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The persistent dynamic secrets of senescence

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While the beneficial versus detrimental implications of the senescence-associated secretome remain an issue of debate, time-resolved analyses of its composition, regulatory mechanisms, and functional consequences were largely missing. The dynamic activity of NOTCH is now shown to direct two distinct senescence phenotypes, by first promoting a pro-senescent TGF-β1-dependent secretome, followed by a second wave of pro-inflammatory, senescence-clearing cytokines.

The molecular investigation of cellular senescence has unveiled an increasingly complex cellular condition the central features of which include the permanent cessation of cell division and a massive secretory phenotype composed of a plethora of factors, thereby underscoring its cell-autonomous and non-cell-autonomous implications[1]. Proliferating cells enter senescence in response to physiological cues during embryonic patterning and organogenesis, to pathophysiological signals related to aging or imminent malignant transformation, or to exogenous causes of damage like injury or cytotoxic therapies[2]. Senescence has important clinical ramifications, especially for age-related disorders such as arteriosclerosis, diabetes, hepatic and pulmonary fibrosis, and

operates as a critical barrier to cancer development as well as an effector principle of anticancer therapy[2].

Oncogene-induced senescence (OIS) prevents full-blown cancer formation by terminating the proliferative expansion of aberrant mitogen-driven, pre-malignant cells, and, subsequently, by promoting their clearance through immune cells which are attracted by components of the senescence-associated secretory phenotype (SASP)[3]. However, the simplistic view of a solely tumor-suppressive process is challenged when put into the context of a heterologous tissue composition, in which secreted factors may impinge on bystander cells in the tumour environment, or SASP-primed host immune cells may cross-talk with non-malignant cellular components at the tumour site, possibly resulting in a pro-tumorigenic rather than a tumor-controlling net effect[1]. Although senescence, when compared to apoptosis, has always been considered a slow-onset response to the initiating insult, the order of events eventually leading to full-featured senescence, and the kinetics of distinct steps of the senescence process have remained largely unaddressed in the field so far.

Employing plasma membrane proteomics (PMP), time-course analyses and senescence-state-specific genetic targeting, Narita and colleagues report in this issue of *Nature Cell Biology* a dynamic pattern of the OIS-related secretome composed of two biochemically distinct and functionally opposing subsets of factors[4]. Using induction of an oncogenic Ras-G12V allele in human diploid fibroblasts as a model system of OIS, PMP identified the NOTCH1 receptor, previously implicated in replicative senescence[5], as being upregulated during Ras-induced senescence, which was confirmed by flow cytometry in both OIS and DNA damage-induced senescent cells. Starting at around day 2 after induction of oncogenic Ras, the time when cells begin to exhibit first signs of senescence, the authors observed continuously increased surface expression of NOTCH1, reflecting the parallel development of full senescence features in the cells, based on a variety of senescence markers. Interestingly, although pro-inflammatory SASP components such as IL-1α, IL-1β, IL-6 and IL-8, as well as matrix-degrading proteases (such as MMP1, MMP3 and MMP10), were also

upregulated at full senescence, TGF-β1 was transiently induced at around day 2 to 4, coinciding with the time when the cleaved, active NOTCH1 intracellular domain (N1ICD) and NOTCH1 target genes such as HES1 became detectable (Fig. 1a). Introduction of a dominant-negative form of the N1ICD co-activator MAML1 (dnMAML1) to block N1ICD action abrogated TGF-β1 induction and led to an even stronger increase of pro-inflammatory SASP factors, thereby suggesting that NOTCH1 might operate as a repressor of the pro-inflammatory SASP. Indeed, the authors demonstrated that N1ICD was able to repress pro-inflammatory cytokines by downregulating the transcriptional activity of their master regulator C/EBPβ. Conversely, enforced expression of N1ICD at day 6, *i.e.* during the later, second phase of senescence induction, when the NOTCH1 receptor remains strongly expressed but endogenous levels of N1ICD were no longer detectable, resulted in enhanced TGF-β1 but lowered pro-inflammatory cytokine expression, thus mimicking the first wave of the senescence-associated secretome. Of note, whereas the actual compositions of a N1ICD/TGF-β1-governed versus a pro-inflammatory secretome seemed to change in a dynamic and reciprocal fashion over time, the senescent arrest status of the cells appeared unaffected by the oscillating auto- and paracrine activities of these factors. Constitutive N1ICD expression, with or without concomitant activation of oncogenic Ras, triggered cellular senescence with a distinct, persistently TGF-β1-governed secretome that largely lacked the SASP-typical pro-inflammatory cytokines. Importantly, the secretome of N1ICD-senescent fibroblasts, but not the "second-wave" secretome of Ras- or DNA damage-induced senescent cells, exerted a lastingly senescence-like growth arrest in normal, proliferating "target" cells (Fig. 1b).

Co-culture experiments of N1ICD-senescenct with normal fibroblasts demonstrated that both TGF-β and NOTCH signaling became detectable in bystander fibroblasts as well. Because NOTCH is activated through membrane-bound ligands, N1ICD-driven upregulation of the NOTCH target and ligand JAG1 may account for the cell-cell contact-dependent "lateral induction" of NOTCH activity, which further adds to the secretome-mediated effects in adjacent target

cells. The underlying principles of these findings were recapitulated *in vivo*, especially in Ras-G12V-driven hepatocyte senescence in the mouse, where Ras-positive cells and, through "lateral induction", surrounding Ras-negative cells, presented as NOTCH1/HES1-positive. Senescent Ras-dnMAML1 hepatocytes, destined for premature inhibition of the N1ICD/TGF-β1-governed first-wave secretory program, and, presumably, a switch to a more pro-inflammatory type of SASP, disappeared faster, which correlated with an accelerated attraction of $CD3^+$ T-cells, the infiltration of which into the site was possibly facilitated by second-wave SASP-enhanced endothelial lymphocyte adhesion. Rather unexpectedly, Ras-senescent hepatocytes that co-expressed N1ICD did not persist for extended periods of time, but quantitatively disappeared due to an increased rate of apoptosis. Surprisingly, it was this genotype that ultimately turned out to be prone to liver cancer development.

