

Review

Molecular pathology of low malignant bladder transitional cell carcinoma: a current perspective

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Summary. Bladder transitional cell carcinoma (BTCC) actually has two phenotypes: low malignant and aggressive. Most previous molecular and cytogenetic analyses of bladder cancer were focused on aggressive BTCC. Little is known about the events that lead to the development of low malignant BTCC. This review mainly introduces the concept of two types of bladder tumors and then focuses on the molecular pathology of low malignant BTCC in particular. It is hoped that further understanding of the molecular pathology of low malignant BTCC may provide novel therapies and many other clinical benefits in patients with this disease.

Key words: Bladder cancer, Well-differentiated, Oncogene, Tumor suppressor genes, Urothelial hyperplasias

Introduction

A cornerstone in the investigation of the bladder transitional cell carcinoma (BTCC) is the recognition of the two phenotypic tumors: low malignant and aggressive BTCC (Koss, 1992; Jones and Droller, 1993). Recent genetic analyses have demonstrated that the different genetic defects may underlie the two bladder tumorigenesis pathways (Chang et al., 1994; Spruck et al., 1994; Knowles, 2002). Experimental evidence also strongly supports the existence of these two pathways (Zhang et al., 2001). Most previous molecular and cytogenetic analyses of urinary bladder cancer were focused on aggressive BTCC. Little is known about the events that lead to the development of low malignant BTCC. This review mainly introduces the concept of two types of bladder tumors and then focuses on the molecular pathology of low malignant bladder cancer in particular.

The concept of two types of bladder tumors

The low malignant BTCC, which accounts for 70-80% of the urothelial carcinomas, presents as superficial, papillary lesions that has a propensity to recur, but which only infrequently progresses to muscle-invasive stage or metastasize (Grossman, 1996). If treated promptly, the 5-year survival rate of this variant can approach 90%. The pathological characteristic of this type of BTCC is low-grade/well-differentiated neoplasms, previously classified as grade I-II, non-invasive papillary transitional cell carcinoma (TCC), but classified as papillary urothelial neoplasms of low malignant potential (PUNs-LMP) and low grade papillary urothelial carcinomas (LG-PUCs) in the 1998 and 1999 revised World Health Organization (WHO) classification.

The aggressive BTCC accounting for 20-30%, presents as an invasive tumor at diagnosis, and has a very high risk of progressing to incurable distant metastases (Steinberg et al., 1992). Clinical and histopathological data strongly suggest that the majority of the invasive urothelial carcinomas are not derived from the superficial papillary tumors, but from a unique precursor lesion, i.e., at carcinoma in situ (CIS), or can arise denovo (Schalken et al., 1992; Steinberg et al., 1992). Their pathological morphology is the high-grade lesion that begins as dysplasia or carcinoma in situ (CIS), and occasionally as high-grade papillary carcinoma.

Thus, urothelial carcinomas appear to develop and progress via two distinctive phenotypic pathways with drastically different biological behavior and clinical outcome. Recent genetic analyses have provided strong evidence that different genetic defects may underlie the two bladder tumorigenesis pathways. Low malignant BTCC is frequently accompanied by alterations in chromosome 9 and FGFR3, whereas dysfunction of p53 and pRb is primarily associated with aggressive BTCC (Spruck et al., 1994; Knowles, 2002; Figure 1).

Both types of neoplasm can develop in the same patient, either simultaneously or sequentially. Predicting which patients with low malignant BTCC will ultimately

develop the more aggressive type of bladder cancer and will recur more easily are two major challenges facing bladder cancer investigations.

The pathological counterparts of low malignant BTCC

The 1973 WHO system has been the most used grading system for bladder carcinoma (Mostofi et al., 1973). Unfortunately, criteria for the 3 different grades are rather vague, resulting in considerable interobserver variability (Schapers et al., 1994). The consensus meeting organized by the World Health Organization/International Society of Urological Pathology (WHO/ISUP) in 1998 proposed the categorization of the papillary lesions of the urinary bladder as papilloma, PUNs-LMP, LG-PUNs, or high-grade papillary urothelial carcinomas (HG-PUCs) (Epstein et al., 1998). However problems in the differential diagnosis between PUNs-LMP and LG-PUNs remain (Helpap and Kollermann, 2000), and the clinical significance of defining a group of PUNs-LMP is still questionable.

