An update on molecular genetics of gastrointestinal stromal tumours

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Gastrointestinal stromal tumours (GISTs) are the most common primary mesenchymal tumours of the gastrointestinal tract. Most of them show activating mutations of the genes coding for KIT or platelet-derived growth factor receptor α (PDGFRα), two receptor tyrosine kinases (RTKs). The RTK inhibitor Imatinib (Gleevec®, Novartis, Switzerland), induces regression of the tumour. The level of response to treatment, together with other clinicopathological parameters is related to the type and site of the activating mutation, thus suggesting that these tumours should be classified according to the molecular context. This is confirmed also by the phenomenon of the resistance to treatment, which arises because of different mechanisms (second mutation, amplification, activation of other RTKs) and can be fought only by specific RTK inhibitors, that are at present under development. RTK activation involves an homogeneous transduction pathway whose components (MAPK, AKT, PI3K, mTOR and RAS) are possible targets of new molecular treatment. A new paradigm of classification integrating the classic pathological criteria with the molecular changes will permit personalised diagnosis and treatment.

Gastrointestinal stromal tumours (GIST), although relatively rare, are the most common primary mesenchymal tumours of the gastrointestinal tract, with an incidence of nearly 20/1 000 000/year. Their biological behaviour is difficult to predict, ranging from benign to malignant. The most reliable prognostic factors are size and mitotic index. On the basis of these factors (and to some extent on anatomical location), two risk classifications are proposed (tables 1 and 2).

Stromal tumours of the gastrointestinal tract were regarded as smooth-muscle tumours (leiomyoma, leiomyoblastoma) until electron microscopy and immunohistochemistry analysis showed that only a small fraction of these tumours showed smooth-muscle differentiation. Therefore, in 1983 Mazur and Clark proposed the non-committal designation, stromal tumour, which now encompasses tumours with schwannian or neuronal differentiation (gastrointestinal autonomic nerve tumours). We now know that GISTs may have either a well-developed or an incomplete myoid, neural, autonomic nerve or mixed phenotype, or may remain undifferentiated. In the late 1990s, it was shown that GISTs share morphological, immunophenotypical and genetic characteristics with the interstitial cells of Cajal (ICC), the pacemaker cells of the gut. They have immunophenotypical and ultrastructural features of both smooth muscle and neuronal differentiation, and regulate peristalsis. Most GISTs express the tyrosin kinase KIT oncoprotein that is also the immunohistochemical marker of ICC. The expression of KIT is so strong and specific that it was claimed to be required for the diagnosis, whereas it is now clear that a small, but significant fraction of GISTs (5–10%) are indeed KIT negative.

KIT is normally expressed in several cell types other than ICC. In particular, KIT expression has a crucial role in embryogenesis, encouraging differentiation of primitive mesenchymal progenitor cells towards ICC and is essential to the formation of a functional ICC network. It belongs to the type III receptor tyrosine kinase (RTK) subfamily, whose members include platelet-derived growth factor receptors α and β (PDGFRα and PDGFRβ). All RTKIIs contain five immunoglobulin-like domains in their extracellular ligand-binding region followed by a single transmembrane domain and a cytoplasmic tyrosine kinase domain interrupted by a large kinase insert. The ligand of KIT is known as stem cell factor. As in other RTK, stem cell factor induces dimerisation of KIT followed by transautophosphorylation of the cytoplasmic tyrosine kinase domain, leading to activation of multiple signalling pathways, such as the PI3K/AKT and e-Jun N-terminal kinase/STAT pathways (fig 1). The constitutive activation of KIT is one of the earliest transforming events in GISTs and occurs mainly through activating mutations in the kit gene, but there is evidence of alternate activating mechanisms in a subset of tumours. Activating mutations of kit gene in GIST occur in exons 11, 9, 13 and 17 (fig 1), corresponding to the juxtamembrane intracellular regulatory domain, the extramembrane domain and the two intracytoplasmic tyrosine kinase domains, respectively. In the first phase, the presence of activating mutations seemed to be related to a malignant behaviour. Subsequently, it was shown that most GISTs, even the tumours <1 cm in size that were found incidentally, do harbour KIT mutations. The meaning of KIT activation is highlighted by the recent introduction of...
It is associated with small intestinal localization and aggressive behaviour. Its mechanism probably affects an antidermisation motif in the extracellular domain.

