

Cytogenetic and immunological alterations of recurrent bladder cancer

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Abstract: *In this review, we discussed genetic and immunological changes of bladder cancer along with relapse of this disease; however, it should be noted that in most cases, multiple genetic and immunological changes occur simultaneously or mutually dependent on each other. Many genetic mutations disrupt the function of genes involved in regulation, and conversely, chromosomal aberrations lead to changes in transcription. Pathogenesis and transition of normal urothelium of bladder into cancer are multifactorial processes. Chronic inflammation causes the initiation and progression of the main pathophysiology of invasive and metastatic cancer. A dichotomy is observed in the role of immune cells in bladder cancer. Although the immune response protects the bladder by suppressing tumor growth, certain immune cells including neutrophils, macrophages, and T-lymphocytes contribute the development and progression of the tumor.*

Keywords: *bladder cancer, relapse, immune cells, cytogenetics, chromosomal aberrations*

Introduction: According to the world health organization, bladder cancer is the most common malignant tumor of the urinary tract and is ranked 7th in the structure of oncopathology in men and 17th in women. Every year, more than 380 thousand new cases of BC are diagnosed. This disease causes the death of 150 thousand people, while the ratio between the gender is 3.8:1. 2.7 million people have a history of BC. Recently, the US conducted an analysis of the General and stage-specific incidence of BC adjusted for age (5-year survival and mortality rates between 1973 and 2009 y [1].

Although there were some limitations in the analysis of the Surveillance, Epidemiology and End Results (SEER) database, it should be noted that over the past 30 years, the mortality rate from BC has not changed significantly, reflecting shortcomings in the identification, monitoring and treatment of this group of patients [2].

In recent years, the incidence of bladder cancer has been steadily increasing in Uzbekistan. In 2005, 997 patients with bladder cancer were registered by oncology center, in 2001 - 1078, and by the end of 2018 - 1401 patients respectively. The frequency of newly diagnosed diseases also increases from 201 cases in 1995 to 280 in 2000 and to 303 cases in 2017 year [3].

Although epithelial carcinomas are the most common among malignancies, their karyotype characteristics and genetic pathway remain poorly understood. Most of the data presented are quantitatively limited and do not have karyotype accuracy [4].

GENETIC ABERRATIONS. Cancer cells have growth advantages over normal cells, which have historically been considered the result of a series of genetic mutations. As with most cancers, the exact causes of bladder cancer remain unclear. Somatic genetic mutation is one of the most important leading factors of oncogenesis and progression of bladder cancer. Bladder cancer is usually not inherited, but rather the result of the accumulation of somatic mutations of the bladder cells over time. The number of genetic changes has increased enormously thanks to advances in second generation DNA sequencing techniques [5].

Frequently mutated genes in BC (bladder cancer) include TP53 (41%), KDM6A (28%), ARID1A (22%), PIK3CA(18%), MLL2 (17%), CREBBP (15%), RB1 (15%), STAG2 (13%), FGFR3 (13%), EP300 (13%), TSC1 (8%), and HRAS (8%) [6].

Recently, Hedegaard J and coauthor. [7] reported that BC (bladder cancer) can be grouped into 3 subclasses (classes 1, 2, and 3) based on whole genome expression profiles. Class 1 tumors have a lower risk of progression and better prognosis than class 2 and 3 tumors. Class 1 tumors show increased regulation of early cell cycle genes (CCND1, ID1, and RBL2), while late cell cycle genes (CDC20 , CDC25A, CDK, and PLK1) and cancer stem cell markers (ALDH1A1, ALDH1A2 , PROM1, NES, and THY1) are highly expressed in class 2 tumors. The keratin (CRT) gene family shows increased expression in class 2 and/or 3 tumors compared to class 1 tumors. Most BC (86%) show mutations in chromatin remodeling genes, including histone methyltransferases (58% of cases), histone-lysine demethylases (54%), SWI / SNF complexes (40%), and histone acetyltransferases (32%). Overall, 76% of all primary bladder tumors exhibit mutations in at least 1 chromatin regulatory gene [6].

