EDUCATIONAL SERIES Blue series*

Clin Transl Oncol (2007) 9:5-12
DOI 10.1007/s12094-007-0003-x

Molecular biology of bladder cancer

Nuno M. Luis[a], Elena López-Knowles[a] and Francisco X. Real[a,b]

[a]Unitat de Biologia Cel.lular i Molecular. Institut Municipal d'Investigació Mèdica. Barcelona. Spain.
[b]Universitat Pompeu Fabra. Barcelona. Spain.

Bladder cancer is a major cause of health expenses and it presents formidable clinical challenges. Two types of tumors have been identified, papillary and non-papillary. The former are mainly characterized by *FGFR3* and chromosome 9 alterations and a low frequency of *Tp53* alterations. The latter are characterized by a high frequency of alterations in genes in the p53 and Rb pathways. Chromosome 9 alterations, specially in 9q, are crucial to bladder cancer development and occur in both types of tumors. Progression of some superficial tumors (mainly TaG3 and T1G3) to high-grade, invasive, carcinomas provides evidence of some overlap between the two pathways. Distinct gene expression profiles have been identified in superficial and invasive tumors. The stage is now ready for the clinical application of this knowledge.

Key words Bladder • FGFR3 • Genetic alterations • p53 Rb • Urothelial carcinoma

Luis NM, López-Knowles E, Real FX (2007) Molecular biology of bladder cancer. Clin Transl Oncol 9:5-12

INTRODUCTION

Bladder cancer is a major health burden in the Western world. In 2002, approximately 139,000 new bladder cancer cases were diagnosed in Europe, the male:female ratio being 3.7:1. In the same year, approximately 51,000 patients died from this disease[1]. In Spain, it is the 5th leading cause of cancer-related death in men and the 13th in women. Bladder cancer generates the highest medical cost per patient and it is the fifth most expensive cancer in terms of total medical care expenditures.

The two best established lifestyle/environmental risk factors for bladder cancer are smoking and occupational exposure to aromatic amines (i.e. dye, textile, leather, chemical and rubber industries)[2]. Therefore, this tumor is a largely preventable disease.

BLADDER TUMORS

Approximately 95% of malignant bladder tumors are urothelial cell carcinomas (UCC) which can be classified as papillary (most common type, tend to grow slowly towards the lumen), solid (less frequent, infiltrate the bladder wall and are more agressive) or Carcinoma In Situ (CIS, a very agressive kind of cancer that involves only the inner lining of the bladder).

Staging and grading

The bladder is a hollow and distensible organ that sits on the pelvic floor and collects urine from the kidneys. A scheme of the bladder wall is represented in figure 1.

Tumors are classified according to depth of invasion into superficial (Ta and T1) and invasive (T2, T3, T4). pTa tumors are defined as those which have an exophytic «fingerlike» growth and do not grow beyond the urothelium; pT1 tumors invade the *lamina propria* but not the *muscularis propria*; pT2 tumors as those invading the *muscularis propria*; pT3 tumors as those invading perivesical tissue; and pT4 tumors as those invading other organ structures.

Grading is used to evaluate the cytological and/or growth pattern characteristics of the tumor and it is an important predictor of its biologic potential[3].

1. Flat and papillary hyperplasia. Flat hyperplasia consists of a markedly thickened mucosa without cytologic atypia. Papillary hyperplasia is characterized by urothelium of variable thickness exhibiting a slightly undulating growth.

*Supported by an unrestricted educational grant from Sanofi-Aventis.

Correspondence: F.X. Real.
Unitat de Biologia Cel.lular i Molecular.
Institut Municipal d'Investigació Mèdica.
Carrer del Dr. Aiguader, 88.
08003 Barcelona. Spain.
E-mail: preal@imim.es

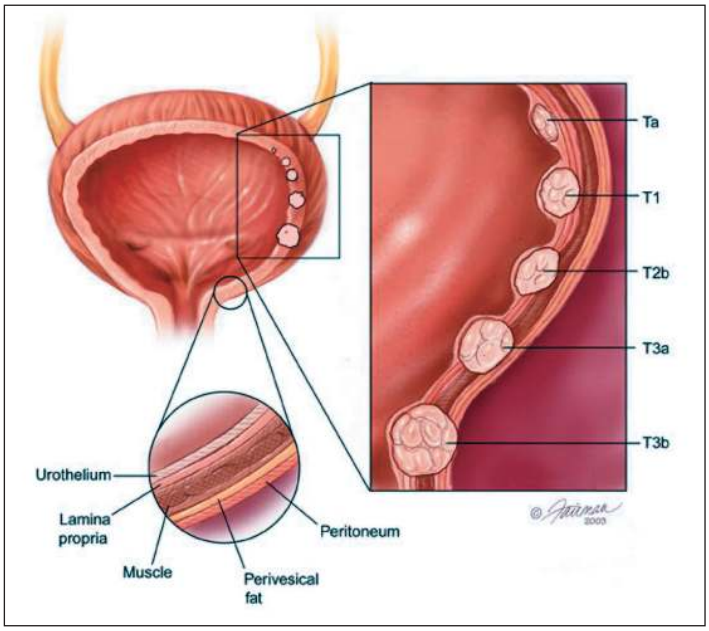

Fig. 1. Bladder tumors are classified on the basis of the degree of involvement of the bladder wall and the nuclear grade. Ta tumors do not invade the basal membrane and T1 tumors do not invade muscle. This classification is of prognostic and therapeutic importance (Adapted from The University of Michigan, Department of Urology at the Michigan Urology Center).

