Molecular cytogenetic characterization and diagnostics of bladder cancer

L. HOUSKOVA[1*], Z. ZEMANOVA[1], M. BABJUK[2], J. MELICHERCIKOVA[3], M. PESL[2], K. MICHALOVA[1]

[1]*Center of Oncocytogenetics, Institute of Clinical Biochemistry and Laboratory Diagnostics, General Faculty Hospital and 1st Medical Faculty, Charles University, Prague, e-mail: LuHouskova@seznam.cz*
[2]*Department of Urology, General Faculty Hospital and 1st Medical Faculty, Charles University, Prague*
[3]*Institute of Hematology and Blood Transfusion, Prague*

Received March 26, 2007

Bladder cancer is a heterogenous malignancy with wide scale of clinical manifestation. Different chromosomal aberrations have been already identified in bladder tumors. These aberrations can be detected by multicolor interphase fluorescence *in situ* hybridization (I-FISH) or comparative genomic hybridization (CGH). The aim of this study was to determine the diagnostic benefits of non-invasive I-FISH method and to comprehensively characterise genetic alterations using CGH in selected patients with bladder tumors. We examined 128 urine samples and correlated our results with histological findings. I-FISH using UroVysion kit ® showed positivity in 63,6 % of G1 tumors, 64,3 % of G2 tumors and 91,7 % in G3 tumors. We examined also 12 bladder tissue samples by means of CGH and various genetic alterations were ascertained independent on tumor grade. The most frequent gains and losses of DNA material were detected on chromosomes 1, 8, 9, 10, 11, 13, and 14. The contribution of I-FISH is in an early and non-invasive detection of bladder cancer recurrences during follow up of patients after the surgery. CGH provides information about further genetic alterations and some of them could be ascertained as recurrent changes with prognostic significance.

Keywords: bladder cancer, interphase fluorescence in situ hybridization (I-FISH), comparative genomic hybridization (CGH)

Bladder cancer is the sixth most common malignancy detected in men and the thirteenth detected in women in the Czech Republic. More than 2500 of new cases of bladder cancer is diagnosed every year which means that the incidence of this disease has almost doubled in comparison to the data quoted in 1970. From the global point of view the estimations show that there is more than 250 000 of bladder cancer cases newly diagnosed every year over the world and the incidence is gradually increasing in all industrial countries.

Around 70 % of the patients with bladder tumor develop superficial forms (Ta, T1, Tis) that are accompanied by a high rate of recurrence up to 80 %. Therefore long-term follow up is needed to prevent progression to invasive, potentially lethal bladder tumor. The risk of progression varies between 2-50 % according to the type of primary tumor. Invasive tumors (T2-T4) occur in 20-30 % of patients and develop often lethal metastases (more than 50 %). Many observations clarified that bladder cancer is a disease with variable behavior. Some tumors seem to be aggressive, but their further course is favourable and vice versa.

Our understanding of the genetic changes that accompanies bladder cancer initiation and progression is being increasingly out-ravelled. Cytogenetic studies revealed frequent alteration of various chromosomes in bladder cancer that predetermined its behaviour [1–3]. For example, homozygous deletion of *CDKN2A* gene in 9p21 locus occurs in early stages of bladder tumor development. Alternative transcripts from this gene, proteins INK4A and ARF play very important role in the cell cycle regulation. INK4A protein controls the RB-regulated G1-S transition, whereas ARF protein stabilizes, enhances the p53 protein level and blocks its degradation. As the tumor progresses into invasive or metastatic stage, further chromosomal changes as loss of 8p, 11p, 17p, gains of 1q, 8q, 11q or aneuploidy of various chromosomes appear. Mechanisms generating aneuploidy are based on defects of mitotic checkpoints due to reduction of the checkpoint component levels. Weakening of the major cell cycle

*Corresponding author

control mechanism that prevents chromosome missegregation leads to near-diploid aneuploidy and therefore to the change in the gene dosage [4]. Aneuploidy of various chromosomes is often accompanied by centrosomal defects (80 % of all bladder tumors) and correlates with higher tumor grade and stage [5].

