O-GlcNAc regulates Tyrosine phosphorylation 105 site on PKM2: A Mechanistic insight linking O-GlcNAc to Warburg effect in human alveolar adenocarcinoma

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Abstract

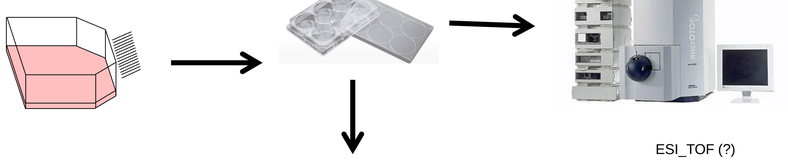
Pyruvate kinase (PK), a rate-limiting enzyme during glycolysis, catalyzes the production of pyruvate and adenosine 5′-triphosphate (ATP) from phosphoenolpyruvate (PEP) and adenosine 5′-diphosphate (ADP). PKM2 is crucial for aerobic glycolysis and provides a growth advantage to tumors by diverting glucose to anabolic synthesis.Cancer cells have to alter their metabolism to allow the production of metabolic intermediates that are the precursors for biomass production. Growing evidences have shown that a nutrient-sensitive protein modification as O-GlcNAc can modulate cancer cell metabolism. O-GlcNAc is a dynamic post-translational modification that can occur in the same residue or in an adjacent site of phosphorylation.Recently, our group revealed increased O-GlcNAc levels during epithelial to mesenchymal transition (EMT) in A549 cells as well as increased glucose flux through the hexosamine biosynthesis pathway (HBP). Here, we investigated the possible interplay between O-GlcNAc and phosphorylation of PKM2 during EMT.

Methodology

A549 DMEM 25 mM glucose
10% SFB

Epithelial to mesenchymal transition
(TGF-β 5 ng/ mL)

Mass Spectrometry



ESI_TOF (?)

Western Blot
PKM2,PY105PKM2, β-actin ,OGA, OGT, CTD110.6

Results

TGF-β induces HBP activation

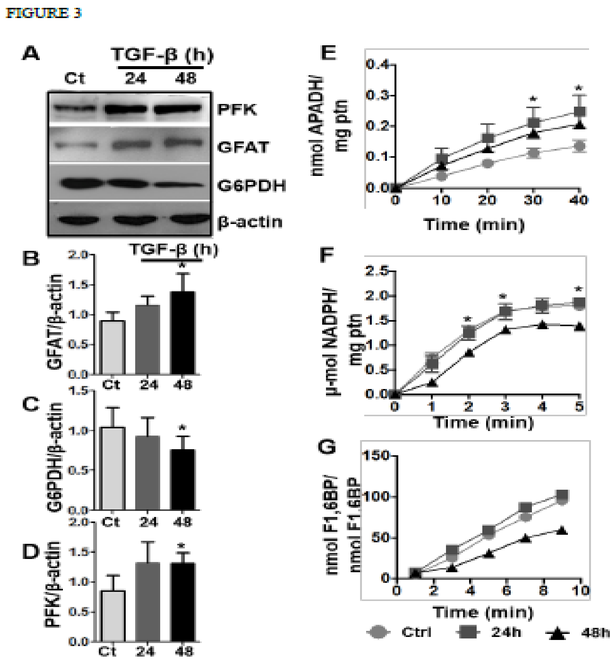


FIG 3.**TGF-β modulates rate-limiting enzymes of glucose metabolic pathways.** *A,* A549 cells were incubated without (Ct) or with 5ng/ml of TGF-β over 24 h and 48 h. Western blot of cell lysate loads analyzing expression levels of GFAT, G6PD, PFK and β-actin. Signal intensities were normalized, using β-actin as loading control, and relative intensities of GFAT (*B*), G6PD (*C*), PFK (*D*) were presented. GFAT (*E*), G6PDH (*F*) and PFK (*G*) activities in cell lysates were measured as described in Experimental Procedures. Results are shown as mean ± standard deviation. P values were calculated using the Student's t-test and ANOVA test. *P<0.01.

Results

O-GlcNAc levels increase during TGF-β-induced Epithelial-Mesenchymal Transition (EMT) in A549 cells.

