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Research Article

Detection of HER-2/neu, c-myc amplification and p53 inactivation by FISH in Egyptian patients with breast cancer

Nachweis von HER-2/neu, c-myc-Amplifikation und p53-Inaktivierung durch FISH bei ägyptischen Patienten mit Brustkrebs

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Abstract

Breast cancer is a leading cause of cancer-related deaths in women worldwide. The clinical course of this disease is highly variable and clinicians continuously search for prognostic parameters that can accurately predict prognosis, and indicate a suitable adjuvant therapy for each patient. Amplification of the two oncogenes HER-2/neu and c-myc and inactivation of the tumor suppressor gene p53 are frequently encountered in breast carcinomas. The purpose of this study was to use the fluorescence in situ hybridization (FISH) for the assessment of HER-2/neu and c-myc amplification and p53 inactivation and to relate these molecular markers with the commonly used clinical and pathological factors. The study was conducted on 34 tissue samples obtained from 33 females and 1 male with breast carcinomas and 17 samples obtained from 16 females and 1 male with benign breast lesions. Results revealed that the level of HER-2/neu, c-myc and p53 in the malignant group was significantly increased as compared to the benign group. On relating the level of the molecular markers to clinicopathological factors, p53 was significantly associated with increased patient's age. The sensitivity of the investigated markers significantly increased with larger tumor size. Concerning tumor grade, HER-2/neu and p53 showed a significant increase in low-grade tumors whereas c-myc showed a highly significant increase in high-grade tumors. With regard to disease staging, HER-2/neu and c-myc were the only markers that showed significant increase at late stages of disease. p53 and HER-2/neu were significantly associated with positive lymph nodal status. A significant correlation was obtained between the levels of the three biomarkers to each other. Conclusively, the combination of HER-2/neu, c-myc and p53 can stratify patients into different risk groups.

Keywords: breast cancer, fluorescent in situ hybridization (FISH), genetic alterations, HER-2/neu, p53, c-myc

Zusammenfassung

Brustkrebs ist weltweit die häufigste Ursache für krebsbedingte Todesfälle bei Frauen. Der klinische Verlauf bei dieser Erkrankung ist sehr unterschiedlich, und Kliniker sind bemüht, prognostische Parameter zu finden, die für jeden Patienten zuverlässig den Verlauf voraussagen und eine geeignete adjuvante Therapie vorgeben können. Die Amplifikation der zwei Onkogene HER-2/neu und c-myc und die Inaktivierung des Tumorsuppressorgens p53 treten bei Brustkrebs häufig auf. Das Ziel dieser Studie war es, mit dem Verfahren der Fluoreszenz-in-situ-Hybridisierung (FISH) die Amplifikation der beiden Onkogene HER-2/neu und c-myc sowie die Inaktivierung des Tumorsuppressorgens p53 zu messen und diese molekularbiologischen Marker mit den gebräuchlichen klinischen und pathologischen Angaben zu korrelieren.

Die Studie wurde an 34 Gewebeproben, die von 33 Frauen und einem Mann mit Brustkrebs stammen, und 17 Proben, die von 16 Frauen und einem Mann mit benignen Brustgewebeveränderungen gewonnen wurden, durchgeführt. Die Ergebnisse zeigen, dass die Werte von HER-2/neu, c-myc und p53 in der Krebsgruppe signifikant verändert waren im Vergleich zu der Gruppe mit gutartigen Tumoren. Bei der Beziehung der Gehalte der molekularen Marker zu den klinisch-pathologischen Befunden waren die Befunde bei p53 signifikant mit dem Alter der Patientin korreliert. Die diagnostische Sensitivität der untersuchten Marker war signifikant korreliert mit der Größe der Tumoren. Im Hinblick auf das Tumorgrading zeigten HER-2/neu und p53 eine signifikante Zunahme bei low-grade-Tumoren, während c-myc eine signifikante Zunahme bei high-grade-Tumoren aufwies. Bezüglich des Disease-Staging waren HER-2/neu und c-myc die einzigen Tumormarker, die einen signifikanten Anstieg im letzten Stadium der Erkrankung aufwiesen. p53 und HER-2/neu waren signifikant assoziiert mit positivem Lymphknotenstatus. Eine signifikante Korrelation bestand bei den Werten der drei Biomarker untereinander.
Zusammenfassend kann die Bestimmung von HER-2/neu, c-myc und p53 in den Gewebeproben die Patienten in verschiedene Risikogruppen einteilen.

Schlüsselwörter: Brustkrebs, Fluoreszenz-in-situ-Hybridisierung (FISH), genetische Veränderungen, HER-2/neu, p53, c-myc

