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Vimentin is an important ACE2 co-receptor for SARS-CoV-2 in epithelial cells

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Highlights

- SARS-CoV-2 upregulates vimentin surface expression
- SARS-CoV-2 spike interacts with vimentin and ACE2 at the cell surface
- Cell-surface vimentin favors SARS-CoV-2 infection
- Vimentin inhibition protects viral-induced cytotoxicity and modulates host response

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Article

Vimentin is an important ACE2 co-receptor for SARS-CoV-2 in epithelial cells

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SUMMARY

Vimentin is a type III intermediate filament protein, widely expressed in mesenchymal cells. Mainly located in the cytoplasm, vimentin can also appear at extracellular locations, where it may interact with bacterial or viral pathogens. In this study, we aimed at investigating the implication of vimentin in SARS-CoV-2 viral entry and the consequences on viral replication and cellular response. We showed that upon infection, vimentin was upregulated at the cell surface, where it interacts with ACE2 for SARS-CoV-2 entry. We demonstrated a direct interaction between SARS-CoV-2 spike protein, ACE2, and vimentin in epithelial cells. Inhibition of cell-surface vimentin availability resulted in reduced viral entry and cytopathogenic effects. Finally, we showed that the expression of inflammatory cytokines and chemokines was modulated by vimentin—SARS-CoV-2 interaction. In conclusion, our data suggest that cell-surface vimentin acts as a co-receptor for SARS-CoV-2.

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the current pandemic of the coronavirus disease 19 (COVID-19). SARS-CoV-2 is an enveloped single-stranded RNA virus. As of August 2022, it has infected over 578 million people and is the cause of 6.4 million deaths worldwide (https://covid19.who.int). SARS-CoV-2 is a member of the Coronaviridae family, belonging to the β-coronavirus genera closely similar to SARS-CoV and Middle East respiratory syndrome (MERS)-CoV.1 SARS-CoV-2, SARS-CoV, and MERS-CoV are zoonotic and particularly pathogenic coronaviruses triggering global and restricted outbreaks.2 SARS-CoV, MERS-CoV, and SARS-CoV-2 are more likely to infect lower airway, causing respiratory distress and in worst cases septic shock and multiple organ failures.1 Severe COVID-19 results from the inability to mount an antiviral response but also from the induction of a cytokine storm, characterized by the secretion of large amounts of cytokines and chemokines including among others interleukin (IL)-6, tumor necrosis factor α (TNF-α), IL-2, IL-1β, chemokines (C-X-C motif) ligand (CXCL)10, chemokines (C-C motif) ligand CCL2, CCL3, CCL4.1 IL-6, CXCL10 and CCL5 are one of the headliners of the cytokine storm as they are greatly produced by epithelial cells during the SARS-CoV-2 infection, acting as a signal to recruit immune cells to the infection sites.4

Both SARS-CoV and SARS-CoV-2 are able to bind to angiotensin-converting enzyme 2 (ACE2), which catalyzes the cleavage of angiotensin II into the vasodilator angiotensin 1–7.5–7 ACE2 is a membrane bound metallopeptidase, which is the key receptor for the entry of SARS-CoV-2 into host cells. The S1 subunit of the spike (S) protein of SARS-CoV-2 binds to ACE2, which initiates the priming of the SARS-CoV-2 S protein by transmembrane serine protease 2 (TMPRSS2), allowing virus-cell fusion and cell entry.1 However, ACE2 is poorly expressed throughout the respiratory tract,1 suggesting that other cofactors may compensate and facilitate the interactions between S protein and ACE2. For example, neuropilin-1 (NRP1) has been shown to enhance the interaction between ACE2 and S protein.10 CD209L/L-SIGN, CD209/DC-SIGN, and heparan sulfate may also facilitate SARS-CoV-2 entry into lung cells.11,12 Finally, vimentin was shown to interact with SARS-CoV-2 S protein at the cell surface.13 The high level of similarities between SARS-CoV and SARS-CoV-2 implies that SARS-CoV-2 is able to bind to vimentin as well, as recently suggested.14–16

Vimentin is a type III intermediate filament cytoskeletal protein expressed in nonmuscle cells, including fibroblasts, endothelial cells, macrophages, melanocytes, Schwann cells, and lymphocytes.17,18 The basic structure of vimentin consists of a central α-helical rod domain flanked by unstructured head and tail...
domains. Vimentin forms a vast intracellular, dynamic, and flexible network surrounding the nucleus and spanning toward the cell periphery that allows maintenance of the cell’s organelles and plays an important role in several cell events.

