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REVIEW

Inborn and acquired metabolic defects in cancer

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Abstract The observation that altered metabolism is the fundamental cause of cancer was made by Otto Warburg nearly a century ago. However, the subsequent identification of oncogenes and tumor suppressor genes has displaced Warburg's theory pointing towards genetic aberrations as the underlining cause of cancer. Nevertheless, in the last decade, cancer-associated mutations have been identified in genes coding for tricarboxylic acid cycle (TCA cycle, also known as Krebs cycle) and closely related enzymes that have essential roles in cellular metabolism. These observations have revived interest in Warburg's hypothesis and prompted a flurry of functional studies in the hope of gaining mechanistic insight into the links between mitochondrial dysfunction, metabolic alterations, and cancer. In this review, we discuss the potential pro-oncogenic signaling role of some TCA cycle metabolites and their derivatives (oncometabolites). In particular, we focus on their effects on dioxygenases, a family of oxygen and α-ketoglutarate-dependent enzymes that control, among other things, the levels and activity of the hypoxia-inducible transcription factors and the activity of DNA and histone demethylases.

Keywords Cancer · Metabolism · Mutation · Succinate dehydrogenase · Fumarate hydratase · Isocitrate dehydrogenase

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Introduction

At the beginning of the 20th century, Otto Warburg observed that cancer tissues have high rates of glycolysis even in the presence of oxygen, a metabolic phenotype that he labeled "aerobic glycolysis" ([1] and reviewed in [2]). Warburg attributed these metabolic changes to defects in mitochondrial respiration and ATP production (oxidative phosphorylation). The aerobic glycolysis of cancer cells has been widely investigated throughout the years, and it is now considered as one of the metabolic hallmarks of cancer transformation and has been exploited to develop novel diagnostic and therapeutic tools [3]. Nevertheless, in subsequent decades after Warburg's pioneering findings, belief in his theory waned, partly due to the identification of mutations in cancer predisposition genes including oncogenes (pro-tumorigenic) and tumor suppressor genes (anti-tumorigenic). Furthermore, convincing evidence that mitochondrial dysfunction is the actual cause of the metabolic switch in cancer and of tumorigenesis in general was scarce [2]. However, in the last decade, the identification of loss- or gain-of-function mutations in key metabolic enzymes that have a causal role in tumorigenesis has awakened interest in Warburg's hypothesis [4, 5].

Genetic evidence for the involvement of metabolic enzymes in tumorigenesis

SDH mutations in hereditary paragangliomas and pheochromocytomas and other sporadic tumors

Mitochondrial succinate–coenzyme Q oxidoreductase, which catalyzes the conversion of succinate to fumarate in the TCA cycle (Fig. 1a) while simultaneously transferring

electrons from succinate to coenzyme Q (complex II of the respiratory chain), is comprised of four subunits: succinate dehydrogenase (SDH) *SDHA*, *SDHB*, *SDHC*, and *SDHD* all of which are nuclear genes encoding mitochondrial enzymes. A decade ago, heterozygous germline mutations in *SDHB*, *SDHC*, and *SDHD* subunits were identified as the causal tumor suppressor genes in hereditary paragangliomas and pheochromocytomas (hPGL), a rare hereditary cancer predisposition syndrome of the chromaffin tissue arising in the adrenal medulla pheochromocytoma (PCC) or derived from the parasympathetic tissue of the head and neck paraganglioma (PGL) [6–8]. Also, more recently, mutations in *SDHA* and the SDH assembly factor *SDHAF2* (formerly known as *SDH5* and required for flavination of SDH) have been described in hPGL [9–11]. Though primarily associated with hPGL, *SDHB* mutation carriers have additional increased susceptibility to renal cell cancers (RCC) [12–14]. In all cases, the loss-of-function germline mutations are followed by a somatic "second hit" of the second allele (usually deletion) in the tumor cells [15]. Furthermore, somatic mutations in several *SDH* genes are increasingly appreciated in sporadic PGL, PCC, and RCC [16]. Recently, a role for *SDH* mutations in gastrointestinal stromal tumors (GISTs) was also proposed. In particular, it was found that mutations in *SDHB*, *SDHC*, and *SDHD* are correlated with the rare development of a combination of hPGL and GIST, defined as Carney–Stratakis syndrome, and with the nonfamilial Carney triad, characterized by the presence of extra-adrenal paragangliomas, GIST, and pulmonary chondromas (reviewed in [17]).

