# Characterizing The Functions of The Novel NME1-NME2 Readthrough Transcript

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## Introduction

Novel readthrough transcript NME1-NME2 has been identified as being significantly overexpressed in inflamed gingival tissues of children with aggressive form of periodontitis by RNA library sequencing. A readthrough transcript occurs during transcription when the original termination site was not correctly identified and transcription continues. The eight-fold overexpression of the NME1-NME2 readthrough transcript in inflamed tissues is perplexing and needs further investigation for its role in inflammation.

## Objective

This study aims to understand the role(s) of the NME1-NME2 readthrough transcript in inflammation and other pathologic conditions.

## Methods

A full-length cDNA representing the novel NME1-NME2 readthrough transcript was cloned into pCMV6 Plasmid vector with a Myc-DDK tag. This plasmid was stably transfected into HEK cells and total RNA from transfected and wild type HEK cells was harvested and sequenced. U2OS cells that express TLR 4 receptors were also stably transfected with the NME1-NME2 readthrough plasmid and challenged with LPS (1µg/ml) for 24h. Total RNA was extracted and analyzed by RT-PCR for effects of NME1-NME2 on the expression of proinflammatory cytokine IL-6 upon LPS stimulation. Immunofluorescence microscopy of transfected cells and immunohistochemistry of inflamed and healthy gingival tissues were also carried out.

## Results

Table I: Genes Upregulated & Downregulated by NME1-NME2 Readthrough Transcript

| Upregulated | | | Downregulated | | |
|---|---|---|---|---|---|
| *Gene Name* | *Q Value* | *Log2FC* | *Gene Name* | *Q Value* | *LogFC* |
| MAOA | 7.15E-80 | 3.45 | HSP1A1 | 3.16E-29 | -2.09 |
| NEFL | 1.28E-55 | 2.51 | LCP1 | 1.39E-23 | -1.58 |
| BEX5 | 7.24E-41 | 5.70 | ESRP2 | 3.55E-16 | -1.69 |
| PPP1RC | 1.98E-36 | 1.35 | IDL1 | 1.16E-13 | -1.19 |
| CHAC1 | 6.05E-36 | 2.62 | CGA | 1.69E-12 | -1.38 |
| MAGED1 | 9.76E-20 | 1.46 | PCK1 | 1.65E-10 | -1.56 |
| SLC43A1 | 3.76E-14 | 1.36 | AC099329.1 | 3.32E-08 | -9.43 |
| MAL2 | 8.31E-14 | 1.25 | RMRP | 3.65E-07 | -1.96 |
| PTN | 2.24E-13 | 1.50 | RN7SL1 | 2.71E-06 | -1.00 |
| PDCD | 1.04E-11 | 1.12 | RPL7AP66 | 2.71E-06 | -1.73 |
| PSMB1 | 6.27E-11 | 1.15 | NR4A2 | 5.42E-05 | -1.01 |
| RIPK4 | 2.10E-10 | 1.19 | ZEB1 | 1.09E-05 | -1.06 |
| EPCAM | 5.80E-10 | 1.49 | SYP | 2.77E-05 | -1.06 |
| DDIT3 | 6.02E-09 | 1.19 | RN7SK | 0.002 | -1.19 |
| SNCG | 7.49E-07 | 1.57 | | | |
| CEBPB | 9.64E-07 | 1.10 | | | |
| XBP1 | 1.95E-05 | 1.03 | | | |
| WFDC2 | 4.11E-05 | 1.59 | | | |
| SH3BGR | 0.0004 | 1.05 | | | |
| FBXO2 | 0.0008 | 1.24 | | | |
| SMIM11B | 0.0008 | 1.02 | | | |
| CFD | 0.0010 | 1.12 | | | |

## Results

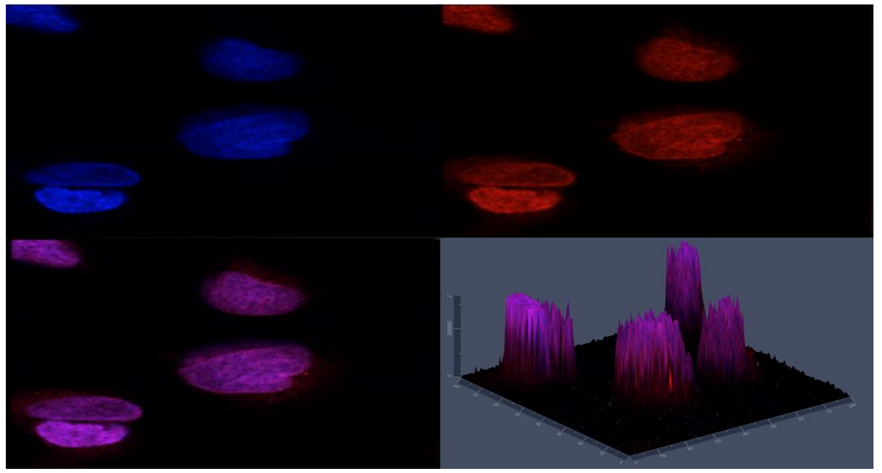

Figure 1: Immunofluorescence Images of U20S cells transfected with NME1-NME2 plasmid and stained with rabbit mab to FLAG tag. The NME1-NME2 protein appears predominantly in the Nucleus with trace amounts in the perinuclear cytoplasm.

