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Review

The multifaceted contribution of α-ketoglutarate to tumor progression: An opportunity to exploit?

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HIGHLIGHTS

- αKG is at the center of metabolic reactions essential for cells.
- αKG regulates enzymes involved in hypoxic adaptation and epigenetic modifications.
- Elevated intracellular levels of αKG impinge on tumor progression.
- Increase of αKG levels may represent a possible anti-cancer strategy.

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ABSTRACT

The thriving field that constitutes cancer metabolism has unveiled some groundbreaking facts over the past two decades, at the heart of which is the TCA cycle and its intermediates. As such and besides its metabolic role, α-ketoglutarate was shown to withstand a wide range of physiological reactions from protection against oxidative stress, collagen and bone maintenance to development and immunity. Most importantly, it constitutes the rate-limiting substrate of 2-oxoglutarate-dependent dioxygenases family enzymes, which are involved in hypoxia sensing and in the shaping of cellular epigenetic landscape, two major drivers of oncogenic transformation. Based on literature reports, we hereby review the benefits of this metabolite as a possible novel adjuvant therapeutic opportunity to target tumor progression. This article is part of the special issue "Mitochondrial metabolic alterations in cancer cells and related therapeutic targets".

1. Introduction

Tricarboxylic acids (TCA) cycle resumes a succession of metabolic oxidative reactions of carbohydrates-derived carbon taking place in the mitochondrial matrix to provide reducing equivalents NADH and $FADH_2$ necessary for the mitochondrial electron transport chain to

Abbreviations: Ac-CoA, Acetyl Coenzyme A; ACO2, Aconitase 2; AML, Acute Myeloid Leukemia; ARNT, Aryl Hydrocarbon Receptor Nuclear Translocator; CI, Complex I; CPT-1, Carnithine Palmitoyl Transferase-1; CREB, cAMP Response Element-Binding Protein; CIT, Citrate; EGF, Epidermal Growth Factor; Fga, Fatiga; FH, Fumarate Hydratase; FIH-1, Factor Inhibiting HIF-1α 1; GBBH, ɤ-Butyrobetain Hydroxylase; GDH, Glutamate Dehydrogenase; GLS, Glutaminase; GOT1, Glutamate Oxaloacetate Transaminase 1; GOT2, Glutamate Oxaloacetate Transaminase 2; GPT1, Glutamate Pyruvate Transaminase 1; GPT2, Glutamate Pyruvate Transaminase 2; GS, Glutamine Synthetase; HG, Hydroxyglutarate; HIF, Hypoxia Inducible Factor; ICT, Isocitrate; IDH, Isocitrate Dehydrogenase; IkB-α, NF-kB Inhibitor Alpha; IKKβ, Inhibitor Of Nuclear Factor Kappa-B Kinase Subunit Beta; Jmjc, Jumonji-C; KDM, Histone Lysine Demethylases; mTORC1, mammalian Target Of Rapamycin Complex 1; NF-kB, Nuclear Factor Kappa-Light-Chain-Enhancer Of Activated B Cells; ODDD, Oxygen Dependent Degradation Domain; OG, Oxoglutarate; OGDD, Oxoglutarate Dependent Dioxygenase; OAA, Oxaloacetate; P4H, Collagen Proline 4-Hydroxylase; PHD, Prolyl Hydroxylase; SA, Succinate; SC, Stem Cell; SCoA, Succinyl Co-A; SDH, Succinic Dehydrogenase; Sima, Similar; TCA, Tricarboxylic Acid; TET, Ten Eleven Translocation; Tgo, Tango; VDAC, Voltage Dependent Anion Channel; VHL, Von Hippel Lindau; αKG, α-ketoglutarate; αKGDH, α-ketoglutarate dehydrogenase

