UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE

Control of Glucose Metabolism Is Important in Tenogenic Differentiation of Tendon Progenitors Derived from Injured Tendons

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Objectives

Current treatments for tendon injuries are still fraught with complications such as re-rupture, adhesions and scar formation. Tendon progenitors reside in tendons and likely participate in tendon healing. It is important to control tendon progenitors to differentiate into tendon cells but not into other lineage, such as chondrogenic cells that potentially induce cartilaginous metaplasia.

Increases in glucose uptake and glucose metabolites have been found in healing Achilles tendons. High glucose environment can disturb tendon stem /progenitor cell function.

We hypothesize that glucose metabolism may be critical to determine the fate of tendon progenitors during tendon healing.

The objectives of this study are to determine changes in glucose metabolism during differentiation of tendon progenitors and to examine the significance of glucose metabolic changes in differentiation of tendon progenitors.

Methods

Human injured tendon-derived progenitor cells (hITPCs): They were isolated from lacerated hand flexor tendons (3 males and 2 females) at the time of surgery, expanded and stored after FACS analysis of stem cell markers (CD90, CD73 and CD105). The hITPCs were cultured in micromass to induce chondrogenic differentiation. BMP12 (100ng/ml) was added in monolayer culture to induce tenogenic differentiation. The culture was treated with 2-deoxy-D-glucose (2DG, 1mM), an inhibitor of glycolysis.

^{13}C-metabolic flux analysis: The monolayer +/- BMP12 and micromass cultures (n=3) were incubated with [1,2 -^{13}C] glucose (25mM) for 48 hours and the cells were extracted by 4% perchloric acid. Isotopomer enrichment and concentrations of ^{13}C- glucose metabolites or intermediates were analyzed by GC-MS and/or LC/MS/MS.

Cytochemical study: The cultures were fixed with 4% paraformaldehyde for 10 min and stained with Alcian blue (pH 1.0).

Statistical Methods: Statistical analysis was performed using Prism 6. The threshold for significance for all tests was set as $p < 0.05$.

Results

Figure 1. Tenogenic and chondrogenic cultures

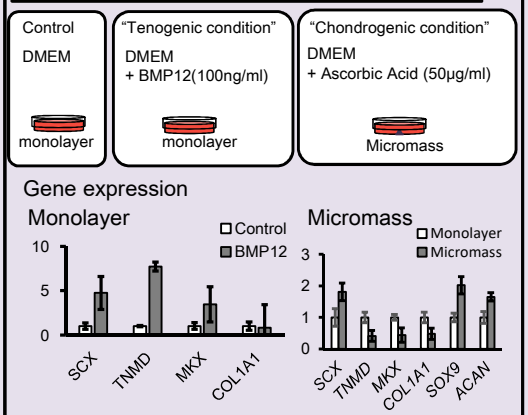
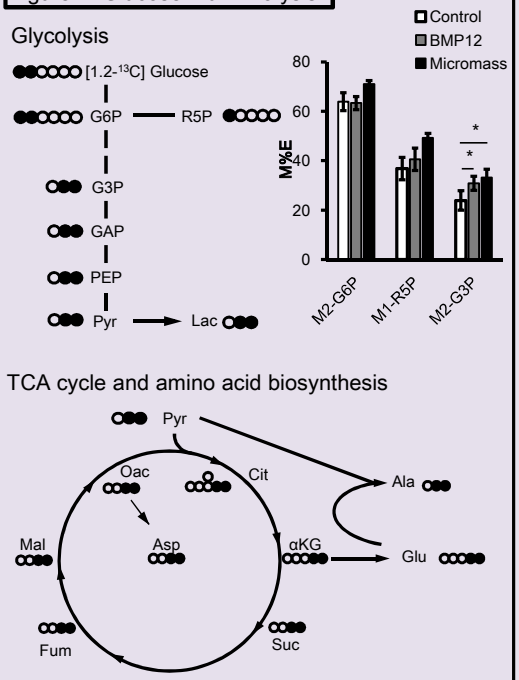


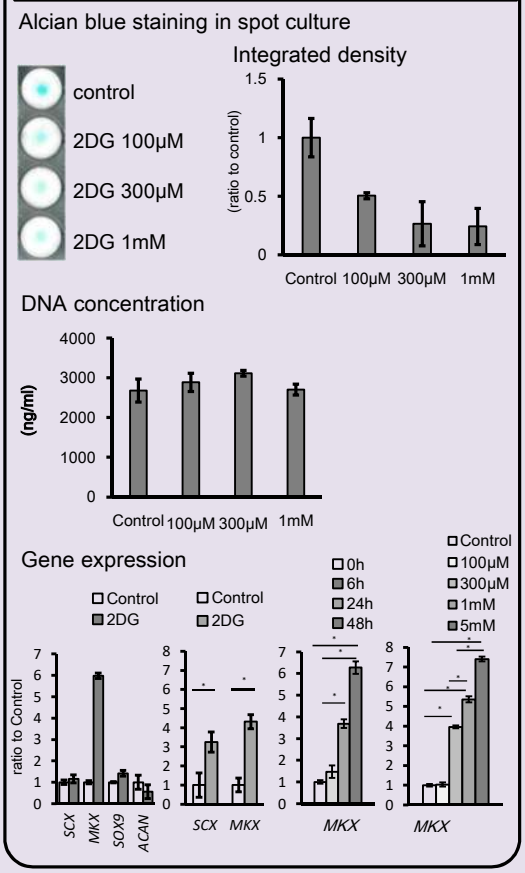
Figure 2. Glucose Flux Analysis



The progenitors stimulated glycolysis toward tenogenic differentiation. They strongly stimulated glycolysis, TCA pathway and biosynthesis of amino acids from the TCA cycle intermediates toward chondrogenic differentiation.

Results

Figure 3. Inhibition of Glucose use by 2-deoxy-glucose (2DG) Inhibited GAG accumulation and stimulated gene expression of MKX



Discussion and conclusion

- Glucose metabolism was altered during tenogenic and chondrogenic differentiation of tendon progenitors.
- Chondrogenic differentiation is associated with strong stimulation of glycolysis and TCA cycle pathways and a significant increase in biosynthesis of amino acids from the intermediates of the TCA cycle.
- Inhibition of glycolysis by 2DG inhibited chondrogenic differentiation in cultured hITPCs while stimulated gene expression of tenogenic transcription factor Mkx.
- These findings indicate that control of glucose metabolism is critical to determine the fate of tendon progenitors.
- Control of glucose metabolism may be beneficial for the treatment of injured tendons.

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