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Glucose levels dictate cell fate following oxidative stress exposure by regulation of the sirtuin 3/p53 pathway

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High blood sugar has been identified as a risk factor for a number of musculoskeletal disorders including tendinopathy[1]. Oxidative damage is also a common feature of degenerative diseases[2]. Musculoskeletal tissues are regularly exposed to oxidative stress as a result of mechanical loading[3]. We hypothesized that high extracellular glucose levels would hinder the ability of cells to withstand oxidative stress leading to cell dysfunction and predisposing to tissue degeneration.

Primary human tendon-derived fibroblasts (tenocytes) were cultured in either 15 mM (high) or 5 mM (normal) glucose media and treated with 100 μM hydrogen peroxide. Peroxide treatment of tenocytes grown in high glucose led to a robust increase in level of apoptosis which was mediated by the pro-apoptotic protein bim. There was no increase in apoptosis in peroxide-treated cells cultured in normal glucose. To establish how glucose level was influencing cell fate, we examined the activity of the FOXO and p53 transcription factors, known regulators of the oxidative stress response. Levels of FOXO1 were higher in peroxide treated cells regardless of extracellular glucose level. In contrast, levels of acetylated p53 were only elevated in peroxide-treated cells grown in high, not normal, glucose. Knockdown of either p53 or FOXO1 using RNAi prevented the increase in bim RNA and protected against apoptosis induction in peroxide-treated cells grown in high glucose indicating both factors were involved in apoptosis induction. Bim is a direct transcriptional target of FOXO1[4] but not of p53. We found p53 co-operated with FOXO1 to increase bim levels by inhibiting expression of the bim repressor miR17-92.

Surprisingly, knockdown of FOXO1 using RNAi prevented the increase in levels of acetylated p53 in peroxide-treated cells grown in high glucose suggesting FOXO1 facilitated p53 acetylation. To confirm this, we over-expressed FOXO1 in tenocytes using an adenoviral vector (adFOXO1) and found levels of acetylated p53 were higher in adFOXO1-infected cells compared to controls. Levels of sirtuin 3 (sirt3), a p53 deacetylase and regulator of mitochondrial function, were lower in peroxide-treated cells grown in high (but not normal) glucose and in adFOXO1-infected cells compared to controls. Over-expression of sirt3 in peroxide-treated cells grown in high glucose prevented the increase in acetylated p53 and protected against apoptosis indicating FOXO1 promotes p53 activation by inhibiting expression of the p53 deacetylase sirt3. In cells grown in high glucose, FOXO1 activity results in altered mitochondrial activity, increased intracellular reactive oxygen species and preliminary data indicate it may also promote expression of a sirt3-targeting miRNA; effects not seen in cells grown in normal glucose.

In conclusion, extracellular glucose profoundly influences the ability of cells to survive oxidative stress. In high glucose following oxidative stress exposure, FOXO1-mediated inhibition of sirt3 expression facilitates p53 activation leading to apoptosis. However in normal glucose, sirt3 levels are maintained and the p53-FOXO1 axis disrupted, preventing apoptosis. High blood glucose levels may predispose to degenerative disease by inhibiting the ability of cells to withstand oxidative stress.

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