Senescence in Aging and Regeneration

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With age, senescent cells accumulate in various tissues, which is causatively linked to a state of low-grade chronic inflammation, aging and age-related diseases. As a possible therapy for age-related diseases, the pharmacological ablation of senescent cells using senolytics was proven to be efficient in numerous preclinical disease models and first clinical pilot studies are ongoing. The herein used first generation senolytics, targeting senescent cell anti-apoptotic pathways (SCAPs), are mainly repurposed drugs that lack efficacy, selectivity and often have an unfavorable safety profile. Recently, we discovered an alternative target pathway for the development of novel senolytic compounds that is based on an altered lipid metabolism in senescent cells.

We found that the enzymatic phospholipase A2 activity as well its produced metabolite arachidonic acid are significantly upregulated intracellularly in senescent cells. Since high levels of arachidonic acid can induce apoptosis, we tested, if inhibition of enzymes that use arachidonic acid as a substrate would increase its levels further to cytotoxic dosis specifically in senescent cells. Indeed, a range of various cell types were selectively (therapeutic index of up to 700-fold) eliminated in vitro when senescent. In vivo, the treatment of 27 months old C57BL6 mice resulted in decreased markers of senescence in various tissues as well as markedly improved neuromuscular functionality and frailty index score. **In summary**, we here provide a promising novel strategy based on lipid metabolism to eliminate senescent cells for counteracting functional decline and diseases associated with aging.

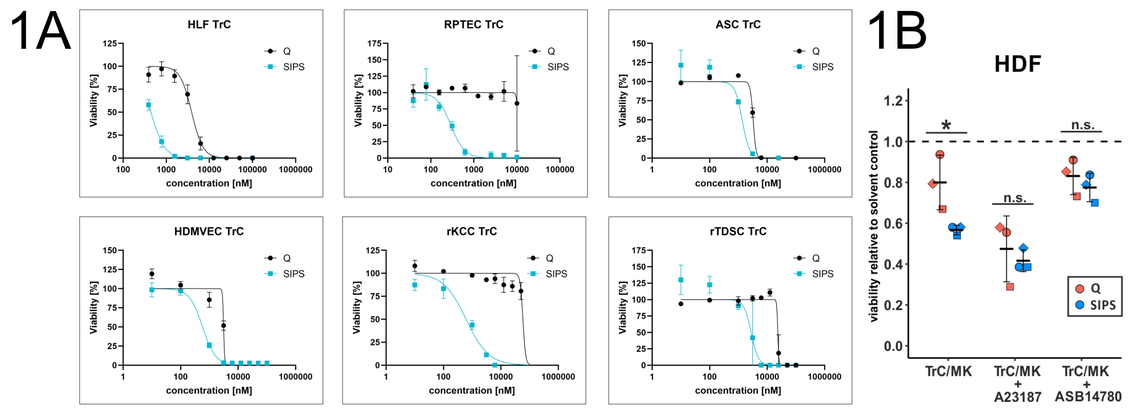


Fig. 1: IAAC is senolytic in vitro. (**A**) A variety of human and rat cell types were induced to senescence using doxorubicin (SIPS) and tested for cytotoxicity as compared to quiescent cells (Q). (**B**) Sensitivity to IAAC-senolytics is dependent on phospholipase A_2 activity and can be modified by inducing (A23187) or inhibiting (ASB14780) $cPLA_2$. HLF: human lung fibroblasts; RPTEC: human renal proximal epithelial cells; HDF: human dermal fibroblasts; ASC: human adipose derived mesenchymal stroma cells; HDMVEC: human dermal microvascular endothelial cells; rKCC: rat knee chondrocytes; rTDSCs: rat tendon-derived stem cell.