2. Flat lesions with atypia can be classified as reactive atypia, dysplasia or CIS. Dysplasia indicates cytologic and architectural abnormalities.
3. Papillary neoplasms can be classified as papilloma, papillary neoplasm of low malignancy potential (PUNLMP), low grade papillary carcinoma and high grade papillary carcinoma. In papilloma, normal appearing urothelium lines papillary fronds. PUNLMP refers to thickened urothelium in the absence of cytologic features of malignancy. Low grade papillary carcinoma exhibits an overall orderly appearance and has minimal anomalies in architecture and/or cytologic features. High grade papillary carcinoma has a disorderly appearance with marked architectural and cytologic abnormalities.

Natural history of bladder tumors: two pathways, clonal origin

UCC is a heterogeneous disease with a variable natural history. Low-grade Ta tumors have a very low progression rate and rarely present a threat to the patient. At the other extreme, high-grade tumors imply a high risk of progression and cancer death.

Two pathways

It has been proposed that there may be at least two separate pathways leading to bladder cancer, papillary and nonpapillary[4]. Papillary lesions arise in hyperplastic urothelium whereas invasive tumors arise from dysplastic urothelium. The overlap between both pathways is reflected by progression of superficial papillary tumors to high grade invasive carcinomas in some cases. Papillary tumors are commonly associated with alterations in genes involved in the RAF/MEK/ERK pathway (i.e. *FGFR3*)[5,6] and in *PIK3CA*[7]. By contrast, non-papillary invasive tumors are commonly associated with p53 and pRb pathway alterations (fig. 2).

Clonal origin

The development of multiple tumors in either a synchronous or metachronous manner in the same patient is a common characteristic of UCC. Two theories have been proposed to account for these findings: tumors may be monoclonal in origin (multifocal tumors evolving from a single transformed cell) or they may be oligoclonal (a change in the urothelium giving rise to multiple clones of initiated cells subsequently evolving into independent tumors). Current evidence supports the notion that most tumors are monoclonal but up to 30% of tumors may indeed be oligoclonal[8,9].

Genetic alterations in bladder cancer

For many years, it has been clear that UCC represents a complex disease and that it is a paradigm of tumor progression. The current status of knowledge on the molecular biology of bladder cancer is presented, pointing out the differences between papillary and invasive bladder tumors.

MOLECULAR GENETICS OF PAPILLARY BLADDER TUMORS

Fibroblast growth factor receptor 3 (FGFR3)

FGFRs (1-4) regulate proliferation, differentiation, angiogenesis and embryonic development. They display distinct tissue expression, ligand specificity, signal

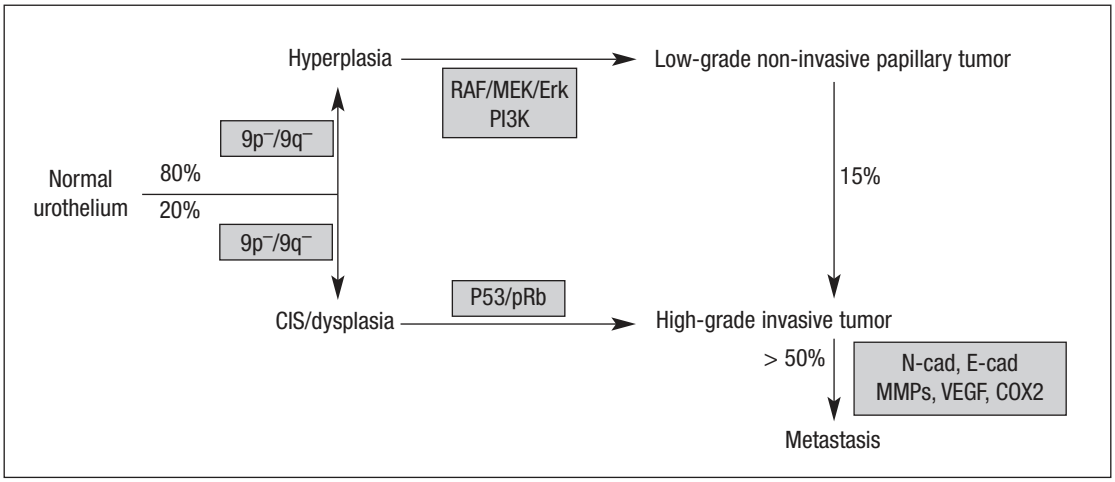

Fig. 2. Bladder tumors arise through two different molecular pathways (dual track concept). Papillary tumors are preceded by urothelial hyperplasia whereas high grade, muscle-invasive tumors, are preceded by dysplasia and/or carcinoma in situ. Chromosome 9 alterations are thought to be an early event of both pathways. Mutations in genes involved in the MEK pathway (i.e. *FGFR3*) and *PIK3CA* are common in low-grade papillary tumors. Mutations in genes involved in the p53 and Rb pathways are crucial for muscle-invasive tumors. Altered expression of genes involved in cell adhesion, proteases, and angiogenesis plays an important role in metastases.

