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Gerometabolites

The pseudohypoxic aging side of cancer oncometabolites

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Oncometabolites are defined as small-molecule components (or enantiomers) of normal metabolism whose accumulation causes signaling dysregulation to establish a milieu that initiates carcinogenesis. In a similar manner, we propose the term "gerometabolites" to refer to small-molecule components of normal metabolism whose depletion causes signaling dysregulation to establish a milieu that drives aging. In an investigation of the pathogenic activities of the currently recognized oncometabolites R(-)-2-hydroxyglutarate (2-HG), fumarate, and succinate, which accumulate due to mutations in isocitrate dehydrogenases (*IDH*), fumarate hydratase (*FH*), and succinate dehydrogenase (*SDH*), respectively, we illustrate the fact that metabolic pseudohypoxia, the accumulation of hypoxia-inducible factor (HIFα) under normoxic conditions, and the subsequent Warburg-like reprogramming that shifts glucose metabolism from the oxidative pathway to aerobic glycolysis are the same mechanisms through which the decline of the "gerometabolite" nicotinamide adenine dinucleotide (NAD)$^+$ reversibly disrupts nuclear–mitochondrial communication and contributes to the decline in mitochondrial function with age. From an evolutionary perspective, it is reasonable to view NAD$^+$-driven mitochondrial homeostasis as a conserved response to changes in energy supplies and oxygen levels. Similarly, the natural ability of 2-HG to significantly alter epigenetics might reflect an evolutionarily ancient role of certain metabolites to signal for elevated glutamine/glutamate metabolism and/or oxygen deficiency. However, when chronically altered, these responses become conserved causes of aging and cancer. Because HIFα-driven pseudohypoxia might drive the overproduction of 2-HG, the intriguing possibility exists that the decline of gerometabolites such as NAD$^+$ could promote the chronic accumulation of oncometabolites in normal cells during aging. If the sole activation of a Warburg-like metabolic reprogramming in normal tissues might be able to significantly increase the endogenous production of bona fide etiological determinants in cancer, such as oncometabolites, this undesirable trade-off between mitochondrial dysfunction and activation of oncometabolites production might then pave the way for the epigenetic initiation of carcinogenesis in a strictly metabolic-dependent manner. Perhaps it is time to definitely adopt the view that aging and aging diseases including cancer are governed by a pivotal regulatory role of metabolic reprogramming in cell fate decisions.

Over the past decade, Otto Warburg's initially discarded hypothesis from the 1920s, that mitochondrial dysfunction and the acquisition of a glycolytic phenotype were both metabolic features at the root of cancer, has finally attained the status of a core cancer hallmark.[1-7] The metabolic signatures of cancer cells, which have been frequently perceived by traditional biochemists as indirect, secondary phenomena merely required

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to support oncogene-directed anabolic proliferation and survival,[8] are not passive responses to damaged mitochondria as originally proposed by Otto Warburg; we now know that the metabolism and metabolites of cancer cells can be oncogenic themselves. Thus, proto-oncogenes and tumor suppressors possibly originated through evolution as components of metabolic regulation and, more importantly, so-called oncometabolites can directly impair the normal epigenetic regulation of cell differentiation and alter cell signaling to drive cellular transformation and oncogenesis.[9-22]

If Otto Warburg were alive today, he would surely be surprised not only by the new convention of metabolic reprogramming as a bona fide cancer attribute, but also by the latest discovery that dysfunctional mitochondria and Warburg-like metabolic reprogramming are crucial contributors to aging triggered by the reversible decline of nuclear nicotinamide adenine dinucleotide (NAD^+) metabolite levels.[23,24] We here propose that, given that the term oncometabolite refers to a small-molecule component (or enantiomer) of normal metabolism whose accumulation causes signaling dysregulation to establish a milieu that initiates carcinogenesis,[20,22] it might be reasonable to coin the term gerometabolite to denote a small-molecule component of normal metabolism whose depletion causes metabolic and nonmetabolic dysregulation to establish a milieu that drives aging.

Oncometabolites: Reprogramming the Epigenetic Landscape in Cancer

The term oncometabolite was first coined to describe (R)-2-hydroxyglutarate [(R)-2HG], the reduced form of 2-oxoglutarate (2OG).[9-11,13,19,20,22] (R)-2HG is a byproduct of gain-of-function mutations in the genes encoding the isocitrate dehydrogenases 1 and 2 (*IDH1* and *IDH2*), which normally catalyze the reversible $NADP^+$-dependent oxidative-decarboxylation of isocitrate to produce 2OG in the cytoplasm or mitochondria, respectively. There has been increasing interest in (R)-2HG due to its apparent novelty as a rare metabolite found only in trace amounts in non-diseased mammalian cells under normal conditions; in addition, (R)-2HG is truly pathogenic and not just an indolent byproduct of a loss-of-function mutation in human malignancies. The pathological accumulation of small organic acids such as (R)-2HG due to the acquisition of neomorphic enzymatic activity by cancer-associated mutations in cytosolic IDH1 and mitochondrial IDH2 is apparently sufficient to impair cellular differentiation by competing with the normal functioning of α-ketoglutarate, which is produced in part by wild-type *IDH* and is used as a substrate for dioxygenase enzymes that modify nuclear epigenetic marks.[4,20,22] For example, (R)-2HG can inhibit certain members of the ten-eleven translocation (TET) family of dioxygenase enzymes, including TET2 DNA hydroxylase, which converts 5′-methylcytosine (5mC) to 5-hydroxymethylcytosine, an intermediate in either passive or active DNA demethylation. In addition, (R)-2HG can inhibit specific Jumonji C domain-containing histone lysine demethylase (KDM) enzymes, which demethylate the lysine residues on histone tails (e.g., histone H3 lysine 9 [H3K9]). The ability of (R)-2HG to competitively inhibit chromatin-modifying α-ketoglutarate-dependent dioxygenase enzymes alters histone and DNA methylation in a synergistic manner, thus drastically impairing normal epigenetic regulation by promoting hypermethylation at CpG islands in some cases. Indeed, the oncogenic activity of the (R)-2HG oncometabolite likely relies on its ability to epigenetically block the acquisition of differentiation markers while inducing the expression of stem cell-maintenance genes.[4,20,22,25,26]