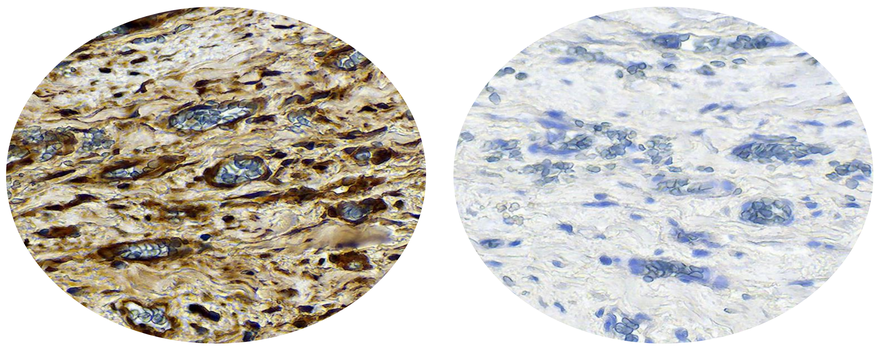

Figure 2: Immunohistochemistry of Inflamed gingival tissues showing enhanced expression of NME1-NME2 protein in microvascular endothelial cells.

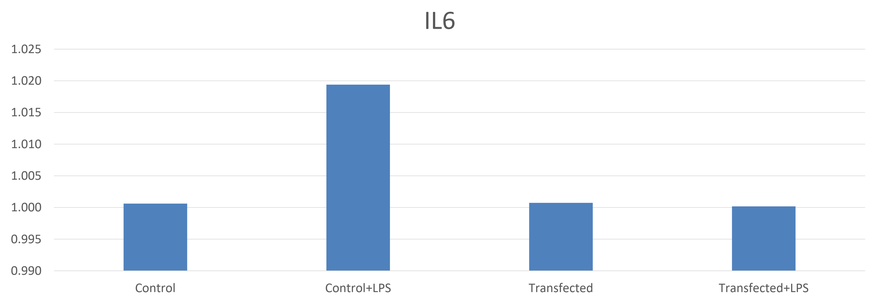


Figure 3: Preliminary RT-PCR results of U2OS transfected and wild type cells stimulated with LPS for 24. Primers for human IL-6 were used to assess expression levels. This preliminary experiment shows NME1-NME2 transfected cells showing suppressed IL-6 levels. These RT-PCR experiments are still in progress.

A total of 466 genes were differentially expressed (DE) in cells transfected with NME1-NME2 readthrough transcript compared to the wild type controls. Many of the upregulated genes were directly or indirectly involved with inflammation. Some of these dealt with oxidative stress while others were inhibitors of protein phosphatase activity. Finally, there were transcription factors that regulated inflammation (Table I). NME1-NME2 overexpressing cells when challenged by LPS showed some inhibitory effect on IL-6 expression (Fig.3). Immunofluorescence analysis of transfected cells showed NME1-NME2 protein located mostly in the nucleus with trace amounts in the cytoplasm (Fig. 1).

## Discussion

Table II: IPA of Genes Regulated by NME1-NME2 Readthrough Transcript

| Ingenuity Pathway Analysis of Genes Regulated by NME1-NME2 Readthrough Transcript | | |
|---|---|---|
| *Diseases & Disorders* | | |
| Name | p-value range | # Molecules |
| Connective Tissues Disorders | 2.36E-03 – 7.72E-10 | 84 |
| Inflammatory Disease | 2.64E-03 – 7.72E-10 | 88 |
| Inflammatory Response | 2.92E-03 – 7.72E-10 | 79 |
| Organismal Injury and Abnormalities | 3.15E-03 – 7.72E-10 | 388 |
| Skeletal and Muscular Disorders | 2.36E-03 – 7.72E-10 | 99 |
| *Molecular & Cellular Functions* | | |
| Cellular Movement | 3.18E-03 – 2.56E-08 | 101 |
| Cellular Development | 3.11E-03 – 1.42E-06 | 107 |
| Cellular Growth and Proliferation | 3.11E-03 – 1.42E-06 | 102 |
| Cellular Assembly and Organization | 2.40E-03 – 1.42E-06 | 25 |
| Cellular Function and Maintenance | 2.91E-03 – 1.42E-06 | 15 |

## Conclusions

This study is ongoing and preliminary observations indicate NME1-NME2 readthrough transcript is a fully translated protein that shows prominent location in the nucleus and maybe serving important regulatory role(s) in inflammation.

## Acknowledgments

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## References

1) Gemma S Puts, M Kathryn Leonard, Nidhi V Pamidimukkala, Devin E Snyder, David M Kaetzel, Nuclear functions of NME proteins, Laboratory Investigation, Volume 98, Issue 2, 2018, Pages 211-218, ISSN 0023-6837, https://doi.org/10.1038/labinvest.2017.109.