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MINI REVIEW

The role of metabolic enzymes in mesenchymal tumors and tumor syndromes: genetics, pathology, and molecular mechanisms

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Abstract

The discovery of mutations in genes encoding the metabolic enzymes isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH), and fumarate hydratase (FH) has expanded our understanding not only of altered metabolic pathways but also epigenetic dysregulation in cancer. *IDH1/2* mutations occur in enchondromas and chondrosarcomas in patients with the non-hereditary enchondromatosis syndromes Ollier disease and Maffucci syndrome and in sporadic tumors. *IDH1/2* mutations result in excess production of the oncometabolite (D)-2-hydroxyglutarate. In contrast, SDH and FH act as tumor suppressors and genomic inactivation results in succinate and fumarate accumulation, respectively. SDH deficiency may result from germline *SDHA*, *SDHB*, *SDHC*, or *SDHD* mutations and is found in autosomal-dominant familial paraganglioma/pheochromocytoma and Carney-Stratakis syndrome, describing the combination of paraganglioma and gastrointestinal stromal tumor (GIST). In contrast, patients with the non-hereditary Carney triad, including paraganglioma, GIST, and pulmonary chondroma, usually lack germline *SDH* mutations and instead show epigenetic SDH complex inactivation through *SDHC* promoter methylation. Inactivating *FH* germline mutations are found in patients with hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome comprising benign cutaneous/uterine leiomyomas and renal cell carcinoma. Mutant IDH, SDH, and FH share common inhibition of α-ketoglutarate-dependent oxygenases such as the TET family of 5-methylcytosine hydroxylases preventing DNA demethylation, and Jumonji domain histone demethylases increasing histone methylation, which together inhibit cell differentiation. Ongoing studies aim to better characterize these complex alterations in cancer, the different clinical phenotypes, and variable penetrance of inherited and sporadic cancer predisposition syndromes. A better understanding of the roles of metabolic enzymes in cancer may foster the development of therapies that specifically target functional alterations in tumor cells in the future. Here, the physiologic functions of these metabolic enzymes, the mutational spectrum, and associated functional alterations will be discussed, with a focus on mesenchymal tumor predisposition syndromes.

The role of metabolic enzymes in cancer

Metabolic alterations are regarded as one of the hallmarks of cancer [1] and include the phenomenon of "aerobic glycolysis", which was first described by Otto Warburg in the 1920s and termed the "Warburg effect" [2]. It has been shown that even in the presence of oxygen, cancer cells switch from generating ATP by the highly energy-efficient process of oxidative phosphorylation to the much less efficient process of glycolysis [3, 4]. However, cancer metabolism may also be affected by various other mechanisms, such as dysregulated signaling through activated oncogenes (e.g., *RAS*, *MYC*) and mutant tumor suppressors (e.g., *TP53*) [1]. To date, it is still not entirely clear whether aerobic glycolysis is a true cause or rather a consequence of cancer.

The metabolic enzymes isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH), and fumarate hydratase (FH) play important roles in the tricarboxylic acid cycle (also known as the citric acid cycle or Krebs cycle). The discovery that mutations in the encoding genes can lead to cancer development offers the opportunity to further characterize the complex regulatory mechanisms of energy metabolism in cancer. Further, the roles of these enzymes beyond regulation of metabolic pathways provide important

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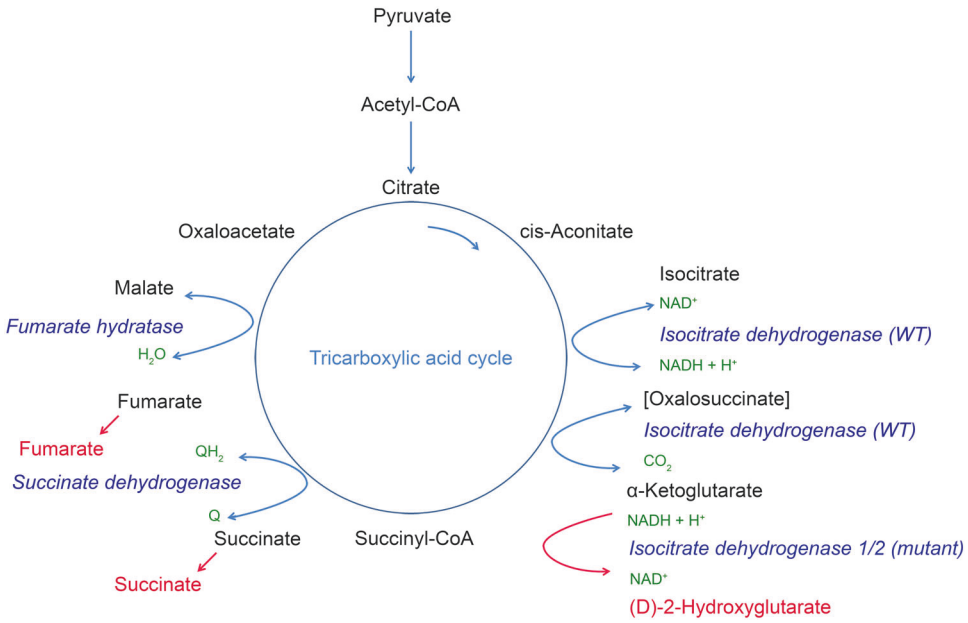

Fig. 1 The tricarboxylic acid cycle. Shown are the biochemical reactions catalyzed by isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH), and fumarate hydratase (FH) and oncometabolites resulting from the respective mutations. NAD indicates nicotinamide adenine dinucleotide, Q ubiquinone, and QH_2 ubiquinol

insights into epigenetic changes as potential drivers in cancer.

Change-of-function *IDH* mutations occur during post-zygotic divisions and lead to somatic mosaicism. They are associated with the development of multiple enchondromas in non-hereditary Ollier disease and Maffucci syndrome, which may transform to chondrosarcoma in a subset of cases [5, 6]. SDH deficiency resulting from inactivating germline mutations of the SDH subunits *SDHA*, *SDHB*, *SDHC*, or *SDHD* is associated with hereditary paraganglioma and pheochromocytoma as well as Carney-Stratakis syndrome, the latter comprising gastrointestinal stromal tumor (GIST) and paraganglioma [7, 8]. Finally, inactivating germline mutations of *FH* are associated with the hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome, which includes benign cutaneous and uterine leiomyomas and renal cell carcinoma [9].