Diagnostics of bladder cancer and prediction of its further course is routinely carried out according to histopathology and cytology findings i.e. changes in morphology and immunophenotype of the cell. However modern molecular cytogenetic methods offer the possibility to examine the initial cause of the tumor development i.e. changes in genetic information.

Genetic aberrations indicating malignancy can be found by interphase fluorescence *in situ* hybridization (I-FISH). This method enables the examination of the non-dividing cell nuclei obtained from the urine. SOKOLOVA et al. [6] developed UroVysion kit specific for detection of specific chromosomal aberrations occuring in bladder cancer. This kit uses fluorescently labelled centromeric and locus-specific DNA probes for chromosomes 3, 7 , 17 and locus 9p21. Probes are hybridized to nuclei of the cells obtained from urine samples. I-FISH method is non-invasive, very fast and has significantly higher sensitivity than conventional urine cytology i.e. up to 81 %.

Another approach to genetic characterization of bladder cancer is comparative genomic hybridization (CGH) [7]. This method enables the detection of changes in DNA copy numbers – gains (e.g. amplifications) and losses (e.g. deletions) in the whole genome within a single experiment. CGH technique helped in the assesment of recurrent genetic alteration and therefore in the development of UroVysion kit [8].

The aim of this study was to perform molecular cytogenetic examination of urine and tissue samples of patients with urological disorders, to correlate these results with histological findings and to evaluate the contribution of I-FISH and CGH method to diagnostics and genetic characterization of bladder cancer.

Materials and methods:

Patients. We examined prospectively 128 urine samples from 97 patients, who had symptoms suggestive of bladder cancer or came for a check-up with recurrent bladder cancer event, using I-FISH method. Control group was formed by 31 donors. Voided urine samples (max volume 35 ml) were mixed in a 2:1 ratio with Carbowax® (2% polyethylene glycol in 50% ethanol) within 30 min of colletion and refrigerated (2°C to 8°C). The urine sediments were fixed in Carnoy´s fixative and slides were prepared according to standard cytogenetic protocols. Furthermore, we examined tissue samples from bladder tumors or normal urothelium from 10 men and two females. These samples were processed by CGH for characterization of genetic alterations within the whole genome. Unfortunately, due to the absence of urine samples from these patients, we were not able to perform also I-FISH examination as in previous patients and to make a comparison of our findings.

I-FISH. Multicolor I-FISH was performed according to the guidelines of the manufacturer (Abbott-Vysis™, Downers Grove, IL, USA). Briefly, slides were pretreated with 2x saline/sodium citrate (SSC) at 73°C, 0,5 mg/ml pepsin at 37°C/ 0,01N hydrochloric acid (HCl) in a waterbath at 37°C, 1% formaldehyde, washed in PBS at room temperature and dehydrated in 70%, 85% and 100% ethanol. The UroVysion® probe mix was placed on the slides, co-denaturated (73°C/2 min) and hybridized (39°C/24 hrs). After hybridization slides were washed using 0,4xSSC/0,3% NP-40, 2xSSC/0,1% NP-40 and air dried. Diaminophenyloindole II. (DAPI II.) was used as a counterstaining.

Slides were scored for fluorescence signals using fluorescence microscope (Zeiss Axioplan 2 Imaging, Germany) with filter set including DAPI single bandpass (counterstain), aqua single bandpass (chromosome 17), gold single bandpass (9p21 locus), red single bandpass (chromosome 3) and green single bandpass (chromosome 7). Enumeration and evaluation of the I-FISH signals were done on morphologically abnormal nuclei with abnormal „DAPI pattern“ according to manufacturer recommendations as follows i.e. 25 morphologically abnormal nuclei were counted until ≥4 of the 25 nuclei showed gains for 2 or more chromosomes in the same nucleus or ≥12 of the 25 nuclei have zero 9p21 signals. If there were no abnormal nuclei, 200 morphologically normal nuclei were counted.

Sensitivity and specifity were calculated considering the findings from histology and cystoscopy.

