

# Site-dependent differential *KIT* and *PDGFRA* expression in gastric and intestinal gastrointestinal stromal tumors

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**In gastrointestinal stromal tumors (GISTs), mutually exclusive gain-of-function mutations of *KIT* and *PDGFRA* are associated with different mutation-dependent clinical behavior. Taking into account the well-known different clinical behavior of GISTs from the stomach or the intestine, the aim of the current study is to evaluate the mutation- and site-dependent effects on mRNA and protein expression of *KIT* and *PDGFRA* in a large series of primary GISTs. Fresh-frozen tissue of 53 primary GISTs from gastric (75%) or intestinal (25%) sites were analyzed for mutation of *KIT* or *PDGFRA* using direct sequencing. Furthermore, *KIT* and *PDGFRA* mRNA and protein expression were determined using quantitative RT-PCR and quantitative densitometric evaluation of Western blot data. Each tumor either had a mutation of *KIT* (79%) or *PDGFRA* (21%). All GISTs with *PDGFRA* mutation were from gastric sites. Mutation-dependently, GISTs with *KIT* mutation had a significantly higher expression of *KIT* and at the same time a significantly lower expression of *PDGFRA* compared to GISTs with *PDGFRA* mutation. Site-dependently, gastric GISTs had a significantly higher expression of *PDGFRA* and a significantly lower expression of *KIT* compared to intestinal GISTs. Additionally, even if the *KIT*-mutated GISTs alone were considered, a significantly higher expression of *PDGFRA* could be observed in gastric than in intestinal tumors. We also found a significant correlation between a higher protein expression of *PDGFRA* and longer disease-free survival. The correlation of gastric site and *PDGFRA* mutation with higher *PDGFRA* expression and longer disease-free survival suggests different regulatory roles of *KIT* and *PDGFRA* gene expression on the control of cell proliferation, and, thereby on clinical behavior. The higher *PDGFRA* expression in gastric GISTs possibly contributes to the well-known site-dependent clinical behavior.**  
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Gain-of-function mutations of the type III receptor tyrosine kinases *KIT* (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) and *PDGFRA* (platelet-derived growth factor receptor,  $\alpha$  polypeptide) have been identified as the central event in the tumorigenesis of gastrointestinal stromal tumors (GISTs).<sup>1,2</sup> The interstitial cells of Cajal located in the muscular layer of the gastrointestinal

tract are regarded as the precursor cells for GISTs,<sup>3</sup> and ligand-independent constitutive receptor tyrosine kinase signaling presumably initiates neoplastic formation in interstitial cells of Cajal through elevated cell proliferation.<sup>4</sup> Mutations of *KIT* and *PDGFRA* have been demonstrated to occur mutually exclusively. About 60–70% of GISTs have activating mutations of *KIT*, whereas mutations of *PDGFRA* are less frequent and can be found in only 10–20% of GISTs.<sup>5–9</sup> GISTs with *KIT* mutation and *PDGFRA* mutation are characterized by distinct histomorphologic phenotypes, and GISTs with *PDGFRA* mutation appear clinically less aggressive.<sup>9–15</sup> Two similar studies analyzing gene expression in GISTs with *KIT* or *PDGFRA* mutation reported that the

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mRNA and protein expression of *KIT* and *PDGFRA* are highly different between these two types of tumors.<sup>16,17</sup> Although both genes were basically expressed in all tumors, the quantitative regulation was different, in that tumors with *KIT* mutation had a higher expression of *KIT*, whereas the tumors with *PDGFRA* mutation had a higher expression of *PDGFRA*. These findings suggest an important impact of the activating mutation on the expression of the mutated gene itself, providing a possible explanation for mutation-dependent differences in clinical behavior.

It has also been observed that GISTs from the intestine generally exhibit a more aggressive clinical course compared to GISTs from the stomach.<sup>18,19</sup> Correspondingly, Antonescu *et al*<sup>20</sup> found an anatomical site-dependent gene expression in GISTs, and *PDGFRA* was among the genes that were expressed higher in the gastric tumors.

The aim of the current study was to determine the mRNA and protein expression of *KIT* and *PDGFRA* in a large series of 53 primary GISTs with mutation of *KIT* or *PDGFRA* from different anatomical sites, to evaluate quantitatively site-dependent differences in the expression levels of *KIT* and *PDGFRA*. Furthermore, we compared the impact of mutated gene, anatomical site and gene expression on the clinical behavior, to determine whether the expression of *KIT* and *PDGFRA* would be a valuable independent factor for the prognosis of GISTs.

