Gene alterations in the PI3K/PTEN/AKT pathway as a mechanism of drug-resistance (Review)

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Abstract. The most common therapeutic approach for many cancers is chemotherapy. However, many patients relapse after treatment due to the development of chemoresistance. Recently, targeted therapies represent novel approaches to destroy cancer cells. The PI3K/PTEN/AKT pathway is a key signaling pathway involved in the regulation of cell growth. Dysregulated signaling of this pathway may be associated with activating mutations of PI3K-related genes. Analyses of these mutations reveal that they increase the PI3K signal, stimulate downstream Akt signaling, promote growth factor-independent growth and increase cell invasion and metastasis. In this review, we summarize the PI3K/PTEN/AKT pathway genetic alterations in cancer and their potential clinical applications.

Contents

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1. Introduction

Phosphatidylinositol 3-kinases (PI3Ks) are lipid kinases that phosphorylate phoshoinositides at the D-3 position of the inositol ring generating second messengers that govern cellular activities and promote various biological properties including proliferation, survival, motility and morphology changes. Members of the PI3K family are grouped into three classes according to sequence homology, substrate preference and tissue distribution (1). In terms of regulating cell division and tumorigenesis, the most important PI3K proteins are those that belong to class IA, the catalytic subunit p110α and its associated regulatory subunit p85. In quiescent cells, the regulatory subunit p85 maintains the p110α catalytic subunit in a low-activity state. Upon growth factor stimulation, the SH2 domain [Rous-sarcoma (src) oncogene homology-2 domain] of the p85 subunit binds to phosphorylated tyrosine in receptor tyrosine kinases or their substrate adaptor proteins. This binding relieves the inhibition of p110α subunit and mediates recruitment of this subunit to the plasma membrane (2). Activation of p110α leads to the production of phosphatidylinositol 3,4,5-triphosphate (PIP3), which recruits adaptor and effector proteins containing a pleckstrin homology domain (PH domain) to cellular membranes including the protein kinase B (PKB/Akt), phosphoinositide-dependent kinase 1 (PDK-1) (3). Once at the membrane PKB/Akt is phosphorylated at Thr308 and Ser473 by PDK1 and the mTORC2 complex, respectively. Once activated PKB/Akt phosphorylates and actives target proteins involved in many different cellular functions, which span cell cycle progression, cell survival, metabolism, ribosome biogenesis, protein translation, RNA transcription and cell motility (4,5). PIP3 is a substrate of the phosphatase PTEN (phosphatase and tensin homologue, deleted on

chromosome 10), which dephosphorylates PIP3 to generate phosphatidylinositol 4,5-bisphosphate (PIP2), therefore, it is a negative regulator of PI3K signaling and functions as a tumor suppressor (6). This phosphatase is often mutated, deleted or downregulated in various tumors leading to constitutive activation of the PI3K pathway (7,8).

PI3K signaling pathway is altered in human cancers, it is caused by alterations in the control and activity of genes that regulate cell growth and differentiation, leading to abnormal cell proliferation (Fig. 1). These 'cancer-related genes' fall into two major classes that have opposite effects on normal cell proliferation and, sometimes, these altered genes have important effects in promoting cancer. Tumor suppressor genes normally repress cell growth and are inactivated in cancer, while oncogenes, such as protein kinases, which normally stimulate cell growth, become hyperactivated in cancer (9). Deregulation of the PI3K pathway has been directly implicated in several human cancer types. The best known genetic alterations of this pathway are loss of the tumor suppressor PTEN, amplification of genomic region containing Akt, and activating point mutations at PI3K. The most frequent mutations in PI3K, PTEN and AKT genes are reported in Table I by search in the COSMIC website (http://www.sanger.ac.uk/genetics/CGP/cosmic/) (Table I).

