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Characterisation of senescent renal epithelial cells in experimental renal disease reveals transcriptional heterogeneity at a single cell level

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Introduction:

Senescent cells accumulate in multiple organs including kidneys with ageing and following injury or irradiation.[1] Senescent cell burden associates with worsened clinical outcomes in renal disease including transplantation and glomerulonephritis.[2-4] The depletion of senescent cells improves organ function in several animal models of aging and irradiation thereby making renal senescent cells a potential therapeutic target for nephrologists.[5-7] Despite this, the mechanisms underlying the detrimental effects of senescence remain poorly understood. In-vitro work suggests these cells are heterogenous, exhibiting varying phenotypes at different timepoints and in response to different modes of senescence induction.[8,9] Here we perform the first characterisation of the in vivo renal epithelial cell senescent transcriptome at single-cell resolution.

Methods:

Single cell libraries from healthy and injured murine kidneys were prepared using a high-throughput droplet-based library preparation workflow (10x). Sequencing was performed on a NextSeq 550 High Output Kit v2 (Illumina). Alignment was performed by splicing aware aligner STAR 2.5.1b within the cellranger 2.1.0 wrapper before downstream analysis in the R environment.

Results

We generated 27,444 single cell transcriptomes from kidneys of multiple experimental groups: uninjured 8-week-old mice, aged 2-year-old mice, ischemia reperfusion injury, unilateral ureteric obstruction, cisplatin nephropathy, irradiated nephropathy and mice receiving a senescent cell depleting agent, ABT-263. Senescent epithelial cell burden ranged from 2.7% (uninjured) to 37% (cisplatin) of the epithelial cells. Senescent epithelial cells demonstrated a mean of 475 ± 207 differentially expressed genes compared to their non-senescent counterparts. Over enrichment analysis revealed shared gene ontology terms across groups including expected pathways such as “regulation of cell death”,” regulation of apoptotic process”, and notable over enrichment for genes involved in cell motility, mitochondrial reactions and ATP metabolism. Network topology-based analysis suggests complex overlapping gene networks governing cellular aging, stress response and cell cytoskeletal control.
Pooling the senescent epithelial cells, we used an unsupervised shared nearest neighbour clustering algorithm to reveal heterogeneity within the senescent cell population. Several of the clusters displayed equal cell contributions from multiple injury models, suggesting they represent a shared senescent subtype. In contrast other clusters are distinct and are unique to certain injuries. Intriguingly, senescent cells from the ABT263 treated mice and irradiated mice cluster together, separate from the other groups. Figure 1 shows these clusters annotated on a t-Distributed Stochastic Neighbour Embedding plot.

Discussion

We show for the first time that senescent renal epithelial cells display transcriptional heterogeneity, influenced in part by the nature of the renal injury. Even within a single experimental condition distinct transcriptional groups can be described. Senescent cells isolated from ABT-263 treated kidneys are transcriptomically similar to a subset of irradiated cells. In our hands ABT-263 reduced senescent epithelial cells and reduced progressive renal fibrosis. This suggests a senescent subtype which may resistant to the drug effect may also be present in some disease conditions. Novel pathways altered in senescence include glycolysis, mitochondrial ATP synthesis and cytoskeletal formation. Work is ongoing to characterise these renal senescent subtypes, to better understand pathogenic mechanisms and search for future therapeutic targets in renal disease.