READING BEYOND THE RED:

What Fellows Are Reading in Other Journals

AMP Kinase Activation Attenuates Cardiac Remodeling in Pulmonary Hypertension due to Heart Failure with Preserved Ejection Fraction; Lung Epithelial Progenitor Cells in Alveolar Regeneration; and Drug Discovery and Novel Therapies for Lung Cancer

Andrea R. Levine¹, William Bain¹, Joseph S. Bednash¹, Mark T. Gladwin^{1,2}, and Bryan J. McVerry^{1,2}

¹Division of Pulmonary, Allergy, and Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; and ²Pittsburgh Heart, Lung, and Blood Vascular Medicine Institute, Pittsburgh, Pennsylvania

Recommended Reading from the University of Pittsburgh Pulmonary and Critical Care Medicine Research Fellows; Mark T. Gladwin, M.D., Program Director, and Bryan J. McVerry, M.D.

Gélinas R, et al. AMPK Activation Counteracts Cardiac Hypertrophy by Reducing O-GlcNAcylation. *Nat Commun* (1)

Reviewed by Andrea R. Levine, M.D.

AMP-activated protein kinase (AMPK) is a cellular energy sensor that is activated in the setting of energy derangements such as starvation and exercise, directing cellular metabolism toward catabolism (2). AMPK is the target of metformin and regulates the mechanistic target of rapamycin (mTOR), a drug used in organ transplantation, lymphangioleiomyomatosis, and tuberous sclerosis. AMPK deficiency has been identified in metabolic syndrome-associated pulmonary hypertension due to heart failure with preserved ejection fraction (PH-HFpEF), and AMPK has been studied in cardiac hypertrophy, where cardiac AMPK activation occurs in an adaptive response to pressure or volume overload (3–5). Early intervention with AMPK activators was shown to prevent phenylephrine-induced hypertrophy in cardiomyocytes *in vitro*, and to reduce cardiac hypertrophy in mice subjected to transaortic constriction or isoproterenol (3, 4). Yet, we lack a mechanistic understanding of how AMPK attenuates cardiac hypertrophy.

The hexose biosynthesis pathway (HBP) is an accessory glucose metabolic pathway that is regulated by the aminotransferase glutamine fructose-6-phosphate aminotransferase (GFAT). HBP synthesizes the carbohydrate O-linked β -N-acetyl glucosamine (O-GlcNAc), which, via post-translational modification, is added to proteins in a process called O-GlcNAcylation. Diabetes and cancer are associated with an abundance of O-GlcNAc and upregulation of O-GlcNAcylation. Cardiac hypertrophy is associated with increased O-GlcNAcylation in rodent models of transaortic

constriction, spontaneous hypertension, and phenylephrine-induced cardiomyopathy (6).

Gélinas and colleagues explored the potential for AMPK activation to abate cardiac hypertrophy through modulation of O-GlcNAc. Using neonatal rat ventricular myocytes, they demonstrated that phenylephrine-induced cardiomyocyte hypertrophy was ameliorated by treatment with the AMPK activators A769662, AICAR, and phenformin (a metformin analog), and this occurred independently of traditional downstream targets of AMPK that have been implicated in cardiac hypertrophy, including NFAT, ERK, and p70S6K. The authors further demonstrated that phenylephrine-treated neonatal rat ventricular myocytes hypertrophied in association with increased GFAT protein and O-GlcNAc levels. Treatment with A769662 phosphorylated and inhibited GFAT, which downregulated the HBP and reduced O-GlcNAc.

Using an AMPK α 2 knockout (KO) mouse, Gélinas and colleagues tested their hypothesis *in vivo*. Angiotensin II (AngII) treatment, a known inducer of cardiac hypertrophy, increased O-GlcNAc and O-GlcNAc transferase activity in wild-type mouse hearts without any significant change in the KO hearts. Metformin increased AMPK signaling, reduced cardiac O-GlcNAc and O-GlcNAc transferase, and phenotypically blunted the development of AngII-induced left ventricular hypertrophy in wild-type mice, an effect that was absent in the AMPK KO animals.

