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Tissue transglutaminase expression promotes castration-resistant phenotype and transcriptional repression of androgen receptor ☆

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Abstract Many studies have supported a role for inflammation in prostate tumour growth. However, the contribution of inflammation to the development of castration-resistant prostate cancer remains largely unknown. Based on observations that aberrant expression of the proinflammatory protein tissue transglutaminase (TG2) is associated with development of drug resistance and metastatic phenotype in multiple cancer types, we determined TG2 expression in prostate cancer cells. Herein we report that human prostate cancer cell lines with low expression of androgen receptor (AR) had high basal levels of TG2 expression. Also, overexpression of TG2 negatively regulated AR mRNA and protein expression and attenuated androgen sensitivity of prostate cancer cells. TG2 expression in prostate cancer cells was associated with increased invasion and resistance to chemotherapy. Mechanistically, TG2 activated nuclear factor (NF)-κB and induced epithelial–mesenchymal transition. TG2/NF-κB-mediated decrease in AR expression resulted from transcriptional repression involving *cis*-interaction of NF-κB in a complex with TG2 with the 5′-untranslated region of *AR*. Negative regulation of AR could be partially abrogated by repression of TG2 or NF-κB (p65/RelA) by gene-specific small interfering RNA. These results suggested that a novel pathway links androgen dependence with TG2-regulated inflammatory signalling and hence may make TG2 a novel therapeutic target for the prevention and treatment of castration-resistant prostate cancer.

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1. Introduction

Prostate cancer is a common malignancy and the second leading cause of cancer-related deaths in the Western world [1]. Normal prostate cells and early-stage prostate cancer cells require the presence of androgens for growth and survival. Hence, androgen deprivation via surgical or chemical castration is a well-established treatment option for prostate cancer at various stages. Although hormonal ablation therapies initially induce remissions in most cases of prostate cancer, some cases progress and evolve to an androgen-independent state, known as castration-resistant prostate cancer (CRPC) [2]. CRPC is characterised by clinical and molecular heterogeneity and is the main cause of prostate cancer-associated mortality [1]. Treatment options for CRPC are extremely limited mainly because of the intrinsic chemoresistance acquired by the cancer cells during disease progression while the patient is receiving therapy. Understating the molecular mechanisms associated with progression of prostate cancer is critical to development of novel therapeutic approaches for CRPC.

Androgen resistance mechanisms linked with the development of CRPC can be grouped into three categories. The first consists of DNA-based alterations, such as mutation or amplification of the androgen receptor (*AR*) gene. These occur in only a minority of patients [2]. The second mechanism is AR signalling that remains active even with castration levels of serum testosterone in patients without *AR* mutations or amplification. In these patients, alternative pathways can lead to AR signalling activation. For example, *Her-2/neu*-induced activation of AKT [3] or nuclear factor (NF)-κB [4] can promote AR activation. The third mechanism is complete bypass of AR pathways, allowing cancer cells to survive in the absence of androgen-dependent or -independent AR activation [2]. Indeed, large numbers of samples of metastatic tumours obtained from prostate cancer patients have exhibited lack of AR expression [5]. Moreover, tumour-initiating cells (TICs) isolated from human prostate tumours have lack of AR expression and had increased NF-κB activity [6]. Importantly, these TICs possessed stem cell characteristics and recapitulated the prostate tumour heterogeneity when injected into mice [6].

Investigators have proposed that inflammation can contribute to the progression of CRPC [7]. For example, androgen ablation therapy, which is accompanied by increased cytokine production owing to infiltration of immune cells in shrinking tumours, is known to support hormone-independent growth and survival of prostate cancer cells [8]. In addition to this effect of androgen ablation therapy, CRPC is resistant to chemotherapy and becomes metastatically competent. In light of these findings and recent observations that expression of proinflammatory protein, tissue transglutaminase (TG2) is aberrantly upregulated in multiple drug-resistant and metastatic cancer cell types [9–11], we examined TG2 expression in hormone-dependent and -independent prostate cancer cell lines. Herein we present evidence that overexpression of TG2 promotes AR-independent growth and survival of prostate cancer cells by inducing constitutive activation of NF-κB. TG2 expression resulted in transcriptional repression of androgen receptor (AR) via interaction with the 5′-untranslated region of *AR* gene in a complex with NF-κB. Moreover, TG2 expression promoted the invasiveness of prostate cancer cells and their resistance to doxorubicin by inducing epithelial–mesenchymal transition (EMT). Taken together, these results suggest that aberrant expression of TG2 is a novel pathway that contributes to the progression of androgen-dependent prostate cancer to CRPC and thus may represent a promising target for treatment and prevention of this aggressive form of prostate cancer.

2. Materials and methods

2.1. Cell lines, vectors and reagents

The prostate cancer cell lines LNCaP, V-CaP, DU 145, MDA-PCA-2b (A10), MDA-PCA-2a (A11), C4-2B and PC-3 were provided by Dr. Nora Navone (The University of Texas MD Anderson Cancer Center). Cells were maintained in Roswell Park Memorial Institute medium (RPMI 1640) or Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) supplemented with 10% foetal-calf serum (FCS), 2 mM L-glutamine and antibiotics. The MEM/F-12 medium was also supplemented with vitamins (Sigma–Aldrich, St. Louis, MO), non-essential amino acids (BioWhittaker, Walkersville, MD) and 1 mM/l sodium pyruvate (BioWhittaker).

Full-length TG2 (TG2-WT) was subcloned into a pCDH lentiviral vector (System Biosciences, Mountain View, CA) as described previously [12]. Stable clones of TG2-transfected LNCaP cells were selected via growth in a puromycin-containing medium (1 μg/ml). For transient transfection of PC-3 cells with small interfering RNA (siRNA), SignalSilence TG2-specific and control siRNAs were purchased from Cell Signaling Technology (Danvers, MA). Anti-TG2 and anti-β-actin antibodies were purchased from Abcam (Cambridge, MA), and anti-E-cadherin, anti-N-cadherin and anti-fibronectin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Lipofectamine 2000, Oligofectamine and Stealth siRNA (negative control) were obtained from Invitrogen (Carlsbad, CA). All media were purchased from Fisher Scientific (Pittsburgh, PA).

2.2. TG2 activity and expression

Prostate cancer cells grown in 25-cm^2 flasks were harvested at 80% confluence, washed twice with