## Materials and methods

### Tumor Samples

This study comprises both snap-frozen as well as formalin-fixed and paraffin-embedded tissue samples from 53 primary GISTs. All tumors were removed surgically without other prior treatment. None of the patients (Table 1) had received imatinib mesylate (Gleevec, Novartis, Switzerland) before operation or disease progression. No adjuvant treatment has been carried out. Mitoses were counted in 50 high-power fields (HPFs), while proliferation rate was estimated as the percentage of Mib1-positive nuclei from tumor areas with the highest mitotic activity. Risk of clinically aggressive behavior was evaluated according to the consensus approach published by Fletcher *et al*,<sup>21</sup> and the malignant potential was evaluated according to the proposal of Miettinen *et al*.<sup>22</sup>

### Mutation Analysis of *KIT* and *PDGFRA* Genes

Mutation analysis of *KIT* exons 9, 11, 13 and 17, as well as *PDGFRA* exons 12, 14 and 18 was performed on snap-frozen tumor tissue from all 53 primary GISTs using direct sequencing of PCR products as described previously.<sup>23</sup>

### RNA Isolation and Reverse Transcription

Total RNA was isolated from snap-frozen tumor tissue from all 53 GISTs using ~50 mg frozen tissue per ml TRIzol (Invitrogen Life Technologies, Karlsruhe, Germany) as described previously.<sup>23</sup> Total RNA concentration was quantified with the RNA 6000 nano LabChip using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

### Quantitative PCR

Gene specific primers for *KIT* (5'-ATGTTGTCCCAA CCAAGG-3' and 5'-CTTCTAAGTCTAGGGCCAAC TC-3') and *PDGFRA* (5'-TGTCTGTTGTCATTG GA-3' and 5'-CTTCAACCACCTTCCCAAAC-3') were designed on different exons with a melting temperature of 60°C for PCR products with a length of 114 and 200 nucleotide pairs, respectively. PCR was run in 20 µl reactions in triplicates on an iCycler (Bio-Rad Laboratories GmbH, München, Germany) using the QuantiTect SYBR Green PCR Kit (Qiagen, Hilden, Germany) and gene-specific primers in a final concentration of 300 nM. The temperature profile consisted of (i) an initial step at 95°C for 10 s for *Taq* activation; (ii) 40 cycles at 95°C for 15 s and 60°C for 60 s; and (iii) a final melting curve analysis with a temperature ramp from 60 to 95°C and a heating rate of 3°C/min. PCR efficiencies were calculated with a relative standard curve derived from a cDNA mixture (a twofold dilution series with seven measuring points in triplicates) and gave regression coefficients >0.95 and reproducible primer-specific efficiencies of 85–99%. Gene-specific amplification was confirmed by a single peak in melting curve analysis and a single band in high-resolution agarose gel electrophoresis (SeaKem LE agarose, BMA Rockland, ME, USA). No template controls (no cDNA in PCR) and genomic controls (no enzyme in reverse transcription reaction) were run for each gene to detect unspecific and genomic amplification or primer dimerization. Relative expression levels were calculated from the relative standard curve.<sup>24</sup> The reference genes 18S rRNA and *actin beta* (*ACTB*) could be confirmed to be equivalently expressed within most of the examined groups as described previously.<sup>25</sup> Expression of *KIT* and *PDGFRA* mRNA was calculated in relation to the mean expression of 18S and *ACTB*, and logarithmized to obtain approximately normally distributed data. The fold-change comparisons for the expression of *KIT* and *PDGFRA* between different groups were calculated as the ratio between the means.

### SDS-PAGE and Western Blot Analysis

Total lysates of 49 snap-frozen tumor tissues were separated on 10 or 12% SDS-polyacrylamide gels (SDS-PAGE), and the proteins were electrophoretically

