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Association of Molecular Localization of Human Cytomegalovirus (HMCV) and P110 ,CDK2 & P15 Genes Expression Products in Tissues from Iraqi Female Patients with Breast Tumors .

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Abstract

A total number of (120) formalin-fixed, paraffin embedded breast tissues. The mean of age of the patients with breast carcinoma was higher (41.75±8.763 years) than the mean age of the benign tumors group (39.47 ±7.825 years) and the mean age of those females in the group of healthy control (37.33± 7.556years). The poorly differentiated grade (III) of breast cancer (BC) constituted 42.5% (17/40) whereas those with moderately(II) and well (I) differentiated grades BC constituted 30% (12/40) and 27.5% (11/40), respectively. The DNA of HCMV was detected in 37.5 %(15/40) of tissues from breast cancers while HCMV DNA in the tissues from benign breast tumors was detected in 17.5% (7 /40). No HCMV positive – ISH reaction was detected in healthy ovarian tissues of the control group. The positive P110-IHC reactions were detected in 42.5% (17 out of 40 cases) of malignant breast tumor. While ,in benign breast tumor was expressed in 47.5% . No signal was reported in the tissues of control group .The positive CDK2-IHC reactions were disclosed in 47.5% (19 / 40) of breast cancer cases. Whereas, in benign breast tumor the positive results was 50% (20 / 40) of CDK2- protein expression. No signal was reported in the tissues of control group. The positive P15-IHC reactions were detected in 45% (18 / 40) of malignant breast tumors .While, 35% (14 / 40) of benign ovarian tumors. No signal was reported in the tissues of control group.

Our results indicate that the HCMV might contribute to the development of subset of breast tumors. The present results of the rates of defects or mutations in the P110;CDK2& P15 genes in relation to the grade of breast cancer tissues also could point for their occurrence and contribution as early events in breast carcinogenesis.

Keywords: HCMV , P110, CDK2, P15, CISH, IHC.

Introduction:

Breast carcinoma is the second-most-common cause of cancer-related death among women worldwide. It is relatively slow growing tumors and the most frequently diagnosed non –skin malignancy of women in many populations. The incidence increase with age, raising concern that it was an un identified environmental cause[1][2].

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As in all cancers, the cause of breast cancer remains unknown. Research into its etiology has documented risk factors of breast carcinoma include early age of menarche, late age of menopause, a positive family history of breast cancer, hormone replacement therapy, age, sex, and nulliparity[3][4]. The International Agency for Research on Cancer reports that biological carcinogens cause 18-20% of cancers, suggesting the tremendous potential of controlling microbe-related processes for cancer prevention[5].At present, the etiologic role of viral infections in some human cancers—for example, the role of hepatitis B and C viruses in hepatocellular carcinoma, and the role of the Epstein–Barr virus (EBV) in nasopharyngeal carcinoma—has been confirmed [6].The three most studied viruses that could possibly cause breast cancer in humans are: mouse mammary tumor virus (MMTV), the Epstein-Barr virus (EBV) and the human papilloma (HPV)[7].

Human cytomegalovirus(HCMV) (Also known human herpesvirus 5) is a member of herpesviridae family (subfamily betaherpesvirinae) which can remain latent lifelong after the primary infection and shows recurring activation[8].

HCMV infects nearly 50–90% of the population worldwide. HCMV infection is either asymptomatic or causes mild discomfort. It has large protein repertoire that can promote or initiate neoplastic changes in human cell with dysregulatory effects on the proliferative cell cycle, resulting in a block of the apoptotic pathways, tumor suppressor proteins dysfunction, and DNA mutation[9].

HCMV genome and antigens has been reported in several kinds of human cancers. These human malignancies include breast cancer, brain cancer, prostate cancer, colon cancer, and salivary gland cancer [10]. So, the virus may have the potential to spread to the adjacent breast epithelial cells.Moreover, HCMV has been found to infect the macrophages and induce an atypical phenotype of M1/M2 macrophages. This phenotype of macrophage releases cytokines, which are involved in cancer initiation or promotion[11].

Richardson hypothesized that incidence of breast cancer could be raised by late exposure to HCMV[12].

This hypothesis was based on the incidence of breast cancer and its correlation with the seroprevalence of HCMV. Cox and colleagues investigated the correlation between levels of HCMV IgG with the development of breast cancer. They enrolled 399 women with invasive breast cancer and 399 controls. Results of their study suggest a statistically significant correlation with the elevation of HCMV IgG levels and development of breast cancer in some women [13].

