Human & Veterinary Medicine
International Journal of the Bioflux Society HVM

OPEN ACCESS
Review

Etiopathogenesis of bladder cancer. Risk factors, genetic aspects and novel diagnosis biomarkers

[1,2,3]Anamaria Truţa, [4]George Saraci, [5]Tudor-Adrian Hodor-Popon, [6]Liviu Ghervan, [1]Ioan V. Pop

[1] Department of Medical Genetics, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [2] Research Center for Functional Genomics Biomedicine and Translational Medicine, "Iuliu Hatieganu " University of Medicine Pharmacy, Cluj -Napoca, Romania; [3] Oncology Institute, Cluj-Napoca, Romania; [4] Department of Internal Medicine "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; [5] Department of Urology-Robotic Surgery, Clinical Municipal Hospital, Cluj-Napoca, Romania; [6] Clinical Institute of Urology and KidneyTtransplant, "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca.

Abstract. Bladder cancer is a complex pathologic process with multifaceted etiology and etiopatogenesis dependent of various factors: chemical carcinogens (smoking, professional exposure, artificial sweeteners, pelvic radiation, diet, and industrial carcinogens), drug abuse, chronic treatments with analgetics and ciclofosfamide, menopausal hormone therapy). Genetic factors (genetic polymorphisms, miRNA) are involved in bladder cancer etiopathogenesis, evolution, prognosis, response to specific therapy or survival rates. Dietary factors are mentioned as part of bladder carcinogenesis (meat consumption, total fluids intake, vegetables, artificial sweeteners) as well as various disorders in patient's medical history (chronic infection and inflammation of urinary bladder, schistosomiasis, chronic lithiasis, catheterism, HPV infection, pelvic radiation). Family history of cancers (bladder and kidney cancers, cervix cancers) along with bladder chronic inflammations and bladder dysplasia are among risk factors. Genetic component is very important in bladder carcinogenesis (XPC, ERCC2 and ERCC5 genetic variants, NRF2 and NRF2 target genes, p53 and Rab oncogene). Current paper focuses on the pathogenesis of bladder cancer stressing upon modern genetic markers used in early diagnosis, prognosis and virtual therapeutic targets.

Key Words: bladder cancer, etiopathogenesis, risk factors, genetic polymorphism, miRNA, novel diagnostic biomarkers, recent advances.

Corresponding Authors: G. Saraci, email: gsaraci@yahoo.com

Introduction

Bladder cancer is defined as a real public health problem worldwide so it is important to understand its complex etiopathogenesis and identify new biomarkers which might be used in screening programmes , early diagnosis, evaluation of progression's and recurrence's rates in order to decrease bladder cancer incidence or improve clinical outcome of these patients (Burger et al 2013). In the efforts to understand better the etiopathogenesis of bladder cancer and its mechanisms in pathological malignant processes current review underline the importance of defining new non invasive tumoral biomarkers in bladder malignancies. These new tumoral non invasive biomarkers (miRNA, genetic polymorphisms) might represent useful tools in developing new diagnostic strategies, new therapeutic options, novel techniques in monitoring bladder cancer evolution after surgical treatment or in monitoring non muscle invasive cancers, but also for screening programmes applied on risk population and significantly decrease bladder cancer incidence worldwide. Screening and prophylactic programmes which aim to diagnose diseases in early stage using clinical and genetic laboratory parameters and questionnaires applied to risk population are designed to be available worldwide after clinical validation. miRNA can be part of screening programmes after clinical validation and will offer opportunity of more efficient non invasive screening programmes easily accepted by risk population than current screening programmes based on invasive procedures (cystoscopy, biopsy). The new non invasive tumoral biomarkers might be included in new precise therapeutic strategies. The aim is to optimize therapy response, minimize side effects of oncologic therapies but also to increase life quality of oncologic patients and to reduce recurrences rates of bladder carcinoma (Ye et al 2014; Goodison et al 2013). Describing the complete etiopathogenesis in bladder cancer and its complex pathological mechanisms by altering control processes and DNA repair mechanisms and also the disorders which underlay bladder cancer development will make us understand belter this pathology and find new diagnostic strategies .All these findings can lead us to early diagnosis and real rates of positive diagnosis. Many cases of bladder cancer are not diagnosed correctly or in early stages, being often confused with chronic inflammation, fibrosis, other bladder pathologies or prostate pathology due to urinary tract contiguity and similar symptoms (polyuria, pollakiuria, hematuria, abdominal pain) (Urquidi et al 2012).

