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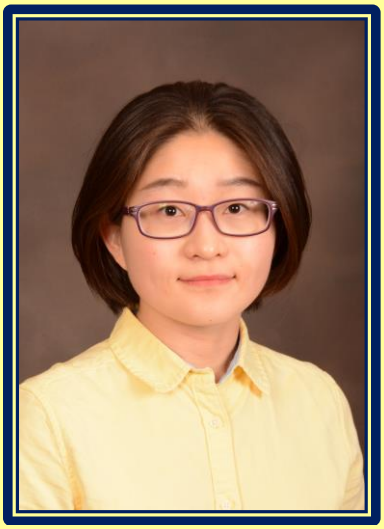

Shuai Hu

Medicinal Chemistry, Ph.D. Candidate
(Mentor: Dr. Nouri Neamati)

"Discovery and Mechanistic Studies of Novel Redox Modulators for Treatment of Pancreatic Cancer"

Wednesday May 13, 2:00 p.m.
Remote, Blue Jeans https://bluejeans.com/287068993

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Public Oral Examination
For the Degree of Doctor of Philosophy

Shuai Hu

"Discovery and Mechanistic Studies of Novel Redox Modulators for Treatment of Pancreatic Cancer"

Wednesday, May 13, 2020
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Committee Members

Dr. Nouri Neamati (Chair)
Dr. Amanda L. Garner
Dr. Mats Ljungman
Dr. Zaneta Nikolovska-Coleska
Dr. Maureen Sartor
Dr. Duxin Sun

Abstract

Pancreatic cancer remains a devastating disease and conventional chemotherapy shows modest efficacy because of drug resistance and systemic toxicity. The reprogramming of energy metabolism and oxidative stress are two hallmarks of cancer, and redox modulators have been developed as an attractive approach. At low or moderate levels, reactive oxygen species (ROS) serve as signaling molecules to mediate cellular functions; while at high levels, ROS induce oxidation of lipids, proteins, and DNA, ultimately leading to cell death. In this dissertation project, I aimed to identify novel redox modulators and provide a preclinical characterization of their mechanisms of action (MOAs) in pancreatic cancer cells.

Through lead optimization of a previously studied quinazolinedione-based redox modulator, we identified QD394 with significant cytotoxicity in a panel of pancreatic cancer cell lines. Bru-seq technique and clustering analysis revealed remarkably similar post-treatment transcriptomic profiles between QD394 and napabucasin. Both compounds inhibited STAT3 phosphorylation, induced DNA damage, increased cellular ROS, and decreased the GSH/GSSG ratio. Moreover, QD394 caused an iron- and ROS-dependent, GPX4-mediated cell death, suggesting ferroptosis as a major mechanism. QD394 also decreased the expression of mitochondrial proteins, including LRPPRC and PNPT1 involved in mitochondrial RNA catabolic processes. A derivative QD394-Me was synthesized with improved plasma stability and reduced toxicity in mice compared to QD394. These results demonstrate that QD394 and QD394-Me represent novel ROS-inducing drug-like compounds warranting further development for the treatment of pancreatic cancer.

Mito-Chlor, a mitochondrial-targeting triphenylphosphonium derivative of the nitrogen mustard chlorambucil, was identified to inhibit transcription of the mitochondrial genome through Bru-seq analysis, which is similar to a new ROS inducer SQD1 featuring a styrylquinoline-5, 8-dione core. Both Mito-Chlor and SQD1 decreased the mRNA levels of mitochondrial genes. However, only Mito-Chlor reduced their protein expression, and interfered with mitochondria membrane potential and oxidative phosphorylation. Both compounds increased cellular and mitochondrial ROS and stimulated similar downstream signaling related to oxidative stress and AP-1 transcription factors. These results establish SQD1 and Mito-Chlor as novel mitochondrial transcription inhibitors and redox modulators that may be applied to study cancer cell death related to mitochondrial function and redox signaling.

Finally, a medium-throughput phenotypic screen of 20,000 diverse drug-like compounds produced a quinolin-chlorobenzothioate, QCBT7, as a potent hit with submicromolar cytotoxicity. Its structure is similar to 8-quinolinethiol hydrochloride (8TQ) that inhibits the regulatory subunit of the proteasome. As a more stable derivative of 8TQ, QCBT7 caused the accumulation of ubiquitylated proteins, indicating its proteasome inhibitory activity. Additionally, QCBT7 increased the expression of a set of genes (PFKFB4, CHOP, HMOX1, and SLC7A11) at both nascent RNA and protein levels, similar to the known proteasome inhibitors MG132 and ixazomib. We have also identified PFKFB4 as a potential biomarker of proteasome inhibitors that can be used to monitor treatment response. Together, this study discovers that QCBT7 induces proteasome inhibition, hypoxic response, endoplasmic reticulum stress, and glycolysis, leading to cell death.

In summary, the work as a whole provides a detailed characterization of redox modulators and their effects on cell death, mitochondria, or proteasome activity. We also identify novel genes and pathways that ROS signaling could be involved to be beneficial for cancer therapeutics, especially in pancreatic cancer. This thesis contributes to the overall understanding of ROS signaling in pancreatic cancer and the validity of ROS-modulating therapies. This collective work provides the foundation to improve the redox modulators discovered for testing *in vivo*.

Publications

Hu, S., Jin, Y., Liu, Y., Ljungman, M., and Neamati, N. (2018) Synthesis and mechanistic studies of quinolin-chlorobenzothioate derivatives with proteasome inhibitory activity in pancreatic cancer cell lines, *Eur J Med Chem*, *158*, 884-895.

Li, H. *, **Hu, S.** *, Neamati, N., and Guan, Y. (2018) TAIJI: approaching experimental replicates-level accuracy for drug synergy prediction, *Bioinformatics*, 35 (13), 2338-2339. (*Contributed equally to this work)

Shergalis, A. G., **Hu, S.**, Bankhead, A., 3rd & Neamati, N. (2020) Role of the ERO1-PDI interaction in oxidative protein folding and disease, *Pharmacol Ther*, 107525.

Hu, S., et al. A novel redox modulator induces a GPX4-mediated cell death dependent on iron and reactive oxygen species. In preparation for submission to Journal of Medicinal Chemistry.

Chen, W.*, **Hu, S.***, et al. Identification and characterization of novel mitochondrial transcription inhibitors. In preparation for submission to molecular cancer therapeutics. (*Contributed equally to this work)

Select Presentations

2019 ***"Identification of a novel mitochondrial transcription inhibitor"***, Student Talk, 39th Annual Pharmacological Sciences and Bio-related Chemistry Symposium, Ann Arbor, MI, March 2019. (oral presentation)

2019 ***"Identification of novel ROS-signaling genes as potential therapeutic targets for pancreatic cancer using next-generation sequencing and survival analysis"***, Fourth Annual RNA Symposium, Ann Arbor, MI, March 2019

2018 ***"Synthesis and mechanistic studies of quinolin-chlorobenzothioate derivatives with proteasome inhibition activity in pancreatic cancer cell lines"***, AAPS PharmSci 360, Washington, DC, November 2018.

2017 ***"Discovery of Novel Small-Molecule Reactive Oxygen Species Inducers for Treating Pancreatic Cancer***", Drug Discovery and Development in Michigan Symposium, Detroit, MI, October 2017