Histopathology

Histopathology 2012 DOI: 10.1111/j.1365-2559.2012.04300.x

Loss of succinate dehydrogenase subunit B (SDHB) expression is limited to a distinctive subset of gastric wild-type gastrointestinal stromal tumours: a comprehensive genotype–phenotype correlation study

Leona A Doyle, Dylan Nelson,[1] Michael C Heinrich,[2] Christopher L Corless[1] & Jason L Hornick

Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, [1]Department of Pathology and Knight Cancer Institute, Oregon Health and Science University, and [2]Division of Hematology and Medical Oncology, Portland VA Medical Center and Knight Cancer Institute, Oregon Health and Science University, Portland, OR, USA

Date of submission 16 February 2012
Accepted for publication 7 April 2012

Doyle L A, Nelson D, Heinrich M C, Corless C L & Hornick J L

(2012) *Histopathology*

Loss of succinate dehydrogenase subunit B (SDHB) expression is limited to a distinctive subset of gastric wild-type gastrointestinal stromal tumours: a comprehensive genotype–phenotype correlation study

Aims: Gastrointestinal stromal tumours (GISTs) typically harbour *KIT* or *PDGFRA* mutations; 15% of adult GISTs and >90% in children lack such mutations ('wild-type' GISTs). Paediatric and occasional adult GISTs show similar, distinctive features: multinodular architecture and epithelioid morphology, indolent behaviour with metastases, and imatinib resistance. Recent studies have suggested that these tumours can be identified by loss of succinate dehydrogenase subunit B (SDHB) expression. The aim of this study was to validate the predictive value of SDHB immunohistochemistry in a large genotyped cohort.

Methods and results: SDHB expression was examined in GISTs with known genotypes: 179 with *KIT* mutations, 32 with *PDGFRA* mutations, and 53 wild type. Histological features were recorded without knowledge of genotype or SDHB status. SDHB was deficient in 22 (42%) wild-type GISTs. All other tumours showed intact SDHB expression. All SDHB-deficient GISTs with known primary sites arose in the stomach, and had multinodular architecture and epithelioid or mixed morphology. None of the wild-type GISTs with intact SDHB showed multinodular architecture, and only four (13%) had epithelioid morphology.

Conclusions: SDHB-deficient GISTs are wild-type gastric tumours with distinctive histology. Immunohistochemistry for SDHB can be used to confirm the diagnosis of this tumour class. SDHB expression is retained in all GISTs with *KIT* and *PDGFRA* mutations.

Keywords: gastrointestinal stromal tumour, KIT, PDGFRA, soft tissue sarcoma, succinate dehydrogenase

Abbreviations: GIST, gastrointestinal stromal tumour; IHC, immunohistochemistry; NF1, neurofibromatosis 1; SDH, succinate dehydrogenase; SDHB, succinate dehydrogenase subunit B; WT, wild-type

Introduction

The succinate dehydrogenase (SDH) complex is located in mitochondria, and participates in the electron transport chain (complex II) and tricarboxylic acid cycle by catalysing oxidative dehydrogenation of succinate to fumarate. This complex consists of four subunit proteins (A to D) as well as additional groups such as iron–sulphur centres and ubiquinone.[1] SDH subunit B (SDHB) is normally ubiquitously expressed; loss of SDHB expression in tumours reflects dysfunction of the SDH complex, either because of mutations in the

Address for correspondence: Jason L Hornick, MD, PhD, Department of Pathology, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA. e-mail: jhornick@partners.org

genes coding for any of the four subunit proteins, or because of deficient SDH complex activity secondary to other, as yet unknown, mechanisms. In this respect, the SDH complex functions as a tumour suppressor, whereby loss of any of the subunit proteins leads to destabilization of the complex and tumour formation.[1,2] Loss of SDHB detected by immunohistochemistry (IHC) has been shown to be 100% sensitive for detecting paragangliomas or pheochromocytomas with mutations in *SDHB*, *SDHC*, or *SDHD.*[3] More recently, it has been shown that loss of SDHB also identifies a clinicopathologically distinctive subset of gastric gastrointestinal stromal tumours (GISTs), which have been referred to as 'paediatric-type', 'type 2' or 'SDH-deficient' GISTs.[4–7]

The majority of GISTs harbour mutations in *KIT* or *PDGFRA*, resulting in activation of transcriptional, mitogenic and anti-apoptotic pathways.[8,9] The efficacy of the tyrosine kinase inhibitor imatinib mesylate in blocking the effects of such activating mutations has dramatically improved the survival of patients with metastatic or locally advanced GIST.[10] However, 15% of GISTs in adults and >90% in children lack *KIT* and *PDGFRA* mutations, and are the so-called 'wild-type' (WT) GISTs.[11–13] In contrast to *KIT* and *PDGFRA* mutant GISTs, WT GISTs are poorly responsive to imatinib.[14] WT GISTs also include those tumours arising in the setting of the Carney triad, the Carney–Stratakis syndrome, and neurofibromatosis 1 (NF1). Carney triad-associated and Carney–Stratakis syndrome-associated GISTs and a subset of non-syndromic WT gastric GISTs have been referred to as 'paediatric type', on the basis of clinical, pathological and genetic similarities to paediatric GISTs,[4] and 'type 2' on the basis of loss of SDHB expression.[6] Specifically, these tumours arise exclusively in the stomach (particularly the antrum), where they may be multifocal, show a distinctive multinodular/plexiform growth pattern, often have epithelioid morphology, and are wild type for *KIT* and *PDGFRA*, features that are frequently seen in paediatric GISTs.[13,15] Furthermore, also similar to GISTs in children, these tumours commonly metastasize to lymph nodes, which is an extremely rare occurrence in conventional GISTs. They tend to pursue an indolent clinical course, even with metastatic disease, despite being imatinib-resistant.[13,15] Recent studies have suggested that the mechanism underlying tumour formation in this distinctive group of GISTs is dysfunction of the SDH complex, which is reflected in consistent loss of SDHB expression. This finding appears to be characteristic of this class of tumours, and has led to the alternative designation 'SDH-deficient' GIST.[7] SDHB expression in a large series of GISTs with known *KIT* and *PDGFRA* mutation status has not been previously evaluated. The aim of this study was to validate the predictive value of SDHB IHC for identifying this subset of WT GISTs in a large genotyped cohort of tumours.

