Nephrology Dialysis Transplantation 33 (Supplement 1): i7–i8, 2018
doi:10.1093/ndt/gfy104

D. AKI EXPERIMENTAL

FO014 TUBULAR CELL HYPERTROPHY *VIA* ENDOCYCLE AND PROLIFERATION OF TUBULAR PROGENITORS ARE CENTRAL MECHANISMS OF RESPONSE AFTER AKI

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INTRODUCTION AND AIMS: Acute kidney injury (AKI) is considered a reversible disease because of the capacity of all survived tubular cells to proliferate as demonstrated by proliferation markers. However, as this mechanism does not explain why even mild AKI episodes imply a substantial risk of developing chronic kidney disease (CKD), we questioned the high intrinsic regenerative capacity of tubules.

METHODS: To investigate the proliferative capacity of tubular cells after AKI, we developed two conditional transgenic mouse models: 1. Pax8.rtTA;TetO.Cre;R26.FUCCI2aR (Pax8/FUCCI2aR) to track all tubular cells; 2. Pax2.rtTA;TetO.Cre;R26.FUCCI2aR (Pax2/FUCCI2aR) to track a putative scattered tubular progenitor population. Administration of doxycycline for 10 days drove the reporter expression in Pax8+ and Pax2+ cells in healthy and after 30'unilateral ischemia injury followed by a 30 day reperfusion period (IRI). The FUCCI2A technology provides an elegant instrument to monitor the cell cycle phasing as follows: mCherry+cells are in G1 phase, mVenus+ cells are in S/G2/M phase of cell cycle.

RESULTS: Confocal analysis of Pax8/FUCCI2aR mice demonstrated that immunostaining for "proliferation markers" such as KI-67, PCNA and p-H3 didn't mirror exactly cell-cycle phase, indicating that proliferation markers do not predict cell division, but rather that tubular cells are able to undergo cell cycle variant without cell division, such as endocycle, after IRI. Endocycling is an evolutionary conserved cell cycle variant, recently found in the regenerative processes of some mammalian organs. During endocycling, G1 and S phases proceed alternatively without passing mitosis, generating mononuclated cells with an increased cell size and DNA content and therefore developing a cell cycle dependent hypertrophy. The latter is a conserved method for terminally differentiated cells to increase biosynthetic capability in order to respond to the immediate requirement to maintain organ function after injury. Endocycling was investigated by combining cell-cycle phase FUCCI2aR expression and DNA content assessment by flow cytometry. This analysis revealed that 11.5±0.8% of Pax8+tubular cells underwent endocycles instead of mitosis at day 30 after IRI. The majority of endocycling cells were in S1-S2 segments of the cortex with a higher cell surface area in comparison to cells in G1 phase. Accordingly, tubular cell hypertrophy *via* endocycle is the dominant feature in the cortex of human biopsies with CKD after AKI. By contrast, Pax2+ tubular progenitors, that represent a small subset of tubular cells, didn't undergo endocycle but rather complete mitosis at day 30 after IRI. Automatic quantification analysis by flow cytometry demonstrated that while Pax8+ tubular cells are irreversibly lost, Pax2+ tubular cells are the only proliferating cells generating new tubular cells after AKI.

CONCLUSIONS: This study suggests the existence of two crucial mechanisms of response to AKI that take place in tubular cells with a different differentiation state. Only Pax2+tubular progenitors is able to divide, generating new tubular cells and therefore driving a real tubular regeneration. On the other hand, the differentiated tubular cells undergo endocycle-mediated hypertrophy, in order to retain organ function after injury. These results revise the currently accepted view on tubular regeneration and suggest the endocycle-mediated hypertrophy as a prognostic indicator of the risk for CKD progression.