Loss-of-function mutations in tumor suppressor genes encoding the Krebs cycle enzymes fumarate hydratase (*FH*) and succinate dehydrogenase (*SDH*) lead to an accumulation of the oncometabolites fumarate and succinate, respectively.[12,14,16-18,21] Abnormal accumulation of fumarate and succinate has potential tumorigenic effects via several mechanisms including the following: overproduction of reactive oxygen species (ROS), which may participate in oncogenic signaling and tumor progression by the irreversible modification of DNA and oxidation of proteins; irreversible modification of cysteine residues in proteins by succination, which may result in the constitutive activation of the NRF2-mediated antioxidant defense pathway that can promote tumorigenesis not only by enhancing ROS detoxification, but also by producing a reductive milieu that can promote cell survival and proliferation; and dysregulation of the enzymatic reactions involved in the biosynthesis of arginine and purine. As observed for (R)-2HG, the aberrant accumulation of fumarate and succinate can also inhibit the activities of α-ketoglutarate-dependent TET and the KDM family of 5mC hydroxylases, thus remodeling the cancer epigenome toward undifferentiated and aggressive phenotypes (e.g., neuroendocrine differentiation and epithelial-to-mesenchymal transition). Accordingly, there is an overlap in the hypermethylation patterns of tumors containing *IDH* and *SDH* mutations,[16] strongly suggesting a shared role for (R)-2HG, succinate, and fumarate oncometabolites in the reprogramming of the epigenetic cancer landscape.

Oncometabolites and Pseudohypoxia: A Metabolic Link to Gerometabolites?

As mentioned above, a common feature of the mutations in IDH, FH, and SDH enzymes is the reduced activity of α-ketoglutarate-dependent dioxygenases such as TET and KDMs, which leads to an inhibition of histone and DNA demethylation. The (R)-2HG, succinate, and fumarate oncometabolites also target hypoxia-inducible factor α (HIFα) prolyl hydroxylases (PHDs). Early studies showed that (R)-2HG cannot only reduce levels of α-ketoglutarate, but also inhibit HIFα-PHDs, leading to decreased HIFα degradation and an enhanced HIFα-orchestrated "pseudohypoxic" response (**Fig. 1**); accordingly, HIFα has been shown to be upregulated in cells treated with exogenous (R)-2HG and in cells that overexpress mutant *IDH1*. Conversely, later studies suggested that in contrast to (S)-2HG, which acts as an inhibitor of HIFα-PHD, (R)-2HG stimulates the activity of this enzyme;[27,28] accordingly, the expression of mutant *IDH1* has

been shown to enhance HIFα degradation and diminish HIFα response levels, whereas the loss of HIFα-PHD activity can block the transformation ability of mutant *IDH*. In certain cellular contexts, therefore, HIFα or other specific targets of hydroxylation by HIFα-PHD appear to suppress the oncogenic potential of (R)-2HG. Thus, it remains somewhat unclear whether (R)-2HG has an agonistic or antagonistic effect on HIFα-PHD at tumor-relevant concentrations and whether HIFα, which has traditionally been viewed as oncogenic, could act as a tumor suppressor in some *IDH*-mutated tumors. Nevertheless, these data were generated in vitro and highlight contradictory (and difficult to integrate with analyses of tumors) but not mutually exclusive results, obtained from different models, in which (R)-2HG-induced stabilization of HIFα as a consequence of competitive inhibition of PHDs, the 2OG-dependent dioxygenases that regulate HIFα, is a potential mechanism for oncogenesis closely related to HIFα-driven glycolytic response.

SDH- and *FH*-deficient cells and tumors have been reported to exhibit the activation of an HIFα-orchestrated "pseudohypoxic" response, which could, at least in part, be attributed to the allosteric inhibition of HIFα-PHD by elevated levels of succinate or fumarate (**Fig. 1**).[29-36] Succinate was initially found to impair PHD2, the α-ketoglutarate-dependent enzyme regulating HIFα stability, through product inhibition, and subsequent work confirmed that succinate could also inhibit the related enzyme PHD3. Succinate impedes PHD activity by product inhibition and prevents the decarboxylation of α-ketoglutarate to succinate, an essential co-reaction in the hydroxylation of targets by PHDs. Inhibited PHDs can no longer hydroxylate HIFα, resulting in its stabilization. Because fumarate can similarly inhibit PHD2, these observations link the

