RESEARCH ARTICLE

Phosphatidylinositol 3-kinase (*PI3KCA*) Oncogene Mutation Analysis and Gene Expression Profiling in Primary Breast Cancer Patients

Mahesh Kandula[1], Kalyan Kumar Chennaboina[1]*, Ammi Raju YS[1], Suryanarayana Raju[2]

Abstract

Background: The phosphatidylinositol 3-kinase (PI3K) pathway plays a significant role in apoptosis, cellular proliferation and motility. The aim of the present study was to analyze mutations and gene expression profiles of the *PI3KCA* gene to determine any role in breast carcinomas. Materials and Methods: We analyzed 38 breast cancers for mutations in the two PIK3CA hotspots in exons 9 and 20 by direct sequencing of DNA obtained from biopsy samples. We have also analyzed expression of the *PI3KCA* gene in 38 breast carcinoma tumor and corresponding control tissue samples at the mRNA level by RT-PCR. The Fisher's exact test (2×2 only) was performed using MedCalc software for to examine associations with mRNA levels. Results: In the present study a total of 13 cases demonstrated somatic mutations. In 9/13 cases 1633 G>A (E545K) were found in exon 9, whereas in exon 20, 4/13 cases had 3140A>G mutation. Our combined analysis showed *PI3KCA* mutations present in 34% of human breast cancer patients. In our study, we have also clearly found significantly higher expression in breast cancer tissues in comparison with control tissues (p=0.001). Conclusions: PIK3CA mutation is an emerging tumor marker that, in the future, might be used in the process of choosing a treatment. The detection of *PI3KCA* mutation might have important clinical implications for diagnosis, progression and therapy.

Keywords: Phosphatidylinositol 3-kinase - breast cancer - gene expression - mutations

Asian Pac J Cancer Prev, **14 (9)**, 5067-5072

Introduction

Phosphatidylinositol 3-kinase/AKT/mTOR pathway plays an important role in proliferation, migration and survival. Activation of the phosphatidylinositol 3-kinase/AKT/mTOR pathway is attained through the mutation in the p110α subunit of the *PI3KCA* gene which leads to the development of cancer (Samuels, 2012). Mutations in the *PI3KCA* gene play a significant role in carcinogenesis and progression which occurs in many cancers including breast cancer (Vivanco, 2002). It is evident from previous studies that mutation of *PI3KCA* occurs in 18-40% of the breast cancer patients (Bachman et al., 2004; Campbell, 2004; Levine, 2005; Saal, 2005). The *PI3KCA* gene consists of 20 exons and most common frequent mutations of *PI3KCA* associated with breast cancer are seen in exon 9 and exon 20 which encodes a helical domain and a COOH-terminal kinase domain respectively (Lee, 2005). Mutations affecting the two hot-spots have recently been demonstrated to be functionally different (Zhao, 2008). These mutations resulted in the modulation of the amino acid E545K in the helical domain and H1047R in the kinase domain which eventually led to the increased activity of the *PI3KCA* (Samuels, 2004). A preclinical study has demonstrated that breast tumors with the mutations in the *PI3KCA* gene are resistant towards the PI3K inhibitors due to RAS/RAF/MEK pathway activation (Ihle et al., 2009). The occurrence of *PI3KCA* mutations are more commonly seen in the ER-α positive breast cancer patients than in ER- α negative patients(Samuels, 2004). Studies have demonstrated a better overall survival (OS) and disease free survival (DFS) in breast cancer patients with *PI3KCA* mutations when compared with that of breast cancer patients without these mutations (Maruyama, 2007; Stemke-Hale et al., 2008; Kalinsky et al., 2009; Dupont, 2011). However, others showed that *PI3KCA* mutations in breast cancer patients are associated with poor clinical outcome (Li, 2006; Barbareschi et al., 2007; Lai et al., 2008). Our aim of this study is to identify mutational status of the two hotspot regions of *PIK3CA* (exons 9 and 20) in breast cancer. We also hypothesized that mutations resulting in the activation of the *PIK3CA* pathway may play a role in the progression of breast cancer. To test this hypothesis, we analyzed gene expression profiles

*[1]Department of Biotechnology, Krisani Biosciences Private Limited, [2]Nizams Institute of Medical Sciences, India *For correspondence: kalyan.ch@krisanibio.com*

of *PI3KCA* in both primary breast tumoral tissue and corresponding normal tissue.

