Development of a model of renal cell senescence

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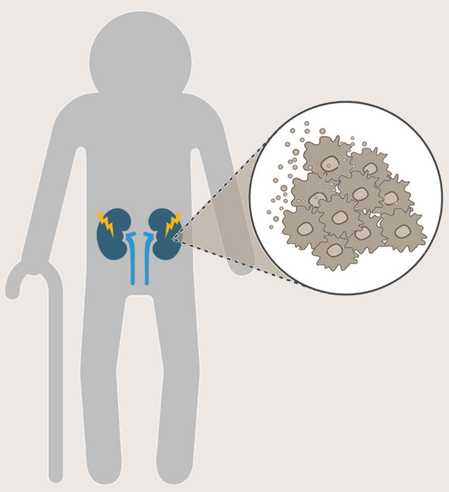
INTRODUCTION



Chronic kidney disease (CKD) is a major and increasing public health issue affecting more than 10% of the global population. CKD is characterised by a slow, gradual, and irreversible **loss of renal functions**. Dialysis or transplantation are required in the terminal stages to supplement renal function.

Growing evidence suggests that **cell senescence** plays a crucial role in the pathogenesis of CKD. Indeed, the **persistence and accumulation of senescent cells** is involved in the development of **kidney fibrosis** through the secretion of pro-inflammatory and pro-fibrotic factors called **senescence-associated secretory phenotype (SASP)**.

In kidneys, senescence-associated alterations seem to **affect mostly proximal tubular epithelial cells (PTEC)**. However, limited information exists to explain the molecular mechanisms underlying these disturbances in renal senescent cells.

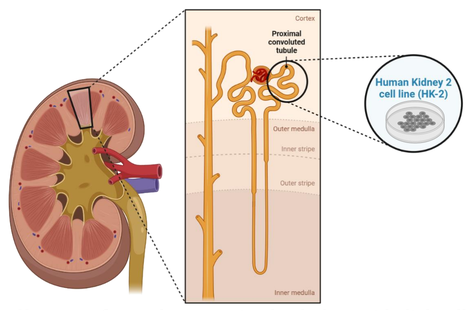
In this context, our laboratories are **studying the emerging roles of senescence in the kidney**, and more specifically the underlying **metabolic dysfunctions** in PTEC.

AIMS & METHODS

Aims

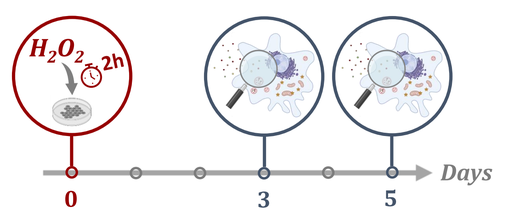
1. To develop a **model of renal senescence** *in vitro*
2. To **characterize** this model using **different senescence markers**

Material



Here, we focused on proximal tubular epithelial cells (PTEC). Experiments were performed on a human immortalized cell line called Human Kidney 2 (HK-2).

Senescence induction



HK-2 cells were incubated for 2 hours with 300 or 500 µM of H_2O_2. Senescence markers were evaluated 3 and 5 days after H_2O_2 treatment.

