Comparison of Expression and Localization of Casein Kinase 2 in Prostate and Breast Cancer

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Abstract: Background CK2α enzyme is up regulated in many cancers suggesting its link in cancer development. Objective to evaluate and compare the expression pattern and localization of CK2α in breast and prostate cancer; to predict aggression in two most prevalent forms of cancers in females and males, at early stages. Methods immunohistochemistry was done to evaluate the expression of CK2α in prostate and breast cancer phenotype. Invasive as well as non-invasive cases were included in the study. Benign Prostatic Hypertrophy and normal breast tissue were used as controls. Independent sample t test was carried out to determine the statistical significance between the groups. Results significant increase in expression levels of CK2α was observed in both types of cancers studied (Prostate cancer and breast cancer) as compared to their respective controls. However, there was no difference in CK2α expression and localization between invasive and non-invasive cases. Conclusion CK2 can be used as biomarker for predicting cancer phenotype in both prostate and breast cancer patients, in early stages. There was no indication of this protein having any significant difference in expression between invasive and non-invasive carcinoma.

Key words: CK2α · Survivin · c-Myc · Ca Prostate · Ca Breast

INTRODUCTION

All over the world, the top ten well known causes of death include cancer. In 2012, among the total diagnosed cancers, 15% males were diagnosed with prostate cancer, making it the second most common cancer among men. Whereas in women, among total cancer cases include 25.2% for the breast, the most frequent form of cancer among women [1].

Two frequent diseases of men in old age are prostate cancer and benign prostatic hyperplasia that occur in prostate gland [2]. There are limited prognostic biomarkers for clinical use e.g. serum PSA [3]. An advance in the occurrence of the disease has been noticed recently, partially because of Prostate-specific antigen screening [4]. At present there are numerous clinically important challenges accompanying this conservative paradigm for the diagnosis and treatment of prostate cancer. Each of them expressively influences effective management of the prostate cancer and is subject of investigation in the basic research on biology of prostate tumor. The extensive use of PSA test has led to a massive increase in diagnosis of patients having clinically restricted and low Gleason graded carcinomas, which might not require treatment, as their tumors are comparatively indolent. Particularly patients with Gleason configuration of 3 or less practically never relapse after the local therapy, and very possibly could be managed conservatively with vigilant waiting, however, a small portion of these tumors will advance fast and require speedy treatment [5]. Subsequently, a big clinical challenge is presented by current inability to enthusiastically differentiate indolent from the aggressive tumors in, prostate cancer patients presenting with low Gleason graded tumors on biopsy [6]. The lack of this prognostic material has led to a substantial “overtreatment” of the patients who would then require conservative management only.

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Tumor markers are becoming increasingly important in breast cancer research because of their impact on diagnosis, prognosis, treatment, and survival, and because of their relation to breast cancer subtypes [7]. Although consistent mammography screening has aided and improved diagnosis of breast cancer but a fresh study has reported that annual mammography in the women aged between 40-59, does not decrease mortality, from the breast cancer further than that of physical examination or the usual care, when there is adjuvant therapy for breast cancer, freely available. Overall 22% of screen identified invasive breast cancers were found to be over-diagnosed, showing one over-diagnosed breast cancer case for every 424 women who had received mammography screening in trial.

CK2α is present in cytoplasm and nucleus of eukaryotic cells, having more than 300 substrates, out of which many are implicated in signal transduction and cell division [8]. Protein kinase CK2 is notably up-regulated in all the cancers studied so far, thus supporting neoplastic phenotype [9]. Keeping this fact in mind, we wanted to compare the expression pattern of CK2α in prostate and breast cancer, hypothesizing that it might be a common denominator deregulated in both types of cancers. In addition we hypothesize that CK2α behaves in a similar manner in both caners and thus can be used to differentiate between the invasive and non-invasive forms of prostate and breast cancer.

MATERIAL AND METHODS

The study was cross sectional analytical study conducted at the Department of Biochemistry and Molecular Biology, Army Medical College Rawalpindi, Armed Forces Institute of Pathology Rawalpindi. Tissue specific expression and localization of CK2α was determined by immunohistochemistry (IHC) in prostatic and breast cancer specimens.

Patients and Samples: Paraffin embedded tissue sections of diagnosed patients of prostate cancer (n=30), benign prostatic hyperplasia (n=30) and breast cancer (n=30), were taken from Armed Forces Institute of Pathology, Rawalpindi.

Material: The goat polyclonal casein kinase IIα Antibody (C-18) was purchased from Santa Cruz Biotechnology (Cat # sc-6479), (LSAB Kit/HRP, and Rb/Mo/Goat (DAB+) system from DAKO cat #K0679. Antibody Diluting Reagent Solution: Ready to Use, Invitrogen Ref NO 003218 (contains 0.1%Sodium Azide). All the other chemicals were acquired from Sigma Aldrich.

