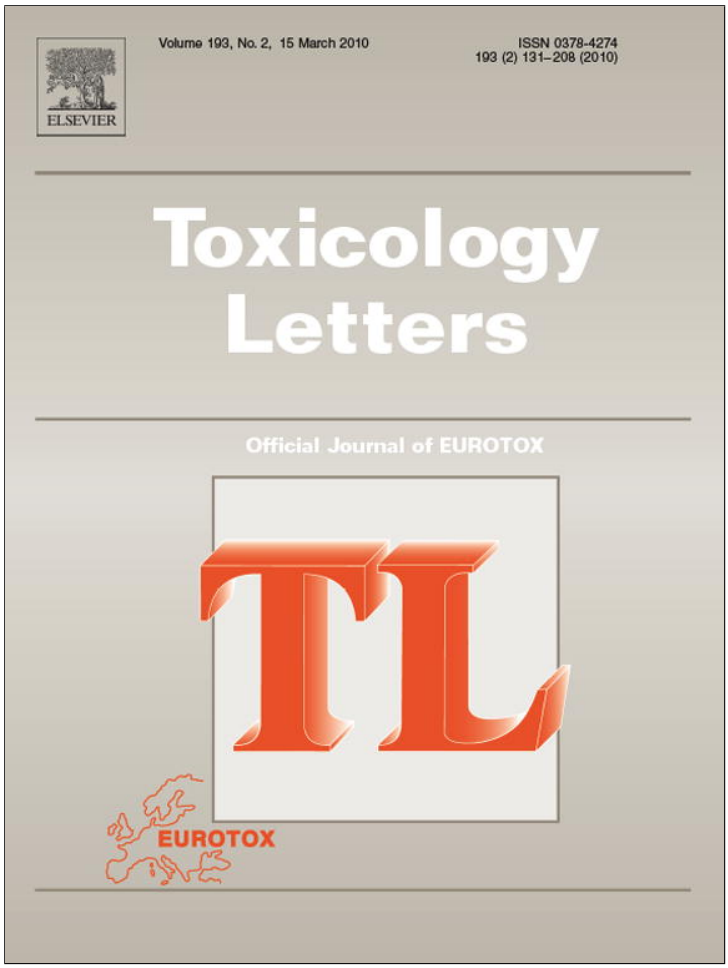

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Mini review

# Environmental factors and genetic susceptibility promote urinary bladder cancer

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ABSTRACT

Cancer of the urinary bladder is the second most common malignancy of the genitourinary tract, currently accounting for up to 5% of all newly diagnosed tumours in the western world. Urinary bladder carcinogenesis seems to develop from the interaction of environmental exposure and genetic susceptibility. Smoking, specific industrial chemicals, dietary nitrates and arsenic represent the most important exogenous risk factors. Chromosomal abnormalities, silencing of certain genes by abnormal methylation of their promoter region, alterations in tumour suppressor genes and proto-oncogenes that induce uncontrolled cell proliferation and reduced apoptosis, are molecular mechanisms that have been reported in bladder carcinogenesis. In this article, we discuss the environmental risk factors of bladder cancer and we review the genetic and epigenetic alterations, including aberrant DNA methylation and deregulation of microRNAs expression. We also discuss the role of p53 and retinoblastoma suppressor genes in disease progression. Finally, we present recent reports on the use of molecular profiling to predict disease stage and grade and direct targeted therapy.



**Contents**



## 1. Introduction

Cancer of the urinary bladder is the 5th most common cancer in the Western world, responsible for approximately 14,000 cancer-related deaths in the United States each year (Jemal et al., 2009). Bladder cancer (BC) can occur at any age; however it is generally a disease of the middle-aged or elderly. The median ages at diagnosis are 69 years in males and 71 in females and it is about 3 times more common in men than in women. The increased life expectancy of the Western population has led to a concomitant rise in the incidence of bladder cancer during the last 20 years, currently accounting for up to 5% of all new cancers. Ninety-eight percent of all bladder cancers are epithelial malignancies, with the vast majority being transitional cell carcinomas (TCC). Other rare histologic types include squamous-cell carcinomas, adenocarcinomas, and nonepithelial cancers. The most common finding in

