ORIGINAL ARTICLE

Overexpression of p53 in Nigerian breast cancers and its relationship with tumour grade and oestrogen / progesterone expressions

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ABSTRACT

Background: Mutation of the tumour suppressor gene, p53, is implicated in most cancers. This gene has also been associated with high tumour grade in breast cancers. African women are known to generally have high grade tumours. This study sought to determine the expression of p53 protein as well as the relationship with oestrogen receptor (ER) and progesterone receptor (PR) proteins.

Methodology: Formalin-fixed, paraffin-embedded tissue samples of diagnosed invasive breast cancer were obtained from the Department of Anatomic and Molecular Pathology, Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos, Nigeria between 2002 and 2005. The clinical details of the patients were obtained from the histological request forms. Immunohistochemical studies were carried out in the Department of Histopathology, Royal Cornwall Hospital, Truro, United Kingdom with the automated Vision Biosystems Bond-Max Machines. The statistical analysis was done with SPSS version 12.

Results: Overexpression of p53 is seen in (86/116) 73.1% in Nigerian breast cancers and 89.6% of these cancers were of higher grade. The study also showed that (27/35)77.1% of ER positive patients also showed p53 overexpression (*p=0.592*). We also found that (64/93) 68.8% of PR negative patients overexpressed p53 while (*21/23*) 91.3% of PR positive cases overexpressed p53 (*p=0.036*).

Conclusion: Most Nigerian breast cancer cases were of high grade and showed p53 overexpression. We found no significant relationship between p53 overexpression and ER status but, there was a significant relationship between PR status and p53 overexpression. Further studies are advocated to determine the prognostic value of p53 in Nigerian breast cancer cases.

Keywords: *High grade, immunohistochemistry, low grade, tumour suppressor gene, well differentiated*

DISCLOSURES: NONE

INTRODUCTION

The tumour-suppressor gene, p53, is the single most common target for genetic alteration in human tumors.[1] A little over 50% of human tumours contain mutations in this gene. Homozygous loss of the p53 gene is found in virtually every type of cancer, including carcinomas of the lung, colon and breast - three leading causes of cancer deaths.[2] The p53 gene is called to apply emergency brakes, when deoxyribonucleic acid (DNA) is damaged by irradiation, ultraviolet light, or mutagenic chemicals.[1] Through poorly understood mechanisms, there is a rapid increase in p53 levels and activation of p53 as transcription factor. The accumulated wild-type p53 binds to DNA and stimulates transcription of several genes that mediate the two major effects of p53: cell-cycle arrest and apoptosis.

The arrest of p53-induced cell cycle occurs late in the G1 phase and is caused by the p53-dependent transcription of the cyclin D kinase (CDK) inhibition p21. The p21 gene, inhibits the cyclin/CDK complexes and thus, prevents the phosphorylation of pRb necessary for cells to enter the S phase.[1] Such a pause in cell cycling is welcome because it allows the cells time to repair the DNA damage inflicted by the mutagenic agent. With loss of normal p53 (common in many tumours), cells exposed to mutagenic agents replicate the damaged DNA and thus, mutations become fixed in the genome.

Hormone receptor status, proliferative activity, loss of differentiation, inactivation of tumour suppressor genes, and over-expression of oncogenes are related events that may affect the prognosis of patients with breast cancer.[3] Studies have been variously carried out on patients in advanced countries and on Africans in diaspora on this subject matter.[3,4,5]

The present study is aimed at finding out overexpression of p53 and its relationship with hormone receptors as expressed in native Nigerian breast cancers.

METHODOLOGY

Formalin-fixed, paraffin-embedded tissue samples of invasive breast cancer were obtained from the Department of Anatomic and Molecular Pathology, Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos, Nigeria. One hundred and nineteen cases of evaluable tissue samples of invasive breast cancers were selected for this study from the cases reported between the year 2002 and 2005. The study was approved by the Clinical Research and Ethics Committee of LUTH in 3rd April, 2006. The samples were those obtained mainly from patients seen at the surgical clinics and theatres in LUTH, peripheral State hospitals, private health institutions in Lagos State as well as other health institutions in nearby Ogun State during the period of study. The clinical details of the patients were obtained from the histological request forms. Further analysis for the immunohistochemistry was carried out in the Department of Histopathology, Royal Cornwall Hospital, Truro, United Kingdom. This was because the facility was not available in our centre.

Tissue Microarrays Construction

The tissue blocks were melted and the tissues re-embedded in paraffin wax by the first author. Sections from the new blocks were stained with Haematoxylin and Eosin (H and E) stain. The H and E sections were re-evaluated for histological type and grade using Nottingham criteria – a modified Bloom - Scarf Richardson classification.

