











Reciprocal regulation between the molecular clock and kidney injury

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Tubulointerstitial fibrosis is the common pathological substrate for many etiologies leading to chronic kidney disease. Although perturbations in the circadian rhythm have been associated with renal disease, the role of the molecular clock in the pathogenesis of fibrosis remains incompletely understood. We investigated the relationship between the molecular clock and renal damage in experimental models of injury and fibrosis (unilateral ureteral obstruction, folic acid, and adenine nephrotoxicity), using genetically modified mice with selective deficiencies of the clock components *Bmal1*, *Clock*, and *Cry*. We found that the molecular clock pathway was enriched in damaged tubular epithelial cells with marked metabolic alterations. In human tubular epithelial cells, *TGFβ* significantly altered the expression of clock components. Although *Clock* played a role in the macrophage-mediated inflammatory response, the combined absence of *Cry1* and *Cry2* was critical for the recruitment of neutrophils, correlating with a worsening of fibrosis and with a major shift in the expression of metabolism-related genes. These results support that renal damage disrupts the kidney peripheral molecular clock, which in turn promotes metabolic derangement linked to inflammatory and fibrotic responses.

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Introduction

Organ fibrosis is an unfavorable consequence of chronic inflammation characterized by an excessive accumulation of ECM. In the kidney, fibrosis is the ultimate stage of the cellular response to chronic inflammation and the converging pathological substrate for several entities of different origins, leading to chronic kidney disease (CKD) (1). Whereas the understanding of the molecular and cellular basis of organ fibrosis, including that of the kidney, has experienced substantial progress in the last decade (2, 3, 4), options for its prevention or treatment are scarce, with only a few drugs

offering limited therapeutic advantage in the case of idiopathic pulmonary fibrosis (5). Renal tubulo-interstitial fibrosis is characterized by a profound alteration of metabolism within the tubular epithelial cell (TEC), majorly related to a drastic reduction in fatty acid oxidation (FAO) (6). We recently showed that genetically mediated FAO enhancement in the renal tubule resulted in significant protection from fibrosis in several experimental models (7). This and other studies support an important role for metabolic failure in kidney fibrosis, whereby inflammation and mitochondrial dysfunction contribute to perpetuate a vicious cycle that results in ECM deposition, fibrosis, and progression of CKD.

Many physiological functions of most tissues and cells across living organisms exhibit daily periodic fluctuations related to the circadian rhythm, majorly conditioned by predictable environmental cues such as the light/dark cycle. The circadian rhythm is composed by a master clock, located in the suprachiasmatic nucleus of the hypothalamus, which is entrained by light and can synchronize tissue and cell peripheral clocks via neuronal and humoral signals (8). At the molecular level, the peripheral clocks are regulated by a transcriptional feedback loop in which clock components such as *Arntl1* (*Bmal1*) and *Clock* activate the transcription of their own repressors including *Per* and *Cry* (9). Perturbations in the circadian rhythm, including the molecular clock, have been associated with many pathologies including renal disease (10, 11, 12, 13, 14). In addition, there is an important crosstalk between the circadian clock and metabolism (15). Nutrient flux in the bloodstream fluctuates considerably throughout the day as a consequence of diurnal behavioral rhythmicity. Consequently, the levels of most metabolites, including glucose, amino acids, and lipids oscillate in the blood in a synchronous manner with the environmental time. In the kidney, the circadian clock modulates blood flow, glomerular filtration rate, and ion and water excretion. However, the bidirectional influence between fibrosis and alterations of the molecular clock remains incompletely understood. By using several models of kidney injury in the setting of mice genetically modified for key components of the molecular clock, we aimed to clarify this question. In addition, we

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