Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.03.028

PI3K pathway gene polymorphismin breast cancer tissues proliferation with synergistic effect of EBV- LMP1 oncogene activity among breastcancer patients in Basrah, southern of Iraq

Rasha N. Jawad[1*], Hassan J. Hasony[2], Jasim Al- Diab[3]
[1,2] Microbiology Department, College of Medicine, University of Basrah, Iraq
[3] Pathology Department, College of Medicine, University of Basrah, Iraq

***Corresponding author:** Rasha N. Jawad, Microbiology Department, College of Medicine, University of Basrah, Iraq, Email: rashanoorijawad@gmail.com

Submitted: 25 November 2022; Accepted: 12 December 2022; Published: 14 January 2023

ABSTRACT

PI3K is an intracellular heterodimer of two subunits regulater and catalytic one, induces many signalling pathways after activation. Specific primers used to detect PI3K in breast tissues of 186 patients with 84 benign of fibroadenoma and 72 invasive ductal carcinoma. conventional PCR detects that 97% of PI3K genome identity with standard strain and from 42 samples send to sequencing , there were (38.1%) ofPI3KCA samples were mutated, when 15 (93.75%) of them in malignant and just one (6.25%) was benign. PI3KCA mutation increased with the advancing grades of malignant tissue especially in grade III, and the variation increased directly with the age. It has been noticed that PI3K pathways mutation and EBV-LMP1 have a directly effect on tumor progression . PIK3CA mutationswere positively corelated with EBV-positive invasive ductal carcinoma and also associatedwith the mutation loci where those in exon 9 (ΔE542K), and exon 20 (ΔH1047R)are frequently detected and associated with greater PIK3CA mutations .

Keywords: *PI3K,Invasive ductal carcinoma, Breast cancer*

.

INTRODUCTION

phosphatidylinositol 3phosphate(PI3K) is one of the intracellular heterodimer proteins consisting of regulatory sub-unit protein p85 by which can link to the transmembrane receptor and another catalytic protein SU p110.1, 25 Its intracellular signaling pathway plays an essential role in the regulation of cell growth, apoptosis, and the cell cycle. Many factors, can increase the activity of the PI3K/Akt pathway as cancer-related viruses specially EBV oncoproteins2. Mutations of genes in these pathways produced mutated proteins, overexpressed or deleted base pair, depending on the case, resulting in biological effects which increased proliferation, genomic instability, changed cytoskeleton and deminsed apoptosis and many cancerous process.3

The PI3K pathway is one of the major signaling pathways with oncogenic properties in a variety of malignancies specifically, breast cancer tumorigenesis is believed to be related on the PI3K pathway, most are depend on the fact that at least one molecular mechanism that potentially activate the pathway. Themutations of the PI3Kgene is the main PI3K-promoting mechanisms, speciallyPIK3CA gene mutations.

The frequency reported to be 20 -30% suggests an important role of this gene in tumorigenesis4. The modifications of genes in many signaling pathways such as PI3K/AKT/mTOR promote the cancer dangerous by provoking critical cellular functions5. Depending to different studies, PIK3CA gene mutations in breast cancer are studied worldwidly.6,7,8,9Although Epstein Bar Virus EBV oncogenes can affect many signal pathways, such as NF-κB, MAPK, and JNK, but it seems that the PI3K/Akt pathway is the most essential one. This article focus on the role of PI3Kmutations in breast tissues and examines PI3K mutations and its association withEBV-LMP1 gene polymorphism.

