Immunohistochemical Targeting of p110β Isoform of phosphatidylinositol 3-kinase co-associated with Cyclin-Dependent Kinase 1 in a Group of Tissues from Iraqi Patients with Breast Cancer

Mohammed A.M. Al-Qurtas *

ABSTRACT

Background: While two-thirds of breast cancers express hormone receptors for either estrogen (ER) and/or progesterone (PR) , genetically altered PI3K pathway was found in more than 70% of ER-positive breast cancers.An aberrant activity of cyclin-dependent kinase 1 (CDK1) in a wide variety of human cancers has selectively constituted an attractive pharmacological targets in MYC-dependent human breast cancer cells.

Aim of the study: Role of p110-beta as well as and CDK 1 in the pathogenesis of subset of breast cancers and contribution in their carcinogenesis.

Type of the study: is a retrospective study

Methods: This retrospective research enrolled 70 paraffin embedded breast tissue blocks which were retrieved from archives of the period 2011 till 2017 at major hospitals and private histopathological laboratories as well as Forensic Medicine Institute in Baghdad. They comprised 30 breast cancers, 25 benign breast tumors and 15 apparently normal breast autopsies. Two 4 mm - thick sections were specified on positively charged slides for monoclonal primary p110 as well as and CDK 2 antibodies using immune-enzymatic antigen detection system for immunohistochemistry (IHC) techniques.

Results: Seventeen out of 30 (56.7%) of the total breast cancer cases in this study showed positive immunohistochemistry reaction(IHC) for detection of P110-beta gene expression in these tissues. In the benign group, 6 out of 25 cases (24%) revealed positive IHC signals. None of control group presented positive signals. The differences begroup tween the percentages of P110-beta in breast cancers and each of control group and benign breast tumors group are statistically very highly significant (P value = < 0.0001). The expression of CDK1 was detected in 53.3% (16 out of 30) of breast cancers tissues and in 44% (11 out of 25) benign breast tumors, whereas none of control group of tissues showed CDK1- expression.

Conclusions: The present data indicate that p110-beta as well as and CDK 1 could have a role in the pathogenesis of subset of breast cancers and contribution in their carcinogenesis.

Keywords: Breast cancers; p110-beta;PI3K; CDK1; Immunohistochemistry.

Al-Kindy College Medical Journal 2017: Vol.13 No.2
Page: 127-136

*College of Alkindy Medical / Baghdad University, Iraq

Received 1th Dec 2017, accepted in final 30th Dec 2017
Corresponding to : Mohammed A.M. Al-Qurtas

Two-thirds of breast cancers are expressing hormonal receptors for either estrogen (ER) and/or progesterone (PR). Adjuvant anti estrogen therapies have a great successful role in these hormonal-dependent cancers, yet a respective bulk of patients developed resistant metastatic disease (1).A genetically altered PI3K pathway [its products, phosphatidylinositol 3,4,5 triphosphate (PIP3)] was found in more than 70% of ER-positive breast cancers (2,3). Activation of PI3K led to anti-estrogen resistance of such cancers (4). Tumor suppressor phosphatase and PTEN dephosphorylate PIP3 and then antagonize PI3K .Thus, combined inhibition of both p110α and p110β, was required for sustained therapeutic effect in cases of PTEN-deficient, ER-positive breast cancers . Herein, PI3K inhibitors are being developed for the treatment of breast and other cancers (5,6). The p110α is essential for growth of tumors driven by PIK3CA mutations while p110β can mediate tumorigenesis in some of those with PTEN-deficient cancers (7,8). Therefore, p110β-selective inhibitors have focused on those cancers which frequently harbor PTEN alterations, such as prostate cancers, lung squamous cell carcinomas, and the triple-negative breast cancers (9).Inhibition of PI3K suppresses anti-estrogen-resistant growth of ER-positive breast cancer cells (10, 11).Phosphatidylinositol 3-kinase a has specific function in cell survival and phosphatidylinositol 3-kinase b in DNA synthesis of human colon carcinoma cells (12).Phosphoinositide 3-kinases (PI3K) are lipid kinases that arerapidly produced upon cell stimulation and play a role of second messenger during cell growth, survival, migration and membrane trafficking (13). A specific function for phosphatidylinositol 3-kinase a (p85 α-p110 α) in cell survival and for phosphatidylinositol 3-kinase b (p85 α-p110 β) in de novo DNA synthesis of human colon carcinoma cells. The gain of function by p110‧mutation or overexpression was common in human cancers (14- 16). In contrast, no somatic mutations of the genes encoding of the β, γ, and δ isoforms and as such, their correlations to human cancers were much less reported (17), although increased expression of the β and δ isoforms occurred in some bladder and colonic tumors as well as glioblastoma (18, 19). The γ isoform controls migration of breast cancer cells, is requirement for regulation of the chemotaxis in cancer progression in carcinoma cells (20),and involved in tumor angiogenesis (21).Herein, the chemotaxis in macrophages requires PI3K_ and Class IA enzymes (i. e. p85/p110alpha and p85/p110 β) (22).
To date, twenty different CDKs have been reported in mammalian cells and about the same number of cyclins

(23). Three interphase cyclin-dependent kinases (CDK2, CDK4 and CDK6) and the mitotic (CDK1), were found asthe "master regulators" of cell cycle progression, molecular engines that drive cell cycle transitions (24,25).Expression of cyclin-D in association and activation of CDK4 and CDK6 promote phosphorylation of the Retinoblastoma pocket protein, de-repressing E2F transcription factors, thereby expression of genes for G1/S transition, including Cyclin E. (26,27). Phosphorylation of Cdk1 will inhibit its activity during G2-phase and Cyclin B1 (allosteric activator of Cdk1) is influenced by this phosphorylation (28,29),and later the anaphase promoting complex must destroy this cyclin B to allow proceeding of mitosis (30).Targeting cyclin-dependent kinase 1 (CDK1) but not CDK4/6 or CDK2 is selectively lethal to MYC-dependent human breast cancer cells.The MYC oncogeneamplification has been found in approximately 15% of human breast cancers (31,32). MYC over-expression results in transcriptional amplification andcell cycle progression (33).However, gene amplification was not only the reason of the observed MYC over-expression . In breast cancer cells, increased cyclin E-CDK2 activity has contributed to MYC-induced G1-S phase transition (34),possibly through suppression of CDK inhibitor, namely p21. However, consequently, cancerous cells have other genes or pathways to overcome the anti-tumorigenic effects of activation of MYC, whereby several cell cycle kinases identified as MYC-synthetic lethal genes in different types of cancer, including CDK2 (35) and CDK1 (36).Aberrantly activated CDK could induce unscheduled cellular proliferation that led to genomic as well as chromosomal instability in cancer cells and as such, series of specific CDK inhibitors have been developed for tumorous cells (37).This study is aiming to analyze the rate of concordance ofp110βand CDK1 translational expression in breast tissues from a group ofpatients with malignant and benign breast tumors.

