




ORIGINAL ARTICLE

NEAT1 and MALAT1 are highly expressed in saliva and nasopharyngeal swab samples of COVID-19 patients

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Funding information

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Projetos Individuais no Combate à Covid-19 (Proind 2020); Conselho Nacional de Desenvolvimento Científico e Tecnológico

Abstract

COVID-19, caused by the SARS-CoV-2 virus, has become a significant global public health problem, with a wide variety of clinical manifestations and disease progression outcomes. lncRNAs are key regulators of the immune response and have been associated with COVID-19 risk infection. Previous studies focused mainly on in-silico analysis of lncRNA expression in the lungs or peripheral blood cells. We evaluated the expression of lncRNAs NEAT1, MALAT1, and MIR3142 in saliva and nasopharyngeal swab from SARS-CoV-2 positive (n = 34) and negative patients (n = 46). A higher expression of the lncRNAs NEAT1 and MALAT1 ($p < 0.05$) were found in positive samples. NEAT1 had a higher expression mainly in saliva samples ($p < 0.001$), and MALAT1 was upregulated in nasopharyngeal samples ($p < 0.05$). Area under the ROC curve for NEAT1 in saliva was 0.8067. This study was the first to investigate the expression of lncRNAs in saliva and nasopharyngeal samples of COVID-19 patients, which gives new insights into the initial response to infection and infectivity and may provide new biomarkers for severity and targets for therapy.

KEYWORDS

COVID-19, lncRNA, MALAT1, nasopharyngeal samples, NEAT1, saliva, SARS-CoV-2

1 | "HOT TOPICS"

lncRNAs (long noncoding RNAs) are transcripts larger than 200 nucleotides in length that do not appear to have protein-coding potential (Cabili et al., 2011), although some of them may produce small functional peptides (Choi et al., 2019). Further to complex regulation in multiple cell processes, lncRNAs are emerging as critical regulators of immune responses (Bocchetti et al., 2021; Heward & Lindsay, 2014), and some may control innate and adaptive cell types (Chen et al., 2017).

Antiviral immune responses are also influenced by lncRNAs (Liu et al., 2020; Ouyang et al., 2016; Qiu et al., 2018), but information regarding a role in COVID-19 patients is limited.

In 2020, Vishnubalaji et al. performed a more in-depth analysis of transcriptome data from primary normal human bronchial epithelial cells during SARS-CoV-2 infection and lung biopsies derived from COVID-19 patients. They included the lncRNA transcriptional portrait in response to SARS-CoV-2 infection in lung cells. Among the regulated lncRNAs were NEAT1, MALAT1, and miR3142HG all of

