

## **TORCing to secretory senescence**

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**Abstract**

Cellular senescence is often accompanied by the extensive production of many secretory proteins, which mediate the diverse effects of senescence on the tissue microenvironment. The mechanistic target of rapamycin (mTOR), a master regulator of protein synthesis, controls this secretory phenotype of senescence through modulating translation, transcription and stabilization of mRNA.

A simplistic description of cellular senescence is a state of ‘permanent’ proliferative arrest. This cell-autonomous aspect of senescence has implications in cancer and ageing: roles attributed to a tumour suppressive function and a reduction in tissue regenerative capacity, respectively. Increasing attention, however, has been focused on the non-cell-autonomous activities of senescent cells, which occur primarily through the senescence-associated secretory phenotype (SASP)<sup>1</sup>. The SASP involves a large number of secretory factors, including pleiotropic cytokines, growth factors, and extracellular matrix (ECM) proteins and proteases, providing profound and diverse impacts on the tissue microenvironment. The SASP modulates many aspects of tumorigenesis, including: the immune reaction; the proliferative capacity of neighbouring cells (and of themselves); ECM integrity, and; vascularity. Therefore, senescence as a whole is not merely a tumour suppressor, but rather a complex ‘tumour modulator’. It has become evident that the SASP is also an integral part of more physiological processes such as embryonic development and wound healing<sup>1</sup>. Therefore, understanding how the SASP is regulated and how to manipulate the SASP is a central issue in cancer biology and perhaps any other pathology that involves disruption of tissue homeostasis. Two recent studies<sup>2,3</sup>, including one in this issue<sup>3</sup>, now provide evidence that the mechanistic target of rapamycin (mTOR) kinase, a master regulator of protein synthesis, controls SASP regulatory modules, which involve mRNA translation, transcription and stabilisation.

mTOR complex 1 (mTORC1) senses nutrients, growth factors and other environmental cues, and controls cell growth and proliferation through promoting anabolic metabolism<sup>4</sup>. mTORC1 has been implicated in senescence, but the outcome of manipulating mTOR activity during senescence appears to vary depending on the

model<sup>5-8</sup>. In addition, although a positive relationship between mTORC1 activity and the SASP has been suggested<sup>6,9</sup>, the mechanism(s) for this correlation were unclear. Using rapamycin on human fibroblasts, Laberge *et al.* first show that the secretion of major components of the SASP, including the pro-inflammatory cytokine IL-6, are mTOR dependent<sup>2</sup>. This is not simply due to a general reduction of protein synthesis occurring upon mTOR inhibition, since these rapamycin-sensitive SASP components are mostly modulated at the mRNA level<sup>2</sup>. The transcription of the SASP components is largely regulated through complex positive feedback loops between pro-inflammatory cytokines and the transcription factor NF- $\kappa$ B, conferring local amplification on the pro-inflammatory cascade. In the context of senescence, the same group previously showed that IL-1 $\alpha$ , which is mostly bound to the cell surface, is an upstream regulator of a pro-inflammatory network within the SASP through activation of NF- $\kappa$ B<sup>10</sup>. Consistent with these observations, Laberge *et al.* show that rapamycin treatment attenuates the up-regulation of IL-1 $\alpha$  during senescence but, in contrast to other rapamycin-sensitive SASP components, this reduction is primarily due to translation rather than transcription. They proposed that mTOR facilitates *IL1A* (encoding IL-1 $\alpha$ ) translation, thereby activating NF- $\kappa$ B. This triggers the amplification of the downstream pro-inflammatory cytokine network through positive feedback between NF- $\kappa$ B and the cytokines, including IL-1 $\alpha$  (tentatively termed ‘IL-1 $\alpha$  model’ in this review, Figure 1b).

