

1 | **Oncometabolites: unconventional triggers of oncogenic signalling cascades**

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32 **Abstract**

33 Cancer is a complex and heterogeneous disease thought to be caused by multiple genetic lesions.
34 The recent finding that enzymes of the tricarboxylic acid (TCA) cycle are mutated in cancer
35 rekindled the hypothesis that altered metabolism might also have a role in cellular
36 transformation. Attempts to link mitochondrial dysfunction to cancer uncovered the unexpected
37 role of small molecule metabolites, now known as oncometabolites, in tumorigenesis. In this
38 review, we describe how oncometabolites can contribute to tumorigenesis. We propose that
39 lesions of oncogenes and tumour suppressors are only one of the possible routes to
40 tumorigenesis, which include accumulation of oncometabolites triggered by environmental cues.

41 **Keywords**

42 Mitochondria, cancer, oncometabolites, FH, SDH, IDH, fumarate, succinate, 2-hydroxyglutarate

43 **Background**

44 Cancer is a complex and multifactorial disease. Although its malignant features have been known for
45 centuries, it was not until the advent of modern biology that the molecular determinants of cancer
46 transformation have been elucidated. In 1911, pioneering work from Peyton Rous showed that avian
47 sarcomas were transmissible to other healthy fowls through a filtrate of the tumours devoid of cells
48 [1, 2]. Later on, the agent present in those extracts and responsible for tumour formation was
49 identified as a retrovirus, later called Rous Sarcoma Virus (RSV). This important discovery started the
50 field of tumour virology and led to the identification of the first oncogene *v-Src* [3-6], first in
51 retroviruses and then in normal avian DNA [7]. The emerging idea was that cancer was caused by
52 alterations of the genome. Since then, other oncogenes, including *MYC*, *RAS*, *ERBB*, *PI3K* [2, 6] and
53 the first tumour suppressor gene *RB1* [8, 9] were discovered. The causative role of *RB1* inactivation
54 in retinoblastoma formation reinforced the concept of cancer initiation driven by genomic
55 alterations. These discoveries led Knudson and colleagues to hypothesise a “multiple-hit” model of
56 tumorigenesis, where multiple genetic alterations are required to achieve full blown transformation
57 [8]. We now know that tumorigenesis requires the acquisition of multiple enabling features, the
58 hallmarks of cancer, among which metabolic rewiring is becoming increasingly recognised [10, 11].
59 In a seminal paper, Shim et al. showed that Lactate Dehydrogenase A is a target of the proto-
60 oncogene Myc and is required for *c-Myc*-induced anchorage-independent growth in both human
61 and mouse cellular models [12]. Since then, scientists have uncovered several aspects of the
62 metabolic reprogramming of cancer, and realised that not only dysregulated metabolism is required
63 to sustain proliferation but it also affects tumour microenvironment and the immune response [11].
64 The discovery that mutations in the metabolic genes *Fumarate Hydratase (FH)* [13], *Succinate*
65 *Dehydrogenase (SDH)* [14-17], and *Isocitrate dehydrogenase (IDH)* [18-21] lead to cancer further

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66 supported a primary role of metabolic alterations in tumorigenesis. Thanks to these discoveries, a
67 novel paradigm is emerging whereby mitochondrial metabolites that accumulate in these conditions
68 act as oncogenic signalling molecules, becoming bona fide *oncometabolites*. Recent data suggest
69 that reprogramming of cellular metabolism occurs both as direct and indirect consequence of
70 oncogenic mutations and that environmental cues, such as hypoxia, could also affect the abundance
71 of oncometabolites, amplifying oncogenic cascades. In this review we describe the main oncogenic
72 functions of oncometabolites and how their abundance can be affected by genetic mutations and
73 environmental cues.