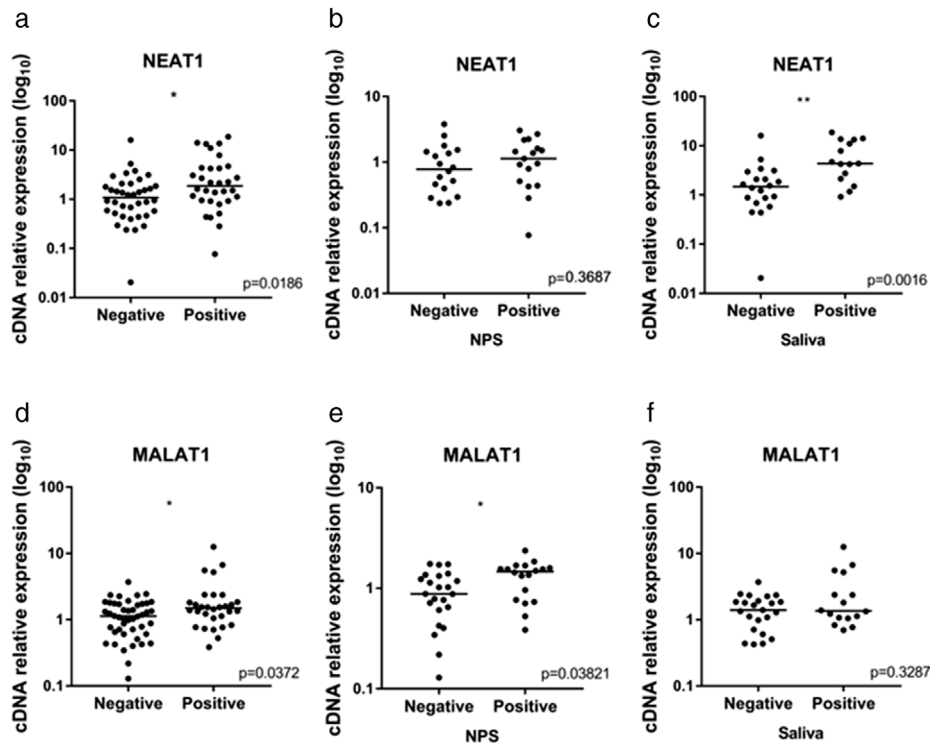


FIGURE 1 Relative expression levels of lncRNAs in negative and positive samples for SARS-CoV-2. (a) NEAT1 expression including nasopharyngeal swab (NPS) and saliva samples. (b) NEAT1 expression in NPS samples. (c) NEAT1 expression in saliva samples. (d) MALAT1 expression including NPS and saliva samples. (e) MALAT1 expression in NPS samples. (f) MALAT1 expression in saliva samples. * $p < 0.05$ and ** $p < 0.01$, Mann-Whitney test

which have been previously associated with immune response and viral infection.

Considering the relevance of saliva and nasopharyngeal cells in infectivity and initial SARS-CoV-2 infection, we analyzed the expression of those lncRNAs in nasopharyngeal swab (NPS) and saliva samples from 41 individuals (39 NPS/saliva paired samples, 2 NPS without saliva counterpart). The National Research Ethics Committee approved the study (CAAE: 31687620.2.0000.0096), and samples were collected from healthcare workers in a tertiary public hospital in Curitiba, Brazil, after obtaining written informed consent. The samples were designated according to SARS-CoV-2 detection by RT-qPCR as “negative” ($n = 23$ individuals and 46 samples) or “positive” ($n = 18$ individuals and 34 samples). All positive samples were wild type SARS-CoV-2, using genotyping methodology previously described (Adamoski et al., 2021). The median age of the negative group was 40 (± 10.9) and 41.5 (± 10.3) for the positive samples. For NEAT1, MALAT1, and miR3142HG lncRNAs expression, RNA was extracted from 200 μ L of sample with Trizol Reagent (Invitrogen). A total of 70 ng of total RNA was treated with DNase I (1U), and the absence of DNA contamination was confirmed by a retrotranscription (RT) minus reaction. For RT, the Superscript III kit (Invitrogen) was used. qPCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) and 10 nM of each primer. The relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method with two internal controls (B-actin/RNU6).

Positive samples for SARS-CoV-2 exhibited a significant upregulation of the lncRNAs NEAT1 and MALAT1 ($p < 0.05$) (Figure 1). For NEAT1, this difference was observed mainly in saliva samples ($p < 0.001$), when comparing only NPSs from negative and positive samples, this difference was not observed. In contrast, MALAT1 was observed with higher expression only in NPS positive samples ($p < 0.05$), when considering only saliva samples, no difference was observed. MIR3142HG expression was detected in less than 17.5% of the samples analyzed and it was removed from further analysis.

When analyzing paired samples, including NPS and saliva from the same patient, NEAT1 showed a higher expression in saliva samples, when comparing negative and positive samples together ($p < 0.001$), only negative ($p < 0.05$), and only positive samples ($p < 0.002$). The expression of MALAT1 was also higher in saliva samples, but this difference was only observed among all (negative and positive) samples ($p < 0.001$) and negative samples only ($p < 0.001$). As swab positive samples had high MALAT1 expression, differences between swab and saliva samples were not evidenced among positive individuals.