This appears to be only one side of the story, however. In this issue, Herranz *et al.* provide a distinct and unexpected view on mTOR’s regulation of the SASP<sup>3</sup>. They identified rapamycin through a small molecule screen for SASP inhibitors. Similar to the study by Laberge *et al.*<sup>2</sup>, they also observed that a reduction of mRNA levels

upon mTOR inhibition is more prominent than the effect on translation for most SASP components analysed. However, this reduction in mRNA levels appears to be caused by enhanced mRNA degradation. They identified MAPKAPK2 (also known as MK2) as a specific target of mTOR-regulated translation during senescence. MAPKAPK2, a downstream effector of p38 $\alpha$  MAPK, has been shown to phosphorylate the RNA-binding protein ZFP36L1 to inhibit its AU-rich element (ARE)-mediated mRNA decay (AMD) activity<sup>11</sup>. They propose a model that mTOR specifically promotes *MAPKAPK2* translation during senescence, thereby inhibiting the AMD activity of ZFP36L1, which targets some SASP components (tentatively termed ‘MK2 model’ in this review, Figure 1b).

How can we reconcile these two models? Although Herranz et al. demonstrate a significant down-regulation of *IL1A* mRNA upon mTOR inhibition in the ‘MK2 model’, they also show a preferential reduction of translation within SASP components, including IL-1 $\alpha$  (although IL-1 $\alpha$  is reportedly localised at cell surface, we include IL-1 $\alpha$  as a SASP components in a broad sense in this review)<sup>3</sup>. Although exactly which mRNAs encoding SASP components are directly degraded through ZFP36L1-mediated AMD remains to be elucidated, *IL1A* appears to have a relatively ARE-rich 3’ UTR<sup>3</sup>. Conversely, in the ‘IL-1 $\alpha$  model’, the levels of *IL1A* mRNA appear to be slightly reduced by rapamycin treatment, at least at an early time point after senescence induction<sup>2</sup>. These results perhaps imply a cooperative contribution of both mechanisms. It is notable that the primary senescence models used in these studies are different: DNA damage- (the IL-1 $\alpha$  model) or oncogene-induced (the MK2 model) senescence. Thus it is possible that the relative contributions of these two models might be different between the experimental systems. In addition, similar

to mTOR activity, MAPKAPK2 activation is dynamic during OIS establishment<sup>3</sup>, thus the balance between these models might shift during the course of senescence. Although both studies use several means to inhibit mTOR activity, the IL-1 $\alpha$  model mostly relies on the use of rapamycin, an allosteric inhibitor of mTORC1, which partially inhibits the phosphorylation of eIF4E-binding proteins (4E-BPs), and is thus a weaker inhibitor of mTOR-mediated Cap-dependent translation initiation compared to the ATP-competitive mTOR kinase inhibitors, such as Torin1, which was the primary compound used to define the MK2 model<sup>4</sup>. It would be important to systematically compare the effects of both types of inhibitors on differential regulation of translation during senescence.

In both models, it is not entirely clear how the specificity of mTOR-dependent regulation of *IL1A* and *MAPKAPK2* translation, is achieved, since both transcripts lack the 5' terminal oligopyrimidine (TOP) or TOP-like motifs, which characterises the mRNAs more sensitive to mTORC1 inhibition<sup>2,3,12</sup>. Nevertheless, a recent study, which performed a genome-wide profiling of Torin1-sensitive mRNA translation in mouse embryonic fibroblasts using the Ribo-seq technology, identified *Mapkapk2* as one of the most affected transcripts<sup>12</sup>. Interestingly, instead of the TOP(-like) motifs, Laberge *et al.* predict a highly stable secondary structure downstream of the AUG in the *IL1A* transcript, and suggested that the RNA helicase activity downstream of mTORC1 might be involved in regulating such structured mRNAs. This idea requires experimental validation. It would be interesting to know whether *MAPKAPK2* and other 'non-TOP(-like)' mRNAs that are sensitive to mTOR inhibition, identified in the Ribo-seq studies, contain such a secondary structure<sup>12,13</sup>.