Materials and methods

The study group included 264 GISTs with known *KIT* and *PDGFRA* genotypes. Genotyping was performed as follows. Tumour DNA was extracted from five to ten 5-μm unstained slides. The samples were treated with xylene and ethanol, vortexed, and centrifuged. The pellet was air-dried and incubated with proteinase K at 56°C for 48 h, and this was followed by several wash and vortex treatments as directed by the Qiagen DNA Mini kit (Qiagen, Valencia, CA, USA). The sample was purified with a column, quantified by measuring the optical density 260/280 nm absorption ratio, and stored at −80°C. Mutation screening of exons 12, 14 and 18 of *PDGFRA* and exons 9, 11, 13 and 17 of *KIT* was carried out using high-resolution melting curve analysis on a Roche LightCycler 480 (Roche, Indianapolis, IN, USA) or denaturing high-performance liquid chromatography on a Transgenomic Wave System (Transgenomic, Omaha, NE, USA). Samples with mutations were confirmed with Sanger sequencing (Applied Biosystems, Carlsbad, CA, USA).

SDHB expression was examined by IHC following pressure cooker antigen retrieval (0.001 M citrate buffer; pH 6.0), using a mouse anti-SDHB monoclonal antibody (1:100 dilution; clone 21A11AE7; Abcam, Cambridge, MA, USA) on 4-μm-thick formalin-fixed paraffin-embedded whole tissue sections. SDHB expression was evaluated by two of the authors (L.A.D. and J.L.H.) blinded to both clinical features and genotype. SDHB was scored as 'intact' when any granular cytoplasmic staining was observed in tumour cells, or 'deficient' when there was a complete absence of granular cytoplasmic staining in tumour cells with positive internal controls. Non-neoplastic cells, such as endothelial, smooth muscle and epithelial cells, served as internal positive controls. Histological features, specifically tumour growth pattern and tumour cell morphology, were recorded where possible and without knowledge of genotype or SDHB status.

Results

SDHB EXPRESSION IN GIST ACCORDING TO GENOTYPE

Among the 264 tumours, there were 179 tumours with *KIT* mutations (154 in exon 11, 17 in exon 9, four

Table 1. Summary of immunohistochemical staining for succinate dehydrogenase subunit B (SDHB) in gastrointestinal stromal tumours

Genotype (no.)	SDHB-intact, no. (%)	SDHB-deficient, no. (%)
Wild type (53)	31 (58)	22 (42)
KIT mutant (179)	179 (100)	0 (0)
Exon 11	154 (100)	0 (0)
Exon 9	17 (100)	0 (0)
Exon 13	4 (100)	0 (0)
Exon 17	4 (100)	0 (0)
PDGFRA mutant (32)	32 (100)	0 (0)
Exon 18 (25)	25 (100)	0 (0)
Exon 12 (4)	4 (100)	0 (0)
Exon 14 (3)	3 (100)	0 (0)

in exon 13, and four in exon 17), 32 tumours with *PDGFRA* mutations (25 in exon 18, four in exon 12, and three in exon 14), and 53 WT tumours. SDHB expression was deficient in 22 (42%) WT GISTs. All other tumours showed intact SDHB expression, including 100% of *KIT* and *PDGFRA* mutant GISTs and 31 (58%) WT GISTs (Table 1). The cytoplasmic staining pattern for SDHB was granular, reflecting its mitochondrial localization, and was often variable in intensity and extent (Figure 1). In most cases staining was moderate in intensity, but some cases showed weak staining, requiring examination under high magnification. In occasional cases with intact SDHB expression, SDHB staining was observed in <50% of tumour cells, with some regional variability on individual slides, suggesting that uneven tissue fixation may affect staining. SDHB-deficient tumours showed a complete absence of staining in all tumour cells (Figure 2). Two patients with SDHB-deficient GISTs were known to have germline *SDHC* mutations, and one had a germline *SDHB* mutation.

CLINICOPATHOLOGICAL FEATURES OF SDHB-DEFICIENT GISTS AND WT GISTS WITH INTACT SDHB EXPRESSION

Of the 22 patients with SDHB-deficient GISTs, 12 (55%) were female and 10 (45%) were male. Age was known for 18 patients; the mean and median ages of patients were 33 and 31 years, respectively (range 18–56 years). All SDHB-deficient GISTs with known primary sites ($N = 21$) arose in the stomach and showed a multinodular or plexiform growth pattern (Figure 3; Table 2). The primary site of one SDHB-deficient tumour located in the omentum was unknown; this tumour also showed a multinodular growth pattern. Five tumours showed a focally infiltrative growth pattern as well, consisting of strands of tumour invading into the surrounding muscularis propria (Figure 3). The tumour cell morphology of the SDHB-deficient GISTs was either predominantly epithelioid (68%) or mixed epithelioid and spindle cell (32%) (Figure 3). None showed predominantly spindle cell morphology. Within individual nodules, the tumours were hypercellular with minimal intervening stroma (Figure 3). The clinical and pathological features of a subset of the evaluated SDHB-deficient GISTs have been described previously.[4] Two patients had radiographic findings suggestive of pulmonary chondroma, and one patient had a pheochromocytoma. A fourth patient had a family member with a history of paraganglioma. These findings suggest the possibility of Carney triad and Carney–Stratakis syndrome, respectively.

