HOW COULD MOLECULAR BIOLOGY MODIFY THE MANAGEMENT OF UPPER URINARY TRACT TUMOURS?

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Summary.- Background: Upper urinary tract urothelial carcinomas (UUT-UCs) account for only 5-10% of urothelial carcinomas and the gold standard treatment is open radical nephroureterectomy. Strong differences exist regarding tumor behaviour between the upper and the lower urinary tract.

Objective: To demonstrate how the current knowledge in molecular biology of UUT-UCs is likely to modify the management of these tumors.

Acquisition of evidence: A MEDLINE search was performed on UUTUC using the following terms: urinary tract cancer; urothelial carcinomas; upper urinary tract; molecular markers; renal pelvis; ureter; ureteroscopy; nephroureterectomy; adjuvant treatment; neoadjuvant treatment; recurrence; risk factors and survival.

Evidence synthesis: Conservative surgery for low-risk UUT-UCs allows for preservation of the upper urinary renal unit, while sparing the patient the morbidity associated with open surgery. Such surgical strategy might be more appropriate in tumors displaying certain molecular markers: microsatellite instability, E-cadherin, MET, Aurora-A, and Ki-67. These markers could help to identify more candidates to nephron-sparing treatment without compromising the oncologic outcome. Susceptibility means an increase in risk conferred by one or more polymorphisms (allele types) of a given gene or genes, which expose the individual to the genotoxic effects of environmental carcinogens. The variant allele SULT1A1*2 with reduced sulfotransferase activity and the T allele of rs9642880 on chromosome 8q24 enhance the risk of UUT-UCs. If an at-risk genetic profile could be established, it might be possible to prevent urothelial carcinomas in some patients.

Conclusions: Surgical practice is gradually moving towards minimally invasive techniques which spare the functional unity of the kidney and urinary tract. The ongoing identification of distinct carcinogenic mechanisms for UUT-UCs might open the way to specific treatments adapted to the molecular pattern of each tumor. The next era might hopefully be that of chemoprevention.


Resumen.- OBJETIVO: Los carcinomas de células uroteliales del tracto urinario superior (TUS) suponen sólo el 5-10% de los carcinomas uroteliales y el tratamiento de referencia es la nefroureterectomía abierta. Hay grandes diferencias respecto al comportamiento de los tumores entre el tracto urinario superior e inferior.

Demostrar cómo el conocimiento actual en biología molecular de los tumores uroteliales del TUS es probable que modifique el manejo de estos tumores.
Adquisición de Evidencia: Se realiza una búsqueda en MEDLINE sobre tumores uroteliales del TUS utilizando los siguientes términos: cáncer del tracto urinario, carcinomas uroteliales, tracto urinario superior, marcadores moleculares, pelvis renal, ureter, ureteroscopia, nefroureterectomía, tratamiento adyuvante, tratamiento neoadyuvante, recurrencia, factores de riesgo y supervivencia.

Síntesis de Evidencia: La cirugía conservadora para los tumores uroteliales del TUS permite la preservación de la unidad renal a la vez que evita al paciente la morbilidad asociada con la cirugía abierta. Dicha estrategia quirúrgica podría ser más apropiada en tumores que muestren ciertos marcadores moleculares: inestabilidad de microsatélites, Ecadherina, MET, Aurora-A y Ki67. Estos marcadores podrían ayudar a identificar a más candidatos a tratamiento conservador sin comprometer el resultado oncológico. La susceptibilidad significa un aumento de riesgo otorgado por uno o más polimorfismos (tipos de alelos) de un determinado gen o genes, los cuales exponen al individuo a los efectos genotóxicos de los carcinógenos ambientales. El alelo variante SULT1A1*2 con actividad reducida de sulfotransferasa y el alelo T de rs9642880 en el cromosoma 8q24 aumentan el riesgo de tumores uroteliales del TUS. Si se pudiera establecer un perfil genético en riesgo, podría ser posible prevenir los carcinomas uroteliales en algunos pacientes.

