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Laurenti, Giulio; Tennant, Daniel

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Isocitrate dehydrogenase (IDH), Succinate dehydrogenase (SDH), Fumarate hydratase (FH): Three players for one phenotype in cancer?

Giulio Laurenti and Daniel A. Tennant

Institute of Metabolism and Systems Research, University of Birmingham, Birmingham UK

In the early 1920s Otto Warburg observed that cancer cells have altered metabolism and from this, posited that mitochondrial dysfunction underpinned the aetiology of cancers. The more recent identification of mutations of mitochondrial metabolic enzymes in a wide range of human cancers has now provided a direct link between metabolic alterations and cancer. In this review we discuss the consequences of dysfunction of three metabolic enzymes involved in or associated with the Tricarboxylic Acid (TCA) cycle: succinate dehydrogenase (SDH), fumarate hydratase (FH) and isocitrate dehydrogenase (IDH) focusing on the similarity between the phenotypes of cancers harboring these mutations.

Keywords: Isocitrate dehydrogenase, succinate dehydrogenase, fumarate hydratase, cancer metabolism, hypoxia, ROS.

Correspondence should be addressed to:

Giulio Laurenti

Institute of Metabolism and Systems Research, University of Birmingham

Birmingham, B15 2TT, UK

Email:g.laurenti@bham.ac.uk

Telephone: +44 121 414 3768

Introduction

Metabolic reprogramming is now a recognized hallmark of cancer transformation. The first observation of this phenotype was in the early 1920's, when Otto Warburg described the concept of aerobic glycolysis in tumours. However, almost a century later, it is still unclear whether in most tumours this is the cause or a consequence of the transformation itself. A number of seminal discoveries since 2000 have shown that in some cases, metabolic transformation at least accompanies the genesis of a tumour. Dysfunction of one of three enzymes involved in the tricarboxylic acid cycle (TCA): isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH) and fumarate hydratase (FH) have been shown to be directly responsible for the initiation of cancer in some hereditary and sporadic malignancies. We discuss here whether the knowledge gained from the study of mutations in any of these three enzymes in tumours can lead us to a general view of the role of metabolic dysfunction in oncogenesis. In this review, we discuss the role of mutations in these enzymes on a number of aspects of cellular phenotype: specifically, tumour metabolism and its downstream effects.

Function of wild type IDH, SDH and FH.

Cellular metabolism can be simplified into two main functions: anabolism, which supports the synthesis of macromolecules to sustain processes such as cellular repair or proliferation, and catabolism, supporting the breakdown of macromolecules to obtain energy or new anabolic substrates. The TCA cycle, which functions within the mitochondria of the cell, supports both functions. Catabolism of macromolecules provides metabolic intermediates that are incorporated into the TCA cycle, providing reducing equivalents in the form of reduced adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$) to the electron transport chain (ETC) for the production of ATP. Anabolism is also supported through the production of the necessary metabolic building blocks for the synthesis of macromolecules like fatty acids, amino acids and nucleotides [1]. IDHs are a key family of enzymes responsible for the incorporation of glucose and fatty acid carbons into the TCA cycle. They catalyze the decarboxylation of isocitrate to α-ketoglutarate (α-KG) with the concomitant reduction of NAD(P) to NAD(P)H. Mammals express three isoforms of the enzyme: cytosolic NADP-dependent IDH1, mitochondrial NADP-dependent IDH2 and mitochondrial NAD-dependent IDH3 [2]. IDH1 and IDH2 are homodimers that catalyze the reaction reversibly, while IDH3 is a heterotetramer that only oxidizes isocitrate. Also within the TCA cycle, SDH, also known as complex II, is an enzyme with roles both in the TCA cycle and ETC. In the former, SDH catalyzes the oxidation of succinate to fumarate, using the electrons generated to reduce ubiquinone to ubiquinol in the ETC. SDH is a heterotetrameric enzyme composed of four subunits (SDHA, SDHB, SDHC and SDHD) and unlike many other enzymes of the TCA cycle, does not have a cytoplasmic counterpart [3]. The adjacent enzyme in the TCA cycle, which catalyzes the reversible hydration of fumarate to malate is FH, a homotetrameric enzyme that has both mitochondrial and cytosolic forms.