Of the 31 WT GISTs with intact SDHB expression, gender was known for 26 patients and age for 18. Sixteen patients (62%) were female and 10 (38%) were male. The mean and median ages of patients with WT GISTs showing intact SDHB expression were 50 and 51 years, respectively (range 17–80 years). Ten tumours (42%) arose in the small intestine, eight (33%) in the stomach, five (21%) in the colon, and one (4%) in the oesophagus; the primary site was unknown in seven cases. Seven cases had insufficient tumour in the slides available for review to enable accurate assessment of growth pattern. Of the remaining 24 cases, all showed a diffuse or sheet-like architecture, in contrast to the multinodular growth pattern of the SDHB-deficient GISTs. The most common cytomorphology in the WT GISTs with intact SDHB expression was spindle cell (65%), followed by mixed epithelioid and spindle cell (22%). Only four cases (13%) had purely epithelioid morphology, all gastric tumours. Two of the cases with epithelioid morphology showed focally notable pleomorphism. Two WT GISTs with intact SDHB expression arose in patients with NF1.

Four of the *KIT* exon 11 mutant GISTs had a multinodular architecture, two with epithelioid, one with spindle cell and one with mixed morphology. Two of these tumours were located in the stomach; the anatomical sites of the other two tumours were unknown. All of these tumours showed intact staining for SDHB. None of the remaining *KIT* or *PDGFRA*

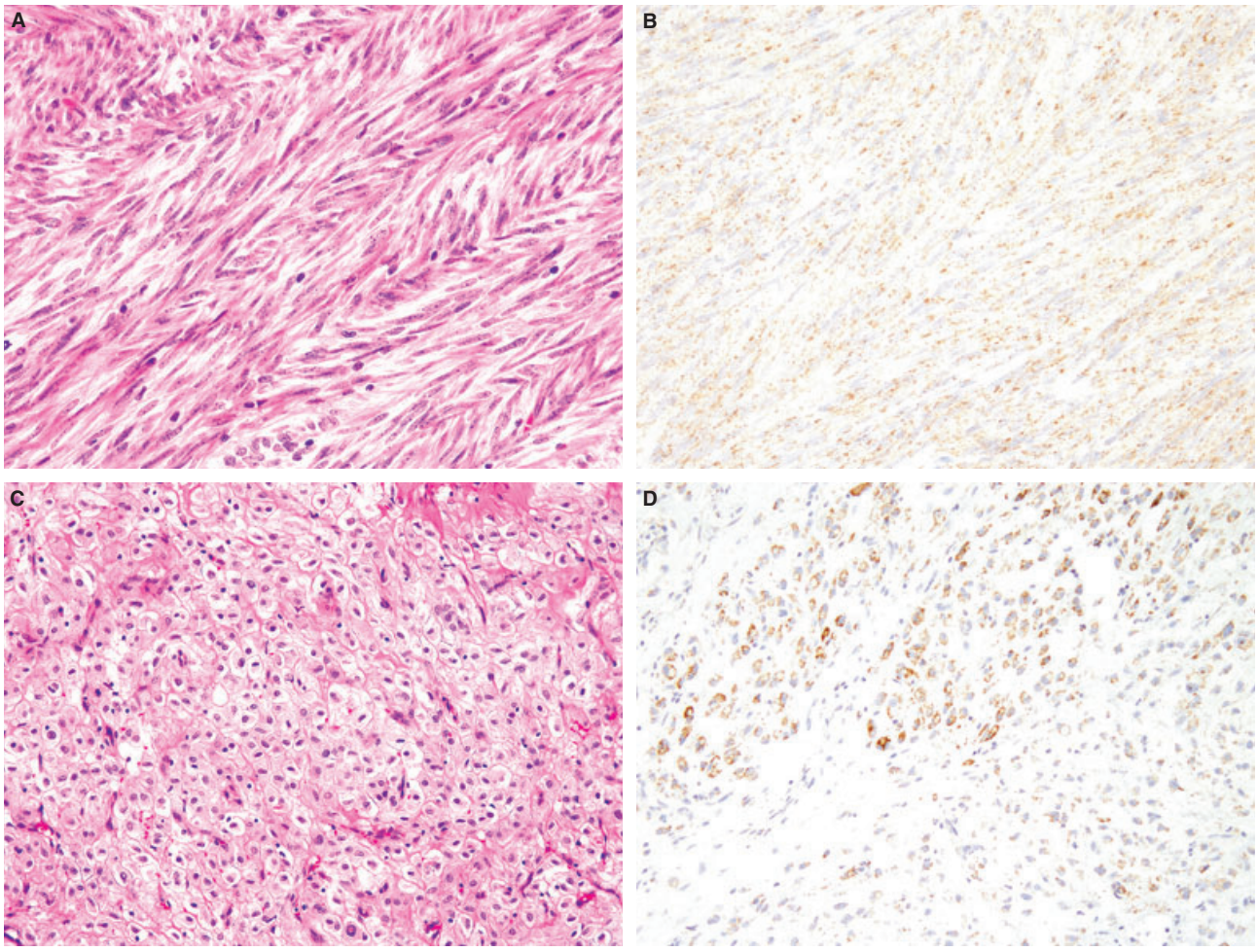

Figure 1. *KIT* exon 17 mutant spindle cell gastrointestinal stromal tumour (GIST) of the stomach (**A**). Tumour cells show diffuse granular cytoplasmic staining for succinate dehydrogenase subunit B (SDHB) (**B**). *PDGFRA* exon 18 mutant epithelioid GIST of the stomach (**C**). Variable expression of SDHB is observed in tumour cells (**D**).

mutant tumours showed a multinodular growth pattern. The sensitivity and specificity of a multinodular/plexiform architecture for predicting SDHB deficiency were 100% and 98%, respectively, with a positive predictive value of 85%.