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bladder cancer is microscopic or gross haematuria. However, the clinical manifestations are associated with stage and thus range from asymptomatic disease to uremic coma. For instance weight loss, skeletal pain and oedema of the lower extremities can rarely be found in non-muscle invasive tumours, whereas they represent common findings in locally advanced and metastatic disease. At presentation 75–85% of patients have tumours confined to the urothelial mucosa (stage Ta) or lamina propria (stage T1). Nevertheless, more than 60% of these tumours will recur at least once and progress to less differentiated or invasive neoplasms (≥T2) in 10–15% of all cases. The remaining patients present with muscle invasive tumours (stages T2–T4) or with *de novo* metastatic disease (5%), having less favourable prognosis.

Bladder cancer follows the general concept of multi-step carcinogenesis and it is likely that multiple lesions on the DNA of target cells are required for malignant transformation. Moreover, similar carcinogens may facilitate the development of different genetic alterations due to the microenvironment of transitional epithelium. Bladder carcinogenesis is thought to develop from the interaction of environmental exposures and genetic susceptibility. Herein, we present the environmental risk factors of bladder cancer and we review the genetic and epigenetic alterations and the key signalling molecules involved. Furthermore, we present the molecular profiling, a novel technology based on simultaneous determination of multiple molecular markers by using microarrays and focus on its application to predict disease stage, progression and clinical outcome.

## 2. Environmental risk factors

The most important environmental factors associated with bladder cancer are listed on Table 1. Tobacco smoke is considered the most important exogenous risk factor for bladder cancer (Stewart et al., 2008). It is estimated that 50% of TCCs are directly attributable to tobacco smoke. Arylamines and more than 60 other carcinogens present in tobacco smoke are associated with DNA adduct formation and alterations in tumour suppressor genes expression (Wallerand et al., 2005). Smokers present with higher grade and stage tumours, ultimately having higher bladder cancer-specific mortality compared to non-smokers. Cessation of smoking leads to a 40% reduction of bladder cancer risk within one year, however the risk remains increased for as many as 25 years.

Occupational exposure accounts for approximately 20% of all bladder cancer cases. Aromatic amines (e.g. benzidine, β-napthylamine, and 4-aminobiphenyl) present in aniline dyes and many other industrial chemicals have been reported to be causally related to bladder cancer (Rouissi et al., 2009; Golka et al., 2004). Occupational urinary bladder cancer has been observed in painters, varnishers, hairdressers, textile, rubber and leather industry workers. The latency period between exposure and clinical presentation is related to cumulative dose and ranges from 30 to 50 years. Perchloroethylene is a chlorinated hydrocarbon found mostly in dry-cleaning solvents that has been associated with the development of bladder cancer. A review of the epidemiologic literature demonstrated a substantial higher risk for the development of bladder cancer in laundry and dry-cleaning workers (Mundt et al., 2003). Similarly, polycyclic aromatic hydrocarbons have been related with the development of respiratory and urinary tract cancers (Bosetti et al., 2007).

The uncontrolled use of nitrogen-based fertilizers and pesticides has led to nitrate contamination of soil and groundwater. The relation between dietary nitrates and the development of TCC is controversial. A recent study conducted in Taiwan residents demonstrated a significant positive relationship (Chiu et al., 2007), whereas a cohort study in Dutch population did not support an association between dietary nitrates and bladder cancer (Zeegers et al., 2006). Many other epidemiologic studies relate the high levels of chloride and arsenic in drinking water with increased risk of developing bladder cancer (Villanueva et al., 2003; Chiou et al., 2001). The presumable mechanisms for arsenic carcinogenesis are DNA methylation changes, aberrant gene expression, altered cell metabolism and oxidative stress (Huang et al., 2004).