METHODS

Breast cancer specimens in formalin fixed blocks were collected during the period between January 2019 to December 2021 from Al -Sadder teaching hospital. Total of 186patients with different age range from teens to elderly of 79 years old: on pathologic diagnosis basis, 84 with fibroadenoma, 72 with invasive ductal carcinoma and 5 benign cases , negative for EBV-LMP1. The tissue sample blocks were brought to Microbiology Departments\ Virology labs, Central laboratories of Basrah Medical CollegeforDNA extractionby grinding the tissue and dewaxing for preparinng PI3K genome by using G-DEX™IIc Genomic DNA Extraction Kit of Intron company. All samples submitted to conventional PCR technique using of four specific primers PI3K to cover all part of gene (1-770); (760-1550); (1538-2378) and (2364-3201). (Table 1), PI3KCA was splitted in to four pieces to be sequenced, then the DNA sequencing company removed overlap areas and compined the parts to get the whole gene sequencing by assembly PCR. The PCR products waspassing in gel electrophoresis to detect accurate bandssize which explained in detail procedure by10then sending it for DNA sequencing company.DNA sequencing:Cloned DNAs were purified and identified by DNA sequencing (GATC-Biotech Ltd (Germany)). Using online tools for Sequence analyzing which performedwith (https://www.gatc-biotech.com/en/mygatc4/single-readsequencing/mywatchbox/mysinglerun.html#watchboxlist_watchbox_div),(http://blast.ncbi.nlm.nih.gov),(http://www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html).

TABLE 1: Oligonucleotides primers and their sequences

Gene Target	Sequence (5'→3')	Company
PI3KC (1-770)	F- 5' TACGGAGGTGCTGGTAGTAGTCC '3 R- 5' GTAGGTGTGTGAAAATTTTATATGAACGGG '3	Invitrogen
PI3KC (760-1550)	F- 5' TGTGTGGATGTGATGAATAC '3 R- 5' TCAGTCCTGCGTGGGAATAGC '3	Invitrogen
PI3KC (1538-2378)	F- 5' CTCATTGTCTGATCGATCTCTG '3 R- 5' AGTAACTCTGACATGATGTCTGGG '3	Invitrogen
PI3KC (2364-3201)	F-5' TACAGTCTCAATGACAAAGTCTTG '3 R-5' CAATGCATGCTGTTTAATTGTGTGG '3	Invitrogen

J Popul Ther Clin Pharmacol Vol 30(3):e265–e272; 14 January 2023.

RESULTS

Breast cancer samples were amplified by PCR by using and 42 samples that showed typical bandsfor the four primers (Figure 1)and adequate quantity were selected to DNA sequencing of PI3KCA and EBV-LMP1. PI3KCA mutations were detected in 16 samples(38.1%) among them 15(93.75) in malignant and just 1 (6.25%) in benign and none of the control group. The differences was statistically significant (P<0.01). (Table2). Wild PIK3CA was the dominant (61.9%) among them 53.8% in malignant cases compared to 26.6% among the benign samples and 19.2% in the control .