Discussion

The role of the SDH complex as a tumour suppressor was first described in familial paraganglioma syndromes, in which patients have germline inactivating mutations in the genes coding for SDHA, SDHB, SDHC, or SDHD.[16–20] Interestingly, germline mutations in such genes have also been identified in 12–16% of patients with apparently sporadic paragangliomas.[21,22] The mechanism by which SDH complex dysfunction leads to tumorigenesis has not yet been fully elucidated; one possibility is induction of hypoxia-inducible factor 1-α (HIF1-α), which results in transcription of genes involved in tumour formation.[16] Loss of SDHB expression is observed in paragangliomas from patients with germline mutations in any of the *SDH* subunit genes. Indeed, loss of SDHB expression is 100% sensitive for detecting paragangliomas with mutations in *SDHB*, *SDHC*, or *SDHD*.[3] SDHB IHC has therefore become an invaluable screening tool for patients with paraganglioma/pheochromocytoma; genetic testing for *SDH* mutations can be limited to patients with SDHB-deficient tumours.[3] *SDHB* mutations have recently also been described in a small number of renal cell carcinomas, which correspondingly show loss of SDHB expression.[23]

SDH complex dysfunction was first identified as an alternative mechanism of tumour formation in GISTs lacking *KIT* or *PDGFRA* mutations in patients with Carney–Stratakis syndrome.[24,25] This syndrome

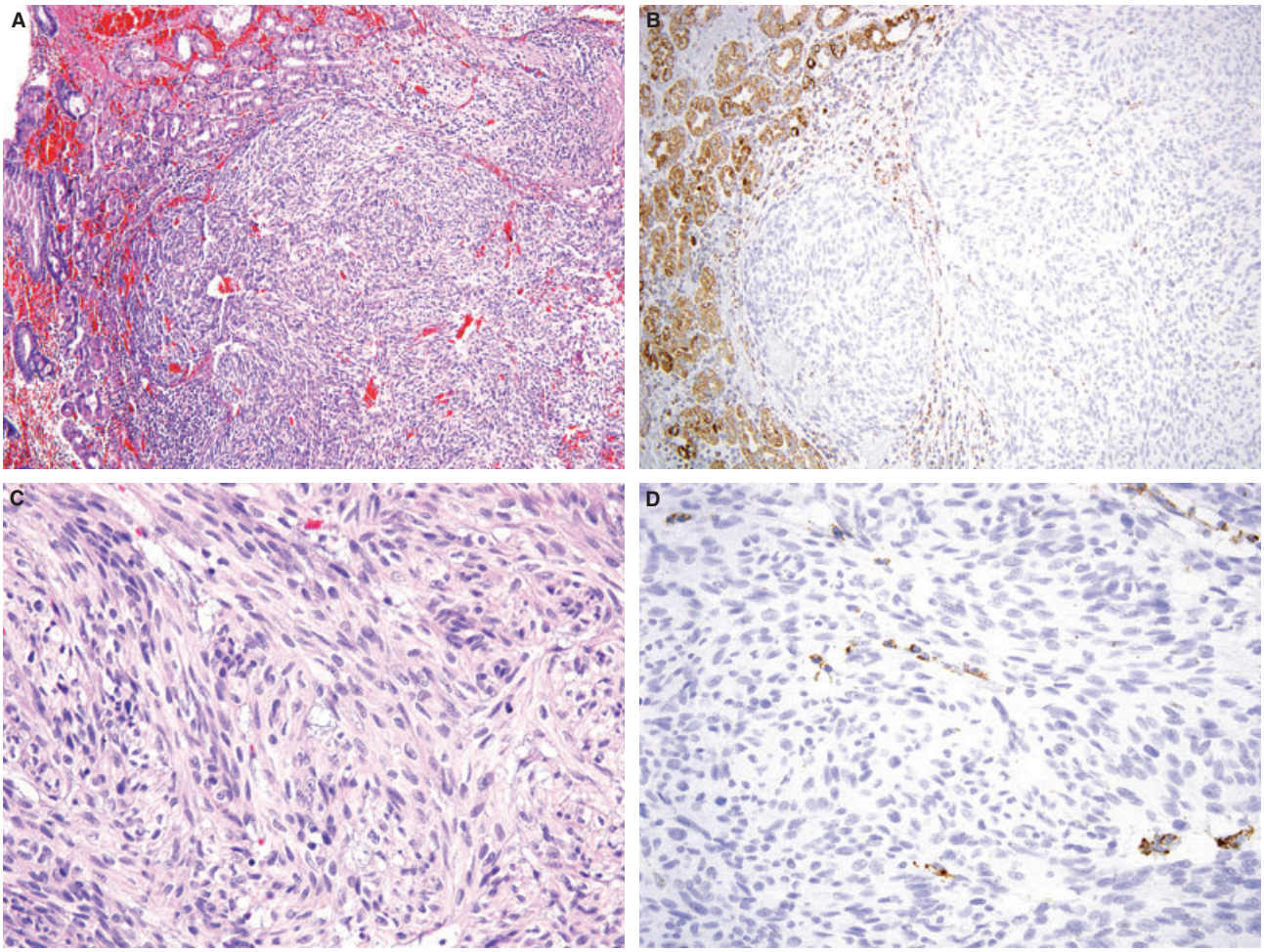

Figure 2. Wild-type gastrointestinal stromal tumour (GIST) of the stomach with characteristic multinodular architecture (**A**). Tumour cells show loss of succinate dehydrogenase subunit B (SDHB) expression, in contrast to the overlying gastric epithelium (**B**). Tumour cells show mixed spindle cell and epithelioid cytomorphology (**C**). Expression of SDHB in vascular endothelium serves as an internal positive control; in contrast, the surrounding tumour cells are negative for SDHB (**D**).

consists of the dyad of gastric GIST and paraganglioma, is inherited in an autosomal dominant fashion, shows variable penetrance, and presents at a young age (median 19 years).[25,26] As in hereditary paraganglioma syndromes, loss-of-function germline mutations in *SDHB*, *SDHC* or *SDHD* are found in patients with Carney–Stratakis syndrome.[24,25] Loss of the WT allele in the GISTs of affected patients is associated with decreased mitochondrial respiratory chain enzyme activity,[24] suggesting that, as in paraganglioma, the mechanism for tumour development is related to deficient SDH activity. GISTs occurring in Carney–Stratakis syndrome arise exclusively in the stomach, are often multifocal, and frequently spread to lymph nodes.[26]

Carney triad is a non-hereditary syndrome that most commonly affects young women, and consists of gastric GIST, paraganglioma, and pulmonary chondroma.[27,28] Affected patients may also develop adrenocortical adenomas and oesophageal leiomyomas.[29] Carney triad-associated GISTs lack *KIT* and *PDGFRA* mutations, and show SDH complex dysfunction and loss of SDHB expression; however, in contrast to Carney–Stratakis syndrome, *SDH* mutations have not been identified in patients with Carney triad.[6,30–32] The mechanisms underlying deficient SDH complex function in Carney triad are unknown.[30,31] In a large series of 104 GISTs arising in patients with Carney triad, tumours occurred exclusively in the stomach, most commonly the antrum (61%), were often multifocal, and usually had epithelioid morphology.[32] Lymph node metastases were found in 29% of patients, and 25% and 13% had liver and peritoneal metastases, respectively. Tumours showed either no response or weak partial responses to imatinib.

