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Project Title: Investigating Roles of a Conserved Matrix Protein in Adhesion and Injury Response

Introduction/Objectives: Tubulointerstitial nephritis antigen (TIN-ag) is a glycoprotein localized in the renal proximal tubule. Studies suggest TIN-ag anchors renal tubular cells to the basement membrane. TIN-ag shares 51% similarity with Dead man walking (Dmw) in *Drosophila*, where preliminary genetic results suggest that Dmw serves as a ligand for integrins containing the α PS3 subunit. The aims of this project are: 1. To ascertain whether cells expressing α PS3 bind to Dmw, and 2. To determine if rat proximal tubule cells (RPTCs) induce expression of TIN-ag when exposed to the chemotherapeutic drug cisplatin.

Methods: Dmw: *Drosophila* S2 cells were stably transfected with genes coding for α PS3, α PS1, α PS2, and Dmw. These cells were incubated in media containing V5-tagged Dmw protein. The presence of bound V5-tagged Dmw was detected by antibodies and fluorescence activated cell sorting (FACS). TIN-ag: RPTCs were incubated in different cisplatin concentrations: 10 μ M, 2 μ M, 0.4 μ M, 0.08 μ M, 0.016 μ M, and 0 μ M. TIN-ag mRNA was isolated from each well. Real-time PCR was performed, and a gel was run to determine relative amounts of TIN-ag mRNA.

Results: Dmw: FACS analysis revealed the following percentages of cells bound to V5-tagged Dmw: Control, 0.51%; α PS1, 1.89%; α PS2, 1.14%; α PS3, 1.35%. TIN-ag: The bands representing TIN-ag mRNA at concentrations of cisplatin described above were indistinguishable from one another in terms of quantity.

Summary/Discussion: Dmw: Although more S2 cells expressing α PS3 integrin bound to V5-tagged Dmw than did the control cells, fewer α PS3-expressing cells bound than did α PS1-expressing cells. The overall low percentage of Dmw-binding cells suggests that Dmw does not function as an integrin ligand. TIN-ag: The lack of elevated amounts of TIN-ag mRNA in cisplatin-exposed renal cells suggests cells damaged by cisplatin do not induce expression of TIN-ag compared to control cells.