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ORIGINAL ARTICLE

Protein expression of KIT and gene mutation of *c-kit* and *PDGFRs* in Ewing sarcomas

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Abstract

Ewing sarcoma is a highly malignant tumor of bone preferentially arising in children and young adults. Its 5-year survival rate is only 50% despite the use of multimodal therapeutic approaches, requiring a search for new therapeutic targets and the development of novel therapeutic modalities. KIT and PDGFRs are type III receptor tyrosine kinases, and activating mutations in *c-kit* (which encodes KIT) and *PDGFRs* have been reported as oncogenic events in many malignancies. Imatinib is a selective inhibitor of KIT, PDGFR, and ABL tyrosine kinase activity and exerts different anti-tumor effects according to the regions of mutations in *c-kit* and *PDGFR* genes. Thus, we evaluated the immunohistochemical expression of KIT protein and the mutational status of exons 9, 11, 13, and 17 of the *c-kit* gene, exons 12 and 18 of the *PDGFRA* gene, and exon 12 of the *PDGFRB* gene in 71 formalin-fixed, paraffin-embedded Ewing sarcomas to increase our understanding of the potential, if any, of imatinib treatment for this malignancy. Of the 71 samples, 27 (38%) were immunohistochemically positive for KIT; however, activating mutations in *c-kit* were found in only 2 of 71 Ewing sarcomas (2.6%) within exon 9. No activating mutations in the *PDGFRA* and *PDGFRB* genes were found, but pleomorphism was identified in exon 18 of the *PDGFRA* gene. Our results for KIT protein expression agree with those of previous studies. This is the largest series of *c-kit* mutational analysis in Ewing sarcoma to date, and the results definitively show that *c-kit* activating mutations are not coincident with KIT protein expression in Ewing sarcoma in most samples. These findings imply other mechanisms for KIT activity and leave open the question of whether imatinib would be efficacious in the treatment of Ewing sarcoma.
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Keywords: Ewing sarcoma; c-Kit; KIT; PDGFRA; PDGFRB

Introduction

Ewing sarcoma represents the second-most common primary bone malignancy in children and adolescents. Ewing tumor is a highly aggressive neoplasm with 5-year survival rates of only 50% despite the use of multimodal therapeutic approaches [4]. Approximately 25% of patients have detectable metastases to lung, bone, and bone marrow at diagnosis. Furthermore, nearly all patients have micrometastases, as evidenced by a 10% cure rate with local therapy alone [32]. The standard of

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care is systemic therapy combined with surgery or radiotherapy for local control. The most important negative prognostic factor is tumor dissemination at the time of diagnosis [32]. A particularly unfavorable prognosis is related to metastases to bone and/or bone marrow. Treatment of metastatic tumors has been unsuccessful despite high-dose chemotherapeutic agents [16]. A search for new therapeutic targets and the development of novel therapeutic modalities are therefore needed for the treatment of Ewing sarcoma.

The lack of new effective drugs in the treatment of Ewing sarcoma, together with the side effects of high doses, supports the need for innovative therapeutic strategies. These therapies include targeting molecules that seem to be critical for the pathogenesis and progression of Ewing sarcoma [27]. Imatinib (STI-571, Gleevec®, Basel, Switzerland) is a tyrosine-kinase inhibitor that selectively blocks tyrosine phosphorylation of ABL, KIT, and platelet-derived growth factor (PDGF) receptor-A and -B (PDGFRA and B). Imatinib inhibits BCR–ABL kinase activity in patients with chronic myeloid leukemia (CML) and is also active in patients with gastrointestinal stromal tumors (GISTs) in which mutations of the c-*kit* gene result in a constitutively activated receptor [2,5]. This compound may have clinical use in other neoplasms in which KIT and PDGFRs are involved in proliferative or antiapoptotic responses [8].

The *c-kit* proto-oncogene encodes a 145- to 165-kD membrane-bound glycoprotein of type III receptor tyrosine kinase (KIT, CD117) [7,38]. The ligand for *c-kit* is stem cell factor (SCF), alternatively known as mast cell growth factor, steel factor, or kit ligand [36]. KIT is normally expressed on mast cells, melanocytes, germ cells, interstitial cells of Cajal, and hematopoietic progenitor cells [14,29,33]. Binding of ligand SCF to KIT receptor results in KIT dimerization and subsequent activation of the Janus family kinase-signal transducer and activator of transcription (JAK-STAT), phosphatidylinositol 3′-kinase (PI-3 K), and mitogen-activated protein (MAP) kinase pathways that promote cell growth and differentiation [19]. In addition to its normal role, deregulation of KIT is widely believed to play a role in certain human tumors, including germ cell tumors, mast cell tumors, and gastrointestinal stomal tumors (GISTs), as well as small cell lung cancers (SCLCs), melanoma, breast cancer, and neuroblastoma [3,6]. In a number of other types of tumors, KIT-mediated growth occurs via mutation of *c-kit*, which results in ligand-independent activation of the receptor [3,6,10]. Oncogenic KIT mutation is found most commonly in exon 11, the region encoding part of the KIT juxtamembrane region. Gain-of-function mutation of exons 9, 13, and 17 have also been reported [9,25]. Imatinib exhibits great efficacy in GISTs with a mutation in exon 9 or 11 of *c-kit* [9].

Similar to KIT, PDGFR is a type III receptor tyrosine kinase and acts as a receptor for the relevant ligand PDGFs [37]. Overexpression of PDGFs and PDGFRs has been reported for many solid tumors, including glioblastoma, meningioma, melanoma, ovarian cancer, prostate cancer, lung cancer, pancreatic cancer, and gastric carcinoma [6,23,35]. Imatinib exhibits high efficacy in tumors with a mutation in exon 12 of *PDGFRA* [11].

Ewing sarcoma proliferation and survival are also determined by autocrine and paracrine activation of growth factor receptors and their ligands, including insulin-like growth factor I, gastrin-releasing peptide, SCF, and their receptors [17,18,24,26]. It has also been reported that PDGFRB and PDGF-C mediate motility and growth of Ewing sarcoma cell lines [34,39]. These discoveries have led to a better understanding of the mechanisms involved in the pathogenesis of this neoplasm and allowed the identification of some biologic targets.

Although previous studies have examined *c-kit* mutations in Ewing sarcoma, the series have been quite small. Here, in what is, to our knowledge, the largest series to date to analyze *c-kit* mutations in Ewing sarcoma, we sought to characterize the protein expression of KIT and the mutational status of the *c-kit* and *PDGFR* genes. Because imatinib appears to exhibit greater efficacy in GISTs that carry mutations in exons 9 or 11 of c-*kit* or exon 12 of *PDGFR*, we focused here on the clinical relevance of *c-kit* and *PDGFR*s in Ewing sarcoma. Our goal was to elucidate the role of KIT protein and mutational activation of these genes as a basis for determining the efficacy of using imatinib as a therapy against this malignancy.

Materials and methods

Archival tissue material

We collected 71 formalin-fixed, paraffin-embedded Ewing sarcomas from Korea, Italy, Brazil, and Argentina. All of the hematoxylin–eosin stained slides were reviewed by two independent pathologists (PYK, DI). Samples did not come from patients who had received chemotherapy or radiotherapy. This protocol was reviewed and approved by IRB of Kyung Hee University, Seoul, Korea.

Immunohistochemical analysis

Immunohistochemical staining was performed on 4-μm-thick, formalin-fixed, paraffin-embedded tissue sections. Tissue sections were deparaffinized three times in xylene for a total of 15 min and subsequently rehydrated. Endogenous peroxidase activity was blocked in

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