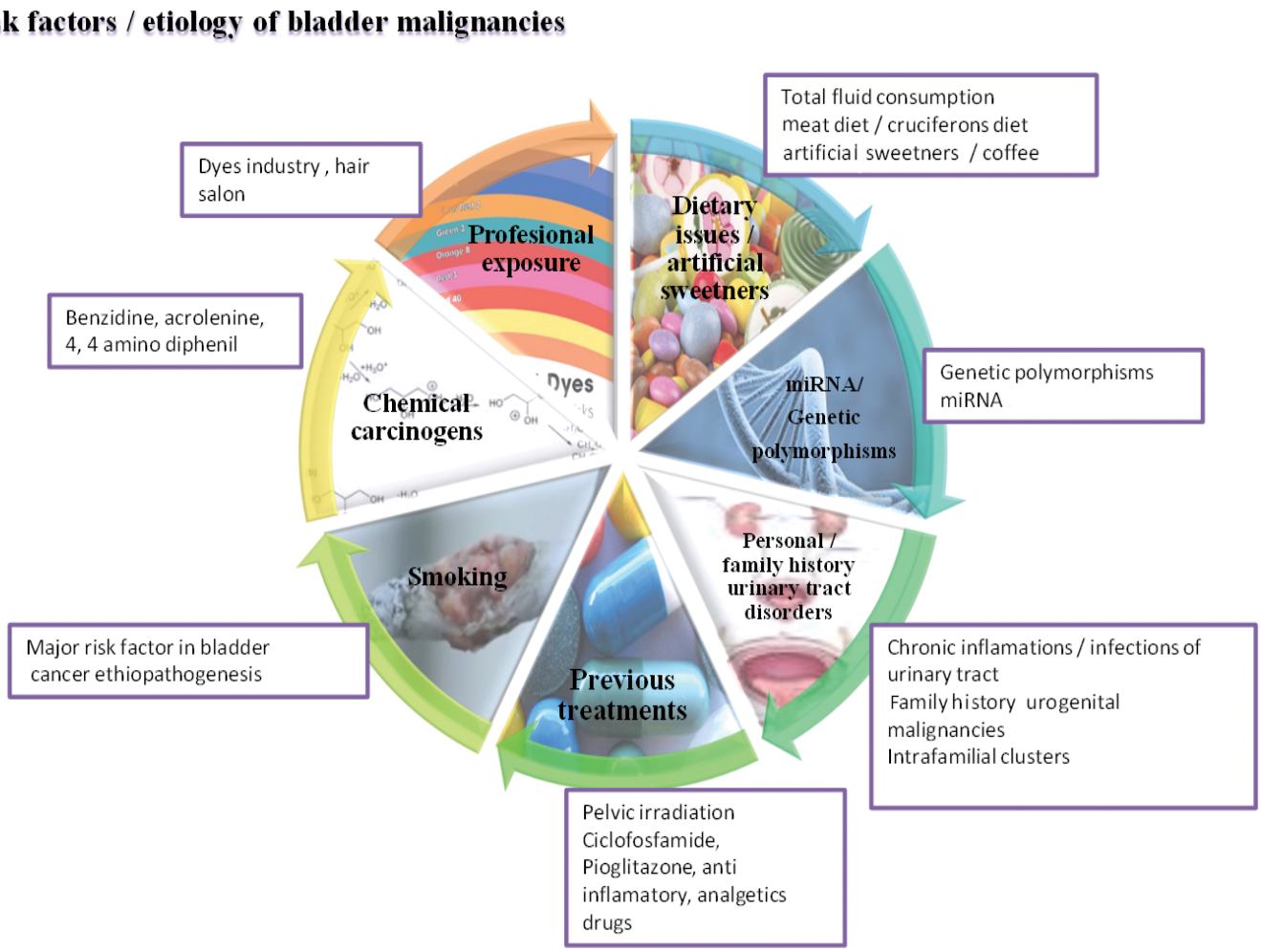

Figure 1. Risk components in bladder cancer- personal synthetic approach.

Epidemiology. Risk factors. Etiopathogenesis

Bladder cancer is the most common malignancy of the urinary tract with the incidence being four times higher in men than in women (Schned et al 2012). Caucasians have higher incidence of bladder cancer but the reason is not known yet. Development of urothelial carcinoma is also related to age with a 70 – 73 years median age of diagnosis (Walker et al 2014). North America leads in incidence while lower incidence is reported in Japan and Finland. It will be interesting to prove if geographic distribution is dependent on environmental risk factors, diet, occupational exposure or genetic differences (Working Groups 2014).

Table1. Bladder cancer incidence (Walker et al 2014)

Bladder cancer incidence	Men ≥ 85 years old	Women ≥85 years old	Women ≤85 years old
	142 / 100.000	74/100.000	33/ 100.000

Table 2. Bladder cancer incidence and mortality in US 2015 (www.cancer.org)

Bladder cancer US statistics in 2015	Number of cases	Men	Women
incidence	74	56.32	17.68
mortality	16	11.5	4490

Intrafamilial clusters (perinatal mother-to-infant or early horizontal transmission) have been reported in bladder cancer pathology and recent studies suggested the role of genetic polymorphisms in bladder carcinogenesis. This hypothesis opens new research pathways in finding new diagnostic biomarkers in bladder cancer but also in predicting bladder cancer evolution, recurrence, prognosis and define new therapeutic solutions according to molecular profile of bladder tumors (Hemminki et al 2011). Family and personal clinical history of bladder cancer is a well known risk factor in bladder cancer etiopathogenesis and susceptibility. New data emerged on the influence of family history tumours rather than bladder cancer. Family history of bladder tunours influenced survival rates, prognosis and evolution (Hemminki et al 2011; Blaveri et al 2005).

Many genetic polymorphisms are identified in bladder cancer etiopathogenesis which influence bladder tumors susceptibility, prognosis or therapy response in oncologic patients (Reszka et al 2014).

Infectious and inflammatory disorders. Neutrophils, macrophages, lymphocytes promote tumor development and rapid progression of carcinogenetic process. Neutrophils are responsible of secretion of neutrophil peptides 1-3 which promotes carcinogenesis. Primary response of macrophages is induced by pro inflamatory cytokines: IL 6 and TNFα (Thompson et al 2014). All these mechanisms explain part of bladder carcinogenesis, correlation wth chronic inflammatory disorders and treatment or

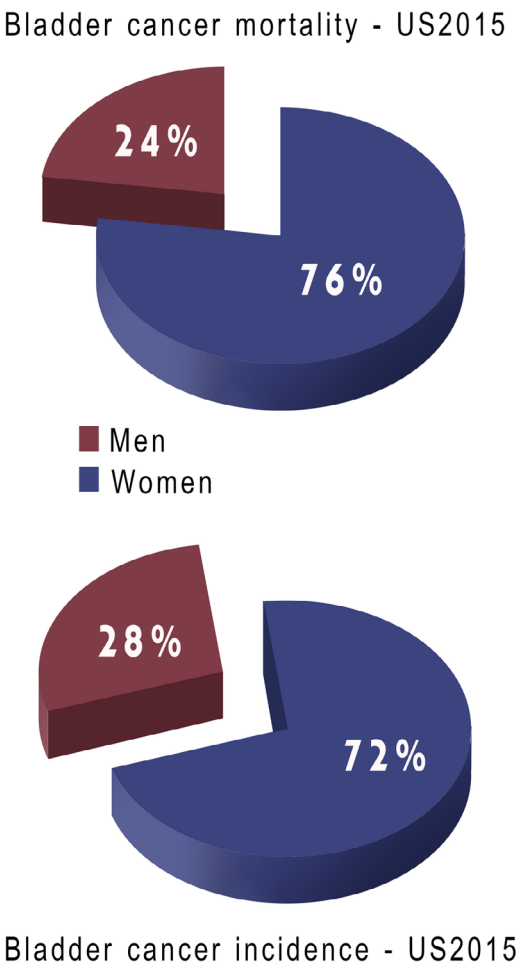

Figure 2. Bladder cancer incidence and mortality in US – 2015 (www.cancer.org)

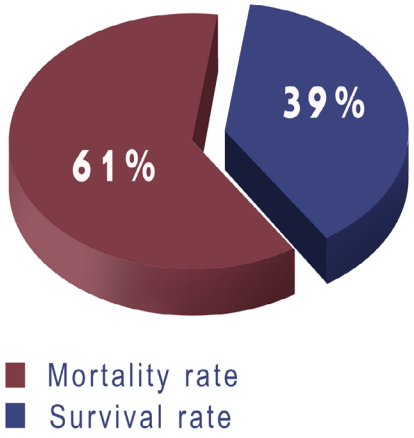

Figure 3. Worldwide mortality in bladder cancer (Walker et al 2014)

cytokine-modified tumor effects (Desai et al 2014). Cytokines levels are correlated with incidence, recurrences, therapy response, evolution, some genetic polymorphisms, survival rates and response at BCG therapy. Lymphotoxin β receptor is up-regulated in bladder cancer via activation of Nuclear Factor - Kb, then NF – k B can explain part of pathogenessis. NF – kB explains correlation between those two bladder pathologies. Levels of proteins are correlated with histologic grade, clinical stage, lymph node metastasis. Consequently they are possible candidates in non invasive tumoral biomarker diagnosis of bladder cancers (Thompson et al 2014; Shen et al 2014; Luo et al 1999; Kamat et al 2007).

