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CK2 and c-Myc co-Expression or Correlation: Pathway to Human Prostate Cancer

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Abstract: The CK2, a serine/threonine, protein kinase, targets over and above 300 substrates including c-Myc. CK2 expression is elevated in human cancers including prostate cancer. c-Myc expression is also up-regulated in the prostate cancer. The objective was to evaluate the co expression and correlation of CK2 and c-Myc in prostate cancer. Study Design: Cross Sectional Analytical Study. Duration: Study was conducted at Army Medical College and AFIP, duration was two years. Methods: a retrospective study of immunohistochemical analysis, approved by Armed Forces Institute of Pathology Ethical Committee. Paraffin embedded tissues of diagnosed prostate cancer, 30 in number and 30 cases of Benign Prostatic Hyperplasia (BPH) were included in the study. The tissue sections were subjected to immunostaining for CK2 and c-Myc and staining intensity was measured for each protein expression. Data was analysed through SPSS version 20. Pearson correlation coefficient was applied to correlate expressions of CK2 and c-Myc and p-value calculated.Results; significant expression observed in prostate cancer tissue as compared to BPH. Strong correlation was observed between the CK2 and c-Myc nucleus and amid the c-Myc and CK2 total, as compared to BPH. Conclusion.CK2 and c-Myc expressions are highly and significantly correlated in prostate cancer in invasive as well as non-invasive stages as compared to BPH as control.

Key words: CK2 · c-Myc · Prostate Cancer · Immunohistochemistry

INTRODUCTION

The protein kinase CK2 is a ubiquitous protein, which is present in cytoplasm and nucleus. An established kinase to phosphorylate over 100 substrates [1] has catalytic subunits either α or α' which associate with β subunits [2] CK2 is involved in cell differentiation, proliferation, transformation and apoptosis [3]. Increased activity of CK2 has been recorded in all malignancies that have been investigated up till now [4] CK2 has been a target of molecular therapy [5]. It is evident that the decontrolled expression of CK2α subunit imparts an oncogenic potential [6]. Of many substrates of enzyme CK2, one is c-Myc. It is renowned proto-oncogene, obligatory for a normal development of cells, but in case of cancers, it is up-regulated. The cause for the up-regulation is unknown [7].Tissues having increased proliferation have been seen to be over expressing c-Myc [8]. A radical increase in the lymphogenesis is observed, when transgene of c-Myc has a co-expression with enzyme CK2, showing strong intercommunication connecting CK2 and c-Myc. Since c-Myc is proficient to be phosphorylated by CK2, the association amid CK2 and c-Myc might be due to a communication amidst the two molecules.

MATERIALS AND METHODS

Patients and Samples: Paraffin embedded tissues with 30 diagnosed cases, each of prostate cancer and benign prostatic hypertrophy were taken from Armed Forces Institute of Pathology, Rawalpindi. Study included 6

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(20%) non-invasive cases, 15 (50%) perineural invasive and 9 (30%) lymphovascular invasive cases of prostate cancer (Data under publication).

Material: Mouse monoclonal anti-Human c-Myc unconjugated antibody was purchased from Invitrogen (Cat # AHO0062) and goat, polyclonal, casein kinase Iiα, Antibody (C-18) was obtained from the Santa Cruz Biotechnology, (Cat # sc-6479). All other chemicals obtained from the Sigma Aldrich.

Immunohistochemistry: Tissue sections with a thickness of 2-3 micron were heated to 56°C, then deparaffinised, followed by rehydration in xylene, in absolute alcohol, in the 80% and finally in 70% alcohol respectively. Slides plunged in the distilled water. Antigen retrieval was done by heating them in the 10X EDTA + TRIS Antigen Retrieval Solution, at 100°C in the Electric De cloaking Chamber, for a period of 25 minutes. Washing performed, using distilled water, then PBS, three times (5 min). Slides, applied with the peroxidase block, then washed with PBS. Incubation with a primary antibody (100µg/0.5ml dilution for c-Myc and 1:200 dilution for CK2) and then washing with PBS, done. Incubation with the (LSAB Kit/HRP, Rb/Mo/Goat(DAB+)system from DAKO cat#K0679, Secondary Antibodies and washing with PBS done. Streptavidin applied for 15 minutes, followed by PBS washing and then DAB staining, 10 minutes. Washed with distilled water, thrice. Counterstained with Hematoxylin and washing with distilled water. Dehydration of the sections was done using descending concentrations of 90%, 80% and 70% of alcohol and finally treated with xylene. Slides were mounted using DPX.

