LONG NON-CODING RNA REGULATES IFN-I RESPONSE AND SARS-CoV-2 REPLICATION

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BACKGROUND

SARS-CoV-2 infection triggers immune reprogramming through global transcriptomic changes that drive the development of Coronavirus disease 2019 (COVID-19). Although differential expression and functions of protein-coding transcripts have been extensively studied in SARS-CoV-2 infection, less than 2% of the human genome encodes protein-coding transcripts. The majority of the transcriptome consists of non-protein-coding RNAs (ncRNAs). Long noncoding RNAs (lncRNAs), which constitute a large proportion of the transcriptome, regulate protein-coding gene expression and function, impact immunity, response to infections, and have prominent roles in health and disease. However, the impact of lncRNAs on SARS-CoV-2 pathogenesis is poorly understood.

METHODS

- We identified cellular lncRNAs with significant differential expression (DE) in SARS-CoV-2 infected vs. uninfected cells from published transcriptomic data. We silenced expression of a virus-induced lncRNA, ***Bre-AS1,*** in a human lung epithelial cell model using lncRNA-specific ASO (BAsi) or negative control (NC) and compared viral replication in BAsi vs. NC cells using a plaque assay.
- We also determined the effect of *Bre-AS1* expression on IFN-I response in cells infected with SARS-CoV-2.
- Using various cellular and molecular methods, we determined how *Bre-AS1* inhibits phosphorylation of a transcription factor STAT3 and enhances type I interferon (IFN-1) response and inhibits SARS-CoV-2 replication

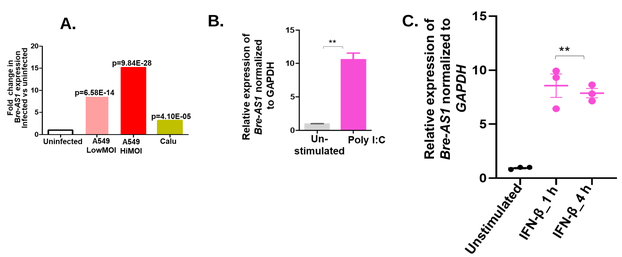


Figure. 1. (A) We identified lncRNAs with significant differential expression in SARS-CoV-2 infected vs. uninfected cells across two cell types (A549-hACE2 and Calu) from published transcriptome data[1]. lncRNA *Bre-AS1* expression levels were significantly higher in SARS-CoV-2 infected vs. uninfected cells[1]. **(B)** *Bre-As1* is expressed at significantly higher levels in polyI:C stimulated A59 cells than unstimulated cells. **(C)** IFN-β stimulation of A549 cells induced significant *Bre-AS1* expression.

Our data show that virus infection and IFN-I stimulation induce expression of lncRNA *Bre-AS1. Bre-AS1* enhances Interferon-stimulated gene expression and inhibits viral replication.

RESULTS

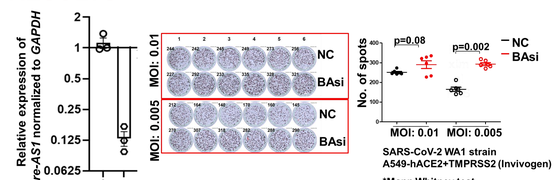


Figure 2. Effect of *Bre-AS1*-silencing on SARS-CoV-2 replication. **(A)** A549-hACE2-TMPRSS2 (A549^{AT}) cells were transfected with specific ASO to silence BA (BAsi) or negative control ASO (NC). Silencing of lncRNA was confirmed by qPCR. **(B,C)** We transfected A549^{AT} cells in triplicates with ASO, infected with SARS-CoV-2 virus (MOI 0.01 or 0.005). We incubated the cells further for 24 h, and fixed them using 10% formalin. Following fixation, we performed immunostaining using the anti-SARS-CoV-2 antibody and the Vectastain ABC kit and DAB Peroxidase Substrate kit (Vector Laboratory, Inc., CA, USA) for development. *Bre-AS1*-silencing (BAsi) significantly increased virus replication at MOI 0.005. Non-parametric Wilcoxon-Mann-Whitney tests were used for statistical comparisons and two tailed p values are indicated.

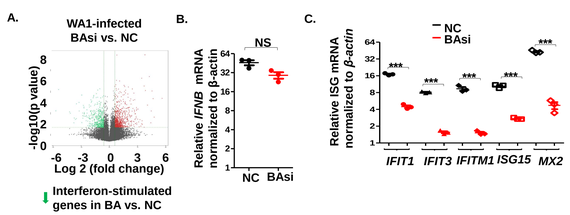


Figure 3. Effect of *Bre-AS1* silencing on IFN-I response. We transfected A549-hACE2-TMPRSS2 cells in triplicates with ASO (BAsi vs NC), infected with SARS-CoV-2 (MOI 0.005) and collected cells after 24 h. **(A)** Total RNA was extracted from BAsi or NC transfected and SARS-CoV-2-infected A549^{AT} cells and whole transcriptome analyses was performed (RNA-seq). Several IFN-stimulated genes (ISGs) were significantly downregulated in BA vs. NC [p<0.05, FC≥1.5] **(B,C)** *IFNB* and *ISG* mRNA were measured by qPCR and normalized to a housekeeping gene *β-actin*. Non-parametric t-test for statistical comparisons and two tailed p values are indicated *p<0.05; **p<0.01. ***p<0.001.