74 **TCA cycle enzymes mutations and the emerging paradigm of oncometabolite-
75 driven tumorigenesis**

76 SDH was the first mitochondrial enzyme found mutated in cancer [14]. It was the first time that
77 mutations of a mitochondrial enzyme, once thought to be incompatible with life [22], were linked to
78 tumour predisposition. This and the subsequent discovery of FH mutations in renal cancer catalysed
79 a substantial effort to elucidate the molecular links between mitochondrial dysfunction and
80 tumorigenesis. These major findings are reported below.

81 **Succinate Dehydrogenase (SDH)**

82 SDH is an enzyme of the TCA cycle involved in the conversion of succinate to fumarate and a key
83 component of the mitochondrial respiratory chain. This enzyme is composed of four subunits, SDHA,
84 SDHB, SDHC, SDHD; and two assembly factors, SDHF1 and SDHF2 [23, 24]. Mutations in *SDH* are
85 found in familial paragangliomas and pheochromocytomas [14-17], renal carcinomas [25], T-Cell
86 leukaemia [26], and gastrointestinal stromal tumours [27]. SDH deficiency causes profound
87 metabolic changes. Recent work from Gottlieb and co-workers showed that mouse *Sdhb*^{-/-} cells have
88 a high demand of extracellular pyruvate and utilise glucose-derived carbons for aspartate
89 biosynthesis through pyruvate carboxylation [28].

90 One of the most striking features of SDH-deficient cells is the accumulation of succinate [29],
91 a metabolite implicated in tumorigenesis and, for this reasons, recently defined an oncometabolite
92 [30]. Among its many functions, succinate is a competitive inhibitor of α -ketoglutarate (aKG)-
93 dependent dioxygenases (aKGDD), a class of enzymes involved in a plethora of biological processes.
94 For instance, succinate inhibits prolyl-hydroxylases (PHDs), aKGDDs involved in the degradation of
95 Hypoxia Inducible Factor (HIF), leading to the aberrant stabilisation of HIFs even when oxygen is
96 abundant, a condition called pseudohypoxia [31]. Succinate inhibits other aKGDDs, including Ten-
97 Eleven Translocation proteins (TETs), enzymes involved in DNA demethylation, [32], leading to CpG
98 island hypermethylation [33]. Succinate also causes the inhibition of Histone Lysine Demethylases
99 (KDMs), aKGDDs involved in histone demethylation [32], causing even further epigenetic changes

100 [33, 34]. Interestingly, DNA hypermethylation phenotype was associated with dedifferentiation and
101 increased invasion potential of SDH-deficient tumours [33, 35]. However, the molecular mechanisms
102 behind this phenotypic switch are still under investigation.

103 **Fumarate Hydratase**

104 FH is an enzyme of the TCA cycle that converts fumarate to malate. Whilst homozygous FH
105 mutations cause fumaric aciduria, a condition associated with infantile encephalopathy and brain
106 malformations [36], heterozygous *FH* mutations followed by the loss of heterozygosity of the second
107 allele cause Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) [13, 37]. *FH* is also mutated in
108 paragangliomas, pheochromocytomas [38, 39], downregulated in sporadic clear cell carcinomas [40]
109 and deleted in neuroblastoma [41]. Cristal structure of human FH showed that clinically-relevant
110 mutations affect evolutionary conserved regions involved in either the catalytic activity or the
111 folding and stability of the protein [42], leading to abnormal accumulation of fumarate [43-45]. The
112 loss of FH also leads to a complex rewiring of cell metabolism. For instance, FH loss leads to an
113 increased uptake of glutamine that is diverted into haem synthesis and bilirubin excretion to
114 maintain mitochondrial NADH production and mitochondrial potential. Also, the accumulation of
115 fumarate in FH-deficient cells leads to the reversal of the urea cycle enzyme argininosuccinate lyase,
116 causing the production of argininosuccinate [44, 45]. More importantly, the reverse activity of
117 argininosuccinate lyase makes FH-deficient cells auxotrophic for arginine and sensitive to its
118 depletion[45]. In normal condition, argininosuccinate is produced from aspartate and citrulline by
119 argininosuccinate synthase (ASS1). It is possible to speculate that in FH-deficient cells ASS1 activity is
120 somehow inhibited by arginosuccinate accumulation and therefore aspartate diverted into other
121 pathways such as pyrimidine biosynthesis, favouring tumour growth[46].