There are new proven correlation between urogenital schistosomiasis and bladder carcinogenesis. There is no molecular mechanism described which links schistosomiasis to bladder carcinogenesis. New experimental studies on animal models must prove molecular mechanisms induced by Schistosoma hematobium in bladder carcinogenesis (Fu et al 2012). The parasite induces inflammation, fibrosis, dysplasia and increases transcription of collagen genes COL 17A1, but also metalloproteinases 9, 10 and 13. TIMP 1 (tissue inhibitor of metalloproteinase) is also part of fibrotic and displastic process in bladder pathology. Other genes are inhibited by Schistosoma infection: COL 6 A3, COL8A2, COL4A5, COL3A1 (Honeycutt et al 2014; Botelho et al 2011).

New clinical researches revealed the correlation between bladder cancer and diabetes mellitus, actually between diabetic patients under pioglitazone therapy and bladder cancer. ϒ -peroxisome-proliferator receptor (PPARγ) is detected in normal urothelium but over expressed in bladder cancers and represent part of differentiation and tumor proliferation. This new findings reveal importance of developing screening tests in type 2 diabetes patients treated with Pioglitazone as part of bladder cancer prevention (Mylona et al 2009). Neurogenic bladder, urinary lithiasis, bladder anomalies (bladder diverticula), along with invasion of colorectal carcinoma are cited among risk factors (Chung et al 2013). It will be important to reveal pioglitazone´s side effects and metabolic pathways in order to avoid next generation treatments with similar carcinogenesis risks.

In bladder cancer smoking is correlated with number of cigarettes, duration of smoking, intensity, type of administration. It was proven to be main part of bladder carcinogenetic process and it was also correlated with poor prognosis, high recurrence rates, poor response to therapy and low survival rates. Smoking cessation improves quality of life in oncologic patients while smoking was correlated with bladder cancer recurrences (Sosnowski et al 2014).

Chemical carcinogenesis. It was proven that MBT (mercapto benzothiazole), PBN (phenyl - ɑ naphtylamine), ortho-toluidine, acroleine or benzidine are part of bladder carcinogenesis (Sorahan et al 2008). Exposure to arsenic in drinking water, aromatic amines , 4 ‘4 ‘methylenbis 2- chloroaniline are involved in bladder cancer etiopathogenesis as well as chemical carcinogens derived from smoking like benzidine, nitrosamine or acroleine. Exposure to hair dyes is also part of bladder carcinogenesis, benzidine proved to be an important risk factor (Xiang et al 2007). Expression of mutant p53 protein after benzidine exposure was studied by immuno PCR assay in order to prove its carcinogenetic role in bladder malignancies. Ifosfamide caused often hemorrhagic recurrent cystitis through its metabolite acroleine and this mechanism could explain bladder carcinogenesis via preneoplastic disorders induced mainly by COX 2 inhibition (Letasiova et al 2012; Macedo et al 2011).

Diet. It seems that higher fluid intake per day might reduce bladder cancer incidence but proofs are inconsistent and need more trials (Bai et al 2014). Alcohol consumption and bladder malignancies correlation are still inconsistent and needs molecular mechanisms to explain pre neoplastic bladder disorders (Kim et

al 2014). There was a positive correlation between coffee consumption and bladder cancer in studies adjusted for sex , age and smoking status. Bladder cancer risk is 80% increased in coffee drinkers of more than 10 cups per day versus non coffee drinkers (Sala et al 2000). Green tea consumption was not associated with bladder carcinogenesis and had anti-inflammatory effect on urothelium (Henriques et al 2014). Meat consumption is associated with increased risk of various human malignancies, including bladder cancer while fruits and vegetables, especially cruciferous might have protective effects. Soy is rich in isoflavones like genistein and daidzein and is correlated with lower incidence of bladder cancers. Vitamins A and E and selenium have protective effects in bladder cancer as well as multivitamins combinations (Wennersten et al 2014).

Genetic polymorphism and diagnostic biomarkers

CHEK2 -is a suppressor gene localized on chromosome 2 which encodes protein CHEK2 involved in DNA repair processes functioning as a suppressor of carcinogenesis in bladder cancer. Main genetic polymorphisms of CHEK2 gene involved in bladder cancer are: IVS 2, IG≥A, 1100 del C, del 5395, I 157 T(Liu et al 2014). CYP1B1- is localized on chromosome p22-21 and encodes a protein made of 543 aminoacids which controls estradiol and xenobiotics metabolic pathways . Banaszkiewiczet al (2013) described Arg4Gli (m1), Ala119Ser (m2- in SRS 1), Leu432Val (m3), Asn453Ser (m4) as main genetic polymorphisms involved in bladder carcinogenesis (Jiang et al 2014; Scheffer et al 2014). HPV infection is also mentioned as part of etiopathogenesis in various malignant tumors. It contains 2 oncoproteins: E6 and E7 which create complex interactions with p53 protein and its tumoral suppression function (Banaszkiewicz et al 2013). HPV carcinogenetic pathways are related with E6 and E7 oncoprotein which explain various malignant conditions induced by HPV infection. E7 is an oncoprotein which alter mainly cell growth physiological process and often induce benign transformation of infected tissues. Rb protein binding may promote carcinogenesis and explain molecular mechanisms which underlay various carcinogenetic mechanisms induced by HPV infection. E6 oncoprotein is interacting with p53 gene and is altering suppressor carcinogenetic physiological mechanisms and may induce various malignancies in infected organs, including bladder cancer (Liu et al 2014; Banaszkiewicz et al 2013). Same oncogenic proteins E6, E7 are involved in the etiopathogenesis of multiple malignancies like cervix cancer, oral cancer or bladder tumoral pathology (Whiteside et al 2008). It represents also a good therapeutic target in malignancies where HPV is part of etiopathogenesis (Li et al 2015). Using E6, E7 as therapeutic targets might lead to new therapeutic and prophylactic options in these malignancies where HPV is implicated (Li et al 2010). They could explain differences in therapeutic response and may represent a new option in oncologic therapies and vaccines (Zhou et al 2004). It was proven that HPV positive cancers had better response to therapy comparing with HPV negative malignancies (Ganguly et al 2003). This hypothesis might be considered also in bladder cancer therapeutic option (Ramqvist et al 2011; Dalianis et al 2014).

Increased serum levels of 14ARF will alter MDM protein function. This will lead to increased levels of p53 protein which frequently create complex interaction with E6 oncoprotein and will generate uncontrolled proliferation and alter apoptosis (Ha et al 2010). P53 mutations produced by direct or indirect mechanisms as result of interactions with chemical carcinogens derived from smoking process are also mentioned as important carcinogenetic pathway in bladder cancer (Goodison et al 2013). Altering genomic integrity as result of carcinogens exposure seems to be the main pathway of etiopathogenesis of bladder cancer (Shen et al 2012).

