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BRIEF COMMUNICATION

Predicting the response of the dental pulp to SARS-CoV2 infection: a transcriptome-wide effect cross-analysis

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Abstract

Pulpitis, inflammation of the dental pulp, is a disease that often necessitates emergency dental care. While pulpitis is considered to be a microbial disease primarily caused by bacteria, viruses have also been implicated in its pathogenesis. Here, we determined the expression of the SARS-CoV2 receptor, angiotensin converting enzyme 2 (ACE2) and its associated cellular serine protease TPMRSS2 in the dental pulp under normal and inflamed conditions. Next, we explored the relationship between the SARS-CoV-2/human interactome and genes expressed in pulpitis. Using existing datasets we show that both ACE2 and TPMRSS2 are expressed in the dental pulp and, that their expression does not change under conditions of inflammation. Furthermore, Master Regulator Analysis of the SARS-CoV2/human interactome identified 75 relevant genes whose expression values are either up-regulated or down-regulated in both the human interactome and pulpitis. Our results suggest that the dental pulp is vulnerable to SARS-CoV2 infection and that SARS-CoV-2 infection of the dental pulp may contribute to worse outcomes of pulpitis.

Introduction

SARS-CoV2 is a highly aggressive coronavirus, which has infected over 6 million people to date [1]. Most infections (81%) produce only mild symptoms or are asymptomatic. Fifteen percent of infections are severe and require hospitalization. Transmission of this disease is produced by asymptomatic carriers, symptomatic patients, as well patients who are in the incubation period. Human-to-human transmission occurs via close contact of respiratory droplets, direct contact with infected individuals, or by contact with contaminated objects and surfaces.

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Viral entry into the target cells requires angiotensin converting enzyme 2 (ACE2) and its associated cellular serine protease TPMRSS2 [2]. ACE2, a negative regulator of the renin-angiotensin system, functions as the key SARS coronavirus receptor and stabilizer of neutral amino acid transporters [3]. Viruses found to use this protein for cell entry include Influenza virus and the human coronaviruses HCoV-229E, MERS-CoV, SARS-CoV, and SARS-CoV-2. It is postulated that the pattern of expression of ACE2 in human respiratory epithelia and oral mucosa explains the rapid human–human transmission [4]. TMPRSS2, a serine protease, facilitates entry of viruses into host cells by proteolytically cleaving and activating viral envelope glycoproteins. While a number of studies have examined the expression of ACE2 and TPMRSS2 in various tissues, their expression in the dental pulp is yet to be examined.

Pulpitis, inflammation of the dental pulp, is a disease that often necessitates emergency dental care. Over 90% of dental emergency visits are due to pulpitis pain [5]. In the United States alone, over 22 million procedures are performed annually to treat diseased dental pulps [6]. While pulpitis is considered to be a microbial disease primarily caused by bacteria [7], viruses have also been implicated in its pathogenesis [8]. The complex interaction between the 31 SARS-CoV2 and human proteins has been recently

reported [9], but the correlation between this interactome and pulpitis is yet to be explored.

Here, we first used existing gene expression data (53,000 genes) from our previously published study on pulpitis (GEO NCBI accession number GSE77459; ID: 20007745) [10]. Then, with the data and methods used in a recently published study on the transcriptome-wide effects on coronavirus infection in human cells [11], a master regulator analysis (MRA) [12] was performed to identify genes whose expression values are correlated (upregulated or downregulated) in both the human interactome [11] and pulpitis datasets [10]. With these techniques, we endeavored to answer two important questions: how would the dental pulp respond to SARS-CoV2 infection, and how would ACE2 and TPMRSS2 expression change during SARS-CoV2 infection?

Results and discussion

We identified 75 relevant genes whose expression values are either upregulated or downregulated in both human interactome and pulpitis datasets (Table 1). Of particular interest amongst these genes are ACE2 and TMPRSS2, which were shown to be exploited by SARS-CoV-2 for cell entry and for spike (S) protein priming, respectively [9].