CGH. Tissue was homogenized using liquid nitrogen and DNA was extracted with DNA isolation kit for mammalian blood (Roche Diagnostics, Germany). Isolated test DNA was labelled by nick translation with SpectrumGreen-dUTP™, reference DNA was labelled with SpectrumOrange-dUTP™. Inverse CGH was used to test the performance of CGH hybridization. This procedure was performed with opposite labelling, i.e. test DNA was labelled with Spectrum Orange-dUTP™ and reference DNA was labelled with Spectrum Green-dUTP™. Analysis of nick-translated products was accomplished by separation using electrophoresis in 1% agarose gel (final lenght of fragments cca 1000bp). We prepared a probe by precipitation of an equal amount of labelled test and reference probes, Cot-1 DNA with 3M NaAc and 100% EtOH and dissolved the probe in hybridization buffer. Denaturation of metaphase slides was proceeded in formamide solution (70% formamide, 20x SSC, H_20). The probe was denaturated separately in a water bath. The slides with probes were hybridized in a moist chamber at 42°C for 3 days. Post-hybridization wash was carried out in 0,4 x SSC/0,3% NP40 and 2x SSC/0,1% NP40. Diaminophenylindole II. (DAPI II). was used as a counterstain.

For each CGH analysis at least 15-20 metaphase spreads were captured using a fluorescence microscope (Axioplan

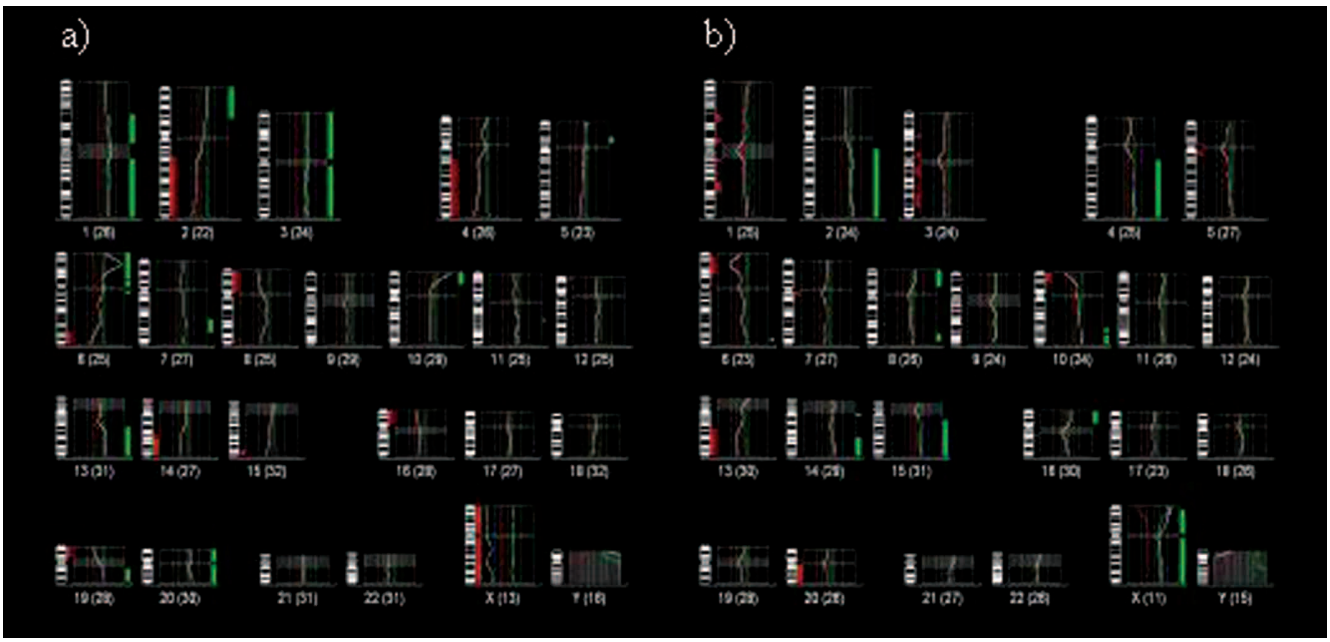

Figure 1. CGH result of patient No.11
a) CGH proved DNA copy number changes on chromosomes 1, 2, 3, 4q, 5p, 6, 7q, 8, 10p, 11q, 12p, 13q, 14q, 15q, 16p, 19 and 20.
b) Inverse CGH did not prove DNA copy number changes on chromosomes 2p, 5p, 7q, 10q, 11q and 19, therefore these were excluded from the result as the hybridization artefacts. The final result was concluded as follows: 46,XY,cgh ish enh(1)(p13-p31),enh(1) q12-qter),dim(2)(q14-qter),enh(3),dim(4)(q21-qter),enh(6)(p11-pter),dim(6)(q24-qter), dim(8)(p11-pter),enh(10)(p12-pter),dim(10)(q26-qter),enh(13)(q21-qter),dim(14)(q22-qter), dim(15)(q26-qter), dim(16)(p11-pter),enh(20).

