

Current views on cell metabolism in SDHx-related pheochromocytoma and paraganglioma

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Abstract

Warburg's metabolic hypothesis is based on the assumption that a cancer cell's respiration must be under attack, leading to its damage, in order to obtain increased glycolysis. Although this may not apply to all cancers, there is some evidence proving that primarily abnormally functioning mitochondrial complexes are indeed related to cancer development. Thus, mutations in complex II (succinate dehydrogenase (SDH)) lead to the formation of pheochromocytoma (PHEO)/paraganglioma (PGL). Mutations in one of the *SDHx* genes (*SDHx* mutations) lead to succinate accumulation associated with very low fumarate levels, increased glutaminolysis, the generation of reactive oxygen species, and pseudohypoxia. This results in significant changes in signaling pathways (many of them dependent on the stabilization of hypoxia-inducible factor), including oxidative phosphorylation, glycolysis, specific expression profiles, as well as genomic instability and increased mutability resulting in tumor development. Although there is currently no very effective therapy for *SDHx*-related metastatic PHEOs/PGLs, targeting their fundamental metabolic abnormalities may provide a unique opportunity for the development of novel and more effective forms of therapy for these tumors.

Key Words

- ▶ SDHx
- ▶ glycolysis
- ▶ Warburg effect
- ▶ reactive oxygen species
- ▶ succinate dehydrogenase
- ▶ pheochromocytoma
- ▶ paraganglioma
- ▶ renal cell carcinoma
- ▶ gastrointestinal stromal tumor
- ▶ hypoxia
- ▶ pseudohypoxia

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Introduction

In previous innovative work, Hanahan & Weinberg (2000) determined the unique hallmarks of cancer that together constitute a fundamental principle that provides a logical framework for understanding the remarkable diversity, yet nevertheless similarity, of various cancers. Six hallmarks of cancer, namely sustaining proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, promoting angiogenesis, and resisting cell death, are the driving

forces that ultimately cause cancer cell development and spread, leading to patient death (Hanahan & Weinberg 2000). Recently, Hanahan & Weinberg (2011) have added two new emerging hallmarks: evading immune destruction and reprogramming energy metabolism.

Additional scientific studies have also shown that altered energy metabolism is as widespread in cancer cells as many of the other cancer-associated traits that have been well accepted as the hallmarks of cancer

(Levine & Puzio-Kuter 2010). This raises the question of whether deregulating cellular energy metabolism could be a core hallmark of cancer cells. In fact, redirection of energy metabolism is largely orchestrated by proteins that are involved in one way or another in programming the core hallmarks of cancer. When viewed in this way, abnormal oxidative phosphorylation (OXPHOS) is simply another phenotype that is caused by altered oncogenes or tumor suppressor genes (Levine & Puzio-Kuter 2010). Multiple lines of evidence indicate that the process of tumorigenesis is often associated with altered metabolism. In 1926, Otto Warburg reported that cancer cells produce most of their ATP via 'aerobic glycolysis' (Warburg *et al.* 1926). A significant glycolytic production of ATP despite aerobic conditions, referred to as the Warburg effect, was found to be the characteristic of most cancer cells (Warburg 1956). Warburg reasoned that respiration must be damaged in cancers because high levels of O₂ are unable to suppress the production of lactic acid by cancer cells (known as the Pasteur effect). However, new studies have demonstrated that tumor mitochondria are fairly functional with regards to respiration and ATP synthesis, exhibiting almost normal respiratory control ratios and capabilities for the oxidation of respiratory substrates (Eakin *et al.* 1972, Bensinger & Christofk 2012, Krejci 2012, Nakajima & Van Houten 2013).

Although mitochondria are fairly functional in the majority of cancers, some cancers were found with mutations in the genes linked to paramount mitochondrial processes, the Krebs cycle (tricarboxylic acid cycle (TCA); Linehan & Rouault 2013, Zhang *et al.* 2013) and OXPHOS. Mitochondrial complex II, also known as succinate dehydrogenase (SDH), is one such protein involved in both TCA and OXPHOS. This membrane complex catalyzes the oxidation of succinate to fumarate in TCA and serves as an electron donor to complex III via CoQ (Eng *et al.* 2003, Gottlieb & Tomlinson 2005). Succinate oxidation results in the reduction of ubiquinone (CoQ) to ubiquinol at the mitochondrial inner membrane as one part of the respiration electron transfer chain (Sun *et al.* 2005a). SDH is composed of four subunits (SDHA–D), all encoded by nuclear genes (Baysal 2003, 2008, Yankovskaya *et al.* 2003, Sun *et al.* 2005b, Cascon *et al.* 2008). The large SDHA subunit is catalytic. The conversion of succinate to fumarate is accomplished by SDHA through the reduction of a flavin adenine dinucleotide, a molecule bound to its protein moiety. This reaction is measured as SDH activity. Electrons are then passed to three Fe–S centers bound to SDHB, which eventually transfers them to ubiquinone (coenzyme Q). The smaller

subunits, SDHC and SDHD, bind ubiquinone and anchor the entire complex to the inner membrane of the mitochondria (Rustin *et al.* 2002).

