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ORIGINAL ARTICLE

ADHFE1 and C-MYC Association and Over-Expression in Different Stages of Human Breast Cancer Tissues

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ABSTRACT

Chief enantiomer in breast tumors is D-2HG. Mitochondrial Alcohol Dehydrogenase (ADHFE I) produces D-2HG. It is the CA breast oncogene which leads to decreased patient survival. c-MYC can upregulate ADHFE I by changes in iron metabolism whereas co expression of c-MYC and ADHFE I greatly augment ort hotopic growth of tumor in MCF7 cells.

Aims: To determine the expression levels and localization of c-Myc and ADHFE I in different stages of breast cancer tissue**.**

Study Design: Cross-sectional comparative study.

Methodology: Present study was conducted to enhance knowledge of c-Myc function in breast cancer by examination of relation between nuclear c-Myc and iron containing ADHFE I enzyme protein expression, using immunohist cichemistry. All this information was recorded on performa.

Statistical analysis: Data was analyzed using SPSS version 25. Analysis of variance (ANOVA) test, was employed to observe mean difference between groups**.**

Results: Nuclear c-Myc and cytoplasmic ADHFE I levels were significantly higher than controls and were positively correlated with each other in advancing stages of breast cancer.

Conclusion: It was concluded that the association between the expression levels of c-Myc oncogene and ADHFE-1 enzyme existed in all advancing stages of CA Breast and the expression of both increased as the breast cancer advanced.

Keywords: Breast cancer, c-Myc, ADHFE-I and Immunohistochemistry.

INTRODUCTION

Unbalanced metabolism is a symbol of cancer, playing a substantial role in leading the course of disease[1]. Reprogramrning of metabolic nature, occurring in the breast tumors can be linked to increase in alleged oncogenic metabolites. Such oncogenic products may contribute to malignant transformation[2]. Metabolic remodeling leads to higher plasticity[3] and change the chromatin in the cells along with changes in rnicroenvironment of tumor or alteration in antitumor immunity[4]. A significant breakthrough from the recent studies, is the reflection that the 2- hydroxyglutarate gets accumulated in the breast cancers[5]. Nuclear transcription factor c-Myc is an oncoprotein that triggers tumorigenesis[6]. It has been observed that the tissues which develop increased proliferation also develop over expression of MYC as it u p-regulates in the patients of MAFLD and related HCC[7]. In case of lymphomas such as Burkitt lymphoma, the translocation of MYC transcription factor to the immunoglobulin or to the locus on T cell receptor can lead to deregulated expression and thus transformed lymphocytes. Its upregulation has been witnessed in several malignancies[8]. A noteworthy association exists between raised levels of 2HG and stimulation of M YC in breast cancer[9]. Studies have shown a global raise of DNA methylation in the 2HG-high tumors in African American patients. Such tumors showed stem cell-like transcription with WNT and MYC pathway activation and over-expression of glutaminase[10].

Mitochondrial enzyme alcohol dehydrogenase is a D-2HG—generating enz yme, it comprises iron- binded protein I, and is named as ADHFE I. It is an oncogene of CA breast, which leads to decreased patient survival[11]. ADHFE I promotes formation of reactive oxygen, D-2HG and reductive metabolism of glutamine. It aims cell dedifferentiation, mesenchymal change and phenocopied change that appears with an increased D-2HG level in the cancerous cells[12]. D-2HG is a metabolite with oncogenic potential, a prospective driver in the disease progress[13].

Objectives: To determine the expression levels and localization of c-Myc and ADHFE I in different stages of breast cancer tissue**.**

METHODOLOGY

Sample Collection: Paraffin embedded tissue sections of clinically diagnosed patients with known stage of breast cancer were selected from the Department of Pathology, Army Medical College. Rawalpindi. Data availability and protocol were granted by Institution's Ethical Committee. The Procedures and techniques performed were in accord with " 1964 Helsinki declaration and its later amendments". Prior notified consent from participants in study, was taken. Samples of CA breast tissue were acquired from the Pathology Department of Combined Military Hospital, Rawalpindi.

