POSSIBLE ROLE OF NUCLEAR FACTOR κB DETECTED BY IN SITU HYBRIDIZATION IN THE PATHOGENESIS OF TRANSITIONAL CELL CARCINOMA OF THE BLADDER

Haider Sabah KADHIM¹, Tariq I. AL-JEBOORI¹, Mohammed S. TAWFIK²


RÉSUMÉ • OBJECTIF : Le carcinome cellulaire transitionnel (CCT) de la vessie demeure une pathologie majeure à travers le monde. Les mécanismes moléculaires de formation et de développement des tumeurs sont complexes. Ils incluent vraisemblablement l’interaction de gènes suppresseurs de tumeur, d’oncogènes, de protéines régulatrices du cycle cellulaire et d’autres facteurs. D’où cette étude qui tente d’explorer le rôle du facteur nucléaire κB (FN-κB) dans le CCT de la vessie en corrélation avec différents critères clinico-pathologiques tels que le grade tumoral, l’invasion du muscle par la tumeur, la schistosomiase et le CCT à la présentation en tant que tumeur primaire ou récurrente.


RÉSULTATS : Une corrélation significative a été trouvée (p < 0,05) pour l’invasion musculaire et la schistosomiase mais pas pour les autres critères.

CONCLUSION : La présente étude tend à prouver le rôle potentiel du facteur de transcription FN-κB dans le CCT de la vessie.

plays an important role in the regulation of immune response, cell apoptosis, cell-cycle progression, inflammation, and oncogenesis. A wide range of stimuli, including cytokines, mitogens, environmental particles, and viral or bacterial products, activate NF-κB [2].

Activated NF-κB translocates into the nucleus where it modulates the expression of a variety of genes, including those encoding cytokines, growth factors, acute phase response proteins, cell adhesion molecules, other transcription factors, and several cell apoptosis regulators [2].

NF-κB is expressed at a low level in all normal cells, as an inactive form and sequestered in the cytoplasm by the specific inhibitory IκB protein. It is present at high levels in a large fraction of human tumors, promoting both cell survival and proliferation. It also influences the transcription of a wide range of immune response genes, like adhesion molecules, chemokines, and cytokines [3-4]. It plays a central role in inflammation through its ability to induce transcription of pro-inflammatory genes [5].

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INTRODUCTION

Transitional cell carcinoma (TCC) of the urinary bladder is the second most common tumor of the genitourinary tract; it is also the second most common cause of death from these cancers [1]. Conventional histopathologic evaluation of bladder cancer through tumor stage and grade is not adequate enough to predict the exact behavior of most bladder tumors, but it is becoming apparent that the accumulation of genetic and molecular changes ultimately determines a tumor’s phenotype and subsequent clinical behavior [1].

The nuclear factor -κB family of transcription factors

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The ability of NF-κB to suppress apoptosis and to regulate cell-cycle transition clearly indicates that NF-κB may participate in many aspects of oncogenesis. Indeed, elevation of NF-κB activity is evident in a number of human cancers, including breast cancer, non-small cell lung carcinoma, thyroid cancer, T- or B- lymphocyte, leukemia, melanoma, colon cancer, bladder cancer, and several virally induced tumors [2]. The earliest evidence for a role of NF-κB in oncogenic transformation has been derived from the fact that v-Rel, a highly oncogenic retroviral homologue of c-Rel, causes carcinogenesis in avian lymphoid cells. Later studies suggested that v-Rel also has the capacity of transforming mammalian cells in vivo [6-7].

Regarding the role of NF-κB in bladder cancer, Sumitomo et al. [8] (1999) showed that overexpression of κ may express low basal NF-κB activity that was inducible after exposure to stress (hypoxia or acidosis) and led to the up-regulation of IL-8 expression [3]. Blockade of NF-κB by mutant IκB prevented the induction of IL-8 and resulted in inhibition of angiogenesis and metastasis in human TCC xenografts growing in the bladder of nude mice. These data suggest that the heterogeneity of constitutive NF-κB activity and induction observed in this study might correlate with the histological grade of TCC cells: well to moderately differentiated TCC cells may express low basal NF-κB activity and IL-8 expression (which is inducible in response to appropriate stimuli); whereas poorly differentiated TCC cells may express IL-8 constitutively due to constitutively active NF-κB [3]. Interestingly, these observations are in contrast to findings in human melanoma cells, in which IL-8 mRNA and protein are inducible in the highly aggressive and metastatic cells, but not in the poorly aggressive cells [9].