Deleterious mutations in any of the *SDH* genes invariably result in decreased SDH activity or a significant reduction or complete absence of the protein (Rustin *et al.* 2002, van Nederveen *et al.* 2009, Gill *et al.* 2011, Korpershoek *et al.* 2011, Yang *et al.* 2012). Inherited defects in particular SDH subunits in humans are associated with variable clinical presentations ranging from early-onset devastating encephalomyopathy to tumor susceptibility or optic atrophy. Homozygous or compound heterozygous mutations in *SDHA* cause metabolic neurodegenerative disorders like congenital Leigh syndrome and late-onset optic atrophy, ataxia, and myopathy (Birch-Machin *et al.* 2000, Parfait *et al.* 2000, Horvath *et al.* 2006, Burnichon *et al.* 2010, Levitas *et al.* 2010). Recently, Alston *et al.* (2012) have presented the first patient with hypotonia and leukodystrophy due to a novel homozygous *SDHB* mutation. Heterozygous mutations in *SDHA–D* predispose to tumorigenesis (Fig. 1; Maher & Eng 2002, Astuti *et al.* 2003, 2004, Eng *et al.* 2003, Schiavi *et al.* 2005, Bayley *et al.* 2006, Benn *et al.* 2006, Cascon *et al.* 2008). The detailed molecular and cellular mechanisms linking these latter *SDH* mutations and tumorigenesis have not been fully elucidated. Thus, consistent with Knudson's two-hit hypothesis for tumorigenesis, a heterozygous germline mutation in an *SDH* gene is associated with a loss of the WT allele, or other silencing mechanisms (e.g. methylation) of the WT allele are present in a tumor (Baysal *et al.* 2000, Astuti *et al.* 2003, 2004, Baysal 2003, 2004, 2008, Eng *et al.* 2003, Gimenez-Roqueplo *et al.* 2003, Ni *et al.* 2008, 2012, Sandgren *et al.* 2010, Bardella *et al.* 2011, Killian *et al.* 2013, Letouze *et al.* 2013) as the starting point for tumor development. Moreover, the pathophysiology of distinct clinical phenotypes associated with abnormalities in SDH subunits remains to be determined (Timmers *et al.* 2009a). Detailed knowledge about *SDH* mutations is available in a database (LOVD, v.2.0 (Leiden Open Variation Database), <http://www.lovd.nl/2.0>; Bayley *et al.* 2005).

Although these findings led to a renewed interest in cancer metabolism, our knowledge on the specifics of tumor metabolism is still fragmented. Nevertheless, multiple lines of evidence indicate that the process of tumorigenesis is often associated with altered metabolism. In this review, we show and discuss how mutations in *SDH* subunits can lead to reprogramming of cancer-related metabolism. Also, this paper reviews recent findings

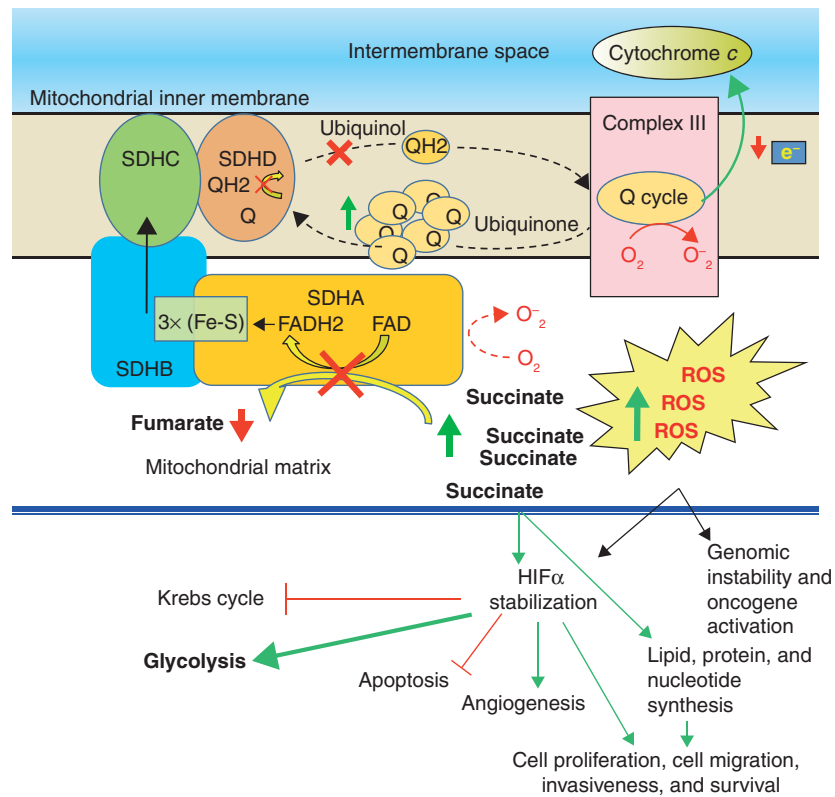


Figure 1

The succinate dehydrogenase complex (SDH), as a member of the tricarboxylic acid cycle (TCA), catalyzes the oxidation of succinate to fumarate. In this reaction, two hydrogen atoms are removed from succinate by FAD. These electrons from the reduced SDH-FADH₂ complex are then transferred to ubiquinol-ubiquinone (coenzyme Q), a soluble component of the electron transport system of complex II. In the Q cycle, the sequential oxidation and reduction of the lipophilic electron carrier, coenzyme Q, generates protons that are transferred to complex III, with the ultimate generation of ATP (complex V). Coenzyme Q, beside its function in the respiratory chain as an electron carrier mediating electron transfer between the various dehydrogenases and the cytochrome pathway, also works as a powerful antioxidant in biological membranes.

Dysfunction of SDH inactivates the electron transport chain and the Krebs cycle. A lack of suboptimal level of SDH activity will not only cause decreased ATP production, but will also result in increased ROS with succinate accumulation. An increase in ROS, like the accumulation of succinate, leads to stabilization of HIF α . HIF α stabilization subsequently activates glycolysis, cell proliferation, cell migration, invasiveness, and angiogenesis and inhibits apoptosis. The overexpression of ROS triggers genomic instability, oncogene activation, and tumor suppressor inactivation. e⁻, electron; FAD, flavin adenine dinucleotide; FADH₂, FAD hydroquinone; ROS, reactive oxygen species; Q, ubiquinone; QH₂, ubiquinol; SDHA, B, C, and D, succinate dehydrogenase complex subunits A, B, C, and D.

related to key metabolites, transcription factors, and enzymes that play an important role in the regulation of cancer metabolism, and that blocking these metabolic pathways or restoring altered pathways can lead to new approaches in cancer treatment.

Pheochromocytoma and paraganglioma

Pheochromocytomas (PHEOs)/paragangliomas (PGLs) are rare neuroendocrine tumors that produce catecholamines (Lenders et al. 2005). PHEOs/PGLs arise from three distinct parts of the neural crest: the adrenal medulla (PHEOs) and the sympathetic and parasympathetic paraganglia (extradrenal PGLs) (Papasprou et al. 2012).

