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SPOTLIGHT

Putting the DOT on IL1A

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IL-1α is an upstream component of the senescence-associated secretory phenotype. In this issue, Leon et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202008101) show that DOT1L-mediated H3K79 methylation at the *IL1A* gene plays a key role in its induction during oncogene-induced senescence.

Senescence is a heterogeneous phenotype, often characterized by the ability to secrete diverse functional molecules (senescence-associated secretory phenotype [SASP]). The quantity and quality of those SASP components are key determinants of the short-term and long-term impacts of senescent cells in vivo (1). The inflammatory SASP is cooperatively mediated by two transcription factors (TFs), nuclear factor-κB (NF-κB) and CCAAT/enhancer-binding protein β (C/EBPβ), which target inflammatory cytokines (including IL-1, IL-6, and IL-8) that can, in turn, activate those TFs.

Thus, the overall signal is amplified both cell autonomously and noncell autonomously (1), and depletion of some individual factors can lead to the collapse of the entire inflammatory SASP (2). In particular, IL-1α is proposed to be an upstream cytokine in this feed-forward SASP network (3).

Located in the *IL1* cluster, the *IL1A* gene is a product of *IL1B* gene duplication, and consequently IL-1α and IL-1β share a receptor, IL-1R1, and its downstream signaling (4). Yet, they have distinct regulatory mechanisms for their activities at multiple levels. While both are produced as pro-forms, IL-1β, but not IL-1α, is a well-established substrate of the inflammasome/Caspase-1 complexes (5). Unlike IL-1β, IL-1α has additional functions, represented by the presence of a nuclear localization signal within its pro-domain (4).

In the senescence context, IL-1α–specific regulatory mechanisms have also been proposed: Mammalian target of rapamycin (mTOR) preferentially facilitates IL-1α translation and GATA4 can, probably indirectly, promote IL-1α induction upstream of NF-κB (1). In contrast to IL-1β, IL-1α is a substrate of noncanonical inflammatory caspases (5). Functionally, expression of downstream SASP components, IL-6 and IL-8, depends on IL-1α, but not IL-1β (3). Compared with the differential regulation through the mostly post-transcriptional steps listed above, studies directly comparing *IL1* transcriptional regulation appear limited. This is perhaps, in part, because transcriptional regulation of *IL1A* is less explored compared with that of *IL1B* and other SASP genes. Transcription at the *IL1* locus is mainly driven by NF-κB and C/EBPβ, and both *IL1A* and *IL1B* have been linked to "super-enhancers" or de novo enhancers in the senescence context (6). However, evidence suggesting differential regulatory mechanisms for these *IL1* genes is now emerging. Olan et al. (7) recently showed that, while *IL1A* and *IL1B* share enhancers upon acute induction by TNFα in fibroblasts, the enhancer utilization at the *IL1* locus becomes more specialized through 3D chromatin reorganization during oncogene-induced senescence (OIS), distinguishing the transcriptional activation of *IL1A* and *IL1B*.

In this issue, Leon et al. (8) introduce a new mechanism for how *IL1A* expression is distinctly regulated. In their epiproteomics survey of OIS culture models, they focus on an increase in histone H3K79me2/3, among others. H3K79 methylation is associated with gene activation, and DOT1L is the only known H3K79 methyltransferase (9). DOT1L and H3K79me2/3 are involved in diverse biological processes, including development, reprograming, and cell cycle (9), but their role in senescence was unknown.

Besides a global increase in H3K79me2/3 level during OIS, the authors also showed a focal increase in DOT1L binding and DOT1L-dependent H3K79me2/3 deposition at the *IL1A* locus. Curiously, in contrast to *IL1A*, H3K79me2/3 levels were even reduced at other major SASP gene loci (*IL1B*, *IL6*, and *CXCL8*) in OIS cells (Fig. 1). This preferential H3K79me2/3 deposition at the *IL1A* locus also occurs when DOT1L is ectopically expressed, supporting the specific association between DOT1L and *IL1A*, at least among the SASP genes tested. In addition, the authors showed a close correlation between *IL1A* expression and H3K79me2/3 accumulation at this locus. Notably, *DOT1L* knockdown resulted in the down-regulation of *IL1A*, as well as other SASP genes. This is consistent with the idea that IL-1α is an upstream regulator of the SASP network (3). Indeed, ectopic expression of IL-1α in DOT1L-depleted OIS cells restored expression of *IL1B* and other SASP genes. DOT1L also appears necessary for *CEBPB* (encoding C/EBPβ) up-regulation during OIS. While ectopic DOT1L can induce *CEBPB* and the SASP genes without the obvious senescence phenotype, only *IL1A* is associated with H3K79me2/3 alteration. The authors also demonstrated that *DOT1L* itself is up-regulated during OIS and acts downstream of stimulator of interferon genes

