model of osteoporosis induced by ovariectomy (OVX), the authors showed that Dnmt3a has a role in pathological bone loss[7].

To identify genes that are repressed by Dnmt3a-mediated DNA methylation, Nishikawa *et al.*[7] then carried out a genome-wide approach using transcriptomics coupled with methyl-CpG binding domain (MBD) sequencing—a technique analyzing genome-wide DNA methylation patterns—to identify genes whose expression was repressed and that were methylated after RANKL stimulation in cell culture. This approach indicated that *Irf8*, a negative regulator of osteoclastogenesis[2], has increased methylation at CpG sites downstream of the gene body and concomitant altered gene expression (**Fig. 1**).

The epigenetic silencing of a negative regulator to stabilize osteoclast differentiation introduces a new concept into the myeloid differentiation field. Importantly, DNA methylation occurred after *Irf8* expression had already been downregulated, approximately 12 h after RANKL stimulation. Thus, it is likely that Dnmt3a stabilized *Irf8* expression at a low level at an early phase of osteoclast precursor differentiation into osteoclasts, which typically requires 3–4 d of RANKL stimulation in cell culture. Signals that initially downregulate *Irf8* expression after RANKL stimulation remain to be identified but are probably related to Notch-RBP-J signaling[8] which also regulates osteoclastogenesis. Downregulation of *Irf8* expression may also be mediated by chromatin-based mechanisms, as changes in histone methylation in *Irf8* that can alter gene expression and potentially recruit Dnmt3a were observed in this study[7]. This work further supports the notion that IRF8 is a key negative regulator of osteoclast phenotype that needs to be epigenetically silenced for osteoclastogenesis to proceed.

Another striking finding of the study, on the basis of Dnmt3a overexpression in osteoclast precursors, was that Dnmt3a expression alone was not sufficient to suppress *Irf8* or promote osteoclastogenesis. The methyl donor for Dnmt3a is *S*-adenosylmethionine (SAM), and hence the authors analyzed its concentrations[7]. They found that SAM levels were significantly increased after RANKL stimulation, and that increased SAM concentrations augmented Dnmt3a activity and methylation at the *Irf8* locus[7]. SAM is generated from methionine and ATP, and Nishikawa *et al.*[7] attributed the increase in SAM to increased cellular ATP levels. They then linked this to RANKL-induced increased flux through the tricarboxylic acid cycle and oxidative phosphorylation, which is in accord with increased mitochondrial biogenesis previously observed in osteoclastogenesis[7,9]. This change in cell respiration resulted in elevated concentrations of ATP, leading to increased SAM production[7]. A role for cellular metabolism in regulating the production of substrates for epigenetic regulators has been established[4], and the current study supports a RANKL-induced metabolic-epigenetic coupling in which mitochondrial biogenesis and efficient ATP generation are linked to *de novo* DNA methylation by Dnmt3a.

This study also has important translational and therapeutic implications, as treatment of mice with the theaflavin derivative TF-3, which selectively inhibits Dnmt3 methyltransferase activity, abrogated bone loss in the OVX model of osteoporosis[7]. Together with two recent studies showing that inhibition of BET bromodomain-containing epigenetic regulators by the small-molecule compounds JQ-1 and IBET-151 suppresses pathological bone remodeling in OVX, inflammatory arthritis, and tumor models[10,11], these results support a role for epigenetic therapy in bone diseases. Although these inhibitors did not exhibit apparent toxicity, given the broad expression and importance of their targets in fundamental cell processes, a thorough examination of side effects and off-target effects is warranted.

The work by Nishikawa *et al.*[7] establishes Dnmt3a-mediated DNA methylation and silencing of *Irf8* to be an important epigenetic mechanism that promotes osteoclastogenesis. Epigenetic mechanisms such as DNA methylation can thus potentially stabilize components of myeloid lineage differentiation to make them difficult to reverse by environmental cues. Such 'irreversible' phenotypes could be important for committed functions such as bone resorption. This work also shows that epigenetic regulation of osteoclast differentiation is linked to cell metabolism and is susceptible to therapeutic targeting. It will be necessary to identify additional epigenetic mechanisms that regulate osteoclastogenesis, such as tissue-specific remodeling of the enhancer landscape, which has an important role in myeloid cell differentiation[12,13]. Future work in this area to identify key epigenetic regulators and osteoclast-specific enhancers, as well as the signaling pathways, metabolic programs and transcription factors that regulate them, will dramatically enhance a new therapeutic space for drug development to treat pathological bone resorption.

