SURVIVIN AND CK2 CO-EXPRESSION IN HUMAN PROSTATE CANCER

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ABSTRACT

Objective: To check the co-expression of survivin and CK2 in prostate cancer patients as compared to the Benign Prostatic Hyperplasia (BPH) patients.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: This study was conducted at Armed Forces Institute of Pathology, Army Medical College Rawalpindi, from Dec 2012 to April 2014.

Material and Methods: The study was designed as a cross-sectional analytical study and conducted at Armed Forces Institute of Pathology, Army Medical College Rawalpindi, from Dec 2012 to April 2014, after approval from institutional ethical committee. Expression of survivin was analyzed by immunostaining in paraffin-embedded sections from 30 diagnosed cases of resected prostate cancer and 30 BPH cases that were also immune stained for CK2.

Results: The CK2 and survivin were found to overexpress in prostate cancer as compared to BPH. Total score of CK2 survivin were strongly positive and significantly correlated in non-invasive cases as compared to BPH.

Conclusion: There is a strong positive correlation between survivin and CK2 over-expression in prostate cancer patients specifically non-invasive cases suggesting the coordination between the two proteins in early stages of prostate cancer progression.

Keywords: Casein Kinase 2, Co-expression, Prostate Cancer, Survivin.

INTRODUCTION

The protein kinase CK2, aser/thr kinase and a ubiquitous protein, is present in cytoplasm and nucleus. It phosphorylates over 100 substrates and several are involved in regulating signal transduction and cell division[1] The CK2 enzyme consists of catalytic subunits either α or α′ which associate with β subunits[2] and form a tetrameric complex[3]. The possible combinations of subunits include $\alpha_2\beta$, $\alpha\alpha'\beta_2$, $\alpha'_2\beta_2$[4]. CK2, having broad supply of substrates, plays important roles in significant cellular functions such as differentiation, proliferation, transformation and apoptosis[5]. Increased activity of CK2 has been observed in all malignancies investigated uptill now and it can be declared that CK2 control is coordinated with carcinogenesis[6]. CK2 over-expression in many human cancers has been a target of molecular therapy[7]. It is evident that irregular expression of CK2α subunit results in an oncogenic potential. Recent studies also showed that elevated expression of CK2 in cancer cells reflects tumor cell proliferation as well as pathobiological features of cancer. It is also possible that CK2 deregulation might influence apoptosis in tumor cells[8]. survivin expression has been observed in various malignancies in the nucleus and/or cytoplasm of tumor cells. Nuclear survivin is crucial for complete mitosis and cytoplasmic survivin plays a role in apoptosis inhibition[9]. The mechanism involved in Inhibitor of Apoptosis (IAP) regulation in cells during tumor development is not clear[10]. Survivin is found to have implications in apoptosis inhibition and mitosis regulation in tumor cells[11]. Survivin overexpression has been observed in

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various tumors including prostate, breast, pancreas, ovarian and lung cancer[12]. The elevated expression of both of these proteins in cancers suggests their co-expression and functional correlation. Hence this study was designed to evaluate the co-expression of survivin and CK2 in the prostate cancer samples.

MATERIAL AND METHODS

Paraffin embedded tissues of 30 diagnosed cases of prostate cancer and Benign Prostatic Hyperplasia (BPH), were carefully chosen, by non- probability convenience sampling. The study was designed as a cross-sectional analytical study and conducted at Armed Forces Institute of Pathology, Army Medical College Rawalpindi, from Dec 2012 to April 2014. Each of the prostate cancer and BPH cases analyzed for CK2 expression (data under publication), were immunostained for survivin. Age of prostate cancer patients was between 60 to 70 years, BPH patients were between 50 to 65 years of age. The study included 6 (20%) non-invasive cases, 15 (50%) peri-neural invasive and 9 (30%) lymphovascular invasive patients.

Material

Goat, polyclonal, casein kinase IIα antibody (C-18) was procured from Santa Cruz Biotechnology, (Cat#sc-6479), monoclonal mouse anti-human survivin antibody was obtained from Dako(Clone 12C4) 1:100. For immunostaining, universal LSAB Kit/HRP, Rb/Mo/Goat (DAB+) (Cat # 0679), Antibody Diluting Reagent Solution, Ready to Use, Cat no 003218 was used. All other chemicals were obtained from Sigma Aldrich.

