Expression of Early Growth Response Genes in Prostate Cancer

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Abstract

Prostate cancer is the most prevalent malignancy and the second leading cause of cancer mortality in men. Early growth response gene-1 (Egr-1) plays a crucial role in the development and progression of prostate cancer. We investigated the expression of Egr-1 in fifty eight cases of prostatic adenocarcionoma and ten cases of benign prostatic hyperplasia using Immunohistochemistry technique. Human prostate cancer specimens demonstrated higher levels of Egr-1 in malignant acini located predominantly in the cytoplasm, with no expression in benign prostatic hyperplasia. The intensity of the staining was positively correlated with the Gleason grading. These results show that Egr-1 is over-expressed in prostate cancer and suggest a role for Egr-1 in prostate cancer growth.

Introduction

Prostate cancer is the most common form of cancer in the male. It is the second leading cause of cancer deaths in men in the UK and USA, after lung cancer [1]. The prevalence of prostate cancer is so high that it could almost be considered a normal age-related phenomenon. An estimated 164,690 new cases, with 29,430 deaths in the United States in 2018, the mortality of this disease is due to metastasis to the bone and lymph nodes [2]. With the various treatments available, 90% of patients with localized disease expect to live for 15 years, whereas 30% of patients with disseminated cancer have 5 year survival rate [3,4]. Prostate cancer progression is thought to proceed from multiple defined steps through prostatic intra-epithelial neoplasia, cancer, and progression to androgen-independent and refractory invasive terminal phase [5,6].

Although the molecular mechanism of prostate cancer progression remains largely unknown, a few genes such as E-cadherin, catenin, TGF-1 and insulin- like growth factors I and II have been shown to be aberrantly expressed and are markers of prostate cancer [7,8]. To clearly understand the multistep progression of this disease many other genes remain to be identified.

One of the over-expressed genes found in prostate cancer tissue is the transcription factor early growth response gene 1 (Egr-1) [9,10]. In prostate cancer, Egr-1 levels are constitutively high and closely linked to cancer development and progression as measured by the Gleason grade of the tumor but is low in or absent from normal prostate tissue [10]. This seems to be specific to prostate tumor cells, because in mammary and lung tumors, as well as most normal tissues Egr-1 expression is low [11,12].

Egr-1 is a multifunctional transcription factor regulating a remarkable spectrum of cellular responses from survival to apoptosis, growth to growth arrest, differentiation to transformation, senescence as well as memory and learning effects. This zinc-finger protein is a short-lived, immediate early growth response gene known to be induced by a large number of extracellular stimuli such as irradiation, hypoxia, hyperoxia and chemotherapy agents [13].

Because Egr-1, Egr-2, and Egr-α appear to be involved in regulation of cell proliferation and Egr-1 seems to play a role in malignant transformation of prostate cells, we investigated the expression of Egr-1 in prostate cancer. Mechanistic insight into how prostate cancer develops yields molecular targets that are needed for realizing chemopreventive strategies and the identification of prostate cancer mediators provides biomarkers of disease and indicators of progression [14].

Materials and methods

Case selection

Fifty eight prostate adenocarcinoma and ten benign prostatic hyperplasia specimens were obtained from histopathology archives of the Tripoli Medical center. The prostatic carcinoma was evaluated for Gleason grading as 10 cases were well differentiated, twenty three cases were moderately differentiated and twenty four cases were poorly differentiated. Surgical procedures were performed during the period 2007-2009 and patients were selected randomly. In this study all cases used were prostatectomy or prostatic ships

Specimens were routinely fixed in 10% neutral buffered formalin and embedded into paraffin wax after routine processing. Sections from each tissue block in each case were stained with haematoxylin and eosin to confirm the original diagnosis. The block containing the most tumour was selected for each case. Sections of 4 μm thickness were taken from each block for immunohistochemical staining. The sections were stained for the early growth response-1 (Egr-1). Negative controls were included to ensure the accuracy of the staining.

Immunohistochemistry

The mouse monoclonal Egr-1 antibody (abcam 54966) was raised to amino acid 444-544 of human Egr-1 and this antibody is specific to Egr-1 as recommended by the manufacture. Sections were stained using the Catalysed Signal Amplification system (Dako, UK) which is based on streptavidin-biotin-horseradish peroxidase complex formation according to the manufacturer's instructions. Briefly, 4μm-thick serial sections were mounted onto premium slides, deparaffinized and rehydrated by passage through a series of graded alcohols. Endogenous peroxidase was inhibited for 20 min by incubation with freshly prepared 3% hydrogen peroxide in methanol. The slides were treated with target retrieval solution, EDTA (1.21g Tris base + 0.37g EDTA in 1000ml dH2O pH9.0) in a microwave at high temperature for 8 minutes. After washing in PBS sections were treated with 5% blocking serum (Dako, UK) in PBS for 20 min to reduce nonspecific antibody binding.