Antibodies: The anti-ADHFE I antibody, employed was obtained from Abcam (ab229 146; I : 1000), c-Myc antibody, Catalog NoAHO0062, was from Invitrogen.

Immunohistochemistry: Paraffin fixed tissue sections, each with 3 pm thickness, were transferred to histo-grip coated slides, and then placed in an oven for I hour, at temperature 5f› 'C. De-waxing with solution of absolute xylene for 10 min and rehydration in ethanol (absolute, 80°/c, 70°/c for 3 min each), was carried out. Complete rehydration with water followed by antigen retrieval in l0X EDTA and TRIS Antigen Retrieval solution, was done for 25 minutes, at 100 'C, in the decloaking chamber. Addition of hydrogen peroxidase was done followed by PBS washing, thrice for 5 minutes each. Incubation with the primary antibody, 60 minutes and secondar y antibody, 15 minutes was done. Treatment with streptavidin-HRP (15 minutes), followed by the chromogen DAB (10 min) was carried out. Distilled water slide washing and hematox ylin counter staining for 1 minute was done. Slides dehydration in xylene and alcohol and mounting with DPX coated cover slips was done.

Pathology and Scoring: Slides were stained for ADHFE I and c Myc. Each slide was then scored by three histopathologists. For ADHFE I staining, the tumor ADHFE I lev'els were scored according to staining intensit y. A staining intensit y score of 0 was negative, score of I was low, score of 2 was intermediate and score 3 was taken as strong. Percentage of positive nuclei was calculated[14]. c-Myc scoring comprised of intensit y from 0 to 3 (0,1 , 2, 3). Percentage score was given I. I - 25°/c, while 2. 26-50°/c, 3.5 I -75°/c and 4.76- 100°/c. An intensit y score greater than I , was considered High. A percentage score > 3 was considered as High.

Image analysis: Tissue-stained image of each slide with positive result was obtained. Multi-head microscope (Olympus BX4I) was used.

Statistical Analysis: The analysis was carried out using SPSS-25. Analysis of variance (ANOVA) test, was employed to observe mean difference between groups whereas post-hoc test was applied to the see the inter-group comparisons.

RESULTS

Total 131 patients were recruited. There were 10 healthy controls, 30 patients were of stage- I CA breast, 32 patients of stage-2, 29 patients of stage-3 and there were 30 patients of stage-4 CA breast. The mean c-Myc transcription factor in stage- I patients was 4.80*3.52, in stage-2 patients it was 5.8 I*2.83, stage-3 patients had mean c-Myc value of 6.90*2.61 while mean c-Myc in stage-4 patients was 7.27*2.33 as shown in Fig- I and this result was statistically significant (p= 0.005).

Table 1: Mean c-Myc and ADHFE1 in study groups (n=131)

Variables	Stage-1	Stage-2	Stage-3	Stage-4	p-Value
c-Myc (mean*SD)	4.80*3.52	5.8 I*2.83	6.90*2.6 I	7.27*2.33	0.005*
ADHFE-1 (mean*SD)	3.57*2.67	5.38*2.32	6. I 0*2.36	7.30*2.03	<0.00 I*

*Statistically significant

Likewise when ADHFE I was checked, stage- I patients had mean ADHFE I 3.57*2.67, stage-2 patients 5.38*2.32, in stage-3 patients it was 6.I0*2.36 while mean ADHFE I in Stage-4 patients was 7.30*2.03 as shown in Fig-2, and that was significant statistically (p<0.00 I) shown in the table-1.

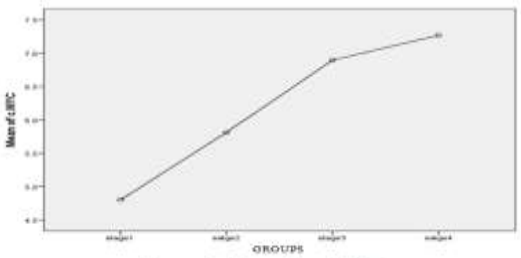

Figure-1: Means Plot (c-Myc)

Post-hoc test was applied to the see the inter-group comparisons. It showed similar statistically significant results with respect to c-Myc and ADHFE I between stage- I & stage-3 groups and stage- I & stage-4 groups but ADHFE I was also statistically significant in stage- I & stage-2 groups as shown in Table-2**.**

