

Expression of proto-oncogene c-Myc in patients with urinary bladder transitional cell carcinoma

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Abstract

Background: c-Myc is a proto-oncogene located on human chromosome 8. It encodes a transcriptional factor which regulates the expression of approximately 10% to 15% of human genes, playing a crucial role in cell growth, differentiation, cellular metabolism, apoptosis, and cell transformation. The aim of this study is to correlate the expression of c-Myc in patients suffering from urinary bladder transitional cell carcinoma with tumor grade, stage, and lymph node metastases.

Materials and methods: Formalin-fixed, paraffin-embedded tissue samples were obtained from 54 consecutive patients who underwent transurethral resection of bladder tumor or radical cystectomy (RC) as treatment for urinary bladder transitional cell carcinoma. Immunohistochemistry was performed using c-Myc monoclonal antibody and c-Myc expression was then analyzed for correlation with tumor stage, grade, and lymph node metastases.

Results: From a total of 54 patients, 42 (77.8%) had c-Myc positive staining and 12 (22.2%) were c-Myc negative. In the c-Myc positive group, 28 patients (66.7%) had low grade tumors and 33 (78.6%) presented with non-muscle-invasive disease ($p < 0.05$). In the c-Myc negative group, 10 patients (83.3%) had high-grade disease and 8 (66.7%) presented with muscle-invasive disease ($p < 0.05$). Lymph node metastases were evaluated in 17 patients who underwent RC. Of these, 5 had lymph node metastases, 4 of whom had c-Myc negative staining ($p < 0.05$).

Conclusions: In our study, c-Myc negative staining was associated with higher grade and higher stage disease. On the contrary, most c-Myc positive tumors were low grade and non-muscle-invasive disease. In patients who underwent RC, c-Myc negative staining was associated with lymph node metastases.

Keywords: Bladder cancer; c-Myc expression; c-Myc proto-oncogene; Transitional cell carcinoma; Urothelial carcinoma

1. Introduction

Urinary bladder transitional cell carcinoma (TCC) is the second most common malignancy of the urinary system after prostate cancer.^[1] It is estimated that 78% of bladder cancer cases are diagnosed in patients aged 55 years and older, and 70% of patients present with non-muscle-invasive disease and have a fairly good prognosis.^[2] Concerning treatment for non-muscle-invasive bladder cancer, in T1 tumors at high risk of progression or patients who have failed intravesical treatment, radical cystectomy (RC) is a valid option.^[3] On the other hand, when muscle-invasive bladder cancer is diagnosed, RC is the gold standard treatment of choice, providing a 5-year survival of 50%.^[4] In men, standard RC includes removal of the bladder,

prostate, seminal vesicles, distal ureters, and regional lymph nodes.^[5]

c-Myc is a proto-oncogene located on human chromosome 8. It is a member of the Myc family. The c-Myc gene encodes a transcriptional factor that dimerizes with MAX and other factors. The c-Myc-MAX complex binds and regulates the expression of approximately 10% to 15% of human genes, playing a crucial role in cell growth, differentiation, cellular metabolism, apoptosis, and cell transformation.^[6] c-Myc gene expression is regulated not only by growth factors, hormones, and their respective signaling pathways, but also by the concentration of nutrients. Actions of c-Myc include stimulation of energy and enzyme substrate production in order to satisfy the increased metabolic needs of growing and proliferating cells, formation of new organelles, especially ribosomes and mitochondria, stimulation of DNA replication, and G1/S progression of the cell cycle.

In cancer cells, deregulated c-Myc gene combined with lots of tumor suppressor genes, like TP53, can lead to uncontrolled cell growth independent of nutrient concentration.^[7,8] c-Myc overexpression is a characteristic of the majority of human cancers and contributes to the development of at least 40% of tumors.^[8] As analyzed in genomic studies, c-Myc gene amplification has been identified in approximately 25% of breast cancers, 30% of ovarian cancers, and 8% of prostate tumors. Upregulated expression of c-Myc can also occur with translocations between

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Current Urology, (2021) 15, 231–233

Received April 27, 2020; Accepted September 17, 2020.

<http://dx.doi.org/10.1097/CU9.0000000000000053>

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chromosomes, placing the gene under control of unrelated enhancers, such as in Burkitt lymphoma and multiple myeloma. Deregulation of signaling pathways in chronic myeloid leukemia, breast, and colorectal cancer can enhance protein stability of c-Myc and increase c-Myc gene transcription.^[9] The aim of this study is to investigate the expression of c-Myc in patients suffering from urinary bladder TCC and its correlation with tumor grade, stage, and lymph node metastases.