Several studies have shown that vimentin is not exclusively expressed intracellularly and that it can be localized at the cell surface and even secreted. Interestingly, the presence of vimentin at the cell surface is necessary for the entry of pathogens into the host cells such as Escherichia coli, Streptococcus pyogenes, enterovirus 71, dengue virus, or SARS-CoV. Given the high similarities between SARS-CoV and SARS-CoV-2 S proteins, we wondered whether cell-surface vimentin could serve as a co-receptor for SARS-CoV-2 infection. We demonstrated here that SARS-CoV-2 transiently upregulates the expression of vimentin and that vimentin and ACE2 interact in the presence of SARS-CoV-2. Inhibition of vimentin results in decreased viral uptake, increased cell viability, and decreased inflammatory cytokine and chemokine expression. Altogether, our results highlight the role of vimentin during SARS-CoV-2 infection and suggest that drug targeting vimentin may represent a novel avenue for the prevention of SARS-CoV-2 infection.

RESULTS
Cellular expression of vimentin, ACE2, and TMPRSS2 in Vero E6, A549, and Caco-2 cells
In a first set of experiments, we wanted to determine the expression levels of ACE2, TMPRSS2, and vimentin in epithelial cells from various origins, including Vero E6, a green monkey kidney epithelial cell line, Caco-2, a human colon epithelial cell line, and A549 cells, a human alveolar basal epithelial cell line, using primers indicated (Table 1). We found that transcript levels for ACE2 were significantly higher in Vero E6 cells compared with Caco-2 and A549 cells (Figure 1A). TMPRSS2 was expressed at similar levels and significantly higher in Vero E6 and Caco-2 cells than in A549 cells (Figure 1A). Finally, Caco-2 cells expressed very low levels of vimentin as compared with Vero E6 and A549 cells and Vero E6 expressed higher levels than A549 cells (Figure 1A).

We next wanted to address the cellular distribution of vimentin in resting cells. First, surface vimentin was quantified by flow cytometry in nonpermeabilized condition and expressed as mean fluorescence intensity for the different cell types. We found that A549 cells displayed the highest expression of vimentin at the surface, whereas Vero E6 and Caco-2 expressed intermediates and low levels, respectively (Figures 1B and 1C). These findings were further confirmed by confocal microscopy. Resting cells were fixed and stained for vimentin in the absence of permeabilization. In agreement with flow cytometry data, we found a marked expression of vimentin at the cell surface of A549 cells (Figure 1C). Vero E6 cells expressed intermediate levels of cell-surface vimentin, whereas it was barely detectable in Caco-2 cells (Figure 1C). In addition, we found that ACE2 was expressed by Vero E6 and Caco-2 cells but not by A549 cells (Figure 1C). Overall, these data indicate that epithelial cells express various amounts of vimentin and ACE2; Vero E6 cells express intermediate levels of both vimentin and ACE2; A549 cells express high levels of vimentin but no ACE2; and Caco-2 cells express intermediate levels of ACE2 but no vimentin.
SARS-CoV-2 uptake is associated with vimentin, ACE2, and TMPRSS2 expression

We next evaluated whether differential expression of vimentin and ACE2 in Vero E6, Caco-2, and A549 cells was associated with differential SARS-CoV-2 cell entry.

We then infected Vero E6, Caco-2, and A549 cells with SARS-CoV-2 IHU-MI2129 (Wuhan) strain at an MOI of 1 over 10, 30, and 120 min and quantified viral entry by one-step qRT-PCR and normalized to actin expression (Figure 2A). In Vero E6 cells, we observed a 3-fold increase in the number of SARS-CoV-2 between 10 min and 2 h of infection. In Caco-2 cells, the number of viral copies was similar to that of Vero E6 cells after 10 min but did not increase over the 2-h period. Finally, SARS-CoV-2 was much less efficient in infecting A549 cells because viral copies were 300 times lower in A549 than in Vero E6 or Caco-2 cells (Figure 2A). Thus, considering the respective expression of vimentin, ACE2, and TMPRSS2 in these different cell types, these data suggest that the expression of vimentin at the cell surface may favor the infection in Vero E6 cells.

SARS-CoV-2 infection increases surface vimentin expression

We next addressed whether the expression of vimentin at the cell surface was affected by viral infection. We then infected Vero E6, Caco-2, and A549 cells with SARS-CoV-2 for 10, 30, and 120 min and monitored surface expression of vimentin by flow cytometry. In Vero E6 cells, we found that cell-surface vimentin increased after 10 and 30 min of infection by SARS-CoV-2 in comparison with uninfected cells (Figure 2B). After 2 h of infection, surface vimentin expression decreased to reach levels found in uninfected cells. A similar trend was observed for A549 cells (Figure 2B). In contrast, in Caco-2 cells, vimentin expression at the surface was not affected by SARS-CoV-2 infection (Figure 2B). Vimentin staining after permeabilization revealed that indeed Caco-2 cells express negligible levels of vimentin as compared with A549 or Vero E6 cells (Figure S1). Overall, these data showed that infection is associated with an increase of vimentin expression at the cell surface in Vero E6 and A549 cells.