There are only slight morphological differences in PUNs-LMP and LG-PUNs, as defined and illustrated in the 1998 WHO/ISUP. When pathologists were required to discriminate between PUNs-LMP and LG-PUNs, the discrepancies were 50% after education compared with

39% before education. In contrast, there were no discrepancies when the discrimination was between PUNs-LMP and HG-PUCs or carcinoma *in situ* (Murphy et al., 2002). Biologically the progression rates in patients in these 2 groups seem similar at 8% to 15% if it is permissible to gauge by at least 1 study (Jordan et al., 1987). Even the death rates are not too different at 0% to 4% in patients presenting with a PUNs-LMP and 4% to 12% in those in whom the initial tumor is interpreted as LG-PUCs (Jordan et al., 1987; Holmang et al., 1999). Furthermore, it has been our experience that urologists usually treat low grade, noninvasive papillary urothelial neoplasms as the same whether these tumors are interpreted as PUNs-LMP or LG-PUCs.

Therefore, several studies (Jordan et al., 1987; Schapers et al., 1994; Murphy et al., 2002) have suggested a two-tier grading system: PUNs-LMP and LG-PUCs can be combined into low pathological grade of urothelial neoplasms (low malignant BTCC); and CIS and HG-PUCs as high pathological grade (aggressive BTCC) (Murphy et al., 2002; Table 1). This two type classification can definitely induce the interobserver variability. In addition, from a molecular perspective, papillomas, PUNs-LMP and LG-PUCs should be classified together as a group of urothelial neoplasms with good differentiation (van Rhijn et al., 2002). Activating-point mutations in the FGFR3 gene occur frequently in low-grade and low-stage bladder

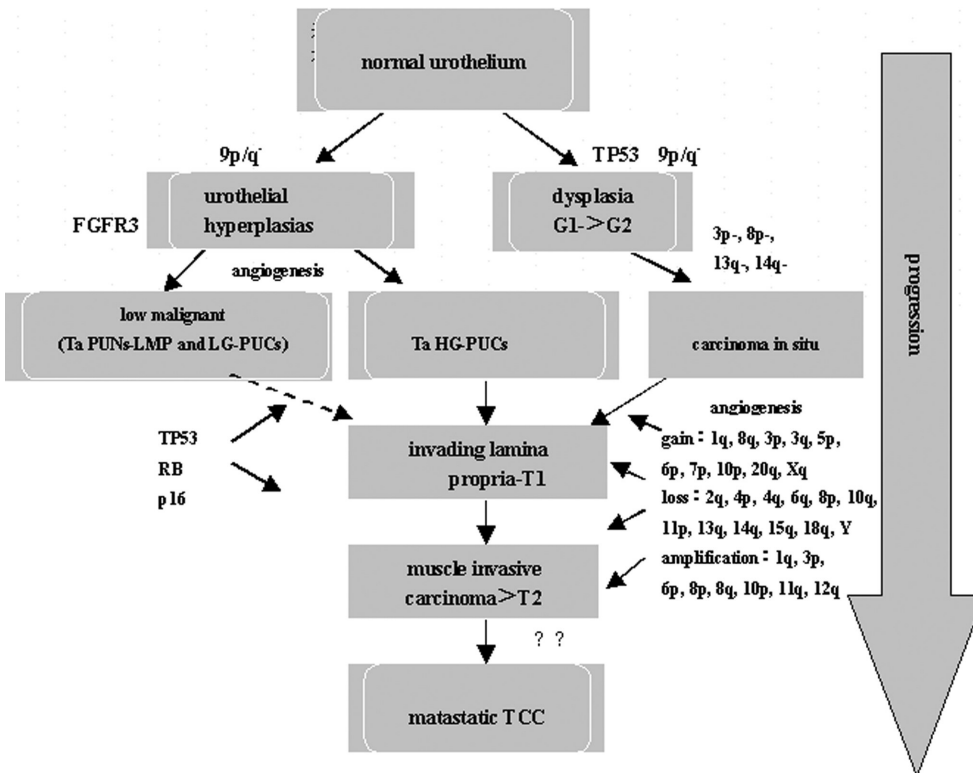


Fig. 1. A model for TCC progression based on histopathological observations and genetic data. Only chromosome 9 loss and FGFR3 mutation are found at significant frequency in superficial papillary tumours. Many gains, losses and amplifications occur at or after Ta.T1 progression. TCC, transitional cell carcinoma. PUNs-LMP, papillary urothelial neoplasms of low malignant potential. LG-PUCs, low grade papillary urothelial carcinomas, HG-PUCs, high grade papillary urothelial

carcinomas, whereas they are rare in high-grade carcinomas.