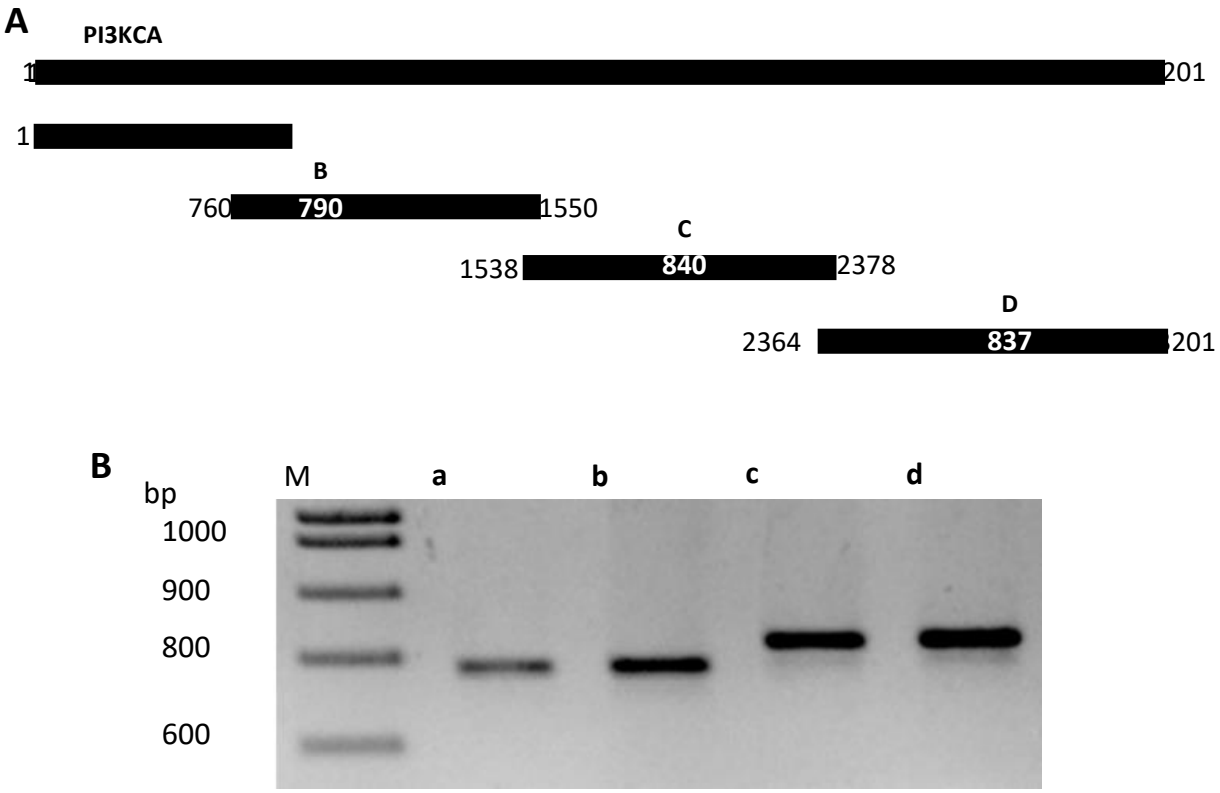

FIGURE 1: PI3KCA parts to cover the gene. A) diagram showed the parts that used to sequence PI3KCA. B) bands of PI3KCA products showed by gel elctrophoersis. M= Marker 1000bp; a= (1-770); b (760-1550); c= (1538-2378); d (2364-3201)

TABLE 2: Frequency of PI3KCA mutation among malignant , benign and control group.

PI3K AC	B		M		Control		Total		X2	P value
	No	%	No	%	No	%	No	%		
Mutants	1	6.25	15	93.75	0	0	16	38.1	68.0028	0.01
Wild	7	26.9	14	53.8	5	19.2	26	61.9	18.5148	0.01
Total	8	19	29	69	5	19.2	42	100		

The study showed that PI3KCAmutation increased significantly (P<0.01)by age of patients (Table 3) with 8 out of 15 (53.3%) of all mutation samples were foundin patients above fifties, while 6 samples (40%of malignant) weredetected at age group 30 to 49 years old and just one sample(7.6%) showed mutated PI3KCA at age group 10-29 years old (Table 2). The majority of wild type were detected at age group of 30-49 years (50%) which showed no significant differences (P>0.05).

TABLE 3: Distribution of PI3KCA mutations according to patient age and PI3K type:

Age groups	No.	%	PI3K				Control		X2	P value
			Wild		Mutants					
			n	%	N	%	N	%		
10-29	8	19.04	6	27.3	1	7.6	1	2	28.7138	0.01
30-49	19	45.24	11	50	6	40	2	40	1.36594	NS
> 50	15	35.72	5	22.7	8	53.3	2	40	12.3556	0.01
Total	42	100	22	100	15	100	5	100		

According to pathologic diagnosis 28 pools of DNA from malignant samples withinvasive ductal carcinoma were sequenced forPI3KCA andfound that a significant($P<0.01$) numbers of PI3KCA mutation appeared among patients with gradeIII in 8 out of 13 (61.5%), while grade 2 in 4/13(30.7%) and just one sample in grade 1 (7.7%). On the other hand, the non-mutated showed rise among the grades I and II (Table 4).

TABLE 4: Occurrence of PI3K mutation in relation to BC grading.

PI3KCA	Total		BC grade						X2	P value
			G1		G2		G3			
	No	%	n.	%	n	%	n	%		
Mutants	13	46.4	1	7.7	4	30.7	8	61.5	43.7646	0.01
Non-mutated	15	53.6	1	6.7	7	46.7	7	46.7	38.9094	0.01
TOTAL	28	100	2	100	11	100	15	100		

Table 5 showed that mutation of PI3KCAgenome significantly associated($P<0.01$) with occurrence ofdeletion in EBV-LMP1 as there were 13 mutated PI3K out of 15(86.7%)with EBV-LMP1 deletion mutationindicating the impact of LMP1 deletion in enhancing PI3KCA mutation, while 25 samples out of 27 (92.6%) of the wildtype of PI3KCA associated with non-deletion mutation in EBV-LMP1.

