



Mutation analysis concerning possibility of cancer of unknown primary new treatment

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Abstract

Anti-epidermal growth factor receptor monoclonal antibodies are approved and effective only in KRAS wild-type patients with advanced colorectal carcinoma. The present retrospective study investigated the role of KRAS gene mutations and other clinical features in patients with cancer of unknown primary site (CUP). The metastases from two male patients of an unknown primary tumor were included in this retrospective analysis. Both cases were determined cancer of unknown primary by clinicopathologic findings. The KRAS, BRAF, and PI3KCA mutational analyses were performed by means of PCR using genomic DNA for each PCR reaction. All PCR products were directly sequenced. PIK3CA mutation was found in case 1 at exon 9 in this study. This study further investigated the role of KRAS, PIK3CA, and BRAF gene mutations and clinical features for possibilities for new therapies in patients with additional CUP cases. It has been discussed protocols of EGFR blockade and gene-mutation status for extension of indication. The possibility for EGFR blockade to be effective for new indications has been revealed.

Keywords: EGFR, KRAS, cancer of unknown primary

Background

Various guidelines have been suggested for appropriate evaluation of metastatic cancers when the primary tumor site is not readily identifiable based on the clinical presentation, tissue histology and immunohistochemical staining. After such evaluation, cancer of unknown primary (CUP) is the diagnosis made in about 4–5% of invasive cancers [1–3]. The development of epidermal growth factor receptor (EGFR)-targeting drugs, used both in conjunction with standard care and also as a single therapy, has drastically changed the management of colorectal cancer. Recently, EGFR blockade has become a major research focus, and a number of clinical researches on this new treatment have been started in the field of clinical oncology [4]. Certain mutations in the KRAS gene are currently the most widely used predictors for the effectiveness of EGFR-inhibiting drugs in colorectal cancer. The present retrospective study investigated the role of KRAS gene mutations and other clinical features in patients with cancer of unknown primary site (CUP) [5,6]. We have analyzed additional cases with the recently improved methods for KRAS analysis and report the data of these cases.

Patients and Methods

The metastases from two male patients of an unknown primary tumor were included in this retrospective analysis. The definition of CUP syndrome covers patients with histologically confirmed metastatic cancer, when the combination of a complete medical history, accurate physical examination and laboratory tests, and conventional diagnostic imaging procedures (i.e. chest X-ray, abdominal ultrasonograph, and, on the basis of suspected primary disease, CT, magnetic resonance imaging, PET, mammography, and endoscopic procedures) have failed to identify the primary site. The histological types by needle biopsy and sites of our patients' metastases are summarized in **Table 1**. The blood concentrations of CA19-9, CA15-3, CA125, and carcinoembryonic antigen (CEA) and the most commonly used markers—the leukocyte common antigen, cytokeratins, chromogranin, and vimentin—were assessed for histopathological diagnosis.

Each of these cases was found as a large abdominal tumor and from diagnostic imaging it was not clear whether it was developed intraperitoneally or retroperitoneally. Histopathological diagnosis was conducted in each case by needle biopsy, and in the Case 1, development of poorly-

Table 1. Clinicopathologic findings.

Case	sex	age	PS	Pathological diagnosis	Positive marker	Treatment	Prognosis	Tumor location
Case 1	M	75	1	undifferentiated?	vimentin, LCA, CD3, MDM2	ADM, IFM	4M	abdomen
Case 2	M	70	1	adenocarcinoma	ND	GEM	3M	abdomen

Table 2. PIK3CA mutations were found in case 1 at exon 9.

Case	KRAS *	BRAF exon 15V600E	PI3KCA exon 9 E542K	PI3KCA exon 9E545K/D	PI3KCA exon 20 H1047R
1	ND	GTG	GAA	AAG	CAT
2	ND	GTG	GAA	GAG	CAT

* Scorpion-arms method

differentiated tumor and non-epithelial tumor were suspected by HE staining and immunostaining respectively. With the Case 2, it was found to be an adenocarcinoma by HE staining, but nothing was found positive in immunostaining and tumor-marker-in-the-blood tests. It has not been detected any lesions that was considered primary lesion in such tests as various diagnostic imagings and esophagogastroduodenoscopy, and existence of any blood disorders such as malignant lymphoma was denied. From the above, following the definition, both cases were determined cancer of unknown primary.

