Biology of gastrointestinal stromal tumour and mechanisms of imatinib resistance

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Abstract

The introduction of imatinib therapy for GISTs has represented a major breakthrough of oncology in the last decade, improving dramatically the prognosis of GIST patients. The discovery of an oncogenic event (kinase mutations) having a major predictive value underlines the need for a paradigm of classification combining thorough pathological examination and molecular analysis. The response to therapy with imatinib is indeed determined by the type of mutation in kinase genes. Genetic analysis should be therefore performed in all cases deserving such a therapy. The study of the resistance has enabled us to discover other important oncogenetic events (e.g. dysregulation of downstream pathways) that are important in the genesis and therapy of other tumours as well. Genetic studies have also allowed a molecular classification of GISTs, thus identifying subsets of GISTs (e.g. SDH-negative, paediatric) that do not behave as the "classical", RTK-mutated tumours, probably representing different "entities".

Keywords CKIT; GISTs; imatinib; PDGFRA; RTK; targeted therapy

Introduction

Gastrointestinal stromal tumours (GISTs) are the most frequent mesenchymal tumours of the gastrointestinal tract, with an incidence between 15 and 20 new cases/10^6/year.[1] It is however possible that the true incidence be underestimated. Autopsy studies have indeed shown that many GISTs are simply incidental findings at the autopsy and do not give any symptomatology.[2,3] The most frequent localization is the stomach, followed by the small intestine, the colon-rectum and the oesophagus. The existence of true extragastrointestinal GISTs has been proposed, but it is questioned by many authors. Although the concept that mesenchymal tumours of the gastrointestinal tract with leiomyomatous morphology are 'bizarre or blastomatous' had been recognized since the 40s by Stout, the particular nature of these tumours was defined first in the 80s, when Mazur et al, based mainly on electron microscopy findings, proposed the non committed term of "Gastrointestinal stromal tumours" (Figure 1). This concept was further developed with the help of immunohistochemistry (positivity of CD34 in 70% of cases, while the expression of smooth muscle markers was found only in a minority of cases). At the end, in 1998, two groups[4,5] showed independently that most GISTs show constitutively activating mutations of the *CKIT* gene that encode an important receptor tyrosine kinase (RTK) type III. KIT (CD117) immunohistochemical expression was contemporarily reported in more than 95% of GIST cases, thus becoming an important tool for the diagnosis[6] (Figure 2a–d). In 2003, it was shown that a substantial fraction of *KIT* wild-type GISTs show mutations in the gene coding for *PDGFRA* (platelet-derived growth factor alpha), another RTK type III (Figure 2e–h) and that these mutations are mutually exclusive with mutations in *CKIT*.[7,8] Oncogenesis of GISTs is therefore probably related mainly to early activation of RTKs. The importance of this discovery is also underlined by the fact that KIT and PDGFRA are very good targets for the tyrosine kinase inhibitor imatinib mesylate (Gleevec®, Novartis Pharma AG, Basel, Switzerland). Imatinib is now the first-line drug in the treatment of inoperable or metastatic GISTs and may be used before and/or after surgical treatment.[9] Imatinib is indeed approved in US and in Europe for adjuvant therapy and may be used also in a neoadjuvant setting to reduce the tumour mass.[10] The effect of imatinib on sensitive GISTs is dramatic, as it was shown in the first communications of Joensuu et al.[11] However, primary and secondary resistance to targeted therapy remains, in spite of this great success, a problem to solve. Beneath a minority of GISTs that simply do not respond to the therapy with imatinib (primary resistance), it is now well known that about half the patients develop disease progression by 2 years of treatment with imatinib.[12] The main predictor of the response to therapy is represented by the type and localization of the mutation in the RTK genes.[13,14] Therefore, the genetic alterations in the RTK genes are not only important early events in the genesis of GISTs; they also define the response to targeted therapy. In the last years, alternative/additional oncogenetic events have been identified, that could have at least partially a prognostic/predictive meaning, such as mutations in the small G-proteins BRAF and KRAS and hyperexpression of the transcription factor ETV1.[15–17]

As outlined above, in the last decade GISTs have represented an important model for the targeted therapy of solid tumours. The understanding of their biology has been of paramount importance for the developing of RTK inhibitors. Moreover, GISTs represent the perfect example for the need of a change in the paradigm of classification of disease, integrating the "classical" clinicopathologic parameters with the molecular alterations, a process that has been always put forward for haematologic malignancies.

This review will therefore focus on the biology and molecular pathology of GISTs, and on the mechanisms of resistance to the targeted therapy.

RTK III

KIT and PDGFRA belong to the group of RTK III, together with PDGFRB, macrophage colony-stimulating-factor receptor (CSFR1), Fl cytokine receptor (FLT3). RTK III are characterized by 5 Ig-like extracellular domains, one transmembrane domain, one intracellular juxtamembrane regulatory domain and two intracellular tyrosine kinase domains, which have also autophosphorylating capacity[9] (Figure 3). *KIT* and *PDGFRA* genes are located on the same chromosomal region (4q12) and their