Chromosomal rearrangements. Especially the concomitant result of aberrant non-homologous end attachment [8], can lead to the formation of an oncogene and, therefore, can initiate oncogenesis [9] or increase the expression of the oncogene. Deletions in both branches of chromosome 9 are often observed in both non-invasive cancer and invasive bladder cancer, and in patients with bladder cancer with tumors that have deletions of 9p22, 9q22.3, 9q33 or 9q34, relapse is faster than in patients without these deletions[10].

Deletions of chromosome 9 also affect some tumor suppressor genes, including cyclin-dependent kinase inhibitor 2 A (CDKN2A) and 2B (CDKN2B), as well as TSC1 . Amplifications were often detected in 6p22. 3 (E2 F3), 8p 12 (FGFR1), 8q22.2 (CMYK), 11q13 (CCND1, EMS 1, INT 2), and 19q13.1 (CNE), and homozygous deletions were detected in 9p21.3 8p23. 1 and 11p13 [11].

Genetic and epigenetic changes as therapeutic targets in cancer. Recent technological advances have enabled the discovery of genetic and epigenetic alterations that have led to a better understanding of the mechanisms of bladder cancer at the molecular level, and have provided a huge number of specific biological and molecular targets for therapy. As a result, p53, FGFR3, ERBB2, and PI3K were subjected to immuno and / or chemotherapy in clinical trials [12].

Unlike genetic changes, epigenetic changes can be reversed through pharmacological treatment. Thus, epigenetic treatment offers a new strategy for anti-cancer therapy. Epigenetic drugs for clinical use mainly include DNMT inhibitors (5-azacitidine and 5-Aza-2'-deoxycytidine) and histone deacetylase inhibitors (HDAC) (SAHA, valproic acid and romidepsin) [13].

Tazemetostat (an EZH2 inhibitor) is currently being evaluated in ongoing clinical trials. Bladder cancer has been considered for epigenetic therapy, namely the use of DNMT and HDAC inhibitors to treat bladder cancer [14]. Clinical trials using these epigenetic drugs for bladder cancer are continuing. Cytogenetic studies have shown that transitional carcinoma cells are characterized by multiple chromosomes [15].

Translocations are rare, at least in the early stages, and do not appear to play an important role in the initiation of uroepithelial carcinomas. Instead, the cytogenetic profile is dominated by non-random chromosome increments and especially, losses with the latter indicating that the loss of the tumor suppressor gene may be the most important event in the pathogenesis of uroepithelial carcinomas. The fact that chromatin loss dominates the picture of imbalance indicates that the loss of the tumor suppressor gene (s) is the most important pathogenetic consequence of chromosomal aberrations associated with uroepithelial

carcinoma. Changes involving chromosome 9 [-9, del (9p), del (9q)] are the most common chromosomal aberrations in uroepithelial carcinomas. Rearrangements of chromosome 9 are considered as the only change in cases with simple karyotypes in early and superficial carcinomas, but also persist in massively complex karyotypes of advanced muscle invasive tumors [16]

In addition to the loss of the entire copy of the chromosome, material loss on both sides is often observed, which may indicate the presence of at least one pathogenetically important TSG in each branch. therefore, the loss of chromosome 9 material has been widely recognized as an early widespread, pathogenetically important, and early event in the carcinogenesis of urinary tract transition cells that the loss of 9P material may be associated with the development of tumors with more aggressive biological behavior, or alternatively, they may be associated with early disease progression [6]. Although it has been reported that some attractive candidates, such as p16 / CDKN2 in 9p21 and TSC1 in 9q34, are homozygously removed in the superficial TCC of the bladder the major consequences of these chromosomal aberrations at the gene level remain unknown[17].