Methods:

Study Groups: This study was designed as a retrospective research. Therefore, a collective number of (70) formalin-fixed, paraffin embedded breast tissue blocks enrolled in this study which comprised both patients and control samples that their age ranged from 38 to76 years. These retrospective paraffin-embedded samples were retrieved from the archives of the period from 2011 till 2017 belonging to major hospitals and private histopathological laboratories in Baghdad. The diagnoses were based on their accompanied pathological reports of the corresponding patients. These blocks included a group of (30) biopsies from patients who had undergone surgical operation or biopsies for their breast cancers (BC), (25) biopsies from patients who had undergone surgical operation or biopsies for their benign breast tumors and (15) autopsies from apparently normal breast tissues control group. These breast tissues were properly subjected to fixation as well as paraffin embedding and used for this research work as an age- and sex- matched groups.

Laboratory methods:

SLIDE PREPARATION: Tissue sectioning of the tissue blocks was conducted at the histopathological department of Teaching laboratories / Medical City and a confirmatory histopathological evaluation of each obtained tissue blocks was done by a consultant pathologist. One paraffin embedded (4 mm) thick-tissue section was prepared and mounted on ordinary glass slide and stained with hematoxyline and eosin, while other (4 mm) thick-tissue sections were stuck onto positively charged slide to be used for detection of p110-antigen using Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC kit that was purchased from (Abcam, UK) , an immunoenzymatic antigen detection system for immunohistochemistry techniques, using specificRabbit Monoclonal primary p110 antibody as well as and CDK 2 antibody which were also purchased from (Abcam, UK).The details of methods for performing IHC reaction with these antibodies were conducted according the instructions of that manufacturing company, and were done in the Research Laboratory of the Clinical Communicable Diseases Research Unit, at College of Medicine, University of Baghdad

Histopathological Analysis: According to the specification of the used kits, the proper use of IHC detection system gave an intense blue signal at specific sites of the antibody reaction in positive test tissues. The signal was evaluated microscopically using × 100 lens for counting the positive cells. The IHC results were given intensity and percentage scores based on intensity of positive signals and number of cells that gave these signals, respectively. Positive cells have been counted in 10 different fields of 100 cells for each sample where the average percentage of positive cells within the 10 fields was determined. A scale of 0-3 was applied for relative intensity with 0 corresponding to no detectable ISH reaction, and 1, 2, 3 equivalents to low, moderate, and high intensity of reaction respectively. The results were assigned to one of the following percentage score categories: 1%-25% (score 1), 26%-50% (score 2) or > 50% (score 3) (38).

Statistical Analysis:T test, ANOVA test, and Chi square have been applied for statistical examination of results obtained in this research. The statistical analysis was done by using Pentium-4 computer through the SPSS program (version-19) and Excel application.

Results

Distribution of patients with breast tumors group according to their Age .

The archival specimens collected in this study were related to breast tumor patients whom ages were ranged from fifteen years to seventy five years. The mean age of the patients with breast carcinoma (45.9 $\pm$ 11.9 years) was higher than the mean age of the benign group (42.5 $\pm$ 14.4 years) and the mean age of those females in the group of healthy control (36.8$\pm$ 14.4 years). There are significant statistical differences ($p< 0.05$) between different groups according to age (Table 1). In malignant breast tumors, the most commonly affected age stratum was (41-60) years which constituted 53.3% , while the age stratum (31-40 years) was constituted 30% , followed by 8.33 % for each of age stratum (15 - 30years) and (61 -75 years) . In benign breast tumors, the most affected age stratum (35 - 60years) constituting 56% followed by 22 % for each of age stratum of (15 -34 years) and (61-72 years) . Statistical comparison of these age strata revealed significant differences ($p< 0.05$).

Histopathological Grading of Breast Carcinomas:

On distributing breast carcinoma group according to their grading of breast cancers(Table 2) ,the results of present study show that poorly differentiated grade

breast carcinomas constituted 40% (12 of total 30 cases) , whereas cases with moderately and well differentiated grades constituted 33.3% (10 out of 30 cases) and 26.7 % (8 out of 30 cases) , respectively .The statistical analysis of grading distribution of breast carcinoma shows significant differences ($p<0.05$)between poorly differentiated grade and well differentiated grade, while non-significant difference was noticed between poorly differentiated and moderately differentiated breast carcinomas .

P110 Beta- Associated Breast Tumors . It was found after application and analysis of (IHC) for detection of P110- beta gene expression in the tissues obtained from patients with breast cancers as well as benign breast tumors that seventeen (17) out of thirty (30) patients with carcinoma of breast showed positive immunohistochemistry reaction where it constituted 56.7% of the total breast cancer cases of this study (Table 3 and Figure 1). The benign group revealed 24% positive signals which represented 6 out of 25 cases in this group. None of control group presented positive signals for P110-beta-IHC test However, in comparison to the percentage of P110-beta in healthy control group as well as in the group of benign breast tumor, the differences between the percentages of P110-beta in tissues of patients with breast cancers and each of these above mentioned groups are statistically very highly significant (P value = < 0.0001).