**Table 1** Clinicopathologic data in 53 primary GISTs

Patient no.	Age/gender	Site	Mutation	Tumor diameter (cm)	Mitotic count/50HPFs	DFS (months)	OS (months)
1	45/F	Gastric	<i>PDGFRA</i> D842V	<5	<5	104	NED 104
2	84/M	Gastric	<i>PDGFRA</i> D842V	<5	<5	11	NED 11
3	53/F	Gastric	<i>PDGFRA</i> D842V	<5	<5	9	NED 9
4	61/M	Gastric	<i>PDGFRA</i> Dup 556-565	<5	<5	40	NED 40
5	69/F	Gastric	<i>PDGFRA</i> D842V	<5	<10	19	NED 19
6	66/M	Gastric	<i>PDGFRA</i> D842H, Del 843-846	<5	>10	69	NED 69
7	53/M	Gastric	<i>PDGFRA</i> D842A, Del 843-846	<10	<5	81	NED 81
8	58/M	Gastric	<i>PDGFRA</i> D842V	<10	<5	19	NED 19
9	65/M	Gastric	<i>PDGFRA</i> D842V	<10	<5	64	NED 64
10	66/M	Gastric	<i>PDGFRA</i> D842V	<10	<5	29	NED 29
11	66/M	Gastric	<i>PDGFRA</i> D842V	<10	<5	50	NED 50
12	66/M	Gastric	<i>KIT</i> Del 553-558	<5	<5	12	NED 12
13	77/F	Gastric	<i>KIT</i> Del 557	<5	<5	9	DOO 9
14	78/F	Gastric	<i>KIT</i> Del 560	<5	<5	14	NED 14
15	52/M	Gastric	<i>KIT</i> K550L, Del551-556	<5	<5	28	NED 28
16	80/F	Gastric	<i>KIT</i> V559D	<5	<5	62	NED 62
17	69/M	Gastric	<i>KIT</i> V559D	<5	<5	8	NED 8
18	73/M	Gastric	<i>KIT</i> V560D	<5	<5	90	NED 90
19	79/F	Gastric	<i>KIT</i> V560D	<5	<5	58	DOO 58
20	52/M	Gastric	<i>KIT</i> W557E, Del 558	<5	<5	26	NED 26
21	86/F	Gastric	<i>KIT</i> W557R	<5	<5	28	NED 28
22	82/F	Gastric	<i>KIT</i> Del 557-558	<5	>10	57	NED 57
23	72/F	Gastric	<i>KIT</i> Del 558-562	<5	>10	72	NED 72
24	53/M	Gastric	<i>KIT</i> Del 557-558	<10	<5	18	NED 18
25	69/M	Gastric	<i>KIT</i> Del 557-558	<10	<5	11	NED 11
26	67/M	Gastric	<i>KIT</i> Dup 577-580	<10	<5	51	NED 51
27	70/F	Gastric	<i>KIT</i> V559D	<10	<5	112	NED 112
28	47/M	Gastric	<i>KIT</i> V559G	<10	<5	78	NED 78
29	85/F	Gastric	<i>KIT</i> V560D	<10	<5	35	NED 35
30	54/M	Gastric	<i>KIT</i> V560D	<10	<5	0	AWD 14
31	64/F	Gastric	<i>KIT</i> Del 552-555	<10	<10	92	NED 92
32	64/M	Gastric	<i>KIT</i> Del 555-559	<10	>10	39	NED 39
33	58/M	Gastric	<i>KIT</i> Del 557-558	<10	>10	34	AWD 72
34	72/M	Gastric	<i>KIT</i> Dup 502-503	<10	>10	25	NED 25
35	49/F	Gastric	<i>KIT</i> Dup 571-579	>10	<5	1	AWD 1
36	78/F	Gastric	<i>KIT</i> Dup 502-503	>10	<10	65	NED 65
37	68/M	Gastric	<i>KIT</i> Del 550-558 <sup>a</sup>	>10	>10	38	NED 38
38	63/M	Gastric	<i>KIT</i> K558R, Del 559-565	>10	>10	17	AWD 54
39	64/M	Gastric	<i>KIT</i> V559D	>10	>10	84	AWD 111
40	61/F	Gastric	<i>KIT</i> W557C, Del 558-559	>10	>10	23	AWD 78
41	68/F	Intestinal	<i>KIT</i> Del 555-556	<5	<5	21	NED 21
42	54/F	Intestinal	<i>KIT</i> Del 557-558	<10	<5	19	NED 19
43	82/M	Intestinal	<i>KIT</i> V559D	<10	<5	42	NED 42
44	54/M	Intestinal	<i>KIT</i> V559G	<10	<5	62	NED 62
45	74/F	Intestinal	<i>KIT</i> Dup 502-503	<10	<10	13	NED 13
46	67/M	Intestinal	<i>KIT</i> K558N, Del 559 (hemizygot)	<10	<10	41	AWD 46
47	56/M	Intestinal	<i>KIT</i> Del 556-558	<10	>10	0	DOTD 19
48	62/F	Intestinal	<i>KIT</i> Del 557-558	<10	>10	0	AWD 47
49	54/F	Intestinal	<i>KIT</i> Del 559-561	<10	>10	0	AWD 36
50	43/M	Intestinal	<i>KIT</i> K558N, Del 559	<10	>10	13	AWD 76
51	61/M	Intestinal	<i>KIT</i> V559D	<10	>10	19	AWD 95
52	73/M	Intestinal	<i>KIT</i> W557G	<10	>10	0	AWD 26
53	57/M	Intestinal	<i>KIT</i> V560E	>10	<5	86	NED 86

AWD, alive with disease; DFS, disease-free survival; DOO, died of other causes; DOTD, died of tumor disease; F, female; GIST, gastrointestinal stromal tumor; M, male; NED, no evidence of disease; OS, overall survival.

<sup>a</sup>additional *KIT* exon 9 del C1461 in codon 480.

transferred to nitrocellulose filters. Uniform blotting was checked by staining the nitrocellulose filter with Ponceau S (Sigma, Taufkirchen, Germany). Blots were cut horizontally at the 72-kDa marker band. The upper share of the blots were probed with anti-*KIT* antibody (no. 3392; Cell Signalling

Technology, New England Biolabs, Frankfurt, Germany) or anti-*PDGFRA* antibody (no. 3164; Cell Signalling Technology), and the lower share with an anti- $\beta$ -actin antibody (no. 4967; Cell Signalling Technology) in a 1:1000 dilution. The immunoreactive proteins were visualized using the

chemiluminescence ECL plus detection system (Amersham Bioscience, Freiburg, Germany) after incubation with a horseradish peroxidase-conjugated goat anti-rabbit antibody (no. A0545; Sigma). Stripping of blotting membranes for reprobing was carried out by incubation of the membranes in stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl, pH 6.7) for 20 min at 60°C with occasional agitation. Bands on the ECL film were quantified using the 1Dscan Ex gel analysis software (Scanalytics, Rockville, MD, USA). The different Western blots were adjusted by the presence of one calibrator sample on every single gel. The raw densitometric intensities for *KIT* and *PDGFRA* varied between 2 and 15 000 for the different tumor samples. In those cases where the protein amount was under the detection limit, a value of 1 was assigned. Relative protein amounts of *KIT* and *PDGFRA* were calculated in relation to  $\beta$ -actin, and logarithmized to obtain approximately normally distributed data. The fold-change comparisons for the expression of *KIT* and *PDGFRA* between different groups were calculated as the ratio between the means.

