**Lung alveolar regeneration by p16 deletion**

**in alveolar epithelial cells**

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*Introduction* Alveolar regeneration involves dynamic changes of

a major progenitor cell in alveoli, alveolar type 2 cells (AT2) that

may be altered in alveolar diseases such as emphysema. Targeting

p16, a cell cycle inhibitor involved in stem cell fate, in AT2 could be

a way to restore AT2 plasticity and promote alveolar regeneration.

*Methods* In vivo, morphological analysis (mean linear intercept),

AT2 (pro-SpC) number and KRT8+ cells were quantified in wild type

(WT) or p16-/- mice at D21, D90 and D150 after elastase (lung destruction)

or PBS instillation. In vitro alveolar organoids were used to

test the regenerative properties of EpCAM+ cells (from p16-/- or WT

mice lung). Number and size of organoids were quantified at D14 of

culture. bgalactosidase staining was used to quantified senescence.

Senolytics (Dasatinib + Quercetin) were used in vitro on organoids

and in vivo between D21 and D90 (5/7 days) after elastase instillation.

Single cell RNAsequencing was used to determine EpCAM+ cells

associated with a regenerative process in the lung and organoids.

*Results* In vivo, p16 was overexpressed in AT2 of WT mice with a

lung destruction compared to control. Deletion of p16 was not protective

for alveolar architecture at D21 in the elastase model, but

p16-/- mice had less alveolar destruction than WT mice at D90 and

D150, highlighting a regeneration process occurring after D21. At

D21, p16 deletion in elastase mice was associated with an increase

in number of AT2, increase of pro-SPC + KRT8+ cells and decrease

of senescence. Organoids made with epithelial cells from injured

lung of WT mice were fewer and smaller than PBS injected mice.

Number and size of organoids made with lung epithelial p16-/- elastase

mice cells were more numerous and bigger than organoids

made with EpCAM+ WT elastase mice. Supplementation of organoids

medium with senolytics decrease bgalactosidase staining of

organoids and increase the number and size of organoids. Senolytics

in vivo demonstrate a regenerative alveoli process occurring in

a curative manner between D21 and D90. Single cell analysis showed

an increase, exclusively in p16-/- emphysematous lung, of a specific

cluster of AT2 cells expressing KRT8 that need to be described more

precisely.

*Conclusions* Targeting p16 may reverse alveolar epithelial cell

dysfunction and increases endogenous alveolar regeneration by

increase a new subpopulation of AT2 characterized by KRT8+ markers

and associated with p16possenescent cells elimination.

*Disclosure of interest* The authors declare that they have no competing

interest.

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