Seeing Through the Smoke: How E-cigarettes Affect the JAK/STAT6 Pathway

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Introduction

- The popularity of e-cigarettes is outpacing that of traditional tobacco products ("E-cigarette use among youth…" 2016).
- JUUL is a popular e-cigarette which accounted for 63% of e-cigarette sales in 2020 (Conway 2020).
- Use of e-cigarettes is significantly associated with worsened symptoms of asthma and chronic obstructive pulmonary disorder (COPD) (Osei et al. 2020).
- JAK/STAT6 is an inflammatory signal transduction pathway involved in the development of asthma and COPD (Walford and Doherty 2013).
- A colorimetric assay of HEK 293 IL-13 Sensor cells can be used to measure the effect of JUUL aerosol extract and e-liquid on the signaling activity of JAK/STAT6 for which IL-4 and IL-13 are the ligands.

Questions Asked

- **Can JUUL aerosol extract or e-liquid activate JAK/STAT6 in the absence of ligand?**
- **Does JUUL aerosol extract or e-liquid have a synergistic or antagonistic effect on JAK/STAT6 signaling activity?**
- **Do different JUUL flavors impact JAK/STAT6 signaling?**

Literature Cited

Conway J. Vaping market share by brand U.S. 2020. Statista. 2020 Sep 18.

Osei AD, Mirbolouk M, Orimoloye OA, Dzaye O, Uddin SMI, Benjamin EJ, Hall ME, DeFilippis AP, Bhatnagar A, Biswal SS, et al. Association Between E-Cigarette Use and Chronic Obstructive Pulmonary Disease by Smoking Status: Behavioral Risk Factor Surveillance System 2016 and 2017. American Journal of Preventive Medicine. 2020;58(3):336–342. doi:10.1016/j.amepre.2019.10.014

U.S. Department of Health and Human Services. E-Cigarette Use Among Youth and Young Adults. A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2016.

Walford HH, Doherty TA. STAT6 and lung inflammation. JAK-STAT. 2013;2(4). doi:10.4161/jkst.25301

Image Sources

https://www.forbes.com/sites/kathleenchaykowski/2018/11/16/the-disturbing-focus-of-juuls-early-marketing-campaigns/?sh=23c004a614f9

https://www.invivogen.com/hek-blue-il4-il13

Methods

Aerosol Extract (10, 5, 2.5, 1.25 v/v% in DMEM) → **IL-13** / **No IL-13**

E-liquid (0.4, 0.2, 0.1, 0.05 v/v% in DMEM) → **IL-13** / **No IL-13**

Treatments applied to HEK293 IL-13 Sensor Cells
Cells incubated for 18 hours

Quanti-Blue assay (signaling activity) and Cell Titer-96 assay (number of cells)

***All treatments applied in quadruplicate**

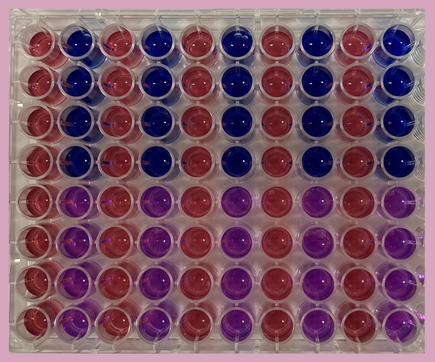

Figure 1. 96-well plate of Quanti-Blue and Cell Titer-96 assays applied to HEK293 IL-13 Sensor Cells

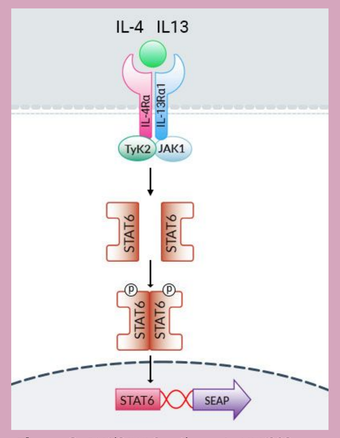

Figure 2. JAK/STAT6 pathway in HEK293 IL-13 Sensor Cells
https://www.invivogen.com/hek-blue-il4-il13