SARS-CoV-2 colocalizes with vimentin and ACE2 at the cell surface

As the expression of both vimentin and ACE2 was the highest in Vero E6 cells, all the subsequent experiments were carried out with these cells. We first wondered whether Vero E6 cells spontaneously secrete vimentin and if infection with SARS-CoV-2 induced vimentin secretion. Cells were infected or not with SARS-CoV-2, and vimentin was assayed in the cell supernatant by Western blot. We were not able to detect vimentin in the culture media alone; however, when cell supernatants were not cleared before lyophilization, vimentin was detected in the supernatant of uninfected cells and the signal slightly increased after 6 h of infection (Figure S2A). However, when supernatants were cleared before lyophilization, vimentin was not detected (Figure S2B), suggesting that Vero E6 cells do not spontaneously secrete vimentin. To further examine whether expression of vimentin at the cell surface serves during the infection process, we monitored vimentin and ACE2 expression relative to SARS-CoV-2 viral particles. Hence, Vero E6 cells were infected with SARS-CoV-2 for 30 min, and vimentin, ACE2, and SARS-CoV-2 spike protein were observed by confocal microscopy after immunofluorescence staining of nonpermeabilized cells. We found that SARS-CoV-2 spike protein colocalized with both ACE2 and vimentin (Figure 3A), suggesting that SARS-CoV-2 spike protein, ACE2, and vimentin physically interact during cell infection. To further confirm this hypothesis, the interaction of vimentin with SARS-CoV-2 was examined by immunogold labeling and transmission electron microscopy. Vero E6 cells were infected with SARS-CoV-2 and labeled with antibodies directed against vimentin coupled with gold particles of approximately 10 nm diameter. On the images we can visualize certain viral particles labeled with the dense particles showing the fixation of vimentin on the viral particles of SARS-CoV-2 (Figure 3B). Lastly, we performed co-immunoprecipitation experiments. Vero E6 cells were infected or not with SARS-CoV-2 for 10, 30, or 120 min, and cell lysates were co-immunoprecipitated with anti-vimentin antibodies and detected with anti-ACE2 antibodies or inversely co-immunoprecipitated with anti-ACE2 antibodies and detected by immunogold labeling and transmission electron microscopy.
with anti-vimentin antibodies. As shown in Figure 3C, ACE2 interacted with vimentin after 10 min, and this interaction lasted for at least 120 min. Interestingly, no interaction was observed between vimentin and ACE2 when cells were not noninfected, suggesting that vimentin acts as a co-receptor facilitating internalization of SARS-CoV-2 virions.

Figure 2. SARS-CoV-2 transiently upregulates vimentin expression at the cell surface in Vero E6 and A549 cells
Vero E6, Caco-2, and A549 cells were infected with SARS-CoV-2 (MOI = 1) for 10, 30, and 120 min. (A) SARS-CoV-2 entry was quantified by qPCR targeting the viral E gene and expressed as Relative Quantity (RQ) normalized to the housekeeping ACTB gene as endogenous control. (B) Expression of extracellular vimentin was monitored by flow cytometry and expressed as MFI. (C) Representative histograms are shown. The experiments were performed in triplicates (N = 3), and the values represent the mean ± standard deviation. *, **, ***, and **** represent p < 0.05, p < 0.01, p < 0.001, and p < 0.0001, respectively, by one-way ANOVA and the Tukey test for post-hoc comparisons.
Vimentin inhibition reduces viral infection

We next wondered whether vimentin was required for SARS-CoV-2 infection of Vero E6 cells. Cells were pretreated with withaferin A (WFA), a steroidal lactone that binds and induces vimentin aggregation.

Figure 3. Vimentin interacts with ACE2 and SARS-CoV-2

(A) Vero E6 cells were infected with SARS-CoV-2 for 30 min, and vimentin, ACE2, and SARS-CoV-2 spike protein cell distribution was analyzed by confocal microscopy (scale bar: 10 μm for the merge picture and 5 μm for the individual channel pictures).

(B) TEM image illustrating the interaction between SARS-CoV-2 and vimentin, using gold-conjugated anti-secondary antibodies following anti-vimentin antibody staining.

(C) Cells were infected or not for the indicated times with SARS-CoV-2, and whole-cell lysates were subjected to immunoprecipitation with anti-Vimentin (top) or anti-ACE2 (bottom) antibodies. Co-IP ed products were analyzed by western blot using anti-vimentin and anti-ACE2 antibodies. The experiments were performed in triplicates (N = 3); representative results are shown.

Vimentin inhibition reduces viral infection

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Figure 4. Vimentin inhibition results in reduced SARS-CoV-2 entry in Vero E6 cells

Vero E6 cells were treated with withaferin A (WFA, 15 µM) for 4 h or with anti-vimentin antibody (1 µg) for 1 h and then infected with SARS-CoV-2 (MOI = 1) for 30 min.