The correlation between the karyotype and the level and stage of the tumor A strong correlation was observed between the degree / stage of tumors and the karyotypic profile. Most superficial and well - differentiated tumors (TaG1) were pseudo-or almost diploid and showed simple karyotypes (5 or less chromosomal changes)). A progressive increase in the number of chromosomal aberrations depending on the degree and / or stage of the tumor was evident in most recorded series, where TaG1 tumors showed less abnormal karyotypes than T1G2 tumors, which in turn were less abnormal than T2G3 tumors. This is consistent with the view that uroepithelial carcinomas follow multi-stage carcinogenesis and that their clinical development is due to the synergistic effect of accumulated genetic changes.

Immune cells play opposite roles in the pathogenesis of bladder cancer. The adaptive immune system appears to have an antitumor effect, while the innate immune system has an antitumor effect. In the following sections, we critically examined the role of key immune cells in regulating the pathophysiology of bladder cancer.

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Myeloid suppressor cells (MDSCS) are important immune precursors for the formation of granulocytes, macrophages, and dendritic cells. MDSCs play a role in angiogenesis and metastasis, supporting angiogenesis and lymphangiogenesis and promoting tumor cell survival [18]. MDSCS have been shown to stimulate angiogenesis by releasing factors including VEGF, TNF, IL-1 β , and major fibroblast growth factor (bFGF).

Neutrophils Dichotomy is observed when neutrophils are exposed to bladder cancer. On the one hand, neutrophils target cancer cells that perform several important anti-cancer functions. But they also stimulate excessive inflammation, release growth factors into malignant cells, secrete matrix-destroying enzymes, and they are the main VEGF-containing cells [19]

Macrophages. It has been shown that tumor-related macrophages (TAM) contribute to the development of angiogenesis in bladder cancer and may even be required [20]. Macrophages can be differentiated into subsets M1 and M2 depending on the type of cytokines present. Under inflammatory conditions, macrophages change their phenotype and become more M1-

like or classically activated macrophages (CD86 + iNOS + TLR2 + MHCII high) that produce reactive oxygen species and secrete cytokines, including TNF- α and IL-12, and activate T H 1 cells to release IFN- γ and IL-2 to activate cytotoxic T cells harmful to the body's homeostasis [21].

Interleukin 6 IL-6 is a primary Pro-inflammatory cytokine in humans and is produced mainly by T-lymphocytes and macrophages. Levels of IL-6 are elevated in both the urine and serum of patients with urothelial bladder carcinoma compared to the control group IL-6 is also elevated in the urine of UCC patients, relative to people with less malignant non-muscle-invasive bladder cancer. This suggests that IL-6 may contribute to the development of malignant cancer phenotypes. Poor prognosis and increased metastasis were associated with elevated serum IL-6 levels in bladder, ovarian, and prostate cancers [22].

TNF- α is mainly produced by activated macrophages, and two TNF- α gene polymorphisms have been found to increase the risk of bladder cancer [23]. This could potentially be due to TNF- α -induced regulation of thymidine phosphorylase, an enzyme that has been shown to promote the development of bladder cancer. TNF- α also contributes to angiogenesis and the development of several types of tumors. TNF- α genes are located on chromosome 6 in the region of the main histocompatibility complex (MHC) adjacent to the lymphotoxin locus. Sixteen gene polymorphisms have been identified, two of which are TNF + 488A and TNF-859T. TNF + 488A polymorphism is found in 28.1% of cases of bladder cancer and in 14.9% of healthy people, while TNF-859T is present in 26.6% of cases of bladder cancer and only in 14.4% of healthy patients. The role of these different polymorphisms in cancer is not fully understood due to conflicting results from various studies. However, TNF + 588A is implicated in rheumatoid arthritis along with General variable immunodeficiency. TNF polymorphisms are associated with several types of cancer, including non-Hodgkin's lymphoma, myeloma, and prostate cancer.