TABLE 5: Relation of PI3KCA mutation to EBV-LMP1 deletions.

PI3K	EBV-LMP1				Total	X2	P value
	deletion		Non-delet.				
	n.	%	n.	%	No. (%)		
Mutants	13	86.7	2	13.3	15 (35.7)	53.8756	0.01
Non-Mutated	2	7.4	25	92.6	27 (64.3)	72.5904	0.01
Total	15	35.71	27	64.28	42 (100%)	8.16327	0.05

Different kinds of PI3KCA mutation were observed in the results of DNA sequencing. Five mutants showed ΔH1047R variants from them 4 were positive EBV-LMP1(80%) and one were negative EBV-LMP1,their frequency was 33.3% (Table 6) and Four mutants ΔE542K was detected in 4 of EBV-LMP1 positive samples(100%) , their frequency 26.6% which represent a significant relation between the occurrence of specific locus hotspot mutations and EBV-LMP1 infection specially those with deletions. Another 4 substitution mutations ΔR88Q; ΔR93Q; ΔC420R and ΔE545K were appeared once in malignant samples. For second type of mutation there were deletion mutations identified in 2 samples, one including 20 base pair in area of 593 to 602 EBV-LMP1 positive sample and the other 9 base pair between 330-350 in EBV-LMP1 negative sample(Table 6).

PI3KCA hotspot mutations at positions of ΔH1047R and ΔE542K are associated with EBV-LMP1 positive cases which presented with 26.6% for each indicating their relation to EBV-LMP1 variants .

TABLE 6: local positions of PI3K mutation of BC in Basrah

Type of mutation	No	%	EBV-LMP1			
			+ve		-ve	
			n.	%	n.	%
330-350	1	6.7	0	0	1	6.7
593-602	1	6.7	1	6.7	0	0.0
ΔH1047R	5	33.3	4	26.6	1	6.7
ΔE542K	4	26.6	4	26.6	0	0.0
ΔR88Q	1	6.7	1	6.7	0	0.0
ΔR93Q	1	6.7	1	6.7	0	0.0
ΔC420R	1	6.7	1	6.7	0	0.0
ΔE545K	1	6.7	1	6.7	0	0.0

DISCUSSION

In this study, we analyse the correlation between presence of EBV-LMP1 and PI3KCA mutation in breast cancer cells11. EBV-LMP1 was used to detect presence of EBV in tissue 12,13. The study analyse DNA sequencing of 42 samples, 37 of them were positive to EBV-LMP1 and the other 5 were benign and negative to LMP1 and used as a negative control for sequencing of PI3KCA. The result showed that mutation of PI3KCA increased in malignant which was observed in 93.75% which is greater than the reported figure in other studies where the mutations of PI3K were noticed in 20–30% of all patients with breast cancers 14,15. The PI3KCA that showed mutation in breast cancer, was proved in several studies 16,17. PI3K involved in several signaling pathways to induce proliferation or regulation of cell apoptosis, survival and diffrentiaion 16. Compatibile with this study result, PI3KCA was shown to be muatated in about 93.7% of malignant breast cancer samples and most of them recorded in samples of elderly 18. Advanced grades of tumor asgrade IIIthat shown in this study has contributed to more mutation rate in PI3KCA in the presence of EBV- LMP1. Some other studies explained this phenomenon due to the patient taking of different kinds of drugs and chemotherapies that may affect the PI3KCA mutation rates19. However, different ways that the oncoprotein LMP1 and LMP2 were found to perform in order to disrupt cell signalling pathways one of these is AKT pathway20 which is in agreement with the finding of this study.