Immunohistochemistry: Tissue sections, cut with 2-3 micron thickness, heated at 56°C, then deparaffinized and rehydrated in the xylene and then absolute alcohol, 80% and 70% alcohol. Slides dipped in distilled water. Antigen retrieval by heating in 10X EDTA + TRIS Antigen Retrieval Solution, 100°C, in Electric DE cloaking Chamber, for 25 minutes. Washing of the slides with distilled water, cooling for 20 minutes, washing with PBS thrice (5 min each). Slides treated with peroxidase block solution, washed with PBS. Incubation with primary antibody (1:200 dilutions for CK2) and washing with PBS. Incubation with (LSAB Kit/HRP, Rb/Mo/Goat (DAB+) system, DAKO, Secondary Antibody for 15 min, washing with PBS. Treatment with Streptavidin for 15 minutes and PBS washing, then DAB staining for 10 minutes, was done. Washing with distilled water thrice and sections were counterstained with the Hematoxylin for 1 min, followed by washing with distilled water. Dehydration of tissue sections, using descending concentration of 90%, 80% and 70% alcohol and final treatment with xylene, was done. Slides mounted, using DPX.

CK2 staining was assessed by using score, 0= no staining, 1+= weak staining, 2+ = moderate staining, 3+ = strong staining. Nuclear and cytoplasmic distribution was observed by scoring both the nuclear and cytoplasmic cancer. The sum of nuclear and cytoplasmic scores shows total expression levels of CK2 e.g. 1+ in nucleus and cytoplasmic 3+, makes a total of 4.

RESULTS

Histologically the prostate cancer was of adenocarcinoma type and breast cancer was of Invasive Ductal Type. The tissue specific CK2α levels were high in prostate and breast cancer patients as compared to their controls but no difference was found in the expression of CK2α between invasive and non-invasive .However total CK2α expression was high (p < 0.05) in non-invasive and invasive cases as compared to BPH (Mean scores 2.93±1.23) respectively. Cytoplasmic localization of CK2α in noninvasive group and invasive group was also high as compared to BPH group (1.33±0.61) with no significant difference among three groups (p > 0.05). Nuclear localization was significantly different (p < 0.05) among the groups with highest in non-invasive cases than invasive and lowest in BPH cases (0.80±1.06). The expression of CK2α was compared in prostate and breast cancerous paraffin embedded tissues of invasive as well as non-invasive cases. The findings are shown in Table-I.
DISCUSSION

Previous studies show that CK2 is overexpressed in all proliferative states. Experimental studies by Tawfic et al. show that deregulated expression of a subunit of CK2 indicates oncogenic potential in cells so that in collaboration with some oncogenes it causes a reflective enhancement of tumor phenotype [10]. CK2, is a known pleiotropic serine/threonine protein kinase [11]. participates in an array of cellular processes targeting more than 300 substrates [12]. CK2 expression is reported to be elevated in human cancers [13], but its role in the up-regulation of carcinogenesis is yet to be determined [14].

In the present study, we have observed elevated expression and localization of CK2α in nucleus of prostate and breast cancer tissues as compared to their respective control cases. But no significant difference in expression and localization was observed between invasive and non-invasive cases. Previous studies have reported that CK2α localization is diffused in both nucleus and cytoplasm in normal cells but in cancer cells, CK2α is intensely localized in nuclear compartment [15]. The global profiling of gene expression shows that CK2 has been marked as a marker for prognosis in patients having lung squamous cell carcinoma [14]. CK2 activity elevation has also been observed in human prostate tissues [16].

Present study has added to the existing knowledge about the distribution pattern of CK2α within the cell. The main difference found between benign proliferative states and cancerous phenotype was the nuclear localization of CK2α in both types of cancers studied. We propose that this distribution is critical in transition from benign to malignant conditions regardless of the nature of malignancy. As we did not observe any significant difference between nuclear localization of CK2α and the invasive and non-invasive forms, thus we propose that even at early and localized forms nuclear localization of CK2α is the key contributory factor for cancerous phenotype.

It has been established that a large variety of different types of cancer cells depend on raised CK2 level for their continued existence [17]. We have seen the continuous increased expression of CK2α in non-invasive as well as invasive stages of prostate as well as breast cancers.

CONCLUSION

CK2 can be used as biomarkers in both prostate and breast cancer patients. There was no indication of these proteins have any significant difference in expression between invasive and non-invasive carcinoma.

REFERENCES


