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RAPID COMMUNICATION

Mediator complex subunit 12 is a gatekeeper of SARS-CoV-2 infection in breast cancer cells

Cancer patients are at increased risk for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and mortality. Like other viruses in the SARS family, SARS-CoV-2 employs two host proteins, angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), for viral entry.[1] Recent studies showed that many of the host proteins identified as potential targets for developing COVID therapies are dysregulated in cancer,[2] prompting us to investigate whether human cancer cells are susceptible to SARS-CoV-2 infection, and whether chemotherapy could modulate a cancer patient's risk for infection.

Breast cancer is one of the most frequent cancer diagnoses worldwide. The expression levels of ACE2 and TMRPSS2 among cell lines and tissues are shown in Figure S1A–C. CAL-51, a breast cancer cell line expressing detectable level of ACE2 and TMPRSS2 proteins (Fig. S1D), was found permissive to SARS-CoV-2. To our knowledge, CAL-51 is the first breast cancer cell line susceptible to SARS-CoV-2 infection. To determine whether chemotherapy affects SARS-CoV-2 infectivity, we measured ACE2 protein levels after treating CAL-51 cells with fluorouracil (5-FU), doxorubicin, paclitaxel, and docetaxel (Fig. S2A–D). Western blotting showed that 5-FU and doxorubicin significantly increased ACE2 levels, whereas paclitaxel and docetaxel had no effect in CAL-51 (Fig. 1A, S2E). Interestingly, pre-treatment of cells with clinically relevant concentrations of 5-FU and doxorubicin significantly and modestly increased SARS-CoV-2 infectivity, respectively (Fig. 1B, S2F) in accordance with the increased ACE2 levels induced by these drugs. On the contrary, paclitaxel and docetaxel treatment slightly inhibited the infectivity of SARS-CoV-2 in CAL-51 cells at lower doses and had no effect at higher doses (Fig. S2G, H). The differential effects of chemotherapies on ACE2 expression and SARS-CoV-2 Spike-mediated entry imply that paclitaxel and docetaxel may be safer options than 5-FU and doxorubicin for breast cancer treatment.

To investigate how chemotherapy increases ACE2 expression and modulates SARS-CoV-2 infectivity, we focused on the role of Mediator complex subunit 12 (MED12) because MED12 levels are strongly linked to chemosensitivity, and mediator proteins were among the proteins downregulated by SARS-CoV-2 infection which, in turn, affect the SARS-CoV-2 life cycle.[3] We measured the protein levels of MED12 in response to chemotherapy drugs. 5-FU and doxorubicin treatments gradually decreased MED12 levels in CAL-51 cells over time (Fig. 1A, Fig. S2E). The decrease of MED12 was not observed in response to paclitaxel and docetaxel treatments (Fig. S2E). To examine whether MED12 regulates ACE2 expression, we knocked out MED12 using CRISPR/cas9 in CAL-51 cells (Fig. S3A). The ACE2 mRNA and protein levels were dramatically increased in MED12 null CAL-51 cells as compared to the parental cells (Fig. S3B, C). Importantly, 5-FU treatment has no effects on the regulation of ACE2 levels in MED12 null CAL-51 cells (Fig. S3D). These results support a model where chemotherapy (e.g., 5-FU) treatment decreases MED12 levels, which results in the elevated ACE2 levels.

To examine if MED12 regulates SARS-CoV-2 infectivity in breast cancer cells, we infected MED12 null and CAL-51 parental cells with SARS-CoV-2 at a multiplicity of infection (MOI) of 2 and measured SARS-CoV-2 M protein expression using immunofluorescence (IF). MED12 KO CAL-51 displayed 60% SARS-CoV-2 M-positive cells as compared to 20% in parental CAL-51 cells (Fig. 1C, D). To assess if MED12 regulates viral entry, we performed a pseudovirus infection assay and observed that MED12 KO increased Spike-mediated pseudovirus entry, but had no effects on control pseudovirions bearing vesicular stomatitis virus G (VSV-G) protein that transduced cells equivalently (Fig. 1E, F). This result supports that the increased infection of MED12 null

