ISSN 0006-2979, Biochemistry (Moscow), 2025, Vol. 90, No. 6, pp. 650-670 © Pleiades Publishing, Ltd., 2025.
Published in Russian in Biokhimiya, 2025, Vol. 90, No. 6, pp. 695-719.

REVIEW

The Role of m^{6}A-RNA Methylation in the Development, Progression, and Treatment Response of Bladder Cancer

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Received December 11, 2024
Revised April 20, 2025
Accepted April 23, 2025

Abstract—Bladder cancer (BCa) remains a significant clinical challenge because of high recurrence rates and variable response to immunotherapy and chemotherapy. Recent studies have highlighted the role of N6-methyladenosine (m^{6}A) modification in RNA in the regulation of various cellular processes, including tumor progression and drug resistance. The review examines the impact of m^{6}A methylation on BCa pathogenesis, with a particular special focus on the role of m^{6}A pathway factors and m^{6}A-modified RNAs in tumorigenesis, proliferation, invasion, and migration of cancer cells. The mechanisms of m^{6}A-mediated chemotherapy resistance in BCa cells are discussed, including single nucleotide polymorphisms in m^{6}A-associated patterns. Significant advances in the high-throughput analysis of m^{6}A methylation have enabled development of novel m^{6}A-based biomarkers for the risk assessment, early diagnostics, and prediction of relapse and treatment response in BCa. The review outlines the prospects of the m^{6}A-based molecular diagnostics in BCa.

DOI: 10.1134/S0006297924604441

Keywords: m^{6}A, N6-methyladenosine, bladder cancer (BCa), biomarker, prognosis

INTRODUCTION

Bladder cancer (BCa) remains the most common malignancy of the urinary tract. According to the World Health Organization (WHO) data for 2022, BCa ranks 6th in prevalence among cancers in men, 9th – among both sexes, and 13th – in global mortality rates [1]. In the Russian Federation, it holds the 6th position in prevalence among cancers in men, 9th – among both sexes, and 16th – in mortality rates among all oncological diseases [1].

BCa is categorized according to the depth of invasion into the muscular layer and is subdivided into non-muscle-invasive BCa (NMIBC) and muscle-invasive BCa (MIBC). Approximately 75% of newly diagnosed cases are NMIBC. The overall 5-year survival rate for NMIBC exceeds 70%, but remains below 6% for MIBC [2]. The ‘gold standard’ treatment for NMIBC

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is transurethral resection of bladder tumor (TURBT), followed by adjuvant intravesical chemotherapy or immunotherapy. MIBC requires more aggressive therapy, such as trimodal treatment, which combines TURBT, radiotherapy, and systemic chemotherapy. The recurrence rate for NMIBC varies widely, with the reported rates between 40 and 90% [3-5]. The rate of NMIBC progression to MIBC ranges from 5 to 55% [6].

One of the cancer biomarkers that has attracted significant attention is N6-methyladenosine (m^{6}A), a dynamic and reversible epitranscriptomic RNA modification involved in various cellular processes. m^{6}A methylation occurs exclusively within the context-specific sequences known as DRA*CH motifs (where D = A, G, or U; R = A or G; H = A, C, or U). A unique distribution and density of m^{6}A in RNA molecules is typically referred to as an m^{6}A pattern. The distribution of m^{6}A patterns across RNA transcripts is non-random; they are highly conserved but can vary depending on the RNA type and cellular context, tissue, developmental stage, and disease. m^{6}A patterns can be assessed within individual RNA transcripts or in the entire transcriptome. DRA*CH and alternative m^{6}A consensus motifs are typically evolutionary conserved and appear mostly near 3′- and 5′-untranslated regions (UTRs), whereas their presence in coding regions is less frequent [7]. m^{6}A methylation can occur in specific mRNAs or non-coding RNAs (ncRNAs), affecting their stability, splicing, translation, and decay. The presence, location, and density of m^{6}A modifications in individual transcripts can determine their fate and functions. Together, these factors influence a complex network of m^{6}A-mediated regulation, which impacts all aspects of RNA metabolism.

