Peptidylarginine deiminase 4 (PAD4) is a key factor for SARS-CoV-2 replication and SARS-CoV-2-induced pro-inflammatory responses

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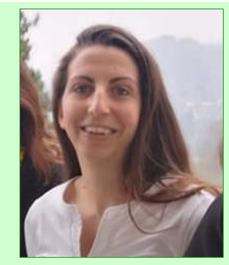

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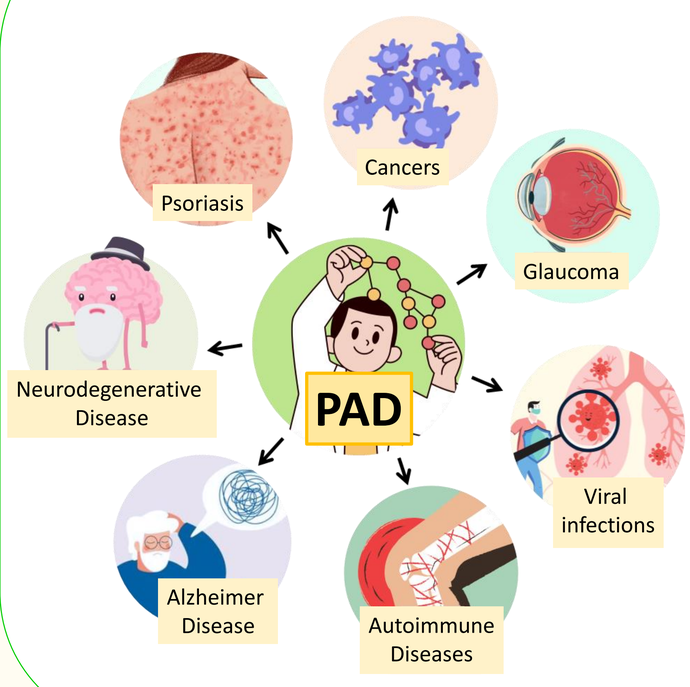
VIRUSES 2024 TRAVEL AWARD

BACKGROUND



- Peptidyl arginine deiminases **(PAD)** are a family of enzymes witch catalyzed the hydrolization of the guanidinium group of a peptidyl-arginine to form peptidyl-citrulline. This post-translational modifications is known as **citrullination**.
- Five PAD isozymes (PADs 1-4 and 6) are expressed in mammalians.
- The **dysregulation of PADs expression** and/or an aberrant profile of citrullinated protein were observed in many **inflammatory conditions.**
- PADs inhibitors have been tested in several in vivo models of inflammatory and autoimmune diseases in which they have demonstrated high therapeutic efficacy.
- Based on some similarities between the clinical outcome observed in autoimmune/autoinflammatory disease and **COVID-19**, including aberrant cytokine release, it has been hypothesized that PADs activity may be an important factor to the severity of SARS-CoV-2 infection.

OUR GOALS

- ❖ Clarify whether SARS-CoV-2 infection affects PADs expression.
- ❖ Assess the antiviral activity of PAD inhibitors both *in vitro* and *in vivo.*
- ❖ Investigating PAD inhibitors potential to control the hyperproduction of pro-inflammatory markers associated with COVID-19 disease.
- ❖ Define the changes in proteome of citrullinated proteins (citrullinome) during infection.