IV. Distribution of grading of breast carcinomas according to the IHC results for P110-beta detection. The P110-betapositive results of IHC were detected in 61.5% (8 out of 13) of these tissue with breast cancers showing moderate differentiated grade, followed by tissues showing the well differentiated grade (i.e. 5out of 9) where it comprised 55.6% of the total number of this grade , and lastly by tissues with poor differentiated grade where it constituted 50% of total number of this grade (i.e.4 out of 8). Statistically, the distribution of IHC results for detection of **P110-beta** according to the grading of breast carcinoma shows non-significant differences (P>0.05) (Table 4).

V. Results of CDK1- IHC Signal Scoring: The expression of CDK1 protein was detected as a brownish discoloration at nuclear localization (Figure 2). The expression of CDK1 was detected by in 53.3% (16 out of 30) cells with breast cancers and in 44% (11 out of 25) cases benign breast tumor , while none of control group showed CDK1- expression . A high percentage (56.2% :9 out of 16 cases) was involving cases with malignant breast tumors that have moderate score (score II) .While, in benign breast tumor group, 45.5%(5 out of 11) were found to have either weak score (score I) or moderate score (score II). Statistically, significant differences (p <0.05) were found on comparing the results (according to score) when each group of breast cancers and benign breast tumors were compared to control group, but the difference between the group of malignant and benign breast tumors was statistically not significant(Table 5).

VI. The relation of CDK1-tumor suppressor gene expression to histopathological grading of breast carcinoma:- Table (6) shows the relation of CDK1 expression to the grade of breast carcinoma of this study. It was found that the percentage of positive - IHC reactions in the well differentiated grade was (55.5%; 5 cases out of 9) while the percentage of those tissues with moderately differentiated grade that showed positive CDK1-IHC reactions was (61.5% ;8 cases out of 13) and lastly the percentage of these cancers with poorly differentiated grade that showed such positive IHC reactions was (37.5%; 3 cases out of 8). Statistically, there are non-significant differences regarding the distribution of CDK1-IHC reactions according to tissue differentiation of breast cancer in the present study (P> 0.05). There are strong positive relationships (with highly significant correlation) between the results of P110-beta and CDK1markers (p <0.01).Also, there are no-significant correlations among the results of P110-beta &CDK1 and grade of breast cancer (Table 7).

Table (1): Distribution of breast tumor patients according to their age .

The Patients	N	Mean Age	S.D	S.E	Minimum	Maximum
Malignant Breast Tumors	30	45.9	11.9	1.05	16.0	75
Benign Breast Tumors	25	42.5	14.4	2.94	15.0	72
Healthy Breast Tissues Control	20	36.8	14.4	3.23	17.0	72
Statistical Analysis	(P <0.05) = 0.009					

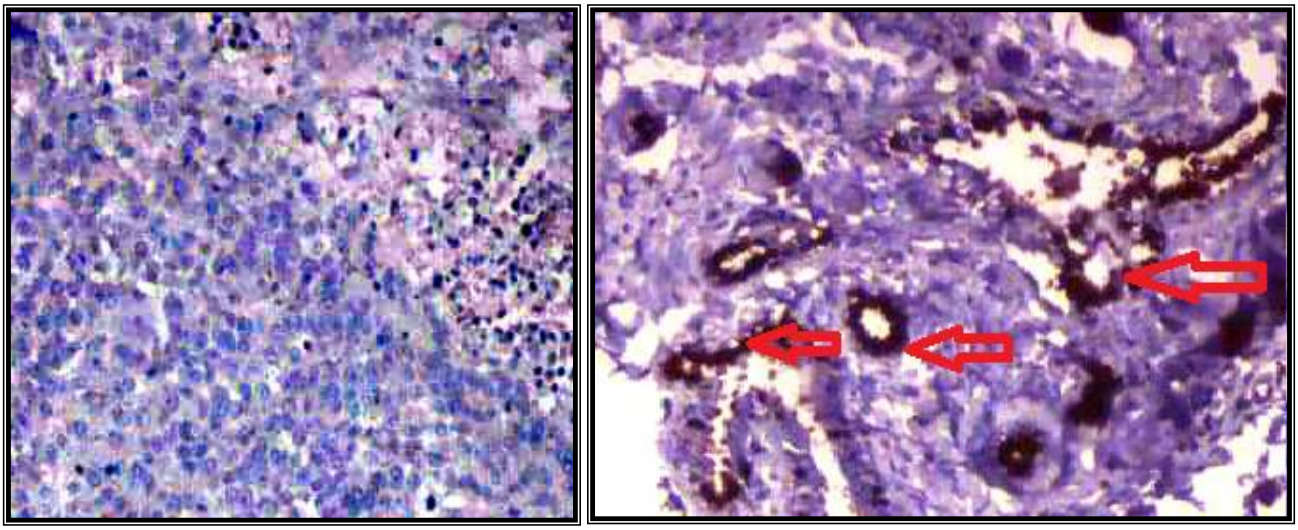
Table (2) : Grading of Breast cancers group.

Grading / Differentiation	N	%
Well *	8	26.7
Moderately **	10	33.3
Poorly	12	40
Total	30	100.0

* Significant differences when well grade compared to poorly grade. **Non significant difference when moderate grade compared to poorly grade.

Table (3): Results of immunohistochemistry for detecting P110-beta in tissues with breast tumors.

Studied groups		P110-beta immunohistochemistry		Total	Comparison of significant	
		Positive	Negative		P-value	Sig.
Breast Cancers	N	17	13	30	0.001	Highly Sig. (P<0.01)
	%	56.7	43.3	100		
Benign Breast tumors	N	6	19	25		
	%	24	76	100		
Healthy control	N	0	15	15		
	%	0	100	100		