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produce energetic molecule ATP [1]. TCA intermediates are also required for amino acids, nucleotides and lipids biosynthetic process. In this context, α-ketoglutarate (αKG) or 2-oxoglutarate (2-OG) is recognized as a metabolite with pleiotropic activity being at the center of a wide range of physiological processes besides cellular metabolism. The αKG molecule comprises two carboxylic groups displaying weak acidic properties as well as a ketone group in the α-position, which influences the compound's reactivity. This ketoacid is the product of the reversible reaction of isocitrate decarboxylation catalyzed by NADP-dependent isocitrate dehydrogenase isoforms (IDH1/2), localized in the cytoplasm and mitochondria, respectively. Furthermore, the heterotrimeric IDH3 complex located in the mitochondria provides the not reversible NAD-dependent conversion of isocitrate to αKG. This enzyme is evolutionarily distinct from the other two isoforms and is allosterically regulated by energy charge and NADH levels [2]. An additional metabolic reaction providing αKG is glutaminolysis, in which deamination of glutamine by glutaminase (GLS) gives rise to glutamate and subsequent deamination of this latter by glutamate dehydrogenase (GDH) produces αKG and ammonia [3] (Fig. 1). αKG can also be produced *via* glutamine transamination to an intermediate ketoacid, namely α-ketoglutaramate, which after an ω-amidase-catalyzed hydrolysis releases αKG and ammonia [4]. Equally important but often overlooked, ω-amidase activity is detected in both mitochondrial and cytosolic compartments especially in mammalian liver and kidney. It is thought to be involved in cancer biology as a consequence of the strictly anaplerotic nature of its reaction [5]. Through the glutamine/αKG axis, this ketoacid is tightly involved in amino acids metabolism, particularly important in fast growing cancer cells [6] (Fig. 1). Likewise, glutamate pyruvate transaminases (GPT1/2) and glutamate oxaloacetate transaminases (GOT1/2) produce αKG from glutamate/pyruvate and oxaloacetate transamination reactions, respectively (Fig. 1) [7]. Like most of the TCA cycle intermediates, αKG is transported from the mitochondrial matrix to the cytoplasm and *vice versa*. It crosses the outer mitochondrial membrane through the voltage-dependent anion channel (VDAC), and the inner mitochondrial membrane through the αKG/malate antiporter [8]. In the past years, αKG raised much attention as one of the central metabolic regulators of tumor fate and it has been recognized as a critical player during development. For these reasons, we here reviewed the multifaceted role of αKG in cell physiology and human pathology, focusing on its impact on cancer and suggesting possible adjuvant therapeutic strategies based on this metabolite. We will also discuss the advantages of the use of Drosophila melanogaster as a valuable model for the study of cancer metabolism and for the identification of novel metabolic-based therapeutic intervention.

2. αKG at the crossroad of physiological cell processes

Besides its metabolic role as TCA intermediate, αKG is directly involved in several cell processes as substrate for biosynthetic reactions or by regulating certain enzymes involved in the most diverse cell functions (Fig. 2). It contributes to the regulation of protein synthesis being the substrate of the reversible GDH reaction for glutamate biosynthesis that can be subsequently converted to proline, arginine or glutamine. In particular, the latter reaction is catalyzed by glutamine synthetase (GS). In the past years, αKG gained much attention as a precursor of glutamine since this is a nutritionally semi-essential amino acid necessary for proper growth in most cells and tissues. The functional peculiarities of αKG reside in the fact that it is more stable than glutamine in water solution, does not increase nitrogen load in the organism and is not neurotoxic like glutamate [3]. Several studies have shown that intravenous administration of αKG-ornithine salt is effective in chronically malnourished subjects [9], ameliorates wound healing in severe burn patients [10], sustains protein synthesis in skeletal muscle and reduce sarcopenia after surgery [11,12]. In this context, it has been recently demonstrated that dietary supplementation with αKG induced skeletal muscle hypertrophy in mice through the activation of the mTORC1 mediated protein synthesis [13] and prevent muscle wasting in a mouse model of Duchenne muscle dystrophy [14]. By controlling L-carnitine biosynthesis from ε-N-trimethyl-lysine, αKG is also involved in regulating lipid metabolism [15]. In particular, when pyruvate import into the mitochondria is inhibited and glucose catabolism is slowed down, anaplerotic reactions produce αKG from glutamine to generate Ac-CoA supporting biosynthetic pathways including fatty acid synthesis to sustain cell growth and proliferation [16,17]. These data highlight the metabolic flexibility of cells to fuel the TCA cycle in order to maintain the cellular homeostasis. Furthermore, non-enzymatic oxidative decarboxylation of αKG neutralizes hydrogen peroxide (H_2O_2) and restores cellular redox state to prevent oxidative stress damage [18]. αKG ketone group on the α-carbon reacts with the oxidative agent releasing CO_2, H_2O and succinate (SA) in mammalian cells, as well as in other models including *D. melanogaster* [19]. In addition, αKG reduces both ammonia- and ethanol-induced oxidative stress and provides detoxification in cyanide poisoning in animals' liver and brain by binding cyanide with its carbonyl group to form the intermediate a-ketoglutarate-cyanohydrin [20] and enhancing antioxidant enzymes activities and glutathione levels [21,22].