2, Zeiss, Germany) equipped with CCD camera. Chromosomal regions were defined as over-represented when the green/red signal ratio exceeded 1,25 and under-represented when the ratio was below 0,8. Images and ratio profiles were interpreted using software *ISIS* for CGH analysis (MetaSystems™ GmBH, Altlussheim, Germany) according to previously published criteria [9].

Results

Clinical data. We examined 128 urine samples from 51 females and 77 males (mean age 65 years, ranging from 23 to 93 years). Histological examination proved transitional cell carcinoma in 97 patients from our cohort i.e. concretely 33x grade 1, 28x grade 2 and 36x grade 3. Control group was formed by 31 donors, ten of which were healthy and 21 had benign urological disease (benign prostatic hyperplasia, nephrolithiasis, cystitis etc.).

Data of I-FISH analysis. Results of fluorescence *in situ* hybridization are given in Table 1. Molecular cytogenetic findings correlate well with histological results. I-FISH proved positivity in 63,6 % of samples with grade 1 (G1), in 64,3 % samples with grade 2 (G2) and in 91,7 % of samples with grade 3 (G3). False positive results were found in 5 negative controls (all with benign prostatic hyperplasia) and always included only biallelic deletion of 9p21 locus.

Biallelic deletion of locus 9p21 was detected in 41,7 % of the patients with bladder cancer i.e. 51,5 % in G1, 28,5 % in G2, 13,9 % in G3, respectively. This aberration was mostly detected in patients with low grade bladder cancer. Aneuploidy for chromosomes 3, 7 and 17 was identified in 48,6 % of the patients i.e. 3 % in G1, 25 % in G2 and 75 % in G3. These aberration are

Table 1. Correlation of I-FISH results with histological findings in 97 patients with bladder cancer

Tumor grade	I-FISH proved positivity n/N (N=97 patients)	Sensitivity of I-FISH (%)	Distribution of chromosomal aberrations i.e. biallelic deletion of locus 9p21/ aneuploidy of chromosomes 3, 7 and 17 / both clones
G1	21/33	63,6	17 / 1 / 3
G2	18/28	64,3	8 / 7 / 3
G3	33/36	91,7	5 / 27 / 1

Table 2. DNA copy number changes detected by CGH in 12 patients with bladder cancer

Patients	Grade	DNA losses	DNA gains
No.1	bph	none	none
No.2	G2	5p15-pter, **9q21-qter**[a], **11p11-pter**[a], **11q22-qter**[a], **14q23-q32**[a]	9p21, 20
No.3	G2	**8p21-pter**[a], **11q22-q32**[a], **14q**[a],	**8q11-qter**[a]
No.4	G2	1p31-pter, 3p14-pter, 6q25-qter, 9p12-p23, **9q21-qter**[a], **10q21-qter**[a], 17p11-pter	**1q12-qter**[a], 3q12-qter, 4q12-q28, **13q14-q31**[a]
No.5	G3	1p21-p31	5p11-pter, 12p11-pter, 16q23-qter
No.6	G3	none	9q13-q21
No.7	G3	**8p21-p23**[a], **11p12-p15**[a]	7, 21q11-qter, 19, 20
No.8	G1	6p12-pter, **8p11-p23**[a], 8q21-qter, **9**[a], **10q11-qter**[a], **11**[a], 12, **14q21-qter**[a]	**1q12-qter**[a], 4q12-qter, 5q11-5q21, **13q13-q32**[a]
No.9	G1	1p34-pter, 2q37-qter, **9q33-qter**[a], 16p11-pter,17, 19, 20q11-qter, 22q11-qter	**1q25-q32**[a], 2q32-q33, 3q25-q26, 4p11-p13, 4q12-q34, 5q15-q22, 6q11-q23, 7q31, **8q12-q13**[a], 9p13-pter, 12q14-q22, **13q14-q33**[a]
No.10	G3	1q32-q42, 3q24-qter, **8p12-pter**[a], **11p15-pter**[a],11q23-q24, **14q22-qter**[a], 15q22-q25, 16p13-pter, 20q11-qter	1p21-p31, 6q12-q24**, 8q11-qter**[a], 16q12-qter
No.11	G3	2q14-qter, 4q21-qter, 6q24-qter, **8p11-pter**[a], 10q26-qter, **14q22-qter**[a], 15q26-qter, 16p11-pter	1p13-p31, 1q12-qter, 3p, 3q, 6p11-pter, 10p12-pter, **13q21-qter**[a], 20
No.12	G2	9p21-pter, 10q21-qter, **11p12-pter**[a], **14q22-qter**[a]	**8q21-qter**[a], 10p13-pter, 12q21-qter