RESULTS

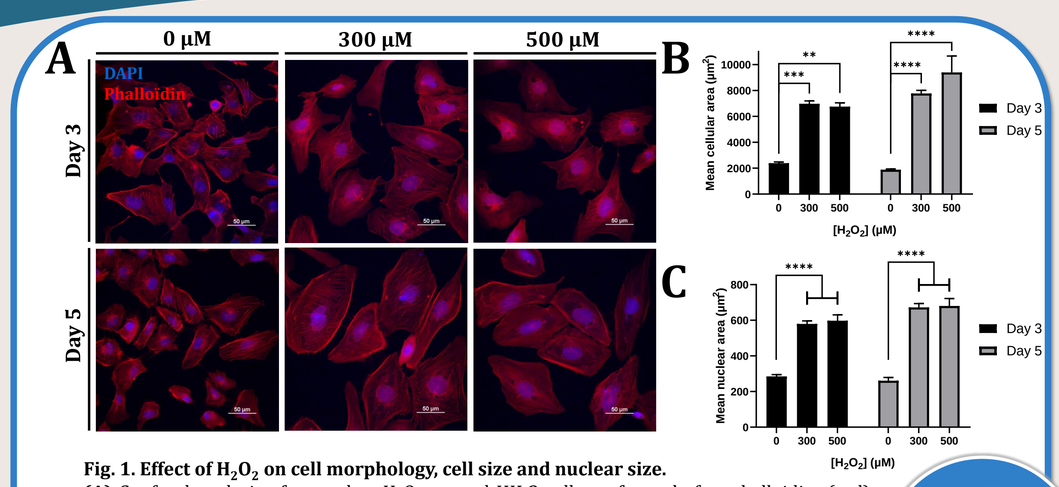


Fig. 1. Effect of H_2O_2 on cell morphology, cell size and nuclear size.
(A) Confocal analysis of control vs H_2O_2-treated HK-2 cells performed after phalloidin- (red) and DAPI- (blue) staining. **(B-C)** Quantification of mean cellular area (µm²) **(B)** and mean nuclear area (µm²) **(C)**. Data presented as Mean +/- SEM ; n = 3 ; Two-way ANOVA + Tukey post hoc test ; ** p < 0,01 ; *** p < 0,001 ; **** p < 0,0001

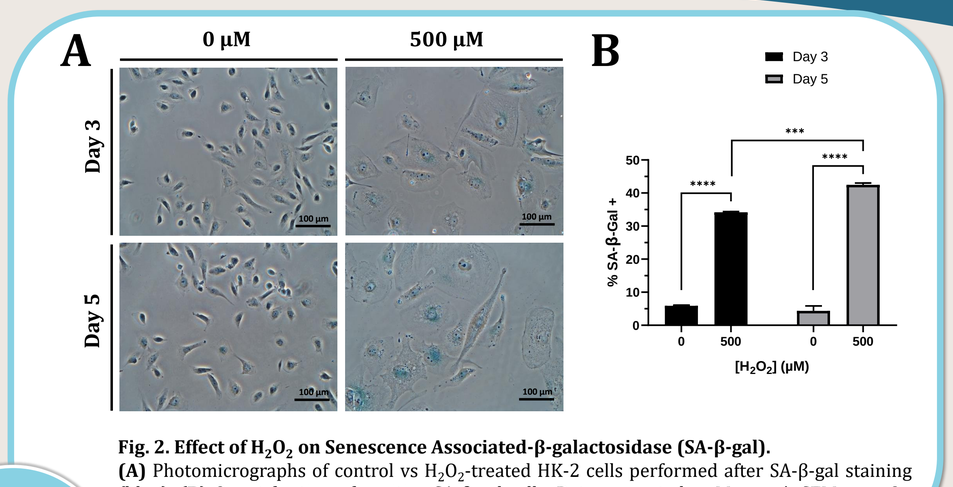


Fig. 2. Effect of H_2O_2 on Senescence Associated-β-galactosidase (SA-β-gal).
(A) Photomicrographs of control vs H_2O_2-treated HK-2 cells performed after SA-β-gal staining (blue). **(B)** Quantification of positive SA-β-gal cells. Data presented as Mean +/- SEM ; n = 3 ; Two-way ANOVA + Tukey post hoc test ; *** p < 0,001 ; **** p < 0,0001

