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UROLOGIC ONCOLOGY

Seminars article

Circulating biomarkers to guide systemic therapy for urothelial carcinoma

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Abstract

There are very few biomarkers used to diagnose bladder cancer and no clinically approved biomarkers for prediction or prognostication of this disease. All currently available biomarkers are based on urine tests, and thus, they may not be applicable to patients with extravesical tumors. Biopsy of metastatic sites requires an invasive procedure, whereas serum-based markers, which can be easily obtained and serially measured, thus have obvious merit. These deficiencies may be overcome with advances in genome sequencing, identification of circulating tumor cells, and RNA-, protein-, and DNA-based biomarkers. Here, progress in circulating biomarkers in both superficial and invasive bladder cancer is described.

Keywords: Bladder cancer; Circulating tumor cells; Cell-free DNA; Circulating tumor DNA

Introduction

New research focusing on identifying circulating biomarkers for urothelial cancer has emerged in the past decade, but work still lags behind progress in other cancers. Lessons can be learned from studies in other neoplasms such as breast cancer where circulating tumor cell (CTC) enumeration was developed for the purpose of assessing disease burden and response to chemotherapy [1]. Newer on the scene are markers that may associate with or are directly biologically implicated in the therapeutic vulnerability to drugs such as kinase inhibitors [2], cytotoxic chemotherapy [3,4], or immune checkpoint blockade [5], for example. Many more tissue-based biomarkers have been studied (although none are approved); however, most of these are not suitable for detection in body fluids for the purpose of prognostication and prediction.

Clinical staging of urothelial cancers of the bladder or upper tracts using transurethral resection of bladder tumor (TURBT) pathology, imaging, and examination is inaccurate, as are risk-adapting strategies for assignment of neoadjuvant chemotherapy in patients with presumptively localized disease [6]. No biomarkers exist for patients with metastatic or unresectable disease to guide initial or subsequent therapies. Trials of targeted and immune-based therapeutics provide hope for patients and oncologists, and many of these studies have identified molecular correlates to response. Most of these correlates are tissue based, but some markers can be identified in serum [7]. Although there is much excitement surrounding circulating biomarkers, their clinical relevance and analytic validity are current shortcomings in the evaluation and management of patients with bladder cancer. Furthermore, it remains to be seen how they would fit into current management algorithms, and most importantly, if they alter the outcome of patients with urothelial cancer. However, standardization and validation across studies are still lacking, limiting potential broad applicability outside of clinical trials and associated correlative studies. Here, we review selected studies of circulating biomarkers and their potential effects and applications.

Circulating tumor cells

Many studies that quantify circulating disease burden in patients with localized, locally advanced, or metastatic

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urothelial cancer use CTCs. These cells are loosely defined as tumor cells that have detached from a primary or metastatic tumor site, entered the bloodstream, and can be detected there. They are hypothesized to be the source of metastasis, although not all CTCs can form a new tumor. CTCs are an intermediary to disseminated tumor cells, which can be detected residing in tissue sites where clinically evident metastasis may occur, such as the bone marrow, lung, and liver [8]. These 2 population groups, however, are likely different biologically in their ability to extravasate, recirculate, multiply, and respond to therapy.

Many methods to purify intact CTCs prospectively are available based on positive and negative selection with antibodies to transmembrane antigens, size exclusion/inclusion, and a variety of other electrical/chemical/physical properties of cancer cells. These cells can then be used for biomarker annotation or grown in culture in the laboratory or *ex vivo* in immunocompromised mice for further experimental projects [9]. Caution should be maintained when interpreting these studies, however, because not all purported CTCs are tumoral in origin. Genetic or epigenetic evidence of the tumor origin of putative CTCs such as detection of a somatic mutation or genomic copy number alteration, which is also present in the tumor, would convincingly prove the derivation of CTCs, but these are often omitted from studies measuring or identifying CTCs. Lack of definite proof of the origin of putative CTCs has implications in the sensitivity and specificity of the biomarker test; putative CTCs identified without validation may be circulating epithelial cells, which do not originate from tumors, rather than true CTCs. Lastly, several CTC detection tests rely on identifying epithelial cell adhesion molecule (EpCAM) or similar molecules on the membrane of circulating cells. This molecule is frequently lost during the epithelial-mesenchymal transition [10], and this may be required for metastasis [11], suggesting that methods relying on detection of membrane antigens alone are likely to underestimate the presence and quantity of CTCs or potentially miss important subsets of cells with metastatic potential.