By using automated sequencing technology, it was demonstrated that the PI3K genes are mutated in human cancers. These studies have identified cancer-specific somatic mutations in several tyrosine kinase and tyrosine phosphatase genes as well as in the PIK3CA gene which encodes the p110α catalytic subunit of phosphatidylinositol 3-kinase. PIK3CA is a 34 kb gene located on chromosome 3q26.3 that consists of 20 exons coding for 1068 amino acids yielding a 124 kDa size protein.

The first study on PIK3CA gene mutation in human cancers was published by Samuels and colleagues (10). The authors initially analysed the sequence of eight PI3K and eight PI3K-like genes of primary colorectal tumors and discovered that PIK3CA was the only PI3K gene harboring somatic mutations. Subsequently, somatic mutations in the PIK3CA gene have been reported in many human cancer types including cancers of the colon, ovary, breast, brain, liver, stomach and lung. The PIK3CA mutations are somatic, cancer-specific and heterozygous and can be divided into four classes defined by the four domains of the catalytic subunit in which they occur, the adaptorbinding domain (ABD), C2 domain, helical domain and catalytic domain. Analysis of the PIK3CA gene in human tumor samples has identified somatic mutations that affect a total of 38 residues.

The majority of the mutations, 'hot-spot' mutation, map to three sites, E542 and E545 in the helical domain (exon 9) and H1047 in the kinase domain (exon 20). E542 and E545 are commonly changed to lysine, whereas H1047 is frequently substituted with arginine (Fig. 1). The crystal structure of the complex between the catalytic subunit of PI3Kα, p110α, and its regulatory subunit, p85α, revealed that many of the mutations occur at residues lying at the interfaces between p110α and p85α or between the kinase domain of p110α and other domains within the catalytic subunit affecting the regulation of kinase activity by p85 or the catalytic activity of the enzyme (11). It was well documented that these mutations cause a gain of protein enzymatic function and induce oncogenic transformation when expressed in primary chicken-embryo fibroblasts and in NIH3T3 cells (12).

2. PIK3CA mutations in human cancer

High frequency of somatic mutations is observed in the gene encoding the p110α catalytic subunit of PI3K are frequently detected in exon 9, encoding residues of the helical domain, and in exon 20, encoding the kinase domain. These mutations affect preferentially residues that are highly conserved and therefore of critical functional importance. By introducing mutations in three hot-spots of the human PIK3CA gene into the corresponding residues of the chicken PIK3CA gene, Kang and colleagues (12), demonstrated that these mutated p110 proteins induced oncogenic transformation when expressed in primary chicken embryo fibroblasts. All three mutants (E542K, E545K and H1047R) display enhanced lipid kinase activity and enhanced enzymatic activity as demonstrated by the high levels of phosphorylated Akt. One year later the same group presented further results demonstrating the oncogenic potential of these mutants also *in vivo*. The mutants of p110 induced tumors in the chorioallantoic membrane of chicken embryo and caused hemangiosarcomas in the animal (2). These results have been confirmed further by a study by Samuels *et al* (13) in which they analyzed the oncogenic activity of H1047R mutation using a different approach. They established an isogenic colorectal cancer cell line in which the mutant allele of PIK3CA gene was disrupted by gene targeting. For this purpose, the colorectal cancer cell line HCT116 was selected because it contains the hot-spot mutation H1047R in exon 20. HCT116 cells exhibited increased phosphorylation of Akt and the forkhead transcription factors FKHR and FKHRL1 in comparison to the PIK3CA WT isogenic HCT116 cells. Moreover, mutated HCT116 cells had a high apoptotic resistance and an increased ability to migrate and metastatize when injected in the tail vein of athymic nude mice. Similar results are observed using the colorectal cancer cell line DLD1 harboring the hot-spot mutation E545K in exon 9. The *in vitro* and *in vivo* oncogenicity of PIK3CA mutants strongly suggests a critical role for these mutated proteins in human malignancies and provides evidence that a kinase with a cancer-specific mutation, such as PI3K, might be an ideal target for specific small molecule inhibitors that could be developed as anticancer drugs. Since, p110α is a critical component of cellular physiology, small molecule inhibitors that discriminate the mutated and wild-type form of PIK3CA might minimize undesirable effects that could arise from interference with the wild-type protein.