Introduction

Breast cancer ranks first among cancers affecting woman throughout the world and its marked impact is not restricted to Western industrialized societies [49]. Latest estimates suggest that more than 1,050,000 new breast cancer cases occur worldwide annually, with nearly 580,000 cases in developed countries and the remainder in developing countries [17]. Breast cancer is the most common malignancy among Egyptian females accounting about 37.6% of all malignancies in women [36]. Over the three years 2000–2002, 1831 breast cancer cases were registered; 1810 females and 21 males with an average of 603 cases of female breast cancer per year.
The progression of malignant tumors is a consequence of multiple alterations of the genome including either activation of oncogenes such as HER-2/neu and c-myc or inactivation of tumor suppressor genes such as p53 [54], [48], [8].
HER-2/neu oncogene is a member of the human epidermal growth receptor family [12]. The gene product is a protein with extracellular and intracellular domain with tyrosine kinase activity involved in signal transduction of cell growth and development [23]. Amplification of the HER-2/neu receptor tyrosine kinase has been implicated in the pathogenesis and aggressive behavior of approximately 25% of invasive human breast cancers. It has been suggested that aberrant HER-2 signaling contributes to tumor initiation and disease progression [52]. Determination of HER-2/neu in serum of patients with metastatic breast cancer has proved clinical utility since it correlates with protein expression in primary tumors [53].
The c-myc oncogene has been shown to be amplified and/or overexpressed in many types of cancer. The frequencies of its amplification, mRNA and protein overexpression in breast cancer vary between 1%–94%, 22%–95%, and 50%–100%, respectively [26], [6]. c-myc protein is a product of c-myc proto-oncogene and exerts diverse effects on cell behavior. c-myc, being a transcription factor, plays important role in cell growth and differentiation and was found to be associated with a more aggressive tumor and a poorer patient survival [33].
The human p53 gene encodes a 53 KD nuclear phosphoprotein consisting of 393 amino acids [30]. Wild-type p53 protein blocks the entry of cells into S phases whereby allowing DNA repair to occur before replicating the genome. p53 can also induce apoptosis when DNA repair fails [25]. However, not only mutant p53 cannot prevent the propagation of genetically damaged cells but also can inhibit apoptosis [27]. More recently, anti-p53 antibodies are of clinical significance as a serological marker in the diagnosis and monitoring of breast cancer [32].
To our knowledge, this is the first report to demonstrate the genetic alterations in Egyptian breast cancer patients using FISH technique.
In the current study, we assessed the genetic alterations in HER-2/neu, c-myc and p53 genes using fluorescence in situ hybridization (FISH) in Egyptian breast cancer patients and investigated the prognostic role of the three markers and their relation to each other and to demonstrate their relation to the classical clinicopathological factors.

Materials and methods

Patients

Patients with primary breast cancer had either an excision biopsy with axillary evacuation or a modified radical mastectomy in the surgical department at the National Cancer Institute, Cairo University. No preoperative radiation therapy or chemotherapy was administered to patients.

Thirty-four consecutive samples were obtained during the period from 1999–2001 from 33 females and 1 male with breast carcinoma (27 with invasive duct carcinoma and 7 with non-invasive duct carcinoma). The age of the patients ranged from 23 to 75 years. Seventeen samples were obtained from 16 females and 1 male with benign breast lesions to serve as a control group (12 with fibroadenoma, 4 with fibrocystic disease and 1 with gynecomastia). The age of the patients ranged from 15–52 years. The histologic grades of the tumor foci were assessed using the modified Bloom-Richardson classification [16]. Clinical staging was done according to TNM classification [3]. Informed consent was obtained for all cases.

Tissue samples were cut into pieces, one was formaline-fixed and paraffin-embedded for subsequent histopathological examination, and the second was shock-freezed in liquid nitrogen and stored at –80°C for the analysis of HER-2/neu, c-myc and p53.

Fluorescence in situ hybridization analysis

Specimen preparation, hybridization, and microscopy were performed as previously described [37], [38] with the following modifications. Frozen-stored tissues were slowly thawed in cold phosphate buffered saline (PBS) at 4°C, light-touched on glass slides. Slides were incubated in 75 mM KCl for 20 min at 37°C, fixed in freshly prepared Carnoy's solution (methanol : acetic acid, 3:1 v/v) and air-dried.

The SpectrumOrange labeled HER-2/neu probe and the SpectrumGreen chromosome 17 centromeric alpha-satellite (chr.17cen.) probe were cohybridized on the tissue specimen. For c-myc hybridization locus specific identifier (LSI) c-myc (SpectrumOrange) and CEP8 (SpectrumGreen) probe and LSI p53 probe were applied. FISH probes were obtained from Vysis, Inc, Downers Grove, IL.

The c-myc and HER-2 gene copy numbers in approximately 200 nuclei in the predominant tumor cell population were estimated in relation with centromere CEP8 and CEP17. Hybridization signals were enumerated by the ratio of orange signals (for c-myc and HER-2) to green signals (for CEP8 and CEP17) in morphologically intact and nonoverlapping nuclei. At least a 3-fold increase of the c-myc signals over CEP8 signals in the tumor cells was considered the criterion for gene amplification. The same criterion was applied to HER-2 amplification analysis using LSI HER-2 probe together with CEP17 probe [24], [9].

Statistical analysis

Cut-off values were determined using receiver operating characteristic curve (ROC curve). The Mann-Whitney and Wilcoxon tests, and univariate analysis were performed using a chi-square test of association or Fisher's exact model to test the association of categorical variables, whereas t-test was used for continuous variables. P values below 0.05 were considered as significant [2].

Results

Cut-off values for HER-2/neu and c-myc

The best cut-off values were estimated to maximize the sum of sensitivity and specificity. Optimal cut-off value of 1.2 for both HER-2/neu and c-myc are shown in Figure 1.