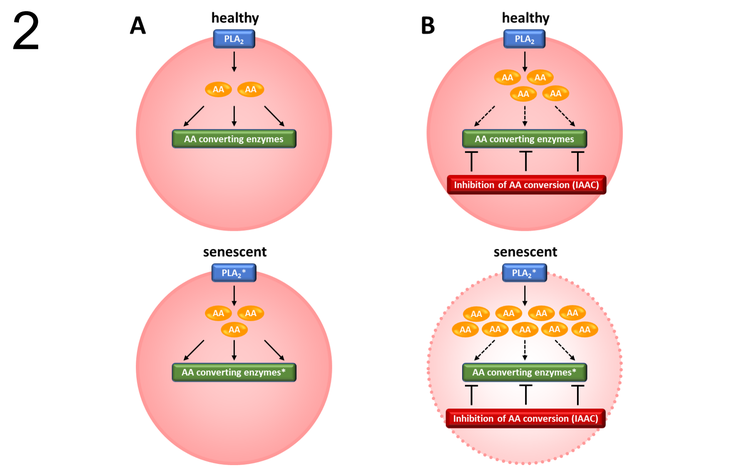
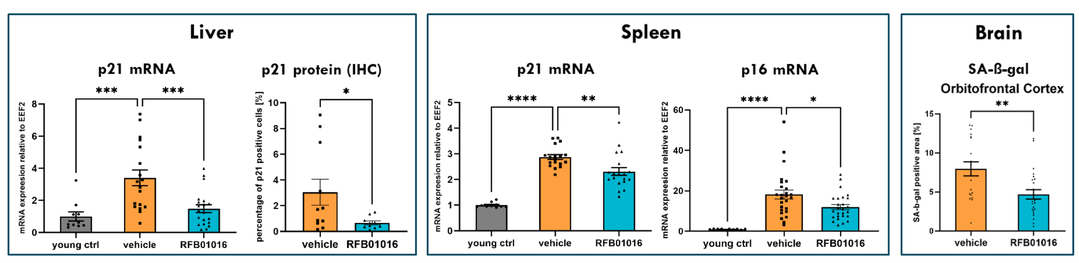


Fig. 2: Scheme of senolysis by inhibition of arachidonic acid conversion. (**A**) Phospholipase A_2-activity is increased in senescent cells leading to increased turnover of arachidonic acid. (**B**) Inhibition of arachidonic acid converting enzymes leads to an accumulation of arachidonic acid in senescent cells that in turn triggers apoptosis.



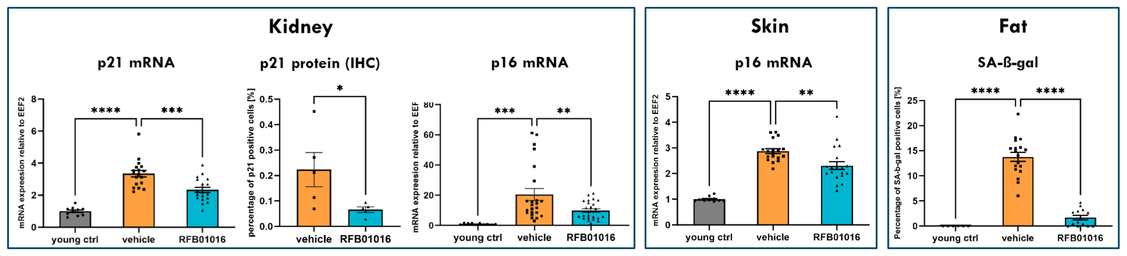


Fig. 3: IAAC is senolytic in vivo. Markers of senescence decrease (p21, p16, SA-β-Gal staining) are reduced after treatment with RFB01016, an inhibitor of arachidonic acid conversion, in several organs, showing systemic senolysis *in vivo*.