The association between pelvic radiotherapy and the development of bladder cancer has been well documented in the past (Kaldor et al., 1995). Moreover, studies performed in the Ukraine population after the Chernobyl accident, linked the chronic exposure to persistent low-dose of ionizing radiation (IR) to the activation of specific molecular pathways and the subsequent initiation of urinary bladder carcinogenesis (Romanenko et al., 2000; 2002; 2003; 2009). More specifically, it has been demonstrated that long-term, low-dose of IR induces oxidative stress, causing DNA damage (Romanenko et al., 2000) significant activation of DNA repair enzymes (base and nucleotide excision repair), 8-hydroxy-2′deoxyguanosine (8-OHdG), 8-oxoguanine-

**Table 1**
Environmental risk factors for the development of bladder cancer.

| Environmental factor | Comment | Reference |
|---|---|---|
| Smoking | 50% of cases | Stewart et al. (2008) |
| | Higher cancer-specific mortality | Wallerand et al. (2005) |
| Aromatic amines | Aniline dyes and other industrial chemicals | Rouissi et al. (2009) |
| | | Golka et al. (2004) |
| Ionizing radiation | Pelvic radiotherapy | Romanenko et al. (2009) |
| | Radio-contaminated areas | Kaldor et al. (1995) |
| Arsenic | Contamination of water | Huang et al. (2004) |
| | | Chiou et al. (2001) |
| Dietary nitrites/nitrates | Fertilizers and pesticides | Chiu et al. (2007) |
| | | Zeegers et al. (2006) |
| Chloride | Chlorinated water | Villanueva et al. (2003) |
| | Weak correlation | |
| Chlorinated hydrocarbons | Dry-cleaning solvents | Mundt et al. (2003) |
| | Contamination of soil | |
| Polycyclic aromatic hydrocarbons | Aluminium production, coal gasification, coke production, tar and tar-ralated products | Bosetti et al. (2007) |
| Alkylating agents | Cyclophosphamide | Knight et al. (2004) |
| | Carcinogenic metabolite (acrolein) | Tanguay et al. (2003) |
| Coal | Coal miners | Lopez-Abente et al. (2006) |
| Schistosomiasis | *Schistosoma haematobium* | Gouda et al. (2007) |
| | Endemic in Africa, Asia and South America | Fedewa et al. (2009) |
| | Squamous-cell carcinoma | |

DNA-glycosylase (OGG1), apurinic/apyrimidinic endonuclease 1 (APE1) and xenoderma pigmentosum A (XPA) (Romanenko et al., 2002), overexpression of p38 mitogen-activated protein (MAP) kinase and cytoplasmic retention of NF-κB (Romanenko et al., 2003), as well as epigenetic alterations, such as aberrant DNA methylation (see Section 3.2) (Franco et al., 2008).

A remarkable observation is that individuals with seemingly equal exposure to environmental carcinogens vary enormously in their risk of developing cancer. This is attributed probably to the fact that the carcinogenic detoxification system and the DNA repair capacity vary within the human population. Much interest is concentrated on the significance of genetic polymorphism in the glutathione S-transferase (GST) supergene family involved in cellular metabolism and in detoxification of cytotoxic and carcinogenic products. In a recent study, involving 731 bladder cancer patients and 740 control subjects, GSTM1, GSTT1 and GSTP1 null genotypes were associated with a 19–48% increase in odds ratio (OR) of bladder cancer (Yuan et al., 2008). Similarly, slow and rapid acetylators of aromatic amines demonstrate variable susceptibility to TCC. Current evidence suggests a relationship between N-acetyltransferase 2 (NAT-2) and more pronounced urothelial damage (Rouissi et al., 2009; Song et al., 2009). In addition, a study designed to evaluate the importance of genetic instability in developing bladder cancer, strongly suggested that individuals better able to resist DNA damage are less susceptible to TCC (Schabath et al., 2003). Accordingly, polymorphisms of the X-ray repair cross-complementing 1 (XRCC1) and the human oxoguanine glycosylase 1 (hOGG1) genes that encode enzymes of the base excision repair (BER) mechanism for repairing DNA damage, are risk factors of bladder cancer (Arizono et al., 2008). In addition, single nucleotide polymorphisms (SNPs) in the 8-oxoG DNA-glycosylase (OGG1), the poly(ADP-ribose) polymerase family member 1 (PARP1) and the major gap filling polymerase-beta (POLB) BER genes also showed significant associations with bladder cancer risk (Figueroa et al., 2007). Some cytokines, such as the vascular endothelial growth-factor (VEGF) and the tumour necrosis factor-alpha (TNF-alpha) which are crucial for angiogenesis, local invasion and metastasis, are encoded by genes that are also polymorphic. Recently, there has been an association between the aggressiveness of TCC and certain VEGF and TNF-alpha genotypes (Kim et al., 2005). Accordingly, a large-scale evaluation of candidate cancer genes has identified three SNPs in the promoter region of VEGF that were associated with increased risk for bladder cancer (García-Closas et al., 2007).