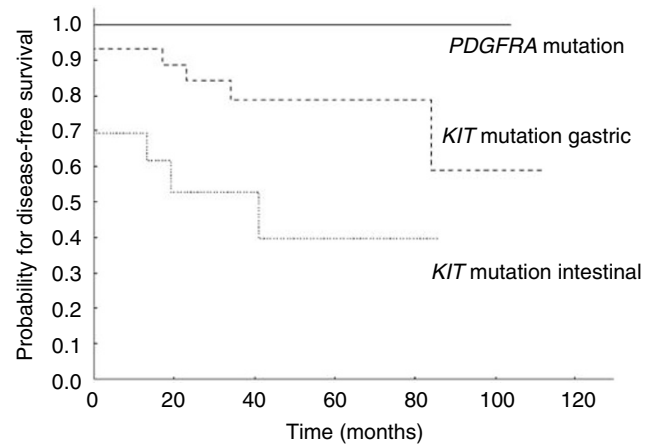
### Statistics

Descriptive statistics, tests and graphs were performed with Statistica 6.0 (StatSoft, Hamburg, Germany), and the statistical software system R.<sup>26</sup> Associations between the clinicopathological and the molecular genetic parameters were evaluated using the Wilcoxon test or the Fisher test in the case of categorical variables. Disease-free survival rates were plotted by the Kaplan–Meier method. Associations of patient and tumor parameters with disease-free survival times were assessed using the log-rank test.

## Results

### Clinicopathologic Analysis and Disease-Free Survival

The current study comprised 53 primary GISTs, including 40 (75%) from gastric and 13 (25%) from intestinal sites (Table 1). Mutation analysis revealed *KIT* mutation in 42 (79%) cases and *PDGFRA* mutation in 11 (21%) cases. No cases were observed with both *KIT* and *PDGFRA* mutation. All 11 cases with *PDGFRA* mutation were from gastric sites, while 29 (69%) and 13 (31%) of the cases with *KIT* mutation were from gastric and intestinal sites, respectively ( $P=0.03$ ). Six (11%) patients had synchronous metastatic disease at the time of operation. Seven (13%) patients developed metachronous progressive disease after a mean of 33 months (range: 13–84 months). Forty (75%) patients had no disease progression after a mean follow-up of 44 months (range: 8–112 months). Patients with gastric tumors had significantly longer disease-free



**Figure 1** Mutation- and site-dependent differences in disease-free survival. *PDGFRA*-mutated GISTs were exclusively from gastric sites, and none of these patients had tumor progress. Patients with gastric *KIT*-mutated tumors had significantly longer disease-free survival compared to patients with intestinal *KIT*-mutated tumors ( $P=0.03$ ). There was no statistically significant difference in disease-free survival for patients with *PDGFRA*-mutated tumors and gastric *KIT*-mutated tumors.