One-third or more of PHEO/PGL cases have a familial etiology (Neumann et al. 2002, Erlic et al. 2009, Gimenez-Roqueplo et al. 2012). This group is heterogeneous with diverse hereditary backgrounds due to germ line mutations in 16 susceptibility genes to date. Some of these include neurofibromatosis type 1 (*NF1*; Viskochil et al. 1990), the ret proto-oncogene (*RET*; Mulligan et al. 1993), the von Hippel-Lindau (*VHL*; Latif et al. 1993) tumor suppressor, the SDH subunits (*SDHA/B/C/D*; Baysal et al. 2000, Niemann & Muller 2000, Astuti et al. 2001, Burnichon et al. 2010), SDH complex assembly factor 2 (*SDHAF2*; Hao et al. 2009), transmembrane protein 127 (*TMEM127*; Qin et al. 2010, Yao et al. 2010, Jiang & Dahia 2011), the MAX protein (*MAX*; Comino-Mendez et al. 2011), kinesin

family member 1B (*KIF1B*; Schlisio *et al.* 2008, Yeh *et al.* 2008), the 2-oxoglutarate (2OG)-dependent prolyl hydroxylase enzymes (*PHD2*, Ladroue *et al.* 2008, Eltzschig *et al.* 2009), isocitrate dehydrogenase 1 (*IDH1*; Gaal *et al.* 2010), and most recently hypoxia-inducible transcription factor 2 α (*HIF2A*; Zhuang *et al.* 2012, Toledo *et al.* 2013), fumarate hydratase (*FH*; Castro-Vega *et al.* 2013), and H-RAS protein (*H-RAS*; Crona *et al.* 2013). Somatic mutations of these genes are also involved in PHEO/PGL tumors (Burnichon *et al.* 2012a, Weber *et al.* 2012, Crona *et al.* 2013, Dahia 2013). Hereditary and sporadic PHEOs/PGLs can be divided into two groups based on their transcription profile revealed by genome-wide expression microarray analysis (Lopez-Jimenez *et al.* 2010, Burnichon *et al.* 2011, Galan & Kann 2013, Vicha *et al.* 2013). The first group (cluster 1) includes tumors carrying *VHL* and *SDHx* (*SDHD*, *SDHB*, *SDHC*, *SDHA*, and *SDHAF2*) mutations and also accounts for about 30% of sporadic tumors (Dahia *et al.* 2005, Lopez-Jimenez *et al.* 2010, Burnichon *et al.* 2011). The second group (cluster 2) represents tumors carrying *NF1*, *RET*, and *KIF1B* mutations, and also includes about 70% of sporadic tumors (Burnichon *et al.* 2011, Gimenez-Roqueplo *et al.* 2012, Shah *et al.* 2012, Galan & Kann 2013). The newly discovered *TMEM127* and *MAX* genes are most likely associated with cluster 2, and *HIF2 α* with cluster 1 (Burnichon *et al.* 2011, 2012b, Lorenzo *et al.* 2012, Zhuang *et al.* 2012). However, a subset of *MAX*-related tumors may have impaired SDH activity, and metabolomics in these tumors could uncover new data that could be very useful clinically for their diagnosis (Rapizzi *et al.* 2012).

In cluster 1, *VHL/SDHx* mutations lead to impaired degradation and accumulation of HIF1/2 α and display signatures of pseudohypoxia, angiogenesis, increased reactive oxygen species (ROS), and reduced oxidative response resulting in changes in cell metabolism (energy metabolism regulation). *VHL* and *SDH* subunit mutations distribute tumors to separate subclusters within cluster 1 (Eisenhofer *et al.* 2004, Dahia *et al.* 2005, Burnichon *et al.* 2009, Lopez-Jimenez *et al.* 2010). Cluster 2-related PHEOs/PGLs are linked together by the activation of kinase signaling pathways driven by oncogenes that are involved in kinase signaling, translation, initiation, protein synthesis, and genes involved in neural/neuroendocrine identity (Dahia *et al.* 2005, Powers *et al.* 2007, Yeh *et al.* 2008, Burnichon *et al.* 2011, Jiang & Dahia 2011, Shah *et al.* 2012). Cluster 1 is characterized by immature catecholamine phenotypic features of associated tumors (Eisenhofer *et al.* 2004). The immature phenotype involves reduced or absent expression of numerous catecholamine

biosynthetic and secretory pathway components, mainly phenylethanolamine *N*-methyltransferase, the enzyme that converts norepinephrine to epinephrine (Eisenhofer *et al.* 2011a, 2012). Also, *SDH*-related tumors often produce dopamine. Thus, cluster 1 tumors can be distinguished from cluster 2 tumors by the absence of epinephrine production (Eisenhofer *et al.* 2004, 2011a,b, Burnichon *et al.* 2012b, Eisenhofer *et al.* 2012). Most recently, Imperiale *et al.* (2013) evaluated metabolic characteristics of PHEOs/PGLs tumors, using ^1H high-resolution magic angle spinning nuclear magnetic resonance (HRMAS-NMR) spectroscopy. *SDHx*-related tumors were characterized by an increase in succinate levels, significantly lower values of glutamate, and lower values of ATP/ADP/AMP in *SDHx*-related tumors compared with other subtypes. *VHL* tumors were found to have the highest values of glutathione (GSH) compared with other PHEOs/PGLs. This study showed that HRMAS-NMR spectroscopy is a future promising method for investigating the metabolomic profile of various PHEOs/PGLs.

SDH dysfunction and metabolic changes

Succinate accumulation

It is well documented that abnormal SDH function induces an accumulation of succinate (Selak *et al.* 2005, King *et al.* 2006, Hobert *et al.* 2012, Rao *et al.* 2013). Very recently, Lendvai *et al.* (2013) showed that tissue levels of succinate in PGLs due to *SDHB/D* mutations were several-fold higher. Their results showed that the mean fumarate concentration in *SDHB*-related PGLs is significantly lower than in the apparently sporadic PHEO/PGL group. Lendvai *et al.* (2013) also demonstrated a significantly increased succinate:fumarate ratio in *SDHB*-related PGLs and suggested that this ratio may be used as a new metabolic marker for the detection of *SDHB*-related PHEOs/PGLs. Thus, mass spectrometric-based measurements of succinate:fumarate ratios in PHEO/PGL tumor tissue may provide a novel method to identify patients to be tested for *SDHB/C/D* mutations. The measurements could also be useful for assessing metabolic factors responsible for variable clinical presentations of tumors resulting from mutations of different *SDHx* genes. Also, plasma organic acid analysis may provide an effective and inexpensive screening method to determine the presence of *SDHx* mutations in the near future (Hobert *et al.* 2012).