(A) Viral entry was evaluated by qPCR and expressed as percentage of entry relative to untreated cells.
(B) Viral replication was also quantified by qPCR in untreated, WFA- and anti-vimentin antibody-treated cell supernatants after 24 and 48 h post-infection and expressed as Ct values.
in vitro, or with anti-vimentin antibodies and then infected for 30 min. Viral entry was quantified by qPCR and visualized by immunofluorescence and confocal microscopy. When cells were treated with WFA or anti-vimentin antibodies, we observed a significant decrease (>50%) in SARS-CoV-2 entry as compared with untreated cells (Figure 4A). These data were further confirmed by confocal microscopy, which showed a decrease of SARS-CoV-2 staining in Vero E6 cells treated with WFA or anti-vimentin antibodies (Figure 4C). We next asked whether replication of SARS-CoV-2 was affected by vimentin inhibition. Vero E6 cells were treated with WFA or anti-vimentin antibodies, and viral replication was monitored by qPCR 24 and 48 h after infection. When cells were treated with WFA, we found that the Ct values were significantly higher than in untreated cells at 24 h. However, at 48 h, the Ct values decreased and were comparable to those obtained in untreated cells (Figure 4B). A similar trend was observed when cells were treated with anti-vimentin antibodies (Figure 4B), suggesting that viral replication is not severely affected by vimentin inhibition and that differences probably rely on decreased uptake. Immunofluorescence and confocal microscopy experiments using anti-double-stranded (ds)RNA antibodies that label replicating viruses supported this observation and revealed that although the number of infected cells was reduced in the presence of WFA or anti-vimentin antibodies, the staining of dsRNA remained similar in individual infected cells (Figure 4D). These data suggest that vimentin favors SARS-CoV-2 infection of cell but is not necessarily required for viral replication.

**Vimentin inhibition protects viral-induced cytotoxicity and modulates host response**

Finally, we next evaluated the effects of vimentin inhibition on host cell survival and host responses. Vero E6 cells were treated with WFA or anti-vimentin antibodies and infected with SARS-CoV-2 for 24 and 48 h. Cell viability was quantified by MTT assay. No differences were observed after 24 h (Figure 5A). However, 48 h after infection, we found a significant reduction of cell viability, which may reflect increased apoptosis or cytopathic effects (Figure 5A). Pretreatment of cells with WFA or anti-vimentin led to an increase of cell viability as compared with untreated, infected cells (Figure 5A), suggesting that vimentin inhibition may protect host cell from virus-induced cell death.

We next wondered whether vimentin inhibition altered inflammatory cytokine and chemokine expression. Cells were pretreated or not with WFA or anti-vimentin antibodies and stimulated with SARS-CoV-2. After 24 h, transcript expression was evaluated by qRT-PCR. Expression of interferon α (IFN-α) and CXCL5 was not significantly modulated by vimentin inhibition (Figure 5B). In contrast, we found that expression of IL-6, CCL5, and CXCL10 was not affected or slightly decreased in WFA-treated cells, whereas pretreatment with anti-vimentin led to a 15-fold decrease in IL-6 expression and a 10-fold reduction in CCL5 and CXCL10 expression (Figure 5B).

Altogether, these data suggest that vimentin is involved in SARS-CoV-2-mediated cell death and epithelial inflammatory response.

**Effects of vimentin inhibition during infection with the omicron variant**

Finally, we wondered whether vimentin was also required during infection with another SARS-CoV-2 variant expressing a distant spike protein. Vero E6 cells were then pretreated with WFA or anti-vimentin antibodies and infected with the B.1.1.529 (omicron) variant, which is characterized by 30 amino acid substitutions, three short deletions, and one insertion in the spike protein, compared with the original strain of SARS-CoV-2.31 We found that vimentin inhibition resulted in a significant reduction of viral entry as revealed by qPCR, reaching 50% and 60% when cells were treated with WFA or with anti-vimentin antibodies, respectively (Figure 6A). We next quantified cell viability after infection with the omicron variant. We found that cell viability decreased by 20% after 24 h of infection and approximately 50% after 48 h (Figure 6B). When cells were treated with WFA or anti-vimentin antibodies, no significant change was observed in...
cell viability after 24 h of infection. After 48 h, we saw a significant increase of 10% in cell viability in cells treated with WFA compared with untreated cells, whereas cells that were treated with anti-vimentin antibodies showed a slight increase in cell viability compared with untreated cells (Figure 6B). Regarding viral replication and similar to what already observed with the original strain, omicron variant replication did not appear affected by anti-vimentin treatment at 24 and 48 h (Figures 6C and 6D). Finally, as previously observed with the original strain, we found that the expression of CXCL5 and IFN-α was not affected by anti-vimentin treatment or WFA (Figure 6E), whereas that of IL-6, CCL5, and CXCL10 was significantly decreased by anti-vimentin antibodies, but not by WFA treatment (Figure 6E).

Overall, these results showed that anti-vimentin treatment of Vero E6 cells led to similar results between the original strain and the omicron variant regarding infection, viral replication, cell viability, and cellular response; this suggests that the mutations that occur in the spike protein of the omicron variant do not affect interaction with and the function of vimentin during SARS-CoV-2 infection.