Most important, αKG is the obligate co-substrate of Fe(II)/2-oxoglutarate-dependent dioxygenases (OGDD), a superfamily of enzymes that catalyze the oxidative decarboxylation of αKG producing succinate

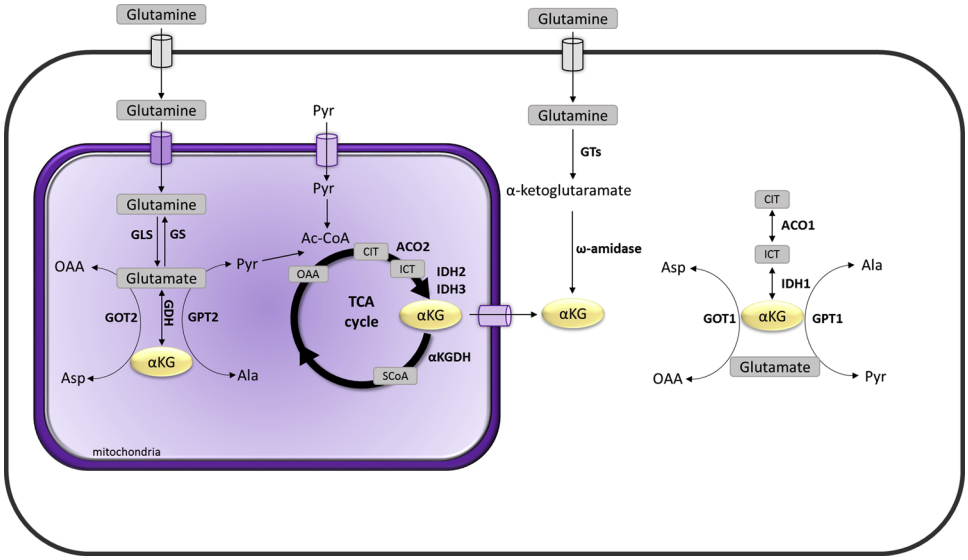

Fig. 1. αKG biosynthetic pathways. αKG derives from glutamine and glutamate deamination in the mitochondria, mediated by glutaminase (GLS) and glutamate dehydrogenase (GDH), respectively. In the cytosol, it can be produced from glutamine *via* generation of α-ketoglutaramate, catalyzed by glutamine transaminases (GTs), that is subsequently converted into αKG by ω-amidase. Moreover, αKG originates from transamination of glutamine by GTs or glutamate by GPT1/GOT1, in the presence of Pyr/OAA respectively. Inside the mitochondria, GPT2 and GOT2 isoforms catalyze the same reactions. Lastly, αKG derives from the conversion of isocitrate catalyzed by isocitrate dehydrogenases IDH2 and IDH3 in the mitochondria or IDH1 in the cytosol.

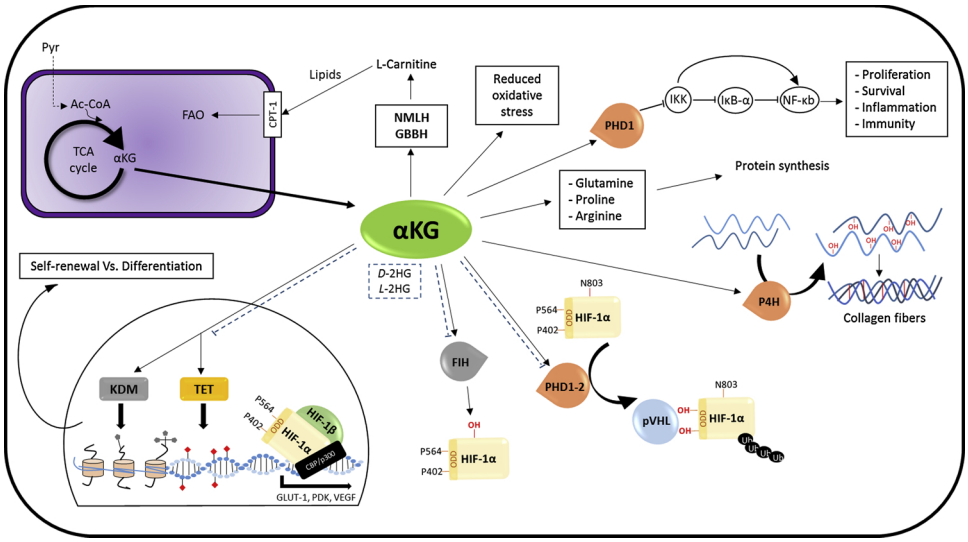

Fig. 2. Overview of αKG impact on cell homeostasis. αKG is involved in multiple cellular processes in both physiological and pathological conditions. It protects against oxidative stress, modulates protein and lipid biosynthesis, and it is involved in fatty acids β-oxidation (FAO). As substrate of 2-OG dependent dioxygenases, it activates hydroxylases (PHDs and FIH) to limit HIF-1α signaling, by stimulating pVHL mediated protein poly-ubiquitination and proteasomal degradation in normoxia. The metabolite also affects gene expression and cell stemness by stimulating epigenetic DNA and histone demethylation by TET and JmjC enzymes. Finally, αKG activates PHD1 to induce NF-κB degradation, and collagen fibers hydroxylation at proline residues by αKG-dependent proline 4-hydroxylase allows collagen monomers stabilization.

