Research Progress in Bladder Cancer Diagnosis and Therapy

Xuanhe Chen¹, Xue Tan², Fangjing Fan², Chong Li²*

¹Department of Biological Chemistry, Biological Chemistry Major, Grinnell College, Grinnell, IA 50112, United States
²Core Facility for Protein Research, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

Abstract: Bladder cancer is one of the most frequent malignancies associated with high morbidity, high mortality, as well as inconvenient post-operative recurrence. Typical symptoms of bladder cancer include hematuria, frequent urination, nocturia, and dysuria. Most of the primary bladder tumors are non-muscle invasive, which do not grow in muscularis propria, and are often treated with surgery. Muscle-invasive tumors are more common in recurrent diseases, which have higher risk of metastasis. In addition, the treatments of these tumors have more serious side effects such as radical cystectomy with neoadjuvant chemotherapy. The mechanisms of pathogenesis and metabolisms of bladder cancer are yet unclear. However, several genetic mutations and influences from external environment have been proven to be the risk factors for bladder cancer. Along with the developments in researches, the understanding about bladder cancer formation and treatment has been improved over the years. Further studies on medical detection through new technologies are beneficial to the diagnosis and treatment of bladder cancer. This review aimed to provide a general view of some recent progresses of bladder cancer’s pathogenesis, diagnosis, treatment, and developments on research methods.

Keywords: Bladder cancer, Pathogenesis, Diagnosis, Treatment, Translational medicine, Precision medicine

1. Introduction

Bladder cancer is a tumor of the urinary system which has high recurrence rate, pathological progression, and low survival rate after advanced metastatic disease. As one of the most common causes of cancer death, bladder cancer has about 70,000 annual new cases and about 15,000 deaths in the USA annually. The number of diagnosed cases worldwide is more than 430,000 each year. Typically, bladder cancer has different morbidity and mortality rates between men and women. Approximately 75% of bladder cancer patients are men, and the death rate of male patients is about 2 times higher than that of female patients[1]. The status of bladder cancer in China is similar. An analysis of China’s bladder cancer data between 1998 and 2008 shows that the incidence of bladder cancer is 7.49 over 100,000, that is, 2.5% of total malignant tumors. Death rate of all registered bladder cancer cases is about 2.63 over 100,000. The incidence in male patients is 3.3 times higher than that in female patients and the death rate in male patients is 2.9 times higher than that in female patients. The incidence in rural area is 2.4 times higher than that in urban area; however, the death rate of urban patients is 1.6 times as high as that of rural patients[2,3]. Although the results from the analysis indicate that China has mediocre bladder cancer incidence and death rate, there is a trend that bladder cancer becomes more severe over the years[2,3].
Most bladder cancers are transitional cell carcinomas and could be either low grade or high grade. The diseases could also be divided into non-muscle invasive and muscle invasive according to the tumor’s invasion of the muscularis propria. About 75% of bladder cancer cases are non-muscle invasive and 25% are muscle invasive or other metastatic diseases. Among the diagnosed diseases, about half of the non-muscle-invasive tumors and almost all muscle-invasive tumors are high grade. Bladder cancers can be classified into papillary, solid, and mixed types morphologically. The papillary type is predominant, particularly in non-muscle-invasive tumors\[^{[4]}\]. For low-grade disease, local therapy and surveillance are the common management strategies, while neoadjuvant chemotherapy, radiotherapy, and radical surgery are necessary for effective treatment. Long-term follow-up is required for bladder cancer management due to its high recurrence rate. The associated expenses of disease monitoring are so high that only a fraction of bladder cancer patients can be treated professionally\[^{[5]}\]. There are limited numbers of effective tumor markers for diagnosis, staging, monitoring, and predicting prognosis. Thus, novel therapies for advanced metastatic bladder cancer are still under assessment.