This work has important ramifications for our understanding of OIS as a tumor-prohibitive safeguard program reminiscent of the natural *ad integrum* response to tissue injury, where the cell-matrix defect requires acute stabilization by pro-fibrotic secretion from regeneration-supportive senescent cells in an early phase. However, these senescent cells must be immunologically cleared in a subsequent phase before excessive scars would form, thus contributing to tissue homeostasis[2,6]. The model presented by Narita and colleagues consists of a pro-senescent, pro-fibrotic NOTCH1/TGF-β1-governed first wave followed by a C/EBPβ-driven pro-inflammatory, matrix-degrading and senescence-clearing second wave, and resembles a (patho-)physiological two-phase tissue repair program that could be enlisted to counter oncogenic activation as a distinct type of tissue insult. It is interesting to speculate whether the induction of NOTCH1 in senescence may equip the cells with a latent stemness capacity that might eventually help to replenish a challenged tissue when the damaging stress no longer applies, possibly contributing to cancer formation if NOTCH signaling cannot be terminated (as known from activating NOTCH1 mutations in T-cell acute lymphoblastic leukemia[7]). However, such hypotheses await future experimental investigation.

The findings of Narita and colleagues give rise to many interesting questions. It is currently unclear, whether this reciprocal, two-secretome model is generalizable in terms of different senescence-inducing triggers and alternative tissue contexts. The regulator residing at the tip of the hierarchical signaling network also awaits conclusive identification, and it would be interesting to probe the roles of IL-1α, the inflammasome, or NOTCH1 (Ref. [8]). One also wonders where to position NF-κB, which shares an overlapping SASP-typical set of target genes with C/EBPβ□□□□□ [9]), but unlike C/EBPβ, is positively regulated by NOTCH signaling, or, may actually drive NOTCH signaling through induction of JAG1 (refs[10,11]). In addition, the signal in response to which NOTCH1/N1ICD gets turned off again, and whether there is a feedback loop with transducers from the second-wave network remain unclear.

It would also be important to address whether stressed cells would follow the time-dependent two-wave model in a synchronous manner, in non-engineered, real tissue damage scenarios or pre-neoplastic lesions *in vivo*. What net impact should one expect from two reciprocal secretomes if they co-occur in different phases (and strengths) within the same local environment, ultimately neutralizing opposing signals, for instance, towards host immune cells? And, how different are homotypic responses of adjacent (pre-)cancer cells as compared to heterologous interactions with various stroma cell types evoked by secreted factors or lateral induction? Only deep-phenotyping single-cell analyses such as multi-color flow cytometry, multiplexed *in situ*-imaging or "Drop-seq" transcriptomics will eventually be able to scan the uniformity/heterogeneity of the secretome state over many individual cells of distinct origins within the (pre-)tumor site, although tracking a multitude of individual cells over time remains a technical challenge *in vivo*[12].

These open questions notwithstanding, the study by Narita and colleagues has succeeded in uncovering the time-dependent two-wave composition of the senescence-associated secretome, furthering our current understanding of the SASP beyond what has been generally thought to be a rather static program. When considering the functionally highly discriminative secretomes

complementing the fluctuating numbers of senescent cells at a given time, and at a given site due to paracrine or lateral senescence induction on one hand, and immune cell-mediated clearance on the other, the previously thought terminal and persistent senescence condition is increasingly revealed to be dynamic and changeable – a "moving target". Given that the elimination of senescent cells is a therapeutic objective[13,14], especially in cancer, the findings presented here open the possibility of developing treatment strategies to stabilize the beneficial side of the two waves, the TGF-β-governed senescence-reinforcing phase[15], or to further exploit the repressive impact of N1ICD signaling on C/EBPβ activity in settings where senescent cells seem to be accountable for lasting and possibly tumor-promoting local inflammation.

COMPETING FINANCIAL INTERESTS

The author declares no competing financial interests.

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Legends

Figure 1 A NOTCH1-C/EBPβ-controlled two-wave senescence secretome phenotype. (**a**) Ras-senescent cells present with two distinct secretomes: an early-phase ("1st wave") NOTCH1/TGF-β-governed signaling network that reinforces senescence in an auto-/paracrine fashion and in neighboring cells ("lateral induction") through JAG1 ligand/NOTCH1 receptor cell-cell interactions. A C/EBPβ-driven, largely pro-inflammatory "2nd wave" secretome is suppressed as long as NOTCH1 signaling is actively mediated through the N1ICD moiety. (**b**) Early-phase Ras-senescent cells, with an activated NOTCH1/TGF-β signaling program, may face distinct fates: if NOTCH1/N1ICD activity persists, lateral induction results in senescence spreading, enhanced apoptosis, and occasional tumor development, as these cells do not promote immune clearance due to their rather immunosuppressive secretome. In contrast, late-phase senescent cells that switched to the C/EBPβ-driven pro-inflammatory secretome bear the risk of

paracrine bystander growth promotion until they get cleared by host immune cells. A pink outline around senescent cells reflects a Ras-active status.

Figure 1