RESULTS

PADs induction by SARS-CoV-2 infection

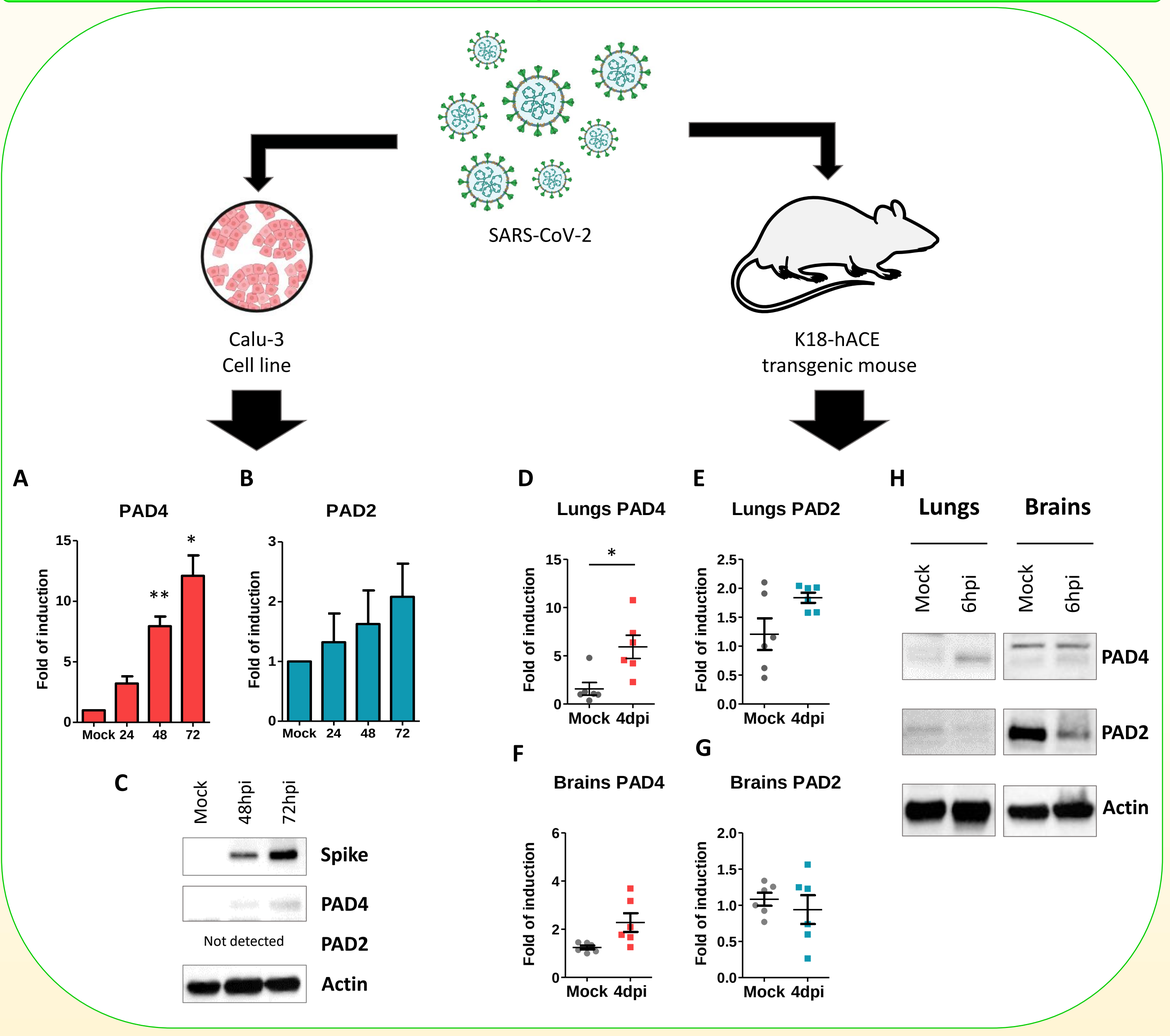


Figure 1. PADs induction by SARS-CoV-2 infection *in vivtro* and *in vivo*

(A-B)Comparative RT-PCR. Calu-3 airway epithelial cells were infected with SARS-CoV-2 delta strain and PADs mRNA was assessed by quantitative RT-PCR at indicated time points. Data were normalized with housekeeping gene PGK1 and are presented relative to uninfected cells.
(C) Western Bolt. Calu-3 airway epithelial cells were infected with SARS-CoV-2 delta strain and PAD4 and PAD2 protein expression was assessed by immunoblot.
(D-G)Comparative RT-PCR. K18-ACE2 transgenic mice were infected with SARS-CoV-2 delta strain and PADs mRNA was assessed by quantitative RT-PCR at indicated time points. Data were normalized with housekeeping gene Actin and are presented relative to uninfected cells.
(H) Western Bolt. K18-ACE2 transgenic mice were infected with SARS-CoV-2 delta strain and PAD4 and PAD2 protein expression was assessed by immunoblot.