Genetic alterations of low malignant BTCC 1 tumor suppressor genes and candidate tumor suppressor genes

Chromosome 9

Chromosome 9 loss of heterozygosity (LOH) has been observed in 34% of superficial Ta tumors (Chi et al., 1999). Homozygous deletions at 9p21, commonly resulting in codeletion of the cell cycle regulatory genes p15, p16 and p15ARF, are common as in other tumour types, and these are widely believed to represent the targets of deletion of 9p (Williamson et al., 1995; Orlov et al., 1999). But other evidence showed that deletions were more frequent on 9q than on 9p, furthermore, the latter being mostly associated with 9q deletion, suggesting that alteration of genes on 9q may be an early event associated with superficial papillary tumors (Simoneau et al., 1999). However, localization of tumor suppressor genes on 9q has been hampered by the low frequency of subchromosomal deletions. This could indicate that there are multiple relevant tumour suppressor genes on this chromosome. Until very recently, the candidate genes included DBCCR1 (9q32-33), TSC1 (9q34) and PTCH(9q22).

DBCCR1 (9q32-33)

A few tumours were found with deletions focused on a very small region of <800kb, located at 9q32-33, close to the marker D9S195 (Habuchi et al., 1997). To date, no mutations in the second allele have been found in tumour samples with 9q32-33 LOH at sufficient frequency to conclude that these genes play a major role. Transcriptional silencing by CpG island hypermethylation of gene regulatory regions is the possible mechanism for inactivation of this gene, because expression of DBCCR1 was silenced by promoter hypermethylation in 50% of bladder cancer cell lines analysed, while no obvious methylation was detected in the normal urothelium in association with

ageing (Habuchi et al., 2001). In addition, Nishiyama et al. (2001) provided functional evidence to authenticate DBCCR1 as a tumour suppressor by using gene-transfer methods. Exogenous expression of DBCCR1 protein or an HA epitope-tagged fusion protein, HA-DBCCR1 in NIH3T3 cells and human bladder tumour cell lines resulted in suppression of proliferation due to an increase in the number of cells in the G(1) phase of the cell cycle, thereby supporting the hypothesis that this is the tumour suppressor gene targeted by 9q32-33 deletion in bladder cancer.

PTC (9q22)

Deletion mapping in the 9q22 region identifies a 0.5 CM common minimal region of deletion between markers D9S280 and D9S1809, encompassing PATCHED (PTC), a gene identified as a tumour suppressor in basal cell carcinoma and in medulloblastoma. Mutation analysis was only found in two patient in a report (McGarvey et al., 1998), and others did not find it (Xie et al., 1997; Aboukassim et al., 2003). However, average expression of PTC mRNA measured by semiquantitative RT-PCR was significantly decreased in tumours with LOH in the 9q22 region, compared to normal urothelium, suggesting that the PTC gene is a putative suppressor at the 9q22 locus and that haploinsufficiency of this gene may be an early event in the development of papillary bladder tumours (Aboukassim et al., 2003). More recently, Hamed et al. (2004) provided further evidence to support this hypothesis of PTC as acting as a tumor suppressor gene in bladder cancer. They submitted Ptc(+/-) heterozygous mutant mice and their wild-type littermates to chemical carcinogenesis by adding N-butyl-N- (4-hydroxybutyl) nitrosamine to their drinking water. Preneoplastic and neoplastic changes were observed significantly earlier in the PTC(+/-) than in the wild-type mice.

TSC1 (9q34)

TSC1, located at 9q34, related to the tuberous sclerosis complex. Mutations of TSC1 were found in approximately 13% of tumours (Edwards et al., 2002)

Table 1. Two types of bladder transitional cell carcinoma according to their genetic and biological potential.

