Reciprocal regulation of metabolic and stress sensors

Melissa Huestis, Sadia Rahmani, Warren W. Wakarchuk & Costin N. Antonescu

Department of Chemistry and Biology, Ryerson University, 350 Victoria Street, Toronto ON, Canada, M5B 2K3

Cancer cells use stress signalling pathways to support survival

STRESSOR

Poor tumor vascularization → Hypoxia → Reactive Oxygen Species (ROS)

Nutrient Deprivation

Chemotherapy or targeted therapies

→ Oxidative Stress, Metabolic Stress, Genotoxic Stress

RESPONSE

Activation of stress signalling

Cell Adaptation
(Drug resistance, etc)

Tumor Environment

Crucial stress sensors and their role in cells

AMP-activated Protein Kinase (AMPK)
Energy Sensor
Responds to: Metabolic Stress
↑ ADP:ATP

p38 Mitogen-activated Protein Kinase (p38 MAPK)
Stress Sensor
Responds to: Oxidative Stress, Osmotic Stress, Heat Shock

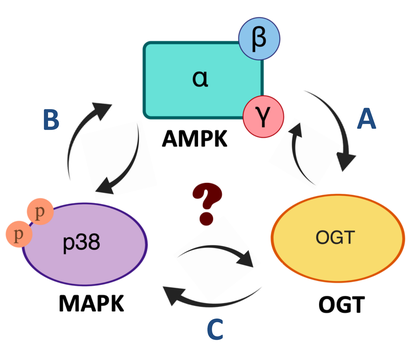
O-GlcNAc Transferase (OGT)
Sugar Sensor
Responds to: Metabolic Stress
UDP-GlcNAc → O-GlcNAc

Evidence of cross-talk has been previously established between these sensors, but little is known about their relationship during the cells stress response.

Objective and Hypothesis

Overall Objective

Determine the degree of interplay, if any, between AMPK, p38 and OGT during the cells stress response.

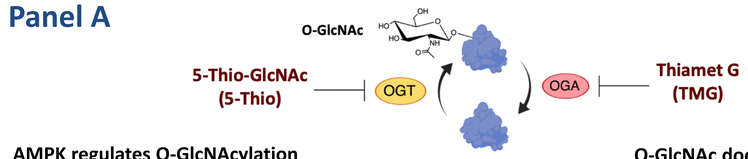


Hypothesis

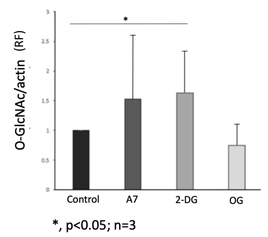
Upon induced stress, AMPK and p38 signaling pathways will together, or separately, reciprocally interact with OGT to aid in the re-establishment of homeostasis.

AMPK activity acutely increases O-GlcNAcylation

Panel A



AMPK regulates O-GlcNAcylation



*, p<0.05; n=3

Figure 1. ARPE-19 cells were treated with A-769662 (A7), 2-deoxyglucose (2DG) and 5 µM oligomycin (OG) and probed for global O-GlcNAc via western blotting. Shown are the mean ± SE of 3 independent experiments (n=3).

O-GlcNAc does not impact AMPK

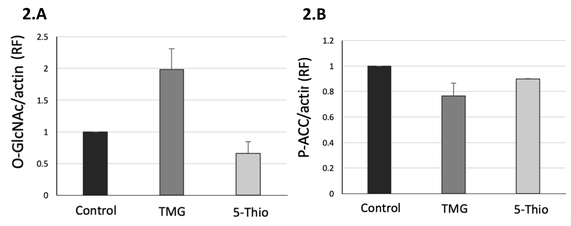


Figure 2. ARPE-19 cells were treated with Thiamet G (TMG) or 5-Thio-GlcNAc (5-Thio). Western blotting to probe for levels of **A)** O-GlcNAc and **B)** phosphorylated Acetyl-CoA carboxylase (P-ACC) was conducted and relative band intensities were quantified.

The p38 stress response relies on AMPK activity

Panel B

CC = Compound C = AMPK inhibitor
SB = SB-202190 = p38 inhibitor

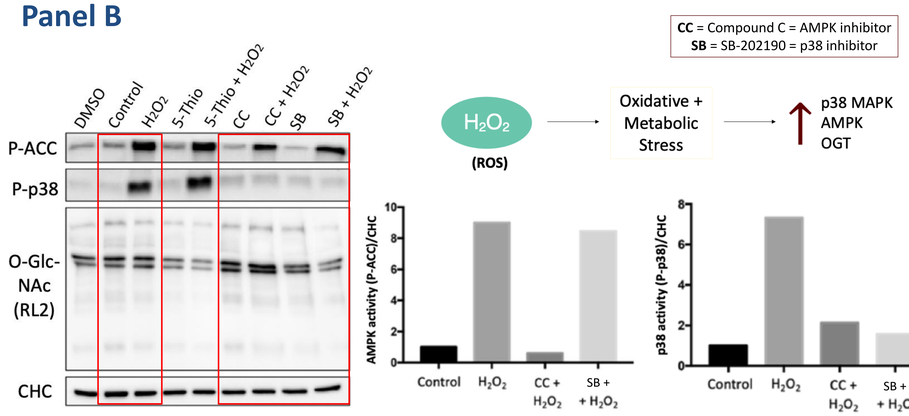


Figure 4. ARPE-19 cells were treated with various inhibitors in the presence and absence of hydrogen peroxide (H_2O_2). Western blot quantifications are graphically represented, and all data was normalized to levels of the loading control, clathrin heavy chain (CHC), measured.