**DISCUSSION**
Pathogens and more specifically viruses have the ability to infect target cells following interaction with specific cell surface receptors. Importantly, assistance of nonspecific co-receptors may increase receptor affinity, favor infection efficiency, and contribute to the tropism. Based on its high homology with SARS-Co-V, it was rapidly demonstrated that SARS-CoV-2 is endocytosed after engagement of ACE2 by its spike protein. However, several reports have revealed that depending on the cell type, SARS-CoV-2 entry may
Figure 6. Vimentin inhibition results in reduced viral entry, increased cell viability, and modulates cell transcriptional response against the Omicron variant

(A) Vero E6 cells were treated with WFA (15 μM) for 4 h or with anti-vimentin antibody (1 μg) for 1 h and infected with B.1.1.529 Omicron SARS-CoV-2 variant (MOI = 1). Viral entry was quantified after 30 min infection by qPCR and expressed as percentage of entry relative to untreated cells. (B) Cell viability was assessed by MTT test after 24 and 48 h and expressed as percentage of viability relative to uninfected cells. (C) Viral replication was quantified by qPCR in cell supernatant after 24 and 48 h post-infection and expressed as Ct values.
that vimentin inhibition was accompanied by a significant reduction of SARS-CoV-2-induced cytopathic and which also surround the viral factories to form vimentin cages ensuring efficient synthesis of the viral spike protein. This is also supported by previous observations that showed that both a chicken polyclonal antibody and a rabbit anti-vimentin antibody that respectively binds to multiple epitopes of the SARS-CoV-2 spike protein. This Spike is also believed to be responsible for the interaction of the Spike protein with the host receptor ACE2. However, it has been shown that in the presence of vimentin, the SARS-CoV-2 Spike protein fails to interact with ACE2, suggesting that vimentin, ACE2, and SARS-CoV-2 Spike protein form a trimolecular complex. Evidence that ACE2 and vimentin interact directly and that this interaction only occurred in the presence of SARS-CoV-2, suggesting that vimentin, ACE2, and SARS-CoV-2 Spike protein form a trimolecular complex, as previously hypothesized with SARS-CoV. Given the fact that the rod II domain of vimentin is localized at the cell surface and possesses lectin-like properties and that glycans shield approximately 40% of the SARS-CoV-2 Spike protein surface, one can suspect that vimentin interacts with the Spike protein through its lectin-like domain. We also showed that the pretreatment of cells with WFA significantly reduced SARS-CoV-2 entry in Vero E6 cells. WFA is a steroidal lactone with anti-inflammatory properties, which binds soluble vimentin, perturbs its expression, and causes aggregation of vimentin filaments, highlighting the requirement of cell-surface vimentin for efficient viral entry. However, we cannot rule out that WFA inhibits TMPRSS2 activity and thus viral entry because it was recently shown that WFA binds and stably interacts at the catalytic site of TMPRSS2. Nevertheless, when cells were pretreated with anti-vimentin (V9) monoclonal antibodies, similar results were obtained. Interestingly, the V9 monoclonal antibody recognizes an epitope located in the C-terminal “tail” domain of the vimentin protein, suggesting that this region is exposed at the cell surface and involved in the interaction with ACE2 and/or SARS-CoV-2 Spike protein. This is also supported by previous observations that showed that both a chicken polyclonal anti-vimentin antibody and a rabbit anti-vimentin antibody that respectively binds to multiple epitopes of the vimentin C-terminus and targets amino acids 425–466 of the vimentin C-terminus significantly decreased uptake of SARS-CoV-2. Interestingly, vimentin antibodies targeting the vimentin C-terminus and stained for vimentin (red) and dsRNA (green) (scale bar: 10 μm). (E) The expression of cytokine-encoding genes (IL6 and INFα) and chemokines (CCL5, CXCL5, CXCL10) was investigated after 24 h by qRT-PCR and normalized to the housekeeping ACTB gene as endogenous control. The experiments were performed in triplicates (N = 3), and the values represent the mean ± standard deviation. *, **, ***, and **** represent p < 0.05, p < 0.01, p < 0.001, and p < 0.0001, respectively, by one-way ANOVA and the Tukey test for post-hoc comparisons.

Figure 6. Continued

(D) Representative confocal microscopy image representing Omicron-infected Vero E6 cells (24 h) pretreated or not with WFA or anti-vimentin antibodies and stained for vimentin (red) and dsRNA (green) (scale bar: 10 μm). (E) The expression of cytokine-encoding genes (IL6 and INFα) and chemokines (CCL5, CXCL5, CXCL10) was investigated after 24 h by qRT-PCR and normalized to the housekeeping ACTB gene as endogenous control. The experiments were performed in triplicates (N = 3), and the values represent the mean ± standard deviation. *, **, ***, and **** represent p < 0.05, p < 0.01, p < 0.001, and p < 0.0001, respectively, by one-way ANOVA and the Tukey test for post-hoc comparisons.
effects and by an increase of cell viability. Thus, the role of vimentin at later stages of SARS-CoV-2 infection, including viral replication, assembly, and egress, needs to be further explored.