Immunohistochemistry

Tissue sections with thickness of 2-3 microns were heated at a temperature of 56 ^{0}C, then deparaffinized and rehydrated with xylene, absolute alcohol, 80% and then 70% alcohol respectively. Slides were then plunged in distilled water. The antigen retrieval was done by heating in 10X EDTA + TRIS Anitgen Retrieval Solution, at 100^{0}C in the Electric Decloaking Chamber for 25 minutes. Washing of slides with distilled water was done. The slides were allowed to cool for 20 minutes and washed with PBS, three times (for5 min each). The slides were then treated with peroxidase block solution and washed with PBS again. Sections were then incubated with primary antibody for survivin with dilution 1:100 and for CK2 1:200 and washed with PBS. The sections were then subjected to incubation with (LSAB Kit/HRP,Rb/Mo/Goat (DAB+) system from DAKO cat#K0679, secondary antibodies for 15 mins and washed with PBS again. Slides were

Table-1a: Non-Invasive group: Correlation between CK2 and survivin expression and localization.

Proteins/ Localization	CK2_nuc	CK2_cyt	CK2 total	survivin_nuc	survivin_cyt	survivin total
CK2_nuc	1					
CK2_cyt	.280	1				
CK2_total	.633*	.827**	1			
survivin_nuc	.610*	.671*	.835**	1		
survivin_cyt	.697*	.677*	.932**	.919**	1	
survivin_total	.708*	.729*	.957**	.373	.423	1

Table-1b: Invasive group: Correlation between CK2 and survivin expression and localization.

Proteins/ Localization	CK2_nuc	CK2_cyt	CK2 total	survivin_nuc	survivin_cyt	survivin total
CK2_nuc	1					
CK2_cyt	.184	1				
CK2_total	.827**	.601**	1			
survivin_nuc	.373	.251	.580**	1		
survivin_cyt	.423	.247	.585**	.679**	1	
survivin_total	.436	.272	.636**	.905**	.927**	1

treated with Streptavidin for 15 minutes followed by PBS washing and DAB staining for 10 minutes. Washing was done with distilled water three times (5 min each). Sections were counterstained with haematoxylin for 1 min and washed 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 mounting medium.

We previously reported the nuclear and cytoplasmic distribution of CK2 by scoring both the nuclear and cytoplasmic prostatic cancer. The staining intensity ranges from 0 to 3+. The score 0= no staining,1+=weak staining,2+=moderate staining,3+=strong staining. The sum of nuclear and cytoplasmic scores shows total expression levels of CK2. For example 1+ in nucleus and cytoplasmic 3+,makes a total of 4+[13]. Survivin scoring,was done as the percentage of positive cells, as follows I: 1-10% positive cells, II: 11-50%, III: 51-80% and IV: >80% positive cells. Staining intensity was scored as 1: weak, 2: moderate and 3: intensive. Scores for percentage of positive cells and scores for expression intensities were multiplied to calculate an immunoreactive score (IRS) as follows; 0-2 = no staining; 3-4 = weak staining, 6-8 = moderate staining; 9-12 = strong staining[14].

Data analysis

Data was analyzed through SPSS version 20. Descriptive statistics were used to describe the results i.e. mean and standard deviation (SD) for quantitative variables while frequency along with percentages for qualitative variables. Pearson's correlation coefficient was calculated to study relationship between different variables. ANOVA test was applied for the comparison between three groups. Independent t-test was applied for the comparison between two groups. Post hoc Tuckey's test was applied for comparisons. A *p*-value < 0.05 was considered as significant.

RESULTS

Average Gleason score of 30 patients of prostate cancer (adenocarcinoma) was 7.33 (SD ±1.124) with range of 5 to 9. Lymphovasular invasion was a seen in 9 (30%) patients while perineural invasion was seen in 15 (50%) patients. So a total of 19 invasive cases and 11 non-invasive cases were analyzed. It was also reported that CK2 expression was high in non-invasive (mean score 5.4545 ± 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=0.01). Mean difference between invasive and non invasive group by Post hoc Tuckey's test was insignificant p>0.05 ,while comparison between invasive and BPH p<0.05 and between non-invasive and BPH was significant p< 0.05. Cytoplasmic localization of

