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 SDH 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

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 programing 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 paramout 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, Koppershoek 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...
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) (Papaspyrou et al. 2012).

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 or 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 HIFα. HIFα 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.

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

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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 2α (HIF2α; Zhuang et al. 2012, Toleda 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 KIF1Bβ 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 HIF2α 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 HIF1α/2α 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/ endocrine 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 severalfold 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 HIF1α 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 substrates 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 HIFz 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 HIFz–PHD interaction leads to the stabilization of HIFz and activation of the HIF complex (Lee et al. 2005). HIFz 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 HIF1α and/or HIF2α (Dahia et al. 2005, Selak et al. 2005, Mole et al. 2009, Favier & Gimenez-Roqueplo 2010, Semenza 2010, 2011, 2012, Keith et al. 2012). HIF1α and HIF2α 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). HIF1α 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 HIF2α-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 (Holmiquist-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 HIF2α overexpression in VHL PHEOs and PGLs, whereas in SDHx-related tumors nuclear HIF1α staining was more prominent, Gimenez-Roqueplo et al. (2001, 2002) described overexpression of HIF2α 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 HIF2α 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 HIF2α 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 HIF2α 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 HIF1α 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.
Endocrine-Related Cancer

ROS exposure can result in oxidative damage to mitochondrial and cellular proteins, lipids, and nucleic acids. The activity of the ROS-generating enzyme Nox1 is capable of causing chronic metabolic oxidative stress

Reactive oxygen species

A lack of SDH activity results in increases in steady-state levels of O2 to H2O2 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 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.

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). HIFα 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 SDH-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 HIF1α 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 HIF1α. PKM2 interacts with HIF1α in the nucleus and functions as a transcriptional coactivator to enhance the expression of HIF1α 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 (Fanti 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, Valenta & 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 HIFα, 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 anaerobic 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
Endocrine-Related Cancer consistently very low in all P/AMP, because the concentration of ATP/ADP/AMP was tumours does not lead to a full restoration of ATP/AD- related tumours than in PHEOs/PGLs harboring 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-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 pseudo- hypoxia 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 tumours (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 HIFα 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 HIF1α and upregulation of HIF2α. 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 HIF1/2α 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.
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 was due to a reduced half-life of mutant protein by rapid degradation of SDHB-mutated protein resulting in its sequent stabilization of the HIF1/2a. 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 HIF1/2a 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 HIF1/2a (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 HIFα but dependent on the presence of PHD3 (Tennant & Gottlieb 2010).

Direct activation of PHD by the activator KRH102053 increases HIF1/2α hydroxylation and promotes its degradation (Choi et al. 2008, Nepal et al. 2011). Targeting HIF1/2α with their specific inhibitors (e.g. currently by either the direct inhibitor PX-478 or the indirect inhibitor PX-12; both targeted to HIF1α) 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 HIF2α 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 HIF1/2α-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 HIF1α 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 SDHxvar+ 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 HIF1/2α and promotes its stability by preventing ubiquitination and proteasomal degradation of HIF1/2α (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 (alespimycin), 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.

Disruption of pH regulators

In addition, activation of the HIF1α 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 HIF2α over HIF1α in SDH-related tumors (Eisenhofer et al. 2004, Favier et al. 2009, Jochmanova et al. 2013), suggesting a leading role for HIF2α in SDHx-related tumorigenesis. Thus, the identification of subgroups of patients with preferential or combined activation of HIF1α or HIF1/2α, 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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