2. Materials and methods

Formalin-fixed, paraffin-embedded tissue samples were obtained from 54 consecutive patients (51 males and 3 females). All patients in the study had urothelial bladder cancer. Patients with histological variants or mixed histology were excluded from the study. Patients were divided into two groups according to their c-Myc proto-oncogene expression (c-Myc positive and c-Myc negative groups). In terms of risk factors, all 54 patients were smokers. Immunohistochemistry was performed using a recombinant, monoclonal anti-c-Myc antibody Y69, a well-established anti-c-Myc monoclonal antibody which has demonstrated high batch-to-batch consistency and reproducibility, sensitivity, and specificity. c-Myc expression status was then analyzed for correlation with tumor stage, grade, and lymph node metastases. *T* test was performed for both groups including all the tested parameters listed above using GraphPad software.

3. Results

The median age of the patients was 72.8 years (74.1 years in the c-Myc positive group and 68.2 years in the c-Myc negative group). Thirty-seven patients underwent transurethral resection of the bladder tumor (TURBT). Seventeen patients underwent RC as treatment for muscle-invasive urinary bladder urothelial carcinoma. Out of a total of 54 patients, 42 (77.8%) had c-Myc positive staining and 12 (22.2%) were c-Myc negative. Fourty of 42 patients in the c-Myc positive group were male. Eleven of 12 patients in the c-Myc negative group were male.

In the c-Myc positive group, 28 patients underwent TURBT and 14 patients underwent RC. Twenty-eight patients (66.7%) had a low grade tumor and 33 (78.6%) presented with non-muscle-invasive disease ($p < 0.05$).

In the c-Myc negative group, 9 patients underwent TURBT and 3 patients underwent RC. Ten patients (83.3%) had high-grade disease and 8 (66.7%) presented with muscle-invasive disease ($p < 0.05$).

Lymph node metastases were evaluated in patients who underwent RC. As a result, out of a total of 17 patients who underwent cystectomy, 5 patients had lymph node metastases, the majority of whom (4 patients) had c-Myc negative staining ($p < 0.05$) (Table 1).

4. Discussion

Bladder cancer is the second most common malignancy of the genitourinary system. c-Myc gene amplification is present in up to 30% of patients with bladder cancer. The mechanism of the association between c-Myc expression and pathological and prognostic features of urothelial bladder cancer patients has not yet been clearly determined, as data from several trials have reported conflicting results.

In a study of 64 hospitalized patients diagnosed with non-muscle-invasive TCC, Yunfei et al. found that c-Myc RNA expression was significantly higher as compared to normal bladder mucosa tissue samples. However, no difference was found in terms of the level of c-Myc RNA between patients with low and high-grade TCC and between patients with Ta and T1 tumors. They also found that c-Myc protein concentration was elevated in TCC samples as compared to normal bladder samples, but these protein levels were not significantly different between the 64 patients, based on the different grade and pathological stage of each patient's tumor.^[10]

In another study, Watters et al. examined the correlation between c-Myc gene amplification and progression of non-muscle-invasive TCCs to muscle-invasive disease. Bladder cancer tissue samples were taken from patients with \geq pT2 cancer (group 1) and from patients with pT1 or pTa cancer that progressed to \geq pT2 (group 2). Samples in the latter group were taken before and after progression of the TCC; thus, 45 samples were examined in total. The results of fluorescence in situ hybridization showed that 93% of tumors from group 1 had elevated copy numbers of c-Myc and chromosome 8, but none of these tumors demonstrated gene amplification. In the second group, 93% of samples taken during pTa/pt1 stage disease and 87% of those taken during \geq pT2 stage disease had increased copy numbers of c-Myc, and 90% of all samples from group 2 had polysomy 8. However, only 13% of the \geq pT2 tumors in group 2 demonstrated c-Myc gene overexpression. The authors suggested that an increased c-Myc copy number might predict future invasive tumor development.^[11]

