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Review

An update on the molecular pathology of urinary bladder tumors

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ABSTRACT

Urothelial carcinoma is the fourth most common tumors after prostate cancer, lung, and colorectal carcinoma but the second most common urologic malignancy. Urothelial carcinoma composed more than 90% of bladder tumors while squamous cell carcinoma and adenocarcinomas composed 5% and 2% respectively. The intense research involving the different molecular aspects of bladder cancer has provided a great insight into identifying more about molecular profiling and pathways of bladder cancer.

In this review, we will highlight the general concepts of the molecular features; profiling and classification as well as the molecular pathways for bladder carcinomas, especially urothelial carcinoma. Also, we will discuss the advances of molecular biomarkers for screening, early diagnosis, surveillance and potential prognosis of urothelial carcinoma of the bladder. Studies showed that accumulation of genetic alterations involving the clonal expansion of altered cells with growth advantages through sequential multi-step pathways results in progression of bladder tumors.

The accumulated research data from literature has revealed that the genomic signatures of urothelial carcinoma are required to subclassify bladder cancer into genetically distinct subgroups. These findings could improve the understating of pathogenesis as well as will provide new therapeutic modules e.g. targeted therapy.

Introduction

Histopathological and molecular studies indicate that urothelial carcinomas (UC) may evolve along two different pathways with distinct biological behavior and clinical prognosis that presented as a heterogeneous group of tumors. Urothelial tumor has two common subtypes. The most common is the papillary, low-grade, non-invasive subtype (70%), believed to arise from areas of urothelial papillomas or hyperplasia and often multifocal with high recurrence rate. These tumors (10%–15%) are infrequently progress to muscle invasion. Stage pT1 tumors that have lamina propria invasion without muscle invasion represent 10% to 20% of cases. Interestingly, there is a subset of pT1 tumors with aggressive behavior and recurrence with the muscle-invasive disease. This subset has mixed molecular features that are related to both pT1 and the invasive subtype. The second group is presents with aggressive muscle-invasive disease (Stages pT2–pT4). This represents about 20% of urothelial cancers which will spread and develop metastases in about half of the cases and less than 50% 5-year survival rate. Most of the invasive tumors arise through the subsequent sequences of events start from normal to dysplasia, CIS and then invasive tumors.

1. Overview of the molecular pathogenesis of urothelial cancer

The recent identification of Cancer Stem Cells (CSCs) in urothelial carcinoma has shed more light into the two-pathway model of carcinogenesis of UC. Evidence suggests that the urothelium is a hierarchically organized tissue containing urothelial stem cells that can give rise to biological heterogeneity within a tumour by differentiating into downstream differentiated tumour cells. Ck14, p63 and ALDH1A1 are some of the CSCs markers which may have a role in developing aggressive type of UC. A study showed that CD44 positive CSCs show higher tumorigenic potential than CD44 negative cells. Also, a panel of 477 genes were found to be up regulated in CD44+ CSCs (referred to as a bladder CSCs gene signature [1] This signature was found to have prognostic significance. Furthermore, stem cell-related genes can highlight subgroups of patients with non-invasive bladder cancers who are at risk of developing aggressive disease with shorter survival. It has also been reported that high-grade UC that is poorly differentiated is characterized by overexpression of embryonic stem-cell genes [2]. Collectively, all the research findings could help to identify candidate genes associated with the clinical behaviour of aggressive bladder cancers. This could potentially lead to the establishment of standardized microarray-based tests as pioneered for breast cancer and the

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development of a personalized approach to the clinical management of bladder cancer [3].

1.1. The molecular basis of multifocality of urothelial tumors

Urothelial carcinoma is characterized by the high frequency of synchronous and metachronous multifocal primary cancers. Synchronous tumors are defined as multiple primary tumor in which the cancer occur at the same time. And metachronous tumors are defined as the cancers develop consequently sometimes years after resection of the first primary. In addition; dysplastic changes are also seen in the surrounding urothelium. There are two potential explanations for multifocality. The “field cancerization effect” is postulated, where exposure to carcinogens leads transforming genetic alterations that occurs simultaneously at different areas of the urothelium resulting in multiple genetically unrelated tumors [4].

Alternatively, the “monoclonal theory” suggests that a single transformed progenitor cell proliferates and spreads throughout the urothelium either by intraepithelial migration or by intraluminal implantation resulting in multifocality [5]. Multiple tumors might be characterized by early genetic instability and loss of cell adhesion, leading to the migration of neoplastic cells through wide areas of the urothelium [5]. After initial dysplastic changes, tumor cells can accumulate additional genetic aberrations, resulting either in either intratumoral heterogeneity or distinct subclones with different genetic alterations [6]. The discovery of CSCs added a new dimension for the occurrence of multifocality and recurrence of UC, whereas cancer stem cells (CSCs) that are remaining after gross tumor ablation will eventually result in tumor recurrence.

1.2. Urothelial carcinoma phenotypes and their behavior

Morphologically, urothelial carcinoma encompasses a wide range of divergent differentiation to that includes squamous or glandular epithelial differentiation. In addition, sarcomatoid differentiation is also reported in up to 10% of invasive high grade bladder cancer. The molecular basis of these patterns and their potential relationship to outcome is a matter of vibrant research [7]. One hypothesis is that these patterns represent independent tumor clones that are derived from separate CSCs. Alternatively, it has been also proposed that urothelial cancer starts as a monoclonal proliferation derived from a single multipotent CSC, which subsequently diverts into these different patterns. This divergence theory is supported by the identification of a “transition zone” between the histologically dissimilar areas to indicate divergence from a monoclonal origin.