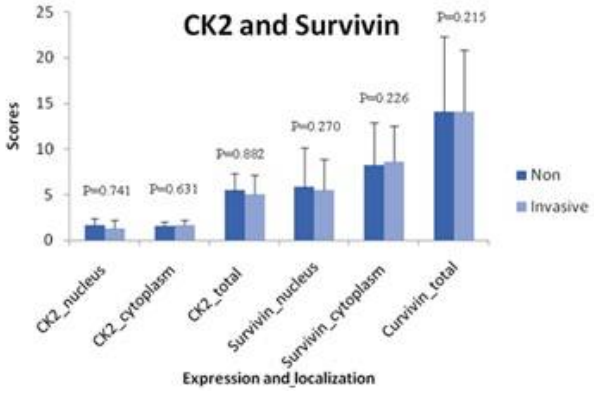


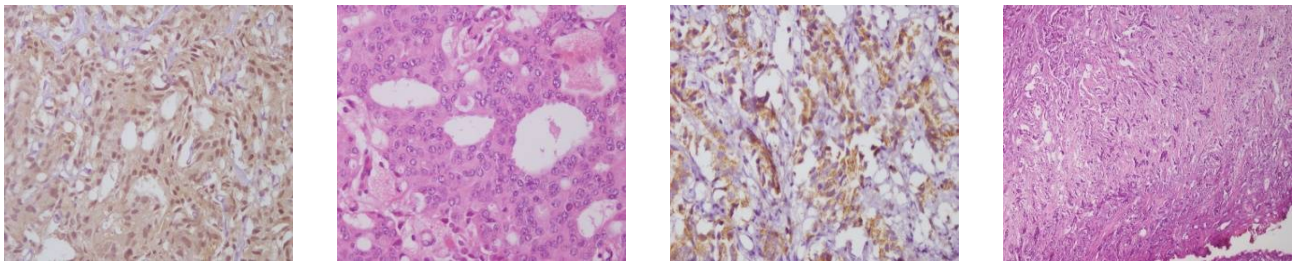
Figure-1: CK2 and survivin: Invasive vs Non-invasive.

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 among the three groups (p> 0.05). Nuclear localization was significantly different among the groups with highest in 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 prostate cancer tissues as compared to BPH subjects.

We checked the expression of survivin in these cancer tissues (BPH, survivin expression was not detected, data not shown. In non-invasive cases, mean nuclear survivin score was 5.8182 (SD ± 4.30855), mean cytoplasmic survivin score 8.2727 (SD ± 4.62798) and total survivin

score 14.0909 (SD ± 8.26383). In invasive cases, mean score for nuclear survivin expression was 5.4737 (SD ± 3.45396), mean score for cytoplasmic survivin 8.6316 (SD ± 3.91877) and mean score for total survivin expression 14.1053 (SD ± 6.75685).

Comparing invasive subjects with non-invasive subjects, we found no significant difference between CK2 and survivin expression and localization. The *p*-value was found to be above 0.05 at 95% confidence interval (as shown in fig-1).



(a) (b) (c) (d)

Figure-2: Photomicrograph showing moderate grade 2 nuclear and cytoplasmic staining (neu+2, Cyt +2) of CK2 immunomarker in prostate carcinoma (magnification 400X).

A positive, strong and significant correlation was found between CK2 expression/ localization and survivin expression/localization in non-invasive cases. In non-invasive cases, there was strong and significant correlation between CK2 nucleus and survivin nucleus, CK2 cytoplasm v/s survivin cytoplasm and CK2 total v/s survivin total. The total CK2 expression was very strong positively and significantly correlated with nuclear, cytoplasmic and total survivin expression as shown in table-1a. In invasive cases, no correlation was observed between survivin expression and localization versus CK2 expression and localization. Nuclear, cytoplasmic and total expression of survivin was strongly correlated with total expression levels of CK2 as shown in table-1b. (Data under publication).

DISCUSSION

Survivin has been widely implicated in tumorigenesis because of its activity related to apoptosis inhibition, cell cycle progression, metastasis and increased angiogenesis. The CK2 is observed to promote viability of tumour cell by enhancing survivin expression. Moreover, survivin overexpression alone was enough to revert CK2 inhibition effects on cell viability[15]. It has been observed that CK2 promotes tumor cell survival and inhibits apoptosis and in this regard survivin has been identified as a critical downstream target of CK2 in tumors [16].

In the present study, it was observed that increased expression of CK2 and survivin was present in invasive as well as non-invasive cases of prostate cancer. CK2α overexpression has been correlated with survivin expression previously[17]. CK2 modulates apoptotic activity via IAPs (apoptosis inhibitory proteins)[18] and it has been observed that CK2-mediated survivin up-regulation leads to enhanced cell survival and tumorigenesis[19]. In prostate cancer cells CK2 inhibition study has also proved the link between CK2 expression and survivin, showing that inhibiting CK2 also led to decrease in survivin expression[20]. Previously, we observed elevated expression and localization of CK2 in the nucleus of prostate cancer tissues as compared to BPH cases. But no significant difference in expression and localization was observed between invasive and non-invasive cases. The expression of survivin was not observed/ very weak in normal cells and it was found to be intense in cancerous tissues, in both invasive and non-invasive cases. The correlation was very strong between CK2 and survivin expression in non-invasive cases as compared to invasive cases. Hence our observations were similar to the studies

conducted earlier stating that the CK2 and survivin overexpression are linked. We suggest that strong correlation between CK2 and survivin in non-invasive cases link their co-expression at early stage of prostate cancer i.e., non-invasive prostate cancer.

CONCLUSION

There is a strongly positive and significant correlation between survivin and CK2 overexpression and localization in non-invasive prostate cancer patients suggesting their coordination in early stages of prostate cancer.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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