pathway activation, and biological effects. FGFR mRNAs are commonly subject to alternative splicing. There are two major isoforms of FGFR3 transcripts that are generated from a mutually exclusive splicing event in which the second half of the third Ig-like domain is encoded by either the 151 bp of exon 8 (FGFR3b, predominant in epithelia) or the 145 bp of exon 9 (FGFR3c, predominant in mesenchymal cells). Inherited skeletal dysplasias have been linked to activating germline point mutations in *FGFR1*, 2 and 3. Germline point mutations in *FGFR3* cause the short limb syndromes of achondroplasia, hypochondroplasia and thanatophoric dysplasia. Mutations lead to constitutive receptor activation; increasing activity is associated with increasing severity of the disease[10,11]. Activating *FGFR3* mutations occur in 50-60% of UCC and are associated with papillary, low grade, superficial tumors[5]. Somatic mutations are restricted to a few codons, essentially the same that are mutated in the germline in short limb syndromes. Mutations in the extracellular domain (i.e. codons 248, 249) or within the transmembrane domain and its vicinity (i.e. codons 372, 375, 382, 393) lead to receptor dimerization in the absence of ligand; mutations in the intracellular domain (codon 652) confer constitutive kinase activity. Mutations are associated with an increased risk of recurrence and a lower risk of progression among patients with superficial tumors, indicating that they are a marker of tumors with good prognosis[12].

Ras genes

H-*ras*, K-*ras*, and N-*ras* code for monomeric GTPases that can activate the RAF/MEK/ERK and PI3K/AKT/PTEN cascades. Oncogenic Ras mutations make the GTPase insensitive to the action of activating proteins (GAPs) and lock it in the GTP-bound, active state. In UCC, point mutations have been identified in codons 12, 13 and 61 in H-*ras* and in N-*ras* at an overall frequency of 10-20%. *FGFR3* and *Ras* mutations are mutually exclusive, possibly because they both activate the MAPK pathway. *Ras* mutations do not seem to be associated with stage or grade[13].

Amplification of the gene coding for Raf1, a kinase involved in Ras protein signalling, has been associated to grade, stage and poor survival and overexpression has been associated to grade, but not to stage[14,15].

PIK3CA and TSC1

The phosphatidylinositol-3-kinase (PI3K) pathway is crucial to many aspects of cell growth and survival. Class IA proteins are most important in proliferation and tumorigenesis; they are constituted by a heterodimer of p110, the catalytic subunit, and an adaptor subunit (p85, p50 or p55). *PIK3CA*, which encodes p110α, maps to 3q26, a region amplified in UCC. *PIK3CA* mutations have been reported in 20% of tumors, in association with low stage and grade tumors and with *FGFR3* mutations[7]. There is some evidence that UCC can also overexpress PI3K in comparison with adjacent normal epithelium.

TSC1 (9q34) encodes hamartin, a GAP that negatively regulates cell cycle progression. Its action is blocked by AKT and activation of the PI3K pathway. Inactivating TSC1 mutations are found in 12% of UCC and LOH at 9q34 occurs in 32% of cases[16,17]. Stage and grade do not seem associated with these alterations.

