Diabetic Complications Consortium

Application Title: p53-Regulated Metabolic Fitness of Self-Renewing Nephron Progenitor Cells (NPC)

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1. Project Accomplishments:

This project has established that:

1. p53-null NPC have decreased basal and maximal oxidative respiration rate, and are dependent on glycolysis for energy.
2. Pharmacological inhibition of glycolysis results in decreased Cited1 and Six2 expression, and increased Wnt4 expression in NPC. Ex vivo, 24h treatment of E12.5 kidneys with YN1 resulted in accelerated nephrogenesis.

The data obtained in this study demonstrate that NPC renewal and differentiation are sensitive to changes in energy metabolism.

2. Specific Aims:

Specific Aim: *Test the hypothesis that p53-driven metabolic fitness regulates the self-renewal capacity of Cited1+/Six2+ progenitor cells.*

Demonstrate that Cited1+/Six2+ progenitors respond via an adaptive metabolic response to extracellular cues to maintain self-renewal.

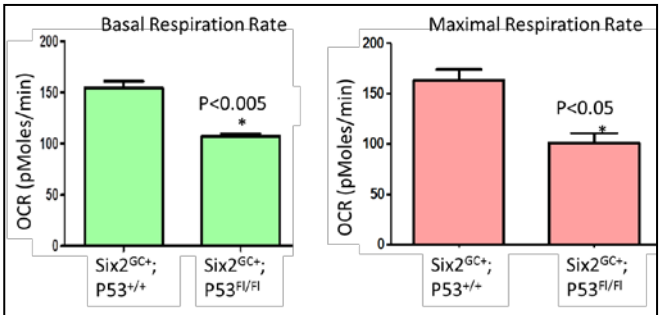

Fig. 1. Decreased basal and maximal Oxidative Phosphorylation in p53-null NPC.

A) Determine the metabolic profile of Cited1+/Six2+ cells before and after conditional genetic deletion of p53 from Six2+ cells. Rates of glycolysis and oxidative phosphorylation and levels of ATP and ROS will be established under normal conditions and in the context of stress (high/low glucose).

Results: Energy metabolism profiles of wild-type and conditional p53-null Six2+ nephron progenitor cells (NPC) were compared. Compared to wild-type P53-null NPC have decreased oxidative respiration, indicated by lower basal and maximal measurements of oxygen consumption rate (Fig. 1, OCR). This is in line with decreased expression of genes in the OxPhos pathway uncovered by RNASeq profiling of the mutant cells (1).

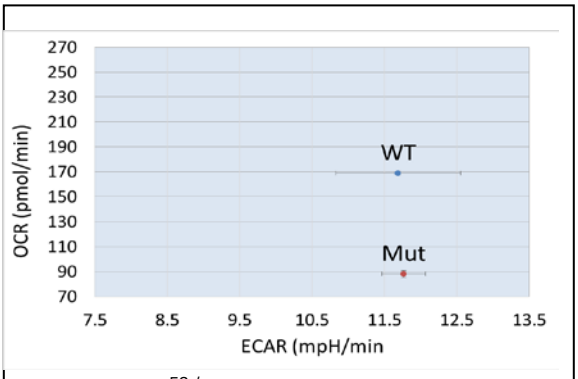

Fig. 2. Six2$^{p53-/-}$ NPC have suppressed OxPhos (OCR, oxygen consumption rate), but unchanged basal glycolysis rate (ECAR), compared to wild type NPC.

Comparison of basal glycolysis rate revealed p53-null NPC have a similar basal glycolytic rate (ECAR, extracellular acidification rate; Fig. 2) to wild-type NPC. Suppressed OxPhos (OCR) and unchanged glycolysis rate (ECAR) (Fig. 2) suggest that mutant cells would be dependent on glycolysis as their main energy source, and would be predicted to have lower energy production. Both these points are true – mutant cells have 2-fold lower ATP levels (1) than wild-type, and inhibition of glycolysis in Six2$^{p53-/-}$ kidneys inhibits growth and nephrogenesis (Fig. 3). In contrast, wild-type kidneys continue to grow and surprisingly show increased nephrogenesis (top panel, and see Fig. 5). ATP and ROS measurements in the context of stress are in progress.

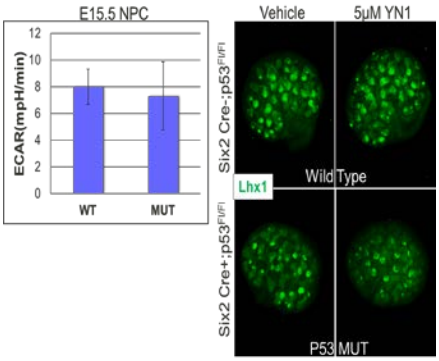

Fig. 3. Glycolysis flux (ECAR) is unchanged in p53-null NPC compared to WT (littermate) NPC. Pharmacological inhibition of glycolysis (see Fig. 12 for details) in the mutant kidneys results in decreased nephrogenesis compared to wild-type littermate kidneys, suggesting increased dependence of Six2$^{p53-/-}$ cells on glycolysis for energy metabolism.

B) Determine the cell fate of NPC after a pharmacologically induced metabolic switch in Cited1+/Six2p53+/+ cells. NPC will be evaluated for proliferation and maintenance of Cited1, or differentiation to Cited1- cells, or cell death. P-ATF2 and P-Akt, key regulators of NPC proliferation, will also be measured by immunostaining and quantified by Western blot. The effects of pharmacologic inhibition of glycolysis vs glucose deprivation on OxPhos rate will also be determined.