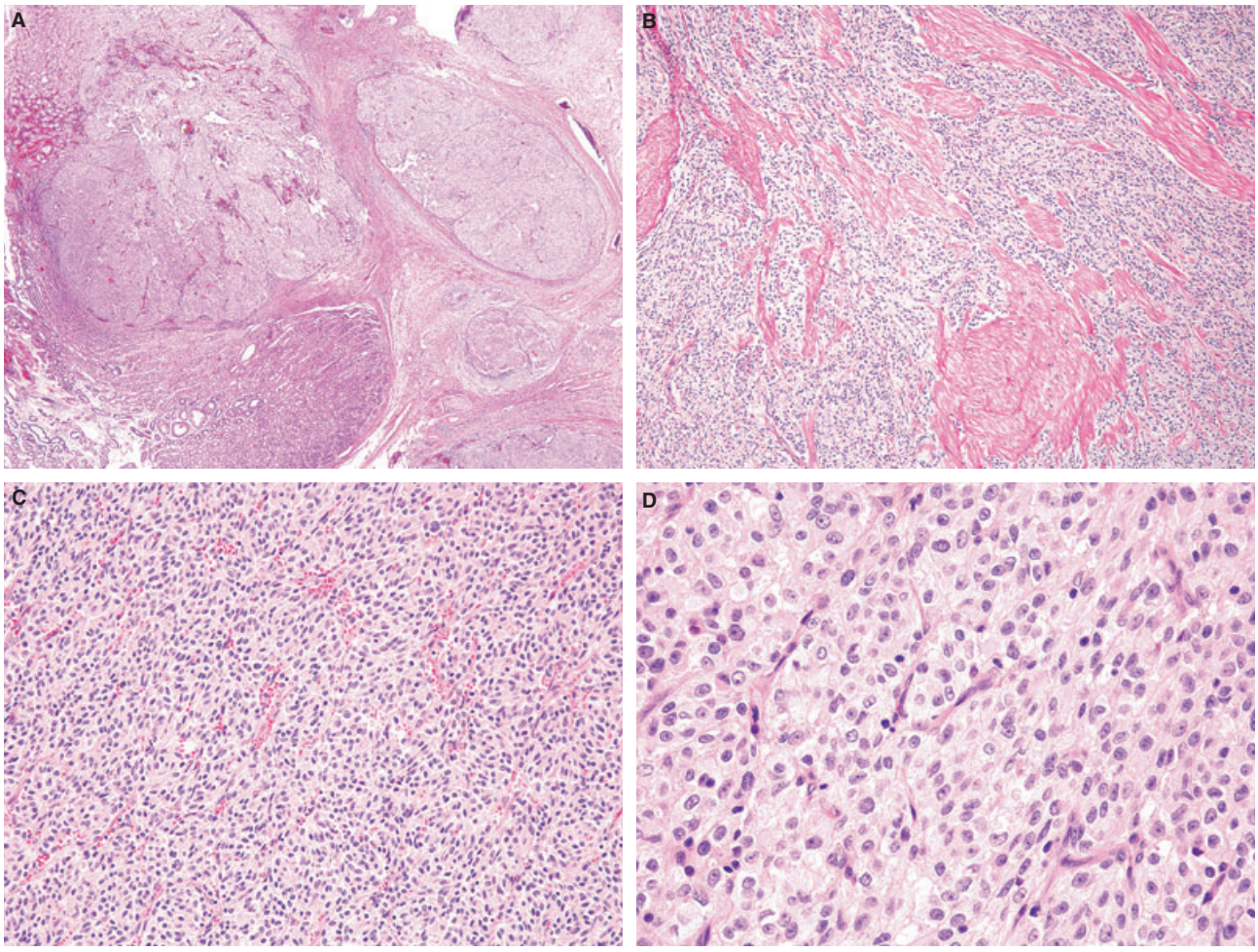

Figure 3. Succinate dehydrogenase subunit B (SDHB)-deficient gastrointestinal stromal tumour (GIST) with a multinodular/plexiform architecture (**A**). Infiltrative areas of growth into the muscularis propria in an SDHB-deficient GIST (**B**). SDHB-deficient GIST showing hypercellularity with minimal intervening stroma (**C**) and epithelioid tumour cell morphology (**D**).

The clinical and histological features of GISTs arising in Carney triad and Carney–Stratakis syndrome therefore differ from those of most conventional adult gastric GISTs, which occur in an older age group (50–80 years) and show a slight male predominance.[33] Gastric tumours are more common in the corpus, but are also commonly epithelioid; approximately 50% of adult gastric GISTs will have either purely epithelioid or mixed morphology. The typical pattern of tumour progression is liver and peritoneal dissemination, and in a large study of 1765 patients with gastric GISTs, no patients developed lymph node metastases.[33]

'Paediatric-type' GIST refers to WT tumours that occur in adults and have similar clinical and pathological features to those of GISTs arising in children and in patients with Carney triad and Carney–Stratakis syndrome.[4,6] Paediatric GISTs show a marked female predominance and nearly always arise in the stomach, where they are often multifocal.[13,15] In a study of 44 patients under 21 years of age with gastric GISTs, 76% of tumours had purely or predominantly epithelioid morphology, 41% were hypercellular with minimal stroma, and many showed a plexiform growth pattern.[15] Almost all paediatric GISTs are wild type for *KIT* and *PDGFRA*, and accordingly tend to be poorly responsive to imatinib.[13,15] Despite the WT genotype, paediatric GISTs show evidence of KIT pathway activation.[13] In contrast to conventional adult GISTs, lymph node metastases from paediatric GISTs are common. The clinical behaviour is unpredictable, but patients with metastatic disease typically have an indolent clinical course.

