Human Pathology (2013) **44**, 1271–1277

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Use of potassium channel tetramerization domain-containing 12 as a biomarker for diagnosis and prognosis of gastrointestinal stromal tumor

Tadashi Hasegawa MD, PhD[a,*], Hiroko Asanuma PhD[a], Jiro Ogino MD, PhD[a], Yoshihiko Hirohashi MD, PhD[b], Yasuhisa Shinomura MD, PhD[c], Hiroyuki Iwaki MD, PhD[d], Hironobu Kikuchi MD, PhD[e], Tadashi Kondo MD, PhD[f]

[a]*Department of Surgical Pathology, Sapporo Medical University School of Medicine, Chuo-ku, Sapporo 060-8543, Japan*
[b]*Department of Pathology, Sapporo Medical University School of Medicine, Chuo-ku, Sapporo 060-8556, Japan*
[c]*Department of Internal Medicine, Sapporo Medical University School of Medicine, Chuo-ku, Sapporo 060-8543, Japan*
[d]*Department of Pathology, Sunagawa City Medical Center, Sunagawa 073-0196, Japan*
[e]*Department of Surgery, Sunagawa City Medical Center, Sunagawa 073-0196, Japan*
[f]*Division of Pharmacoproteomics, National Cancer Center Research Institute, Chuo-ku, Tokyo 104-0045, Japan*

Received 17 August 2012; revised 12 October 2012; accepted 17 October 2012

Keywords:
KCTD12;
Diagnosis;
Prognosis;
Immunohistochemistry;
Gastrointestinal stromal tumor;
GIST

Summary Previously, we showed that the expression of potassium channel tetramerization domain-containing 12 (KCTD12), which was discovered by a proteomics approach, is associated with high-risk behavior of gastrointestinal stromal tumors (GISTs). Here, we examined the distribution and expression of this protein by immunostaining with a commercially available polyclonal KCTD12 antibody in GISTs (n = 64) and other types of malignancy (n = 168) to clarify its diagnostic and clinical significance. Diffuse KCTD12 immunoreactivity was found in most GISTs (52 cases; 81%). KCTD12 expression was observed primarily in vascular endothelial cells, Purkinje cells of the cerebellum, and some neurons scattered throughout the cerebral cortex. KCTD12 was absent from not only the interstitial cells of Cajal but also interstitial cells of Cajal hyperplasia that was encountered incidentally in colon diverticulitis. KCTD12 immunostaining was also seen in malignant peripheral nerve sheath tumors (2/10 cases; 20%), synovial sarcomas (2/10; 20%), solitary fibrous tumor (1/8; 13%), angiosarcoma (1/7; 14%), and colon adenocarcinoma (1/24; 4%). In survival analyses, the 5-year recurrence-free survival rate of patients without KCTD12 expression was only 16.7% compared with 95.6% in those with KCTD12 expression ($P < .0001$). Ki-67 and KCTD12 were significant predictors of recurrence-free survival, and KCTD12 expression provided additional information about recurrence-free survival after accounting for Ki-67 status. Overall, KCTD12 expression was specific for GISTs from neoplastic and nonneoplastic adult tissues other than brain and served as a predictor of GIST recurrence. These findings suggest that KCTD12 is a useful and reliable biomarker for both the diagnosis and prognosis of GIST.

* Corresponding author. Department of Surgical Pathology, Sapporo Medical University School of Medicine, South 1 West 16, Chuo-ku, Sapporo 060-8543, Japan.
E-mail address: hasetada@sapmed.ac.jp (T. Hasegawa).

0046-8177/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humpath.2012.10.013

1. Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract and occur most frequently in the stomach, followed by the small intestine, colon, and extragastrointestinal sites such as the omentum and mesentery [1]. They emerge from the interstitial cells of Cajal (ICCs) and are characterized by the presence of gain-of-function mutations in c-kit (*KIT*) or platelet-derived growth factor receptor α (*PDGFRA*) [2,3]. These mutations result in the constitutive activation of signaling pathways downstream of receptor tyrosine kinases. The malignant potential of GISTs varies from virtually benign tumors to aggressive sarcomas. Some patients are at a significant risk for tumor recurrence and progression to metastatic disease, even after complete excision of their tumors. A few risk-stratification schemes are available for operable GISTs [4-7]; tumor size, mitosis count, and tumor site are considered established risk factors for recurrence.