In vitro antiviral activity of PADs inhibitors

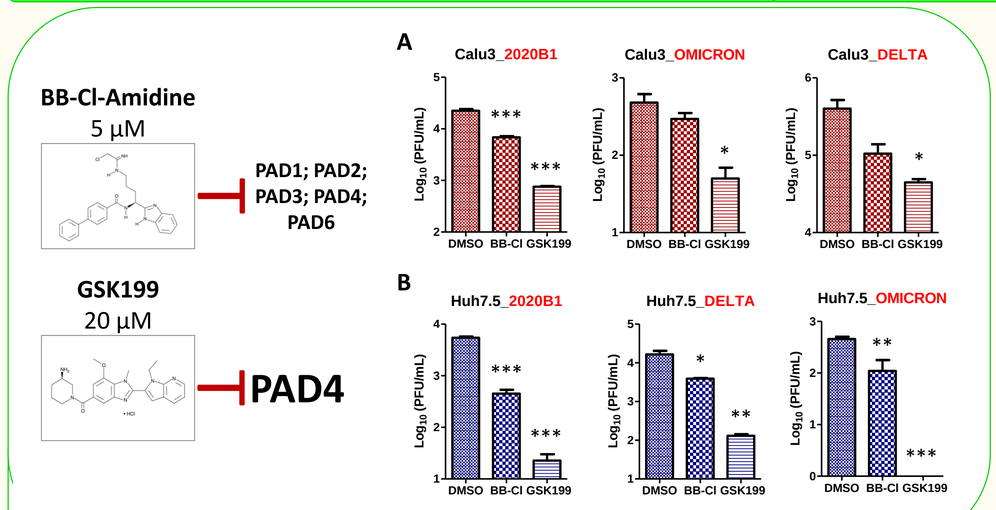


Figure 2. In vitro antiviral activity of PADs inhibitors

(A) Plaque assay. Calu-3 cells were pre-treated for 1h with not-toxic concentration of BB-Cl and GSK199 (or vehicle DMSO) and then infected with the indicated SARS-CoV-2 strains. After 2 h of incubation viral inoculum were removed and substituted with new medium whit BB-cl and GSK199. Viral productions were harvested at 24hpi and titrated by plaque assay.
(B) Plaque assay. Huh7.5hepatocyte derived cellular carcinoma cells were pre-treated for 1h with not-toxic concentration of BB-Cl and GSK199 (or vehicle DMSO) and then infected with the indicated SARS-CoV-2 strains. After 2 h of incubation viral inoculum were removed and substituted with new medium whit BB-cl and GSK199. Viral productions were harvested at 48hpi and titrated by plaque assay.
(C-D) Comparative RT-PCR. Calu-3 airway epithelial cells were infected with SARS-CoV-2 delta strain and IL-6/TNF-α mRNA was assessed by quantitative RT-PCR at indicated time points. Data were normalized with housekeeping gene PGK1 and are presented relative to uninfected cells.

