

## NOVEL NON INVASIVE DIAGNOSTIC STRATEGIES IN BLADDER CANCER

ANAMARIA TRUTA<sup>1,2,3</sup>, TUDOR ADRIAN HODOR POPON<sup>4</sup>,  
GEORGE SARACI<sup>5</sup>, LIVIU GHERVAN<sup>6</sup>, IOAN VICTOR POP<sup>1</sup>

<sup>1</sup>Medical Genetics Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

<sup>2</sup>Research Center of Functional Genomics Biomedicine & Translational Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

<sup>3</sup>I. Chiricuta Oncology Institute, Cluj-Napoca, Romania

<sup>4</sup>Department of Urology - Robotic Surgery, Clinical Municipal Hospital Cluj-Napoca, Romania

<sup>5</sup>Internal Medicine Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

<sup>6</sup>Urology Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania, Clinical Institute of Urology and Kidney Transplant Cluj-Napoca, Romania

### Abstract

*Bladder cancer is one of the most commonly diagnosed malignancies worldwide, derived from the urothelium of the urinary bladder and defined by long asymptomatic and atypical clinical picture. Its complex etiopathogenesis is dependent on numerous risk factors that can be divided into three distinct categories: genetic and molecular abnormalities, chemical or environmental exposure and previous genitourinary disorders and family history of different malignancies. Various genetic polymorphisms and microRNA might represent useful diagnostic or prognostic biomarkers. Genetic and molecular abnormalities - risk factors are represented by miRNA or genetic polymorphisms proved to be part of bladder carcinogenesis such as: genetic mutations of oncogenes TP53, Ras, Rb1 or p21 oncoproteins, cyclin D or genetic polymorphisms of XPD, ERCC1, CYP1B1, NQO1C609T, MDM2SNP309, CHEK2, ERCC6, NRF2, NQO1Pro187Ser polymorphism and microRNA (miR-143, -145, -222, -210, -10b, 576-3p). The aim of our article is to highlight the most recent acquisitions via molecular biomarkers (miRNAs and genetic polymorphisms) involved in bladder cancer in order to provide early diagnosis, precise therapy according to the molecular profile of bladder tumors, as well as to improve clinical outcome, survival rates and life quality of oncological patients. These molecular biomarkers play a key role in bladder carcinogenesis, clinical evolution, prognosis and therapeutic response and explain the molecular mechanisms involved in bladder carcinogenesis; they can also be selected as therapeutic targets in developing novel therapeutic strategies in bladder malignancies. Moreover, the purpose in defining these molecular non invasive biomarkers is also to develop non invasive screening programs in bladder malignancies with the result of decreasing bladder cancer incidence in risk population.*

**Keywords:** bladder cancer, molecular diagnostic biomarkers, miRNA, genetic polymorphism, early diagnosis

## Introduction

Bladder cancer has a complex etiopathogenesis dependent on various factors: chemical carcinogens (smoking, professional exposure: industry carcinogens), diet (artificial sweeteners, coffee consumption and meat consumption, total fluid intake), previous treatments (pelvic radiation, drug abuse, chronic treatments with analgesics and anti-inflammatory drugs, hormone therapy) or genetic factors (genetic polymorphisms, microRNAs) [1,2]. These risk factors are involved in bladder cancer etiopathogenesis, clinical evolution, prognosis, response to specific therapy or survival rates [3]. Dietary factors are mentioned also as part of bladder carcinogenesis (meat consumption, total fluid intake, vegetables, artificial sweeteners) and various disorders in the patient's medical history (chronic infections and inflammations of urinary bladder, Schistosomiasis, HPV, chronic lithiasis, long term catheterization) [4,5,6,]. We can also mention as important etiological risk factors: the patient's clinical and family history - previous disorders (chronic inflammations and fibrosis, uro-genital malignancies, congenital abnormalities of urinary tract) [7] and various malignancies [8]. The genetic component is very important in bladder carcinogenesis (CCND1, CHEK2, CYP1B1, XPC, ERCC2 and ERCC5, MDM2SNP309 genetic variants, NRF2 and NRF2 target genes, p53 and Rab oncogene) [9,10,11]. It will be important to identify and define various miRNA and genetic polymorphisms involved in bladder carcinogenesis and use these genetic variants as diagnostic or prognostic biomarkers or as useful non invasive parameters in patients surveillance or screening programs, but also as important parameters in the improvement of the EORTC scale (European Organization for Research and Treatment of Cancer) in predicting progression and recurrences in bladder cancer [12,13].