Materials and Methods

Study population

Breast carcinoma patients were assessed on the basis of clinical and pathological examinations. This Study is a Hospital-based case-control study conducted in South Indian population. All incidents of breast cancer cases were newly diagnosed during the study period Ethical committee approved the study for the benefit of humans in general. The procedures followed were in accordance with the ethical standards of responsible committee of the Institutes/Hospitals, to participate in a face-to-face interview using a structured questionnaire.

Selection criteria

Senior pathologists confirmed all diagnoses. We interviewed and collected the data about the patient's demographic factors; we collected the information on age, smoking, chewing, usual alcohol intake, and previous cancer diagnoses. Participants were also asked about their family history of cancer, and the clinical information for these cases was obtained from medical records like tumor size, stage, and whether they were receiving chemotherapy, and radiotherapy. Patients were recruited following certain inclusion and exclusion criteria, which were determined before the beginning of the study.

Inclusion and exclusion criteria

All new cases of clinically confirmed breast cancer would be taken for study. Patients of confirmed breast cancer who give their consent were included. All patients who refuse to give consent were excluded.

Sample collection

For mutation analysis, based on the above criteria, a total of 38 breast cancer patients were enrolled in the study. About 3ml Blood samples were collected in from pathology lab after diagnosis. All the samples diagnosed mainly as invasive Ductal Carcinoma (IDLS). For gene expression study, human breast tumor tissues and corresponding normal tissues were collected after surgery in RNAlater (Ambion). All collected tissue and blood samples were preserved and stored at 37°C overnight. All samples were obtained from the Indo American cancer Hospital & NIMS Hospital, Hyderabad, between August 2011 - June 2012. The patient cohort consisted of 38 Invasive ductal carcinoma. Informed consent was obtained from all the individuals prior to collection of samples. The ethics committee approved the study from where the samples were collected.

DNA isolation

DNA was isolated from the tissue samples from breast cancer patients and blood samples from healthy volunteers by a rapid non-enzymatic method by salting out cellular proteins with saturated solution and precipitation by dehydration (Alluri et al., 2008). The red blood cells were lysed completely using RBC lyses solution. The lysate were then treated with cell lysis solution in order to lyse the cell components. The protein content is removed by protein precipitation solution. The precipitated DNA was suspended in 70% ethanol in order to remove the salts. The DNA was then dissolved in TE buffer and stored at 4°C .Cell lysis, protein precipitation, DNA precipitation and DNA hydration were carried out in the experiment.

PCR and direct sequencing

One sample of the genomic DNA was used per 25m l of PCR mixture, PCR buffer with 15 mM $MgCl_2$, 0.15 mM dNTPs, 1U/ML Taq polymerase and 12.5 pmol of each primer (Table 1). After mixing all the contents, PCR tubes were kept in thermal cycler and a 3 step PCR was performed with an initial denaturation at 94°C for 5 minutes followed by cycling at 94°C for 30 seconds, annealing for 30 seconds at appropriate temperature, 72°C for 45 seconds and a final extension at 72°C for 5 minutes was carried out for about 35 cycles. PCR products were analyzed on a 2% agarose gel and photographed on a UV light transilluminator. PCR products were purified by QIAquick PCR purification kit (Qiagen) and sense and antisense sequences were obtained by using forward and reverse internal primers, respectively. Each exon was sequenced using the BigDye Terminator Cycle sequence following the PE Applied Biosystem strategy and Applied Biosystems ABI PRISM3100 DNA Sequencer (Applied Biosystem, Forster City, CA). All mutations were confirmed by performing two independent PCR amplifications.

