Metabolic enzymes (IDH, FH, SDH) and mesenchymal tumor(syndrome)s

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Introduction

Tumours show various metabolic aberrations but perhaps the most central to tumour proliferation is the process originally described by Otto Warburg in the 1920's [1]. This phenomenon in which cancer cells reprogram their energy metabolism by switching to glycolysis, producing excessive levels of lactate, even under normoxic conditions ("aerobic glycolysis") is now recognized as the 'Warburg effect'. While this is a common phenomenon in cancer as highlighted by "the hallmarks of cancer"[2], the molecular basis for increased glycolysis and defective respiration is an area that is still widely being investigated. Also certain oncogenes (e.g. MYC) and tumour suppressor genes (e.g. TP53) affect metabolism. A group of tumour syndromes with mutations in metabolic enzymes may shed new light on defective energy metabolism in cancer. Inactivating germline mutations can be identified in subunits of mitochondrial complex II in patients with head and neck paragangliomas and pheochromocytomas [3]. Interestingly, germline mutations in these *succinate dehydrogenase* genes are also found in patients with Carney-Stratakis syndrome, combining gastrointestinal stromal tumors and paragangliomas [4]. In addition, inactivating germline mutations of another tricarboxylic acid cycle gene, *fumarate hydratase* (FH), were shown to cause autosomal dominant HLRCC syndrome (hereditary leiomyomas and type 2 papillary renal cell carcinoma), including benign cutaneous and uterine leiomyomas and renal cell cancer [5]. A third metabolic enzyme affected by somatic mutations in cancer is *isocitrate dehydrogenase* (IDH), first identified in gliomas [6], and in up to 81% of patients with multiple enchondromas (Ollier disease / Maffucci syndrome), thereby establishing a third tumour syndrome related to mutations in a metabolic enzyme [7,8].

IDH

Somatic mutations in IDH are found in ~50% of chondrosarcomas [7,9,10] and cause epigenetic changes [11-13]. As a consequence of the mutation, the enzyme gains a new function and catalyzes the reduction of α-ketoglutarate to D-2-hydroxyglutarate (D2HG)[14-16]. D2HG is considered as an oncometabolite and inhibits α-ketoglutarate dependent oxygenases like

TET2 [17,18]. This results in inhibition of DNA demethylation, causing hypermethylation. Indeed, a hypermethylation phenotype was found in *IDH1* mutant cartilage tumors [7]. D2HG also inhibits other α-ketoglutarate dependent oxygenases [19,20] such as the Jumonji domain histone demethylases, thereby increasing histone methylation as well [12]. These epigenetic changes are presumed to affect differentiation. When mesenchymal stem cells are treated with D2HG, or when an *IDH* mutation is introduced, osteogenic differentiation is inhibited and instead chondrogenic differentiation is stimulated, which explains the development of enchondromas during bone development [21,22].

Chondrosarcoma is the second most frequent primary bone malignancy, predominantly affecting adults[23]. The development of chondrosarcoma occurs through the acquisition of additional genetic alterations (multistep genetic progression model) [24], involving amongst others the pRb and Hh pathway, COL2A1, NRAS and YEATS2 [25-28]. In high grade chondrosarcomas, the IDH mutation is no longer essential for tumor growth [29,30].

Detection of hotspot mutations in *IDH1* or *IDH2* can be usefull in the differential diagnosis. Distinction between chondrosarcoma and chordoma or chondroblastic osteosarcoma can sometimes be challenging. *IDH* mutations are present in 87% of Ollier- associated enchondromas, 86% of secondary central chondrosarcoma, 38-70% of primary central chondrosarcoma, ~15% of periosteal chondrosarcoma and 54% of dedifferentiated chondrosarcoma [7-9,31] and are absent in peripheral chondrosarcoma, osteosarcoma and in chordoma [7,9,32,33].

FH

Fumarate hydratase (FH) is also known to be mutated in cancer and to cause defective energy metabolism as well as epigenetic deregulation in cancer. Inactivating germline mutations cause autosomal dominant HLRCC syndrome (hereditary leiomyomas and type 2 papillary renal cell carcinoma), including benign cutaneous and uterine leiomyomas and renal cell cancer [5], while somatic mutations are rare. The accumulated fumarate, a consequence of the mutation, inhibits histone demethylases and the TET family of 5 hydroxymethylcytosine (5mC)-hydroxylases [34,35] thereby causing global hypermethylation [36]. Using immunohistochemistry, loss of 5hmC and increased H3K9me3 could be shown in *FH* mutant tumor cells [37].

Morphologically, HLRCC-associated uterine leiomyomas are characterized by eosinophilic cytoplasmic inclusions, prominent eosinophilic nucleoli, and perinucleolar halos [38]. Moreover, the accumulation of fumarate induces aberrant succination of proteins and positive staining for (S)-2-succinocysteine (2SC) can be used as a robust biomarker for mutations in *FH* [37-41]..

SDH

Inactivating mutations in subunits of mitochondrial complex II including the succinate dehydrogenase subunit D (SDHD), C (SDHC) and B (SDHB) genes, are found in patients with paragangliomas and pheochromocytomas [3]. Also, a subset of gastrointestinal stromal tumors (GIST), lacking mutations in *KIT* or *PDGFRA*, carry mutations in one of the *SDH* genes [4] or an SDHC epimutation [42]. Similar to fumarate, the accumulated succinate, caused by the mutation, inhibits histone demethylases and the TET family of 5 hydroxymethylcytosine (5mC)-hydroxylases [34,35,43] thereby causing global hypermethylation [36].

SDH deficient GIST is predominantly found in the stomach of children and young adults, with a female predominance [44]. Its estimated frequency is ~7.5% of gastric GISTs. Lymph node metastases are found in 20-59% of the cases and gastric recurrence is common. Despite this, SDH deficient GIST typically displays an indolent clinical behavior, which can not be predicted using the conventional risk assessment schemes for GIST[44,45]. They typically show epithelioid morphology and a multinodular and plexiform growth pattern in the muscularis propria [44].

Mutations in one of the SDH subunits destabilize the complex, causing degradation and loss of staining for SDHB. Immunohistochemistry for SDHB is therefore a surrogate marker for mutations in one of the SDH subunits [46]. Using immunohistochemistry, loss of 5hmC and increased H3K9me3 could be shown in *SDH* mutant tumor cells [37]. In *SDH* mutant GIST, 5-hmC was staining was also low to absent [47].

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