Molecular genetic features and risk assessment in a series of 30 patients who underwent an operation for gastrointestinal stromal tumours

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Background: The objective of the study was to investigate the relationship between molecular genetic features and the standard criteria of risk assessment in patients affected by gastrointestinal stromal tumours (GISTs).
Methods: A review was conducted of a series of 30 patients, with a mean age of 67 years, who underwent surgery for primary GISTs. R0 resection was accomplished in 27 patients. CD117, CD34 desmin, vimentin, S-100 and smooth muscle actin were immunohistochemically tested to achieve a diagnosis of GIST. The loss of wild-type KIT or platelet-derived growth factor receptor alpha (PDGFRα) genes was investigated by sequencing the tumour DNA.
Results: Tumour genes mutations were reported in 23 patients (77%), and wild-type in seven. Mutations on the KIT gene occurred in 18 patients, and mutations on the PDGFRα gene in five. The average sizes of the GIST were 8.7 cm, 5.4 cm and 5.9 cm for KIT gene-mutated, PDGFRα gene-mutated and wild-type tumours, respectively. KIT gene mutations were detected in 50% of gastric and in 70% of extragastric GISTs. Moreover, 70% of tumours with a mitotic rate ≥ 5 x 50 high-power fields (HPFs) underwent KIT gene mutations. Conversely, PDGFRα mutations were observed only in gastric GISTs with a mitotic rate ≤ 5 x 50 HPFs. By stratifying GISTs according to classes of risk, KIT mutation was shown in most of the high-risk tumours. PDGFRα mutations occurred exclusively in lower classes of risk.
Conclusion: Molecular analysis data might have a role as a prognostic variable in models of risk assessment for patients with GISTs.

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Gastrointestinal stromal tumours (GISTs) are the most common nonepithelial neoplasm of the gastrointestinal tract. Because GISTs share CD117 positivity with the interstitial cells of Cajal, they were assumed to originate from that cell type.[1] GISTs almost uniformly express the tyrosine kinase receptor, KIT, which is encoded by the proto-oncogene KIT, located on chromosome 4q11–12.[2] Activation of KIT by its ligand leads to the activation of networks of signal transduction pathways which control cell proliferation, survival, apoptosis and other cell functions.[3] Tyrosine kinase receptor [platelet-derived growth factor receptor alpha (PDGFRα)] mutations have been documented in a small group of GISTs. Mutually exclusive gain-of-function KIT or PDGFRα mutations are central events in the pathogenesis of GISTs.[4,5] So far, predictive factors for the prognosis of patients with GISTs are mainly based on the macroscopic and microscopic features of the tumours.[6] The predictive value of the gene mutations is still unclear. The aims of the study were to analyse the relationship between the expression of KIT and PDGFRα mutations and the phenotype of the GISTs in a series of resected patients, and to explore the potential role of gene mutations as an adjunctive prognostic factor.

Method

Patient population

The study included 30 patients who underwent an operation for a GIST. There were 15 women and 15 men, with a mean age of 67 years, and an age range of 16–90 years. The mean size of the tumours was 8 cm in diameter, with a range of 0.5–21.0 cm. The tumours were located in the stomach (n = 16), small bowel (n = 9), duodenum (n = 1), left colon (n = 1), peritoneum (n = 2) and spleen (n = 1). The GISTs were discovered incidentally in 18 patients during diagnostic imaging studies or surgeries performed for other reasons. Acute tumours were diagnosed in 12 patients because of a life-threatening condition (gastrointestinal bleeding,

bowel obstruction or perforation). On presentation, three patients exhibited hepatic metastasis. Peritoneal and omental metastases were reported in two patients, and there was tumour involvment in both the liver and peritoneum in one patient.

Histology and immunohistochemistry

The specimens were examined by using light microscope morphology analysis after haematoxylin-eosin staining. The diagnosis of GIST was confirmed by immunohistochemical investigations, including antibody tests for CD34, CD117, vimentin, desmin, smooth muscle actin (SMA) and S-100 tumour markers.

Molecular biology

The molecular profiles were screened to indicate genetic mutations. Exons 9, 11, 13 and 17 of KIT, and exons 12 and 18 of PDGFRα, were evaluated by PCR and the Sanger sequencing method for tumour DNA. Molecular analysis of the tumour samples was performed by DNA extraction from paraffin-embedded tissue, the amplification of the DNA by PCR, direct genomic sequencing according to Sanger's procedure, and data examination. The genomic sequencing was performed by using Genetic Analyzer® 3130, with Sequencing Analysis® Software, version 5.2.