RNA extraction and cDNA synthesis

The samples were thawed and homogenized using omini THq electric homogeniser (Inkarp) Total RNA was extracted from biopsy of breast tumors and corresponding non-tumor tissues using the RNAeasy Mini Kit (Qiagen, Germany). According to the manufacturer's instructions. RNA was digested with DNase I (Invitrogen). The concentration and purity of extracting RNA were determined by measuring the absorbance at 260 nm and 280 nm. All samples whose concentration was a minimum of 100ng/μl and 260/280 between 1.8-2 were included in the study. Alternately the samples were run on agarose to check for the integrity. Reverse transcription was performed in a personal Master cycler (Bio-Rad CFX 96), using 1µg of total RNA in the presence of Random Hexamer (50ng/µl) and Reverse Transcriptase (50U/µl) in a total volume of 20µl, including also: 10×TaqMan RT Buffer, $MgCl_2$ solution (25mM), dNTPs mixture (10mM),

Table 1. PCR were Performed by using Specific Primers

Gene	Position	Primer Sequence
PI3KCA mutation analysis		
PI3KCA	Exon 9	F: GGGAAAAATATGACAAAGAAAGC
		R: CTGAGTCAGCCAAATTCAGTT
PI3KCA	Exon 20	F: CTCAATGATGCTTGGCTCTG
		R: TGGAATCCAGAGTGAGCTTTC
PI3KCA gene expression		
PI3KCA	cDNA	F: GGACAATCGCCAATTCAG
		R: TGGTGGTGCTTTGATCTG

an RNAse Inhibitor (20U/µl) and nuclease-free water. The reaction mixture was incubated for 10 minutes at 25°C, 60 minutes at 42°C, heated for 5 minutes to 95°C and then at 4°C for a minimum of 2 minutes. The resulting cDNA was stored at -20°C until further use.

Gene expression analysis

In assessing the relative gene expression on the SYBR chemistry of, *PI3KCA* gene, real time - polymerase chain reaction (qRT-PCR) was performed, with1 µl of cDNA, 12.5µl SYBR Green, and with specific primers (Table 1) were synthesized at Bioserve Biotechnologies Ltd. (Hyderabad, India). Primers were designed in different exons for all the genes to avoid amplification from contaminating DNA. A three-step PCR assay was standardized using a Bio-Rad thermo cycler and carried out with an initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30s, annealing at 58°C for 30s, and extension at 72°C for 45s. A final extension at 72°C for 5 min was carried out. Each sample was run in triplicates and each PCR experiment included one non-template control wells. A melt curve was also added to the PCR program for confirming a single intact PCR product without any non-specific amplification. An NTC was also added with all the experimental setups. Gene expression levels were normalized to the expression of the housekeeping gene GAPDH. In addition to SYBR green fluorescent detection, the amplified products were also resolved on 1.8% agarose gels and visualized by staining with ethidium bromide. In the present study, increased mRNA expression was defined as ≥2.0 folds, "normal" expression was ranging from 0.5001-1.9999 folds and decreased mRNA expression was ≤0.5 folds The data collection step was performed at a temperature that was 3°C lower than the melting temperature of each amplicon and higher than that of primer-dimers of each primer pair. This cycle was followed by a melting curve analysis, ranging from 55°C to 95°C, with temperature increasing steps of 0.5°C every 10 s. Baseline and threshold values were automatically determined for all plates using the Bio-Rad iQ5 Software 2.0.

Statistical analysis

The fisher's exact test (2×2 only) was performed by using MedCalc software for Windows (version 7.4.1.0; Mariakerke, Belgium) to examine the association of mRNA levels between breast cancer tumoral tissue and blood samples and different clinical and pathological parameters. The difference between the groups was considered significant if the p value was 0.05.

Table 2. Clinical and Pathological Characteristics of Breast Cancer Patients

All Patients		38 (100%)
	Age Range	29-73
	Median	47
Nuclear Grade	II	28 (74%)
	III	10 (26%)
Tumor Size		32 (80%)
4 above		6 (15%)
Lymph Node	Negative	20 (53%)
	Positive	18 (47%)
Hormonal Status	ER+/PR+	14 (37.5%)
	ER-/PR-	24 (63%)
Her-2 Status	Positive	22 (60%)
	Negative	16 (40%)
Menopausal Status	Postmenopausal	12 (37%)
	Premenopausal	25 (63%)

Results

Clinical and pathological characteristics of breast cancer patients

The clinical and pathological data of breast cancer patients were showed in Table 1. The median of patients age was 47 (ranged from 29-73). All 38 cases were invasive ductal carcinomas. The majority samples were of histological grade II with 15 cases, and ten cases which were grade III. The percentages of ER/PR negative tumors (63%) were higher when compared with ER/PR positive tumors (37.5%) (Table 2).