The aim of recent research studies in bladder cancer pathology is to identify novel diagnostic and prognostic biomarkers in bladder cancer using medical genetics and functional genomics technology and provide early diagnosis, precise therapy and improve clinical outcome, life quality and survival rates of patients with bladder cancer [14].

## Novel genetic biomarkers

Bladder cancer is a highly heterogenous malignancy derived from the urothelium of the urinary bladder with profound genetic valences (genetic polymorphisms, miRNA) that could characterize etiopathogenesis, evolution, prognosis, response to specific therapy or survival rates.

Much interest has been given to urine tests in developing non invasive diagnosis biomarkers for bladder cancer [8]. The usual diagnostic tools, such as cystoscopy, require experience, while histopathological examination represents the golden standard nowadays in bladder cancer

positive diagnosis. Urine cytology has a high specificity but lack of sensitivity in low-grade urothelial carcinomas. Studying non invasive molecular biomarkers (miRNA or genetic mutations) in bladder carcinogenesis and harvesting biomarkers from the blood of bladder cancer patients and compare with their tumors and normal tissue profiling could lead to a relatively non-invasive, cost-effective test with equivalent or improved sensitivity and specificity [12,13]. Identifying miRNA and genetic polymorphisms involved in bladder cancer etiopathogenesis using functional genomic technologies, recent research studies aim at: developing new diagnostic and prognostic strategies based on non invasive tumoral biomarkers, novel therapeutic/chemoprevention strategies, improvement of the clinical evolution of these patients and also improved EORTC scale for bladder cancer Genetic polymorphisms. Intra-familial clusters have been reported in bladder cancer and recent studies documented the influence of genetic polymorphisms in bladder carcinogenesis. This hypothesis opens new research ways in finding novel diagnostic biomarker in bladder cancer, but also in predicting bladder cancer evolution, prognosis and develop novel therapeutic strategies according to molecular profile. Family history of bladder cancer is a well-known risk factor in bladder cancer etiopathogenesis and susceptibility, new data emerging under the influence of family history tumors rather than bladder cancer. Recent research studies identified tumors in whose case family history influenced survival rates, prognosis and clinical evolution. Many genetic polymorphisms are identified in bladder cancer etiopathogenesis which influence tumor susceptibility, prognosis or therapy response [13,14].

Studying *genetic polymorphisms* involved in bladder cancer has proved that NQO1Pro187Ser is an important part of bladder carcinogenesis involved in the etiopathogenesis of urinary system malignancies: bladder cancer, prostate cancer or renal cell carcinomas [15,16], but also a risk factor for other malignancies like: breast cancer, colorectal cancer or esophageal carcinoma [17,18,19].

Relevant clinical research studies have shown that NQO1C609T is involved in bladder cancer and after clinical validation, this genetic polymorphism might represent an important diagnostic or prognostic biomarker in clinical oncology [19,20,21,22].

MDM2SNP309 promotes various genetic mutations of p53 involved in bladder carcinogenesis and is correlated with poor prognosis and fast evolution for T1 stage bladder malignancies. MDM2SNP309 also represents an important risk factor in developing bladder cancer and might be seriously considered in the oncological evaluation. It may help to develop precise therapy in bladder malignancies according to the molecular profile of the tumour [22,23].

P53Arg72Pro genetic polymorphism might be considered an important prognostic tool for invasive tumors versus superficial types of bladder cancer and could

represent a useful parameter in bladder cancer surveillance or EORTC scale of prediction progression and recurrences of bladder cancer [24,25].

