REVIEW ARTICLE

Urinary long noncoding RNAs in nonmuscle-invasive bladder cancer: new architects in cancer prognostic biomarkers

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Several reports over the last 10 years provided evidence that long noncoding RNAs (lncRNAs) are often altered in bladder cancers. lncRNAs are longer than 200 nucleotides and function as important regulators of gene expression, interacting with the major pathways of cell growth, proliferation, differentiation, and survival. A large number of lncRNAs has oncogenic function and is more expressed in tumor compared with normal tissues. Their overexpression may be associated with tumor formation, progression, and metastasis in a variety of tumors including bladder cancer. Although lncRNAs have been shown to have critical regulatory roles in cancer biology, the biological functions and prognostic values in nonmuscle-invasive bladder cancer remain largely unknown. Nevertheless, a growing body of evidence suggests that several lncRNAs expression profiles in bladder malignancies are associated with poor prognosis, and they can be detected in biological fluids, such as urines. Here, we review current progress in the biology and the implication of lncRNAs associated with bladder cancer, and we discuss their potential use as diagnosis and prognosis biomarkers in bladder malignancies with a focus on their role in high-risk nonmuscle-invasive tumors. (Translational Research 2017;■:1–10)

Abbreviations: BlCa = bladder cancer; BTA = bladder tumor antigen; circRNAs = circular RNA; FDA = United States of America's Food and Drug Administration; FDP = fibrin degradation product; FISH = fluorescent in situ hybridization; GAS-5 = growth arrest-specific 5; GHET 1 = gastric carcinoma highly expressed transcript 1; HGMI = high-grade muscle invasive tumors; ImmunoCyt = immunocytometry; ISH = in situ hybridization; ISUP = International Society of Urological Pathology criteria; Linc-UBC1 = upregulated in bladder cancer 1; lncRNAs = long noncoding RNAs; MALAT-1 = metastasis-associated lung adenocarcinoma transcript 1; MEG3 = maternally

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expressed gene 3; miRNAs = microRNAs; mRNA = RNA mature; NBE = normal bladder epithelium; ncRAN = noncoding RNA expressed in aggressive neuroblastoma; ncRNAs = noncoding RNA; NEAT 1 = nuclear paraspeckle assembly transcript 1; NMIBC = nonmuscle-invasive bladder cancer; NMP22 = nuclear mitotic apparatus; PBS = phosphate buffered saline; PCAT-1 = prostate cancer–associated transcript 1; PCR = polymerase chain reaction; PSA = prostate-specific antigen; PUNLMP = papillary urothelial neoplasm of low malignant potential; PVT-1 = plasmacytoma variant translocation gene lncRNA; qRT-PCR = quantitative real-time polymerase chain reaction; RIP = RNA immunoprecipitation; RNA-Seq = RNA sequencing; shRNA = small hairpin RNA; SNHG16 = snoRNA host gene 16; SPRY4-IT1 = protein sprouty homolog 4 lncRNA; TCC = transitional cell carcinoma; TNM = tumor-node-metastasis; T-UCRs = transcribed ultraconserved regions; TUG1 = taurine-upregulated gene 1; UBC = urinary bladder cancer; UBC1 = upregulated in bladder cancer 1; UCA1 = urothelial cancer associated-1; UCRs = ultraconserved regions; UEs = urinary exosomes; UNMIBC = upregulated in nonmuscle-invasive bladder cancer; WHO = World Health Organization

INTRODUCTION

Bladder cancer (BlCa) is one of the most common malignancies of the urinary tract, with a significant morbidity and mortality.[1]

Around 75–85% of the patients are diagnosed as having nonmuscle-invasive bladder cancer (NMIBC). Despite the treatment, these patients have a probability of recurrence at 5 years ranging from 50 to 70% and of progression to muscle-invasive disease of 10–15%.[2]

The high rate of disease recurrence of BlCa requires lifelong surveillance in many patients, consisting of cystoscopy, which is invasive and expensive, and cytology, which shows poor sensitivity for low-grade cancers.[3]