FH mutations in hereditary leiomyomatosis and renal cell cancer

Fumarate hydratase (FH) catalyzes the reversible conversion of fumarate to malate in the TCA cycle (Fig. 1a). Loss-of-function germline mutations in *FH* predispose to hereditary leiomyomatosis and renal cell cancer (HLRCC), inherited leiomyomas (generally benign tumors of the smooth muscle), and renal (type II papillary and collecting duct) carcinoma [18, 19]. There is evidence to suggest that *FH* mutations may also be involved in the pathogenesis of breast, bladder, and testicular (Leydig cell) cancers [20, 21]. Similar to SDH in hPGL, enzymatic activity of FH is absent in HLRCC tumors and loss of the wild-type allele is observed in the majority of tumors [19].

IDH mutations in glioma and AML

Recently, mutations in isocitrate dehydrogenase (*IDH*) have been identified in gliomas and acute myelogenous leukemia (AML) [22–26]. IDH1 is one of three human IDH enzymes that catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (aKG, also known as 2-oxoglutarate). *IDH1* mutations are somatic and unlike *SDH* and *FH* mutations in hPGL and HLRCC respectively, no loss of heterozygosity has been demonstrated (i.e., all tumors retained one wild-type allele). Furthermore, the majority of *IDH1* mutations in gliomas are strictly confined to a single residue, R132, whereas mutations in *SDH* and *FH* occur throughout the gene and though predominantly missense, changes also manifest as truncating, insertion, and deletion mutations. Sequence analysis of gliomas that are wild type for IDH1 R132 revealed a subset of tumors harboring mutations in the homologous exon of *IDH2* and at the equivalent residue R172 [26]. Therefore, from a genetic point of view, *IDH1*/*IDH2* mutations in glioma and AML appear dominant, oncogenic gain-of-function mutations. Indeed, a recent study demonstrated that these specific point mutations in IDH change the activity of the enzyme which no longer produces α-ketoglutarate but rather uses (reduces) α-ketoglutarate to a less familiar metabolite, 2-hydroxyglutarate (2HG) [27] (see below and Fig. 1a).

Biochemical and biological consequences of mutations in the cancer-associated metabolic enzymes

SDH and *FH* mutations in cancer: a tale of PHDs and HIFs

The observation that tumors derived from *SDH* or *FH* mutations are genetically and histologically characterized by a strong hypoxic signature and are significantly more vascularized [28–30] suggested a causal link between TCA cycle dysfunctions and the activation of the hypoxia-inducible transcription factors (HIFs), master regulators of the response to low oxygen. Importantly, HIF activation can also explain some of the metabolic alterations observed in these tumors and it may play a supportive role in the tumorigenic process. In fact, HIF is known to orchestrate the metabolic and genetic reprogramming required to sustain tumor cell growth, vascularization, and proliferation [31, 32]. The molecular link between TCA cycle dysfunction and HIF activation was initially proposed by Selak and colleagues who demonstrated that the accumulation of succinate in SDH-deficient cells causes the inhibition of the HIF prolyl 4-hydroxylases (PHDs), important regulators of the stability of the α subunit of HIF [33]. In normoxic conditions, PHDs hydroxylate two proline residues on the oxygen-dependent degradation domain of HIFα, targeting it to the ubiquitin–proteasome degradation machinery (Fig. 1b). This hydroxylation requires oxygen and α-ketoglutarate and produces carbon dioxide and succinate. For that reason, the accumulated succinate in SDH-deficient cells impairs PHDs activity and leads to HIFα stabilization under normoxic conditions, a phenomenon that has been defined as pseudohypoxia [15]. Importantly,