P53 is well-known as a suppressor oncogene responsible for apoptotic processes, cell senescence, proliferation and control [26]. Its genetic mutation is frequently involved in various carcinogenetic processes including bladder malignancies [27].

*miRNA*. MiRNAs are non coding small molecules made of 19-22 nucleotides involved in gene regulation and part of various malignancies etiopathogenesis. miRNAs might correlate malignancies with clinical evolution, prognosis or survival rates [28].

Studying bladder cancer etiopathogenesis has proven that miRNA-222 is correlated with the presence of in situ carcinoma, progression rate, fast evolution and poor survival rate [29].

miRNA-143 is also prognostic biomarker correlated with survival rates in bladder malignancies [30].

MiRNA-145 is involved in bladder cancer and might represent an important diagnostic biomarker in low grade, non muscle invasive bladder cancers [30]. MiRNA-21 is up-regulated in high grade bladder cancer and might represent a trustable diagnosis biomarker which can differentiate high grade bladder cancer from low grade [30,31,32,33].

MiRNA-137 might represent an important prognostic biomarker in bladder malignancies, which is correlated with cell proliferation, progression, tumor invasion and metastasis. Like

miRNA-137, miRNA-10b is a biomarker associated with tumor progression, metastatic processes but also important target in defining precise therapy in bladder cancers [34]. MiRNA-29c, 10b and miRNA-210 might also represent diagnostic biomarkers in bladder malignancies

and after clinical validation might provide early diagnosis in bladder cancer, improve clinical outcome and survival rates of these patients [34,35,36,37]. Being aware of the diagnostic difficulties in bladder cancer due to its long atypical asymptomatic clinical evolution (common genitourinary symptoms: painless hematuria, dysuria, urgency, frequency) and limited values of medical imaging techniques (cystoscopy, CT urography, ultrasonography) especially in superficial bladder malignancies, research studies try to define novel diagnostic biomarkers which might provide early diagnosis, precise therapy in bladder cancer, improve clinical outcome, quality of quality and survival rates [38,39,40,41,42,43].

Nowadays bladder cancer diagnosis is based on: cystoscopic examination mainly, urine cytology and histological examination [43]. Except for medical imaging tests and clinical examination, we mention complementary tests used in the positive diagnostic of bladder malignancies: UroVysion test (FISH), ImmunoCyt, BTA ( bladder tumor antigen) and NMP22 [43,44,45,46].

*BTA (bladder tumor antigen)* is a qualitative, non invasive, easy to perform diagnostic test in bladder cancer which measures complement factor H related protein and might be used along with urine cytology, cystoscopic and histopathological examination in bladder cancer diagnostic evaluation [46,47,48,49].

*NMP22 (nuclear matrix protein 22)* is an important biomarker and together with UroVysion test (fluorescence in situ hybridization) might represent useful non invasive surveillance instrument in bladder malignancies [50,51].