Conclusiones: La práctica quirúrgica se está moviendo gradualmente hacia las técnicas mínimamente invasivas que conservan la unidad funcional del riñón y tracto urinario. La identificación en marcha de distintos mecanismos carcinogénicos para los tumores uroteliales del TUS podría abrir el camino para tratamientos específicos adaptados al patrón molecular de cada tumor. La próxima era podría ser la de la quimioprevención.


INTRODUCTION

The urothelium is the epithelium that lines the upper and lower urinary tract. Over 95% of urothelial malignancies are derived from urothelium. They can be located either in the lower tract (bladder, urethra) or in the upper tract (pyelocaliceal cavities, ureter). The estimated annual incidence in western countries is about 1-2 new cases/100,000 inhabitants. Pyelocaliceal tumours are about twice as common as ureteral tumours (1,2). The gold standard treatment for UTUC is open radical nephroureterectomy (NUT) with removal of a bladder cuff despite morbidity and the systematic loss of a renal unit (3,4). In some imperative cases (i.e., a patient with a solitary kidney, bilateral disease, or renal insufficiency), endoscopic management has been proposed with promising results (5,6). The natural history of UTUC differs from that of bladder cancer: 60% of UTUC are invasive at diagnosis compared to only 15% of bladder tumours. Moreover, UTUC invading the muscle wall usually have a very poor prognosis. The 5-year specific survival is less than 50% for pT2/pT3 and less than 10% for pT4. Although the mechanisms of carcinogenesis are thought to be similar throughout the urinary tract, recent epidemiological data and genetic studies suggest otherwise (7). It is now obvious that strong differences exist regarding tumor location and behavior between the upper and the lower urinary tract (8). In the current article, our objective was to depict how the knowledge of molecular pathways and molecular biology involved in UTUC is likely to modify the management of these tumours.

Evidence Acquisition

A MEDLINE search was performed on urothelial malignancies and UTUC management using combinations of the following terms: urinary tract cancer; urothelial carcinomas; upper urinary tract; molecular markers; renal pelvis; ureter; ureteroscopy; nephroureterectomy; adjuvant treatment; neoadjuvant treatment; recurrence; risk factors and survival. The publications concerning UTUC were mostly retrospective, including some large multicentre studies. Due to the scarcity of randomized data, articles were selected for this article based on the following criteria: evolution of concepts; accordance with current topic; study quality and relevance. Older studies were included selectively if they were historically relevant or in case of scarce data in recent publications.

Carcinogenesis, genetic pathways and molecular markers

Urothelial tumours can develop in a synchronous or metachronous multifocal manner at different urinary tract sites. It belongs to the so-called “clonal development theory” where two hypotheses have been put forward to explain this (9):

- the field change hypothesis, which considers that mutagenic agents in the urine, in contact with the entire urothelium, can transform cells at several sites and thus induce the development of several tumour clones;

- the clonal development theory, which attributes the development of tumours to the transformation of multiple clones at multiple sites.
• the intraluminal seeding and implantation hypothesis supports the view of the clonal development of a multifocal cancer. Multiple sites and recurrences would be due to migration within the lumen, then to grafting of tumour cells to the urinary tract wall, or to the intraepithelial expansion of cells from the primary tumour.

Several genetic studies support a clonal origin for UCs. The analysis of p53 gene mutations and of the pattern of genetic changes has also established the clonal nature of synchronous urothelial tumours in most urinary tract cancers. Heterogeneity or oligoclonality could be explained by clonal divergence and the selection of different cell populations originating from an initial common tumour cell (10). Some of the changes occur in non invasive and are associated with tumour onset; others are specific of infiltrating or high-grade tumours and are involved in tumour progression. The proposed schemes for the sequence of genetic events are neither exhaustive nor identical, but all agree that the tumour develops in several stages, each of which is associated with specific genetic changes. Differences between upper tract and lower tract urothelium have been described in the biological characteristics of the urothelium in the ureter and renal pelvis compared to the bladder (8). These results support the fact that UTUC and bladder UC have different carcinogen pathways.