Table 2: Mean c-Myc and Mean ADHFE1 in the Inter-group comparisons (n=131)

Inter-Group Comparison	Stage-1 Group Vs. Stage-2	Stage-2 Group Vs. Satge-3	Stage-1 Group Vs. Stage-3	Satge-2 Group Vs. Stage-4	Stage-1 Group Vs. Stage-4
C-myc (mean*SD)	0.507	0.434	0.029*	0.194	0.006*
ADHFE-1 (mean*SD)	0.016*	0.605	<0.001*	0.600	<0.001*

*Statistically significant

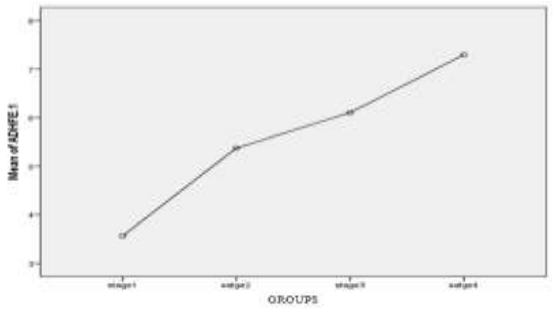

Figure-2: Means Plot (ADHFEI)

DISCUSSION

There is ample evidence that c-Myc is a protooncogene. There is ample evidence for the critical role of c-Myc in the pathogenesis of virtually all types of human cancers[15]. Our study was in coordination with one researcher whose research showed that miR-22/Spl/c-Myc networking regulates the CD147 up-regulation in CA breast and also miR-22 represses CA breast invasiveness and metastatic capabilities[16].

We indicated in our study that as the cancer advances there is a constructive correlation built between the oncogene c-Myc, and the enzyme ADHFE- 1. A similar study was performed by Mishra et al, that showed that alcohol dehydrogenase (iron-cADHFE 1), is CA breast oncogene which lessens the survival of the patient. They claimed that c-MYC upregulated enzyme ADHFE1 by alteration in the metabolism of iron, while the co-expression of c-Myc and ADHFE1 effectively boosted orthotopic progress of the tumor in the MCF7 cell lines[17]. Our research indicated statistically significant results with respect to c-Myc and ADHFE1 between stage-1 & stage-3 groups and stage- 1 & stage-4 groups. ADHFE 1 was also statistically significant in stage-1 & stage-2 groups. Stage-wise comparison between c-Myc and ADHFE- 1 using immunostaining can be very promising for research in other types of cancers. On the other hand one researcher found that enzyme ADHFE I had commonly a downregulation and hyper-methylation in different cancers and cancer cell lines and also in varying tissue samples. They found that a high tissue manifestation of enzyme ADHFE I was definitely linked to good prognosis of patients with colon cancer, gastric cancer and breast cancers[18]. Whereas our study agrees with the work of researchers, whose research showed that the accumulation of R-2HG in the cells could possibly lead to amplification of the processes taking part in the cancer development. R-2HG, is a consequence product of numerous metabolic enz ymes, including the ones in the mitochondrial. It was found that the production of mitochondrial 2HG was elevated in the CA breast cell lines. There was active competition for the preliminary substrate 2OG, between alcohol dehydrogenase ADHFE- I and isocitrate dehydrogenase IDH2[19]. The significant association between ADHFE-I and c-Myc in the later stages of CA breast is indicative of poor prognosis. Furthermore AHDFE- I mRNA expression analysis and methylation of DNA can be potentially used as diagnostic markers in the cancers and can prove to be of great benefit in predicting survival of the patients with cancer.

Limitations: Our study had limitations like financial constraints, lack of resources and short duration of study.

CONCLUSION

It was concluded that the association between the expression levels of c-Myc oncogene and ADHFE-1

enzyme existed in all advancing stages of CA Breast and the expression of both increased as the breast cancer advanced.

Authors' Contribution: SS&UA: Conceptualized the study, analyzed the data, and formulated the initial draft.

NI&AF: Contributed to the proof reading.

RS,RH&AS: Collected data.

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