The accumulation of specific metabolites has been illustrated in different tumor models with inherited and acquired alterations of enzymes of the TCA cycle, such as

fumarate in cases of *FH* gene mutations (Isaacs *et al.* 2005) and 2-hydroxyglutarate in mutations in one of the two *IDH* genes (*IDH1/2*) (Dang *et al.* 2009). These findings have important implications for our understanding of tumorigenesis because these metabolites convey oncogenic signals (oncometabolites; Kaelin & McKnight 2013).

Succinate that accumulates in the mitochondrial matrix due to SDH dysfunction leaks out into the cytosol, where it inhibits the activity of HIF1/2 α PHDs (PHD1, 2, and 3, also known as EGLN2, 1, and 3 respectively) that hydroxylate two prolyl residues (Dann & Bruick 2005). PHDs are members of a large superfamily of α -ketoglutarate-dependent dioxygenases. PHD action normally requires oxygen and α -ketoglutarate as cosubstrates and ferrous iron and ascorbate as cofactors. (Hewitson *et al.* 2003, Kaelin & Ratcliffe 2008). Succinate competes with α -ketoglutarate in binding to the PHD enzyme. Therefore, increasing succinate levels offset the effect of PHD activity. A lack of SDH activity inhibits succinate-ubiquinone activity; thus, electrons that would normally transfer through the SDHB subunit to the ubiquinone pool are instead donated to molecular oxygen to give a superoxide anion with a subsequent increase in ROS production and oxidative stress. ROS exposure also inhibits the interaction of HIF α and PHDs, similar to the accumulation of succinate, but it is proposed that such an inhibition of this interaction by ROS may be more important for tumorigenesis (Yankovskaya *et al.* 2003, Guzy *et al.* 2008, Majmundar *et al.* 2010). The inhibition of the HIF α -PHD interaction leads to the stabilization of HIF α and activation of the HIF complex (Lee *et al.* 2005). HIF α regulates the transcription of a number of genes that are known to be involved in tumorigenesis and angiogenesis, extracellular matrix elements, and coordinated suppression of oxidoreductase enzymes, all processes that would be directly or indirectly regulated by the activation of HIF1 α and/or HIF2 α (Dahia *et al.* 2005, Selak *et al.* 2005, Mole *et al.* 2009, Favier & Gimenez-Roqueplo 2010, Semenza 2010, 2011, 2012, Keith *et al.* 2012). HIF1 α and HIF2 α regulate both shared and unique target genes and pathways. The common shared targets are vascular endothelial growth factor (VEGF), GLUT1, GLUT3, and hexokinase 2 (HK2). HIF1 α exclusively stimulates the expression of several glycolytic enzymes, whereas the embryonic transcription factors Oct4, cyclin D1, platelet-derived growth factor, and erythropoietin are activated in a HIF2 α -dependent manner (Fig. 2; Rankin *et al.* 2007, Patel & Simon 2008, Furlow *et al.* 2009, Florczyk *et al.* 2011, Koh *et al.* 2011, Franke *et al.* 2013, Singh *et al.* 2013). The differential effects of these two transcription factors

in numerous cellular systems are now well established and reviewed, including their link to the pathogenesis of PHEO and PGL (Holmquist-Mengelbier *et al.* 2006, Koh *et al.* 2011, Branco-Price *et al.* 2012, Chiavarina *et al.* 2012, Keith *et al.* 2012, Semenza 2012, Jochmanova *et al.* 2013). Despite the fact that Pollard *et al.* (2005, 2006) found relatively more common HIF2 α overexpression in *VHL* PHEOs and PGLs, whereas in *SDHx*-related tumors nuclear HIF1 α staining was more prominent, Gimenez-Roqueplo *et al.* (2001, 2002) described overexpression of HIF2 α and VEGF in patients with PHEOs and PGLs carrying *SDHB* and *SDHD* mutations compared with sporadic PHEOs and PGLs, and Favier *et al.* (2009) found overexpression of HIF2 α mRNA in both *VHL* and *SDH*-related PHEO and PGL. Also, Eisenhofer *et al.* (2004) and Koh *et al.* (2011) support the leading role of HIF2 α in the tumor development and progression in cluster 1 tumors as well as their unique noradrenergic phenotype (Jochmanova *et al.* 2013). The important role of HIF2 α in various developmental issues is also supported by previous observations performed in fetal paraganglia and neuroblastoma (Tian *et al.* 1998, Favier *et al.* 1999, Nilsson *et al.* 2005, Jochmanova *et al.* 2013).

Similarly, the mechanism of PHD inhibition by succinate is likely to extend to other numbers of a large superfamily of α -ketoglutarate-dependent dioxygenases. One of them is the factor inhibiting HIF, which normally hydroxylates HIF1 α on the asparagine 803 residue. This blocks its interaction with the coactivators histone acetyltransferase p300 (p300) and cAMP-response element-binding protein under normoxic conditions (Mahon *et al.* 2001, Lando *et al.* 2002) and thus inhibits the transactivation of HIF target genes (Khan *et al.* 2011, Cascon & Tennant 2012). Also, *SDHx* mutations inhibit the activity of the jumonji-domain (JmjC) histone demethylases (Cervera *et al.* 2009, Xiao *et al.* 2012). These enzymes use α -ketoglutarate to remove the methyl groups found on arginines and lysines of histones H3 and H4 (Agger *et al.* 2008). *SDHx* mutations decrease histone demethylase activity (specifically the JMJD3 demethylase) and lead to increased methylation of histone H3 (H3K27me3) (Cervera *et al.* 2009). Similarly, very recently Letouze *et al.* (2013) showed that increased tumor levels of succinate lead to DNA hypermethylation, a process causing global changes in gene expression as a critical tumorigenic mechanism. These modulations in the pseudohypoxic signature observed in *SDHx*-related tumors can distinguish the gene expression phenotypes observed in the two subgroups of tumors in cluster 1.

