ANTIBIOTIC MECHANISMS

PAS is doubly poisonous

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Several enzymes of the folate biosynthesis pathway are targets of antibacterial drugs, including PAS, a close structural analog of the folate precursor *p*-aminobenzoic acid (PABA), and are effective against *Mycobacterium tuberculosis*. Because of its structure and from genetic studies, it has long been presumed that PAS functions as a competitive inhibitor of DHPS, the second folate biosynthetic pathway enzyme, displacing the DHPS substrate PABA. However, PAS is only a modest inhibitor of *M. tuberculosis* DHPS *in vitro*. Chakraborty *et al*. took a metabolomic approach to gain new insight into the antibiotic mechanism of PAS. Though PABA was among the metabolites that accumulated when cultures were treated with PAS, as expected, they also found that PAS induced specific patterns of changes in metabolite levels that were distinct from those caused by sulfonamide antibiotics that also target the folate biosynthetic pathway. Looking closely at the time courses of antibiotic uptake and PABA accumulation and by performing chromatography on treated samples and subsequent *in vitro* assays of enzyme function, the authors concluded that products of PAS metabolism were capable of inhibiting the folate pathway downstream of DHPS. These results suggest that PAS poisons the folate biosynthetic pathway by acting as a surrogate DHPS substrate and serving as replacement substrates and/or inhibitors of subsequent enzymes. *MB*

PLURIPOTENCY

Oleate dependency

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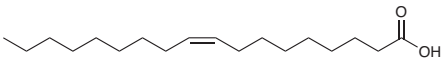

Hopes for regenerative medicine derive in part from pluripotent stem cells; however, these cells also have tumorigenic properties that limit their use in medicine. Mechanisms to eliminate residual pluripotent stem cells for clinical applications are therefore needed to overcome this limitation. Ben-David *et al.* now perform a high-throughput small-molecule screen and a smaller-scale counterscreen to identify 15 compounds that are selectively cytotoxic to pluripotent cells. Interestingly, nine of the compounds shared a common phenylhydrazine moiety, and therefore the authors anticipated the molecules might share a common mechanism of action. Gene expression changes resulting from exposure to one compound were consistent with an endoplasmic reticulum stress phenotype. In prior work, this compound, as well as three of the others, scored positive as inhibitors of stearoyl-CoA-desaturase (SCD1), an endoplasmic reticulum membrane protein. Biochemical assays confirmed inhibition of SCD1 in pluripotent stem cells by these compounds. Genetic or chemical disruption of SCD1 activity in stem cells yielded massive cell death, whereas exogenously added oleic acid—the product of SCD1—rescued cells from death, confirming the importance of this enzyme for cell survival. Collectively, these data indicate that pluripotent cells depend on SCD1 activity and suggest that inhibition of SCD1 to eliminate residual pluripotent cells may reduce the risk of tumor formation from stem cell–derived therapeutics. *AD*

NEUROBIOLOGY

A pain paradox

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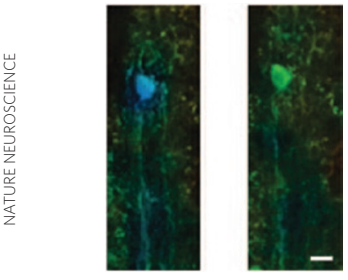

Morphine is a major component of pain-treatment strategies, targeting μ-opioid receptors, but opiate use can lead to tolerance as well as hyperalgesia, a paradoxical sensitization to pain. In their current work, Ferrini *et al.* found maximal morphine-induced hyperalgesia (MIH) occurred within seven days of twice-daily dosing of morphine in rats. To understand the mechanism of MIH and to determine whether outputs from spinal lamina I neurons were at play, the authors tested whether the Cl^- gradient in these neurons, which are the target for morphine's analgesic effect, was affected by morphine. Indeed, repeated or high-dose morphine inhibited Cl^- extrusion (causing Cl^- accumulation) in these neurons. Stabilizing the anion gradient reversed the hyperalgesia. Morphine treatment led to a decrease in expression of the main regulator of Cl^- homeostasis in these neurons, the K^+-Cl^- cotransporter KCC2. Spinal microglial cells, which are thought to disrupt lamina I neuron Cl^- homeostasis, were necessary and sufficient for MIH. Microglial P2X4 receptors were upregulated with repeated morphine treatment, which in turn resulted in release of the growth factor BDNF, and this was sufficient to induce MIH. Notably, mice with a deletion of BDNF did not show MIH. These results suggest that MIH is driven by upregulation of P2X4Rs in microglia, leading to BDNF release and ultimately downregulation of KCC2 and disruption of Cl^- homeostasis in lamina I neurons. *MB*

METABOLISM

AMPing up life

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A link between metabolism and life span is emerging. For example, maintaining the derivatives of adenine metabolism at relatively constant amounts is important for longevity, but the mechanism underlying this connection is not known. Stenesen *et al.* performed a forward genetic screen in *Drosophila*, identifying the gene encoding adenylosuccinate synthetase, an enzyme involved in AMP biosynthesis, as a negative regulator of longevity. Genetic depletion of other AMP biosynthetic enzymes provided further evidence that this pathway affects lifespan. The authors also confirmed that AMP and ADP concentrations were elevated in long-lived mutant flies; the activity of AMP kinase (AMPK), an enzyme that senses cellular energy and is regulated by AMP and ADP, is elevated, and lifespan gains in AMP biosynthetic mutants depend on the expression of AMPK. In addition, the authors demonstrated that dietary supplements of adenine restored wild-type amounts of AMP and ADP and reverted lifespan gains in mutant flies. On the basis of these findings, they hypothesized and confirmed that reduced adenine consumption contributes to the life-extending effects of caloric restriction. Taken together, these data indicate that restriction of a single dietary factor, adenine, could recapitulate longevity gains derived from caloric restriction. *AD*

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