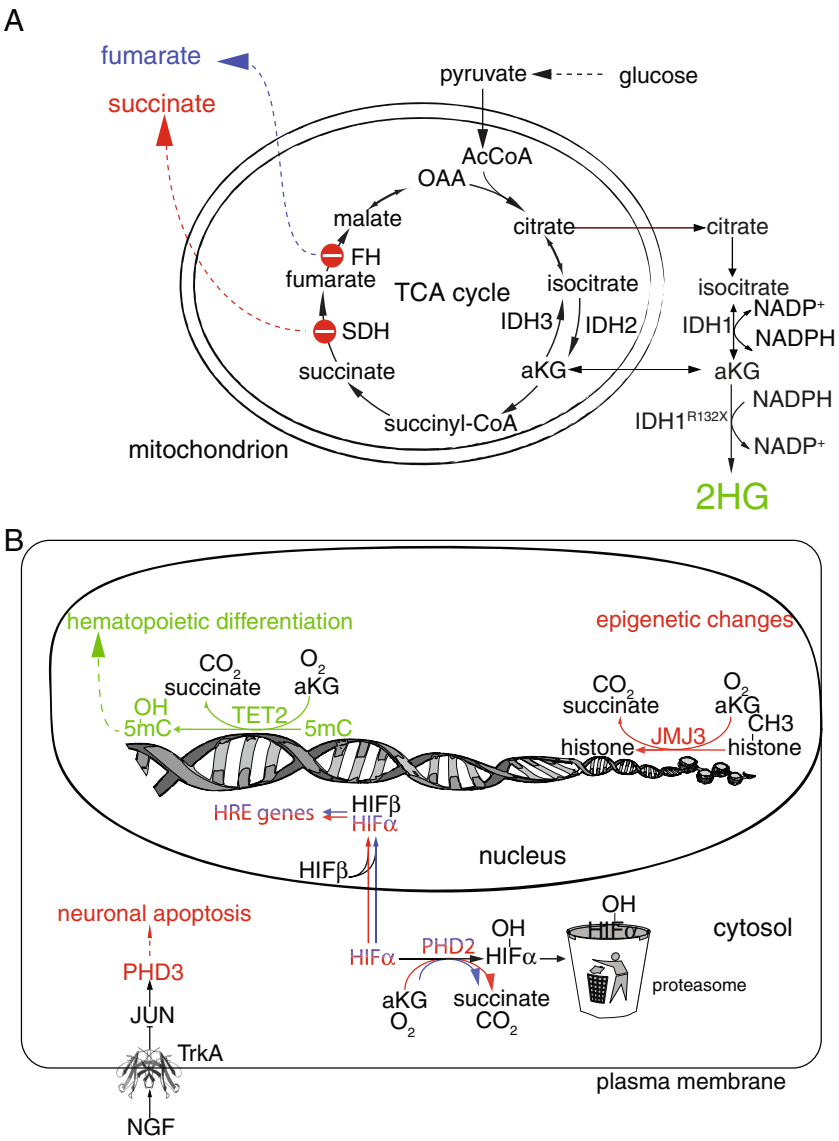

Fig. 1 The biochemistry and pathophysiology of oncometabolites accumulation in cancer. **a** Fumarate and succinate accumulate in the mitochondria and in the cytosol of cells expressing loss-of-function mutants of SDH or FH. 2-Hydroxyglutarate is accumulated as a consequence of neomorphic mutations in IDH1 in the cytosol and IDH2 in the mitochondria. **b** Biochemical effects of the accumulated oncometabolites in the cell. The effects are color coded: *red* for succinate, *blue* for fumarate, and *green* for 2HG. The accumulation of succinate impairs the enzymatic activity of several aKG-dependent dioxygenases: JMJd3, which regulates chromatin structure; PHD3, which is involved in promoting neuronal apoptosis in response to NGF withdrawal; and PHD2, which primarily regulates HIFα stability. Similarly, fumarate inhibits PHD2 enzymatic activity causing HIF stabilization. 2HG accumulation impairs DNA demethylation via the inhibition of the aKG-dependent dioxygenase TET2 and affects hematopoietic cells differentiation

the metabolic inhibition of PHDs is not unique to succinate as fumarate, which is accumulated in HLRCC cells, was also demonstrated to be a potent inhibitor of PHDs [34]. The accumulation of succinate and fumarate in the relevant cancer tissues as well as the accompanied PHD inhibition and pseudohypoxia was confirmed both in HLRCC leiomyomas and PGL tissues by Pollard et al. [35]. The biochemical characterization of the inhibition of PHDs by succinate and fumarate, as opposed to other TCA cycle metabolites, has been reported [36]. Furthermore, other biochemical studies showed that PHD activity is competitively inhibited by succinate or fumarate and, therefore, the ratio between α-ketoglutarate and succinate (or fumarate) rather than the absolute concentrations of these metabolites dictates PHD activity. In line with this, MacKenzie et al. used cell-permeable esters of α-ketoglutarate to reactivate PHDs enzymatic activity and thus alleviated pseudohypoxia caused by the accumulation of succinate or fumarate [37, 38].

