Journal of Morphology and Anatomy

Research Article Open Access

Mini Chromosomes Maintenance Complex Binding Protein as an Alternative Breast Cancer Cells Proliferative Marker to Monoclonal Ki67

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Abstract

Background: Cancer is the last stage of a genetic mutation of a cell with capacity to divide uncontrollably. MKi67 is the gold standard for the study of proliferation of cells and determine the prognosis of breast cancer patients. MKi67 is not a direct agent of cell replication thus making direct therapeutic effect on the gene and its product difficult, unlike MCMBP which is involved in cell replication directly. Most studies on MCMBP were done at mRNA level on colorectal carcinoma. This study focused on the immunohistochemical correlation of MCMBP as an alternative breast cancer cells proliferative marker by comparing it with Mki67 and the tumor grade.

Methods: Sixty (60) cancerous breast tissues were used for the test and 45 breast benign lesion tissues as control. The tissue sections were stained with haematoxylin and eosin (H&E) staining technique; and Horseradish peroxidase diaminobenzidine (HRP/DAB) immunohistochemical procedure using antibody to MKi67 and MCMBP. H&E grading was done using Elston-Ellie modification of Scaff-Bloom-Richard Methods. The percentage of tumor cells stained brown by immunohistochemical procedure was scored according to St. Gallen 2013 recommendation for MKi 67 for breast cancer. Tumor grades and values (absolute and scored) obtained for the antibodies were compared using Paired Samples T Test and Pearson's correlation.

Results: Genetic expression showed that the mean value of MKi67 was 21.88, median 15.00 that of the MCMBP was 27.73, median 20.00. There was a strong relationship between the staining characteristics of the two antibodies (r=0.850, P=0.001). MCMBP as well as MKi67 was able to differentiate benign tumor from malignant breast tissue (p<0.001; p<0.001) respectively. The scored value of MKi67 and MCMBP were moderately correlated with the tumor grade (r=0.526, r=0.486) respectively.

Conclusion: MCMBP like MKi67 is a good marker for the study of proliferative index of cells.

Keywords: Breast tumors, MKi67, MCMBP, Correlation

Introduction

Breast lesions encompass various breast diseases ranging from inflammation to malignancy. Malignancy is an uncontrolled growth of cells with tendency to invade another organ while benign tumor is localized in growth. Most cancers in the breast arise from cells that make up the internal structures of the breast involved in secreting milk. These structures are known as ducts and lobules. Molecular characterization of breast cancer using immunohistochemistry had made individual personalized medicine possible [1] especially to people living in the poor developing world [2]. Immunohistochemical characterization of gene expression is nearly equivalent to the mRNA characterization both in specificity and sensitivity [3]. Monoclonal ki67 (MKi67) as an aid of cell proliferation prevents chromosomes from collapsing into a single chromatin mass by forming a steric and electrostatic charge barrier; its protein has a high net electrical charge and acts as a surfactant to dispersing chromosomes, thus enabling independent chromosome motility and efficient interactions with the mitotic spindle [4,5]. Minichromosome Maintenance Complex–Binding Protein [MCMBP] is directly involved in the dynamics of the minichromosome maintenance complex by unwinding the MCM2-7 from the sister chromatids paving way for mitosis. MCM family was actively involved in nuclear replication, the role of which may later translate to a new chemotherapeutic drug for cancer. According to Lewandowska [6], the use of MCM proteins allows one to assess the response to therapy in a number of common tumor types. Ishimi et al. [7] and Simon and Schwacha al. [8] studied the utility of MCM in the design of new anti-cancer therapies by inhibiting DNA replication by direct effect of heli-quinomycin and ciprofloxacin on MCM 2-7 complex by Kim et al. [9] reported that MCMBP helped *Trypanosome brucei* to evade host immunity (Figures 1-3).

Few studies had been done on MCMBP. Gene expression analysis had shown that alternation on MCMBP transcripts levels correlated with an increment in the relapse of human carcinoma and great increase in colorectal carcinoma [10]. Therefore, protein expression of MCMBP was studied by comparing it with the MKi67 which had been described in literature as a gold standard marker of proliferation immunohistochemically [11].

Materials and Methods

Area of study and sample collection

Formalin Fixed Paraffin Embedded (FFPE) tissue blocks of samples of patients attending Ladoke Akintola University Teaching Hospital

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Received June 12, 2018; **Accepted** February 02, 2019; **Published** February 09, 2019

Citation: Iyiola S, Enweani IB, Ngokere AA, Avwioro OG, Komolafe AO, et al. (2019) Mini Chromosomes Maintenance Complex Binding Protein as an Alternative Breast Cancer Cells Proliferative Marker to Monoclonal Ki67. J Morphol Anat 3: 119.