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Taming the inflammasome

Maayan Levy, Christoph A Thaiss & Eran Elinav

The NLRP3 inflammasome is involved in the molecular etiology of multiple autoinflammatory diseases. Two studies identify inhibitors of NLRP3 activation and might pave the way for new treatment options for a variety of diseases.

A decade ago, the NLRP3 inflammasome was identified as a multi-protein complex of the innate immune system, consisting of the NOD-like receptor (NLR) NLRP3, the adaptor protein ASC and caspase-1. The NLRP3 inflammasome functions primarily in myeloid cells. It is activated by a variety of stimuli, including bacterial pore-forming toxins and molecules related to cellular damage, such as HMGB1. Upon oligomerization, caspase-1 becomes active and cleaves the pro-inflammatory cytokines IL-1β and IL-18 into their active secreted forms (**Fig. 1**). The discovery that this complex can also be activated by a variety of inducers of sterile inflammation, such as ATP, solid crystals, and environmental irritants, has provided the missing link between these substances and a range of IL-1β–driven autoinflammatory disorders, among them gout, atherosclerosis, obesity, and

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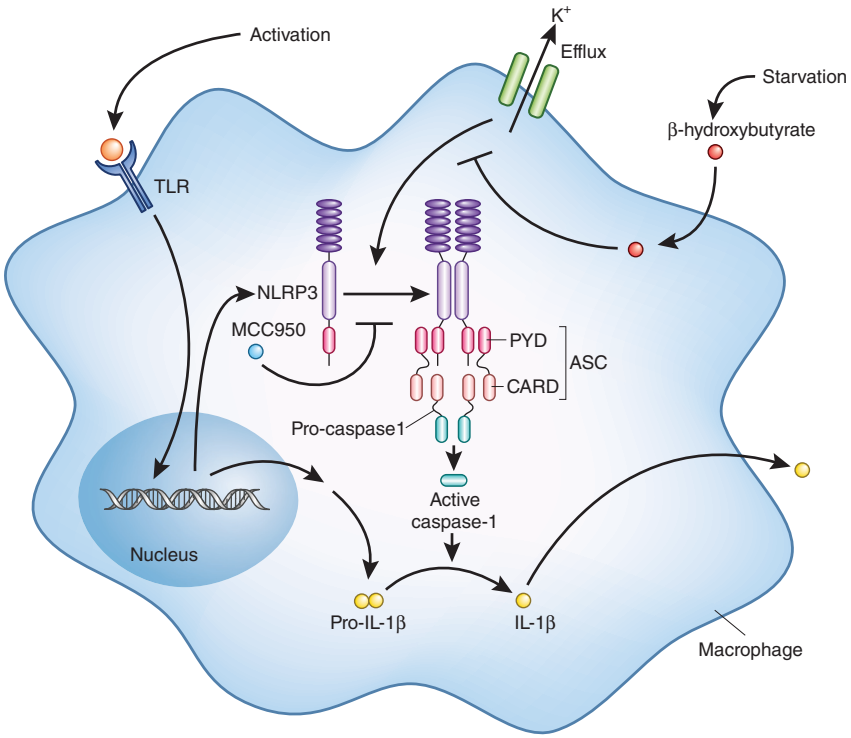

Figure 1 Inhibitors of the NLRP3 inflammasome. In response to activation of innate immune receptors by stimuli such as microbial ligands, transcription of pro-inflammatory genes including those encoding NLRP3 and pro-IL1β is induced. Transcription of proinflammatory genes primes components of the NLRP3 inflammasome complex. Upon stimulation with a variety of endogenous and exogenous signals, a common characteristic of which is the induction of K^+ efflux from the activated cell, the NLRP3 inflammasome assembles as a complex with ASC and pro-caspase-1. As a consequence, caspase-1 cleaves pro-IL-1β into its active form for secretion. Youm *et al.*[6] suggest that β-hydroxybutyrate inhibits K^+ efflux and prevents NLRP3 activation. Coll *et al.*[7] describe the small molecule MCC950 as a specific inhibitor of NLRP3 inflammasome assembly. PYD, pyrin domain; CARD, caspase activation and recruitment domain.

neurodegenerative diseases[1]. Furthermore, gain-of-function mutations in NLRP3 cause cryopyrin-associated periodic syndromes (CAPSs), including familial cold autoinflammatory syndrome (FCAS) and Muckle–Wells syndrome (MWS), rare human conditions that are characterized by chronic fever, rash, joint pain, and neurological complications[2]. Deletion of the NLRP3 inflammasome was shown to ameliorate atherosclerosis, multiple sclerosis, Alzheimer disease, type 2 diabetes, and gout in mouse models of these diseases[3].

