Prognostic value of apoptotic activity in muscle-invasive bladder cancer

Prognostička vrednost apoptotske aktivnosti u mišićnoinvazivnom karcinomu mokraće bešike

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Key words: urinary bladder neoplasms; apoptosis; tumor markers, biological; cystectomy.

Introduction

Transitional cell carcinoma (TCC) of the bladder is a common malignancy worldwide that is associated with significant morbidity and mortality. The increasing number of patients diagnosed with urothelial cancer, year after year, and obvious limitations in standard diagnostic, therapeutic and prognostic approaches, created a strong interest in applying immunohistochemistry in the field of uropathology.

A hallmark of bladder cancer is its variable prognosis. However, aggressive behavior is generally restricted to those tumors that are high grade, that have penetrated the lamina propria extensively and that are accompanied by carcinoma in situ either nearby to the tumor or at distant sites 1–3.

Conventional clinical and pathologic parameters such as tumor grade, stage, size, multifocality, as well as vascular and lymphatic extension provide important diagnostic and prognostic information 1. The presence or absence of numerous histological parameters, such as thickness of urothelium, polarity, cytoplasmic clearing, nuclear size, nuclear crowding, nuclear chromatin distribution, nucleoli, mitoses, accompanying inflammation and neovascularity, undoubtedly, helps coming to the proper diagnosis for a given case 5,6. Yet, they have a limited ability to predict the clinical outcome of many patients with bladder cancer and extensive efforts have been made to identify markers that could foretell recurrence and progression of the disease, development of metastases, response to treatment and patient survival 7. To become of clinical use, new prognostic markers must add some predictive capability beyond what current clinical and pathologic parameters offer 5.

Prognostic mystery of muscle-invasive urothelial cancer

It has been established that patients with high-grade, muscle-invasive tumors at primary diagnosis or recurrence have poor prognosis 7. Fifty percent of these patients develop distant metastases within two years and 60% die within five years of initial treatment. Thus, new questions are being proposed: How do we best monitor patients for recurrence and progression of disease? How do we identify patients who need adjuvant therapy after radical cystectomy? Which patients will have benefit from adjuvant therapy?

Radical cystectomy with pelvic lymph-node dissection is currently the mainstay of treatment for patients with muscle-invasive cancer. However, 10-year survival rates for individuals treated by radical cystectomy are only 37%–45% 8,9. It is widely accepted that presence of the clinically undetectable micrometastases is the main reason of the above statistics. In theory, the use of preemptive or neoadjuvant chemotherapy should, therefore, offer a chance to control distant disease and improve long-term survival. Nevertheless, the results of numerous clinical trials have been more disappointing than promising, no approach for treating advanced bladder cancer has proved to be optimal, with a large meta-analysis failing to show any survival benefit. Most recently, an international multicentre trial of 976 patients comparing radical treatment with or without neoadjuvant cisplatin, methotrexate and vinblastine showed only a 5.5% absolute difference in survival at 3 years, which was less than the 10% that was required for introduction into clinical use 10,11.

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The latest large international cohort which included 4,257 patients treated with radical cystectomy for bladder TCC presented that a 5-year recurrence-free and cancer specific survival was only 29%. Patients with pT4 bladder urothelial carcinoma had very variable outcomes. Furthermore, Tilki et al.\textsuperscript{17} pointed out that female patients with pT4 bladder TCC had worse outcome than male patients with the same stage of the disease.

These findings represent a step apart but there is a doubt whether all patients should be exposed to the toxic effects of chemotherapy. Perhaps, the answer could be found in the patient selection which, in fact, might be the best way of improving treatment outcome in the future\textsuperscript{9, 10}.

**Controversial expression of members bcl-2 family proteins in muscle-invasive bladder cancer**

Bladder cancer can occur at any age, but it is generally a disease of the middle-aged and elderly population, with a median age at diagnosis of 66 years\textsuperscript{13}. Like other malignancies that arise in later life, bladder cancer is a result of acquired alterations in DNA, which may manifest as induction of oncogenes, loss of tumor-suppressor genes, impairment of normal DNA repair mechanisms or interruption of cell cycle homeostasis\textsuperscript{14}.

The most recent studies have indicated that the entire apoptotic cascade in bladder cancer suffer genetic changes\textsuperscript{9, 14,15}. Many of the proteins that conduct apoptosis are constitutively expressed in viable cells, but under favorable growth conditions, their pro-apoptotic activities are held in check by bcl-2 and its pro-survival relatives\textsuperscript{16–20}. Some members of the bcl-2 family, such as bcl-2 and bcl-xL, are mighty inhibitors of apoptosis, whereas other family members, such as bak and bax, promote this cell death process\textsuperscript{20–22}.

In histologically normal urothelium, weak bcl-2 immunostaining is detected in basal cell compartment which reflects the need of the progenitor cells for bcl-2 protection against apoptosis in order to ensure survival of the entire epithelium. Bcl-2 oncoprotein absence in the suprabasal layers of normal urothelium demonstrates that bcl-2 is not necessary during completion of the differentiation process\textsuperscript{23}. Since bcl-2 expression was found to be absent in the normal adjacent bladder tissues examined, the hypothesis that this gene expression may have large impact on bladder cancerogenesis has been proposed\textsuperscript{24}. Some earlier studies suggested that bcl-2 expression was an independent prognostic factor associated with favorable clinical outcome in some human cancers including urinary tract cancers, but not in the invasive bladder cancer\textsuperscript{25}. Thus, although bcl-2 itself does not appear to have a major prognostic importance in invasive bladder cancer, a persistent presence of excess bcl-2 protein might be associated with a poor prognosis to some extent.