Here, mutations in these metabolic enzymes, their impact on metabolic homeostasis, and the associated tumor syndromes will be discussed after a brief overview of their physiologic function in the tricarboxylic acid cycle. Their biologic roles in cancer development will be reviewed, with a focus on mesenchymal tumors, as well as opportunities for therapeutic targeting and future studies.

Physiologic roles of IDH, SDH, and FH in the tricarboxylic acid cycle

First described by Hans Adolf Krebs in 1937 [10], the tricarboxylic acid cycle is the primary metabolic pathway for all aerobic processes in animal tissues [11]. It involves a series of reactions (Fig. 1) that generate essential substrates as building blocks for biosynthesis while (indirectly) utilizing about two-thirds of the total consumed oxygen and generating about two-thirds of the total energy [11]. The tricarboxylic acid cycle is located within mitochondria, represents the final common pathway for the oxidation of carbohydrate, protein, and lipids, and plays important roles in gluconeogenesis, transamination, deamination, and lipogenesis [11].

IDH isoforms are crucial enzymes for the incorporation of glucose and fatty acid carbons into the tricarboxylic acid cycle. The homodimeric $NADP^+$-dependent enzymes IDH1 and IDH2 reversibly catalyze the oxidative decarboxylation of isocitrate to produce α-ketoglutarate, NADPH, and CO_2 [4, 12]. SDH is an enzymatic complex composed of the SDHA, SDHB, SDHC, and SDHD subunits embedded in the inner mitochondrial membrane and is involved not only in the tricarboxylic acid cycle but also in the electron transport chain (as complex II). SDH catalyzes the reversible oxidation of succinate to fumarate, using the electrons generated through reduction of ubiquinone to ubiquinol in the electron transport chain [12]. Finally, FH (which has both mitochondrial and cytosolic isoforms) catalyzes the reversible hydration of fumarate to (L)-malate [12].

IDH mutations lead to changes in enzyme function and generation of the oncometabolite (D)-2-hydroxyglutarate

Mammals express three IDH isoforms [13] with distinct intracellular location: IDH1 and IDH2 isoforms are $NADP^+$-dependent enzymes that reversibly catalyze the

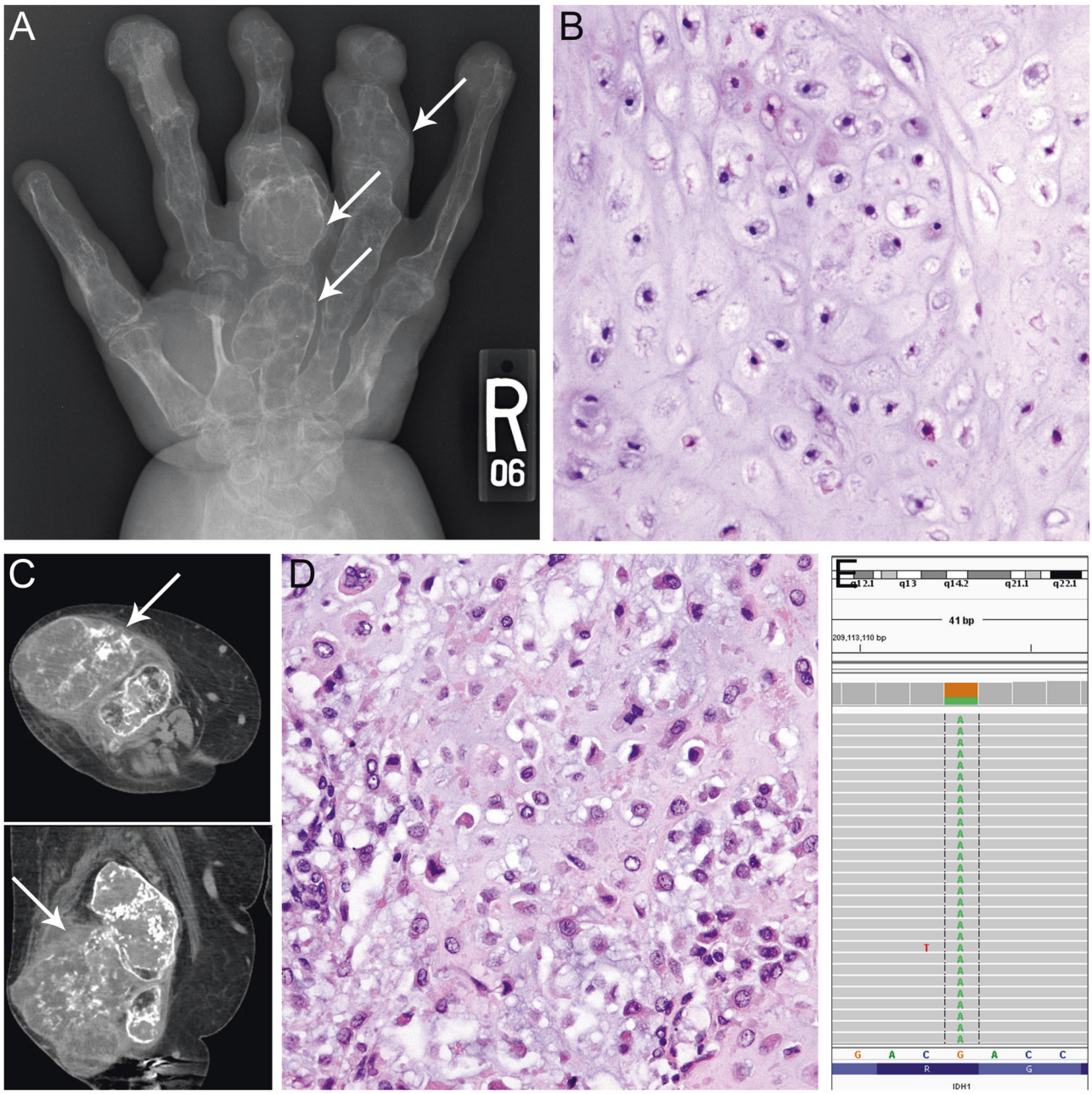

Fig. 2 Tumors characterized by isocitrate dehydrogenase (IDH) deficiency. A female patient with Maffucci syndrome developed multiple enchondromas of the distal extremities (**a** x-ray image of the right hand performed at age 25), histologically characterized by a benign cartilaginous proliferation (**b**). At age 34, CT imaging (**c**, coronal (top) and frontal (bottom) views) identified the presence of a chondrosarcoma adjacent to the right patella (**c**, arrows), measuring up to 21.6 cm in greatest dimension and histologically displaying chondrosarcoma (**d**) with moderate cytologic atypia. Targeted sequencing revealed a heterozygous *IDH1* p.R132C (c.394C>A) missense mutation (**e** allele fraction 31%)