DNA extraction from formalin-fixed, paraffin-embedded (FFPE) tumor tissue sections

Tumor cell-rich areas of hematoxylin and eosin-stained sections were marked under a microscope, and tissues were scratched from the same areas of deparaffinized unstained sections. DNA for pieces of the scratched tissue samples was isolated using the QIAamp FFPE Tissue Kit (QIAGEN KK, Tokyo, Japan).

Mutational analyses

The KRAS, BRAF, and PI3KCA mutational analyses were performed by means of PCR using 100 ng of genomic DNA for each PCR reaction. All PCR products were directly sequenced using an ABI Prism 377 (Applied Biosystems, Foster City, CA) and were then evaluated by means of Sequence Navigator software (Applied Biosystems). Each sequence was carried out at least twice, starting from an independent amplification reaction.

KRAS

Exon 1 of the KRAS gene was amplified in order to seek potential mutations on the two foremost codons (12 and 13), which have been reported as mutated in CRC. PCR amplification was carried out using the Scorpion-arms method and previously described primers and conditions [7].

BRAF

Exons 11 and 15 of the BRAF gene, including the classical mutation V600E, were amplified using previously described conditions and primers [6].

PI3KCA

Exons 9 and 20 of the alpha polypeptide (the catalytic subunit of the PI3K protein, PI3KCA), which are frequently mutated in CRC, were amplified using a standard PCR protocol and previously described primers [7].

Results

Clinicopathologic findings

Both specimens were adenocarcinomas. These two cases received chemotherapy (Table 1). Regarding the clinical data in our cases, it was found that in most of these present cases, histological types could be diagnosed under light microscope, and tissue (including EGFR) and markers in the blood served as auxiliary diagnosis. Markers were particularly useful in diagnosing neuroendocrine tumors.

KRAS, BRAF, and PI3KCA mutations

Analyzing one representative tumor sample in each patient, we could not find any mutations in the KRAS codon 12 and 13, or BRAF mutations at codon 600. PI3KCA mutations were found in case 1 at exon 9 (Table 2).

Discussion

KRAS mutations occur in different tumor types, and are particularly important in colorectal, pancreatic, and NSCLC carcinogenesis [8-12]. KRAS mutations predict a lack of therapeutic benefit of anti-EGFR monoclonal antibodies in colorectal cancer, but this dynamic is more equivocal in lung cancer.

Anti-epidermal growth factor receptor monoclonal antibodies (panitumumab and cetuximab) are approved and effective only in KRAS wild-type patients with advanced colorectal carcinoma [13-16]. The purpose of our meta-analysis was to evaluate the real effects of cetuximab and panitumumab in KRAS wild-type patients treated in randomized trials.

Panitumumab has exhibited promising antitumor activity in several clinical trials, and in 2006 gained FDA approval for the treatment of patients with EGFR-expressing metastatic CRC with disease progression following chemotherapy regimens containing fluoropyrimidine, oxaliplatin, and irinotecan [14].

Some clinical studies were performed for the new indication

Table 3. KRAS, BRAF, and PIK3CA mutations.