and CO_2 from O_2 [23]. These enzymes require Fe(II) as cofactor and ascorbate to reduce iron in the active site. Several enzymes providing different biological functions belong to this superfamily, including hypoxia-sensing system, DNA and histone demethylases, collagen stabilizing enzymes, carnitine biosynthesis, ankyrin and EGF hydroxylation [24] (Fig. 2). Among these processes, αKG regulates the stabilization of collagen fibers under the effect of two types of OGDD, namely proline 4(*R*)-hydroxylase (P4H) and proline 3(*S*)-hydroxylases. These enzymes provide the hydroxylation of prolines at positions Yaa and Xaa respectively of the triplet Xaa-Yaa-Gly composing collagen, favoring the folding of collagen triple helixes thus setting the pavement to other post-translational modifications [25]. The hypoxia sensing system and enzymes responsible for epigenetic modifications regulated by αKG will be discussed in details in the context of cancer, although they play a critical role also in physiological processes. For example, αKG regulates the signaling cascade of transcription factor NF-κB implicated in inflammatory response, cell survival and proliferation by stimulating the oxygen-dependent prolyl hydroxylase PHD1 that inactivates IKKβ kinase *via* hydroxylation of Pro191, which in turn phosphorylates Inhibitory κB-α (IκB-α) triggering NF-κB [26]. On the other hand, αKG produced by glutaminolysis regulates polarization of macrophages toward M2 subpopulation [27]. Moreover, histone and DNA demethylation are mediated by enzymes belonging to the OGDD family, respectively, Jumonji-C domain-containing family (JmjC) and Ten Eleven Translocation proteins (TET1-3) [28–30]. In this frame, high levels of αKG were found to favor self-renewal in naïve mouse embryonic stem cells (SCs) promoting histone/DNA demethylation which contribute to the regulation of pluripotency-associated gene expression [31]. Conversely, high levels of αKG induced global histone and DNA demethylation in primed human or mouse epiblast SCs leading to differentiation, supporting the idea that αKG modulates stemness properties depending on the pluripotent state [32]. Overall, αKG is involved in a plethora of physiological reactions, reflecting the dynamism of intracellular metabolic reactions and the centrality of this metabolite in the regulation of physiological processes.

3. Role of αKG in tumor progression

3.1. Involvement in cancer cell metabolism

In cancer cells, glycolysis and TCA cycle intermediates are deviated from energy production to provide the necessary precursors for macromolecules biosynthesis and to support cell proliferation. Such metabolic reprogramming is one of the hallmarks of cancer cells that use a series of anaplerotic reactions to replenish the TCA cycle intermediates [33]. One of the most important is glutaminolysis, an anaplerotic pathway that refills the TCA cycle with αKG in fast proliferating cells. In these cells, the TCA cycle suffers a strict deficit at the level of citrate that is shuttled to the cytosol for the purposes of lipid biosynthesis. As a general metabolic setting, cancer cells prevalently oxidize αKG derived from glutaminolysis to succinyl-CoA in order to feed the TCA cycle for their energy supply and NADPH, instead of engaging into oxidative decarboxylation. Indeed, glutamine becomes the main source of OAA lost for biosynthetic activities and thereby necessary to maintain the cycle ongoing. However, glutamine-derived αKG can also undergo mitochondrial reductive carboxylation as the main carbon source to maintain citrate levels and support lipid synthesis, under specific stress conditions, such as hypoxia [34,35], oxidative phosphorylation defects [6] or impairment of mitochondrial pyruvate import [16,17]. Cytoplasmic and mitochondrial NADPH-dependent isocitrate dehydrogenases (IDH1 and IDH2, respectively) were shown to support this mechanism. Furthermore, it has been proposed that energy availability and the mitochondrial membrane potential can control the TCA flux tuning IDH3 and IDH2 activity, as IDH3 is inhibited by high energy charge and NADH levels while IDH2 requires NADPH, which is provided by the H+-transhydrogenase reaction and driven by the mitochondrial electrochemical gradient [36]. The reductive carboxylation of αKG depends also on αKG oxidation reaction and on αKG/citrate ratio rather than on single metabolites levels alone [6,37]. Moreover, it has been shown that αKG oxidation prevails on reductive decarboxylation even in presence of mitochondrial dysfunction and that the balance between these two routes depends on the oxidative phosphorylation fitness [38], highlighting the pivotal role of αKG in feeding at the same time both reductive and oxidative pathways of the TCA cycle.

