Review

Morphological and Molecular Profiles and Pathways in Bladder Neoplasms

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Abstract. *After several decades of use of the Wealth Health Organization (WHO) 1973 grading system for bladder neoplasms (1973 WHO), a new grading system was adopted in 2004 by the WHO (2004 WHO). Some leading experts in the field of urogenital pathology have expressed their concerns with the 2004 WHO categorization system, both with regard to the nomenclature and the grading categories used. Several molecular and genetic studies have provided another perspective on the classification of urothelial neoplasms. The two most important developments are the identification of a mutation in the fibroblast growth factor receptor 3 gene in more than 50% of urothelial carcinomas and the discovery of cDNA profiles characteristic of different subsets of bladder cancer. There is strong correlation between the molecular findings and the 2004 WHO classification and prognosis.*

After several decades of use of the 1973 World Health Organisation (WHO) grading system for bladder neoplasms (1), a new grading system proposed in 1998 by the International Society of Urological Pathology (2) (1998 ISUP) was adopted in 2004 by the WHO (2004 WHO) (3, 4). Two factors elicited the call for a change: Firstly, the use of the term "carcinoma" for a subset of the WHO 1973 grade 1 noninvasive (pTa) papillary carcinomas was not considered appropriate given the indolent biological nature of most of these tumours and secondly there was a lack of a clear definition of the three WHO 1973 grades. It was thought that the latter was responsible for the well-recognized large inter-observer variation for grading of bladder cancer, as well as the very large proportion of WHO grade 2 low stage (pTa, pT1) bladder cancer. This "garbage bin" function of WHO 1973 grade 2 was thought to reduce its prognostic significance.

Several molecular and genetic data, partially driven by technological innovations such as high throughput cDNA and DNA profiling, may provide another perspective on the classification of urothelial neoplasms. The two most important developments are the identification of a mutation in the *fibroblast growth factor receptor 3* (*FGFR3*) gene in more than 50% of urothelial carcinomas (5) and the discovery of cDNA profiles characteristic of different subsets of bladder cancer (6). The strong correlation of some molecular findings with clinical behaviour might benefit their combined use with morphological data for the development of a clinically and scientifically relevant classification system for urothelial neoplasms.

Morphological and Molecular Profiles

Flat urothelial lesions

The classification of the flat pre-neoplastic urothelial lesions of the urinary tract, as redefined by the 2004 WHO classification, comprise: hyperplasia, dysplasia and carcinoma *in situ* (CIS). Each of these lesions may occur either isolated or associated with papillary neoplasms and/or invasive urothelial carcinomas. Their prognosis is reported in Table I.

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Table I. *Prognosis of the flat urothelial lesions (based on the published literature).*

	Reactive atypia	Flat hyperplasia	Dysplasia	Carcinoma *in situ*
Recurrence	No	Unknown	*Unknown **73% *vs.* 43% in cases without dysplasia	*Unknown **Unknown
Progression	No	Unknown	*13%-19%° **30%-36%°°	*28% **42%-83%°°

*Primary, **secondary, °progression to CIS, °°progression to muscle invasive carcinoma.

Urothelial hyperplasia. In the 2004 WHO classification, it is recognized that hyperplasias may be flat or pseudopapillary. Flat urothelial hyperplasia consists of a markedly thickened urothelium, greater than seven cell layers thick, without cytological atypia (7). The observation that there is no disturbance of the layering and the nuclei are inconspicuous help to establish the diagnosis. This lesion may be seen in the mucosa adjacent to low-grade papillary urothelial lesions. Hyperplasia with a pseudopapillary architecture refers to a slight tenting or undulation of the urothelium lacking a well-defined central fibrovascular core, although small vessels may be present at the base of the papillae. When seen by itself, there are no data proving that it has any premalignant potential (8, 9).