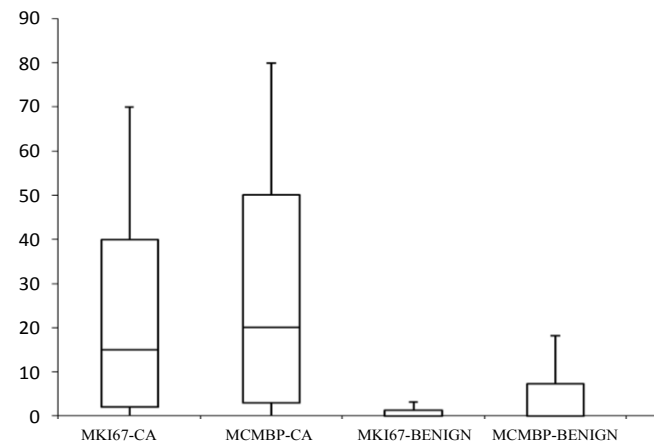

Figure 1: Boxplot comparing the absolute values of cancer and benign tumors for MKi67 and MCMBP. The plot showed that both antibodies were able to distinguish benign lesion from malignant lesions. The MCMBP actual values were higher than the values obtained from Mki67 antibody.

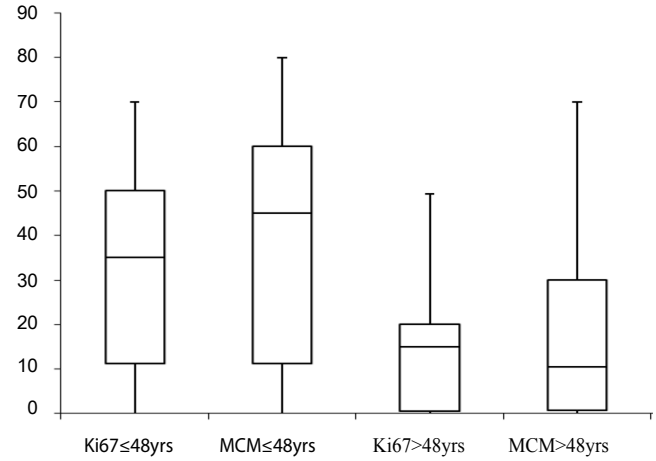

Figure 2: Boxplot comparing MKi67 and MCMBP for women stratified by age 48 years.

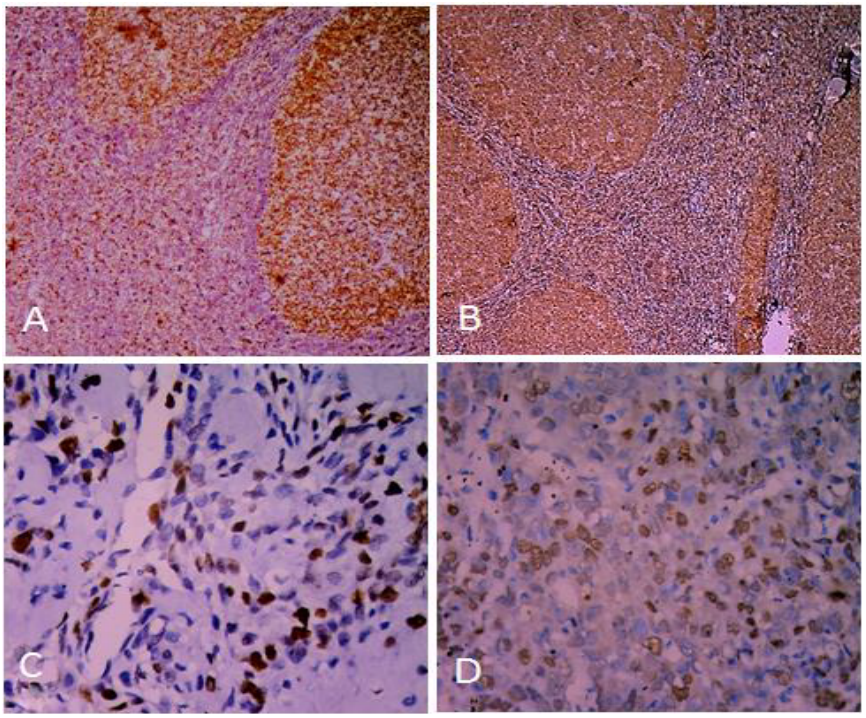

Figure 3: A and B: MKi67 and MCMBP staining of tonsil for control x100; C and D: MKi67 and MCMBP staining for invasive ductal carcinoma of the breast cancer respectively x400.

located in southwestern Nigeria were retrieved from histopathological archives. Approval for this research was obtained from the Ethical Clearance Committee of the Hospital (Table 1).

