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**Molecules, Stress Signaling and Wnt Responsiveness**

Small molecules are important in chemical genetics exploration of intracellular pathways involved in normal and pathological processes. Wnt signaling plays a central role in tissue maintenance and cancer. This report showed a mechanism by which mitotic and genotoxic stress modulates Wnt responsiveness to control cell shape and renewal. The paper was featured in the journal *Cell Chemical Biology.*

Medicinal chemists and stem cell biologists at the Human BioMolecular Research Institute (HBRI), in San Diego, CA, and Stanford University, in Stanford, CA, respectively, have reported on a compound that revealed a molecular cascade linking stress signaling through HIPK2 and p53 to the regulation of TCF/LEF transcriptional activation. This may have importance for tissue morphogenesis and anti-cancer drug discovery efforts.

Writing on January 26, 2021, in the journal *Cell Chemical Biology,* the study used recently discovered small molecules **PAWI-1** and **PAWI-2** to identify a molecular cascade linking stress signaling through ATM, HIPK2, and p53 to the regulation of TCF/LEF transcriptional activation. **PAWI-1** and **PAWI-2** delineated a mechanism involving mitotic assembly stress to modulate Wnt signaling.

“The finding that mitotic stress or DNA damage regulate Wnt responsiveness is important because it explains why stressed cells cannot regenerate and heal tissue damage” explains co-author Dr. Mark Mercola. “By blocking the ability to respond to

Wnt signaling, cellular stress prevents cells from migrating, replicating and differentiating."

Given its activity, this new compound is of utility as an anti-cancer drug candidate. Ongoing studies show its utility against models of pancreatic, breast, prostate and colon cancer.

The report describes two compounds named **PAWI-1** and **PAWI-2** (**p**53 **a**ctivator and **W**nt **i**nhibitor-**1** or **-2**, respectively). The initial "hit" compound, **PAWI-1,** was identified in a high throughput screen of ~76,000 drug-like compounds designed to regulate the ability of Wnt/β-catenin to induce target genes. **PAWI-2** was developed based on an extensive structure-activity relationship study for inhibition of Wnt3a in HEK293T cells. After numerous rounds of medicinal chemical refinement, **PAWI-2** was discovered. **PAWI-2** is more potent than **PAWI-1** and has many advantages in terms of improved pharmaceutical properties. **PAWI-1** and **PAWI-2** inhibited the expression of genes regulated by endogenous β-catenin signaling in SW480 colorectal adenocarcinoma cells, including *c-MYC*, *CCND* and *AXIN2.* In other studies, **PAWI-1** did not cause a general inhibition of transcriptional activity. As a follow up, **PAWI-2** was examined to inhibit colon cancer but in other studies inhibited pancreatic, prostate, and breast cancer cells and was potent across the board. In addition, **PAWI-2** also dramatically decreased pancreatic, prostate, breast and colon cancer in xenograft models *in vivo*.

"*The PAWI-2 compound may lead to treatment applications in a whole host of cancers that may prove efficacious in clinical trials*"… explains co-author Dr. John Cashman. "*As anti-cancer PAWI-2 drug development progresses, we expect PAWI-2 to be less toxic than current therapeutics for pancreatic cancer, and patients will benefit from improved safety, less side effects and possibly with significant cost-savings."*

**How the compound works**

Chemists and biologists at HBRI, Stanford, Sanford-Burnham-Prebys Medical Discovery Institute, University of California, San Diego, Christian-Albrechts-University of Kiel, Germany, and University of Washington are among the first group to apply small molecules and a high throughput assay to obtain pharmacologically active compounds of use in cancer and tissue regeneration. **PAWI** compounds block Wnt signaling and simultaneously activate p53 indirectly by binding to the colchicine site on tubulin. To our knowledge, microtubule function or dynamics has not been shown to impact Wnt signaling. Microtubules mediate diverse processes from DNA replication to cell motility and cytoarchitecture that play key roles in development, tissue homeostasis, and disease. Hence, revealing links between microtubule function and Wnt inhibition and p53 induction might be fundamentally important.

**Publication**

“Small Molecule Probe Reveals a Kinase Cascade that Links Stress Signaling to Wnt Responsiveness.” Jiongjia Cheng, Masanao Tsuda, Karl Okolotowicz, Mary Dwyer, Paul J. Bushway, Alexandre R. Colas, Joseph Lancman, Dennis Schade, Jaechol Lee, Nirmal Vadgama, Isaac Perea Gil, Arne A.N. Bruyneel, Justine Quach, Wesley L. McKeithan, Travis L. Biechele, Joseph C. Wu, Randall T. Moon, Duc Dong, Ioannis Karakikes, John R. Cashman and Mark Mercola, *Cell Chemical Biology*, Volume **28**, 1-11 (2021).

**Media contacts:** To arrange on-site, phone, or Skype interviews with the researchers involved in this study, please contact John Cashman at HBRI (858) 458-9305 or JCashman@hbri.org or Mark Mercola at Stanford University at 650-721-3281.

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**About Human BioMolecular Research Institute**
The Human BioMolecular Research Institute is a non-profit research institute conducting basic research focused on unlocking biological and chemical principles related to diseases of the human brain, cardiovascular disease and cancer. The Institute conducts fundamental studies of central nervous system disorders, heart disease and cancer including stem cell approaches and translates findings into new drug development to address human illness. In addition, the institute promotes scientific learning through community service and public access by disseminating information and sharing research with collaborators, colleagues and the public. For more information, visit www.HBRI.org.

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