Grant Title: Deciphering Liver Glycome Regulation by the PGC-1/FN3K Axis

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Abstract

Glycation, the spontaneous non-enzymatic modification of proteins by free reducing sugars has been demonstrated to contribute to the pathophysiology of metabolic disorders, cardiovascular disease and cognitive decline. Yet, the mechanisms governing the cellular adaptive response to glycation-induced alterations in protein function are not fully understood. The removal of monosaccharide moieties from proteins, one form of protein de-glycation, has been demonstrated to be involved in modulating protein function. Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) and 1β (PGC-1β) are pivotal metabolic transcriptional co-activator proteins that associate with numerous transcription factors and boost their ability to induce expression of their cognate target genes in liver and in additional tissues. As PGC-1s promote glucose production (hepatic gluconeogenesis) and utilization (glycolysis), we postulated PGC-1s may act to counter the proteotoxic effects of glucose intracellularly by boosting anti-glycation mechanisms.

Based on preliminary results, implicating PGC-1s in the regulation of the de-glycase FN3K, we aimed (a) to explore the mechanism of PGC-1 mediated FN3K activation and (b) to elucidate the PGC-1 regulated glycome in liver cells. To obtain better insight regarding possible transcription factors involved in the regulation of FN3K via PGC1-s we employed both siRNA knockdown and chemical inhibitor approaches to examine candidate transcription factors involved. We utilized mice with liver specific PGC-1s knockout and liver-derived cell lines as model systems. In order to identify proteins and biological pathways regulated by PGC-1/FN3K mediated deglycation, a mass spectrometric analysis focused on glycation was performed on protein extracts from cells with or without forced expression of PGC-1s in combination with Fn3k knockdown. Targets for PGC-1 mediated changes in glycation were quantitatively identified.

A cell autonomous role for PGC-1s in induction of Fn3k was confirmed in several liver-derived cell lines and primary hepatocytes by overexpression of either PGC-1α or PGC-1β . Of note, we identified a correlation between PPARGC1A mRNA expression levels and FN3K mRNA expression levels in liver tumor samples from two cohorts of human patients.

Furthermore, PGC-1α overexpression induced FN3K protein expression in HepG2 cells.

Knockdown of the transcription factor HNF4α failed to affect PGC-1α mediated induction of Fn3k, however, two separate inhibitors of the transcription factor FOXO1 strongly blunted PGC-1α mediated induction of Fn3k expression. Phenylborate (PB) binds to glycated (and with lesser affinity to glycosylated) proteins and phenylborate affinity purification can be used to isolate such modified proteins. PB based purification of proteins from liver-derived cells with or without forced expression of PGC-1s in combination with Fn3k knockdown revealed the PGC-1α regulated glycome via its effects on Fn3k. Thus, PGC-1α controls the feeding-induced induction of a critical liver deglycase, FN3K, as well as levels of protein glycation in liver cells.