PI3KCA mutation analysis of Exon 9 and 20

The frequency of observed mutations is shown in Table 3. In the present study 38 primary breast cancer samples were used, total 13 cases contained somatic mutations. 9/13 cases 1633 G>A (E545K) (Figure 1A and 2A) were found in exon 9, whereas in exon 20, 4/13 cases found 3140A>G mutation (Figure 1B and 2B). Our combined analysis shows that *PI3KCA* mutations are present in 34% of human breast cancer patients.

Expression of PI3KCA gene in breast cancer tumoral tissue and corresponding normal tissue

The Relative gene expression patterns of *PI3KCA* gene were quantified in 38 breast cancer tumor samples and corresponding blood samples of the same individual by real time PCR. Gene expression levels were shown ratio between *PI3KCA* gene and the reference gene GAPDH to correct for the variation in the amount of RNA. The mRNA expression of the investigated genes of breast cancer tissue and blood samples was reported in Table 4 and depicted in Figure 1B. In this study, *PI3KCA* mRNA levels showed up regulation in 17 of 38 (21%) cases in tumoral tissues (range 2.10-9.15) and 6 of 38 (58%) cases in normal tissues (range 2.01-8.80). The increased expression of the *PI3KCA* was significantly greater (p=0.001) in the tumoral tissue when compared with normal tissues. In our , we found that all the 17 samples who showed

Table 3. List of *PIK3CA* exon 9 and exon 20 Mutations Identified in Breast Cancer

Exon	*PI3KCA* Mutation	Cases (n=38)
Exon-9	1633G>A (E545K)	9 (18.5%)
Exon-20	3140A>G	4 (10.5%)

Table 4. RT-PCR Analyses of mRNA Expression in Breast Cancer Patients

PI3KCA Expression	Tumoral Tissue (%) n=38	Normal Tissue (%) n=38	p value
Altered Expression	17 (64%)	6 (16%)	0.001
Normal Expression	14 (36%)	32 (84%)	

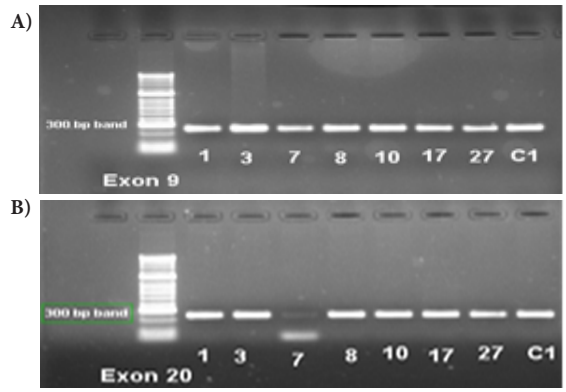

Figure 1. Agarose Gel Picture of A) exon-9 and B) exon-20 Primers Amplification

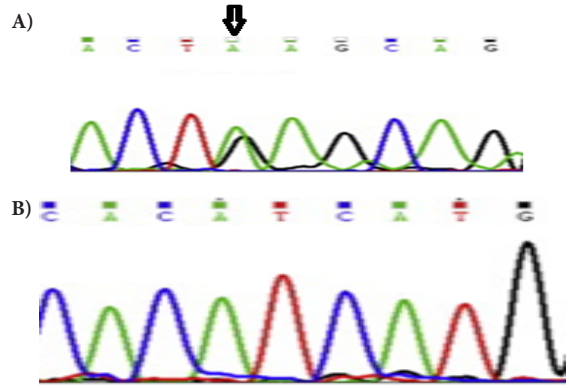

Figure 2. *PI3KCA* Mutation in A) exon-9 1633 G>A (E545K) and B) exon-20 3140A>G

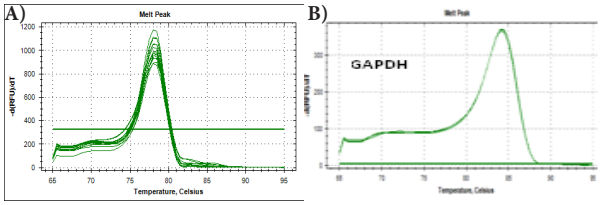

Figure 3. A) *PI3KCA* Melt Curve and B) GAPDH Melt Curve

Table 5. Correlation of the *PI3KCA* exon 9 and exon 20 Mutations and mRNA Levels with Clinical Data