Abbreviations: bph, benign prostatic hyperplasia; G1, histological grade 1; G2, histological grade 2; G3, histological grade 3
[a] In boldface are emphasized recurrent aberrations occuring at least in four patients out of 12 (≥30 %).

clearly associated with higher grade bladder cancer. Clones with both aberrations were found in 9,7 % of the patients.

Data of CGH analysis. Results of comparative genomic hybridization are given in Table 2. Only genetic alterations confirmed by inverse CGH were included into this study, other changes were considered to be the hybridization artefacts (see Figure 1). Tissue samples for CGH were obtained from 12 patients (2x G1, 4x G2, 6x G3), control sample was obtained from a patient with benign prostatic hyperplasia. The CGH analysis proved high number of genomic alterations. DNA copy number changes varied from one to 22 in our cohort. We have not any change in DNA copy number in the control sample. The most frequent changes (at least four cases out of 12) were observed on chromosomes 1, 8, 9, 10, 11, 13 and 14. Gains of material were detected on long arms of chromosomes 1, 8 and 13 and losses of material on short arms of chromosomes 8 and 11 and on long arms of chromosomes 9, 10, 11 and 14.

Discussion

Biological potential of each tumor can not be determined only by routinely used methods such as cystoscopy, urine cytology or histology. Thus, there is a strong need for new prognostic factors that would clarify the cause of bladder tumor development, progression and prognosis. Genetic alterations in bladder cancer seem to be promising prognostic markers, especially in early detection of high grade tumors and prediction of recurrence in low grade tumors [10].

Molecular cytogenetic studies have identified various chromosomal aberrations during bladder cancer development. The UroVysion kit® contains the set of DNA probes providing the highest sensitivity in detecting the bladder cancer. The group that developed UroVysion kit® achieved the overall sensitivity 81 % and specifity up to 96 % in the cohort of 265 patients with the combination of centromeric probes for chromosomes 3, 7, 17, and locus-specific probe for 9p21 region [8]. We examined 99 patients with these probes and reached the sensitivity 73,2 % and specifity 83,8 %. Although these values are lower likely due to small number of patients in our cohort, they correlate with other studies performed on similar cohort of patients [11].

Biallelic deletion of locus 9p21 is considered to be an early change in neoplastic processes and can be often found even in histopathologically normal urothelium [12]. We found false positive results in five patients with benign prostatic hyperplasia, but with regard to the fact that these patients belong to the group with higher risk of bladder tumor due to the age (mean age 67,2 years), they will stay under further surveillance. This method could be also very useful in follow up of patients with recurrent bladder cancer, where the relaps of the disease could be detected earlier. But still it is important not to underestimate the specificity during the surveillance [10].

Comparative genomic hybridization performed on our cohort of patients showed the most frequent DNA copy number changes of chromosomes 1, 8, 9, 10, 11, 13 and 14. These results correlate well with findings described in the literature [13]. We observed the loss of 14q as the most frequent genetic change (six cases i.e. G1 2x, G2 3x, G3 1x) in our cohort. Loss of heterozygosity (LOH) on 14q was described in more than 40 % of urothelial carcinomas and is characteristic for the invasive disease [14, 15]. Despite no concrete tumor suppressor has been found in 14q, at least two frequent breakpoints in 14q23 and 14q12 were identified [16].