Initial studies of SDHB expression in GIST showed loss of expression in tumours associated with both Carney triad and Carney–Stratakis syndrome, as well as in a small number of gastric tumours with similar pathological

Table 2. Clinicopathological features of succinate dehydrogenase subunit B (SDHB)-deficient and SDHB-intact wild-type gastrointestinal stromal tumours

	SDHB-deficient wild-type GIST, no. (%)*	SDHB-intact wild-type GIST, no. (%)*
Median age (years)	31	51
Sex		
Female	12 (55)	16 (62)
Male	10 (45)	10 (38)
Anatomical location		
Stomach	21 (100)	8 (33)
Small intestine	0 (0)	10 (42)
Colon	0 (0)	5 (21)
Oesophagus	0 (0)	1 (4)
Growth pattern		
Multinodular/plexiform	22 (100)	0 (0)
Sheet-like	0 (0)	24 (100)
Tumour cell morphology		
Spindle cell	0 (0)	20 (65)
Epithelioid	15 (68)	4 (13)
Mixed spindle cell and epithelioid	7 (32)	7 (22)

*Percentages calculated for all cases with available information.

features.[5,6] The control groups of sporadic GISTs in these studies showed intact staining for SDHB, but genotypic data were limited; *KIT* or *PDGFRA* genotype was reported for only five of the tumours with intact SDHB expression. A large study of 756 gastric GISTs estimated the frequency of SDHB-deficient tumours at 7.5%.[7] The SDHB-deficient GISTs showed similar clinicopathological features to 'paediatric-type' GISTs. Focused mutation testing of the SDHB-deficient GISTs in that study did not identify mutations in *SDHB*, *SDHC*, or *SDHD*, and all were wild type for *KIT* and *PDGFRA*. The genotypes of the gastric GISTs with intact SDHB expression were not reported. An additional 378 non-gastric GISTs showed intact expression of SDHB, indicating that loss of SDHB is limited to gastric GISTs. Loss of SDHB expression is also seen in most paediatric GISTs.[2,6] In a study of WT GISTs occurring in patients without a personal or family history of paraganglioma, germline mutations in *SDHB* or *SDHC* were found in 12% of patients.[2] No somatic mutations in *SDHB*, *SDHC* or *SDHD* were identified in the tumours tested. However, almost all of the WT tumours (apart from those associated with NF1) showed loss of SDHB expression by IHC and reduced SDH activity as compared with *KIT* mutant GISTs, suggesting that SDH dysfunction contributes to tumour formation in many WT GISTs even in the absence of germline or somatic mutations in *SDHB*, *SDHC*, or *SDHD*.

In the current study, we have expanded the evaluation of SDHB expression in GIST by examining a large cohort of genotyped tumours to determine the specificity of SDHB as a biomarker. All *KIT* and *PDGFRA* mutant GISTs ($N = 211$) showed intact expression of SDHB. As in previous studies, the intensity and extent of staining were variable, and any granular staining, however focal, was considered to represent intact expression. It is of note that occasional cases showed regional variability in staining on individual slides, with focal areas of completely negative staining, suggesting that tissue fixation may significantly impact on SDHB staining. However, in such areas, the internal controls (i.e. endothelial cells) also lacked SDHB staining. It is therefore important to emphasize that appropriate staining of internal controls is essential in order to evaluate the expression of SDHB in tumour cells. This is particularly relevant for small mucosal or core needle biopsy specimens, which must be examined carefully to avoid misinterpretation.

Of the WT GISTs, 42% showed absence of SDHB expression. Of these tumours, two had germline mutations in *SDHC* and one in *SDHB*; however, mutation analysis of the *SDH* genes was not performed as part of this study. The group of SDHB-deficient GISTs had distinct clinical and morphological features as compared with WT GISTs with intact SDHB expression (Table 2). SDHB-deficient GISTs occurred in a significantly younger age group than the WT GISTs with intact SDHB expression, with a median age of 31 versus 51 years ($P < 0.0001$). There was no difference in gender distribution between the two groups, with both showing a slight female predominance. SDHB-deficient GISTs arose exclusively in the stomach, showed a multinodular growth pattern, occasionally with areas of more diffusely infiltrative growth, and were composed of epithelioid tumour cells, either entirely or combined with a spindle cell component.

WT GISTs showing intact SDHB expression most commonly arose in the small intestine (42%), followed by the stomach (33%), colon (21%), and oesophagus (4%). This group of tumours showed a diffuse, sheet-like growth pattern similar to that of *KIT* mutant GISTs, and the most common tumour cell morphology was

pure spindle cell. Little is known about the tumour-initiating events in WT GISTs that show intact SDHB expression. It is of note that WT GISTs associated with NF1 have been shown to express SDHB consistently,[6,34] and, in the current study, two of the WT tumours with intact SDHB expression were from NF1 patients. Between 3.5% and 13% of WT GISTs harbour *BRAF* mutations;[35–38] whether this subset retains SDHB expression remains to be determined.

Our findings confirm those of prior studies, suggesting that loss of SDHB expression is a useful marker for identifying a clinicopathologically and biologically distinctive group of WT GISTs ('paediatric-type' or 'type 2' GISTs). In addition, our study indicates that loss of SDHB expression is highly specific for this class of GIST; all *KIT* and *PDGFRA* mutant GISTs showed intact staining for SDHB. WT GISTs with intact SDHB expression have some clinical and pathological features that help to distinguish them from SDHB-deficient GISTs, although further characterization of the former group was limited by the available clinical and histological data. Although SDHB-deficient GISTs show a distinctive multinodular/plexiform growth pattern and epithelioid morphology, rare *KIT* mutant GISTs also present a multinodular architecture. Furthermore, many gastric GISTs are epithelioid, particularly those with mutations in *PDGFRA*.[33] Therefore, although morphological features may suggest the diagnosis of 'SDH-deficient' GIST, IHC may be advisable to confirm the diagnosis.