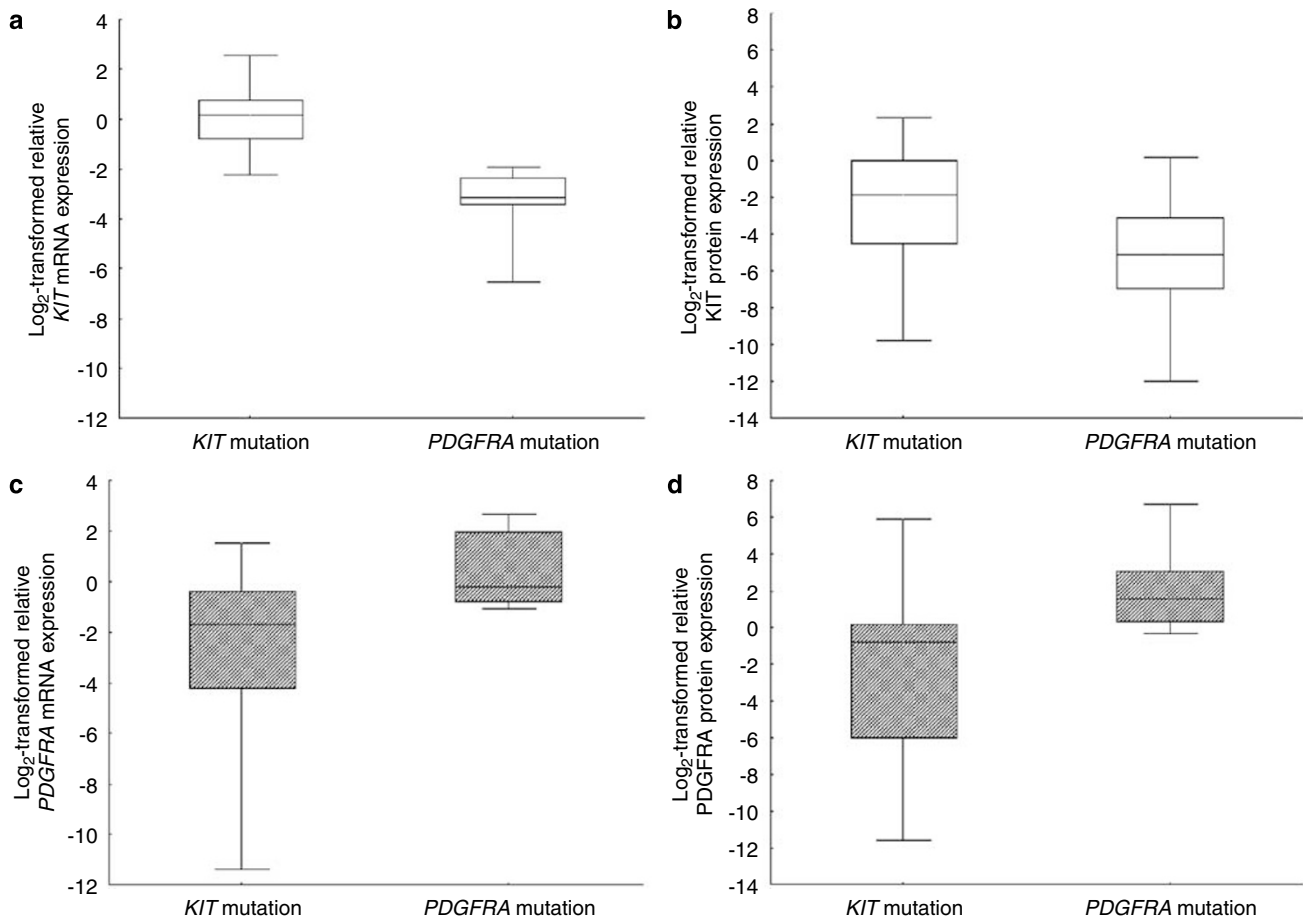
survival than patients with intestinal tumors ( $P=0.004$ ), and patients with *PDGFRA*-mutated tumors had a significantly longer disease-free survival than patients with *KIT*-mutated tumors ( $P=0.04$ ). Additionally, *KIT*-mutated tumors from gastric sites were associated with significantly longer disease-free survival compared to *KIT*-mutated tumors from intestinal sites ( $P=0.03$ , Figure 1). On the other hand, there was no significant difference in disease-free survival when comparing gastric tumors with *PDGFRA* and *KIT* mutation.

### Mutation-Dependent Expression of *KIT* and *PDGFRA*

The mean *KIT* expression was 10.3-fold higher on the mRNA level ( $P=6 \times 10^{-7}$ ) and 7.5-fold higher on the protein level ( $P=0.03$ ) in both gastric and intestinal GISTs with *KIT* mutation compared to GISTs with *PDGFRA* mutation (Figure 2a and b). On the other hand, the mean *PDGFRA* expression was 7.3-fold lower on the mRNA level ( $P=5 \times 10^{-4}$ ) and 25.7-fold lower on the protein level ( $P=0.001$ ) in GISTs with *KIT* mutation (Figure 2c and d).

### Site-Dependent Expression of *KIT* and *PDGFRA*

In addition to the mutation-dependent expression of *KIT* and *PDGFRA*, the expression of these two genes was also site-dependent. The mean *PDGFRA* expression was significantly higher in gastric GISTs compared to their intestinal counterparts (8.2-fold higher on the mRNA level,  $P=8 \times 10^{-4}$ ; and 86.2-fold higher on the protein level,  $P=3 \times 10^{-5}$ , Figure 3a and b). In contrast, the mean *KIT* expression was significantly lower in gastric GISTs (2.6-fold lower



**Figure 2** Mutation-dependent mRNA and protein expression of *KIT* and *PDGFRA*. (a) *KIT* mRNA expression; (b) *KIT* protein expression; (c) *PDGFRA* mRNA expression; and (d) *PDGFRA* protein expression. *KIT* mRNA and protein expression (open box) were significantly higher in GISTs with *KIT* mutation. *PDGFRA* mRNA and protein expression (hatched box) were significantly higher in GISTs with *PDGFRA* mutation. Horizontal line, median; inner box, 25 and 75% interval; outer spread, minimum and maximum.

on the mRNA level,  $P=0.02$ ; and 5.3-fold lower on the protein level,  $P=0.006$ , Figure 3c and d).