Cystoscopy identifies most papillary and solid lesions but is invasive. Furthermore, cystoscopy may be inconclusive, false positive or negative. Urine cytology has a reasonable sensitivity and specificity for the detection of high-grade BlCa, whereas the sensitivity for detection of low grade tumors ranges only from 4% to 31%. The overall sensitivity and specificity of urine cytology range from 7 to 100 and from 30 to 70%, respectively.[4] Cystoscopy has been reported to have up to a 30% false negative and a 17% false positive rate.[5-7] Procedures used to diagnose BlCa may include cystoscopy, biopsy, urine cytology, and imaging test. The estimated cost per diagnosis is about ten-fold higher for cystoscopy compared with urine-based markers. However, this cost-effectiveness has to be confirmed on the basis of the biomarker ability to reduce cystoscopy requirements.[8,9]

An accurate biomarker can reduce the number of the invasive and expensive cystoscopies performed each year. Cytology is tumor specific, but it is not very sensitive. Therefore, a biomarker with better sensitivity and comparable specificity to cytology could eventually replace cytology and significantly improve the management of NMIBC.

Recent studies have demonstrated that long noncoding RNAs (lncRNAs) play important roles in carcinogenesis and cancer metastasis,[10] and aberrant expression of lncRNAs has been identified in BlCa, where they may function as oncogenes or tumor suppressors.[11-14]

In the present review, we summarize recent progress in the application of urinary lncRNAs for the diagnosis, assessment, and treatment of BlCa. We propose that urinary lncNRAs represent a new informative tool in the management of BlCa patients.

LITERATURE SEARCH

We searched the Medline/PubMed databases of the National Library of Medicine for the comprehensive information on the lncRNAs as biomarkers introduced for BlCa. The terms used for this research included lncRNAs, BlCa, biomarker, early detection, prognosis, and the combinations of these words.

LONG NONCODING RNAS: BIOLOGY AND FUNCTIONS

The ENCODE project estimated that the majority of the genome produces >10.000 unique long noncoding RNAs (lncRNAs),[15,16] and new lncRNAs will continue to be discovered with the advent of the high-throughput transcriptome sequencing known as RNA-Seq. The little translation potential due to the presence of numerous stop codons in the RNA mature transcript[17-20] gives them the definition of nonprotein coding transcripts, whereas the arbitrary limit of 200 bases in length distinguishes long from short ncRNAs.[21,22] According to their genomic locations and relationship with flanking protein-coding genes, lncRNAs are classified as 1) sense or antisense, depending on the transcription direction (same or opposite) compared with the neighboring coding genes; 2) intronic, when they are localized within the introns of

protein-coding genes, do not overlap with their exons and are transcribed in either orientation relative to adjacent protein-coding genes; 3) divergent, when they are transcribed in the opposite direction with respect to promoters of protein-coding genes; 4) intergenic, when they are transcribed between protein-coding genes and do not share promoters, exons or introns with protein-coding genes. The majority of lncRNAs are transcribed by RNA polymerase II and share properties with coding mRNA, such as splicing and polyadenylation. In addition, most lncRNAs are exported to the cytosol, although some are found in both cytoplasm and nucleus. In general, lncRNAs are less conserved and more tissue- and cell-specific than protein-coding genes,[15,23] with the exception of the members of particular class of them named ultraconserved regions (UCRs). These elements are transcribed as lncRNAs from sequences of DNA 100% conserved in human, mice, and rat genome and are often associated with malignant states.[24] The global characterization of ncRNA functions is a crucial task and multiple approaches have been used to identify the putative function of lncRNAs. One approach proposed by Guttman et al.[25] called guilt-by-association, is based on the association between expression pattern of groups of lncRNAs in cell types and tissues and biological processes, mRNA expression profiling data, and gene ontology or functional pathway analyses. In this approach, groups of lncRNAs of unknown function are linked with groups of protein-coding mRNAs known to be involved in a specific cellular process based on a common expression pattern across cell types and tissues. Thus, a positive correlation between the expression profile of a lncRNA and mRNAs suggests a common function in the same cellular process. For example, lincRNA-p21 was predicted to be associated with p53-mediated DNA damage responses. Subsequently, lincRNA-p21 was validated as p53 target that modulates apoptotic responses on DNA damage.[26] This approach is part of a useful tool for global prediction of lncRNA function, and also provides a working hypothesis for targeted perturbation experiments. In addition, several studies have shown that some lncRNAs are bound by polycomb repressive complexes or other types of chromatin remodeling complexes and mediate gene silencing (eg, HOTAIR, HOTAIRM1, ANRIL, KCNQ1OT1, AIR, XIST) or activation (eg, HOTTIP or mistral).[27] Linear or circular lncRNAs can bind microRNAs (miRNAs), thus preventing them from regulating their mRNA targets.[28-31] Several authors[30,31] provide powerful evidence that circRNAs, covalently linked by the head-to-tail splicing of exons, can function as miRNA sponges to suppress miRNA activity. A circular RNA encoded as hsa_circ_001569 has been determined to be significantly upregulated in colorectal cancer tissues. Functional experiments revealed a strong association of hsa_circ_001569 with increased colorectal cancer cell proliferation and invasion. Thus, the main function of hsa_circ_001569 was demonstrated as a circRNA sponge, by upregulating miR-145 target transcripts (E2F5, BAG4, and FMNL2) and, subsequently, their protein amounts.[32] Recent studies also revealed that circRNAs have a great value in cancer diagnosis. Li et al[33] first discovered a significant negative correlation between hsa_circ_002059 and gastric metastasis, indicating their potential as novel diagnostic tools.