decarboxylation of isocitrate to yield α-ketoglutarate, NADPH, and CO_2, whereas IDH3 is a structurally unrelated heterotetrameric NAD^+-dependent enzyme that *irreversibly* decarboxylates isocitrate and produces α-ketoglutarate, NADH, and CO_2 [4, 12]. Whereas IDH1 is located in the cytoplasm and peroxisomes, IDH2 and IDH3 are located in the mitochondrial matrix [4].

Among IDH, SDH, and FH, genetic alterations of *IDH1/2* are most frequent: mutations have been observed in tumors of various origins, such as glioma (80–90% of cases) [14], acute myeloid leukemia (AML) (~20 of cases) [15, 16], sinonasal undifferentiated carcinoma (55% of cases) [17], cholangiocarcinoma (10–20% of cases) [18], chondrosarcoma (50–70% of cases), and spindle cell hemangioma (70%) [19, 20].

Table 1 Summary of metabolic enzymes and their role in mesenchymal tumor syndromes

Enzyme	Gene	Locus	Oncometabolite/substrate	Syndrome	Inheritance	Associated tumors
IDH1/2	*IDH1*	2q34	(D)-2-hydroxy-glutarate	Ollier disease	Sporadic	Multiple enchondromas, chondrosarcoma, gliomas, juvenile granulosa cell tumors
	IDH2	15q26.1		Maffucci syndrome	Sporadic	Multiple enchondromas, chondrosarcoma, spindle cell hemangioma, angiosarcoma, astrocytoma, pituitary adenoma, juvenile granulosa cell tumor, pancreatic adenocarcinoma, others
SDH	*SDHA*	5p15.33	Succinate	Familial paraganglioma/ pheochromocytoma	AD	Paraganglioma/pheochromocytoma, renal cell carcinoma
	SDHB	1p36.13				
	SDHC	1q23.3		Carney-Stratakis syndrome	AD	GIST, paraganglioma (rare: renal cell carcinoma, pituitary adenoma)
	SDHD	11q23.1		Carney triad	Sporadic	GIST, paraganglioma, pulmonary chondroma
				Hereditary renal cell carcinoma	AD	Renal cell carcinoma
FH	*FH*	1q43	Fumarate	HLRCC	AD	Renal cell carcinoma, uterine, and cutaneous leiomyoma
				NA	AD	Paraganglioma/pheochromocytoma

AD autosomal dominant, *FH* fumarate hydratase, *GIST* gastrointestinal stromal tumor, *HLRCC* hereditary leiomyomatosis and renal cell cancer, *IDH* isocitrate dehydrogenase, *SDH* succinate dehydrogenase

Mutations in the IDH3 isoform have not been reported in cancer. It has been shown that the prognostic significance of *IDH1/2* mutations varies in between different tumor types [4]: for instance, in glioblastoma, *IDH1/2* mutations are associated with favorable outcome [21–23], whereas in AML, cholangiocarcinoma and chondrosarcoma, the prognostic significance of *IDH1/2* mutations is less clear [24–26]. In myelodysplastic syndromes and myeloproliferative neoplasms, *IDH1/2* mutations confer a poor prognosis [4].

Of note, the spectrum of *IDH1* and *IDH2* mutations varies among the different tumor types: for instance, in grade II/III gliomas and glioblastomas, the most common *IDH1* mutation is p.R132H (85–90% of *IDH* mutations); *IDH1* mutations are much more common than *IDH2* mutations. Cholangiocarcinoma shows a predominance of *IDH1* over *IDH2* mutations (ratio 10:1), with the *IDH1* mutation p.R132C as the most common alteration (50–60% of *IDH* mutations). In AML, the p.R140Q mutation in *IDH2* is the most frequent alteration (30–50% of *IDH* mutations), with *IDH1* and *IDH2* mutations occurring at a ratio of 1:1–2. Chondrosarcoma harbors a spectrum of *IDH* mutations similar to cholangiocarcinoma, with predominance of *IDH1* over *IDH2* mutations (ratio 20:1) and p.R132C representing the most common amino acid exchange (40–50% of *IDH* mutations). Thus, the use of the R132H *IDH1* mutation specific antibody, commonly applied by pathologists in glioma diagnostics [27], is not useful for the other tumor types in which other *IDH1* or *IDH2* mutations prevail.

Chondrosarcoma is the second most frequent primary malignant bone neoplasm [28]. During progression, chondrosarcomas accumulate genetic alterations that include dysregulation of the cell cycle and hedgehog signaling pathway as well as genetic alterations in *COL2A1*, *NRAS*, and *YEATS2* [29–33]. Secondary chondrosarcomas may also arise in enchondromas.

The non-hereditary enchondromatosis syndromes Ollier disease and Maffucci syndrome are rare, usually show unilateral involvement, and are associated with somatic mosaic heterozygous *IDH1* or *IDH2* mutations (Fig. 2 and Table 1) [5]. Ollier disease comprises multiple enchondromas, and occasionally gliomas or juvenile granulosa cell tumors [34]. Maffucci syndrome includes enchondromatosis and spindle cell hemangioma, but patients may also develop chondrosarcoma, angiosarcoma, and occasionally other tumors such as astrocytoma, pituitary adenoma, juvenile granulosa cell tumor, and pancreatic adenocarcinoma [28]. The risk of developing secondary chondrosarcoma is dependent on the location of the tumors and estimated at ~40% [35]. Approximately 40–90% of tumors in these cancer predisposition syndromes harbor mutations of Arg132 of *IDH1* or, less frequently, Arg172 of *IDH2* [5, 6].