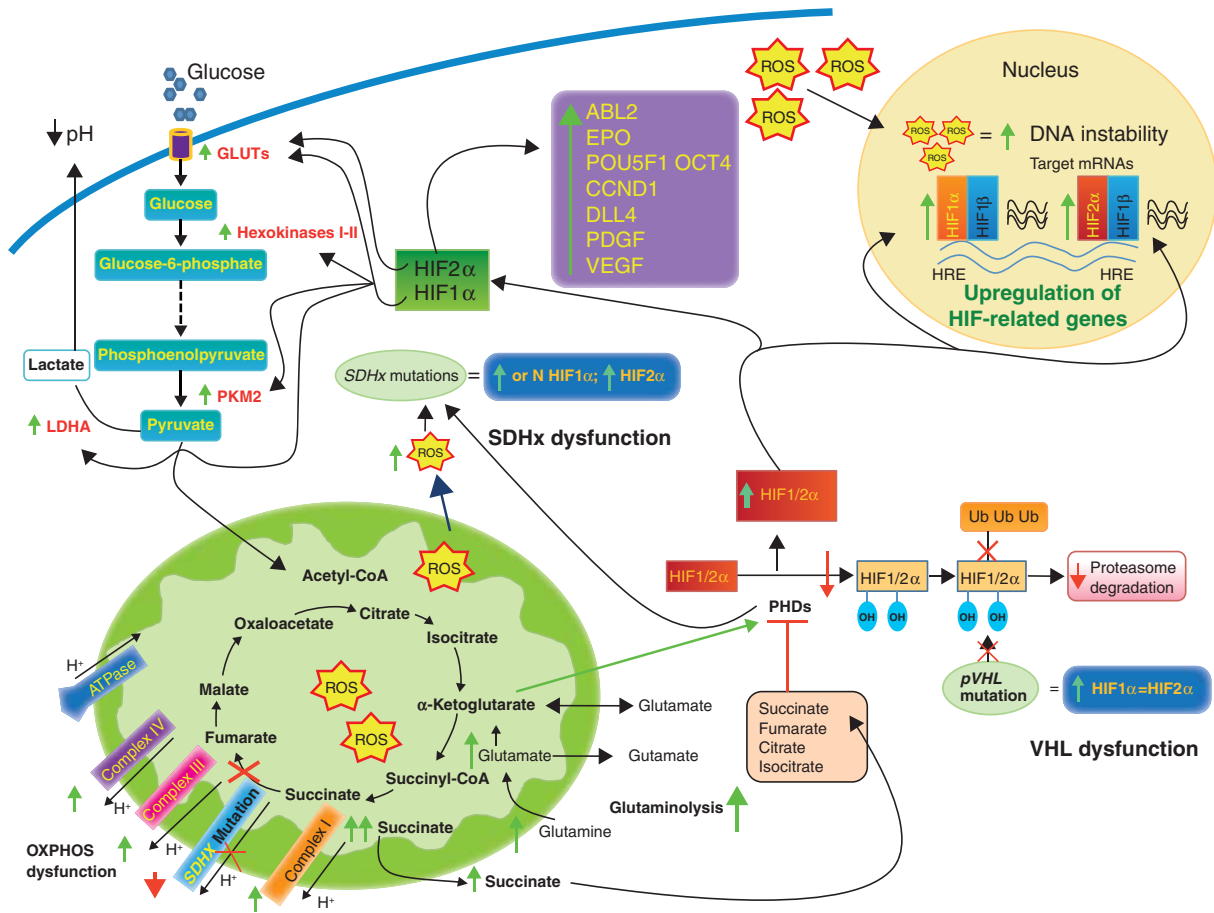


Figure 2

Different mechanisms have been proposed to explain the link between *SDHx* mutations and tumorigenesis. First, the loss of function of *SDH* causes an accumulation of succinate and the overproduction of ROS. Second, inactivation or dysfunction of *SDH* inhibits the activity of *HIF* α PHDs. Inhibition of PHDs results in an insufficient hydroxylation of *HIF1/2* α . The unhydroxylated *HIF1/2* α protein cannot be degraded by the proteasome, and *HIF1/2* α is stabilized. This stabilization can be overcome by α -ketoglutarate. *VHL* mutations result in similar inadequate *HIF1/2* α proteasome degradation and *HIF1/2* α stabilization. The stabilization of *HIF1* α rather than *HIF2* α increases glycolysis (due to overexpression of some glycolytic enzymes) and can regulate glutaminolysis. *HIF2* α stabilization is involved in the direct or indirect activation of a number of genes that are known to be involved in the inhibition of apoptosis, tumorigenesis, and angiogenesis. The stabilization of *HIF1/2* α leads to an upregulation of *HIF*-related genes due to binding to HREs and to an overexpression

of hypoxia-related genes. Increased ROS accumulation results in oxidative DNA damage and genomic instability and inhibits PHDs, similarly to the accumulation of succinate. Increasing activity of complex I, III, and IV may be a compensatory reaction to a lack of, or decreased, complex II activity in *SDHx*-related tumors. *ABL2*, *ABL2* protein tyrosine-protein kinase; *CCDN1*, cyclin D1; *DLL4*, delta-like protein 4; *EPO*, erythropoietin; *GLUTs*, glucose transporters; *HIF*, hypoxia-inducible factor; HREs, hypoxia-responsive elements; *LDH-A*, lactate dehydrogenase A; OXPHOS, oxidative phosphorylation; *PDGF*, platelet-derived growth factor; PHDs, prolyl hydroxylases; *PKM2*, pyruvate kinase muscle isozyme 2; *POU5F1 OCT4*, POU domain, class 5, transcription factor 1 isoform; *pVHL*, protein of the von Hippel-Lindau tumor suppressor gene; ROS, reactive oxygen species; *SDH*, succinate dehydrogenase; Ub, ubiquitin; *VEGF*, vascular endothelial growth factor.

Reactive oxygen species

A lack of *SDH* activity results in increases in steady-state levels of O_2^- to H_2O_2 that could then form more powerful oxidants, such as hydroxyl radicals, through Haber-Weiss-driven Fenton chemistry as well as organic hydroperoxides capable of causing chronic metabolic oxidative stress (Slane *et al.* 2006, Spitz 2011, Owens *et al.* 2012). Chronic ROS exposure can result in oxidative damage to mitochondrial and cellular proteins, lipids, and nucleic acids.