The central involvement of the inflammasome in human disease has incited efforts to identify potent and specific ways to interfere with NLRP3 activation in the setting of auto-inflammatory diseases. Although both endogenous[3,4] and synthetic[5] inflammasome inhibitors have been described, the only clinically available therapeutics for NLRP3-driven autoinflammatory diseases are antibodies targeting IL-1β signaling, namely anakinra, rilonacept, and canakinumab, which are not specific to NLRP3 activity and cause general immunosuppression[2]. In this issue of *Nature Medicine*, Youm *et al.*[6] and Coll *et al.*[7] describe two new molecules that function as potent and specific inhibitors of NLRP3, thus constituting promising candidates for clinical testing.

During starvation, systemic metabolic alterations induce the production and utilization of ketone bodies, small acidic metabolites that serve as sources of ATP in periods of glucose shortage[8]. Youm *et al.*[6] report that one such ketone body, β-hydroxybutyrate, but not the structurally related acetoacetate or butyrate, specifically inhibits NLRP3 inflammasome activation and downstream cytokine production by numerous known NLRP3 activators in mouse bone marrow–derived macrophages and human monocytes *in vitro*. When administered at physiological concentrations to mice in complex with nanolipogens that improve its bioavailability, β-hydroxybutyrate blocked NLRP3 inflammasome activation in response to monosodium ureate (MSU) crystals, the causative agent of gout. Similarly, in mouse models with human gain-of-function mutations leading to the manifestations of MWS and FCAS, β-hydroxybutyrate potently reduced IL-1β secretion.

This finding extends previous reports of the close interconnection between the metabolic and innate immune systems[9]. Secreted metabolites are sensed by the immune system to mediate a concerted whole-organismal energy and defense program. One such example is the inhibitory effect of short-chain fatty acids on macrophage inflammatory responses[10]. The identification of β-hydroxybutyrate as an NLRP3 inhibitor provides a rationale for the investigation of the effectiveness of a ketogenic, anti-inflammatory diet for the treatment of NLRP3-dependent autoinflammatory diseases. Indeed, Youm *et al.*[6] observed an amelioration of inflammatory symptoms in the mouse model of FCAS when mice were fed a ketogenic diet. This mouse-based study is an exciting development in the emerging field of dietary approaches as modulators of immunity[11] and should prompt further investigation in human studies. However, to avoid the long-term effects that a ketogenic diet may impose on other aspects of metabolic homeostasis in humans, further modification of the ketogenic diet may be needed to enable elevation of ketones while avoiding systemic side effects.

Coll *et al.*[7] report the identification of a synthetic NLRP3 inhibitor of the inflammasome, MCC950, which was first described in a screen of IL-1β–processing inhibitors more than a decade ago[12]. Similarly to β-hydroxybutyrate, MCC950 blocks NLRP3 in mouse macrophages in response to canonical *in vitro* activators and prevents ASC speck formation, a characteristic microscopic feature of inflammasome assembly and ASC oligomerization. Interestingly, and in contrast to β-hydroxybutyrate, Coll *et al.*[7] find that MCC950 also inhibits the recently discovered caspase-11–dependent noncanonical pathway of IL-1β release and pyroptosis induction. In mice, MCC950 potently reduces serum IL-1β levels in response to lipopolysaccharide (LPS) and improves disease manifestations in a mouse model of multiple sclerosis, including the suppression of disease-driving pathogenic T cell responses in the brain. In addition, Coll *et al.*[7] use MCC950 to ameliorate the symptoms of MWS in a mouse model and to block NLRP3 activation in blood cells *in vitro* from a small cohort of people with MWS.

Despite their structural dissimilarity, both β-hydroxybutyrate and MCC950 seem to be specific inhibitors of NLRP3, not affecting Toll-like receptor (TLR) signaling or other inflammasome-forming NLRs. The effect of β-hydroxybutyrate seems to be independent of any common immunomodulatory mechanism of starvation, such as autophagy or the production of reactive oxygen species (ROS). At a molecular level, the numerous endogenous and microbial stimuli that activate the NLRP3 inflammasome converge at the induction of potassium

(K^+) efflux from activated cells, suggesting that this molecular event might initiate inflammasome complex formation[13]. Youm *et al.*[6] find that β-hydroxybutyrate blocks K^+ efflux from macrophages and prevents ASC complex formation (**Fig. 1**). It remains to be studied whether β-hydroxybutyrate may directly regulate K^+ channels, or whether it regulates K^+ homeostasis through other intracellular processes. Coll *et al.*[7] find that MCC950 also blocks ASC assembly, but in this case the inhibitory mechanism seems to be independent of intracellular ion levels or NLRP3 oligomerization, pointing toward the possibility that this small molecule acts downstream of known NLRP3 activators and interferes with the process of mature inflammasome complex formation (**Fig. 1**). Further understanding of the mechanisms of action of these newly identified NLRP3 inhibitors will shed new light on the various intracellular regulatory steps controlling inflammasome assembly and signaling.

