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c-Myc and CK2 Correlation: A Passageway to Human Breast Cancer

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Abstract: c-Myc transcription factor is a protooncogene and a substrate of Casein Kinase 2 enzyme, both of these are upregulated in breast cancer. A Cross Sectional Analytical study with breast cancer paraffin embedded tissue sections (N=30) was conducted using Immunohistochemistry, cytoplasmic and nuclear expression of c-Myc and CK2 enzyme was determined and correlation between the two was found out. Scoring was performed by three histopathologists, blindly, any incongruity in results, corrected by using nearest readings. A high expression of Total CK2 in invasive cases was seen as compared to non-invasive. Nuclear and cytoplasmic localization was also higher in invasive group as compared to non- invasive.Total c-Myc expression was high in the invasive group, in comparison with non- invasive. In invasive cases, there was very strong and significant correlation between c-Myc and CK2 total, between c-Myc and CK2 cytoplasm and between c-Myc and CK2 nucleus. c-Myc and CK2 as biomarkers can help predicting the cancer phenotype and aggression.

Key words: c-Myc · CK2 · Ca Breast · Correlation

INTRODUCTION

Breast cancer is the commonest among women, under developed and developed world, causing death of over 508000 females in 2011 [1]. CK2 has over 100 substrates, many of which are connected with signal transduction and cell division [2]. Up-regulated in cancers, protein kinase CK2 supports neoplastic phenotype [3]. A ubiquitous serine / threonine kinase, particularly conserved, having a regulatory subunit (28 kDa β), the catalytic subunits (42 kDa α and 38 kDa α') and α2β2, α'2β2, αα'β2 tetramers [4]. The finding that CK2 displays dual specificity kinase action, has opened new venues for research its cellular activities [5]. CK2 is consistently deregulated in different cancers [6]. Regulatory proteins are protected by CK2, from degradation by caspases. This activity can lead to tumorigenesis [5]. CK2 elevated levels in cancers, support to tumorigenesis in transgenic mice, makes it a prospective candidate for molecular targeted therapy [7]. One of the substrates of CK2 is c-Myc. It is a recognized proto-oncogene. c-Myc is necessary for normal development but in cancers, it is upregulated. The reason for the upregulation in cancer is not known but is thought to take part in development of tumor [8]. c-Myc is a nuclear transcription factor that is essential for mammalian development [9]. Tissues with increased proliferation have been found to over express c-Myc [10]. In lymphomas including Burkitt lymphoma, translocation of c-Myc to immunoglobulin or locus on T cell receptor can cause deregulated expression and transformation in

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lymphocytes [11]. A drastic increase in lymphogenesis is seen when a transgene of c-Myc is co-expressed with CK2, indicating harmonious intercommunication involving CK2 and c-Myc. When activity of the CK2 in cell lines was inhibited pharmacologically, their proliferation was decreased and accordingly the steady levels of c-Myc also fall. Hence CK2 can be regarded as a significant controller of the steadiness of the c-Myc protein. Given that c-Myc is capable of being phosphorylated via CK2, it is hypothesized that the harmony amid the CK2 and c-Myc may be because of an operating communication among the two molecules [12]. It has been observed that co-expression of c-Myc and CK2 in transgenic models accelerated lymphogenesis dramatically as CK2 promotes stability of c-Myc [13].

MATERIALS AND METHODS

This study conducted at Department of Biochemistry and Molecular Biology, Army Medical College Rawalpindi and Armed Forces Institute of Pathology Rawalpindi, was a Cross Sectional Analytical study. Duration of the study was two years.

Sample Collection: Breast cancer tissue in paraffin embedded form were selected (N=30). Samples were obtained from Armed Forces Institute of Pathology. Breast cancer was of invasive ductal type.

Statistical Analysis: Data analysis was done by SPSS version 20. Descriptive statistics used to describe results i.e. mean, standard deviation (SD) for the quantitative variables, frequency along with the percentages for qualitative variables. Pearson correlation coefficient was used to find relationship among different variables. A p-value < 0.05 was considered, significant.

Ethical Approval: Research protocol was approved from Ethical Committee, Armed Forces Institute of Pathology. Study was conducted according to Good Clinical Practices, as approved by FDA, 1996 (FDA, 1996) and Declaration of Helsinki (WMA,2000).