**Microsatellite instability**

MSI is rarely encountered in bladder UCs (about 3%). Since MSI occurs in more than 15% of sporadic UTUC, somatic inactivation of mismatch repair genes (MMR) might be an initiating event in non-HNPCC tumours (11,12). In fact, a deficient MMR system might be involved in the pathogenesis of both hereditary and sporadic cancers. Tumours with stable microsatellites do not show loss of mismatch repair gene expression. The genetic risk of developing UTUC is thus multifactorial. A high-penetrance germline mutation (as in HNPCC) is rarely (<5%) at the origin of an UTUC. A proportion of UTUC would occur in individuals with a significant inherited multifactorial susceptibility to urothelial cancer that is not obviously familial. MSI incidence is high in UTUC (>20%) but very low in bladder cancer (<3%), confirming that UTUC belong to the category of HNPCC-related tumours but that bladder cancer does not.

Significant advances have been made in UTUC management on the basis of MSI status. In patients with UTUC displaying high-MSI levels, the cancer is likely to be hereditary if the patient is under 60 years of age or has a personal or family history of a HNPCC-type cancer (13). MSI screening identifies an extra 4% of hereditary cancers (14). When gene mutations are detected, the patient benefits from multidisciplinary management. The presence of other HNPCC-associated cancers is sought and close monitoring of patients is undertaken. Genetic counselling is provided to the patient’s family. In addition, high MSI is a sign of a better prognosis. It identifies a subset of patients with better survival. Moreover microsatellite markers also predict response to adjuvant treatment in patients with colorectal cancer. This is based on the observation that in vitro MSI-High cell-lines respond better to chemotherapy agents than stable cell-lines do. In a near future, MSI might help select patients with invasive UTUC with enhanced sensitivity who might as well benefit from adjuvant chemotherapy (14). This would be an important step forward as adjuvant treatments offer only modest results currently.

**E-Cadherin**

Epithelial Cadherin (E-CD) is a transmembranous cell-cell adhesion receptor, responsible for the maintenance of tissue architecture. It is expressed in most epithelial tissues and in some types of malignancies. In fact, loss of E-CD is believed to be responsible for de-differentiation and invasiveness due to a dissociation of cells from tissue structures. E-CD expression can be easily detected and quantified by immunohistochemistry. Loss of E-CD correlates with an increased risk of recurrence and a poorer survival rate in UTUC. It can be used to identify a group of patients that require a thorough follow-up (15-17).

**Ki-67**

This protein is a marker of cell proliferation. The fraction of Ki-67 positive cells (labelling index) has been shown to correlate with clinical outcomes in tumours. The labeling index can be determined by immunohistochemistry. In several studies, Ki-67 correlated with grade and stage. It was a predictor of disease free survival in univariate and multivariate analysis (15,16).

**MET**

The tyrosine kinases MET is a member of the MET proto-oncogene family. It has been shown that they can encourage invasive growth process, tumour growth and metastazation. In UTUC samples, MET was significantly associated with clinicopathological findings (17). High MET correlated with higher occurrences of vascular invasion (18).

**Aurora-A**

Aurora-A, located on 20q13, is a member of the serine/threonine kinase and is essential for mitosis. Aurora-A regulates spindles assembly before
mitosis, centrosome maturation and chromosome segregation. Therefore, Aurora-A plays a key role in cell division and in maintaining genomic integrity. In UTUC, Aurora-A influences the development of vascular invasion and tumor aggressiveness via a mechanism not clearly elucidated yet (19).