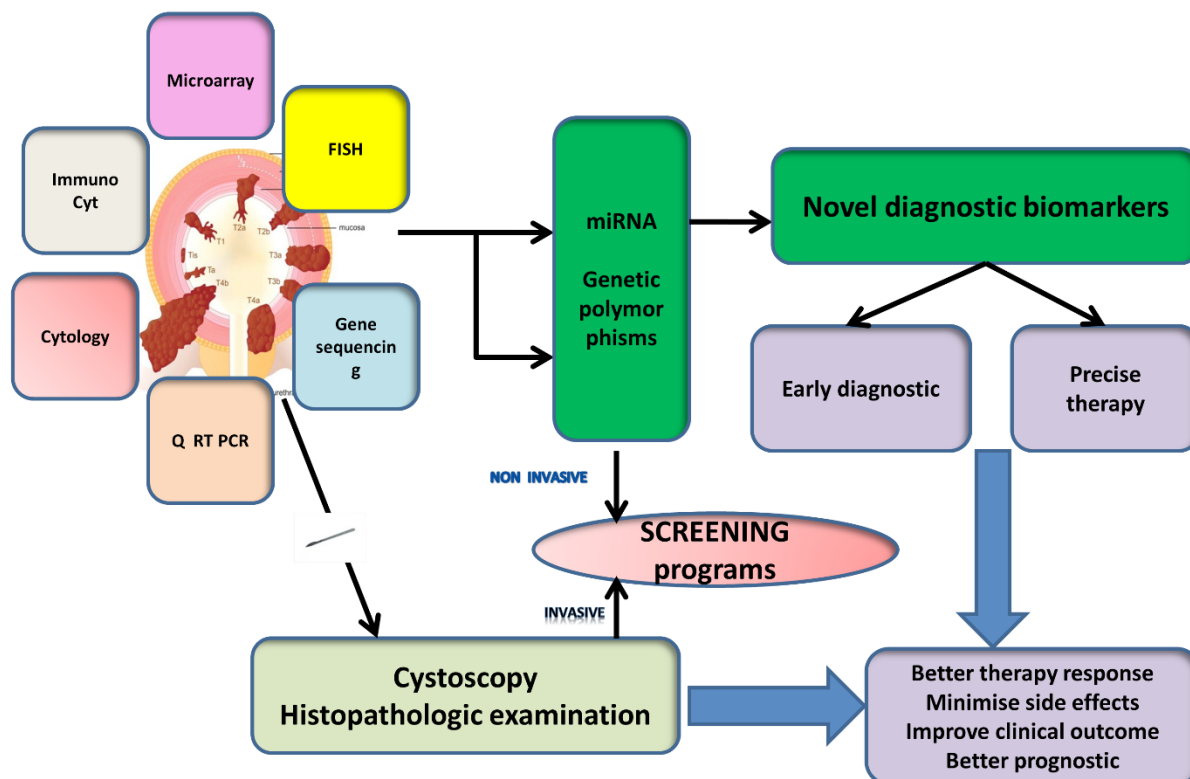
*ImmunoCyt* is another non invasive diagnostic tools in bladder cancer which combines immuno- fluorescence technique with urine cytology and its characterized by a high rate of false positive results induced by genitourinary benign disorders [52,53,54].

**Table I.** Diagnostic value of miRNAs in bladder malignancies [30,31,32,33].

MiRNA	Diagnostic value in bladder cancer	Specifications
MiRNA-145	+	Diagnostic biomarker in muscle invasive bladder cancers
MiRNA-21	+	Diagnostic biomarker in high grade bladder cancers

**Table II.** Prognostic value of miRNAs in bladder malignancies [34,35,36,37].

miRNA	Prognostic value in bladder cancer	Specifications
MiRNA-137	++	Poor prognostic biomarker for metastatic processes and fast clinical evolution
MiRNA-143	++	Poor prognostic biomarker
MiRNA-222	++	Poor prognostic biomarker
MiRNA-10b	++	Poor prognostic biomarkers for invasion and metastatic processes
MiRNA-29c	+	Prognostic biomarker Suppresses cell growth in bladder cancer



**Figure 1.** Bladder cancer diagnostic techniques-personal synthetic approach.

## Conclusions

Bladder cancer is a real health problem worldwide because of its incidence, prevalence, high recurrence rate, with a long silent clinical evolution, which is diagnosed using medical imaging techniques and histopathological approach. A wide range of non invasive genetic biomarkers have been evaluated that can provide early diagnosis and, above all, may estimate and characterize bladder malignancies evolution, prognosis, survival rate, response to therapy, and can be also included as useful parameters in non invasive screening programs. Blood profiling for bladder cancers pathology might represent an interesting non-invasive test, showing accurate information about the tumor grade, therapy response and patients prognosis. Using-these molecular biomarkers in clinical practice might provide early diagnosis in bladder malignancies, precise therapy and improve clinical outcome patients.

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