All Patients		Exon 9	Exon 20	Altered Expression
Nuclear Grade	II	6 (66%)	2 (50%)	11 (64%)
	III	3 (34%)	2 (50%)	6 (36%)
Tumor Size	1-4	9 (100%)	4(100%)	12 (70%)
	≥4	0 (0)	0 (0)	5 (30%)
Lymph Node	Negative	5 (55%)	3 (75%)	7 (41%)
	Positive	4 (45%)	1 (25%)	10 (59%)
Hormonal Status	ER+/PR+	7 (77%)	3 (75%)	9 (52%)
	ER-/PR-	2 (23%)	1 (25%)	8 (48%)
Her-2 Status	Positive	7 (77%)	4(100%)	8 (48%)
	Negative	2 (23%)	0 (0)	9 (52%)
Menopausal Status	Postmenopausal	6 (66%)	3 (75%)	8 (48%)
	Premenopausal	3 (34%)	1 (25%)	9 (52%)

mutations in exon 9 and exon 20 were showed increased expression of *PI3KCA* in tumoral tissues when compared with corresponding normal tissues.

Correlation of the PI3KCA mRNA levels with clinical data

In the present study, Postmenopausal women with ER positive tumors showed high frequency of mutations in exon 9 (66%, 6 of 9) and exon 20 (75%, 3 of 4). ER, PR positive and negative tumors showed equal distribution 77% (7 of 9) of *PI3KCA* gene expression. Her-2 neu positive tumors showed high frequency of mutations in exon 9 (77%, 7 of 9) and exon 20 (100%, 3 of 4). *PI3KCA* gene expression is high in grade II tumors, these tumors also showed high frequency of exon 9 and exon 20 mutations in grade II cases. Tumor size between 1-4 and lymph node positive showed high frequency of gene expression and lymph node negative tumor showed high frequency of exon 20 mutations (Table 5).

Discussion

The *PIK3CA* pathway is frequently activated in different human cancers *PIK3CA* gene is mutated in an average of 15% of human cancers it is evident that cancer of the breast harbor the most *PIK3CA* mutation with average mutation frequency of 25%. In this study we have reported the presence of *PIK3CA* gene mutation in 28% of breast cancer tumors. The incidence of *PIK3CA* mutations found in our study 28% is high compared with previous reports. These somatic mutations may increase the kinase activity of *PIK3CA* contribution to cellular transformation. These mutations were predominantly found in kinase and helical domain of *PIK3CA*. Frequently occurring mutation were found in exon-9 and exon-20 Bachman et al. (2004). Reported that average 25% of breast cancers harbors mutation in either kinase or helical domain. Campbell et al. (2004) reported 40% of mutation frequency in all 20 coding exons (new as well as previously reported mutations). Bachman et al. (2004) and Campbell et al. (2004) studies noted no association of the presence of *PIK3CA* mutations with other clinical factors. Saal et al. (2005) reported 25% of *PIK3CA* mutation rate, in this study author found significant correlation between *PIK3CA* mutation and ER/PR positive and lymph node metastasis and Her2. Levine et al. (2005) reported 18% of mutation in exon-9 and exon-20 for breast cancer with no correlation between *PIK3CA* mutation and clinical data. Kang et al. (2005) found over expressed cDNA containing the common E545K mutations in chick embryo fibroblast. Sammul (2005) reported that common mutation of *PIK3CA* may be in increased activity of the *PIK3CA* enzyme as maintained by increased cell signaling, cell growth and invasion. *PIK3CA* mutation is an important pathway in cancer cell growth (Areumnuri, 2013).