TUMOR TYPE OF BTCC	PATHOLOGY	PATHOLOGY (1998)	TNM (1973)	PRECANCEROUS LESION	CHARACTERISTIC GENETIC ALTERATION
Type I Low malignant)	Papilloma PUNs-LMP LG-PUCs	G1-2	Ta	Urothelial hyperplasia	Chromosome 9q (DBCCR1, TSC1, PTCH) FGFR3, Ras
Type II (aggressive)	HG-PUCs CIS	G2-3	>T1 Tis	diaplasia	TP53, RB

BTCC: bladder transitional cell carcinoma; CIS: carcinoma in situ.

raising the possibility that this represents a bladder tumor suppressor. However, reports have shown big differences in TSC1 mutation analysis (Habuchi et al., 1999; van Tilborg et al., 2001). More recently, Knowles et al. (2003) carried out mutation analysis of 62 bladder tumors and 33 bladder tumor-derived cell lines. Twelve percent of samples contained mutations. There are only 3 cases with mutation retained heterozygosity for TSC1 despite the tumors they chose mostly with 9q LOH (>80%) for that study, indicating that haploinsufficiency for TSC1 can contribute to the development of bladder cancer. Functional analysis should confirm the authenticity of TSC1 as a bladder tumour suppressor.

In all, in recent years efforts have been directed towards identifying the postulated tumour suppressor genes on this chromosome arm by deletion mapping and mutation analysis. However, no convincing candidate genes have been identified. So it is possible that haploinsufficiency of one or both genes is all that is required. It has also been suggested that deletion of chromosome 9 may be related to the extent of genome-wide hypomethylation (P<0.0001) and be a consequence rather than a cause of tumour development (Kimura et al., 2001). However, if this is the case it is not clear why this should happen in bladder but not other tumour types and what the selection pressures that result in clonal

populations of tumour cells with specific regions of chromosome 9 LOH might be!

Oncogene

FGFR3

Fibroblast growth factor receptor 3 (FGFR3) gene mutations were reported recently at a high frequency in low-grade superficial urothelial cell carcinoma (UCC) ranging from 35%-75%, contrasting sharply with the much lower frequency in superficially invasive (stage pT1) and invasive (stages pT2 to 4) tumors and the absence of mutation in carcinoma in situ (Billerey et al., 2001; Kimura et al., 2001; Sibley et al., 2001; van Rhijn et al., 2001). All were missense mutations located in exons 7, 10 or 15 and all had been previously described as germline mutations in skeletal dysplasia syndromes (Webster and Donoghue, 1997). Interestingly, FGFR3 mutation appears to be not only significantly more frequent in tumors of low grade and stage, but also identifies those with a low recurrence rate (van Rhijn et al., 2001). This finding could potentially have significant application for identifying the large group with favorable disease characteristics in which reduced surveillance may be appropriate.