Genetic studies on urothelial hyperplasia carried out on patients with an associated papillary urothelial carcinoma showed frequent genetic alterations in chromosome 9, while chromosomal changes more specifically associated with aggressive bladder cancer (loss of 17p, 2q, 4, 11p) were uncommon (10). Microsatellite analysis suggested a clonal relationship between hyperplasia and concomitant urothelial carcinoma in 50% of cases. In a study on patients with urothelial hyperplasia in association with low-and/or high-grade urothelial carcinomas, an *FGFR3* mutation was identified in 50% of hyperplasias accompanied by low-grade papillary urothelial carcinoma (11). The molecular data therefore suggest that a lesion with the morphology of urothelial hyperplasia in patients with bladder cancer may indeed represent a (pre)neoplastic lesion. Clinical follow-up data after detection of an isolated flat urothelial hyperplasia have not been reported.

Urothelial dysplasia (also known as low-grade intra-urothelial neoplasia). In urothelial dysplasia, the thickness of the urothelium is usually normal (four to seven layers) but may be increased or reduced. There is loss of clearing of the cytoplasm, nucleomegaly, irregularity of nuclear contours and altered chromatin distribution. Nucleoli are usually not conspicuous; only a minor degree of pleomorphism is allowable in dysplasia and the mitotic activity is variable though usually not in the higher layers. Loss of polarity is evidenced by crowding and nuclei with their long axis parallel to the basement membrane (7).

Dysplastic lesions are typically seen in bladders with urothelial neoplasia and are uncommon in patients without it (12). There is some evidence, largely genetic, that dysplasia shares some abnormalities with CIS and therefore likely represents a precursor lesion. One study that applied the 1998 ISUP/WHO criteria indicated a 19% risk of developing cancer with a mean follow-up of 4.9 years (13).

The few molecular studies specifically carried out on lesions with the morphology of dysplasia show frequent DNA aneuploidy and frequent (75%) deletions in chromosome 9, while in about 50% of cases of dysplasia, a *p53* mutation has been detected, a finding in line with the view that dysplasia represents a genetically unstable neoplastic process (14).

Carcinoma in situ (high-grade intra-urothelial neoplasia). Carcinoma *in situ* is histologically characterized by unequivocal severe cytological atypia, *i.e.* the type of atypia usually seen in invasive urothelial carcinoma (7). CIS frequently shows diffuse, strong cytoplasmic reactivity for CK20 and diffuse nuclear reactivity for p53 throughout the full thickness of the urothelium. CD44 reactivity is limited to a residual basal cell layer of normal urothelium when present, but is absent in the neoplastic cells. A panel consisting of these three antibodies is important as not all cases of CIS consistently exhibit the characteristic immunostaining.

Primary (or isolated) CIS has a 28% risk of progression in 5 years, which is lower than the 40-59% 10-year risk of progression of CIS secondary to a (usually high-grade) urothelial carcinoma (8, 9).

An equally high proportion of about 80% of CIS lesions display mutant *p53* and chromosome 9 deletions (14) and increased proliferative activity, while *FGFR3* mutations are very uncommon (15). Both cytokeratin 20 expression pattern and MIB-1 staining pattern can help distinguish reactive changes from dysplasia and CIS (16).

Non-flat urothelial neoplasms

The classification of the non-flat urothelial neoplasms includes papillary and endophytic lesions. The urothelial lining of the papillary lesions shows a continuum of changes and abnormalities similar to that seen in the flat lesions, ranging from normal and hyperplastic urothelium to dysplasia and CIS (7). The classification includes papilloma and inverted papilloma, papillary urothelial neoplasm of low malignant potential, low- and high-grade papillary

Table II. *Prognosis of urothelial papillary lesions**.