Risk assessment

The risk assessment of the tumours was evaluated by applying the criteria of Miettinen and Lasota/ Armed Forces Institute of Pathology criteria. Patients were divided into four prognostic groups (very low, low, intermediate and high risk) by tumour size, location and mitotic rate as variables in order to evaluate the likelihood of GIST malignant behaviour (Table 1).[6]

Results

Patients received laparoscopic or open surgery, depending on the tumour size, primary location of the GIST and the presence of metastases (Table 2). R0 resection was achieved in 27 patients (90%), but three of them required a hepatic resection to remove liver metastases. Only a gross resection of the primary malignancy was obtained (R2) in the remaining three patients because there was extensive spread of the tumour to the peritoneal cavity. Extended node dissection is not indicated in surgery for GISTs, and it was never performed. Postoperative mortality did not occur. Morbidity was reported in three patients [pulmonary embolism (n = 1), perihepatic abscess treated with percutaneous ultrasound-guided drainage (n = 1) and postoperative hemorrhage requiring relaparotomy (n = 1)].

Morphologically, the tumours displayed a spindle cell type (n = 13), epitheloid type (n = 10) and mixed populations cells (n = 7). CD117 expression was immunohistochemically documented in 100% of the GISTs, and CD34 in 24 patients (80%). Other markers, such as vimentin, desmin, SMA and S-100, were detected in a sporadic manner. The mitotic rate of the tumours was ≥ 5/50 high-power fields (HPFs) in 10 patients, and ≤ 5/50 HPFs in 16. There was no detectable mitotic activity in the tumours of four patients.

Twenty-three tumours (77%) proved to be affected by KIT (n = 18) or PDGFRα mutations (n = 5), while seven tumours were wild type. Exon 11 was involved in 15 cases, and exon 9 and exon 17 in one case each, in the GISTs with KIT mutation. Both exon 9 and exon 17 were involved in a further case of KIT mutation. There was exon 18 involvement in all five GISTs that harboured PDGFRα mutations.

The average diameter of the tumours carrying mutations in KIT was 8.7 cm, compared to 5.4 and 5.9 cm for tumours carrying PDGFRα mutations and the wild type, respectively. KIT gene mutations were shown in nine of the 10 patients with a tumour exceeding 10 cm in diameter. There was a mutatated KIT gene in 50% of the gastric GISTs, and in ≥ 70% of the bowel and extravisceral GISTs. KIT gene mutations were found in seven of the 10 tumours with a mitotic rate ≥ 5/50 HPFs (70%), 10 of 16 with a mitotic rate ≤ 5/50 HPFs (63%), and one of four tumours with no mitotic activity (25%). In

Table 1: Risk assessment of gastrointestinal stromal tumours, using Miettinen and Lasota/Armed Forces Institute of Pathology criteria[6]

Risk class	Features
Very low, if any, malignant potential	≤ 2 cm and ≤ 5 (mitotic index)*
Low malignant potential	Gastric: ≥ 2 cm and ≤ 10 cm, and ≤ 5 (mitotic index) ≤ 2 cm and ≥ 5 (mitotic index) Intestinal: ≥ 2 cm and ≤ 5 cm, and ≤ 5 (mitotic index)
Intermediate malignant potential	Gastric: ≥ 10 cm and ≤ 5 (mitotic index) ≥ 2 cm and ≤ 5 cm, and ≥ 5 (mitotic index) Intestinal: ≥ 5 cm and ≤ 10 cm, and ≤ 5 (mitotic index)
High malignant potential	Gastric: ≥ 5 cm and ≥ 5 (mitotic index) Intestinal: ≥ 10 cm or ≤ 5 (mitotic index)

*: Mitotic index = number of mitoses per 50 high-power fields

Table 2: Primary location and surgeries in patients with gastrointestinal stromal tumours

Primary GIST location	Surgery	Cases (n = 30)
Stomach (n = 16)	Wedge resection	8
	Partial gastrectomy	5
	Partial gastrectomy plus hepatic resection	1
	Total gastrectomy	1
	Total gastrectomy plus hepatic resection	1
Small bowel (n = 10)	Jejunoileal resection	6
	Jejunoileal resection plus hepatic resection	1
	Wedge resection	2
	Ileocolic resection	1
Other (n = 4)	Peritonectomy plus small bowel resection	2
	Left colectomy	1
	Splenectomy	1