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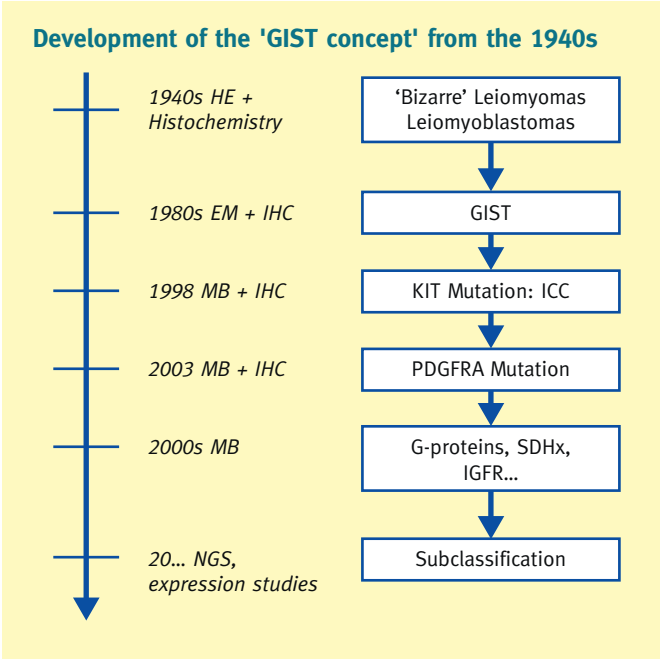

Figure 1 The influence of the techniques on the classification is evident. EM, electron microscopy, IHC, immunohistochemistry, ICC, interstitial cells of Cajal, MB, molecular biology, SDH, succinate dehydrogenase, IGFR, insulin growth factor receptor, NGS, next generation sequencing.

products show a high level of structural and sequence homology. Binding of the ligand (SCF for KIT and PDGF for PDGFRA) induces homodimerization of the receptor, with autophosphorylation and activation of the metabolic pathways of RAS-RAF-MAPK, PIK3CA-AKT and signal transducer and activator of transcription 3 (STAT3)[8] (Figure 4).

CKIT

Under physiological conditions, activation of KIT plays an important role in the development of several cell types, in particular melanocytes, haematopoietic progenitor cells, mast cells, primordial germ cells and interstitial cells of Cajal, the probable cells of origin of GISTs. The presence of KIT-activating mutations has been reported in the pathogenesis of several human tumours, besides GISTs thus suggesting a pivotal role of KIT in oncogenesis.

Activating mutations of *KIT* in GISTs are prevalently somatic, but some very rare germline mutations have been described, representing familial GISTs (Table 1). Activating mutations result in ligand-independent dimerization of KIT and subsequent tyrosine kinase activation. Activating mutations are most commonly (60–70% of cases) found in exon 11 of *KIT* gene, corresponding to the juxtamembrane intracellular regulatory domain of the protein (Figure 3). These mutations let the conformation of the protein change into the active state. The different mutations can be in-frame deletions, insertions, substitutions, or various combinations.[13,14] The type and localization of the genetic change is related to clinical parameter such as prognosis and localization. For instance deletions, above all those involving codons 557–558, are associated with shorter overall and disease-free survival,[18] whereas internal tandem duplications are associated with a relatively indolent course[19] (Table 1).

Mutations in exon 9, corresponding to the extracellular domain of the KIT molecule (Figure 3), are found in about 10% of GIST cases[13] and are almost exclusively duplications of six nucleotides, corresponding to the A502_Y503 residues of the proteins. Their effect is probably due to the same conformational changes that are caused by ligand binding. Mutations in exon 9 of *KIT* are associated with localization in small intestine or colon and poorer prognosis.[13] Moreover, exon 9-mutated GISTs show different gene expression signatures from exon 11 mutated ones.[20]

Primary mutations in tyrosine kinase domain (exon 17) and ATP-binding site (exon 13) are uncommon[13] (Table 1 and Figure 3). They are prevalently found as secondary mutations arising in patients treated with receptor tyrosine kinase inhibitors (RTKI), as cause of secondary resistance (see below). Most *KIT* exon 13 mutations represent single substitutions leading to K642E in the aminoacydic sequence.[13] However, other substitutions have been described, and some of them are also sensitive to imatinib.

There are several experimental evidences of the role of activating *KIT* mutations in the development of GISTs. For instance, phospho-KIT is invariably found in tumour extracts from GISTs and mutant KIT is clearly oncogenic in vivo. As recalled above, there are clear evidences that activating mutations of KIT trigger downstream pathways such as MAPK, PIK3CA-AKT1 and STAT3 (for a comprehensive review, see Antonescu, 2011[10] and Corless, 2011[9]) (Figure 4). The MAPK pathway, through many transcriptional regulators (MYC, ELK, CREB), can trans-activate the cell cycle. The PIK3CA pathway, through stimulation of AKT and PDK1, has an antiapoptotic effect and indirectly interferes with the cell cycle. Following RTK activation, STAT3 is phosphorylated and translocated in the nucleus, where it acts as a transcription factor, stimulating proliferation and inhibiting apoptosis (Figure 4).

Recently, an elegant study of Chi et al.[16] has suggested that ETV1 (ETS translocation variant 1) may play an important role in GIST tumorigenesis, probably "collaborating" with KIT. The clinical importance of ETV1, however, has not been confirmed.[21]

The importance of pathways downstream RTKs has been underlined by functional and pharmacological studies, who have shown that some factor downstream of PIK3CA, but probably upstream of MTOR play a crucial role for the cell survival.[9,22]

On physiological conditions, the levels of KIT are tightly regulated by endocytosis and degradation via proteasome. Half-life of mutated KIT is longer than that of wild-type KIT, probably because of physical interaction with heat-shock protein 90 (HSP90). This is also underlined by the efficacy of HSP90 inhibitors in experimental models of GISTs.

PDGFRA

PDGFRA is another RTK III, sharing sequence homologies and functional homologies with KIT. It is mutated in GISTs and AML and is fused with FILP1 in systemic mastocytosis and hypereosinophilic syndrome.[10] In GISTs, activating mutations in *KIT* and *PDGFRA* are mutually exclusive whereas *PDGFRA* is mutated in exons 12, 14 and 18, corresponding to the juxtamembrane regulatory domain and the tyrosine kinase domain of the protein,