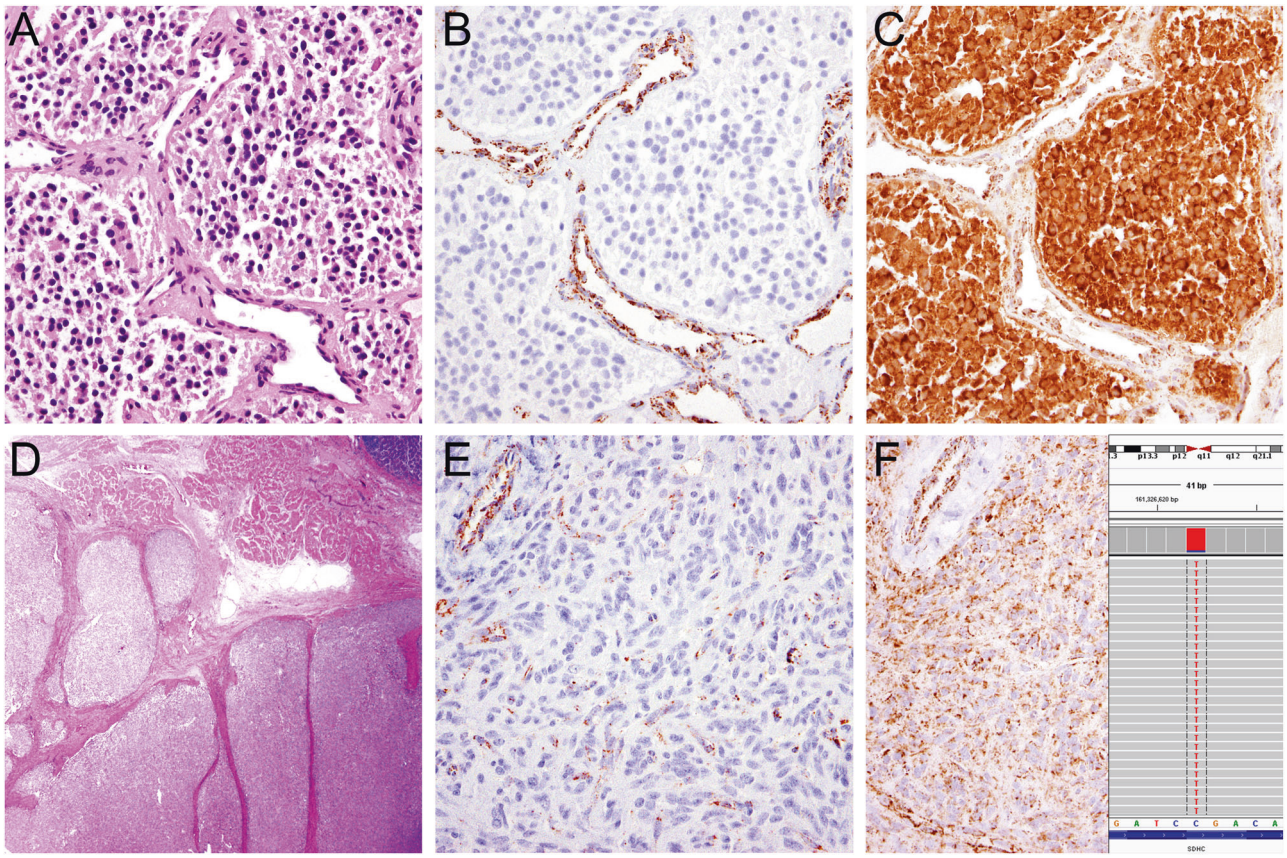

Fig. 3 Tumors characterized by succinate dehydrogenase (SDH) deficiency. A paraganglioma arising in the retroperitoneum (**a**) showing loss of SDHB expression (**b**) and retained SDHA expression (**c**) in tumor cells (admixed small vessels serve as an internal control). A gastrointestinal stromal tumor (GIST) arising in the stomach, showing the typical multinodular architecture of SDH-deficient GIST (**d**). Another example of an SDH-deficient GIST with loss of SDHB expression (**e**) and retained SDHA expression (**f**) in tumor cells. This tumor harbored a homozygous *SDHC* p.R133* (c.397C>T) nonsense mutation (**f**, inset, allele fraction 91%)

In contrast to *SDH* and *FH*, which harbor loss-of-function mutations and act as *bona fide* tumor suppressor genes (see below), *IDH1/2* mutations confer a gain of function and change the catalytic activity of the enzyme (Fig. 1): mutant *IDH1/2* catalyzes the reduction of α-ketoglutarate and results in high-level production of the oncometabolite (D)-2-hydroxyglutarate [36–38]. In normal cells, (D)-2-hydroxyglutarate levels are very low and tightly regulated [4]. Characterizing the oncogenic properties of (D)-2-hydroxyglutarate is an area of active investigation: it has been hypothesized that (D)-2-hydroxyglutarate may promote cellular transformation by altering the redox state of cells or leading to metabolic and epigenetic changes, in particular affecting tumor suppressor enzymes that depend on the structurally related α-ketoglutarate as cosubstrate [4]. (D)-2-hydroxyglutarate inhibits α-ketoglutarate-dependent oxygenases such the TET family of 5-methlycytosine (5mC) hydroxylases (see below) [39, 40]. Also, loss of physiologic IDH activity may result in changes in mitochondrial function and promote the metabolic switch to glycolysis [4]. Experimental treatment of mesenchymal stem cells with (D)-2-hydroxyglutarate or introduction of an *IDH* mutation inhibits osteogenic differentiation and stimulates chondrogenic differentiation—providing a possible explanation for the development of enchondromas during bone development [41, 42].

Interestingly, preservation of the wild-type *IDH1* allele seems to be necessary to allow for formation of heterodimers with mutant *IDH1* and provide mutant *IDH1* with (cytoplasmic) α-ketoglutarate as a substrate for generating (D)-2-hydroxyglutarate [4]. Accordingly, gliomas with homozygous *IDH1* mutations show much lower levels of oncogenic (D)-2-hydroxyglutarate than those with heterozygous mutations [43], and tumors associated with Ollier disease and Maffucci syndrome generally harbor heterozygous (rather than homozygous) *IDH* mutations [5].