An alternative model that links SDH and FH deficiency with PHD inactivation and HIF stabilization was proposed: Relying on a previously characterized role of respiratory chain-derived reactive oxygen species (ROS) as signals for HIF stabilization under hypoxia [39, 40], Guzy and colleagues showed that cells expressing mutant *SDHB*, but not mutant *SDHA*, are characterized by significant mitochondrial ROS production required, together with succinate, for a complete inactivation of PHDs [41]. Interestingly, these results fit better with earlier observations when SDHA was the only subunit of SDH that

appeared not to be associated with hPGL. However, as discussed above, SDHA was recently added to the list of tumor suppressors in hPGL [11]. Nevertheless, it is still plausible that ROS accumulation in some SDH-mutated tumors may have an additive modifying role that affects the type and severity of the tumor (*SDHB* mutations are associated with RCC and more aggressive PCC). Of note, ROS accumulation was observed also in FH-deficient cells though the underlining mechanism appeared to be different. In fact, Sudarshan et al. demonstrated that in an FH-deficient cell line, defects in oxidative phosphorylation cause upregulation of glycolysis that initiates ROS generation by NADPH oxidase [42]. Unfortunately, conflicting findings do not allow for defining a clear biological picture of the role of ROS in these tumors. For instance, the findings of Selak and colleagues could not support the need of ROS for HIFα stabilization in SDH-deficient cells [43]. In addition, the reduction of fumarate levels, without the recovery of mitochondrial function obtained by reconstituting a cytosolic-confined FH into FH-deficient cells, was found to be sufficient for reactivating PHDs and for HIFα degradation [44].

Recently, the relevance of succinate as a regulator of HIF stability was extended also to hypoxia. Puisségur and colleagues found that the HIF-dependent expression of microRNA-210 (miR-210) targets several subunits of the respiratory chain and, importantly, SDHD, which causes accumulation of succinate [45]. This amplification loop suggests that the stabilization of HIFα under hypoxia could be modulated not only by the concentration of oxygen but also by the concentration of succinate allowing a further control of PHDs activity.

Is HIF the only relevant target?

While the key function of PHDs is to hydroxylate and destabilize HIFα, it is likely that other substrates of PHDs are playing roles in the response to hypoxia. Therefore, when PHDs are inhibited by succinate or fumarate, the inhibition of hydroxylation of these PHD substrates may contribute to tumorigenesis regardless of HIF activity. Two studies proposed a novel, HIF-independent role of PHD3 in neuronal apoptosis [46, 47]. These investigators found that apoptosis of neuronal or neuroendocrine-derived cells (like PGL and PCC) at early stages of development requires PHD3 activity (Fig. 1b). In this scenario, the accumulation of succinate due to SDH deficiency would impair PHD3-dependent apoptosis, therefore setting the stage for their neoplastic transformation. These findings also help to explain why SDH mutations predispose patients to PGL and PCC. However, direct PHD3 targets that may mediate the apoptotic response have not been identified. A similar PHD3-dependent developmental defect in FH-deficient cells has not been reported. In contrast, a genome-wide transcriptomic analysis revealed that FH-deficient leiomyomas are characterized by a significant downregulation of the serum response factor (SRF) and its target genes *FOS* and *JUNB* [48]. This genetic signature suggests that the cause of leiomyoma formation might be a defective SRF-dependent smooth muscle differentiation. However, whether PHDs could have a role in SRF-mediated differentiation is not known.