Dendritic cells. There are three types of antigen-presenting cells (APC), dendritic cells (DC), macrophages, and B cells. Of the APC, dendritic cells are the most effective inducers of antitumor immunity. Immature DCS were found in the urine of people with bladder cancer. Dendritic cells are also present and minimally activated in the bladder tumor tissue. Beatty and coauthor suggested, that DCS are found in the urine because they migrate there from ex vivo bladder tumors, in fact, immature and minimally activated DCS are found in the urine and bladder of cancer patients [24]. One theory suggests that this may be the result of tumor cells inhibiting the release of DC stimulation and maturation factors. This leads to accelerated tumor development, since immature DCS are less able to initiate activation of T-lymphocytes, which leads to T-cell anergy. While DCS have been used to treat tumors with various immunotherapy treatments, their exact function in cancer remains poorly understood.

T-lymphocytes are usually found in the tumor microenvironment and can contribute to oncogenesis in the bladder [25]. In fact, T-cell subsets can perform both tumor and suppressive functions in bladder cancer. Levels of the T H 17 subgroup are elevated in bladder tumors compared to peripheral blood. Conversely, t-regulatory lymphocyte (Treg) levels were higher in peripheral blood compared to bladder tumors [26]. The presence of elevated levels of Tregs in tumor tissue is also associated with a poor prognosis. Granulocyte and monocyte cells observed in bladder cancer express elevated levels of PD-1, which is believed to be an immunosuppressive protein, compared to healthy people. PD-1 is secreted by Tregs, again indicating their potential role in bladder cancer. However, the mechanisms underlying the effects of Tregs and T H17 in bladder cancer have yet to be determined.

Furukawa and coauthor [27] showed that IL-21 has an antitumor effect in MBT-2 cells. They found that the effect of IL-21 in bladder cancer is mediated by cytotoxic T-lymphocytes. Bladder carcinomas have developed a mechanism to avoid immuno-induced apoptosis. Under normal conditions, the Fas / Fas ligand system provides programmed cell death in infected and cancerous tissues. The Fas ligand is found mainly on T-lymphocytes and natural killer cells (NK) [28].

Activation of Fas by binding the FAS ligand leads to apoptosis of the cell carrying the receptor. Bladder carcinomas have developed a mechanism to avoid this immune response by removing Fas, effectively avoiding apoptosis. In addition, low-grade bladder cancers have been shown to develop resistance to Fas-ligand-induced apoptotic events below Fas. One possible mechanism for explaining this phenomenon is related to the X-chromosome inhibitor of the apoptosis protein (XIAP), which is a member of the IAP gene family. XIAP binds to the proteases of caspase-3 and caspase-7, effectively blocking the induction of FAS apoptosis. Some cancers, including liver, colon, and pancreatic cancers, use similar mechanisms. It is assumed that the production and secretion of soluble Fas (sFas) produced by all cell lines of bladder cancer can block the action of T-lymphocytes and even induce apoptosis in immune cells [29]

Conclusion: Despite extensive research on bladder cancer, the basic cellular and molecular mechanisms remain unclear. In addition, the role of individual cell types, cytokines and chemokines, as well as cytogenetic changes in the pathogenesis of recurrent bladder cancer has yet to be determined. It is extremely important to characterize the factors that are primarily involved in the onset of the disease process, invasion, proliferation and metastasis of tumor recurrence. At the same time, identifying regulatory mediators that inhibit urothelial cell differentiation and maintain normal structure is key to developing more effective and efficient therapeutic approaches.

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References

- [1] Babjuk M, Burger M, Zigeuner R, et al; members of the EAU Guidelines Panel on Non-muscle-invasive bladder cancer. Guidelines on Non-muscle-invasive bladder cancer (Ta, T1 and CIS). Edition presented at the EAU Annual Congress Stockholm 2017. ISBN 978-90-79754-65-6.
- [2] Gakis G, Witjes JA, Compérat E, et al; members of the EAU Guidelines Panel on Muscle-invasive and Metastatic Bladder Cancer. Guidelines on primary urethral carcinoma. Edition presented at the EAU Annual Congress 2016 Milan. ISBN 978-90-79754-71-7.
- [3] Tillyashaykhov M. N., Rakhimov N. M. Khasanov Sh. T., Features of Clinical Manifestation of the bladder cancer in young people// Doctor Bulletin. - Samarkand, 2019. - №2. - P. 108-113
- [4] Mitelman F. www.Mitelmansdatabase.org. 2018.
- [5] Jokinen, M. P., & Seely, J. C. (2018). Urinary Bladder, Ureter, and Urethra. Boorman's Pathology of the Rat, 167–188. doi:10.1016/b978-0-12-391448-4.00012-5
- [6] Cancer Genome Atlas Research Network Comprehensive molecular characterization of urothelial bladder carcinoma. Nature. 2014;507:315–22.

