CHRD 2021: Abstract & Poster Submission Form

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Research Category:

- ◉ Basic Science
- ○ Clinical
- ○ Community Health / Policy

What was your role in the project?

- ☑ Design
- ☑ Perform Experiments
- ☑ Analyze Data
- ☑ Write Abstract
- ☐

Presenter Status:

- ○ Undergraduate Students
- ○ Masters Student
- ○ PhD Student
- ◉ Post-Doctoral Fellows
- ○ Residents
- ○ Non-Trainee

Title

DNA-sensing: a potential link between first hit stimuli and inflammatory response in experimental congenital diaphragmatic hernia

Background

Recent scientific advances indicate the immune response to viral infection or pollution could be the first hit stimuli in the pathogenesis of congenital diaphragmatic hernia (CDH). Activation of the DNA-sensing cGAS-STING pathway by increases of double-stranded DNA (dsDNA) presents a possible link between relevant external factors and immune response in the developing embryo.

Objective

We hypothesized that DNA-sensing plays a crucial role in the pathophysiology of CDH.

Methods

To investigate the activation of the cGAS-STING pathway, we treated human BEAS-2B cells with Nitrofen for 24h and performed immunofluorescence staining of cGAS. We further used the Quant-IT™ PicoGreen™ assay to assess dsDNA concentration in cells after 24h Nitrofen treatment. We harvested Nitrofen treated fetal rats at embryonic days E15 and E18. Lungs were sectioned after paraffin-embedding and immunofluorescent staining of cGAS was performed.

Results

Treatment of BEAS-2B cells for 24h revealed increased fluorescence intensity of cGAS in cells (area threshold ROI / pixel Control 15.6±3.6, Nitrofen 36.4±12.3; p:0.007). Quant-IT™ PicoGreen™ assay in BEAS-2B cells after 24h treatment with Nitrofen revealed a higher concentration of dsDNA (N=3; Controls: 6.85x10-4±3x10-5 ng/ml, Nitrofen: 8.02x10-4±1.8x10-5; p = 0.01). Immunofluorescent staining for cGAS showed higher relative fluorescence in lung sections of Nitrofen treated pups at E15 (N=4, 1.6-fold increase; p = 0.009) and E18 (N=5, 2.3-fold increase; p<0.0001).

Conclusion

Our data show that Nitrofen treatment is leading to the upregulation of cGAS in the rat model of CDH and human BEAS 2B cells. Furthermore, Nitrofen treated cells showed higher dsDNA concentrations. These results indicate that DNA sensing and dysregulation of the cGAS-STING pathway is a possible link between variable, external activators of an immune response contributing to the pathophysiology of CDH.

Authors

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