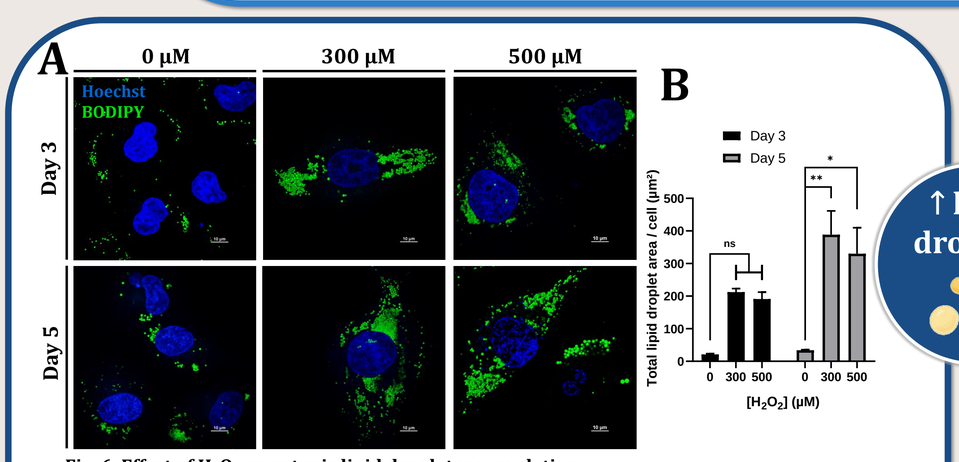
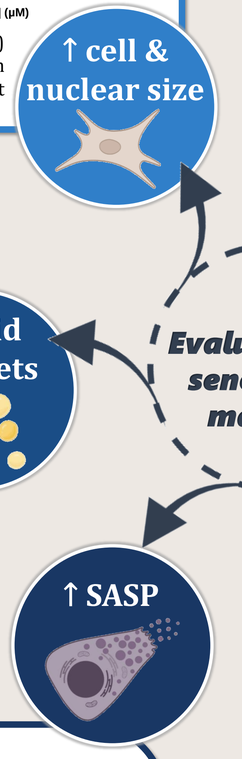


Fig. 6. Effect of H_2O_2 on ectopic lipid droplet accumulation.
(A) Confocal analysis of control *vs* H_2O_2-treated HK-2 cells performed after BODIPY- (green) and Hoechst- (blue) staining. **(B)** Quantification of the total lipid droplet area per cell (µm²). Data presented as Mean +/- SEM ; n = 3 ; Two-way ANOVA + Tukey post hoc test ; * p < 0,05 ; ** p < 0,01 ; ns = not significant (p>0,05)



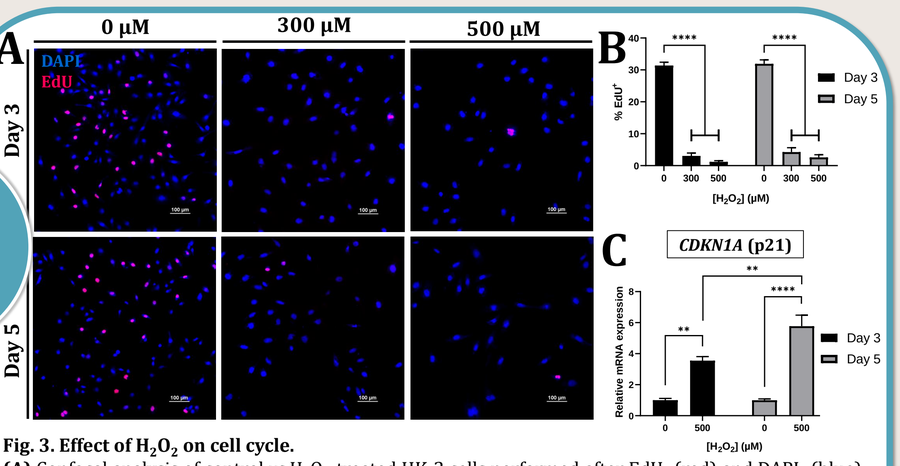


Fig. 3. Effect of H_2O_2 on cell cycle.
(A) Confocal analysis of control *vs* H_2O_2-treated HK-2 cells performed after EdU- (red) and DAPI- (blue) staining. **(B)** Quantification of EdU positive cells performed using ImageJ. **(C)** Relative mRNA expression of *CDKN1A* gene, normalized with 18S, in control *vs* H_2O_2-treated HK-2 cells. Data presented as Mean +/- SEM ; n = 3-6 ; Two-way ANOVA + Tukey post hoc test ; ** p < 0,01 ; **** p < 0,0001