In addition, Sauter et al. showed that c-Myc overexpression is associated with bladder tumors of low histological grade and low stage. Less than half of grade 3 tumors exhibited c-Myc overexpression, whereas 82% of grade 1 and 2 tumors exhibited overexpression. Moreover, pTa/pT1 tumors tended to overexpress c-Myc when compared to pT2–4 tumors, but the difference was not statistically significant.^[12] In contrast with c-Myc overexpression, it was found that c-Myc gene copy number gains were associated with tumors of higher malignant behaviour. The higher the pathological stage and the grade of the tumor, the higher c-Myc gene copy number it had; these differences were statistically significant. In addition, an association between c-Myc gene copy number and polysomies of chromosomes 7, 8, and 17 was found ($p < 0.001$), indicating that

Table 1
Results

Groups	n	Type of surgical procedure		Grade		Stage	
		TURBT	RC	Low	High	NMIBC	MIBC
cMyc ⁺	42	28 (66.7%)	14 (33.3%)	28 (66.7%)	14 (33.3%)	33 (78.6%)	9 (21.4%)
cMyc ⁻	12	9 (75%)	3 (25%)	2 (16.7%)	10 (83.3%)	4 (33.3%)	8 (66.7%)

MIBC = muscle-invasive bladder cancer; NMIBC = non-muscle-invasive bladder cancer; RC = radical cystectomy; TURBT = transurethral resection of bladder tumor.

tumors of higher grade and advanced stage had increased genomic instability.^[12,13]

In contrast with our study, Schmitz-Dräger et al. investigated 185 urothelial tissue specimens and showed that only 18% of Tis tumors exhibited c-Myc overexpression whereas approximately 60% of Ta, T1, and \geq T2 had overexpression of c-Myc. However, they found no correlation between c-Myc overexpression and tumor grade.^[14] Another study that examined the prognostic value of c-Myc in muscle-invasive urothelial carcinoma of the bladder showed c-Myc expression in 37% of patients with advanced stage urothelial carcinoma and concluded that c-Myc is a negative prognostic factor and its expression is associated with recurrent disease within less than 2 years of diagnosis.^[15]

In view of the findings above, data regarding c-Myc expression and its correlation with tumor grade, stage, and prognosis are conflicting. As c-Myc is a proto-oncogene, we would expect that its activation would lead to high grade and high stage tumors. However, results from our study as well as results from similar studies suggest that the expression of c-Myc is instead associated with lower stage and grade disease. This could be explained by the fact that carcinogenesis is a complex process, and although c-Myc expression may be associated with development of cancer, it is not sufficient in and of itself to cause high-grade disease, as other molecular pathways may also be involved. Accordingly, bladder cancer heterogeneity requires further molecular classification, which may now be possible. A consensus report suggesting six molecular subtypes has been proposed by Kamoun et al. based on MIBC.^[16] In order to develop their consensus report, several molecular pathways were investigated. More specifically, oncogenic mechanisms associated with positive expression of FGFR3, which may be associated with c-Myc expression, allow classification of tumors as luminal papillary tumors presenting with better prognosis as compared to other molecular subtypes.

5. Conclusion

In our study, c-Myc negative staining was associated with higher grade and higher stage disease. On the contrary, the majority of c-Myc positive tumors were low grade and non-muscle invasive. In patients who underwent RC, c-Myc negative staining was associated with lymph node disease. There is increasing interest in identifying prognostic markers for bladder cancer patients which may assist us in choosing the best therapeutic strategy based on an individualized approach. c-Myc expression may act as such a marker, as it is easy to be identified by immunohistochemistry in paraffin-embedded tumor samples. More studies are necessary in order to clarify the best use of c-Myc expression as a potential marker in bladder cancer patients.

Acknowledgments

None.

Statement of ethics

The study was registered and approved by the Ethics Committee of the General Hospital of Athens, G.N.A. "G. Gennimatas". The study complied with the principles of the Declaration of Helsinki for protection of human subjects. All patients were informed in

detail by the treating physician before inclusion in the study and gave written informed consent prior to participation.

Conflict of interest statement

The authors declare that they have no relevant conflict of interest related to the publication of this paper.

Funding source

There was no funding support for this project.

Author contributions

All authors contributed equally to conception and design of the study, drafting, and critically revising the article for important intellectual content, final approval of the version to be published.

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How to cite this article: Fragkoulis C, Ntoumas G, Glykas I, Papadopoulos G, Stathouros G, Kostopoulou A, Choreftaki T, Ntoumas K. Expression of proto-oncogene c-Myc in patients with urinary bladder transitional cell carcinoma. *Curr Urol* 2021;15(4):231–233. doi: 10.1097/CU9.0000000000000053