**Exon 17 (activation loop)**

The activating mechanism of these rare mutations (0.6% of cases) affecting codons 820 and 822, is unclear. A mutation occurring at codon 817, highly activating and frequently observed in other tumours (mastocytosis, acute myelogenous leukaemia), was never observed in GISTs, implying that the transforming mechanisms in the genesis of GIST are different from those of other tumours.

**MUTATION IN THE PDGFRα GENE**

They are observed in 7–12% of cases, occurring more often in exon 18 (activation loop) and rarely in exons 12 (juxtamembrane domain) and 14 (kinase I domain). pdgfra Mutants are prevalently epithelioid, located in the stomach and show weak or no immunohistochemical reactivity for KIT, but are functionally similar to kit mutants. The mutations occur in homologous domains, and activation of the downstream signalling pathways seem to be largely similar in the two mutant subtypes. Some degree of difference in gene expression may exist, but these data need confirmation in larger series.

**Exon 18 (activation loop)**

Mutations occur at codons 842–849. Some of them (D842V, R842–842KI and D842–843IM) have shown considerable resistance to treatment with imatinib.

**Exon 12 (juxtamembrane domain)**

Mutations occur at codons 561–571 and are associated with good response to imatinib.
Exon 14 (kinase I domain)
A single rare mutation is described (N659K). It showed in vitro sensitivity to imatinib that is comparable to that observed in kit exon 13 mutants.

GISTS IN THE PAEDIATRIC AGE GROUP
Most GISTs (95%) arise in adults over 40 years of age. Some GISTs in children (6–14 years) and young adults (15–24 years) occur in connection with Carney’s triad or neurofibromatosis type 1. Rare cases of familial GISTs are described, which carry a kit or pdgfra germline mutation. Sporadic paediatric GISTs
Two series of paediatric GISTs showed that these tumours occur without mutations in both kit and pdgfra. They show mainly an indolent course, with treatable recurrence. A specific gene expression signature was found in five cases, including overexpression of phosphatase kinase alpha 1 (PHKA1), previously reported in a subset of acute myelogenous leukaemia in elderly women.

Paediatric GISTS associated with syndromes
GISTS associated with neurofibromatosis type 1 do not have mutations in the kit or pdgfra gene, except in rare cases, not corresponding to the hot spots of sporadic GISTs. They show an indolent course, preferential location in the small bowel and the colon and a tendency for multiple tumours. Carney’s triad is an association of GIST, paraganglioma and pulmonary chordoma. The genetic basis is unknown. In

Table 3 Summary of most frequent kit and pdgfra mutations in sporadic gastrointestinal stromal tumours

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Frequency (%)</th>
<th>Mutation</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>kit</td>
<td>11</td>
<td>20–60</td>
<td>Deletion–insertion 550–561&lt;br&gt;Point mutations 557, 559, 560, 576&lt;br&gt;Internal tandem duplications beyond 570 (3' end)</td>
<td>Deletion is often associated with bad prognosis. Good response to imatinib</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>&lt;5</td>
<td>Point mutation 642</td>
<td>Bad response to imatinib</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>Rare (&lt;1)</td>
<td>Point mutation 820</td>
<td>Bad response to imatinib</td>
</tr>
<tr>
<td>pdgfra</td>
<td>12</td>
<td>Roughly 1</td>
<td>Point mutation 561&lt;br&gt;Deletion–insertion 560–571&lt;br&gt;Insertion 582–586</td>
<td>Good response to imatinib</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>&lt;0.5</td>
<td>Point mutation 659</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>2–3</td>
<td>Deletion–substitution 842–847</td>
<td>Mutation 842 (D842V) resistant to imatinib&lt;br&gt;Other sensitive</td>
</tr>
</tbody>
</table>
all, 85% of patients are women. The diagnosis is generally made at a young age or in infancy. GISTs associated with Carney’s triad do not harbour mutations in the kit or pdgfra genes.18 98

Familial GISTs are rare.57 45–93 Most affected families carry a kit germ-line mutation, inherited as autosomal dominant. One family showed a mutation in the pdgfra gene. Tumours are usually multiple and multifocal and arise at earlier ages than sporadic GISTs. They are associated with urticaria pigmentosa, melanocytic nevi, melanomas, achalasia or neuronal hyperplasia of the myenteric plexus.57 85–93 Genetic mechanisms of progression are similar in familial and sporadic GISTs in adults.59

Cyto genetic changes in GISTs

The cytogenetic changes in GISTs were extensively studied by using different techniques (table 4).99–108