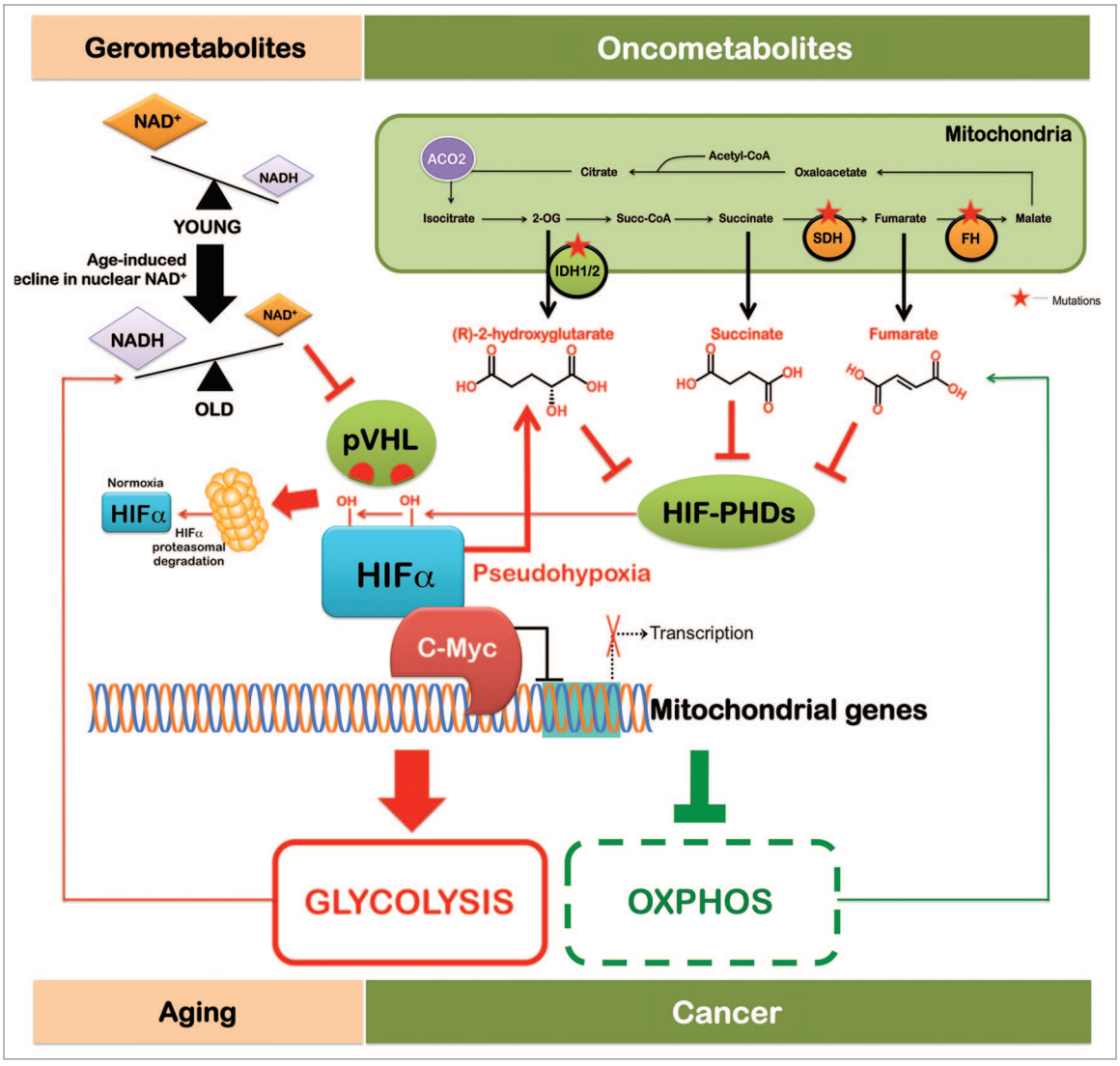

Figure 1. The pseudohypoxia-switching hub: A unifying link between gerometabolites and oncometabolites. Transition from oxidative metabolism into Warburg-like aerobic glycolysis sets a cell metabotype commonly shared by aging and cancer. The intriguing convergence of gerometabolites and oncometabolites on *Myc*, the so-called "oncogene from hell", provides not only evidence for a key molecular "funnel factor" linking metabolism with aging–cancer signatures, but also provocatively implicates *Myc* as a distinctive mechanistic target to decelerate aging and postpone age-related diseases such as cancer without the emergence of resistance phenomena.

elevated levels of HIFα-dependent oncogenic pathways observed in SDH- and FH-deficient tumors to the anti-HIFα-PHD activity induced by elevated levels of the oncometabolites succinate and fumarate.

A landmark study by David Sinclair's group at Harvard revealed that a decrease in a small-molecule component of normal metabolism (i.e., NAD^+) causally triggers an HIFα-driven metabolic reprogramming that disrupts mitochondrial homeostasis in normal tissues during aging.[23] The biogenesis of complex I, the largest enzyme of the mammalian mitochondrial oxidative phosphorylation (OXPHOS) respiratory chain, is a very complex process due to the large size and number of subunits (45 in humans). The situation is further complicated, because the complex I subunits have a double genomic origin; some of the genes encoding complex I subunits have been retained in the genome of the mitochondrion, the ancient symbiont of eukaryotes, thus complicating the transcriptional regulation of OXPHOS complex I. The functional communication between the nucleus and mitochondria necessary for the formation of stoichiometric OXPHOS complexes, which is largely driven by the peroxisome proliferator-activated receptor-γ coactivators α and β (PGC-1α and PGC-1β),[37] is lost during the aging process, causing a specific loss of mitochondrial, but not nuclear, encoded OXPHOS subunits. Gomes et al.[23] described the existence of a previously unrecognized PGC-1α/β-independent pathway of nuclear–mitochondrial communication that is induced by a decline in nuclear NAD^+, a central metabolic cofactor, by virtue of its redox capacity. NAD^+ is consumed as a co-substrate by the so-called sirtuins to deacetylate proteins in different subcellular compartments for a variety of functions, such as optimizing mitochondrial function and biogenesis and stabilizing HIF-1α under normoxic conditions,[38-46] thus causing a pseudohypoxia-driven imbalance between nuclear- and mitochondrial-encoded OXPHOS subunits (**Fig. 1**). This process is accelerated by deleting the NAD^+-dependent histone deacetylase sirtuin 1 (SIRT1), whereas treatment with a compound that boosts NAD^+ levels is sufficient to restore the mitochondrial homeostasis in old mice to a state similar to that of young mice in a SIRT1-dependent manner.[23]

