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Laboratory-Based (Basic, Wet Bench) / Postdoctoral Fellow
Pediatrics / Allergy and Immunology

Proteomic Analysis Reveals Omeprazole's Modulation of Inflammatory, Proliferative, and Metabolic Pathways in Eosinophilic Esophagitis

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Background: Eosinophilic esophagitis (EoE) is a chronic allergic disease characterized by esophageal dysfunction, type 2 inflammation, and eosinophilic infiltration. Although proton pump inhibitors (PPIs) like omeprazole are used to manage EoE, their underlying mechanisms remain unclear.

Methods: Whole-cell proteomics of air-liquid interface (ALI) cultures of esophageal epithelial cells (EPC2-hTERT) was used to investigate the mode of action of omeprazole in IL-13-treated cells. EPC2 cells grown at ALI were treated with 100 ng/ml IL-13 and/or 50 µM acid-activated omeprazole. Cell pellets were lysed, digested using S-Trap, and peptides were desalted, dried, and reconstituted with iRT peptides. Proteomic analysis was performed using an Exploris 480 mass spectrometer coupled with an Ultimate 3000 nano UPLC, employing data-independent acquisition (DIA). Western blotting of ALI cultures was conducted to validate protein-level changes. Cell proliferation was assessed using EdU incorporation in monolayer cultures, and mitochondrial respiration was evaluated using the Seahorse XF Cell Mito Stress Test.

Results: Unsupervised clustering revealed distinct separation between groups, with the first principal component (PC1) accounting for 37.81% of the variation, primarily reflecting omeprazole's impact. Differentially accumulated proteins (DAPs) were identified using FDR < 0.05 and $\log_2 FC \geq |1|$. We identified 108 DAPs in IL-13-treated vs. untreated ALI, 190 in omeprazole-treated vs. untreated, and 155 in IL-13 and omeprazole-treated vs. IL-13-only ALI. Omeprazole significantly downregulated pathways associated with inflammation (e.g., interferon responses) and cell proliferation (e.g., E2F targets, G2M checkpoint), including suppression of key proliferation markers such as TOP2A and MCMs. Reduced EdU incorporation confirmed decreased proliferation. Seahorse analysis showed that omeprazole attenuated IL-13-induced increases in basal respiration, ATP production, and maximal respiration, indicating modulation of mitochondrial function and metabolic stress. Concurrently, it upregulated pathways related to metabolism and cellular homeostasis, including cholesterol homeostasis, oxidative phosphorylation, fatty acid metabolism, xenobiotic metabolism, and hypoxia response.

Discussion: These findings suggest that omeprazole modulates inflammatory, proliferative, and metabolic pathways in esophageal epithelial cells. Further studies are warranted to determine how these molecular effects contribute to its therapeutic benefit in EoE.