Editorial

Predicting the biological behavior of non-muscle-invasive bladder cancer: from histology to molecular taxonomy

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Urinary bladder cancer represents the seventh most common cancer worldwide; the most common type of bladder cancer is urothelial carcinoma (1). About 75% of patients are initially diagnosed with non-muscle-invasive bladder cancer (NMIBC), whose treatment includes a surveillance regimen based on risk stratification for recurrence and progression (1,2). The need for intensive and long-term follow-up, mainly represented by cystoscopic examinations with biopsies, makes bladder cancer the most expensive cancer to treat (3).

To predict the risk of recurrence and progression for NMIBC patients, the European Organization for Research and Treatment of Cancer (EORTC) has designed a weighted calculator based on six different morphological variants; grade, stage, presence of carcinoma in situ (CIS), multiplicity, size, and prior recurrence rate (4). This calculator is widely used for addressing clinical decisions and has been validated by a recent study (5). Recently, it has been clarified that tumors with similar morphological features can have distinct genetic profiles and may belong to different molecular subgroups with different biological behavior (1). Interestingly, many attempts in these last years have demonstrated the promising predictive value of molecular markers in NMIBC (1,6). For example, a so-called "molecular grade" based on *FGFR3* mutation and MIB-1 expression has been proposed and validated, identifying three subgroups at favorable (mutation; normal expression), intermediate (no mutation; normal expression or mutation; high expression) or poor (no mutation; high expression) prognosis (5).

Interestingly, a Danish group has firstly identified a 16-gene classifier for CIS (7) and then a 45-gene signature of disease progression for superficial bladder carcinoma (8), both strictly correlated with clinical outcome (7,8). These first classifiers were independent from classical histopathological features, highlighting the importance of a molecular integration of morphological parameters for a better prognostic stratification of NMIBC. In 2012, the same research group performed a comprehensive analysis taking into account also the issue of intra-patient variation of molecular profile. Considering indeed a specific panel of 96 genes associated with disease aggressiveness (38 from published genes signature and 58 from reanalysis of microarray data) in 39 metachronous tumors from 17 patients, the researchers obtained a PCR-based 12-gene signature for NMIBC progression (9). This 12-gene panel incorporated seven genes up-regulated (*KPNA2*, *BIRC5*, *UBE2C*, *CDC25B*, *COL4A1*, *MSN* and *COL18A1*) and five

genes down-regulated (*COL4A3BP*, *MBNL2*, *NEK1*, *FABP4* and *SKAP2*) in progressing tumors. Different combinations among these genes constitute a so called "progression score".

Although this genetic panel demonstrated a high prognostic value also in multivariable analysis, a definitive validation of such panel on a larger cohort was urgently needed before considering it for a potential utilization in clinical practice. To this aim, 1,224 patients (of which 750 fulfilled the inclusion and sample quality criteria) from ten different European centers were enrolled for a subsequent study, that represents the prospective multicenter validation of the 12-gene panel of NMIBC progression (10). Firstly, the researchers analyzed the progression score using the first tumor included from each patient. A high progression score was significantly associated with old age, high stage and grade, concomitant presence of CIS, Bacillus Calmette-Guérin (BCG) treatment, progression to muscle invasion and high EORTC score. The progression score revealed to contain independent prognostic information when compared to clinical risk/EORTC parameters. Notably, combining the progression with the EORTC score in a combined score, the predictive accuracy was significantly increased. The combined score also revealed a better specificity.