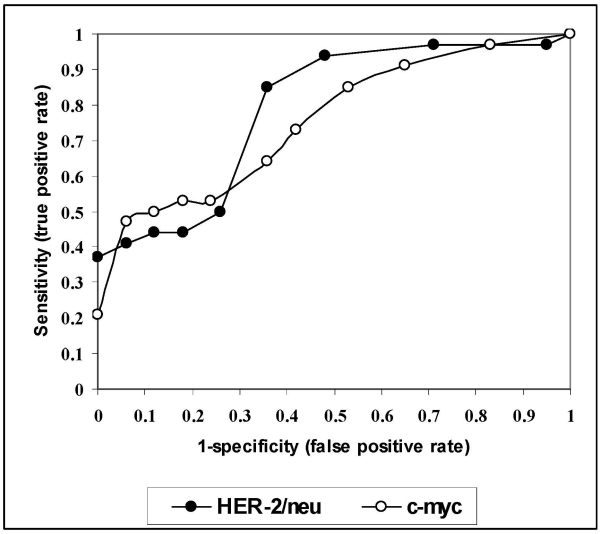

Figure 1: ROC curve of both HER-2/neu and c-myc. The area under the curve is 0.789 and 0.752 for HER-2/neu and c-myc, respectively.

Expression of HER-2/neu, c-myc and p53 in benign and malignant groups

Genetic alteration in HER-2/neu, c-myc and p53 were significantly higher in the malignant group when compared to the benign one as show in Table 1. In the malignant group, the levels (mean, median and positivity rates) for HER-2/neu are 2.8±0.17, 1.435 (0.9–4.2) and 85% respectively, versus 1.25±0.11, 1.06 (0.86–2.5) and 29% in the benign group.

For c-myc, the levels (mean, median and positivity rates) are 1.89±0.13, 1,825 (0.95–3.53) and 73% respectively in the malignant group as compared to 1.2±0.08, 1.1 (0.87–2.02) and 35% respectively in the benign group. 0 (0–1) and 64% are the median and positivity rate, re-

Table 1: The level of HER-2/neu, c-myc and P53 in benign and malignant groups

Parameters	Benign group	Malignant group	Statistics	
			Test	p-value
HER-2/neu - median (range) - mean ± SE - positivity rate	 1.06 (0.86–2.5) 1.25 ± 0.11 5 (29%)	 1.435 (0.9–4.2) 2.08 ± 0.17 29 (85%)	 Z = 1.88 t = 3.26 χ^2 = 16.94	 0.002 0.002 0.00004
c-myc - median (range) - mean ± SE - positivity rate	 1.1 (0.87–2.02) 1.20 ± 0.08 6 (35%)	 1.825 (0.95–3.53) 1.89 ± 0.13 25 (73%)	 Z = 1.49 t = 3.36 χ^2 = 11.64	 0.024 0.002 0.0006
p53 - median (range) - % positivity	 1 (0–2) 3 (17%)	 0 (0–1) 22 (64%)	 Z = 1.58 χ^2 = 14.44	 0.013 0.0001

% positivity = % of positive cases for p53 deletion
1 and 0 refer to one or no signal indicating deletion of one or two copies of p53 gene, respectively.
2 refer to two FISH signals from normal nuclei.

spectively for p53 in the malignant group in comparison to 1 (0–1) and 17% in the benign group.

Relation between the investigated molecular markers and clinicopathological findings

The levels of the three investigated genes in relation to the clinicopathological factors were shown in Table 2 and Table 3. The results show positivity rate of HER-2/neu, c-myc and p53 and their relation to clinicopathological factors. At age <45, positivity rate of HER-2/neu, c-myc and p53 were 12 (75%), 10 (62.5%) and 4 (25%) respectively, whereas at age >45 positivity rate of HER-2/neu c-myc and p53 were 17 (94.44%), 15 (83.33%) and 18 (100%) respectively.

In relation to tumor size, the positivity rate of HER-2/neu, c-myc and p53 were 7 (70%), 4 (40%) and 5 (50%) for tumor size less than 3 cm respectively. For the tumor size more and equal to 3 cm, the positivity rate of HER-2/neu, c-myc and p53 were 22 (91.66%), 21 (87.5%) and 17 (70.83%) respectively. On the other hand the positivity rate of HER-2/neu, c-myc and p53 were 25 (89.28%), 19 (67.85%) and 19 (67.85%) respectively in grade I & II cases, whereas it was 4 (75%), 6 (100%) and 3 (50%) respectively in grade III. Concerning disease staging, HER-2/neu and c-myc were the only markers that showed significant expression at late stages of disease (HER-2/neu: 2.86 (1.27–4.2), 2.63±0.23; c-myc: 88.88%) than in early stages (HER-2/neu: 1.35 (1.22–3.24), 1.69±0.2; c-myc: 56.25%).

Regarding nodal status, p53 and HER-2/neu were significantly expressed in patients with positive lymph nodes (p53: 77.3%, HER-2/neu: 2.49±0.22) when compared to those with negative lymph nodal status (p 53: 41.7%, HER-2/neu: 1.69±0.25).

Correlation tests

On relating the level of the three biomarkers to each other, they exhibited a significant correlation with each other (HER-2/neu and c-myc r=0.511, p=0.002; p53 and c-myc r=0.356, p=0.03; p53 and HER-2/neu r=0.432, p=0.01) (Table 4) .

Positivity of the molecular markers in benign and malignant groups

The number of cases that give positive results for the three markers together (HER-2/neu, c-myc and p53) constitute 46% and 12% in malignant and benign cases, respectively. On the other hand, the percentage of negativity for the three markers is 3% and 52% in the malignant and benign cases, respectively.

