Study of *PIK3CA*, *BRAF*, and *KRAS* mutations in breast carcinomas among Chinese women in Qinghai

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ABSTRACT. Phosphatidylinositol-3-OH kinase and RAS-activated signaling pathways play an important role in tumor formation. Abnormalities in relevant genes play essential roles in the occurrence and development of many human cancers. Studies of breast cancer have mainly focused on the women in western countries, but few studies have examined the frequency of mutations in *PIK3CA*, *BRAF*, and *KRAS* in Chinese breast cancer patients. In this study, we conducted sequence analysis of *PIK3CA*, *BRAF*, and *KRAS* and determined relationships with the occurrence of breast cancer in women from Qinghai. DNA was extracted from 25 cases of human breast cancer tissue samples. *PIK3CA*, *BRAF*, and *KRAS* mutation analysis was performed by polymerase chain reaction and DNA sequencing. No mutations were found in *PIK3CA*, *BRAF*, and *KRAS* of adjacent tissues. However, *PIK3CA* mutations were observed in 32% (8) of the 25 breast cancer tissues examined, in which exon 9 accounted for 4% (1), exon 20 accounted for 28% (7), and no mutations were found in exon 1 of *PIK3CA*. Sequencing of exon 2 of *KRAS* suggested that 20% (5) of the 25 samples harbored a mutation and 16% (4) of *BRAF*

harbored a mutation. Any mutation in these 3 oncogenes may induce the occurrence and development of breast cancer.

Key words: BRAF; Breast cancer; KRAS; Mutation; PIK3CA

INTRODUCTION

Breast cancer is one of the most common malignant tumors. In women, breast cancer is the most frequent causes of cancer death (Lai et al., 2008). The occurrence and development of breast cancer are complicated processes involving multiple factors and steps resulting from a series of gene alterations.

PIK3CA plays a vital role in coding P110 catalytic subunit of the class I phosphatidylinositol-3-kinases (PI3K). *PIK3CA* mutations result in activation of the PI3K/AKT signaling pathway, which regulates cell metabolism, growth, proliferation, and survival (Osaki et al., 2004; Workman, 2004; Board et al., 2008; Lin et al., 2009). *PIK3CA* mutations occur mainly in 2 hotspots, including the kinase domain from exon 20 and helical domain from exon 9, as well as occasionally in exon 1 (Board et al., 2008; Simi et al., 2008; Stemke-Hale et al., 2008).

The *BRAF* gene is frequently activated in the mitogen-activated protein kinase/extracellular signal-regulated kinase signaling pathway, which affects cell division and differentiation (Feng et al., 2005). Studies have showed that the *BRAF* gene encodes for the 32-kDa BRAF protein composed of 783 amino acids (Fransén et al., 2004; Kim et al., 2006). The KRAS protein is also an essential regulatory factor upstream of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway (Ji et al., 2007). Although *BRAF* and *KRAS* are both involved in the signaling pathway, mutations in their 2 genes were found to be mutually exclusive (Ahlquist et al., 2008). The cellular and molecular mechanisms conferring oncogenic effects and activating *KRAS* mutations remain incompletely understood (Simi et al., 2008). Studies have showed that activation of the mitogen-activated protein kinase pathway was important for the development of colorectal cancer via *BRAF* or *KRAS* mutations (Popescu et al., 1985). Thus, in this study, we examined whether the *BRAF* and *KRAS* gene have the same effect in breast cancer.

We detected mutations of *PIK3CA*, *BRAF*, and *KRAS* in 25 breast cancer tissues to determine whether the mutations were related to the occurrence of breast cancer.

MATERIAL AND METHODS

Samples

Breast cancer tissues samples and the corresponding adjacent tissues were collected from 25 patients, and all cases were confirmed by pathological diagnosis. Tissue samples were conserved in -20°C.

Polymerase chain reaction (PCR) amplification and sequencing

Because *PIK3CA* gene mutations in human cancers are frequently observed in exons 9 and 20, and occasionally in exon 1, we focused on these 3 exons in *PIK3CA*. Additionally,

primers were designed to detect mutations in exon 2 of *KRAS* and exon 15 of *BRAF*. The primers used for sequencing *PIK3CA*, *BRAF*, and *KRAS* (are shown in Table 1). PCR was carried out to amplify fragments of these 3 genes. PCR was conducted in 25-µL volume containing: 2.5 µL PCR buffer (Takara, Shiga, Japan), 4 µL 2.5 mM dNTPs, 2 µL 25 mM $MgCl_2$, 0.4 µL 10 µM of each forward and reverse primer (Generay Bioengineering, Shanghai, China), 0.25 µL rTaq DNA polymerase. PCR was performed at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, with a final extension for 10 min. The PCR products were evaluated by 1.5% agarose gel electrophoresis, dyed with ethidium bromide, and visualized under UV light using a gel-imager. PCR products were purified using a PCR Purification kit (Invitrogen). The purified products were sequenced in both directions using an ABI 3730 (Applied Biosystems, Foster City, CA, USA).