p38 regulates OGT and overall O-GlcNAcylation levels during stress

Panel C

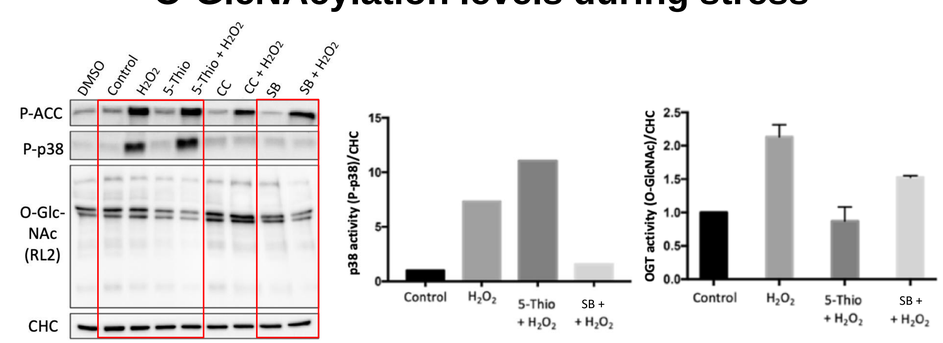
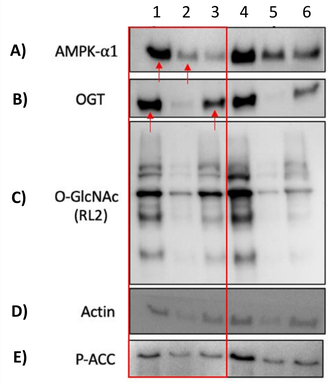


Figure 5. ARPE-19 cells were subject to various conditions and proteins were quantified from western blots. Quantifications are graphically represented, and all data was normalized to levels of the loading control, CHC, measured.

Reciprocal regulation of expression of AMPK by OGT

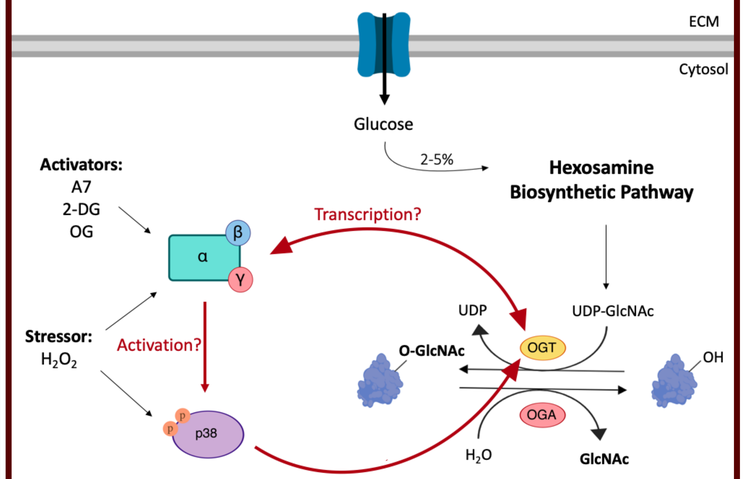
Panel A



1- siControl
2- siOGT
3- siAMPK
4- siControl + TMG
5- siOGT + TMG
6- siAMPK +TMG

Figure 3. ARPE-19 cells were transfected with OGT, AMPK or Con 7 (control) siRNA alone and with TMG and probed for **A)** total AMPK (AMPK-α), **B)** O-GlcNAc Transferase (OGT), **C)** cellular O-GlcNAcylation, **E)** P-ACC and **D)** Actin via western blotting. Degree of OGT and AMPK knockdown was approximately 90 and 70%, respectively.
*Data obtained from Sadia Rahmani, 2019

Working Model



- OGT and AMPK reciprocally regulate each other
- p38 activation may solely depend on the presence of AMPK
- p38 appears to increase O-GlcNAcylation during the stress response

All stressors regulate each other simultaneously to ensure an appropriate response to cellular stress.

Future Directions

- Explore the relationship between O-GlcNAc hydrolase and AMPK.
- Investigate the long-term effect of the O-GlcNAc modification on AMPK enzymatic activity.

Acknowledgements

NSERC CRSNG

Ryerson University