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

Moritz Markel[1,2], Wai Hei Tse[1], Nolan DeLeon[1], Jaida Albrechtsen[1], Shana Khanamouizadeh[1], Daywin Patel[1], Martin Lacher[2], Richard Keijzer[1]

[1]University of Manitoba, Department of Surgery, Division of Pediatric Surgery and Children's Hospital Research Institute of Manitoba, Winnipeg, Canada
[2]University of Leipzig, Department of Pediatric Surgery, Leipzig, Germany

MiRACLA MicroRNAs • And • Congenital • Lung • Anomalies

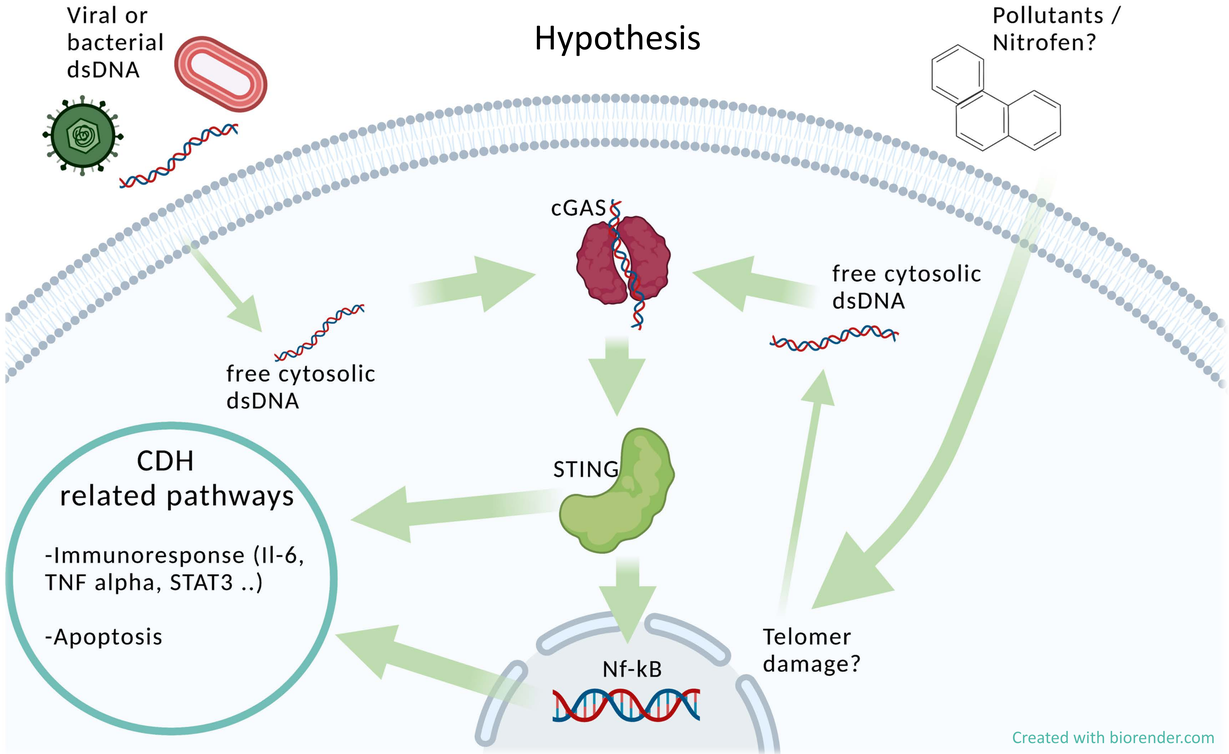
BIOLOGY OF BREATHING Research Theme

Introduction

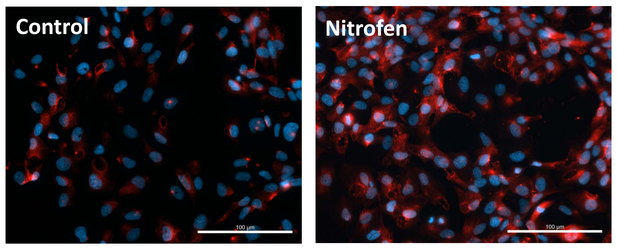
- The immune response to viral infection or pollution could be the first hit stimulus in the pathogenesis of congenital diaphragmatic hernia (CDH).
- DNA-sensing cGAS-STING pathway and increase of double-stranded DNA (dsDNA) is a possible link between relevant external factors and immune response in the embryo.