2. Pathogenesis of bladder cancer

Cancer originates from abnormal replication of cells. The specificity or mutation of genes is one of the major causes of cancer. The carcinogenesis of bladder comes from long-term accumulation of genetic mutation. The genes related to bladder carcinogenesis include oncogenes, tumor suppressor genes, DNA mismatch genes, and some genes encoding drug-metabolizing enzymes. The products of proto-oncogenes include kinases, growth factors, or their receptors. Under normal circumstances, these products help maintain cell signaling and proliferation. Oncogenes cause cancer through activation mechanism. When a proto-oncogene is converted to an oncogene, it would have abnormal expression and lead to an overproduction of gene products or protein products. On the other hand, the mutations on tumor suppressor genes cause cancer by affecting inactivation mechanism of the cells. The proliferation of cells requires harmonious promotion and inhibition. The cell growth would be out of control if the tumor suppressor genes are inactivated. The proteins produced by DNA mismatch repair genes play an important role in the recognition and excision of mismatched DNA during replication. Instability of signals related to DNA mismatch repair genes can be used as marker of gene mutants to reflect abnormal replication and repair functions of bladder cancer phenotype. Some reports also indicate that the genes of drug metabolic enzymes exist in urethral epithelial cells could affect the formation of DNA adducts, which positively relate to the formation of cancerous tumors\[^{[6]}\].

In addition to the genetic abnormalities that may lead to cancer formation, some genes are also involved in the maintenance of cancer cell function. For example, cancer stem cells are believed to be responsible for tumor self-renewal, initiation, drug resistance, and metastasis. During recent years, several researches related to cancer stem cells have made progress. One of the studies conducted single-cell sequencing on 59 cells including human bladder cancer stem cells, bladder cancer non-stem cells, bladder epithelial stem cells, and bladder epithelial non-stem cells from three bladder cancer specimens. The result showed that human bladder cancer stem cells had clonal homogeneity which suggested that the cells had biclonal origin from the other two types of stem cells. This study also identified 21 genes involved in the functions of cancer stem cells\[^{[7]}\]. Moreover, there are studies on bladder cancer stem cells which found associations between specific genes and characteristics of cancer stem cells. One study on bladder cancer stem cells and normal bladder stem cells found that the C228T mutation of telomerase reverse transcriptase promoter, whose activation had been observed in almost all human tumor histotypes\[^{[8]}\], occurred in the cancer stem cells but could not be observed in normal cancer stem cells. The experimental result concluded that the C228T mutation would lead to telomerase reverse transcriptase overexpression and cancerization. In contrast, the restoration of C228T mutation can recover the telomerase reverse transcriptase expression and abolish tumor formation\[^{[9]}\]. Particular enzymes could also influence on the self-renewal of bladder cancer stem cell. For example, it has been found that GALNT1, a glycotransferase, mediates glycosylation and activation of a signaling pathway which could maintain the self-renewal of cell and tumor initiation\[^{[10]}\].

Besides, the genetic causes discussed above, certain behaviors or characteristics have been proven to increase the risk for bladder cancer. Smoking is considered to be one of the most important risk factors for bladder cancer because multiple molecules in cigarette could promote the formation of cancerous urinary cells. Studies have shown that men who never smoke would face much less risk of having bladder cancer compared with smokers, and ceasing smoking would rapidly decrease the risk at any period of life\[^{[11]}\]. Over 60 kinds of carcinogens which can cause direct DNA damage are detected in cigarette smoke. The smoking caused DNA damage on bladder cancer patients had been confirmed by a study between people with and without smoking habit. The result indicated that these two groups of patients had different molecular characteristics, and the difference could affect the ways of treatment\[^{[12]}\]. Some other general risk factors for cancer, such as aging, could also increase the incidence of bladder cancer\[^{[13]}\].