The mechanism by which AMPK activation abrogates cardiac hypertrophy has been unknown. This paper further supports AMPK activation as a potential therapeutic focus in cardiac hypertrophy and defines O-GlcNAc as a downstream target of AMPK activation. O-GlcNAc signaling has been well studied in diabetes and metabolic syndrome, where the AMPK activator metformin is routinely used, and recent evidence supports an O-GlcNAcylation derangement in the skeletal muscle of humans with type 2 diabetes (7). Furthermore,

(Received in original form August 26, 2018; accepted in final form November 19, 2018)

Correspondence and requests for reprints should be addressed to Bryan J. McVerry, M.D., Division of Pulmonary, Allergy, and Critical Care Medicine, School of Medicine, University of Pittsburgh, UPMC Montefiore NW 628, 3459 Fifth Avenue, Pittsburgh, PA 15213. E-mail: mcverrybj@upmc.edu.

Am J Respir Cell Mol Biol Vol 60, Iss 2, pp 244–247, Feb 2019

Copyright © 2019 by the American Thoracic Society

Originally Published in Press as DOI: 10.1165/rcmb.2018-0280RO on November 26, 2018

Internet address: www.atsjournals.org

aberrant skeletal muscle AMPK activity has been demonstrated in PH-HFpEF associated with metabolic syndrome in ZSF1 rats, and treatment with metformin successfully reversed the phenotype of both hyperglycemia and PH-HFpEF (5). Collectively, these data identify AMPK as a prominent target in cardiac hypertrophy, diabetes, and pulmonary vascular disease related to metabolic syndrome via the downstream target O-GlcNAc, and clinical trials are planned to investigate the effect of metformin on cardiac hypertrophy and PH-HFpEF (ClinicalTrials.gov Identifier: NCT03629340).

Drug discovery efforts to develop novel therapeutic agents to treat metabolic syndrome and the various end-organ dysfunctions that occur as sequelae of metabolic syndrome have focused on AMPK as a potential molecular target. Investigators have identified numerous AMPK activators either by using a high-throughput screen (compound 13) or by synthesizing small molecules to target components of the known crystal structure of AMPK (compound 911) (8). However, to further investigate these drugs, researchers need to use a truly bidirectional bench-to-bedside approach, studying the particular AMPK isoform that is targeted, the downstream signaling cascade that is activated, and the *in vitro* and *in vivo* ramifications of AMPK activation. ■

References

1. Gélinais R, Mailleux F, Dontaine J, Bultot L, Demeulder B, Ginion A, *et al.* AMPK activation counteracts cardiac hypertrophy by reducing O-GlcNAcylation. *Nat Commun* 2018;9:374.
2. Herzig S, Shaw RJ. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat Rev Mol Cell Biol* 2018;19:121–135.
3. Bairwa SC, Parajuli N, Dyck JRB. The role of AMPK in cardiomyocyte health and survival. *Biochim Biophys Acta* 2016;1862:2199–2210.
4. Byrne NJ, Sung MM, Dyck JRB. The role of AMPK in the control of cardiac hypertrophy. In: Lopaschuk G, Dhalla N, editors. *Cardiac energy metabolism in health and disease*. Advances in biochemistry in health and disease. Vol. 11. New York, NY: Springer; 2014. pp. 199–220.
5. Lai Y-C, Tabima DM, Dube JJ, Hughan KS, Vanderpool RR, Goncharov DA, *et al.* SIRT3-AMP-activated protein kinase activation by nitrite and metformin improves hyperglycemia and normalizes pulmonary hypertension associated with heart failure with preserved ejection fraction. *Circulation* 2016;133:717–731.
6. Mailleux F, Gélinais R, Beauloye C, Horman S, Bertrand L. O-GlcNAcylation, enemy or ally during cardiac hypertrophy development? *Biochim Biophys Acta* 2016;1862:2232–2243.
7. Shi H, Munk A, Nielsen TS, Daughtry MR, Larsson L, Li S, *et al.* Skeletal muscle O-GlcNAc transferase is important for muscle energy homeostasis and whole-body insulin sensitivity. *Mol Metab* 2018;11:160–177.
8. Kim J, Yang G, Kim Y, Kim J, Ha J. AMPK activators: mechanisms of action and physiological activities. *Exp Mol Med* 2016;48:e224.