Furthermore, few of them can also produce small biological active peptides.[34] In the last 2 years, several studies have identified functionally important micropeptides translated from lncRNAs. Anderson et al.[35] for instance, described a lncRNA that coded for myoregulin, a 46-amino acid micropeptide that reduces muscle performance by inhibiting the calcium ATPase SERCA in muscle cells. Myoregulin was derived from a lncRNA called LINC00948 in human and AK009351 in mouse. More recently, another lncRNA-derived micropeptide, DWORF, which activates SERCA to boost muscle performance, has been described. The lncRNA responsible for this micropeptide is called LOC100507537 in human and NONMMUG026737 in mouse.[36] These findings further support the role of some RNAs, which are currently classified as noncoding, in producing micropeptides with biological impact.

In summary, lncRNAs participate in various mechanisms including guidance of proteins to specific genomic loci, structural roles, and as molecular decoys in various cellular contexts.

LONG NON-CODING RNAS AND BLADDER CANCER: ABERRANT EXPRESSION AND REGULATORY FUNCTIONS

As multiple reports suggest, lncRNAs play an important role in urothelial carcinogenesis by modulating cellular pathways of cell transformation. As for translated genes, they can be functionally categorized in oncogenic or tumor-suppressor lncRNAs.[37] The general strategy to find cancer-associated lncRNAs is to compare lncRNA expression profiles in bladder cancer tissues with adjacent non-neoplastic tissues, using conventional molecular biology techniques (eg, subtractive suppression hybridization technique, cDNA microarrays, and polymerase chain reaction–based assays) (Table I). Therefore, we can expect that more new lncRNAs will be discovered in BlCa by using RNA-seq technology.

Table I. Bladder cancer (BlCa) expressed long noncoding RNAs (lncRNAs): features and localization

lncRNA	Location	Expression	Platform	Sources	References
HOX-AS-2	Chr7p15.2	Up	qRT-PCR	Urine exsosome	11
HOTAIR HOTAIR M1	Chr12q13.13	Down	RIP, qRT-PCR	Urine exsosome, Nonmuscle-invasive BlCa	11,27,38
LincRNA-p21	Chr17p13.1	Down	RIP, qRT-PCR	BlCa	26
T-UCR 8+	Chr1p36.22	Up	qRT-PCR, ISH	BlCa	29
UCA1	Chr19p13.12	Up	Subtractive	BlCa, urine sediments	39-42
UCA1a			Hybridization, PCR		
H19	Chr11p15.5	Up	PCR	BlCa	14,43-46
NEAT1	Chr11q13.1	Up	PCR	BlCa	44
Linc-UBC1	Chr1q32.1	Up	Microarray, qRT-PCR	Invasive bladder cancer	47
MALAT-1	Chr11q13.1	Up	PCR	BlCa, urine exsosome	48,49
TUG1	Chr22q12.2	Up	qRT-PCR	Bladder urothelial carcinoma	50,51
UNMIBC	Chr1p31.1	Up	RIP, qRT-PCR	NMIBC	52
PVT1	Chr8q24.21	Up	qRT-PCR	BlCa	53
ncRAN	Chr17q25.1	Up	qRT-PCR	BlCa	54
PCA-T	Chr8q24.21	Up	qRT-PCR	BlCa	55
GHET 1	Chr7q36.1	Up	qRT-PCR	BlCa	56
SPRY4-IT1	Chr5q31.3	Up	qRT-PCR	BlCa	57
MEG3	Chr14q32.3	Down	qRT-PCR	BlCa	58-60
GAS 5	Chr1q25.1	Down	qRT-PCR	BlCa	61