3. PTEN mutations in human cancer

The most extensive evidence for the involvement of the PI3K pathway in human cancer stems from studies of the *PTEN* tumor suppressor gene. PTEN loss of function occurs in a wide spectrum of human cancers through mutations, deletions, transcriptional silencing, or protein instability at a frequency that can rival p53 alterations in particular settings (14). A lack of redundancy of PTEN might explain the high frequency of mutations. Despite its potential serine, threonine, and tyrosine phosphatase activity, the lipid phos-

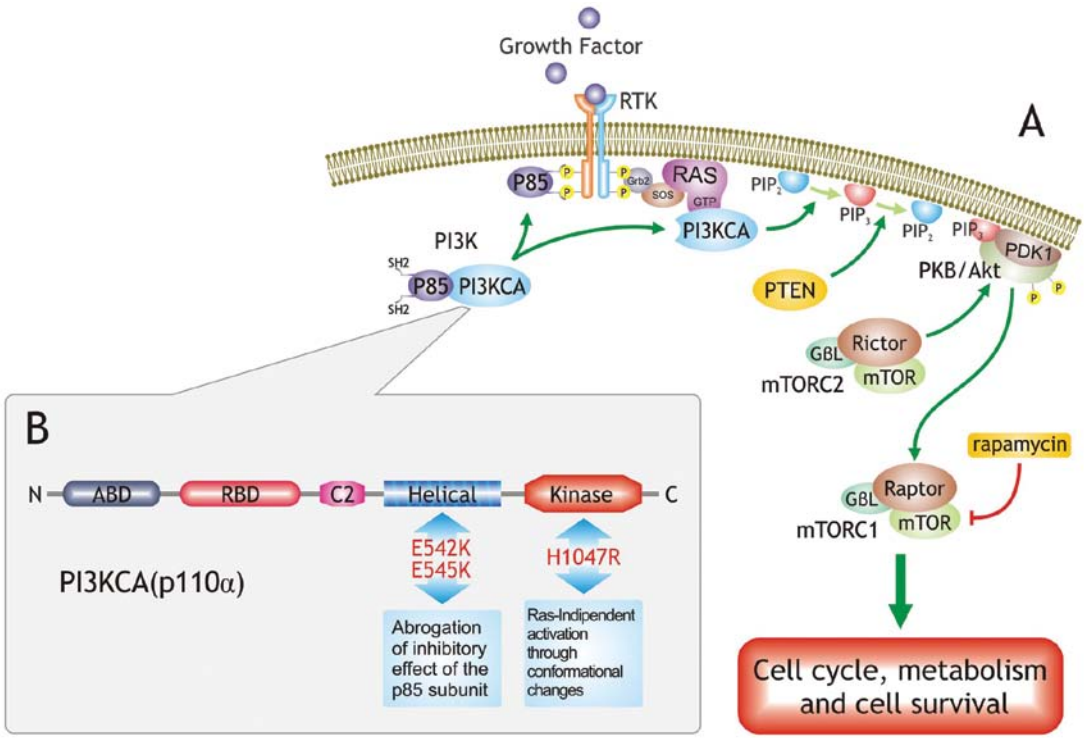

Figure 1. The PI3K/PTEN/Akt pathway. (A) Activation of PI3K/PTEN/Akt pathway could result in increased transcription of many genes that promote cellular growth and malignant transformation. Thus, targeting this signaling pathway may prevent cancer progression. (B) Description of PIK3CA and its functional domains with the most common somatic mutations.

phatase function of PTEN has been shown to be the major driving force in tumor suppression (15,16). A cancer-derived mutation G129E, which abrogates the lipid phosphate activity of PTEN but spares its protein phosphatase activity, results in inactivation of PTEN tumor-supressor function *in vitro* (17).