IDH, SDH and FH mutations in cancer

Of the three enzymes described above, the most commonly mutated in cancer is thought to be IDH1 and IDH2. Mutations in these enzymes were first shown in colon cancers [4], and successively detected in other cancers such as gliomas [5], acute myeloid leukemia (AML) [6] and choloangiosarcoma [7]. Interestingly mutations in IDH3 have yet to belinked to tumourigenesis. While mutations in IDH in gliomas are associated with a better prognosis than those patients with IDH wild-type tumours, in a specific subtype of cytogenetically normal AML (CN-AML) they appear to be associated with a worse outcome [8], suggesting that despite a common metabolic profile, the biological consequences of IDH mutations can vary with cell type or location. The most common IDH mutation found in cancer is the substitution of a single arginine in the catalytic site of the enzyme, R132 in IDH1 and R140 or R172 in IDH2, which results in a gain of function. Instead of the oxidative decarboxylation of isocitrate to α-KG, the mutant form is unable to bind isocitrate, instead reducing α-KG to produce the new metabolite 2-hydroxyglutarate (2-HG) [9]. Mutations in SDH, associated with succinate accumulation to millimolar concentrations, were discovered to be causative of hereditary paraganglioma/pheochromocytoma (HPGL/PCC) [10]. Since then have been observed in a number of other cancers including thyroid, neuroblastoma and ovarian cancer [11]. FH inactivating mutations, which are associated with the accumulation of fumarate, are the main characteristic of germline mutations found in patients affected by hereditary leiomyomatosis and renal cell carcinoma (HLRCC) [12], a neoplastic syndrome that predisposes patients to the development of uterine and skin leiomyomas as well as a very aggressive form of renal cancer. More recently, mutations in FH have also been demonstrated in bladder, breast and testicular cancer [13].

How do mutations in IDH, SDH and FH support malignant transformation?

Metabolic reprogramming

To meet the needs of highly proliferative cells, tumours undergo metabolic remodeling [14], generally summarized as an increase in glucose and glutamine uptake and consumption, and increased production of glycolytic ATP and lactate. Due to having central roles in cellular metabolism, alterations of enzymes in the TCA cycle result in significant perturbations in cellular metabolism, resulting in metabolic pathway re-wiring in order for the cells to synthesize anabolic building blocks such as amino acids, fatty acids and nucleotides. Mutations in SDH are characterized by high levels of succinate and low concentrations of fumarate and malate. However, the metabolic perturbations extend further into central carbon metabolism, reducing concentrations of other amino acids, nucleotides, glycolytic and pentose phosphate pathway intermediates. In an ovarian cancer model, mutations in SDH correlate with an increased contribution of glucose to ATP production and redirection of glycolytic intermediates into the pentose phosphate pathway for nucleotide production. In addition, increased glutamine uptake and contribution to TCA cycle intermediates has also been observed [15]. Recently, it has been demonstrated that SDH defective cells increase their reliance on the activity of pyruvate carboxylase (PC), which converts pyruvate into the

TCA cycle intermediate oxaloacetate (OAA). From this, cells can synthesize aspartate despite the truncated TCA cycle present in SDH-deficient cells [16]. In kidney cancers, mutations in FH were also shown to induce an increase in glucose uptake, glycolytic rate and contribution of glucose to the pentose phosphate pathway [28]. Interestingly, these cells also exhibit a lower but still significant oxidative TCA cycle activity, supported by increased glutamine metabolism [17]. A further study showed that FH mutations in kidney cancer are associated with a reduction in the activity of the metabolic sensor, AMP-activated protein kinase (AMPK), which led to increased synthesis of fatty acids and proteins to support ongoing cellular anabolism [18]. Similarly, fatty acid synthesis is likely perturbed in IDH1 mutated cells, as one of the consequences of this mutation is the decrease in NADPH production, the reducing potential from which is absolutely required for this metabolic process [19]. Mutations in IDH, SDH and FH have therefore been shown as resulting in significant metabolic re-wiring, especially of the mitochondrial metabolic network, and these alterations directly support tumorigenesis (Figure 1).

ROS production

Reactive oxygen species (ROS) are a family of highly reactive molecules derived from oxygen that contain an unpaired electron, conferring on them the ability to oxidize other molecules, thereby altering their function [20]. Increased ROS production can result in the oxidation of reactive amino acid residues within proteins, fatty acids, and the irreversible modification of DNA, all of which can induce the activation of oncogenic pathways that contribute to cancer transformation and progression. Due to their considerable reactivity, levels of ROS are maintained at low steady-state by a series of detoxification systems; one of the most important of which is the glutathione system. In the last few years, a number of studies have shown that mutations in IDH, SDH and FH can contribute directly (by increasing ROS production) or indirectly (by inactivating antioxidant pathways) to the high levels of ROS often observed in cancer (Figure 1). IDH mutations have been shown to reduce NADPH production while increasing its consumption through the conversion of α-KG to 2-HG. NADPH is essential for the maintenance of the reduced gluthatione (GSH) pool, a major anti-oxidant system within cells. Insufficient NADPH to maintain a favorable GSH:GSSG ratio results in increased steady-state ROS and therefore oxidation of cellular constituents (Figure 1). Consequently, cells with IDH mutations have higher DNA mutation rates, lipid peroxidation and reduced survival in the presence of oxidative stress [21]. In glioma cells, IDH mutations are associated with reduced GSH, increased ROS levels and increased sensitivity to chemotherapy [22]. Alterations in the glutathione system are also associated with FH mutations. Accumulation of fumarate in FH mutant cells induces the production of succinic-glutathione (GSF), which can act as an alternative substrate for glutathione reductase, resulting in reduced NADPH and GSH levels and increased ROS production (Figure 1) [23]. Mutations in SDH, on the other hand, have been associated with a more direct increase in mitochondrial ROS production (Figure 1). Mutations in SDHC and SDHB but not SDHA subunits have been shown to directly lead to increased ROS production and in the case of SDHC, elevated rates of DNA mutation [24, 25].