The viable, representative areas of each tumour specimen were selected and marked on the H and E stained slide. The corresponding tissue block was sampled for the tissue microarrays (TMAs). Core needle biopsy specimen were retrieved from the original tumour blocks using a manual arrayer and positioned in a recipient paraffin wax array block previously prepared. Three cores of tissues per tumour were obtained with a 2 mm diameter tissue cylinder.

Immunohistochemical Study

Immunohistochemical studies were carried out with the automated Vision Biosystems Bond-Max Machines. Tissue sections cut at 3–5µm and the pre-treatment for all slides included dewaxing in xylene, hydration in alcohol and washing with distilled solution.

Individual antigen retrieval systems were heated at 100^0C as follows:

p53, epitope retrieval solution 2(pH 9.0) for 30 minutes
ER, epitope retrieval solution 2(pH 9.0) for 20 minutes
PR, epitope retrieval solution 2(pH 9.0) for 20 minutes

Endogenous peroxidase activity was inactivated in 3% hydrogen peroxide for 10 minutes. Several other steps were followed according to Bondmax protocol.[6] Tissue sections were then incubated with primary antibodies for 20 minutes at room temperature. All primary antibodies used were commercially available mouse monoclonal IgGs. Table 1 shows the detailed data on the antibodies.

Mixed diaminobenzidine (DAB) refine substrate chromogen solution was used in the protocol, then, washed with Bond wash solution again, dehydrated and mounted. Negative controls for each series were performed leaving out the primary antibody. The positive control is as indicated in Table 1.

Nuclear staining of p53 was considered and diffuse cytoplasmic staining was ignored. The expressions of the antibody are shown in the figures 2, 3 and 4. Quick score method was adopted for the antibody, using the method devised by Allred, *et al*.[7] It is based on intensity and proportion of cell staining as follows:

Intensity

- Negative (*no staining of any nuclei at high magnification*) = 0
  Weak (*only visible at high magnification*) = 1
- Moderate (*readily visible at low magnification*) = 2
- Strong (striking positive at low magnification) = 3

Proportion of Cells Positive (Tumour nuclei)

- 0% = 0
- <1% =1
- 1 – 10% = 2
- 11 – 33% = 3
- 34 – 66% = 4
- 67 – 100% = 5

The Quick score is the sum of the two component score and gives a score in the range 0-8. In this study, score of $\leq$ 2 was considered negative; score of 3 and 4 was considered low positive expresser and score of 5 and above was considered as strongly positive expresser.[7]

Both the low positive and strongly positive expressers were regarded as having p53 overexpression and hence, regarded as positive. Tumours with no staining and scores of less than 2 were regarded as negative.

Statistics

The statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 16. The relationship between the degrees of expression was calculated using a Spearman correlation test. Cross tabulation and x^2 test were used for univariate comparison of data.

RESULTS

Out of the 124 histologically confirmed cases of invasive breast cancers, 122 were successfully made into TMA cores. The evaluable tumours from the TMAs varied for different antibodies; these were between 116 and 119, hence 116 was analysed in this study.

Distribution of p53 Overexpression in Breast Cancers

Table 2 shows that most 86/116 (73.1%) of the breast cancers showed p53 overexpression.

Comparison of p53 Overexpression with Tumour Grade

Table 3 summarises the relationship between p53 expression and tumour grade. Eighty-five out of one hundred and sixteen (73.3%) cases of breast cancers showed p53 overexpression and most 104/116 (89.6%) were of higher grade. However, there is no significant association between the grade of breast cancer and overexpression of p53 (*p = 0.400*)

Figure 1. H&E stain of invasive breast cancers *(x40)*

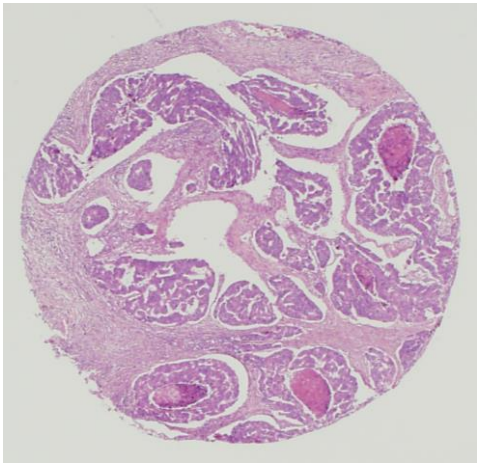
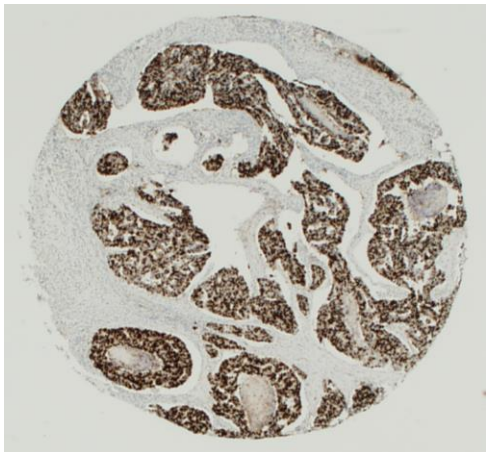


