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DISCLOSURES

None.

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See related article, “Haploinsufficiency of the Transcription Factor Ets-1 Is Renoprotective in Dahl Salt-Sensitive Rats,” on pages 3239–3250.

New Insights into Fuel Choices of Nephron Progenitor Cells

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Metabolic flexibility (*i.e.*, the ability to use different fuels for energy) is an inherent property of all cells. Glucose, glutamine, and fatty acids can each meet cellular ATP demands; how, why, and when that choice occurs ultimately may determine cell fate. Nearly a century ago, Otto Warburg reported that tumor cells use glucose to fuel their survival and proliferation, even in aerobic conditions. Glycolysis, although a relatively primitive process, is the most rapid way for a cell to meet its energy needs, despite generating only 2 mol ATP for every mole of glucose.¹ Glucose metabolism *via* the pentose phosphate shunt also promotes nucleotide and lipid synthesis, essential building blocks for eventual differentiation.² Fatty acids, however, can be oxidized *via* the citric acid cycle (TCA) in mitochondria to yield 36 mol ATP per mole of glucose, although this process also generates reactive oxygen species, a potential source of cellular damage. Glutamine can be used as an alternate energy source for neoplastic and mesenchymal cells; it enters the TCA cycle *via* α -ketoglutarate to generate 9 mol ATP per mole glucose. Transcriptional programming as well as external growth factors can promote fuel switching, and it is this flexibility that ultimately governs cell fate.³ That process has recently undergone careful scrutiny as new tools have become available to study the biochemistry of single cells.

In this issue of the *Journal of the American Society of Nephrology*, Liu *et al.*⁴ show that fuel usage by nephron progenitor cells (NPCs) changes over developmental time and that this change influences their renewal-differentiation balance. During development, nephrons continually differentiate through aggregation and epithelialization of NPCs, and it is essential that a sufficient NPC population is maintained for successive waves of nephrons to form. This self-renewal program is controlled by a complex interplay of FGF, BMP, and Wnt signaling within the NPC niche.⁵ Late in kidney development, the renewal-differentiation balance becomes skewed in favor of differentiation, leading to a final wave of nephron formation, in which all NPCs are depleted.⁶ Competitive repopulation experiments

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show that NPCs isolated from embryonic day 19 (E19) mouse kidneys rapidly differentiate when engrafted into NPC niches of E12 kidneys, whereas E12 NPCs engrafted into E12 niches generally renew in their undifferentiated state.⁷ These studies suggest that there are intrinsic differences between NPCs at distinct developmental ages. The paper by Liu *et al.*⁴ sheds light on a key difference, namely that young NPCs use glycolysis to a greater extent than old NPCs. To understand if utilization of glycolysis in young NPCs promotes their renewal, they inhibited glycolysis in explanted kidneys from E13 mouse embryos. Interestingly, they found that NPC differentiation accelerated, supporting a causal association between glycolysis and maintenance of the self-renewing state. To provide an explanation, they tested the effect of inhibiting glycolysis on the response of NPCs to Wnt/ β -catenin signaling, which is the pathway that initiates their differentiation in the developing kidney. Inhibition of glycolysis lowered the threshold for differentiation by Wnt/ β -catenin, suggesting that aging NPCs with reduced glycolytic capacity become sensitized to epithelial induction. Therefore, why do young NPCs preferentially use glycolysis? PI3K-AKT signaling is required for NPC renewal, and the authors asked if glycolysis intersects with this pathway. Indeed, they found that inhibition of PI3K-AKT signaling reduces glycolysis, indicating that this pathway influences metabolic choices of the cell. This leads to a model in which PI3K-AKT signaling promotes glycolysis, which lowers sensitivity to the inductive Wnt signal in the young NPC. As development progresses, glycolysis decreases, and NPCs become sensitized to Wnt/ β -catenin, skewing the balance in favor of differentiation.

Several different stem and progenitor cell types, such as mesenchymal stem cells (MSCs) and hematopoietic progenitor cells, use glycolysis as their primary fuel source, with a transition from glycolysis to mitochondrial metabolism associating with differentiation.^{8,9} Although attributing causality is experimentally challenging, there is strong support for glycolysis promoting the undifferentiated state and mitochondrial metabolism promoting differentiation.¹⁰ Although the paper by Liu *et al.*⁴ shows that the NPC is similar to several other stem and progenitor cells with respect to its fuel choice, one important difference is that increased mitochondrial metabolism does not push it out of the progenitor state and into the differentiation cascade. Instead, it lowers the threshold for responsiveness to the Wnt/ β -catenin differentiation signal, allowing the cell to remain a progenitor but increasing the likelihood that it differentiates out of the progenitor niche. What could be regulating the choice between fuel sources in the NPC? The paper by Liu *et al.*⁴ shows that developmental age is the primary culprit, with the shift from glycolysis to mitochondrial metabolism occurring over the course of just a few days in the developing mouse. Interestingly, mammalian target of rapamycin (mTOR) pathway activity in NPCs increases with biologic age.⁷ mTOR activity is known to increase mitochondrial biogenesis and mitochondrial metabolism, and an attractive hypothesis is that increasing mTOR activity over developmental time promotes mitochondrial respiration and

reduces glycolysis.¹¹ This effect could be compounded by the loss of FGF20 seen in aging NPCs, which would be predicted to reduce PI3K-AKT promotion of glycolysis.⁷