Presence of EBV-LMP was confirmed to be an activation factor for PI3K and cause mutation in it. It showed that EBV-LMP1 and 2 play a critical role in malignant metastasis20,21,22. These findings clearly distinguish the presence of LMP1 might triggered the PI3K mutation and that was clearly appeared in results of this study which showed that about 31% of samples were mutated in PI3K and LMP1 togather in same samples (Table 5). 93.7% of malignant samples with PI3K mutation, when others14,15 found these mutation in 20-30%. Theexon 9 and 20were the important hot spots of oncogenic mutations, which code for activation kinase and helical domains of the enzyme and result in over-enhancing of this protein 4. In the helical domain of p110, there are exon 9 mutations which enable p110 escape from the inhibitory effect of p85 via the Src-homology 2 (SH2) domain. mutations of exon 20 are located near the activation loop of the kinase domain. thisstudy reported 10% frequency of PI3K somatic mutations in breast cancer, but later studies reported 30%when found PIK3CA was the most frequent mutation, associated with an increased kinase activity of the PI3K pathway. Mutant PIK3CA promotes cell growth and invasion of human cancer cells.

23 that almost consistent with the frequencies of 33.3% (H1047R) and 26.6% (E542 K) found in this study for exon 20 and exon 9 mutations respectively, although some studies reported lower frequency (16% for H10487R location)24. Which agree with Karacas et. Al who mentioned that PI3K p110 subunit were mutated in breast cancer25. These mutationsperformed by single nucleotide substitutions that determine amino acid substitutions, E542 K, E545 K and H1047R thatprompt transformation and tumorigenicity26. However, many tumor sequencing studies reported somatic mutations of PI3K, represented in certain hotspots, which lead to cancer progression by gaining a function for PIk3CA 27.Since, PI3K mutations in breast cancers, at E542K in exon 9 and H1047R in exon 20, have been noticedin studies utilyzinging MCF10A cell lines as immortalized breast epithelial cells. This mutation in PI3K was found to be synergistically associated with mutation in LMP1 gene of EBV in same samples . Barbareschi et al. 28observed different effects based on mutation loci. They mentioned that mutations in exon 9 (ΔE542K), were associated with poor prognosis, while those occurring in exon 20 (ΔH1047R) wereachieved better prognosis.Four other PI3K variants were detected in this study; ΔR93Q was recorded once and it was mentioned in a study and show no effect in PI3K activation 30. Furthermore , additional variants of ΔR88Q, C420R and E545K were detected in this study once and it was recorded before and showed to be contracted with phosphorylation of AKT, MEK1, MEK2 and protein S6 and these will increase proliferation and cell metastasis 30.31,32,33.

CONCLUSIONS

PIK3CA mutation were very important to study after the results emphasize their roles in malignant breast tissues, and its functional correlate, PI3K signaling strength, EBV-LMP1 deletion in determiningthe cellular mutational activation of this pathway.These mutations lead to enhanc the kinase activity of the PI3K pathway, resulting in abnormal deregulated cell proliferation. In our study, we provided evidence that PIK3CA mutation in E542K or H1047R, gave a more aggressive phenotype in breast cancer cells. Importantly, proved that PIK3CA mutation could lead to the constitutive activation of the PI3K/AKT/mTOR pathway which suppression of apoptosis, especially when coupled to EBV-LMP1 oncogene which may contributed in the overlapingwith the treatment and development of cancersto chemotherapy resistance in BC .