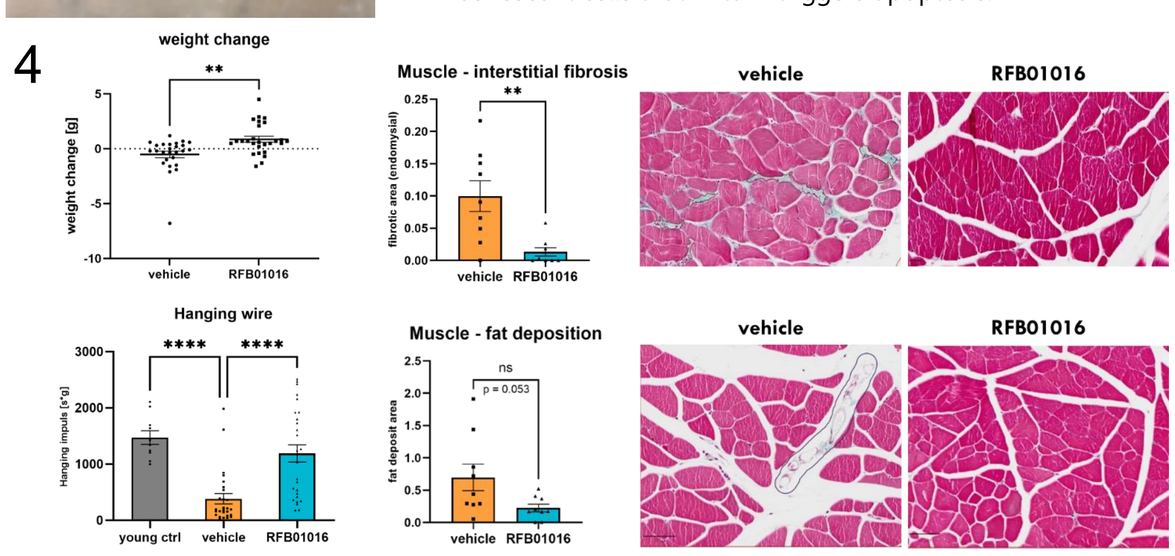


Fig. 4: IAAC senolysis improved muscular function in aged mice. Senolytic treatment with RFB01016 increased body weight and muscle strength as measured with the hanging wire test and reduced interstitial fibrosis and fat deposition in muscle tissue.

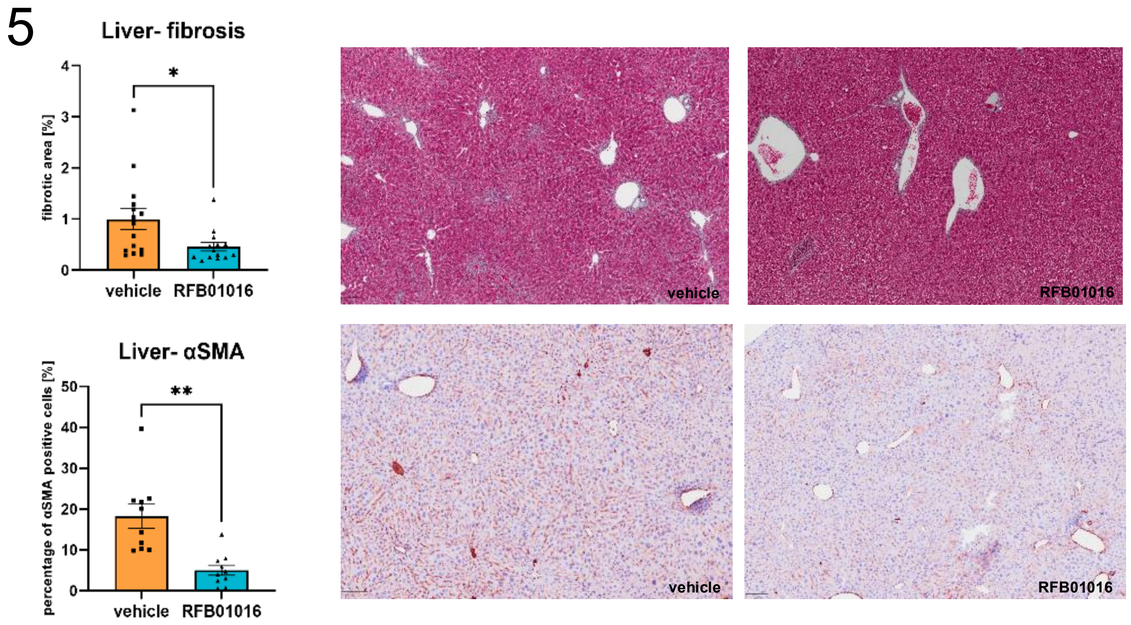


Fig. 5: IAAC senolysis reduced liver fibrosis in aged mice. Staining of liver tissue for collagen (Masson's trichrome staining) and αSMA reveals reduced collagen deposition and myofibroblast activation after senolytic treatment with RFB101016.