Given its role in immune response and inflammation, we next wondered whether vimentin was involved in host cell responses to SARS-CoV-2 infection. We showed that treatment of cells with anti-vimentin antibodies reduced the expression of chemokines and cytokines, including CCL5, CXCL10, and IL-6. IL-6 is a pleiotropic cytokine with multiple activities on inflammation and immunity. It has been shown that SARS-CoV-2 spike protein induces IL-6 expression in an angiotensin II type 1 (AT1)-dependent signaling resulting from ACE2 downregulation. Importantly, IL-6 is one of the major headliners of the cytokine storm that accelerates the severity of the infection by SARS-CoV-2. In addition, vimentin has been shown necessary for the assembly and activation of the NLRP3 inflammasome, which is involved in the production of pro-inflammatory cytokines. Overall, our results suggest that beside reducing viral entry, targeting vimentin may also reduce the risk of cytokine storm syndrome and thus evolution toward severe COVID-19.

In summary, our study showed that vimentin inhibition reduced viral uptake, protected against virus-mediated cell cytotoxicity, promoted cell survival, and reduced pro-inflammatory response. Interestingly, similar data were obtained when cells were infected with the Omicron variant. It was previously shown that anti-vimentin antibodies also reduced the uptake of pseudoviruses expressing spike proteins from the variants UK B.1.1.7 and Brazil P.1. Hence, drugs targeting vimentin, including ALD-R491, a novel oral, fast-acting, and noncytotoxic molecule, appear as promising and lasting candidates for the treatment of SARS-CoV-2 infections and COVID-19.

**Limitations of the study**

Although our study provides significant insights into the role of surface-associated vimentin during infection with SARS-CoV-2, there are some limitations that should be addressed to fully elucidate its mode of action. For example, experiments aiming at characterizing the molecular domains involved in this tripartite interaction would further reveal important mechanistic understandings. In addition, the role of intracellular vimentin should be further explored, particularly in the late stage of viral replication. Finally, this study was performed using cell lines. It would be relevant to investigate the role of vimentin during SARS-CoV-2 infection of primary epithelial cells.

**STAR METHODS**

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**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105463.
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AUTHOR CONTRIBUTIONS
Conceptualization and supervision, B.D.; Investigation, J.A., P.A.A., L.J., and Y.S.; Formal Analysis, J.A. P.A.A., J-L.M., and B.D.; Writing—Original Draft, J.A. and P.A.A.; Writing—Review & Editing, J-L.M. and B.D.

DECLARATION OF INTERESTS
The authors declare no competing interests.

INCLUSION AND DIVERSITY
We support inclusive, diverse, and equitable conduct of research.

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Mouse monoclonal Anti-Vimentin (Clone V9) (AF647/AF488) | Santa Cruz Biotechnology | Cat# sc-6260; RRID: AB_628437 |
| Mouse monoclonal Anti-ACE2 | Santa Cruz Biotechnology | Cat# sc-390851; RRID: AB_2861379 |
| Mouse monoclonal Anti-dsRNA | Jena Bioscience | Cat# RNT-SCI-10010; RRID: AB_2922431 |
| Mouse monoclonal Anti-SARS-CoV-2 spike | R&D Systems | Cat# MAB105403-100; RRID: N/A |
| Goat polyclonal Anti-Mouse IgG HRP | Thermo Fisher Scientific | Cat# 32430; RRID: AB_1185566 |
| Goat polyclonal Anti-Mouse IgG Alexa fluor™ 488 | Thermo Fisher Scientific | Cat# A28175; RRID: AB_2610666 |
| Goat polyclonal Anti-Mouse IgG Alexa fluor™ 555 | Thermo Fisher Scientific | Cat# A32727; RRID: AB_2633276 |
| Polyclonal Anti-mouse IgG Ultrasmall 0.8nm | Aurion | Cat# 800.022; RRID: AB_2632382 |
| Goat polyclonal Anti-Mouse IgG FITC | Jackson ImmunoResearch Labs | Cat# 115-095-003; RRID: AB_2338589 |
| **Bacterial and virus strains** | | |
| SARS-CoV-2 IHU-MI2129 (wuhan) | Isolated from patient (Andreani et al. [59]) | N/A |
| SARS-CoV-2 B.1.1.529 (Omicron) | Isolated from patient (Andreani et al. [59]) | N/A |
| **Chemicals, peptides, and recombinant proteins** | | |
| Witaferin A | Santa Cruz Biotechnology | sc-200381; CAS: 5119-48-2 |
| Protein G Agarose | Abcam | ab193258 |
| Thiazolyl Blue Tetrazolium Bromide (MTT) | Sigma-Aldrich | M5655; CAS: 298-93-1 |
| DAPI (4',6-diamidino-2-phenylindole, dichlorhydrate) | Thermo Fisher Scientific | Cat#D1306 |
| **Critical commercial assays** | | |
| Quick-RNA™ MiniPrep | Zymo research | Cat#R1055 |
| One-Step RT-PCR SuperScript™ III Platinum™ | Thermo Fisher Scientific | Cat#12574026 |
| RT-PCR MMLV | Thermo Fisher Scientific | Cat#28025013 |
| PCR smart SYBRGreen fast Master kit | Roche Diagnostics | Cat#09242404001 |
| Substrat HRP Immobilon Western | Millipore | WBKLS0500 |
| **Experimental models: Cell lines** | | |
| A-549 | ATCC | RRID: CVCL_0023 |
| Caco-2 | ATCC | RRID: CVCL_0025 |
| Vero E6 | ATCC | Cat# CRL-1586; RRID: CVCL_0574 |
| **Oligonucleotides** | | |
| Primers for IL6, see Table 1 | From 62 | N/A |
| Primers for IFNA, see Table 1 | This Paper | N/A |
| Primers for CCL5, see Table 1 | From 61 | N/A |
| Primers for CXCL5, see Table 1 | This Paper | N/A |
| Primers for CXCL10, see Table 1 | From 62 | N/A |
| Primers for VIM, see Table 1 | This Paper | N/A |
| Primers for ACE2, see Table 1 | This Paper | N/A |
| Primers for TMPRSS2, see Table 1 | From 63 | N/A |
| Primers for ACTB, see Table 1 | From 64 | N/A |
| Primers for E, see Table 1 | From 65 | N/A |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Benoit Desnues (benoit.desnues@univ-amu.fr)