REFERENCE

1. Ortega, M. A., Fraile-Martínez, O., Asúnsolo, Á., Buján, J., García-Honduvilla, N., & Coca, S. (2020). Signal transduction pathways in breast cancer: the important role of PI3K/Akt/mTOR. Journal of oncology, 2020. vol, no. page ??
2. Zhang, D., Yuan, Y., Zhu, J., Zhu, D., Li, C., Cui, W., ... & Liu, B. (2021). Insulin-like growth factor 1 promotes neurological functional recovery after spinal cord injury through inhibition of autophagy via the PI3K/Akt/mTOR signaling pathway. Experimental and Therapeutic Medicine, 22(5), 1-9.
3. Chen, J. (2012). Roles of the PI3K/Akt pathway in Epstein-Barr virus-induced cancers and therapeutic implications. World journal of virology, 1(6), 154.
4. Elwy, F.; Helwa, R.; El Leithy, A.A.; Shehab El din, Z.; Assem, M.M.; Hassan, N.H. PIK3CA mutations in HER2-positive Breast Cancer Patients; Frequency and Clinicopathological Perspective in Egyptian Patients. Asian Pac. J. Cancer Prev. 2017, 18, 57–64
5. Barbareschi, M.; Buttitta, F.; Felicioni, L.; Cotrupi, S.; Barassi, F.; Del Grammastro, M.; Ferro, A.; Dalla Palma, P.; Galligioni, E.; Marchetti, A. Di_erent prognostic roles of mutations in the helical and kinase domains of the PIK3CA gene in breast carcinomas. Clin. Cancer Res. 2007, 13, 6064–6069.
6. Ding,W.J.; Zeng, T.;Wang, L.J.; Lei, H.B.; Ge,W.;Wang, Z. Genes with mutation significance were highly associated with the clinical pattern of patients with breast cancer. Oncotarget 2017, 8, 98094–98102.
7. Hu, Z.Y.; Xie, N.; Tian, C.; Yang, X.; Liu, L.; Li, J.; Xiao, H.;Wu, H.; Lu, J.; Gao, J.; et al. Identifying Circulating
8. Tumor DNA Mutation Profiles in Metastatic Breast Cancer Patients with Multiline Resistance. EBioMedicine 2018, 32, 111–118.
9. Perez-Tenorio, G.; Alkhori, L.; Olsson, B.; Waltersson, M.A.; Nordenskjold, B.; Rutqvist, L.E.; Skoog, L.;Stal, O. PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. Clin. Cancer Res. 2007, 13, 3577–3584. [CrossRef] [PubMed]