A monoclonal antibody to Egr-1 (3μg/ml) was applied overnight for at $4C^0$, washed, and incubated for 30 min at room temperature in secondary biotinylated antibody (Santa Cruz Biotechnology). After washing in buffer for 10 min, slides were incubated for 30 min in avidin-biotin enzyme reagent diluted as suggested by the manufacturer and rinsed in buffer. Peroxidase activity was visualized after a 5-min incubation with freshly prepared diaminobenzidine tetrahydrochloride substrate solution (Vector Novared). Sections were lightly counter-stained with Gill's haematoxylin, dehydrated, and mounted. Negative controls were included in each run where the antibody was omitted and the same IgG isotype of the antibody was applied.

Statistical analysis

Non-parametric statistical analysis of data was performed using the statistical software package SSPS version 22.0. Statistics. Levels of EGR-I in benign and cancer patients were compared using the two-sample Mann-Whitney rank-sum test. A value of $P \leq 0.05$ was considered statistically significant.

Results

Immunocytochemistry was used to localize the Egr-1 protein expression in prostatic adenocarcioma. Results indicate expression of Egr-1 protein levels was lower in normal prostate tissue and benign prostatic hyperplasia (Fig. 1 A & B) compared with prostate cancer

tissues ($p < 0.001$). The expression was vary from weak, moderate to strong levels in well, moderate and poorly differentiated adenocarcinoma respectively (Fig. 2, A, B and C).

EGR-1 expression is more widely distributed in the cytoplasm (Fig. 3A). Furthermore, the staining in the nuclei was few and limited but the entire nucleus is uniformly stained (Fig. 3B). Negative controls in these experiments included incubation of sections with primary antibody in the presence of excess antigen (Fig 4).

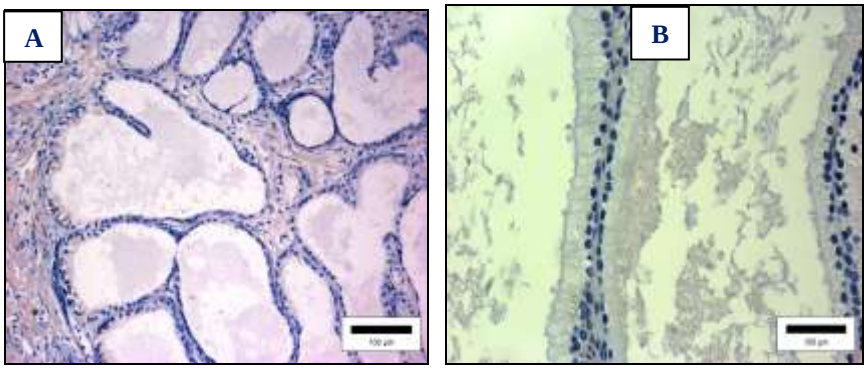
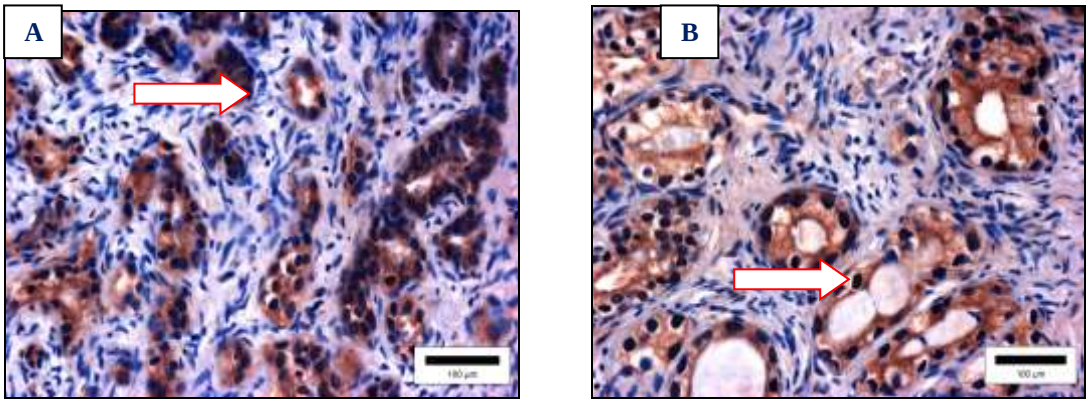