Aggressive behavior in urothelial cancer is associated with molecular-morphological changes. Superficial urothelial tumors almost invariably display an “epithelial” phenotype, whereas muscle-invasive tumors are heterogeneous and approximately evenly divided between “epithelial” and “mesenchymal” phenotypes. Muscle-invasive tumors show a mixed population of “epithelial” and “mesenchymal” phenotypes, in contrast to low grade lesions that are formed entirely of an epithelial phenotype. At the molecular level, these tumors exhibit epithelial to mesenchymal transition (EMT) changes [8]. Such clinically aggressive tumors are also characterized by pronounced aneuploidy and complex chromosomal abnormalities [8].

1.3. Towards a molecular classification of urothelial carcinoma

A number of studies have shown possibility of classifying bladder tumors based on the molecular alterations including study from The cancer Genome Atlas Research Network [9]. Also recent studies have shown that molecular signatures can accurately classify urothelial carcinoma into two distinct groups regardless of morphology [10]. Different types of molecular changes have been reported including chromosomal aberrations, gene expression changes, and epigenetic changes. Low complexity chromosomal changes reported in low grade pTa tumors include frequent FGFR3 mutation, infrequent p53 mutations and LOH of 9q and 9p [7]. Muscle invasive tumors, on the other hand, generally show more complex chromosomal changes with frequent TP53 mutation and rare FGFR3 mutation. It should be noted, however, that chromosome 9 deletions cannot distinguish between non-invasive and high grade invasive types [11]. LOH of the PTEN locus on chromosome 10 appears to be much more common in muscle invasive as compared with superficial tumors [12].

Gene expression profiling was able to subclassify urothelial carcinoma in clinically relevant subgroups. High-grade stage T1 tumors were classified based on hierarchical clustering into three distinct subgroups, with each having a unique copying number alterations, FGFR3, and p53 mutation status. The first group was characterized by frequent FGFR3 mutation. On the other hand, the third group did not have FGFR3 mutation, a strikingly low frequency of chromosome 9 loss but have prevalence of p53 mutant. The second group had fewer chromosomal aberrations compared to the thirds [7].

miRNAs are small class of non-coding regulatory genes. Differential miRNA expression and their potential clinical utility as diagnostic markers for urothelial cancer has been reported [13]. Hanke and colleagues reported that miR-126: miR-152 ratio enabled the detection of bladder cancer in urine samples [14]. In addition to the diagnostic utility, recent evidence has shown a prognostic potential for these miRNAs. For instance, the upregulation of miRs-126, 182, 199a was found to distinguish bladder cancer patients from disease-free controls. The combination of miR-126 and 182 identified up to 77% of bladder cancer cases. Larger scale validations are necessary to further define these markers [15,16]. Other chromosomal aberrations have been reported in bladder cancer, as reviewed previously [17,18].

2. Molecular pathways of urothelial carcinoma

Recent studies suggest the presence of two distinct pathways of urothelial carcinoma including superficial non-invasive low-grade tumors (70%) and tumors infrequently progress to muscle invasion (10–15%). A number of serious molecules/pathways were reported to be associated with noninvasive superficial urothelial carcinoma including *FGFR3*, PI3K/AKT pathway and RAS [19] cell cycle pathway. Activating fibroblast growth factor receptor 3 (*FGFR3*) mutations are detected in 70–80% of noninvasive urothelial carcinoma compared to 10–20% of invasive tumors. Mutations between the IgII and IgIII domains (exon7) are by far the most common mutations of FGFR3 [10]. Activating point mutations of FGF receptor 3 (*FGFR3*) are found in up to 80% of low-grade and stage urothelial carcinoma (UC) of the bladder. One of the studies mentioned that 42% of tumors with no detectable mutation showed over-expression of the wild type receptor, including many muscle-invasive tumors [20].

Activated FGFR3 triggers the downstream PI3K pathway. *PIK3CA* mutations tend to occur in a subset of cases harboring *FGFR3* mutations, supporting the notion that they do not represent an alternative pathway of tumor progression. PI3KCA hotspot mutations in condons (542, 545 and 1047) have been found in approximately 20% of superficial bladder tumors in contrast to a very low incidence in invasive tumors. The lower prevalence of *PIK3CA* mutations in muscle-invasive tumors further strengthens the notion that papillary non-invasive and muscle-invasive tumors are two different molecular entities [22]. Individual mutations in FGFR3 or PIK3CA and the different mutated combinations FGFR3-PIK3CA/AKT1 and PIK3CA-RAS can activate the AKT but not the MAPK pathway. Some combinations of mutated genes in the RAS-MAPK and PI3K-AKT signaling pathways represent mutually exclusive events.

On the other hand, the pathways of cell cycle in invasive urothelial carcinoma include mainly tumor suppressor genes: TP53, p16 and RB. TP53 mutations induce a series of downstream effects, including decreased expression or loss of p21 (cell cycle arrest). This important downstream target of p53 is downregulated in the majority of urothelial carcinomas with TP53 mutations. Several codons seemed to be preferentially mutated, including codons 280 and 285. These two