A B

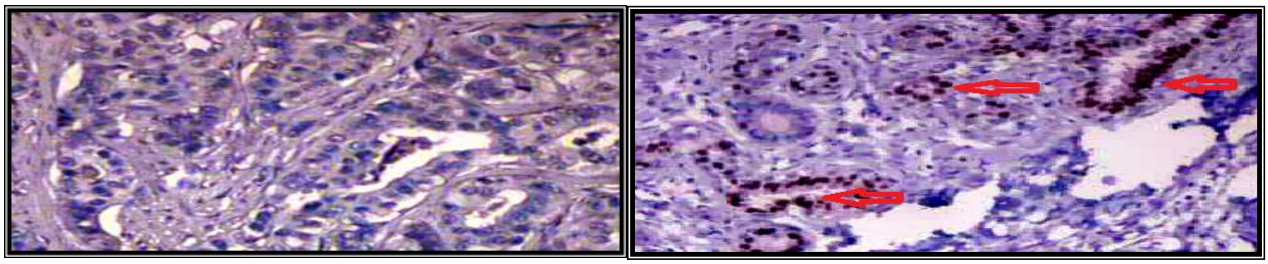
FIGURE 1: Immunohistochemical reactions (IHC) for detection of **P110-beta** usingspecific primary antibodies for **P110-beta** and biotinylated -labeled anti- **P110-beta** ;Stained with HRP/DAB (brown) and counter stained by hematoxyline (Blue).A. Invasive breast ductal carcinoma with negative **P110-beta** -IHC reaction(40X). B. Invasive breast cancer with positive **P110-beta** -IHC reaction that revealed high score and strong signal intensity (40X).

Table (4): The distribution of IHC results for P110-beta detection according to the grading of breast carcinoma :-

Grade		P110-beta immunohistochemistry		Total	Comparison of significant	
		Positive ISH	Negative ISH		P-value	Sig.
well	N	5	4	9	0.387	Non Sig. (P>0.05)
	%	55.6	44.4	100		
moderate	N	8	5	13		
	%	61.5	38.5	100		
poor	N	4	4	8		
	%	50	50	100		
Total	N	17	13	30		
	%	56.7	43.3	100		

Table (5): Frequency distribution of immunohistochemistry results of CDK1 **protein according to the signal scoring.**

CDK1 expression		Healthy breast Tissues (n=15)		Benign breast Tumors (n=25)		Breast Cancers (n=30)		P
		N	%	N	%	N	%	
Negative		15/15	100.0	14/25	56.0	14/30	46.7	< 0.004 significant
Positive		0	0.00	11/25	44.0	16/30	53.3	
Scoring	I	0	0.0	5/11	45.5	3/16	18.8	
	II	0	0.0	5/11	45.5	9/16	56.2	
	III	0	0.0	1/11	9.00	4/16	25	
Mean Rank		100.1				91.5		



A B

FIGURE 2: Immunohistochemical reactions (IHC) for detection of **CDK1** usingspecific primary antibodies for **CDK1**and biotinylated -labeled anti-**CDK1**;Stained with HRP/DAB (brown) and counter stained by hematoxyline (Blue).A. Invasive breast ductal carcinoma with negative **CDK1**-IHC reaction(40X). B. Invasive breast cancer with positive **CDK1**-IHC reaction that revealed high score and strong signal intensity (40X).

Table (6):The distribution of CDK1 expression tests results according to tumor grade/ differentiation of breast carcinoma .

Tumor grade		CDK1 over expression		Total
		Positive CDK1 IHC	Negative CDK1 IHC	
Well	Count	5	4	9
	% within grade	55.5%	44.5%	100.0%
	% within CDK1	31.2%	28.6%	29.6%
Moderate	Count	8	5	13
	% within grade	61.5%	38.5%	100.0%
	% within CDK1	50%	35.7%	44.4%
Poor	Count	3	5	8
	% within Grade	37.5%	62.5%	100.0%
	% within CDK1	18.8%	35.7%	25.9%
Total	Count	16	14	30
	% within grade	53.3%	46.7%	100.0%
	% within CDK1	100.0%	100.0%	100.0%

Table (7): Correlations among studied markers P110-beta &CDK1, within Grade and Age in patients with Breast Cancer.

Assay				
Spearman's rho		Age groups /Year	Grade	CDK1
Grade	r.	.063		
	P-value	.697		
CDK1	r.	.009	.209	
	P-value	.956	.196	
P110-beta	r.	-.027	.093	-.066
	P-value	.867	.566	.685*

***Correlation is significant (P<0.05).**

VII. Correlations among studied markers (P110-beta, CDK1) within Grade and Age in patients with Breast Cancers:There are strong positive relationships (with highly significant correlation) between the results of P110-beta and CDK1markers (p <0.01).Also, there are no-significant correlations among the results of P110-beta &CDK1 and grade of breast cancer (Table 7).

Discussion:

In Iraqi females, breast cancers are on the top of their commonest ten cancers in Iraq, where they account for one third of female cancers (39).Most of these Iraqi patients were diagnosed in the younger aged patients who were at late stage of presentation whom breast tissues have showed more aggressive tumor behaviors, where these could be related to the life style as well as many hereditary and environmental factors (40). The initial reports have noticed an association between breast cancers and cervical intraepithelial neoplastic lesions. However, molecular events in the genesis of the majority of breast cancers are yet unknown (41). Aging was generally noticed as a risk factor that increases a possibility of malignant changes in the breast epithelial tissues. The present preliminary results might also point for the importance of such risk factor in the studied breast cancers tissues where they could be related to the effect of hormonal changes during long exposure of these breast epithelial tissues to them (42). In the current study, the age of patients from where these tissues were obtained was ranging between 38-69 years and their mean age was 52.8 + 8.6 years. The present results are consistent with many Iraqi as well as global reports that breast malignant tumors are usually targeting females aged over forty 29-32. (43). The present results could also point for such importance of age factor, along with the prolonged effect of hormonal changes, in the carcinogenesis of this group of breast cancers (44). The estrogen and its derivatives were incriminated in breast carcinogenesis, and as such estrogen receptor – positive breast carcinomas are more sensitive to anti-estrogen therapies (45). In addition, it was found that cancers in young- aged women have higher grade and proliferation fractions, more vascular invasion and expressed fewer estrogen and progesterone receptors when compared to cancers in older women (46). Various previous studies have analyzed the importance of histopathological grading as an important parameter for risk assessment in BC patients (47).Herein, this study has followed the most popular grading system, Nottingham modification of Scarf-Bloom-Richardson system (which depends on tubular formation, nuclear pleomorphic and mitotic figures), to evaluate the histopathological grading of our series of breast cancers. Regarding grading of the studied tissues, our results are consistent to the percentages of other Iraqi study by AL-Anbari in (48) who found 11% of their BC tissues were in grade I , 48% in grade II and 41% in grade III. Likewise, another study by AL-Khafaji in (49) revealed a similar percentages;11% , 58% and 31% for grade I , II , and III , respectively , while in the group of BC tissues from Syrian patients were 4.7% for grade I, 66.6%for grade II and 28.8% for grade III. However, in the study of Zubair et al ., (50) only 4.17% of tissues have grade I whereas grade II constituted 75.8% and 20% were grade III .In Iraq, it was demonstrated that poorly differentiated grades have associated with high frequency of hormonal receptor-negative tumors and DNA aneuploidy; and in turn were indicating for poor prognosis (51). It was reported that survival rate for10-years in patients with grade I cancers reached 80% and 45% for those with grade III cancers (Rosai and Ackermans,2004)while death due to breast carcinoma was 90% among patients with BC tissues graded as III cancers (52). In the current study , was found 56.7%(17 out of 30 cases) of the total breast cancer cases in this study showed positive immunohistochemistry reaction(IHC) for detection of P110- beta gene expression in these tissues. The combined inhibition of p110-α most effectively inhibits AKT/mTORC1 signaling,cell growth and survival, and tumor growth in PTEN-deficient, ER+ breast cancers. While, p110-δ inhibition suppressed the majority of AKT activation, where PTEN-deficient cancer cells have a large excess of PI3K/AKT signaling and only a small fraction is required to maintain mTORC1 activation. Herein, it is unclear whether the anti-estrogen treatment was uniformly increase the anticancer effects of PI3K inhibition in PTEN-deficient, ER+ breast cancers (53). Recently, it was demonstrated by Costa *et al.* that combined p110 δ inhibition did more effectively decrease PIP3levels and cell viability than the single-isoform inhibition in *PIK3CA* mutant ER+ breast cancer cells. However, inhibition of p110β maximally suppressed P-AKT levels, suggesting that p110α drives AKT-independent pro growth signaling in *PIK3CA*-mutant cells (54). It was found that increased activity may involve an overexpression of p110and p110β in some of those cancers studied by Backer et al., (55) and Rodriguez-Viciana et al., (56). Overexpression of the catalytic subunit alone was sufficient to generate a fully

active enzyme which was revealed by p85α excess in these cancer cells. However, additional mechanisms may also participate in enzymatic deregulation, including tyrosine kinase overexpression and mutation of Ras or the p85 regulatory subunit. Many functions were previously attributed for PI3Ks in cell division, survival, cell differentiation, migration and tumor invasion (57). However, most of these reports did not discriminate between p110δ and p110α, precluding from specific functions. Overexpression of the wild-type catalytic subunits p110 β, -γ, or -δ of class I PI3K is sufficient to induce an oncogenic phenotype in cultured cells. In contrast, wild-type p110αlacks this transforming potential but could be acquired by point mutations or by myristylation or farnesylation (58). There are reports pointing for an elevated expression of p110 β and p110 δ in various human cancers (59).In contrast to the prevalence of p110α mutations detected in various tumor types, there have been no reports of cancer-specific mutations in p110β, -γ, or -δ (60) where absence of the mutations in these non- α isoforms might point for their oncogenic potential as wild-type proteins. It appears that differential expression of wild-type p110β, -γ, or -δ made re-examining the expression profiles of various cancers for possible up-regulation of non-_ isoforms at the RNA and protein levels worthwhile .

However, the reasons for this oncogenic potential of the non- α isoforms of p110 are not known. The oncogenicity of all isoforms of class I p110 depends on kinase activity. For the tumor-suppressive effect of the lipid phosphatase PTEN, it is strongly argue in favor of a dominant, if not exclusive, role of lipid kinase activities in the oncogenic transformation induced by p110 isoforms.The requirements for upstream and downstream signaling in the transformation process induced by the p110 isoforms are in accord with published literature. These requirements divide the isoforms into two groups: one consisting of p110_ and -_, the other encompassing p110α and -δ.Bothp110α and -δ are linkedto upstream receptor tyrosine kinases (61).

However, p110 p110β and -γ have Ras-binding domains that can bind to Ras, yet, mutations that abolish Ras binding of oncogenic p110α do not interfere with transformation. Mutations of p110α and the wild-type protein p110δ are strong stimulators as well as activators of Akt signaling. The β isoform can be activated by G protein-coupled receptors and by receptor tyrosine kinases (62).

The overexpressed p110α but not of p110β may be toxic (63), and the inability of wild-type p110α for inducing oncogenic transformation was attributed to the low levels of expression. (49, 52, 53). Expression of a particular p110 isoform of class IA tends to affect the expression levels of other isoforms ; herein, the endogenous levels of p110α are down-regulated in cells overexpressing the β or δ isoform. The expression of CDK1 was detected by in 53.3% (16 out of 30) cells with breast cancers and in 44% (11 out of 25) cases benign breast tumor , while none of control group showed CDK1- expression . CDK1 has role in mitosis as well as self-renewal and differentiation of human embryonic stem cells (64).Participation of CDK1 depends on the association with cyclin A or B to progress into G1/S and G2/M phases of cell cycle via the phosphorylation of > 500 various candidate substrates for CDK1 that are G2 and M phase-specific (65-67).In contrast to CDK4, CDK6 and CDK2 (are redundant in the mammalian cell cycle) CDK1 (which is essential for cell division) is sufficient to drive cell cycle in all cell types (68). Hyperactivation of CDK1 was reported to have poor prognosis in some types of cancers such as those in lung (69), ovaries (70), kidney (71),and breast (72). The inhibition of CDK1 in lymphomas, hepatoblastomas, and breast cancers has induced MYC-dependent apoptosis (73). In this respect, Goga *et al.* found that CDK1 inhibition led to synthetic lethality in mouse lymphoma and hepatoblastoma (74) They also showed that breast cancer have selective sensitivity to CDK1 inhibition(75) .The loss of CDK1 leads to substantial mitotic catastrophe (76) which possibly increases MYC-induced replication and subsequently result in cell death. Therefore, specific targeting of CDK1 might be effective for breast tumors dependent on MYC activation (77-79).