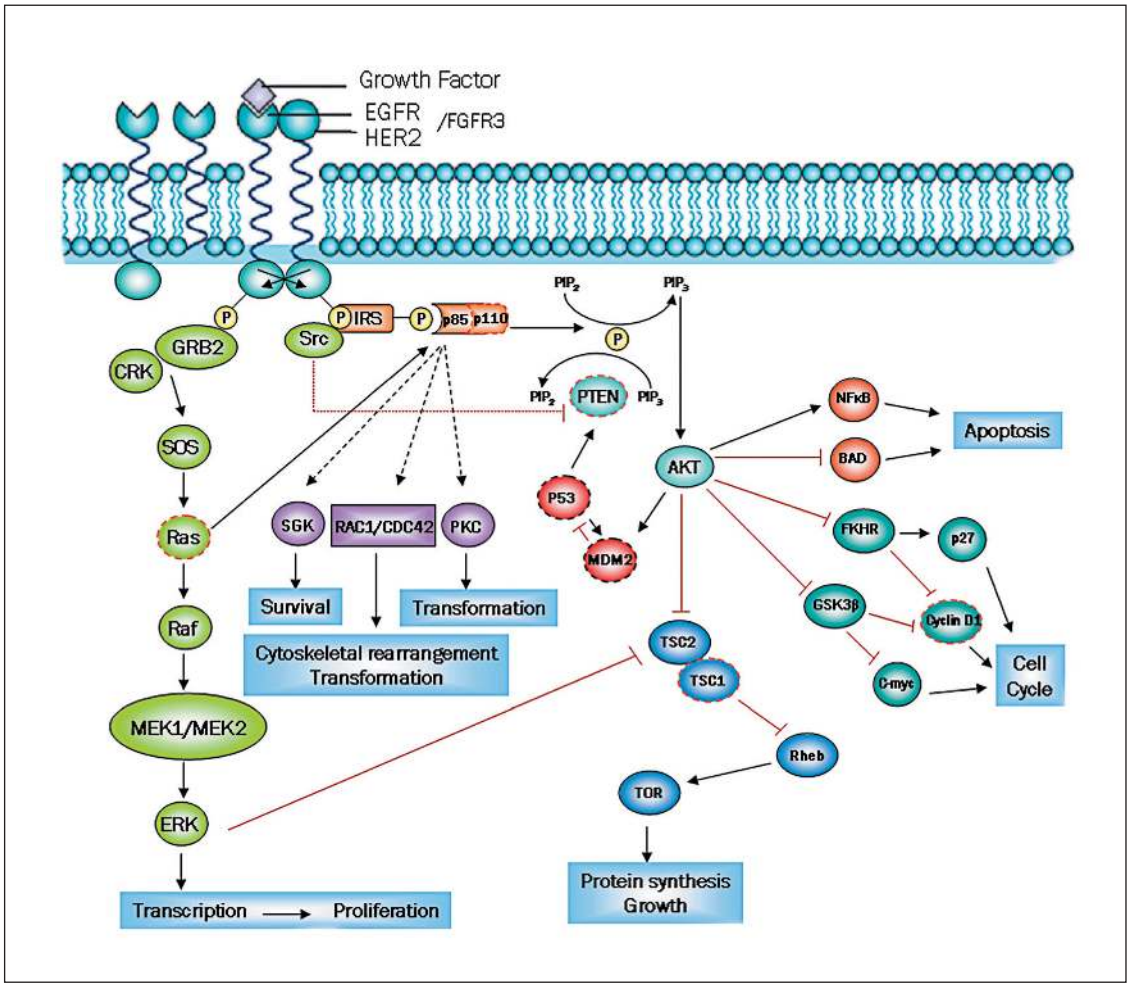

Fig. 3. Receptor tyrosine kinases are activated by ligand and, through the recruitment of adaptor proteins containing SH2 domains, signal to Ras and the ERK/MAPK pathway which is mainly involved in proliferation. In the case of FGFR3, specific adaptors such as Frs-2 have been implicated. In addition, they can signal to the PI3K pathway thus leading to survival and inhibition of apoptosis. The Akt kinase mediates these effects by modulating the activity of several proteins such as NF-κB, GSK-3β, and mTOR. PI3K is also involved in the regulation of cell motility and cytoskeletal remodelling.

MOLECULAR GENETICS OF INVASIVE TUMORS

Virtually all human tumors deregulate either the Rb or p53 pathway or both and this is also the case for invasive UCC.

Rb pathway alterations

The Rb pathway is responsible for regulating the passage from G1 to S phase. Phosphorylation of pRb by CDK4/6 and CDK2 dissociates the pRb-repressor complex, leading to the release of bound E2F from pRb. «Free» E2F is then active and drives the transcription of S-phase genes encoding for proteins required for G1 to S phase transition and DNA replication.

Mutations of the *Rb1* gene have been described in UCC but the studies are not extensive due to the large gene size. There is controversy about the prognostic value of pRb expression in tumor tissues[18-20].

p16, encoded by the *INK4A/ARF* locus, is a CDK4/6 inhibitor which blocks the phosphorylation of pRb. The *INK4A/ARF* locus plays a central role in tumor suppression and it is inactivated in approximately 50% of human cancers. mRNA levels of $p16^{INK4A}$ and $p14^{ARF}$ are undetectable in normal urothelium and increase with stage and grade[21]. At the protein level, loss of p16 has been associated to minimally invasive bladder cancer. $p16^{INK4A}$ and $p14^{ARF}$ methylation have been proposed as biomarkers of stage, clinical outcome, and prognosis.

E2F1 expression has been correlated with proliferation in UCC, having a growth promoting effect. The ***E2F3*** locus is amplified in invasive tumors[22] and its product is overexpressed in 33% of UCC[23].

Cyclin D1 overexpression occurs frequently in UCC and has been proposed to be associated with the growth of low-grade papillary tumors[24].

p53 pathway alterations

The p53 pathway plays a major role in the response to DNA damage, oncogenic stress, and other types of cellular stress. p53 induces G1 arrest by upregulating p21, G2 arrest by upregulating GADD45, 14-3-3σ and p21, and apoptosis by upregulating genes such as Bax, NOXA, and PUMA. In addition, it can repress genes such as *c-myc* to promote G1 arrest and *cyclin B1* to promote G2 arrest[25].

The *Tp53* gene is frequently altered in UCC: 270 different mutations have been registered in the IARC database[26] up to October 2006, of which 262 (97%) are in exons 4 to 9. The most common mutations are missense (72.5%), 12.2% and 5.55% being nonsense and silent, respectively. The main hotspots are codons 285, 248, 280, 175 and 213. *Tp53* mutations are significantly more frequent among high grade and stage tumors; their prevalence ranging from 14-52% depending on the T and G subgroup considered[27]. Overal, *Tp53* mutations and p53 nuclear overexpression are uncommon in TaG1/G2 tumors and are frequent in high grade/invasive tumors[28.] *HDM2*, a p53 target gene and regulator, is gained/amplified in approximately 9% of high grade tumors.

p63 and *p73* are members of the *p53* family. *p63* maps to 3q27-29, a region that is amplified in UCC; this locus encodes multiple proteins resulting from alternative splicing that transactivate p53 responsive elements or act as dominant negatives towards p53 and p73. p63 is lost in most invasive tumors. A decrease of the levels of p73 has also been associated to bladder cancer stage[29].

Two additional important genes in the p53 pathway are Aurora A kinase *(STK6)* and *p21*. Overexpression of Aurora A (20q13), often associated with gene amplification, leads to increased degradation of p53, causing down-regulation of checkpoint-response pathways and facilitating oncogenic transformation. Expression of p21 is reduced in muscle invasive tumors compared to non-invasive tumors[30].

A few studies have combined the analysis of p53, p16, p21 and pRb expression in bladder tumors and described that they act in cooperative or synergistic ways to promote progression: p53/p16, p53/p21, p53/pRb or p53/p21/pRb[18,31-34].

PTEN

This gene maps to 10q23 and it encodes a lipid phosphatase that acts as a negative regulator of PI3K pathway by hydrolyzing 3,4,5-PIP3 to 4,5-PIP2. PTEN physically interacts with p53 in the nucleus, leading to p53 stabilization and increased transcriptional activity. *PTEN* is mutated or deleted in 14% of invasive bladder cancers with 40% LOH at 10q[35,36]. *PTEN* downregulation has been described in 13% of tumors, mainly muscle-invasive tumors.

