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SHORT REPORT

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Mutational analysis of *BRAF* and *K-ras* in gastric cancers: absence of *BRAF* mutations in gastric cancers

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Abstract Recently, *BRAF* mutations were found in a variety of human cancers. Interestingly, the most common of *BRAF* mutation (V599E) has not been identified in tumors with *K-ras* mutations. Whereas the majority of human cancer types has been screened for *BRAF* mutations, no detailed studies on gastric cancers have been investigated. Thus, we decided to investigate the incidence of *BRAF* mutations in gastric cancers, and the relationship between *BRAF* and *K-ras* mutations in such cancers. Three nonpathogenic *BRAF* polymorphisms and seven *K-ras* missense mutations were found in 66 gastric cancers and 16 gastric cancer cell lines. Although only 9% of our gastric cancer panels had *K-ras* mutations, the incidence of *BRAF* mutations was not high. Thus, *BRAF* mutations, which are present in a variety of other human cancers, do not seem to be involved in gastric cancer development.

Introduction

Gastric cancer is the most common cancer in east Asian countries such as Korea (Bae et al. 2001). *CDH1* germline mutations have been reported in patients with the diffuse type of familial gastric cancers (Guilford et al. 1998; Yoon et al. 1999). However, *CDH1* mutations are not recognized as a major cause of familial gastric cancer in Asian countries (Yoon et al. 1999; Kim et al. 2003). Recent reports indicate truncation mutations predominate in patients of Western origin, whereas only a few missense mutations are found in patients from Asian countries (Guilford et al. 1998; Yoon et al. 1999). Recently, we reported a *MET* germline missense mutation in a diffuse type of familial gastric cancer patient (Kim et al. 2003). However, the *MET* mutation frequency is low (5%, 1/21), suggesting that it is not a major cause of familial gastric cancer. Although somatic mutations have been reported in some genes, such as *p53* and β-catenin (Fenoglio-Preiser et al. 2003; Woo et al. 2001), the genetic mechanisms underlying the development of gastric cancers have not been sufficiently identified. *BRAF* mutations have recently been found in a variety of human cancers (Davies et al. 2002). BRAF is one of three serine/threonine RAF kinases, is RAS-regulated, and participates in cell growth and malignant transformation kinase pathways (Brose et al. 2002; Smith et al. 2003). *BRAF* somatic mutations have been identified in 66% of malignant melanomas (Davies et al. 2002), 35.8%–69% of papillary thyroid carcinomas (Cohen et al. 2003; Kimura et al. 2003), 5.1%–10% of colorectal tumors (Rajagopalan et al. 2002; Yuen et al. 2002), 33% of low-grade ovarian serous carcinoma (Singer et al. 2003), and at a relatively low frequency in a wide range of other cancers (Davies et al. 2002). A high frequency of *BRAF* mutations has also been reported in 82% of nevi (Pollock et al. 2003). Reported *BRAF* mutations are confined to exons 11 and 15 (kinase domain), and up to 80% of *BRAF* mutations involve a V599E substitution (Davies et al. 2002). Interestingly, this most common of *BRAF* mutations has not been identified in tumors with *K-ras* mutations (Rajagopalan et al. 2002; Yuen et al. 2002; Kimura et al. 2003; Singer et al. 2003). This mutually exclusive relationship supports the hypothesis that *BRAF* (V599E) and *K-ras* mutations exert equivalent effects in tumorigenesis (Rajagopalan et al. 2002; Singer et al. 2003).