As previously discussed, there are important predictive and prognostic implications of the diagnosis of an 'SDH-deficient' GIST. These tumours frequently metastasize, often to lymph nodes, yet pursue a relatively indolent clinical course despite metastatic disease. WT GISTs are relatively resistant to therapy with imatinib, but may respond better to second-generation and third-generation tyrosine kinase inhibitors such as sunitinib, sorafenib, nilotinib, and dasatinib.[4,13,14] Furthermore, risk stratification based on the most commonly used system for assessing the malignant potential of GISTs (Armed Forces Institute of Pathology) does not seem to predict the clinical behaviour of these tumours.[4,7,32]

Recognizing this distinctive group of GISTs also identifies a group of patients for whom genetic testing and follow-up for the development of additional gastric GISTs or other tumour types is warranted. Although patients with Carney–Stratakis syndrome or Carney triad appear to represent a small subgroup of SDH-deficient GISTs,[7] in many cases GIST is the sentinel tumour in these syndromes; the time interval before development of a second tumour may be years or decades.[13,28,29] Very recently, loss-of-function *SDHA* mutations were reported in four gastric WT GISTs (germline mutations confirmed in three patients), suggesting that mutations in this subunit can represent an alternative mechanism for SDH complex dysfunction in the absence of mutations in *SDHB*, *SDHC*, or *SDHD*, and that screening for *SDHA* mutations should also be performed in SDHB-deficient GISTs.[39,40] It should also be noted that some familial GISTs arise because of germline mutations in *KIT* or *PDGFRA*.[41,42] SDHB IHC may therefore also be useful for guiding mutation testing in such affected individuals.

In summary, SDHB-deficient GISTs are WT gastric tumours with distinctive features that can be recognized histologically. SDHB IHC can be used to confirm the diagnosis of this class of tumour, which has prognostic, therapeutic and syndromic implications. SDHB expression is retained in GISTs with *KIT* and *PDGFRA* mutations; therefore, SDHB IHC may also be useful for guiding appropriate mutation analysis. 'SDH-deficient' is a unifying terminology that reflects the pathogenesis of this clinically and pathologically distinctive group of WT GISTs.

Acknowledgements

This work was supported in part by a Merit Review Grant from the Department of Veterans Affairs (M. C. Heinrich), and funding from the Life Raft Group (M. C. Heinrich and C. L. Corless) and the GIST Cancer Research Fund (M. C. Heinrich and C. L. Corless).

References

1. Gottlieb E, Tomlinson IP. Mitochondrial tumour suppressors: a genetic and biochemical update. *Nat. Rev. Cancer* 2005; **5;** 857–866.
2. Janeway KA, Kim SY, Lodish M *et al.* Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. *Proc. Natl Acad. Sci. USA* 2011; **108;** 314–318.
3. van Nederveen FH, Gaal J, Favier J *et al.* An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. *Lancet Oncol.* 2009; **10;** 764–771.
4. Rege TA, Wagner AJ, Corless CL *et al.* 'Pediatric-type' gastrointestinal stromal tumors in adults: distinctive histology predicts genotype and clinical behavior. *Am. J. Surg. Pathol.* 2011; **35;** 495–504.
5. Gaal J, Stratakis CA, Carney JA *et al.* SDHB immunohistochemistry: a useful tool in the diagnosis of Carney–Stratakis and Carney triad gastrointestinal stromal tumors. *Mod. Pathol.* 2011; **24;** 147–151.
6. Gill AJ, Chou A, Vilain R *et al.* Immunohistochemistry for SDHB divides gastrointestinal stromal tumors (GISTs) into 2 distinct types. *Am. J. Surg. Pathol.* 2010; **34;** 636–644.
7. Miettinen M, Wang ZF, Sarlomo-Rikala M *et al.* Succinate dehydrogenase-deficient GISTs: a clinicopathologic, immunohistochemical, and molecular genetic study of 66 gastric GISTs with