Abnormal m^{6}A methylation patterns have been observed in various pathological conditions, such as neurodevelopmental and neurological disorders [8, 9], cardiovascular diseases [10, 11], viral infections [12], metabolic disorders [13], and immune response [14]. Altered m^{6}A methylation patterns have been observed and extensively studied in various cancers, including breast, lung, liver, and colorectal cancers, as it was found that specific m^{6}A modifications correlate with tumor progression, metastasis, and patient prognosis [15].

m^{6}A modifications have been identified as a significant factor in cancer biology, affecting various aspects of tumorigenesis and cancer progression. m^{6}A methylation of mRNA serves as a gene regulatory mechanism and influences proliferation and invasion of tumor cell, as well as tumor immune evasion and metastasis, by coordinating and modulating gene expression in a reversible and highly dynamic manner [16].

The review discusses the role of m^{6}A methylation in the development of BCa, the pro- and anti-oncogenic functions of m^{6}A pathway factors, m^{6}A methylation of mRNAs and ncRNAs related to oncogenes and tumor suppressors, and their potential diagnostic and prognostic significance. We also explored how m^{6}A methylation contributes to the development of resistance to chemotherapeutic agents.

CURRENT CHALLENGES IN THE DIAGNOSTICS AND TREATMENT OF NMIBC

At present, BCa diagnosis is confirmed exclusively by histological examination of biopsies obtained during the TURBT. Urine cytology is used to detect exfoliated tumor cells. This method is highly sensitive in the detection of carcinoma *in situ* and high-grade (G3) tumors, but is much less efficient in the identification of low-grade (G1) tumors [17]. Thus, the sensitivity of urine cytology test in detecting carcinoma *in situ* varies widely, ranging from 28 to 100% [18]. The positive result indicates the presence of transitional cell carcinoma, which can originate in any part of the urinary tract, but the negative result does not rule out the possibility of BCa [19].

Due to the low sensitivity of urine cytology in the diagnostics of low-grade BCa, numerous methods for analyzing BCa molecular markers have been developed. However, none of these markers have been widely adopted as a standard with a high prognostic value [20].

Currently, biomarkers can be categorized into two distinct groups: (i) diagnostic markers, which help confirm a BCa diagnosis; (ii) prognostic markers, which assess the risks of disease progression and recurrence in patients already diagnosed with BCa [21].

Five diagnostic test systems have been approved by the FDA: NMP22 test kit, NMP22 BladderChek Test, BTA TRAK, BTA stat, and UroVysion [22, 23]. Despite the established efficacy of these test systems in the identification of BCa markers, their application in clinical practice remains limited [24].

At present, there are no urinary BCa biomarkers that can substitute for cystoscopy or reduce the frequency of cystoscopic examinations [20], indicating an ancillary role of non-invasive urine analysis methods conducted prior to definitive diagnosis.

A standard of treatment for NMIBC is transurethral bladder resection followed by adjuvant chemo- or immunotherapy. The two techniques for bladder resection are TURBT (resection of the bladder wall with the tumor as a single block) or piecemeal tumor resection [19].

Current clinical guidelines recommend a single instillation of a chemotherapeutic agent (most commonly, mitomycin C, epirubicin, or pirarubicin) after TURBT, which has been proven to significantly reduce

the recurrence rates. A comprehensive review by Sylvester et al. [25] reported that this treatment lowers the five-year recurrence rate from 59 to 45%. Such improvement in the outcome (14%) means that approximately one in seven patients will benefit from the therapy, while the remaining six will be unaffected but still exposed to its potential side effects and risks. Hence, a single instillation of a chemotherapeutic agent cannot be considered a definitive solution, thus emphasizing the need for continued research aimed to further reduce the risk of tumor recurrence and metastasis.

