PERSPECTIVES

CANCER

Clearing stressed cells

Cell cycle arrest produces a p21-dependent secretome that initiates immunosurveillance of premalignant cells

By **Virinder Reen**[1,2] and **Jesús Gil**[1,2]

Complex organisms repair stress-induced damage to limit the replication of faulty cells that could drive cancer. When repair is not possible, tissue homeostasis is maintained by the activation of stress response programs such as apoptosis, which eliminates the cells, or senescence, which arrests them (*1*). Cellular senescence causes the arrest of damaged cells through the induction of cyclin-dependent kinase inhibitors (CDKIs) such as p16 and p21 (*2*). Senescent cells also produce a bioactive secretome (the senescence-associated secretory phenotype, SASP) that places cells under immunosurveillance, which is key to avoiding the detrimental inflammatory effects caused by lingering senescent cells on surrounding tissues. On page 577 of this issue, Sturmlechner *et al.* (*3*) report that induction of p21 not only contributes to the arrest of senescent cells, but is also an early signal that primes stressed cells for immunosurveillance.

Senescence is a complex program that is tightly regulated at the epigenetic and transcriptional levels. For example, exit from the cell cycle is controlled by the induction of p16 and p21, which inhibit phosphorylation of the retinoblastoma protein (RB), a transcriptional regulator and tumor suppressor. Hypophosphorylated RB represses transcription of E2F target genes, which are necessary for cell cycle progression. Conversely, production of the SASP is regulated by a complex program that involves super-enhancer (SE) remodeling and activation of transcriptional regulators such as nuclear factor κB (NF-κB) or CCAAT enhancer binding protein–β (C/EBPβ) (*4*).

SEs are large enhancers enriched in specific chromatin modifications and genes regulated by SEs often modulate cell fate decisions. Sturmlechner *et al.* searched for senescence-associated SEs (SASEs) that were conserved across species and cell types, and were activated by various senescence inducers. One of 11 conserved SASEs was in proximity to *CDKN1A*, which encodes p21. Suppressing p21 expression in mouse and human cells in vitro as well as mouse hepatocytes in vivo allowed senescent cells to reenter the cell cycle while decreasing the expression of multiple SASP components. Indeed, p21-expressing cells can swiftly secrete proinflammatory factors that partially overlap with the SASP. The authors called this the p21-activated secretory phenotype (PASP).

Sturmlechner *et al.* found that activation of p21 following stress rapidly halted cell cycle progression and triggered an internal biological timer (of ~4 days in hepatocytes), allowing time to repair and resolve damage (see the figure). In parallel, C-X-C motif chemokine 14 (CXCL14), a component of the PASP, attracted macrophages to surround and closely surveil these damaged cells. Stressed cells that recovered and normalized p21 expression suspended PASP production and circumvented immunosurveillance. However, if the p21-induced stress was unmanageable, the repair timer expired, and the immune cells transitioned from surveillance to clearance mode. Adjacent macrophages mounted a cytotoxic T lymphocyte response that destroyed damaged cells. Notably, the overexpression of p21 alone was sufficient to orchestrate immune killing of stressed cells, without the need of a senescence phenotype. Overexpression of other CDKIs, such as p16 and p27, did not trigger immunosurveillance, likely because they do not induce CXCL14 expression.

In the context of cancer, senescent cell clearance was first observed following reactivation of the tumor suppressor p53 in liver cancer cells. Restoring p53 signaling induced senescence and triggered the elimination of senescent cells by the innate immune system, prompting tumor regression (*5*). Subsequent work has revealed that the SASP alerts the immune system to target preneoplastic senescent cells. Hepatocytes expressing the oncogenic mutant $NRAS^{G12V}$ (Gly^{12}→Val) become senescent and secrete chemokines and cytokines that trigger $CD4^+$ T cell–mediated clearance (*6*). Despite the relevance for tumor suppression, relatively little is known about how immunosurveillance of oncogene-induced senescent cells is initiated and controlled.