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 [14]

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A genetically altered PI3K pathway [its products, phosphatidylinositol 3,4,5 triphosphate (PIP3)] was found in more than 70% of ER-positive breast cancers[15].Activation of PI3K led to anti-estrogen resistance of such cancers[16].Tumor suppressor phosphatase and PTEN dephosphorylate PIP3 and then antagonize PI3K[17].

Inhibition of PI3K suppresses anti-estrogen-resistant growth of ER-positive breast cancer cells[18].

Phosphatidylinositol 3-kinase a has specific function in cell survival and phosphatidylinositol 3-kinase b in DNA synthesis of human colon carcinoma cells[19]. Thus, combined inhibition of both p110α and p110β, was required for sustained therapeutic effect in cases of PTEN-deficient, ER-positive breast cancers[1]. Herein, PI3K inhibitors are being developed for the treatment of breast and other cancers[20]. 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[21].

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[22].The gain of function by p110_mutation or overexpression was common in human cancers. In contrast, no somatic mutations of the genes encoding of the p110β and α isoforms and as such, their correlations to human cancers were much less reported , although increased expression of the p110β and αisoforms occurred in some bladder and colonic tumors as well as glioblastoma[23].

CDK2 is a member of CDKs family that is important in malignant transformation of human breast epithelial cells, probably by complexing with cyclinD1 or with the assistance of low molecular weight cyclinE[24].Inhibition of CDK2 activity could effectively restrain the proliferation of breast cancer cells[25],including those resistant to endocrinotherapy[26].

Cyclin-dependent kinase 2 associate protein 1(CDK2-AP1) is a specific negative regulatory protein for CDK2, which is mainly responsible for degrading CDK2 and interacting with DNA polymerase α to affect DNA replication of S-phase cells. CDK2-AP1 also seems to have the potential to control cancer cell growth and modify the functioning of the androgen-responsive pathway, described by[27].In spite of its important role in cancer suppression, work on CDK2-AP1 in breast cancer is insufficient. Only one single report had indicated that P12 can inhibit growth of breast cancer cells in vivo and in vitro by regulating the cell cycle[28].

p15*INK4b*, which also functions as a Cdk4/6 inhibitor and is strongly induced by transforming growth factor b (TGF-b)[29][30].Both loci, *INK4a* and *INK4b*, are frequently deleted in a variety of tumors and cell lines . In addition, these proteins can also be inactivated by point mutations or methylation[31].

The expression of proteins p16*INK4a*, p15*INK4b*, and p19*ARF* can be decreased by hypermethylation of the CpG island upstream of corresponding exon 1 in both humans and rodents[32][33].In epithelial cells, TGF-b causes upregulation of p15*INK4B* mRNA and increased binding of a 15-kDa protein to cdk6[34].

This study is aiming to analyze the rate of concordance of P110 ,CDK2 & P15-genes translational expression and HCMV in breast tissues from a group of Female Iraqi patients with breast tumors .

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Materials and Methods:

This study was designed as a retrospective case-control study research. A total number of (120) formalin-fixed, paraffin embedded breast tissues was included. These cases were distributed on the following groups: **40** blocks of malignant breast tumor tissues; **40** blocks of benign breast tumor tissues; **40** blocks breast tissues as apparently healthy controls. The diagnosis of these tissue blocks were based on their accompanied records. A consultant pathologist reexamined all these cases to further confirmation of their diagnosis. One section was mounted on ordinary glass slide and stained with hematoxyline and eosin, while other slides were mounted on charged slide to be used for CISH as well as IHC.

The detection of HCMV by CISH kit (Zyto Vision GmbH. Fischkai, Bremerhaven. Germany) was performed on 4µm - paraffin embedded tissue sections. **Immunohistochemistry / Detection system** (Abcam. England) was used to demonstrate the P110 ,CDK2 & p15 gene expression (protein) in cells using a specific monoclonal antibodies, i.e. Primary antibody for that specific epitope which binds to nuclear targeted protein .The bound primary antibody is then detected by secondary antibody which contains specific label peroxidase labeled polymer conjugated to goat anti mouse immunoglobulin. The substrate is DAB in chromogen solution produced a positive reaction resulting in a brown- color precipitate at the antigen site in these tissues.

