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Utility of immunohistochemical investigation of SDHB and molecular genetic analysis of *SDH* genes in the differential diagnosis of mesenchymal tumors of GIT

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Summary. Loss of expression of beta subunit of succinate dehydrogenase (SDHB) was proved to be present in a subgroup of *KIT*/*PDGFRA* wt gastrointestinal stromal tumors (GISTs). To evaluate possible diagnostic utility of SDHB immunohistochemistry in the differential diagnostics of mesenchymal tumors of gastrointestinal tract (GIT), 11 cases of *KIT*/*PDGFRA* wt GISTs, 12 gastric schwannomas (GSs), 20 solitary fibrous tumors (SFTs), 4 leiomyomas (LMs), 16 leiomyosarcomas (LMSs), 5 synovial sarcomas (SSs), 3 endometrioid stromal sarcomas (ESSs), and 1 ileal inflammatory myofibroblastic tumor (IMT) were investigated for SDHB immunoexpression together with molecular genetic analysis of genes encoding succinate dehydrogenase (*SDH*). Three recent cases of *KIT*/*PDGFRA* mutant GISTs were used as controls. Among the 11 *KIT*/*PDGFRA* wt GISTs, 6 expressed SDHB, 1 of them harboring a sequence change of *SDHD*. All SDHB-negative cases were *SDHB-D* wt. In 1 of the control GIST cases molecular genetic analysis revealed an *SDHD* sequence change in addition to a mutation in *KIT* exon 11. No SFT was truly SDHB-negative, but in 2 of them the staining was impossible to analyze. Furthermore, 1 SFT carried an *SDHB* and another 1 *SDHD* sequence change. All GSs, LMs, LMSs, SSs, ESSs, and IMT were SDHB-positive or non-analyzable, and *SDHB-D* wt.

Additional factors may play a role in regulating expression of SDHB. Furthermore, SDHB immunohistochemistry alone may be misleading in excluding tumors other than GIST (especially SFT) in the differential diagnosis of *KIT*/*PDGFRA* wt mesenchymal tumors of GIT.

Key words: GIST, Stromal tumor, KIT, PDGFRA , SDH

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Introduction

Succinate dehydrogenase (SDH), also known as mitochondrial complex II, is an enzyme complex located in the inner mitochondrial membrane, which consists of four main subunits (SDHA, SDHB, SDHC, and SDHD), assembly factors (SDHAF1, SDHAF2), iron-sulphur centers, and ubiquinone. It participates in the electron transport chain and Krebs cycle by catalyzing oxidative dehydrogenation of succinate to fumarate (Gottlieb and Tomlinson, 2005). As immunoexpression of SDHB is dependent on complete assembly of the whole SDH complex, the immunohistochemical investigation of SDHB represents an important source of information on function of the enzyme complex. The SDHB protein is normaly ubiquitously expressed, whereas its loss reflects dysfunction of the SDH complex. Such a dysfunction caused by loss-of-function mutations of the genes encoding individual subunits of SDH (i.e. *SDHx* genes) was first described in familial paraganglioma/pheochromocytoma syndrome (Baysal et al., 2000; Niemann and Muller, 2000; Astuti et al., 2001a,b; van Nederveen et al., 2009).

In 2007, germline mutations of *SDHx* and loss of SDHB expression were identified in gastrointestinal

stromal tumors (GISTs) in patients with Carney-Stratakis syndrome (McWhinney et al., 2007; Pasini et al., 2008). Since then, loss of SDHB expression was reported to occur also in other GISTs lacking mutations of genes encoding receptor tyrosine kinases KIT and platelet-derived growth factor receptor alpha (PDGFRα), i. e. so-called *KIT* and *PDGFRA* wild-type (*KIT*/*PDGFRA* wt) GISTs, and immunoexpression of SDHB became a standard tool used to discriminate between SDHB-positive GISTs driven by activation of KIT/ PDGFRα pathway, and SDHB-deficient GISTs which represent a different clinical, genetical and therapeutical entity (Agaimy et al., 2009; Gill et al., 2010, 2011a; Gaal et al., 2011; Miettinen et al., 2011; Rege et al., 2011; Barletta and Hornick, 2012; Doyle et al., 2012).

However, little is known about expression of SDHB in other *KIT*/*PDGFRA* wt mesenchymal tumors of gastrointestinal tract (GIT). Therefore, we performed the study on expression of SDHB in GISTs and their mimics. Furthermore, we correlated SDHB-status with molecular genetic profile of the tumors.

Materials and methods

Eleven cases of *KIT*/*PDGFRA* wt GISTs (7 gastric, 4 intestinal), 12 gastric schwannomas (GSs), 20 solitary fibrous tumors (SFTs), 4 leiomyomas (LMs), 16 leiomyosarcomas (LMSs), 5 synovial sarcomas (SSs), 3 endometrioid stromal sarcomas (ESSs), and 1 ileal inflammatory myofibroblastic tumor (IMT) were retrieved from our archives. Three recent cases of *KIT* or *PDGFRA* mut GISTs were used as control cases.

Tissue for light microscopy was fixed in 4% formaldehyde and embedded in paraffin using routine procedures. Five micrometer-thick sections were cut from the tissue blocks and stained with hematoxylin and eosin.