Zacharias WJ, *et al.* Regeneration of the Lung Alveolus by an Evolutionarily Conserved Epithelial Progenitor. *Nature* (9)

Reviewed by William Bain, M.D.

Alveolar epithelial cells can be subjected to a multitude of injurious and cytotoxic stimuli that may damage the alveolar–capillary barrier and impair gas exchange (10). The lung is a quiescent organ under homeostatic conditions; therefore, progenitor cells must proliferate

after injury to restore the critical functions of the alveolar epithelium (11). It has long been suggested that surfactant-producing epithelial alveolar type 2 (AT2) cells support restoration of the lung barrier by serving as a progenitor cell for AT1 cells, and this has been supported by recent experiments (12). However, others have suggested that Keratin5⁺ (Krt5⁺) cells from the bronchial epithelium repopulate and colonize the alveolar airspace after severe injury (13). Because alveolar repair mechanisms hold tremendous importance for the study of acute lung injury and regenerative medicine, Zacharias and colleagues sought to better understand the biology of alveolar regeneration (9). The authors identified a novel alveolar epithelial progenitor (AEP) cell subset of the AT2 lineage that expresses the gene *Axin2*, which is critical for AT2 cell expansion in response to Wnt signaling during alveolar development (14). These cells remain stable during homeostasis in the adult lung but proliferate rapidly in response to injury to restore the alveolar epithelium.

Using a Cre-recombinase conditional reporter mouse for cell lineage tracing, the authors demonstrate that *Axin2*⁺ cells express the AT2 marker *Sftpc* (the gene for surfactant protein C) and are restricted to the distal airspace, accounting for ~20% of the total AT2 cell population. The AEP population is stable during lung homeostasis but expands rapidly in response to epithelial injury modeled by murine PR8H1N1 influenza infection that causes extensive lung epithelial cell death. In regions of histologically scored moderate and severe lung injury 1 month after influenza infection, the authors found increased levels of proliferating AEP cells (as measured by Ki67 positivity). However, 1 month after infection in the regions of most severe injury, described as “total alveolar destruction,” only Krt5⁺ cells that likely migrated from the bronchial epithelium were present. However, Krt5⁺ cells were not sufficient to reestablish mature lung epithelium, as few SFTPC⁺ cells were found in the regions of most severe lung injury until 3 months after injury, when AEP cells colocalized with Krt5⁺ cells to repopulate the alveolar epithelium. Using FACS of mouse lungs after influenza injury, the authors demonstrated stable numbers of AEP cells in both control and influenza-treated mice, suggesting self-renewal of the AEP pool in both homeostasis and injury. In contrast, the authors found a significantly increased pool of AEP cells differentiating into AT2 cells after influenza injury compared with controls.

Using RNA-sequencing data in combination with FACS and immunohistochemistry, the investigators identified the putative AEP cell-surface marker Tm4sf1. Using human lung tissue from rejected transplant donors, the authors identified a pool of human AEPs that were positive for the Tm4sf1 surface marker as well as AT2 (Tm4sf1⁺ HTII-280⁺)- and epithelial (EPCAM⁺)-specific markers. Human AEPs were able to grow into three-dimensional alveolar organoids when cultured in the presence of fibroblasts. Notably, Wnt signaling favored AT2 proliferation and Wnt inhibition favored AT1 differentiation, suggesting that *Axin2*⁺ AEPs are Wnt responsive, which was supported by RNA-sequencing data showing AEP gene expression enriched for Wnt signaling targets.