Abbreviations: GAS 5, growth arrest-specific 5; *GHET 1,* gastric carcinoma highly expressed transcript 1; *MALAT-1,* metastasis-associated lung adenocarcinoma transcript 1; *MEG3,* maternally expressed gene 3; *ncRNA,* noncoding RNA; *NEAT 1,* nuclear paraspeckle assembly transcript 1; *TUG1,* taurine-upregulated gene 1; *UBC1,* upregulated in bladder cancer 1; *UCA1,* urothelial cancer associated-1; *UNMIBC,* upregulated in nonmuscle-invasive bladder cancer.

LNCRNAS WITH TUMOR-ONCOGENE FUNCTIONS IN BLADDER CANCER

Similar to protein-coding oncogenes, lncRNAs with oncogenic function are more expressed in tumor cells compared with the normal ones Fig 1. For example, the lncRNA urothelial cancer associated-1 (UCA1) of 1442 bp in length has been screened and cloned from the human bladder (transitional cell carcinoma, TCC) cell line BLZ-211.[39] UCA1 is highly specific in BlCa and in embryonic tissues, but not in adult tissues or adjacent non-neoplastic tissues, indicating that UCA1 may be involved in embryonic development and reactivated during adult tumourigenesis. Furthermore, after exogenous UCA1 expression in BLZ-211 bladder cell lines, the proliferation, migration, invasion, and drug resistance behaviors were increased. In addition, when BLZ-211 cells-expressing UCA1 were inoculated into nude mice, their capacity of tumor formation was increased,[39] suggesting that UCA1 has a pivotal oncogenic role in BlCa development. Recently, it has been proven that UCA1 confers resistance to cisplatin/gemcitabine via activating miR-196a-5p through the transcription factor CREB in bladder cancer cells and in a xenograft mouse model.[40-42] lncRNA H19 is one of the first discovered noncoding RNAs in mammalian genome[43]; it has been found to be highly expressed in human embryos and fetal tissues, whereas its expression is completely lost in adults.[44] It is re-expressed in a number of tumors, including bladder carcinoma, demonstrating that it is an onco-fetal RNA.[44] In particular, H19 expression levels were remarkably increased in bladder cancer tissues as compared with adjacent normal control tissues.[14,45,46] The ectopic expression of H19 promotes bladder cancer cell proliferation in vitro[14] and enhances bladder cancer cell migration, both in vitro and in vivo.[45] On the basis of these findings, H19 appears to be an onco-lncRNA and serves as tumor marker in BlCa. More recently, a new lncRNA, linc-UBC1 (upregulated in bladder cancer 1) was found to be up regulated in ~60% of invasive bladder cancer tissues and physically associated with polycomb repressive complexes 2 complex to regulate histone modification status of target genes.[47] MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) was the first to be characterized in metastatic small cell lung cancer,[48] but recent studies showed that MALAT1 is also upregulated in BlCa, and its expression levels correlate with tumor grade and metastatic stage.[49,50] MALAT1 is highly conserved among mammals (MALAT1) and in bladder cancer cells, which is able to promote epithelial-mesenchimal transition and subsequent cell migration, by activating the Wnt/β-catenin signaling pathway.[50] Taurine-upregulated gene 1 (TUG1; also known as TI-227H; Linc00080; ncRNA00080) was initially detected in a genomic screen for genes in response to taurine treatment in