Figure 2. Overexpression p53 in invasive breast cancers *(x40)*



Overexpression of p53 and ER Status

Most of the patients 58/81 (71.6%) with p53 overexpression were ER negative. Similarly, 27/35 (77.1%) of ER positive patients also overexpressed p53, as shown in *Table 4*. This shows p53 is overexpressed in the patients regardless of the ER status. Thus, there is no significant association between p53 overexpression and ER in breast cancers *(p = 0.592)*.

Figure 3. Oestrogen receptors expression in invasive breast cancers *(x40)*

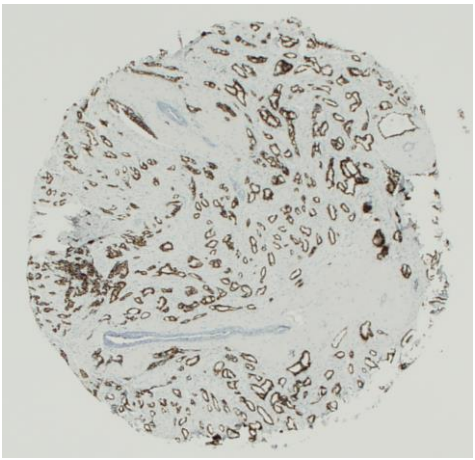
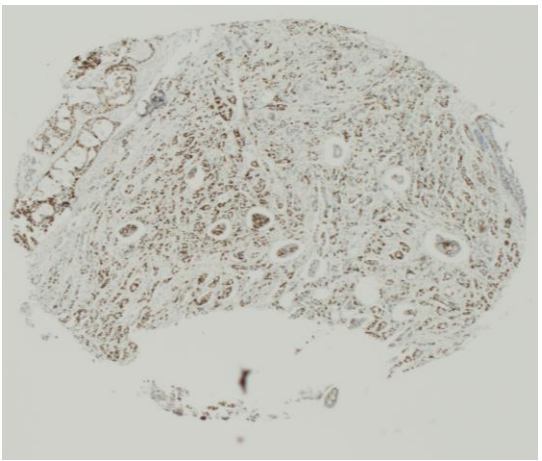


Figure 4. Progesterone receptors expression in invasive breast cancers *(x40)*



Overexpression of p53 and PR Status

Table 5 shows that 64/93 (68.8%) of PR negative patients overexpressed p53 while (21/23) 91.3% of PR positive cases overexpressed p53. In all, (85/116) 73% of the PR study group overexpressed p53, and a significant relationship exists between p53 overexpression and PR status of breast cancers *(p = 0.036)*.

Table 1. Antibodies used to immunostain breast cancers

Antibody	Clone	Working Dilution	Source	Positives Control	Staining Pattern
Oestrogen Receptor *(ER)*	NCL-L-ER-6F 11	1:200	Novocastra Laboratories, Newcastle Upon Tyne, U.K.	Endometrium	Nuclear
Progesterone Receptor *(PR)*	NCL-L-PGR	1:150	Novocastra Laboratories, Newcastle Upon Tyne, U.K.	Endometrium	Nuclear
p53	NCL-L-p53-DO7	1:75	Novocastra Laboratories, Newcastle Upon Tyne, U.K.	Colon carcinoma	Nuclear

Table 2. Distribution of p53 Overexpression in breast cancers

p53 expression	No. of Cases	%
Negative	30	26.9
Low Positive	14	12.1
Strongly Positive	72	62.0
Total	**116**	**100**

