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The non-functional ACE2 isoform, but not the SARS-CoV-2 receptor, is induced as an interferon-stimulated gene, in SARS-CoV-2 infected adults

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ABSTRACT

The recently discovered truncated, non-functional, ACE2 transcript (dACE2), but not the full-length ACE2 (f-lACE2), is induced by IFNs in differentiated airway cells. We measured expression of both ACE2 isoforms in SARS-CoV-2 positive and negative subjects, in relation to Interferon-stimulated genes. A significant activation of dACE2 transcript was found, in SARS-CoV-2 positive adults either hospitalized or not, showing a positive correlation with ISG15; f-lACE2 expression was weakly activated and not ISG-related. We confirmed a specific activation of dACE2 transcript in nasopharyngeal cells, related to the mucosal IFN response.

1. Introduction

Infection with SARS-CoV-2 starts with the binding to its main receptor, the angiotensin-converting enzyme 2 (ACE2) in respiratory mucosal cells [1]. *ACE2* relative rate of expression in specific cell types may be a determinant of the effectiveness of SARS-CoV-2 spread in the respiratory tract [2,3]; conversely, ACE2 exerts protective functions from acute lung injury and plays a role in COVID-19 outcome [4]. *ACE2* expression has been proposed to be enhanced by the mucosal interferon (IFN) response, raising the concern that this may enhance efficiency of SARS-CoV-2 infection [5]. Subsequently, the existence of a short, truncated *ACE2* isoform also named deltaACE2 (dACE2) was demonstrated [6–8]. The truncated protein, not able to serve as SARS-CoV-2 entry receptor, is unstable and of unknown functions [6–8]. This truncated isoform, but not the full-length ACE2 (f-lACE2), was shown to be induced by IFNs and viruses in several, but not all, cell types [6–10]. Other studies found that IFNs activated the f-lACE2 transcript also, but to a lesser extent than dACE2, in primary airway cells [11,12]. Here, we measured the differential expression of *ACE2* isoforms in nasopharyngeal cells, obtaining samples from SARS-CoV-2 infected children and adults, including hospitalized patients that were not previously tested for dACE2 expression and including control individuals, in relation to the activation of IFN-stimulated genes (ISG).

Subjects (children and adults reporting contacts with a SARS-CoV-2 positive case, asymptomatic medicine students and health workers attending periodic screening, adults presenting with respiratory symptoms) were enrolled during February-May 2021 when attending emergency departments (ED) of Sapienza University Hospital, to perform the SARS-CoV-2 molecular tests. Deidentified data were collected after an informed consent was signed; the study was approved by the Hospital Review board and the Ethics Committee (Rif. 5836, Prot. 0690/2021). Nasopharyngeal swabs (NPS) were taken for SARS-CoV-2 detection using RealStar® SARS-CoV-2 RT-PCR Kit 1.0 (Altona Diagnostics, Germany), targeting E and S viral genes. Within 3 h from SARS-CoV-2 molecular testing, total RNA was extracted from NPS residual cells. Levels of f-lACE2 and dACE2 transcripts using isoform-specific primers and probes validated for specificity in previous studies [6,9], and of the well-known markers of IFNs' activation, ISG15 and ISG56, were measured by quantitative Reverse Transcription-Real time PCR assays, in co-amplification with the beta-glucuronidase (GUS) invariant gene [9]. The mRNA copy number of target genes was calculated using the threshold cycle (Ct) relative quantification to the GUS Ct of the same

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sample (the $2^{-\Delta Ct}$ method), as described [9]. A gene transcript was considered not detectable when the Ct value was > 40. ISG56 transcript was not measured in the hospitalized patients because the NPS sample was not sufficient.

Relative expression values of f-lACE2, dACE2, ISG15, ISG56 and the f-lACE2/dACE2 ratio were log transformed and compared using multivariable regression analysis, controlling for age and sex. For categorical variables (including the f-lACE2/dACE2 ratio stratified as < 0 or ≥ 0), Pearson's chi-square test was used to test significance. Pearson's coefficients were calculated controlling for the confounding variables age and sex, to assess partial correlations between f-lACE2, dACE2 and their ratio, with ISG15 and age in years. Analysis was performed with SPSS v.27 (IBM).

