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Original article

Differential up-regulation of erythropoietin and its receptor in benign and malignant prostatic tissue☆

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Abstract

Purpose: To investigate differential up-regulation of erythropoietin and its receptor in benign and malignant prostatic tissue.

Materials and methods: An immunohistochemical analysis of EPO and EPOR expression was performed on 30 cases of prostate carcinoma (PCa) with 16 high-grade prostate intraepithelial neoplasia (PIN), 50 cases of benign prostatic hyperplasia (BPH) lesions, and 30 normal prostatic tissue samples as control.

Results: Over-expression of EPOR was only shown in PCa and high-grade PIN tissue but over-expression of EPO was also shown in BPH tissue. There was significant relationship between EPO and EPOR score in BPH, high-grade PIN, and PCa but no association between EPOR or EPO score and Gleason score in PCa.

Conclusions: Up-regulation of EPOR has a more important role in comparison with EPO in prostate carcinogenesis. Differential over-expression of EPOR and EPO in benign and malignant prostatic tissue is ascribed to different mechanisms involved in up-regulation between EPO and EPOR.

Keywords: Prostate carcinoma; Erythropoietin; Erythropoietin receptor; Hypoxia

1. Introduction

Hypoxia is a common phenomenon in solid tumors, resulting from inadequate blood supply with impaired vascular function. Hypoxia stress has not always been considered to be adverse to the survival and growth of tumors. Actually, once tumor cells adapt to the hypoxia environment, they become more aggressive as hypoxia directs the remodeling of tumor vasculature or phenotypic changing of tumor cells.

Hypoxia regions has been demonstrated in human prostate carcinoma (PCa) by analyzing the extent of hypoxia in tumor tissues with Eppendorf pO2 microelectrode, and increasing levels of hypoxia in prostate carcinomas were reported to be closely associated with increasing clinical stage and patient age [1,2]. Moreover, over-expression of hypoxia-inducible factor-1α (HIF-1α), the surrogate marker of hypoxia, was shown in human PCa, and its expression was considered as an early event for prostate carcinogenesis [3].

Once the transcription factor HIF-1α is activated, more than 60 putative target genes will actively participate in tumor cells' response to hypoxia stress. Of them, erythropoietin (EPO) with its receptor (EPOR), a member of the type I cytokine receptor superfamily, has recently been reported to be expressed in human PCa [4]. Previously, we have demonstrated up-regulation of EPOR in human PCa and high-grade prostate intraepithelial neoplasia (high-grade PIN) [5]. In the present study, we further investigated the differential up-regulation of EPO and EPOR in normal, benign and malignant human prostatic tissue.

2. Materials and methods

2.1. Patients and samples

Study protocols involving human materials were approved by the institutional ethics committee at Changhai

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Table 1
Characteristics of patients with PCa, $n = 30$, 2003–2004

PSA(ng/ml)		Tumor stages			Gleason score			
Median	range	pT2	pT3a	pT3b	2–4	5–7	8	9
15.6	4.1–23.6	16	8	6	7	16	6	1

PSA = prostate-specific antigen; PCa = prostate carcinoma.

Hospital. All the samples were from specimens archived in the Department of Pathology at the Changhai Hospital between 2003 and 2004. PCa tissue samples were obtained from 30 patients with an age range from 60 to 77 years (average 70 years), who had undergone radical retropubic prostatectomy in the hospital. BPH tissue samples were from 50 patients (aged from 62 to 79 years, average 72 years) with prostate-specific antigen (PSA) level less than 4 ng/ml, who underwent transurethral resection of the prostate. A total of 30 normal prostate tissue samples were obtained by autopsy from the patients aged from 20 to 30 years (average 24 years). Patients with PCa did not receive any treatment such as radiation, chemotherapy, or hormonal therapy before the removal of the prostate. Tumors were scored with the Gleason system. PSA, tumor stage, and Gleason score of patients with PCa are shown in Table 1. Some PCa samples had adjacent high-grade PIN lesions and some BPH samples contained inflammation lesions. Hematoxylin and eosin (H & E) stained slides of all cases were reviewed, and the diagnosis was confirmed by two senior pathologists.

2.2. Immunohistochemical staining

Four-μm-thick paraffin sections were serially cut from formalin-fixed, paraffin-embedded tissues. One of these sections was stained with H & E for histopathologic diagnosis. Immunohistochemical assays were performed on formalin-fixed, paraffin-embedded sections as previously described [6]. The primary antibodies used were rabbit polyclonal antibodies purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Sections of 4 μm on glass slides were deparaffinized in Hemo-D and rehydrated in graded alcohols, followed by endogenous peroxidase block in 3% H_2O_2 and antigen retrieval in boiling 10% citrate buffer. Then, slides were incubated with the polyclonal antibody against EPO, EPOR (1:200 dilution) overnight at 4°C, and subsequently with horseradish peroxidase labeled dextran polymer coupled to anti-rabbit antibody (DAKO Envision+ System HRP, DAKO Inc., Carpinteria, CA) for 30 minutes at room temperature after 3 washes with tris buffered saline containing Tween 20 (TBST, pH 7.6, DAKO). Finally, slides were developed with diaminobenzidine for 10 minutes and counterstained with hematoxylin after washing three times with TBST. The specificity of staining was confirmed by processing sections from the same paraffin block with omission of the primary antibody (negative control). As a positive control, reactions with sections of breast cancer archived in the pathological department of our hospital were used. Cytoplasmic or membrane staining that was clearly distinguishable from the background was considered positive.

2.3. Semiquantitative analysis and interpretation of staining

At least 500 epithelial cells within each area showing positive immunoreactivity were evaluated in normal prostate, BPH, high-grade PIN, and PCa. The percentage of cells with no staining (0) or weak (1), moderate (2), or intense staining (3) was analyzed by visual inspection under $\times 100$ magnification and a staining score was calculated using the formula: weighted mean of stain intensity = (Σintensity $\times$ percentage of cells)/total percentage of cells. The scoring system took into account not only the staining intensity but also the percentage of the cells that exhibit EPO, EPOR staining. EPO and EPOR expression was graded semiquantitatively according to the results of staining score. In the present study, EPO and EPOR staining were also classified into overexpression, which was defined as moderate or strong staining shown in any prostatic epithelium within benign or malignant tissues, and normal expression, which included weak or negative staining of EPO or EPOR. These analyses were performed using a Nikon E-400 microscope with computer-aided image analysis system, and digital images were captured using a digital camera (Nikon DU100; Tokyo, Japan) at $\times 200$ magnification [7]. Slides were evaluated twice at different times by 3 investigators who were unaware of the pathologic characteristics of the samples, and the resulting mean levels were used for the statistical analyses.

2.4. Statistics

Kruskal-Wallis test was used to compare differences in EPO, EPOR staining scores among groups. Spearman test was used to analyze the relationship between EPO and EPOR score for each group, and to relate EPO or EPOR score to Gleason score in PCa. Computations were performed using SAS 9.1.3 software (SAS Institute Inc., Cary, NC). A two-sided P value less than 0.05 was considered to be statistically significant.

3. Results

PCa, high-grade PIN, and BPH lesions were identified on H & E staining sections according to morphological changes (Fig. 1). A total of 16 high-grade PIN lesions were found in 16 PCa samples, and no PCa sample showed multiple high-grade PIN lesions.

EPO and EPOR expression were observed mainly in the glandular epithelium. A predominantly cytoplasmic pattern of staining for EPO and EPOR was observed, but membrane