Toward enhanced indications of conservative management

Conservative surgery for low-risk UTUC allows for preservation of the upper urinary renal unit, while sparing the patient the morbidity associated with open surgery. With advances in fiberoptic technology and ever smaller calibre instruments, open surgery is being superseded progressively by less invasive endoscopic procedures. Consequently, first-line conservative endoscopic management is also being increasingly performed in patients with a normal contralateral kidney (1,2,20). Endoscopic management is currently used by several teams in elective indications, due to better experience and enhanced material dedicated to conservative approaches. It is even becoming the preferred option for some patients in this situation. Over the past 15 years, rigid ureteroscopy has been established as a minimally invasive modality for the treatment of upper tract tumours. Advances in distal-tip deflection and scope durability have expanded the role of flexible ureteroscopy from a diagnostic to a therapeutic procedure (6). This improvement in technology has built on existing experience to expand the potential indications of flexible ureteroscopy to include UTUC.

According to the most recent European guidelines, conservative management of UTUC can be considered in imperative cases (renal insufficiency, solitary functional kidney) or in elective cases (i.e. when the contralateral kidney is functional) for low-grade, low-stage tumours. The choice of technique depends on technical constraints, the anatomical location of the tumour and the experience of the surgeon (Table I) (1,2).

Conservative management is increasingly being advocated instead of nephroureterectomy, the gold-standard treatment, for UTUC with a good prognosis. Such surgery might be most appropriate in patients with tumours displaying aforementioned molecular markers (e.g., MSI). Obviously, all patient should undergo first a diagnostic ureteroscopy with multiple biopsies to screen for these markers. Thus, a better molecular knowledge of these tumours could

<table>
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<tr>
<th>Indications for conservative management of UUT-UCC</th>
<th>Grade of recommendation</th>
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<tr>
<td>• Unifocal tumour</td>
<td>B</td>
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<tr>
<td>• Small tumour</td>
<td>B</td>
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<tr>
<td>• Low-grade tumour (cytology or biopsies)</td>
<td>B</td>
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<tr>
<td>• No evidence of an infiltrative lesion on CT urography</td>
<td>B</td>
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<tr>
<td>• Understanding of close follow-up</td>
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Techniques used in conservative management of UUT-UCC

| • LASER should be used in case of endoscopic treatment                   | C                       |
| • Flexible ureteroscopy is preferable over rigid ureteroscopy            | C                       |
| • Open partial resection is an option for pelvic ureteral tumours        | C                       |
| • A percutaneous approach remains an option in small, low-grade, caliceal tumours unsuitable for ureteroscopic treatment | C                       |
help to identify more candidates to nephron-sparing treatment without compromising the oncologic outcome.

In order to make it understandable, we propose the flow-chart in Figure 1 to assess how markers have the potential to advance soon from the laboratory to the bedside of our patients.

**Further perspectives: polymorphism and genetic susceptibility**

Genetic polymorphism is defined as the presence of different allele sequences for a single gene; this is sometimes linked to variations in the expression of constitutive DNA. Some genetic polymorphisms are associated with an increased risk of cancer or faster disease progression. Most identified risk factors concern urothelial carcinomas in general and are similar for UTUC and bladder cancers. Most of the chemicals that initiate urothelial carcinomas, including those found in tobacco smoke, are activated in the kidney to genotoxic metabolites which interact with DNA. Besides, there are UTU-specific risk factors which include kidney disorders due to abuse by analgesics and Chinese herbs, and Balkan endemic nephropathy (21). UTU tumours develop once symptoms of kidney disorders have appeared. Deleterious changes in the renal parenchyma are at the origin of 8-10% of UTUC. Kidney disorders are more common in women than in men and occur after prolonged exposure to analgesics. Phenacetin, in particular, has been incriminated as it is sometimes involved in the onset of papillary necrosis. The implication of Chinese herbs in UTUC is a recent discovery (21). Although herbalists may recommend these herbs for their hepatic properties, rapidly progressive renal fibrosis occurs on prolonged intake (22). The carcinogens contained in these herbs are degraded by renal cell microsome enzymes. Kidney toxicity is due to the metabolites of Aristolochic Acid (AA) which intercalate into DNA and induce breaks. Because of the high inter-individual variability in the response to Chinese herbs, it has been hypothesised that some