Whereas the majority of human cancer types have been screened for *BRAF* mutations, no primary gastric cancers have been examined, and only six gastric cancer cell lines have been investigated (Davies et al. 2002). Since between 0% and 28% of gastric cancers have *K-ras* mutations (Lee

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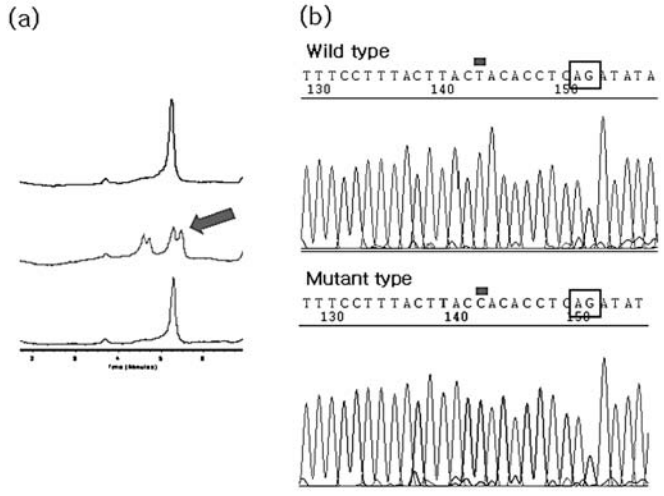

Fig. 1a, b *BRAF* polymorphism in intron 14 of tissue sample S45. **a** DHPLC chromatograms. *Arrow* Abnormal pattern in intron 14 of S45. The other two peaks originate from normal controls. **b** Automatic sequencing showing the nucleotide substitution (IVS14-10T→C)

et al. 1995; Arber et al. 1997), we decided to investigate the incidence of *BRAF* mutations in gastric cancers, and the relationship between *BRAF* and *K-ras* mutations in such cancers. Our approach was to screen for *BRAF* and *K-ras* mutations in gastric tumors and cell lines to determine whether *BRAF* was involved in the development of gastric cancers. In addition, 20 familial gastric cancer patients without *CDH1* and *MET* germline mutations were investigated for the presence of *BRAF* germline mutations.

Materials and methods

We screened *BRAF* and *K-ras* mutations in 66 gastric cancer tissues and 16 gastric cancer cell lines (SNU-1,-5, -16, -216, -484, -520, -601, -620, -638, -668, -719, AGS, KATO III, MKN45, MKN74, and NCI-N87). Criteria for selection of familial gastric cancers were at least two first- or second-degree relatives affected with gastric cancer, at least one of which was diagnosed with cancer prior to age 50 (Kim et al. 2003). Blood samples from probands of each of these families were collected from Seoul National University Hospital. Informed consent was obtained from all participants before testing. Pathological data were available on 62 of the 66 gastric cancer tissues and showed that 32 were diffuse types of gastric cancers, 24 were intestinal types, and six were mixed types. Of 20 familial cancer probands, eight represented families suffering from diffuse types of gastric cancer, four represented families suffering from intestinal types, and there was no histological data available for the remaining eight. DNA from tumor samples and from peripheral blood lymphocytes was extracted by using TRI reagent (Molecular Research Center, Cincinnati, Ohio, USA) according to manufacturers' instructions. Codons 12 and 13 of the *K-ras* gene were screened by bi-directional sequencing with the *Taq* dideoxy terminator cycle sequencing kit and an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, Calif., USA). The following polymerase chain reaction (PCR) primer sequences were used for amplification of *K-ras* exon 1; F: 5'-GGTGGAGTATTTGATAGTGTA-3', R: 5'-GGTCCTGCACCAGTAATATGC-A-3'. Exons 11 and 15 of the *BRAF* gene were screened with previously described primer sets (Kimura et al. 2003) by both PCR-SSCP (single-strand conformational polymorphism) and DHPLC (denaturing high performance liquid chromatography; WAVE, Transgenomic, Omaha, Nb., USA), as previously described (Kim et al. 2000, 2003). The melting temperatures of each exon were optimized by analyzing melting curves with WAVEMAKER software (Transgenomic). All samples with abnormal PCR-SSCP bands or DHPLC patterns were subsequently sequenced. Lung adenocarcinoma cell line NCI-H1395 and colorectal cancer cell line HT-29 were used as positive controls for exon 11 and exon 15 *BRAF* gene mutations, respectively (Smith et al. 2003). Reverse transcription (RT)-PCR was performed with two primer sets to examine alternative splicing of the *BRAF* gene; F1: 5'-AAATGTTGAATGTGACAGCA-3', R1: 5'-CAAAATGGATCCAGACAACT-3', F2: 5'-TCCACAGAGACCTCAAGAGT-3', R2: 5'-GCACTCTGCCATTAATCTCT-3'. Complementary DNA was synthesized by using the SuperScript RT II system (Invitrogen, Carlsbad, Calif., USA).