GENOMIC LEVEL ALTERATIONS

Chromosomal aberrations can be primary, related to the cause of a tumor, or secondary, involved in progression. Deletions and gains/amplifications contribute to altered expression of tumor suppressor genes and oncogenes, respectively. Higher rates of genomic alterations are present in pT1 than in papillary pTa tumors. The most consistent alterations in advanced-stage UCC are gains of 1q, 8q and 20q and losses of 8p, chromosome 11 and 9. Array CGH analysis has been applied to the study of UCC[37-39]. A summary of the most common alterations reported, classified on the basis of their chromosomal location, follows.

Alterations common to both pathways (chromosome 9)

The q arm of chromosome 9 is lost both in low and high grade tumors, suggesting it is a primary event in the genesis of bladder cancer[40,41]. Losses at 9q cover 3 major deleted regions (9q22, 9q32-33, and 9q34) and one or several tumor suppressor genes may be located in them. Candidate genes therein include *Netrin*, *TSC1*, *PTCH* and *DBCCR1*[17,42-44]. Allelic loss at 9q has been reported as an early occurrence in the development of bladder cancer but it has also been associated with invasive disease and with disease recurrence in superficial bladder tumors. Deletions of 9p are also common in bladder tumors and affect mainly 9p21, where the *INK4A/ARF* locus maps[45].

Other alterations – invasive tumors

Alterations in chromosome 8 often involve loss of the p arm, gain of the q arm and amplification of a small region at 8p12. LOH in 8p is associated with a more aggressive tumor phenotype indicating the possible presence of a tumor suppressor gene. The minimal region of 8p21-22 contains several candidate genes: *TRAIL-R2*, *DBC2* and *LZTS1*[46,47]. A commonly gained region in 8q contains *c-myc*. 8p12 amplicons contain *FGFR1*.

5p amplification is one of the few alterations occurring more frequently in muscle invasive tumors than in early invasive cancers[48]. The most common site of amplification (5p12) contains *TRIO*; 5p13-12 has also been defined as a critical region of allele losses associated with tumor progression and a marker of adverse prognosis independent of stage and grade[49].

Amplification of 6p22 affects up to 20% of high grade, invasively growing tumors[39]. This region has been narrowed down to 1.6 Mb at 6p22.3 and contains potential oncogenes such as *SOX4*, *CDKAL*, *DEK*, *ID4*, and *E2F*, the latter being a strong pathogenic candidate[23,50-52].

The regions containing *EGFR1* (7p12) and *EGFR2* (17q11) are amplified in 4.6% and 3.4% of UCC, respectively; protein overexpression has been described in 48%[53] and 41%[54] of tumors, respectively. Amplification/overexpression of EGFR is associated with tumor proliferation, aggressive behaviour and poor prognosis[55].

Other common alterations in UCC are gains of 1q and 20q, amplifications of 11q13 and 12q14 (candidate genes *cyclin D1* and *HDM2*, respectively) and losses of 11p.

METHYLATION

As in other tumors, promoter methylation constitutes a common mechanism of silencing of tumor suppressor genes in UCC[56]. The frequencies of methylation of the best studied genes are: *cadherin-1* (36%), RAS-associated domain family *(RASSF1A)* (35%), *CDH13* (29%), secreted *Frizzled-related protein 1 (sFRP1)* (29%), *FHIT* (16%), *retinoic acid receptor β* (15%), $p16^{INK4A}$ (7%), and *death-associated kinase* (4%)[57]. Hypermethylation of *APC*, $p14^{ARF}$ and *RASSF1A* has also been described in exfoliated cells in the urine of patients with UCC. A recent study showed that methylation of promoter regions of *p16*, *p14*, *E-cadherin*, *RARβ2*, *RASSF1a* and *GSTP1* occurs in both normal and CIS samples from patients with UCC and increases with progression[58]. *sFRP* gene silencing by methylation has been shown to be associated to invasive bladder cancer and to overall survival[59].

EXPRESSION ANALYSES WITH MICROARRAYS

The first study analyzing gene expression in UCC arrays showed different gene expression profiles in superficial and invasive tumors[60]. The same group subsequently identified expression profiles distinguishing stages, as well as a similar expression profile between CIS and invasive tumors[61]. Data from microarrays with 10368 cDNAs allowed to identify 25 genes able to classify tumors as superficial or invasive with 90.5% accuracy[62]. This classifier had an 82.5% accuracy when used on the data set from Dyrskjot et al. The gene-classifiers reported by Blaveri and Dyrskjot had no genes in common. Other studies have also reported minimal overlap for the genes identified for clinically similar tumors. Sánchez-Carbayo et al separated superficial from invasive tumors with 82.2% accuracy and stratified tumors on the basis of clinical outcome with 82% (all tumors) or 90% accuracy (when considering only invasive tumors)[63].

APOPTOSIS

In the bladder, as in other tissues, failure of the regulatory genes involved in apoptosis may result in survival of cells with genomic abnormalities, tumorigenesis and resistance to anticancer agents. Low levels of FAS and FASL have been associated with higher grade, stage and a poor prognosis[64]. Overexpression of the antiapoptotic protein BCL-2, involved in mitochondrial permeabilization, is associated with p53 overexpression and with poor outcome[65]. Survivin, an inhibitor of caspase-3 and caspase-7, is detectable in urine and has been proposed as a biomarker for the detection of bladder cancer[66]. FHIT protein has been shown absent or reduced in 61% of UCC and its expression correlated with pathological and clinical status[67].