Although *IDH* mutations have been shown to be no longer essential to promote tumor growth in high-grade chondrosarcoma [44, 45], the detection of canonical *IDH* mutations may be of diagnostic value, for instance, when chondroblastic osteosarcoma enters the differential diagnosis. *IDH* mutations have been reported in 87% of

enchondromas in patients with Ollier disease, 77% of tumors in patients with Maffucci syndrome, 86% of secondary central chondrosarcoma, 38–70% of primary central chondrosarcoma, 15% of periosteal chondrosarcoma, and 54% of dedifferentiated chondrosarcoma [5, 6, 19, 46], but are absent in peripheral chondrosarcoma, osteosarcoma, and chordoma [5, 19, 47, 48].

Identical *IDH* mutations in multiple tumors have been identified in 88% of patients with Ollier disease and Maffucci syndrome [5].

Inactivating *SDH* mutations lead to accumulation of succinate

Loss-of-function alterations of the SDH complex, also representing the mitochondrial complex II, can be observed in familial paraganglioma/pheochromocytoma [7] with autosomal-dominant inheritance (Fig. 3 and Table 1), the autosomal-dominant Carney-Stratakis syndrome (GIST and paraganglioma) [49, 50], and the non-hereditary Carney triad [51] (GIST, paraganglioma, and pulmonary chondroma). The SDH complex is composed of proteins encoded by *SDHA*, *SDHB*, *SDHC*, and *SDHD*, and loss of function of any of these four components leads to complex inactivation and loss of SDHB expression detectable by immunohistochemistry (Fig. 3) [8].

Paraganglioma and pheochromocytoma are rare tumors that mostly affect middle-aged adults between the third and fourth decades. While the majority of these tumors behaves in a benign fashion, the excessive production of catecholamines or local mass effect may result in high morbidity and mortality in a subset of patients.

Familial paragangliomas were first described in 1933 [52]. Up to 30% of paragangliomas and pheochromocytomas harbor germline mutations in predisposing genes [53], with *SDH* subunit mutations being most common, followed by mutations in *VHL* (4–10% of cases), *RET* (1–5% of cases), and *NF1* (1–5% of cases) [54–56]. More recently, germline mutations in *TMEM127* [57] and *MAX* [58] have been identified. Germline *SDH* mutations are found in ~10% of apparently sporadic and >80% of familial paragangliomas/pheochromocytomas, respectively [55, 59]. Inactivating mutations in *SDHA* [56], *SDHB* [60], *SDHC* [61], *SDHD* [62], and *SDHAF2* [63] follow an autosomal-dominant inheritance pattern (Table 1). *SDHAF2* encodes a mitochondrial protein that is required for the flavination (i.e., enzymatic activation) of SDHA [63]. The penetrance of disease in these paraganglioma syndromes appears to be highly variable [64]. For instance, paraganglioma syndrome 1 (caused by *SDHD* germline mutation) and 2 (*SDHAF2*) are notable for high penetrance, multifocality, and parent-of-origin inheritance, with disease usually manifesting in cases of paternal origin [64]. Paraganglioma syndrome 4 (*SDHB*) confers increased risk of malignancy, whereas paraganglioma syndromes 3 (*SDHC*) and 5 (*SDHA*) are infrequent and show lower penetrance [64]. It remains to be determined why mutations affecting one enzymatic complex may lead to such variable clinical presentations [64].

Rarely, germline mutations in *FH* have been reported to predispose to malignant paraganglioma/pheochromocytoma, pointing towards a (partial) functional overlap with SDH deficiency [65–67].

Also, an association of germline *SDH* mutations and development of renal cell carcinoma has been reported: [68, 69] SDH deficiency has been shown to impair oxidative phosphorylation which renders SDH-deficient renal cell carcinoma dependent on aerobic glycolysis [68, 70]. SDH-deficient renal cell carcinomas are usually indolent tumors with a predilection for younger patients [71] and distinct morphologic features that include a predominantly solid architecture, uniform eosinophilic tumor cells with vacuolated or flocculent cytoplasmic inclusions and admixed mast cells [72]. A morphologic overlap with FH-deficient renal cell carcinoma has been described for a subset of cases [73].

In both paraganglioma and GIST, loss of SDHA expression predicts germline *SDHA* mutation [74–76]. While a combination of GIST and paraganglioma (Carney-Stratakis syndrome) seems to be rarely associated with *SDHA* germline mutation [74], few such cases have been observed. Carney-Stratakis syndrome is rarely associated with pituitary adenomas [77] and renal cell carcinoma [78].

SDH-deficient GISTs are characterized by several features that help distinguish these tumors from conventional *KIT/PDGFRA*-mutant GISTs: they virtually always arise in the stomach, show epithelioid or mixed epithelioid and spindle cell morphology, and a characteristic multinodular or plexiform growth pattern that facilitates their recognition on conventional hematoxylin and eosin-stained slides [79].

SDH-deficient GISTs show either inactivating mutations in genes encoding the SDH subunits or alternate *SDHC* promoter methylation causing epigenetic inactivation (Table 1) [80, 81]. Patients with Carney-Stratakis syndrome have been reported to harbor germline mutations in *SDHA*, *SDHB*, *SDHC*, and *SDHD*, whereas Carney triad is a non-hereditary condition with a predilection for females, and patients only rarely (<10%) harbor *SDH* subunit germline mutations [82]. Carney triad can be complete, comprising GIST, paraganglioma, and pulmonary chondroma, or incomplete with only one or two of these three manifestations.

SDH-deficient GISTs show loss of SDHB expression by immunohistochemistry: GISTs with *SDHB*, *SDHC*, *SDHD* mutation or *SDHC* promoter methylation show retained SDHA expression, whereas *SDHA*-mutant cases can be identified by additional SDHA loss.