- [7] Hedegaard J, Lamy P, Nordentoft I, Algaba F, Høyer S, Ulhøi BP, et al. Comprehensive transcriptional analysis of early-stage urothelial carcinoma. *Cancer Cell*. 2016;30:27] e [Cancer Genome Atlas Research Network Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature*. 2014;507:315–22
- [8] Ghezraoui H, Piganeau M, Renouf B, Renaud JB, Sallmyr A, Ruis B, et al. Chromosomal translocations in human cells are generated by canonical nonhomologous end-joining. *Mol Cell*. 2014;55:829–42
- [9] Williams SV, Hurst CD, Knowles MA. Oncogenic FGFR3 gene fusions in bladder cancer. *Hum Mol Genet*. 2013;22:795–803
- [10] José Batista da Costa, Ewan A. Gibb, Trinity J. Bivalacqua, Yang Liu, Htoo Zarni O Molecular characterization of neuroendocrine bladder cancer // *Clin Cancer Res* July 1 2019 25 (13) 3908-3920; DOI:10.1158/1078-0432.KBH-18-3558.
- [11] G9a Inhibition Has Antitumor Activity in Bladder Cancer *Cancer Discov* September 3 2019 9 (9) OF6-OF6; DOI:10.1158/2159-8290.CD-RW2019-105
- [12] Samuel L. Washington III, John Neuhaus, Maxwell V. Meng and Sima Social Determinants of Appropriate Treatment for Muscle-Invasive Bladder Cancer *P. Porten Cancer Epidemiol Biomarkers// Prev* August 1 2019 28 (8) 1339-1344; DOI:10.1158/1055-9965.EPI-18-1280.
- [13] C. J. Willson, G. P. Flake, R. C. Sills, Immunohistochemical Expression of Cyclin D1, Cytokeratin 20, and Uroplakin III in Proliferative Urinary Bladder Lesions Induced by o-Nitroanisole in Fischer 344/N Rats//First Published August 28, 2015 Research Article Find in PubMed <https://doi.org/10.1177/0300985815603432>.
- [14] You JS, Jones PA. Cancer genetics and epigenetics: two sides of the same coin? *Cancer Cell*. 2012;22:9–20
- [15] Yang FL, Hong K, Zhao GJ, et al. Construction of prognostic model and identification of prognostic biomarkers based on the expression of long non-coding RNA in bladder cancer via bioinformatics *Beijing Da Xue Xue Bao Yi Xue Ban*. 2019; 51(4):615-622.
- [16] Angeline S. Andrew, Margaret R. Karagas, Florian R. Schroeck, Carmen J. Marsit, Alan R. Schned, Jason R. Pettus, David A. Armstrong and John D. Seigne MicroRNA Dysregulation and Non-Muscle–Invasive Bladder Cancer Prognosis// *Cancer Epidemiol Biomarkers Prev* April 3 2019 28 (4) 782-788; DOI:10.1158/1055-9965.EPI-18-0884
- [17] Shingo Maeda, Kohei Murakami, Akiko Inoue, Tomohiro Yonezawa and Naoaki Matsuki. CCR4 Blockade Depletes Regulatory T Cells and Prolongs Survival in a Canine Model of Bladder Cancer // *Cancer Immunol Res* July 1 2019 7 (7) 1175-1187; DOI:10.1158/2326-6066.CIR-18-0751
- [18] Zhu Z, Shen Z, Xu C. Inflammatory pathways as promising targets to increase chemotherapy response in bladder cancer. *Mediators Inflamm*. 2012;2012:1–11
- [19] Darryl T. Martin, Hongliang Shen, Jill M. Steinbach-Rankins, Xi Zhu, Katelyn K. Johnson, Jamil Syed, W. Mark Saltzman and Robert M. Glycoprotein-130 Expression Is Associated with Aggressive Bladder Cancer and Is a Potential Therapeutic Target // *Weiss Mol Cancer Ther* February 4 2019 18 (2) 413-420; DOI:10.1158/1535-7163.MCT-17-1079
- [20] Van der Horst G, Bos L, van der Pluijm G. Epithelial plasticity, cancer stem cells, and the tumor-supportive stroma in bladder carcinoma. *Mol. cancer Res*. 2012;10(8):995–1009
- [21] Mantovani A, Locati M. Tumor-associated macrophages as a paradigm of macrophage plasticity, diversity, and polarization: lessons and open questions. *Arterioscler. Thromb. Vasc. Biol*. 2013;33(7):1478–1483