GIST: gastrointestinal stromal tumour

Table 3: The clinicopathological features of patients with gastrointestinal stromal tumours (*n* = 30)

Mutation	Patients, *n* (%)	Primary location	Points	Tumour size (cm)	Points	Mitotic index	Points
None	7 (23)	Stomach	3	≤ 2	1		
		Small bowel	3	2–5	3	≤ 5 x 50 HPFs	4
		Peritoneum	1	5–10	1	≥ 5 x 50 HPFs	3
				≥ 10	2		
c-KIT	18 (60)	Stomach	8	≤ 2	1		
		Small bowel	7	2–5	6	≤ 5 x 50 HPFs	11
		Peritoneum	1	5–10	3	≥ 5 x 50 HPFs	7
		Spleen	1	≥ 10	8		
		Large bowel	1				
PDGFRα	5 (17)	Stomach	5	≤ 2	1		
				2–5	1	≤ 5 x 50 HPFs	5
				5–10	3	≥ 5 x 50 HPFs	0
				≥ 10	0		

HPFs: high-power fields, PDGFR: platelet-derived growth factor receptor alpha

addition, four of the six tumours that metastasised expressed KIT mutations. The five cases with mutation of the PDGFRα gene were all located in the stomach, and had a mitotic rate ≤ 5/50 HPFs. The allocation of tumours on the basis of the risk assessment criteria for GISTs by Miettinen and Lasota is shown in Table 3, together with the molecular analysis.[6] Accordingly, nine patients were classified as having very low-, five as low-, five as intermediate-, and 11 as high-risk tumours (Table 4). High- and intermediate- risk patients were given target therapy with imatinib mesylate or sunitinib following an oncological consultation.

Discussion

GISTs are the most common mesenchymal tumours of the gastrointestinal tract. Since they were recognised as a distinct neoplasm from myogenic and neurogenic tumours, many studies have focused on classification criteria to subdivide this heterogeneous tumour group into benign and malignant GISTs. Since the introduction of the therapeutic armamentarium of

Table 4: The distribution of c-KIT and PDGFRα mutations in four risk classes, according to Miettinen and Lasota[6]

Risk class	Wild type	c-KIT	PDGFRα	Total
Very low	3	4	2	9
Low	0	2	3	5
Moderate	1	4	0	5
High	3	8	0	11
Total	**7**	**18**	**5**	**30**

PDGFRα: platelet-derived growth factor receptor alpha

imatinib (a tyrosine kinase inibitor which blocks the kinase activity of KIT and PDGFRα genes), the risk assessment of GISTs has become increasingly important. Selected patients with resectable primary GISTs may be offered imatinib treatment after surgery, but it has to be demonstrated that the tumours have features suggestive of an aggressive course.

The USA National Institute of Health consensus criteria were first applied to estimate the risk of aggressive GIST behaviour.[7] Applying these criteria, the high-risk category includes tumours ≥ 10cm, regardless of mitotic activity, tumours of any size when the mitotic activity exceeds 10/50 HPFs, and tumours ≥ 5cm when the mitotic count exceeds 5/50 HPFs. Miettinen and Lasota proposed a further model of risk stratification, which takes into account the site of the tumour as an adjunct to tumour size and mitotic activity. Generally, gastric GISTs are associated with a better outcome than intestinal GISTs.[6] Recently, tumour rupture on presentation or during surgery was proposed as a further independent prognostic factor for survival in patients with GIST.[8]

The detection of gain-of-function mutations in the gene encoding the KIT receptor, and rare mutations in PDGFRα, was the hallmark of understanding the biology of GISTs.[4,5] Gene mutation rates in GIST have been estimated to range from 21–57% for KIT,[9,10] and from 5–15% for PDGFRα.[11] It was shown in data from this study that 77% of patients with GISTs had gene mutations, of which 60% were in KIT, and 17% in the PDGFRα genes. Whether or not KIT/PDGFRα mutations influence the outcome of patients with GIST has been previously investigated, but definitive results are still lacking. The aim of investigations was to specifically evaluate the relationship between the presence of mutations in the KIT gene, mostly involving exon 11, and the malignant behaviour of the GIST.[12] A poor prognosis for exon 11 mutations has been suggested by the findings in several studies,[9,10,13] but has not been confirmed universally.[14–17] In addition, the prognostic significance of the type of mutation of exon 11 (missense versus others) is unclear, although deletions and insertions have been shown to carry a greater risk with respect to recurrence-free survival. By contrast, the presence of PDGFRα gene mutations, mostly affecting exon 18, relates to less aggressive GIST behaviour.[18]