PTEN somatic mutations occur in a large percentage of human cancers, with the highest numbers found in endometrium, central nervous system, skin, and prostate cancers.

Germline PTEN mutations are present in ~80% of patients with Cowden syndrome (18). CS is an autosomal dominant disorder characterized by multiple hamartomas and a risk of breast, thyroid, and endometrial carcinomas. Renal cell carcinoma, certain brain tumors, and melanoma may occur at increased frequency in CS, although this association is less clear (19). Mutations have been reported to occur at PTEN in breast cancer in varying frequencies (5-21%) (20,21). Loss of heterozygosity (LOH) is probably more common (30%) (21). Mutations at certain residues of PTEN, that are associated with Cowden's disease, affect the ubiquitination of PTEN and prevent nuclear translocation. These mutations leave the phosphatase activity intact (22). Inhibition of PTEN activity leads to centromere breakage and chromosome instability (23). Thus, PTEN has diverse activities.

4. AKT alteration in human cancer

Akt/protein kinase B (PKB) is a 57 kD Ser/Thr kinase. Human genome contain three Akt genes, encoding the isoforms Akt1(PKBα), Akt2(PKBβ) and Akt3(PKBγ) (24) which are encoded by genes located on 14q32, 19q13.1-13.2 and 1q44 (25-27). Each gene encodes a protein containing an amino-terminal pleckstrin homology (PH) domain that serves to target the membrane for activation. The roles that Akt plays in cancer are complex. Akt can be activated by genetic mutations, genome amplifications and more commonly by mutations in upstream signaling components. Amplification of Akt-2 was observed in human ovarian carcinomas (26). Increased levels of Akt are detected in carcinomas of the breast, ovary and prostate and are associated with a poorer prognosis in comparison with tumors that do not display increased levels of expression. *AKT1* has been reported to be mutated in some breast, colorectal, melanoma and ovarian cancers (28,29). *AKT2* is not mutated frequently in human cancer. *AKT2* is amplified in certain cancers (e.g., 12.1% ovarian and 2.8% breast carcinomas) (29). A recent study documents the mutation of AKT3 in some melanoma samples (30).

Carpten *et al* (28) identified a transforming E17K PH-domain mutation in *AKT1* in breast (8%), colorectal (6%), and ovarian (2%) cancers. This study documented an Akt mutation that results in a glutamic acid (E) for a lysine (K) substitution at amino acid 17 (E17K) in the PH domain. This E17K-*AKT1* mutant exhibits transforming activity *in vitro* and *in vivo* owing to its constitutive PIP3-independent recruitment to the membrane. However, identification of frequent missense mutations causing increased activity of any of the three AKT isoforms has been elusive (Table I).

Cells with this *AKT1* mutation have not been observed to have mutations at *PIK3CA*. Akt and PI3KCA mutations were mutually exclusive, a similar scenario is also frequently observed with *RAS* and *BRAF* mutations (31). This *AKT1* mutation alters the electrostatic interactions of Akt-1 which

Table I. Frequency of PI3K, PTEN and AKT somatic mutations by tumor type.

Gene	Cancer type	Mutated/tested cases (%)*	Most frequent gene mutation/all mutations (%)
PI3K			**3140A>G**
	Breast	1493/5835 (26)	621/1493 (42)
	Large intestine	753/6011 (12)	144/753 (19)
	Endometrium	233/927 (25)	52/233 (22)
	Ovary	136/1525 (9)	43/136 (32)
	Urinary tract	189/942 (20)	26/189 (14)
PTEN			**388C>G**
	Endometrium	169/1822 (9)	54/169 (32)
	Ovary	15/845 (2)	3/15 (20)
	CNS	52/3523 (2)	3/52 (6)
	Lung	4/1325 (0.3)	2/4 (50)
AKT			**49G>A**
	Breast	86/1749 (5)	47/86 (55)
	Urinary tract	11/343 (3)	11/11 (100)
	Thyroid	10/351 (3)	10/10 (100)
	Lung	6/1539 (0.3)	6/6 (100)
	Endometrium	5/241 (2)	5/5 (100)

*Data are obtained from the COSMIC website.

allows it to form new hydrogen bonds with the natural PtdIns ligand (28).