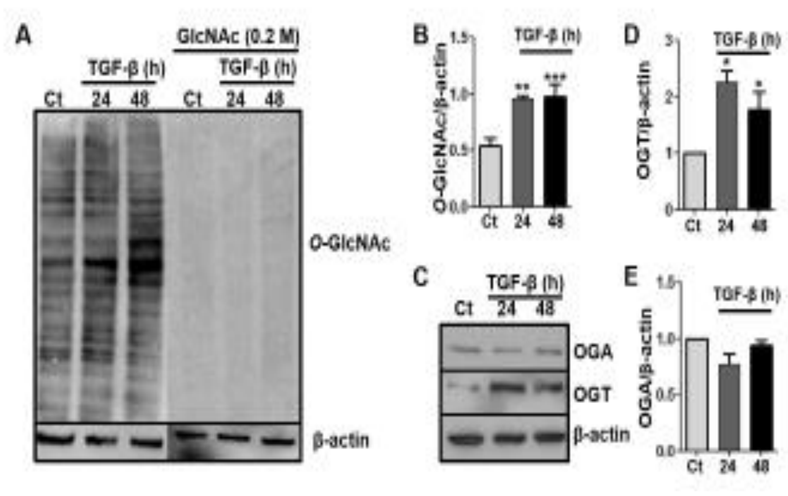
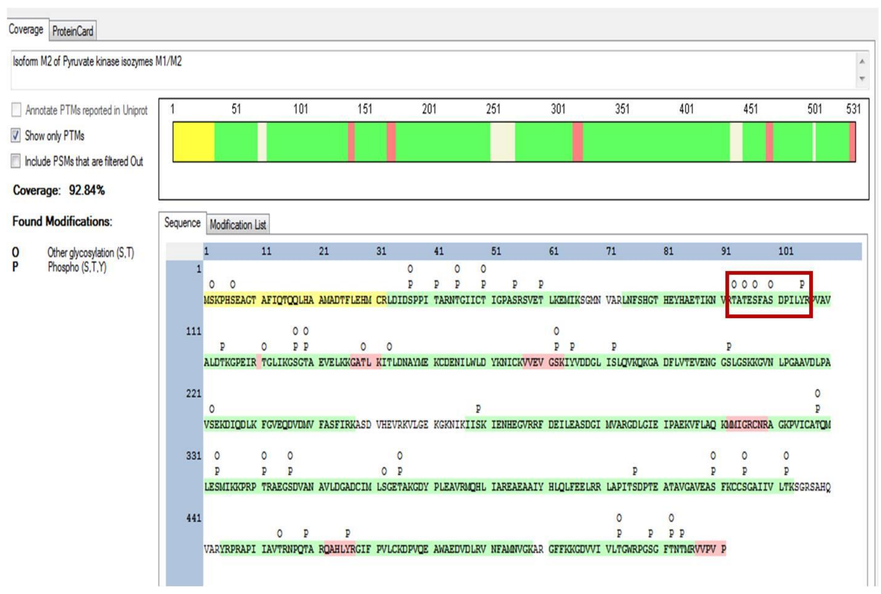


FIG. 6. ***O*-GlcNAc levels, OGT and OGA alterations during TGF-β-induced EMT in A549 cells.** *A*, Western blot of OGT, OGA and *O*-GlcNAc levels; *O*-GlcNAc competitive assay was conducted by pre-incubation of the antibody with 0.2 M free GlcNAc before membrane labelling to confirm CTD 110.6 specificity. *B, C, D,* Histograms represent densitometric analyses of western blots of *O*-GlcNAc levels, OGA and OGT respectively. Signal intensities were normalized with β- actin as loading control. Quantitative analyses are shown as mean ± standard deviation. P values were calculated using Two-way ANOVA and the appropriate post-test.* *P<0.05, .** P<0.01, .*** P<0.001.*

O-GlcNAc sites on PKM2 were mapped by high resolution mass spectrometry during TGF-β-induced Epithelial-Mesenchymal Transition (EMT) in A549 cells.