	Papilloma	Papillary neoplasm of low malignant potential	Low-grade papillary carcinoma	High-grade papillary carcinoma
Recurrence	0-8%	27-47%	48-71%	55-58%
Grade progression	2%	11%	7%	Not applicable
Stage progression	0%	0-4%	2-12%	27-61%
Survival	100%	93-100%	82-96%	74-90%

*From A Lopez-Beltran and R Montironi (21).

carcinoma. Their prognosis is reported in Table II. A series of urothelial lesions ranging from hyperplasia to carcinoma can have an exclusively endophytic pattern of growth. The molecular studies have basically been performed on papillary neoplasms.

Papilloma. Urothelial papilloma is defined as a discrete papillary growth with a central fibrovascular core lined by urothelium of normal thickness and cytology. This is a rare benign condition typically occurring as a small isolated growth, commonly, but not exclusively, seen in younger patients. It is considered a benign lesion with a low recurrence rate but no progression (1, 17-19).

A total of 75% of papillomas harbour the *FGFR3* gene mutation (19). In addition, papillomas do not display features of genetic instability. In fact, chromosomal changes, as manifested by loss of heterozygosity, are reported to be lacking in the four samples investigated (20).

Inverted papilloma. Inverted papilloma applies to a benign urothelial tumor that has an inverted growth pattern with normal to minimal cytologic atypia of the cells. Most cases are solitary nodular or sessile lesions, smaller than 3 cm, and arise in the bladder trigone but can also be found along the urinary tract (4, 21, 22). At histology, inverted papilloma has a smooth surface covered by normal urothelium and endophytic cords of urothelial cells invaginating extensively from the surface urothelium into the subjacent lamina propria but not into the muscular bladder wall. Focal minor cytological atypia may be present but mitotic figures are not seen or very rare (22). When completely excised, inverted papillomas have a very low risk of recurrence. In a minority of cases, they may be associated with urothelial carcinoma, occurring either concurrently or subsequently. Rarely, cases of urothelial carcinoma arising in inverted urothelial papillomas have been described.

Molecular analysis of larger series of these lesions provided evidence that they commonly lack genetic alterations, including loss of heterozygosity (LOH). They also lack the *FGFR3* gene mutation, distinguishing them from most urothelial carcinomas (23). The identification of a single case showing non-random X-chromosome inactivation was considered by one research group as sufficient evidence for their neoplastic rather than reactive nature (24). It could be argued, however, that this case may have originated from a larger patch of urothelium of which multiple progenitor cells share the same X-chromosomal inactivation.

Papillary urothelial neoplasm of low malignant potential (PUNLMP). Such a neoplasm is a papillary urothelial lesion with an orderly arrangement of cells with minimal architectural abnormalities and minimal nuclear atypia, irrespective of cell thickness. The urothelium lining the papillae is similar to that in flat hyperplasia (2, 25). The major distinction from papilloma is that in PUNLMP the urothelium is much thicker and/or nuclei are significantly enlarged. Current prognostic information suggests that PUNLMP has lower recurrence and progression rates than low-grade papillary carcinoma (4, 21, 26-29). Tumour recurrence, stage progression and tumour-related mortality occur in approximately 35%, 4% and 2% of patients, respectively.

One paper demonstrated a frequency of *FGFR3* gene mutations in PUNLMP equal to that in low-grade urothelial carcinomas (19) while another (30) showed that LOH occurs in about 80% of PUNLMP, comparable with its frequency in higher grade urothelial carcinomas (31). PUNLMP and low-grade urothelial carcinomas could not be separated by cytokeratin 20 expression pattern (32, 33), MIB-1 proliferation index, or p53 overexpression (32, 34), although for the latter two markers, higher average indices were found in low-grade urothelial carcinomas. Currently, no cDNA profiling data of PUNLMP *versus* low-grade urothelial carcinomas are available, since they were not separately analysed in a large multicenter study on nonmuscle invasive urothelial carcinomas (35).