Discussion

Breast cancer is a disease with a tremendous heterogeneity in its clinical behavior. It is well known that the clinical outcome of breast cancer patients depends on different clinicopathological factors, including the metastatic status of the lymph nodes, tumor size, tumor grade, and histopathological subtype, but there are other molecular markers that can aid the selection of patients for adjuvant therapies. These molecular markers split the apparently uniform population into clear subpopulations so that the therapies offered to patients may become a reflection of their individual therapeutic needs, rather than the assumption that all patients behave as the population median [22].

In our study, we assessed the genetic alterations in HER-2/neu, c-myc and p53 genes that are commonly reported in human breast carcinomas. In the present study, the gene alterations were studied using FISH technique. FISH

Table 2: Positivity rate of HER-2/neu, c-myc and the % positivity of p53 and their relation to clinicopathological factors

Clinicopathological factors	Parameters		
	HER-2/neu >1.2	c-myc >1.21	p53 positive
Age: <45 (n=16) ≥45 (n=18) Statistics	12 (75%) 17 (94.44%) χ^2=0.86, p=0.35	10 (62.5%) 15 (83.3 %) χ^2=1.0, p=0.32	4 (25%) 18 (100%) χ^2=8.91, p=0.002
Tumor size <3 cm (n=10) ≥3 cm (n=24) Statistics	7 (70%) 22 (91.66 %) χ^2=7.76, p=0.005	4 (40%) 21 (87.5%) χ^2=11.56, p=0.0006	5 (50%) 17 (70.83%) χ^2=6.54, p=0.01
Grade: I & II (n=28) III (n=6) Statistics	25 (89.28%) 4 (75%) χ^2=15.21, p=0.00009	19 (67.85%) 6 (100%) χ^2=6.76, p=0.009	19 (67.85%) 3 (50%) χ^2=11.63, p=0.0006
Stage I & II (n=16) III & IV (n=18) Statistics	12 (75 %) 17 (94.44 %) χ^2=0.86, p=0.35	9 (56.25%) 16 (88.88%) χ^2=6.76, p=0.009	9 (56.25%) 13 (72.22%) χ^2=0.72, p=0.39
Lymph nodes positive (n=22) negative (n= 12) Statistics	8 (36.4%) 11 (91.7%) χ^2=2.94, p=0.37	7 (31.8%) 10 (83.3%) χ^2=6.76, p=0.39	17 (77.3%) 5 (41.7%) χ^2=5.54, p=0.05

Table 3: The level of HER-2/neu, c-myc and their relation to clinicopathological factors

Clinico-pathological factors	Parameters			
	HER-2/neu >1.2		c-myc >1.21	
	Median (range)	Mean ± SE	Median (range)	Mean ± SE
Age: <45 ≥45 Statistics	2.05 (1.22–3.44) 2.22 (1.27–4.2) Z=0.41, p=0.99	2.21 ± 0.27 2.26 ± 0.25 t=0.13, p=0.89	2.05 (1.22–3.41) 2.11 (1.25–3.53) Z=0.57, p=0.9	2.06 ± 0.22 2.25 ± 0.21 t=0.61, p=0.53
Tumor size <3 cm ≥3 cm Statistics	1.31 (1.22–4.2) 2.71 (1.27–4.15) Z=1.76, p=0.07	1.73 ± 0.41 2.41 ± 0.19 t=1.64, p=0.11	1.72 (1.33–3.44) 2.11 (1.22–3.53) Z=0.22, p=0.82	2.06 ± 0.49 2.20 ± 0.16 t=0.35, p=0.73
Grade: I & II III Statistics	2.22 (1.22–4.2) 2.21(1.27–3.32) Z=0.63, p=0.82	2.24 ± 0.19 2.25 ± 0.56 t=0.02, p=0.98	2.1 (1.25–3.53) 1.77 (1.22–2.76) Z=0.67, p=0.75	2.27 ± 0.17 1.86 ± 0.27 t=1.4, p=0.42
Stage I & II III & IV Statistics	1.35 (1.22–3.24) 2.86 (1.27–4.2) **Z=1.52, p=0.02**	1.69 ± 0.20 2.63 ± 0.23 **t=2.84, p=0.008**	1.75 (1.22–3.21) 2.15 (1.25–3.53) Z=0.88, p=0.41	2.01 ± 0.27 2.27 ± 0.18 t=0.82, p=0.42
Lymph nodes positive (n=22) negative (n=12) Statistics	2.71 (1.27–4.2) 1.31 (1.22–3.24) Z=1.44, p=0.032	2.49 ± 0.22 1.69 ± 0.25 **t=2.18, p=0.038**	2.11 (1.25–3.53) 2.1 (1.22–3.21) Z=0.5, p=0.96	2.18 ± 0.16 2.16 ± 0.41 t=0.052, p=0.96

Table 4: Correlation between p53, c-myc and HER-2/neu in the malignant group

	p53		c-myc		HER-2/neu	
	r	p	r	p	r	p
p53	-	-	-	-	-	-
c-myc	**0.358**	**0.03***	-	-	-	-
HER-2/neu	**0.432**	**0.01***	**0.511**	**0.002***	-	-

technique is a useful method to evaluate metaphase chromosomes or interphase nuclei to detect genetic aberrations in a variety of solid tumor. FISH analysis of interphase nuclei detects genetic aberrations that are difficult to identify by conventional cytogenetic analysis [55], [7].

