Pathways to Mesangial Cell (MC) Extracellular Matrix (ECM) Production in a Model for Chronic GLUT1 Overexpression

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We previously developed MC with persistent 10-fold overexpression of GLUT1 (i.e. MCGT1 cells). Control MC (i.e. MCLacZ cells) were also produced for comparison. Here we investigated potential pathways to the persistent ECM production in chronic GLUT1-overexpressing MC (MCGT1 cells grown for more than 2 months in culture). ERK1/2 activation was examined by immunoblotting with phosphospecific antibodies; PKC isoforms were examined by immunoblotting, and suppression of PKC was induced by PMA; TGFβ1 expression was examined by Northern analyses and ELISA assay. Chronically maintained MCGT1 cells were passed and grown 7 days to confluence in 8mM glucose medium, placed in 1% serum medium for 24 or 48 hours, then harvested. ERK1/2 phosphorylation was found to be unchanged in MCGT1 vs MCLacZ cells (1.13±0.13 vs. 1.0±0.07; n=6), TGFβ1 mRNA levels were similar in MCGT1 vs MCLacZ cells, and total TGFβ1 protein levels were lower in MCGT1 vs MCLacZ (26± 13pg/mL vs 51±19; n=3). In contrast, PKCα and PKCβ1 active protein fractions were both increased in MCGT1 cells. The PKCβ2 protein was not detectable in the MC. Subsequently, PMA suppression (100nM, 24h) of PKC inhibited the 1.5-fold excess FN expression of MCGT1 cells. In contrast, the elevated FN mRNA levels of MCGT1 were not suppressed by 90% inhibition of aldose reductase (AR) activity. Angiotensin II (ATII, 1μM) treatment of MCLacZ control cells increased TGFß1 mRNA by 63% at 8 hours, and increased FN mRNA levels by 180% at 8 hours. In contrast, ATII treatment of GLUT1-underexpressing cells (i.e. MCGT1AS antisense-GLUT1 cells) did not significantly stimulate either TGFβ1 or FN expression at these time points. ATII did not stimulate ERK1/2 phosphorylation in either MCLacZ or MCGT1AS cells.

Conclusions :

1) Inhibition of AR did not block excessive FN expression by MCGT1.

2) Chronic overexpression of GLUT1 selectively activated the α and β1 isoforms of PKC, while TGFβ1 and the MAPK pathways were not activated, suggesting PKC may mediate the excessive ECM production of MCGT1 cells. This was further supported by PMA suppression of high FN transcript levels in MCGT1.

3) Antisense-GLUT1 treatment of MC inhibited ATII stimulation of TGFβ1 and FN, suggesting glucose uptake is also important to this ECM response.