Genetic polymorphism XPD 751Glu is also part of etiology in bladder cancer while XRCC1-399 was not proven as part of etiologic processes in this malignant tumor (Gao et al 2010). Recent genetic research studies proved that IL – 1RN and IL – 4 gene polymorphisms are also important genetic markers of susceptibility to bladder cancer. Studies have the role to define precisely involvement of these genetic biomarkers in etiopathogenesis, diagnosis, evolution, recurrences and use these tumoral non -invasive genetic biomarkers in positive diagnosis of cancer. This will allow us to get an early diagnosis, improve life quality and survival rates, decrease number of recurrences and define precise therapy in oncology (Zhang et al 2014). Recent meta-analysis studied relation between MMP (matrix metalloproteinase) and bladder cancer. Results are still contradictory and will open new perspectives in proving complex etiopathogenesis in bladder cancer: genetic, environment factors, dyes, industrial and chemical carcinogenetic factors. Studies of MMP-I-1607 1G/2G , MMP -2-1306 and MMP -9-1562 C/T and their role in bladder cancer have already proven the correlation between MMP –I-1607 1G/2G genetic polymorphism and bladder cancer but only in recessive models and a significant correlation between MMP -2-1306 C/T in homozygote models of bladder cancer .There are no correlation between MMP -9-1562C /T and bladder cancer (Chu et al 2013). Case control studies aiming to complete etiopathogenesis proved that serum levels of MMP 9 and MMP 7 are higher in patients with bladder cancer than in controls, while TIMP -1 (tissue inhibitor of matrix metalloproteinase) is lower in patients diagnosed with bladder cancer (Kim et al 2013). Other recent researches tried to define correlation between Lys939Glu polymorphism of xeroderma pigmentosum group XPC and bladder cancer susceptibility. Based on large meta-analysis studies results reported Lys939Glu as important factor in bladder cancer in homozygous models (Zhang et al 2014). MDM 2 SNP 309 T > G genetic polymorphisms are also involved. New meta-analysis proved that genotype for MDM 2 309 T > G is associated with important risk of bladder cancer (Shen et al 2012). MMP 8 is surely not correlated with higher risk of bladder cancer in Caucasian population (Zhang et al 2014; Wang et al 2012; Vasudevan et al 2011).

MiRNAs are small non coding molecules with an approximately 22 nucleotides which act as post transcriptional regulators of gene expression. They are important in carcinogenesis, tumor progression, evolution, metastasis and prognosis (Xiu et al 2014). They might represent important diagnostic biomarkers in various oncologic disorders but also a prognosis biomarker in evaluating tumor progression, recurrences or metastasis. They could be useful parameters in developing new therapeutic strategies in oncology by selecting individualized therapy in order to optimize response to therapy, obtain better response, minimize side effects and above all increase life quality of oncologic patients (Zhang et al 2014; Vasudevan et al 2011; Uppal et al

2014). MIR145 is correlated with different histological types of bladder cancer, evolution, prognosis and recurrence. It was under expressed in low grade and non invasive bladder carcinoma and overexpressed in high grade urothelial bladder carcinoma (Song et al 2014). It targets socs7 oncogene and promotes interferon β, so miRNA 145 can be considered an important candidate as diagnostic biomarker in bladder carcinoma, tumour recurrence or prognosis (Dip et al 2013).

New research studies tried to identify new diagnostic biomarkers in oncology using genomics technology. The aim was obtain early diagnosis and define new therapeuthic strategies according to molecular profile of bladder tumors. which definitely will improve prognosis, clinical evolution, life quality of these patients and minimize side effects of precise therapies used nowadays.

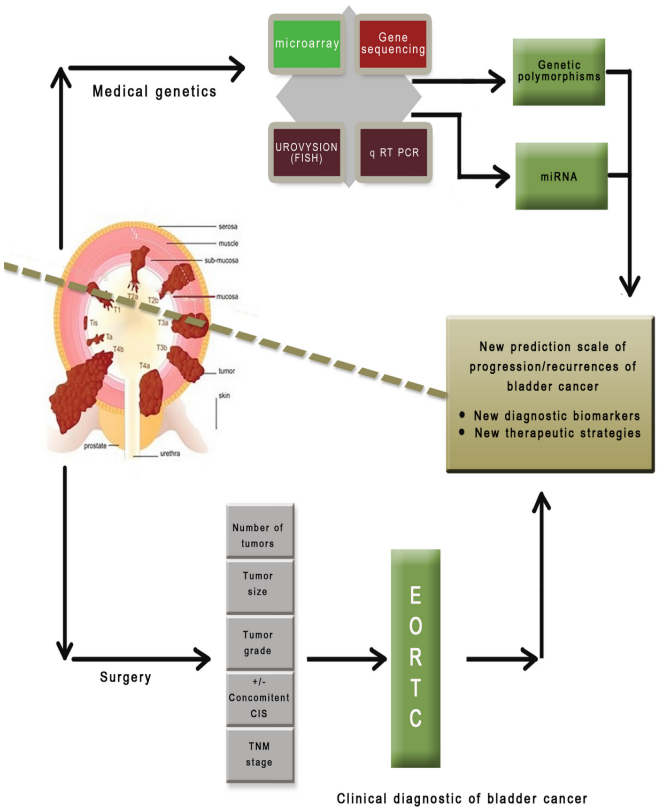

Figure 4. Diagnostic, therapeutic, staging and monitoring approach in bladder cancer- personal integrative opinion

miRNA-133 expression proved to be altered in urologic malignancies, includding bladder carcinoma. Aberrations in MIR expression appeared in different forms for muscle invasive bladder cancer and non muscle invasive type. This fact will define accurate tumoral biomarkers in bladder cancer which can predict clinical features, histologic type, evolution and also prognosis (Noguchi et al 2013; Guancial et al 2014). MiRNA -133a and miRNA-133b are located both on the same cistonic unit of chromosome 18 and are important biomarkers for various human malignancies: bladder cancer, pancreatic ductal carcinoma, hepatocellular carcinoma, esophageal carcinoma, carcinoma of the tongue. Experimental studies which used bladder cancer xenografts in mouse models confirmed that miR -133 and miR-218 effectively suppressed bladder cancer evolution (Zhao et al 2013). MIR-99a, also evaluated as potential tumoral diagnostic cancer biomarker was significantly down regulated in bladder cancer tissues. Even in low expression in bladder carcinoma it was correlated with aggressive phenotypes, rapid progression and poor prognosis albeit some studies suggested that miRNA 99a inhibits bladder cancer cell growth by arresting G1/S transition (Wu et al 2014). MiRNA200c is part of bladder carcinogenesis, correlated with disease progression and poor prognosis in T1 bladder cancer (Ichimi et al 2009). MIR-200c/141 cluster was upregulated in bladder urothelial carcinoma versus normal bladder urothelium and its expression was reduced in invasive bladder cancer versus non invasive bladder tumour. It functions as a promoter of hypermethilation mechanism which can define bladder carcinogenesis (Shan et al 2013; Feng et al 2013). It also controls dysplastic process and sensitivity to EGFR therapy. MiRNA 200c expression includes EGFI (novel receptor for EGFR) which can lead to new therapy protocols in bladder cancers and optimize therapy response using well defined etiopathogenetic pathways (Rosenberg et al 2013). MiRNA -200c is also correlated with bladder cancer invasion-metastatic process. It is part of other carcinogenetic processes in colorectal, lung, endometrial carcinoma, hepatocellular carcinoma, epithelial ovarian cancer, pancreatic carcinoma, or pre neoplastic disorders like Barrett esophagus. It can be used as a poor prognosis biomarker and predicts poor response to specific chemotherapy. MiR -200 c family, miR – 155, miR-192 and miR-209 are depressed in bladder cancer (Shan et al 2013; Wang et al 2012). MiR 200 c, 200 b, 30a, 30 e are also related to cancer progression and poor prognosis (Baffa et al 2009).