Table 1. List of primers and PCR conditions used in this study.

Gene	Primer sequence (5'→3')	Annealing temperature	Fragment
PIK3CA			
Exon 20	F: CAGGAGATGTGTTACAAGGCTTAT R: TCAGTTCAATGCATGCTGTTTTAAT	55°	267
Exon 9	F: AGTAACAGACTAGCTAGAGACAAT R: CCATTTTAGCACTTACCTGTGAC	55°	141
Exon 1	F: CTCCACGACCATCATCAGG R: GATTACGAAGGTATTGGTTTAGACAG	55°	420
KRAS			
Exon 2	F: AGGCCTGCTGAAAATGACTGAA R: AAAGAATGGTCCTGCACCAG	55°	168
BRAF			
Exon 15	F: AATGCTTGCTCTGATAGGAAAA R: AGCATCTCCAGCGCCAAAAAT	55°	230

RESULTS

We analyzed 25 human breast cancer tissues to detect mutations in *PIK3CA*, *BRAF*, and *KRAS* genes by PCR amplification and direct bilateral sequencing (Table 2). The results showed that *PIK3CA* mutations (exons 20, 9, and 1) were detected in 32% (8) of the 25 patients with breast cancer, while 28 and 4% of mutations were in exons 20 and 9, respectively. No mutation was found in exon 1 of the 25 samples, but in the intron, we indetified a mutation in A390G. Mutations in *PIK3CA* were located in 2 primary hotspots (1633G→A, 3140A→G), *BRAF* in 1808 (G→C) and *KRAS* in 100 (C→A). The frequencies of *BRAF* and *KRAS* mutations were 16 and 20% (Table 2), respectively. Moreover, 2 mutant sites, 3140 (A→G) of *PIK3CA* and 100 (C→A) of *KRAS*, coexisted. The 1808 (G→C) mutations in *BRAF* and the 100 (C→A) mutations in *KRAS* have not been reported previously in breast cancer. All mutations in these 3 genes were in cases of invasive ductal cancer. Previous studies suggested that mutations in *KRAS*, *BRAF*, and *PIK3CA* were frequently observed in several types of human cancers, including colon, breast, and lung cancers. However, mutual mutations in these 3 genes in different types of cancer have not been thoroughly investigated. Xu et al. (2011) found that only 5.9% of mutations were in both *PIK3CA* and *KRAS* in human cancer of cholangiocarcinoma. In our study, we found that *PIK3CA* mutations coexisted with *KRAS* mutations in one sample. We unexpectedly detected mutual mutations of 3140A-G and 3134A-C in PIK3CA (Figure 1).

Table 2. *PIK3CA*, *BRAF*, and *KRAS* mutations identified in breast cancer tissues.

Sample/test result	Mutation result		
	PIK3CA nucleotide change	*BRAF* nucleotide change	*KRAS* nucleotide change
1 Mutation detected	None	None	Exon 2 100C→A
2 Mutation detected	None	None	None
3 Mutation detected	None	None	Exon 2 100C→A
4 Mutation detected	Exon 20 3140A→G	Exon 15 1808G→C	None
5 Mutation detected	Exon 20 3140A→G	None	None
6 Mutation detected	Exon 20 3140A→G	Exon 15 1808G→C	None
7 Mutation not detected	-	-	-
8 Mutation detected	Exon 20 3140A→G	None	None
9 Mutation detected	Exon 9 1633G→A	Exon 15 1808G→C	None
10 Mutation not detected	-	-	-
11 Mutation not detected	-	-	-
12 Mutation detected	Exon 20 3140A→G	None	None
13 Mutation detected	Exon 20 3140A→G	None	Exon 2 100C→A
14 Mutation not detected	-	-	-
15 Mutation detected	None	None	Exon 2 100C→A
16 Mutation detected	None	Exon 15 1808G→C	None
17 Mutation not detected	-	-	-
18 Mutation detected	None	None	Exon 2 100C→A
19 Mutation not detected	-	-	-
20 Mutation not detected	-	-	-
21 Mutation not detected	-	-	-
22 Mutation not detected	-	-	-
23 Mutation not detected	-	-	-
24 Mutation not detected	-	-	-
25 Mutation detected	Exon 20 3140A→G	-	None
Mutated (%)	8 (32%)	4 (16%)	5 (20%)