RESULTS

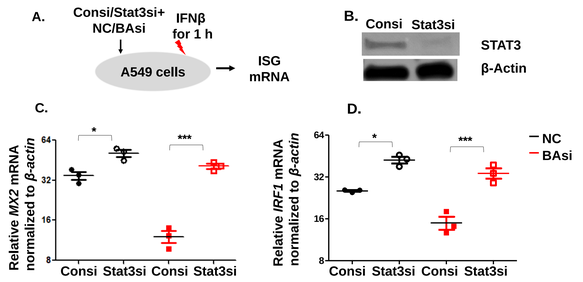


Figure 5. STAT3-silencing rescued ISG-expression in BA-silenced cells: (A) We co-transfected siRNA targeting STAT3 (STATsi) or Control (Consi) along with BAsi or NC ASO in triplicates. **(B)** We confirmed STAT3-knockdown by Western blot. Cells were stimulated with IFNβ (100u/ml; 1 h) and **(C)** *MX2* (ISRE-driven); **(D)** *IRF1* (GAS-driven) expression was measured by qPCR and normalized to β-actin. t-test; two-sided p *<0.05, ***<0.001 was considered significant.

References

1. Blanco-Melo D, Nilsson-Payant BE, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. Cell. 2020;181(5):1036-45 e9.
2. Zhang L, Liu B, Deng QH, Li JX. LncRNA BRE-AS1 acts as a tumor suppressor factor in bladder cancer via mediating STAT3. Eur Rev Med Pharmacol Sci. 2020;24(10):5320-8
3. Tsai MH, Pai LM, Lee CK. Fine-Tuning of Type I Interferon Response by STAT3. Front Immunol. 2019;10:1448.

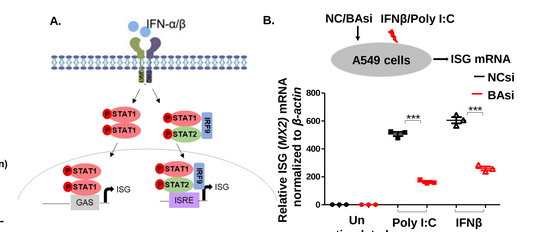
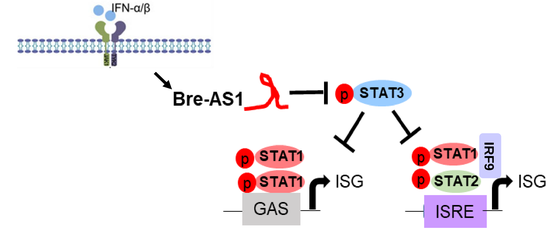


Figure 4. (A) IFN-I stimulation triggers phosphorylation of STAT1 and STAT2 proteins that then recruit IRF9 to form the trimeric IFN-stimulated gene factor 3 (ISGF3) complex. The pSTAT1 homodimers or ISGF3 complex then translocate into the nucleus where they binds to GAS or ISRE regions within the promoter regions of ISGs inducing ISG transcription. **(B)** We transfected A549 cells in triplicates with ASO, stimulated with IFNβ (100u/ml for 1 h) or Poly I:C (100u/ml; 24 h). ISG (*MX2*) mRNA measured by qPCR and normalized to a housekeeping gene (β-actin). t-test; ***p<0.001 **(C)** Overexpression of *Bre-AS1* was shown to inhibit Tyr705 phosphorylation of STAT3[2]. We transfected A549 cells with BAsi or NC, collected cell lysates and determined Tyr705 pSTAT3, STAT3 and B-actin expression by Western blot. **(D)** pSTAT3 sequesters pSTAT1 and prevents it from forming homodimers that activate ISG containing GAS element in the promoter. STAT3-PLSCR2 complex prevents IGSF3 from binding to ISRE element in ISG promoters and inhibits ISG transcription. pSTAT3 homodimers suppress expression of STAT1, STAT2 and IRF9 thus reducing function IGSF3 complex and ISG-transcription [From Tsai et al 2019)[3]**.**

CONCLUSIONS



Our data show that virus infection and IFN-I stimulation induce expression of lncRNA *Bre-AS1*. Phosphorylated STAT3 inhibits transcription by both GAS and ISRE elements in the promoter of ISG genes. Bre-AS1 inhibits STAT3 phosphorylation and enhances ISG expression.

ADDITIONAL KEY INFORMATION

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