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Serine Metabolism Links Tumor Suppression to the Epigenetic Landscape

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Intermediary metabolism provides substrates that shape epigenetic status, but whether this interaction can be responsible for oncogenesis is largely unknown. In a recent issue of *Nature*, Kottakis et al. (2016) now show that the common tumor suppressor gene *LKB1* can function by mediating this connection through an LKB1/AMPK/mTOR signaling axis.

Cells require mechanisms that allow for the communication of nutritional status to other elements of the cellular machinery. One of the most evolutionarily conserved systems that accomplishes this task is the AMP activated kinase (AMPK), which senses energy status and when primed by its upstream kinase Liver Kinase B1 (LKB1) coordinates numerous programs, including the suppression of growth signaling by mTOR (Shackelford and Shaw, 2009). Nutrient sensing also coordinates metabolism with epigenetic status by mediating the flux of posttranslational modifications on histones and nucleic acids that together contribute to gene regulation. Central carbon metabolism generates acetyl-coenzyme A involved in histone acetylation, and amino acid metabolism, particularly serine and methionine metabolism, provide methyl groups for histones and nucleic acids (Gao et al., 2016). Since the Michaelis constants of the enzymes that participate in these reactions are often commensurate with the concentrations of these substrates, many of these reactions are under the direct regulation of nutrient availability. Epigenetic reprogramming is an early event in tumorigenesis, and the enzymes involved in mediating histone and DNA modifications are frequently mutated in cancer (Feinberg et al., 2016). Furthermore, *IDH1 and IDH2*, genes encoding the metabolic enzyme isocitrate dehydrogenase, are frequently mutated in cancer (Gao et al., 2016). These mutations confer a neomorphic enzyme activity that results in the accumulation of the oncometabolite 2-hydroxyglutarate, which inhibits several alpha-ketoglutarate-dependent dioxygenases regulating the epigenetic state of histones and DNA. Thus, there is substantial genetic evidence that epigenetic states can be selected during cancer, as can the link from metabolism to epigenetics in cancer pathogenesis (Gao et al., 2016). However, beyond mutations in *IDH1/2* and a few other cases, it is not known whether this interaction is responsible for tumorigenesis. Kottakis et al. (2016) now report that loss of LKB1 in the pancreas (in cooperation with KRAS mutation) alters the serine, glycine, and one-carbon (SGOC) metabolic network through an LKB1/AMPK/mTOR signaling axis. They further show that the key mediator within the network appears to be altered DNA methylation at retrotransposon elements (Kottakis et al., 2016).

LKB1, a serine/threonine kinase, is the enzyme encoded by a gene that causes an inherited autosomal dominant proliferative disorder, Peutz-Jeghers syndrome, that is characterized by gastrointestinal polyps and increased cancer incidence (Shackelford and Shaw, 2009). It is known to be frequently mutated in human lung adenocarcinomas and in cervical and pancreas cancers (Kottakis et al., 2016; Shackelford and Shaw, 2009). Although LKB1 phosphorylates numerous substrates, in many cases it acts by phosphorylating AMPK, and its mutations are often linked to dysregulated energy homeostasis.

Consistent with what has been previously found in lung, Kottakis et al. showed that loss of LKB1 exacerbated tumorigenesis in genetically engineered mice expressing a pancreas-specific $KRAS^{G12D}$ allele (Kottakis et al., 2016). These $KRAS^{G12D/+}$;$LKB1^{-/-}$ (KL) cells had increased glycolysis compared to $KRAS^{G12D/+}$ (K) cells, indicating that LKB1 induces metabolic reprogramming in this context. In addition, genome-scale