PATIENTS AND METHODS

Twenty patients (13 males and 7 females) with TCC of the bladder, which had been confirmed by histopathology, were included in this study. They ranged in age from 38-72 years. Patients were diagnosed clinically by consultant urologists at Al-Kadhimiya Teaching Hospital, Baghdad.

Eleven patients presented for the first time and the rest presented with recurrent bladder tumors, most of which had been treated surgically. Information was obtained about each patient through a questionnaire including age, sex, address, time of presentation, and relevant medical history. Schistosomal infection was ascertained either from the patient’s history based upon clinical manifestations and management or by cystoscopy finding of bilharzioma or the parasite eggs in histopathological sections.

Control group

Five patients with bladder diseases other than cancer were considered as control group. Normal urothelium was taken for biopsy with permission of the patients.

Tumor biopsy specimens

All patients had transurethral resection of bladder tumor (TUR-BT). The specimens taken were multiple pieces, 1-5 mm in thickness, and were immersed in 10% formalin in order to make a paraffin block.

Procedure

Serial tissue sections were cut 4-6 μm thick and were positioned on positively charged slides. The slides were then heated at 80°C overnight. The tissue sections were deparaffinized by standard methods. The slides were treated with Proteinase K solution and dehydrated. One drop of the Biotinylated long DNA probe for human NF-κB (MaximBiotech Cat. No. IH-60031) hybridization solution was placed on the tissue section in oven or heating block at 70°C for 8-10 minutes to denature the secondary structure of RNA. After that slides were placed in a humid chamber and incubated at 37°C for 3-4 hours to allow hybridization of the probe with the target nucleic acid. The slides were soaked in detergent wash at 37°C until the cover slips fell off, then treated with RNase A and the conjugate. One to two drops of substrate were placed on tissue section at room temperature for about 10 minutes, or until color development was complete, the latter was monitored by viewing the slides under the microscope. A blue colored precipitate will form at the site of the probe in positive cells. Slides were then counterstained using nuclear fast red and sections were mounted with a permanent-mounting medium (DPX). Finally the examination and scoring were done under light microscope by a pathologist at power X400 (Figure 1) according to the scoring system shown in table I.

<table>
<thead>
<tr>
<th>MARKER</th>
<th>Negative</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-κB</td>
<td>&lt; 5%</td>
<td>5-25%</td>
<td>25-50%</td>
<td>&gt; 50%</td>
</tr>
</tbody>
</table>

Statistical analyses

Data analysis was performed using Chi² test which was used to find out the effect of different patients’ criteria on the reading of in situ hybridization for NF-κB detection.

RESULTS

This is the first time that NF-κB has been detected by the in situ hybridization technique in bladder cancers in Iraq. The results are limited due to the limitation of materials, i.e., 20 cases, 10 patients with muscle invasion bladder cancer, 7 patients were schistosomal bladder cancer, 3 patients with non-schistosomal bladder cancer.
The results of normal urothelium were negative for the control group. Nuclear factor κB was positive in 9 patients (45%). From those positive cases, 6 patients (66.7%) had positive history of schistosomiasis. High-grade tumor was the most frequent grade represented in 7 patients (77.8%). Eight patients had invasive tumor (88.9%). Only 4 patients (44.4%) of the NF-κB-positive cases were presented as primary tumor. Regarding the statistical analyses of these results, only history of schistosomiasis and tumor invasiveness showed a significant correlation in the positive cases with p < 0.05 as shown in table II. The results of frequency distribution of NF-κB scores showed no significant correlation between each score and any of patients’ criteria, namely, history of schistosomiasis, tumor grade, muscle invasion and presentation whether primary of recurrent.

DISCUSSION & CONCLUSION

Evidence for the involvement of NF-κB in oncogenesis is not new. Numerous studies have indicated that NF-κB activation is required to protect cells from the apoptotic cascade induced by TNF [10]. Furthermore, NF-κB could promote cell-cycle transition by a direct transcriptional up-regulation of the cyclin D1 gene [11]. The key role that NF-κB plays on multiple steps of oncogenesis makes this factor a central and favorable target for therapeutic intervention of cancer [12]. Indeed, experimental data suggest that inhibition of NF-κB could enhance the efficacy of cancer chemotherapies and radiation [13].