These normoxic ROS accelerate the DNA-damaging processes as a 'mutator phenotype', causing genomic instability, as well as an increase in glucose consumption and sensitivity to glucose deprivation-induced toxicity and slower growth rates. ROS are also involved in Ras-Raf-MEK signaling. Ras-Raf-MEK signaling activation causes the mediation of protection against apoptotic cell death induced by increased oxidative stress (Jiang *et al.* 2005). The activity of the ROS-generating enzyme Nox1 is

required for VEGF, a potent stimulator of tumor angiogenesis (Rustin *et al.* 2002, Dudkina *et al.* 2005, Slane *et al.* 2006, Pan *et al.* 2009). Fliedner *et al.* (2012) detected elevated superoxide dismutase 2 expression in *SDHB*-derived PHEOs/PGLs that is an indirect evidence for increased ROS production and may reflect elevated oxidative stress.

Warburg effect

A lack of SDH activity and consequent other changes lead to the Warburg effect in *SDHx*-related tumors. Because metabolic control over the glycolytic rate can be applied at many steps in the glycolytic pathway (Dang *et al.* 1997, Gatenby & Gillies 2004), most studies in cancer support the hypothesis that control over glycolytic flux primarily resides at the transport and phosphorylation steps (upregulation of glucose transporters (notably GLUT1 and GLUT3) and HK2; Gatenby & Gillies 2004, Mathupala *et al.* 2009, Choi *et al.* 2013). HIF α enhances the glycolytic pathway by increasing target gene expression from GLUT1, GLUT3, through HK2 and pyruvate kinase variant M2 (PKM2) to lactate dehydrogenase-A (LDH-A) and other glycolytic and anabolic enzymes and metabolites (Osthus *et al.* 2000, Soga 2013). Some expression studies have not found the overexpression of GLUT1 in *SDHx*-related tumors (Favier *et al.* 2009, Lopez-Jimenez *et al.* 2010, Fliedner *et al.* 2012). Moreover, increased expression of *GLUT3* and *HK2* mRNAs observed in *SDHx*-related tumors (Favier *et al.* 2009, Fliedner *et al.* 2012) can explain the high sensitivity of [¹⁸F]-FDG PET for *SDHx*-related tumors, mainly observed in *SDHB*-related PHEOs/PGLs (Timmers *et al.* 2007, 2009b, 2012, Zelinka *et al.* 2008, Taieb *et al.* 2009). Fliedner *et al.* (2012) detected the M2 isoform of *PKM2* mRNA, which appeared to be possibly elevated in *SDHB*-mutant tumors. *PKM2* is generated by increased transcription and alternative splicing of the *PKM2* gene through a HIF1 α and c-Myc-mediated process. *PKM2* catalyzes the final and also rate-limiting reaction in the glycolytic pathway and promotes tumorigenesis by regulating the Warburg effect. *PKM2* also possesses a positive feedback regulation toward HIF1 α . *PKM2* interacts with HIF1 α in the nucleus and functions as a transcriptional coactivator to enhance the expression of HIF1 α target genes that promote the shift from OXPHOS to glycolytic metabolism (Luo & Semenza 2011, 2012, Luo *et al.* 2011). Also, overexpression of LDH-A has been found in *SDHx*-related tumors (Favier *et al.* 2009, Fliedner *et al.* 2012). In proliferating cancer cells, the majority of the pyruvate generated from glucose (>90%) is converted to

lactate by LDH-A, where it is readily secreted into the extracellular environment. By converting pyruvate to lactate, LDH-A recovers the NAD⁺ needed to maintain glycolysis and ATP production. This step is critical for the maintenance of tumor proliferation *in vivo* (Fantin *et al.* 2006, Jones & Thompson 2009). LDH-A may be upregulated by a high glycolytic flux through the carbohydrate-response elements (ChoREs) by binding HIF or myc products (Semenza 2002a,b, Walenta & Mueller-Klieser 2004). Moreover, both LDH-A and mitochondria activity are mutually regulated at the level of metabolites. They depend on the availability of pyruvate and on the NADH:NAD⁺ ratio. The generation of lactate and the export of intracellular acid lead to an acidic tumor microenvironment, which is correlated with a poor prognosis and may facilitate tumor invasion and metastasis leading to the stimulation of cell migration and angiogenesis (Chiche *et al.* 2010, Vegran *et al.* 2011). Thus, activation of HIF α , c-myc, and other proteins stimulates many processes that result in the Warburg effect in these tumors (Vogelstein & Kinzler 2004, Deberardinis *et al.* 2008, Yuneva 2008, Jones & Thompson 2009, Gogvadze *et al.* 2010, Levine & Puzio-Kuter 2010, Cairns *et al.* 2011, Koppenol *et al.* 2011).

Glutamine metabolism

Tannahill *et al.* (2013) showed a dysfunctional TCA cycle pointed toward an alternative source of succinate. The microarray study showed a significantly higher concentration of glutamine transporter *SLC3A2* mRNA. Thus, substantial increases in succinate accumulation have been demonstrated through processes involving increased import and metabolism of glutamine (Tannahill *et al.* 2013). Therefore, we suggest that glutamine metabolism can be involved in *SDHx*-related tumors. Succinate can be derived from glutamine through anaplerosis by α -ketoglutarate. Recently, Imperiale *et al.* (2013) found significantly lower values of glutamate in *SDHx*-related tumors compared with other subtypes. These catabolic pathways are reversible and involve the removal of nitrogen as part of the mechanism that regulates nitrogen homeostasis; the carbon skeleton from glutaminolysis may eventually enter anabolic or anaplerotic processes (including the formation of nucleotides, lipids, and proteins; Yuneva 2008, Dang 2010, Eng & Abraham 2010).

Meng *et al.* (2010) observed that nitrogen source restriction repressed carbon metabolic pathways, including glucose utilization. Therefore, the interconversion between glutamine and α -ketoglutarate serves as the

bridge connecting nitrogen and carbon metabolism. Thus, glutaminolysis and the Warburg effect become two integral parts of the cellular machinery to balance the carbon and nitrogen metabolism. Glutaminolysis also supports the production of molecules, such as GSH and NADPH, which protect cells from oxidative stress.