Results and discussion

We screened 20 familial gastric cancer patients in order to identify *BRAF* germline mutations. No such mutations were found. We also screened 66 gastric cancer tissues and 16 gastric cancer cell lines and found a total of three *BRAF* polymorphisms. No clear pathogenic *BRAF* mutations were identified in exons 11 and 15. The SNU-638 gastric cancer cell line harbored a silent P452P mutation (CCT→CCC) in exon 11. Gastric tissues S40 and S45 showed the same sequence changes (IVS14-10T→C) 10 bp upstream of the acceptor site invariant AG of intron 14. These variations were also found in the matched normal tissues of S40 and S45 (Fig. 1). Ninety-six unrelated healthy individuals with two positive controls (S40 and S45) were screened by DHPLC in order to identify possible IVS14-10T→C polymorphisms. However, none of these healthy controls showed an aberrant diagram in DHPLC, whereas

Table 1 *BRAF* and *K-ras* mutations in gastric cancers

Sample	Classification	Type	TNM	Gene	Sequence change
S40	Tumor	Diffuse	II	*BRAF*	IVS14-10T→C
S45	Tumor	Diffuse	IV	*BRAF*	IVS14-10T→C
SNU-638	Cell line	–	–	*BRAF*	P452P, CCT→CCC
S22	Tumor	Diffuse	IV	*K-ras*	G12V, GGT→GTT
S23	Tumor	Intestinal	III	*K-ras*	G12V, GGT→GTT
121	Tumor	Diffuse	III	*K-ras*	G12D, GGT→GAT
221	Tumor	Diffuse	III	*K-ras*	G12V, GGT→GTT
SNU-1	Cell line	–	–	*K-ras*	G12D, GGT→GAT
SNU-601	Cell line	–	–	*K-ras*	G12D, GGT→GAT
AGS	Cell line	–	–	*K-ras*	G12D, GGT→GAT

two positive controls showed abnormal patterns. Analysis involving RT-PCR followed by direct sequencing showed this intronic change did not result in alternative splicing. All 16 gastric cancer cell lines were re-screened by direct sequencing of exons 11 and 15 of *BRAF* to confirm the results obtained by PCR-SSCP and DHPLC analysis. In the *K-ras* mutation analysis, we identified seven *K-ras* (9%, 7/82) somatic mutations in the 66 gastric cancers and 16 gastric cancer cell lines. All seven *K-ras* mutations were found in codon 12. The results of the *BRAF* and *K-ras* mutation analyses are summarized in Table 1. Most *K-ras* mutations in gastric cancers were found in codon 12, in accordance with previous reports (Lee et al. 1995; Arber et al. 1997).

Although *BRAF* mutations have been found in many human cancer types, little is known of their possible presence in gastric cancers. Moreover, the exclusive relationship between *BRAF* and *K-ras* mutations suggests gastric cancers without *K-ras* mutations may contain *BRAF* mutations. We found that, although only 9% of our gastric cancer panels had *K-ras* mutations, there was not a high incidence of *BRAF* mutations. Thus, *BRAF* mutations, which are found in a variety of other human cancers, do not seem to be involved in gastric cancer development.