HIF stabilization and pseudohypoxia

The growth of most solid tumours is associated with reduced oxygen availability, known as hypoxia. Much of the adaptation to hypoxia is mediated by a family of heterodimeric transcription factors known as Hypoxia Inducible Factors (HIFs), which consist of an oxygen-labile α subunit and a constitutively expressed β subunit [26]. Under normoxic conditions the α subunit undergoes constitutive degradation by a process that involves a series of post-translational modifications. The first step of this process is mediated by a family of α-KG dependent enzymes known as HIF prolyl hydroxylases (PHDs), which hydroxylate two highly conserved prolyl residues on the HIFα subunit. PHDs catalyze a reaction that couples the hydroxylation of the target prolyl residue to oxidative decarboxylation of α-KG to succinate in a process that utilizes O_2 and requires Fe^{2+}. After hydroxylation, HIFα subunits are recognized by the von Hippen-Lindau (pVHL) E3 ubiquitin ligase, which mediates ubiquitylation of the hydroxylated protein, permitting proteasomal degradation [27]. As PHDs depend on oxygen as co-substrate, they are inactive under hypoxic conditions, resulting in HIFα translocation into the nucleus, its dimerization with HIFβ and induction of the transcription of genes involved in processes such as glucose metabolism and angiogenesis (Figure 2). More recently, it has been shown that tumours with defects in SDH, FH and perhaps IDH stabilize HIF-1α even under normoxic conditions: a phenotype known as pseudohypoxia (Figure 2). Mutations in IDH are associated with the production of 2-HG: a metabolite structurally similar to α-KG that can interact with PHDs (Figure 2). However, its effect on these enzymes is disputed: it has been reported to inhibit, activate directly, and activate indirectly (through degradation to α-KG). It is therefore not clear the result of IDH1 mutations on HIF-1α activity in tumours, although it has been reported to increase its expression [28]. Selak and colleagues demonstrated that loss of SDH activity leads to the inhibition of PHD enzymes and stabilization of HIF-1α through the accumulation of succinate (which is structurally similar to α-KG) (Figure 2) [29]. Activation of the HIF-1α pathway following inactivation of SDH has also been demonstrated in a series of familial PGL tumours [30]. Finally, activation of the HIF-1α pathway due to PHD enzyme inhibition has been demonstrated in cells with inactivating mutations of FH. The raised fumarate observed as a result of inactivation of this enzyme competes with α-KG for binding to the PHDs, resulting in their inhibition (Figure 2). HIF-1α is therefore stabilized and, as a result, high levels of HIF-1α transcription factor and its target genes have been observed *in vitro* and in patients affected by HLRCC leiomyomas [31].

NRF2 pathway activation: a new entry in the phenotype induced by mutations in metabolic enzymes?

In the last few years, it has become apparent that the activation of a further transcription factor, called Nuclear factor erythroid2-related factor 2 (NRF2), may constitute another common signaling pathway used by tumours formed through mutations in SDH, FH, IDH and perhaps other metabolic enzymes. NRF2 is a key player in the activation of a cellular anti-oxidant defense transcriptional profile, and although it is well known to have protective

effect in neurodegenerative disease, aging and cancer, in the last few years many studies revealed a darker side to NRF2, which may be tumour-promoting [32]. Under physiological conditions, NRF2 is sequestered in the cytosol by its inhibitor, Kelch-like ECH-associated protein 1 (KEAP1). During oxidative stress, modification of cysteine residues of KEAP1 results in the release of NRF2, which accumulates in the nucleus (Figure 2) [33]. In FH-inactivated cells, fumarate accumulation induces succination of cysteine residues on KEAP1 and activation of the NRF2 pathway (Figure 2). Papillary type 2 renal cells carcinoma that harbor an inactive FH have been shown to upregulate NRF2 target genes in both *in vitro* [34] and *in vivo* [35]. Although a direct relation between SDH inactivation and NRF2 pathway activation has not yet been identified, SDH mutations are highly likely to result in NRF2 pathway activation – perhaps through increased ROS production (Figure 2). In support of this hypothesis, the pharmacological inhibition of SDH by 3-nitropropionic acid (3-NPA) has been shown to be able to induce nuclear translocation of NRF2 in human astrocytes, showing directly that SDH inhibition can result in NRF2 pathway activation [36]. Kanamory and colleagues have also demonstrated that in anaplastic gliomas with IDH1/2 mutations, high levels of NRF2 target genes were associated with a poor prognosis, while in IDH wild-type tumours NRF2 status had no apparent influence on the prognosis of patients [37], suggestive of a co-operation between other phenotypes induced by IDH1 mutations, and NRF2 pathway activation.

