Biochemical and Biophysical Research Communications 395 (2010) 158–162

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The frequency of KRAS mutation detection in human colon carcinoma is influenced by the sensitivity of assay methodology: A comparison between direct sequencing and real-time PCR ☆

Takashi Kobunai [a,b,*], Toshiaki Watanabe [a], Yoko Yamamoto [a], Kiyoshi Eshima [b]

[a] *Department of Surgery, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan*

[b] *Tokushima Research Center, Taiho Pharmaceutical Co. Ltd., 224-2 Ebisuno, Hiraishi, Kawauchi-cho, Tokushima 771-0194, Japan*

ARTICLE INFO

Article history:
Received 25 March 2010
Available online 31 March 2010

Keywords:
Colon cancer
Detection method
Frequency
KRAS mutation

ABSTRACT

Purpose: Kirsten rat sarcoma (KRAS) gene mutations occur early in the progression of colorectal adenoma to carcinoma. The mutation status of the KRAS gene determines the benefits of molecular targeting drugs in patients with advanced colorectal cancer, although many methods are available to detect such mutations. The purpose of this study was to evaluate the influence of assay sensitivity on the detection frequency of mutated genes. *Methods:* Colorectal tumors in 224 colorectal cancer patients were characterized for KRAS mutations using PCR amplification following by direct sequencing as well as a peptide nucleic acid (PNA)-clamp real-time PCR-based assay. *Results:* KRAS mutations were observed in 32.1% (72/224) patients by direct sequencing, and 43.3% (97/224) by PNA-clamp PCR. The chi-square test revealed that the difference in the frequency of KRAS mutations determined by direct sequencing and PNA-clamped PCR (threshold for 1% detection) was statistically significant ($p < 0.015$). *Conclusions:* Our data suggest that assay method sensitivity clearly influences the detection frequency of mutated genes. As more sensitive assays detect more mutated genes in clinical samples, this must be taken into consideration when determining KRAS gene status in clinical practice.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Mutations in the Kirsten rat sarcoma (KRAS) oncogene are frequently found in human cancers, particularly those of the pancreas, gall bladder, bile duct, thyroid gland, non-small cell lung cancer, and colorectal cancer [1–4]. The presence of these mutations may determine the prognosis and drug response to new cancer therapies targeting the K-*ras* protein pathway [5].

Cetuximab and panitumumab, the monoclonal antibodies (mcAbs) used to target the epidermal growth factor receptor (EGFR), were recently approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) for use as single agents or in combination with other chemotherapeutic drugs in the treatment of metastatic colorectal cancer (mCRC). However, the McAbs only benefit a subset of patients that express the wild-type K-*ras* protein; tumors with mutated K-*ras* do not respond to this treatment modality [6–9]. A significant improvement in overall survival and progression-free survival was observed in patients with wild-type K-*ras* tumors following treatment with cetuximab compared with supportive care alone, but not in those with mutated K-*ras* tumors [8]. It is therefore important that the KRAS mutation status be determined precisely to maximize the patient's benefit in a clinical setting.

While a variety of methods are available for the detection of KRAS mutations, nested PCR followed by direct sequencing and allele-specific real-time PCR have been widely utilized so far. We hypothesized that differences in the sensitivity of mutation screening methods may influence mutation detection frequency. To test this, we compared the frequency of KRAS mutations detected in clinical colon cancers by two discrete methods. The first involved classical nucleotide sequencing analysis in which PCR amplification is followed by direct sequencing, and the second is a more sensitive method involving the peptide nucleic acid (PNA)-clamp real-time PCR-based assay [10].

2. Materials and methods

2.1. Cell culture

SW480 and HCT116 cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD). Cells were

☆ *Financial support:* Ministry of Education, Science, Sports, Culture, and Technology of Japan; the Ministry of Health, Labour, and Welfare; in part by TAIHO Pharmaceutical Co., Ltd.

* Corresponding author at: Department of Surgery, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan. Fax: +81 3 5375 6097/+81 88 665 6554.

E-mail address: azteca_tk@yahoo.co.jp (T. Kobunai).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.03.167