For immunohistochemical investigations the following primary antibodies were used: SDHB (polyclonal, 1:100, Santa Cruz Biotechnology, Santa Cruz, CA), SDHB (21A11, 1:100, Abcam, Cambridge, MA), DOG-1 (K9, RTU, Novocastra, Newcastle, UK), Stat6 (S20, polyclonal, 1:100, Santa Cruz Biotechnology, Santa Cruz, CA). No special pretreatment was used. The primary antibodies were visualized using the supersensitive streptavidin-biotin-peroxidase complex (Biogenex, San Ramon, CA). Appropriate positive and negative control slides were employed. Furthermore, non-neoplastic mucosal epithelial or endothelial cells were used as internal positive control. Samples negative in staining with the polyclonal anti-SDHB antibody by Santa Cruz were then stained with the monoclonal antibody by Abcam. Only those samples that did not stain with any of the antibodies, but showed indubitable granular cytoplasmic positivity of intratumoral endothelial cells with at least one of the antibodies, were regarded as SDHB-negative. In the absence of endothelial staining, the sample was labeled as non-analyzable (NA). The diagnosis of SFT was then proved by immunoexpression of Stat6 and absence of staining with DOG-1 antibody in SDHB- cases.

DNA for molecular genetic investigation was extracted from formalin-fixed, paraffin-embedded tissues. Several 5 μm thick sections were placed on the slides. Hematoxylin and eosin stained slides were examined for determination of area of tumor tissue. Then, tumor tissue from unstained slides was scraped and DNA was isolated by the NucleoSpin® Tissue Kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) according to the manufacturer's protocol. Mutational analysis of coding sequence, including exon-intron junctions of *SDHB, SDHC,* and *SDHD* genes was performed by PCR and direct sequencing. In GIST cases, analysis of exons 9, 11, 13 and 17 of the *KIT* gene (accession number U63834), exons 12, 14 and 18 of the *PDGFRA* gene (accession number D50017) was performed as well.

In GIST cases, their pattern (spindled, mixed, epithelioid) was compared with previous markers.

Results

GISTs

All GIST cases used in the study were previously defined on the basis of their morphology and pattern of immunoexpression of KIT (CD117), desmin, and S-100 protein, either during routine daily service or in consultation practice. Basic clinical and morphological features of the investigated GISTs are summarized in Table 1. SDHB status was investigated in two steps. In the first step, the Santa Cruz anti-SDHB antibody was used in all cases. As the second step, all SDHB-negative or questionable cases were stained with the Abcam monoclonal anti-SDHB antibody.

Immunohistochemical and molecular genetic

Table 1. Clinicopathologic features of GISTs.

No.	Localization	Gender and age	Pattern	Size (cm)	MI
1	stomach	F, 47	mixed	14	1
2	stomach	F, 63	spindle	?	10
3	stomach	F, 43	mixed	7	5
4	stomach	F, 76	mixed	2	2
5	stomach	F, 70	spindle	10	2
6	stomach	M, 62	spindle	7	0
7	stomach	F, 48	epithelioid	6	2
8	stomach	F, 12	mixed	?	10
9	small intestine	M, 75	epithelioid	6	133
10	small intestine	F, 30	epithelioid	7	0
11	small intestine	M, 46	spindle	5	5
12	small intestine	F, 51	mixed	2	4
13	small intestine	M, 60	mixed	2.5	0
14	mediastinum	M, 52	epithelioid	15	25

M, male; F, female; MI, mitotic index (number of mitoses per 5 mm^2); ?, unknown

features of the tumors are summarized in Table 2. Briefly, of the 11 GIST *KIT/PDGFRA* wt cases, 6 tumors were SDHB+ and 5 were SDHB- when stained with the Santa Cruz antibody. Three of the SDHB- tumors were localized in stomach, 2 of those showed mixed pattern and 1 displayed epithelioid morphology. Two SDHB- GISTs were located in the small intestine, 1 of them being of mixed, the other one of epithelioid cell morphology (Fig. 1). SDHB negativity was confirmed by the Abcam antibody in only one female pediatric gastric GIST of mixed morphology, which was found to be *SDHB-D* wt (Fig. 2). On the contrary, one of the SDHB+ gastric GISTs harbored an SDHB sequence change in exon 1 (G12S). Other tumors showed no SDHB-D genetic changes.

Of the three *KIT* or *PGDFRA* mut GISTs, which were used as control cases, only 1 was SDHB-negative (Fig. 3), although this case was also shown to bear G12S change in exon 1 of *SDHD* gene in addition to W557_K558 deletion in exon 11 of *KIT* gene.

All cases were KIT-positive, regardless of their SDHB-status. Membranous pattern was more prominent in tumors composed of spindle-shaped cells (Cases 2, 5, 6 and 11), whereas in epihelioid tumors or epithelioid cells of mixed tumors the dot-like cytoplasmic pattern was more eye-catching. There was no significant difference in KIT staining between SDHB- cases 8 and 14 and other cases.

Table 2. Immunohistochemical and molecular genetic features of GISTs.