The mutant $KRAS^{G12V}$ oncoprotein induces mitogenic stress that increases p21 expression, initiates senescence, and triggers immunosurveillance. Sturmlechner *et al.* show that $KRAS^{G12V}$ expression in mouse hepatocytes can attract macrophages and subsequently cytotoxic T cell clearance in a p21-dependent manner. Upon p21 ablation in hepatocytes,

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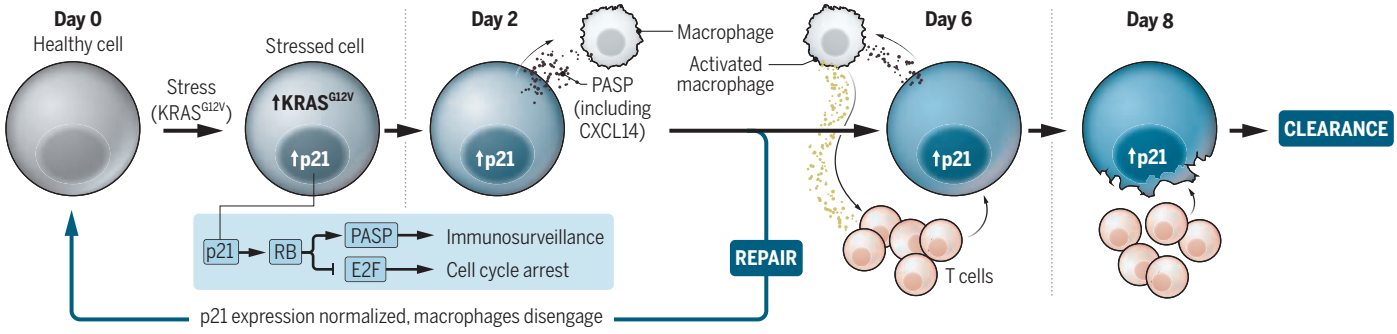
Policing stressed cells

Cell stress (such as that elicited by oncogenic $KRAS^{G12V}$) induces p21 activation, which can orchestrate cell cycle arrest and immunosurveillance through retinoblastoma (RB) hypophosphorylation. p21 induction triggers the p21-associated secretory phenotype (PASP), including secretion of C-X-C motif chemokine 14 (CXCL14), which attracts macrophages. p21 initiates a biological timer that gives stressed cells time to repair damage and normalize p21. If repair is not possible, the timer expires: Macrophages are activated and recruit T cells to clear stressed cells.



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these stressed cells fail to promote immunosurveillance, and small clusters of proliferating $KRAS^{G12V}$-expressing premalignant cells become apparent in vivo.

In addition to controlling the cell cycle, p21 is a regulator of vital cellular processes including apoptosis, DNA repair, and cell motility (*7*). Sturmlechner *et al.* now find that p21 is responsible for placing stressed cells under immune control, which prevents the earliest stage of neoplastic transformation. Strategies that can enhance immune infiltration within the tumor microenvironment are of considerable interest. Therefore, PASP or CXCL14 induction could be explored as a potential therapeutic approach to augment anticancer immune responses. In this context, the repercussions of prolonged PASP-mediated immune stimulation on T cell exhaustion would be worth investigating. Conversely, studies have reported chronic p21 expression in the liver to be a marker of poor prognosis for liver cancer (*8*), raising questions about how the role of p21 and PASP changes once tumors have been established.

The induction of senescence in mutant KRAS-driven tumors in the lung or the pancreas elicit different immune responses (*9, 10*). Whether the findings of Sturmlechner *et al.* extend beyond the liver will need to be examined. In addition, the SASP induces senescence in neighboring cells in a paracrine manner (*11*). Whether in the right context the PASP can also cause paracrine growth arrest of adjacent cells is currently unclear. Another conundrum is the expression (or lack thereof) of p53, which often occurs in tumors. Previous studies have reported that continuous p21 expression in p53-null cells can paradoxically be oncogenic, resulting in DNA replication deregulation and genomic instability (*12*). Whether p21-dependent immune clearance can be reactivated in p53-null tumors requires investigation. Such future studies should allow for therapeutic exploitation of stress-related immunosurveillance for a variety of senescence-associated diseases, such as cancer, fibrosis, and beyond. ■