Conclusions

The identification of a number of metabolic enzymes as underpinning the formation of some hereditary and sporadic malignancies represented a step forward in understanding cancer biology, and confirmed that Warburg's hypothesis of mitochondrial dysfunction underlying cancers is correct at least in a subset of tumours. Mutations in some enzymes of the TCA cycle: IDH, SDH and FH are associated with the accumulation of metabolites that are able to influence many aspects of cancer development and progression and for this reason are termed onco-metabolites. Due to their structural similarity with α-KG, the metabolites 2-HG, succinate and fumarate can interact and inhibit a series α-KG-dependent dioxygenase enzymes, among which PHD family is currently one of the best described. PHD inactivation has been shown to induce HIF stabilization and the activation of a hypoxic response under normoxic conditions, known as a pseudohypoxic phenotype.

Dysfunction in IDH, SDH and FH are also involved, directly or indirectly, with the higher levels of ROS often found in cancer cells. Because of the central role of the TCA cycle in cellular metabolism, alteration in its activity through enzyme mutation induces metabolic reprogramming in order to maintain the energetic and anabolic demands of the proliferating cancer cell. New evidence also suggests that dysfunction in metabolic enzyme activity might play a role in the activation of the NRF2 pathway, which in the last few years has been demonstrated to support malignant aspects of cancer transformation.

Cancers harboring IDH, SDH or FH therefore appear to be highly dependent on the induction of a common phenotype which comprises the activation of a hypoxic cellular response and increased intracellular levels of ROS, while maintaining cell proliferation through metabolic re-wiring. In addition, it is highly likely that they all also activate the NRF2 pathway, providing protection against oxidative stress and toxins, resulting in phenotypes such as therapy resistance. Understanding the similarity between the phenotypes induced by mutations of metabolic enzymes could therefore provide therapeutic targets valid for a wide range of different tumours, and a holistic approach that investigates the effects of mutations in these enzymes in parallel may be warranted.

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Figures legend

Figure 1 Influence of mutant IDH1/2 and dysfunctional SDH and FH on ROS production and metabolic reprogramming. Both mutant IDH1/2 and dysfunctional FH induce a reduction in reduced glutathione (GSH) levels causing an indirect increase of ROS while dysfunction in SDH has been suggested to directly induce an increase in ROS production. Increased ROS level then activates signaling pathways that support tumorigenesis. IDH1/2 mutant and dysfunctional SDH and FH directly support the switch towards a metabolism that can support tumorigenesis in presence of and remodeled TCA cycle.
FH, fumarate hydratase; GSH, reduced glutathione; IDH, isocitrate dehydrogenase; ROS, reactive oxygen species; SDH, succinate dehydrogenase.

Figure 2 Mutations in IDH1/2, SDH and FH result in the accumulation of the metabolites 2-HG, succinate and fumarate, respectively. Because these metabolites have a structure similar to α-KG, they can interact and inhibit α-KG dependent dioxygenases such as the HIF Prolyl hydroxylases (PHDs). PHD activity in normoxia induces hydroxylation of HIF-1α, which is then recognized by the E3 ubiquitin ligase, pVHL, inducing ubiquitylation and proteasomal degradation. The inhibition of PHDs by hypoxia or the metabolites shown results in the stabilization of HIF-1α, allowing it to translocate into the nucleus, dimerize with HIF-1β and activate the transcription of its target genes. Fumarate accumulation also induces succination of cysteine residues on KEAP1 inducing its conformational changes and the release of NRF2 which can translocate into the nucleus and activate the transcription of target genes. The activation of NRF2 could also be induced by alterations in SDH activity which can promote an increase in ROS levels, modification of cysteine residues on KEAP1 and release of NRF2.
ARE, antioxidant response element; FH, fumarate hydratase; IDH, isocitrate dehydrogenase; HIF, hypoxia inducible factor; HRE, hypoxia responsive element; 2-HG, 2-hydroxyglutarate; α-KG, α-ketoglutarate; KEAP1, Kelch-like ECH-associated protein 1; NRF2, Nuclear factor erythroid2-related factor 2; OAA, oxaloacetate; PHD, prolyl hydroxylases; mROS, mitochondrial reactive oxygen species; ROS, reactive oxygen species; SDH, succinate dehydrogenase; pVHL, von Hippen-Lindau protein;