Chi –square test was used to detect the significance between variables of our study. All the statistical analysis was done by SPSS program (Version– 19) &P value was considered significant when $p < 0.05$.

Results:

I.Distribution of patients with breast tumor and healthy control group according to their Age .

The archival specimens collected in this study were related to breast tumor patients whom ages were ranged from nineteen years to seventy five years. The mean age of patients with malignant breast tumor (41.75 ±8.763 years) was higher than the mean age of the benign breast tumors (39.47 ±7.825 years) . While, the mean age of apparently healthy control (AHC) was (37.33 ± 7.556) years. However,there was no significant differences ($p<0.05$) between different groups in age distribution Table (1).

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

Studied groups	N	Mean (Age/ Year)	Std. Deviation	Std. Error	Range		P-value	
					Mini.	Maxi.	ANOVA Test	LSD test
A.H. Control	40	37.33	7.556	1.195	27	56	P=0.241 Non	P^1=0.152 NS

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Benign Breast Tumor	**40**	**39.47**	**7.825**	**1.237**	**19**	**58**	**sign. (P>0.05)**	**P^2=0.956 NS**
Breast Cancer	**40**	**41.75**	**8.763**	**1.386**	**25**	**67**		**P^3=0.137 NS**
Total \ Mean	**120**	**38.04**	**8.04**					

P^1= A.H. Control Vs Benign tumor, P^2= A.H. Control Vs breast cancer & P3= Benign tumor Vs breast cancer

***II. Histological Grade of Breast Cancer*:**

The results of present study show that poorly differentiated grade breast (grade III) carcinomas constituted 42.5% (17 out of 40 cases) , whereas cases with moderately grades (grade II) constituted 30% (12out of 40 cases) and well differentiated (grade I) 27.5% (11 out of 40 cases) , respectively . The results revealed non-significant differences at (P>0.05) between poorly differentiated grade and well differentiated grade, also non-significant difference was noticed between poorly differentiated and moderately differentiated breast carcinomas Table (2) .

Table (2) :Tumor Grading ofbreast cancers group.

Breast Cancer Grades(Differentiated)	**N**	**%**	**P-value**
Well (I)	11	27.5	**P=0.584 Non sign. (P>0.05)**
Moderately (II)	12	30	
Poorly (III)	17	42.5	
Total	40	100.0	

III. Human Cytomegalovirus (HCMV) - Associated Breast Tumor by Chromogenic In Situ Hybridization Technique (CISH).

***I. HCMV associated with apparently healthy breast control tissues using DNA-CISH detection*:**

In this study, the apparently healthy breast control tissues were tested by using HCMV -DNA-CISH detection. All cases were negative; therefore they are excluded from the statistical analysis.

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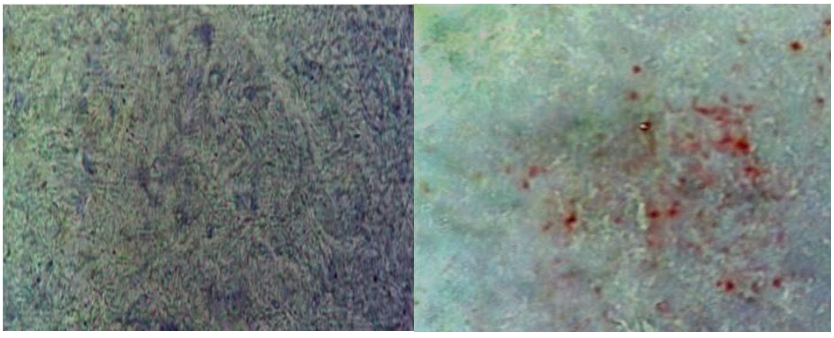
***II .Results of HCMV in Female Patients with Breast Tumor*:**

Table (3) shows the positive results of HCMV-CISH detection in malignant breast tumor, where 37.5% (15 out of 40 cases) showed positive signals, while, 62.5% negative signals which represented 25 out of 40 cases in this group. Whereas ,in the benign breast tumor group was 17.5% (7 out of 40 cases). Negative signals which in benign group represented 33 out of 40 cases constituted 82.5%. Statistically, significant difference ($p<0.05$) was found on comparing the percentages of HCMV DNA among the study groups.

Table (3): Distribution of HMTV –DNA Signals in Female Patients with Breast Tumor by Using CISH Technique.