In a second analysis, Dyrskjøt and colleagues applied the 12-gene panel considering all tumors of all patients. Since the risk of progression may change during the disease course and since recurrent multifocal tumors may have distinct genetic profiles as well as different biological behaviors, they classified patients using the highest score obtained in the disease course for cases with multiple tumors analyzed. With this approach, the progression score changed from low to high risk for 20% of patients, remaining stable for the 80% of patients. Although a stable score for the majority of patients was observed, these results highlight the importance of a continuous analysis during the disease course, representing the 20% of up-graded patients a not negligible proportion. From the molecular point of view, this is in line with the known theory of the genetic field cancerization of the bladder. Indeed, recurrent tumors share multiple clonal mutations, but, at the same time, new mutations may accumulate during disease course potentially changing the risk of progression as observed in 20% of patients of this study. Interestingly, the 12-gene progression score showed also a significant correlation with another very promising molecular classifier (11), in which tumors were subdivided into three categories (low, intermediate and high risk of progression) on the basis of transcriptional analysis and the presence of basal or luminal phenotypes. Indeed, tumors with high-risk progression score were classified mostly as class 2 (high risk), while tumors with low-risk progression score were classified mostly as class 1 (low risk) or class 3 (intermediate risk) (10). This validation further highlights the potential predictive value of the 12-gene panel; based on PCR analysis, furthermore, its feasibility appears more suitable also for the application in clinical routine practice than more complex molecular analyses. Another point of strength of this method is represented by its validation on both fresh-frozen and formalin-fixed paraffin-embedded tissue, making of this 12-gene panel a versatile and robust method of molecular analysis.

The genes considered by this panel are important genes in cancer (with features of drivers) and may be incorporated in specific panels for next-generation sequencing. For example, among genes up-regulated in aggressive bladder tumors, the study of *KPNA2* may be of interest for oligoastrocytomas (down-regulated in long-term survivors) (12), and among genes down-regulated in progressive bladder tumors, the determination of mutational status of *SKAP2* appears of importance in non-small-cell lung cancer, where its iper-expression is associated with poor prognosis (13).

A key point for the application of genetic analysis in the future will be represented by the possibility of investigating few but highly significant mutated genes in different tumors, also from different organs, with the same genetic panel; the design of these panels is already started and is also very promising (14). Taking into consideration the potential limitations of the validation of the 12-gene NMIBC classifier (10), the first regards to the low progression rate (37 patients out of 750, 5%): a longer follow-up may guarantee a more precise indication about recurrence and muscle invasion. Furthermore, future methods should consider not only inter-tumor heterogeneity (partly represented in this work by the analysis of different tumors of same patients), but also intra-tumor heterogeneity.

The future perspective of the molecular taxonomy of bladder cancer is encouraging, and integration for different applications of molecular classifier are at the same time urgently needed. For example, a new field of application of such genetic analyses is represented by the so-called liquid biopsies. A recent work has indeed indicated that cell-free tumor DNA can be detected in plasma and urine even in patients with noninvasive tumors. With high levels of tumor DNA detectable before progression, and above all in urine samples (15), this tool may become fundamental in a near

future, maybe used in parallel with a robust genetic classifier like the 12-gene panel described, not only for patients monitoring but also for addressing the most appropriate clinical decisions.

Another very important future perspective regarding the predictive power of genetic tools is represented by the possibility through molecular analysis to predict the probability of nodal metastasization of bladder cancer. Indeed, in patients with high-risk NMIBC and muscle-invasive bladder cancer undergoing radical cystectomy, accurate prediction of the presence of nodal metastasis could provide guidance for selecting patients who have an imperative need for perioperative systemic chemotherapy integrated with extended lymph nodes dissection at the time of radical surgery (16). Smith and colleagues, for example, studying clinically lymph-node negative, muscle-invasive bladder cancer patients, developed a 20-gene panel able to stratify patients into low or high risk of lymphovascular invasion and of nodal metastases (17).

Notably, the extranodal extension (ENE) is a morphological feature of nodal metastasis that has been recently indicated as prognostically important in many cancer types (18-22), including bladder and other genitourinary tumors (23,24). ENE indicates that neoplastic cells, through a rupture of nodal capsule, have reached perinodal adipose tissue, increasing local aggressiveness and metastatic potential. Since for some tumors, for example the squamous cell carcinoma of head and neck, ENE-predictive genetic panel already exists (25), future genetic analysis may consider this parameter as an important prognostic moderator that can be predicted, earmarking ENE a subgroup of node-positive cancer patients with significantly poorer prognosis. These genetic models may also address future strategies of targeted therapy.

In conclusion, genetic taxonomy, in particular the 12-gene classifier, is potentially useful predictive tools for NMIBC. Notably, the panel by Dyrskjøt and colleagues has been validated in a large cohort of patients, and its predictive power is enhanced when it is used in conjunction with histomorphological parameters. The potential of a combined approach (molecular profile plus histological level) is promising and may represent the decisive tool for precision medicine.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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