In the human dental pulp, both ACE2 and TMPRSS2 are expressed consistently in biopsies of normal and inflamed tissues (Fig. 1a, b). The expression levels of both ACE2 and TMPRSS2 remain unchanged under conditions of inflammation. These findings are similar to expression patterns in other tissues. For example, both of these viral entry molecules are expressed in the ileum and colon and, expression levels in mucosal biopsies of these tissues do not differ between patients with active Irritable Bowel Disease and those of control patients [13]. Similarly, the expression level of ACE2 in lung tissues does not differ between biopsies taken from patients with chronic respiratory diseases such as chronic obstructive pulmonary diseases and asthma compared to those from healthy volunteers [14].

It is important to note that the consistent expression of both ACE2 and TMPRSS2 in the dental pulp confers its vulnerability to SARS-Cov2 infection. The oral cavity stands at the entry of the respiratory system where its fluids like saliva have been reported to harbor and transmit SARS-CoV2 [15]. This vulnerability may also increase the risk of infection in dental personnel. While several studies have documented the risk of COVID-19 in health care workers, they have been mostly limited to those in medicine and surgery. To our knowledge the risk of acquiring SARS-CoV2 or developing COVID-19 in dental personnel is yet to be documented.

Table 1 Master regulator analysis (MRA) between the gene microarray datasets of inflamed human dental pulp tissue cells and SARS-Cov2 transcriptome in human lung tissue cells.

	MRA_INTEGRATED	NAME
EEF1A1	-5.080949116	EEF1A1
RNF128	-4.50987272	RNF128
MIF4GD	-4.371542513	MIF4GD
EIF4B	-4.290264493	EIF4B
DCTN2	-4.244374978	DCTN2
NDUFA10	-4.005673362	NDUFA10
ACE2	-3.8876092	ACE2
EIF3F	-3.881669285	EIF3F
SFTPD	-3.825013544	SFTPD
OCIAD2	-3.630532698	OCIAD2
BTF3	-3.287748138	BZW2
BCL2L2	-3.036538169	BRF1
RPS20	-2.967143456	RPS20
PFDN5	-2.942695302	PFDN5
VKORC1	-2.911783488	VKORC1
CAV1	-2.659287172	CAV1
PPIH	-2.362712184	PPIH
CAMLG	-2.171439376	CAMLG
C20orf27	-2.165842753	C20orf27
PPIA	-2.097660662	PPIA
TMPRSS2	-1.950913709	TMPRSS2
MARK3	-1.720091248	MARK3
FAHD1	-1.694196448	FAHD1
UBE2I	-1.625592879	UBE2I
MNAT1	-1.596010101	MNAT1
ATP6V1G1	-1.584289102	ATP6V1G1
YWHAE	-1.392488514	YWHAE
SGTA	-1.290957934	SGTA
BCL2L1	-1.176653696	BCL2L1
CHMP2B	-1.102816779	CHMP2B
LAS1L	-1.095536157	LAS1L
CHEK2	-1.082909434	CHEK2
IKBKB	-1.082420145	IKBKB
CLEC4G	-1.070002283	CLEC4G
NAE1	-1.035671767	NAE1
ISLR	-0.980194767	ISLR
CD209	-0.8171606	CD209
SLC46A3	-0.790643856	SLC46A3
PSMA2	-0.717908742	PSMA2
RCAN3	-0.711262906	RCAN3
TERF1	-0.597173885	TERF1
SERPING1	-0.590351578	SERPING1
C11orf74	-0.513118793	C11orf74
NPHP3	-0.381046915	NPHP3-ACAD11
POLR2B	-0.350218246	POLR2B
XPA	-0.335997822	XPA
LCP1	-0.333421252	LCP1
H2AFY2	-0.279673123	H2AFY2
TBCB	-0.264829439	TBCB
N4BP2L2	-0.096942088	N4BP2L2
CLEC4M	0.156476249	CLEC4M
IRF3	0.171350307	IRF3
BAP1	0.521077364	BAP1
ALB	0.578252442	ALB
NMB	0.617598222	NMB
MKRN2	0.680489923	MKRN2
DDAH2	0.691965724	DDAH2
BRF1	0.697516152	BTF3
RYBP	1.0083968	RYBP
PPIG	1.049955368	PPIG
ARL4D	1.085916348	ARL4D
TPSAB1	1.203096629	TPSAB1
DEDD2	1.279555758	DEDD2
BCL2	1.627276472	BCL2
HGS	1.701685134	HGS
KPNA2	1.812827618	KPNA2
ATF5	1.877899235	ATF5
SMOC1	1.984103579	SMOC1
BCL2A1	2.039171802	BCL2A1
NCOA5	2.497359696	NCOA5
MARK2	2.54098886	MARK2
ZNF410	2.79859011	ZNF410
PLEKHO1	2.820533045	PLEKHO1
DDX5	3.367639793	DDX5
MCL1	4.043126478	MCL1

Underexpressed genes are highlighted in blue, overexpressed genes in red.

