Clinica Chimica Acta 477 (2018) 141–153

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Review

mRNA, microRNA and lncRNA as novel bladder tumor markers

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ARTICLE INFO

Keywords:
Bladder cancer
Molecular markers
qRT-PCR
mRNA
microRNA
lncRNA

ABSTRACT

Early detection of bladder cancer (BC) is essential for improvement of the patient's prognosis and general survival rates. Current diagnostic methods are still limited, so new specific and cost-effective biomarkers are emerging as the noninvasive tools in treatment decisions in recurrent BC.

Gene expression and epigenetic profile can be analysed using quantitative real-time-PCR (qRT-PCR) method in urine, blood and tissue. This review provides an update of recent findings on BC molecular profile as novel markers in diagnosis and prognosis of bladder tumors. We describe mRNA-, microRNA- and lncRNA-based biomarkers involved in the BC detection, diagnosis, prediction of recurrence and monitoring after treatment.

1. Introduction

Bladder cancer (BC) is an asymptomatic disease with a high tendency to recur and progress, despite intensive and prolonged intravesical treatment. Especially, the patients with non-muscle invasive bladder cancer (NMIBC) require lifelong monitoring due to high recurrence rates. The potential of NMIBC to progress to muscle invasive bladder cancer (MIBC) is highly unpredictable. The standard treatment for NMIBC is transurethral resection (TUR). Urologists observed frequent recurrence of BC after primary TUR and a subsequent tumor progression [1,2].

Guidelines on NMIBC for individual patients have been updated by the European Association of Urology. The risk of recurrence and progression can be forecasted using the risk tables and scoring system developed by The European Organization for Research and Treatment of Cancer (EORTC). They can be used to stratify patients for treatment decision, into three groups: with a low, intermediate, and high risk of recurrence and progression [3].

Current standard clinical diagnostics for BC detection include invasive cystoscopy combined with noninvasive cytology. Cystoscopy is not only an unpleasant procedure, entailing complications such as urinary tract infections or haematuria but it is also an expensive approach. Urinary cytology is thought to be a highly specific diagnostic method, but it exhibits low sensitivity with respect to detection of low-grade (G1) tumors and its accuracy depends on the pathologist's experience. Moreover, urine cytology cannot completely rule out the presence of a tumor. BC patient's monitoring with periodic cystoscopy and urine cytology are more useful for monitoring cancer recurrence or progression [4].

Early detection of BC is essential for improvement of the patient's prognosis and general survival rates. Current diagnostic methods are limited, so new specific and cost-effective biomarkers are needed as the noninvasive tools. BC testing based on molecules released into the urine by tumor cells might contribute to reducing the use of cystoscopy. Novel BC biomarkers can also be of value for patient stratification for better BC management and highly invidualised treatment adapted to patient's needs. Appropriate stratification of BC patients will help in treatment selection between adjuvant intravesical immunotherapy or chemotherapy, or radical cystectomy. Unfortunately, to this date, no sufficiently sensitive and specific molecular tests have been developed and adopted by clinicians [3,5].

The best known molecular tests such as nuclear matrix protein 22 (NMP22™), or UroVysion are the markers of BC risk, but they are still not recommended for routine use [5]. Moreover, the molecular markers based on gene expression level are cytoskeletal protein (Cytokeratin 20) and telomerase (hTERT), but these new markers are not been widely applied either [6].

The purpose of this review is to summarize the most recent scientific advances regarding new markers such as mRNA, microRNA (miRNA, miR) and long non-coding RNA (lncRNA) profile analysed using quantitative real-time polymerase chain reaction (qRT-PCR). To achieve this goal, searching was initiated using multiple approaches: 1. Mainly using the PubMed search engine; 2. Search string: “bladder cancer” and “mRNA” or “miRNA” or “lncRNA”; 3. Original articles; 4. Articles published in English, full version; 5. Period covered: 1 January 2016 through 5 June 2017. Altogether, 16 manuscripts concerning mRNA, 34 miRNA and 37 lncRNA were selected and retrieved for data extraction. The present article reviews and summarizes state-of-the-art

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https://doi.org/10.1016/j.cca.2017.12.009
Received 21 August 2017; Received in revised form 5 December 2017; Accepted 5 December 2017
Available online 07 December 2017

research on blood, urine, and tissue from BC patients or in combination with cell study. In the review, we focused on studies that reported on the association between the expression levels of genes, miRNA, lncRNA and the major pathological characteristics of the tumor as well as the clinical outcomes.

2. Importance of new markers in bladder cancer

BC is the second most common urooncological disease after the prostate cancer [7]. It has a significant impact on public health in industrialized countries due to the high mortality rates as a result of late diagnosis at advanced tumor stage (T) [8]. It is a very heterogeneous disease, with various combinations of the risk factors. Common risk factors include tobacco smoking, chronic urinary tract infections, arsenic exposure, environmental, occupational exposure to carcinogens and genetic factors [9,10]. Nowadays BC recurrence and progress to MIBC is highly unpredictable also after appropriate treatment [3]. Tumors that are pathologically similar may behave differently; therefore, to diagnose the progression or relapse of BC, the patients require lifelong invasive monitoring and treatment [11]. New markers have to meet this condition and a broad range of cases is required for the method to be validated. These problems are a potential challenge for the detection of bladder tumor markers (BTMs). New genetic biomarkers may provide more important information about BC than the protein markers which reflect mainly an infection or inflammation.