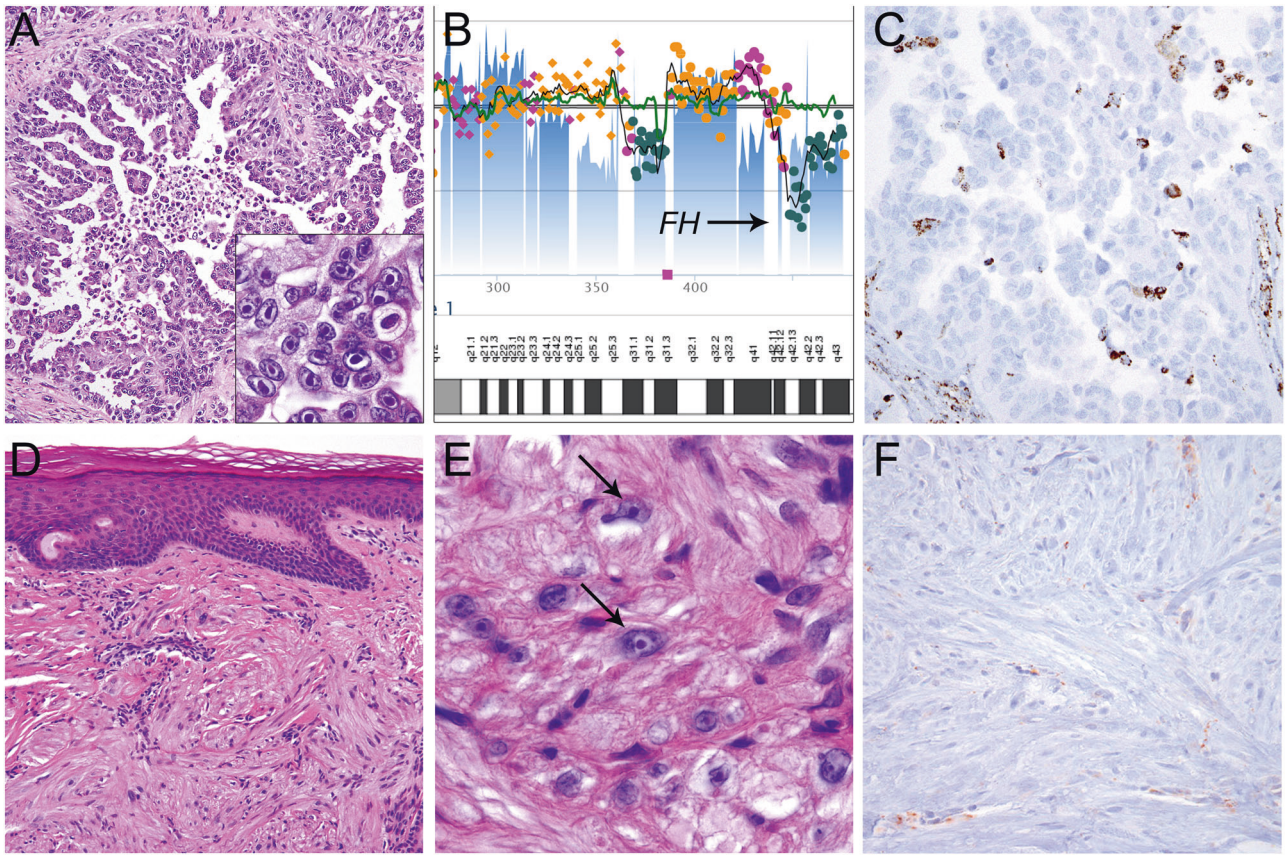

Fig. 4 Tumors characterized by fumarate hydratase (FH) deficiency. A renal cell carcinoma with a papillary growth pattern (**a**) exhibiting prominent nucleoli surrounded by a perinucleolar halo (**a**, inset). This tumor was shown to have a homozygous deletion of *FH* at 1q43 (**b**, arrow), leading to loss of FH expression in tumor cells (**c**). A pilar leiomyoma (**d**) showing the typical nuclear features (**e**, arrows) and loss of FH expression (**f**); endothelial cells and inflammatory cells serve as internal controls. Case courtesy of Dr. Michelle Hirsch, Department of Pathology, Brigham and Women's Hospital

Although SDH-deficient GISTs show expression of activated KIT [8], the mechanism of KIT activation remains unclear. They lack the canonical chromosomal alterations observed in *KIT/PDGFRA/NF1*-mutant GISTs (i.e., loss of 14q, 22q, 1p, and 15q) and instead, may show 1q deletion presumably involving the *SDHC* locus [50, 51]. SDH-deficient GISTs show a propensity for multifocality and lymph node metastasis, but usually follow an indolent clinical course. As demonstrated recently, risk stratification systems initially established for *KIT/PDGFRA*-mutant GISTs fail to predict disease progression in SDH-deficient GISTs [83].

SDH complex inactivating mutations result in accumulation of the substrate succinate (Fig. 1) which has been shown to inhibit various α-ketoglutarate-dependent dioxygenases, including the TET family of 5mC hydroxylases [84–86], leading to decreased hydroxylation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and subsequent decrease in 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) formation, thereby establishing a hypermethylation program (see below) [81, 87]. At the same time, levels of fumarate and malate decrease. 5hmC expression has been shown to be low to absent in SDH-deficient GISTs by immunohistochemistry [88], which is similar to observations made in other SDH- and FH-deficient tumors, corresponding to genome-wide hypermethylation [89].

Inactivating *FH* mutations lead to accumulation of fumarate

Mutations in *FH* lead to inactivation of the enzyme and subsequent accumulation of the substrate fumarate and also, to a lesser degree, succinate. High levels of fumarate cause changes in metabolism and epigenetic regulation, similar to the effects of *IDH* and *SDH* mutations (see below). By immunohistochemistry, loss of 5hmC and increased H3K9me3 levels can be detected in *FH*-mutant tumor cells [89].