Our results revealed that HER-2/neu was amplified in 85% of malignant breast tumors, but only in 29% of benign tumors. Similar results were obtained by Grushko et al. [21] and Park et al. [35]. In addition, to evaluate the HER-2/neu status at the mRNA and DNA level of breast carcinomas, Vanden Bempt et al. [51] studied 32 invasive duct carcinomas for the detection of HER-2/neu mRNA levels by RT-PCR. Corresponding paraffin sections were examined by FISH. Only 28 cases showed increased levels of HER-2/neu mRNA. FISH analysis showed corresponding gene amplification in all 28 cases with 2 cases showing peculiar amplification pattern. In almost all cases, amplification of the HER-2/neu gene results in protein overexpression and poor prognosis. Patients whose tumors have HER-2/neu gene amplification have a shorter disease-free survival time than patients whose tumors exhibit a normal HER-2/neu gene copy number [20]. In addition, Yassin et al. [57] studied the expression of HER family in Egyptian breast cancer patients and reported that HER-2 expression was encountered in 79.5% by immunohistochemistry versus 81.6% by RT-PCR. Tubbs et al. [50] using primary FISH testing in 742 consecutive cases of breast cancer, reported 80% of the cases not amplified for HER-2 (HER-2/CEP17=0.8–1.9), whereas 19% (142/742) of cases were HER-2 amplified (HER-2/CEP17>or=2.0).

As shown in our results, HER-2/neu positivity significantly associates with tumor size and grade. Similar results were obtained by Almasri and Al Hamad [1] and Prati et al. [42]. Regarding nodal metastasis, our results are in agreement with other studies which reported that HER-2/neu amplification has been strongly associated with positive lymph nodes [44], [29]. They suggested that HER-2/neu oncogene may be a marker for aggressive clinical behavior in breast cancer patients. In relation to stage, HER-2/neu amplification was shown to be significantly associated with late stages of the disease, confirming previous reports [43], [47] that detected increased frequency of HER-2/neu amplification, by FISH analysis, in patients suffering from stages later than IIB based on TNM staging system. HER-2/neu amplification detected by FISH also predicted disease-related death independent of classical clinicopathological factors in breast cancer patients who received adjuvant therapy [15].

Matrix metalloproteinases (MMPs), in particular the gelatinases MMP2 and MMP9, are important mediators of tumour invasion and metastasis. Decock et al. [14] demonstrated that patients with HER-2/neu overexpressing tumours showed an increase of 27% in plasma MMP2 activity, but not in MMP9, compared with patients without overexpression, suggesting a role for HER-2/neu in the signalling pathways of MMP2 activation in carcinogenesis.

In our study, results showed that c-myc was amplified in 73% of malignant breast tumors, but only in 35% of benign lesions. Similarly, Blancato et al. [11] reported a high proportion (70%) of breast carcinoma were amplified for the c-myc gene. Also, Park et al. [35] found that 15.2% of the breast cancer specimens were c-myc positive. Blancato et al. [11] reported that c-myc gene amplification using FISH was correlated with the percentage of tumor cells which expressed high levels of its protein, as detected by immunohistochemistry in invasive cells.

Results of the current study demonstrated a significant relation between c-myc positivity and tumor size, grade and stage of the disease. Regarding tumor size, Aulmann et al. [5] examined c-myc oncogene amplification using FISH in a series of 96 pure ductal carcinoma in situ (DCIS) and observed that c-myc oncogene amplification was significantly associated with larger tumor size. They concluded that c-myc oncogene appears to be involved in the development of a more aggressive phenotype of DCIS. Shanmugham et al. [46] reported that c-myc expression, assessed by immunohistochemical staining, showed a positive association with increasing grade. With regard to disease staging, Yang et al. [56] and Aulmann et al. [4] showed a significant association between c-myc expression and amplification, respectively, and advanced clinical stage of the disease. They implicated the role of c-myc in tumor development and consequently disease progression.

Results of the present study revealed that p53 mutations, assessed by FISH, were detected in 64% of malignant cases but only in 17% of benign tumors. This finding is in agreement with the study of Lukas et al. [28], which investigated p53 mutation in breast carcinoma in situ (CIS) using immunohistochemical method. They have shown that p53 protein overexpression was identified in 22% of pure intraductal breast carcinomas and in 35% of breast CIS with invasive disease. Recently, Peng et al. [39] reported an increase in the level of p53 gene expression in breast cancer using molecular beacon imaging

technology. In our study, genetic alterations in p53 were detected in benign breast tumors. This is consistent with the study of Rohan et al. [45] who demonstrated that accumulation of p53 protein in benign disease was associated with an increased risk of progression to breast cancer.