In summary, the authors have identified an alveolar epithelial progenitor cell subset of AT2 cells that can self-renew and respond rapidly to injury to regenerate alveolar epithelium in a Wnt-modulated response. Further work is required to determine whether Wnt pathways, which have been implicated in cancer and lung fibrosis, can be safely modulated to foster alveolar repair after acute lung injury. Nevertheless, this fascinating study has tremendous translational implications for pulmonary medicine, including

exciting possibilities for regenerative medicine in acute lung injury and lung transplantation. ■

References

9. Zacharias WJ, Frank DB, Zepp JA, Morley MP, Alkhaleel FA, Kong J, *et al.* Regeneration of the lung alveolus by an evolutionarily conserved epithelial progenitor. *Nature* 2018;555:251–255.
10. Matute-Bello G, Liles WC, Steinberg KP, Kiener PA, Mongovin S, Chi EY, *et al.* Soluble fas ligand induces epithelial cell apoptosis in humans with acute lung injury (ARDS). *J Immunol* 1999;163: 2217–2225.
11. Beers MF, Morrissey EE. The three R's of lung health and disease: repair, remodeling, and regeneration. *J Clin Invest* 2011;121: 2065–2073.
12. Barkauskas CE, Counce MJ, Rackley CR, Bowie EJ, Keene DR, Stripp BR, *et al.* Type 2 alveolar cells are stem cells in adult lung. *J Clin Invest* 2013;123:3025–3036.
13. Ray S, Chiba N, Yao C, Guan X, McConnell AM, Brockway B, *et al.* Rare SOX2⁺ airway progenitor cells generate KRT5⁺ cells that repopulate damaged alveolar parenchyma following influenza virus infection. *Stem Cell Rep* 2016;7:817–825.
14. Frank DB, Peng T, Zepp JA, Snitow M, Vincent TL, Penkala IJ, *et al.* Emergence of a wave of Wnt signaling that regulates lung alveologenesis by controlling epithelial self-renewal and differentiation. *Cell Rep* 2016;17:2312–2325.

Kategaya L, *et al.* USP7 Small-Molecule Inhibitors Interfere with Ubiquitin Binding. *Nature* (15)

Reviewed by Joseph S. Bednash, M.D.

In the United States, lung cancer is the current leading cause of cancer mortality, accounting for ~25% of deaths from malignancy (16). The tumor suppressor protein p53 plays an important protective role against all cancers, including lung cancer. Mutations in the *TP53* gene, which encodes p53, are found in up to 80% of small cell lung carcinomas and ~50% of non-small cell lung carcinomas (17). Primarily a transcription factor, activated p53 induces cell cycle arrest, cellular senescence, or apoptosis in response to DNA damage. Although it was previously thought to be “undruggable,” new strategies are emerging to target p53 and associated malignancies.

The primary molecular antagonist of p53 is E3 ubiquitin-protein ligase Mdm2 (MDM2) (18, 19). Briefly, ubiquitination is a post-translational modification that modulates cellular protein trafficking. MDM2 ubiquitinates p53, tagging p53 for proteasomal degradation (18, 19). MDM2 overexpression occurs in ~25% of non-small cell lung cancer tumors that retain wild-type p53 (19). Given its central role in tumorigenesis, p53 and its regulators (e.g., MDM2) are attractive therapeutic targets for the development of novel antineoplastic compounds.

To target p53, Kategaya and colleagues developed a small-molecule inhibitor of the deubiquitinase (DUB, which removes ubiquitin) ubiquitin carboxyl-terminal hydrolase 7 (USP7), which targets MDM2 as one of its substrates (15). As such, depletion or inhibition of USP7 increases ubiquitination and degradation of MDM2. As mentioned above, degradation of MDM2 directly

stabilizes p53. In short, increased abundance or activity of USP7 means less MDM2 and more p53.

To develop a novel USP7 inhibitor, the authors used two parallel methods for drug discovery: a target-based approach and a phenotypic approach. A target-based approach assays compounds for their ability to bind to a known target. In this study, Kategaya and colleagues used nuclear magnetic resonance (NMR) assays to screen a library of 4,871 small molecules for their ability to bind to USP7. A phenotypic approach uses functional or cell-based assays to test compound efficacy to achieve a certain outcome or phenotype. The authors used a fluorescence-based deubiquitination assay to screen a separate library of ~1.37 million compounds for their ability to inhibit cleavage of a fluorescent ubiquitin substrate by USP7. Using hits from the NMR (target-based) screen, they further optimized parent fragments using the fluorescence-based DUB (phenotypic) assay, and identified two lead compounds for further testing.