The current results of this study indicate thatp110-beta as well as and CDK 1 could have a role in the breast pathogenesis and carcinogenesis.

References:

1. Early Breast Cancer Trialists' Collaborative G, Davies C, Godwin J, Gray R, Clarke M, Cutter D, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet. 2011;378:771-84.
2. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490:61-70.
3. Gonzalez-Angulo AM, Ferrer-Lozano J, Stemke-Hale KA, Sahin AA, Liu S, Barrera JA, et al. PI3K Pathway Mutations and PTEN Levels in Primary and Metastatic Breast Cancer. Mol Cancer Ther. 2011.
4. Miller TW, Hennessy BT, Gonzalez-Angulo AM, Fox EM, Mills GB, Chen H, et al. Hyperactivation of phosphatidylinositol-3 kinase promotes escape from hormone dependence in estrogen receptor-positive human breast cancer. J Clin Invest. 2010;120:2406-13.
5. Mayer IA, Abramson VG, Isakoff SJ, Forero A, Balko JM, Kuba MG, et al. Stand up to cancer phase Ib study of pan-phosphoinositide-3-kinase inhibitor buparlisib with letrozole in estrogen receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer. J ClinOncol. 2014;32:1202-9.
6. Chon HS, Marchion DC, Xiong Y, Chen N, Bicaku E, Stickles XB, et al. The BCL2 antagonist of cell death pathway influences endometrial cancer cell sensitivity to cisplatin. GynecolOncol. 2012;124:119-24.
7. Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH, et al. Essential roles of PI(3)K-p110beta in cell growth, metabolism and tumorigenesis. Nature. 2008;454:776-9.
8. Utermark T, Rao T, Cheng H, Wang Q, Lee SH, Wang ZC, et al. The p110alpha and p110beta isoforms of PI3K play divergent roles in mammary gland development and tumorigenesis. Genes & development. 2012;26:1573-86.
9. Graupera M, Guillermet-Guibert J, Foukas LC, Phng LK, Cain RJ, Salpekar A, et al. Angiogenesis selectively requires the p110alpha isoform of PI3K to control endothelial cell migration. Nature. 2008;453:662-6.
10. Miller TW, Forbes JT, Shah C, Wyatt SK, Manning HC, Olivares MG, et al. Inhibition of mammalian target of

rapamycin is required for optimal antitumor effect of HER2 inhibitors against HER2-overexpressing cancer cells. Clin Cancer Res. 2009;15:7266-76.
11. O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res. 2006;66:1500-8.
12. BeÂnistant C, Chapuis H and Roche S (2000).A specific function for phosphatidylinositol 3-kinase a (p85a-p110a) in cell survival and for phosphatidylinositol 3-kinase b (p85a-p110b) in de novo DNA synthesis of human colon carcinoma cells. Oncogene (2000) 19, 5083 – 5090.
13. Vanhaesebroeck B and Waterfield MD. (1999). Exp. Cell Res., 253, 239 -254.
14. Samuels, Y. &Velculescu, V. E. (2004) Cell Cycle 3**,** 1221-1224. [PubMed]
15. Samuels, Y., Wang, Z., Bardelli, A., Silliman, N., Ptak, J., Szabo, S., Yan, H., Gazdar, A., Powell, S. M., Riggins, G. J., *et al.* (2004) Science 304**,** 554.
16. Kang, S., Bader, A. G., Zhao, L. & Vogt, P. K. (2005) Cell Cycle 4**,** 578-581.
17. Samuels, Y., Wang, Z., Bardelli, A., Silliman, N., Ptak, J., Szabo, S., Yan, H., Gazdar, A., Powell, S. M., Riggins, G. J., *et al.* (2004) Science 304**,** 554.
18. Benistant, C., Chapuis, H. & Roche, S. (2000) Oncogene 19**,** 5083-5090.
19. Knobbe, C. B. &Reifenberger, G. (2003) Brain Pathol. 13**,** 507-518.
20. Hill K, Welti S, Yu J, Murray JT, Yip SC, Condeelis JS, Segall JE and Backer JM. (2000). J. Biol. Chem., 275,3741 ± 3744.
21. Hamada, K., Sasaki, T., Koni, P. A., Natsui, M., Kishimoto, H., Sasaki, J., Yajima, N., Horie, Y., Hasegawa, G., Naito, M., *et al.* (2005) Genes Dev. 19**,** 2054-2065.
22. Wymann, M. P., Zvelebil, M. &Laffargue, M. (2003) *Trends Pharmacol. Sci.*24, 366-376.
23. Malumbres, M.; Barbacid, M. Mammalian cyclin-dependent kinases. *Trends Biochem. Sci.* 2005, *30*, 630-641.
24. Dorée, M.; Hunt, T. From Cdc2 to Cdk1: When did the cell cycle kinase join its cyclin partner2002, *115*, 2461-2464.