HCMV-CISH			Studied groups		Pearson Chi-Square (P-value)
			Benign tumor	Breast Cancer	
	Positive	N	7	15	P=0.012 Sign. (P<0.05)
		%	17.5%	37.5%	
	Negative	N	33	25	
		%	82.5%	62.5%	
Total		N	40	40	
		%	100.0%	100.0%	



A ***B***

Figure (1): Microscopic appearance of HCMV-positive CISH signals of breast tumors. Using Digoxigenin-Labeled HCMV Probe ;Stained with 3-Amino 9-Ethyl Carbazole (Red) and Counter Stained by Nuclear Blue Solution (Blue). Red signal are detected at complementarity sequences sites (arrows). surface epithelial breasttumor with negative HCMV –ISH reactions (40X) B.Positive HCMV-ISH signals of surface epithelial breast cancer (40X) .

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IV. The Evaluation Results of P110 in Breast Tumor Groups:

***I.The results of P110- protein expression in the apparently healthy breast control tissues*.**

In this study, the results of immunohistochemistry staining of 110-protein using biotinylated anti-P110 protein- antibodies in the apparently healthy breast control tissues showed no signal was expressed to all cases; therefore they are excluded from the statistical analysis.

II.*The results of p110- protein expression in breast tumor*:

Table (4) shows the results of immunohistochemistry staining of P110 protein using biotinylated anti- p110 protein- antibodies in breast tumor tissues which were detected at high power field examination as a brownish discoloration at nuclear and cytoplasmic localizations .The results showed that 42.5% (17 out of 40 cases) of p110- protein expression were positive in malignant breast tumor. While ,in benign breast tumor was expressed in 47.5%.Statistical analysis of the p110- immunohistochemical assay tests show non-significant difference (p>0.05) .

Table (4).The Results of p110- Protein Expression in Breast Tumors By Immunohistochemistry Technique.

P110-IHC			Studied groups		Pearson Chi-Square (P-value)
			Benign tumor	Breast Cancer	
	Positive	N	19	17	P=0.653 Non sign. (P>0.05)
		%	47.5%	42.5%	
	Negative	N	21	23	
		%	62.5%	57.5%	
Total		N	40	40	
		%	100.0%	100.0%	

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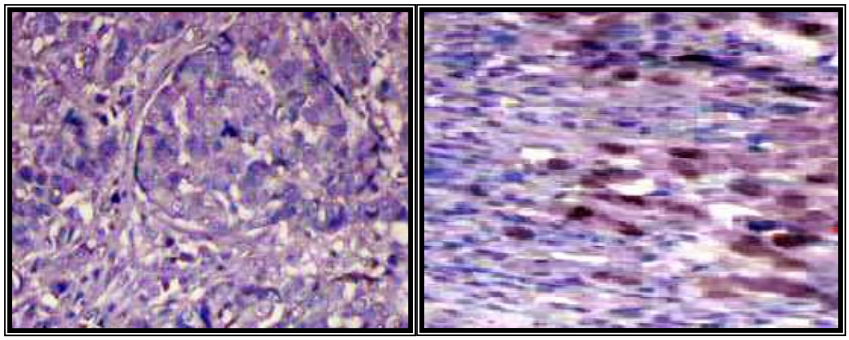

A **B**

Figure (2): The Results of Immunohistochemical Staining of P110 Protein Expression in Surface Epithelial BreastTumors. Using Biotinylated -Labeled Anti-P110 Protein Antibody, Stained By DAB-Chromogen (Brown) and Counter Stained By Mayer's Hematoxyline (Blue). A. Breast Tumors with negative P110 –IHC reactions(40X) B. Positive P110 –IHC reaction of Breast cancer (40X).

V : The Evaluation Results of Cyclin Depend Kinase -2 (CDK2) in BreastTumor Groups:

***I.The results of CDK2- protein expression in the apparently healthy breastcontrol tissues*.**

In this study, the results of immunohistochemistry staining of CDK2 protein using biotinylated anti-CDK2 protein- antibodies in the apparently healthy breast control tissues showed no signal was expressed to all cases; therefore they are excluded from the statistical analysis.

***II .The results of CDK-2 protein expression in breast tumor*:**

The results showed that 47.5 % (19 out of 40) of CDK2- protein expression were positive in breast cancer cases. While, no signal was expressed in (52.5%: 21 out of 40 cases). Whereas, in benign breast tumor the positive results was 50% (20 out of 40 cases) of CDK2- protein expression. Statistical analysis of the CDK2-immunohistochemical assay tests show non-significant difference (p>0.05)Table (5).