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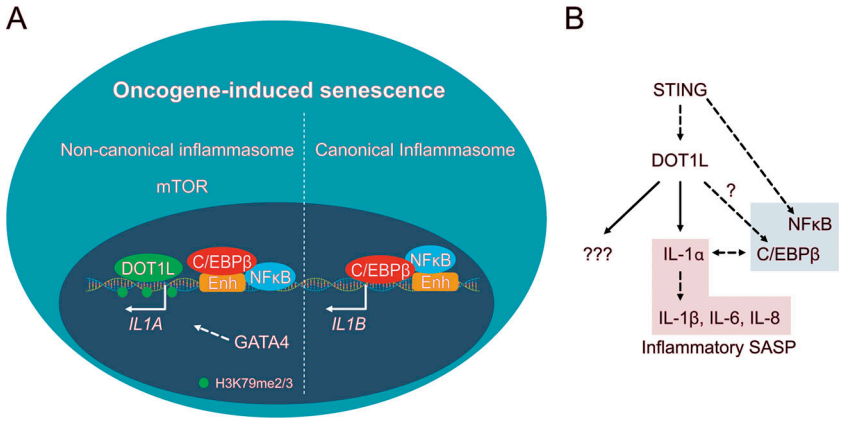

Figure 1. **IL1 and inflammatory SASP regulation during oncogene-induced senescence. (A)** IL-1α and IL-1β are differentially regulated at several stages during OIS: transcriptomically, both genes are regulated by the TFs C/EBPβ and NF-κB but, unlike in the context of acute induction, they use distinct enhancers (Enh) during OIS. Now DOT1L/H3K79me2/3 is proposed to be a direct mediator of *IL1A*, but not *IL1B* or other SASP components. Note, GATA4 is a transcription factor, but it appears indirectly involved in the IL-1α induction during senescence; post-transcriptionally, *IL1A* mRNA is preferentially translated via mTOR, and IL-1α is a substrate of the noncanonical inflammasome, whereas IL-1β is processed by the canonical inflammasome. **(B)** SASP regulation during OIS. DOT1L plays a key role in coordinating the entire inflammatory SASP through *IL1A* induction. It also can induce C/EBPβ, but it is unknown whether this is through IL-1α and/or other targets. Note, *DOT1L* itself is transcriptionally upregulated during OIS downstream of STING, where the cyclic GMP-AMP synthase–STING–NF-κB pathway is the cytoplasmic DNA sensing effector implicated in triggering the inflammatory SASP.

(STING). Collectively, *IL1A* is potentially the primary target of DOT1L, feeding forward to the downstream SASP components (Fig. 1).

Leon and colleagues (8) provide new mechanistic insights into the transcriptional/epigenetic regulation of the *IL1* genes, but the study also opens up outstanding questions. For example, how DOT1L finds its targets remains unclear. As the authors discussed, the epigenetic environment affects DOT1L/Dot1 activities, exemplified by H2BK120Ub and H4K16ac, which allosterically activate DOT1L/Dot1 (10). Further understanding of such DOT1L-associated epigenetic crosstalk should clarify and consolidate the hierarchical view of the SASP network. It is also important to know how general these findings are. Are they specific to senescence or even only to OIS? As mentioned earlier, the high-order chromatin environment of the *IL1* locus is highly different between acute and chronic inflammatory conditions in fibroblasts, the latter represented by OIS (7). Is DOT1L involved in acute inflammation? If not, does DOT1L contribute to the chromatin rewiring of the *IL1* locus during OIS or other chronic inflammation conditions? This possibility is exciting, particularly considering that, while DOT1L activity is modulated by preexisting epigenetic marks, DOT1L can also modulate the local and global epigenetic landscape through inhibition of local histone deacetylation and heterochromatinization (11), as well as potentially through global heterochromatin redistribution (12). Finally, although the authors showed a positive correlation between DOT1L and *IL1A* in 7,12-dimethylbenz[a]anthracene/12-O-tetradecanoylphorbol-13-acetate–induced murine papillomas, which contain OIS preneoplastic lesions, further validation is required to determine the physiological relevance of this study. Interestingly, unlike directly targeting SASP components (2), the senescence arrest is preserved after DOT1L depletion, hinting at a "senomorphic" potential of targeting DOT1L.

Leon et al. (8) indeed show that the treatment with pinometostat (EPZ-5676), the first selective DOT1L inhibitor used in clinical trials in leukemia (9), leads to a decrease in H3K79me2/3 and reduction of the SASP. The mechanism of how DOT1L inhibition blocks the SASP without senescence reversal is unknown, but it is conceivable that other DOT1L/H3K79me2/3-controlled genes may, directly or indirectly, contribute to the maintenance of the arrest. It has been shown that a reduced level of DOT1L leads to growth arrest and cell death (13). Indeed, it was also reported that DOT1L depletion promotes senescence induction in endothelial cells (14). Comprehensive genome-wide analysis of DOT1L/H3K79me2/3 and their crosstalk with other chromatin features might reveal a new epigenetic landscape in senescence, potentially relevant for considering new therapeutic targets.

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