Materials And Chemicals: Casein Kinase IIα Antibody(C-18), polyclonal, IgG 200µg/ml, Santa Cruz(cat#6479) with 1:200 dilution, Hela and Jurkat cell lysates used, as positive controls. c-Myc Antibody, Mouse Monoclonal antihuman c-Myc unconjugated Catalog NoAHO0062, 100µg/0.5ml, from Invitrogen. Detection Kit, LSAB Kit/ HRP, Rb/ Mo/ Goat (DAB+) system from (DAKO) Ref: K0679.Antibody Diluting Reagent Solution, Ready to Use, Invitrogen, Ref No 003218, contains 0.1%Sodium Azide.

Immunohistochemistry: Tissue section of 3-4 micron thickness, cut from paraffin block, kept in water bath at (40°-45°C), fixed on positively charged slides, having histogrip coating, kept at 56°C for 2hrs in oven. The slides then treated with absolute xylene for 10 minutes, absolute alcohol for 3 minutes, 80% alcohol for 2 minutes and 70% alcohol for 2 minutes and then dipped in water. 10X EDTA + TRIS Anitgen Retrieval Solution was used to treat slides, at 100°C,in electric de cloaking chamber, for 25 minutes, washing with distilled water was done. Slides were treated with Phosphate Buffer Solution and blocked by use of Peroxidase Blocking Solution S2023 DAKO. Washing was done thrice with Phosphate Buffer. Incubation using Primary antibody for 1 hour, was carried out followed by washing with PBS and incubation with secondary antibody for 15 minutes. After washing again Streptavidin-HRP treatment done for 15 minutes, followed by washing. DAB Chromogen was then applied for 10 minutes, washing with distilled water and counterstaining with Hematoxylin for 1 minute, was done. This was followed by washing with distilled water, counterstaining for 1 minute with Hematoxylin, washing and treatment with alcohol 90%, 80%, 70%, Xylene 90%, 80%, 70%. Mounting with coverslips having DPX coated coating.

Scoring: Scoring by three histopathologists was done blindly, discrepancies adjusted by using nearest readings.CK2 Scoring was done as 0= no staining, 1+ = weak staining, 2+ = moderate staining, 3+ = strong staining. The sum of nuclear staining and cytoplasmic staining shows total expression of the CK2. In c-Myc scoring, intensity was given 0, 1, 2, 3. Intensity score >1 was High. Percentage score was allocated 1.1-25%, 2. 26-50%, 3.51-75%, 4.76-100%. Intensity score > 1 was reflected as High and a percentage score > 3 was deliberated as High.

RESULTS

Tissue specific expression/localization of CK2α and c-Myc in breast cancer tissue was determined by IHC.

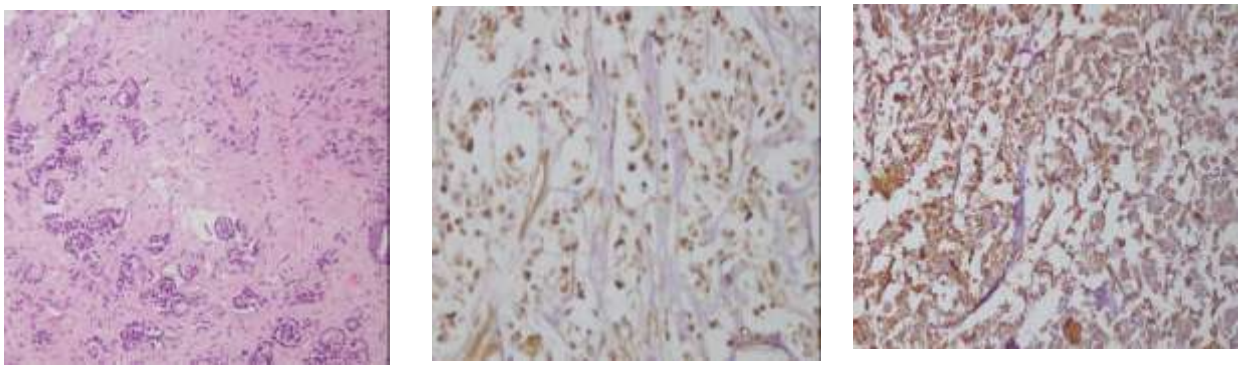
Comparison of c-Myc and CK2α Expression among Histopathological Groups: The study included 30 patients of Invasive Ductal Carcinoma. In 10 patients, there was no perineural invasion evident, but in 20 patients, it was seen. In invasive cases, score of average Nottingham Index was 6.0 (SD = 1.16691) and in the noninvasive