## 3. Genetic and epigenetic alterations

### 3.1. Chromosomal aberrations

The characteristic behaviour of bladder cancer has given the opportunity to investigate the genomic alterations present in newly diagnosed and recurrent tumours, of various grades and stages. Numerous structural and numerical changes have been identified, some representing an early step, while others seem to be related with progression. Two general observations that came from studies based on comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) arrays are that tumours of different stages demonstrate different chromosomal aberrations and that higher rate of chromosomal aberrations are present in muscle invasive tumours (stages ≥T2) compared to superficial tumours (stages Ta, T1) (Blaveri et al., 2005; Koed et al., 2005). Moreover, it seems that gains and amplifications generally predominate over deletions in advanced-stage tumours. The above mentioned aberrations could lead to fusion products and possibly to altered expression of oncogenes or tumour suppressor genes (see Section 3.4).

The most frequent chromosomal aberrations found in all grades and stages of papillary and solid TCC, is loss of heterozygosity (LOH) at chromosome 9 (Hirao et al., 2005). Deletions of chromosome 9 have also been demonstrated in histological normal, hyperplastic and dysplastic urothelium. These findings support the hypothesis that aberrations of chromosome 9 probably represent an early, or even the initial event in urinary bladder carcinogenesis (Abraham et al., 2007). In most of the cases the chromosome's long arm is affected (9q22, 9q32–33, 9q34). According to Knudson's hypothesis, the above observations suggest that one or more tumour suppressor genes could be located on the long arm of chromosome 9. Candidate genes include the *ptch1*, the *tsc1* (already implicated in other cancers) and the *dbc1*. The *ptch1* gene, also known as the Gorlin Syndrome gene, is found within a small region of deletion at 9q22 and is a tumour suppressor of the hedgehog pathway (Aboulkassim et al., 2003). The tuberous sclerosis complex gene 1 (*tsc1*) which is located at 9q34 encodes hamartin, a protein that together with tuberin negatively regulate the mTOR (mammalian target of rapamycin) pathway. Germline mutations of *tsc1* have been associated with tuberous sclerosis and familial hamartoma syndrome. Finally, the *dbc1* gene, at 9q33, probably exerts non-apoptotic cell death and stall in the G1 phase (Lopez-Beltran et al., 2008).

Deletions at the short arm of chromosome 9 have been also found in TCC of the urinary bladder. Studies are focused on *cdkn2a* at 9p21, a tumour suppressor most frequently inactivated by homozygous deletion (Chapman et al., 2005). The *cdkn2a* locus encodes two proteins, p16 and $p14^{ARF}$, both of which are considered key cell cycle regulators. Other chromosomal aberrations found in early-stage tumours include deletions of 8p, 11p and gains of 1q and 17q (Qin et al., 2006).

As mentioned above, the progression of a superficial bladder tumour to muscle invasive disease is associated with the occurrence of a wide spectrum of genetic alterations. This results in major genetic divergence even in synchronous, adjacent tumours. Yet, strong genetic similarities have been documented among invasive TCCs irrespectively of the depth of invasion. The above observation suggests that invasion of the lamina propria could be the hallmark of a more aggressive behaviour, with subsequent further local invasion and ultimately formation of distant metastases. In general, the chromosomal aberrations found predominantly in invasive tumours are deletions of 6q, 11p, 18q and gains of 1q, 8q and 17q. Some of these aberrations reflect alterations in known genes (deletions of 17p13 and 13q14 result in inactivation of *p53* and *Rb1* respectively), whereas the significance of others remains vague (Chan et al., 2009).