The PH domain mutation confers many different properties to the *AKT1* gene. Namely the mutant *AKT1* gene has: i) an altered PH domain conformation, ii) is constitutively-active, iii) has an altered cellular distribution as it is constitutively-associated with the cell membrane, iv) morphologically transforms Rat-1 tissue culture cells and v) interacts with c-Myc to induce leukemia in Eμ-Myc mice (Eμ, enhancer of immunoglobulin μgene; Myc, Myc oncogene originally isolated in avian myelocytomatosis virus) (28). This PH domain mutated *AKT1* gene does not alter its sensitivity to ATP competitive inhibitors, but does alter its sensitivity to allosteric kinase inhibitors (28).

5. Mutations in PI3K pathway and chemoresistance

In vitro evidence suggests that PI3K activation is associated with decreased sensitivity to several different chemotherapeutic agents, including paclitaxel, doxorubicin and 5-fluorouracil. It was demonstrated that ovarian cancer cells overexpressing constitutively active Akt or containing AKT gene amplification were highly resistant to paclitaxel as opposed to cancer cells expressing low Akt levels. Constitutively active Akt inhibits the release of cytochrome c normally induced by paclitaxel thereby promoting apoptosis resistance (32,33). Breast cancer cell lines that express both HER2 and HER3 appear to have a higher degree of Akt activation. Moreover, transfection of HER2 in MCF7 breast cancer cells that express HER3 caused a PI3K-dependent activation of Akt, and was associated with an increased resistance to chemotherapeutic agents including paclitaxel, doxorubicin, 5-fluorouracil, etoposide and camptothecin. Selective inhibition of PI3K or Akt activity with their respective dominant-negative expression vectors sensitized the cells to chemotherapy-induced apoptosis (34). Liedtke *et al* (35) examined whether there is a correlation between activating mutations in the catalytic subunit of PI3K and response to therapy in stage II-III human breast cancer treated with preoperative chemotherapy. They hypothesized that activation of this pathway through somatic mutations may be associated with decreased response to cytotoxic treatment and increased residual cancer volume after chemotherapy.

They examined PIK3CA mutation status in 140 patients with stage II-III breast cancer and correlated the results with clinical and pathological variables, including response to chemotherapy. They did not observe any association between PIK3CA gene status and response to anthracycline-based or anthracycline-containing and paclitaxel-containing chemotherapies. They demonstrated that the frequencies of PIK3CA mutations were similar in patients with extremely chemotherapy-sensitive tumors and those with decreased responses (RCB-I or RCB-II) or even with extensive residual cancer (RCB-III). They also examined whether the effect of PIK3CA mutation on chemotherapy response was different among ER-negative and ER-positive tumors. PIK3CA mutation status was not predictive of response in either ER-positive or ER-negative tumors.

The PI3K pathway as a mediator of monoclonal antibody therapy resistance, one of the most successful examples of targeted therapies for epithelial cancers has been the demonstration that breast cancers with amplification of the ERBB2/HER2 oncogene are responsive to trastuzumab (Herceptin), a humanized monoclonal antibody directed against the transmembrane domain of the HER2 protein. Patients whose breast cancer