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CORONAVIRUS

Defective viral RNA sensing linked to severe COVID-19

Genetic variation in a sensor of double-stranded RNA can exacerbate COVID-19

By **John Schoggins**

Why do some people with COVID-19 get sicker than others? Maybe exposure to a particularly high dose of the causative virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), accounts for the difference. Perhaps deficiencies in diet, exercise, or sleep contribute to worse illness. Although many factors govern how sick people become, a key driver of the severity of COVID-19 appears to be genetic, which is common for other human viruses and infectious agents (*1*). On page 579 of this issue, Wickenhagen *et al.* (*2*) show that susceptibility to severe COVID-19 is associated with a single-nucleotide polymorphism (SNP) in the human gene 2′-5′-oligoadenylate synthetase 1 (*OAS1*).

The authors reasoned that SARS-CoV-2 should be inhibited by interferon-mediated antiviral responses, which are among the first cellular defense mechanisms produced in response to a viral infection. Interferons are a group of cytokines that induce the transcription of a large cadre of genes, many of which encode proteins with the potential to directly inhibit the invading virus. Wickenhagen *et al.* interrogated many hundreds of these putative antiviral proteins for their ability to suppress SARS-CoV-2 in cultured cells and found that OAS1 was particularly potent against SARS-CoV-2.

OAS1 is an enzyme that is activated in the presence of double-stranded RNA, which is scattered along an otherwise single-stranded SARS-CoV-2 genome because of an assortment of RNA hairpins and other secondary structures. Once activated, OAS1 catalyzes the polymerization of adenosine triphosphate (ATP) into a second messenger, 2′-5′-oligoadenylate. This then triggers the conversion of ribonuclease L (RNaseL) into its active form so that it can cleave viral RNA, effectively blunting viral replication (*3*). Wickenhagen *et al.* found that OAS1 is expressed in respiratory tissues of healthy donors and COVID-19 patients and that it interacts with a region of the SARS-CoV-2 genome that contains double-stranded RNA secondary structures (see the figure).

OAS1 exists predominantly as two isoforms in humans—a longer isoform (p46) and a shorter version (p42). Genetic variation dictates which isoform will be expressed. In humans, p46 is expressed in people who have a SNP that causes alternative splicing of the *OAS1* messenger RNA (mRNA). This results in the utilization of a terminal exon that is not used to translate p42. Thus, the carboxyl terminus of the p46 OAS1 protein contains a distinct four–amino acid motif that forms a prenylation site. Prenylation is a posttranslational modification that targets proteins to membranes. In cell culture experiments, Wickenhagen *et al.* showed that only OAS1 p46, but not p42, could inhibit SARS-CoV-2. However, when the prenylation site of p46 was engineered into p42, this chimeric p42 protein was able to inhibit SARS-CoV-2, which strongly implicates a role for OAS1 specifically at membranes.

Why are membranes important? SARS-CoV-2, like all coronaviruses, co-opts cellular membranes at the endoplasmic reticulum to form double-membrane vesicles, in which the virus replicates its genome. Thus, membrane-bound OAS1 p46 may be specifically activated by RNA viruses that form membrane-bound vesicles for replication. Indeed, the unrelated cardiovirus A, which also forms vesicular membranous structures, was inhibited by OAS1. Conversely, other respiratory RNA viruses, such as human parainfluenza virus type 3 and human respiratory syncytial virus, which do not use membrane-tethered vesicles for replication, were not inhibited by p46.

Wickenhagen *et al.* examined a cohort of 499 COVID-19 patients hospitalized in the UK. Whereas all patients expressed OAS1, 42.5% of them did not express the antiviral p46 isoform. These patients were statistically more likely to have severe COVID-19 (be admitted to the intensive care unit). This suggests that OAS1 is an important antiviral factor in the control of SARS-CoV-2 infection and that its inability to activate RNaseL results in prolonged infections and severe disease, although other factors likely contribute.

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