### 3.2. Aberrant DNA methylation

Many genes have CpG-rich regions that are grouped in clusters known as CpG islands, found within or near the promoter regions. Hypermethylation of these regions results in loss of gene expression and gene silencing. Several tumour suppressor genes associated with TCC of the urinary bladder demonstrate aberrant methylation and subsequent down-regulation and silencing of gene expression. Among them, $p16^{INK4A}$ is the one most thoroughly studied. This tumour suppressor alts progression from G1 to S phase by inactivating cyclin-dependent kinase 4 and cyclin-dependent kinase 6. Methylation of $p16^{INK4A}$ has been found in frequencies ranging from 7% to 60% of TCCs of the urinary bladder. On the contrary, methylation of the $p15^{INK4b}$ gene which is located in the same locus has been reported to occur only in a small subclass of TCCs, suggesting that there are other mechanisms responsible for its inactivation (Brait et al., 2008; Catto et al., 2005).

The methylation status of 10 different genes was examined from 98 bladder cancer samples (Maruyama et al., 2001). According to

the results of this study, four genes were most frequently methylated: *cdh1*, *cdh13*, *rassf1a* and *apc*. Moreover, methylation of *cdh1*, a member of the cadherin family encoding a calcium dependent cell to cell adhesion glycoprotein, was associated with shortened survival. It is presumed that loss of *cdh* function leads to cancer progression by increasing proliferation rate or by promoting local invasion and metastasis. In a second study, both tissue and urine samples were used to examine the methylation status of *lama3*, *lamb3* and *lamc2* (Sathyanarayana et al., 2004). These genes encode a family of extracellular matrix glycoproteins (laminins) implicated in cell adhesion, differentiation, migration and signalling. According to the results of this study, there was an association between methylation of these genes and overall poor prognosis. Moreover, the methylation status of specific p53 target genes was determined by performing quantitative methylation-specific real-time PCR (Christoph et al., 2006). Hypermethylation of the promoter region in tumour samples from 110 tumour patients was detected for apoptotic protein-activating factor (APAF-1) (100%), death-associated protein kinase (DAPK-1) (74%) and insulin-like growth-factor-binding protein-3 (IGFBP-3) (66%), but not for Caspase 8 (CASP-8) (3.6%). Importantly, the APAF-1 and IGFBP-3 methylation levels were able to distinguish tumours with higher recurrence risk from low-risk tumours, thus acting as valuable prognostic markers for reappearance and progression of the disease. Similar results were presented in a recent study, performed in 34 patients with TCC of the bladder (Christoph et al., 2007).

To conclude, methylation takes place early in the process of carcinogenesis and can be detected by non-invasive methods in both serum and urine samples. Furthermore, methylation always occurs in the same DNA location and thus it is easier to be detected compared to other genetic biomarkers (e.g. gene mutations). These characteristics render it as a potential diagnostic tool for the early diagnosis of TCC. Compared to conventional urine cytology, the use of methylation markers seems more sensitive and specific especially for low grade tumours, where cancer cells are more cohesive and not readily shed into the urine. The methylation profile of the promoters of several tumour specific genes has been analysed, as described above, providing invaluable information regarding the progress and the outcome of the disease. Nevertheless, further investigation is needed for routine clinical application of methylation markers (Esteller, 2005).