-	KRAS mutation
CUP(Ohta et al)	9%
pancreatic	56%
colorectal	51%
uterine	15%
ovary	11%
-	BRAF mutation
CUP(Ohta et al)	9%
melanoma	44%
thyroid	8%
colorectal	6%
ovary	5%
-	PIK3CA mutation
CUP(Ohta et al)	9%
squamous cervical	36%
uterine	25%
breast	21%
colorectal	17%

The summary of gene analysis described in the literature and our previous report.

of cetuximab as follow. During a Phase III trial involving 424 patients of untreated head and neck squamous cell carcinoma, the group receiving treatment with a combination of cetuximab and radiation (211 cases) had a median survival period of 49 months and a local control period of 24.4 months, which exceeded the results of radiotherapy-alone group (213 cases) with the median survival period of 29.3 months and the local control period of 14.9 months [17,18]. Furthermore, during the Phase III trial involving 442 cases of untreated head and neck squamous cell cancer (relapse or metastasis cases), the group treated with a combination of cetuximab and chemo (222 cases) had a median survival period of 10.1 months, a progression-free survival period of 5.6 months, and a response rate of 36 percent, all of which predominantly exceeded the results of the chemotherapy-only group (220 cases) with the survival period of 7.4 months, the progression-free period of 3.3 months, and the response rate of 20 percent.

During a Phase II trial involving 66 cases of previously treated advanced non-small cell lung cancer, the cetuximab-only treatment resulted in a response rate of 4.5 percent and a disease control rate of 30.3 percent [11,12]. As for the results of EGFR-positive cases (60 cases) alone, the cetuximab-only treatment had a response rate of 5.0 percent and a disease control rate of 30.0 percent.

Increasing numbers of studies have reported that information on EGFR mutation is useful when choosing the regimen for treating CRC [7]. Currently several methods are used to investigate EGFR mutation. The characteristics of these methods need to be clarified. For surgically excised specimens consisting mostly of cancer cells, direct sequencing is a good choice to test the mutation. The cancer cell content of the cytological specimens is often much less. When the presence of the cancer cells is confirmed by a pathological examination, the content of

cancer cells is usually more than 1%, and high-sensitivity detection methods, like the Scorpion ARMS method are able to detect mutations. A high-sensitivity detection method should be used to detect EGFR mutations from cytological specimens.

Functional RAS may also be potentially important for regulating the actin cytoskeleton, which was suggested as being a critical driver of oncogenic transformation [19]. We analyzed the mutation of same part of these 3 genes in 9 cases of CUP in our previous reports [6] (Table 3), and mutation was detected in one case each of KRAS and BRAF. In the 2 cases analyzed in the present study, mutation was detected in 1 case of PIK3CA (Table 3). The dimension of gene analysis described in the literature is larger and I cannot compare them simply, but I consider that the incidence rates of these gene mutations in CUP are low. Janku *et al.*, found a high prevalence of KRAS mutations in pancreatic (56%), colorectal (51%), uterine (15%), and ovarian (11%) cancers, which is similar to previously published findings and data from the COSMIC database (Table 3) [6, 11].

BRAF mutations have been mainly reported in melanoma, colorectal, papillary thyroid, and ovarian cancer (Table 3) [20,21]. In colorectal cancer they are associated with a poor prognosis; however, unlike KRAS mutations, BRAF mutations might not be predictive of a lack of benefit of cetuximab [22]. In agreement with previously published data, our study showed a high prevalence of BRAF mutations in melanoma (44%) and, to much lesser extent, in thyroid, colorectal, and ovarian cancer (8%, 6%, and 5%, respectively). In regard to individual aberrations, oncogenic mutations in two hot-spot regions (exons 9 and 20) of PIK3CA have been identified in various malignancies, including common tumors such as breast, lung, colorectal, ovarian and uterine (Table 3) [6,11]. In this study, PIK3CA mutations were identified in 11% of diverse tumor types. Tumors with a high prevalence of PIK3CA mutations were squamous cell cervical (36%), uterine (25%), breast (21%), colorectal (17%), squamous cell head and neck (15%), and ovarian cancers (11%).

Conclusions

We analyzed KRAS, BRAF, and PIK3CA genes in two CUP cases. PIK3CA mutation was found in case 1 at exon 9 in this study. We have analyzed nine cases of CUP in our previous report and found mutation in one case each of KRAS and BRAF. It has been discussed protocols of EGFR blockade and gene-mutation status for extension of indication. The possibility for EGFR blockade to be effective for new indications has been revealed.

Competing interests

The authors declare that they have no competing interests.

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