No.	Mutational profile	SDHB Santa Cruz	SDHB Abcam
1	*KIT* wt, *PDGFRA* wt, *SDHD* p.G12S	+	
2	*KIT* wt, *PDGFRA* wt, *SDHB-D* NA	+	
3	*KIT* wt, *PDGFRA* wt, *SDHB-D* NA	NA	NA
4	*KIT* wt, *PDGFRA* p.D842V, *SDHB-D* wt	+	
5	*KIT* wt, *PDGFRA* wt, *SDHB-D* wt	+	
6	*KIT* wt, *PDGFRA* wt, *SDHB-D* NA	+	
7	*KIT* wt, *PDGFRA* wt, *SDHB-D* NA	NA	NA
8	*KIT* wt, *PDGFRA* wt, *SDHB-D* wt	-	-
9	*KIT* wt, *PDGFRA* wt, *SDHB-D* NA	+	
10	*KIT* wt, *PDGFRA* wt, *SDHx* wt	+	
11	*KIT* wt, *PDGFRA* wt, *SDHB-D* NA	NA	NA
12	*KIT* p.W557_E561del, *PDGFRA* wt, *SDHB-D* wt	-	+
13	*KIT* wt, *PDGFRA* wt, *SDHB-D* NA	NA	NA
14	*KIT* p. W557_K558del, *PDGFRA* wt, *SDHD* p.G12S	-	-

NA, not analyzable; *SDHB-D*, genes *SDHB*, *SDHC*, and *SDHD*; wt, wild type

Table 3. Schwannomas.

No.	Sex and age	Size (cm)	*SDHB-D* status	SDHB Santa Cruz	SDHB Abcam
1	F, 77	4.5	wt	+	
2	F, 74	7	wt	+	
3	M, 16	?	NA	+	
4	F, 88	2.5	wt	+	
5	F, 64	4	wt	+	
6	M, 82	3	wt	+	
7	F, 46	3.5	wt	+	
8	F, 80	?	wt	+	
9	M, 47	?	wt	+	
10	M, 43	3.5	wt	+	
11	F, 26	?	wt	NA	NA
12	M, 63	?	wt	NA	NA

M, male; F, female; ?, unknown; NA, not analyzable; *SDHB-D*, genes *SDHB*, *SDHC*, and *SDHD*; wt, wild type

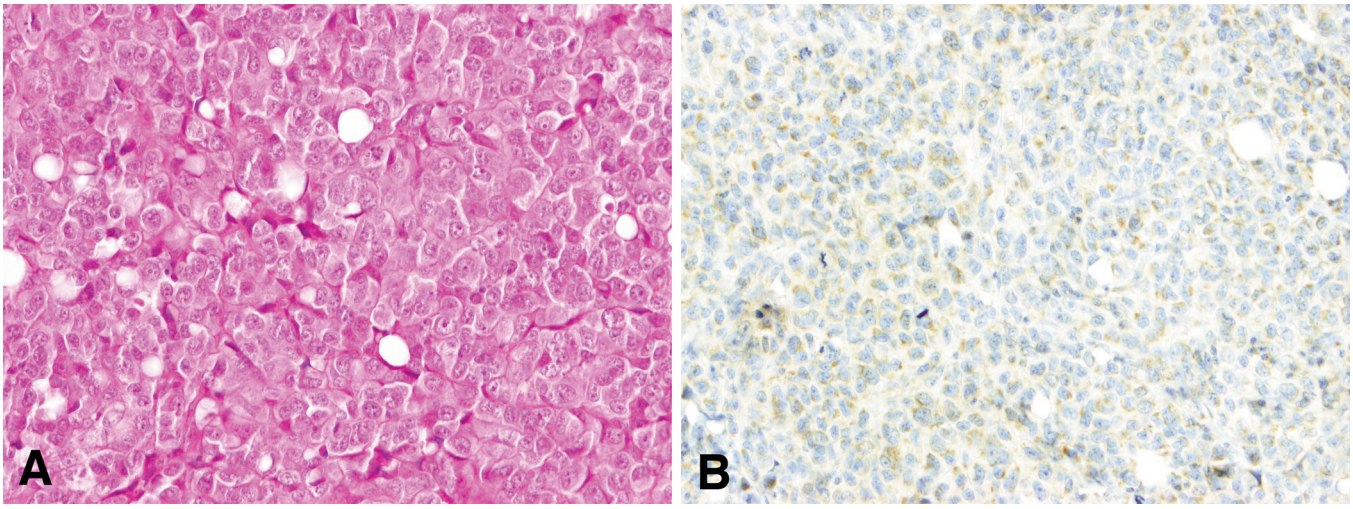

Fig. 1. GIST Case 9 was located in the small intestine, composed of epithelioid cells, with numerous mitoses (**A**, H&E), and with striking granular SDHB positivity (**B**, SDHB Santa Cruz). x 200

Gastric schwannomas

Of the 12 GSs used in the study, 10 showed at least focal granular staining of neoplastic cells with at least one anti-SDHB antibody (Fig. 4). The 2 remaining cases were found to be impossible to analyze due to the lack of staining of endothelial cells. Eleven tumors were *SDHB-D* wt, only 1 sample was non-analyzable due to poor quality of DNA (Table 3).