Low- and high-grade papillary urothelial carcinoma. Low-grade papillary carcinoma is a papillary urothelial lesion with an overall orderly appearance but with easily recognizable variation of architectural and or cytological features seen at scanning magnification (7). Variation of polarity and of nuclear size, shape and chromatin texture are the hallmarks of the lesion. Mitotic figures are infrequent and usually seen in the lower half of the urothelium. The urothelium lining the papillae is similar to that in flat dysplasia. Tumour recurrence, stage progression and tumour-related mortality is approximately 50%, 10% and 5%, respectively.

High-grade papillary urothelial carcinoma is a papillary urothelial lesion with predominantly or totally disorderly appearance at low magnification, with both architectural and cytological abnormalities. The epithelium is disorganized and there is a spectrum of nuclear pleomorphism ranging from

moderate to marked. The nuclear chromatin tends to be clumped and nucleoli may be prominent. Mitotic figures, including atypical forms, are frequently seen at all levels (7). The urothelium lining the papillae is similar to flat CIS. High-grade papillary urothelial carcinomas have a high risk of progression, with figures varying from 15% to 40% , and of association with invasive disease at the time of diagnosis (36, 37).

A number of molecular and genetic studies separately analysed the noninvasive (pTa) and superficially invasive (pT1) carcinomas. *FGFR3* gene mutations are very common in pTa carcinomas (>70%), while *p53* gene mutations occur in fewer than 5% (15, 38, 39). The latter are associated with high-grade pTa carcinomas. A significant difference with regard to the occurrence of both the *p53* and *FGFR3* mutations was noted in pTa urothelial carcinomas *versus* pT1 urothelial carcinomas (40). Strikingly, in papillary pTa urothelial carcinomas, the presence of *p53* and *FGFR3* mutations are virtually mutually exclusive (38). Mutations of the *ras* genes have been found in a small proportion of pTa urothelial carcinoma. These tumours do not carry a mutant *FGFR3* gene (41). Zieger *et al*. showed that pTa carcinomas are generally characterized by genetic stability, related to their common absence of *p53* inactivation, with chromosomal changes limited to chromosome 9, while pT1 urothelial carcinomas mostly exhibit increased genetic instability with chromosomal changes extending to 17p, 13q, and 8p (39). Previous studies had also shown that pTa urothelial carcinomas are characterized by a limited number of chromosomal changes, most notably loss of (parts of) chromosome 9, as well as by gain of 1q (42). Single nucleotide polymorphism array analysis on a limited series of cases extended this observation, as it showed frequent allelic imbalance in pTa cancer for 20p and q, as well as (somewhat less frequently) for 18q (43), separating them statistically from pT1 cancer. In an initial cDNA expression profiling study on bladder cancer, about 80% of pTa cancer could be correctly classified, while pTa cancer wrongly classified as pT1 or pT2 proved to have a worse prognosis (6). A separate study using unsupervised clustering of gene expression profiles of a set of superficial bladder carcinomas (pTa, pT1) reported their successful separation into two clusters of genes, one mainly containing low-grade pTa tumours and the other containing all pT1 tumours as well as a substantial proportion of (mostly high-grade) pTa tumours (44). The classification success with their multigene classifiers to separate pTa from pT1 carcinomas was about 75% in their study.

Invasive urothelial carcinoma

There is considerable confusion in the terminology applied to invasive urothelial carcinoma. Various terms include "superficial muscle invasion," "deep muscle invasion," "muscle invasion (not otherwise specified)", and "superficial bladder cancer". The latter term is particularly confusing as it could be applied to CIS, noninvasive papillary neoplasms, or truly invasive urothelial carcinoma (3, 4). Due to variations in treatment and prognostic significance related to the depth of invasion of bladder tumours, the WHO 2004 developed several recommendations to provide clinicians with this essential information in an unambiguous manner (3, 4). WHO 2004 recommended invasive urothelial carcinomas be graded as low or high grade (3, 4, 21, 45).