Table (5).The Results of CDK2- Protein Expression in Breast Tumors by Immunohistochemistry Technique.

CDK2			Studied groups		Pearson Chi-Square (P-value)
			Benign tumor	Breast Cancer	
	Positive	N	20	19	P=0.115

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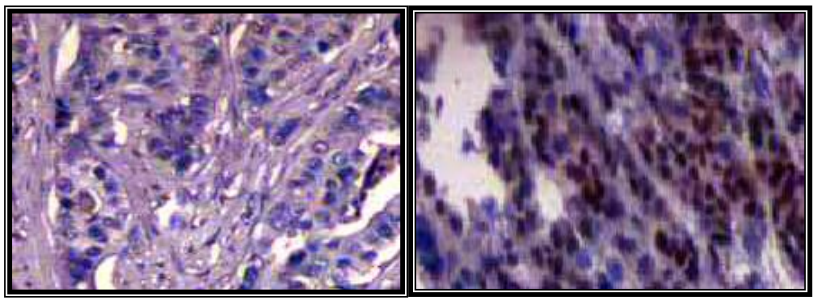
		%	50%	47.5%	Non sign. (P>0.05)
	Negative	N	20	21	
		%	50%	52.5%	
Total		N	40	40	
		%	100.0%	100.0%	



A **B**

Figure (3) : Microscopic appearance shows over expression of CDK2 protein in breast tumors. Stained by DAB chromogen (brown) and counter stained with Mayer's heamatoxylin. A. Surface Epithelial BreastTumors with negative CDK2 –IHC reactions(40X) B. Positive CDK2 –IHC reaction of Breast cancer (40X).

***VI. The Results of Evaluation of p15 in Breast Tumor Groups*:**

Protein expression of P15 protein expression was evaluated by using biotinylated anti- p15 protein- antibodies in breast tumor tissues which were detected at high power field examination as a brownish discoloration at cytoplasmic localizations .

***I.The results of P15- protein expression in the apparently healthy breastcontrol tissues*.**

In this study, the results of immunohistochemistry staining of P15 protein using biotinylated anti- p15 protein- antibodies in the apparently healthy breast control tissues showed no signal was expressed to all cases; therefore they are excluded from the statistical analysis.

***II. The results of P15- protein expression in Breast Tumor*:**

P15- protein expressionwas detected by IHC test in 45% (18 out of 40 cases) of malignantbreast tumors .While, 35% (14 out of 40 cases) of benign breast tumors

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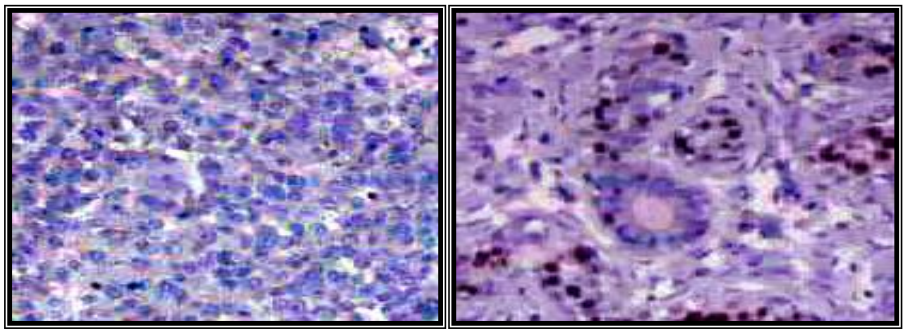
but no signal was reported in the tissues of control group. Statistical analysis of the p15 immunohistochemical assay tests show non-significant difference (p>0.05) Table (6) .

Table (6).The results of P15- protein expression in BreastTumor

P15			Studied groups		Pearson Chi-Square (P-value)
			Benign Tumor	Breast Cancer	
	Positive	N	14	18	P=0.228 Non sign. (P>0.05)
		%	35.0%	45%	
	Negative	N	26	22	
		%	65.0%	55%	
Total		N	40	40	
		%	100.0%	100.0%	



A **B**

Figure (4) : Microscopic appearance shows over expression of P15 protein in breast tumors. Stained by DAB chromogen (brown) and counter stained with Mayer's heamatoxylin. A. Breast Tumors with negative p15 –IHC reactions(40X) B. Positive p15 –IHC reaction of Surface Epithelial Breastcancer (40X).