3.2. Regulation of hypoxic response

The maintenance of appropriate oxygenation is necessary to sustain physiologic cell functions [39], although cells, tissues and even organisms are continuously exposed to reduced oxygen level (hypoxia). Hypoxia plays a critical role in both physiological and pathological contexts, where its effects can be more or less severe, depending on the O_2 levels, duration and context [40]. Of particular relevance is the contribution of hypoxia in tumor progression, selection of cancer cells and metastasis formation. In fact, cancer cells are frequently exposed to chronic or intermittent acute hypoxia triggering complex transcriptional responses to adapt to environmental conditions. Hypoxia Inducible Factors (HIFs), a family of heterodimeric transcription factors consisting of an oxygen sensitive α-subunit and a constitutively

expressed β-subunit, control such transcriptional response. Three paralogs of HIF-α (HIF-1α, HIF-2α, and HIF-3α) and two paralogs of the HIF-β subunit (ARNT, ARNT2) have been found in human [39]. HIF-1α is ubiquitously expressed, while HIF-2α and HIF-3α are preferentially expressed in specific tissues [41]. Although intracellular abundance of HIF-1α can be regulated in a O_2 independent fashion [42], the canonical regulation of HIF-1α involves the hydroxylation of Pro402 and Pro564 in the oxygen dependent degradation domain (ODDD) by prolyl hydroxylases (PHDs) in response to oxygen tension. Hydroxylation of these conserved residues triggers the pVHL-mediated ubiquitination and the consequent proteasomal degradation of HIF-1α subunit (Fig. 2). The parallel hydroxylation of Asn803 mediated by the factor inhibiting HIF-1 (FIH-1) prevents the transactivation function blocking the interaction of HIF-1α with p300/CREB [43]. Under hypoxic conditions (0.05–5% O_2), PHDs and FIH-1 are inhibited, HIF-1α is stable, translocates into the nucleus, dimerizes with HIF-1β and binds with p300/CREB activating the hypoxic transcriptional program. PHDs and FIH-1 belong to the already described OGDD superfamily and hydroxylate the target residues of HIFα [44,45]. These enzymes are O_2 sensors since their K_m is around 100 μM while oxygen concentrations in tissues is around 10–30 μM and, thus, intracellular O_2 is rate limiting for enzymatic activity under physiological conditions [41,46,47]. Similarly, abundance of αKG regulates PHDs activity thus controlling the response to hypoxic environment. The PHDs K_m for αKG is about 50 μM, very close to its physiological concentration. Hence, even modest variations in αKG abundance may interfere with PHDs activity leading to profound modifications of cancer cells metabolism [24,48]. In line with this, we demonstrated that the co-occurrence of reduced oxygen consumption and increased levels of αKG in respiratory Complex I (CI) deficient cells boost PHDs activity leading to a chronic HIF-1α destabilization even in hypoxic environment, a condition defined *pseudonormoxia* [49–53]. These data are also corroborated by the fact that αKG can allosterically stimulate PHD activity by increasing its affinity for oxygen, allowing the destabilization of HIF-1α also at very low concentrations of oxygen [42,54,55].