3. Diagnosis of bladder cancer

At present, bladder cancer is commonly diagnosed by accident or due to symptoms. For example, the common and typical symptoms, including painless and intermittent
macroscopic hematuria, are found in about 75% of the patients. However, as hematuria can also be the result of other diseases such as urinary tract infection, nephrolithiasis, or benign prostatic hyperplasia, particular inspections are required for accurate diagnosis. For instance, diagnostic cystoscopy could be used for identifying lesions within the bladder or urethra, whereas imaging of upper urinary tract by ultrasonography, intravenous urogram, and computed tomography (CT) is effective methods to identify lesions within the ureter, renal pelvis, and renal parenchyma. An analysis of Chinese bladder cancer diagnosis showed that 74.3% of patients in China were diagnosed by white light cystoscopy with biopsy. Fluorescence and narrow-band imaging cystoscopy had extra detection rate of 1.0% and 4.0%, respectively. Diagnostic transurethral resection provided detection rate of 16.9%. These data did not differ much from western countries[13]. The diagnostic efficiency of bladder cancer by symptoms alone is limited. Data showed that a number of bladder cancer patients had few symptoms. The only presenting symptoms in them were urinary urgency or frequency. In such cases, diagnosis based on symptoms was not effective[14]. Due to the urgent needs, many recent studies related to bladder cancer diagnosis have focused on developing effective diagnostic tools.

Studies of some diagnostic tools are based on the characteristics of bladder cancer cells. For example, tumor-initiating cell is a subpopulation of cells with stem cell-like characteristics and could affect tumor growth, recurrence, and metastasis. This kind of cell is valuable for its application in clinical diagnosis and therapy. One of the recent studies pertaining to tumor-initiating cells isolated from different bladder cancer cell lines confirmed that the cells from different resource were heterogeneous[14]. The group analyzed the gene expression profiles of the tumor-initiating cells and then developed a transcription factor gene regulatory network that included key transcription factors involved in the cell function, which could be a potent foundation of the future researches on diagnosis[14]. Some other studies about bladder cancer diagnostic tools are related to characteristics of the disease. Although cystoscopy is used most commonly, the body invasion and cost of cystoscopy make it inconvenient. It has been observed that urinary microRNAs of bladder cancer patients are different from healthy people. In this case, urinary microRNAs could be reliable biomarkers for bladder cancer diagnosis as they could be operated in vitro. The accuracy of this potential biomarker is testified in one analysis of data from corresponding researches[15].

In addition, bladder cancer diagnosis is a long-term comprehensive process which would still be necessary even after treatment. New findings could also help in determining how treatments work in patients. Progress of the DNA sequencing related to bladder cancer recurrence would hopefully contribute to the diagnosis of recurrence after surgery. Certain exomes, such as a transcriptional coactivator, MLL which regulates gene expression in early embryo development is proven to have the ability to alter the bladder cell recurrence[16]. Neoadjuvant chemotherapy, as a novel and mainly used bladder cancer treatment, requires further understanding of its related cell responses too. Several studies have focused on the genetic profiling of bladder cancer and neoadjuvant chemotherapy. For example, one of the studies found that mutation of FGFR3 gene and strong expression of ERCC1 gene were correlated with neoadjuvant chemotherapy response in muscle-invasive bladder cancer patients[17].

4. Treatment of bladder cancer

As the pathogenesis of bladder cancer is complex, there could be multiple aspects for its treatment. Part of the targeting agents is developed aiming to block the tumor formation. KMP1 is a novel mouse monoclonal antibody particularly related to bladder cancer. Its expression results in significant inhibition of proliferation, migration, adhesion, and tumor cell growth in bladder cancer cell of nude mice. The mechanism of KMP1 antibody is discovered to block the function of antigen CD44. The characteristic of KMP1 makes it valuable to be specific diagnostic biomarker or target agent of bladder cancer treatment[18].

There are certain studies of bladder cancer treatment focusing on the self-renewal of cancer cells. Substantial works have been done in this area. A novel Hedgehog inhibitor, iG2, developed from Streptomyces roseofulvus, has a remarkable ability to block the activation of certain genes in bladder cancer cells. It has been observed that iG2 could also repress the growth, attenuate the proliferation, and enhance apoptosis of bladder tumor cells. iG2 reduces the self-renewal ability of bladder cancer stem cells as well as the tumor formation[19]. New findings related to bladder cancer stem cell self-renewal signals, such as the KMT1A-GATA3-STAT3 circuit and the inhibition of GALNT1 with Sonic Hedgehog signaling, are hopefully to be used in the development of novel therapeutic agents[20].