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122 Fumarate has been implicated in tumorigenesis of HLRCC and, for this reason, included in
123 the list of “oncometabolites” [47]. Similarly to succinate, fumarate inhibits several aKGDDs, including
124 PHDs, leading to pseudohypoxia [48]. Of note, the non-canonical activation of NF- κ B signalling by
125 fumarate also contributes to the pseudohypoxic phenotype in FH-deficient cells [49]. Although
126 pseudohypoxia has been considered an important driver of tumorigenesis, recent data showed that
127 *HIFs* are dispensable for the formation of benign pre-tumorigenic lesions in Fh1-deficient mice [50].
128 Recent findings identified the Since Abelson murine leukaemia viral oncogene homolog-1 (ABL-1) as
129 another potential driver in fumarate-dependent tumorigenesis[51]. Interestingly, ABL-1 inhibitors
130 suppress the invasion capabilities of FH-deficient cells both *in vitro* and *in vivo*. Mechanistically,
131 through ABL-1 activation, fumarate stimulates an antioxidant response mediated by transcription
132 factor NRF2-related factor 2 (NRF2) and a metabolic rewiring through activation of mammalian
133 target of rapamycin (mTOR)-HIF1A axis.[51] Other mechanisms have been proposed to explain

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134 fumarate-dependent tumorigenesis, which include broad epigenetic changes [52], caused by
135 inhibition of histone and DNA demethylases [32] and post translation modification caused by
136 fumarate. Indeed, fumarate is a mild electrophilic molecule and can react with thiol residues of
137 proteins through a process called *succination* [53-56]. This post-translational modification is a
138 distinctive feature of FH-deficient tumours and now used for diagnostic purposes [54]. Although
139 several succinated proteins have been identified in FH-deficient cells [56, 57], the biological roles of
140 this process are still under investigation. It was recently reported that succination of mitochondrial
141 Aconitase (ACO2) impairs its enzymatic activity [57] and succination of Kelch-Like ECH-associated
142 protein-1 (KEAP-1) inhibits its negative modulatory effect on the transcription factor [NFE2-related](#)
143 [factor-2 \(NRF2\)](#) [50, 58]. NRF2 activation has been previously reported as pro-tumorigenic event
144 [59], but is role in FH-deficient tumours is still debated. The thiol residue of the antioxidant
145 tripeptide Glutathione (GSH) is also subject of succination [60, 61] and its depletion causes oxidative
146 stress, which induces senescence in primary FH-deficient kidney cells. Of note, genetic ablation of
147 *p21*, a major player in senescence induction, in FH-deficient mice induces transformation of benign
148 cysts confirming the tumour-suppressive role of senescence in FH-mediated tumorigenesis [61].
149 [However, the role of fumarate in ROS regulation is still under investigation. For instance, a recent](#)
150 [work by Jin and collaborators showed that fumarate is required for activation of the antioxidant](#)
151 [protein Glutathione peroxidase \(GPx\) by direct binding](#) [62].