3.3. Oncometabolites and control of epigenetic modifications

As already described cancer cells adapt to adverse microenvironmental conditions and oncogene activation by modulating their metabolic routes. Such metabolic reprogramming is often associated to the accumulation of specific metabolites that have been termed "oncometabolites" for which a role in the progression toward malignancy has been demonstrated [56]. To date, three TCA intermediates are considered true oncometabolites, namely succinate, fumarate and D-2-hydroxyglutarate (D-2HG). Their role in tumor progression has been discovered after the identification in different cancers of mutations in genes encoding for succinate dehydrogenase (SDH) [57–59], fumarate hydratase (FH) [60] and isocitrate dehydrogenase (IDH) [61,62], respectively. Loss of function mutations in SDH and FH genes lead to the accumulation of succinate and fumarate that act as competitive inhibitors of αKG-dependent dioxygenases, including PHDs. The inhibition of PHDs prevents HIF-1α hydroxylation and the consequent degradation even in normoxia, a condition called *pseudohypoxia* [63,64]. Heterozygous somatic mutations affecting *IDH1/2* genes have been found in glioma and acute myeloid leukemia (AML) [61,62,65]. Mutations of R132 in *IDH1* and R172 or R140 in *IDH2* impair the isocitrate binding inducing a shift in the enzyme's substrate affinity and catalytic activity [66] and in turn a neomorphic function. Indeed, this translates in a loss of normal activity and a gain of a NADPH-dependent function: IDH reduces αKG to D-2G causing accumulation of the latter to supraphysiological levels [65,67,68]. Increased levels of D-2HG may also derive from glutamine reductive carboxylation under hypoxic conditions in IDH *wild type* cells [34]. The enantiomer L-2HG has also been appointed as an oncometabolite [69,70] and can be accumulated in hypoxia or in presence of severe mitochondrial defects [6,70,71]. Being structural analogues of αKG, both enantiomers are weak competitive inhibitors of OGDD occupying the enzyme's catalytic site, with different levels of potency [72] (Fig. 2). In particular, increased D-2HG levels inhibit αKG-dependent lysine demethylases and inhibits the TET family of 5-methylcytosine hydroxylases, inducing histone and DNA hypermethylation in different models [73]. Experimental and clinical data support the hypothesis that D-2HG induced dysregulated histone and DNA methylation sustains stemness of cancer cells favoring their progression toward malignancy [74,75]. Moreover, although 2HG effect on PHDs is still debated, both enantiomers markedly reduced FIH-catalyzed hydroxylation in HIF-1α C-terminal transactivation domain [76,77].

Interestingly, hydroxyglutaric acidurias (single or combined) are inherited neurological disorders characterized by accumulation of D-2HG or L-2HG or both enantiomers and presenting with a complex clinical manifestation that includes developmental delay, seizures and hypotonia, plus other miscellaneous symptoms (reviewed in [78]). D-2HG aciduria can be found in the autosomal recessive Type I form caused by loss of function mutations in *D2HGDH* encoding for D-2HG dehydrogenase that converts D-2HG in αKG, or the autosomal dominant Type II form generated by gain of function missense mutations in *IDH2* [79]. Autosomal recessive L-2HG aciduria instead is caused by loss of function missense mutations in *L2HGDH* encoding for L-2HG dehydrogenase that catalyzes the FAD dependent conversion of L-2HG to αKG [78]. Moreover, autosomal recessive combined D-2HG and L-2HG aciduria has been found to be induced by loss of function mutations in *SLC25A1* and *SLC25A4* encoding respectively for the mitochondrial citrate carrier and the adenine nucleotide translocase 1 [80,81]. In particular, in the case of L-2HG aciduria, a co-morbidity with development of malignant brain tumors has been reported [82,83], highlighting a pro-tumorigenic role of elevated levels of L-2HG.

4. Is αKG an anticancer agent?

Given the central role of αKG in cancer cell metabolism and transcriptional program, the possibility of using this metabolite as an anticancer agent to counteract oncogenic processes has emerged over the past years. Several strategies attempted to increase αKG intracellular levels in order to prevent tumor progression toward malignancy. Taking into account the affinities for OGDDs [46], studies reported the competitive inhibitory effect is not only mediated by the accumulation of oncometabolites but is rather dependent on the ratio metabolite/αKG. For example, it has been demonstrated that 2-HG inhibition of histone demethylases can be reversed by increasing αKG amounts [72]. Similarly, we demonstrated that defective respiratory CI induced the increase of αKG/SA ratio leading to HIF-1α destabilization in hypoxia and reduced tumor growth both *in vitro* and *in vivo* [51,53], indicating that targeting respiratory CI may represent a feasible strategy to prevent tumor progression. However, lack of this enzyme does not induce a complete eradication of the tumor, but rather a slowdown of proliferation. In fact, cancer cells missing CI were unable to adapt to hypoxic conditions but they recruit pro-tumorigenic M2 macrophages that support tumor growth at a late stage [53]. Whether the polarization of macrophages toward M2 population is due to secretion of αKG, in agreement with what previously reported [27], or to other molecular mechanisms, is still not known. However, the prevention of hypoxic adaptation surpass the ability of αKG to recruit M2 macrophages, at least in the initial phases of tumor progression, inhibiting tumor growth and clearly opening a temporal window for further therapeutic approaches. Accumulation of αKG due to αKGDH inactivation triggers TET1 and TET3 protein expression and enzymatic activity in breast cancer, limiting cell migration and epithelial-mesenchymal transition [84]. Based on these evidences, it has been proposed to use increase αKG intracellular levels as a possible anticancer strategy. Treatment with exogenous αKG was shown to induce a dose-dependent HIF-1α