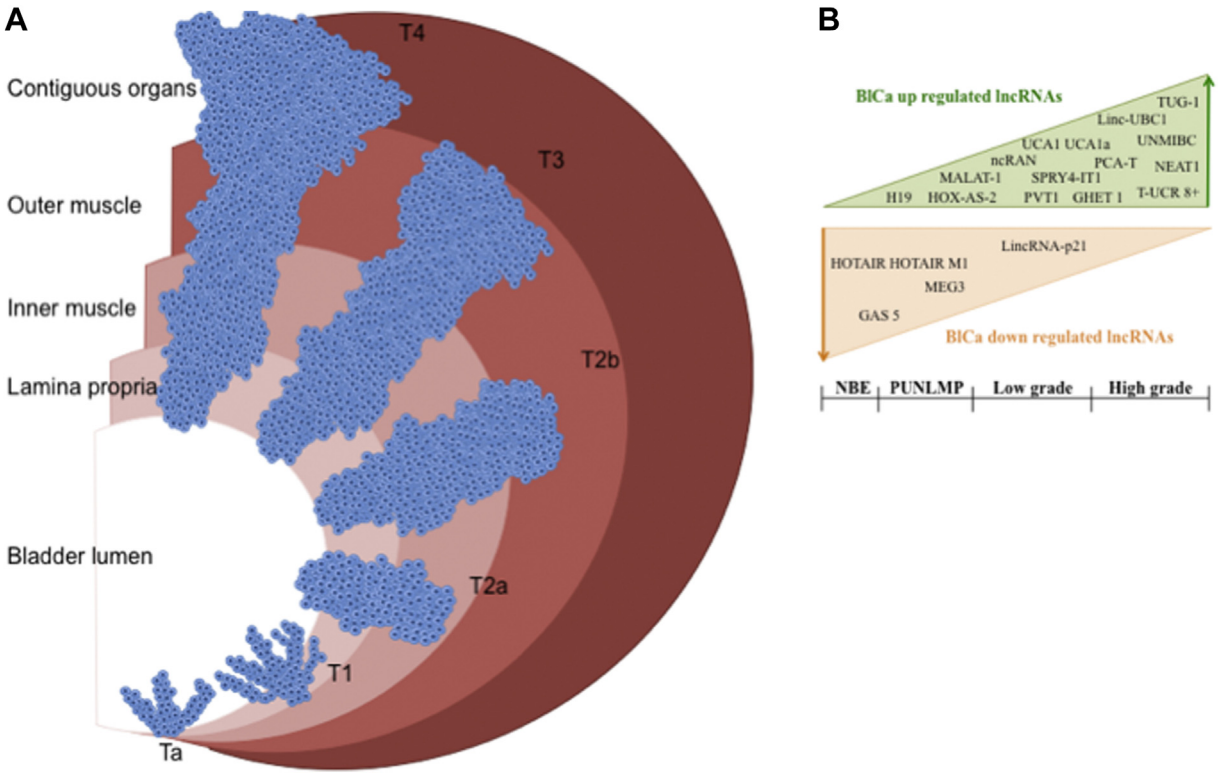

Fig 1. LncRNAs involved in bladder cancer progression. **(A)** Graphical description of a sagittal view of the bladder wall showing the progression of lesions associated with the staging of BlCa according to the tumor-node-metastasis (TNM) system. **(B)** Schematic representation of deregulated lncRNAs described in the manuscript and their expression correlated to grading, according to 2004 WHO/International Society of Urological Pathology (ISUP) criteria. Papillary urothelial neoplasm of low malignant potential (PUNLMP) is equivalent to grade 1 of the older system (1973 World Health Organization [WHO]). NBE, normal bladder epithelium.

developing mouse retinal cells. After these initial observations, expression levels of TUG1 have been found to be deregulated in a large panel of tumors including BlCa. In particular, TUG1 was found upregulated in bladder urothelial carcinoma compared with paired normal urothelium, and high expression levels of TUG1 were associated with high grade and stage carcinomas. More recently, it has been observed that TUG1 levels were significantly increased in metastatic tumors and were associated with shorter overall survival of MIBC patients. TUG1 silencing in vitro led to a decrease in cancer cell proliferation and a reduction in migration capacity of cancer cells.[51] The expression levels of TUG1 were upregulated in bladder urothelial carcinoma compared with paired normal urothelium. High TUG1 expression levels were associated with high grade and stage carcinomas. Cell proliferation inhibition and apoptosis induction were observed in TUG1 siRNA-transfected bladder urothelial carcinoma T24 and 5637 cells.[52] The lncRNA termed lncRNA-UNMIBC (upregulated in nonmuscle-invasive bladder cancer) was evaluated in NMIBC compared with normal mucosa. The upregulation of this lncRNA has been associated with tumor growth and may indicate a negative prognostic factor of recurrence.[53] More recently, it has been demonstrated that noncoding RNA transcripts originate from UCRs, named transcribed ultraconserved regions (T-UCRs), have different expression profiles and play functional roles in the pathophysiology of multiple cancers. By using a microarray approach, it has been identified the ultraconserved RNA (uc.) 8+ as the most upregulated T-UCR in BlCa tissues, although its expression was lower than the one in pericancerous bladder tissues.[29] In addition, in vitro experiments, evaluating the effects of uc.8+ silencing, showed significant decreased capacities for bladder cancer cell invasion, migration, and proliferation. Plasmacytoma variant translocation gene lncRNA (PVT-1) is an oncogene encoding a lncRNA, which maps to chromosome 8q24. It has been demonstrated that PVT1 is upregulated in bladder cancer tissues and cells, functioning as an oncogene. Its silence suppresses proliferation of bladder cancer cells. In addition, shRNA sequences, targeting PVT1 controlled by