CTCs can also be detected retrospectively in the absence of purification, using highly specific expressed tumor markers. These tests often rely on polymerase chain reaction (PCR) for detection of rare cells (i.e., 1 in 10^{-6} cells) that express the marker of interest. For instance, detection of prostate-specific antigen mRNA in patients with prostate cancer or a tumor-specific genomic rearrangement in other cancers in circulating cells could identify CTCs. Although PCR is highly sensitive, its use is limited by the ability to further characterize identified cells as they are destroyed in the process of their identification. As such, the earliest descriptions of putative urothelial CTCs used this method to retrospectively identify cells with expression of uroplakins and epidermal growth factor receptor (EGFR) in patients with metastatic or completely resected disease [12]. Interestingly, patients with circulating cells containing uroplakin/EGFR mRNA after complete resection were at higher risk for recurrence than those without putative CTCs [12]. Circulating cells expressing tumor-specific cytokeratins in patients with or without bladder cancer have also been used as a way to identify putative CTCs. For example, cytokeratin 20^{+} circulating cells were found only in patients with cancer and correlated with increased clinical stage/disease burden [13]. Although these biomarkers are more cancer sensitive than cancer specific, they are relevant to urothelial cancer because they are expressed in most cases.

Early studies that prospectively identified urothelial CTCs used the CellSearch platform. This device uses negative selection of $CD45^{+}$ cells to deplete immune cells and positive selection with EpCAM and cytokeratins to isolate CTCs. Naoe et al. [14] showed that purported CTCs were present in 8 of 14 patients with metastatic urothelial carcinoma, but no patients with nonmetastatic cancer. Flaig et al. [15] elaborated the study of urothelial CTCs by showing that CTC presence in patients with metastatic disease was associated with death within 1 year. CTCs in this group of 44 patients harbored aneuploidy as measured using fluorescence *in situ* hybridization with the Urovysion, convincingly proving these cells were true CTCs. Furthermore, studies in a 55-patient cohort with advanced or metastatic urothelial cancer using this platform showed that CTCs were more frequently present in patients with metastatic disease compared with localized disease and that presence of CTCs adversely correlated with progression-free survival and cancer-specific mortality [16]. CTCs identified in these further studies did not use orthogonal tests to confirm tumor derivation, although they likely found true CTCs as the Flaig study proved that CTCs identified with CellSearch were aneuploid.

IsoFlux uses antibody capture as does CellSearch, but IsoFlux also incorporates microfluidics to improve capture of labeled cells [17]. Cells are labeled with an immunomagnetic antibody to EpCAM as they are passed through an isolation chamber containing a magnetic roof. Putative CTCs are drawn up into the chamber to be used for downstream analysis. As opposed to Cellsearch, which uses CD45 negative selection, waste cells continue to flow through the microfluidic device unselected. This platform was used to enumerate and study CTCs in patients receiving neoadjuvant chemotherapy, patients with metastasis, and healthy volunteers [18]. Among 20 patients with paired samples, the number of CTCs per 5- to 10-ml sample decreased from 13 to 5 cells after neoadjuvant chemotherapy in patients with muscle-invasive bladder cancer (MIBC). Lower CTCs after chemotherapy correlated with better response to chemotherapy. Patients with metastasis harbored 29 cells per sample. Importantly, the investigators performed next-generation sequencing on a panel of 50 cancer genes in 8 patient samples and found mutations in only 4 patients. This is somewhat lower than expected given the previously reported high frequency of alterations in genes tested (*TP53*, *PIK3CA*, *FGFR3*, *ERBB2*, *RB1*, etc.),