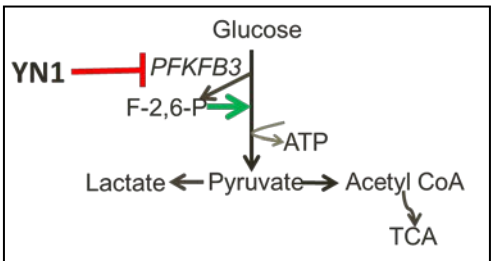

Fig. 4. YN1 inhibits PFKFB3, a potent activator of glycolysis.

Results: Inhibition of glycolysis in E12.5 kidneys with 5μM YN1, an inhibitor of glycolysis activator PFKFBP3 (6-Phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 3) (2) (Sigma), (Fig. 4) results in increased nephrogenesis (Fig. 5C, F) and a smaller, dispersed CM (Fig. 5D), decreased Cited1 and Six2 expression in isolated NPC with concomitant increase in Wnt4 expression (Fig.5B). Inhibition of glycolysis was confirmed by measuring glycolysis rate (ECAR) on YN1-treated NPC (Fig. 5A) cultured in NPC expansion medium (3). Quantitation of nascent nephrons (Lhx1+) and tips in YN1 treated kidneys shows increase in nephrogenesis is independent of tip number (Fig. 5E).

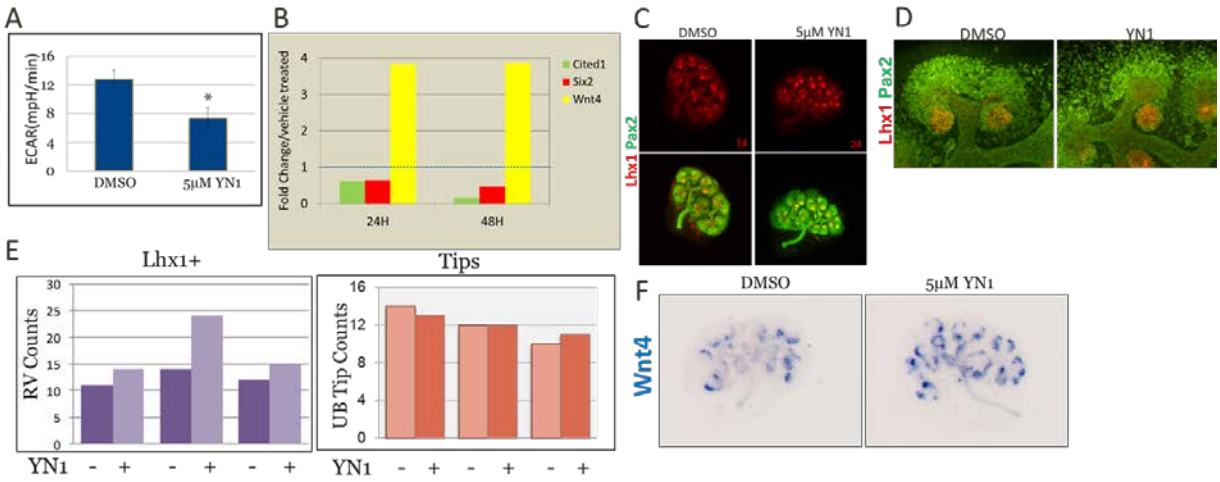

Fig. 5. Glycolysis inhibition accelerates nephrogenesis. **A**.YN1 inhibits ECAR; **B**. Nearly two-fold decrease in Cited1and Six2 expression concomitant to increased Wnt4 expression in isolated NPC cultured in NPC media and treated with 5uM YN1 for 24h relative to control (DMSO). After 48h treatment Cited1 and Six2 expression decreased further. **C**. E12.5 wild-type kidneys treated with YN1 (5μM) for 24h. Whole mount IF for Lhx1 (nascent nephrons, bright red) and ureteric tips (faint red), and Pax2 (CM and derivatives and UB, green). N = 10 kidney pairs. **D**. Change in CM morphology and size in YN1 treated E12.5 kidneys after 24h.**E**. Quantitation of nascent nephrons (Lhx1+) and tips in YN1 treated kidneys shows increase in nascent nephrons is independent of increase in tip number. N= 3 kidney pairs. **F.** YN1-induced nascent nephrons are Wnt4+, shown by whole mount in situ hybridization.

Effect of glycolysis inhibition on signaling pathways (P-Akt and P-ATF2) and on OxPhos rate are in progress.

3. Publications:

Li Y, Liu J, Li W, Brown A, Baddoo M, Li M, Carroll T, Oxburgh L, Feng Y and *__Saifudeen Z__. p53 Enables Metabolic Fitness and Self-Renewal of Nephron Progenitor Cells. *Development, 2015* Apr 1; 142(7):1228-41. PMID: 25804735

Bibliography

1. Li Y, Liu J, Li W, Brown A, Baddoo M, Li M, Carroll T, Oxburgh L, Feng Y, Saifudeen Z. p53 enables metabolic fitness and self-renewal of nephron progenitor cells. Development. 2015;142(7):1228-41. doi: 10.1242/dev.111617. PubMed PMID: 25804735; PMCID: PMC4378244.
2. Seo M, Kim J-D, Neau D, Sehgal I, Lee Y-H. Structure-Based Development of Small Molecule PFKFB3 Inhibitors: A Framework for Potential Cancer Therapeutic Agents Targeting the Warburg Effect. PLoS ONE. 2011;6(9):e24179. doi: 10.1371/journal.pone.0024179.
3. Brown AC, Muthukrishnan SD, Oxburgh L. A Synthetic Niche for Nephron Progenitor Cells. Dev Cell. 2015;34(2):229-41. doi: 10.1016/j.devcel.2015.06.021. PubMed PMID: 26190145; PMCID: PMC4519427.