Solitary fibrous tumors

Clinicopathological, immunohistochemical and molecular genetic data are shown in Table 4. In summary, none of the cases was proved to be SDHB-, although 2 cases had to be categorized as non-analyzable due to the lack of staining of endothelial cells. However, it has to be stressed that interpretation of SDHB staining was extremely difficult in some cases because of small

Table 4. Solitary fibrous tumors.

No.	Sex and age	Size (cm)	*SDHB-D* status	SDHB Santa Cruz	SDHB Abcam
1	M, 33	?	wt	+	
2	M, 59	?	wt	+	
3	F, 45	2	wt	-	+
4	F, 80	"large"	wt	+	
5	M, 74	21	wt	+	
6	F, 55	2.2	wt	+	
7	F, 59	5.5	wt	+	
8	M, 51	6.7	wt	+	
9	M, 77	11	*SDHB* p.S163P	-	+
10	M, 70	7	wt	+	
11	M, 66	17	wt	+	
12	F, 48	?	NA	-	+
13	F, 73	7	NA	NA	NA
14	M, 48	12	*SDHD* p.G12S	+	
15	F, 71	19	wt	+	
16	F, 74	?	wt	+	
17	F, 55	?	wt	NA	NA
18	F, 29	17	wt	+	
19	F, 28	"large"	wt	+	
20	F, 67	4	wt	+	

M, male; F, female; ?, unknown; NA, not analyzable; *SDHB-D*, genes *SDHB, SDHC,* and *SDHD*; wt, wild type

Table 5. Smooth muscle tumors.

No.	Sex and age	Diagnosis	Size (cm)	*SDHB-D* status	SDHB Santa Cruz	SDHB Abcam
1	F, 69	LM	4.7	wt	+	
2	M, 36	LM	10	wt	-	+
3	M, 61	LM	7	wt	+	
4	F, 58	LM	1.2	NA	+	
5	F, 35	LMS	4	wt	+	
6	F, 38	LMS	3.5	wt	-	+
7	F, 60	LMS	12	wt	+	
8	F, 56	LMS	?	wt	+	
9	F, 65	LMS	?	NA	+	
10	F, 77	LMS	?	wt	+	
11	F, 49	LMS	9	wt	+	
12	F, 75	LMS	12	wt	+	
13	M, 41	LMS	?	wt	+	
14	M, 74	LMS	?	wt	-	+
15	M, 71	LMS	7.5	wt	+	
16	M, 59	LMS	2	NA	+	
17	F, 69	LMS	3.5	wt	+	
18	M, 65	LMS	?	NA	+	
19	F, 77	LMS	?	NA	+	
20	F, 66	LMS	5	wt	+	

M, male; F, female; LM, leiomyoma; LMS, leiomyosarcoma; ?, unknown; NA, not analyzable; *SDHB-D,* genes *SDHB, SDHC,* and *SDHD*; wt, wild type

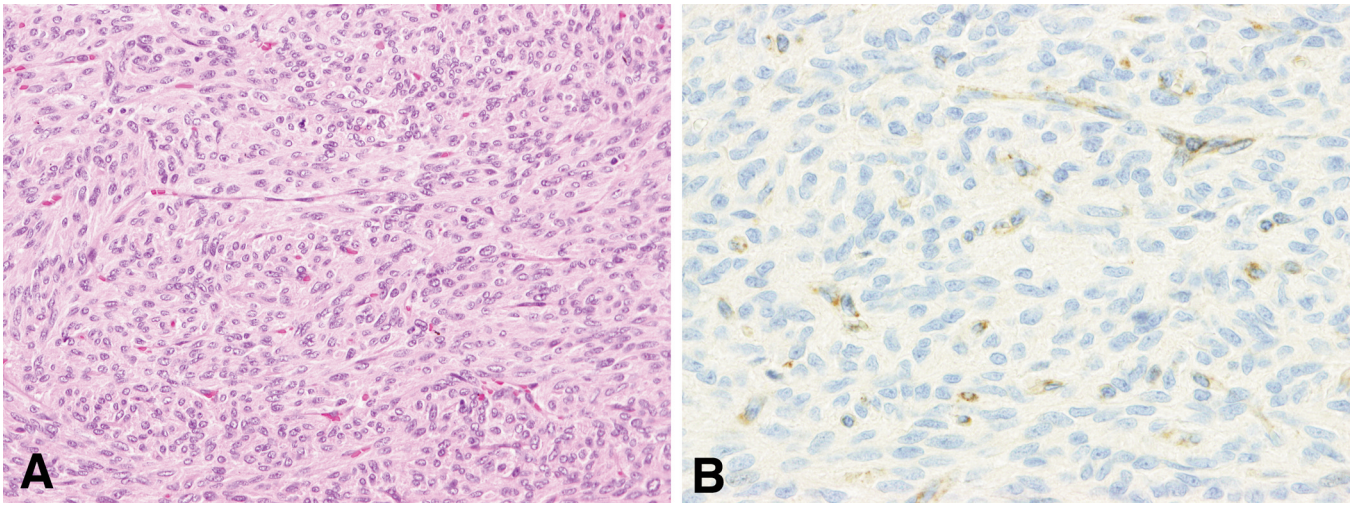

Fig. 2. Gastric pediatric GIST Case 8 displayed mixed cellular morphology (**A**, H&E), with the neoplastic cells showing no SDHB staining in contrast to positive endothelial cells and scattered infiltrating leucocytes (**B**, SDHB Abcam). A, x 100; B, x 200

volume of the cytoplasm of neoplastic cells. DNA quality was sufficient for *SDHB-D* analysis in 18 cases. Of those, 2 showed gene sequence changes, namely G12S change in *SDHD* (Fig. 5) and S163P in *SDHB*. Both cases were SDHB-positive.