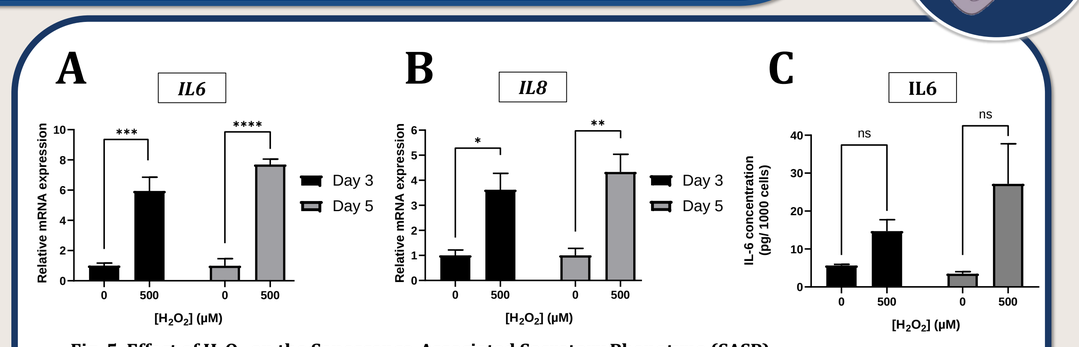


Fig. 5. Effect of H_2O_2 on the Senescence-Associated Secretory Phenotype (SASP).
(A-B) Relative mRNA expression of *IL6* **(A)** and *IL8* **(B)** genes in control *vs* H_2O_2-treated HK-2 cells.
(C) IL6 level in conditioned media collected from control vs H_2O_2-treated HK-2 cells. Data presented as Mean +/- SEM ; n = 4 ; Two-way ANOVA + Tukey post hoc test ; * p < 0,05 ; ** p < 0,01 ; *** p < 0,001 ; **** p < 0,0001 ; ns = not significant (p>0,05)

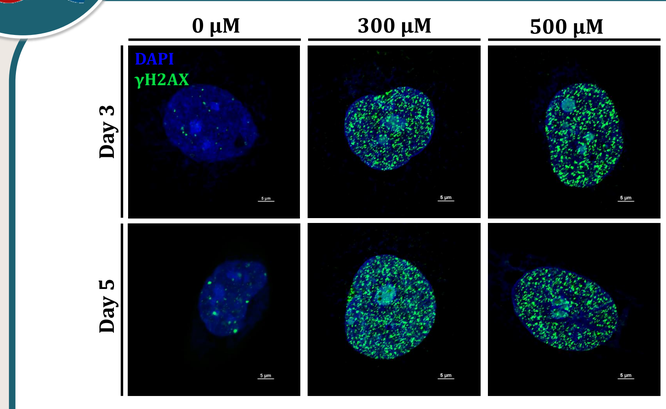


Fig. 4. Effect of H_2O_2 on DNA damage.
Confocal analysis of control *vs* H_2O_2-treated HK-2 cells performed after γH2AX-(green) and DAPI- (blue) staining.

TO REMIND...

We developed a cellular model of senescence by exposing HK-2 cells to 300 and 500 µM of H_2O_2 for two hours. Three and five days after exposure to H_2O_2, HK-2 cells exhibited several senescence markers, such as enlarged cell and nuclear sizes, increased SA-β-gal- and γH2AX-staining, overexpression of *IL6, IL8* and *CDKN1A* (p21) genes and decreased EdU incorporation. Interestingly, disturbances in lipid metabolism were also measured, notably through the appearance of lipid droplets in senescent cells. This may therefore participate to kidney dysfunction. However, additional investigations in this model of renal senescence are necessary to further characterize the contribution of the disturbance of lipid metabolism in senescence-induced CKD.

CONCLUSION & PROSPECTS

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