KRAS and BRAF Mutations Predict Primary Resistance to Imatinib in Gastrointestinal Stromal Tumors

Claudia Miranda1, Martina Nucifora4, Francesca Molinari4, Elena Conca2, Maria Chiara Anania1, Andrea Bordoni5, Piercarlo Saletti6, Luca Mazzucchelli6, Silvana Pilotti2, Marco A. Pierotti3, Elena Tamborini2, Angela Greco1, and Milo Frattini4

Abstract

Purpose: Gastrointestinal stromal tumors (GIST) are characterized by gain-of-function mutations in KIT/PDGFRα genes leading to a constitutive receptor activation which is well counteracted by imatinib. However, cases in which imatinib as first-line treatment has no effects are reported (primary resistance). Our purpose is to investigate alterations in downstream effectors, not reported so far in mutated GIST, possibly explaining the primary resistance to targeted treatments.

Experimental Design: Two independent naive GIST cohorts have been analyzed for KIT, PDGFRA, KRAS, and BRAF mutations by direct sequencing. Cell lines expressing a constitutively activated and imatinib-responding KIT, alone or in combination with activated KRAS and BRAF, were produced and treated with imatinib. KIT receptor and its downstream effectors were analyzed by direct Western blotting.

Results: In naive GISTs carrying activating mutations in KIT or PDGFRA a concomitant activating mutation was detected in KRAS (5%) or BRAF (about 2%) genes. In vitro experiments showed that imatinib was able to switch off the mutated receptor KIT but not the downstream signaling triggered by RAS–RAF effectors.

Conclusions: These data suggest the activation of mitogen—activated protein kinase pathway as a possible novel mechanism of primary resistance to imatinib in GISTs and could explain the survival curves obtained from several clinical studies where 2% to 4% of patients with GIST treated with imatinib, despite carrying KIT-sensitive mutations, do not respond to the treatment.

Introduction

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the gastrointestinal tract. This uncommitted term, formally referred to tumors showing smooth muscle differentiation, identifies after 1998 the most representative example of "simple sarcomas" (1), in which a single receptor tyrosine kinase (RTK) mutation plays a crucial role in dictating both pathogenesis and predictivity. Hirota and colleagues (2) in fact showed for the first time that a significant subset of GISTs harbored mutations in the RTK KIT gene. Subsequently, in 2003, the gene encoding for the homologous receptor PDGFRA was showed to be mutually exclusively mutated in these tumors (3). Currently, we know that KIT alterations (principally deletions, point mutations, and insertions) affect exons 11 and 9 and rarely exons 13 and 17. Cumulatively, KIT alterations are carried by approximately 70% to 80% of GISTs. PDGFRA mutations, deletions, and point mutations in exons 18, 12, and 14 are present in about 5% to 10%. The rate of GISTs carrying wild-type KIT and PDGFRA genes accounts for 10% to 20% of cases (4). As result of KIT and PDGFRA mutations, these tumors harbor constitutively activated KIT and/or PDGFRA receptors which, in turn, upregulate 2 main signal pathways, where the RAS–RAF–MEK–ERK and the PI3K–AKT–mTOR transducer protein kinases are involved.

It is widely reported that GIST respond well to imatinib (5), a selective tyrosine kinase inhibitor able to interfere...