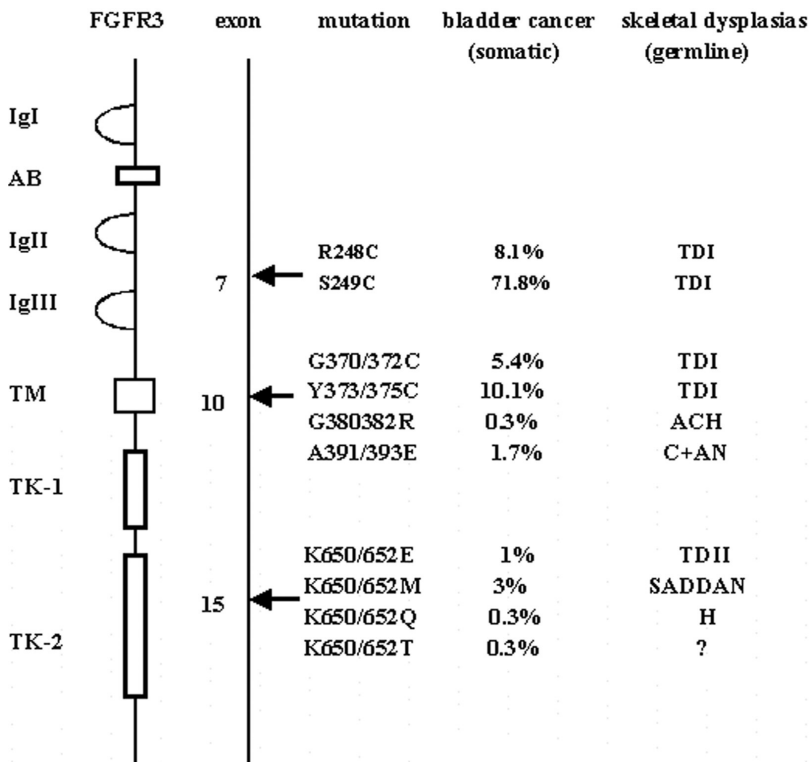


Fig. 2. Structure of FGR 3 protein showing positions of exons, and positions and frequency of mutations identified in bladder transitional cell carcinoma AB, acid box. TM, transmembrane domain, TK-1 and TK-2 tyrosine kinase domains. TD1, thanatophoric dysplasia type I, TDIF, thanatophoric dysplasia type II. C+AN, craniofacial dysostosis with acanthoma nigricans. SADDAN, severe archondroplasia with developmental delay and acanthosis nigricans. H, hypochondroplasia with milder radiological features than N540H. ACH, achondroplasia. The positions are numbered according to the FGRF3c/FGR3b numbering.

In the 6 mutation analyses of transitional cell carcinoma to date 10 mutations were identified (Fig. 2). By far the most common is S249C (70% of cases) (van Rhijn et al., 2002). This mutation converts a serine residue between Ig domains II and III to a cysteine, generating an uneven number of cysteines in the extracellular domain. This may prevent the formation of normal intrachain disulfide bonds or enable the formation of interchain bonds resulting in ligand-independent dimerization and signaling. Of the mutations identified in transitional cell carcinoma, three are located in the activation loop of the kinase domain and are presumed to stabilize the active conformation.

To date, mutations in other epithelial tumors have been found only in the cervix at low frequency and in 2 cases of colorectal cancer 19. Thus, FGFR3 mutation appears to be significantly bladder-specific. Several researchers have demonstrated the potential values of FGFR3 mutation in the early noninvasive diagnosis and molecular grading of bladder cancer (van Rhijn et al., 2003; Rieger-Christ et al., 2003). Nevertheless the putative oncogenic importance in bladder cancer is still unknown. Recently, Yehnew et al. (2003) demonstrated that constitutively activated FGFR3 can transform NIH-3T3 cells, and that the Ras-ERK and p13K-Akt signaling pathways, which are positively regulated by SHP2, are important for FGFR3-induced transformation. Further study should observe the effect of mutant FGFR3 on the phenotype of urothelial cells *in vitro* and involving signaling pathways.

H-ras

Although ras activation was first identified in bladder cancer (Capon et al., 1983), controversies have existed regarding its precise role in urothelial tumorigenesis. First, the frequency of Ha-ras mutations in bladder cancer ranged anywhere from 6 to 70% in different reports (Saito, 1992). Second, the stage in which ras mutation occurs varies from early to late or to no stage-correlation (Theodorescu et al., 1990; Knowles and Williamson, 1993; Fitzgerald et al., 1995). Third, transfection of an activated ras into primary cultured fibroblasts provoked premature senescence which could be overcome by the inactivation of tumor suppressor genes p53 and p16; this suggests that the activated ras requires the cooperative activity of another oncogenic event to fully transform primary cultured fibroblasts (Serrano et al., 1997). However, recently, by expressing an activated Ha-ras in mouse urothelium, Zhang et al. (2001) found that mice harboring low copy of mUPII/Ha-ras-M developed urothelial hyperplasia followed by superficial papillary tumors; mice harboring high copy of mUPII/Ha-ras-M developed early-onset superficial papillary tumors. This report provides strong evidence that urothelium-specific expression of an activated Ha-ras causes urothelial hyperplasia and superficial papillary bladder tumors. Activating mutation

of Ha-ras, concurrent with overexpression, represents a nonclonal event which is sufficient to elicit papillary noninvasive bladder tumors. These findings therefore establish that ras activation can be an early event in low malignant BTCC formation.

Gene panels by highthroughout technologies

Multiple molecular events take place when normal epithelia are transformed into tumor tissue and later acquire an invasive potential. Previously, these events had been examined at the single-gene level, however, the newly developed array technology has made it possible to simultaneously monitor thousands of genes during tumor evolution and progression.