synthetic "tetracycline-on" switch, suppressed the expression of PVT1 in response to different concentration of doxycycline and inhibited progression of BlCa.[54] Nuclear paraspeckle assembly transcript 1 (NEAT1) is an lncRNA that is specifically present in the paraspeckles. NEAT1 has been demonstrated to be involved in several cancers. A recent study describes that NEAT1 is highly expressed in bladder cancer tissues compared with the matched tissues and in bladder cancer cell lines compared with the normal bladder epithelial cells. It has been shown that the inhibition of NEAT1 suppresses cell proliferation, migration and induces apoptosis, thus supporting its oncogenic role.[44] ncRAN (noncoding RNA expressed in aggressive neuroblastoma) also plays an important role in bladder cancer cells growth and invasion. ncRAN, also named SNHG16 (snoRNA host gene 16), is overexpressed in BlCa and positively correlates with aggressiveness. The silencing of SNHG16 can improve chemotherapy sensitivity in BlCa cell lines.[55] Prostate cancer–associated transcript 1 (PCAT-1) is a lncRNA that promotes cell proliferation in prostate cancer. More recently, it has been demonstrated that PCAT-1 also plays a role in BlCa. PCAT-1 was found to be upregulated in BlCa compared with paired normal urothelium. Cell proliferation inhibition and apoptosis induction were also observed in PCAT-1 small hairpin RNA (shRNA)-transfected bladder cancer T24 and 5637 cells. Data suggest that PCAT-1 plays oncogenic roles in human BlCa.[56]

Another lncRNA, gastric carcinoma highly expressed transcript 1 (GHET1), has recently been shown to act as an oncogenic ncRNA. GHET1 is upregulated in bladder cancer tissues as compared with adjacent normal tissues and is correlated with tumor size, advanced tumor, lymph node status, and poor survival. GHET1 knockdown suppresses the proliferation and invasion of bladder cancer cells, and the inhibition of GHET1 expression reverses the epithelial-mesenchimal transition in bladder cancer cells.[57] Protein sprouty homolog 4 lncRNA (SPRY4-IT) (708 bp) is a lncRNA derived from an intronic region within the SPRY4 gene. SPRY4-IT1 has been found to be upregulated in esophageal squamous cell carcinoma and breast cancer, suggesting that SPRY4-IT1 has a tissue-specific expression pattern and may function as oncogene or tumor suppressor in different cancers. SPRY4-IT1 expression has been found to be increased in bladder cancer tissues, and SPRY4-IT1 levels were highly positively correlated with histological grade, tumor stage, and lymph node metastasis and reduced overall survival. In addition, in vitro assays show that the suppression of SPRY4-IT1 expression in bladder cancer cells significantly inhibits cell proliferation, migration, and invasion.[58]

LNCRNAS WITH TUMOR-SUPPRESSOR FUNCTION IN BLADDER CANCER

A tumor suppressor lncRNA is identified as a gene whose product normally inhibits tumor initiation and progression. Accordingly, a tumor suppressor lncRNA is downregulated or not expressed in cancer tissues. Maternally expressed gene 3 (MEG3) is an imprinted gene that encodes for a lncRNA expressed in many normal tissues, whereas its expression is lost in an expanding list of primary human tumors and tumor cell lines as well as in BlCa.[59] Multiple mechanisms contribute to the tumor suppressive roles of MEG3, including gene deletion, promoter hypermethylation, and hypermethylation of the intergenic, differentially methylated region.[60] The re-expression of MEG3 inhibits tumor cell proliferation in culture and the colony formation in soft agar. The underlying mechanism of growth inhibition is partly the result of MEG3-induced apoptosis.[61] Moreover, a recent study has identified that low expression of serum MEG3 in BC patients, was associated with poor recurrence-free survival.[62]