In the aging scenario, therefore, the decline in nuclear NAD^+ is the causal inducer of the accumulation of HIF-1α under normoxic conditions, which promotes a pseudohypoxic state that disrupts nuclear–mitochondrial communication and contributes to the decline in mitochondrial function with age. Mechanistically, the decline in nuclear NAD^+ levels reduces the activity of SIRT1 in the nucleus, causing the levels of the von Hippel–Lindau (*VHL*) gene product (pVHL), an ubiquitin ligase that recognizes and ubiquitylates HIF to promote its degradation by the proteasome,[47-50] to decline and HIF-1α to be stabilized (**Fig. 1**). Although Gomes et al.[23] failed to detect SIRT1-related changes in the hydroxylation of HIF-1α, it was clear that SIRT1 regulated the pVHL status at the posttranscriptional level, suggesting that SIRT1 is constantly required to maintain mitochondrial homeostasis by inducing pVHL and by ensuring that HIF-1α is degraded efficiently. Because pseudohypoxia will undoubtedly shift glucose metabolism from the oxidative pathway to aerobic glycolysis, the reprogramming toward an HIFα-directed, Warburg-like, pseudohypoxic state appears to be a metabolic phenomenon shared by the most common aging diseases, including cancer,[51,52] type 2 diabetes and metabolic syndrome,[53-55] high fat diet-induced liver steatosis,[56] white adipose tissue-related dietary obesity,[57] and likely also affects other key organs such as the heart and brain during aging.[23]

Oncometabolites, Gerometabolites, Gerogenes, and Gerosuppressors: An HIFα-Driven Pseudohypoxic Metabolic Signature

The fact that the accumulation of oncometabolites and the decline of gerometabolites similarly activate the same metabolic, pseudohypoxic response to elicit oncogenic and aging effects is, intriguingly, consistent with the paradoxical ability of the best-characterized gerogene (i.e., the mechanistic target of rapamycin [mTOR]) to contribute, when stimulated, to the transformation and growth of cancer cells (oncogenesis) while leading to stem cell depletion through the activation of senescence programs (aging).[58,59] The prolonged stimulation of mTOR in normal cells can lead to stem cell depletion and reduced organismal health and life span, and mTOR activation is a common feature in most human malignancies. Although the ultimate molecular mechanism(s) underlying the aimless continuation of developmental growth driven by nutrient-sensing, growth-promoting signaling pathways such as the mTOR gerogene are not completely understood, it should be noted that the hyperfunctions (aging), loss of homeostasis, and age-related disease (cancer) triggered by the continuous post-developmental activity of such gerogenic pathways[60-71] can be easily linked to the pseudo-hypoxic scenario triggered by the accumulation of oncometabolites and the decline of gerometabolites.

HIFα homeostasis is controlled by its rate of translation and degradation (**Fig. 2**). Under physiological conditions in young tissues, HIFα is constantly degraded in a process dependent on oxygen, prolyl hydroxylation by HIFα-PHDs, and ubiquitylation mediated by pVHL. In cancer, HIFα levels are elevated owing to hypoxia (lack in oxygen) or pseudohypoxia (PHD inhibition or pVHL mutation), which inhibits HIFα degradation. We now know that HIFα-PHDs respond to stimuli other than oxygen, including the oncometabolites (R)-2HG, succinate, or fumarate, as illustrated by the pseudo-hypoxic response in *SDH*- and *FH*-deficient tumors. Declining NAD^+ gerometabolite levels also induce a pseudohypoxic state during aging by blocking pVHL-dependent HIFα stability in a SIRT1-dependent manner. Levels of HIFα in cancer can also be elevated due to accelerated translation. In this regard, a pseudohypoxic response in tumors can be achieved by accelerated mTOR-dependent translation of HIFα.[72-78] The mTOR-dependent translation of HIFα is induced by the loss of the *TSC1*, *TSC2,* or *LKB1* tumor-suppressor/gerosuppressor genes and possibly by the activation via

the AKT pathway. Indeed, mTOR inhibition has been shown to lead to a profound attenuation of HIFα protein levels in the majority of primary and cancer cells that have been studied. Under severe hypoxia, however, no influence of mTOR inhibitors on HIFα expression status has been observed; thus, stimulation of HIFα by gerogenic mTOR signaling may only be relevant under mild hypoxia or normoxia. The pseudo-hypoxic hypothesis of the gerogenic activity of mTOR is further supported by the fact that the function of the PHD–HIF feedback loop (hypoxia inactivates PHDs, causing accumulation of HIF-1α; in turn, HIF-1α further transactivates PHDs) has been suggested to limit the induction of HIF-1α by gero-promoters (i.e., mTOR activators) such as insulin, growth factors, hormones, cytokines, and nutrients under normoxia.[79-82] The failure to limit mTOR-dependent induction of HIF-1α may therefore contribute to age-related diseases (**Fig. 1**). Intriguingly, given that 2OG is a limiting co-substrate for PHD activity during normoxia, and that 2OG levels depend on amino acid availability, it is plausible that PHD activity depends not only on oxygen or oncometabolites, but also on amino acid availability, suggesting a global metabolic sensor function for PHDs as a signal for both HIFα and mTOR.[83]