There are agents developed to eliminate bladder cancer after it has formed. A new monoclonal antibody, BCMab1, is against bladder cancer. It specifically identifies the aberrantly glycosylated integrin a3b1 epitope. The BCMab1 antigen expression was consistent with the severity and prognosis of bladder cancer. The antibody could block integrin engagement to inhibit its signaling, this leads to cell cycle arrest as well. By contrast, BCMab1 antigen could be a new biomarker for bladder cancer[21]. The advanced research on BCMab1 has led to the generation of a new antibody-based drug BCMab1-Ra, which was generated by conjugation of BCMab1 with the ricin A chain. A volunteer with bladder cancer received intravesical administration of BCMab1-Ra treatment. No tumor was observed by cystoscope examination after 30 weeks. No local or systemic side effects were observed during the study. Moreover, human anti-mouse antibody
was not detectable in the circulatory system. Although the effectiveness of the new drug is impressive, there is a long way before BCMab1-Ra can be applied in the clinical setting as further data collection is required.

As a complement, the treatment of bladder cancer includes management of the sequelae of other therapies. For example, intravesical bacillus Calmette-Guérin is a widely used vaccine for high-risk, non-muscle-invasive bladder cancer. It has been reported that the spectrum of intravesical bacillus Calmette-Guérin-induced complications vary from granulomatous prostatitis to sepsis. For two patients with systemic illness, the symptoms resolved after adding prednisone at first, indicating that inflammation in systemic disease plays a principal role in the complications. T-cell response and mycobacterium growth inhibition were tested in vitro on patients with similar systemic diseases. The test results indicated that the inhibition of bacillus Calmette-Guérin is more effective on patients with mild symptoms, whereas patients with sepsis and organ involvement had high response in T-cell, but the disease cannot be treated successfully. Based on these observations, it is believed that immunological assays should provide a better insight into the pathogenesis of bacillus Calmette-Guérin infection in individual patients, and this warrants further research.

5. Technology developments of bladder cancer-related works

Advances in technology could efficiently facilitate bladder cancer research. In general, new technologies could make improvement in two aspects: aggrandizing information access and data analysis. Diagnostic tools are important to get effective information from the disease. Narrow-band imaging is a newly developed technology that serves as a tool to provide additional endoscopic information for bladder cancer patients. The current results showed that narrow-band imaging has an advantage in sensitivity compared to conventional white light cystoscopy; however, there was still risk of lower specificity and higher false-positive results. The treatment effect of transurethral cystectomy with narrow-band imaging technique was satisfactory. Residual tumor and tumor recurrence were reduced according to follow-up records. In the future, the application of narrow-band imaging might refine the treatment and follow-up protocol in patients with non-muscle-invasive bladder cancer. However, this new technology requires large-scale prospective studies to confirm whether it is cost effective.

The improvement of data analysis could be interdisciplinary. Researchers need methods that can help them come up with the result as well as evaluate the outcomes. Computer science and machine learning have already been used for bladder cancer research. Tumor budding is considered to be an independent prognostic feature in several tumor types. A research group with machine learning-based methodology reported the relationship between tumor budding and survival evaluated in patients with muscle-invasive bladder cancer. The machine learning technology was applied to quantify tumor buds across immunofluorescence labeled images of samples derived from 100 muscle-invasive bladder cancer patients. A specific classification and regression tree model were constructed to stratify the patients into three staging groups based on disease survival. The decision tree model reported tumor budding features for the stratification of non-metastatic patients into high or low risk of disease-specific death. No clinical feature was utilized to categorize these patients. The findings of this research not only demonstrated that tumor budding, quantified with automated image analyses, provided prognostic value for muscle-invasive bladder cancer but also proved that the new model is feasible to approach research results.