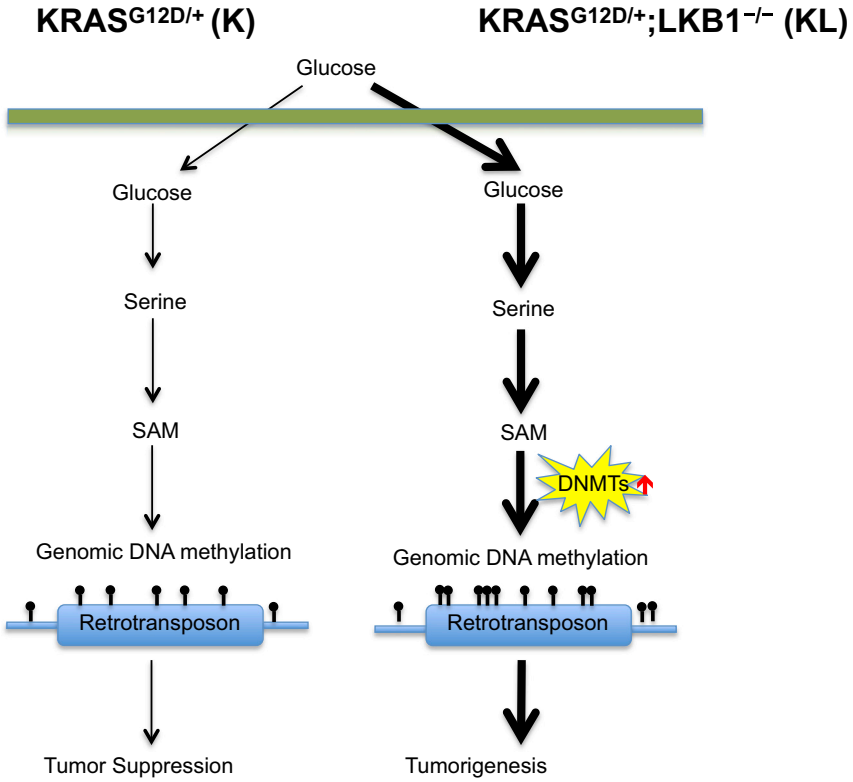

Figure 1. LKB1 Loss Promotes Tumorigenesis through Interlinked Metabolic and Epigenetic Reprogramming

In comparison to KRAS$^{G12D/+}$ (K) cells, KRAS$^{G12D/+}$;LKB1$^{-/-}$ (KL) cells were highly glycolytic, with glucose diverted for serine biosynthesis that supports synthesis of *S*-adenosyl methionine (SAM) and eventually leads to increased DNA methylation, particularly the methylation at retrotransposon elements. Loss of LKB1 leads to elevated DNA methyltransferase (DNMT) activity.

analysis identified a set of genes defining the SGOC network (Mehrmohamadi et al., 2014) as one of the most altered pathways in KL cells. Interestingly, the authors found that proliferation of KL cells was affected by inhibition of enzymes in the serine synthesis pathway (Kottakis et al., 2016), indicating the importance of the de novo serine synthesis pathway in the proliferation of KL cells. Furthermore, the authors showed that this genetic interaction occurs through a signaling axis via LKB1/AMPK/mTOR, which regulates activities of both glucose metabolism and the SGOC network (Ben-Sahra et al., 2016).

SGOC metabolism supports NADPH production and redox homeostasis and also provides one-carbon units for nucleotide synthesis (Locasale, 2013). While lung tumors require LKB1-AMPK signaling for enhanced NADPH production under energy stress (Jeon et al., 2012), Kottakis et al. found that interruption of serine synthesis in pancreas-derived KL cells did not affect either of these metabolic functions, indicating tissue-specific requirements of SGOC metabolism in KRAS-driven tumors (Kottakis et al., 2016). One-carbon units derived from serine metabolism also fuel the methionine cycle, generating the universal methyl-donor S-adenosylmethionine (SAM). Thus, they further show that disruption of serine synthesis decreased SAM levels, indicating a significant contribution from serine metabolism to SAM production in KL cells. Consequently, the authors reported higher DNA methylation in KL cells, which was attributed to the upregulation of DNA methyltransferase activity (Kottakis et al., 2016). Whole-genome bisulphite sequencing revealed methylation at repetitive retrotransposon repeats was found to be most impacted, likely altering gene regulation, and importantly these cells had increased sensitivity to DNA methyltransferase inhibitors indicating that serine synthesis, SAM, and DNA methylation are key components of the pathway connecting *LKB1* loss with epigenetics (Figure 1). Recently, serine uptake was also shown to alter cellular SAM levels and DNA methylation through regulating ATP levels (Maddocks et al., 2016), which suggests a reciprocal interaction back to LBK1-AMPK signaling as well.