destabilization in hypoxia due to proteasomal degradation [85,86]. Moreover, a recent work showed that exogenous supplementation of αKG is able to prevent tumor growth and metastasis formation of triple negative breast cancer cells by switching the metabolism from glycolytic to oxidative [87]. In these models, increased levels of αKG trigger succinate dehydrogenase and fumarate hydratase levels while switching off glycolytic enzymes, inducing a decrease in fumarate and succinate abundance ultimately leading to HIF-1α destabilization [87]. However, αKG cell permeability is generally low and thus cell permeable αKG ester derivatives were developed by coupling the ketoacid with hydrophobic carriers [54,55]. Supplementation with these derivatives was able to reverse pseudohypoxia through reactivation of PHD2 inhibited by fumarate and succinate or to impede hypoxic adaptation, leading to HIF-1α destabilization regardless pO_2 and preventing tumor progression [54,55]. Upon treatment with αKG derivatives, HIF downstream targets were downregulated preventing neoangiogenesis, dramatic metabolic alterations were reported and apoptosis was triggered in cancer cell [54,85,86,88–90]. In this frame, αKG was also found to have an anti-proliferative effect even in normoxia by preventing DNA synthesis and inducing a cell cycle arrest in G1 phase [91]. Moreover, increased intracellular levels of αKG counterbalances succinate-mediated inhibition of OGDD [92] and stimulates histone demethylation by JMJD to induce senescence [93]. Lastly, αKG was found to upregulate H3/H4 histone acetylases, TET3 as well as DNA demethylation in IDH-knockdown colon cancer cells overcoming their resistance to apoptosis [94]. Hence, αKG represents an intriguing tool to tackle malignant transformation and tumor progression also considering that a metabolic intermediate is supposed to present with a low toxicity in normal cells. The few studies here reported highlight the importance of αKG and the need of more extensive investigations to unveil the real potential of this metabolite as a possible therapeutic strategy for those tumors in which hypoxic adaptation and epigenetic modifications induced by hypoxia are important to progress toward malignancy.

5. *Drosophila* as an alternative model for the study of cancer metabolism and hypoxic response

As already mentioned, αKG is a hydrophilic molecule with a low propensity to permeate into cells and a strategy to increase its permeability is to conjugate the ketoacid with hydrophobic carriers [54,55]. In this context, the availability of a wide number of hydrophobic molecules and the possibility to link αKG with hydrophobic compounds with a possible synergistic anti-cancer effect is extremely appealing. Hypothetically, a huge number of αKG derivatives can be synthesized, but the screening of such molecules may not only rely on *in vitro* studies and requires suitable *in vivo* models for cancer. In this frame, *D. melanogaster* is an excellent platform for cancer onset and progression studies, as most of the signaling pathways and genes deregulated during tumorigenesis are closely preserved [95]. Furthermore, the events undergoing cancer evolution are conserved from *Drosophila* to humans, such as increased cell proliferation, resistance to cell death, metastases formation and spread, tumor associated angiogenesis and energy metabolism reprogramming [96]. Similar to human, *Drosophila* cancer cells are exposed to a low oxygen environment [97,98] and adapt to this condition by reprogramming their metabolism towards aerobic glycolysis [99,100]. The up-regulation of glycolytic enzymes occurs in a HIF-1α dependent manner, which plays a central role in hypoxia response also in flies [101] (Fig. 3A–B). The whole process is highly conserved in *Drosophila* and its components are known as Similar (Sima, the HIF-1α orthologue), Tango (Tgo, the HIF-1β orthologue), Fatiga (the only orthologue of human PHDs) and dVhl (*Drosophila* von Hippel-Lindau) [101–104] (Fig. 3C–D). Nuclear accumulation of Sima/HIF-1α in *Drosophila* epithelial tumor models with consequent cascade activation involved in vessel remodeling for oxygen supply of growing tumor mass has been reported [98,105]. Moreover, cell migration and invasion were stimulated by chronic hypoxia characterized by nuclear accumulation of Sima/HIF-1α, while acute hypoxia blocked or delayed the migratory wave [106]. In this context, we have recently demonstrated that the down-regulation of respiratory CI subunit (NDUFV1, the ND51 mammalian orthologue) not only prevents Sima/HIF-1α nuclear accumulation, but it also markedly reduces tumor growth without any signs of vessel associated tumor remodeling [53]. Our *Drosophila* model recapitulate what we previously observed in human cancer cells in which respiratory CI impairment leads to an accumulation of NADH and αKG causing the destabilization of HIF-1α mediated by PHDs activation, as detailed above. Moreover, it has been found that cancer cells metabolism can be compared to the embryonic cells of *Drosophila* [107], as they share the same metabolic program to support the rapid growth with a coordinated upregulation of glycolytic and OXPHOS genes, although oxidative metabolism seemed to be repressed *via* accumulation of TCA cycle intermediates (*i.e.* citrate, isocitrate and αKG). Nonetheless, the metabolic pathways described above in which αKG plays a central role are also conserved in flies. Interestingly, the supplementation of *Drosophila* standard diet with αKG, reduced oxidative stress and toxicity of cyanide-related compounds and aluminum [19,108] and neutralizes the ethanol toxicity through alcohol dehydrogenase upregulation [109]. Similarly, the antioxidant activity of