Accurate evaluation of experimental data requires specific models. For the evaluation of anti-human bladder cancer immunotoxin approaches, an orthotropic nude murine model that mimics the human counterpart has been developed and characterized to provide preclinical evaluation of new treatment modalities. In the particular study, the established human bladder transitional cell carcinoma cell line, BIU-87, was transplanted orthotopically into immunodeficient nude mice. BIU-87 transitional cell carcinoma cells were incubated in monolayer cell culture, and instilled intravesically into mild acid washed bladders in the form of single-cell suspensions. Tumor growth was assessed weekly by magnetic resonance imaging. The experimental mice were examined using multiple methods timely after tumors were detected. The magnetic resonance imaging results showed that the invasion of tumors could be well identified, which indicated that the orthotopic BIU-87 transitional cell carcinoma model was reproducible and ideal for preclinical studies on experimental intravesical therapies.

6. Conclusion

Bladder cancer is characterized by high morbidity and mortality rates, and this disease is becoming more serious globally during recent years. Recent studies have identified a number of relationships between genetic characteristics and bladder cancer phenotype; however, the pathology and metabolism of bladder cancer are not clear yet. We still lack in the effective methods for diagnosis and treatment of bladder cancer. Based on the recent academic research results, the development of better diagnostic methods and agents is expected. New technologies also open up more possibilities for bladder cancer research. Overall, the biology of bladder cancer has only begun to gain some clarity. Whether it is in the areas of pathology, diagnosis, treatment, or research, bladder cancer is a complex topic to be studied and every single piece of new information regarding this cancer matters.
Acknowledgments

The authors are thankful to the Institute of Biophysics, Chinese Academy of Science for all support provided.

Conflict of interest

The authors declare no potential conflicts of interest.

Author contributions

C.L. conceived the idea for the work and initiated the study, X.C. and C.L designed the study, X.C. wrote the manuscript, X.T. and F.F. revised the manuscript, and all authors contributed to the literature search.