Altogether, this work identifies a novel function for LKB1 and AMPK signaling in tumor suppression and extends the importance of the epigenetic arm of SGOC metabolism to tumorigenesis. Thus, the findings provide genetic evidence for a role of metabolism and epigenetics in cancer that reaches beyond the presence of *IDH1/2* mutations. Given that LKB1 induces dedifferentiation in lung tumors (Shackelford and Shaw, 2009) and that dedifferentiation often requires epigenetic remodeling, it is tempting to speculate on the extent to which this outcome may be mediated by this connection. In light of these exciting findings, questions remain. For example, LKB1 and AMPK (as an energy sensor) are also known to induce mitochondrial alterations, which also regulate epigenetics through modulating alpha-ketoglutarate and succinate levels (which are influenced by mitochondrial metabolism and citric acid cycle [TCA activity]), which affect demethylase function (Pan et al., 2016). How the transfer and removal of methyl units through the interplay of SGOC and TCA cycle metabolism occurs is largely unknown. There was also a surprising amount of specificity directed toward changes in the methylation of retrotransposon elements, and how this is established relative to other epigenetic processes requires further investigation. The principles of how methyl donor availability directs specific epigenetic programs that achieve biological outcomes are largely not known.

Nevertheless, the therapeutic potential established in this landmark study will warrant further translational study and also open many areas of basic inquiry that will further our understanding of the link between intermediary metabolism and epigenetics.

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Assisted Living in the Atheroma: Elderly Macrophages Promote Plaques

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Age dominates as a risk factor for human atherosclerosis, yet the underlying mechanisms remain elusive. A recent report described accumulation of senescent cells in atheromata of hypercholesterolemic mice and provides novel insights into how genes expressed by aging cells associate with the characteristics of human plaques implicated in cardiovascular events.

Only a few decades ago, most viewed the atherosclerotic plaque as an amorphous deposit of lipid on the artery wall that grew progressively to clog arteries, ultimately obstructing blood flow and causing heart attacks, strokes, and the other dreaded complications of this disease. We have now gained increasingly sophisticated information regarding the complex cellular interactions and signaling pathways that contribute to atherogenesis (Libby et al., 2016). We understand details of lipid metabolism, the control of uptake of cholesterol-laden lipoprotein particles by vascular wall cells and invading leukocytes, and the manner in which atherosclerotic lesions progress and produce the thrombotic complications that provoke clinical events such as myocardial infarction and ischemic stroke. Most currently regard atherosclerosis not as a simple plumbing problem due to blockage of arteries by progressive lesion growth, but as a process orchestrated by the insidiously dysregulated behavior of cells in response to increasingly well-understood molecular signals.

Nonetheless, we have much yet to learn about the mechanisms that link risk factors such as hypercholesterolemia and the altered behavior of cells that give rise to lesion formation and complication. Many invoke oxidized lipoproteins as an initiating stimulus, but scant evidence actually supports this view in humans. Although inflammatory pathways likely link many traditional risk factors for atherosclerosis with the pathogenesis of the disease, knowledge of the coupling mechanisms remains incomplete (Libby, 2012). A recent report by Childs and colleagues (2016) helps to address this gap.

In humans, age dominates as a risk factor for the development of the complications of atherosclerosis. Accordingly, investigators have probed aging as a contributor to experimental atherosclerosis, most recently using hypercholesterolemic mice. For example, Rauscher and colleagues "rescued" mice rendered hypercholesterolemic due to deficiency in Apolipoprotein E and consumption of a cholesterol-rich diet from atherosclerosis development by transfer of bone marrow-derived progenitor cells from juvenile animals. Transfer of bone marrow cells from aged atherosclerotic mice proved much less effective in this regard (Rauscher et al., 2003). Aging ultimately ends badly for cells as well as whole organisms: in the extreme, death of macrophages and impaired clearance of their remains have received considerable attention in the context of atheroma formation (Geng and Libby, 1995; Tabas and Bornfeldt, 2016). But senescence, a step short of cell death, has not garnered as much consideration.

The recent work of Childs et al. describes accumulation of cells bearing markers of senescence in atherosclerotic lesions at various stages of development by using hypercholesterolemic *ldlr*$^{-/-}$ mice (Figure 1). The senescent cells produced pro-inflammatory cytokines and matrix metalloproteinases (MMPs) that can degrade the arterial extracellular matrix. Childs et al. systematically sought the effects of cellular senescence at various stages of experimental atherogenesis. Early lesions produced only 9–12 days after initiating a lipid-rich diet contained monocytes with markers of senescence (β-galactosidase) in association with pro-inflammatory mediator mRNA accumulation. Analysis of later lesions (after 88–188 days of consuming an atherogenic