Sauter (1997) analyzed 56 superficial carcinomas of the urinary bladder by comparative genomic hybridization (CGH). Results showed that deletions were most frequently found at 9q (20 out of 56 patients; 36%), chromosome Y (15 out of 43 male patients; 35%); 9p (28%); 11q (16%); 11p (13%); and 10q (11%). DNA sequence copy number gains were most prevalent at 1q (34%); 8q (22%); 17q (15%); 20q (13%); and 12q (11%). Diggle et al. (2003), by combining laser capture microdissection and cDNA suppression subtractive hybridization library construction, have identified genes that may be involved in the low grade papillary urothelial carcinoma. Sequencing of 100 unique clones in SSH library after random selection from the library revealed 17% coding for genes involved in cellular metabolic processes, including lactate dehydrogenase and cytochrome oxidase IV; 44% involved in translation, many of which were ribosomal proteins; 5% coding for structural proteins; and 17% involved in cell signaling, cell growth, and cell transport. The remaining genes either encoded proteins with an unknown function in humans (15%) or did not show homology to any published gene sequence (2%). Further study should explain the precise mechanism of these genes involved in low malignant BTCC.

Conclusions

According to the molecular pathology and biological potential of bladder cancer, we suggest a two-type classification. One is low malignant BTCC and the other is aggressive BTCC. Evidence suggests a critical role for FGFR3, H-ras and chromosome 9 alterations in low malignant BTCC initiation rather than progression. In addition, primary researches have found their potential prediction values for disease detection, disease monitoring and prognosis, while some also represent potential therapeutic targets. Further study should focus on the precise mechanism of those genes and molecular alterations relating to low malignant BTCC. It is hoped that further understanding of the molecular pathology of low malignant BTCC may provide novel therapies and many other clinical benefits in patients with this disease.