### 3.3. Altered microRNAs expression

MicroRNAs (miRNAs) are about 22 nucleotide long non-protein coding RNAs that regulate gene expression by interacting with messenger RNAs (mRNAs) and induce target mRNA cleavage and translational repression (Tang et al., 2008). Aberrant miRNAs expression, up-regulation or down-regulation, has been linked to the process of tumourigenesis (Giannakakis et al., 2007). Recent studies reported that specific miRNAs play a critical role in TCC development and progression. These findings are presented in Table 2. Firstly, the differential expression of these miRNAs can act as a primary invaluable marker to distinguish cancer from normal tissue samples. Moreover, a miRNA expression profile analysis may provide information regarding the stage of the disease and be indicative for cancer progression and prognosis. miRNAs can act either as tumour suppressors (miR-17-5p, miR-21, miR-126, miR-221), or as oncogenes (miR-26a, miR-29c, miR-30c, miR-30e-5p). Although, their exact target genes, the molecular pathways they are involved and the clinopathological outcome of their abnormal expression, have not yet been fully elucidated, the miRNAs still represent attractive candidates for gene therapy (Adam et al., 2009). One delicate approach that has recently been developed is the design and application of synthetic antisense oligonucleotides that specifically target oncogenic miRNAs and suppress their expression. The use of these antisense oligonucleotides, also termed antagomirs, appears to be effective against miR-221/222 overexpression, impairing the growth of prostate carcinoma xenografts in mice (Mercatelli et al., 2008). Similarly, injection of the antagomir-17-5p could abolish tumour growth in a neuroblastoma mice model (Fontana et al., 2008). These promising findings indicate that the antagomir treatment will to be a powerful therapeutic strategy against cancer in the near future.

**Table 2**
Altered expression of miRNAs in bladder cancer.

| miRNA | Putative target gene | Reference |
|---|---|---|
| (a) Up-regulation | | |
| miR-10a | FGFR3 | Veerla et al. (2009) |
| miR-17-5p | c-Myc | Gottardo et al. (2007) |
| miR-21 | PTEN | Dyrskjøt et al. (2009) |
| miR-23a | | Gottardo et al. (2007) |
| miR-23b | | Gottardo et al. (2007) |
| miR-26b | | Gottardo et al. (2007) |
| miR-30-3p | KRT7 | Ichimi et al. (2009) |
| miR-103-1 | HOX | Gottardo et al. (2007) |
| miR-125b | | Veerla et al. (2009) |
| miR-126 | EGFL7 | Saito et al. (2009) |
| miR-129 | GALNT1 and SOX4 | Dyrskjøt et al. (2009) |
| miR-133a | KRT7 | Ichimi et al. (2009) |
| miR-143 | Ras | Lin et al. (2009) |
| miR-182 | | Hanke et al. (in press) |
| miR-185 | | Gottardo et al. (2007) |
| miR-199a* | KRT7 | Ichimi et al. (2009) |
| miR-203 | | Gottardo et al. (2007) |
| miR-205 | | Gottardo et al. (2007) |
| miR-221 | $p27^{kip}$ | Gottardo et al. (2007) |
| miR-222 | $p27^{kip}$ | Veerla et al. (2009) |
| miR-223 | | Gottardo et al. (2007) |
| miR-452 | | Veerla et al. (2009) |
| (b) Down-regulation | | |
| miR-7 | FGFR3 | Veerla et al. (2009) |
| miR-26a | | Wang et al. (in press) |
| miR-29c | | Wang et al. (in press) |
| miR-30c | | Wang et al. (in press) |
| miR-30e-5p | | Wang et al. (in press) |
| miR-31 | | Veerla et al. (2009) |
| miR-145 | | Dyrskjøt et al. (2009) |