So far, succinate and fumarate have been depicted as mitochondria-to-cytosol signaling molecules that activate the HIF pathway via the inactivation of PHDs. However, the activity of other enzymes could be affected by the accumulation of TCA cycle metabolites. In particular, all members of the α-ketoglutarate-dependent dioxygenase family [49] may be inhibited by succinate or fumarate since similar to PHDs, their enzymatic activity requires α-ketoglutarate and they produce succinate. Among the members of this family, the jumonji domain- containing (JMJC) histone demethylases have been recently investigated. These enzymes remove the methyl marks on the arginine and lysine residues of histones after performing an α-ketoglutarate- and oxygen-dependent hydroxylation. It was shown that succinate accumulation in SDH-deficient cells impairs the activity of the histone demethylase JMJD3, leading to changes in the methylation mark of histone H3 on arginine 27 (Fig. 1b) [50]. Similarly, in a yeast model of paraganglioma, another histone demethylase, Jhd1, was found to be inhibited both in vitro and in vivo by succinate accumulation; importantly, this effect was reverted by the addition of exogenous, cell-permeable α-ketoglutarate[51].

Histone methylation is an important epigenetic modification which has been demonstrated to regulate gene expression by modulating chromatin structure and the binding of transcription factors [52]. Interestingly, it was recently shown that HIF1 promotes the transcription of two JMJ domain containing proteins, JMJD1A and JMJD2B [53, 54], possibly to facilitate the binding of HIF to the promoter regions of other HIF target genes. These findings might suggest that HIF transcriptional activity requires chromatin remodeling via the activity of these α-ketoglutarate-dependent enzymes. Importantly, unlike PHDs, these dioxygenases retain their activity even at low oxygen levels [55]. It is therefore likely that the presence of inhibiting concentrations of succinate or fumarate may modulate transcriptional activity of HIF and hence, may be responsible for transcriptional differences between hypoxic and pseudohypoxic conditions. Therefore, it is tempting to speculate that succinate and fumarate could act not only as mitochondria-to-cytosol but also as mitochondria-to-nucleus signaling units with the power to regulate gene expression by regulating chromatin structure.

Extracellular roles of succinate and fumarate: ways to help tumorigenesis?

Beyond the inhibition of α-ketoglutarate-dependent dioxygenases, a potentially additional role in the pathophysiology of TCA cycle metabolites was recently identified. In 2004, it was found that two orphan G protein-coupled receptor, namely GPR99 and GPR91, members of the purinergic P2 receptors family, were not activated by nucleotides, as initially proposed, but were actually responsive to extracellular α-ketoglutarate and succinate, respectively. These studies suggest that α-ketoglutarate and succinate can have a hormone-like role; they can be secreted into the bloodstream and convey specific signals to distal cells and tissues. In particular, it was shown that the stimulation of the GPR91 receptor by succinate triggers the secretion of renin from the kidney and leads to increased blood pressure [56]. More recent reports have shown that several other organs such as liver, adipose tissue, and the retina could sense succinate accumulation via the membrane receptor GRP91 (see [57] for a review). Interestingly, in ischemic retina, succinate activates GPR91 in retinal ganglion cells thus promoting vascular endothelial growth factor secretion and local neovascularization which is HIF- independent [58]. This important observation may suggest that in addition to the PHD-HIF pathway, some of the metabolic and pro-angiogenic effects of succinate in hPGL may actually depend on, or synergize with, the paracrine activation of the succinate receptor. Intriguingly, fumarate was found to have a role in regulating blood pressure and its accumulation in the blood correlates with hypertension in a salt-sensitive rat model [59]. It is tempting to speculate that the hormone-like physiological roles of succinate and potentially of fumarate may complement their intracellular metabolic signaling effects and further alter tumor microenvironment to support tumorigenesis.

IDH: a tumor suppressor or an oncogene?