Over expression of the bcl-2 protein protects against a wide variety of apoptotic attacks including radiation and nearly all chemotherapeutic drugs. This over expression of bcl-2 in cancer cells has been reported to interfere with the therapeutic effect of cisplatin through an inhibition of the apoptotic pathway. The bax protein acts as a determinant of an intrinsic chemosensitivity and a gradual enrichment of the bax-negative tumor cells gives a selective survival advantage presenting them more resistant to a chemotherapy-induced apoptosis after radical cystectomy\textsuperscript{25}.

In 2006, Maluf et al.\textsuperscript{26} presented a study evaluating the expression of bcl2, p53 and MDM2 proteins in patients with muscle invasive TCC treated with cisplatin based neoadjuvant therapy. In this study, altered expression of p53 and bcl2 was seen in approximately half of the tumors with a poor outcome and a 5-year survival of 25% and a median survival of 1.1 years\textsuperscript{26, 27}.

On the contrary, Shiina et al.\textsuperscript{28} found no significant correlation between bcl-2 expression and overall survival in 77 patients who received cystectomy. In an analysis of 109 patients with invasive bladder carcinoma treated with preoperative radiation therapy without concurrent chemotherapy, Pollack et al.\textsuperscript{29} demonstrated that bcl-2 over expression was significantly associated with disease progression and upstaging of the tumor during radiation therapy. However, bcl-2 over expression had no significant effect on response to a combination of platinum-based chemotherapy and radiation therapy.

Li et al.\textsuperscript{23} in their study did not find any relation between bcl-2 expression and the histological stage of progression, lymphovascular infiltration or node invasion. However, when they analyzed the data by individual case, a higher proportion of loss of bcl-2 expression was found in superficial or lower-grade TCC cases than those in muscle-invasive or high-grade TCC cases. They also showed that p53 over expression was changing from negative in dysplasia to positive in carcinoma, which was opposite from bcl-2 expression.

Bax inhibits the function of bcl-2 by inducing a bax-bcl-2 complex or by competing with other bcl-2 targets. When the bax expression was higher than the bcl-2 expression, the results seemed to be protective against an early relapse at an early stage of the disease. According to some studies, a greater expression of bax and a lower expression of bcl-2 lead to a favorable ratio of bcl-2/bax which would induce a greater protection from a recurrence of a bladder cancer\textsuperscript{25, 30}.

The Bladder Tumor Marker Network also evaluated a series of 109 patients with G2 or G3, T2 to T3 TCC bladder disease and found no prognostic value of bax and p53 staining. In a study of 109 patients with pT2N0M0 bilharzial-related bladder cancer, bax, along with MIB-1 (Ki-67), bcl-x, and p53, was an independent predictor of progression-free survival in the urothelial carcinoma group\textsuperscript{4, 30, 31}.

**Diagnostic achievements using not only one marker**

The number of simultaneously altered apoptosis markers is an important prognostic indicator for disease recurrence and bladder cancer-specific survival in patients treated by radical cystectomy. The fact that many steps in the cascade need to be altered to achieve inhibition of apoptosis seems intuitive. However, supporting data to this proposition are scarce. On the contrary, cell-cycle regulators have al-
ready been shown to have superior predictive value for both disease recurrence and disease-specific survival when studied in combination rather than when assessed as single markers. Several proliferation and metastasis-associated molecules, such as Ki-67, Rb, EGFR, E-cadherin, MSH2, cyclins, p21WAF1, Kip1, survivin and p53 and other apoptosis-related molecules have shown potential in providing prognostic information related to tumor grade, tumor growth, metastasis, recurrence, and overall survival and cancer specific survival in urothelial carcinomas. The results of many studies are still contradictory and there is no exact marker yet.

It has been established that p53 expression cannot be used solely for a definite prognosis or a selection for either sensitivity or resistance to anticancer treatments. Similarly, other proteins involved in apoptosis, such as bcl-2, bax, CD95 or specific caspases, cannot currently be used for determining sensitivity or resistance to anticancer therapy.

Karam et al. showed that only a combined expression of the apoptosis markers bel-2, caspase-3, p53 and survivin was in association with oncological outcomes of patients treated by radical cystectomy and bilateral lymphadenectomy for TCC of the bladder. Their results showed that altered expression of bcl-2, caspase-3, p53, and survivin were present in more than two thirds of patients with muscle-invasive TCC of the bladder. The altered status of each of the four markers was associated with advanced pathological stage. P53 and caspase-3 were associated with high tumor grade, p53 and survivin with lymphovascular invasion and all three markers were related to lymph-node metastases.

In accordance with rapid development of diagnostic medicine, it is clear that genomics and proteomics represent powerful and promising tools in current cancer research and it is only a question of time when all those new findings will enter clinical practice and be available to every patient. A major challenge will be the integration of proteomics, genomics and immunohistochemistry with current available prognostic markers. This would surely enable the best patient selection for the concomitant therapy after radical cystectomy.

**Conclusion**

So far, no marker has proven itself to be sufficient in providing reliable prognosis and being a mainstay for therapy decision-making in patients with muscle invasive TCC of the bladder. The prognosis based on apoptotic status can be best provided through immunohistochemical staining of a number of apoptotic markers simultaneously. As we are faced with very inconvenient disease in prognostic and therapeutic sense, more studies on this issue are needed.

**REFERENCES**


Received on March 30, 2010. Accepted on October 11, 2010.