Table 1: CK2 and c-Myc correlation between protein expression and localization in non-invasive cases

	CK2-Nucleus	CK2-Cytoplasm	CK2-Total	c-Myc-Total
CK2-Nucleus	1			
CK2-Cytoplasm	0.612	1		
CK2-Total	0.875**	0.919**	1	
c-Myc-Total	0.458	0.578	0.583	1

Table 2: CK2 and c-Myc correlation between protein expression and localization in Invasive cases

	CK2-Nucleus	CK2-Cytoplasm	CK2-Total	c-Myc-Total
CK2-Nucleus	1			
CK2-Cytoplasm	0.367	1		
CK2-Total	0.833**	0.820**	1	
c-Myc-Total	0.789**	0.674**	0.886**	1



(A) (B) (C)

Fig. 1A: Rrepresentative figure of H and E staining
Fig. 1B: Shows strong nuclear staining for CK2α
Fig. 1C: Representative figure of high c-Myc staining.

cases, it was 4.06 (SD = 0.75011. Invasive cases had a high total CK2 expression (mean score 1.8500±1.38697), whereas noninvasive cases had a mean score (mean score 2.2000± 1.03280), ($p>0.05$). CK2 localization in the cytoplasm, in the invasive group 0.9500± 0.82558 was higher than in noninvasive group 0.8000± 0.63246 ($p>0.05$). Localization to nucleus was higher in invasive (1.9000± 0.85224) than the non-invasive cases (1.4000± 0.51640). c-Myc nuclear expression was also higher in invasive (29.0500± 14.10291) than non-invasive (19.5000± 10.34139, ($p>0.05$) showing CK2 nuclear localization in more invasive cases.(Data under publication)

A positive moderate correlation was found in CK2 expression and localization and c-Myc expression and localization in non-invasive but very strong correlation was found in invasive cases. In non-invasive cases, moderate correlation was seen between c-Myc and CK2 expression. In invasive cases, there was very strong and significant correlation between c-Myc and CK2 total, between c-Myc and CK2 cytoplasm and between c-Myc and CK2 nucleus. (Table 1, 2).

DISCUSSION

There is abundant proof that CK2 enzyme is over - expressed in proliferative states. Research done in past demonstrates that deregulated expression of one CK2 subunit renders oncogenic potential in the cells and in cooperation with few oncogenes it causes augmentation of tumor phenotype [14]. CK2, is an acknowledged pleiotropic, serine/threonine protein kinase [15]. It plays a part in an range of cellular processes, targets over 300 substrates [16]. Human cancer show raised expression of CK2 [12]. It is not clear yet, how this up-regulations participates in carcinogenesis [17]. Many cancers depend on elevated CK2 levels for their sustained survival [18]. Keeping this in view, we investigated the co-expression pattern evaluation of CK2α and c-Myc in the breast cancer. We found a positive correlation of these proteins in breast cancer tissues, when stained immunohistochemically.

The correlation was moderate between CK2α expression and localization and c-Myc expression and

localization in non-invasive breast cancer, while it was very strong in invasive cases. Whereas, in invasive cases c-Myc and CK2α expression showed a strong correlation. Previously, various people have reported independent over expression of CK2 and c-Myc in breast cancer. Landesman-Bollag showed that, both in humans and rodent model system with carcinogen-induced breast tumors, CK2 protein expression is raised, indicating its role in tumour formation [19]. Similarly it was shown by Ruzzene and Pinna that neonatal leukemia in mouse caused by c-Myc transgene, expression was augmented by the co-expression of CK2 [18]. Work done by Kim *et al*., also indicates the collaboration among CK2 deregulated expression and c-Myc oncogene, in development of Lymphoma [20]. The strongly positive correlation of CK2 enzyme and the invasive phenotype of immortalized cancer cells was mirrored by positive correlation of CK2 and c-Myc in the cells, which can be the potential candidates for therapeutic targets, like the work done by Putz *et al.* [21]. Our results are in coordination with the work done by Belguise *et al.* whose work explained that, CK2 and c-Rel cooperate to produce tumors having an extremely invasive behavior [22].

CONCLUSION

There is a positive correlation between c-Myc and CK2α, in the pathogenesis of breast cancer. Combined expression of CK2α, c-Myc can be used as biomarkers for predicting the cancer phenotype and aggression.

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