TNM bladder cancer classification evaluates tumour size and depth of invasion (T), number and localisation of lymphnodes (N) and loco-regional or distance metastasis (M); for stage 0 miRNA seems to have a high impact.

The miRNA-214 levels were significantly higher in bladder smooth muscle of invasive bladder cancer than in controls and can be used in bladder cancer initial diagnostic and recurrences (Kim et al 2013). Together with miRNA-639 can lead to early diagnosis and individualised therapy according to molecular profile (Scheffer et al 2014). Four miRNAs are significantly expressed in muscle-invasive bladder cancer versus non muscle invasive bladder cancer: miR-214, - 205, -141, - 199a -3p (Feng et al 2014; Ratert et al 2013).

MIR-210 is a typical hypoxic micro RNA expressed in hypoxic conditions in HIF-dependent mechanism. miRNA 210 is upregulated in peritoneal macrophages treated with LPS so it may have regulatory role in LPS/TLR4 signaling. MIR 210 overexpression can inhibit TLR 4 induced secretion of proinflammatory cytokines IL6 and TNF, targeting directly TNF -k -B1 (Li et al 2014; Catto et al 2011). It is a pottentially negative feed back regulator in LPS / TLR 4 signaling pathways and its role in urologic malignancies was proved. MIR- 210, - 25b, -146, -55 are controlled by inflammatory signals from cells- part of functionally innate or adaptive immune responses or oncogenic signals (Catto et al 2011; Tili et al 2013; Feng et al 2014). Studying proliferative capacity induced by MIR210 in carcinogenesis, various mechanisms are mentioned. They include production of growth factors ligands, stimulation of cell proliferation in tumors and disruption of negative feed back which attenuates proliferative signaling (Odisho et al 2013).

Table 4. MiRNA's value in bladder cancer etiopathogenesis, diagnosis, prognosis, treatment and screening (Xiu et al 2014;Jiang et al2013;Jin et al2015;Andrew et al2014;Liang et al 2014;Zeng et al 2014;Chen et al 2014;Eissa et al2015;Morais et al 2014)

miRNA	miRNA's value		Characteristics
	Diagnostic	Prognostic	Pathologic associations, etiopathogenetic mechanisms. Diagnostic/prognostic parameters for screening programs in bladder cancer.
miRNA145		+	Correlation with low grade, non invasive bladder cancers
miRNA133		+	Correlation with histopathologic type of bladder cancer
miRNA128		+	
miRNA99a		+	
miRNA200c		+	Poor prognossis of T1 , fast evolution, metastasis of bladder malignancies
miRNA200c/141	+	+	EMT, tumoral invasion
miRNA205	+	+	Corelation with survival rates together with miRNA 141
miRNA30a		+	
miRNA16		+	Inhibits progression of bladder cancer ; better prognostic
miRNA200b	+		DNA methylation inducing bladder carcinogenessis
miRNA210	+		Inflamatory disorders are associated with bladder tumoral pathology
miRNA46	+		Inflamatory disorders associated to bladder tumoral pathology
miRNA192		+	Biomarker of proliferation, progression or bladder tumoral cell apoptosis
miRNA34a		+	Indicates lower risck of bladder cancer recurrence Corelated with muscle invasive bladder cancer
miRNA 576-3p	+	+	Regulatory gene in bladder tumoral pathology Controls G1/S = induce prooliferation / inhibition of bladder carcinogenesis Target:cyclin D1 pathway
miRNA451	+	+	Controls proliferation, progression , apoptosis in bladder cancer pathology InhibitEMT process Corelated with TNM stage
miRNA 133b		+	Controls proliferation, progression , apoptosis in bladder cancer pathology Target :Bcl-w / Akt 1 pathways
miRNA 96	+		Differential diagnostic in bladder benign / malignant pathology
miRNA100	+	+	Controls proliferation,progression, apoptosis in bladder cancer pathology, chromosomal stability.Target:HAP2,BAZ2A,FGRF3 gene in bladder carcinogenesis
miRNA 137		+	Progression , proliferation , invasive bladder cancer Target :PAQR3gene

Table 5. Stage 0 bladder cancer, a prominent application for miRNA as diagnostic tool (AJCC 2010)

TNM			Stage
T	**N**	**M**	**0**
T0	**N0**	**M0**	**0 a / 0 is**
Non invasive bladder carcinoma / in situ associated bladder carcinoma	No lymph node metastasis	No distant metastasis	

Conclusions

Although risk factors in bladder cancer are about the same as they were 20 years ago, the mechanism and their interactions reveal new pathways along with genetic aspects, making this clinical entity a multifaceted issue. Current prognostic factors of bladder cancer progression and recurrence rates are represented by: tumor grade, stage, size, number of bladder tumors, multifocality, associated in situ carcinoma. All those parameters used in EORTC bladder cancer scale cannot reflect clinical outcome of oncologic patients or atypical clinical picture in bladder cancer pathology. Research studies try to identify

new prognostic parameters which can explain etiopathogenesis but also predict prognosis and represent useful tools in bladder cancer individualized therapy. Already proven with important role in bladder carcinogenesis are miRNA-20a,-106b,-141,205, -200a, along with down regulated in bladder cancer pathology: miRNA-130 a, 139-5p, 145, -199a. Nowadays bladder cancer is diagnosed by cystoscopic examination with cytologic and histologic tests. It is easy to understand that we need new diagnostic tools like tumoral non invasive biomarkers from blood or urine like miRNA. They can lead to early diagnosis, individualized therapy according to molecular profile, improved therapy response, minimized side effects of therapy, decreased incidence and recurrence rates. It is important to design new screening programmes where miRNA will play a key role.

Acknowledgments

Dr Truta Anamaria acknowledges financial suport from an POSDRU grant no. 159/1.5/S/138776 with title:"Model colaborativ institutional pentru translatarea cercetarii biomedicale in practica clinica –TRANSCENT " .
The authors thank Tudor Fat for technical assistance in graphic design.