A correlation between the number and type of chromosomal changes and biological behaviour of GISTs was suggested.21 Karyotypes from about 60% of GISTs show a partial or total loss of chromosome 14,21 45 104 109 In particular, 14q11.1–12 and 14q22–24 are frequently deleted and can therefore represent sites for tumour suppressor genes participating early in the genesis of GISTs.108 110 Loss of 22q is observed in about half of GISTs, with a higher frequency in advanced tumours.77 111 It is possible therefore that an unknown gene on 22q may be responsible in the early stages of tumorigenesis and in tumour progression.20 45 111 Intermediate-risk and high-risk GISTs show loss of chromosomes 1p, 9p, 9q, 11p, 11q, 12p, 12q, 14q, 16, 19, 22, 22, 8q and gains of 8q and 17q.100 102 104 106 108 111 and gains of 8q and 17q.100 102 104 105 106 A sort of molecular pathway in the acquisition of genetic aberrations may parallel the progressive acquisition of malignancy.14 The precise role of single changes and their prognostic impact was not elucidated. Probably, cytogenetic changes in GISTs, above all in those with intermediate- and high-risk, are more complex.35 53 61 77 112 113 For instance, 8q changes in GISTs, above all in those with intermediate- and high-risk, are more complex.35 53 61 77 112 113 For instance, 8q gains were described in as many as 57% of metastatic GISTs.103 Gains of c-myc, a well-known oncogene located on 8q24.12–13, in only 3 of 100 GISTs,61 implies that the target of this amplification are other, still unknown, oncogenes.

Cell cycle network and GIST

One possible target on chromosome 9p is the cyclin-dependent kinase inhibitor 2A (cdk2a) gene, located on 9p21, with its two transcripts, p16INK4a and p14ARF, which results from an alternative reading frame on the first exon.115 The cyclin-dependent kinase inhibitor 2A (cdk2a) gene has a central role in the control of cell cycle and apoptosis. p14ARF inhibits mouse double minute 2 (MDM2) from degrading p53.115 p16INK4A binds to the cyclin-dependent kinase 4 and blocks the phosphorylation of RB1 protein, with consequent binding of the RB1 to E2F1, which may influence the expression of thousand genes responsible for the control of proliferation, transcription and apoptosis.116–118 Inactivation of p16INK4 may occur through mutation or promoter hypermethylation.116 117 Molecular genetics and immunohistochemistry showed 113 119 120 that a loss of p16 may have an independent value in identifying a subset of tumours with adverse prognosis. These results are supported by the observation that the absence of other members of the CDKN2a network may be linked to adverse prognosis.16 We61 analysed a series of 100 GISTs by fluorescent in situ hybridisation (FISH) and found amplifications of CyclinD1 (cnd1) and mdm2 genes in a subset of high-risk tumours. Mouse double minute 2 interacts with Raf/mitogen-activated protein kinase /mitogen activated protein kinase121 and phosphatidylinositol-3-kinase/AKT/c-Jun N-terminal kinase122 123 pathways, both of which are triggered by KIT-activation.18 21 124 We also found three cases of amplifications of cnd1 and mdm2.61 125 An immunohistochemical study attempted to relate the cell cycle machinery and prognosis in 80 GISTs.126 Cyclin A, cyclin B1, cdc2 and Ki-67 were associated with a high risk of malignant behaviour and short disease-free survival.

**EXPRESSION STUDIES**

The first study of gene expression in GISTs24 showed that the presence of kit mutations (at that time, the presence of pdgfra mutations was not known) could identify a homogeneous expression profile, distinguishing GISTs from other mesenchymal tumours. In particular, genes that probably participated in the pacemaker function of the ICC (ion channels, receptors, transduction molecules) had a highly discriminant value. One of these protein kinase Cβ (prktb) is constitutively activated in GISTs and could therefore be a therapeutic target.

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**Take-home messages**

- Specific receptor tyrosine kinases (RTK) mutation is correlated with response-to-therapy and other clinico-pathological parameters.
- The prognostic impact of single cytogenetic alterations has not been elucidated.
- Factors different from RTK may regulate signalling in gastrointestinal stromal tumours.
- We need a new paradigm of classification that combines pathological criteria and molecular changes.