Smooth muscle tumors

LMs and LMSs are grouped together in Table 5. Generally, all tumors were SDHB-positive, although usually the staining was difficult to interpret, mainly in slender spindle shaped cells. All samples with sufficient quality of DNA were *SDHB-D* wt.

Synovial sarcomas, endometrial stromal sarcomas, and inflammatory myofibroblastic tumor

Due to the low number of cases, all 5 SSs, 3 ESSs, and 1 IMT are lumped together in Table 6. Briefly, expression of SDHB was difficult to evaluate as all tumors were composed of short spindled cells with diminutive amount of cytoplasm, although on thorough investigation all were finally found to be at least focally SDHB+. All but 1 SS and 1 ESS were successfully tested for *SDHB-D* mutations, with negative results in all tested SSs, ESSs and IMT.

Discussion

The majority of GISTs harbor mutations in *KIT* or *PDGFRA* leading to ligand-independent activation of the respective receptor tyrosine kinases (Hirota et al., 1998, 2003; Heinrich et al., 2003). However, about 15% of GISTs occurring in adults and 90% of GISTs in children lack *KIT* and *PDGFRA* mutations (Corless et al., 2004; Agaram et al., 2008). A considerable number of such cases is associated with SDH complex dysfunction. It is estimated that 7.5% of all GISTs belong to this category, which is characterized by lack of SDHB-immunostaining, and thus referred to as SDHB-deficient GISTs (previously also type 2 GISTs and pediatric type GISTs) (Miettinen et al., 2011). SDHB-deficient GISTs can be further divided according to their clinical and molecular genetic features into several groups. Carney-Stratakis syndrome is a dyad of gastric GIST and paraganglioma inherited in an autosomal dominant trait, caused by a germ-line mutation of *SDHx* (Carney and Stratakis, 2002; Pasini et al., 2008). It affects mainly young people with no sex predilection. On the other hand, Carney triad lacks familial occurrence, shows striking female predominance, and despite its SDHB-

Table 6. Synovial sarcomas, endometrial stromal sarcomas, and inflammatory myofibroblastic tumors.

No.	Sex and age	Diagnosis	Size (cm)	*SDHB-D* status	SDHB Santa Cruz	SDHB Abcam
1	M, 30	biphasic SS	?	wt	+	
2	M, 34	monophasic SS	3	NA	+	
3	M, 41	monophasic SS	?	wt	+	
4	M, 29	biphasic SS	?	wt	-	+
5	M, 32	monophasic SS	7	wt	+	
6	F, 63	ESS	5	wt	+	
7	F, 38	ESS	2	NA	+	
8	F, 61	ESS	8.5	wt	+	
9	F, 7	IMT	?	wt	+	

M, male; F, female; SS, synovial sarcoma; ESS, endometrial stromal sarcoma; IMT, inflammatory myofibroblastic tumor; ?, unknown; NA, not analyzable; *SDHB-D,* genes *SDHB, SDHC,* and *SDHD*; wt, wild type