The frequency of PI3K pathway mutations are different among the different breast cancer subtypes. A recent study has reconfirmed that the *PIK3CA* mutations occur almost exclusively in invasive tumors whereas upstream mutations of the PI3K-AKT pathway (PTEN and K-RAS mutations) occur with equal frequency in early- and advanced-stage tumors. This finding suggests that the *PIK3CA* mutations cooperate with these alterations in the malignant transformation (Oda et al., 2008). In our study Estrogen receptor positive tumors show high frequency of exon 9 and exon 20 mutations. Tumors with PI3K mutations were more likely to be ER/PR positive (Cooper et al., 2013). *PIK3CA* mutation status as an independent prognostic value in patients with ERBB2+ breast cancer. *PIK3CA* mutation status could serve as a new independent

prognostic tool when selecting targeted therapies for patients with ERBB2+ breast cancer (Magdalena et al., 2012). A HER2-positive patient treated with trastuzumab is significantly worse in patients with *PIK3CA*-mutated compared with wild-type tumours (Cizkova et al., 2013). This breast cancer subtype specificity suggests that *PIK3CA* mutations and other PI3K pathway aberrations may play a distinct role in the pathogenesis of breast diseases. *PIK3CA* mutations were also associated with sensitivity to tamoxifen. *PIK3CA* mutations will be important as these patients may respond well to hormonal therapy (Sherene et al., 2010). The presence of helical domain mutations may predict poor outcome whereas the presence of kinase domain mutations predicted an improved outcome. Magdalena et al. (2012) gene expression profiling study suggests that over-expression of genes belonging to the Wnt signaling pathway may also play a pivotal role in *PIK3CA*-mutated breast tumors.

Gene expression profiling is an important tool to evaluate genetic heterogeneity in carcinoma and is useful to develop expression based classification for different type of cancer. *PIK3CA* gene expression is associated with biological effects of aberrant cells proliferation and apoptosis, both of which are directly linked to tumor formation. In this study, we have analyzed the gene expression of *PIK3CA* gene in 38 breast carcinoma tumor and corresponding control tissue gene expression analyses performed at mRNA level by RT-PCR. In our study, we have clearly found significantly higher expression of *PIK3CA* in breast cancer tissues in comparison with control tissues. Moreover genetic alteration of *PIK3CA* gene been proved to increase expression of *PIK3CA* gene. Gene expression and gene mutation in *PIK3CA* gene have been reported in many human cancer types including breast cancer. We also demonstrated a significant correlation between the presence of *PIK3CA* mutation and the presence of *PIK3CA* expression. Sherene, 2013, data reported that the high levels of the *PIK3CA*-Gene Signatures are associated with the*PIK3CA* mutant genotype. It may facilitate better selection of responsive patient populations for mTOR inhibition in combination with letrozole. *PIK3CA* gene expression is associated with tumor aggressiveness in breast cancer pattern interfering that *PIK3CA* deregulation may have important biologic and cellular consequences in oncogenic development pathway from transforming through progression. Poor prognostic impact of *PIK3CA* mutation in breast cancer *PIK3CA* over expression may show short sequence and disease free survival. Brain and bone metastatic recurrence tend to over express *PIK3CA* than those who developed metastatic recurrence. *PIK3CA* expression was increased and was associated with high functional (PI3 kinase) activity.

In conclusion, genomic aberrations can predict responsiveness to targeted therapies, and because multiple PI3K pathway members are frequently aberrant in human breast tumors through mutation and other anomalies, this creates an expectation that targeting this pathway may provide an effective therapeutic approach in breast cancer. *PIK3CA* mutation is also an emerging tumor marker that, in the future, might be used in the process of choosing a treatment. However the detection of *PIK3CA* mutation has important clinical implications for diagnosis, progression and therapy.

References

Areumnuri K, Jung EL, Seung SL, et al (2005). Coexistent mutations of KRAS and *PIK3CA* affect the efficacy of NVP-BEZ235, a dual PI3K/MTOR inhibitor, in regulating the PI3K/MTOR pathway in colorectal cancer. *Int J Cancer*, **133**, 984-96.

Bachman KE, Argani P, Samuels Y, et al (2004). The *PIK3CA* gene is mutated with high frequency in human breast cancers. *Cancer Biol Ther*, **3**, 772-5.

Barbareschi MB, Felicioni L, Cotrupi S, et al (2007). Different prognostic roles of mutations in the helical and kinase domains of the *PIK3CA* gene in breast carcinomas. *Clin Cancer Res*, **13**, 6064-9.

Campbell IG, Russell SE, Choong DY, et al (2004). Mutation of the *PIK3CA* gene in ovarian and breast cancer. *Cancer Res*, **64**, 7678-81.