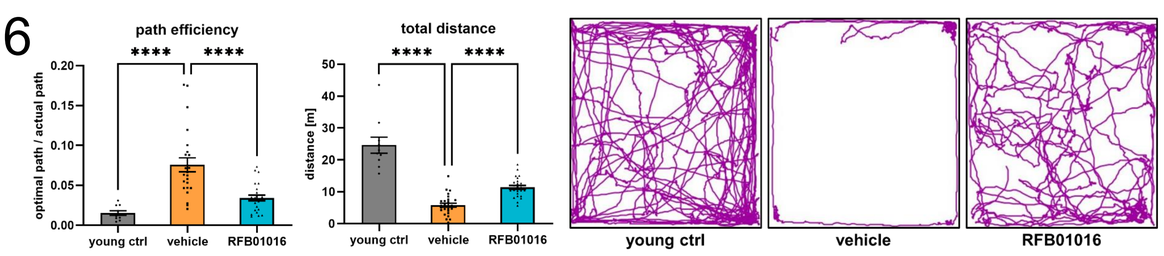


Fig. 6: IAAC senolysis improved exploratory behaviour in aged mice. Senolytic treatment with RFB01016 improved exploratory behaviour and reduced age-associated thigmotaxis in an open field test as measured with path efficiency and distance covered.

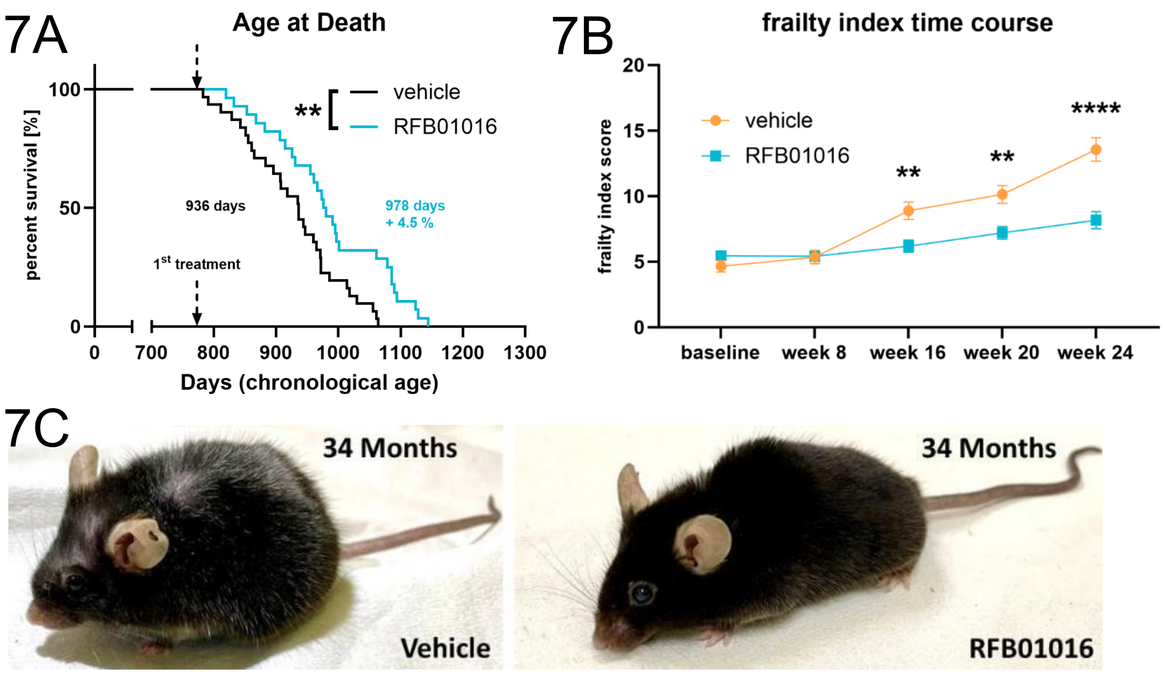


Fig. 7: IAAC senolysis increased fife span and reduced frailty in aged mice. (**A**) Life span of treated animals was observed until death calculated from day of birth. **(B)** Frailty index according to Whitehead et al 2014 is reduced by treatment with RFB01016. (**C**) representative images of mice at 34 months.

Conclusions:

- Inhibition of arachidonic acid conversion shows broad senolytic activity *in vitro* and *in vivo*
- Senolytic treatment with RFB01016 induces tissue regeneration and extends the health and life span in aged mice.

References: Narzt, Pils et al. Epilipidomics of Senescent Dermal Fibroblasts Identify Lysophosphatidylcholines as Pleiotropic Senescence-Associated Secretory Phenotype (SASP) Factors, JID 2021