INVASION AND METASTASIS

Cadherins are the main mediators of cell-cell adhesion in epithelia. Loss of E-cadherin has been described at higher frequency in high-grade, invasive, UCC than in low-grade papillary tumors[68]. Hypermethylation of CpG dinucleotides in the promoter of *CDH1* (encoding E-cadherin) occurs frequently in UCC[69]. The status of E-cadherin has been proposed as an independent prognostic indicator for disease progression.

CONCLUDING REMARKS

The information accumulated in the last few years on the molecular changes associated with papillary and invasive bladder tumors allows a more accurate molecular classification of UCC. This information may help not only in the prediction of patient outcome but in the selection of treatment, as well. Bladder cancer is one of the solid tumors in which molecular studies may soon become part of standard clinical practice.

ACKNOWLEDGEMENTS

Work in the authors' laboratory was funded, in part, by grants C03/010 and G03/174 from Instituto de Salud Carlos III, Ministerio de Sanidad, Madrid. N.M.L. is recipient of a Ph.D. grant from Fundação de Ciência e Tecnologia, Portugal, and E.L. was supported by a Predoctoral Fellowship from the Ramón Areces Foundation.

References

1. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002 (2004) Cancer Incidence, Mortality and Prevalence Worldwide IARC CancerBase No. 5 version 2.0.: IARC Press, Lyon
2. Silverman DT, Devessa SS, Moore LE, Rothman, N (2006) Bladder cancer. Cancer epidemiology and prevention, 3rd edn. Oxford University Press, New York, pp 1101-1127
3. Eble J, Sauter G, Epstein JI, Sesterhenn IA (2004) Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. IARC Press, Lyon, France
4. Pugh RC (1973) Proceedings: The Pathology of Cancer of the Bladder. Cancer 32: 1267-1274
5. Billerey C, Chopin D, Aubriot-Lorton MH et al (2001) Frequent FGFR3 Mutations in Papillary Non-Invasive Bladder (PTa) Tumors. Am. J. Pathol. 158:1955-1959
6. Cappellen D, De Oliveira C, Ricol D et al (1999) Frequent Activating Mutations of FGFR3 in Human Bladder and Cervix Carcinomas. Nat Genet 23:18-20
7. Lopez-Knowles E, Hernandez S, Malats N et al (2006) PIK3CA Mutations Are an Early Genetic Alteration Associated With FGFR3 Mutations in Superficial Papillary Bladder Tumors. Cancer Res 66:7401-7404
8. van Tilborg AA, de Vries A, de Bont M et al (2000) Molecular Evolution of Multiple Recurrent Cancers of the Bladder. Hum Mol Genet 9:2973-2980
9. Hafner C, Knuechel R, Stoehr R, Hartmann A (2002) Clonality of Multifocal Urothelial Carcinomas: 10 Years of Molecular Genetic Studies. Int J Cancer 101:1-6
10. Naski MC, Wang Q, Xu J, Ornitz DM (1996) Graded Activation of Fibroblast Growth Factor Receptor 3 by Mutations Causing Achondroplasia and Thanatophoric Dysplasia. Nat Genet 13:233-237
11. Webster MK, D'Avis PY, Robertson SC, Donoghue DJ (1996) Profound Ligand-Independent Kinase Activation of Fibroblast Growth Factor Receptor 3 by the Activation Loop Mutation Responsible for a Lethal Skeletal Dysplasia, Thanatophoric Dysplasia Type II. Mol Cell Biol 16:4081-4087
12. Hernandez S, Lopez-Knowles E, Lloreta J et al (2006) Prospective Study of FGFR3 Mutations As a Prognostic Factor in Nonmuscle Invasive Urothelial Bladder Carcinomas. J Clin Oncol 24:3664-3671
13. Jebar AH, Hurst CD, Tomlinson DC et al (2005) FGFR3 and Ras Gene Mutations Are Mutually Exclusive Genetic Events in Urothelial Cell Carcinoma. Oncogene 24:5218-5225
14. Mhawech-Fauceglia P, Fischer G, Beck A et al (2006) Raf1, Aurora-A/STK15 and E-Cadherin Biomarkers Expression in Patients With PTa/PT1 Urothelial Bladder Carcinoma; a Retrospective TMA Study of 246 Patients With Long-Term Follow-Up. Eur J Surg Oncol 32:439-444
15. Simon R, Richter J, Wagner U et al (2001) High-Throughput Tissue Microarray Analysis of 3p25 (RAF1) and 8p12 (FGFR1) Copy Number Alterations in Urinary Bladder Cancer. Cancer Res 61:4514-4519
16. Adachi H, Igawa M, Shiina H et al (2003) Human Bladder Tumors With 2-Hit Mutations of Tumor Suppressor Gene TSC1 and Decreased Expression of P27. J Urol 170:601-604
17. Knowles MA, Habuchi T, Kennedy W, Cuthbert-Heavens D (2003) Mutation Spectrum of the 9q34 Tuberous Sclerosis Gene TSC1 in Transitional Cell Carcinoma of the Bladder. Cancer Res 63:7652-7656
18. Shariat SF, Tokunaga H, Zhou J et al (2004) P53, P21, PRB, and P16 Expression Predict Clinical Outcome in Cystectomy With Bladder Cancer. J Clin Oncol 22: 1014-1024
19. Cordon-Cardo C (2004) P53 and RB: Simple Interesting Correlates or Tumor Markers of Critical Predictive Nature? J Clin Oncol 22:975-977
20. Cordon-Cardo C, Wartinger D, Petrylak D et al (1992) Altered Expression of the Retinoblastoma Gene-Product - Prognostic Indicator in Bladder-Cancer. J Natl Cancer Inst 84:1251-1256
21. Frere-Belda MA, Gil Diez de Medina S, Daher A et al (2004) Profiles of the 2 INK4a Gene Products, P16 and P14ARF, in Human Reference Urothelium and Bladder Carcinomas, According to PRb and P53 Protein Status. Hum Pathol 35: 817-824