References

1. Bladder Cancer Symptoms, Tests, Prognosis, and Stages; 2019. Available from: https://www.cancer.gov/types/bladder/patient/about-bladder-cancer-pdq.
2. Han S, Zhang S, Chen W, et al., 2013, Analysis of the Status and Trends of Bladder Cancer Incidence in China. Oncol Prog, 6:89–95.
3. Han S, Zhang S, Chen W, et al., 2013, Analysis of the Status Quo and Trends: Mortality in Patients with Bladder Cancer in China. J Mod Urol, 18:228–32.
4. Kamat AM, Hahn NM, Efstatiiou JA, et al., 2016, Bladder Cancer. Lancet, 388:2796–810.
5. Turo R, Cross W, Whelan P, 2011, Bladder Cancer. Common Cancers, 40:14–9.
6. Zhang R, Zhang J, He Z, et al., 2003, Research Advances on Bladder Cancer Associated Genes. Chin J Cancer, 24:104–7.
7. Yang Z, Li C, Fan Z, et al., 2017, Single-cell Sequencing Reveals Variants in ARID1A, GPRC5A and ML12 Driving Self-renewal of Human Bladder Cancer Stem Cells. Eur Urol, 71:8–12. DOI: 10.1016/j.euro.2016.06.025.
8. Wu S, Huang P, Li C, et al., 2014, Telomerase Reverse Transcriptase Gene Promoter Mutations Help Discern the Origin of Urogenital Tumors: A Genomic and Molecular Study. Eur Urol, 65:274–7. DOI: 10.1016/j.euro.2013.10.038.
9. Li C, Wu S, Wang H, et al., 2015, The C228T Mutation of TERT Promoter Frequently Occurs in Bladder Cancer Stem Cells and Contributes to Tumorigenesis of Bladder Cancer. Oncotarget, 23:19542–51. DOI: 10.18632/oncotarget.4295.
10. Li C, Du Y, Yang Z, et al., 2016, GALNT1-mediated Glycosylation and Activation of Sonic Hedgehog Signaling Maintains the Self-renewal and Tumor-initiating Capacity of Bladder Cancer Stem Cells. Cancer Res, 76:1273–83. DOI: 10.1158/0008-5472.can-15-2309.
11. Brennan P, Bogillot O, Cordier S, et al., 2000, Cigarette Smoking and Bladder Cancer in Men: A Pooled Analysis of 11 Case-control Studies. Int J Cancer, 86:289–94. DOI: 10.1002/(sici)1097-0215(20000415)86:2<289:aid-ijc21>3.0.co;2-m.
12. Joshi M, Millis SZ, Arguello D, et al., 2018, Molecular Characterization of Bladder Cancer in Smokers Versus Nonsmokers. Eur Urol, 4:94–7.
13. Li K, Lin T, Chinese Bladder Cancer Consortium, et al., 2015, Current Status of Diagnosis and Treatment of Bladder Cancer in China Analyses of Chinese Bladder Cancer Consortium Database. Asian J Urol, 2:63–9.
14. Xin Z, Zhao Y, Xu J, et al., 2017, Gene Expression Profiling and Construction of a Putative Gene Regulatory Network of Bladder Cancer Tumor-initiating Cells. Oncotarget, 8:111271–80. DOI: 10.18632/oncotarget.22771.
15. Ding M, Li Y, Lv Y, et al., 2015, Diagnostic Value of Urinary MicroRNAs as Non-invasive Biomarkers for Bladder Cancer: A meta-analysis. Int J Clin Exp Med, 8:15432–40.
16. Song W, Zhao Y, Rui Y, et al., 2015, Novel Variants in MLL Confer to Bladder Cancer Recurrence Identified by Whole-exome Sequencing. Oncotarget, 7:2629–45.
17. Zhao Y, Zhang R, Ge Y, et al., 2018, Somatic FGFR3 Mutations Distinguish a Subgroup of Muscle-invasive Bladder Cancers with Response to Neoadjuvant Chemotherapy. EBioMedicine, 35:198–203. DOI: 10.1016/j.ebiom.2018.06.011.
18. Chen Y, Wang H, Zuo Y, et al., 2018, A Novel Monoclonal Antibody KMP1 has Potential Antitumor Activity of Bladder Cancer by Blocking CD44 in vivo. Cancer Med, 7:1–14. DOI 10.1002/cam4.1446.
19. Zhu L, Chen N, Dong B, et al., 2016, A Novel Hedgehog Inhibitor iG2 Suppresses Tumorigenesis by Impairing Self-renewal in Human Bladder Cancer. Cancer Med, 5:2579–86. DOI: 10.1002/cam4.802.
20. Zhao Y, He L, Lin K, et al., 2017, The KMT1A-GATA3-STAT3 Circuit is a Novel Self-renewal Signaling of Human Bladder Cancer Stem Cells. Clin Cancer Res, 23:1–13. DOI 10.1158/1078-0432.ccr-17-0882.
21. Li C, Zhao Y, Ying D, et al., 2014, BCMab1, a Monoclonal Antibody Against Aberrantly Glycosylated Integrin a3b1, has Potent Antitumor Activity of Bladder Cancer in vivo. Clin Cancer Res, 20:4001–13. DOI: 10.1158/1078-0432.ccr-13-3397.
22. Li C, Yan R, Zhao Y, et al., 2016, BCMab1-Ra, a Novel Immunotoxin that BCMab1 Antibody Coupled to Ricin a Chain, Can Eliminate Bladder Tumor. Oncotarget, 8:46704–5. DOI: 10.18632/oncotarget.13504.
23. Bilsen MP, Meijsgaarden KE, Jong HK, et al., 2018, A Novel View on the Pathogenesis of Complications after Intravesical BCG for Bladder Cancer. *Int J Infect Dis*, 72:63–8. DOI: 10.1016/j.ijid.2018.05.006.

24. Hsueh TY, Chiu AW, 2016, Narrow Band Imaging for Bladder Cancer. *Asian J Urol*, 3:126–9.

25. Brieu N, Gavriel CG, Nearchou IP, et al., 2019, Automated Tumour Budding Quantification by Machine Learning Augments TNM Staging in Muscle Invasive Bladder Cancer Prognosis. *Sci Rep*, 9:5174. DOI 10.1038/s41598-019-41595-2.

26. Li C, Yan R, Bao J, et al., 2006, Characterization of a Novel Transplantable Orthotopic Murine Xenograft Model of a Human Bladder Transitional Cell Tumor (biu-87). *Cancer Biol Ther*, 5:394–8. DOI 10.4161/cbt.5.4.2509.