In vivo antiviral activity of PAD4 inhibitor GSK199

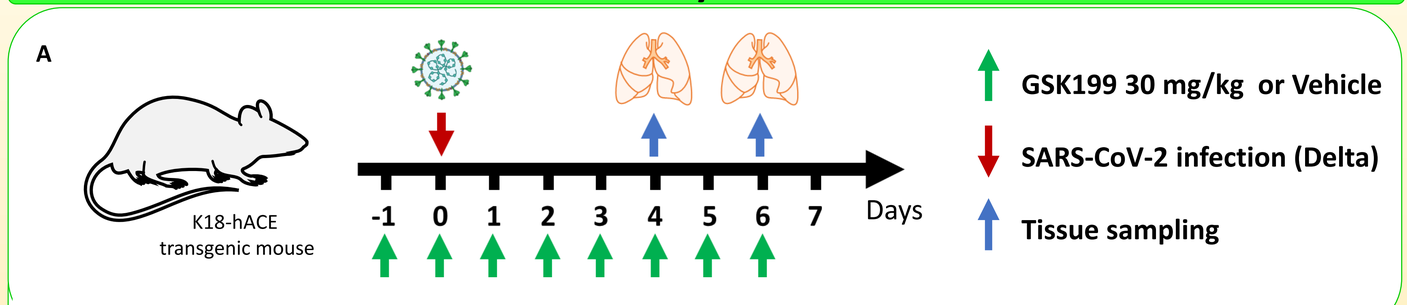


Figure 3. In vivo antiviral activity of PAD4 inhibitor GSK199.

(A) Representation of the treatment and infection scheme of K18-ACE2 transgenic mice. Tissue collected at 4dpi were homogenate in TRI-reagent for RNA extraction. Tissue collected at 6dpi were homogenate in RIPA buffer for protein extraction.

(B-C) Quantitative ddPCR. Viral RNA genome were quantified by droplet digital PCR. Each point represents the amount of viral genome per organ of the treated mouse (n=6 mice per group).

GSK199 affect pro-inflammatory cytokine expression *in vivo*

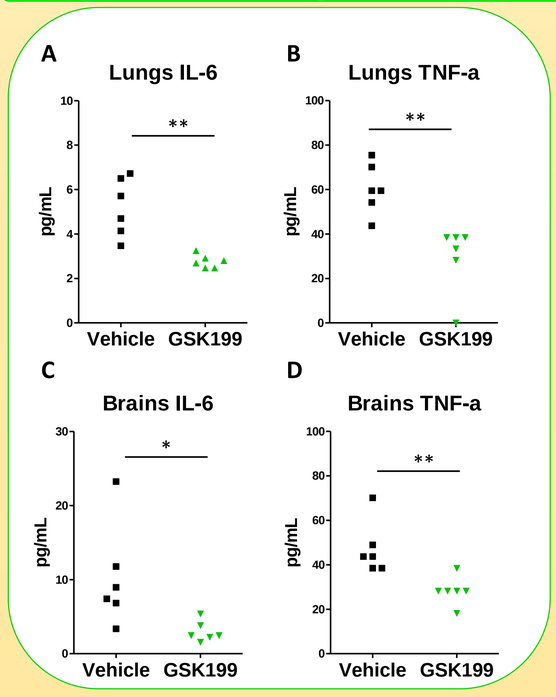


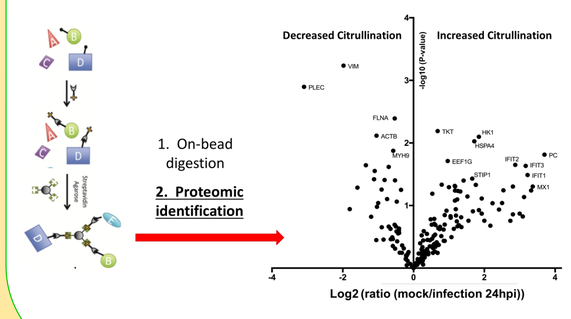
Figure 4. SARS-CoV-2-induced cytokine expression is affected by PAD4 inhibitor *in vivo*

(A-D) Cytokine quantification. Proteins were extracted from Lungs and Brains of mice treated and infected as described in Fig. 3A. proteins extracted in RPA buffer were quantify and concentration of pro-inflammatory cytokine IL-6 and TNF-α were determined by Bio-Plex Multiplex Immunoassay (BIORAD).

Each point represents the average amount of cytokine per organ (n=6 mice per groups) read in experimental duplicate.

Work in progress

Define the proteome of citrullinated protein (*citrullinome*)



CONCLUSIONS

- ❖ **SARS-CoV-2 infection upregulates PAD4 expression at RNA and protein levels.**
- ❖ **PAD4 activity is crucial for SARS-CoV-2 replication *in vivo* and *in vitro*.**
- ❖ **PAD4 inhibitors modulate pro-inflammatory cytokine production**

Would you like to know more about Citrullination and viral infections?

HCoV-OC43 HSV-1 HCMV