

POSSIBLE ROLE OF NUCLEAR FACTOR κ B DETECTED BY *IN SITU* HYBRIDIZATION IN THE PATHOGENESIS OF TRANSITIONAL CELL CARCINOMA OF THE BLADDERHaider Sabah KADHIM¹, Tariq I. AL-JEBOORI¹, Mohammed S. TAWFIK²

Kadhim HS, Al-Jeboori TI, Tawfik MS. Possible role of nuclear factor κ B detected by *in situ* hybridization in the pathogenesis of transitional cell carcinoma of the bladder. J Med Liban 2006 ; 54 (4) : 196-199.

Kadhim HS, Al-Jeboori TI, Tawfik MS. Rôle potentiel du facteur nucléaire κ B détecté par hybridation *in situ* dans la pathogénèse du carcinome cellulaire transitionnel de la vessie. J Med Liban 2006 ; 54 (4) : 196-199.

ABSTRACT • OBJECTIVE : Transitional cell carcinoma (TCC) of the bladder remains a significant health problem worldwide. The molecular mechanisms of tumor development and progression are complicated but likely involve the interaction of tumor suppressor genes, oncogenes, cell cycle regulatory proteins and other factors. Hence, this study attempts to explore the role of nuclear factor- κ B (NF- κ B) in the TCC of the bladder in correlation with different clinicopathological criteria which are tumor grade, muscle invasion by the tumor, schistosomiasis and presentation whether primary or recurrent tumor.

METHODS : Twenty patients with TCC of the bladder were included in the study from June 2003 to June 2004, and were diagnosed by histopathology. The expressions of the transcription factor NF- κ B were studied by *in situ* hybridization technique (ISH).

RESULTS : The results showed that there was a significant correlation ($p < 0.05$) with muscle invasion and schistosomiasis but not with other criteria.

CONCLUSION : The current study showed the possible role of the transcription factor (NF- κ B) in TCC of the bladder.

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 κ B (FN- κ B) dans le CCT de la vessie en corrélation avec différents critères clinicopathologiques 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.

MÉTHODE : Vingt patients avec CCT confirmé de la vessie ont été inclus dans l'étude de juin 2003 à juin 2004. Les expressions du facteur de transcription FN- κ B ont été étudiées par la méthode d'hybridation *in situ*.

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- κ B dans le CCT de la vessie.

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 - κ B family of transcription factors

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- κ B [2].

Activated NF- κ B 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- κ B is expressed at a low level in all normal cells, as an inactive form and sequestered in the cytoplasm by the specific inhibitory I κ B 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].

¹Department of Microbiology, Medical College, ²Medical Research Center, Al-Nahrain University, Baghdad.

Correspondence : Dr Haider Sabah Kadhim. Microbiology Dept. Al-Nahrain Medical College. Al-Kadhimiya, POBox 70056. Baghdad. Iraq.

E-mail : haider_kadhim@yahoo.com

The ability of NF- κ B to suppress apoptosis and to regulate cell-cycle transition clearly indicates that NF- κ B may participate in many aspects of oncogenesis. Indeed, elevation of NF- κ B 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- κ B 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- κ B in bladder cancer, Sumitomo et al. [8] (1999) showed that overexpression of I κ B will induce apoptosis of cytokine-producing bladder cancer cells *in vitro*. While Karashima et al. [3] observed that highly metastatic TCC constitutively expressed high levels of NF- κ B and IL-8, whereas less aggressive TCC cells expressed lower constitutive levels of NF- κ B activity that were inducible after exposure to stress (hypoxia or acidosis) and led to the up-regulation of IL-8 expression [3]. Blockade of NF- κ B by mutant I κ B 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- κ B 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- κ B 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- κ B [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 name, 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 μ m 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- κ B (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 I
SCORING SYSTEM USED IN *IN SITU* HYBRIDIZATION