- [22] Chen M-F, Lin P-Y, Wu C-F, Chen W-C, Wu C-T. IL-6 expression regulates tumorigenicity and correlates with prognosis in bladder cancer. *PLoS One*. 2013;8(4):e61901
- [23] Mandy Berndt-Paetz, Philipp Schulze, Philipp C. Stenglein, Annett Weimann, Reduction of Muscle-Invasive Tumors by Photodynamic Therapy with Tetrahydroporphyrin-Tetratosylat in an Orthotopic Rat Bladder Cancer Model *Mol// Cancer Ther* April 1 2019 18 (4) 743-750; DOI:10.1158/1535-7163.MCT-18-1194
- [24] Beatty JD, Islam S, North ME, Knight SC, Ogden CW. Urine dendritic cells: a noninvasive probe for immune activity in bladder cancer? *BJU Int*. 2004;94(9):1377–1383
- [25] Zhu Z, Shen Z, Xu C. Inflammatory pathways as promising targets to increase chemotherapy response in blade cancer. *Mediators Inflamm*. 2012;2012:1–11
- [26] Chen T, Wang H, Zhang Z, et al. A novel cellular senescence gene, SENEX, is involved in peripheral regulatory T cells accumulation in aged urinary bladder cancer. *PLoS One*. 2014;9(2):e87774.
- [27] Furukawa J, Hara I, Nagai H, Yao A, Oniki S, Fujisawa M. Interleukin-21 gene transfection into mouse bladder cancer cells results in tumor rejection through the cytotoxic T lymphocyte response. *J. Urol*. 2006; 176(3): 1198-1203
- [28] Kai-Jian Wang, Chao Wang, Li-He Dai, Jun Yang, Hai Huang, Xiao-Jing Ma, Zhe Zhou, Ze-Yu Yang, Wei-Dong Xu, Mei-Mian Hua, Xin Lu, Shu-Xiong Zeng, Hui-Qing Wang, Zhen-Sheng Zhang, Yan-Qiong Cheng, Dan Liu, Qin-Qin Tian, Ying-Hao Sun and Chuan-Liang Xu Targeting an Autocrine Regulatory Loop in Cancer Stem-like Cells Impairs the Progression and Chemotherapy Resistance of Bladder Cancer// *Clin Cancer Res* February 1 2019 25 (3) 1070-1086; DOI:10.1158/1078-0432.CCR-18-0586.
- [29] Amirali Salmasi, David A. Elashoff, Rong Guo, Alexander Upfill-Brown, Charles J. Rosser, Jason M. Rose, Louise C. Giffin, Louis E. Gonzalez and Karim Chamie Urinary Cytokine Profile to Predict Response to Intravesical BCG with or without HS-410 Therapy in Patients with Non–muscle-invasive Bladder Cancer// *Cancer Epidemiol Biomarkers Prev* June 3 2019 28 (6) 1036-1044; DOI:10.1158/1055-9965.EPI-18-0893