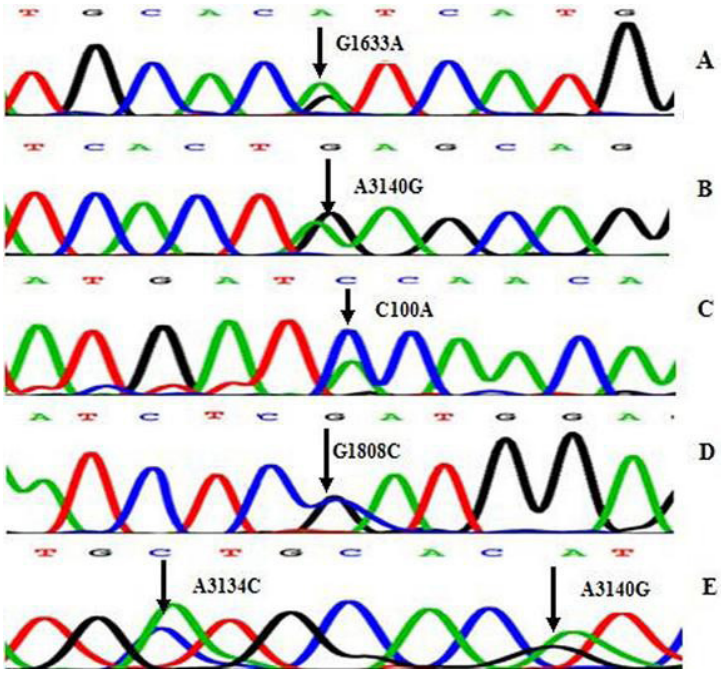

Figure 1. Results of direct sequencing of *PIK3CA* in exon 9 (**A**) and exon 20 (**B**), *BRAF* in exon 15 (**D**) and *KRAS* in exon 2 (**C**). A3134C and A3140G are mutual mutations in *PIK3CA* (**E**).

DISCUSSION

Most previously published studies concerning simultaneous *PIK3CA*, *KRAS*, or *BRAF* mutations were concentrated on colorectal cancer (De Roock et al., 2010). Some studies suggested that *KRAS* and *PIK3CA* mutations coexisted within the same tumor (Velho et al., 2005; Siena et al., 2009; De Roock et al., 2010). *PIK3CA* mutations were coincident with RAS pathway mutations in colorectal cancers (Siena et al., 2009), whereas a previous study found that mutational activation of the PI3K pathway was mutually exclusive with the RAS pathway in breast cancer (Ahlquist et al., 2008).

In this study, we found that frequency of *PIK3CA*, *BRAF*, and *KRAS* mutations were in 32, 16, and 20%, respectively, in breast cancer patients. *PIK3CA* mutations occurred more frequently in conjunction with *KRAS* or *BRAF* mutations than in *PIK3CA* alone, suggesting a possible synergistic effect in the signaling pathways controlled by the oncogenes in colorectal cancer (Velho et al., 2005; Nosho et al., 2008). In 52% of all patients, at least one mutation of these 3 genes was found in the research of breast cancer. Both the mutant locations in *PIK3CA* and the mutation frequency were very similar to previous studies in Caucasian breast cancer (Saal et al., 2005). Regarding histologic types, the mutant types of *PIK3CA* in breast cancer have rarely been reported other than in invasive ductal carcinomas (Lin et al., 2009). Two mutational hotspots (exons 9 and 20) of *PIK3CA* have been identified in various malignancies, including breast, lung, and colorectal cancers (Samuels et al., 2004).

In different tumor types, activated key signals of the PI3K/protein kinase B (AKT)/mammalian target of rapamycin and RAS/RAF/MEK signaling pathways regulate cell proliferation, growth, apoptosis, invasion, and migration (Peyssonnaux and Eychène, 2001; Engelman, 2009). Several studies have suggested that aberrations in PI3K/AKT/mammalian target of rapamycin and the MAP kinase pathway likely coexist, but few studies have examined this, and most were concentrated on colorectal cancer (Engelman, 2009; Ihle et al., 2009). *PIK3CA* expression is directly associated with the expression of epidermal receptor growth factors family members (EGFR and human epidermal growth factor receptor 2). Currently, numerous events are mediated by the over-expression of EGFR and human epidermal growth factor receptor 2, which induce an increase in the PI3K/Akt signaling pathway (Thompson and Thompson, 2004). KRAS is a downstream regulated gene and plays a vital role in the EGFR signal transduction pathway. However, *KRAS* mutation causing EGFR signals to not be accepted may automatically activate the pathway and downstream signals (Soulières et al., 2010). Not all patients could be tested for mutations because of the limited amount of tumor tissues available. Mutations in *KRAS* and *BRAF* were previously found to be mutually exclusive (Davies et al., 2002). Additionally, activation of the RAS/RAF/MEK pathway mediated the resistance to PI3K inhibitors of *PIK3CA* mutation in tumors (Ihle et al., 2009; Janku et al., 2012). Clinical data have shown that a single PI3K/AKT/mammalian target of rapamycin pathway inhibitor may be insufficient for inducing a response, as *PIK3CA* mutations often coexist with other concurrent molecular aberrations (Ihle et al., 2009; Di Nicolantonio et al., 2010). Mutations in *PIK3CA*, *BRAF*, and *KRAS* may trigger sustained activation of the RAS/RAF/MAPK and PI3K/PTEN/Akt signaling pathways.

In conclusion, bidirectional sequencing was used to detect mutations in 3 oncogenes. Gene mutations and activation of molecular pathways play a vital role in tumor formation. Therefore, our results are important for studies aimed at improving the treatment of breast cancer.

Conflicts of interest

The authors declare no conflict of interest.

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