In 2008, a genome-wide screening revealed somatic mutations of the TCA cycle enzyme IDH1 in low grade gliomas and secondary glioblastomas [24]. Just a few months after this seminal discovery, a large parallel DNA sequencing study found *IDH1* mutations in AML patients [60]. Interestingly, subsequent studies demonstrated that also IDH2 is mutated in AML and glioma patients [26, 61, 62]. IDH1 and IDH2 are closely related NADP-linked enzymes, while IDH3 is NAD-linked (Fig. 1a). IDH2 and IDH3 are mitochondrial enzymes that function in the TCA cycle while IDH1 is located in the cytosol and peroxisomes, where it supplies NADPH-reducing equivalents for biosynthetic and other reactions [5]. It should be stressed that the canonical NAD-dependent reaction in the TCA cycle is a specific task of IDH3 which did not appear to be mutated in these cancers. As discussed above, the pattern of mutations (always at the same amino acid residue) and the heterozygosity state of the tumors (no loss of the wild-type allele) suggested an oncogenic, rather than a tumor suppressive role for *IDH* mutants. An unbiased metabolomic analysis of mutant *IDH1*-overexpressing cells found a striking intracellular accumulation of a poorly characterized metabolite, 2HG. In order to explain this finding, Dang et al. demonstrated that the mutant IDH1 acquires a neomorphic catalytic activity that allows a NADPH-dependent reduction of α-ketoglutarate into 2HG [27]. Importantly, this metabolite is significantly accumulated in glioma cells and in the blood of AML patients and therefore, despite its unclear role in tumorigenesis, the definition “oncometabolite” was coined by Dang et al. to portray the potential oncogenic contribution of 2HG (see [5] for discussion).

Other than the obvious genetic and biochemical differences between the loss of SDH and FH functions to the gain of IDH functions in tumors, several biological differences suggested that *IDH* mutations in cancer significantly differ from the genuine TCA cycle dysfunctions. First, as anticipated above, IDH1 and IDH2 appear to be mostly involved in the regulation of the NADP/NADPH ratio in the mitochondria and the cytosol [5] rather than in NADH production, a primary role of the TCA cycle which is executed by IDH3. Of note, the limited roles of IDH1 and IDH2 in the TCA cycle were substantiated by the absence of significant changes in the levels of TCA cycle metabolites in *IDH1*/*IDH2*-mutated cells [27, 62]. In addition, despite the fact that 2HG was proposed to act, similarly to succinate and fumarate, as an inhibitor of PHDs [63, 64], the presence of a HIF signature in *IDH* mutant cells and in gliomas is still debated. For the above reasons, while there may be some overlapping roles of 2HG, succinate, and fumarate, the underlining biochemical features which contribute to tumorigenesis in IDH and *FH*/*SDH* mutant cells seem to differ. A recent study in AML demonstrated genetic redundancy (mutual exclusiveness) between *IDH* mutations and *TET2* deletions [65]. Importantly, TET2 is a recently discovered DNA demethylase which is also an α-ketoglutarate-dependent dioxygenase [66]. Indeed, Figueroa et al. demonstrated that 2HG can inhibit the DNA methylation status of TET2-expressing cells (Fig. 1b).

Summary

So far, alterations in three enzymatic reactions have been reported to possess genetically causal links to cancer formation: (1) Germline mutations in *FH* are associated with HLRCC (leiomyomas and RCC) and potentially with other tumors. (2) Both germline and somatic mutations in

any of the four subunits of *SDH* (*SDHA*, *SDHB*, *SDHC*, *SDHD*) or the SDH assembly factor *SDHAF2* are associated with PGL, PCC, and/or RCC. (3) Somatic mutations in either *IDH1* or *IDH2* are associated with gliomas or AML. While the *FH* and *SDH* mutations are typically loss-of-function mutations and the genes involved behave genetically like tumor suppressors, the *IDH* mutations lead to a gain of a new NADPH-dependent α-ketoglutarate-reductase activity which generates 2HG. Considering the fact that one wild-type *IDH* allele is retained in tumors with *IDH* mutations, and no significant changes in α-ketoglutarate or isocitrate levels were observed in these tumors, it is safe to propose that *IDH1*/*IDH2* mutations are oncogenic gain-of-function mutations.

In all three types of these genetic-metabolic events, it appears that the underlying mechanism of tumorigenesis involved the accumulation of metabolites that convey oncogenic signals (oncometabolites). Although part of this oncogenic activity might be attributed to hormone-like effects of these molecules, strong evidence indicates that the principal oncometabolic activities of succinate, fumarate and 2HG are related to the inhibition of the α-ketoglutarate-dependent dioxygenases, alas with different specificities to different enzymes and therefore, with different biochemical and biological consequences. More generally, these observations reveal a dynamic and bidirectional interaction between the metabolic status of the cell and its genetic profile and propose that small metabolites may be novel and unexplored signaling units.

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