References

Andrew AS, Marsit CJ, Schned AR, et al. Expression of tumor suppressive microRNA-34a is associated with a reduced risk of bladder cancer recurrence. Int J Cancer 2014;doi:10.1002/ijc. 29413.

Baffa R, Fassan M, Volinia S, et al. MicroRNA expression profiling of human metastatic cancers identifies cancer gene targets. J Pathol 2009;219(2):214-21.

Bai Y, Yuan H, Li J, Tang Y, Pu C. Relationship between bladder cancer and total fluid intake: a meta analysis of epidemiological evidence. World J Surg Oncol 2014;12:223.

Banaszkiewicz M, Constantinou M, Pietrusiński M, et al. Concomitance of oncogenic HPV types, CHEK2 gene mutations, and CYP1B1 genepolymorphism as an increased risk factor for malignancy. Cent European J Urol 2013;66(1):23-9.

Bénédicte Clin, "RecoCancerProf" Working Group, and Jean-Claude PaironMedical follow-up for workers exposed to bladder carcinogens: the French evidence-based and pragmatic statement. BMC Public Health 2014;14(1):1155.

Blaveri E, Simko JP, Korkola JE, et al. Bladder cancer outcome and subtype classification by gene expression. Cancer Res 2005;11(11):4044-55.

Botelho MC, Oliveira PA, Lopes C, Correia da Costa JM, Machado JC. Urothelial dysplasia and inflammation induced by Schistosoma haematobium total antigen instillation in micenormal urothelium. Urol Oncol 2011;29(6):809-14.

Burger M, Catto J, Dalbagni G, et al. Epidemiology and Risk Factors of Urothelial Bladder Cancer. European Urology 2013;63(1):234-241.

Catto JW, Alcaraz A, Bjartell AS, et al. MicroRNA in prostate, bladder, and kidney cancer: a systematic review. Eur Urol 2011;59(5):671-81.

Chen XN, Wang KF, Xu ZQ, et al. MiR-133b regulates bladder cancer cell proliferation and apoptosis by targeting Bcl-w and Akt1. Cancer Cell Int 2014;14:70.

Chu H, Wang M, Jin H, et al. EGFR 3'UTR 774T>C polymorphism contributes to bladder cancer risk.Mutagenesis 2013;28(1):49-55.

Chung KT.The Etiology of Bladder Cancer and its Prevention. J Cancer Sci Ther 2013;5:346-361.

Dalianis T. Human papillomavirus and oropharyngeal cancer, the epidemics, and significance of additional clinical biomarkers for prediction of response to therapy (Review). Int J Oncol 2014; 44(6):1799-805.

Desai V, Isharwal S, Pooli A, Lele S, Feloney M. Chronic lymphocytic leukemia of the bladder: an atypical etiology of gross hematuria. Ther Adv Urol 2014;6(5):198–200.

Dip N, Reis ST, Srougi M, Dall'Oglio MF, Leite. KR Expression profile of microrna-145 in urothelial bladder cancer. Int Braz J Urol 2013;39(1):95-101.

Eissa S , Habib H, Ali E, Kotb Y. Evaluation of urinary miRNA-96 as a potential biomarker for bladder cancer diagnosis. Med Oncol 2015;32(1):413.

Feng Y, Liu J, Kang Y, et al. miR-19a acts as an oncogenic microRNA and is up-regulated in bladder cancer. Journal of Experimental & Clinical Cancer Research 2014;33:67.

Feng Y, Kang Y, He Y, et al. microRNA-99a acts as a tumor suppressor and is down-regulated in bladder cancer. BMC Urol 2014;14:50.

Feng Y, L-L Niu,W Wei, et al. A feedback circuit between miR-133 and the ERK1/2 pathway involving an exquisite mechanism for regulating myoblast proliferation and differentiation. Cell Death Dis 2013;4(11):e934. doi:10.1038/cddis.2013.462.

Fu CL, Odegaard JI, DeBroski RH, Hsieh MH. A Novel Mouse Model of Schistosoma haematobium Egg-Induced Immunopathology. PLoS Pathog 2012;8(3):e1002605.

Ganguly N, Parihar SP. Human papillomavirus E6 and E7 oncoproteins as risk factors for tumorigenesis. J Biosci 2009;34(1):113-23.

Gao W1, Romkes M, Zhong S, et al. Genetic polymorphisms in the DNA repairgenesXPDand XRCC1,p53genemutations and bladder cancerrisk. Oncol Rep 2010;24(1):257-62.

Goodison S, Rosser C, Urquidi V. Bladder Cancer Detection and Monitoring: Assessment of Urine and Blood-Based Marker Tests. Mol Diagn Ther 2013;17(2):71–84.

Goodison S, Rosser CJ, Urquidi V. Bladder Cancer Detection and Monitoring: Assessment of Urine- and Blood-Based Marker Tests. Mol Diagn Ther 2013;17(2):71–84.

Guancial EA, Bellmunt J, Yeh S, Rosenberg JE, Berman DM. The evolving understanding of microRNA in bladder cancer. Urol Oncol 2014;32(1):41.

Ha YS1, Kim MJ,Yoon HY, et al. mRNA Expressionof S100A8 as a Prognostic Marker for Progression of Non-Muscle-InvasiveBladder Cancer. Korean J Urol 2010;51(1):15-20.

Hemminki K, Bermejo JL, Ji J, Kumar R. Familial bladder cancer and the related genes. Curr Opin Urol 2011;21(5):386-92.

Henriques A, Arantes-Rodrigues R, I Faustino-Rocha A, et al. The effects of whole green tea infusion on mouse urinary bladder chemical carcinogenesis. Iran J Basic Med Sci 2014;17(2):145-8.

Honeycutt J, Hammam O, Fu CL, Hsieh MH. Controversies and challenges in research on urogenital schistosomiasis-associated bladder cancer. Trends Parasitol 2014;30(7):324-32.

http://www.cancer.org/cancer/bladdercancer/detailedguide/bladder-cancer-key-statistics.

Ichimi T , Enokida H, Okuno Y, et al. Identification of novel microRNA targets based on microRNA signatures in bladder cancer. Int J Cancer 2009;125(2):345-52.

Jiang QQ, Liu B, Yuan T. MicroRNA-16 inhibits bladder cancer proliferation by targeting Cyclin D1. Asian Pac J Cancer Prev 2013;14(7):4127-30.

Jiang W, Sun G, Xiong J, Xi X, Shi Z. Association of CYP1B1 L432V polymorphism with urinary cancer susceptibility: a meta-analysis. Diagn Pathol 2014;9:113.

Jin Y, Lu J, Wen J, Shen Y, Wen X. Regulation of growth of human bladder cancer by miR-192. Tumour Biol 2015;epub ahead of print.