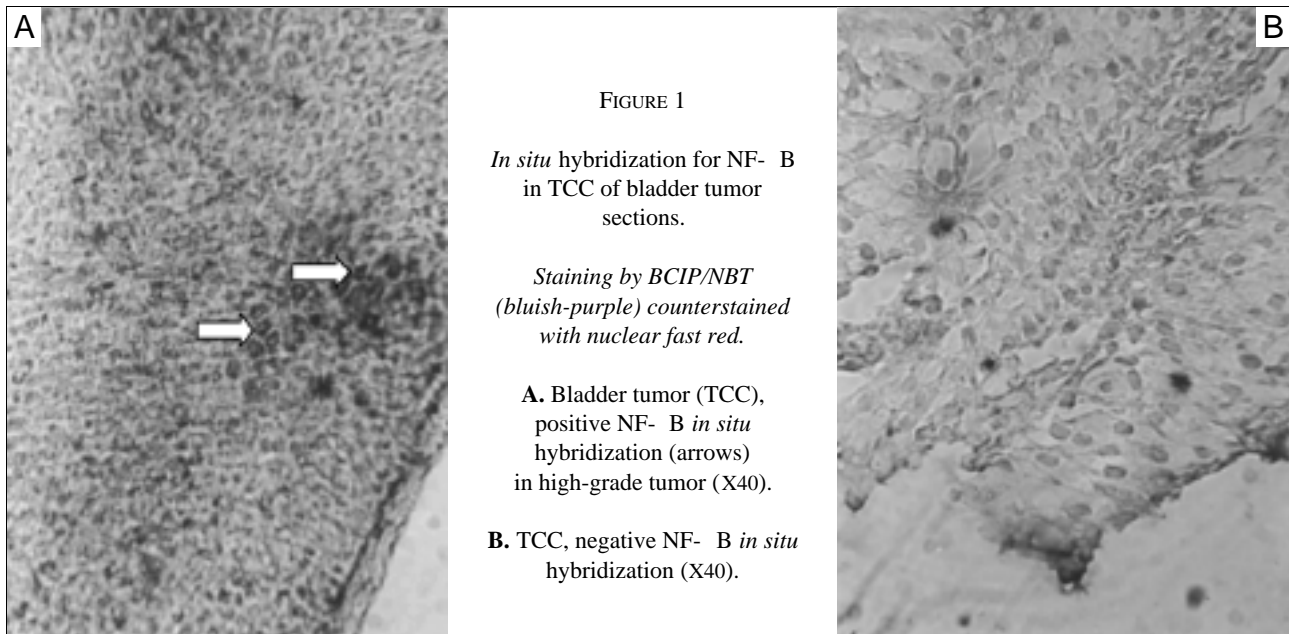
MARKER	Negative	Low	Intermediate	High
NF- κ B	< 5%	5-25%	25-50%	> 50%

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- κ B detection.

RESULTS

This is the first time that NF- κ B 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. (Fig. 1). 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 or recurrent.

DISCUSSION & CONCLUSION

Evidence for the involvement of NF-κB in oncogenesis is not new. Numerous studies have indicated that NF-κB activation can suppress cell death pathways and 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 cor-

relation 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 II
 RESULTS OF *IN SITU* HIBRIDIZATION FOR DETECTION OF NF-κB IN RELATION TO CLINICOPATHOLOGICAL CRITERIA

CRITERIA	NF-κB Expression %	Chi ² value
SCHISTOSOMIASIS		
SBT	66.7%	0.0072
NSBT	33.3%	
HISTOPATHOLOGY		
Low grade tumor	22.2%	0.064
High grade tumor	77.8%	
Invasive tumor	88.9%	0.0056
PRESENTATION		
Recurrent tumor	55.6%	0.5819
Primary tumor	44.4%	
NORMAL UROTHELIUM		
Negative		
Significant p < 0.05 SBT : Schistosomal bladder tumor NSBT : Non-schistosomal bladder cancer		

In regard to schistosomiasis, NF- κ B 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- κ B [16]. Moreover, IL-12-dependent NF- κ B 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- κ B may represent an advanced tumor with poor prognostic sign. Moreover, Abdel-Mageed and Ghoniem [18], in 1998, showed that NF- κ B 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- κ B-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- κ B in urothelial cells. This may be applied to our study, in which almost all SBT showed positive NF- κ B and schistosomiasis is a predisposing factor to bladder cancer [20].