Residual NPS cells from 111 adults and 53 children were tested for the expression of *ACE2* isoforms. In 38/53 children's NPS (71.7 %), dACE2 expression was not detectable and, in several others, very low levels of either dACE2 or f-lACE2 were measured so that we could not include children's samples in the study. This finding is not surprising given that several studies reported lower levels of ACE2 expression in children and related this to the lower susceptibility to severe SARS-CoV-2 infection compared with adults (reviewed in ref 13). In 20 adults' NPS cells, most of which SARS-CoV-2 negative with low cell numbers (as estimated by their high GUS Ct values), dACE2 and f-lACE2 had Ct values > 40; these samples were not further analyzed. Results from 91 cases are reported in Table 1, stratified in three study groups: SARS-CoV-2 negative (n = 41), SARS-CoV-2 positive (n = 22), SARS-CoV-2 hospitalized (n = 28). No significant difference was found when comparing expression levels of the ACE2 isoforms by sex [dACE2 mean ± standard deviation (SD): male = -2.5 ± 1.22 vs female = -3.09 ± 1.42, p = 0.060; f-lACE2 mean ± SD: male = -2.4 ± 0.80 vs female = -2.38 ± 0.61, p = 0.994). f-lACE2, but not dACE2, showed a moderate positive correlation with age (Pearson's = 0.270, p = 0.010).

Expression levels were then compared among three groups adjusting for age and sex (Table 1 and supplementary data); f-lACE2 and dACE2 levels were different among groups with a tendency to be higher in hospitalized patients (Table 1, Fig. 1A and B). Interestingly the f-lACE2/dACE2 ratio was significantly lower and more frequently < 0 (log dACE2 greater than log f-lACE2) in the SARS-CoV-2 positive-subjects, either hospitalized or not (Table 1, Fig. 1C).

Furthermore, a partial Pearson's correlation analysis indicated a moderate positive correlation exists between ISG15 and dACE2 (r = 0.353, p = 0.001; Fig. 1D) but not f-lACE2 (r = 0.157, p = 0.140). Moreover, ISG15 transcripts were significantly higher in the NPS in which the f-lACE2/dACE2 ratio was < 0, than in those with a ratio ≥ 0 [mean ± SD: 0.53 ± 0.34 vs 0.28 ± 0.35; p = 0.001].

Overall, this study detected higher levels of dACE2 expression in SARS-CoV-2 infected adults, in particular in the hospitalized patients, with respect to uninfected controls and confirmed, in the upper respiratory mucosal cells, the relationship between dACE2 and IFN-stimulated genes previously demonstrated in cell lines and tissues [6–10]. Activation of dACE2 was substantial but relatively lower compared to ISG15 and ISG56, in accordance with previous studies [6–10].

We found little difference in f-lACE2 expression between uninfected and SARS-CoV-2 infected subjects either hospitalized or not; hence, f-lACE2 seems not to be activated by the IFN system in response to a SARS-CoV-2 infection [6–8]. An alternative explanation is that a weak f-lACE2 activation by IFNs may occur, as reported [11,12], but is counterbalanced by SARS-CoV-2 downregulation of its own receptor, in analogy to SARS-CoV-1 and NL63-CoV [4,14]. In any case, given that f-lACE2 activation following SARS-CoV-2 infections was not relevant, the early concerns that IFNs might enhance the efficiency of SARS-CoV-2 infection by up-regulating viral receptor expression [5], are not supported by these findings. Nonetheless, f-lACE2 expression, beside a possible regulation by IFNs, is also determined by cell-specific promoters along the airways, and affected by host factors such as age and sex, and underling disease [13,15]. We also found not-detectable or very low expression of both ACE2 isoforms in children, consistent with the positive correlation we observed between f-lACE2 and age in adults, but the number of samples was not sufficient to draw firm conclusions.

We also examined the f-lACE2/dACE2 ratio that is a determinant that could explain the different efficiencies of SARS-CoV-2 infection in different tissues [7]; in most SARS-CoV-2 positive cases, the ratio between the isoforms' log values was < 0, because of the higher dACE2 activation with respect to f-lACE2. In partial disagreement, Blume et al. reported that in airway primary cells, dACE2 is upregulated in response to rhinovirus, but not to SARS-CoV-2 infection [8]; this was attributed to the lack of IFN production and downstream signaling due to the ability of SARS-CoV-2 to inhibit IFN activation. Nevertheless, an IFN-mediated response in the nasopharyngeal mucosa is activated following SARS-CoV-2 infection and can be effective in preventing severe disease [15]. Indeed, in our SARS-CoV-2 infected subjects, we could measure elevated levels of activation of two ISGs' that are considered markers of mucosal IFN response. Thus, we retain that activation of dACE2 transcription could be one of the antiviral weapons of the mucosal IFN response to SARS-CoV-2.