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References

Arber N, Han EK, Sgambato A, Piazza GA, Delohery TM, Begemann M, Weghorst CM, Kim NH, Pamukcu R, Ahnen DJ, Reed JC, Weinstein IB, Holt PR (1997) A K-ras oncogene increases resistance to sulindac-induced apoptosis in rat enterocytes. Gastroenterology 113:1892–1900

Bae JM, Won YJ, Jung KW, Suh KA, Ahn DH, Park JG (2001) Annual report of the central cancer registry in Korea – 1999: based on registered data from 128 hospitals. Cancer Res Treat 33:367–372

Brose MS, Volpe P, Feldman M, Kumar M, Rishi I, Gerrero R, Einhorn E, Herlyn M, Minna J, Nicholson A, Roth JA, Albelda SM, Davies H, Cox C, Brignell G, Stephens P, Futreal PA, Wooster R, Stratton MR, Weber BL (2002) BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res 62:6997–7000

Cohen Y, Xing M, Mambo E, Guo Z, Wu G, Trink B, Beller U, Westra WH, Ladenson PW, Sidransky D (2003) BRAF mutation in papillary thyroid carcinoma. J Natl Cancer Inst 95:625–627

Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA (2002) Mutations of the BRAF gene in human cancer. Nature 417:949–954

Fenoglio-Preiser CM, Wang J, Stemmermann GN, Noffsinger A (2003) TP53 and gastric carcinoma: a review. Hum Mutat 21: 258–270

Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scoular R, Miller A, Reeve AE (1998) E-cadherin germline mutations in familial gastric cancer. Nature 392:402–405

Kim I-J, Ku J-L, Yoon K-A, Heo S-C, Jeong S-Y, Choi H-S, Hong K-H, Yang S-K, Park J-G (2000) Germline mutations of the dpc4 gene in Korean juvenile polyposis patients. Int J Cancer 86:529–532

Kim I-J, Park J-H, Kang H-C, Shin Y, Lim S-B, Ku J-L, Yang H-K, Lee K-U, Park J-G (2003) A novel germline mutation in the MET extracellular domain in a Korean patient with the diffuse type of familial gastric cancer. J Med Genet 40:e97

Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA (2003) High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. Cancer Res 63:1454–1457

Lee KH, Lee JS, Suh C, Kim SW, Kim SB, Lee JH, Lee MS, Park MY, Sun HS, Kim SH (1995) Clinicopathologic significance of the K-ras gene codon 12 point mutation in stomach cancer. An analysis of 140 cases. Cancer 75:2794–2801

Pollock PM, Harper UL, Hansen KS, Yudt LM, Stark M, Robbins CM, Moses TY, Hostetter G, Wagner U, Kakareka J, Salem G, Pohida T, Heenan P, Duray P, Kallioniemi O, Hayward NK, Trent JM, Meltzer PS (2003) High frequency of BRAF mutations in nevi. Nat Genet 33:19–20

Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE (2002) Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature 418:934

Singer G, Oldt R 3rd, Cohen Y, Wang BG, Sidransky D, Kurman RJ, Shih IEM (2003) Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. J Natl Cancer Inst 95:484–486

Smith ML, Snaddon J, Neat M, Cambal-Parrales M, Arch R, Lister TA, Fitzgibbon J (2003) Mutation of BRAF is uncommon in AML FAB type M1 and M2. Leukemia 17:274–275

Woo DK, Kim HS, Lee HS, Kang YH, Yang HK, Kim WH (2001) Altered expression and mutation of beta-catenin gene in gastric carcinomas and cell lines. Int J Cancer 95:108–113

Yoon K-A, Ku J-L, Yang H-K, Kim W-H, Park S-Y, Park J-G (1999) Germline mutations of E-cadherin gene in Korean familial gastric cancer patients. J Hum Genet 44:177–180

Yuen ST, Davies H, Chan TL, Ho JW, Bignell GR, Cox C, Stephens P, Edkins S, Tsui WW, Chan AS, Futreal PA, Stratton MR, Wooster R, Leung SY (2002) Similarity of the phenotypic patterns associated with BRAF and KRAS mutations in colorectal neoplasia. Cancer Res 62:6451–6455