Materials availability
This study did not generate new unique reagents.

Data and code availability
- Data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell lines and cell culture
A549, Caco-2 and Vero E6 were obtained from American Type Culture Collection and were cultured in Eagle’s minimum essential medium (MEM), Dulbecco’s Modified Eagle Medium: Nutrient Mixture F-12 (DMEMF-12) and Dulbecco’s Modified Eagle’s Medium (DMEM), with 10% fetal bovine serum (FBS) and antibiotics (streptomycin [0.1 μg/mL] and penicillin G [100 units/mL], respectively.

Viral production and cell infection
SARS-CoV-2 Wuhan strain and B.1.1.529 Omicron variant were kindly provided by Pr Bernard La Scola. Viruses were produced in 75 cm² culture flasks containing Vero E6 cells in MEM supplemented with 4% FBS and 1% glutamine. Cells were observed daily for cytopathic effect, and after nearly complete cell lysis, supernatant was collected and virus were titrated by median tissue culture infectious dose (TCID50) method after inoculation of the supernatant on Vero E6 cells seeded on 96-well plates. All virus strains were then stored at–80°C until further use. Cells were infected with 20 μL virus suspension at a multiplicity of infection (MOI) of 1 for 10, 30 and 120 min or 24 and 48 h at 37°C in the presence of 5% CO2 and 95% air in a humidified incubator.

METHOD DETAILS

RNA extraction and q-RTPCR
Total RNA was extracted from infected cells (10⁶ cells/well) using RNA extraction Kit (ZYMO RESEARCH) followed by DNase I treatment and evaluated using a spectrophotometer (Nanodrop Technologies). Virus detection was performed using One-Step RT-PCR SuperScript™ III Platinum™ (Life Technologies). Thermal cycling was achieved at 55°C for 10 min for RT, pursued by 95°C for 3 min and then 45 cycles at 95°C for 15 s and 58°C for 30 s using a LightCycler480 system (Roche). The primers and the probe targeted the E gene (Table 1). Viral quantification was expressed as cycle threshold (Ct) values normalized to the actin housekeeping gene. For host gene expression, reverse transcription was done using a Moloney murine leukemia virus-reverse transcriptase kit (Life Technologies) and oligo(dT) primers, and PCR was performed using the Smart SYBRGreen fast Master kit (Roche Diagnostics) and a CFX Touch Detection System (Bio-Rad) using

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Primers for E probe, see Table 1 | From 65 | N/A |

Software and algorithms
- ZEN Digital Imaging for Light Microscopy | ZEISS | RRID: SCR_013672 |
- FlowJo | FlowJo | RRID: SCR_008520 |
- GraphPad Prism v9.0 | GraphPad | RRID: SCR_002798 |
specific primers (Table 1). The results were normalized using the housekeeping endogenous control ACTB gene and expressed as relative quantification (RQ) = 2^(-ΔΔCt) with ΔΔCt = ΔCt_{Target} - ΔCt_{ACTB}.

**Immunofluorescence**

Cells (5 × 10^5 cells/well) grown on coverslips, were fixed with 3% paraformaldehyde for 15 min and permeabilized or not with 0.1% Triton X-100 in PBS for 3 min. Cells were then incubated with anti-Vimentin (V9) Alexa Fluor 647 (Santa Cruz biotechnology), anti-TMPRSS2 Alexa Fluor 594 (Santa Cruz biotechnology) antibodies and 4',6-diamidino-2-phenylindole (DAPI) to label vimentin, TMPRSS2 and nucleus respectively. SARS-CoV-2 virus was labelled using an anti-SARS-CoV-2 spike protein antibody (R&D Systems) or an anti-double stranded (ds)RNA antibody (Jena Bioscience) to evaluate viral replication, followed by a secondary Alexa Fluor 555-conjugated goat anti-mouse IgG (H + L) antibody (Invitrogen). ACE2 was labelled using an anti-ACE2 antibody (R&D Systems) followed by a secondary Alexa Fluor 488-conjugated goat anti-rabbit IgG (H + L) antibody (Invitrogen). Coverslips were washed, mounted with mowiol and observed using an LSM800 Airyscan confocal microscope (Zeiss) and a 63X oil objective.