LncRNA-growth arrest-specific (GAS) 5 has been found downregulated in BlCa as compared with adjacent normal tissues. Knocking down GAS5 expression promotes bladder cancer cell proliferation, whereas the overexpression of GAS5 suppresses cell proliferation. Furthermore, GAS5 knockdown results in an increased percentage of cells in S and G2 phase, and in a decreased percentage of cells in G1 phase, via regulation of chemokine (C-C motif) ligand 1 expression.[63]

APPLICATIONS OF LONG NON-CODING RNAS AS URINARY BIOMARKERS FOR NON-MUSCLE INVASIVE BLADDER CANCER

Bladder tumor markers are secreted in urines and many are tumor cell-associated and can be detected by analyzing exfoliated cells in urine specimens.

An ideal urine marker for BlCa should be noninvasive, easy to interpret, and possess high sensitivity and specificity not only for the diagnosis but also for the reduction of the number of cystoscopies in surveillance of NMIBC.

In the last few years, as more lncRNAs have been discovered and their biological functions clarified, the lncRNAs have been increasingly proposed as important diagnostic and prognostic biomarkers[64] for BlCa. NcRNAs are relatively stable in cells present in urines and their expression in cells isolated from voided urines of BlCa patients has been recently proposed as a noninvasive diagnostic assay.[65]

Approximately 50 ml of urine is spun at 1700*g* for 10 min to pellet exfoliated cells. The pelleted material

Table II. Detailed characterization of available data on potential biomarkers

Ref	N Cases	N control	Validation	Results replication
Zhang et al, 2012[42]	94	144	RT-PCR	Yes (73)
Wang et al, 2006[37]	94	85	RT-PCR	Yes (70)
Srivastava et al, 2014[71]	117	74	RT-PCR	Yes (37, 70)
Zhou et al, 2006[41]	85	10	RT-PCR	Yes (11, 51)

is suspended in 200 μl of PBS. DNA is extracted from the suspension using commercially available Kit, according to manufacturer's instructions.[66] Urine is increasingly studied as it can be obtained noninvasively in large volumes and contains exosomes (UEs) vesicles with diagnostic potential[67] for urological cancers.[68,69] Urinary lncRNAs can be durably detected in urines.[66]

Moreover, RNA is protected by urinary UEs to a greater degree than by cells in urines,[70] and the UEs themselves are remarkably stable.[41] Several reports characterized the performance of lncRNA as potential urinary biomarkers in BlCa (See Table II for details).

Zhang et al[42] evaluated the potential application of UCA1 in urinary sediments from patients with BlCa, and they found that it is particularly valuable for superficial G(2)-G(3) patients at a high risk for muscular invasion with a sensitivity of 86.4% and 92.3%, respectively. UCA1 seems to be a new promising urinary marker for the diagnosis of BlCa. Accordingly, Eissa et al[72] demonstrated that lncRNA-UCA1 has good sensitivity and specificity (91.5 and 96.5%, respectively) for distinguishing BlCa patients from non-BlCa ones.

Some authors[72,73] analyzed the mRNA expression of UCA1 in exfoliated cells in the urines of TCC cases of UBC, finding that UCA1 mRNA expression in TCC-exfoliated cells is not significantly correlated with the clinical characteristics such as patient's age, sex, nodal status, and smoking habits. However, their results indicated that urinary UCA1 gives higher positive results in comparison to cytology, particularly in nonmuscle-invasive low-grade disease. Accumulation of UCA1 in urine sediments could be used as a sensitive and specific diagnostic and follow-up marker for patients with transitional cell carcinoma.

Urine is an excellent source of UEs, 30–150 nM membrane-bound secreted vesicles that represent an excellent source for biomarker discovery.[38,74] Interestingly, UEs are markedly depleted in mRNA but enriched in lncRNAs[75] and lncRNAs show greater specificity than protein-coding mRNA as biomarkers of cancer.[15] Bladder cancer–associated lncRNAs have been isolated from voided cells or free floating in urines. However, no published studies have demonstrated that bladder cancer UE–derived lncRNAs can serve as urinary biomarkers yet.[72] One benefit of using UEs is that the exosome membrane protects the contents from proteases and RNAses, which are ubiquitous in urines.[41] Recently, Berrondo et al[11] showed that HOTAIR and several tumor-associated lncRNAs are enriched in UEs from UBC patients with high-grade muscle-invasive disease (HGMI pT2-pT4).

