Lung Biology Research & Trainee Day
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Category: Staff/Tech/Other
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Title: Targeting ΔNp63α in flavoring-induced airway epithelial cell death
Abstract: Introduction: The airway epithelium is the primary target of most inhaled toxicants. Diacetyl (DA; 2,3-butanedione) is a highly reactive alpha(α)-diketone found in buttery flavorings, which is easily vaporized due to its volatile chemical properties. DA inhalation exposure at occupationally relevant concentrations can cause significant lung disease. Our lab has shown previously that acute DA inhalation exposure causes significant injury to primary human airway basal cells, the progenitor cell of the human airway, with marked decreases in ΔNp63α and integrin beta4 (β4, CD104) expression. Hence, we hypothesized DA exposure causes airway basal cell cytotoxicity through loss of integrin β4 and subsequent anoikis (cell attachment-associated cell death). The purpose of these experiments was to test whether overexpression of ΔNp63α or CD104 prevents DA-induced cell death in airway epithelial cells. Methods: Two human bronchial epithelial cell lines, BEAS-2B and 16HBE14o- (ATCC, Manassas, VA), were grown in submerged cultures for DA exposures. Cells were exposed to various concentrations of DA (2.3, 5.7, 8.6, 11.4mM) for 1 hour. Following exposure, media containing DA was removed, and cultures were replaced with fresh media. Cultures were then monitored for 1, 3, and 5 days after DA exposure to assess for total cell count and cell death by MTT and WST-1 assay. A subset of cultures were transfected with ΔNp63α, CD104 or pcDNA3 (control) (Addgene, Watertown, MA) prior to DA exposure. Immunofluorescence (IF) staining and western blot analyses were then performed on 2,4,6 days after DA exposure for measurement of ΔNp63α and CD104 expression. An ordinary one-way ANOVA with Bonferroni correction for comparing groups ($p<0.05$). Results: A single 1-hr DA exposure at concentrations ≥11.4mM caused significant cytotoxicity by MTT and WST-1 in both BEAS2B and 16HBE cultures (ANOVA; $p<0.001$ and $p<0.05$, respectively). A significant decrease in ΔNp63α and CD104 expression occurred in both cultures at DA concentrations of 5.7 and 8.6mM and prior to cell death, however, returned to baseline levels by Day 4, suggestive of appropriate recovery after lower concentration DA exposures. With overexpression of ΔNp63α but not CD104, a significant improvement in cell viability was seen compared to plasmid transfected controls at equivalent DA exposure concentrations in 16HBE cells (ANOVA, $p<0.05$). Conversely, no significant difference in cell viability was seen with ΔNp63α or CD104 transfection compared to plasmid controls in BEAS2B cultures, despite increased expression of CD104 and ΔNp63α. Conclusions: Overexpression of ΔNp63α in human airway epithelial cells prior to flavoring exposure is protective against DA-induced cytotoxicity. This protection was both concentration- and cell-type dependent, but likely independent of CD104 expression and subsequent anoikis. Future work will identify the specific pathway(s) by which ΔNp63α is cytoprotective to airway epithelial cells exposed to DA and will validate these novel findings in primary human airway basal cells cultures.