Losses of 8p and both arms of chromosome 11 also belong to recurrent genetic changes in bladder cancer. We detected alterations in 8p in four patients of our cohort (all with G3)

and alteration of chromosome 11 in five patients (G2 2x, G3 3x). Deletion of chromosome 8 play an important role in tumorigenesis of bladder cancer. It was proved that LOH of 8p correlates with progression [17]. Few promising candidate tumor suppressor genes were located in 8p. The large telomeric region 8p21.1-pter and more proximal region in 8p11.2-12 were most frequently affected. Stoehr et al. [18] focused on sFRP1 gene (secreted Frizzled-related protein) located in 8p12-11.1 and proved that LOH at this region corellated well with invasive papillary growth. Vecchione et al. [19] examined candidate tumor suppressor FEZ1/LZTS1 located in 8p21-p22 and found that down-regulated FEZ1 correlates with high grade tumors. Regarding the losses on chromosome 11, CDKN1C gene located in 11p15 might be another tumor suppressor candidate due to its ability of inducing senescence in urothelial cells [20]. No bladder cancer related gene has been identified on long arms of chromosome 11 until now. CGH detected losses of 11p and 11q in five resp. four patients of our cohort.

Losses of 9p and 9q are very important changes especially in the early phase in tumorigenesis. LOH on chromosome 9 is associated with activating mutations of the fibroblast growth factor receptor 3 (FGFR3) and is often detected even in urothelial hyperplasias [21]. Biallelic deletion of tumor suppressor p16 located in 9p21 was found in the high percentage of Ta-T1 tumors (59 %) [22]. We proved this aberration by I-FISH in 30 % of our patients (mostly in G1 i.e. 17 %).

CGH revealed loss of 10q in four patients of our cohort. This aberration is characteristic for late-stage events in progression of bladder cancer and is often connected with biallelic deletion or mutation of PTEN tumor suppressor [23].

Gains of genetic material on chromosomes 1q, 8q, and 13q occured the most frequently in our cohort, each gain was detected at least in four patients. DNA copy number changes in 8q might be connected with amplification of CMYC oncogene [24]. Amplification of 1q21-q23 is associated with metastatic dissemination and chemoresistance in bladder cancer. Meza-Zepeda et al [25] identified a novel cyclophilin as a candidate amplified oncogene in 1q21. No candidate tumor suppressor on 13q has been identified in bladder cancer yet.

We can conclude that I-FISH is sensitive non-invasive method useful for detection of bladder cancer from urine samples, monitoring of recurrency and selection of patients for cystoscopy. CGH is very effective experimental method that enables the assesment of DNA copy numbers changes within the whole genome. The contribution of this method to the bladder cancer characterization resides in focusing to specific parts of chromosomes that may be afterwards analysed for the presence of candidate tumor suppressor genes. All findings from the CGH method have to be verified either by inverse CGH or by FISH.

Due to the frequent alteration occuring in bladder cancer we suggest that 11q, 13q, and 14q should undergo detailed molecular examination.

This work was supported by grants from the Ministry of Health MZO 00064165 and MSM 0021620808 and from the Ministry of Education LC535.

References

[1] PANANI AD, FERTI AD, RAPTIS SA, et al. Novel recurrent structural chromosomal aberrations in primary bladder cancer. Anticancer Res 2004; 24: 2967–74.

[2] SARAN KK, GOULD D, GODEC CJ, et al. Genetics of bladder cancer. J Mol Med 1996; 74: 441–445.

[3] STEIDL C, SIMON R, BÜRGER H, et al. Patterns of chromosomal aberrations in urinary bladder tumours and adjacent urothelium. J Pathol 2002; 198: 115–120.

[4] UPENDER MB, HABERMANN JK, McSHANE LM, et al. Chromosome transfer induced aneuploidy results in complex dysregulation of the cellular transcriptome in immortalized and cancer cells. Cancer Res 2004; 64: 6941–6949.

[5] JIANG F, CARAWAY NP, SABICHI AL, et al. Centrosomal abnormality is common in and a potential biomarker for bladder cancer. Int J Cancer 2003; 106: 661–665.