Imatinib is now being explored as an adjuvant therapy in patients with primary resectable GISTs because it increases the time to GIST recurrence [8,9], and patients with a high risk of recurrence have longer survival with 3 years of adjuvant imatinib therapy compared with those with 1 year of treatment [9]. Although imatinib is generally well tolerated, nearly all patients report some adverse effects. To minimize these adverse effects, biomarkers to assess the malignant potential of GISTs are required for the selection of patients who are most likely to benefit from adjuvant imatinib therapy in routine clinical practice.

In our previous report, patients with immunohistochemical positive expression of potassium channel tetramerization domain-containing 12 (KCTD12), which was discovered by a proteomics approach, had better metastasis-free survival than did those not expressing KCTD12 [10]. KCTD12 was originally cloned as a gene that is highly expressed in the fetal cochlea and brain [11], whereas it was recently identified as a component of the $GABA_B$ receptor [12]. The prognostic value of KCTD12 was validated in a multi-institutional series of GISTs by using a monoclonal antibody against KCTD12 that was developed in our laboratory [13,14]. However, this monoclonal antibody is not yet available for clinical application, and KCTD12 expression in normal and abnormal tissues and other types of malignancies remains to be clarified. For this reason, here we studied the immunohistochemical expression of KCTD12 in GISTs and a variety of other soft tissue tumors and carcinomas, some of which appear in the differential diagnosis of GIST, using a commercially available polyclonal antibody. We correlated the expression of KCTD12 with the clinicopathologic characteristics of 64 cases of GIST to establish the diagnostic and prognostic use of the KCTD12 antibody.

2. Materials and methods

2.1. Patients and tumor samples

The medical records of 64 patients with primary, resectable c-kit protein/KIT–positive GIST who were diagnosed and treated between 2000 and 2011 were retrieved from the pathology files of Sapporo Medical University Hospital and Sunagawa City Medical Center. All patients underwent resection with curative intent. In our institutions, administration of adjuvant or neoadjuvant imatinib has been considered for patients with a high-risk tumor, a tumor with a Ki-67 labeling index of 10% or greater, or a marginally resectable tumor since this therapy was approved in July 2009. Patients who received adjuvant/neoadjuvant imatinib were excluded from this analysis because adjuvant imatinib influences the natural course of the disease [8] and morphologic changes such as extensive hyalinization and hypocellularity and even the loss of KIT expression occur in tumor cells after imatinib treatment [15].

In addition, a total of 168 cases of soft tissue tumors and carcinomas diagnosed pathologically at Sapporo Medical University Hospital were selected for study (Table 1): 12 leiomyosarcomas, 6 schwannomas, 10 malignant peripheral nerve sheath tumors (MPNSTs), 6 desmoid-type fibromatoses, 8 solitary fibrous tumors, 7 myxofibrosarcomas, 6 dedifferentiated liposarcomas, 10 synovial sarcomas, 6 Ewing sarcomas, 8 malignant melanomas, 7 angiosarcomas, 10 cutaneous squamous cell carcinomas, 24 breast carcinomas, 24 pulmonary adenocarcinomas, and 24 colon adenocarcinomas. Nonneoplastic adult tissues from a variety of sites in the body were also analyzed. The study protocol

Table 1 Immunohistochemical expression of KCTD12 in 64 cases of GIST, 86 of soft tissue tumors, and 82 of carcinomas

Histologic type	No. of cases	KCTD12-positive cases
GIST	64	52 (81%)
Leiomyosarcoma	12	0 (0%)
Schwannoma	6	0 (0%)
MPNST	10	2 (20%)
Desmoid-type fibromatosis	6	0 (0%)
Solitary fibrous tumor	8	1 (13%)
Myxofibrosarcoma	7	0 (0%)
Dedifferentiated liposarcoma	6	0 (0%)
Synovial sarcoma	10	2 (20%)
Ewing sarcoma	6	0 (0%)
Malignant melanoma	8	0 (0%)
Angiosarcoma	7	1 (14%)
Cutaneous squamous cell carcinoma	10	0 (0%)
Breast carcinoma	24	0 (0%)
Pulmonary adenocarcinoma	24	0 (0%)
Colon adenocarcinoma	24	1 (4%)