Discussion:

Breast cancers have ranked the top on the commonest ten cancers in the Iraqi provinces & districts, accounting for about one third of the registered female cancers[35]. The majority of molecular events in the genesis of breast cancer are unknown. Hereditary, hormonal (estrogenic hormones and derivatives), environmental and life style factors have been attributed to breast carcinogenesis[36].

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On reviewing the 120 cases which were included in this study, it was found the age of the patients with breast cancers was ranging between 19-67 years and their mean age was 38.04years .The present results are consistent with those reported world-wide where these breast malignant tumors were usually affecting females over forty years of age. These results could reflect that age is an important risk factor in tumor changes affecting breast epithelial tissues lesions. In general, aging increase the incidence of the malignant changes in breast epithelial tissues and as such their incidence was found to increase with age[37].

Despite of variability of grading systems (because of its subjective evaluation) yet, the most popular grading system used is Notingham modification of Scarf-Bloom-Richardson (SBR) system which depends on tubular formation, nuclear pleomorphism and mitotic figures [38].

In the current study, it was found that breast carcinoma with poorly differentiated grade breast (grade III) carcinomas constituted 42.5% , whereas cases with moderately grades (grade II) constituted 30% and well differentiated (grade I) 27.5% (11 out of 40 cases) . These results are in disagreement with [39] who showed that only 4.17% of cases have grade I, while grade II breast cancers were having (75.8%). However, grade III- breast cancers were constituted (20%) and are incompatible with the present results.

Histological grading is an important parameter of risk assessment in the patients with breast cancer[40].

In addition, other researchers have reported that tumors in younger women were of higher grade, with higher proliferation fractions, had more vascular invasion and expressed fewer estrogen and progesterone receptors compared to tumors in older women [41].

In this respect, it has been reported that the 10-year survival rate for patients harboring Grade I BC is around 80%, dropping to 45% in Grade III- breast cancers [42].

In the current study, HCMV-CISH detection in malignant breast tumor, where 37.5% showed positive signals. Whereas ,in the benign breast tumor group was 17.5%. In situ hybridization methods for detection of nucleic acid sequences have proved powerful especially for revealing genetic markers and gene expression in a morphological context [43] .

In addition and by courtesy of many previous studies that used ISH techniques had proved ISH as an effective method for detecting and localizing CMV-DNA within the affected tissues. Here about, it has been chosen the molecular design so as to demonstrate the HCMV-DNA of the late gene (that encodes matrix protein of this virus) in Iraqi patients with breast cancer with different grades. In the current study, the CMV DNA-ISH was detected in 34.3% (24 out of 70 cases) malignant breast tumors. None of control group presented positive signals for CMV-ISH test.

Unexpectedly, [44] was found 97% (31 out of 32 cases) of breast carcinoma in their study have evidence of HCMV infection where their expression was based on immunohistochemistry.

From in vitro assays , three regions on the HCMV genome with transforming activity have been identified . Multiple HCMV gene products are known to promote mutagenesis and to dysregulate cell cycle checkpoint controls and drive oncogenic signaling pathways [45].

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Breast feeding is the major route of HCMV transmission during the first year of life in countries where most women are seropositive and breast feeding their infants .[46] showed 60% of cases with breast cancer had antibodies to HCMV.

HCMV could be associated with breast cancer because it is a ubiquitous virus that is shed in breast milk, as well as in saliva, urine, cervical secretion and semen, which implies that HCMV persistently infects epithelial cells [47].

The results showed that 42.5% of p110- protein expression were positive in malignant breast tumor. While ,in benign breast tumor was expressed in 47.5%.
Recently, it was demonstrated by [48] that combined p110α/β inhibition did more effectively decrease PIP3levels and cell viability than the single-isoform inhibition in *PIK3CA* mutant ER+ breast cancer cells. However, inhibition of p110p110αmaximally suppressed P-AKT levels, suggesting that p110βdrives AKT-independent pro-growth signaling in *PIK3CA*-mutant cells.

It was found that increased activity may involve an overexpression of p110alpha and p110beta in some of those cancers studied by[49][50]

Overexpression of the catalytic subunit alone was sufficient to generate a fully active enzyme which was revealed by p85a 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 [51]. However, most of these reports did not discriminate between p110a a and p110b, precluding from specific functions.