After identifying the lead compounds, the authors demonstrated compound specificity and efficacy. Using matrix-assisted laser desorption ionization–time of flight mass spectrometry to evaluate cleavage of ubiquitin chains by a panel of DUBs across all five DUB families, they showed that the two lead compounds were specific inhibitors of USP7. As shown by the NMR techniques used in the screening process, binding of ubiquitin to USP7 was decreased by the USP7 inhibitors. By increasing ubiquitination of MDM2, the USP7 inhibitors decreased the cellular abundance of MDM2 and subsequently increased p53, as predicted. In conclusion, the USP7 inhibitors identified were shown to be effective and specific by both biophysical- and functional-based assays.

Ultimately, the goal of drug discovery is to develop compounds that impact a human disease while simultaneously providing information about the molecular processes that underlie that disease. In 2011, the National Institutes of Health held a workshop on quantitative and systems pharmacology (20), suggesting that many compounds are evaluated and published annually that lack sufficient characterization or specificity to serve as useful pharmacological tools, let alone advance to clinical testing. The National Institutes of Health workshop on quantitative and systems pharmacology suggested the use of a cyclic methodology involving target identification, compound development, computer modeling, and mammalian models of disease that feed back on each other (20). The reviewed study built upon previous work that identified the deubiquitinase USP7 as a druggable target to impact the p53 pathway, and used automated high-throughput screening as well as advanced and robust methods such as NMR and mass spectrometry to evaluate compounds for pharmacological efficacy. The strength of this study lies in its robust characterization of specific USP7 inhibitors, which is necessary to elucidate the systems biology of the tumor suppressor p53 and allow for feedback on the drug discovery process.

The proteasome inhibitors bortezomib (21) and carfilzomib (22) provide proof of concept that targeting the ubiquitin–proteasome pathway can provide therapeutic benefit. However, specifically targeting a DUB as an antineoplastic agent would represent a first-in-class drug for the treatment of malignancy. This study, as well as other efforts to develop a USP7 inhibitor (23), provides insight into the viability of DUB enzymes as therapeutic

targets, an important advancement in the quest for novel agents to combat cancer. Furthermore, drug discovery efforts such as those presented here provide information that will help us understand the systemic effects of USP7 inhibition and to consider DUB inhibitors as potential medicines. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

15. Kategaya L, Di Lello P, Rougé L, Pastor R, Clark KR, Drummond J, *et al*. USP7 small-molecule inhibitors interfere with ubiquitin binding. *Nature* 2017;550:534–538.
16. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018;68:7–30.
17. Duffy MJ, Synnott NC, McGowan PM, Crown J, O'Connor D, Gallagher WM. p53 as a target for the treatment of cancer. *Cancer Treat Rev* 2014;40:1153–1160.
18. Kon N, Kobayashi Y, Li M, Brooks CL, Ludwig T, Gu W. Inactivation of HAUSP *in vivo* modulates p53 function. *Oncogene* 2010;29:1270–1279.
19. Deben C, Deschoolmeester V, Lardon F, Rolfo C, Pauwels P. TP53 and MDM2 genetic alterations in non-small cell lung cancer: evaluating their prognostic and predictive value. *Crit Rev Oncol Hematol* 2016;99:63–73.
20. Stern AM, Schurdak ME, Bahar I, Berg JM, Taylor DL. A perspective on implementing a quantitative systems pharmacology platform for drug discovery and the advancement of personalized medicine. *J Biomol Screen* 2016;21:521–534.
21. Richardson PG, Barlogie B, Berenson J, Singhal S, Jagannath S, Irwin D, *et al*. A phase 2 study of bortezomib in relapsed, refractory myeloma. *N Engl J Med* 2003;348:2609–2617.
22. Jakubowiak AJ, Dytfield D, Griffith KA, Lebovic D, Vesole DH, Jagannath S, *et al*. A phase 1/2 study of carfilzomib in combination with lenalidomide and low-dose dexamethasone as a frontline treatment for multiple myeloma. *Blood* 2012;120:1801–1809.
23. Reverdy C, Conrath S, Lopez R, Planquette C, Atmanene C, Collura V, *et al*. Discovery of specific inhibitors of human USP7/HAUSP deubiquitinating enzyme. *Chem Biol* 2012;19:467–477.