Table 3. Relationship between p53 overexpression and tumour grade

	GRADE	P53		TOTAL
		NEGATIVE	POSITIVE	
1 Low	Count	5	7	12
	% within GRADE	41.7%	58.3%	100.0%
	% within p53	16.1%	8.2%	10.3%
2 Moderate	Count	14	37	51
	%within GRADE	27.5%	72.5%	100.0%
	% within p53	45.2%	43.5%	44.0%
3 High	Count	12	41	53
	%within GRADE	22.6%	77.4%	100.0%
	%within p53	38.7%	48.2%	45.7%
Total	Count	31	85	116
	%within GRADE	26.7%	73.3%	100.0%
	%within p53	100%	100.0%	100.0%

(p= 0.400)

Table 4. Relationship between p53 and oestrogen receptor status

OESTROGEN RECEPTOR		p53		TOTAL
		NEGATIVE	POSITIVE	
Negative	Count	23	58	81
	%within Oestrogen Receptor	28.4%	71.6%	100.0%
	% within p53	74.2%	69.0%	70.4%
Positive	Count	8	27	35
	%within Oestrogen Receptor	22.9%	77.1%	100.0%
	%within p53	25.8%	31.8%	30.2%
Total	Count	31	85	116
	%within Oestrogen Receptor	27.0%	73.0%	100.0%
	%within p53	100%	100.0%	100.0%

(*p= 0.592*)

Table 5. Relationship between p53 and progesterone receptor status

PROGESTERONE RECEPTOR		p53		TOTAL
		NEGATIVE	POSITIVE	
Negative	Count	29	64	93
	% within PR	31.2%	68.8%	100.0%
	% within p53	93.5%	75.3%	80.9%
Positive	Count	2	21	23
	% within PR	8.7%	91.3%	100.0%
	% within p53	6.5%	24.7%	19.1%
Total	Count	31	85	116
	% within PR	26.7%	73.3%	100.0%
	% within p53	100%	100.0%	100.0%

(p =0.036)

DISCUSSION

Most of the breast cancers in this study overexpressed p53 protein, and were of the higher grade. The findings are consistent with the work of Oh, *et al* and Leong, *et al,* who reported that there were less of p53 protein expression in low grade and well differentiated tumours.[8,9] Nevertheless, there was no significant correlation between the grade of tumours and overexpression of p53 protein as recorded in this study (p = 0.400) which is corroborated by the studies of Oh, *et al* and Leong, *et al*.[8,9]

Reed, *et al* reported that 18% of their patients showed positive expression of p53 which is lower than that seen in our study with 73.1% of cases showing positive nuclear staining for p53.[10] This contrasting figure most probably resulted from the fact that majority of the cases from the study cited above were low grade breast cancers whereas in our study majority of the patients presented with higher grade tumours with only 12/116 cases (10.3%) presenting with low grade tumours.

In this study, most of our patients (73.1%) showed low and positive p53 expression and were high grade tumours which is consistent with other studies, they were also ER negative.[3,5,11] However, we found no significant association between p53 overexpression and ER status *(p = 0.592)*; this was also documented by Leong, *et al*.[9] The import of this is that, most times, p53 is expressed in the patients regardless of the ER status.

Interestingly, almost all of our patients (90.9%) with PR positive status showed p53 overexpression, as against 68.8% of PR negative patients showing same. This finding

is significant *(p=0.036)* and therefore, we can conclude that a significant correlation exists between p53 overexpression and PR status of breast cancers. This finding is at variance with the findings of Caleffi, *et al* and Jones, *et al*, which showed a significant p53 overexpression in African-American women which, also, correlates with negative status of ER and PR.[4,5] Dobes, *et al*, in their study, showed significant association of p53 mutation with tumour grades, metastasis, molecular subtypes, Her2 status and inverse correlation with oestrogen and progesterone status.[12] Thus, the PR status of the patients with breast cancer could predict the expression of p53 protein.[12]

Although, our finding contrasts with many studies worldwide, we believe that the tumour biology of Nigerian women with breast cancers needs further evaluation to establish the relationship between PR status and p53 overexpression. This is apart from some limitations of this study which may be as a result of the storage situation of the paraffin embedded tissue blocks that could hamper optimum antigen retrieval for immunohistochemistry, as well as the initial fixative used for the tissue which may have affected the antigens.

CONCLUSION

The overexpression of p53 is more common in high grade breast cancers. The majority of cases in this study are high grade tumours and 73.3% of cases showed positive nuclear staining for p53. Even though, there is no significant relationship between the grade of tumours and p53 overexpression, we can deduce that most high grade tumours show p53 overexpression. Most times, p53 is overexpressed in patients regardless of the ER status but there is a significant correlation between PR status and p53 overexpression. Thus, PR status may predict p53 overexpression in breast cancers of Nigerians. Further studies may be necessary to determine the prognostic value of p53 in breast cancers of Nigerians and Africans.

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