25. Morgan, D.O. Cyclin-dependent kinases: Engines, clocks, and microprocessors. *Annu. Rev. Cell Dev. Biol.* 1997, 13, 261-291. ? J. Cell. Sci.
26. Weinberg, R.A. The retinoblastoma protein and cell cycle control. Cell 1995, 81, 323-330.
27. Giacinti, C.; Giordano, A. RB and cell cycle progression. Oncogene 2006, 25, 5220-5227.
28. Lohka, M.J.; Hayes, M.K.; Maller, J.L. Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. Proc. Natl. Acad. Sci. USA 1988, 85, 3009-3013.
29. Labbe, J.C.; Picard, A.; Peaucellier, G.; Cavadore, J.C.; Nurse, P.; Doree, M. Purification of MPF from starfish: Identification as the H1 histone kinase p34cdc2 and a possible mechanism for its periodic activation. Cell 1989, 57, 253-263.
30. Cox, D., Tseng, C. C., Bjekic, G., and Greenberg, S. (1999) J. Biol. Chem. 274, 1240-1247.
31. Chrzan P, Skokowski J, Karmolinski A, Pawelczyk T. Amplification of c-myc gene and overexpression of c-Myc protein in breast cancer and adjacent non-neoplastic tissue. Clin Biochem.2001;34(7):557-562.
32. Naidu R, Wahab NA, Yadav M, Kutty MK. Protein expression and molecular analysis of c-myc gene in primary breast carcinomas using immunohistochemistry and differential polymerase chain reaction. Int J Mol Med. 2002;9(2):189-196.
33. Nie Z, Hu G, Wei G, Cui K, Yamane A, Resch W, Wang R, Green DR, Tessarollo L, Casellas R, Zhao K, Levens D. c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. Cell. 2012;151(1):68-79.
34. Carroll JS, Swarbrick A, Musgrove EA, Sutherland RL. Mechanisms of growth arrest by c-myc antisense oligonucleotides in MCF-7 breast cancer cells: Implications for the antiproliferative effects of antiestrogens. Cancer Res. 2002;62(11):3126-3131.
35. Molenaar JJ, Ebus ME, Geerts D, Koster J, Lamers F, Valentijn LJ, Westerhout EM, Versteeg R, Caron HN. Inactivation of CDK2 is synthetically lethal to MYCN over-expressing cancer cells.ProcNatlAcadSci USA. 2009;106(31):12968-12973.
36. Goga A, Yang D, Tward AD, Morgan DO, Bishop JM. Inhibition of CDK1 as a potential therapy for tumors over-expressing MYC. Nat Med. 2007;13(7):820-827.
37. Stone A, Sutherland RL, Musgrove EA. Inhibitors of cell cycle kinases: recent advances and future prospects as cancer therapeutics. CritReOncog. 2012;17(2):175-198.
38. Zlobec, R. S. ; René, P .M.;Carolyn, C.; Compton, ;Alessandro Lugl and Jeremy, R . J.(2006). Scoring of p53, VEGF, Bcl-2 and APAF-1 immunohistochemistry and interobserver reliability in colorectal cancer. Modern Pathology ; 19: 1236-1242.
39. Michaelism, M., Doerrm, H.W. and CiantlmJ (2009)The story of human cytomegalovirus and cancer:increasing evidence and open questions.Neoplasia.
40. Stagno, S. and Cloud, G.A. (1994) Working Parents:the impact of day care and breast-feeding on CMV infections in offspring .Proc.Nati.Acad.Sci.USA., 91:2384-2389.
41. Liu Y, Klimberg VS, Andrews NR, Hicks CR, Peng H, Chiriva-Internati M, Henry-Tillman R, Hermonat PL. Human papillomavirus DNA is present in a subset of unselected breast cancers. J Hum Virol. 2001;4:329-34.
42. Key TJ. Serum oestradiol and breast cancer risk. EndocrRelatCancer. 1999;6:175-180.
43. Elkum, N ; Dermime ,S. ;Ajarim, D.;Al-Zahrani, A. ;Alsayed ,A.;Tulbah ,A. ;Al Malik, O. ;Alshabanah, M.; Ezzat, A. and Al-Tweigeri ,T.(2007).Being 40 or younger is an independent risk factor for relapse in operable breast cancer patients: the Saudi Arabia experience. J . BMC Cancer. 5;7:222.
44. Santen RJ, Yue W, Wang J-P. Estrogen metabolites and breast cancer. Steroids. 2015;99:61-6.
45. Pike, M. C., Spicer, D. V. and Dahmoush, L. (1993) Estrogen, progestoycus, normal breast cell profileration and breast cancer risk . Epidemio / Rev., 15:17 – 35.
46. Harvey, J. A ., and Bovbjery, V. E. (2004). Quantitative assessment of mammographic breast density: relationship with breast cancer risk. Radiology ; 230 (1): 29-41.
47. Latinovic, L. and Heinze, G. .(2001).Prognostic grading method in breast cancer. *International J. of*