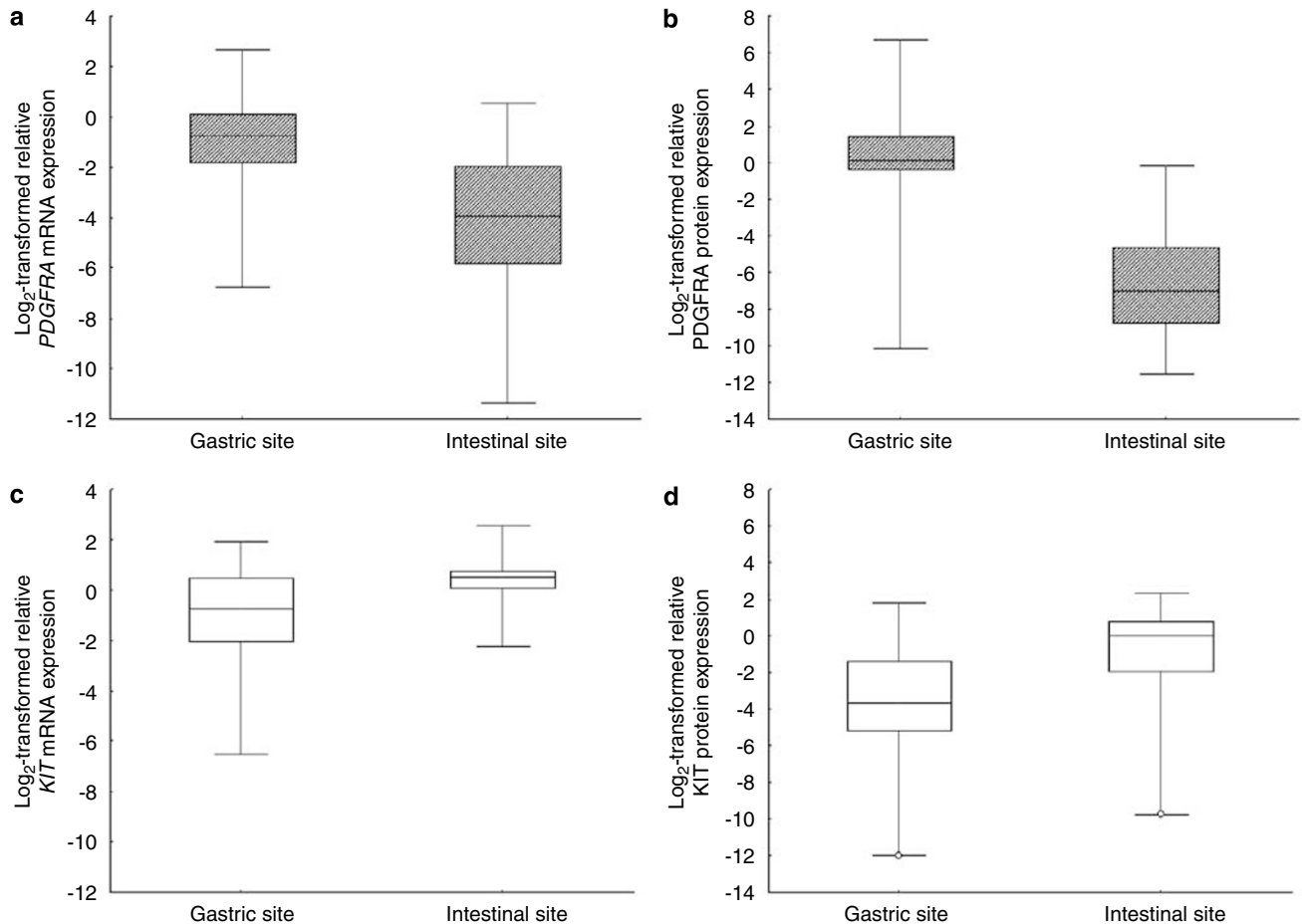
This mutation-independent but site-dependent difference was even more obvious when only *KIT*-mutated GISTs from gastric and intestinal sites were compared. Gastric GISTs with *KIT* mutation expressed *PDGFRA* 5.5-fold higher on the mRNA level ( $P=0.008$ ) and 52.2-fold higher on the protein level ( $P=2 \times 10^{-4}$ ) compared to their intestinal counterparts with *KIT* mutation (Figure 4a and b). On the other hand, although the mean mRNA expression of *KIT* was not significantly different between gastric and intestinal GISTs with *KIT* mutation (Figure 4c), the mean protein expression of *KIT* was 3.7-fold lower in gastric GISTs with *KIT* mutation compared to intestinal GISTs with *KIT* mutation ( $P=0.02$ , Figure 4d). The comparison of mutation-dependent and site-dependent regulation of *KIT* and *PDGFRA* expression revealed that the regulation of *PDGFRA* expression was equally mutation- and site-dependent, whereas the regulation of *KIT* expression was rather mutation-dependent (Figure 4).

### Expression of *PDGFRA* and Disease-Free Survival

A significantly higher mean expression of *PDGFRA* was detected in the 40 GISTs without tumor progress than in the 13 GISTs with tumor progress (2.8-fold higher on the mRNA level,  $P=0.06$  and 27.3-fold higher on the protein level,  $P=0.002$ ). Correspondingly, a two-sided group-wise comparison of tumors with high vs low *PDGFRA* protein expression separated by the mean revealed that higher *PDGFRA* protein expression significantly correlated with longer disease-free survival ( $P=0.01$ , Figure 5).