Figure 1: Egr-1 expression was weak in Benign prostatic hyperplasia (A) or negative (B) (A x20: B x40 immunoperoxidase)



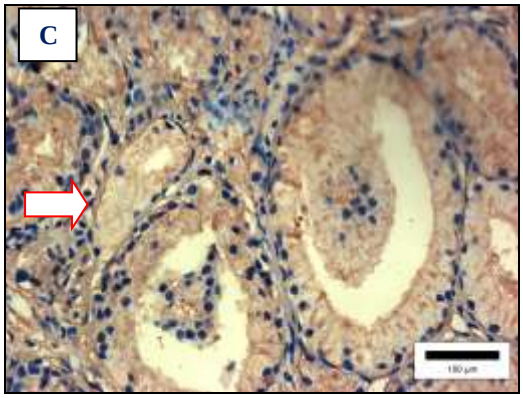


Figure 2: Egr-1 expression was strong in poorly differentiated prostatic adenocarcinoma (A) or moderate
in moderately differentiated adenocarcinoma (B) or weak in well differentiated adenocarcinoma (C) (A x20: B x40 : C x40 immunoperoxidase).

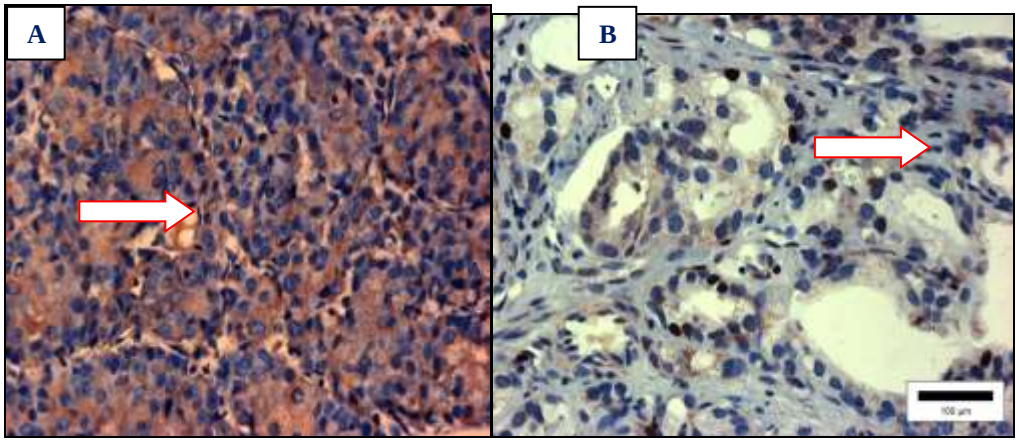


Figure 3: Egr-1 expression shows strong diffuse cytoplasmic staining in prostatic adenocarcinoma (A) and weak focal nuclear staining (B) (A x40: B x40 immunoperoxidase).

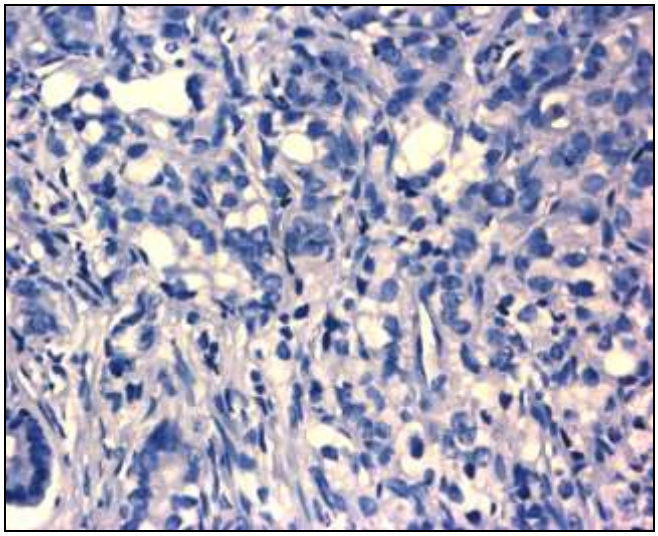


Figure 4: Negative control show no brown staining in prostatic adenocarcinoma (x20, x40).