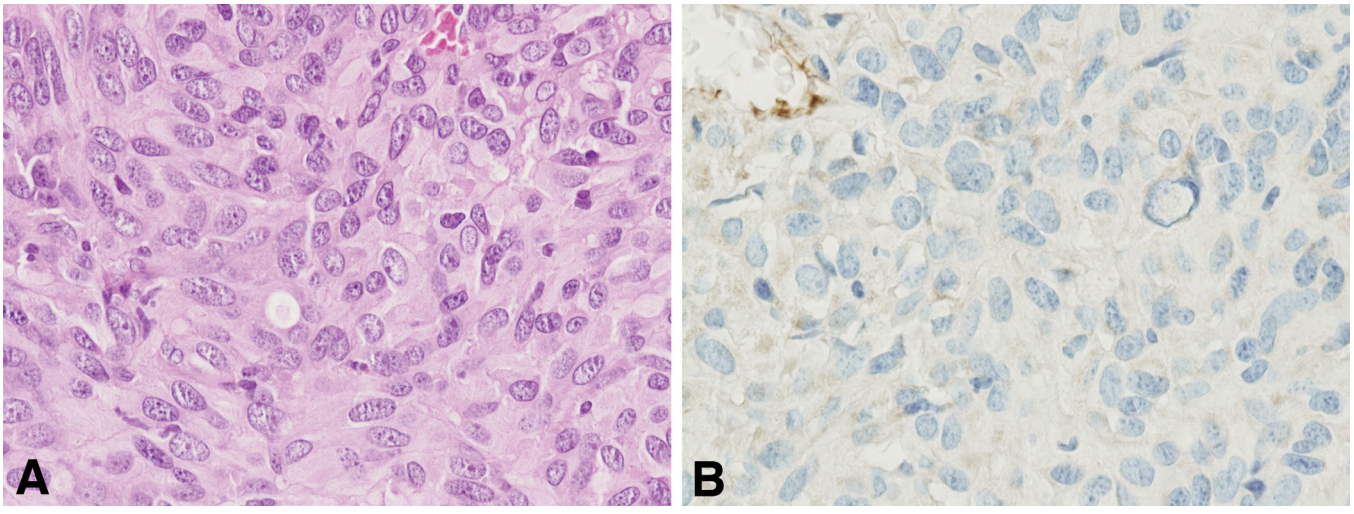

Fig. 3. Mediastinal GIST Case 14, which served as a *KIT*-mutated control case, was composed of epithelioid finely granular oncocyte-like cells (**A**, H&E). The cells showed only faint non-granular SDHB staining much less intensive than in intratumoral endothelial cells or in GIST Case 9 (**B**, SDHB Abcam). x 200

deficiency, all Carney triad-related tumors were reported to be *SDHx* wt (Carney et al., 1977; Carney, 1979, 1983; Matyakhina et al., 2007). The tumors traditionally associated with Carney triad are gastric GIST, pulmonary chondroma and paraganglioma, but esophageal leiomyoma and adrenocortical adenoma may also be related to this syndrome (Carney, 2009). Furthermore, a subset of apparently non-syndromic sporadic *KIT*/*PDGFRA* wt GISTs is also associated with SDH complex dysfunction (Gill et al., 2010, 2011a; Rege et al., 2011). Such tumors occur mainly in children and despite their sporadic nature they are more commonly caused by a germ-line rather than somatic *SDHx* mutation (Janeway et al., 2011; Pantaleo et al., 2011a,b). However, even this category of SDHB-deficient GISTs contains *SDHx* wt tumors, in which the mechanism of their SDH-deficiency remains to be explained (Miettinen et al., 2011; Doyle et al., 2012). Regardless of the exact category of SDHB-deficient GISTs, all such tumors share common clinico-pathological characteristics: gastric localization, epithelioid or mixed cell morphology, multinodular and/or plexiform arrangement, positive KIT immunostaining (but lack of *KIT*/*PDGFRA* mutations), frequent lymph node metastases, and relatively indolent clinical behavior (Gill et al., 2010).

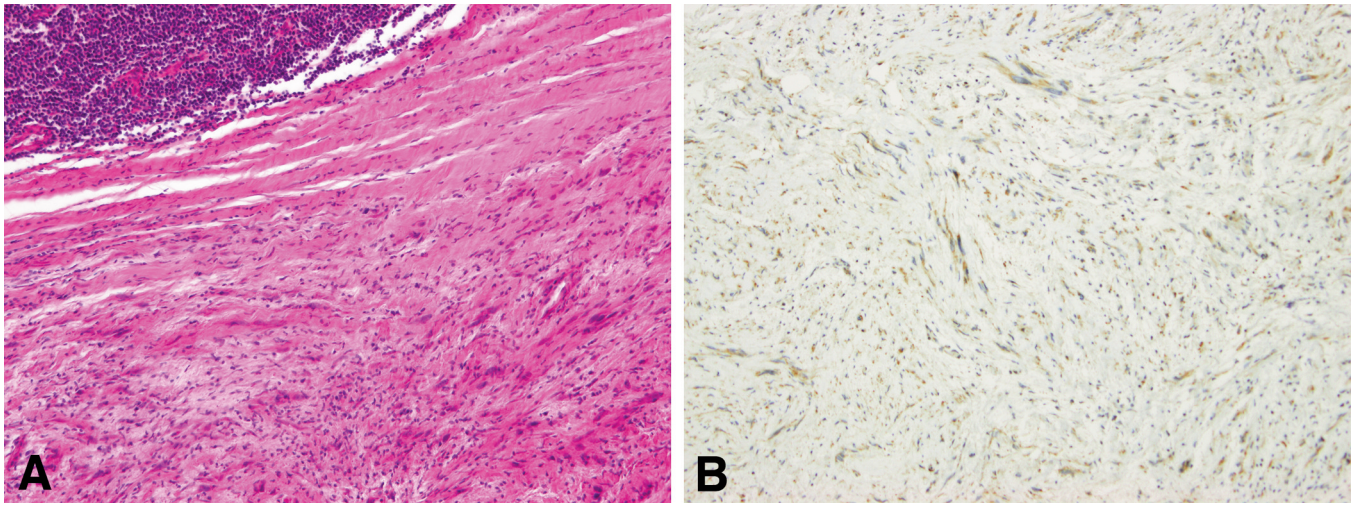

Fig. 4. Gastric schwannoma Case 2 with a peritumoral lymphoid cuff (**A**, H&E) was composed of SDHB-positive spindle shaped cells (**B**, SDHB Santa Cruz). x 100