### Discussion

Apart from the previously described mutation-dependent expression of *KIT* and *PDGFRA*,<sup>16,17</sup> we report here on a site-dependent expression of *KIT* and *PDGFRA*. Gastric GISTs had a 8.2-fold higher mRNA expression and a 86.2-fold higher protein expression of *PDGFRA* compared to GISTs from the

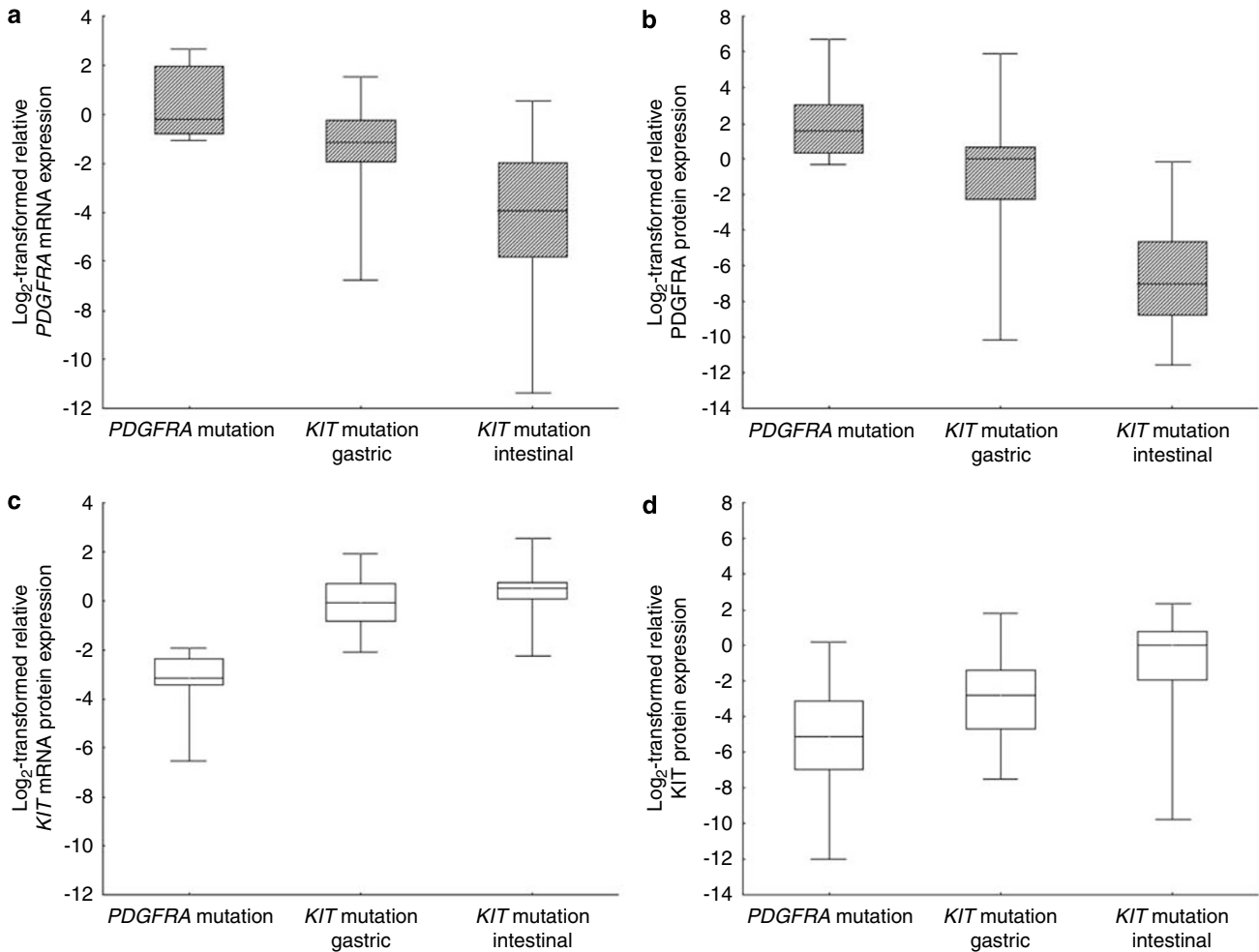


**Figure 3** Site-dependent mRNA and protein expression of *KIT* and *PDGFRA*. (a) *PDGFRA* mRNA expression; (b) *PDGFRA* protein expression; (c) *KIT* mRNA expression; and (d) *KIT* protein expression. *PDGFRA* mRNA and protein expression (hatched box) were significantly higher in GISTs from gastric sites. *KIT* mRNA and protein expression (open box) were significantly higher in GISTs from intestinal sites. Horizontal line, median; inner box, 25 and 75% interval; outer spread, minimum and maximum.