It is readily apparent that by protecting adult cells from initiating premature cell senescence using pharmacological agents that simultaneously prevent oncogenesis, we might delay aging without the potential increase in cancer risk; this scenario is well-recognized for pharmacological inhibitors of mTOR activity, such as rapamycin. By similarly affecting the mTOR gerogenic pathway, calorie restriction (CR) is likely the most-recognized anti-aging intervention that contradicts the paradigm that increasing adult cell function to levels found in the young must be counterbalanced against the risk of higher incidences of cancer. New findings reported by David Sinclair's group strongly suggest that not only long-term CR, but also acute increases in NAD^+ levels and/or small compounds that prevent HIF-1α stabilization (e.g., NMN, a precursor to NAD^+ that increases NAD^+ levels in vivo) can rapidly prevent the decline in pVHL and accumulation of HIF-1α in old mice

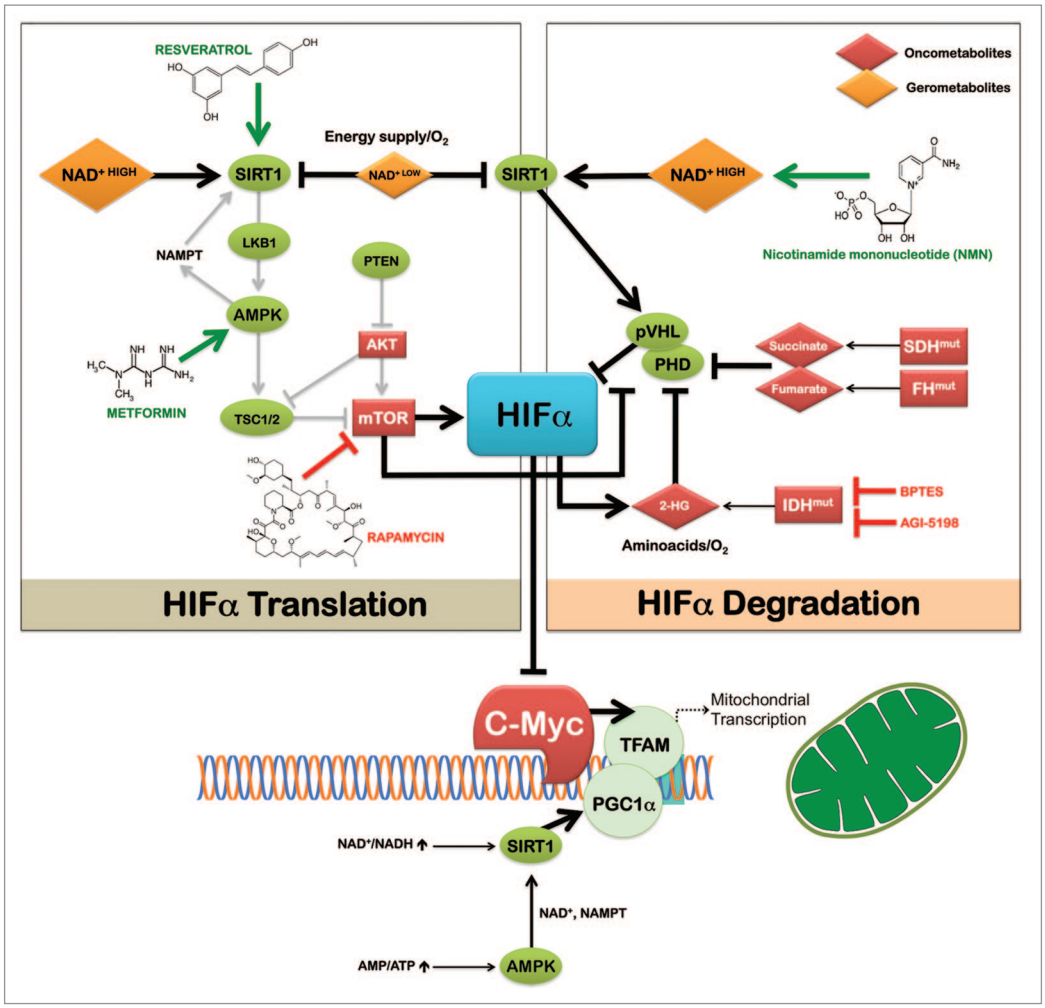

Figure 2. Gerometabolites and oncometabolites could control HIFα homeostasis by its rate of translation (left) and degradation (right). Proteins colored in green represent HIFα downregulators (e.g., gerosuppressors) whereas those in red represent HIFα inducers (e.g., gerogenes, oncometabolites). While it is obvious that the antifungal antibiotic rapamycin, the polyphenol resveratrol, and the biguanide metformin already belong to the family of gerosuppressor-targeting drugs, other SIRT1 activators such as NMN and oncometabolite inhibitors (e.g., BPTES, AGI-5198) should be viewed as drug candidates that could reverse or postpone the pseudohypoxia-switching hub driving aging and cancer.

to restore mitochondrial function and notably reverse many biochemical aspects of aging to that of a young mouse in an SIRT1-dependent manner.[23] Based on the proposed model, which integrates the interconnected signaling of an HIF-1α-driven pseudohypoxic aging/cancer signature by oncometabolites, gerometabolites, gerogenes, and gerosuppressors (**Fig. 2**), we can make several predictions:

First, the activation of metabolic gerosuppressors (e.g., the evolutionarily conserved energy-sensor AMP-activated protein kinase), which critically antagonize the mTOR gerogenic activity,[60-71,84] might exert anti-aging effects by restoring nuclear–mitochondrial communication during aging via the gerometabolite NAD^+. Indeed, AMPK regulates energy expenditure by modulating NAD^+ metabolism and SIRT1 activity. To date, we have evidence that AMPK activation enhances SIRT1 activity by increasing cellular NAD^+ levels, resulting in

