Decline in NRF2 regulated antioxidants in COPD lungs due to loss of its positive regulator DJ-1

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ONLINE DATA SUPPLEMENT

TABLE E1. Age and gender-adjusted ratios (95% confidence intervals) of NRF2, its target antioxidants (NQO1, GCLM and HO1) and its regulators (KEAP1 and DJ-1) mRNA and protein levels in GOLD1-2 (mild COPD), and GOLD 3-4 (advanced COPD) and non-emphysematous lungs

| | Non-COPD (N = 26) | GOLD 1-2 (N = 18) | GOLD3-4 (N = 21) | p-value |
|-----------------------------------|----------------------|----------------------|---------------------|----------|
| NRF2 | | | | |
| mRNA (relative fold change) | 1.00 (reference) | 1.75 (1.20, 2.56) | 1.07 (0.73, 1.56) | 0.593 |
| Protein (arbitrary value) NQO1 | 1.00 (reference) | NA | 0.04 (0.02, 0.06) | <0.0001 |
| mRNA (relative fold change) | 1.00 (reference) | 0.42 (0.33, 0.53) | 0.30 (0.24, 0.37) | < 0.0001 |
| Protein (arbitrary value) GCLM | 1.00 (reference) | NA | 0.57 (0.36, 0.89) | <0.0001 |
| mRNA (relative fold change) | 1.00 (reference) | 0.30 (0.25, 0.37) | 0.25 (0.21, 0.30) | < 0.0001 |
| Protein (arbitrary value) | 1.00 (reference) | NA | 0.09 (0.04, 0.20) | <0.0001 |
| mRNA (relative fold change) | 1.00 (reference) | 0.58 (0.44, 0.76) | 0.27 (0.21, 0.36) | < 0.0001 |
| Protein (arbitrary value) KEAP1 | 1.00 (reference) | NA | 0.23 (0.07, 0.72) | <0.0001 |
| mRNA (relative fold change) | 1.00 (reference) | 1.04 (0.78, 1.38) | 0.85 (0.64, 1.21) | 0.91 |
| Protein (arbitrary value) DJ-1 | 1.00 (reference) | NA ` | 1.19 (0.74, 1.92) | 0.48 |
| mRNA (relative fold change) | 1.00 (reference) | 0.71 (0.62, 0.82) | 0.43 (0.37, 0.82) | < 0.0001 |
| Protein (arbitrary value) GSH | 1.00 (reference) | NA | 0.15 (0.09, 0.26) | <0.0001 |
| (nmole/mg protein) TBARS | 1.00 (reference) | NA | 0.09 (0.03, 0.30) | 0.003 |
| (µmole/mg protein) | 1.00 (reference) | NA | 13.1 (6.5, 26.7) | <0.0001 |

NA: not available; *Ratios (95% CIs) and p-values were obtained from a linear regression model on log₂-transformed mRNA and protein levels comparing GOLD1-2 and GOLD3-4 to non-emphysematous lung samples.

FIGURE E1. Increase in IL-8 secretion, an indicator of stress, with increasing incubation with CSE in Beas2B cells. Human IL-8 ELISA analysis on the culture media for secreted IL-8 levels show temporally increasing levels of IL-8 following CSE (250 $\mu g/ml$) treatment as compared to DMSO vehicle control. The experiment was repeated three times.

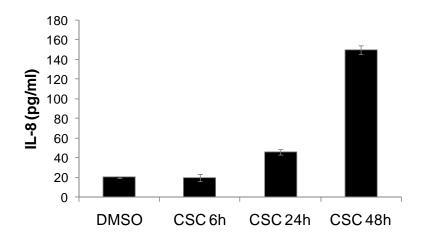


FIGURE E2. Decrease in NRF2 protein levels with increasing incubation with CSE in Beas2B cells. Protein levels of NRF2 protein in Beas2B cells, treated with cigarette smoke condensate (CSE, 250 μ g/ml) for 6 h, 24 h and 48 h time points or DMSO treatment. The experiment was repeated three times.

| | | 6h | | | 24 | 24h | | 48h | | | |
|--------------------|------|----|---|---|----|-----|---|-----|---|---|-------|
| | | 1 | | - | - | d | | - | - | | NRF2 |
| | | | - | _ | | | - | _ | | | GAPDH |
| | DMSO | + | - | - | - | - | - | - | - | - | |
| | CSE | - | - | + | + | + | + | + | + | + | |
| Pre-CSE treatment | NAC | - | + | - | + | - | + | - | + | - | |
| Post-CSE treatment | NAC | - | - | - | - | - | - | - | - | + | |