Cizkova M, Dujaric ME, Lehmann C, et al (2013). Outcome impact of *PIK3CA* mutations in HER2-positive breast cancer patients treated with trastuzumab. *Br J Cancer*, **108**, 1807-9.

Cooper L, Melinda S, Maria G, et al (2013). Clinical and pathologic characteristics of patients with PI3K-mutant breast cancers. *J Clin Oncol*, **31**, 26.

Dupont Jensen J, Laenkholm AV, Knoop A, et al (2011). *PIK3CA* mutations may be discordant between primary and corresponding metastatic disease in breast cancer. *Clin Cancer Res*, **4**, 667-77.

Ihle NT, Lemos R Jr, Wipf P, et al (2009). Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance. *Cancer Res*, **69**, 143-50.

Kalinsky K, Jacks LM, Heguy A, et al (2009). *PIK3CA* mutation associates with improved outcome in breast cancer. *Clin Cancer Res*, **16**, 5049-59.

Kang S, Bader AG, Vogt PK, et al (2005). Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. *Proc Natl Acad Sci*, **102**, 802-7.

Lai YL, Mau BL, Cheng WH, et al (2008). *PIK3CA* exon 20 mutation is independently associated with a poor prognosis in breast cancer patients. *Ann Surg Oncol*, **4**, 1064-9.

Lee JW, Soung YH, Kim SY, et al (2005). *PIK3CA* gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. *Oncogene*, **24**, 1477-80.

Levine DA, Bogomolniy F, Yee CJ, et al (2005). Frequent mutation of the *PIK3CA* gene in ovarian and breast cancers. *Clin Cancer Res*, **11**, 2875-8.

Li SY, Rong M, Grieu F, et al (2006). *PIK3CA* mutations in breast cancer are associated with poor outcome. *Breast Cancer Res Treat*, **19**, 1-5.

Magdalena C, Aurélie S, Sophie V, et al (2012). *PIK3CA* mutation impact on survival in breast cancer patients and in ERa, PR and ERBB2-based subgroups. *Breast Cancer Res*, **14**, 28.

Maruyama N, Miyoshi Y, Taguchi T, et al (2007). Clinicopathologic analysis of breast cancers with *PIK3CA* mutations in Japanese women. *Clin Cancer Res*, **13**, 408-14.

Oda K, Okada J, Timmerman L, et al (2008). *PIK3CA* cooperates with other phosphatidylinositol 3'-kinase pathway mutations to effect oncogenic transformation. *Cancer Res*, **68**, 8127-36.

Saal LH, Holm K, Maurer M, et al (2005). *PIK3CA* mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res*, **65**, 2554-9.

Samuels Y, Diaz J, Schmidt-Kittler O, et al (2005). Mutant *PIK3CA* promotes cell growth and invasion of human cancer cells. *Cancer Cell*, **7**, 561-73.

Samuels Y, Ericson K (2006). Oncogenic PI3K and its role in cancer. *Curr Opin Oncol*, **1**, 77-82.

Samuels Y, Waldman T (2010). Oncogenic mutations of *PIK3CA* in human cancers. *Curr Top Microbiol Immunol*, **347**, 21-41.

Samuels Y, Wang Z, Bardelli A (2004). High frequency of mutations of the *PIK3CA* gene in human cancers. *Science*, **304**, 554.

Sherene L, Benjamin HK, Samira MF, et al (2010). *PIK3CA* mutations associated with gene signature of low mTORC1 signaling and better outcomes in estrogen receptor-positive breast cancer. *PNAS*, **107**, 22.

Sherene L, Stefan M, Jose B, et al (2013). *PIK3CA* genotype and a *PIK3CA* mutation-related gene signature and response to everolimus and letrozole in estrogen receptor positive breast cancer. *J Clin Oncol*, **9**, 8127-36 .

Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, et al (2008). An integrative genomic and proteomic analysis of *PIK3CA*, PTEN, and AKT mutations in breast cancer. *Cancer Res*, **15**, 6084-91.

Vivanco I, Sawyers CL (2002). The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer*, **2**, 489-501.

Zhao L, Vogt PK (2008). Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. *Proc Natl Acad Sci USA*, **105**, 2652-7.