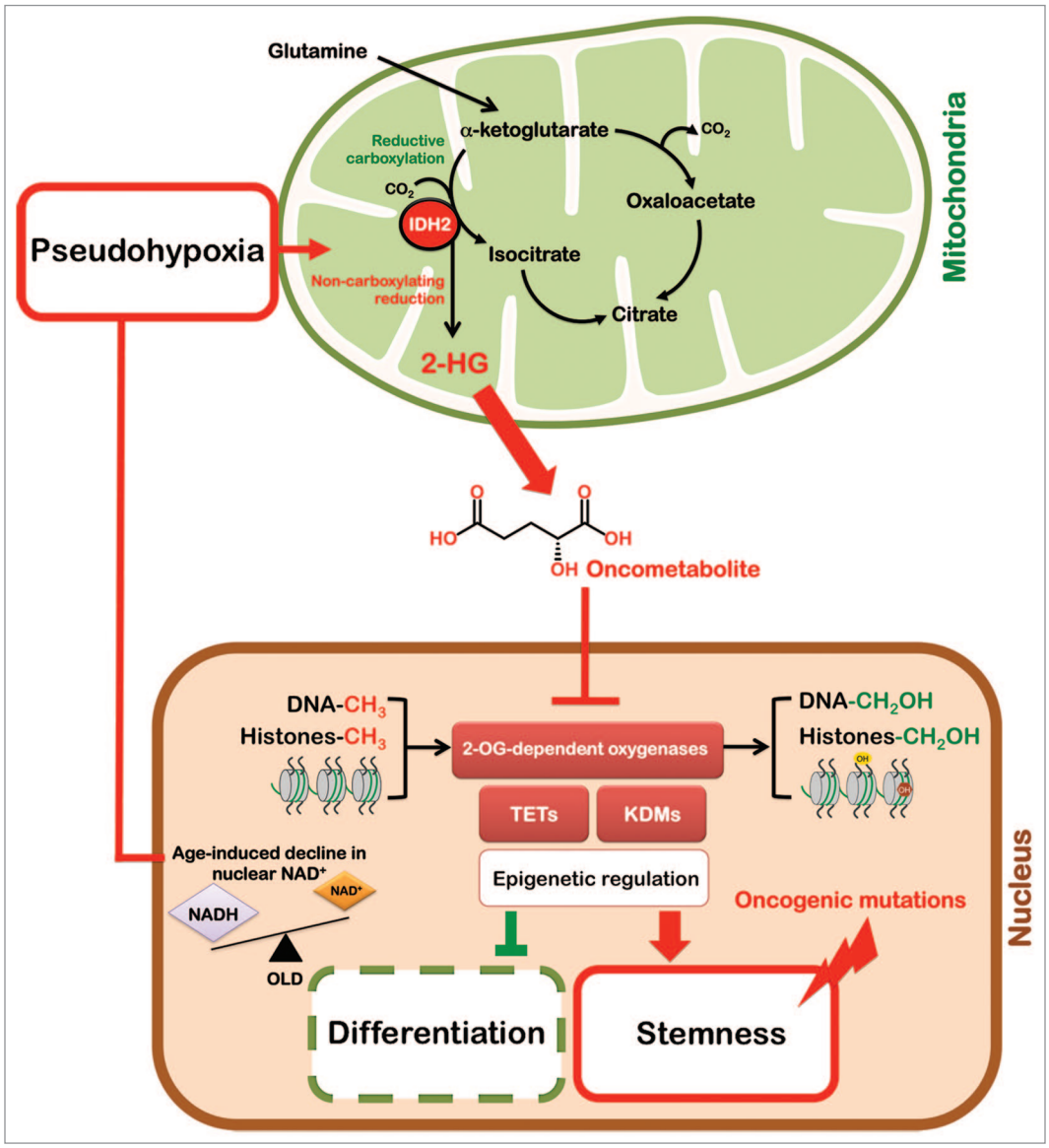

Figure 3. Gero-oncometabolite mitochondrial retrograde-like signaling: A new candidate signaling pathway linking aging with cancer. Mitochondrial retrograde signaling is a pathway of communication from mitochondria to the nucleus under normal and pathophysiological conditions. It involves multiple factors that sense and transmit mitochondrial signals to effect changes in nuclear gene expression; these changes lead to a reconfiguration of metabolism to accommodate cells to defects in mitochondria. Although during aging there is a specific loss of mitochondrial, but not nuclear, encoded OXPHOS subunits, the pseudohypoxic state that disrupts the PGC1-α/β-independent nuclear–mitochondrial communication can contributes not only to the decline in mitochondrial function with age, but can also mediate an undesirable trade-off between Warburg reprogramming and oncometabolites production in normal cells. Glutamine-derived α-ketoglutarate is reductively carboxylated by the NADPH-linked mitochondrial IDH2 to form isocitrate, which can then be isomerized to citrate. However, the increased IDH2-dependent carboxylation of glutamine-derived α-ketoglutarate in hypoxia has been associated with a concomitant increased synthesis of 2-hydroxyglutarate (2HG) in cells with wild-type *IDH1* and *IDH2*. Moreover, reprogramming of metabolism by HIF1α in the absence of hypoxia is sufficient to induce reductive activity on α-ketoglutarate that is uncoupled from carboxylation, thus producing the oncometabolite 2HG. This hypothetical scenario offers a plausible connection between gerometabolites-induced pseudohypoxia and oncometabolites-induced block in differentiation to promote tumorigenesis, likely through epigenetic mechanisms.