Acknowledgements

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Results

- Cells exposed to 10% (3.78, 95% CI = 3.21, 4.36) and 5% (3.70, 95% CI = 3.61, 3.81) JUUL Virginia Tobacco extract with IL-13 had significantly greater mean signaling activity per cell than the control (3.29, 95% CI = 3.17, 3.41).
- Mean signaling activity per cell for those treated with JUUL Mint aerosol extract and with IL-13 was lower than the control (3.50, 95% CI = 3.35, 3.65) for cells treated with 2.5% (3.25, 95% CI = 3.04, 3.46) and 1.25% (3.04, 95% CI = 2.82, 3.26) aerosol extract.
- Cells treated with 0.4% (3.85, 95% CI = 3.51, 4.20) Menthol e-liquid had significantly higher level of mean signaling activity than the control (3.50, 95% CI = 3.18, 3.81).

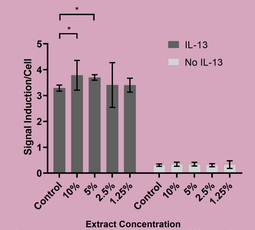

Figure 3. Mean signal induction per cell for HEK293 IL4/IL13 Sensor Cells treated with or without 20 μL IL-13 along with varying concentrations of JUUL Virginia Tobacco e-cigarette aerosol extract (n=4). Asterisks indicate a significant difference between in-group treatments based on a Dunnett's Multiple Comparisons test (* p≤0.05) after a two-way ANOVA. Error bars indicate 95% confidence intervals.

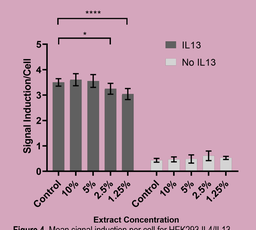

Figure 4. Mean signal induction per cell for HEK293 IL4/IL13 Sensor Cells treated with or without 20 μL IL13 along with varying concentrations of JUUL Mint e-cigarette aerosol extract (n=4). Asterisks indicate a significant difference between treatments in-group treatments based on a Dunnett's Multiple Comparisons test (* p≤0.05, **** p≤0.0001) after a two-way ANOVA. Error bars indicate 95% confidence intervals.

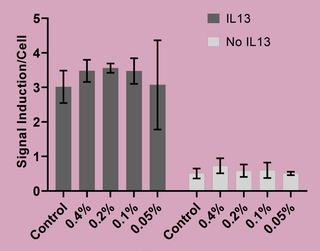

Figure 5. Mean signal induction per cell for HEK293 IL4/IL13 Sensor Cells treated with or without 20 μL IL-13 along with varying concentrations of JUUL Virginia Tobacco e-liquid (n=4). Error bars indicate 95% confidence intervals.

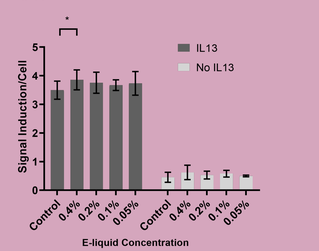

Figure 6. Mean signal induction per cell for HEK293 IL4/IL13 Sensor Cells treated with or without 20 μL IL-13 along with varying concentrations of JUUL Menthol e-liquid (n=4). Asterisks indicate a significant difference between in-group treatments based on a Dunnett's Multiple Comparisons test (* p≤0.05) after a two-way ANOVA. Error bars indicate 95% confidence intervals.

CONCLUSIONS

- **E-cigarette aerosol extract and e-liquid do not activate JAK/STAT6 in the absence of ligand.**
- **JUUL Virginia Tobacco extract and Menthol e-liquid may have a slight synergistic effect with IL-13.**
- **JUUL Mint extract may have a slight antagonistic effect with IL-13.**