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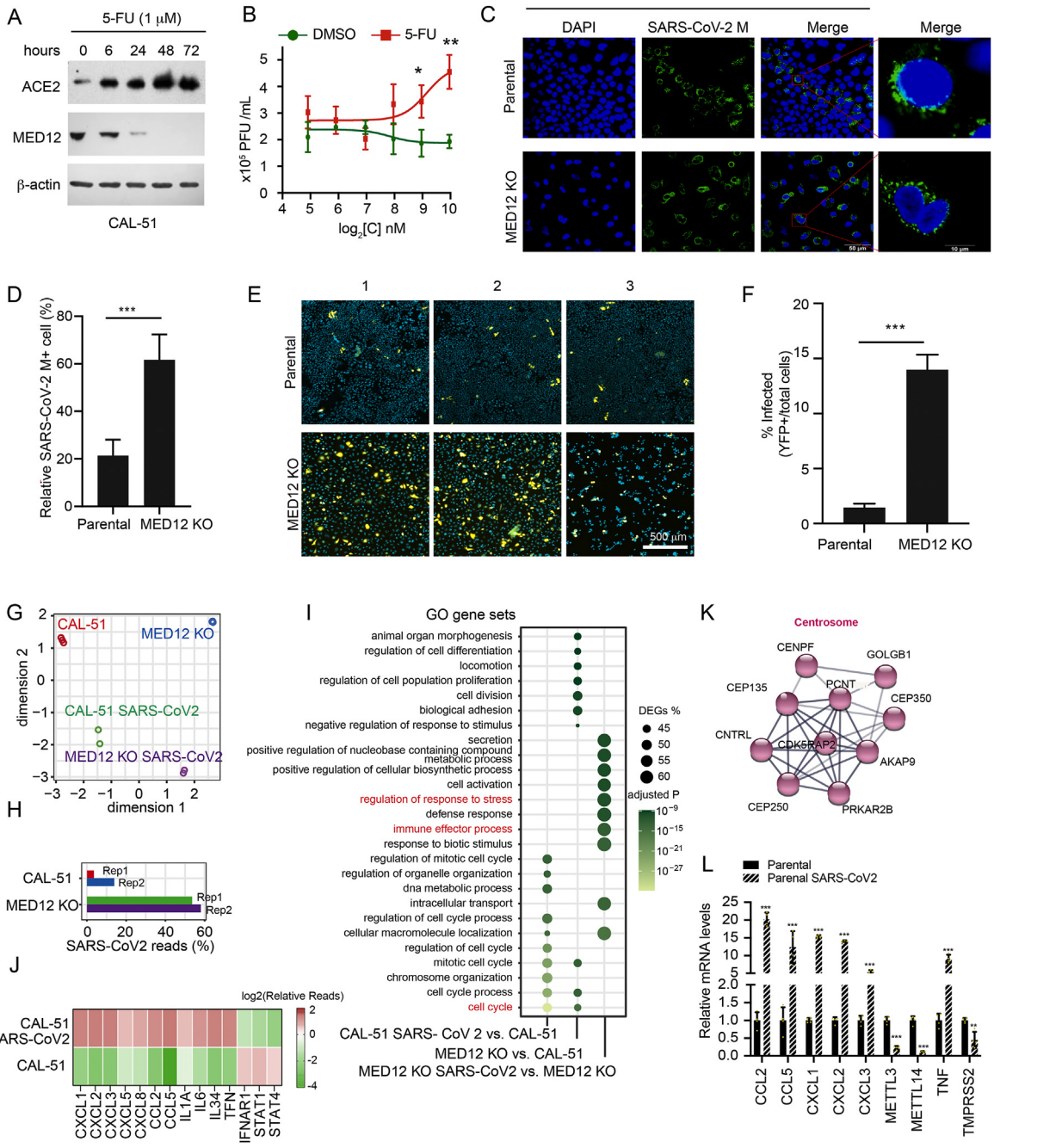

Figure 1 Mediator complex subunit 12 is a gatekeeper of SARS-CoV-2 infection in breast cancer cells. **(A)** Western blotting analysis of ACE2 and MED12 levels in CAL-51 cells after treating with the indicated doses of 5-FU over time. **(B)** SARS-CoV-2 viral titers in CAL-51 cells treated with DMSO or 5-FU. (**C**) Confocal imaging of SARS-CoV-2 M protein expression in parental and MED12 KO cells after 2 days of SARS-CoV-2 infection. **(D)** Quantitative analysis of SARS-CoV-2 M protein-positive cell number in parental and MED12 KO CAL-51 cells. **(E)** Spike-mediated pseudovirus infectivity analysis of parental and MED12 KO CAL-51 cells. **(F)** Quantitative analysis of spike-mediated pseudovirus infectivity of parental and MED12 KO CAL-51 cells. **(G)** Principal component analysis (PCA) for transcriptomic profiles of MED12 KO and parental CAL51 with and without SARS-CoV-2 infection. RNA-seq was performed in duplicate or triplicate and depicted by a circle. **(H)** Percentage of unique mapped reads aligned to the SARS-CoV-2 genome. **(I)** Dotplot visualization of enriched GO terms in the indicated groups. Gene sets for each DE analysis were ranked by their adjusted *p*-value from enrichment analysis. Percentage of DEGs in each gene set is represented as DEGs %. Gene sets were from MSigDB version 7.1 GO biological process collection. **(J)** Heatmap depicting the relative expression levels of DEGs in response to SARS-CoV-2 infection in CAL-51. **(K)** STRING network analysis of the SARS-CoV-2 interacting proteins, whose mRNA levels were changed by SARS-CoV-2 infection in CAL-51. **(L)** QRT-PCR validation of mRNA changes in response to SARS-CoV-2 infection in CAL-51 cells. Significance was determined using a two-tailed Student's *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

cells is, at least in part, due to increased SARS-CoV-2 entry. Together, our data shows that chemotherapeutics, such as 5-FU, reduce MED12 levels, which likely lead to up-regulation of ACE2 to promote SARS-CoV-2 entry in CAL-51 cells.