cells display overexpression of HER2 protein are candidates for this therapy in both the adjuvant and metastatic settings (36). However, not all women whose tumors overexpress HER2 respond to trastuzumab. Only one-third of women with newly diagnosed advanced breast cancer that overexpresses HER2 demonstrate tumor regression with trastuzumab therapy (37). By using an RNA interference (RNAi) genetic screen, Berns and colleagues identified that PTEN is a mediator of trastuzumab resistance in a HER2-overexpressing breast cancer cell line (38). Given that PTEN is a negative regulator of the PI3K pathway, and 25-30% of human breast cancers harbor somatic mutations in PIK3CA gene, activation of this pathway by somatic mutations could also lead to a similar trastuzumab-resistant phenotype. To test this hypothesis Berns and colleagues retrovirally transduced the breast cancer cell line BT-474 with a constitutively active mutant of PIK3CA (H1047R mutant). They demonstrated that expression of this mutant rendered BT-474 cells almost completely insensitive toward trastuzumab. Furthermore, overexpression of PIK3CA (WT) also conferred resistance to trastuzumab, as amplification of this gene has been demonstrated in other malignancies such as ovarian cancers.

These results were also confirmed by a study by Nagata *et al* (39) in which they demonstrated that PTEN activation contributes to the effects of trastuzumab antitumor activity. Trastuzumab treatment increased PTEN membrane localization and phosphatase activity by reducing PTEN tyrosine phosphorylation via Src inhibition. PTEN loss in breast cancer cells by antisense oligonucleotides conferred trastuzumab resistance *in vitro* and *in vivo*.

Patients with PTEN-deficient breast cancers had significantly poorer responses to trastuzumab treatment than those with WT PTEN. Moreover, they demonstrated that PI3K inhibitors restored trastuzumab sensitivity in PTEN-deficient cells, suggesting that PI3K-targeting therapies could overcome this resistance (39). These studies strongly supported the concept that alteration of the PI3K pathway influence trastuzumab resistance, suggesting that combination therapies with PI3K pathway inhibitors and trastuzumab might be more effective for the treatment of patients with breast cancers displaying HER2 overexpression. Jhawer *et al* (40) have demostrated that PIK3CA mutations predict response of colon cancer cells to the cetuximab inhibitory monoclonal antibody directed to the epidermal growth factor receptor (EGFR). Cetuximab is a monoclonal antibody that has been approved for the treatment of colorectal and head/neck cancers. Jhawer and colleagues screened a panel of 22 colon cancer cells and identified cetuximab-resistant and sensitive cells. Cetuximab-sensitive cells were found to be sensitive to EGF-induced proliferation, while cetuximab-resistant cells were not, suggesting that cells dependent on ligand mediated canonical activation of this pathway were sensitive to cetuximab. Moreover, examination of the mutation status of signaling components downstream of EGFR demonstrated that cell lines WT for PIK3CA/PTEN were significantly more sensitive to cetuximab than were cells with activating PIK3CA mutations or loss of PTEN expression. Consistently, the PIK3CA mutant HCT116 cell line was highly resistant to cetuximab, while a modest, although statistically significant response, was observed in the PIK3CA WT isogenic HCT116 cells. Lastly, cell lines mutant for both PIK3CA/PTEN and K-RAS/BRAF were highly resistant to cetuximab, indicating that mutations that constitutively and simultaneously activate the Ras and PI3K pathways confer maximal resistance to cetuximab.

6. Conclusion

Signal transduction pathways interact resulting in the promotion of cell cycle progression, growth and prevention of apoptosis. Elucidating the mechanisms by which signaling pathways cross-regulate each other and how they result in the prevention of apoptosis and promote drug resistance may yield more effective therapy. It has been shown that abnormal expression of signal transduction pathway, such as PI3K/PTEN/AKT, can contribute to drug resistance as well as resistance to targeted therapy. This review highlights in detail PI3K/PTEN/AKT gene alterations as responsible of dysregulated expression of this pathway. Controlling the expression of this pathway may improve cancer therapy.