As shown in our results, p53 positivity is significantly associated with age of the patient, size and histological grade of the tumor and positivity of lymph nodes. Pietiläinen et al. [40] analyzed female breast carcinomas immunohistochemically for p53 expression. They found that high fraction of p53-positive nuclei was significantly related to patient age under 70 years. Moreover, Gion et al. [18] reported that younger age was related to low p53 values in breast cancer patients. Regarding tumor size, Overgaard et al. [34] demonstrated that TP 53 gene mutations were significantly more frequent in tumors of large size. In addition, Montero et al. [31] found a significant relation between p53 positivity and tumor size. With regard to the histological grade, our results showed that p53 mutation was significantly associated with low histological grade, a finding that is contradictory to that of Bertheau et al. [10] and Goel et al. [19]. They reported that p53 positivity is significantly associated with high histological grade. This controversy could be referred to the small number of grade III tumors included in our study. Regarding lymph node status, Pratap and Shousha [41] demonstrated that p53 positivity was significantly related to the presence of extensive axillary lymph node metastasis, a finding that is in harmony with our results. In addition, Cianga et al. [13] reported that 77.2% of the primary breast carcinomas and 75% of the lymph nodes metastases are positive for von Willebrand factor, a marker of the endothelial cells, and this positivity is significantly correlated with the expression of p53, supporting the idea that angiogenesis is a marker for tumor aggressiveness and p53 could be involved in this process. In conclusion, future studies must be completed that include larger databases of patients, which integrate clinical, pathologic, and molecular oncogene parameters. The merging of these data may provide the clinician with an enhancement of prognostic information that accurately predicts the aggressive phenotype for breast cancer. Thereafter, decisions regarding the necessity to administer and delete adjuvant therapies will gain scientific credibility.

Notes

Conflicts of interest

None declared

References

1. Almasri NM, Al Hamad M. Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in breast carcinoma in Jordan. Breast Cancer Res. 2005;7(5):R598-604. DOI: 10.1186/bcr1200
2. Altman DG. Practical statistics for medical research. London: Chapman and Hall; 1991.
3. American Joint Committee on Cancer (AJCC). Manual for staging of cancer. 4th ed. Philadelpha: Lippincott; 1992.
4. Aulmann S, Adler N, Rom J, Helmchen B, Schirmacher P, Sinn HP. c-myc amplifications in primary breast carcinomas and their local recurrences. J Clin Pathol. 2006;59(4):424-8. DOI: 10.1136/jcp.2005.029264
5. Aulmann S, Bentz M, Sinn HP. C-myc oncogene amplification in ductal carcinoma in situ of the breast. Breast Cancer Res Treat. 2002;74(1):25-31. DOI: 10.1023/A:1016061327812
6. Barsyte-Lovejoy D, Lau SK, Boutros PC, Khosravi F, Jurisica I, Andrulis IL, Tsao MS, Penn LZ. The c-Myc oncogene directly induces the H19 noncoding RNA by allele-specific binding to potentiate tumorigenesis. Cancer Res. 2006;66(10):5330-7. DOI: 10.1158/0008-5472.CAN-06-0037
7. Beatty BG, Bryant R, Wang W, Ashikaga T, Gibson PC, Leiman G, Weaver DL. HER-2/neu detection in fine-needle aspirates of breast cancer: fluorescence in situ hybridization and immunocytochemical analysis. Am J Clin Pathol. 2004;122(2):246-55. DOI: 10.1309/X8UP920UF4XM1C5C
8. Benchimol S, Minden MD. Viruses, Oncogenes and tumor suppressor genes. In: Tannock IF, Hill RP, editors. The basic science of oncology. New York: McGraw Hill; 1998. p. 79-105.
9. Bernardino J, Apiou F, Gerbault-Seureau M, Malfoy B, Dutrillaux B. Characterization of recurrent homogeneously staining regions in 72 breast carcinomas. Genes Chromosomes Cancer. 1998;23:100-8. DOI: 10.1002/(SICI)1098-2264(199810)23:2<100::AID-GCC2>3.0.CO;2-6
10. Bertheau P, Steinberg SM, Merino MJ. C-erbB-2, p53, and nm23 gene product expression in breast cancer in young women: immunohistochemical analysis and clinicopathologic correlation. Hum Pathol. 1998;29(4):323-9. DOI: 10.1016/S0046-8177(98)90111-3
11. Blancato J, Singh B, Liu A, Liao DJ, Dickson RB. Correlation of amplification and overexpression of the c-myc oncogene in high-grade breast cancer: FISH, in situ hybridisation and immunohistochemical analyses. Br J Cancer. 2004;90(8):1612-9. DOI: 10.1038/sj.bjc.6601703
12. Brennan PJ, Kumagai T, Berezov A, Murali R, Greene MI. HER2/neu: mechanisms of dimerization/oligomerization. Oncogene. 2000;19:6093-101. DOI: 10.1038/sj.onc.1203967
13. Cianga C, Cianga P, Cozma L, Diaconu C, Carasevici E. Evaluarea angiogenezei in cancerul mamar. Corelatii cu agresivitatea tumorala [Evaluation of the angiogenic process in breast carcinoma--correlations with cancer aggressiveness]. Rev Med Chir Soc Med Nat Iasi. 2004;108(3):657-61.
14. Decock J, Hendrickx W, Wildiers H, Christiaens MR, Neven P, Drijkoningen M, Paridaens R. Plasma gelatinase levels in patients with primary breast cancer in relation to axillary lymph node status, Her2/neu expression and other clinicopathological variables. Clin Exp Metastasis. 2005;22(6):495-502. DOI: 10.1007/s10585-005-3992-2
15. Depowski PL, Brien TP, Sheehan CE, Stylos S, Johnson RL, Ross JS. Prognostic significance of p34cdc2 cyclin-dependent kinase and MIB1 overexpression, and HER-2/neu gene amplification detected by fluorescence in situ hybridization in breast cancer. Am J Clin Pathol. 1999;112(4):459-69.

16. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer: the value of histologic grade in breast cancer: Experience from a large study with long term follow-up. Histopathology. 1991;19:403-10. DOI: 10.1111/j.1365-2559.1991.tb00229.x

17. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide IARC CancerBase No 5, version 2.0. Lyon, France: IARC Press; 2004.