In our study, NF-κB results showed that it was not associated (p > 0.05) with grade as 22.2% and 77.8% of total positive cases were of low and high grade tumor, respectively (Table II). While in muscle invasion the correlation was significant (p < 0.05) as 88.9% of total positive cases showed muscle invasion by tumor. This is in agreement with the concept of NF-κB promoting oncogenesis rather than tumor suppression and with the results of Karashima et al., who suggested that poorly differentiated TCC of the bladder cells might express IL-8 constitutively due to constitutively active NF-κB [3]. The role of IL-8 expression in bladder cancer was known to regulate tumorigenicity and metastasis, as it was proved by Inoue et al. in 2000 [14]. On the other hand the association of NF-κB with the presentation whether primary or recurrent tumor was not significant (p > 0.05). This may denote that the role of NF-κB could be a defect during tumor development or tumor growth.

<table>
<thead>
<tr>
<th>CRITERIA</th>
<th>NF-κB Expression %</th>
<th>Chi² value</th>
</tr>
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<tbody>
<tr>
<td>SCHISTOSOMIASIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBT</td>
<td>66.7%</td>
<td>0.0072</td>
</tr>
<tr>
<td>NSBT</td>
<td>33.3%</td>
<td></td>
</tr>
<tr>
<td>HISTOPATHOLOGY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low grade tumor</td>
<td>22.2%</td>
<td>0.064</td>
</tr>
<tr>
<td>High grade tumor</td>
<td>77.8%</td>
<td></td>
</tr>
<tr>
<td>Invasive tumor</td>
<td>88.9%</td>
<td>0.0056</td>
</tr>
<tr>
<td>PRESENTATION</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent tumor</td>
<td>55.6%</td>
<td>0.5819</td>
</tr>
<tr>
<td>Primary tumor</td>
<td>44.4%</td>
<td></td>
</tr>
<tr>
<td>NORMAL UROTHELIUM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
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</tbody>
</table>

**TABLE II**

RESULTS OF *IN SITU* HYBRIDIZATION FOR DETECTION OF NF-κB IN RELATION TO CLINICOPATHOLOGICAL CRITERIA

**Figure 1**

*In situ* hybridization for NF-κB in TCC of bladder tumor sections.

Staining by BCIP/NBT (bluish-purple) counterstained with nuclear fast red.

A. Bladder tumor (TCC), positive NF-κB *in situ* hybridization (arrows) in high-grade tumor (X40).

B. TCC, negative NF-κB *in situ* hybridization (X40).
In regard to schistosomiasis, NF-κB is known to play a major role in infection and inflammation. In infection with *Schistosoma haematobium*, it was known that in an exaggerated granulomatous response to ova, which is associated with urinary tract pathology, there is an increased TNF-α with diminished IL-10 production [15]. In such condition the immune response would be type 1 with production of TNF-α. However, this cytokine profile does activate NF-κB [16]. Moreover, IL-12-dependent NF-κB activation leads to de novo synthesis and release of IL-8 and TNF-α [17]. Since IL-8 regulates tumorigenesis, angiogenesis and metastasis by human TCC [14], hence, in SBT, NF-κB may represent an advanced tumor with poor prognostic sign. Moreover, Abdel-Mageed and Ghoniem [18], in 1998, showed that NF-κB was predominantly activated in bladder urothelial cells in biopsies from patients with interstitial cystitis compared to controls, and in 2003, Abdel-Mageed found that the NF-κB-induced expression of transcripts of pro-inflammatory factors (TNF and IL-8) correlates with increased protein levels of these factors in the urine of interstitial cystitis patients in comparison to controls [19]. He concluded that these factors are capable of activating NF-κB in urothelial cells. This may be applied to our study, in which almost all SBT showed positive NF-κB and schistosomiasis is a predisposing factor to bladder cancer [20].

Activation of NF-κB can be stimulated in cancers by over-expressed growth factors and cytokines like TNF. In SBT, activators for NF-κB will increase through the effect of chronic schistosomiasis and its cytokine profile that contains the powerful NF-κB activator TNF; hence the condition will be worse.

REFERENCES