It was observed in our study that KIT-mutated GISTs were located in the stomach, as well as at extragastric sites, i.e. the small bowel, large bowel and peritoneum. The latter locations were linked to a more adverse prognosis.[19,20] Four of the six tumours that were metastatic on presentation harboured KIT mutations. Conversely, all the observed cases of GIST with PDGFRα-mutated genes originated from the stomach. where tumours are inclined to be less aggressive. None of them had metastasised. It was reported in a recent study in which the focus was a series of 346 gastric GISTs, that PDGFRα mutations occurred in 35% of the tumours, were associated with a lower mitotic index, and had a benign course in more than 80% of cases.[21] The observed rate of PDGFRα mutation in the intestinal GIST was 3% in the same study. The low potential of PDGFRα-mutated GIST to metastasise has been emphasised in other reports.[22,23]

The size of the tumour is a negative predictor of GIST behaviour.[24] Tumours harbouring mutations on the KIT gene in our study had a greater diameter with respect to PDGFRα-mutated and wild-type GISTs. Mutations in KIT were shown in nine of 10 patients with a GIST size ≥ 10cm, whereas none of the patients with KIT gene mutation had a tumour size ≤ 2cm.

Although based on a limited number of cases, the data from our study also suggest a link between KIT mutation and tumour mitotic index. Mutations were found in 70% of the GISTs with a mitotic index ≥ 5/50 HPFs, 63% of GISTs with a mitotic activity ≤ 5/50 HPFs, and in 25% of GISTs with no mitotic activity.

A more aggressive phenotype for GISTs harbouring KIT mutations has previously been reported. These tumours are larger in size, inclined to invade nearby tissues and organs, are associated with higher mitotic figures and carry a worse prognosis.[10,13] By contrast, the presence of KIT mutations is not constantly linked to phenotypic features predictive of poor outcome. For example, a correlation between KIT mutation and tumour size, mitotic count or a different prognosis, could not be shown in some investigations.[9,17]

KIT mutations were frequently seen in GISTs classsified as high risk in the present study. However, this same molecular alteration was demonstrated even in tumours in lower classes

of risk, making the prognostic role of KIT mutations unclear. Conversely, PDGFRα mutations were expressed by GISTs in the very low- and low-risk classes. A link between this molecular feature and a better outcome is implied by this observation. The observation of a more frequent KIT mutation in high-risk tumours, and the prevalence of PDGFRα mutation in tumours belonging to lower-risk classes, has been confirmed elsewhere.[5,24,25]

Data from our study were insufficient to predict the risk through the analysis of specific subgroups of KIT mutations. A poor oncological outcome associated with KIT exon 9 mutations has been reported in previous studies,[19,20] although this observation has not been confirmed elsewhere.[26,27] The risk of GISTs with exon 9 mutations was linked to the preferred location of this genotype in the small bowel, rather than to underlying mutation, in a recent study.[28] For example, exon 9 KIT-mutated GISTs located in the small bowel were an average size of 7cm, whereas GISTs with the same genotype mutation, but which had developed in the stomach, were an average size of 4cm.

Surgical R0 resection of a localised GIST is still the first choice of treatment. However, tumour recurrence is not a rare event in GISTs where five-year recurrence-free survival ranges from 63–78%. Tumours at high risk of recurrence may benefit from adjuvant therapy, even when completely excised at surgery. With this in mind, efforts should be made to increase the ability to predict the likelihood of recurrence following surgery. The commonly used staging systems for risk assessment have shown to be reliable in predicting the clinical behaviour of tumours, but sometimes they fail. Tumours in either low- and high-risk groups may behave unexpectedly, suggesting that current searching variables employed by the standard prognostic models are unable to assign certain GISTs to a defined risk category.[29-31] Molecular analysis is an additional tool to assist in stratifying and treating GISTs.

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