Overexpression of the wild-type catalytic subunits p110 alpha, beta, or- of class I PI3K is sufficient to induce an oncogenic phenotype in cultured cells. In contrast, wild-type p110_ lacks this transforming potential but could be acquired by point mutations or by myristylation or farnesylation[52][53]. There are reports pointing for an elevated expression of p110 alpha and p110 beta in various human cancers[54].
In contrast to the prevalence of p110 mutations detected in various tumor types, there have been no reports of cancer- specific mutations in p110 [55].

where absence of the mutations in these non- isoforms might point for their oncogenic potential as wild-type proteins. However, the reasons for this oncogenic potential of the non- 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.

Expression of a particular p110 isoform of class IA tends to affect the expression levels of other isoforms ; herein, the endogenous levels of p110alpha are down-regulated in cells overexpressing the p110 isoform.

The results showed that 47.5 % of CDK2- protein expression were positive in breast cancer cases. While, in benign breast tumor the positive results was 50% of CDK2- protein expression. CDK2-AP1 is a direct upstream of CDK2, which can induce cell apoptosis by inactivating CDK2. On losing it CDK2-AP1, an activated downstream signal promotes excessive proliferation of normal cells, which may lead to malignant transformation of normal cells. In cancer, however, loss of CDK2-AP1 could increase proliferation and invasiveness to cancer cells. By immunohistochemistry, the expression of CDK2-AP1 was found to be reduced successively in normal breast tissue, DCIS, invasive breast cancer and metastasized breast cancer. The expression of CDK2-AP1 was also negatively correlated with the expression of CDK2 and cyclin D1.The expression of CDK2 and CyclinD1 changed

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accordingly after downregulation or upregulation of CDK2-AP1 by western blot, suggesting a role of the CDK2-AP1/CDK2/CyclinD1 cell cycle pathway in the initiation and progression of breast cancer. Similar results were obtained in animal assays. The data indicates that CDK2-AP1 can induce sensitivity to docetaxel treatment in breast cancer cells[56].

Furthermore, targeted silencing of Cdk2-induced cell death in LMW-overexpressing breast cancer cell lines, but not in cell lines with no LMW expression. Consequently, mammary epithelial cells expressing LMW-cyclin E are unable to initiate transformation in the absence of Cdk2, whereas the oncogenic LMW-cyclin E/ Cdk2 kinase provides breast cancer cells with the capabilities to resist cell death and as such is required to sustain tumor cell proliferation. Other studies showed that Cdk2 is not required for proliferation and differentiation of hematopoietic cells in vivo[57], nor is it required for neural progenitor cell proliferation, differentiation, and survival of hippocampal granule neurons in vivo[58]. In the adult subventricular zone, it was shown that Cdk2 is critical for proliferation and self-renewal of neural progenitor cells when Cdk4 expression is too low to compensate for loss of Cdk2[59] Consequently, functional redundancies by Cdk1 or Cdk4/6 may compensate for the lack of Cdk2 in those cell types. For example, Cdk1 was shown to bind cyclin E in various tissues and also to regulate G1/S-phase transition in mouse embryonic fibroblasts[60]. However, we show here that although Cdk2 is dispensable for mammary gland development, it is required for LMW-cyclin E tumorigenesis. This reflects the requirement for the phosphorylation of a specific set of substrates that mediate the oncogenic function of LMWcyclin E/Cdk2 kinase. This result suggests that targeting LMW-cyclin E/Cdk2 kinase activity may be a viable and specific strategy for clinical treatment of certain breast tumors.

In the current study ,the p15 was detected in 45% of malignantbreast tumors .While, 35% of benign breast tumors.[61] was found (2%) Methylation of p15 in breast tumors by Multiplex methylation-sensitive PCR.

[62]was found the *p15*^{INK4B} gene was altered in 3 (21%) of the 14 breast cell lines; one had a silent mutation and two had homozygous deletion of the gene.

High levels of p15 would foster p15-cdk4 association and displace cyclin D1 and might inhibit cyclin D1 reassociation. Disruption of cyclin D1-cdk association leads to loss of cyclin D1 stability [63]. In vitro, premixing of p15 with cdk4 prevented added p27 from binding to cdk4 [64].

The significant detection of HCMV along with P110 ,CDK2&P15 genes expression production breast cancer patients are supporting the hypothesis of an etiologic roles for that virus along withmutated and / or defected P110 ,CDK2&P15 genes in breast cancer development.

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