152 ***Isocitrate Dehydrogenase (IDH1/IDH2)***

153 Isocitrate dehydrogenases (IDHs) are homodimeric enzymes responsible for the oxidative
154 decarboxylation of isocitrate to αKG. Three different isoforms of IDH have been described, which
155 have distinct subcellular compartmentalisation. IDH3 is a NAD⁺-dependent mitochondrial enzyme,
156 core component of the TCA cycle. The other two IDH isoforms (IDH1 and IDH2) are NADP⁺-
157 dependent proteins expressed respectively in cytosol and mitochondria [24, 63]. Heterozygous
158 missense mutations affecting *IDH1* and *IDH2* were found in gliomas [18, 19] and in acute myeloid
159 leukaemia (AML) [20, 21]. At odds with *SDH* and *FH*, *IDH* mutations are gain-of-function mutations
160 and confer to the enzyme the ability to produce the oncometabolite R-2-hydroxyglutarate (R-2HG)
161 [64, 65]. 2HG, the reduced form of αKG, is naturally present in two optic isomers, R-2HG and L-2HG.
162 In normal cells, 2HG is a minor by-product of metabolism and its levels are kept low by the activity of
163 a conserved family of proteins, the L/D-2-hydroxyglutarate dehydrogenases (L2HGDH and R2HGDH)
164 [66, 67], which convert 2HG to αKG. Homozygous germline mutations in these two enzymes are
165 responsible for a severe form of 2HG aciduria characterised by developmental abnormalities and
166 premature death in children [67, 68]. Moreover, mutations in L2HGDH have been associated with
167 brain tumours [69] and recently with kidney cancer [70]. Both isomers of 2HG can affect the

168 enzymatic activity of aKGDD. Whilst the role of R-2HG in PHD inhibition and HIF1A stabilisation is still
169 controversial [71, 72], this metabolite was shown to inhibit both TETs and KDMs [72, 73].
170 Consistently, *IDH1*-mutant tumours exhibit DNA hypermethylation in gliomas [74] and leukemia [75],
171 and histone hypermethylation [76]. Evidence that *TET2* and *IDH1/2* mutations are mutually exclusive
172 in AML tumours [75] supports the notion that TETs inhibition and the ensuing DNA
173 hypermethylation are instrumental to IDH-driven tumorigenesis. This hypothesis has been recently
174 corroborated by the finding that DNA hypermethylation causes reduced CCCTC-binding factor (CTCF)
175 binding to DNA, leading to aberrant activation of the oncogene Platelet-derived growth factor
176 receptor (*PDGFRA*) [77] in gliomas. Other mechanisms have been proposed to contribute to IDH-
177 dependent tumorigenesis. For instance, 2HG accumulation inhibits ATP Synthase within the
178 mitochondria activating a series of downstream signals that involve mTOR suppression [78].
179 Moreover, 2HG accumulation is also driven by proto-oncogene *MYC* activation in aggressive breast
180 cancer. Interestingly, those breast tumours are characterised by the same distinctive DNA
181 hypermethylation typical of *IDH* mutant tumours [79]. More importantly, the incubation of cells with
182 R-2HG promotes cytokine independence and alters differentiation in hematopoietic cells,
183 demonstrating for the first time that a small molecule is sufficient to transform cells [80].

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187 **Succinate, fumarate, and 2HG: overlapping and distinct functions.**

188 The brief description above suggests that signalling cascades elicited by fumarate, succinate, and
189 2HG, share some common targets but also exhibit distinct features, which can explain the different
190 types of tumours associated with their accumulation (Fig. 1). The converging signatures, mostly
191 mediated by aKGDD inhibition, include pseudohypoxia and broad epigenetic changes, such a DNA
192 and histones hypermethylation. Interestingly, recent studies showed that pseudohypoxic genes,
193 besides being regulated by HIFs, are also transcriptionally controlled by TETs. Therefore, epigenetic
194 changes are required for a full pseudohypoxic response triggered by these metabolites. However,
195 these metabolites have different IC₅₀ for TETs and KDMs [81], suggesting that their epigenetic effect
196 might have different outcomes, with fumarate being the most effective TETs inhibitor and 2HG the
197 poorest. These metabolites exert other distinct biological functions. As indicated above, fumarate
198 accumulation leads to protein succination, triggering a plethora of biological changes that may
199 synergise with or counteract aKGDDs inhibition [51]. Finally, accumulation of 2HG has been shown
200 to increase protein succinylation via inhibition of SDH and subsequent accumulation of succinyl-CoA
201 [78, 82]. This post translation modification leads to a reprogramming of mitochondrial function and

202 induces resistance to apoptosis. [Interestingly, these are not the only different downstream signals](#)
203 [activated by fumarate and 2HG accumulation. Indeed, fumarate and 2HG elicit opposite effects on](#)
204 [mTOR signalling that could be relevant for the development of specific tumour types \[51, 78\].](#)
205 Therefore, although characterised by a very similar chemical structure, succinate, fumarate, and
206 2HG, appear to have distinct biological roles, well beyond inhibition of aKGDDs.