OXPHOS proteins

OXPHOS proteins include the electron transport chain components, ATP synthase, and the adenine nucleotide translocator. Information about other OXPHOS proteins besides complex II in *SDHx*-related tumors is limited. Favier *et al.* (2009) suggested a lower expression of OXPHOS protein complexes I–IV in *SDHx*- and *VHL*-related tumors than in PHEOs/PGLs harboring *NF1* and *RET* mutations, but complex V expression was relatively similar in all patients. Also, the activity of complexes II, III, or IV was found to be decreased in *SDHx*- and *VHL*-related PHEOs/PGLs, but the differences were smaller for complexes III and IV. In contrast, other groups showed that the activity of SDH or respiratory chain enzyme complex II is low in *SDHx*-related tumors and associated with increased activities of respiratory chain complexes I, III, and IV and citrate synthase. All these factors suggest a compensatory response to the lack of SDH activity (Fliedner *et al.* 2012, Rao *et al.* 2013). However, as shown by Rao *et al.* (2013), the apparently increased activity of complex I, III, IV, and citrate synthase in the *SDHx*-related tumors does not lead to a full restoration of ATP/ADP/AMP, because the concentration of ATP/ADP/AMP was consistently very low in all *SDHx*-related tumors. Rao *et al.* found positive relationships between mitochondrial complex II function, tumor ATP/ADP/AMP content, and tumor catecholamine contents, and suggested the possibility that differences in energy metabolism might also contribute to the lower tumor tissue catecholamine contents in cluster 1 than in cluster 2 tumors. Thus, increased activity of complex I, III, and IV may be a compensatory reaction to a lack of or decreased complex II activity in these tumors.

Thus, the generation of ROS as well as pseudohypoxia and succinate accumulation results in significant changes in key pathways: HIF, glycolysis, angiogenesis, genomic instability, increased cell cycle, and increased mutability.

In summary, increased ROS production has been suggested to contribute to tumorigenesis in *SDHB*-related tumors (Guzy *et al.* 2008, Goffrini *et al.* 2009, Huang & Lemire 2009). *SDHx* mutation-induced increases in ROS

have recently been shown to cause genomic instability that may contribute to tumorigenesis (Slane *et al.* 2006, Owens *et al.* 2012). Second, accumulation of succinate leads to widespread changes, from stabilization of HIF α through inhibition of PHD to DNA hypermethylation, a process causing global changes in gene expression. This accumulation of succinate accompanies low fumarate in malignant *SDHB* tumors. This high succinate:fumarate ratio can be used as a predictor of malignancy in the future. Third, the specific catecholamine phenotype in *SDHx*-related tumors may be due to downregulation of HIF1 α and upregulation of HIF2 α . Fourth, not only glycolysis but also glutaminolysis may be involved in *SDHx*-related tumors.

Future treatment options to attack metabolic alterations in *SDHx*-related tumors

Understanding specific metabolic alterations characteristic and unique to *SDHx*-related PHEOs/PGLs and increasing availability and implementation of molecular profiling and metabolomics in clinical medicine opens new promising options for the use of multiple and personalized metabolic-specific molecular-targeted therapies in the near future, as originally suggested by Eng *et al.* (2003). Several key events involved in the pathogenesis of *SDHx*-related PHEOs/PGLs have been described, such as i) an increase in ROS production resulting in oxidative stress and stabilization of HIF1/2 α and ii) accumulation of succinate which inhibits 2OG-dependent dioxygenases and causes hypermethylated and pseudohypoxic phenotypes. Identification of subgroups of specific molecular-metabolic phenotypes may be especially useful in personalized medicine. Furthermore, targeted therapies hold promise for the treatment of metastatic *SDHx*-related tumors. Thus, although outlined below separately, these approaches are viewed as tightly interconnected and should be combined when appropriate treatments or knowledge are available.

Restoration of SDH activity

An increase in the expression and stabilization of SDH proteins is crucial to prevent various metabolic dysregulations resulting from the absence of *SDHB* protein and therefore dysfunctional mitochondrial complex II in *SDHx*-related tumors. An increase in the expression and stabilization of SDH proteins is crucial to prevent various metabolic dysregulations resulting from the absence of *SDHB* protein and therefore dysfunctional mitochondrial

complex II in SDHx-related tumors. Recently, Yang and colleagues demonstrated that the loss of SDHB function was due to a reduced half-life of mutant protein by rapid proteasome degradation. The authors found that histone deacetylase inhibitors (HDACi) inhibited proteasome degradation of SDHB-mutated protein resulting in its increased stabilization and activity (Yang et al. 2012). However, this approach, although of interest in the near future, cannot be applied to patients with nonsense *SDHx* mutations or *SDHx* gene deletions, because no protein is generated and the second allele is missing by the mechanism of loss of heterozygosity. Furthermore, it is expected that this approach will only partially increase the availability of mutated (and still dysfunctional) protein that will only improve mitochondrial function to a certain degree leading to persistent metabolic dysregulations (although to a lesser degree). Therefore, this therapy most likely will need to be combined with other therapeutic approaches.

Restoration of PHD activity

As described in our study, both succinate and ROS contribute to the inactivation of PHD and subsequent stabilization of the HIF1/2 α signaling pathway. PHD action normally requires oxygen and α -ketoglutarate as co-substrates. PHD inactivation by succinate is competitive with α -ketoglutarate. Therefore, increasing the α -ketoglutarate:succinate ratio levels by treatment with α -ketoglutarate derivatives could critically affect PHD activity and decrease the stabilization of HIF1/2 α (MacKenzie et al. 2007, Tennant et al. 2009, Jokilehto & Jaakkola 2010). Furthermore, esterified α -ketoglutarate induces apoptosis and inhibits tumor growth. These effects are independent of HIF α but dependent on the presence of PHD3 (Tennant & Gottlieb 2010).

Direct activation of PHD by the activator KRH102053 increases HIF1/2 α hydroxylation and promotes its degradation (Choi et al. 2008, Nepal et al. 2011). Targeting HIF1/2 α with their specific inhibitors (e.g. currently by either the direct inhibitor PX-478 or the indirect inhibitor PX-12; both targeted to HIF1 α) has shown antitumoral activity in human tumor xenografts in mice and also seems to be promising for malignant PHEO/PGL (Welsh et al. 2003, 2004, Semenza 2007). However, currently there are no HIF2 α inhibitors that would be preferable in the treatment of *SDHx*-related PHEO/PGL. Nevertheless, it is expected that these compounds will be introduced in the near future (Rogers et al. 2013).

