*Subject line: senescence*

# $NAD^+$: A metabolic knob fine-tuning inflammation during senescence

Pia P. Sogaard[1,2] and Jesús Gil[1,2,*]

[1]MRC London Institute of Medical Sciences (LMS), Du Cane Road, London, W12 0NN, UK.
[2]Institute of Clinical Sciences (ICS), Faculty of Medicine, Imperial College London, Du Cane Road, London W12 0NN, UK.

*Corresponding author: jesus.gil@imperial.ac.uk

**The senescence-associated secretory phenotype (SASP) is responsible for the deleterious effects of senescent cells in aging and cancer. A new study shows that $NAD^+$ metabolism can regulate the pro-inflammatory SASP promoting tumorigenesis.**

Senescence is a stress response characterised by permanent growth arrest and the expression of a range of secreted factors collectively known as the SASP [1]. Senescence can be induced by insults such as replicative exhaustion, DNA damaging agents or oncogenes (the latter leading to what is known as oncogene-induced senescence, OIS). Induction of senescence prevents the replication of old, preneoplastic or damaged cells, thus limiting tumor initiation and fibrotic responses. However, associated with the chronic presence of senescent cells, the pro-inflammatory SASP fuels age-related diseases and can paradoxically contribute to cancer progression. This observation suggests that inhibiting the SASP without disturbing senescent growth arrest may be of benefit for cancer treatment[2]. Consequently, understanding how the SASP is regulated is of utmost importance. In a new study in Nature Cell Biology, Nacarelli et al. [3] show that the strength of the pro-inflammatory SASP depends on cellular $NAD^+$ levels, thus identifying a direct link between cellular metabolism and inflammation.

Senescent cells undergo chromatin remodelling and gene expression changes to irreversible arrest cell proliferation. The chromatin-binding protein High-Mobility Group A 1(HMGA1), usually associated with gene activation, is upregulated in transformed and senescent cells and its overexpression is sufficient to induce senescence [4]. Given the important role of HMGA1 in senescence, Nacarelli et al. set out to investigate the underlying mechanism. By cross-referencing expression and chromatin occupancy data, the authors identified NAMPT as an HMGA1 target upregulated in oncogene-induced senescence (OIS). NAMPT is the rate-limiting enzyme responsible for $NAD^+$ production via the so-called salvage pathway[5]. NAD+ is a well known regulator of cellular metabolism, being an essential coenzyme in redox reactions and a substrate for NAD-dependent enzymes. As expected, knockdown of NAMPT or HMGA1 caused a decrease in the $NAD^+$/NADH ratio in OIS [3]. As NAMPT is

known to be upregulated in colorectal, breast and ovarian cancer, where it is associated with increased inflammation and chemotherapy resistance[5], the impact of this signalling axis on inflammation was also tested. In line with this, inhibition of NAMPT activity through knockdown of HMGA1/NAMPT or using FK866, a specific NAMPT inhibitor, suppresses inflammation. Conversely, the ectopic expression of NAMPT or supplementation with the $NAD^+$ precursor, nicotinamide mononucleotide (NMN), boosted the pro-inflammatory SASP [3]. These findings highlight the central role of $NAD^+$ metabolism downstream of HMGA1/NAMPT in regulating the pro-inflammatory SASP.

To study the role of $NAD^+$ mediated SASP in tumourigenesis the authors assessed its function in pancreatic ductal adenocarcinoma (PDAC), an inflammation-driven tumor. PDAC originates from preneoplastic lesions termed pancreatic intraepithelial neoplasias (PanINs). These lesions are enriched in senescent cells that, through the SASP, contribute to the pro-inflammatory environment supporting progression into PDAC [6]. Nacarelli et al. took advantage of a mouse model in which expression of oncogenic $KRas^{G12D}$ is restricted to the pancreas. These mice develop PanINs which progress into PDAC under stress. Remarkably, the authors demonstrate that interventions that altered the $NAD^+$/NADH ratio, affected the SASP and disease progression: Treatment of mice with NMN increased precancerous and cancerous lesions, and upregulated immune cell infiltration as well as the expression of pro-inflammatory cytokines [3]. By contrast, pharmacological NAMPT inhibition with FK866 suppressed the induction of pro-inflammatory cytokine and senescent markers in the pancreata without reducing immune infiltration or acinar area. These findings confirm that the pro-inflammatory SASP contributes to pancreatic cancer progression and this is aggravated by increasing $NAD^+$ levels with NMN treatment (Figure 1).