### 3.4. Loss of p53 and retinoblastoma gene function

Abnormalities of TP53 have been well described in various malignancies, and germline mutations are associated with the Li-Fraumeni syndrome (Brosh and Rotter, 2009). Alterations of both the P53 and retinoblastoma (RB) molecular pathways play a major role in human bladder carcinogenesis (Shariat et al., 2004). The P53 tumour suppressor protein is encoded by the *p53* gene, located at chromosome 17p13.1. The *p53* gene has multiple functions, with a central role in tumour suppression by initiating apoptosis or inducing cell arrest at the G1/S phase in response to DNA damage through the induction of *p21waf1/cipi*. While most bladder cancers exhibit a loss of a single 17p allele, an additional mutation in the second, wild-type allele can inactivate *p53* leading to increased nuclear accumulation of the corresponding protein and loss of its tumour suppressor function. The most frequent alteration involves exons 5–11 of *p53*, especially codons 280 and 285 and the loss of *Rb* gene function. A recent study performed in 75 non-invasive urinary bladder cancer patients showed that tumour recurrence frequency was 69.4% in patients with wild-type *p53*, and 88.5% in patients with mutant *p53*. Moreover, the progression-free survival was significantly shorter in patients with *p53* mutations, and the frequency of tumour progression was significantly higher in mutated as compared to wild-type tumours (Ecke et al., 2008). Another mechanism for *TP53* gene inactivation is the overexpression of the murine double minutes (*mdm2*) oncogene. The MDM2 protein promotes the

transcriptional inactivation as well as the proteasomal degradation of P53. MDM2 overexpression has been associated with increasing stage and grade of TCC (Simon et al., 2002).

Alterations in the Rb pathway are also commonly found in the development of invasive urinary bladder cancer. The *Rb* gene is located at chromosome 13q14 and its product is a nuclear phosphoprotein, which plays a critical role in several pathways involved in urothelial carcinogenesis, including development, differentiation, cell cycle restriction, senescence and apoptosis. The active form (dephosphorylated) of the RB protein binds to the transcription factor E2F, thereby sequestering it. When RB is competitively phosphorylated, it releases E2F, which is able to bind its target genes and promote cell cycle progression. It has been demonstrated the significant association between *Rb* loss, tumour stage, and tumour grade (Gallucci et al., 2005). In parallel, it has been shown that amplification and overexpression of *E2f3* is also associated with increased tumour stage, grade and proliferation index in human bladder cancer (Olsson et al., 2006). Interestingly, a recent study linked *Rb* inactivation and overexpression of *E2f3* isoforms, *E2f3a* and *E2f3b*, to amplification of 6p22 in bladder tumours (Hurst et al., 2008). This genomic region contains 4 genes (prl, sox4, E2f3 and cdkal1) and appears to play a critical role in tumour progression.

## 4. Molecular profiling

Several investigations have clearly demonstrated that bladder cancer cannot be managed solely on the basis of pathologic staging. In addition, the ability of the current staging system to estimate the risk of adverse clinical outcome for an individual patient is limited. Similarly, the existing therapeutic methods offer long-term recurrence-free survival, only in a subset of patients (Table 3). Namely, transurethral resection (TUR) can achieve complete resection of non-muscle invasive tumours, but has no influence on the natural history of the disease. Radical cystectomy for T2-T4a tumours offers a 5-year recurrence-free survival in just 68% of patients (Stein et al., 2001), whereas the currently used chemotherapeutic regimens offer long-term disease-free survival in only 15% of patients with metastatic disease. Currently, several new therapies are been tested complementary to the established methods of treatment in order to increase effectiveness. A recent study demonstrated increased sensitivity to Bacillus Calmette–Guerin (BCG) induced TRAIL release after intravesical $H_2O_2$ instillations (White-Gilbertson et al., 2009).

Undoubtedly, a thorough insight into the involved pathways and analysis of the combined effects of several validated markers is required to develop clinically valuable tests and new targeted therapies. As carcinogenesis is a multi-step process, synergistic therapeutic regimens that are aimed at multiple targets are more promising than targeting a single step of a pathway. Over the past years, several members of these pathways have proved to be useful for diagnosis, early detection, therapeutic monitoring and staging of bladder cancer. A big challenge in that field is now the use of cDNA microarrays that allows monitoring simultaneously the expression level of thousands of selected known genes as well as cDNAs representing uncharacterized genes, in one hybridization experiment (Sánchez-Carbayo, 2003).