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**Table 4 Summary of cytogenetic changes in gastrointestinal stromal tumours**

<table>
<thead>
<tr>
<th>Changes</th>
<th>Method</th>
<th>Number of cases</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p, 9p, -8q, 9, -10p, -10q, -13, -14q, -22q</td>
<td>CGH</td>
<td>14</td>
<td>Kim, 2000</td>
</tr>
<tr>
<td>1q, -14q, -22q</td>
<td>CGH</td>
<td>12</td>
<td>Breiner et al</td>
</tr>
<tr>
<td>1p, -9q, 1q, -14q, -22q, +5p, +8q, +17q, +20q</td>
<td>CGH</td>
<td>95</td>
<td>El-Rifai et al</td>
</tr>
<tr>
<td>1q, -7l, -9, -13q14 (Rb1), -14q, -15, -22q, +4q, +8q, +15q, +20p, +17q, +20p, +6q, +8q</td>
<td>CGH</td>
<td>22</td>
<td>Debrie-Rychter et al</td>
</tr>
<tr>
<td>1p, -14q, -14, -22, +7</td>
<td>CGH</td>
<td>14</td>
<td>Derré et al</td>
</tr>
<tr>
<td>1p, -9q, -10q, -13q, -14, -15, -22, -5q, +3q, +5q, +8q</td>
<td>CGH</td>
<td>10</td>
<td>Andresson et al</td>
</tr>
<tr>
<td>1p, -9q, -10q, -13q, -14, -15, -22, -5q, +3q, +5q, +8q</td>
<td>CGH</td>
<td>19</td>
<td>Gunawan, 2002</td>
</tr>
<tr>
<td>1p, -9q, -10q, -13q, -14, -15, -22, -5q, +3q, +5q, +8q</td>
<td>CGH</td>
<td>52</td>
<td>Gunawan et al</td>
</tr>
</tbody>
</table>

CGH, comparative genomic hybridisation; FISH, fluorescent in situ hybridisation.
such as KIT.

Another marker that has been identified by gene expression analysis is DOG-1, and it has been proposed also as a possible diagnostic marker. Subsequently gene expression in GISTs may differ according to the presence of mutation in kit or pdgfra, to the type of mutations in kit or pdgfra or to the anatomical location of the tumour. Differentially expressed genes included czen, p70s6k, map2k1, akt, stat3, all of which were in the activating pathways downstream of kit or pdgfra. Koon et al. described by realtime RT-PCR an association between the expression of cell cycle proteins (cyclinB1, centromere protein-F kinetochore protein) and tyrosine kinases with the biological behaviour in a small series of GISTS.

SIGNALLING PATHWAYS

KIT and PDGFRαs in GISTs show a homogeneous transduction pathway consisting of mitogen-activate protein kinase, Akt, p70, STAT1, STAT3, PDGF, mammalian target of rapamycin and RAS. In particular, oncogenic signalling in these tumours differs from haematological diseases, and selective inhibition of the PI3K/mammalian target of rapamycin pathways reduces proliferation and inhibits apoptosis. The degree of activation differs from tumour to tumour, thus suggesting that factors different from KIT may regulate signalling in these neoplasias. The development of new targeted molecular treatments is aimed at selectively blocking these pathways.

MOLECULAR CHANGES AND RESISTANCE TO IMATINIB

Many patients with advanced GISTs develop resistance after variable degrees of initial response to treatment. Two kinds of resistance should be distinguished: (a) primary resistance: evidence of progression within the first 6 months of imatinib treatment, frequently associated with a wild-type KIT protein, mutation in exon 9 of kit or a D842V mutation in pdgfra; (b) secondary resistance: progression of disease after 6 months of treatment. The mechanisms of secondary resistance are heterogeneous: (a) acquisition of a secondary mutation in the kit or pdgfra genes; (b) genomic amplification of kit and overexpression of the protein; and (c) activation of other RTKs. A new generation of tyrosine kinase inhibitors are presently under evaluation to solve this problem.

CONCLUSIONS

GISTs probably do not constitute a single group of tumours; their biological behaviour (the prognosis and above all the response to treatment) depends both on classic clinicopathological parameters (ie, location, size, mitotic activity) and on the molecular changes that are detected in a given tumour (type of mutation in RTK, chromosomal alterations, expression of cell cycle proteins, activation and control of pathways downstream of the RTK, amplification or loss of genes, etc). Moreover, a relationship was found between some pathological characteristics and molecular alterations (for instance, tumours of the small intestine are associated with epithelioid morphology and mutation in exon 9 of kit). This underlines the need for a new paradigm of classification that can combine the old pathological criteria with the molecular changes. In the era of targeted treatments (imatinib is one of the most successful examples), we are forced to change our point of view from the microscopic to the molecular level and to integrate all the data in a coherent schema.

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Molecular changes in GIST