The paper by Liu *et al.*⁴ addresses the effect of developmental age on energetic choices of the NPC, and many questions regarding how fuel source switching may regulate the differentiation of NPCs to nephrons remain open. The resemblance of the metabolic behavior of the NPC with that of the MSC begs the question: could the first steps in the nephron differentiation process be analogous? As MSCs enter their programmed differentiation pathway to become osteoblasts, Wnt/ β -catenin signaling stimulates glycolysis, making it the preferred ATP-generating process for the cell.¹² Mechanistically, Wnt3a signals mainly through LRP5 to activate mTORC2 and AKT, which in turn, acutely increase the abundance of a number of key glycolytic enzymes (*e.g.*, HK2, PFK1, PFKFB3, and LDHA).¹³ Interestingly, although the work by Liu *et al.*⁴ suggests that AKT signaling similarly promotes glycolysis in the undifferentiated NPC, it also shows that inhibiting glycolysis promotes epithelial differentiation of NPCs, pointing to an important difference between these two progenitor cell types in how fuel source utilization regulates differentiation. The role of other features of MSC fuel source selection remains to be studied in the NPC. For example, it has been established that Wnt signaling can stimulate glutamine metabolism *via* the TCA cycle.¹⁴ Also, glutamine efflux in exchange for the import of leucine through the antiporter has been shown to stimulate the ultimate regulator of protein synthesis mTORC1 in these cells.¹⁴ From work on the metabolomics of MSCs and other progenitor cell types, it has become clear that, in any cell lineage, there must not only be temporal control of substrate selection to initiate and promote the process of differentiation, but there must also be some degree of metabolic flexibility, ensuring adaptability to fuel availability. Future studies of the stepwise differentiation of the nephron lineage at a variety of developmental stages will be required to understand how growth factor signaling from surrounding tissue, intrinsic transcriptional changes associated with developmental age, metabolic choices, and differentiation are integrated.

There are, of course, technical caveats worth noting about studies of cellular bioenergetics. First, we are generally limited to studying the behaviors of cultured cells and tissues, and these models may not fully recapitulate *in vivo* processes. Second, although the Seahorse and Oroboros experimental systems have provided many novel insights into cellular metabolism, certain limitations must be acknowledged. For example, although extracellular acidification generally reflects lactate production, as much as one third of the acid generated by cells *in vitro* can occur through the release of CO₂ from the TCA. Recent studies have provided algorithms to help correct for this by determining the precise moles of ATP generated *via* glycolysis versus oxidative phosphorylation. Third, it is unlikely that there is mutual exclusivity in respect to the pathway for ATP generation by a cell; rather, it is likely that one metabolic program predominates but is reinforced by the other. Moreover, the Crabtree effect, in

which glycolysis suppresses oxidative phosphorylation and mitochondrial respiration, may occur, particularly when higher concentrations of glucose are used.¹⁵ Thus, our view of one metabolic pathway taking over from another is largely a reflection of the experimental systems that we use to study them, and in reality, these metabolic pathways are concurrently used. Subtle skewing of the balance between them may have significant consequences for differentiation, but understanding this nuanced systems biology of metabolism may require a new generation of technologies, perhaps featuring *in vivo* reporting.

The paper by Liu *et al.*⁴ breaks new ground and forces us to consider metabolic programming and its temporal nature in the process of nephrogenesis. This work is certain to spawn new and exciting studies to help understand not only the physiology and biochemistry of nephron differentiation but also, the potential aberrancies that initiate and drive renal neoplasia.

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DISCLOSURES

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Extracellular Vesicles in Preeclampsia: Evolving Contributors to Proteinuria

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Preeclampsia remains a condition with high morbidity and mortality, with inherent challenges in diagnosis and treatment. Patients are typically referred to nephrologists to rule out preeclampsia in the setting of nephrotic syndrome or new-onset acute kidney failure during pregnancy. Preeclampsia is thought to be a vascular disease with maternal endothelial dysfunction due to circulating placental antiangiogenic factors.¹ It is currently believed that glomerular capillary damage is the primary factor that leads to proteinuria; however, the precise mechanisms are still being debated. Garovic *et al.* first proposed that loss of glomerular podocytes in the urine that has been observed in many nephrotic syndromes including preeclampsia may be a key factor that contributes to the pathogenesis of proteinuria.²

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