18. Gion M, Boracchi P, Dittadi R, Biganzoli E, Peloso L, Gatti C, Paccagnella A, Rosabian A, Vinante O, Meo S. Quantitative measurement of soluble cytokeratin fragments in tissue cytosol of 599 node negative breast cancer patients: a prognostic marker possibly associated with apoptosis. Breast Cancer Res Treat. 2000;59(3):211-21. DOI: 10.1023/A:1006318112776

19. Goel MM, Goel R, Mehrotra A, Nath P, Agarwal PK, Singh K, Mehrotra R. Immunohistochemical localization and correlation of p53 and PCNA expression in breast carcinoma. Indian J Exp Biol. 2000;38(3):225-30.

20. Gonzalez-Angulo AM, Hortobagyi GN, Esteva FJ. Adjuvant therapy with trastuzumab for HER-2/neu-positive breast cancer. Oncologist. 2006;11(8):857-67. DOI: 10.1634/theoncologist.11-8-857

21. Grushko TA, Blackwood MA, Schumm PL, Hagos FG, Adeyanju MO, Feldman MD, Sanders MO, Weber BL, Olopade OI. Molecular-cytogenetic analysis of HER-2/neu gene in BRCA1-associated breast cancers. Cancer Res. 2002;62(5):1481-8.

22. Hamilton A, Piccart M. The contribution of molecular markers to the prediction of response in the treatment of breast cancer: A review of the literature on HER-2, p53 and BCL-2. Ann Oncol. 2000;11(6):647-63. DOI: 10.1023/A:1008390429428

23. Harari D, Yarden Y. Molecular mechanisms underlying ErbB2/HER2 action in breast cancer. Oncogene. 2000;19:6102-14. DOI: 10.1038/sj.onc.1203973

24. Kallioniemi OP, Kallioniemi A, Kurisu W, Thor A, Chen LC, Smith HS, Waldman FM, Pinkel D, Gray JW. ERBB2 amplification in breast cancer analysed by fluorescence in situ hybridization. Proc Natl Acad Sci U S A. 1992;89(12):5321-5. DOI: 10.1073/pnas.89.12.5321

25. Lane DP. Cancer: p53, guardian of the genome. Nature. 1992;358:15-6. DOI: 10.1038/358015a0

26. Liao DJ, Dickson R. c-Myc in breast cancer. Endocr Relat Cancer. 2000;7(3):143-64. DOI: 10.1677/erc.0.0070143

27. Lotem J, Sachs L. Regulation by bcl-2, c-myc and p53 of susceptibility to induction of apoptosis by heat shock and cancer chemotherapy compounds in differentiation-competent and -defective myeloid leukemic cells. Cell Growth Differ. 1993;4(1):41-7.

28. Lukas J, Niu N, Press MF. p53 mutations and expression in breast carcinoma in situ. Am J Pathol. 2000;156(1):183-91.

29. Maru D, Middleton LP, Wang S, Valero V, Sahin A. HER-2/neu and p53 overexpression as biomarkers of breast carcinoma in women age 30 years and younger. Cancer. 2005;103(5): 900-5. DOI: 10.1002/cncr.20850

30. Miller C, Mohandas T, Wolf D, Prokocimer M, Rotter V, Koeffler HP. Human p53 gene localized to short arm of chromosome 17. Nature. 1986;319(6056):783-4. DOI: 10.1038/319783a0

31. Montero S, Guzmán C, Vargas C, Ballestín C, Cortés-Funes H, Colomer R. Prognostic value of cytosolic p53 protein in breast cancer. Tumor Biol. 2001;22(5):337-44. DOI: 10.1159/000050636

32. Müller M, Meyer M, Schilling T, Ulsperger E, Lehnert T, Zentgraf H, Stremmel W, Volkmann M, Galle PR. Testing for anti-p53 antibodies increases the diagnostic sensitivity of conventional tumor markers. Int J Oncol. 2006;29(4):973-80.

33. Nesbit CE, Tersak JM, Prochownick EV. MYC oncogenes and human neoplastic disease. Oncogene. 1999;18:3004-16. DOI: 10.1038/sj.onc.1202746

34. Overgaard J, Yilmaz M, Guldberg P, Hansen LL, Alsner J. TP53 mutation is an independent prognostic marker for poor outcome in both node-negative and node-positive breast cancer. Acta Oncol. 2000;39(3):327-33. DOI: 10.1080/028418600750013096

35. Park K, Kwak K, Kim J, Lim S, Han S. c-myc amplification is associated with HER2 amplification and closely linked with cell proliferation in tissue microarray of nonselected breast cancers. Hum Pathol. 2005;36(6):634-9. DOI: 10.1016/j.humpath.2005.04.016

36. Parkin DM, Whelan SL, Ferlay J, Teppo L, editors. Cancer incidence in five continents, volume VIII. Lyon (France): International Agency for Research on Cancer; 2002. (IARC Scientific Publication; No. 155).

37. Pauletti G, Godolphin W, Press MF, Slamon DJ. Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence in situ hybridization. Oncogene. 1996;13(1): 63-72.

38. Pauletti G, Slamon DJ. Interphase cytogenetics of the HER-2/neu oncogene in breast cancer. In: Andreeff M, Pinkel D, editors. Introduction to Fluorescence In Situ Hybridization: Principles and Clinical Application. New York, NY: Wiley-Liss; 1999. p. 153-71.