predilection to young age. *Am. J. Surg. Pathol.* 2011; **35;** 1712–1721.
8. Hirota S, Isozaki K, Moriyama Y *et al.* Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998; **279;** 577–580.
9. Heinrich MC, Corless CL, Duensing A *et al.* PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 2003; **299;** 708–710.
10. Demetri GD, von Mehren M, Blanke CD *et al.* Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N. Engl. J. Med.* 2002; **347;** 472–480.
11. Corless CL, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. *J. Clin. Oncol.* 2004; **22;** 3813–3825.
12. Janeway KA, Liegl B, Harlow A *et al.* Pediatric KIT wild-type and platelet-derived growth factor receptor alpha-wild-type gastrointestinal stromal tumors share KIT activation but not mechanisms of genetic progression with adult gastrointestinal stromal tumors. *Cancer Res.* 2007; **67;** 9084–9088.
13. Agaram NP, Laquaglia MP, Ustun B *et al.* Molecular characterization of pediatric gastrointestinal stromal tumors. *Clin. Cancer Res.* 2008; **14;** 3204–3215.
14. Marrari A, Wagner AJ, Hornick JL. Predictors of response to targeted therapies for gastrointestinal stromal tumors. *Arch. Pathol. Lab. Med.* 2012; **136;** 483–489.
15. Miettinen M, Lasota J, Sobin LH. Gastrointestinal stromal tumors of the stomach in children and young adults: a clinicopathologic, immunohistochemical, and molecular genetic study of 44 cases with long-term follow-up and review of the literature. *Am. J. Surg. Pathol.* 2005; **29;** 1373–1381.
16. Burnichon N, Briere JJ, Libe R *et al.* SDHA is a tumor suppressor gene causing paraganglioma. *Hum. Mol. Genet.* 2010; **19;** 3011–3020.
17. Astuti D, Latif F, Dallol A *et al.* Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *Am. J. Hum. Genet.* 2001; **69;** 49–54.
18. Astuti D, Douglas F, Lennard TW *et al.* Germline SDHD mutation in familial phaeochromocytoma. *Lancet* 2001; **357;** 1181–1182.
19. Baysal BE, Ferrell RE, Willett-Brozick JE *et al.* Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 2000; **287;** 848–851.
20. Niemann S, Muller U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. *Nat. Genet.* 2000; **26;** 268–270.
21. Burnichon N, Rohmer V, Amar L *et al.* The succinate dehydrogenase genetic testing in a large prospective series of patients with paragangliomas. *J. Clin. Endocrinol. Metab.* 2009; **94;** 2817–2827.
22. Amar L, Bertherat J, Baudin E *et al.* Genetic testing in pheochromocytoma or functional paraganglioma. *J. Clin. Oncol.* 2005; **23;** 8812–8818.
23. Gill AJ, Pachter NS, Chou A *et al.* Renal tumors associated with germline SDHB mutation show distinctive morphology. *Am. J. Surg. Pathol.* 2011; **35;** 1578–1585.
24. McWhinney SR, Pasini B, Stratakis CA, International Carney Triad and Carney–Stratakis Syndrome Consortium. Familial gastrointestinal stromal tumors and germ-line mutations. *N. Engl. J. Med.* 2007; **357;** 1054–1056.
25. Pasini B, McWhinney SR, Bei T *et al.* Clinical and molecular genetics of patients with the Carney–Stratakis syndrome and germline mutations of the genes coding for the succinate dehydrogenase subunits SDHB, SDHC, and SDHD. *Eur. J. Hum. Genet.* 2008; **16;** 79–88.
26. Carney JA, Stratakis CA. Familial paraganglioma and gastric stromal sarcoma: a new syndrome distinct from the Carney triad. *Am. J. Med. Genet.* 2002; **108;** 132–139.
27. Carney JA, Sheps SG, Go VL, Gordon H. The triad of gastric leiomyosarcoma, functioning extra-adrenal paraganglioma and pulmonary chondroma. *N. Engl. J. Med.* 1977; **296;** 1517–1518.
28. Carney JA. Gastric stromal sarcoma, pulmonary chondroma, and extra-adrenal paraganglioma (Carney Triad): natural history, adrenocortical component, and possible familial occurrence. *Mayo Clin. Proc.* 1999; **74;** 543–552.
29. Carney JA. Carney triad: a syndrome featuring paraganglionic, adrenocortical, and possibly other endocrine tumors. *J. Clin. Endocrinol. Metab.* 2009; **94;** 3656–3662.
30. Matyakhina L, Bei TA, McWhinney SR *et al.* Genetics of Carney triad: recurrent losses at chromosome 1 but lack of germline mutations in genes associated with paragangliomas and gastrointestinal stromal tumors. *J. Clin. Endocrinol. Metab.* 2007; **92;** 2938–2943.
31. Stratakis CA, Carney JA. The triad of paragangliomas, gastric stromal tumours and pulmonary chondromas (Carney triad), and the dyad of paragangliomas and gastric stromal sarcomas (Carney–Stratakis syndrome): molecular genetics and clinical implications. *J. Intern. Med.* 2009; **266;** 43–52.
32. Zhang L, Smyrk TC, Young WF Jr *et al.* Gastric stromal tumors in Carney triad are different clinically, pathologically, and behaviorally from sporadic gastric gastrointestinal stromal tumors: findings in 104 cases. *Am. J. Surg. Pathol.* 2010; **34;** 53–64.
33. Miettinen M, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. *Am. J. Surg. Pathol.* 2005; **29;** 52–68.
34. Wang JH, Lasota J, Miettinen M. Succinate dehydrogenase subunit B (SDHB) is expressed in neurofibromatosis 1-associated gastrointestinal stromal tumors (GISTs): implications for the SDHB expression based classification of GISTs. *J. Cancer* 2011; **2;** 90–93.
35. Agaram NP, Wong GC, Guo T *et al.* Novel V600E BRAF mutations in imatinib-naive and imatinib-resistant gastrointestinal stromal tumors. *Genes Chromosom. Cancer* 2008; **47;** 853–859.
36. Agaimy A, Terracciano LM, Dirnhofer S *et al.* V600E BRAF mutations are alternative early molecular events in a subset of KIT/PDGFRA wild-type gastrointestinal stromal tumours. *J. Clin. Pathol.* 2009; **62;** 613–616.
37. Hostein I, Faur N, Primois C *et al.* BRAF mutation status in gastrointestinal stromal tumors. *Am. J. Clin. Pathol.* 2010; **133;** 141–148.
38. Daniels M, Lurkin I, Pauli R *et al.* Spectrum of KIT/PDGFRA/BRAF mutations and phosphatidylinositol-3-kinase pathway gene alterations in gastrointestinal stromal tumors (GIST). *Cancer Lett.* 2011; **312;** 43–54.
39. Pantaleo MA, Nannini M, Astolfi A *et al.* A distinct pediatric-type gastrointestinal stromal tumor in adults: potential role of succinate dehydrogenase subunit A mutations. *Am. J. Surg. Pathol.* 2011; **35;** 1750–1752.
40. Pantaleo MA, Astolfi A, Indio V *et al.* SDHA loss-of-function mutations in KIT-PDGFRA wild-type gastrointestinal stromal tumors identified by massively parallel sequencing. *J. Natl Cancer Inst.* 2011; **103;** 983–987.
41. Nishida T, Hirota S, Taniguchi M *et al.* Familial gastrointestinal stromal tumours with germline mutation of the KIT gene. *Nat. Genet.* 1998; **19;** 323–324.
42. Chompret A, Kannengiesser C, Barrois M *et al.* PDGFRA germline mutation in a family with multiple cases of gastrointestinal stromal tumor. *Gastroenterology* 2004; **126;** 318–321.