To further investigate the mechanism(s) by which MED12 affects SARS-CoV-2 infection, we compared the transcriptome of MED12 null and parental CAL-51 cells infected with SARS-CoV-2 and analyzed both viral transcripts and host transcriptional response using RNA-seq data. As shown in Figure 1G, SARS-CoV-2 infection elicited significant transcriptional changes, both in parental and MED12 KO cells. Using viral RNA-seq reads to estimate infection levels, SARS-CoV-2 infectivity increased 5-fold in MED12 knockout cells as compared to parental CAL-51 (Fig. 1H). These data confirm that the loss of MED12 leads to increased susceptibility of TNBC cells to SARS-CoV-2 infection. In parental CAL-51, SARS-CoV-2 infection resulted in >8000 gene expression changes (3979 activated and 4520 repressed), encompassing cell cycle, cancer, and cytokine-mediated signaling pathways (Fig. 1I, S3E, and Table S1). Additionally, viral infection triggered chemotactic and inflammatory responses, as indicated by cytokine and chemokine gene response changes (Fig. 1J). SARS-CoV-2 infection-induced gene changes were consistent with induction of cell cycle arrest, p38 MAPK activation, and overproduction of inflammatory cytokines, which has been reported by others in different cell types,[4] underscoring that disruption of significant cellular pathways is repeatedly observed during SARS-CoV-2 infection. Strikingly, comparing the transcriptome of SARS-CoV-2 infected parental and MED12 KO CAL-51 cells revealed a profound impact of MED12 on the viral-induced host transcriptional landscape. Though no pathways were significantly altered by loss of MED12 or SARS-CoV-2 infection alone, concurrent loss of MED12 and SARS-CoV-2 infection affected many cellular pathways, including the MAPK signaling pathway, regulation of response to stress, immune effector process (Fig. 1I, S3E, and Table S1).

We speculated that MED12 KO may affect the expression of host proteins interacting with viral proteins. Among the 322 host proteins identified in proteomics studies that interact with SARS-CoV-2 viral proteins,[3] mRNA levels of 31 viral-dependent host factors were regulated by MED12 (Table S1). For example, the levels of Centromere protein F (CENPF), a centromere protein known to interact with Nsp13 and repress SARS-CoV-2 infectivity,[2,5] was down-regulated in MED12 null CAL-51 as compared to parental cells (Fig. 1K and Table S1). This result implies that CENPF could be a downstream effector of MED12. Moreover, the expression of many fibroblast growth factors (FGFs) was changed in SARS-CoV-2-infected MED12 KO cells (Fig. S3E). Collectively, these findings suggest that MED12 is an essential gatekeeper against SARS-CoV-2 infection. In addition to regulating ACE2 levels, which promotes viral entry, MED12 regulates viral production through intervening broad biological processes.

Comparison of RNA-seq data from SARS-CoV-2 infected *vs*. parental CAL-51 cells and lung tissue from COVID-19 positive patients *vs*. healthy persons[4] identified 341 SARS-CoV-2 infection-responsive host genes shared in both systems, among which 59 genes were activated and 282 were repressed (Fig. S3F, G, Table S1). We validated the expression of some these genes by q-RT-PCR. Consistent with our RNA-seq results, CCL2 (C—C motif chemokine ligand 2), CCL5 (C—C motif chemokine ligand 5), CXCL1 (C-X-C motif chemokine ligand 1), CXCL2 (C-X-C motif chemokine ligand 2), and CXCL3 (C-X-C motif chemokine ligand 3) mRNA levels were increased upon SARS-CoV-2 infection (Fig. 1L), which explains the pro-inflammatory disease state induced by SARS-CoV-2.

To date, the impact of SARS-CoV-2 infection on patients, especially cancer patients, remains poorly understood. Herein we discovered that the Mediator component MED12 links chemotherapy and SARS-CoV-2 infection. Our results demonstrate that MED12 blocks SARS-CoV-2 infection through at least two mechanisms: repressing ACE2 expression to control viral entry, and potentiating broad anti-viral defensive pathways in the host.

Author contributions

W.X, S.Z., and F.L. conceived the project, designed the experiments and wrote the manuscript. S.Z., and F.L. carried out the experiments with the assistance from K.D., and Y.W. P.H. and R.B performed the SARS-CoV-2 and Pseudo-virus entry assays, respectively. P.L., F.L., and O.I. performed the RNA-seq data analysis. F.L., S.Z., R.B., S.M., and P.H. analyzed and visualized the data. K.D., N.S., R.B., P.H., P.L. and Y.K. reviewed and edited the manuscript. W.X. directed and supervised the project. All authors discussed the results and commented on the manuscript.

Conflict of interests

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2021.08.001.

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