![Figure 1. Flow-chart for modern management of UUT Ucs with the help of molecular markers: to enhance the number of candidate for conservative treatment (A) and to screen for hereditary tumors (B) (dashed lines and arrows: research perspective).](image)
Susceptibility means an increase in risk conferred by one or more polymorphisms (allele types) of a given gene or genes, which expose the individual, family or group of individuals (ethnic/geographic variations) to the genotoxic effects of environmental carcinogens, or which promote tumour progression by determining a particular hormone or immune status. Differences in the ability to activate carcinogens may contribute to host susceptibility and be involved in risk of urothelial carcinomas. Genetic polymorphism of enzymes metabolizing carcinogens would yield products of different activity and may determine cancer risk. The kidney microsome enzymes involved in AA; i.e. constituent of the plant species Aristolochia, activation and/or detoxification are an example. The activity of these enzymes varies according to the individual (24). Screening patients and healthy persons for genetic polymorphisms might reveal relationships between genotypes and nephropathy, AA-DNA adduct levels and urothelial cancer risk.

Sulfation is an important step in the detoxification of many environmental chemicals but also in the bioactivation of dietary and other mutagens. It has been recently reported that the variant allele SULT1A1*2 with reduced sulfotransferase activity enhances the risk of UTUC (25). Cytosolic sulfotransferases (SULT) catalyze the conjugation of sulfo group to these substrate, the two major gene families being the phenol (SULT1A1) and hydroxysteroid (SULT2A) sulfotransferases. In the same manner, a polymorphism located at the T allele of rs9642880 on chromosome 8q24 was linked to an enhanced risk of UTUC (26). Although it is not unusual that a genotype confers protection for an organ and increases the risk for another, UUT-UC may share some risk factors or molecular disruption pathways with bladder UC, but each has its own specific features.

Looking ahead, primary prevention would involve, for instance, avoiding exposure of a tissue to a carcinogen. Recently, more active prevention by the administration of inhibitors of carcinogenesis has been proposed for colorectal carcinomas. Recent studies have shown that COX-2 inhibitors can reduce the incidence of colorectal adenomas (premalignant lesions) and the development of sporadic colorectal tumours (27,28). Thus, the characterisation of the molecular features of urothelial carcinomas is still ongoing. The ultimate goal is to relate the tumour genotype with the individual’s phenotype and to offer the patient treatment that is adapted to the specific molecular pattern of their cancer. Yet, it has taken a century to move from the era of treating the ‘urinary apparatus’ to tumour detection and identification by molecular biology techniques. If an atrisk genetic profile could be established, it might be possible to predict, even prevent, urothelial carcinomas in some patients. The next era might hopefully be that of chemoprevention.

**CONCLUSION**

Histologically identical urothelial tumours may arise through different molecular mechanisms. The genetic profiles thus derived may reveal relationships with clinical phenotypes, thus enabling “rational” management of UTUC. The molecular characterisation and the management of urothelial carcinomas has progressed considerably over the last decade. The identification of hereditary tumours of the UUT has, unexpectedly, led to the development of new molecular biology tools useful in diagnosis or prognosis. Microsatellite markers have become part of the urologist’s tool-kit. In addition, surgery is moving towards minimally invasive techniques that spare the kidney’s functional unity as much as possible. The satisfactory cancer control outcomes obtained with conservative endoscopic treatment suggest that endoscopy could be a convincing alternative to NUT of superficial or low-grade UTUC. Surgical practice is gradually moving towards minimally invasive techniques which spare the functional unity of the kidney and urinary tract. The ongoing identification of distinct carcinogenic mechanisms for UTUC might open the way to specific treatments adapted to the molecular pattern of each tumour.

**REFERENCES AND RECOMMENDED READINGS**