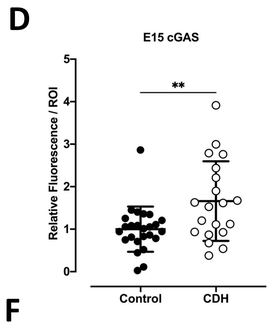
Methods

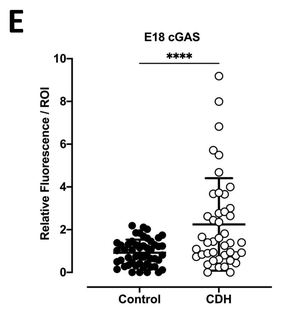
- BEAS-2B cell culture and Nitrofen treatment and immunofluorescence staining cGAS.
- Quant-IT™ PicoGreen™ assay to assess dsDNA concentration in cells after Nitrofen treatment.
- Immunofluorescence staining of Nitrofen treated fetal rat lungs at embryonic days E15 and E18 for cGAS.

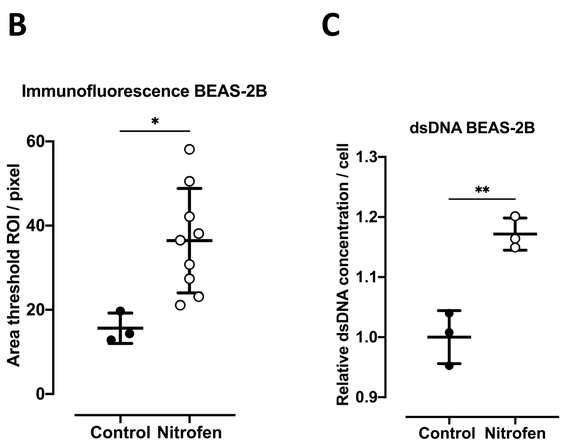


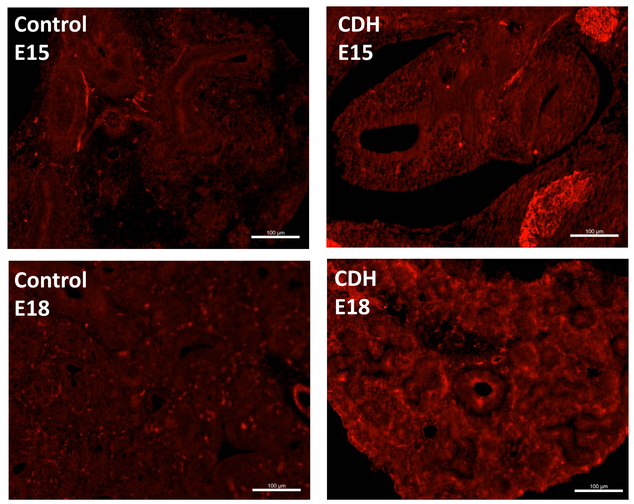
Results











- Treatment of BEAS-2B cells for 24h revealed increased fluorescence intensity of cGAS in cells (**A,B**: 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 (**C**: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 (**D,F**:N=4, 1.6-fold increase; p = 0.009) and E18 (**E,F**: N=5, 2.3-fold increase; p<0.0001).

Conclusion

DNA sensing and dysregulation of the cGAS-STING pathway is a possible new link between variable, external activators of an immune response contributing to the pathophysiology of CDH.

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