39. Peng XH, Cao ZH, Xia JT, Carlson GW, Lewis MM, Wood WC, Yang L. Real-time detection of gene expression in cancer cells using molecular beacon imaging: new strategies for cancer research. Cancer Res. 2005;65(5): 1909-17. DOI: 10.1158/0008-5472.CAN-04-3196

40. Pietiläinen T, Lipponen P, Aaltomaa S, Eskelinen M, Kosma VM, Syrjänen K. Expression of p53 protein has no independent prognostic value in breast cancer. J Pathol. 1995;177(3):225-32. DOI: 10.1002/path.1711770303

41. Pratap R, Shousha S. Breast carcinoma in women under the age of 50: Relationship between p53 immunostaining, tumour grade, and axillary lymph node status. Breast Cancer Res Treat. 1998;49(1):35-9. DOI: 10.1023/A:1005993220824

42. Prati R, Apple SK, He J, Gornbein JA, Chang HR. Histopathologic characteristics predicting HER-2/neu amplification in breast cancer. Breast J. 2005;11(6):433-9. DOI: 10.1111/j.1075-122X.2005.00125.x

43. Regitnig P, Schippinger W, Lindbauer M, Samonigg H, Lax SF. Change of HER-2/neu status in a subset of distant metastases from breastcarcinomas. J Pathol. 2004;203(4):918-26. DOI: 10.1002/path.1592

44. Riou G, Mathieu MC, Barrois M, Le Bihan ML, Ahomadegbe JC, Bénard J, Lê MG. c-erbB-2 (HER-2/neu) gene amplification is a better indicator of poor prognosis than protein overexpression in operable breast cancer patients . Int J Cancer. 2001;95(4):266-70.

45. Rohan TE, Hartwick W, Miller AB, Kandel RA. Immunohistochemical detection of c-erbB-2 and p53 in benign breast disease and breast cancer risk. J Natl Cancer Inst. 1998;90(17):1262-9. DOI: 10.1093/jnci/90.17.1262

46. Shanmugham R, Gopalan R, Shanthi P, Krishnan KB. Tumor angiogenesis and C-myc expression in breast carcinomas. Indian J Pathol Microbiol. 2004;47(3):340-2.

47. Shimada M, Imura J, Kozaki T, Fujimori T, Asakawa S, Shimizu N, Kawaguchi R. Detection of Her2/neu, c-MYC and ZNF217 gene amplification during breast cancer progression using fluorescence in situ hybridization. Oncol Rep. 2005;13(4):633-41.

48. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987;235(4785):177-82. DOI: 10.1126/science.3798106

49. Stewart BW, Kleihues P, editors. World Cancer Report. Lyon (France): International Agency for Research on Cancer; 2003.

50. Tubbs RR, Hicks DG, Cook J, Downs-Kelly E, Pettay J, Hartke MB, Hood L, Neelon R, Myles J, Budd GT, Moore HC, Andresen S, Crowe JP. Fluorescence in situ hybridization (FISH) as primary methodology for the assessment of HER2 Status in adenocarcinoma of the breast: a single institution experience. Diagn Mol Pathol. 2007;16(4):207-10. DOI: 10.1097/PDM.0b013e318064c72a

51. Vanden Bempt I, Vanhentenrijk V, Drijkoningen M, Wlodarsaka I, Vandenerghe P, De Wolf-Peeters C. Real-time reverse transcription-PCR and fluorescence in-situ hybridization are complementary to understand the mechanisms involved in HER-2/neu overexpression in breast carcinomas. Histopathology. 2005;46(4): 431-41. DOI: 10.1111/j.1365-2559.2005.02112.x

52. Wilson CA, Cajulis EE, Green JL, Olsen TM, Chung YA, Damore MA, Dering J, Calzone FJ, Slamon DJ. HER-2 overexpression differentially alters transforming growth factor-beta responses in luminal versus mesenchymal human breast cancer cells. Breast Cancer Res. 2005;7(6):R1058-79. DOI: 10.1186/bcr1343

53. Witzel I, Thomssen C, Krenkel S, Wilczak W, Bubenheim M, Pantel K, Neumann R, Jänicke F, Müller V. Clinical utility of determination of HER-2/neu and EGFR fragments in serum of patients with metastatic breast cancer. Int J Biol Markers. 2006;21(3):131-40.

54. Wyllie AH. The genetic regulation of apoptosis. Curr Opin Genet Dev. 1995;5(1): 97-104. DOI: 10.1016/S0959-437X(95)90060-8

55. Yamamoto D, Senzaki H, Nakagawa H, Okugawa H, Gondo H, Tanaka K. Detection of chromosomal aneusomy by fluorescence in situ hybridization for patients with nipple discharge. Cancer. 2003;97(3):690-4. DOI: 10.1002/cncr.11091

56. Yang J, Zhou L, Hu R, Zhao J, Yao X, Long Q, Fan W, Chen L, Xing T. [Expression of P62c-myc protein in breast cancer and its clinical significance]. Hua Xi Yi Ke Da Xue Xue Bao. 1998;29(4):399-401.

57. Yassin D, Kamel A, Taha H, Lotayef M, Hassan Y, Fawzy A, Akl S. HER family expression in Egyptian breast cancer patients. J Egypt Natl Canc Inst. 2003;15(4):373-80.

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