In conclusion, we showed in NPS cells, a detectable, specific activation of dACE2 transcription that might be considered as part of the complex regulatory mechanisms driving ACE2 expression. Further studies examining differential regulation of ACE2 isoforms in larger groups are needed to clarify the interplay between SARS-CoV-2, its receptor and IFNs, produced following natural infections or administered as a therapy.

Table 1
Demographic and gene expression data of study subjects (n = 91).

Study subjects	SARS-CoV-2 negative n = 41	SARS-CoV-2 positive n = 22	SARS-CoV-2 hospitalized n = 28	p-value
Female/ Male	29/12	14/8	6/22	< 0.001
Age (years)[a]	39.6 ± 14.7	28.3 ± 5.9	57.2 ± 16.9	< 0.001
Gene expression				
Log-f-lACE2[b]	−2.32 (-2.52;-2.10)	−2.77 (-3.07;-2.39)	−2.24 (-2.48;-1.99)	0.02
Log-dACE2[b]	−3.45 (-3.81;-2.88)	−2.82 (-3.43;-2.21)	−2.04 (-2.29;-1.79)	0.04
Log-f-lACE2/dACE2[b]	0.99 (0.60;1.38)	0.03 (-0.44;0.50)	−0.20 (-0.31;0.09)	0.006
Log-ISG15[b]	0.24 (0.14;0.35)	0.56 (0.41;0.71)	0.53 (0.39;0.66)	0.02
Log-ISG56[c]	−0.38 (-0.54;-0.22)	−0.08 (-0.20;0.04)	ND[d]	0.02
Log-f-lACE2/dACE2 < 0[e]	10 (24.4 %)	14 (63.6 %)	22 (78.6 %)	< 0.001

[a] Age is expressed as mean ± standard deviation.
[b] Expression levels of full-length ACE2 (f-lACE2), truncated ACE2 (dACE2), the ratio between expression levels of f-lACE2/dACE2, and of the Interferon stimulated gene (ISG) ISG15, are reported as mean (95 %CI) after log transformation of the values; p-values are reported after controlling for age and gender using regression analysis, as reported in the supplementary data.
[c] ISG56 is reported as mean (95 %CI) after log transformation of the values; p-value is calculated using Student's *t*-test.
[d] Not done.
[e] Number of samples (% to total in the group) in which the ratio between the expression levels of f-lACE2/dACE2 was < 0; p-value is calculated using Pearson's chi-square.