22. Oeggerli M, Tomovska S, Schraml P et al (2004) E2F3 Amplification and Overexpression Is Associated With Invasive Tumor Growth and Rapid Tumor Cell Proliferation in Urinary Bladder Cancer. Oncogene 23:5616-5623
23. Feber A, Clark J, Goodwin G et al (2004) Amplification and Overexpression of E2F3 in Human Bladder Cancer. Oncogene 23:1627-1630
24. Lee CC, Yamamoto S, Morimura K et al (1997) Significance of Cyclin D1 Overexpression in Transitional Cell Carcinomas of the Urinary Bladder and Its Correlation With Histopathologic Features. Cancer 79:780-789
25. Vogelstein B, Lane D, Levine AJ (2000) Surfing the P53 Network. Nature 408:307-310
26. Olivier M, Eeles R, Hollstein M et al (2002) The IARC TP53 Database: New Online Mutation Analysis and Recommendations to Users. Hum Mutat 19:607-614
27. Malats N, Bustos A, Nascimento CM et al (2005) P53 As a Prognostic Marker for Bladder Cancer: a Meta-Analysis and Review. Lancet Oncol. 6:678-686
28. Kelsey KT, Hirao T, Schned A, et al (2004) A Population-Based Study of Immunohistochemical Detection of P53 Alteration in Bladder Cancer. Br J Cancer 90:1572-1576
29. Puig P, Capodieci P, Drobnjak M et al (2003) P73 Expression in Human Normal and Tumor Tissues: Loss of P73alpha Expression Is Associated With Tumor Progression in Bladder Cancer. Clin Cancer Res 9:5642-5651
30. Stein JP, Ginsberg DA, Grossfeld GD et al (1998) Effect of P21WAF1/CIP1 Expression on Tumor Progression in Bladder Cancer. J Natl Cancer Inst 90:1072-1079
31. Chatterjee SJ, Datar R, Youssefzadeh D et al (2004) Combined Effects of P53, P21, and PRb Expression in the Progression of Bladder Transitional Cell Carcinoma. J Clin Oncol 22:1007-1013
32. Cote RJ, Dunn MD, Chatterjee SJ et al (1998) Elevated and Absent PRb Expression Is Associated With Bladder Cancer Progression and Has Cooperative Effects With P53. Cancer Res 58:1090-1094
33. Garcia del Muro X, Condom E, Vigues F et al (2004) P53 and P21 Expression Levels Predict Organ Preservation and Survival in Invasive Bladder Carcinoma Treated With a Combined-Modality Approach. Cancer 100:1859-1867
34. Hitchings AW, Kumar M, Jordan S et al (2004) Prediction of Progression in PTa and PT1 Bladder Carcinomas With P53, P16 and PRb. Br J Cancer 91:552-557
35. Cappellen D, Gil Diez de Medina S, Chopin D et al (1997) Frequent Loss of Heterozygosity on Chromosome 10q in Muscle-Invasive Transitional Cell Carcinomas of the Bladder. Oncogene 14:3059-3066
36. Wang DS, Rieger-Christ K, Latini JM et al (2000) Molecular Analysis of PTEN and MXI1 in Primary Bladder Carcinoma. Int J Cancer 88:620-625
37. Blaveri E, Brewer JL, Roydasgupta R et al (2005) Bladder Cancer Stage and Outcome by Array-Based Comparative Genomic Hybridization. Clin Cancer Res 11: 7012-7022
38. Hurst CD, Fiegler H, Carr P et al (2004) High-Resolution Analysis of Genomic Copy Number Alterations in Bladder Cancer by Microarray-Based Comparative Genomic Hybridization. Oncogene 23: 2250-2263
39. Veltman JA, Fridlyand J, Pejavar S et al (2003) Array-Based Comparative Genomic Hybridization for Genome-Wide Screening of DNA Copy Number in Bladder Tumors. Cancer Res 63:2872-2880
40. Hartmann A, Schlake G, Zaak D et al (2002) Occurrence of Chromosome 9 and P53 Alterations in Multifocal Dysplasia and Carcinoma in Situ of Human Urinary Bladder. Cancer Res 62:809-818
41. Hopman AH, Moesker O, Smeets AW et al (1991) Numerical Chromosome 1, 7, 9, and 11 Aberrations in Bladder Cancer Detected by in Situ Hybridization. Cancer Res 51:644-651
42. Aboulkassim TO, Larue H, Lemieux P et al (2003) Alteration of the PATCHED Locus in Superficial Bladder Cancer. Oncogene 22:2967-2971
43. Amira N, Cancel-Tassin G, Bernardini S et al (2004) Expression in Bladder Transitional Cell Carcinoma by Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction Array of 65 Genes at the Tumor Suppressor Locus 9q34.1-2: Identification of 5 Candidates Tumor Suppressor Genes. Int J Cancer 111:539-542
44. Wright KO, Messing EM, Reeder JE (2004) DBCCR1 Mediates Death in Cultured Bladder Tumor Cells. Oncogene 23: 82-90
45. Williamson MP, Elder PA, Shaw ME et al (1995) P16 (CDKN2) Is a Major Deletion Target at 9p21 in Bladder Cancer. Hum Mol Genet 4:1569-1577
46. Adams J, Cuthbert-Heavens D, Bass S, Knowles MA (2005) Infrequent Mutation of TRAIL Receptor 2 (TRAIL-R2/DR5) in Transitional Cell Carcinoma of the Bladder With 8p21 Loss of Heterozygosity. Cancer Lett 220:137-144
47. Knowles MA, Aveyard JS, Taylor CF et al (2005) Mutation Analysis of the 8p Candidate Tumour Suppressor Genes DBC2 (RHOBTB2) and LZTS1 in Bladder Cancer. Cancer Lett 225:121-130
48. Richter J, Beffa L, Wagner U et al (1998) Patterns of Chromosomal Imbalances in Advanced Urinary Bladder Cancer Detected by Comparative Genomic Hybridization. Am J Pathol 153:1615-1621
49. Bohm M, Wieland I, Schmidt C et al (2002) Loss of Heterozygosity on Chromosome 5p13-12 Predicts Adverse Prognosis in Advanced Bladder Cancer Independent of Tumor Stage and Grade. J Urol 168:2655-2658
50. Aaboe M, Birkenkamp-Demtroder K, Wiuf C et al (2006) SOX4 Expression in