the deacetylation and modulation of the activity of downstream SIRT1 targets that include the PGC-1α transcription factor.[85-87] We know that, in response to metformin, SIRT1 can be activated through an AMPK-mediated increase in gene expression in nicotinamide phosphoribosyltransferase, the rate-limiting enzyme of the salvage pathway for NAD^+.[85,88,89] Moreover, metformin has been shown to inhibit the ability of insulin and IGF-1 to induce HIF-1α expression, and, accordingly, HIF-1α levels appear to decrease significantly in diabetic patients treated with metformin.[90-92] Because NAD^+-dependent SIRT1 can regulate mitochondria homeostasis independent of PGC-1α/β, and AMPK activity dictates the dominant process in response to the energetic state of the cell,[23] further work is necessary to unambiguously elucidate whether gerosuppressant agents with AMPK agonistic activity (e.g., metformin) can elicit their anti-aging (and anti-cancer) effects by restoring nuclear–mitochondrial communication via the suppression of HIF-1α-driven, pseudo-hypoxic cellular states in a PGC-1α/β-independent manner (**Fig. 2**).

Second, if the pseudohypoxic state that disrupts PGC-1α/β-independent nuclear–mitochondrial communication contributes to the decline in mitochondrial function with age, a process that is apparently reversible, it would be relevant to study whether changes in the NAD^+-SIRT1-HIFα-OXPHOS pathway similarly disrupt mitochondrial homeostasis in pre-malignant and cancer tissues. In addition, it would be important to determine whether increasing NAD^+ (e.g., with NMT) is beneficial for cancer prevention and treatment, especially in tumors characterized by the aberrant accumulation of pathogenic oncometabolites (**Fig. 2**).

Third, the direct targeting of (R)-2HG production by inhibiting the conversion of glutamine to α-ketoglutarate using small-molecule inhibitors (e.g., bis-2-[5-phenyl-acetamido-1,2,4-thiadiazol-2-yl] ethyl sulfide)[93] or selective inhibitors directed against the neomorphic forms of mutated *IDH* (e.g., AGI-5198)[94,95] can significantly slow the growth rate of *IDH* mutant cancer cells (**Fig. 2**). This finding suggests that the "starvation" of *IDH* cells of α-ketoglutarate or direct blocking of the ability of the mutant enzyme to produce (R)-2HG may have therapeutic benefits. However, it is also possible that treatment with exogenous α-ketoglutarate may be beneficial due to a reduction in the competitive inhibitory effect of 2-HG on α-ketoglutarate-dependent enzymes. For example, the introduction of cell-permeating α-ketoglutarate derivatives has been shown to restore normal PHD activity, which targets HIF1α for ubiquitylation and proteasomal degradation.[96] This restores normal, low levels of HIF1α in SDH-deficient cells, thus indicating that pharmacological elevation of intracellular α-ketoglutarate alleviates pseudohypoxia in tumor cells with mitochondrial dysfunction of OXPHOS enzymes. Moreover, because increased levels of intracellular α-ketoglutarate have a marked effect on the basal levels of HIF-1α protein (i.e., α-ketoglutarate may be a limiting factor for HIF-PHD activity under normoxia), it is possible that the mTOR-mediated increased translation of HIF-1α can be overcome if HIF-1α degradation can be accelerated by α-ketoglutarate. This scenario raises several new and challenging questions. Can the decline of gerometabolites such as NAD^+ promote the chronic accumulation of oncometabolites in normal cells during aging? Is the sole activation of a Warburg-like metabolic reprogramming in normal tissues able to significantly increase the endogenous production of oncometabolites, even in the absence of mutations in *IDHs* or *OXPHOS* enzymes? If so, oncometabolites, as bona fide etiological determinants in cancer, might then pave the way for the initiation of carcinogenesis in a strictly metabolic-dependent manner (**Fig. 3**). Moreover, it is possible that therapeutic strategies that target oncometabolites might significantly impact the aging process.

Gerometabolites and Oncometabolites Share a Communication Link to the "Oncogene From Hell" (c-Myc)

The extremely powerful gene transcription activator *c-Myc* has long been thought to be a key, if not "the" key, protein target for the development of cell proliferation-inhibiting drugs.[97] The "oncogene from hell",[98] *Myc*, is a critical link between altered cellular metabolism and the genesis of many human cancers.[99-104] *c-Myc* regulates genes involved in the biogenesis of ribosomes and mitochondria as well as the regulation of glucose and glutamine metabolism but can also induce DNA damage, increase reactive oxygen species, and mitigate p53 function to consequently induce genome instability. Moreover, recent publications have revealed that the "evil arsenal" of *Myc* includes the coordination of the crosstalk between tumor and microenvironment as it engages a complex inflammatory response in the tumor stroma and induces angiogenesis.[105,106] This *c-Myc*-dependent connection between a Warburg-like OXPHOS-to-glycolysis shift in cellular bioenergetics and tumor-initiating capacity is well illustrated in the metabolo–genetic processes accompanying the generation of induced pluripotent stem cells (iPSCs).[107-109] The inclusion of the *c-Myc* oncogene in the cocktail of nuclear reprogramming factors OSKM (i.e., *Oct4*, *Sox2*, *Klf4*, and *c-Myc*) potentiates the pluripotent glycolytic behavior and the tumorigenic incidence of derived iPSCs; conversely, *c-Myc* removal decreases the tumorigenicity of iPSCs and facilitates OXPHOS-dependent lineage commitment and terminal differentiation. In cancer, metabolic reprogramming can be mediated by crosstalk between HIF-1α and *c-Myc*. Moreover, SIRT1 is known to directly regulate *c-Myc* transcriptional activity in cancer cells, either via the deacetylation of *c-Myc* or by binding *c-Myc* and promoting its association with *Max*.[110-113] In this regard, it is intriguing that David Sinclair's group now reveals for the first time that the nuclear ability of SIRT1/HIF-1α to inhibit mitochondrial OXPHOS genes functions by decreasing *c-Myc*-regulated transcription of the key mitochondrial transcription factor A (TFAM).[23] TFAM is required for mitochondrial DNA replication, transcription, and maintenance, and low levels of TFAM appear to lead to first-phase OXPHOS dysfunction triggered by the aging-associated decline of NAD^+.[114,115] Although the transition to an irreversible phase 2 OXPHOS dysfunction remains to be characterized, this process might be related to increased ROS production. It is

important to note that mitochondrial dysfunction due to TFAM downregulation been shown to enhance tumorigenesis via mitochondrial genome instability and the production of ROS.[116]