207 **Environmental cues regulates oncometabolite production**

208 The observation that 2HG is sufficient to promote tumorigenesis [80] raised the possibility that
209 environmental cues that increase this metabolite could contribute to tumorigenesis, without
210 underpinning IDH mutations. In support to this hypothesis, it has been shown that hypoxia leads to
211 the production of 2HG (Fig.2), either via reductive carboxylation [83] or via promiscuous substrate
212 usage of lactic dehydrogenase A (LDHA) [84] and malic dehydrogenase 1/2 (MDH1/2) [85]. Since
213 hypoxia is a common feature of solid tumours [86] it is possible that hypoxia-driven production of
214 2HG elicits (epi)genetic changes that drive or amplify the process of tumorigenesis.

215 2-HG is not the only oncometabolite whose levels are altered by hypoxia. During ischemia,
216 succinate significantly accumulates and its oxidation is responsible for ROS generation during
217 reperfusion [87]. Other cues trigger oncometabolite accumulation. For instance, fumarate was
218 shown to accumulate in hyperglycemic conditions [55, 88] (Fig.2), likely as a consequence of
219 mitochondrial dysfunction caused by glucose accumulation [89, 90]. We hypothesise that fumarate
220 accumulation observed in diabetes [88, 90], could, at least in part, explain the increase cancer risk in
221 these patients. Together, these studies seem to suggest that environmental or nutritional cues may
222 cause dysregulation of mitochondrial function, leading to oncometabolite accumulation in the
223 absence of underpinning oncogenic mutations.

224 **Future perspectives**

225 Oncometabolites are emerging as key components of the communication between mitochondria
226 and the nucleus. Chronic accumulation of these small molecules triggered by genetic or
227 environmental cues may alter the epigenetic landscape of the cell eliciting oncogenic signalling
228 cascades. This new paradigm of tumorigenesis challenges the role of gene mutations as the exclusive
229 driving mechanism of tumorigenesis and suggests that the latter may be only one of the possible
230 mechanisms that leads to transformation.

231 **Competing interests**

232 The authors declare no competing interests.

233 **Authors' contribution**

234 MS and CF jointly wrote the manuscript.

235 **Authors' information**

236 MS is a Research Associate in the laboratory of CF. CF is a group leader at the MRC Cancer Unit,
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579

580 Figure legends

581 **Fig.1 Schematic representation of overlapping features of tumours harbouring *FH*, *SDH*, and *IDH*
582 mutations.**

583 Mutations in *FH*, *SDH*, and *IDH* lead to the accumulation of fumarate, succinate, and 2HG,
584 respectively, activating a plethora of signalling cascades. Converging signatures, mainly mediated by
585 aKGDD inhibition, include pseudohypoxia, histone and DNA hypermethylation. The colour of text
586 indicates metabolic alterations (red), epigenetic alterations (blue), post-translational modifications
587 (green) and other pro-tumorigenic alterations (black) elicited by these metabolites.

589

590 **Fig.2 An oncometabolic perspective of tumorigenesis**

591 Schematic representation of how oncometabolite affect the process of tumorigenesis. The indicated
592 oncometabolites can accumulate as a consequence of mutations of TCA cycle enzymes or
593 environmental cues, such as hypoxia or hyperglycemia. These metabolites can act as proper
594 oncogenic triggers and can drive transformation even in the absence of genetic alterations.

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