Invasive carcinomas, including pT1 urothelial carcinomas, frequently display genetic instability as manifested by loss of 1p, 2q, 4q, 5q, 8p, 10q, 11p, 11q and gain of 1q, 2p, 5p, 8q, 11q13, 17q and 20q, in addition to loss of (parts of) 9. The number of chromosomal changes increases with stage, reflecting increasing genetic instability. Muscle invasive (pT2) tumours displayed much more frequent allelic imbalance of chromosome 6, 10p, and 22 as compared to pTa and pT1 carcinomas (43), while *FGFR3* mutations can be shown in 5-15% of pT2-T4 urothelial carcinomas (38, 46, 47).

Pathways in Bladder Cancer Development

Nearly 60% to 90% of patients with "superficial" disease will have a tumor recurrence if treated by transurethral resection (TUR) alone (48-50). A retrospective analysis of 176 patients from Sweden with superficial carcinomas, who were followed until death or for at least 20 years (with no adjuvant therapy), provides some insight into the importance and natural history of this disease when left untreated. An overall recurrence rate of 80% was reported, with 22% of patients dying from the disease if followed long enough; 11% of patients were diagnosed with Ta and 30% of patients with T1 disease (51). In this study, death was directly related to tumour grade, number of tumours and volume of recurrences (51). Combining pathological and molecular findings (Figure 1), a two-pathway model for carcinogenesis has been proposed by Spruck *et al*. (52), distinguishing a genetically stable, prognostically favourable papillary route and a genetically unstable, prognostically unfavourable CIS-invasive carcinoma route, characterized by *p53* mutations. The finding that *FGFR3* mutations are selectively present in the low-grade papillary carcinomas, displaying limited genetic instability as opposed to the flat intraurothelial neoplasias (CIS) strongly substantiates this dual molecular pathway model of bladder carcinogenesis. The strong and selective association of up- and down-regulated genes with *FGFR3* mutation status revealed by recent cDNA profiling studies supports this model (35, 53).

The identification of a subset of 5% of bladder carcinomas carrying both a *p53* and an *FGFR3* mutation, and in particular the observation of both *FGFR3* and *p53*