TGF-β induces increased levels of PY[105]PKM2 levels

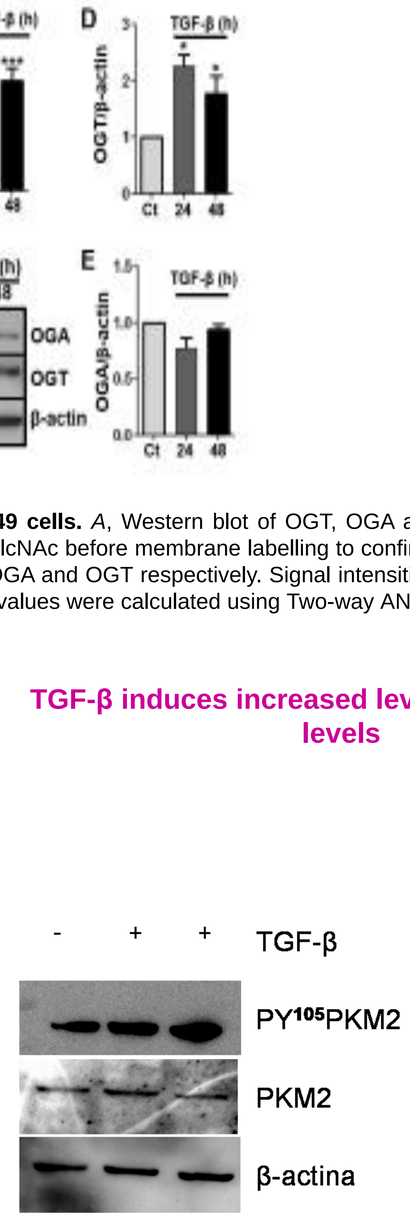


FIG. 6. **TGF-β induces increased levels of PY[105]PKM2 levels.**Western blot of PY[105]PKM2, PKM2 and *β*-actin levels;

OGA inhibitor slightly increases PY[105]PKM2 levels

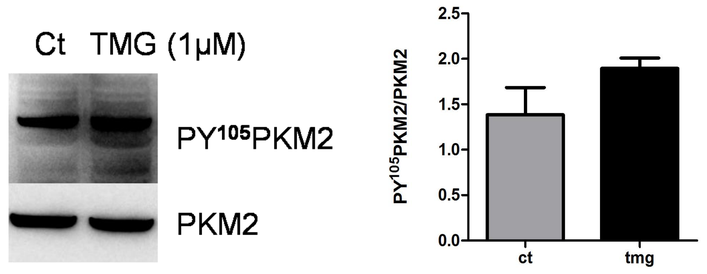


FIG. 6. **OGA inhibitor slightly increases PY[105]PKM2 levels.**Western blot of PY[105]PKM2 and PKM2 levels;

OGA overexpression decreases PY[105]PKM2 levels

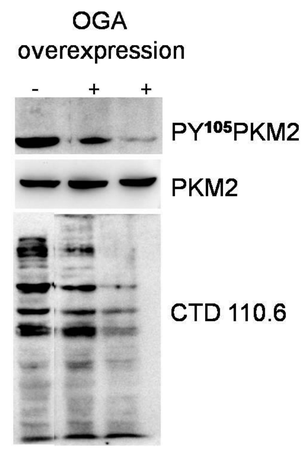
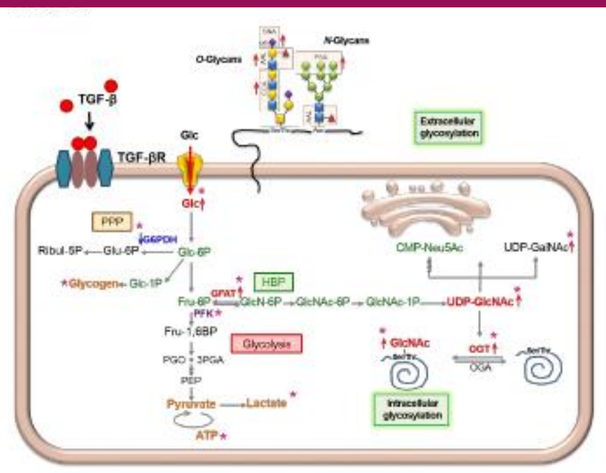


FIG. 6. **OGA overexpression decreases PY[105]PKM2 levels.**Western blot of PY[105]PKM2, PKM2 and CTD110.6 (O-GlcNAc) levels;

Conclusion



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