Prevent ROS damage

A rationale for using antioxidants in HIF1/2 α -driven tumors was recently provided by Gao et al. (2007), who examined the antitumorigenic effect of the antioxidant NAC. They reported that NAC treatment resulted in reduced HIF1 α expression and inhibition of *in vivo* tumor formation in a HIF-driven model of tumorigenesis. Ni & Eng (2012) concluded that the lipid-soluble antioxidant α -tocopherol can selectively protect *SDHx*^{var+} cells from oxidative damage and apoptosis resistance and rebalance the redox metabolites, NAD/NADH, which is a promising opportunity to prevent the development of tumors in patients with *SDHx* mutations. This concept is very unique, introducing prevention for the first time in the treatment of *SDHx* carriers. Thus, α -tocopherol, ascorbic acid, and HDACi could be administered over a long period and could serve as a novel therapeutic paradigm for preventing the development of *SDHx*-related PHEOs/PGLs (Ni & Eng 2012, Yang et al. 2012).

Heat shock protein 90 inhibitors

Malignant *SDHx*-related PHEO/PGL overexpresses heat shock protein 90 (HSP90), a molecular chaperone that assists in binding to HIF1/2 α and promotes its stability by preventing ubiquitination and proteasomal degradation of HIF1/2 α (Liu et al. 2007, Mahalingam et al. 2009, Semenza 2010). Thus, inhibitors of HSP90, such as geldanamycin, and analogs, such as 17-allylamino geldanamycin (17-AAG; tanespimycin), 17-dimethylaminoethylamino-17-demethoxygeldanamycin (alespimicin), or other new analogs, are promising therapeutic agents (Isaacs et al. 2002, Northcott et al. 2012). Giubellino et al. (2013) demonstrated the potent inhibition of proliferation and migration of PHEO cell lines and induced degradation of key Hsp90 clients by 17-AAG and ganetespib. They also showed the efficacy of 17-AAG and ganetespib in reducing metastatic burden and increasing survival in metastatic model of PHEO (Giubellino et al. 2013).

Glycolysis inhibition

In addition, when the TCA cycle is genetically compromised, as is the case in *SDHx*-related PHEO/PGL, glycolytic addiction of the tumor cells is ensured. These tumors are 'glucose addicts' as revealed by their almost 100% of positivity on [¹⁸F]-FDG PET. This may prove to be an Achilles' heel of these tumors. Thus, strategies disrupting glycolytic mechanisms can be used in the future.

A small-molecule inhibitor of GLUT1, such as WZB117 or STF-31, downregulates glycolysis and inhibits cancer cell growth *in vitro* and *in vivo* (Chan *et al.* 2011, Liu *et al.* 2012). In addition, dichloroacetate (DCA) and 3-bromopyruvate reverse cancer-specific aerobic glycolysis (Michelakis *et al.* 2008, Cardaci *et al.* 2012, El Sayed *et al.* 2012, Kluza *et al.* 2012, Kumar *et al.* 2012, Matsushita *et al.* 2012, Sutendra & Michelakis 2013, Sutendra *et al.* 2013). DCA downregulates pyruvate dehydrogenase kinase, which, under normal conditions, upregulates the glycolysis enzyme pyruvate dehydrogenase (Michelakis *et al.* 2008, Kluza *et al.* 2012, Kumar *et al.* 2012, Sutendra & Michelakis 2013, Sutendra *et al.* 2013), shifting metabolism from glycolysis to glucose oxidation and selectively inducing apoptosis in cancer cells (Xie *et al.* 2011). Furthermore, inhibitors of HK2 (Pedersen 2012, Yu *et al.* 2012) may also represent a novel therapeutic approach to malignant SDHx-related PHEO/PGL.

Disruption of pH regulators

In addition, activation of the HIF1 α pathway enhances glycolytic metabolism and generates increased amounts of lactic and carbonic acids. This poses considerable cellular stress and requires a continuous regulation by several pH-regulating systems. It has been shown that disruption of these proteins may provide an effective avenue for future targeted therapies in different cancer models (Parks *et al.* 2013). However, several studies have shown a predominant expression of HIF2 α over HIF1 α in SDH-related tumors (Eisenhofer *et al.* 2004, Favier *et al.* 2009, Jochmanova *et al.* 2013), suggesting a leading role for HIF2 α in SDHx-related tumorigenesis. Thus, the identification of subgroups of patients with preferential or combined activation of HIF1 α or HIF1/2 α , respectively, would help in the development of 'personalized' approaches in this type of therapy. In these patients, disrupting pH-regulating capacities and the export of lactic acid from tumor cells (by disrupting monocarboxylate transporters (MCTs)) could reduce glycolysis and growth rates. Additional strategies could be developed by disrupting glycolytic mechanisms.

Disruption of alternative signaling pathways

Additional treatment strategies could target abnormally functioning pathways, possibly in conjunction with targeting metabolic pathways. For example, the pseudo-hypoxic response and abnormal energy status of tumor cells activate kinase signaling pathways such as

PI3kinase/AKT, RAS/RAF/ERK, and mTOR1/p70s6K, which leads to abnormal cell growth and a lack of apoptotic capacity (Abraham & Eng 2010, Choo *et al.* 2010, Nolting & Grossman 2012). Favier *et al.* (2012) showed that the mTOR pathway was potentially activated in half of PHEO/PGLs. Nolting *et al.* (2012) showed that combination treatment with dual NVP-BEZ235 (a PI3K/mTORC1 inhibitor) and lovastatin (an inhibitor of ERK signaling) had a significant additive effect in mice PHEO MPC and MTT cells and resulted in the inhibition of both AKT and mTORC1/p70S6K signaling without ERK upregulation. However, recently, Ghayee *et al.* (2013) suggested that the use of mTOR inhibitors alone for metastatic SDHB PHEOs/PGLs may not achieve good therapeutic efficacy in these patients.

Summary

Recent advances and insights into SDHx-related PHEOs/PGLs as tumors with significant changes in their metabolism may lead to major advances in the treatment of these tumors.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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Author contribution statement

A Vicha, D Taieb, and K Pacak contributed to the manuscript conception and design, data collection and interpretation, writing, editing, and final proof. K Pacak provided administrative support.

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