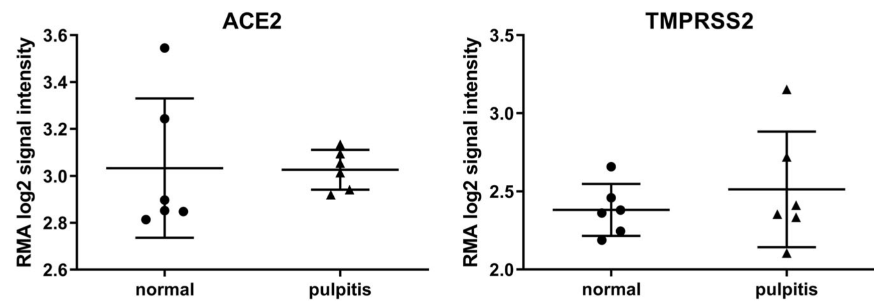

Fig. 1 ACE2 and TMPRSS2 expression in normal and inflamed human dental pulps. Differences in RMA log2 signal intensity between samples were analyzed using Student's *t* test. No statistical difference was found in the expression of both genes in normal and inflamed dental pulps.

The MRA correlation analysis between the pulpitis microarray dataset and the human SARS-Cov2 transcriptome-wide effects shows that both ACE2 and TMPRSS2 values are underexpressed (Table 1). SARS-CoV infection in other tissues have shown marked decrease in ACE2 expression [16]. Furthermore, underexpression of ACE2 was associated with worse outcomes of SARS-CoV2 infection in patients with inflammatory bowel disease [17].

One of the advantages of studies like the present one is that by using omic tools, one can answer several biological questions, without the need of collecting more samples. Our original studies on gene expression in inflamed human pulps was conducted several years ago. We then took advantage of having the sequenced data to explore expression of ACE2 and TPMRSS2 in inflamed and normal human pulps. This was an efficient and quick way to answer a time-sensitive question [10].

Taken together, our results suggest that the dental pulp is vulnerable to SARS-CoV2 infection. The predicted underexpression of ACE2 during SARS-CoV infection in the dental pulp may contribute to worse outcomes of pulpitis.

Materials and methods

Our analysis started by assessing a published study that utilized a dataset describing the transcriptome-wide effects of coronavirus infection in human cells [11]. The study employed a system [18] that probed the transcriptome-wide effects of SARS-CoV2 and its implication on human interactome by applying a MRA, which was performed by comparing infected and mock samples in both MERS and SARS datasets separately with the corto algorithm [18]. With these tools, an MRA correlation analysis of the gene microarray dataset of pulpitis [10] can be performed using a known platform in managing microarray binary data [19].

We first calculated a gene-by-gene signature of differential expression of genes caused by viral infections. In brief, a gene-by-gene signature of viral-induced differential expression is generated, and combined value for each coexpression network is generated by weighting every gene's likelihood in the network, providing a final Normalized Enrichment Score for each genes of the human/Sars-Cov2 integrated interactome. The value is positive when the network is unregulated by the infections, and vice-versa. In this study, we hypothesized that the effects of the viral infection are the same for the dental tissue, therefore we merged the data published in human airway cells [11] with that in dental pulp tissue cells [10]. Consequently, we were able to predict a possible regulator effect for the same genes. This study may therefore suggest some possible effects that should be tested in the future by extracting dental tissue samples from Covid-19 infected patients.

Gene expression analysis of ACE2 and TMPRSS2 in normal and inflamed dental pulps were from our previous study's existing public gene expression database on pulpitis (GEO NCBI accession number GSE77459). Differences in RMA log2 signal intensity between samples were analyzed using Student's *t* test. Statistical significance was set at 0.05.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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