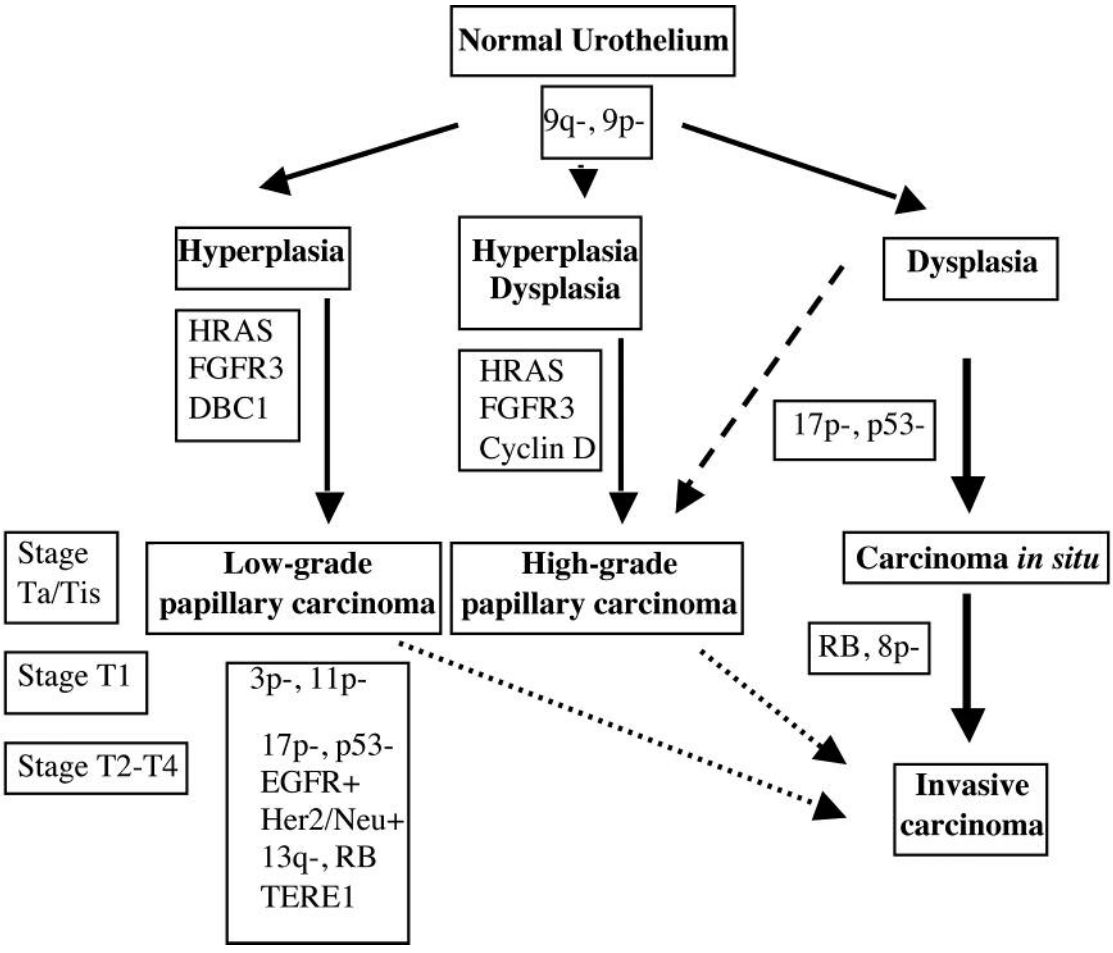

Figure 1. *Molecular pathways in bladder cancer development and progression.*

mutations in occasional pTa carcinomas (53) has led to the proposal of a three pathway model in which the "third" pathway would lead to high-grade pT1 cancer (42). This might also explain why in this subset of pT1 cancer, *FGFR3* and *p53* mutations are not mutually exclusive. An alternative explanation for the existence of this subset of pT1 carcinomas is that they might arise by dedifferentiation from low-grade papillary carcinomas, acquiring a *p53* mutation and losing the *FGFR3* mutation. Indeed, Tomlinson *et al*., by analyzing noninvasive and invasive parts of the same papillary urothelial carcinoma, showed that they may be heterogeneous with regard to *FGFR3* mutations, with most cases showing loss of the *FRGFR3* mutation in the invasive component (46). From a pathological perspective, Cheng *et al*. (8) noted that about 25% of pTa urothelial carcinomas display grade heterogeneity, another argument for the potential of dedifferentiation of noninvasive urothelial carcinomas.

Conclusion

Some leading experts in the field of urogenital pathology have expressed their concerns with the WHO 2004 categorization system both with regard to the nomenclature and the grading categories used (26, 33, 54). Some argued that it is inappropriate to employ a terminology of carcinoma for noninvasive tumours, particularly since their behaviour is less "malignant" than CIS or high-grade intraurothelial neoplasia of the urinary tract, and more or less similar to that of "polyps" of the colorectal tract (2.5% risk of invasive carcinoma after 5 year for polyps >10 mm) (55). Molecular findings comparing the transition of noninvasive (pTa) to invasive (pT1) papillary are also in line with their being separate entities, including expression cDNA profiling studies, stage-specific chromosomal alterations and, most notably, the finding of a switch from polyclonality to monoclonality (43), similarly as shown in tubular adenomas of the colon (56).

Thus, an entity of papillary intraurothelial neoplasia could be envisaged, replacing the terminology of noninvasive papillary carcinomas introduced by the WHO 1973 classification. These papillary intraurothelial neoplasias could comprise both low- and high-grade variants, in perfect symmetry with the flat lesions of the urothelium, *i.e.* low and high-grade intraurothelial neoplasia or dysplasia and CIS. It would also be very much analogous to the nomenclature of low- and high-grade adenomas of the colorectal tract.

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