[6] SOKOLOVA IA, HALLING KC, JENKINS RB, et al. The Development of a multitarget, multicolor fluorescence *in situ* hybridization assay for the detection of urothelial carcinoma in urine. JMD 2000; vol. 2, 3: 116–123.

[7] KALLIONIEMI A., KALLIONIEMI OP, SUDAR D, et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992; 258: 818–821.

[8] HALLING KC. Vysis Uro Vysion for the detection of urothelial carcinoma. Expert Rev Mol Diagn 2003; 3: 507–519.

[9] ISOLA J, DEVRIES S, CHU L, et al. Analysis of changes in DNA sequence copy number by comparative genomic hybridization in archival paraffin-embedded tumor samples. Am J Pathol 1994; 145: 1253–60.

[10] van RHIJN BWG, van der POEL HG, van der Kwast TH. Urine markers for bladder cancer surveillance: a systematic review. Eur Urol 2005; 47: 736–748.

[11] TOMA MI, FRIEDRICH MG, HAUTMANN SH, et al. Comparison of the ImmunoCyt test and urinary cytology with other urine tests in the detection and surveillance of bladder cancer. World J Urol 2004; 22: 145–149.

[12] HARTMANN A, MOSER K, KRIEGMAIR M, et al. Frequent genetic alterations in simple urothelial hyperplasias of the bladder in patients with papillary urothelial carcinoma. Am J Pathol 1999; 145: 721–727.

[13] FADL-ELMULA I, KYTOLA S, EL LEITHY M, et al. Chromosomal aberrations in benign and malignant Bilharzia-associated bladder lesions analyzed by comparative genomic hybridization. BMC Cancer 2002; 2:5.

[14] TZAI TS, CHEN HH, CHAN SH, et al. Clinical significance of allelotype profiling for urothelial carcinoma. Urology 2003; 62: 378–384.

[15] FORNARI D, STEVEN K, HANSEN AB, et al. Under-representation of bladder transitional cell tumour 9q, 11p and 14q LOH in urine and impact on molecular diagnosis. Anticancer Res 2005; 25: 4049–52.

[16] THEOBALD M, CHRISTIANSEN H, SCHMIDT A, et al. Sublocalization of putative tumor suppressor gene loci on chromosome arm 14 in neuroblastoma. Genes Chromosomes Cancer 1999; 26: 40–46.

[17] FORNARI D, STEVEN K, HANSEN AB, et al. Transitional cell bladder tumor: predicting recurrence and progression by analysis of microsatellite loss of heterozygosity in urine sediment and tumor tissue. Cancer Genet Cytogenet 2006; 167: 15–19.

[18] STOEHR R, WISSMANN C, SUZUKI H, et al. Deletions of chromosome 8p and loss of sFRP1 expression are progression markers of papillary bladder cancer. Lab Invest 2004; 84: 465–478.

[19] VECCHIONE A, ISHII H, BALDASSARRE G, et al. FEZ1/LZTS1 is down-regulated in high grade bladder cancer, and its restoration suppress tumorigenicity in transitional cell carcinoma cells. Am J Pathol 2002; 160: 1345–1352.

[20] HOFFMANN MJ, FLORL AR, SEIFERT HH, et al. Multiple mechanisms downregulate CDKN1C in human bladder cancer. Int J Cancer 2005; 114: 406–413.

[21] Van OERS JM, ADAM C, DENZINGER S, et al. Chromosome 9 deletions are more frequent than FGFR3 mutations in flat urothelial hyperplasias of the bladder. Int J Cancer 2006; 119: 1212–1215.

[22] KNOWLES MA. Molecular genetics of bladder cancer: pathways of development and progression. Cancer Surv 1998; 31: 49–76.

[23] WANG DS, RIEGER-CHRIST K, LATINI JM, et al. Molecular analysis of PTEN and MXI1 in primary bladder carcinoma. Int J Cancer 2000; 88: 620–625.

[24] SANCHEZ-CARBAYO M. Recent advances in bladder cancer diagnostics. Clin Biochem 2004; 37: 562–571.

[25] MEZA-ZEPEDA LA, FORUS A, LYGREN B, et al. Positional cloning identifies a novel cyclophilin as a candidate amplified oncogene in 1q21. Oncogene 2002; 21: 2261–2269.