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OBJECTIVES/GOALS: This research aims to identify genetic alterations influencing congenital anomalies of the kidney and urinary tract (CAKUT) and bridge a fundamental gap in understanding the cellular mechanisms underlying kidney development, with the long-term goal of enhancing treatments for congenital renal anomalies. METHODS/STUDY POPULATION: We will use a loss-of-function approach in combination with immunofluorescent microscopy techniques to determine the influence of Dnmbp perturbation on Daam1 localization, actin assembly, and junctional turnover. Additionally, to establish a foundation for delineating the molecular mechanism of DNMBP during kidney development, we will utilize clinical whole exome sequencing data to identify human DNMBP mutations associated with urogenital anomalies. Furthermore, we will determine whether human DNMBP mutations linked to CAKUT lead to disruptions in nephron development through loss-of-function rescue experiments in *Xenopus*. RESULTS/ANTICIPATED RESULTS: Here, we evaluate the dynamics of Dnmbp-mediated transport of Daam1 within the developing kidney and show preliminary data suggesting that Dnmbp and Daam1 directly interact to promote adhesive contact formation between nephron progenitor cells. Furthermore, we propose a model in which Dnmbp functions as a critical regulator of epithelial tissue morphogenesis and provides a functional link between the dynamic processes of actin cytoskeleton regulation, intracellular adhesion, and vesicular transport. Future studies will determine whether Dnmbp interaction with Daam1 facilitates junctional actin assembly by directing Daam1 to cell–cell contact sites via Dnmbp-associated vesicle targeting, enhancing our understanding of the cellular mechanisms influencing tubule morphogenesis. DISCUSSION/SIGNIFICANCE OF IMPACT: This research will establish a previously unknown role for DNMBP in kidney development and provide a comprehensive understanding of the impacts of simultaneously regulating vesicular transport and actin dynamics in nephrogenesis.

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## Repositioning monensin: Enhancing anti-cancer activity and immune modulation in breast cancer cells

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OBJECTIVES/GOALS: Monensin is FDA approved for use in veterinary medicine. Recent studies pointed to its potent anticancer activity. Since de novo drug discovery process typically takes 10 to 15 years and requires an investment of approximately $1.3 to $3 billion, drug repositioning can bypass several steps in this process and increase the potential for success. METHODS/STUDY POPULATION: Cell viability assays were conducted on human MDA-MB-231, MDA-MB-468, and MCF10A breast cancer cell lines and mouse EO771 and 4T1 breast cancer cell lines. MDA-MB-231 cell line was used in all the studies unless specified otherwise. Time course levels of Bcl-2, Bak, p62, and LC3II were assessed via Western blotting with GAPDH as a loading control. Proteomics analysis was conducted by the IDEA National Resource for Quantitative Proteomics. Time course levels of major histocompatibility complex (MHC) I and II and calreticulin were evaluated using flow cytometry. At least three biological replicates have been conducted for each experiment. RESULTS/ANTICIPATED RESULTS: Monensin and several of its novel analogs were potent toward human and mouse breast cancer cell lines. Furthermore, they induced apoptotic cell death as evidenced by Annexin V/PI assay, downregulation of Bcl-2, and upregulation of Bak in MDA-MB-231 cells. Proteomics analysis revealed that several molecular pathways related to MHC class I and II antigen presentation were significantly altered following treatment with these compounds. Additionally, monensin and its analogs significantly increased the expression of MHC class I and II. Our studies also showed that monensin and its analogs increase the surface calreticulin levels. Treatment of MDA-MB-231 cells with these compounds also resulted in an increase in p62 and LC3II expression, suggesting a disruption of the autophagic process. DISCUSSION/SIGNIFICANCE OF IMPACT: These results suggest that monensin and its analogs not only exhibit anti-breast cancer cell activity but also modulate immune-related pathways. By disrupting autophagy and enhancing calreticulin levels, these compounds may potentiate antitumor immune responses, providing a promising avenue for drug repositioning in cancer therapy.

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## Impact of secretome derived from stool samples of patients with multiple system atrophy in alpha-synuclein oligomerization

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OBJECTIVES/GOALS: This study investigates the contribution of the stool secretome (the soluble factors secreted by microbes into extracellular space) to in vitro α-synuclein (αSyn) oligomerization using stool cultures from patients with multiple system atrophy (MSA), a rare neurodegenerative disease hallmarked by pathologic αSyn aggregates. METHODS/STUDY POPULATION: Stool samples from MSA patients (n = 20), household controls (n = 20), and healthy controls (n = 20) will be cultured using an adapted dilution-to-extinction approach. The goal is to reduce microbial complexity progressively to produce random secretome combinations that may affect αSyn oligomerization differentially. The original inoculant and dilutions will be cultured anaerobically to collect conditioned media (CM) enriched with microbial secretomes. CM will be used to expose a fluorescence resonance energy transfer (FRET) biosensor assay and a Gaussia luciferase protein complementation assay – both modified to quantify αSyn-αSyn interaction indicating oligomerization. Any CM-altering αSyn oligomerization will undergo multiomic characterization to identify potential causative agent(s). RESULTS/ANTICIPATED RESULTS: Specific microbe-produced molecules from the literature are anticipated to modulate αSyn oligomerization, identified by targeted, reductionist studies that selected and tested separately single microbial factors on


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