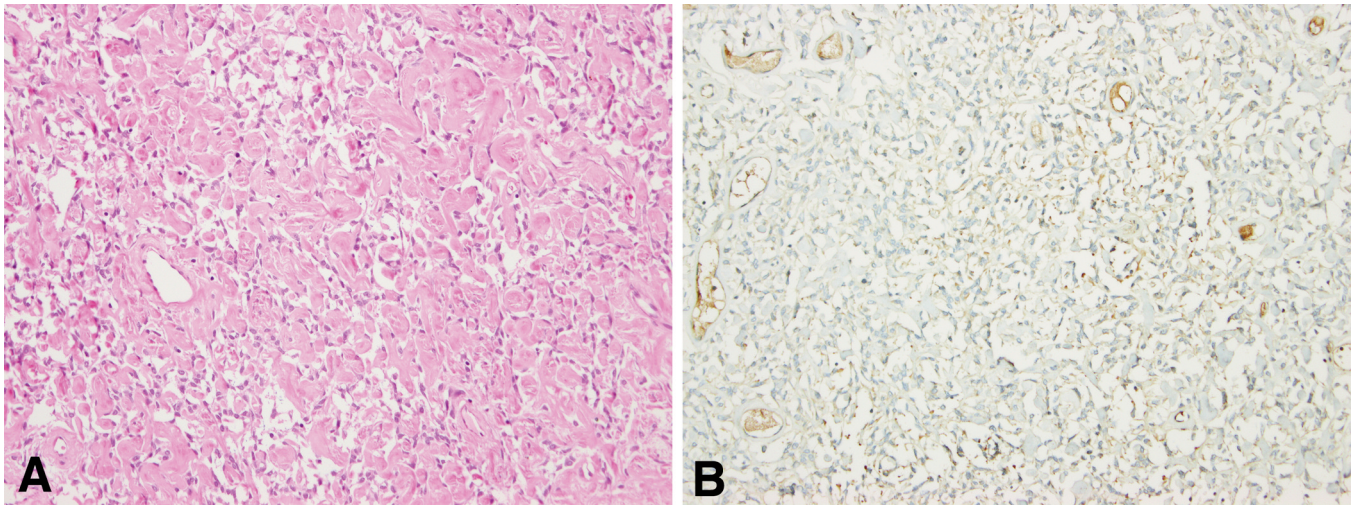

Fig. 5. SFT Case 14 composed of short spindle shaped cells intermingled with ropy collagen bundles (**A**, H&E). The neoplastic cells reacted strongly with anti-SDHB antibody (**B**, SDHB Santa Cruz). x 100

Despite the current focus on SDHB-deficient GIST, it is not the only tumor type associated with SDH complex dysfunction. In addition to paraganglioma/pheochromocytoma, which is also well known to occur in an SDHB-deficient form, either in familial or sporadic setting (Gimm et al., 2000; Amar et al., 2005; Mannelli et al., 2009), several other tumors were recently reported to display signs of SDH dysfunction, namely rare renal cell carcinomas (Vanharanta et al., 2004; Ricketts et al., 2008; Gill et al., 2011b,c), pituitary adenoma (Xekouki et al., 2012), and seminoma (Galera-Ruiz et al., 2008). Besides that, neuroblastoma (Schimke et al., 2010), papillary thyroid carcinoma (Neumann et al., 2004), and renal oncocytoma (Henderson et al., 2009) were suggested to be also possible candidates for SDH-deficient tumors, although with no direct immunohistochemical or molecular genetic support.

As we were unaware of any study on SDHB immunoexpression and/or SDHx mutational analysis of mesenchymal tumors which come into the differential diagnosis of GIST, we collected a short series of the most important GIST mimickers, namely 12 GSs, 20 SFTs, 4 LMs, 16 LMSs, 5 SSs, 3 ESSs, and 1 IMT. For comparison, 11 cases of *KIT*/*PDGFRA* wt GISTs (7 gastric, 4 intestinal) were studied as well. Moreover, 3 recent cases of *KIT* or *PDGFRA* mut GISTs were used as control cases.

Originally, we classified tumors as SDHB-negative if they showed a lack of staining of neoplastic cells by the Santa Cruz antibody despite positive staining of epithelial cells on the mucosal surface. Using this approach, the category of SDHB-negative tumors included: 7 GISTs (3 gastric, 3 intestinal, and 1 mediastinal), 2 GSs, 5 SFTs, 1 LMs, 2 LMSs, and 1 SS. However, on closer inspection in some cases it was found out that intratumoral endothelial cells were also negative. This prompted us to stain the negative cases with monoclonal Abcam antibody. In this setting, endothelial cells were used as internal control. This second round of immunohistochemical investigation reduced the group of SDHB-deficient tumors to 2 GISTs (1 gastric, 1 mediastinal). Others tumors were either re-classified as SDHB-positive or signed out as non-analyzable due to the lack of endothelial staining.

The gastric case, which was SDHB-deficient (GIST Case 8, Fig. 2), occurred in a 12-year old girl with no familial history of a GIST or paraganglioma. At the time of diagnosis, there were no signs of a possible pulmonary chondroma or paraganglioma on record. Molecular genetic analysis ruled out mutations in the hot spots of *KIT, PDGFRA, SDHB, SDHC,* and *SDHD*. According to our current state of knowledge, the tumor should be then ruled out to harbor *SDHA* mutations, either indirectly by SDHA immunohistochemistry or directly by mutational analysis (Pantaleo et al., 2011a,b; Wagner et al., 2013). However, as the issue of SDHA status was beyond the scope of this study (SDHB expression in gastrointestinal mesenchymal tumors), the cost/benefit ratio of such an analysis was found to be unreasonably high.