Patients' data and sample analysis

A total number of 105 breast tumors containing 60 breast cancers, 45 benign breast tumors were obtained from histopathological archives. A representative block was selected for each patient. The tissue blocks were sectioned at 4 µm and stained with H&E [12] to demonstrate general tissue structure and cancer grading. Tumors grading was done using Elston-Ellie modification of Scaff-Bloom-Richard. The result from this was used to determine blocks suitability. To study the effect of age on breast cancer, the age group was classified into age ≤ 48 years and age > 48 years as reported by Okonofua et al. [13] that most women in this area attain menopause at this age group.

Antigen retrieval methodology and immune histochemical staining

The slides were processed for immunohistochemistry as follows:

Tissues were sectioned at 4 µm and floated in water bath onto

Table 1: The correlation and comparison of the values of Mki67 and MCMBP.

Co-relation	MKi67 (n=60)				MCMBP (n=60)				r	p
	Score 1	Score 2	Score 3	Score 4	Score 1	Score 2	Score 3	Score 4	-	-
Cancer	19	15	7	19	18	10	5	27	0.85	0.001
N=60 (%)	-31.7	-25	-11.7	-31.7	-30	-16.7	-8.3	-45		
Benin	38	5	2	-	37	6	2	-	0.81	0.001
N=45 (%)	-84.4	-11.1	-4.4		-82.2	-13.3	-4.4			
Grade1	2	2	--	-	2	2	-	-	-	-
N=4 (%)	-50	-50			-50	-50				
Grade2	13	10	5	4	12	6	2	12	0.67	0.024
N=32(%)	-40.6	-31.2	-15.6	-12.5	-37.5	-18.8	-6.25	-37.5		
Grade3	4	3	2	15	4	2	3	15	0.89	0.016
N=24(%)	-16.7	-12.5	-8.33	-62.5	-16.7	-8.33	-12.5	-62.5		
age≤48	4	6	2	14	5	3	1	17	0.81	0.004
n=26(%)	-15.4	-23.1	-7.7	-53.9	-19.2	-11.5	-3.9	-65.4		
Age>48 n=34 (%)	15	9	5	5	13	7	4	10	0.75	0.88
	-44.1	-26.5	-14.7	-14.7	-38.2	-20.6	-11.8	-29.4		

charged slides. The sections were dried on hot plate at 60°C for 2 hours. The slides were dewaxed and hydrated to distilled water. A litre of retrieval solution was boiled in a pressure pot and the hydrated slides were immersed in it, lid tightened and boiled until full pressure was reached. Boiling was continued for three minutes, after which it was left to cool for thirty minutes at room temperature. Sixty slides in two slides rack were retrieved at a time. Micropolymer detection kit from Abcam (ab80436), Horseradish peroxidase diaminobenzidine method was used. Staining technique was performed according to manufacturer's instruction. Counter staining was done using Iyiola-Avwioro haematoxylin [14] for 1 minute. Polyclonal antibody to MCMBP gene was purchased from Abcam (ab122478) while monoclonal MKi67 (M1B1) was used. Both antibodies were diluted 1:100.

Horseradish peroxidase diaminobenzidine (HRP/DAB) immunohistochemical staining procedure for MKi67 and MCM-BP

Slides were rinsed in distilled water. Tissue section was encircled with solution from hydrophobic pen to prevent reagent from flowing out of the section area. Then each slide was arranged in humidity chamber after each aforementioned treatment and flooded with phosphate buffer saline (PBS) to prevent drying. The slides were drained, two Drops of hydrogen peroxide was added to cover the sections and incubated for 10 minutes. The slides were washed twice in PBS; Protein Block was added and incubated for 10 minutes at room temperature. Slides were washed one time in PBS, two drops of primary diluted antibody (1: 100 for MKi67 and MCM-BP) was added to each slides; one antibody for a slide and incubated at room temperature for 1 hr. They were washed in PBS, complement was added, and incubated for 10 minutes at room temperature. They were washed; HRP conjugate was added and incubated for 15 minutes at room temperature. They were washed; DAB/Substrate was added to the tissue section and incubated for 7 minutes. They were washed, stained with Iyiola-Avwioro haematoxylin [14] for 1 minute. Next was to blue in tap water for 3 minutes, dehydrated, cleared and mounted with resinous mountant.