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References

- Aboukassim T.O., LaRue H., Lemieux P., Rousseau F. and Fradet Y. (2003). Alteration of the PATCHED locus in superficial bladder cancer. *Oncogene* 22, 2967-2971.
- Billerey C., Chopin D., Aubriot-Lorton M.H., Ricol D., Gil Diez de Medina S., Van Rhijn B., Bralet M.P., Lefrere-Belda M.A., Lahaye J.B., Abbou C.C., Bonaventure J., Zafrani E.S., van der Kwast T., Thiery J.P. and Radvanyi F. (2001). Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. *Am. J. Pathol.* 158, 1955.
- Capon D.J., Chen E.Y., Levinson A.D., Seeburg P.H. and Goeddel D.V. (1983). Complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue. *Nature* 302, 33-37.
- Chang J.W., Tengxiang M. and Zjamg S. (1994). *Chinese Journal of Urology* 15, 405-408.
- Chi S.G., Chang S.G., Lee S.J., Lee C.H., Kim J.I. and Park J.H. (1999). Elevated and biallelic expression of p73 is associated with progression of human bladder cancer. *Cancer Res.* 59, 2791
- Diggie C.P., Cruickshank S., Olsburgh J.D., Pellegrin S., Smith B., Banks R.E., Selby P.J., Knowles M.A., Southgate J. and Harnden P. (2003). Identification of genes up-regulated in urothelial tumors. *Am. J. Pathol.* 163, 493-504.
- Edwards J., Duncan P., Going J.J., Watters A.D., Grigor K.M. and Bartlett J.M. (2002). Identification of loci associated with putative recurrence genes in transitional cell carcinoma of the urinary bladder. *J. Pathol.* 196, 380-385.
- Epstein J.I., Amin M.B., Reuter V.R. and Mostofi F.K. (1998). The World Health Organization/International Society of Urological Pathology Consensus Classification of urothelial (transitional cell) neoplasms of the urinary bladder. *Am. J. Surg. Pathol.* 22, 1435-1448.
- Fitzgerald J.M., Ramchurren N., Rieger K., Levesque P., Silverman M., Libertino J.A. and Summerhayes I.C. (1995). Identification of H-ras mutations in urine sediments complements cytology in the detection of bladder tumors. *J. Natl. Cancer Inst.* 87, 129-133.
- Grossman H.B. (1996). Superficial bladder cancer: decreasing the risk of recurrence. *Oncology (Huntingt)* 10, 1617-1624; discussion 1624, 1627-1628.
- Habuchi T., Yoshida O. and Knowles M.A. (1997). A novel candidate tumour suppressor locus at 9q32-33 in bladder cancer: localization of the candidate region within a single 840 kb YAC. *Hum. Mol. Genet.* 9, 913-919.
- Habuchi T., Devlin J., Elder P.A. and Knowles M.A. (1999). Mutation of the 9q34 gene TSC1 in sporadic bladder cancer. *Oncogene* 18, 2657-2661
- Habuchi T., Takahashi T., Kakinuma H., Wang L., Tsuchiya N., Satoh S., Akao T., Sato K., Ogawa O., Knowles M.A. and Kato T. (2001). Hypermethylation at 9q32-33 tumour suppressor region is age-related in normal urothelium and an early and frequent alteration in bladder cancer. *Oncogene* 20, 531-537.
- Hamed S., LaRue H., Hovington H., Girard J., Jeannotte L., Latulippe E. and Fradet Y. (2004). Accelerated induction of bladder cancer in patched heterozygous mutant mice. *Cancer Res.* 64, 1938-1942
- Helpap B. and Köllermann J. (2000). Assessment of basal cell status and proliferative patterns in flat and papillary urothelial lesions: a contribution to the new WHO classification of urothelial tumors of the urinary bladder. *Hum. Pathol.* 31, 745-750.
- Holmang S., Hedelin H., Anderstrom C., Holmberg E., Busch C. and Johansson S.L. (1999). Recurrence and progression in low grade papillary urothelial tumors. *J. Urol.* 162, 702.
- Jones P.A. and Droller M.J. (1993). Pathways of development and progression in bladder cancer: new correlations between observations and molecular mechanisms. *Semin. Urol.* 11, 177-192.
- Jordan A.M., Weingarten, J. and Murphy W.M. (1987). Transitional cell neoplasms of the urinary bladder. Can biologic potential be predicted from histologic grading? *Cancer* 60, 2766.
- Kimura F., Flori A.R., Seifert H.H., Louhelainen J., Maas S., Knowles M.A. and Schulz W.A. (2001). Destabilization of chromosome 9 in transitional cell carcinoma of the urinary bladder. *Br. J. Cancer* 85, 1887-193.
- Kimura T., Suzuki H., Ohashi T., Asano K., Kiyota H. and Eto Y. (2001). The incidence of thanatophoric dysplasia mutations in FGFR3 gene is higher in low-grade or superficial bladder carcinomas. *Cancer* 92, 2555
- Knowles M.A. and Williamson M. (1993). Mutation of H-ras is infrequent in bladder cancer: confirmation by single-strand conformation polymorphism analysis, designed restriction fragment length polymorphisms, and direct sequencing. *Cancer Res.* 53, 133-139.
- Knowles M.A. (2002). *Histopathology* 41(suppl.2), 403-423.
- Knowles M.A., 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
- Koss L.G. (1992) Bladder cancer from a perspective of 40 years. *J. Cell. Biochem. Suppl.* 161, 23-29.
- McGarvey T.W., Maruta Y., Tomaszewski J.E., Linnenbach A.J. and Malkowicz S.B. (1998). PTCH gene mutations in invasive transitional cell carcinoma of the bladder. *Oncogene* 17, 1167-172.
- Mostofi F.K., Sorbin L.H. and Torloni H. (1973). *Histological typing of urinary bladder tumours.* Geneva: World Health Organization.
- Murphy W.M., Takezawa K. and Maruniak N.A. (2002). Interobserver discrepancy using the 1998 world health organization/international society of urologic pathology classification of urothelial neoplasms: practical choices for patient case. *J. Urol.* 168, 968-972.
- Nishiyama H., Gill J.H., Pitt E., Kennedy W. and Knowles M.A. (2001). Negative regulation of G(1)/S transition by the candidate bladder tumour suppressor gene DBCCR1 *Oncogene* 20, 2956-2964
- Orlow I., LaRue H., Osman I., Lacombe L., Moore L., Rabbani F., Meyer F., Fradet Y. and Cordon-Cardo C. (1999). Deletions of the INK4A gene in superficial bladder tumors. Association with recurrence. *Am. J. Pathol.* 155, 105-113.
- Rieger-Christ K.M., Mourtzinou A., Lee P.J., Zagher R.M., Cain J., Silverman M., Libertino J.A. and Summerhayes I.C. (2003). Identification of fibroblast growth factor receptor 3 mutations in urine sediment DNA samples complements cytology in bladder tumor detection *Cancer* 98, 737-744.
- Saito S. (1992). Detection of H-ras gene point mutations in transitional cell carcinoma of human urinary bladder using polymerase chain reaction. *Keio J. Med.* 41, 80-86.
- Sauter G.R. (1997). Molecular cytogenetic analysis of superficial urothelial cancer of the bladder. *Verh Dtsch. Ges. Pathol.* 81, 18-27