With remaining questions about the underlying molecular mechanisms notwithstanding, the discovery of these two NLRP3 inflammasome inhibitors prompts exploration of the clinical potential of both molecules. A therapeutic approach involving specific inhibition of NLRP3 inflammasome signaling may be superior to the currently used antibody-based therapies, by avoiding universal suppression of IL-1β activation by other inflammasome-forming NLRs, which is crucially important in multiple processes such as the response to infection. As a preliminary assessment of the clinical potential of MCC950, Coll *et al.*[7]. demonstrate encouraging parameters of pharmacokinetics and bioavailability of this compound in mice. The exciting discoveries presented by the studies of Youm *et al.*[6] and Coll *et al.*[7] represent a starting point for an era in which using NLRP3-inhibiting therapeutics may serve as a potential future means of prevention and treatment of inflammasome-dependent multifactorial diseases.

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Toward recreating colon cancer in human organoids

Ameen A Salahudeen & Calvin J Kuo

Experimental modeling of cancer typically uses *in vitro* culture of transformed cell lines or *in vivo* animal models. A new study using CRISPR-Cas9 to engineer oncogenic mutations into three-dimensional human colon organoid cultures yields insights into colorectal cancer tumorigenesis.

Conventional experimental models of cancer span a range of *in vitro* and *in vivo* techniques. *In vitro* culture of established cancer cell lines provides human context, robust experimental tractability and scalability; however, the lack of tissue architecture in two-dimensional culture and poorly defined genetic composition are drawbacks. Conversely, animal cancer models have additional disease context that includes three-dimensional organ structure, stromal vasculature and immune components. However, animal systems may not be representative of humans and can be resource intense. The emerging use of three-dimensional organotypic or 'organoid' culture methods now represents a potential advance in cancer modeling that combines the experimental tractability of *in vitro* systems with the tissue context of animal studies[1]. For colon, wild-type organoids containing solely epithelial elements can be generated by culturing primary nontransformed intestinal tissue or stem cells in submerged Matrigel with growth factors that replicate or inhibit stromal signals, such as Wnt, EGF, Notch and BMP. This generates three-dimensional structures that recapitulate tissue-specific multilineage differentiation and aspects of organ architecture[2–4]. Induced pluripotent stem cells can be differentiated to intestinal organoids using similar methodology[5], and air-liquid interface methods allow the growth of gastrointestinal organoids containing both epithelial and mesenchymal components without exogenous growth factor support. These air-liquid interface organoids have been used to successfully model progressive oncogenic transformation of mouse colon, gastric and pancreatic tissue[6,7].

Human colorectal adenocarcinoma arises from a progression of normal colon epithelial cells into hyperproliferative adenomas or polyps, ultimately yielding malignant invasive carcinoma. A series of mutations underlie the adenoma-to-carcinoma progression with initial *APC* inactivation, promoting clonal epithelial outgrowth and mutations of other genes such as *KRAS*, *TP53*, *SMAD4* and *PIK3CA*, then driving the development of metastatic cancer[8]. However, whether mutations in these driver genes is sufficient to elicit the fully malignant phenotype has never been directly tested in primary human cells owing in part to limitations of existing colon cancer models. In this issue of *Nature Medicine*, Matano *et al.*[9] pioneer the *in vitro* organoid modeling of human colon adenocarcinoma by systematically introducing canonical colorectal cancer driver mutations into primary human colon organoid cultures using clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 genome editing. In this manner, synthetic guide RNAs were used to introduce oncogenic mutations into the corresponding wild-type loci within organoids.

In these studies, the authors first expanded normal colon-derived organoids, containing only epithelial components, from normal human colonic mucosa. To achieve this, they used the CRISPR-Cas9 method to induce loss-of-function mutations in *APC*, *SMAD4* and *TP53* individually in colon organoids, followed by growth selection by tailored cell culture conditions (**Fig. 1**). For example, as the *APC* mutations that the authors introduced confer constitutive Wnt pathway activation in colon cancer, they selected for cells that were no longer reliant on extracellular Wnt signaling by omitting the Wnt agonists Wnt3A and R-spondin from the cell culture medium. Similarly, the authors enriched for successful CRISPR-Cas9–mediated introduction of

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