated membranes (MAM). Although, the gene has been annotated, there is no experimental evidence of its transcription and translation. Based on the available data published online, we aimed to investigate the existence of SMPD5 isoform in the human genome in order to understand it role at (MAM) and in the context of Alzheimer disease (AD). The expression of SMPD5 was investigated through molecular biology techniques such as RNA purification, cDNA synthesis, PCR, and qPCR. After further testing, we still not able to concluded that SMPD5 was not transcribed in the cell, which open up new possibilities that SMPD5 indeed is present in human as described in mice.

Introduction

- Alzheimer's disease (AD), the most common neurodegenerative disorder, is characterized by neuronal loss in the brain and alterations in metabolic processes, including perturbed mitochondrial function and changes in lipid metabolism.
- The field is trying to find the link between genetic mutations in genes associated with AD and alterations in lipid homeostasis. Specifically, there is an increase in the activity of sphingomyelinase (SMase), which hydrolyzes sphingomyelin (SM) into ceramide.
- The processing of APP (a protein that, when mutated, causes AD) occurred at an intracellular lipid raft domain that is called mitochondria-associated ER membranes (MAM). Interestingly, sphingomyelinase activity is upregulated in MAMs.
- We aimed to measure the expression the MAM associated sphingomyelinase (SMPD5) which has not been characterized in human and in the context of AD.
- Before purifying RNA from HeLa cells and confirming its quality, we tested our designed primers on genomic DNA. To this end, we performed PCR to amplify DNA fragment containing exonic and intronic regions of SMPD5. With two working primer combinations, we performed PCR using cDNA, synthesized from human RNA.
- Agarose gel electrophoresis and sequence alignments allowed us to confirm that we had a DNA sequence that showed the expected size corresponding with one exonic region of SMPD5 mRNA. However, after analyzing the genomic sequence, we were unable to find the presence of polyadenylation sites and with further molecular tests, we still not sure if SMPD 5 was transcribed.
- The results prompt us to find new methods of investigating the expression of SMPD5 in humans in the near future.

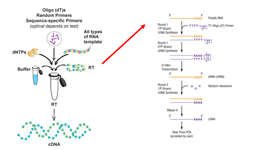
Methods

1. Genomic DNA: was used to try designed primers

F1	CGTTGCTGTACGACGTGGGCAC
R1	CGTGCAGTGCAGGAATCC
F2	TCCGTGCTCCACCAGTCC
R2	TCGTGTCTGTGCGCGCCAGC
F3	GCAACAGTGGACCCACAG
R3	CATGGTACACCTAGCTCTTC
F4	GAGGCAGCGCACACTGTTCG
R4	GTGCATCCGCGCGAGCTG

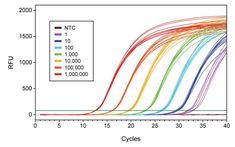
2. RNA extraction and purification

3. cDNA Synthesis



4. Polymerase chain reaction (PCR)

5-Quantitative PCR (qPCR)



Results 1

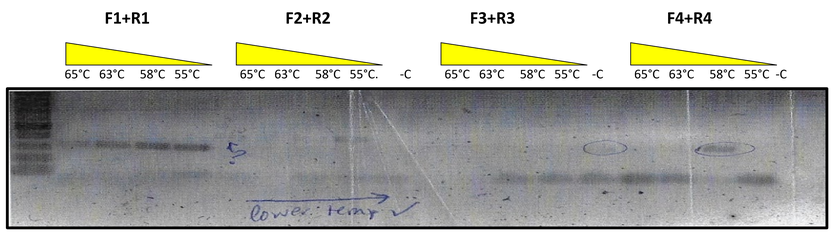


Figure 1. First test of primers on a gradient of 65.0°C to 55.7°C. Note that F1+R1 product was detected at the expected size at all temperatures.

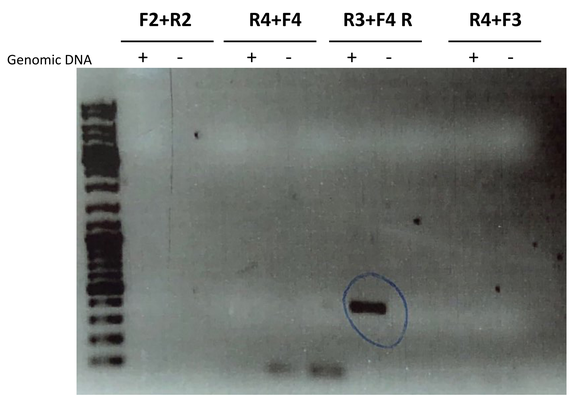


Figure 2. Gel showing the result of retesting F2R2 and F4R4, as well as, trying out combinations of primers at a temperature of 55.0°C. These results suggested that F4 might on genomic DNA.

Results 3

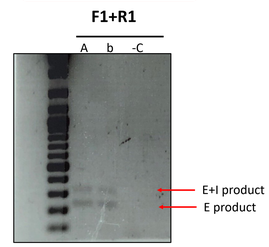


Figure 4. PCR on cDNA Template

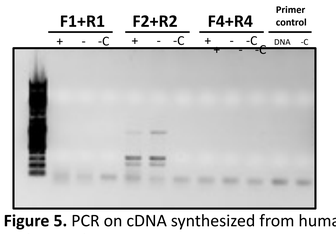


Figure 5. PCR on cDNA synthesized from human RNA digested with or without Dnase.

Figure 6. PCR on cDNA synthesized from human RNA digested with or without Dnase.

Results 2

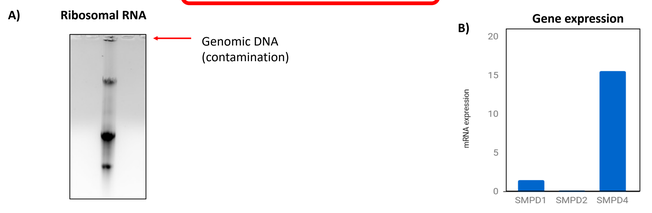


Figure 3. RNA analysis. A) RNA quality and B) Sphingomyelinase isoforms expression using real time qPCR. In A we observed that the RNA quality was preserved by detecting ribosomal RNA. In B we have detected the expression of other SMAse isoforms in our sample.

Discussion

Our results suggest that SMPD5 is transcribed and in human cells.

The results using the F1R1 primers, implied that SMPD5 was being transcribed, because we detected two bands, one showing the size shown at genomic DNA, containing both introns and exons, as well as cDNA, which incorporates only exonic sequences. However, when we replicated this and treated the RNA samples with DNase to make sure we were only amplifying exonic sequences, we did not observe any band. This result suggests that the DNAse enzyme was not properly stopping in our samples during cDNA generation. Thus, we need to perform more tests to make sure if our negative results are accurate or not. This hypothesis is also supported by the the results obtained in Figure 6 when we tested the expression of SOD1 in the same samples, not amplification was found in RNA samples treated with DNAse.

If we do succeed in identifying the human SMPD5, we will clone more SMPD5 which will be an invaluable tool for the lab and in the field of Alzheimer disease

Acknowledgements

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