Scoring: In case of CK2, the score 0= no stain, 1+ = weak staining, 2+ = moderately stained, 3+ = strongly stained. The sum of cytoplasmic and nuclear scores indicates the total expression level of CK2. 1+ in the nucleus and cytoplasm 3+, marks a total of 4+[9]. For c-Myc scoring, the intensity of scores was assigned 0, 1, 2 and 3. Intensity of score >1, considered as high and a percentage score of >3 was considered as high. The percentage score was assigned (1)1-25% (2) 26-50% (3)51-75% (4)76-100% [10].

Data Analysis: Data was analysed through SPSS, (version 20). Descriptive statistics was applied for description of results. Mean, standard deviation, for the quantitative variables whereas frequency along with percentage for the qualitative variables. ANOVA used for comparing the three groups and for multiple comparisons, Post Hoc Tuckey HSD test was used. Pearson correlation coefficient was calculated to determine the correlation of expression. A p-value of< 0.05 reflected to be significant.

RESULTS

Total CK2 expression was high in non-invasive (Mean score5.4545±1.91644) and invasive cases (Mean score 5.0526±2.06757) as compared to BPH (mean scores 2.9333±1.22990) respectively. The difference was found highly significant among the three groups ($p < 0.05$) as calculated through ANOVA. Cytoplasmic localization of CK2 in noninvasive group had score 1.5455± 0.52223, in invasive group 1.6316 ± 0.59726 and BPH group 1.3333±0.60648. The expression was not significantly different amongst three groups (p=0.209). Nuclear localization being significantly different (p=0.044) amongst groups, with the highest inside non-invasive cases (1.6364±0.80904) than invasive (1.2632±0.93346) and BPH cases (0.8000±1.06350). Nuclear expression and localization was found to be significantly higher in non-invasive prostate cancer tissues (p=0.049) but not in invasive cancer tissues (p=0.251) as compared to BPH subjects. c-Myc expression in nucleus, was also not very significant (p=0.840) among the three groups i.e. non-invasive (17.7273 ± 9.22053), invasive (16.3684 ± 9.37615) and BPH (17.7000 ± 6.83878).

At 95% confidence interval, CK2 nucleus expression showed significant difference between the non-invasive and BPH group (p=0.049). Total CK2 levels were also significantly dissimilar between non-invasive and BPH cases ($p<0.001$) and invasive and BPH cases ($p<0.001$).

Positive correlation was present in CK2 expression, localization and expression, localization of c-Myc, from weak to moderate and moderate to strong in non-invasive as well as in invasive cases. In non-invasive cases, there was present, a significant correlation amid c-Myc and CK2 total and amongst c-Myc and CK2 cytoplasm whereas moderate to strong correlation existed between CK2 nucleus and c-Myc. In case of invasive cases, a significant correlation existed amongst c-Myc, CK2 cytoplasmic expression and c-Myc and CK2 total expression. Moderate to strong correlation was visible among c-Myc and CK2 nuclear expression. Weak correlation, observed between c-Myc and CK2 nuclear as well as with CK2 total expression in BPH cases (Shown in Table II a, II b and II c).

Table 1: Multiple Comparisons between the groups

Multiple Comparisons					
Tukey HSD					
Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.
CK2_Nucleus	Non-Invasive	Invasive	.37321	.37226	.578
		BPH	.83636*	.34633	.049
	Invasive	Non-Invasive	-.37321	.37226	.578
		BPH	.46316	.28809	.251
	BPH	Non-Invasive	-.83636*	.34633	.049
		Invasive	-.46316	.28809	.251
CK2_Cytoplasm	Non-Invasive	Invasive	-.08612	.22338	.921
		BPH	.21212	.20782	.567
	Invasive	Non-Invasive	.08612	.22338	.921
		BPH	.29825	.17287	.205
	BPH	Non-Invasive	-.21212	.20782	.567
		Invasive	-.29825	.17287	.205
CK2_Total	Non-Invasive	Invasive	.40191	.62986	.800
		BPH	2.52121*	.58600	.000
	Invasive	Non-Invasive	-.40191	.62986	.800
		BPH	2.11930*	.48744	.000
	BPH	Non-Invasive	-2.52121*	.58600	.000
		Invasive	-2.11930*	.48744	.000
c-Myc_Total	Non-Invasive	Invasive	1.35885	3.08892	.899
		BPH	.02727	2.87378	1.000
	Invasive	Non-Invasive	-1.35885	3.08892	.899
		BPH	-1.33158	2.39045	.843
	BPH	Non-Invasive	-.02727	2.87378	1.000
		Invasive	1.33158	2.39045	.843

Table 2a: Correlation amidst protein expression and localization in the Non-Invasive cases

	CK2 Cytoplasm	CK2 Nucleus	CK2 Total	c-Myc Total
CK2 Cytoplasm	1			
CK2 Nucleus	.280	1		
CK2 Total	.827**	.633*	1	
c-Myc Total	.740**	.535	.908**	1