**Flow cytometry**

For flow cytometry, cells were permeabilized or not with BD Cytofix/Cytoperm kit (BD Biosciences) for 30 min and saturated with 5% bovine serum albumin (BSA) in PBS, for 1 h at 4 degrees. Cells were then washed with FACS buffer (5% FBS, 2 mM EDTA in PBS) and incubated with anti-vimentin (V9) primary antibody (Santa Cruz Biotechnology) for 1 h. Cells were washed again and incubated for 1 h with an Fluorescein (FITC)-conjugated AffiniPure Goat Anti-Mouse IgG (H + L) antibody (Jackson ImmunoResearch). Cells were then analyzed by flow cytometry. Data were collected on a Navios instrument (Beckman Coulter) and analyzed with FlowJo software (FlowJo v10.6.2).

**Immunogold labeling and transmission electron microscopy (TEM)**

Vero E6 cells were fixed for at least 1 h with glutaraldehyde 2.5% in 0.1M sodium cacodylate buffer. Cells were then incubated with anti-vimentin (V9) antibody (Santa Cruz Biotechnology) followed by a secondary goat anti-mouse IgG (H + L) antibody coupled to 10 nm-gold particles (Aurion). For resin embedding, cells were washed three times with a mixture of 0.2M saccharose/0.1M sodium cacodylate. Cells were post-fixed for 1 h with 1% OsO4 diluted in 0.2M Potassium hexa-cyanoferrate (III)/0.1M sodium cacodylate solution. After three 10 min washes with distilled water, the cells were gradually dehydrated with ethanol by successive 10 min baths in 30, 50, 70, 96, and 100% ethanol. Substitution was achieved by successively placing the cells in 25, 50, and 75% Epon solutions for 15 min. Cells were placed for 1 h in 100% Epon solution and in fresh Epon 100% overnight under vacuum at room temperature. Polymerization occurred with cells in 100% fresh Epon for 72 h at 60°C. Ultrathin 70 nm sections were cut using a UC7 ultramicrotome (Leica) and placed on HR25 300 Mesh Copper/Rhodium grids (TAAB, United Kingdom). Sections were contrasted according to Reynolds. 60 Images were obtained by Tecnai G2 TEM (Thermo-Fischer/FEI) operated at 200 keV equipped with a 4096 × 4096 pixels’ resolution Eagle camera (FEI).

**Immunoprecipitation and western blot**

Cell were lysed with RIPA buffer (20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1% Triton X-100, pH 7.5) and lysates were incubated with either anti-ACE2 or anti-vimentin antibody-conjugated protein G agarose beads (Abcam) overnight at 4°C. Beads were then washed with RIPA 1X buffer and bound proteins were eluted with Glycine 0.2M, pH 2.5. Eluted proteins were separated by SDS-PAGE, transferred to nitrocellulose membrane, and analyzed by Western blot. Membranes were saturated using PBS with 0.05% Tween 20 (PBST) supplemented with 3% powdered milk for 45 min and incubated with anti-vimentin (Santa Cruz) or anti-ACE2 (Santa Cruz) antibodies overnight at 4°C on a shaker. Membranes were then washed and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Pierce) for 2 h. Proteins were visualized using the chemiluminescence reaction HRP Substrate (Millipore), and image acquisition was done by using a Fusion Fx imaging system (Vilber Lourmat).

For detection of vimentin secretion, cell supernatants were centrifuged or not to remove cell debris, subjected to lyophilization for 24 h and resuspended in RIPA buffer. Culture medium alone was processed accordingly as negative control. Proteins were then separated by SDS-PAGE, transferred to nitrocellulose membrane, and analyzed by Western blot.
Cell viability
Cell viability was indirectly estimated by assessing cellular metabolism with 3-[4,5-dimethylthiazol-2-yl]-2,5
diphenyl tetrazolium bromide (MTT) assay. Cells were infected with SARS-CoV-2 and after 24 and 48 h,
10 μL of MTT (5 mg/mL, Sigma-Aldrich) were added to the cell cultures and incubated at 37°C for 4 h.
The formed formazan crystals were solubilized with 50 μL of dimethylsulphoxide (DMSO) for 30 min at
37°C and quantified at 540 nm using a Synergy MxF plate reader (Biotek Instruments).

QUANTIFICATION AND STATISTICAL ANALYSIS
Statistical analysis was performed with GraphPad Prism, using the one-way analysis of variance (ANOVA)
with Tukey’s post hoc test for multiple comparisons, and statistical significance was considered for p values
below 0.05. Details for individual analyses are provided in the figure legends. Unless otherwise noted, data
are presented as mean ± standard deviation.