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- Schalken J.A., van Moorselaar R.J., Bringuier P.P. and Debruyne F.M. (1992). Critical review of the models to study the biologic progression of bladder cancer. *Semin Surg Oncol.* 8, 274-278.
- Schapers R.F., Pauwels R.P., Wijnen J.T., Arends J.W., Thunnissen F.B., Coebergh J.W., Smeets A.W. and Bosman F.T. (1994). A simplified grading method of transitional cell carcinoma of the urinary bladder: reproducibility, clinical significance and comparison with other prognostic parameters. *Br. J. Urol.* 73, 625.
- Serrano M., Lin A.W., McCurrach M.E., Beach D. and Lowe S.W. (1997). Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 88, 593-602
- Sibley K., Cuthbert-Heavens D. and Knowles M.A. (2001). Loss of heterozygosity at 4p16.3 and mutation of FGFR3 in transitional cell carcinoma. *Oncogene* 20, 686.
- Simoneau M., Aboukassim T.O., LaRue H., Rousseau F. and Fradet Y. (1999). Four tumor suppressor loci on chromosome 9q in bladder cancer: evidence for two novel candidate regions at 9q22.3 and 9q31. *Oncogene* 18, 157-163.
- Spruck C.H. 3rd, Ohneseit P.F., Gonzalez-Zulueta M., Esrig D., Miyao N., Tsai Y.C., Lerner S.P., Schmutte C., Yang A.S. and Cote R. (1994). Two molecular pathways to transitional cell carcinoma of the bladder. *Cancer Res.* 54, 784-788.
- Steinberg G.D., Trump D.L. and Cummings K.B. (1992). Metastatic bladder cancer. Natural history, clinical course, and consideration for treatment. *Urol. Clin. North. Am.* 19, 735-746.
- Theodorescu D., Cornil I., Fernandez B.J. and Kerbel R.S. (1990). overexpression of normal and mutated forms of HRAS induces orthotopic bladder invasion in a human transitional cell carcinoma. *Proc. Natl. Acad. Sci. USA* 87, 9047-9051.
- van Rhijn B.W., Lurkin I., Radvanyi F., Kirkels W.J., van der Kwast T.H. and Zwarthoff E.C. (2001). The fibroblast growth factor receptor 3 (FGFR3) mutation is a strong indicator of superficial bladder cancer with low recurrence rate. *Cancer Res.* 61, 1265.
- van Rhijn B.W., Montironi R., Zwarthoff E.C., Jobsis A.C. and van der Kwast T.H. (2002). Frequent FGFR3 mutations in urothelial papilloma. *J. Pathol.* 198, 245-251
- van Rhijn B.W., Vis A.N., van der Kwast T.H., Kirkels W.J., Radvanyi F., Ooms E.C., Chopin D.K., Boeve E.R., Jobsis A.C. and Zwarthoff E.C. (2003). Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. *J. Clin. Oncol.* 21, 1912-1921.
- van Tilborg A.A., de Vries A. and Zwarthoff E.C. (2001). The chromosome 9q genes TGFBR1, TSC1, and ZNF189 are rarely mutated in bladder cancer. *J. Pathol.* 194, 76-80.
- Webster M.K. and Donoghue D.J. (1997). FGFR activation in skeletal disorders: too much of a good thing. *Trends Genet* 13, 178.
- Williamson M.P., Elder P.A., Shaw M.E., Devlin J. and Knowles M.A. (1995). p16 (CDKN2) is a major deletion target at 9p21 in bladder cancer. *Hum. Mol. Genet* 4, 1569-77.
- Xie J., Johnson R.L., Zhang X., Bare J.W., Waldman F.M., Cogen P.H., Menon A.G., Warren R.S., Chen L.C., Scott M.P. and Epstein E.H. Jr. (1997). Mutations of the PATCHED gene in several types of sporadic extracutaneous tumors. *Cancer Res.* 57, 2369-2372.
- Yehnew M.A., Movilla N., Ischenko I. and Hayman M.J. (2003). The phosphotyrosine phosphatase SHP2 is a critical mediator of transformation induced by the oncogenic fibroblast growth factor receptor 3. *Oncogene* 22, 6909-6918.
- Zhang Z.T., Pak J., Huang H.Y., Shapiro E., Sun T.T., Pellicer A. and Wu X.R. (2001). Role of Ha-ras activation in superficial papillary pathway of urothelial tumor formation. *Oncogene* 20, 1973-1980.

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