Oncology, 19:1271-77.
48. Al-Anbari S. S .(2009).Correlation of the clinicopathological presentations in Iraqi breast cancer patients with the findings of biofield breast cancer diagnostic system (BDS), Her-2 and Ki-67 immunohistochemical expression, a thesis submitted to the college of medicine and the committee of post graduate studies of the University of Baghdad in partial fulfillment of the requirement for the degree of Ph D in Pathology.
49 .Al-Khafaji , A . H.(2010). Immunohistochemical expression of Estrogen , Progesterone receptors, P53 and Ki67 in Iraqi and Syrian breast cancer patients, clinicopathological study. A thesis submitted to the Collage of Medicine Baghdad university in partial fulfillment of the PhD Degree in medical Pathology .
50. Zubair, A ; Mubarik, A. ;Jamal , S., and Naz, S.(2010) . A comparative analysis of coronary adventitial T-lymphocytes-on autobsy study. *J . Ayup Med . Cool Appottapad*. Vol 22.No.1.p 42-45.
51. Alwan , N. A. S . (2000). DNA Proliferative Index as a marker in Iraqi Aneuploid Mammary Carcinoma. Eastern Mediterranean Health Journal. WHO, Eastern Mediterranean Regional office; 6(5/6), p: 1062-1072.
52. Blamey, R. W. ; Elston, C. W., and Ellis, I. O. (2000).:When is a patient cured of breast cancer? *J.*Mod . Pathol; 13:18A.
53. Miller TW, Balko JM, Fox EM, Ghazoui Z, Dunbier A, Anderson H, et al. ERalpha-dependent E2F transcription can mediate resistance to estrogen deprivation in human breast cancer. Cancer discovery. 2011;1:338-51.
54. Costa C, Ebi H, Martini M, Beausoleil SA, Faber AC, Jakubik CT, et al. Measurement of PIP3 levels reveals anunexpected role for p110beta in early adaptive responses to p110alpha-specific inhibitors in luminal breast cancer.Cancer cell. 2015;27:97-108.
55. Backer JM, Myers Jr MG, Shoelson SE, Chin DJ, Sun XJ, Miralpeix M, Hu P, Margolis B, Skolnik EY and Schlessinger J. (1992). EMBO J., 11, 3469 ± 3479.
56. Rodriguez-Viciana P,Warne PH, Vanhaesebroeck B, Water-®eld MD and Downward J. (1996). EMBO J., 15, 2442 ±2451.
57. Kobayashi M, Nagata S, Iwasaki T, Yanagihara K, Saitoh I, Karouji Y, Ihara S and Fukui Y. (1999). Proc. Natl. Acad. Sci. USA, 96, 4874 ± 4879.
58. Kang, S., Bader, A. G. & Vogt, P. K. (2005) Proc. Natl. Acad. Sci.USA 102**,** 802-807.
59. Knobbe, C. B. &Reifenberger, G. (2003) Brain Pathol. 13**,** 507-518.
60. Samuels, Y., Wang, Z., Bardelli, A., Silliman, N., Ptak, J., Szabo, S., Yan, H., Gazdar, A., Powell, S. M., Riggins, G. J., *et al.* (2004) Science 304**,** 554.
61. Vanhaesebroeck, B. &Waterfield, M. D. (1999) Exp. Cell Res. 253**,** 239-254.
62. Kubo, H., Hazeki, K., Takasuga, S. &Hazeki, O. (2005) Biochem. J.
63. Yip, S. C., El-Sibai, M., Hill, K. M., Wu, H., Fu, Z., Condeelis, J. S. & Backer, J. M. (2004) Cell Motil. Cytoskeleton 59**,** 180-188.
63. Meier, T. I., Cook, J. A., Thomas, J. E., Radding, J. A., Horn, C., Lingaraj, T. & Smith, M. C. (2004)Protein Expr. Purif. 35**,** 218-224.
64. Xiao Qi Wang, Chung Mau Lo, Lin Chen, Elly S-W Ngan, Aimin Xu and Randy YC Poon. CDK1-PDK1-PI3K/Akt signaling pathway regulates embryonic and induced pluripotency. Cell Death and Differentiation (2017) 24, 38-48.
65. Chen, X., Stauffer, S., Chen, Y., and Dong, J. Ajuba phosphorylation by CDK1 promotes cell proliferation and tumorigenesis. J. Biol. Chem. 2016; 291: 14761-14772
66. Petrone, A., Adamo, M.E., Cheng, C., and Kettenbach, A.N. Identification of candidate Cyclin-dependent kinase 1 (Cdk1) substrates in mitosis by quantitative Phosphoproteomics**.** Mol. Cell. Proteomics. 2016; 15: 2448-2461
67. Vantieghem, A., Xu, Y., Assefa, Z., Piette, J., Vandenheede, J.R., Merlevede, W., De Witte, P.A., and Agostinis, P. Phosphorylation of Bcl-2 in G2/M phase-arrested cells following photodynamic therapy with hypericin involves a CDK1-mediated signal and delays the onset of apoptosis. J. Biol. Chem.2002; 277: 37718-37731**.**
68. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer.2009;9(3):153-166.
69. hi, Y.X., Zhu, T., Zou, T., Zhuo, W., Chen, Y.X., Huang, M.S., Zheng, W., Wang, C.J., Li, X., Mao, X.Y. et al. Prognostic and predictive values of CDK1 and MAD2L1 in lung adenocarcinoma. *Oncotarget*. 2016;7: 85235-85243
70. Yang, W., Cho, H., Shin, H.Y., Chung, J.Y., Kang, E.S., Lee, E.J., and Kim, J.H. Accumulation of cytoplasmic Cdk1 is associated with cancer growth and survival rate in epithelial ovarian cancer.*Oncotarget*. 2016; 7: 49481-49497
71. Hongo, F., Takaha, N., Oishi, M., Ueda, T., Nakamura, T., Naitoh, Y., Naya, Y., Kamoi, K., Okihara, K., Matsushima, T. et al. CDK1 and CDK2 activity is a strong predictor of renal cell carcinoma recurrence.*Urol. Oncol.* 2014; 32: 1240-1246
72. Pavlou, M.P., Dimitromanolakis, A., Martinez-Morillo, E., Smid, M., Foekens, J.A., and Diamandis, E.P.Integrating meta-analysis of microarray data and targeted proteomics for biomarker identification: application in breast cancer**.** *J. Proteome Res.* 2014; 13: 2897-2909
73 .Kang, J., Sergio, C.M., Sutherland, R.L., and Musgrove, E.A. Targeting cyclin-dependent kinase 1 (CDK1) but not CDK4/6 or CDK2 is selectively lethal to MYC-dependent human breast cancer cells**.***BMC Cancer*. 2014; 14: 32
74. Goga A, Yang D, Tward AD, Morgan DO, Bishop JM. Inhibition of CDK1 as a potential therapy for tumors over-expressing MYC. Nat Med. 2007;13(7):820-827.
75. Sweeney KJ, Swarbrick A, Sutherland RL, Musgrove EA. Lack of relationship between CDK activity and G1 cyclin expression in breast cancer cells. Oncogene. 1998;16(22):2865-2878.
76. O'Connor DS, Wall NR, Porter AC, Altieri DC. A p34(cdc2) survival checkpoint in cancer. Cancer Cell. 2002;2(1):43-54.
77. Kessler JD, Kahle KT, Sun T, Meerbrey KL, Schlabach MR, Schmitt EM, Skinner SO, Xu Q, Li MZ, Hartman ZC, Rao M, Yu P, Dominguez-Vidana R, Liang AC, Solimini NL, Bernardi RJ, Yu B, Hsu T, Golding I, Luo J, Osborne CK, Creighton CJ,

Hilsenbeck SG, Schiff R, Shaw CA, Elledge SJ, Westbrook TF. A SUMOylation-dependent transcriptional subprogram is required for Myc-driven tumorigenesis. Science. 2012;335(6066):348-353.

78. Yang D, Liu H, Goga A, Kim S, Yuneva M, Bishop JM. Therapeutic potential of a synthetic lethal interaction between the MYC proto-oncogene and inhibition of aurora-B kinase. ProcNatlAcadSci U S A. 2010;107(31):13836-13841.
79. Ferrao PT, Bukczynska EP, Johnstone RW, McArthur GA. Efficacy of CHK inhibitors as single agents in MYC-driven lymphoma cells. Oncogene. 2012;31(13):1661-1672.