Novel BTMs have become especially valuable for NMIBC diagnostics, but as well as for identifying new molecular subtypes to predict recurrence, and NMIBC progression to MIBC or metastasis (Fig. 1). However, a given BTM has to enable differentiation of transitional cell carcinomas (TCC) from other inflammatory conditions. In recent years, research has been focused on molecular markers which are associated with alterations at the molecular level and the course of disease.

3. Molecular biomarkers in the light of the quantitative real-time PCR analysis

New BTMs will help make a distinction between genetic subtypes of BC and should provide a highly sensitive, specific and noninvasive tool for diagnosing and making prognosis for BC. High throughput microarray is a powerful instrument for investigating the biological function of gene expression profile. Detection of transcripts encoding a protein in a cell or tissue can be carried out using the qRT-PCR methods. Moreover, the qRT-PCR data verified the accuracy of microarray results. qRT-PCR has been recognised as quantifying mRNA transcripts, but also miRNA and lncRNA [12]. Therefore, to detect and monitor BC patients we can use an accurate and sensitive method, such as qRT-PCR, and analyze mRNA, miRNA and lncRNA profiles. Statistical analyses revealed associations of individual biomarkers with clinical data. Moreover, analyses of the Receiver Operating Characteristic (ROC) curves with Area Under Curves (AUC) value are used to assess the performance of predictive multivariate models, to show how well each biomarker makes it possible to distinguish between high- and low-risk samples.

Detection of amplicon accumulation can be performed with the use of double-stranded DNA intercalating dyes such as SYBR® Green, EVAGreen®. This allows mirroring the template accumulation and may give false positives due to non-specific product formation such as primer-dimers. Therefore, given a low copy number we should avoid this method and choose, for example, a fluorescent probe like TaqMan®. The TaqMan probes are more specific oligonucleotides than the fluorescent dyes. Other available types of oligonucleotide hybridization probes are molecular beacons and scorpions. All types of the probes are more expensive than reporter dyes but they are often used in BC analysis, especially the TaqMan probes [13–15]. One of the qRT-PCR techniques for miRNA detection is to employ primers with better specificity and sensitivity, like the stem–loop reverse transcription (RT) followed by 5′-nuclease TaqMan PCR analysis [16]. Moreover, the use of the RT-qPCR-D method allows direct application to urine supernatants thus making it possible to quantify miRNAs without RNA extraction, which simplifies the procedure [17].

Nowadays, pathological information regarding BC is useful for predicting prognosis, unfortunately it becomes problematic when it is making into individual clinical decision. Molecular subtypes of BC with predictive potential are currently not clinically applicable. It is believed that molecular stratification of cancer, based on a few common DNA markers, will significantly complement or potentially replace current clinicopathological factors in a future. New unique classification of BC patients will potentially improve treatment efficacy. Moreover, the molecular changes associated with tumor progression during the treatment may have prognostic value in therapeutic responses. By

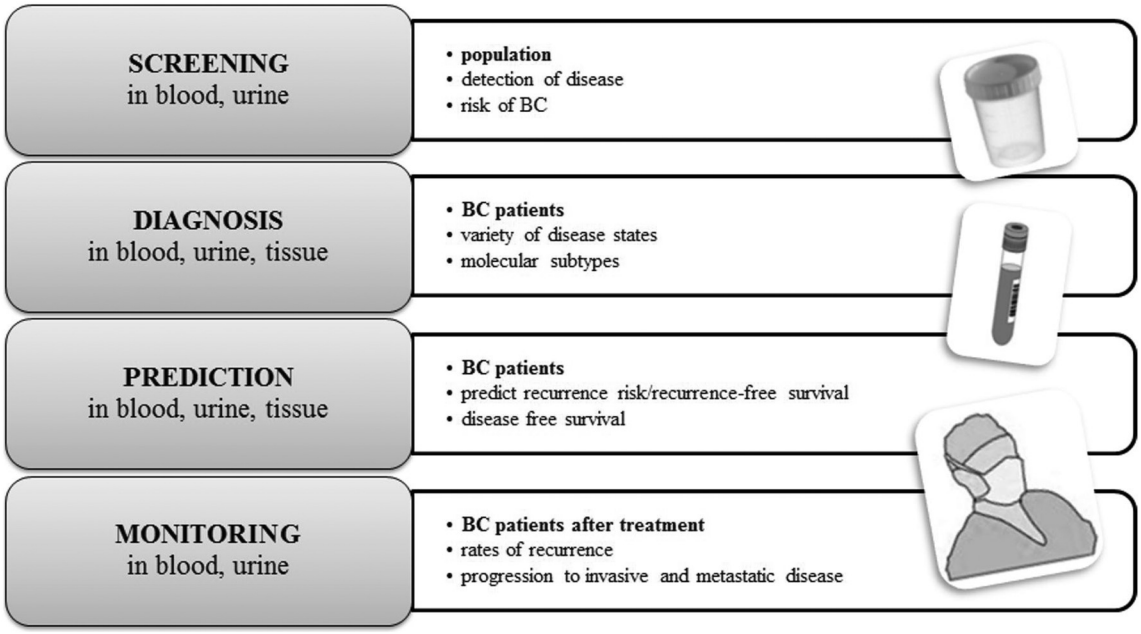

Fig. 1. Usefulness of BTM for population screening and for BC patients diagnosis, prognosis and monitoring.