Summary & Salient features
SUMMARY

Kidney is an organ with limited turnover of cells but exhibits remarkable ability to survive injury and restore function. The precise mechanism by which adult kidney replenishes damaged cells remains to be understood. It is still not clear whether cellular repair in adult kidney is carried out by specialized renal progenitors residing in specific niches or by self-duplication/de-differentiation of mature cells. Understanding the mechanism of cellular de-differentiation in key glomerular subsets like GPECs would further our knowledge of its role in tissue repair, disease progression and enable more effective targeted therapies for acute and chronic kidney diseases. With this perspective, we investigated the de-differentiation potential of murine glomerular epithelial cells, in vitro. We report here that GPECs of adult murine kidney undergo spontaneous EMT to generate cells with metastable phenotype and acquire gene profile and functional properties similar to that of early renal progenitors. More importantly, by clonal assays we demonstrate that these cells adopt metastable phenotype, with attributes of both epithelial and mesenchymal markers. Our study suggests that parietal epithelial cells of the glomeruli act as potential stem cells and EMT could increase this ability.

We ideate that the present study would improve our understanding on the plasticity exhibited by GPECs under EMT stimulation which would further our knowledge of its role in tissue homeostasis and disease progression and for possible intervention.

A remarkable regenerative mechanism exhibited by the kidney is the compensatory renal growth after unilateral nephrectomy. This adaptive growth occurs with an increase in the size of nephrons resulting from both hypertrophy and hyperplasia of the renal cellular components. Although compensatory renal growth is largely referred to as compensatory hypertrophy, the exact mechanism of this adaptive compensatory growth is not known.

Therefore the present studies were undertaken to examine whether there is any up regulation of the genes involved in renal development during CRG after UNx. We report here that in mice, the early phase of compensatory renal growth after unilateral nephrectomy is associated with an active phase of renal hyperplasia and most importantly, involves increased transcript abundance of specific progenitor genes that
mediate early kidney organogenesis as well as EMT like changes indicating cellular de-differentiation. We hope that the present study would help to better understand the complex field of renal pathology viz. repair and regeneration which would ultimately help in devising more efficient targeted therapies for acute and chronic renal diseases.

SALIENT FEATURES

1. GPECS of adult murine kidney undergo spontaneous EMT in vitro to generate cells with characteristics and functional properties of embryonic renal progenitors.
2. Clonal assays demonstrate that these cells adopt a metastable phenotype, with attributes of both epithelial and mesenchymal markers. This suggests that parietal epithelial cells of the glomeruli act as potential stem cells and EMT could increase this ability.
3. Transcript analysis by qRT-PCR demonstrate that consistent with the metastability, these cells exhibit heterogeneous expression of metanephric mesenchymal (Pax2, WT-1, Eya-1, Six-1, GDNF) as well as uteric bud (Hoxb-7, C-ret, K-Cadherin) genes, indicating that these cells might be bipotent renal progenitors.
4. PGE2 mediated EMT blocking experiments show that acquisition of progenitor markers by GPECs was EMT driven and EMT imparts survival and proliferative potential to the terminally differentiated GPECs.
5. Injection of fluorescently tagged CD24+ cells into embryonic kidney organ culture resulted in the integration of the cells to kidney primordia. Additionally, co-culturing of CD24+ cells with E13.5 spinal cord resulted in the formation of embryoid bodies, which on extended culture in matrigel exhibited distinct events of tubulogenesis such as formation of cellular processes, development of branching multi-cellular cords, and establishment of tubules with lumens. These results provide more convincing evidence on the functional progenitor status of these cells.


108
6. FACS sorted PKH-26 labeled CD24+ cells grafted into the kidney capsule of unilateral nephrectomised mice were capable of renal regeneration during compensatory renal growth and expressed markers associated with developing kidney. Co-expression of PKH-26 label with the assessed markers confirmed the donor origin of the graft.

7. Compensatory renal growth after unilateral nephrectomy in adult mice involves de-differentiation of tubules and formation of neo-glomerular like structures indicating active regeneration process.


9. During compensatory renal growth high transcript abundance of kidney progenitor genes such as Pax 2, Lim-1, Wnt6 and Wnt 7b is observed suggesting that embryonic renal development pathways are activated during compensatory renal growth.

10. EMT like changes are observed in regenerating kidney by day 10 of UNx as evidenced by tubular cells co-expressing transient epithelial and mesenchymal markers as well as transcript abundance of major regulators of EMT.

In conclusion, the present study provides evidence that under specific stimuli terminally differentiated epithelial cells of kidney (like GPEC) can undergo EMT and generates cells with traits of renal stem cells. Understanding the regenerative and reparative potential during compensatory growth, this study provide evidence that early phase of CRG is associated with rapid hyperplasia, neo-glomerular formation and up regulation of markers of early nephrogenesis.