Bladder Carcinoma: Clinical Aspects and in Vitro Functional Characterization. Cancer Res 66:3434-3442
51. Evans AJ, Gallie BL, Jewett MA et al (2004) Defining a 0.5-Mb Region of Genomic Gain on Chromosome 6p22 in Bladder Cancer by Quantitative-Multiplex Polymerase Chain Reaction. Am J Pathol 164:285-293
52. Wu Q, Hoffmann MJ, Hartmann FH, Schulz WA (2005) Amplification and Overexpression of the ID4 Gene at 6p22.3 in Bladder Cancer. Mol Cancer 4:16
53. Neal DE, Sharples L, Smith K et al (1990) The Epidermal Growth Factor Receptor and the Prognosis of Bladder Cancer. Cancer 65:1619-1625
54. Ohta JI, Miyoshi Y, Uemura H et al (2001) Fluorescence in Situ Hybridization Evaluation of C-ErbB-2 Gene Amplification and Chromosomal Anomalies in Bladder Cancer. Clin. Cancer Res 7:2463-2467
55. Chow NH, Chan SH, Tzai TS (2001) Expression Profiles of ErbB Family Receptors and Prognosis in Primary Transitional Cell Carcinoma of the Urinary Bladder. Clin. Cancer Res 7:1957-1962
56. Wolff EM, Liang G, Jones PA (2005) Mechanisms of Disease: Genetic and Epigenetic Alterations That Drive Bladder Cancer. Nat Clin Pract Urol 2:502-510
57. Maruyama R, Toyooka S, Toyooka KO et al (2001) Aberrant Promoter Methylation Profile of Bladder Cancer and Its Relationship to Clinicopathological Features. Cancer Res 61:8659-8663
58. Dhawan D, Hamdy FC, Rehman I et al (2006) Evidence for the Early Onset of Aberrant Promoter Methylation in Urothelial Carcinoma. J Pathol 209:336-343.
59. Marsit CJ, Karagas MR, Andrew A et al (2005) Epigenetic Inactivation of SFRP Genes and TP53 Alteration Act Jointly As Markers of Invasive Bladder Cancer. Cancer Res 65:7081-7085
60. Thykjaer T, Workman C, Kruhoffer M et al (2001) Identification of Gene Expression Patterns in Superficial and Invasive Human Bladder Cancer. Cancer Res 61: 2492-2499
61. Dyrskjot L, Thykjaer T, Kruhoffer M et al (2003) Identifying Distinct Classes of Bladder Carcinoma Using Microarrays. Nat Genet 33:90-96
62. Blaveri E, Simko JP, Korkola JE et al (2005) Bladder Cancer Outcome and Subtype Classification by Gene Expression. Clin Cancer Res 11:4044-4055
63. Sanchez-Carbayo M, Socci ND, Lozano J et al (2006) Defining Molecular Profiles of Poor Outcome in Patients With Invasive Bladder Cancer Using Oligonucleotide Microarrays. J Clin Oncol 24:778-789
64. Yamana K, Bilim V, Hara N et al (2005) Prognostic Impact of FAS/CD95/APO-1 in Urothelial Cancers: Decreased Expression of Fas Is Associated With Disease Progression. Br J Cancer 93:544-551
65. Ong F, Moonen LM, Gallee MP et al (2001) Prognostic Factors in Transitional Cell Cancer of the Bladder: an Emerging Role for Bcl-2 and P53. Radiother Oncol 61:169-175
66. Shariat SF, Casella R, Khoddami SM et al (2004) Urine Detection of Survivin Is a Sensitive Marker for the Noninvasive Diagnosis of Bladder Cancer. J Urol 171: 626-630
67. Baffa R, Gomella LG, Vecchione A et al (2000) Loss of FHIT Expression in Transitional Cell Carcinoma of the Urinary Bladder. Am J Pathol 156:419-424
68. Garcia del Muro X, Torregrosa A, Munoz J et al (2000) Prognostic Value of the Expression of E-Cadherin and Beta-Catenin in Bladder Cancer. Eur J Cancer 36:357-362
69. Ribeiro-Filho LA, Franks J, Sasaki M et al (2002) CpG Hypermethylation of Promoter Region and Inactivation of E-Cadherin Gene in Human Bladder Cancer. Mol Carcinog 34:187-198