Discussion

Prostate cancer is the most common malignancy in men. The mortality of this disease is due to metastasis to the bone and lymph nodes. Prostate cancer progression is thought to proceed from multiple defined steps through prostatic intra-epithelial neoplasia, invasive cancer, and refractory terminal phase [15]. The biological basis for the multifocal and histologic heterogeneity attributes of prostate cancer has not been fully elucidated [16]. Egr-1 Transactivator is over-expressed in prostate cancer, and its expression pattern contributes to prostate cancer progression as it regulate a number of steps involved in initiation, mitogenesis, invasiveness, angiogenesis, and metastasis. [13].

In this study we examine the expression of Egr-1 in fifty eight cases of prostatic adenocarcinoma and we found that Egr-1 is over-expressed in all the tissue examined but the intensity of the staining was variable rang from weak stain in well differentiated adenocarcinoma to moderate stain in moderately differentiated adenocarcinoma to strong stain in poorly differentiated adenocarcinoma. These results suggest a role for Egr-1 in prostate cancer growth. Eid & his colleagues 1998 has shown similar result when he examined Egr-1 expression in prostate carcinoma by immunohistochemistry, he

showed predominately basal cell nuclear Egr-1 protein in prostatic acini, nuclear staining was weak in non-malignant tissues, more intense in moderately differentiated carcinoma, and most intense in poorly differentiated carcinoma.

Many other observations support the notion that Egr-1 contributes to prostate cancer progression (previously reviewed in 17). In prostate adenocarcinoma, the mRNA encoding Egr-1 is expressed at higher levels compared with normal tissues [9,10]. The observation that levels of mRNA and protein expression correlate with Gleason scores and inversely correlate with the degree of differentiation of carcinoma cells also suggest that Egr-1 is involved in cancer progression [10]. Moreover NAB2, the regulatory protein that represses the transcriptional activity of Egr-1, is down regulated in human primary prostate carcinomas [18]. Thus, this disruption of the balance between Egr-1 and NAB2 expression results in a high Egr-1 transcriptional activity in prostate carcinoma cells.

Our results of immunohistochemistry for human prostate cancer specimens demonstrated higher levels of Egr-1 in malignant cells located predominantly in the cytoplasm, such results is confirmed by another study showed that Egr-1 differs in cellular localization in benign cells compared with malignant prostate cells and that this localization is critical for the transcriptional activation of Egr-1-dependent genes, whereas benign cells contained lower levels of Egr-1 located predominantly in the nucleus. Benign prostate cells responded to mitogens in vitro, with increased levels of Egr-1, rapid nuclear translocation, and enhanced transcriptional activity, whereas malignant prostate cells did not exhibit the same responses, and that microtubules regulate Egr-l localization in benign prostate cells but not in malignant [19]. These findings suggest that Egr-1 lacks a transactivation function in prostate cancer, it may also indicate that the target genes activated by Egr-1 in prostate cancer tissues are entirely directed to the maintenance of the malignant phenotype rather than functions that antagonize tumor formation.

Recent developments in the generation and analysis of transgenic mouse models have improved the understanding of the early stages of prostate tumorigenesis. Analysis of models based on the zinc finger protein Egr-1 suggests that these transcription factors play distinct roles in the progression of precursor prostatic intraepithelial neoplasia (PIN) lesions. The Egr-1 is overexpressed in human and mouse prostate tumors and PIN lesions and regulates the expression of several genes implicated in prostate tumor progression, including platelet-derived growth factor and insulin-like growth factor

II [20]. Prostate cancer-prone mice lacking Egr1 exhibit a significant delay in tumor progression. Specifically, Egr1 deficiency impairs the transition from PIN to invasive carcinoma. Thus, Egr1 regulate gene programs involved in distinct aspects of prostate tumorigenesis [21]. Another study done by Virolle T and his colleagues 2003 where they identified several new target genes controlling growth, cell cycle progression, and apoptosis such as cyclin D2, P19ink4d, and Fas in TRAMP C2 prostate cancer cells (TRAMP mouse is a well known model of prostate cancer). These results provide a mechanistic basis for the oncogenic role of Egr1 in TRAMP C2 prostate cancer.

Available strategies to antagonize Egr-1 function are still very limited. Silencing of EGR1 using antisense oligonucleotides delays the growth of tumors in vitro and in vivo, validating this transcription factor as a potential target for prostate cancer therapy [23,24]. Further understanding of Egr-1 role in prostatic carcinoma may eventually allow the definition of patients who would be the most likely to benefit from Egr-1 targeted therapies.

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