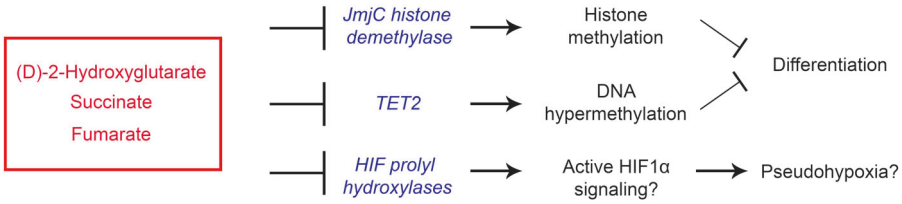

Fig. 5 Simplified overview of proposed consequences of *IDH1/2*, *SDH*, and *FH* mutations for cancer development. While *IDH1/2* mutations lead to functional alterations in enzyme activity and increased production of the oncometabolite (D)-2-hydroxyglutarate, *SDH* and *FH* mutations lead to accumulation of succinate and fumarate, respectively. These inhibit TET family of 5-hydroxymethylcytosine (5mC)-hydroxylases and Jumonji domain histone demethylases leading to genome-wide hypermethylation and alterations in gene transcription with a negative impact on differentiation. In addition, inhibition of hypoxia-inducible factor (HIF) prolyl hydroxylases may lead to activation in HIF-1α signaling inducing pseudohypoxia

FH germline mutations are found in the hereditary HLRCC cancer predisposition syndrome comprising hereditary cutaneous and uterine leiomyomas and renal cell carcinoma (i.e., mostly "type 2" papillary renal cell carcinoma) which follows autosomal-dominant inheritance and shows incomplete penetrance, while somatic *FH* mutations can be found in a small subset of uterine smooth muscle tumors (Fig. 4 and Table 1). Patients with HLRCC develop cutaneous leiomyomas in ~76% of cases, uterine leiomyomas in almost all affected female individuals, and renal cell carcinoma in 10–16% of cases, with a median age of detection of 44 years [90].

In an unselected cohort of smooth muscle tumors, non-atypical uterine leiomyomas showed FH deficiency by immunohistochemistry in 1.6%, cellular leiomyomas in 1.8%, atypical leiomyomas in 37.3%, and none of the leiomyosarcomas tested, whereas the rate of HLRCC was very low in patients with FH-deficient tumors [91]. The median age of patients with FH-deficient uterine leiomyomas was 38 years [91]. HLRCC-associated leiomyomas and renal cell carcinomas are characterized by eosinophilic cytoplasmic inclusions, prominent eosinophilic nucleoli, and characteristic perinucleolar halos (Fig. 4) [92]. Furthermore, characteristic hemangiopericytoma-like vessels have been described in FH-deficient uterine smooth muscle tumors [93]. Loss of FH expression is detectable by immunohistochemistry with relatively high specificity but only moderate sensitivity. The accumulation of fumarate also induces aberrant succination of proteins and positive staining for (*S*)-2-succinocysteine (2-SC) increases the diagnostic sensitivity and can be used as an adjunct biomarker to detect FH deficiency [89, 92, 94–96]. However, the latter marker is not commercially available.

IDH, *SDH*, and *FH* mutations inhibit TET enzymes and lead to genome-wide hypermethylation

Beyond complex changes in metabolism with a shift in substrate dependencies, *IDH*-, *SDH*-, and *FH*-mutant tumors share similar mechanisms of dysregulated DNA methylation and histone modification (Fig. 5): excess (D)-2-hydroxyglutarate resulting from *IDH1/2* mutations, as well as accumulation of succinate in SDH-deficient tumors [84, 85], and fumarate in FH-deficient tumors [85] inhibit TET family enzymes which results in decreased hydroxylation of 5mC to 5hmC and also a subsequent decrease in 5fC and 5caC generation by TET. It has been demonstrated that inhibition of TET2 leads to genome-wide hypermethylation and global changes in gene transcription [4, 89, 97]. Accordingly, *IDH1*-mutant cartilaginous tumors show a hypermethylation phenotype [5]. SDH-deficient GISTs—including GISTs with *SDHx* subunit mutations and *SDHC* promoter methylation—share a common genome-wide hypermethylation phenotype, as the unifying oncogenic mechanism [81]. Methylation profiling confirmed that the epigenetic alterations observed in SDH-deficient GIST are comparable with those in *IDH*-mutant gliomas [97], further highlighting the role of these enzymes for epigenetic maintenance. Intracellular succinate accumulation in SDH-deficient GISTs has been shown to lead to TET inhibition and loss of 5hmC (see below) [97]. Likewise, accumulation of fumarate was shown to induce hypermethylation of DNA [98], confirming that this group of tumors with genetic aberrations in metabolic enzymes share a common mechanism of tumorigenesis through epigenetic changes including hypermethylation.

In addition to DNA hypermethylation, (D)-2-hydroxyglutarate also inhibits other α-ketoglutarate-dependent oxygenases such as the Jumonji domain histone demethylases (JmjC), thereby increasing histone methylation [99–101]. Indeed, increased methylation of H3K9me3 was shown in SDH- and FH-deficient tumors [89], while this was less clear for IDH mutant cartilaginous tumors [26]. Taken together, mutations in metabolic enzymes cause epigenetic changes including both DNA hypermethylation and altered histone methylation which are shown to affect gene expression and to inhibit differentiation [40–42, 99].

Moreover, FH inhibition, together with accumulated fumarate and—to a lesser degree—succinate have been shown to cause intracellular hypoxia-inducible factor (HIF) accumulation and stabilization in part by inhibiting HIF

Table 2 Summary of ongoing clinical trials for the treatment of *IDH-*, *SDH-*, and *FH*-mutant solid tumors