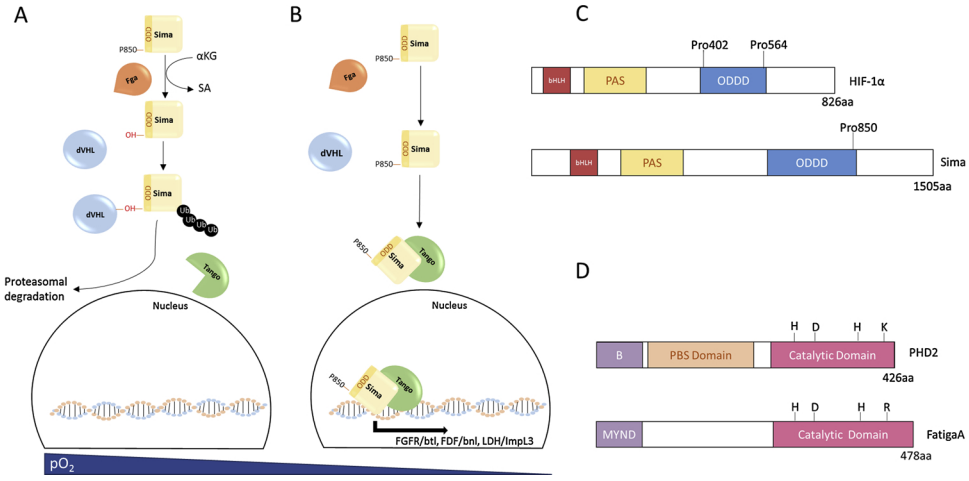

Fig. 3. The conserved hypoxic signaling from Drosophila to human. **(A)** The Sima/ HIF-1α signaling cascade is conserved. In normoxic condition, Fga/PHDs hydroxylate Sima at a single Pro residue (P850). Subsequently, Sima is bound by dVHL that conveys it to proteasome-mediated degradation. **(B)** During hypoxia, Sima is stabilized and, dimerizing with its partner (Tango/HIF-1β), it translocates into the nucleus where induces the transcriptional program in response to oxygen deprivation. **(C)** Functional domains of HIF-1α/Sima are conserved between human and flies. The bHLH domain represents the DNA binding site (red), the PAS domain (yellow) is the dimerization point with their partners (HIF-1β/Tango) and in the oxygen-dependent degradation domain (ODDD, blue) the Proline residues are substrates of the PHDs catalytic domain. **(D)** Functional domains of PHDs/Fatiga are conserved between human and flies. The N-terminal domain is showed in purple (MYND). In the catalytic domain (pink) the His-Asp-His residues are preserved. They are the triad amino acids necessary for iron binding.

αKG was also observed by analyzing the metabolic activity recovery of the fly at low temperatures (0 °C for 15 min). The switch at low temperature triggers a coma-like state of flies with a strong reactive oxygen species production. Treatments with a diet rich in αKG helps the recovery of metabolic activity and motor functions, due to the αKG ability to increase the amino acid production and protein synthesis [109]. Overall, these results suggest that the study of metabolic programs in *Drosophila* on embryonic cells, on some stages of development and in cancer models could provide new information on the coordination of glycolysis, mitochondrial pathways and oxidative metabolism during tumor growth. The many advantages of this *in vivo* model, such as the presence of sophisticated genetic tools, the large number of individuals, the short life cycle, genes and molecular pathways conservation and the possibility of performing drug screening could allow the use of the fruitfly as an excellent model to recapitulate and reveal different aspects of human cancer.

6. Conclusions

Besides its metabolic role as TCA intermediate, αKG is involved in a plethora of physiological reactions and in several cell processes. In the last two decade, metabolomics studies have shown a hub role of αKG in the metabolic reprogramming required for cancer cells survival and proliferation. In addition, as allosteric activator of PHDs αKG is a pivotal regulator of the hypoxic response and epigenetic modifications, two major drivers of oncogenic transformation. This scenario opens the opportunity to exploit the multifaceted contribution of αKG on metabolic rewiring and hypoxic adaptations as a potential adjuvant therapeutic strategy to impinge on tumor progression.

Declarations of interest

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