The other SDHB-deficient tumor was already published as a case report (Daum et al., 2012). Briefly, GIST Case 14 presented as a mass measuring 13.9x7.6x10.4 cm located in the posterior mediastinum of a 52-year-old white man. The tumor consisted of epithelioid cells with abundant finely granular cytoplasm (Fig. 3). Immunohistochemically, the cells were strikingly immunoreactive with CD117 and antimitochondrial antibody. SDHB staining was only focal and faint, without the characteristic granular pattern. Surprisingly, molecular genetic analysis revealed not only p.W557-K558 deletion in exon 11 of the *KIT* gene but also p.Gl2S sequence change in exon 1 of the *SDHD* gene. Although this *SDHD* sequence change is currently of questionable pathogenicity, its prevalence is slightly higher in GISTs or paragangliomas than in the control population (Janeway et al., 2011). Neither existing genetic databases nor current literature provides information on the impact of this sequence change. However, the finely granular appearance of the cytoplasm of the neoplastic cells resembling oncocytes, together with striking contrast between faint immunohistochemical positivity of SDHB and strong granular positivity of antimitochondrial antigen antibody, may mirror pseudohypoxia resulting from partial destabilization of the SDH complex due to the *SDHD* Gl2S sequence change. The incomplete nature of such destabilization might be also responsible for the residual faint focal staining which differs from both typical positive and negative cases. Thus, it is possible that at least some sequence changes in *SDHx* genes and *KIT*/*PDGFRA* mutations are not necessarily mutually exclusive and that they may even cooperate in tumor progression. Nevertheless, the fact that GIST Case 1, which was undoubtedly SDHB-positive, harbored the same *SDHD* sequence, casts doubt on this theory, although the additional effect of several minor factors cannot be ruled out. Furthermore, the striking oncocyte-like appearance was not observed in other *SDHx* sequence change-positive GIST and SFTs.

No other intraabdominal mesenchymal tumor (namely GS, SFT, LM, LMS, SS, ESS, IMT) was found to be SDHB-deficient if strict criteria (negative reaction of neoplastic cells with two antibodies and positive granular intracytoplasmic staining of endothelial cells) were applied. In spite of SDHB-positivity, 2 SFTs revealed SDHB-D sequence changes. SFT Case 9 showed p.S163P of *SDHB*, and SFT Case 14 harbored p.G12S of *SDHD*.

The significance of *SDHB* and *SDHD* sequence changes detected in 2 GISTs and 2 SFTs remains unclear. As 3 of the tumors were SDHB-positive, and 1 GIST harbored concurrent *KIT* mutation, they can hardly be viewed as "real" oncogenic mutations. The non-neoplastic tissue in GIST Case 14 was proven to contain the same gene sequence as the neoplastic cells did, which, in that setting, speaks more for the possibility of polymorphism than for germ-line mutation. But even

this circumstance does not rule out the possible role of detected changes with certainty. Unfortunately, the other *SDHx* sequence change-positive cases were consultation cases and no non-neoplastic tissue was available for analysis. The precise mechanisms by which SDH complex dysfunction leads to tumor formation have not yet been fully elucidated. An important role is believed to be played by succinate accumulation leading to stabilization of HIF1-alpha, overexpression of VEGF (Burnichon et al., 2010), and alteration of DNA methylation profiles (Killian et al., 2013; Mason and Hornick, 2013), resulting in angiogenesis and cell proliferation. Although SDH dysregulation is currently viewed as a consequence of an *SDHx* mutation leading to disruption of the whole complex with resultant loss of SDHB expression, the effects of "immunohistochemically silent" sequence changes have not been sufficiently studied yet. Even those subtle structural changes that do not lead to disruption of the enzyme complex may result in a minor decrease of enzyme activity, which may promote tumor growth initiated by another (e. g. *KIT* or *PDGFRA*) mutation. Although the SDHB- tumors are not driven by gain of function mutations of KIT or PDGFRA, they invariably show immunohistochemical staining for KIT protein, a phenomenon which is currently not fully understood (Miettinen et al., 2011). Our study showed no significant difference in KIT staining between the SDHB-positive and SDHB-deficient GISTs.

In summary, our study found no intraabdominal mesenchymal tumor other than GIST to be SDHB-deficient. However, analysis of SDHB immunoexpression must be performed with caution due to the small amount of cytoplasm in neoplastic cells, mainly in SFTs, smooth muscle tumors, and monophasic SSs. Only intratumoral endothelial cells should be used as internal control because the mucosal epithelium, located usually at the edge of the specimen, may differ in its antigen quality from the more distant neoplastic tissue. In the differential diagnostics, attention also should be paid to other morphological features, as SDHB-deficient GIST should be localized in the stomach, arranged in multinodular/plexiform pattern, composed of epithelioid cells, or mixed in cellular composition. Last but not least, our results raise the suspicion of a possible role of *SDHx* sequence changes of questionable pathogenicity, which may promote tumor growth initiated by another genetic event.

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