Trial no.	Start date	Compound	Study type	Tumor type	Inclusion criteria
NCT03165721	2017	DNA methyl transferase inhibitor SGI-110 (guadecitabine)	Open-label non-randomized phase 2	Wild-type GIST, SDH-deficient paraganglioma/ pheochromocytoma (with germline *SDH* mutation), HLRCC-associated renal cell carcinoma	SDH-deficient GIST: Patients with recurrent or progressive disease, newly diagnosed patients with metastatic disease or residual disease following debulking surgery SDH-deficient paraganglioma/pheochromocytoma: Patients with recurrent or progressive disease, newly diagnosed patients with metastatic disease and/or unresectable disease HLRCC-associated renal cell carcinoma: Patients with recurrent and/or unresectable disease
NCT03212274	2017	Olaparib	Open-label interventional phase 2	Recurrent/progressive *IDH1/2*-mutant glioma, cholangiocarcinoma, or other solid malignant tumors	*IDH1/2*-mutant glioma, cholangiocarcinoma, or other solid malignant tumors: Patients with progression on standard therapy, or for which no effective standard therapy exists Glioma patients ≥12 weeks after chemoradiotherapy
NCT02746081	2016	Mutant IDH-inhibitor BAY1436032	Open-label non-randomized phase 1	*IDH1* R132X-mutant solid tumors	*IDH1* R132X-mutant solid tumors: Patients with disease evaluable as per RECIST 1.1 or RANO (for gliomas), advanced cancer refractory to, have demonstrated intolerance to, or have refused access to, available standard therapies, glioma patients ≥12 weeks after chemoradiotherapy
NCT02496741	2015	Metformin and chloroquine	Open-label interventional phase 1 and 2	Glioma, intrahepatic cholangiocarcinoma, chondrosarcoma	Glioma, intrahepatic cholangiocarcinoma, chondrosarcoma WHO grade ≥II: Patients with newly diagnosed or refractory/ relapsed tumors
NCT02071862	2014	Glutaminase inhibitor CB-839	Open-label non-randomized phase 1	Advanced solid tumors	Part 1: Patients with locally advanced, metastatic and/or refractory solid tumors Part 2: Patients with triple-negative breast cancer, non-small cell lung cancer (adenocarcinoma), renal ell cancer, mesothelioma, FH-deficient tumors, SDH-deficient GIST, SDH-deficient non-GIST, tumors with *IDH1* or *IDH2* mutation, tumors with *MYC* amplification

FH fumarate hydratase, *GIST* gastrointestinal stromal tumor, *HLRCC* hereditary leiomyomatosis and renal cell cancer; *IDH* isocitrate dehydrogenase, *SDH* succinate dehydrogenase

prolyl hydroxylase [102]. An increase in reactive oxygen species and inhibition of HIF-α prolyl hydroxylases in the cytosol, leading to HIF-1α stabilization and activation, has been observed in SDH- and FH-deficient tumors [4, 103]. However, the concept of pseudohypoxia is still controversial and data are limited: while it has been proposed that HIF has tumor suppressor functions in myeloid leukemia, it is not clear whether the HIF response to hypoxia is impaired in *IDH*-mutant tumors in vivo [4], and additional studies are needed to better characterize the functional effects of dysregulated HIF signaling in mesenchymal tumors with mutations in *IDH*, *SDH*, and *FH*.

Therapeutic targeting of cancers driven by *IDH*, *SDH*, and *FH* mutations

Selectively targeting mutant *IDH*, *SDH*, and *FH* while preserving wild-type enzyme function in nonneoplastic cells is challenging; the biologic context in which the mutation occurs further determines sensitivity to treatment. Table 2 summarizes selected ongoing clinical trials for the treatment of *IDH-, SDH-*, and *FH*-mutant solid tumors.

In the past few years, mutant IDH inhibitors have been developed to suppress the production of (D)-2-hydroxyglutarate and have been tested in *in vitro* and *in vivo* models [104–106]. In *IDH1*-mutant chondrosarcoma cell lines, the mutant IDH1 inhibitor AGI-5198 was shown to decrease (D)-2-hydroxyglutarate levels resulting in moderately decreased viability [44, 45]. However, proliferation and migration were not affected, global gene expression, CpG island methylation as well as histone H3K4, -9, and -27 trimethylation levels remained unchanged, indicating that mutant IDH1 inhibition alone may not be sufficient in chondrosarcomas, which seem to no longer rely on these mutations, emphasizing the need for development of alternative or combinatorial strategies, that may exploit the epigenetic changes or the metabolic vulnerability of IDH mutant chondrosarcomas [44].

The oral mutant IDH2-inhibitor enasidenib has recently been approved by the Food and Drug Administration (FDA) for the treatment of relapsed or refractory AML in patients with detectable *IDH2* mutation by FDA-approved genetic testing [107]. It remains to be seen whether enasidenib may also show efficacy in *IDH*-mutant solid tumors.

SDH-deficient GISTs show limited response to first-line imatinib [108] but sunitinib has demonstrated some activity in these patients. Drug screens of >200,000 compounds in SDH-deficient yeast models of paraganglioma identified compounds that were selectively toxic in *SDH*-mutant but not wild-type yeast [109]. The investigators hypothesized that for a subset of these drugs, inhibition of yeast alcohol dehydrogenase (equivalent to human lactate dehydrogenase), which SDH-deficient cells use to generate NAD^+, may be related to the growth inhibitory effect in SDH-deficient yeast and human tumor cell models [109]. Similar to the situation in SDH-deficient GIST, however, clinically effective drugs specifically targeting SDH deficiency in familial paraganglioma/pheochromocytoma remain to be identified.

Novel therapeutic approaches for FH-deficient renal cell carcinoma are subject of ongoing investigation or being tested in clinical trials, and include using metformin to reverse inactivation of AMP-activated protein kinase, inhibition of glucose transport, lactate dehydrogenase A, the antioxidant response pathway, the heme oxygenase pathway, and targeting the tumor vasculature and glucose transport with agents such as bevacizumab and erlotinib [110].

Deeper insights into the metabolic and epigenetic alterations in IDH-, SDH-, and FH-deficient tumors are required in order to identify dependencies and vulnerabilities to develop overarching therapeutic concepts that may be applied to more than one tumor type.

Concluding remarks and perspective

The discovery of mutations in genes encoding the tricarboxylic acid cycle enzymes IDH1/2, SDH, and FH in cancer has expanded our understanding of the complex regulation of metabolism in cancer cells, and provided insight into oncogenic and tumor suppressor properties of mutant metabolic enzymes. Mutant IDH1/2, SDH, and FH share common effects on global DNA methylation and histone modification; further studies are needed to determine the exact mechanisms leading to tumor development and to identify potential targets for treatment.

Biologic context and timing of mutation (for instance, as demonstrated by the model of somatic mosaicism) further play a role in the different clinical presentations of affected individuals. The fact that mutations in the same gene may lead to different phenotypic manifestations (pleiotropy) and that one particular tumor type can be caused by mutations in different genes (genetic heterogeneity) offers the opportunity to better characterize the genetic underpinnings of mesenchymal tumors arising in patient with germline or somatic mosaic mutations in metabolic enzymes.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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