In an independent study, cDNA microarrays containing 17,842 known genes and expressed sequence tags were used to identify differences in gene expression between normal mucosa, non-muscle invasive (early-stage), and muscle invasive (late stage) bladder tumours (Sánchez-Carbayo et al., 2003). Differential expression of several genes, including *cytokeratin 20*, *neuropilin-2*, *p21*, and *p33ING1* was detected and validated by immunohistochemistry using tissue microarrays. Importantly, the expression patterns of the above genes were significantly associated with pathological stage, tumour grade, and altered *Rb* expression. Thus, they represent ideal molecular biomarkers of BC progression and potential targets of novel therapeutics. In a similar study, a high-density oligonucleotide microarrays analysis (59,619 genes and expressed sequence tags) was performed to identify gene clusters associated with BC stage and grade (Dyrskot et al., 2005). Using a cross-validation approach, a 45-gene molecular signature was detected, predicting disease progression and recurrence of pTa tumours. Differentially expressed genes were involved in regulating apoptosis (*birc4* and *birc6*), cell differentiation, and cell cycle progression (*mcm7*, *cdc20*). Significantly, this molecular signature has recently been statistically validated in a multicenter analysis of tumour samples from 404 patients diagnosed with BC (Dyrskot et

**Table 3**
Treatment of TCC of the urinary bladder.

| Stage | Treatment | Comment |
|---|---|---|
| CIS | Intravesical BCG + TUR | Standard treatment |
| | Cystectomy | Patients with incomplete response/recurrence |
| Ta–T1 | TUR | Low-risk patients (followed by a single intravesical dose of chemotherapeutic) |
| | TUR + intravesical chemotherapy | Intermediate-risk patients |
| | TUR + intravesical BCG | High-risk patients |
| | Radical cystectomy | In BCG failure, multiple recurrent, high grade tumours, concomitant CIS |
| T2–T4a | Radical cystectomy | Standard treatment |
| | | 68% recurrence-free survival at 5 years |
| | External beam radiotherapy | Patients unfit for cystectomy |
| | Multimodality treatment | TUR + chemotherapy + radiotherapy (comparable long-term survival) |
| | Neo-adjuvant chemotherapy + radical cystectomy | 5–7% improved overall survival |
| T4b | Radical cystectomy | Palliative |
| N+ | Radical cystectomy + adjuvant chemotherapy | Under debate |
| M+ | Chemotherapy | MVAC (1st line) |
| | | Carboplatin (unfit patients) |
| | | Paclitaxel/gemcitabine (2nd line) |
| Therapeutic prospects | | |
| | Intravesical gene therapy | Non-muscle invasive tumours |
| | | Limitations in gene delivery to the urothelium |
| | Blocking VEGF/VEGFR | Bevacizumab (in phase II clinical trial) |
| | Inhibitors of COX-2 | Chemoprevention |
| | Intravesical $H_2O_2$ | Reverses resistance to TRAIL |
| | | Adjuvant to BCG |

al., 2007). Although more similar studies are needed, that include large number of clinical samples and detailed history data, it is evident that molecular profiling of bladder cancer will become an invaluable tool to accurately predict the progression of the disease and the treatment response, as well as to better guide patient treatment and therapy.

## 5. Conclusion

The behaviour of transitional cell carcinoma of the urinary bladder is highly diverse and defined by two separate, but related processes: tumour recurrence and progression. In order to optimize management, patients are categorized into low, intermediate and high-risk groups. The most useful prognostic parameters are tumour grade, stage, architecture (papillary vs. solid), and the synchronous presence of urothelial dysplasia or cancer in situ. Nevertheless, neither recurrence, nor progression rate can be predicted accurately by physiological means alone. It is apparent, that the elucidation of the molecular mechanisms of the disease is vital. In this vein, a significant amount of studies have been performed over the past years, generating important information regarding the genetic and molecular pathways involved. These findings lead to the development of novel prognostic and therapeutic strategies, such as the antagomirs, synthetic antisense oligonucleotides, that specifically target oncogenic miRNAs and suppress their expression and the molecular profiling, a technology based on microarrays for the simultaneous determination of multiple molecular markers or molecular signatures. Further work is required in order to optimise these methods and validate their efficiency. However, as the molecular basis of the disease becomes rapidly understood, there are high expectations for the development of even more powerful diagnostic and therapeutic tools in the near future.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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