Scoring system

The immunohistochemical slides were reported based on the average percentage of brown staining tumor nuclei as recommended by previous study [15] and manufacturer recommendations. Percentages obtained for MKi67 and MCMBP were scored according to St. Gallen 2013 recommendation [16].

The classification was done as follows:

Scores Proportion

1 0-9%

2 10-18%

3 19-25

4 26+

Statistical analysis

All data were presented using frequencies and percentages. Box plot was used to compare the distribution of variables between malignant and benign tumors stained immunohistochemically with MKi67 and MCM-BP. Pearson correlation and t-test were employed at 95% confidence level to correlate and compare the values. Statistical Package for Social Sciences (SPSS) version 21 was used.

Results

The mean values for women aged 48 years and below were 31.58 and 39.69 for MKi67 and MCMBP respectively while the values for the other groups were 14.47 and 18.59 for Mki67 and MCMBP, respectively. The figure also depicts that both antibodies were able to show that proliferation rate is higher among women below 49 years of age. The mean absolute value was 21.88 and 27.7 for both MKI67 and MCMBP of cancerous samples respectively. The mean absolute value for MKi67 and MCMBP benign breast lesion were 1.84 and 3.95 respectively while the median was 0.

The table showed that the mean of Mki67 antibody was different from MCMBP antibody except among women having age above 48 years but both were strongly related. Both antibodies showed that almost 50% of the cancer samples belong to the two higher grades of the antibodies while just 4% of the benign breast samples were on the same grades. Most women above 48 years of age were on the lower scores of MCMBP and Mki67 while the opposite is the case among patients below 49 years of age. Most of the samples were on the lower half of the scoring system for both antibodies on grade 2 breast cancer while the opposite is the case for grade 3 cancers.

Discussion

This study revealed that MCMBP could be used in the study of proliferative index of breast cancer and appears to be more sensitive in the study of cell division than MKi67. Yousef et al. [17] showed that expression of MCM 2 was higher than MKi67 in both normal breast and breast cancer. Our finding is in conformity with the report of Simon et al. [8] that MKi67 expression could not clearly define slow-growing gastroenteropancreatic neuroendocrine neoplasm when compared with MCM2 and MCM3. The absolute mean value of MCMBP protein expression was significantly higher than that of MKi67 in all the value tested except among the women above 48 yr of age; despite the fact that MKi67 present at nearly all levels of cell proliferation cycle G (1) SG (2) M phase but very less in a resting G (0). MCMBP is highly associated with chromatin in G1/S and S phases, reduced binding to chromatin in G2, and further decreased binding in early M phase, then re-associates with chromatin in late M phase and continue to wane all through to the resting stage G (0). This may be attributed to the fact that the concentration of MKi67 reach the peak at Mitosis Stage which occupies the shortest time of cell cycle and probably disappear immediately the nuclear membrane was formed unlike MCMBP which wanes gradually until the cells formed enter into resting stage G (0). Contrary to this, the higher value may be attributed to the polyclonal nature of the MCMBP antibody used.

Breast cancer histologic tumor grade is based on how an abnormal tissue is closely related to the normal. The advent of MKi67 made the study of mitotic index of formalin fixed paraffin wax embedded tissue block possible. It has been observed that breast tumor grade scoring correlates to MKi67 scoring as it is the case in the present study. Our value is similar to 0.5 correlation obtained by the author [15,17]. The value obtained in this study was different from 0.237 obtained by Petric et al. [18]. Comparison of the values obtained between MCMBP and tumor grade in this study was much similar to the value obtained between MKi67 and tumor grade as both were moderately correlated ($r=0.526$, $r=0.486$, $p<0.05$). During mitosis Ki67 is localized around mitotic chromosomes maintaining and preventing the sister chromatids from being dispersed into the cytoplasm following nuclear envelop disassembly [5].

There is a strong positive relationship between the protein expression of MCMBP and MKi67 ($r=0.830$, $P<0.05$). Similarly, it was found that MCM2 was positively related to MKi67 expression on breast cancer ($r=0.6$, $p<0.05$) [19] but not as strong as the one obtained in this work. This may be attributed to the fact that MCM 2-7 act as a replication agent elongating the coiled DNA for template copy by messenger RNA; the action of which was unloaded by MCMBP in late S phase and G2 phase by interacting with MCM3, MCM5 and MCM7 in a forklike structure paving the way for mitosis to begin with MCMBP continuing to the next cell cycle.

It was observed that inflammatory cells and fibroblasts were not stained by both MKi67 and MCMBP as the cells were not undergoing cell division in the samples studied.

Conclusion

MCMBP has a good prospect to be used as a cell proliferative marker of breast cancer cells but more studies should be carried out on other tumors.

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