Kamat AM, Sethi G, Aggarwal BB. Curcumin potentiates the apoptotic effects of chemotherapeutic agents and cytokines through down-regulation of nuclear factor-κB and nuclear factor-κB–regulated gene products in IFN-α–sensitive and IFN-α–resistant human bladder cancer cells. Molecular Cancer Therapeutics 2007;doi:10.1158/1535-7163.MCT-06-0545.

Kim PH, Sukhu R, Cordon BH, et al. Reflex fluorescence in situ hybridization assay for suspicious urinary cytology in patients with bladder cancerwith negative surveillance cystoscopy. BJU Int 2014;114(3):354-9.

Kim SM, Kang HW, Kim WT, et al. Cell-Free microRNA-214 From Urine as a Biomarker for Non-Muscle-Invasive Bladder Cancer. Korean J Urol 2013;54(11):791-6.

Kim SM1, Kang HW,Kim WT, et al. Cell-Free microRNA-214 From Urine as a BiomarkerforNon-Muscle-InvasiveBladder Cancer. Korean J Urol 2013;54(11):791-6.

Letašiová S , Medveová A, Šovčíková A, et al. Bladder cancer, a review of the environmental risk factors. Environ Health 2012;11 Suppl 1:S11.

Li M, Ma X, Li M, et al. Prognostic role of microRNA-210 in various carcinomas: a systematic review and meta-analysis. Dis Markers 2014;doi:10.1155/2014/106197.

Li YL, Ma ZL, Zhao Y, Zhang J. Immunization with mutant HPV16 E7 protein inhibits the growth of TC-1 cells in tumor-bearing mice. Oncol Lett 2015;9(4):1851-1856.

Li YL, Qiu XH, Shen C, Liu JN, Zhang J. Vaccination of full-length HPV16 E6 or E7 protein inhibits the growth of HPV16 associated tumors. Oncol Rep 2010;24(5):1323-9.

Liang Z, Li S, Xu X, et al. MicroRNA-576-3p Inhibits Proliferation in Bladder Cancer Cells by Targeting Cyclin D1. Mol Cells 2014;doi:10.14348/molcells.2015.2146.

Liu Y, Lin CS, Zhang AM, Song H, Fan CC. The CYP1B1 Leu432Val polymorphism and risk of urinary system cancers. Tumour Biol 2014;35(5):4719-25.

Luo Y, Chen X, Downs TM, DeWolf WC, O'Donnell. MIFN-a 2B Enhances Th1 Cytokine Responses in Bladder Cancer Patients Receiving Mycobacterium bovis Bacillus Calmette-Guerin Immunotherapy. JImmunol 1999;162:2399-405.

Macedo FY, Mourão LT, Palheta RC Jr,et al. Cyclooxygenase 2 contributes to functional changes seen on experimental hemorrhagic cystitis induced byifosfamide in rat urinary bladder. Cancer Chemother Pharmacol 2011;67(4):935-43.

Morais DR, Reis ST, Viana N, et al. The involvement of miR-100 in bladder urothelial carcinogenesis changing the expression levels of mRNA and proteins of genes related to cell proliferation, survival, apoptosis and chromosomal stability. Cancer Cell Int 2014;14(1):119.

Mylona E , Giannopoulou I, Diamantopoulou K, et al. Peroxisome proliferator activated receptor gamma expression in urothelial carcinomas of the bladder: association with differentiation, proliferation and clinical outcome. Eur J Surg Oncol 2009;35(2):197-201

Noguchi S, Yamada N, Kumazaki M, et al. socs7, a target gene of microRNA-145, regulates interferon-β induction through STAT3 nuclear translocation in bladder cancer cells. Cell Death Dis 2013;4(2):e482. doi:10.1038/cddis.2013.11.

Odisho AY, Berry AB, Ahmad AE, Cooperberg MR, Carroll PR, Konety BR. Reflex ImmunoCyt testing for the diagnosis of bladder cancer in patients with atypical urine cytology. Eur Urol 2013;63(5):936-40.

Ramqvist T, Dalianis T. An epidemic of oropharyngeal squamous cell carcinoma (OSCC) due to human papillomavirus (HPV) infection and aspects of treatment and prevention. Anticancer Res 2011;31(5):1515-9.

Ratert N, Meyer HA, Jung M, et al. miRNA profiling identifies candidate miRNAs for bladder cancer diagnosis and clinical outcome. J Mol Diagn 2013;15:695-705.

Reszka E, Jablonowski Z, Wieczorek E, et al. Polymorphisms of NRF2 and NRF2 target genes in urinary bladder cancer patients. J Cancer Res Clin Oncol 2014;140(10):1723–1731.

Rosenberg E , Baniel J, Spector Y, et al. Predicting progression of bladder urothelial carcinoma using microRNA expression. BJU Int 2013;112(7):1027-34.

Sala M, Cordier S, Chang-Claude J, et al. Coffee consumption and bladder cancer in nonsmokers: a pooled analysis of case-control studies in European countries. Cancer Causes Control 2000;11(10):925-31.

Scheffer AR, Holdenrieder S,Kristiansen G,von Ruecker A,Müller SC,Ellinger J. Circulating microRNAs in serum: novelbiomarkers for patients with bladder cancer? World J Urol 2014;32(2):353-8.

Scheffer AR, Holdenrieder S, Kristiansen G, von Ruecker A, Müller SC, Ellinger J. Circulating microRNAs in serum: novel biomarkers for patients with bladder cancer? World J Urol 2014;32(2):353-8.

Schned AR, Lenz P, Moore LE, et al. Analysis of the Distribution and Temporal Trends of Grade and Stage in Urothelial Bladder Cancer in Northern New England from 1994 to 2004. ISRN Pathol 2012:doi:10.5402/2012/283670.

Shan Y, Zhang L, Bao Y, et al. Epithelial-mesenchymal transition, a novel target of sulforaphane via COX-2/MMP2, 9/Snail, ZEB1 and miR-200c/ZEB1 pathways in human bladder cancer cells. J Nutr Biochem 2013;24(6):1062-9.

Shan Y, Zhang L, Bao Y, et al. Epithelial-mesenchymal transition, a novel target of sulforaphane via COX-2/MMP2, 9/Snail, ZEB1 and miR-200c/ZEB1 pathways in human bladder cancer cells. J Nutr Biochem 2013;24(6):1062-9.

Shen M, Duan X, Zhou P, et al. Lymphotoxin β receptor activation promotes bladder cancer in a nuclear factor-κB-dependent manner. Mol Med Rep 2014;doi:10.3892/mmr.2014.2826.

Shen Y, Liu Y, Liu S, Zhang A.The association between -330T/G polymorphism of interleukin 2 gene and bladder cancer.DNA Cell Biol 2012;31(6):983-7.

Song T, Xia W, Shao N, et al. Differential miRNA expression profiles in bladder urothelial carcinomas. Asian Pac J Cancer Prev 2010;11(4):905-11.