CRediT authorship contribution statement

Giuseppe Oliveto: Investigation. **Carolina Scagnolari:**

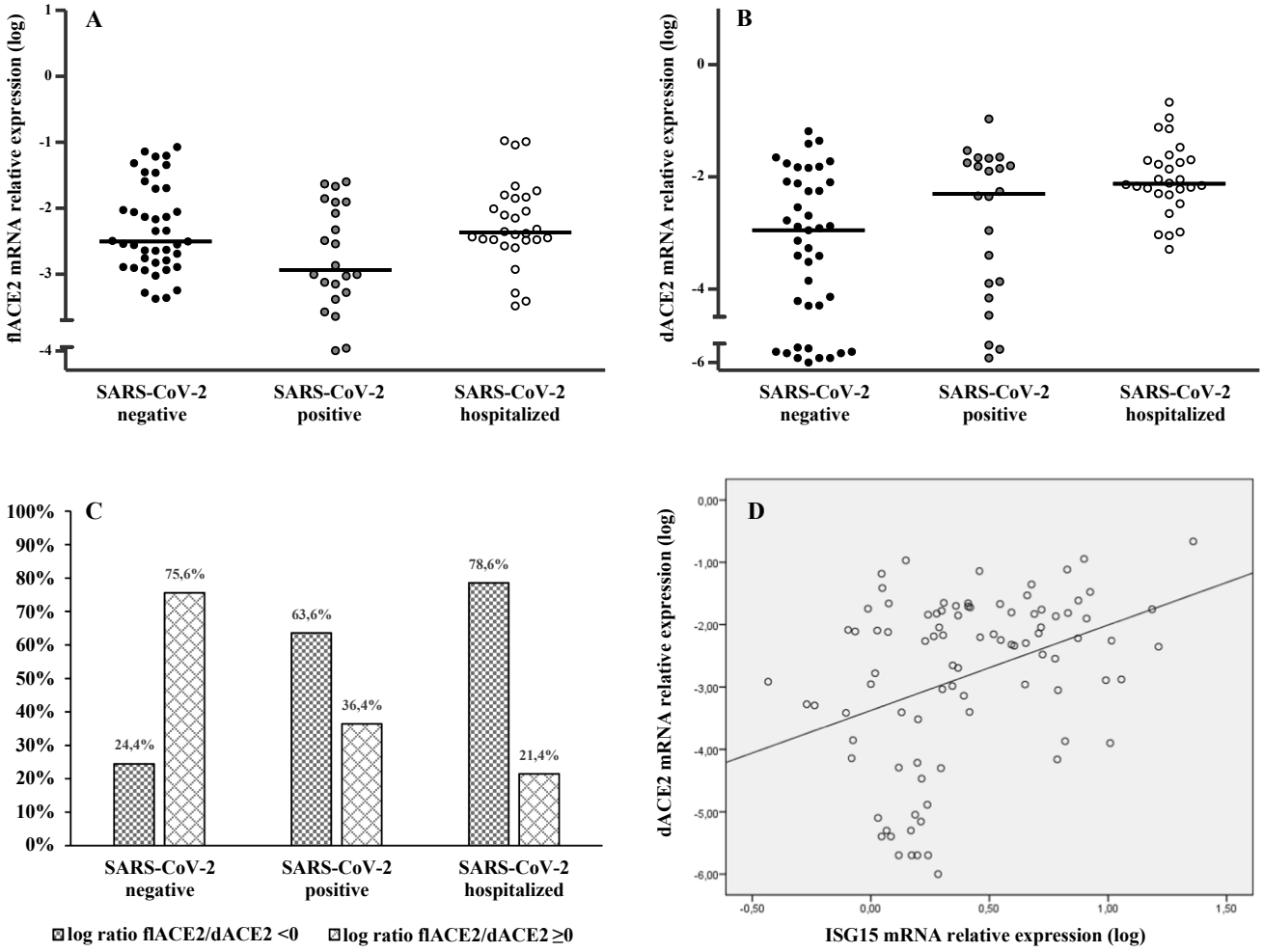

Fig. 1. Full-length ACE2 (f-lACE2) and truncated ACE2 (dACE2) expression, f-l ACE2/dACE2 ratio < 0 or ≥ 0 and partial correlation of dACE2 and ISG15 log values of the three study groups. Expression levels of f-lACE2 (panel A) and dACE2 (panel B) are calculated using the threshold cycle (Ct) relative quantification ($2^{-\Delta Ct}$ method) to the GUS Ct of the same sample and log transformed. Panel C shows the percentage of samples in which the ratio between expression levels of f-l ACE2/dACE2 was < 0 or ≥ 0. Panel D shows the partial correlation (adjusted for age and sex) between dACE2 and ISG15 both expressed as log of fold change ($2^{-\Delta Ct}$) with respect to the β-Glucuronidase (GUS) gene (Pearson's $r = 0.353$, $p = 0.001$).

Conceptualization, Writing – review & editing. **Federica Frasca:** Investigation. **Leonardo Sorrentino:** Investigation. **Luigi Matera:** Data curation. **Raffaella Nenna:** Data curation. **Agnese Viscido:** . **Mirko Scordio:** . **Laura Petrarca:** Data curation. **Anna Maria Zicari:** Data curation. **Elio Gentilini:** Data curation. **Gabriella D'Ettorre:** Data curation. **Giancarlo Ceccarelli:** Investigation. **Fabio Midulla:** Supervision. **Guido Antonelli:** Conceptualization, Formal analysis. **Alessandra Pierangeli:** Conceptualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Data availability

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Sapienza University Hospital (Rif. 5836, Prot. 0690/2021).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.

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