For determining the potential of both lncRNAs as possible biomarkers for the infection, receiver operating characteristic (ROC) curves were calculated. The specificity was significant for MALAT1 in NPS and NEAT1 in saliva. The AUC was 0.8067 ($p < 0.001$) for NEAT1, showing a high power for distinguishing positive and negative saliva samples. A significant positive correlation was also observed, by Spearman's

correlation test, between NEAT1 and MALAT1 expression in both negative and positive samples ($r = 0.48$, $p < 0.01$).

In summary, this study observed a differential expression of NEAT1 and MALAT1 lncRNAs comparing infected and noninfected individuals with SARS-CoV-2 in saliva or NPS samples. For NEAT1, this difference was observed mainly in saliva samples. In contrast, MALAT1 high expression was prominent in positive nasopharyngeal samples.

Both lncRNAs have been associated with immune system responses. NEAT1 is an inflammatory regulator that promotes activation of inflammasomes in macrophages (Zhang et al., 2019) and induces inflammatory cytokines such as interleukin-6 (IL-6) and CXCL8 (Tang et al., 2020). IL-6 and the NLRP3 inflammasome are primary immune components in responses to SARS-CoV-2 infection (Paniri & Akhavan-Niaki, 2020). MALAT1 also exerts various immunological effects including the mediation of NLRP3 inflammasome activation (Menon & Hua, 2020; Yu et al., 2018). Through NF- κ B and HIF-1 α activation, MALAT1 increases production of inflammatory cytokines, such as IL-6 and TNF- α , to promote inflammatory cell infiltration and tissue damage (Tian et al., 2018). Furthermore, MALAT1 has been linked to M1-like activity in macrophages, promoting pulmonary inflammation and injury (Cui et al., 2019).

Most recent studies investigated lncRNA expression in lung cells or in the peripheral blood of COVID-19 patients, and we did not find any analyses of saliva or nasopharyngeal cells. Vishnubalaji et al., in 2020, generated lncRNAs transcriptome data from SARS-CoV-2 infected bronchial epithelial cells and demonstrated upregulation of NEAT1 and MALAT1. Elevated expressions of these lncRNAs were also shown in bronchoalveolar lavage fluid from COVID-19 patients (Moazzam-Jazi et al., 2021). Additionally, in peripheral blood mononuclear cells, increased NEAT1 expression was found in severe COVID-19 patients compared to moderate patients and healthy individuals (Tang et al., 2020). Also, by a single-cell transcriptomic analysis in the respiratory tract and peripheral blood, NEAT1 and MALAT1 were differentially expressed between mild and severe patients (Huang et al., 2021).

SARS-CoV-2's first entry is through mouth and nose cells, and differential expression in these sites may be associated with an initial response to infection, infectivity, viral dosage, and clinical association. The present study presents NEAT1 and MALAT1 high expression in saliva and nasopharyngeal samples in SARS-CoV-2 positive individuals, and these results contribute to a better understanding of COVID-19. NEAT1 and MALAT1 are important components of antiviral immune responses and may provide, in the future, new targets for severity-related diagnostic measures or therapy.

ACKNOWLEDGMENTS

This work was supported by the: PROPLAN/Federal University of Parana, FINEP-Funder of Studies and Projects, Ministry of Science, Technology and Innovation-Brazil, CAPES (001-Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico). We are grateful to the Setor de Ciências Biológicas of the Federal University of Parana and all team of volunteers at SCB-UFPR COVID-19 team.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/omi.12351>

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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How to cite this article: Rodrigues, A. C., Adamoski, D., Genelhould, G., Zhen, F., Yamaguto, G. E., Araujo-Souza, P. S., Nogueira, M. B., Raboni, S. M., Bonatto, A. C., Gradia, D. F., & Carvalho de Oliveira, J. (2021). NEAT1 and MALAT1 are highly expressed in saliva and nasopharyngeal swab samples of COVID-19 patients. *Molecular Oral Microbiology*, 36, 291–294. <https://doi.org/10.1111/omi.12351>