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

REFERENCES

- Williams SG, Buscarini M, Stein JP. Molecular markers for diagnosis, staging and prognosis of bladder cancer. *Oncology* 2001 Nov ; 15 (11) : 1461-84.
- Chen F, Castranova V, Shi X. New insights into the role of nuclear factor κ B in cell growth regulation. *Am J Pathol* 2001 Aug ; 159 (2) : 387-97.
- Karashima T, Sweeney P, Kamat A, Huang S. Nuclear factor κ B mediates angiogenesis and metastasis of human bladder cancer through the regulation of Interleukin-8. *Clin Can Res* 2003 ; 9 : 2786-97.
- Mason N, Aliberti J, Caamano JC, Liou HC, Hunter CA. Cutting edge : identification of c-Rel-dependent and-independent pathways of IL-12 production during infectious and inflammatory stimuli. *J Imm* 2002 : 168 : 2590-5.
- Tak PP, Firestein GS. NF- κ B : a key role in inflammatory diseases. *JCI* 2001 ; 107 (1) : 7-11.
- Gilmore T, Koedood M, Piffat K, White D. Rel/NF- κ B/I κ B proteins and cancer. *Oncogene* 1996 ; 13 : 1367-78.
- Rayet B, Gelinas C. Aberrant Rel/NF- κ B genes and activity in human cancer. *Oncogene* 1999 ; 18 : 6938-47.
- Sumitomo M, Tachibana M, Ozu C, Asakura H, Murai M. Induction of apoptosis of cytokine-producing bladder cancer cells by adenovirus-mediated I κ B overexpression. *Hum Gene Ther* 1999 ; 10 : 37-47.
- Kunz M, Hartmann A, Flory E, Toksoy A. Anoxia-induced up-regulation of interleukin-8 in human malignant melanoma. A potential mechanism for high tumor aggressiveness. *Am J Pathol* 1999 ; 155 : 753-63.
- Barkett M, Gilmore T. Control of apoptosis by Rel/NF- κ B transcription factors. *Oncogene* 1999 ; 18 : 6910-24.
- Joyce D, Bouzazhah B, Fu M et al. Integration of Rac-dependent regulation of cyclin D1 transcription through a nuclear factor- κ B-dependent pathway. *J Biol Chem* 1999 ; 274 : 25245-9.
- Schwartz SA, Hernandez A, Mark Evers B. The role of NF- κ B/I κ B proteins in cancer : implications for novel treatment strategies. *Surg Oncol* 1999 ; 8 : 143-53.
- Baldwin AS. Control of oncogenesis and cancer therapy resistance by the transcription factor NF- κ B. *J Clin Invest* 2001 ; 107 (3) : 241-6.
- Inoue K, Slaton JW, Kim SJ et al. Interleukin-8 expression regulates tumorigenicity and metastasis in human bladder cancer. *Cancer Res* 2000 ; 60 : 2290-9.
- King CL, Malhotra I, Mungai P et al. *Schistosoma haematobium*-induced urinary tract morbidity correlates with increased tumor necrosis factor- α and diminished Interleukin-10 production. *J Infect Dis* 2001 Nov 1 ; 184 (9) : 1176-82.
- Heller RA, Kronke M. Tumor necrosis factor receptor-mediated signaling pathways. *J Cell Biology* 1994 ; 126 (1) : 5-9.
- Al-Mohanna F, Saleh S, Parhar RS, Collison K. IL-12-dependent nuclear factor- κ B activation leads to de novo synthesis and release of IL-8 and TNF- α in human neutrophils. *Journal of Leukocyte Biology* 2002 ; 72 : 995-1002.
- Abdel-Mageed AB, Ghoniem GM. Potential role of rel/nuclear factor- κ B in the pathogenesis of interstitial cystitis. *J Urol* 1998 Dec ; 160 (6 Pt 1) : 2000-3.
- Abdel-Mageed AB. NF- κ B-dependent gene expression of proinflammatory cytokines in T24 cells : possible role in interstitial cystitis. *Urol Res* 2003 ; 31 (5) : 300-5.
- Mostafa MH, Sheweita SA, O'connor PJ. Relationship between schistosomiasis and bladder cancer. *Clinic Micro Rev* 1999 ; 12 (1) : 97-111.

الدور المحتمل لعامل النواة NF- κ B المشخص بطريقة التهجين الموضعي في أمراض سرطان خلايا المثانة الانتقالي

الخلاصة - يعتبر سرطان خلايا المثانة الانتقالي معضلة صحية في كافة أرجاء العالم. وإن التقنيات لجزيئية لتطور وتقدم الورم معقدة جداً وقد تشمل التداخل بين عدة مورثات منها المورثات المثبطة للورم، المورثات المسببة للأورام، البروتينات المنظمة لدورة الخلية بالإضافة إلى عوامل أخرى. وعليه فإن هذه الدراسة تحاول إن تكشف دور عامل النواة NF- κ B في سرطان خلايا المثانة الانتقالي وعلاقتهم مع بعض الخصائص السريرية والمرضية والتي شملت درجة تصنيف الورم النسيجي، انتهاك تاورم للطبقة العضلية من المثانة، الإصابة بمرض المنشقات البولية (البلهارزيا) وحالة الورم كونه أولي أو متواتر. تضمن البحث عشرين مريضاً مصاباً بسرطان خلايا المثانة الانتقالي شخضوا عن طريق الفحص النسيجي في الفترة حزيران (يونيو) 2003. حزيران (يونيو) 2004. تم دراسة عامل النواة NF- κ B بطريقة التهجين الموضعي (in situ hybridization). أظهرت نتائج التهجين الموضعي لعامل NF- κ B إن هناك علاقة ذات مغزى ($p < 0.050$) مع كل من انتهاك الورم للطبقة الفصيصة والإصابة بمرض المنشقات البولية ولم تكن مثل هذه العلاقة مع باقي الخصائص. بينت هذه الدراسة أن هناك دوراً محتملاً لعامل النواة NF- κ B في سرطان خلايا المثانة الانتقالي.