Table 2b: Correlation between protein expression and localization in Invasive cases

	CK2 Cytoplasm	CK2 Nucleus	CK2 Total	c-Myc Total
CK2 Cytoplasm	1			
CK2 Nucleus	.184	1		
CK2 Total	.601**	.827**	1	
c-Myc Total	.660**	.509*	.784**	1

Table 2c: Correlation among protein expression and localization in BPH cases

	CK2 Cytoplasm	CK2 Nucleus	CK2 Total	c-Myc Total
CK2 Cytoplasm	1			
CK2 Nucleus	.214	1		
CK2 Total	.586**	.833**	1	
c-Myc Total	-.058	.290	.129	1

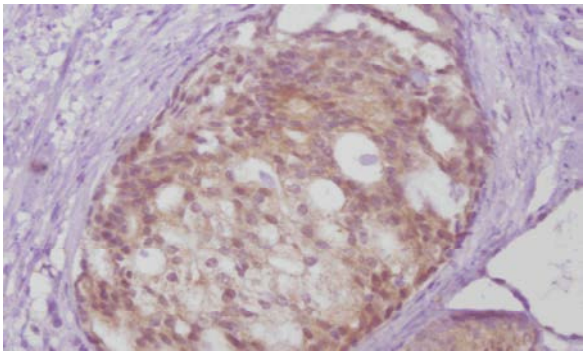

Fig. 1a: Prostatic adenocarcinoma showing strong nuclear and moderate cytoplasmic staining for CK2 immunomarker (400X)

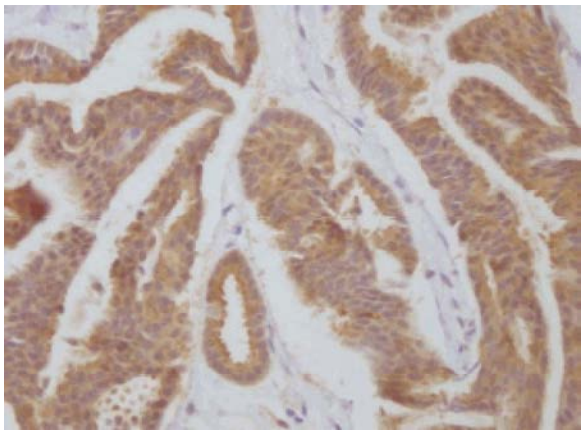

Fig. 1b: Immunohistochemical staining for c-Myc, revealing strong staining (400X), in prostate cancer.

DISCUSSION

CK2, an identified pleiotropic, protein kinase [11]joins in arrange of cellular processes [12]. CK2 expressions are reported to be raised up in human cancers [13] but how this up-regulations plays role in carcinogenesis is yet to be cleared, it has been manifested as marker for the prognosis in patients with squamous cell carcinoma lungs[14]. Diffused CK2 localization both in nuclear and cytoplasmic compartments is present in normal cells but in the cancer cells, CK2 is hugely localized in the nuclear compartment [15]. CK2 activity boost has been witnessed in human prostatic tissues by Laramas *et al previously* and in our study, we also observed higher expression and localization of CK2 in the nucleus of prostate cancerous tissue, as compared to BPH cases.

On the other hand, c-Myc, defined substrate of CK2 is an oncoprotein and transcription factor, regulates cell proliferation [16].

Keeping in view the association of CK2 and c-Myc, we investigated co-expression pattern in prostate cancer. We found positive correlation of these proteins in prostate cancerous tissues. The total CK2 and c-Myc expression was found to have significantly high correlation in prostate cancer tissues including non-invasive prostate tissues as well as invasive cases of prostate cancer tissues as compared to BPH cases. Our findings were consistent with the work previously reported for different cancers suggesting that correlation between expressions of these proteins is followed in prostate cancers. But the difference in our case is that the expression of c-Myc is not excessively elevated. CK2 functional interaction and regulation of c-Myc has been evident in the T cell lymphomas[17]. In lung cancers, a positive correlation has been found between increased expression of c-Myc and CK2 activity[18]. Co-expression of these two genes has been observed in transforming lymphocytes by Seldin and Leder[19]. CK2 was also found to be well-expressed in mammary tumors along with c-Myc (Critical down-stream target) [20]. CK2α suppression by small-interfering RNA (siRNA) inhibited colorectal cancer cells proliferation and resulted in G0/G1 phase arrest and decreased expression of c-Myc[21]. So it is suggested in prostate cancer that CK2 is over-expressed and it may be utilizing more efficiently the constitutive expression of c-Myc for cancer progression.

CONCLUSION

CK2 and c-Myc expression is significantly correlated in prostate cancer, in invasive as well as non-invasive stages as compared to BPH as control.Ck2 and c-Myc connection can help in predicting aggression of cancer.

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