Sorahan T. Bladder cancer risks in workers manufacturing chemicals for the rubber industry. Occup Med (Lond) 2008;58(7):496-501.

Sosnowski R, Przewoźniak K . The role of the urologist in smoking cessation: Why is it important? Urol Oncol 2014;S1078-1439.

Thompson DB, Siref LE, Feloney MP, Hauke RJ, Agrawal DK. Immunological basis in the pathogenesis and treatment of bladder cancer. Expert Rev Clin Immunol 2014;13:1-15.

Tili E, Michaille JJ, Croce CM. MicroRNAs play a central role in molecular dysfunctions linking inflamation with cancer. Immunological Reviews 2013;253:167-184.

Uppal A, Ferguson MK, PosnerMC, et al. Towards a molecular basis of oligometastatic disease: potential role of micro-RNAs. Clin Exp Metastasis 2014;31(6):735–748.

Urquidi V, Rosser C, Goodison S. Molecular Diagnostic Trends in Urological Cancer: Biomarkers for Non-Invasive Diagnosis. Curr Med Chem 2012;19(22):3653–3663.

Vasudevan S. Posttranscriptional Upregulation by MicroRNAs. Wiley Interdiscip Rev RNA 2011;doi:10.1002/wrna.121.

Walker NF, Gan C, Olsburgh J, Khan MS. Diagnosis and management of intradiverticular bladder tumours. Nat RevUrol 2014;11(7):383-90.

Wang G, Chan ES, Kwan B, et al. Expression of microRNAs in the urine of patients with bladder cancer. Clin Genitourin Cancer 2012;10(2):106-13.

Wang M, Chu H, Li P, et al. Genetic variants in miRNAs predict bladder cancer risk and recurrence. Cancer Res 2012;72(23):6173-82.

Wennersten C, Andersson G, Boman K, Nodin B, Gaber A, Jirström K . Incident urothelial cancer in the Malmö Diet and Cancer Study: cohort characteristics and further validation of ezrin as a prognostic biomarker. Diagn Pathol 2014;9:189.

Whiteside MA, Siegel EM, Unger ER. Human papillomavirus and molecular considerations for cancer risk. Cancer 2008;113:2981-94.

Wu D, Zhou Y, Pan H, Zhou J, Fan Y, Qu P. microRNA-99a inhibiting cell proliferation, migration and invasion by targeting fibroblast growth factor receptor 3 in bladder cancer. Oncol Lett 2014;7(4):1219-1224.

Xiang CQ, Shen CL, Wu ZR, et al. Detection of mutant p53 protein in workers occupationally exposed to benzidine. J Occup Health 2007;49(4):279-84.

Xiu Y, Liu Z, Xia S, et al. MicroRNA-137 Upregulation Increases Bladder Cancer Cell Proliferation and Invasion by Targeting PAQR3. PLoS One 2014;9(10):e109734.doi: 10.1371/journal.pone.0109734.

Ye F, Wang L, Castillo-Martin M, McBride R, et al. Biomarkers for bladder cancer management: present and future. Am J Clin Exp Urol 2014;2(1):1–14.

Zeng T, Peng L, Chao C, et al. miR-451 inhibits invasion and proliferation of bladder cancer by regulating EMT. Int J Clin Exp Pathol 2014;7(11):7653-62.

Zhang K, Zhou B, Zhang P, et al. Genetic variants in NAMPT predict bladder cancer risk and prognosis in individuals from southwest Chinese Han group.Tumour Biol 2014:35:4031-40.

Zhao Y, Li Y, Wang L, et al. microRNA response elements regulated TRAIL expression shows specific survival-suppressing activity onbladder cancer. J Exp Clin Cancer Res 2013;32:10.doi: 10.1186/1756-9966-32-10.

Zhou X, Qian X, Zhao Q, Lu Y, Xiong M. Efficient expression of modified human papillomavirus 16 e6/e7 fusion protein and the antitumor efficacy in a mouse model. Biol Pharm Bull 2004;27(3):303-7.

Abbreviations and acronyms: BCG=Bacillus Calmette Guerin, Bcl=B cell lymphoma, C=cytosine, CIS=in situ carcinoma, CYP=cytocrome P, DNA=deoxyribonucleic acid, EGF= epidermal growth factor, EGFR=epidermal growth factor receptor, EMT=epitelial mesenchymal transition, EORTC=The European Organization for Research and Treatment of Cancer Scoring System, ERCC=excision repair cross complement, FISH=fluorescence in situ hybridization, FGFR=fibroblast growth factor receptor, G=guanine, G1=first growth phase of cell cycle. HAP=haemagglutinin protease, HIF=hypoxia inducible factor, HPV=human papilomavirus, IL=interleukin, LPS=lipopolysaccharide, MDM=murine double minute, NF=nuclear factor, NRF=nuclear respiratory factor, PAQR3=progestin and adipoQ receptor family member III, PCR=polymerase chain reaction, RT=reverse transcriptase, S=synthesis phase of cell cycle, T=timine, TLR=toll like receptor, XPC/D=xeroderma pigmentosum complementation group C/D. XRCC=x ray repair cross complementing.

Authors

•Anamaria Truţa, Department of Medical Genetics "Iuliu Hatieganu" University of Medicine and Pharmacy, 6 Louis Pasteur street, Cluj- Napoca, Cluj, Romania, EU, email: ammatruta@yahoo.com

•George Saraci, Department of Internal Medicine "Iuliu Hatieganu" University of Medicine and Pharmacy, 8 V. Babes street, Cluj-Napoca, Cluj, Romania, EU, email: gsaraci@yahoo.com

•Tudor A. Hodor-Popon, Department of Urology-Robotic Surgery, Clinical Municipal Hospital, 11 Tabacarilor street, Cluj-Napoca, Cluj, Romania, EU, email: tudor.hodor@gmail.com

•Liviu Ghervan, "Iuliu Hatieganu" University of Medicine and Pharmacy, Clinical Institute of Urology and KidneyTtransplant, 2 Clinicilor street, Cluj- Napoca, Cluj, Romania, EU, email: liviughervan@yahoo.com

•Ioan V. Pop, Department of Medical Genetics "Iuliu Hatieganu" University of Medicine and Pharmacy, 6 Louis Pasteur street, Cluj- Napoca, Cluj, Romania, EU

Citation	Truţa A, Saraci G, Hodor-Popon TA, Ghervan L, Pop IV. Etiopathogenesis of bladder cancer. Risk factors, genetic aspects and novel diagnosis biomarkers. HVM Bioflux 2015;7(3):206-214.
Editor	Stefan C. Vesa
Received	18 July 2015
Accepted	26 July 2015
Published Online	29 July 2015
Funding	POSDRU grant no. 159/1.5/S/138776 with title:"Model colaborativ institutional pentru translatarea cercetarii biomedicale in practica clinica –TRANSCENT"
Conflicts/ Competing Interests	None reported