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References

1. Kumar A and Carrera AC: New functions for PI3K in the control of cell division. Cell Cycle 6: 1696-1698, 2007.
2. Bader AG, Kang S and Vogt PK: Cancer-specific mutations in PIK3CA are oncogenic in vivo. Proc Natl Acad Sci USA 103: 1475-1479, 2006.
3. Corvera S and Czech MP: Direct targets of phosphoinositide 3-kinase products in membrane traffic and signal transduction. Trends Cell Biol 8: 442-446, 1998.
4. Brazil DP, Park J and Hemmings BA: PKB binding proteins. Getting in on the Akt. Cell 111: 293-303, 2002.
5. Yoeli-Lerner M and Toker A: Akt/PKB signaling in cancer: a function in cell motility and invasion. Cell Cycle 5: 603-605, 2006.
6. Vazquez F and Devreotes P: Regulation of PTEN function as a PIP3 gatekeeper through membrane interaction. Cell Cycle 5: 1523-1527, 2006.
7. Simpson L and Parsons R: PTEN: life as a tumor suppressor. Exp Cell Res 264: 29-41, 2001.
8. Leslie NR and Downes CP: PTEN function: how normal cells control it and tumour cells lose it. Biochem J 382: 1-11, 2004.
9. Weinberg RA: How cancer arises. Sci Am 275: 62-70, 1996.
10. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, *et al*: High frequency of mutations of the PIK3CA gene in human cancers. Science 304: 554, 2004.
11. Huang CH, Mandelker D, Schmidt-Kittler O, Samuels Y, Velculescu VE, Kinzler KW, Vogelstein B, Gabelli SB and Amzel LM: The structure of a human p110alpha/p85alpha complex elucidates the effects of oncogenic PI3Kalpha mutations. Science 318: 1744-1748, 2007.
12. Kang S, Bader AG and Vogt PK: Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. Proc Natl Acad Sci USA 102: 802-807, 2005.
13. Samuels Y, Diaz LA Jr, Schmidt-Kittler O, Cummins JM, Delong L, Cheong I, Rago C, Huso DL, Lengauer C, Kinzler KW, *et al*: Mutant PIK3CA promotes cell growth and invasion of human cancer cells. Cancer Cell 7: 561-573, 2005.
14. Ali IU, Schriml LM and Dean M: Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. J Natl Cancer Inst 91: 1922-1932, 1999.
15. Engelman JA, Luo J and Cantley LC: The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 7: 606-619, 2006.
16. Chow LM and Baker SJ: PTEN function in normal and neoplastic growth. Cancer Lett 241: 184-196, 2006.

17. Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, Hemmings BA, Wigler MH, Downes CP and Tonks NK: The lipid phosphatase activity of PTEN is critical for its tumor suppressor function. Proc Natl Acad Sci USA 95: 13513-13518, 1998.
18. Steelman LS, Bertrand FE and McCubrey JA: The complexity of PTEN: mutation, marker and potential target for therapeutic intervention. Expert Opin Ther Targets 8: 537-550, 2004.
19. Zbuk KM and Eng C: Cancer phenomics: RET and PTEN as illustrative models. Nat Rev Cancer 7: 35-45, 2007.
20. Rhei E, Kang L, Bogomolniy F, Federici MG, Borgen PI and Boyd J: Mutation analysis of the putative tumor suppressor gene PTEN/MMAC1 in primary breast carcinomas. Cancer Res 57: 3657-3659, 1997.
21. Singh B, Ittmann MM and Krolewski JJ: Sporadic breast cancers exhibit loss of heterozygosity on chromosome segment 10q23 close to the Cowden disease locus. Genes Chromosomes Cancer 21: 166-171, 1998.
22. Trotman LC, Wang X, Alimonti A, Chen Z, Teruya-Feldstein J, Yang H, Pavletich NP, Carver BS, Cordon-Cardo C, Erdjument-Bromage H, *et al*: Ubiquitination regulates PTEN nuclear import and tumor suppression. Cell 128: 141-156, 2007.
23. Gupta A, Yang Q, Pandita RK, Hunt CR, Xiang T, Misri S, Zeng S, Pagan J, Jeffery J, Puc J, *et al*: Cell cycle checkpoint defects contribute to genomic instability in PTEN deficient cells independent of DNA DSB repair. Cell Cycle 8: 2198-2210, 2009.
24. Datta SR, Brunet A and Greenberg ME: Cellular survival: A play in three Akts. Genes Dev 13: 2905-2927, 1999.
25. Staal SP, Huebner K, Croce CM, Parsa NZ and Testa JR: The AKT1 proto-oncogene maps to human chromosome 14, band q32. Genomics 2: 96-98, 1988.
26. Cheng JQ, Godwin AK, Bellacosa A, Taguchi T, Franke TF, Hamilton TC, Tsichlis PN and Testa JR: AKT2, a putative oncogene encoding a member of a subfamily of proteinserine/threonine kinases, is amplified in human ovarian carcinomas. Proc Natl Acad Sci USA 89: 9267-9271, 1992.
27. Nakatani K, Thompson DA, Barthel A, Sakaue H, Liu W, Weigel RJ and Roth RA: Up-regulation of Akt3 in estrogen receptor-deficient breast cancers and androgen-independent prostate cancer cell lines. J Biol Chem 274: 21528-21532, 1999.
28. Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S, *et al*: A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 448: 439-444, 2007.
29. Bellacosa A, de Feo D, Godwin AK, Bell DW, Cheng JQ, Altomare DA, Wan M, Dubeau L, Scambia G, Masciullo V, *et al*: Molecular alterations of the Akt oncogene in ovarian and breast carcinomas. Int J Cancer 64: 280-285, 1995.
30. Davies MA, Stemke-Hale K, Tellez C, Calderone TL, Deng W, Prieto VG, Lazar AJ, Gershenwald JE and Mills GB: A novel Akt3 mutation in melanoma tumours and cell lines. Br J Cancer 99: 1265-1268, 2008.
31. Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, *et al*: Patterns of somatic mutation in human cancer genomes. Nature 446: 153-158, 2007.
32. Jin W, Wu L, Liang K, Liu B, Lu Y and Fan Z: Roles of the PI-3K and MEK pathways in Ras-mediated chemoresistance in breast cancer cells. Br J Cancer 89: 185-91, 2003.
33. Page C, Lin HJ, Jin Y, Castle VP, Nunez G, Huang M and Lin J: Overexpression of Akt/AKT can modulate chemotherapy-induced apoptosis. Anticancer Res 20: 407-416, 2000.
34. Knuefermann C, Lu Y, Liu B, Jin W, Liang K, Wu L, Schmidt M, Mills GB, Mendelsohn J and Fan Z: HER2/PI-3K/Akt activation leads to a multidrug resistance in human breast adenocarcinoma cells. Oncogene 22: 3205-3212, 2003.
35. Liedtke C, Cardone L, Tordai A, Yan K, Gomez HL, Figureoa LJ, Hubbard RE, Valero V, Souchon EA, Symmans WF, *et al*: PIK3CA-activating mutations and chemotherapy sensitivity in stage II-III breast cancer. Breast Cancer Res 10: 27, 2008.
36. Hudis CA: Trastuzumab-mechanism of action and use in clinical practice. N Engl J Med 357: 39-51, 2007.
37. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, *et al*: Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 20: 719-726, 2002.
38. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, Linn SC, Gonzalez-Angulo AM, Stemke-Hale K, Hauptmann M, *et al*: A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 12: 395-402, 2007.
39. Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, *et al*: PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 6: 117-127, 2004.
40. Jhawer M, Goel S, Wilson AJ, Montagna C, Ling YH, Byun DS, Nasser S, Arango D, Shin J, Klampfer L, *et al*: PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab. Cancer Res 68: 1953-1961, 2008.