Accumulation of G3P and pEtN drives cellular senescence via altered lipid metabolism

Glycerol-3-Phosphate (G3P) and phosphoethanolamine (pEtN) biosynthetic pathways are modulated during senescence establishment to sustain lipid droplet accumulation and senescence-associated secretome. These findings reveal new targets for an immunomodulatory approach against senescent cells.

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The question

Senescence can be regarded as a cellular stress response that results in stable cell cycle arrest, thus providing a potent tumour suppressor mechanism[1]. The senescence phenotype involves the secretion of immunogenic factors that may lead to the clearance of senescence cells, but can also impair tissue homeostasis and participate in the emergence of age-related disorders, rendering senescence a 'double-edged sword'[2]. Senescence establishment is achieved by profound transcriptional reprogramming together with changes in cell morphology. These alterations are accompanied by important metabolic adaptations, including lipid droplet accumulation[3], although the precise molecular mechanisms underlying lipid changes and its causative link with specific senescence responses, such as cell cycle arrest and the immunogenic secretome, is unknown. Understanding the changes in metabolism that underpin senescence could lead to the identification of therapeutic targets that aim to alleviate senescence burden, such as during ageing.

The observation

Senescence establishment is a dynamic program with different inducer-specific kinetics. We undertook a time-resolved approach to characterize the metabolomic and transcriptomic profiles of human fibroblasts triggered to senesce by four different stresses: DNA damage, overexpression of RAS or RAF proto-oncogenes and replicative exhaustion. Quiescent and proliferative cells were used in parallel as controls (Fig. 1a). We visualized the cellular metabolome — by mass spectrometry — in response to each senescence inducer and compared the results to quiescent cells by principal component analysis correlated to the identified metabolites.

Our analysis led to the identification of metabolites that contribute to senescence-associated metabolic shifts (Fig. 1b). Of these, the accumulation of G3P and pEtN — both of which are lipid precursors — was particularly interesting given that senescent cells accumulate lipid droplets. G3P provides the glycerol backbone for diacylglycerol (DAG), an immediate precursor for triglycerides and phospholipids, whereas pEtN is a precursor of phosphatidylethanolamine. These observations led us to interrogate the lipid profile of senescent cells, revealing that DAG is rewired in these cells to favour triglyceride synthesis at the expense of phospholipids. By performing a gene–metabolite integration analysis, we identified that activation of glycerol kinase was responsible for G3P accumulation, whereas the dephosphorylation of phosphate cytidylyltransferase 2-ethanolamine (PCYT2) caused pEtN increase. By applying overexpression or knock-down approaches in proliferative cells, we found that these two metabolites are interconnected through a metabolic switch that causes senescence and lipid droplet accumulation at the expense of phosphatidylethanolamine de novo synthesis. Finally, we observed that depleting G3P or pEtN from senescent cells significantly reduced their inflammatory response, pointing to G3P and pEtN modulations as novel therapeutic targets.

The implications

We show that lipid droplet accumulation in senescence is a fine-tuned mechanism involving a multi-layered regulation both at transcriptional and post-translational levels. This G3P–pEtN switch underlies an important and physiologically relevant part of the senescence program. Given the effect of G3P or pEtN depletion on the immunogenic secretome, these findings open new angles to target senescent cells in tissues.

Although our study revealed the senescence-associated metabolic signature in several senescence models in vitro and identified positive correlation between glycerol kinase and senescence features in vivo, we remain cautious about the universality of this metabolic signature and the most suitable pathophysiological conditions to target it until appropriate models are developed.

The rapid action of G3P and pEtN on senescence entry opens a new area of investigation into how these small molecules can cause such a potent modification of signalling molecules and epigenetic factors.

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EXPERT OPINION

"The authors identify two important markers — G3P and pEtN — that accumulate in various forms of senescence and show genetic perturbations that can reverse the senescence phenotype. These findings not only help us better understand changes that occur in different senescence programmes but also highlight potential pathways that might be targeted to alleviate the senescence phenotype and other relevant signature changes".
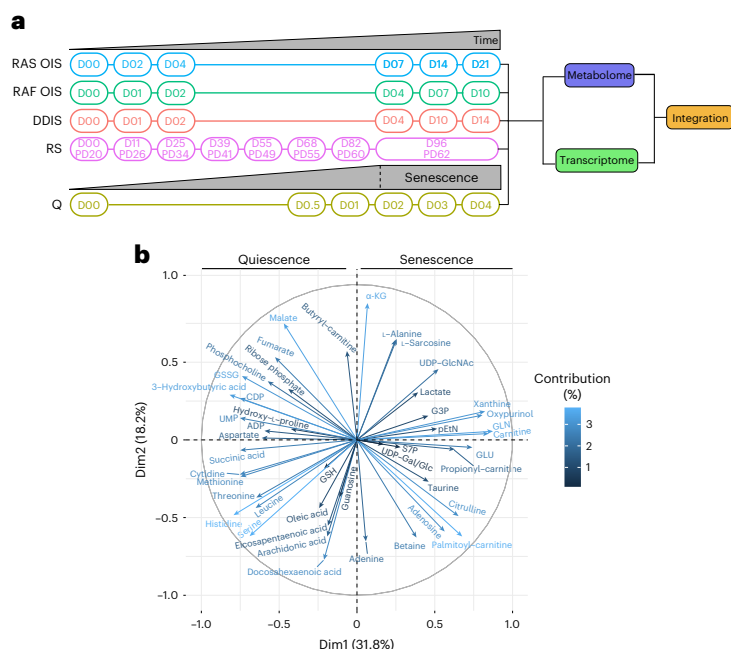**G. Ekin Atilla-Gokcumen, University at Buffalo, SUNY, Buffalo, New York, USA.**

FIGURE

Fig. 1 | G3P and pEtN are part of a senescence-associated metabolic signature. a, Experimental design for the integrative analysis of time-resolved metabolome and transcriptome in human fibroblasts undergoing RAS and RAF overexpression-induced senescence (OIS), DNA damage-induced senescence (DDIS), replicative senescence (RS) and quiescence (Q). D represents 'day'. **b**, Correlation circle for the % contribution of the indicated metabolites to quiescence- and senescence-associated metabolic shifts. Metabolites with dynamics better represented by the two first components are depicted by longer arrows. © 2024, Tighanimine, K., CC BY 4.0.

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BEHIND THE PAPER

Our lab has been interested in cell growth control by the mTOR pathway and its relationship with the cellular senescence programme for more than a decade. This project took an unbiased approach that generated an enormous amount of data. We rapidly focused on G3P and pEtN but initially struggled to understand the origins of these two metabolites in senescent cells. Two back-to-back 'eureka' moments occurred a few days before a meeting with collaborators in May 2022. The first was when a *Nature Metabolism* article on glucose sensing by mTOR[4] inspired us to shift our focus away from mitochondrial G3P metabolism and towards glycerol kinase. In the same week, I borrowed a phos-tag gel from my lab colleague and identified PCYT2 dephosphorylation in senescent cells. Subsequent discussions with my supervisors elucidated the puzzle by drawing a line between G3P and pEtN pathways and proposed the switch as a comprehensive model. **K.T.**

FROM THE EDITOR

"This study stood out to us because the authors use elegant methods to identify and characterize a metabolic shift occurring in senescent cells. These findings strengthen our understanding about cellular senescence and can be harnessed to develop new therapies that may reverse senescence by targeting the identified metabolic changes."
Editorial Team, *Nature Metabolism*.