Despite the pervasive role of *Myc* in human cancer, its requirement for proliferation and maintenance of adult stem cell compartments has raised considerable skepticism regarding the therapeutic value of *Myc,* given the expected toxicity of *Myc* inhibition for healthy tissues. However, the development of the *Myc*-interfering molecule termed Omomyc, which binds c- and N-Myc, Max, and Miz-1 but does not bind Mad or certain HLH proteins, has demonstrated that it might cause edge-specific perturbations in the *Myc* interactome that destroy specific protein interactions of the *Myc* node while leaving others intact.[117-123] This results in a "rewriting" of the *Myc* transcriptome in a manner that produces opposing effects on the 2 arms of Myc activity, namely transactivation and transrepression of gene transcription. Specifically, Omomyc prevents Myc binding to promoter E-boxes and the transactivation of target genes while allowing Myc to retain Miz-1-dependent binding to promoters and transrepression function. Remarkably, Omomyc has a significant effect on the expression of genes encoding metabolic enzymes, including those involved in glycolysis (e.g., phosphoglycerate kinase 1 and lactate dehydrogenase B) and those involved in the expression of oncometabolites such as IDH2. In this scenario, it would be of interest to determine how Omomyc not only impacts the ability of declining NAD^+ to disrupt nuclear–mitochondrial communication with age, but also affects the malignant behavior of tumors with metabolic enzyme mutations that produce oncometabolites. Critically, Omomyc might be utilized in malignant scenarios in which the accumulation of the oncometabolite 2-HG appears in the absence of metabolic enzyme mutations. A recent, pioneering study by Terunuma et al.[124] revealed that the oncometabolite 2-HG accumulates at high levels in a subset of tumors and human breast cancer cell lines.[124] Importantly, the activation of the *Myc* pathway mechanistically phenocopied *IDH* mutations, which were absent in 2-HG-overexpressing breast carcinomas. Myc/2-HG-high tumors exhibited a distinct, increased DNA methylation pattern that was associated with a poor prognosis, a stem cell-like transcriptional signature, and a tendency to overexpress glutaminase, thus suggesting a functional relationship between glutamine and 2-HG metabolism in breast cancer.[124] Because these findings clearly implicate 2-HG as a candidate breast cancer oncometabolite associated with *Myc* activation and poor prognosis, Myc/2-HG-high tumors become an ideal scenario in which to explore whether Myc-disrupting strategies (e.g., Omomyc) can impede the signaling between oncometabolites and pathways resulting in malignancy. Importantly, as is the case for anti-aging interventions, this unique therapeutic strategy may prevent selection for resistance.

Corollary

From an evolutionary perspective, it is reasonable to view the alterations in mitochondrial homeostasis driven by the gerometabolite NAD^+ as a conserved response to changes in energy supplies and oxygen levels. Similarly, the natural ability of 2-HG in the absence of *IDH* mutations to significantly alter epigenetics might reflect an evolutionarily ancient status of certain metabolites to signal for elevated glutamine/glutamate metabolism and/or oxygen deficiency. For example, in human cells proliferating under hypoxia, α-ketoglutarate can accumulate and be metabolized through an enhanced reductive activity of wild-type *IDH2* in the mitochondria, leading to 2-HG accumulation.[125] Crucially, the constitutive activation of HIF-1α is sufficient to recapitulate IDH2-dependent carboxylation of glutamine-derived α-ketoglutarate and the associated, concomitant increased synthesis of 2-HG in cells with wild-type *IDH1* and *IDH2* even in normoxic conditions, thus offering a plausible connection between gerometabolites-induced pseudohypoxia and oncometabolites-induced epigenetic deregulation (**Fig. 3**). As mentioned above, a functional relationship between glutamine metabolism and 2-HG accumulation has also been identified in biologically aggressive, Myc-overexpressing breast carcinomas in the absence of *IDH* mutations.

We are now beginning to understand how the chronic alteration of cellular responses to gerometabolites and oncometabolites became conserved causes of aging and cancer, at least in part by aberrantly connecting a Warburg-like reprogramming of cellular bioenergetics with pseudohypoxia-related (epi)transcriptional circuitries. It appears that a yet-to-be-defined complex, interacting with steps of inhibition, disruption, or activation in metabolic pathways, is likely to be involved in the causal connection between gerometabolites (e.g., those having gerosuppressant activities similar to that of NAD^+ or yet-to-be discovered metabolites with geropromoting activities) and aging as well as between oncometabolites and an enhanced potential for cell transformation to malignancy. This paradigmatic shift in the discussion of the links between cellular metabolism and aging diseases can be further fueled by the identification of well-characterized, cancer-associated mutations in genes encoding enzymes with significant roles in cellular metabolism and by adopting the view that aging and aging diseases such as cancer are governed not only by genetic and epigenetic controllers, but also by a pivotal regulatory role of metabolic reprogramming in cell fate decisions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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