10. Cizkova, M.; Susini, A.; Vacher, S.; Cizeron-Clairac, G.; Andrieu, C.; Driouch, K.; Fourme, E.; Lidereau, R.; Bieche, I. PIK3CA mutation impact on survival in breast cancer patients and in ERalpha, PR and ERBB2-based subgroups. Breast Cancer Res. 2012, 14, R28
11. Wigedan, N. AL-Moosawi, 2017. The relation of TEL-AML1 fusion genes, mutantP53 gene&B19 parvovirus infection in newly diagnosed pediatric Acute Lymphoblastic Leukemia, in Basrah. M.Sc. thesis medical mirobiology. Ch3: 66.
12. Vogt PK, Hart JR, Gymnopoulos M, Jiang H, Kang S, Bader AG, Zhao L, Denley A. Phosphatidylinositol 3-kinase: the oncoprotein. Curr Top Microbiol Immunol. ??????
13. Gulley, M. L. (2001). Molecular diagnosis of Epstein-Barr virus-related diseases. The Journal of molecular diagnostics, 3(1), 1-10.
14. Lee, M. A., Hong, Y. S., Kang, J. H., Lee, K. S., You, J. Y., Lee, K. Y., & Park, C. H. (2004). Detection of Epstein-Barr virus by PCR and expression of LMP1, p53, CD44 in gastric cancer. The Korean journal of internal medicine, 19(1), 43.
15. Bachman, K.E.; Argani, P.; Samuels, Y.; Silliman, N.; Ptak, J.; Szabo, S.; Konishi, H.; Karakas, B.; Blair, B.G.; Lin, C.; et al. The PIK3CA gene is mutated with high frequency in human breast cancers. Cancer Biol. Ther. 2004, 3, 772–775. [CrossRef]
16. Saal, L.H.; Holm, K.; Maurer, M.; Memeo, L.; Su, T.;Wang, X.; Yu, J.S.; Malmstrom, P.O.; Mansukhani, M.; Enoksson, J.; et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Res. 2005, 65, 2554–2559
17. Liu, P., Cheng, H., Roberts, T. M., & Zhao, J. J. (2009). Targeting the phosphoinositide 3-kinase pathway in cancer. Nature reviews Drug discovery, 8(8), 627-644.
18. Janku, F. (2017). Phosphoinositide 3-kinase (PI3K) pathway inhibitors in solid tumors: From laboratory to patients. Cancer treatment reviews, 59, 93-101.
19. Kalinsky, K., Jacks, L. M., Heguy, A., Patil, S., Drobnjak, M., Bhanot, U. K., ... & Moynahan, M. E. (2009). PIK3CA Mutation Associates with Improved Outcome in Breast CancerPIK3CA Mutation and Improved Outcome in Breast Cancer. Clinical cancer research, 15(16), 5049-5059
20. Mosele, F., Stefanovska, B., Lusque, A., Dien, A. T., Garberis, I., Droin, N.,& André, F. (2020). Outcome and molecular landscape of patients with PIK3CA-mutated metastatic breast cancer. Annals of Oncology, 31(3), 377-386.
21. Chen, J. (2012). Roles of the PI3K/Akt pathway in Epstein-Barr virus-induced cancers and therapeutic implications. World journal of virology, 1(6), 154.
22. Liang J, Slingerland JM. Multiple roles of the PI3K/PKB (Akt) pathway in cell cycle progression. Cell Cycle. 2003;2:339–345.
23. Mukohara, T. (2015). PI3K mutations in breast cancer: prognostic and therapeutic implications. Breast Cancer: Targets and Therapy, 7, 111.
24. Samuels, Y.; Diaz, L.A.; Schmidt-Kittler, O.; Cummins, J.M.; Delong, L.; Cheong, I.; Rago, C.; Huso, D.L.; Lengauer, C.; Kinzler, K.W.; et al. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. Cancer Cell 2005, 7, 561–573.
25. Huayu Hu, Junyong Zhu, Yuting Zhong, Rui Geng, Yashuang Ji, Qingyu Guan, et al . PIK3CA mutation confers resistance to chemotherapy in triple-negative breast cancer by inhibiting apoptosis and activating the PI3K/AKT/mTOR signaling pathway. Ann Transl Med 2021;9(5):410 | http://dx.doi.org/10.21037/atm-21-698
26. Karakas, B.; Bachman, K.E.; Park, B.H. Mutation of the PIK3CA oncogene in human cancers. Br. J. Cancer 2006, 94, 455–459.
27. Rand, A.; Yardena, S. PIK3CA in cancer: The past 30 years. Semin. Cancer. Biol. 2019, 59, 36–49
28. Paola Fuso 1,* , Margherita Muratore 1, Tatiana D'Angelo 2, Ida Paris 1 , Luisa Carbognin 1, Giordana Tiberi 1,et al.PI3K Inhibitors in Advanced Breast Cancer: The Past, The Present, New Challenges and Future Perspectives. Cancers 2022, 14, 2161 :1-21
29. Bizhani F, Hashemi M, Danesh H, Nouralizadeh A, Narouie B,Bahari G, et al. Association between single nucleotidepolymorphisms in the PI3K/AKT/mTOR pathway and bladdercancer risk in a sample of Iranian population. EXCLI J 2018;17:3–13.
30. Li, S. Y., Rong, M., Grieu, F., & Iacopetta, B. (2006). PIK3CA mutations in breast cancer are associated with poor outcome. Breast cancer research and treatment, 96(1), 91-95
31. Black, J. D., Lopez, S., Cocco, E., Bellone, S., Altwerger, G., Schwab, C. L., & Santin, A. D. (2015). PIK3CA oncogenic mutations represent a major mechanism of resistance to trastuzumab in HER2/neu overexpressing uterine serous carcinomas. British journal of cancer, 113(7), 1020-1026.

32. Oda, K., Okada, J., Timmerman, L., Rodriguez-Viciana, P., Stokoe, D., Shoji, K.& McCormick, F. (2008). PIK3CA cooperates with other phosphatidylinositol 3′-kinase pathway mutations to effect oncogenic transformation. Cancer research, 68(19), 8127-8136.
33. Gymnopoulos, M., Elsliger, M. A., & Vogt, P. K. (2007). Rare cancer-specific mutations in PIK3CA show gain of function. Proceedings of the National Academy of Sciences, 104(13), 5569-5574.
34. Li, J., Jeong, K. J., Shao, S., Chen, H., Tsang, Y. H., & Mills, G. B. (2018). Systematic functional annotation of somatic mutations in cancer. Cancer cell, 33(3), 450-462