After establishing its physiological relevance, the authors aimed to unravel the molecular mechanism by which NAMPT induces the SASP and inflammation. In agreement with the known association of OIS with a high metabolic activity [7], glycolysis, mitochondrial respiration, and oxygen consumption decreased upon NAMPT inhibition [3]. As a consequence of decreased glycolysis, ADP/ATP ratio increases, resulting in AMPK activation. Interestingly, the authors find this

AMPK activation to increase p53-mediated suppression of p38MAPK, thereby resulting in lower NF-κB-mediated inflammatory signalling [3] (Figure 1). In this way, they identify a direct link between increased metabolism and SASP expression in OIS. Since CEBPβ, another known regulator of the SASP, is unaffected it will be interesting to understand whether $NAD^+$ metabolism controls a specific and distinct subset of SASP factors.

The findings of Nacarelli et al have relevant clinical implications. One is related to the role of NAMPT in cancer progression. NAMPT inhibitors (including FK866) have been shown to reduce growth of solid tumours and leukemias, however they have disappointed as anti-cancer agents in clinical trials [5,8]. In light of these new findings, it remains to be seen if combining NAMPT inhibition with chemotherapy may offer an advantage due to the selective toxicity of FK866 for cancer cells and decreased inflammation. In addition, this study has implications related the role of $NAD^+$ in ageing. The upregulation of $NAD^+$ and NAMPT levels observed in OIS [3] contrasts with the decrease during aging [8], also mirrored in replicative senescence. Supplementation with $NAD^+$ precursors such as NMN and nicotinamide riboside (NR) has been suggested as a strategy to prevent ageing and age-associated disorders, a hypothesis being tested in clinical trials [8]. Importantly, Nacarelli et al show that supplementation with NMN increased the pro-inflammatory SASP and the capacity of senescent cells to support tumorigenesis [3]. This raises the possibility that dietary intake of $NAD^+$ supplementation may have the unintended consequence of exacerbating chronic inflammation and promote progression of precursor lesions such as PanINs into malignant disease. Given the promise that $NAD^+$ supplementation holds, with evidence suggesting improvements on liver and kidney function, age-related muscle atrophy and cardiovascular function in mice [8], it will be critical to understand how to avoid the potential pro-tumorigenic effects suggested by the study of Nacarelli et al. [3].

## COMPETING INTERESTS

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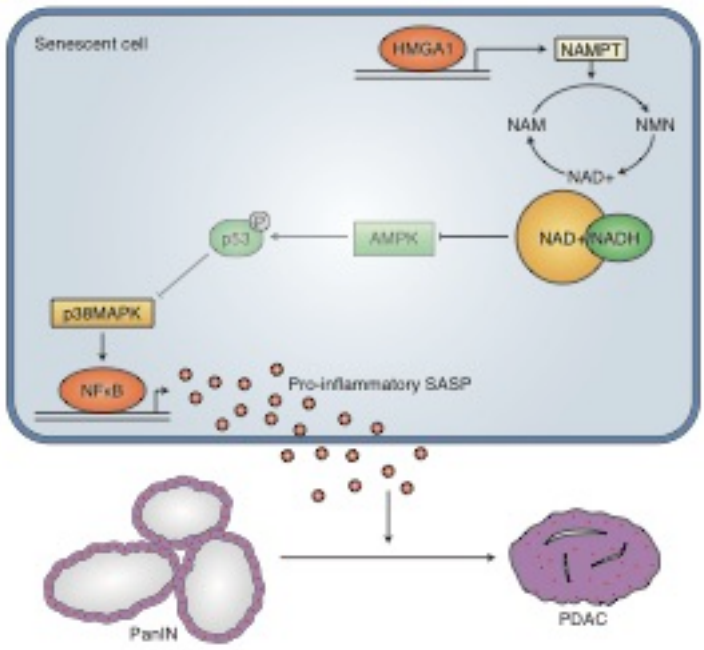



**Figure 1. NAD+ metabolism regulates the pro-inflammatory SASP and can promote tumor progression.** NAD+ metabolism regulates the strength of the pro-inflammatory SASP through modulation of a pathway engaging AMPK, p53, p38MAPK and NF-κB. In cells undergoing OIS, this pathway is hyperactivated due to HMGA1-mediated upregulation of NAMPT. Although this activation is not observed in replicative senescence, dietary NAD+ supplementation (e.g. NMN) also results in SASP induction. Importantly, a proinflammatory SASP facilitates tumorigenesis (e.g. increasing progression from PanIN to PDAC in pancreatic cancer).