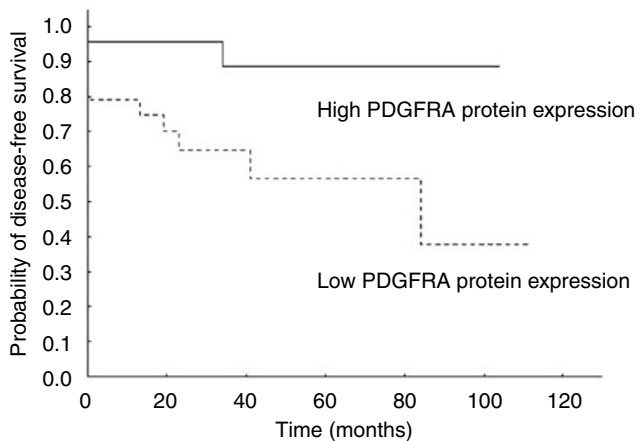
intestine. Furthermore, gastric GISTs had a 2.6-fold lower mRNA expression and a 5.3-fold lower protein expression of *KIT* compared to intestinal GISTs. As *PDGFRA* mutations were found exclusively in gastric GISTs, we performed a subgroup analysis among *KIT*-mutated GISTs to preclude a mutation-dependent bias. This analysis confirmed a further mutation-independent correlation between higher *KIT* expression and intestinal tumor site, and higher *PDGFRA* expression and gastric tumor site. Gastric GISTs with *KIT* mutation had a 5.5-fold higher mRNA expression and a 52.2-fold higher protein expression of *PDGFRA* compared to intestinal GISTs with *KIT* mutation. On the other hand, intestinal GISTs had a 3.7-fold higher protein expression of *KIT*. Therefore, a site-dependent constitutional difference in the expression of *KIT* and *PDGFRA* exists and is maintained irrespective of the type of activating mutation that may occur.

This new finding of a different site-dependent expression of *KIT* and *PDGFRA* is of further relevance for understanding the site-dependent differences in the clinical behavior of GISTs. Tumor

site is an independent prognostic parameter in GISTs, with intestinal GISTs being more clinically aggressive compared to gastric GISTs.<sup>18,19</sup> Although this clinical observation has been correlated with distinct gene expression profiles between gastric and intestinal GISTs,<sup>20</sup> it remains unclear whether this difference is already determined before neoplastic transformation, or is the consequence of a discrete genetic evolution of gastric and intestinal GISTs. GIST tumor cells are dependent on oncogenic *KIT* or *PDGFRA* signaling, permitting increased ligand-independent cell proliferation.<sup>4</sup> Although secondary genetic events are doubtless involved in acceleration of malignant potential in GISTs, it is likely that the simple expression and activation level of *KIT* and *PDGFRA* at least initially controls the intensity of intracellular signal transduction, and, through the balance between cell proliferation and cell survival, the biological tumor behavior. Whereas the previously described mutation-dependent regulation of *KIT* and *PDGFRA* expression might account for the less aggressive clinical behavior of GISTs with *PDGFRA* mutation,<sup>11</sup> the



**Figure 4** Comparison of mutation- and site-dependent effects on mRNA and protein expression of *KIT* and *PDGFRA*. (a) *PDGFRA* mRNA expression; (b) *PDGFRA* protein expression; (c) *KIT* mRNA expression; and (d) *KIT* protein expression. *PDGFRA* mRNA and protein expression (hatched box) were equally mutation- and site-dependent. *KIT* mRNA expression (open box) was strongly mutation-dependent, but only slightly site-dependent. Horizontal line, median; inner box, 25 and 75% interval; outer spread, minimum and maximum.



**Figure 5** Expression of *PDGFRA* and disease-free survival. A two-sided group-wise comparison of tumors with high (continuous line) vs low (dotted line) *PDGFRA* protein expression separated by the mean revealed that higher *PDGFRA* protein expression significantly correlated with longer disease-free survival ( $P=0.01$ ).

current finding of a site-dependent regulation of *KIT* and *PDGFRA* expression provides a reason for the less aggressive clinical behavior of gastric GISTs.<sup>18</sup>

The current study cannot clarify whether *KIT* and *PDGFRA* influence each others' expression reciprocally or are regulated independently. Assuming the latter model, our finding would argue for a derivation of gastric and intestinal GISTs from distinct types of precursor cells, as has already been formulated by Nishitani *et al.*<sup>27</sup> To date, the interstitial cells of Cajal located in the muscular layer of the gastrointestinal tract are regarded as the precursor cell for GISTs. Light and electron microscopy studies have demonstrated that there are morphologically distinguishable types of interstitial cells of Cajal with a site-specific distribution in the gastrointestinal tract.<sup>28</sup> An immunohistochemical analysis revealed that not all interstitial cells of Cajal in the human gut expressed *KIT*.<sup>29</sup> The same authors reported that blocking of *KIT* signaling in newborn mice resulted in the almost complete disappearance

of interstitial cells of Cajal, while the remaining undifferentiated precursor cells developed an ultrastructural smooth muscle cell phenotype.<sup>30</sup> Thus a subset of interstitial cells of Cajal seems to be dependent on *KIT* signaling for the maintenance of their specific phenotype, while the role of *PDGFRA* signaling in these cells remains to be investigated. Taken together, a dependency of different interstitial cells of Cajal in the human gut on *KIT* or *PDGFRA* expression and signaling for maintenance of functionality is likely, and would explain the observed site-dependent differences in the expression of *KIT* and *PDGFRA*.

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## Disclosure/conflict of interest

The authors state that there is no conflict of interest.

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