Martinez-Fernandez et al[76] previously investigated the possibility that HOTAIR expression could serve as a prognostic marker for disease recurrence in NMIBC, demonstrating that patients with higher levels of HOTAIR expression also have earlier recurrence of disease. In addition, they used The Cancer Genomic Atlas data set for bladder cancer to show that HOTAIR expression is correlated with stage of UBC, with the most invasive T4 tumors having the highest level of HOTAIR expression. Given the importance of HOTAIR in bladder tumor progression, there is increased interest in using HOTAIR as a biomarker. Importantly, having a noninvasive way to detect HOTAIR in cancer patients, such as UEs, would be ideal for biomarker development. Further studies are encouraged to assess the clinical utility of UEs HOTAIR content in the management of NMIBC cancer. Furthermore, Berrondo et al[11] also showed that HOX-AS-2, MALAT1, and other lncRNAs are enriched in UEs from UBC patients with HGMI disease (pT2-pT4 on final cystectomy pathology). Therefore, UEs seems to be an amenable field of research for lncRNAs as urinary biomarkers in BlCa.

CONCLUSIONS AND FUTURE PERSPECTIVES

Several bladder urinary tumor markers such as BTA, NMP22, FDP, ImmunoCyt, and FISH (UroVysion)[77] have been developed and approved by FDA for their use in initial diagnosis, monitoring recurrence, and treatment response. The impetus for developing new bladder tumor markers comes from the low effectiveness and invasive nature of cystoscopy and cytology in the management of BlCa patients. Moreover, the urinary markers, currently used, have shown higher sensitivity as compared with cytology, but often they show low specificity.

Literature data collectively indicated the high tissue- and cancer-specific expression of lncRNAs,[78,79] suggesting their diagnostic and prognostic potential in urologic malignancies.

Several lncRNAs have been characterized as potential biomarkers in urine samples. In BlCa, UCA1 (urothelial carcinoma associated 1) transcript detected in urines has shown to be a highly sensitive and specific biomarker of bladder carcinoma.[73] However, the most famous example of such biomarkers is PCA3 in prostate cancer. PCA3 was identified in 1999 as highly overexpressed in almost all prostate cancer tissue specimens, compared with normal or hypertrophied tissue. A PCA3 score, based on the ratio of PCA3 mRNA over PSA mRNA, can be calculated starting from a urine sample collected after digital rectal examination. PCA3 score has been demonstrated to be a more specific predictor of prostate cancer diagnosis compared with the commonly used prostate-specific antigen (PSA).[80-83]

Novel lncRNAs are increasingly being identified to help prognosticate patients with BlCa. More than 30 urinary biomarkers have been reported for use in BlCa diagnosis, but only a few are commercially available, such as BTA and NMP22.[84]

To date, the only one study[71] on diagnostic and prognostic utility of lncRNA as urinary bladder tumor marker concludes that UCA1 mRNA is a better noninvasive biomarker for diagnosis of nonmuscle-invasive low-grade UBC, as compared with the cytology. It may be used in combination with cytology for noninvasive diagnosis of UBC and may increase the interval between monitoring cystoscopy and replacing cystoscopies all together. Further investigations will be encouraged on the UEs, that may represent a noninvasive promising way to detect lncRNAs in cancer patients, as shown by HOTAIR evidence.[11] The widely used commercial microarray platforms in addition to protein coding mRNAs were designed to detect transcripts such as ncRNAs, providing a valuable resource for the identification of novel biomarkers based on miRNAs and lncRNAs. Deep sequencing technology is currently becoming cheaper and more widely accessible, so it is likely that an explosive growth of newly identified lncRNAs differentially expressed in cancers and associated with various clinical outcomes, will be discovered.

In conclusion, the rapidly expanding catalogue of lncRNAs has highlighted their potential value as tumor markers in patient diagnosis and prognosis and holds promises that in the near future lncRNAs will become even more important in cancer patient management.

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