What is in vivo relevance of these studies? An important and often elusive question about the in vivo SASP is the origin of the senescent cells: tumour or stromal, for instance. Both studies first focus on the dark side of the SASP factors derived from stromal senescence (see '1' in Figure 1a). One way to address this question would be using a xenograft mouse model, where co-injected senescent fibroblasts enhance, likely through the SASP, tumour development of the subcutaneously injected tumour cell lines. mTOR inhibition<sup>2,3</sup> or the constitutive activation of ZFP36L1<sup>3</sup> specifically in senescent fibroblasts blunts the enhancement of tumorigenesis in this model. In practice, however, chemotherapy in cancer patients is likely to cause both cell death and senescence either in cancer or stromal cells. This might confer confounding effects through the tumorigenic aspect of the SASP, but a combination therapy of standard chemotherapy and mTOR inhibitors might alleviate the risky part of the senescence induction. Of note, both studies show that mTOR inhibition suppresses the SASP but does not overcome the proliferative arrest. The reason for this is not clear, but, as the authors in these studies speculate, it might be due to the well-recognised anti-proliferative effects of mTOR inhibition and/or the heterogeneous nature of the SASP components. This point is touched on by the Laberge *et al.* study<sup>2</sup>. In a similar xenograft model, the co-administration of rapamycin and a chemotherapeutic drug to the mice injected with a tumour cell line, with or without 'normal' fibroblasts, exhibited a better outcome than chemotherapy alone, in conditions where rapamycin alone has no benefit.

The discovery of the additional benefits of mTOR inhibition, i.e. anti-SASP activity, is encouraging, especially considering that anti-proliferative activity of mTOR inhibitors has already been exploited in clinical and/or preclinical studies in some

types of cancer. However, it is critical to consider both the cell-autonomous and non-cell-autonomous activities of senescence in order to aim for the maximal, or optimal, benefits of senescence-induction during cancer therapy. It has been shown that the SASP can also be tumour suppressive not only in preneoplastic tumours (represented by OIS) but also full-blown cancer contexts, through both anti-proliferative effects (see '2, 3' in Figure 1a) and through the activation of the immune surveillance of senescent cells (see '4' in Figure 1a)<sup>14,15</sup>. Although the data from the current studies suggest that mTOR inhibition does not reverse the proliferative arrest, further investigation would be required to determine the stability of the arrest. Indeed, it was previously suggested that mTOR inhibition diverts senescence to quiescence<sup>5</sup>. mTOR activation triggers several negative feedback signals towards the PI3K pathway, which can be activated by mTOR inhibition<sup>4</sup>. For cancer therapy, this is one rationale for the use of mTOR/PI3K dual inhibitors, and the same logic would also apply to mTOR inhibition to block the SASP, and yet to reinforce senescence arrest. In the mouse liver OIS model, where one can evaluate the induction and immune-mediated elimination of OIS hepatocytes<sup>15</sup>, Herranz et al. showed that rapamycin treatment in mice leads to an accumulation of oncogene-expressing hepatocytes with reduced immune cell infiltration, but the number of cells positive for senescence markers was reduced<sup>3</sup>. The data are consistent with the proposed function of mTOR as a positive regulator of the SASP, but the long-term OIS arrest after rapamycin treatment in this model remains to be validated. Careful consideration of mTOR inhibition of the SASP either by rapamycin analogs, ATP competitors, or even mTOR/PI3K dual inhibitors might extend its applications in cancer therapy.



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## Figure legend

**Figure 1** mTOR regulation of the SASP. **(a)** Schematic view of the diverse downstream effects of the SASP. The SASP can be pro-tumorigenic (1) or tumour suppressive either through reinforcing senescence arrest (2, 3) or facilitating elimination of senescent cells by immune cells (4) (see text for details). The SASP can also affect the tumour microenvironment through modulating the extracellular matrix (ECM) integrity. **(b)** Integral view of the two proposed models: ‘IL-1 $\alpha$  model’ (left) and ‘MK2 model’ (right).