After obtaining histological results and determining the degree of tumor differentiation, patients are stratified into four or three risk groups [19]. Adjuvant therapy is not recommended for patients in the low-risk group. Patients with the intermediate-, high-, or very high-risk NMIBC typically undergo induction and maintenance courses of intravesical BCG (Bacillus Calmette–Guérin) therapy lasting from one to three years [26]. Intravesical chemotherapy may be recommended for intermediate-risk patients with intolerance or contraindications to the BCG therapy [19].

Despite a high efficacy of current treatment options secured in clinical guidelines, the recurrence rate of NMIBC remains significant (from 40 to 90%) [3-5], and the rate of NMIBC progression to MIBC varies between 5 to 55% [6], which emphasizes the ongoing need for new approaches to BCa therapy.

Such high recurrence and progression rates require a deeper understanding of molecular mechanisms driving BCa development. Early detection is critical for improving the clinical outcomes, which is why the development of reliable biomarkers capable of improving the diagnostic and prognostic accuracy and guiding the treatment decisions, has become a major research focus.

In summary, the challenges associated with BCa are multifaceted and include (1) early BCa diagnosis and risk assessment; (2) evaluation of risk of NMIBC progression to MIBC; (3) prediction of NMIBC recurrence; and (4) classification of patients into responders and non-responders to current anti-cancer regimens. While some of these challenges can be partially addressed through existing methods, such as imaging, cytological analysis, and genomic mutation profiling, there is an urgent need to identify novel molecular biomarkers to enhance the diagnostic accuracy and treatment efficacy in BCa. One of the promising research areas is the use of m^{6}A modification as a potential biomarker.

Molecular mechanism of m^{6}A-methylation. m^{6}A is the most prevalent epitranscriptomic modification of both mRNAs and ncRNAs, including lncRNAs (long non-coding RNAs), circRNAs (circular RNAs), miRNAs (microRNAs), and others. m^{6}A methylation is catalyzed by the methyltransferase complex (or 'writer'), consisting of the METTL3/METTL14 (methyltransferase like proteins 3 and 14) heterodimer [27] and auxiliary components, such as WTAP (Wilms tumor 1 associated protein), VIRMA (KIAA1429; vir like m^{6}A methyltransferase associated), RBM15/15B (RNA binding motif protein 15/15B), ZC3H13 (zinc finger CCCH-type containing 13), and others, which lack the methyltransferase activity and play a regulatory role in the complex functioning [28]. Methyl group can be removed by demethylases (or 'erasers'), such as ALKBH5 (AlkB homolog 5) [29] and FTO (fat mass and obesity-associated protein) [30], indicating the reversible and highly dynamic nature of the m^{6}A modification.

m^{6}A-binding proteins, known as 'readers', recognize the m^{6}A mark and determine the fate (stabilization/translation or degradation) of the RNA molecule. Reader proteins belong to various protein families and include YTH-domain proteins (YTHDF1/2/3, YTHDC1/2) [31], heterogeneous nuclear ribonucleoproteins (HNRNPC, HNRNPG, HNRNPA2B1) [32], insulin-like growth factor 2 mRNA-binding proteins (IGF2BP1/2/3) [33], and eukaryotic initiation factors (elF3) [34]. The specificity of reader proteins is determined by a combination of biochemical, spatial, and contextual factor that ultimately make the determination of RNA fate based on m^{6}A marks a highly context-dependent process. Understanding this mechanism is important for the accurate prediction of the outcome of downstream processes, which could shed light on aberrant m^{6}A functions leading to various diseases.

m^{6}A modification can influence expression of both oncogenes and tumor suppressor genes, playing a dual role in cancer progression, which emphasizes its importance in a complex network of molecular interactions that sustain tumor growth and determine its resistance to therapy. m^{6}A modifications can promote accumulation of oncogenic proteins by stabilizing oncogenic mRNAs and inhibiting decay of tumor-promoting mRNAs, which can result in the dysregulation of cell proliferation, promotion of cell cycle progression, inhibition of apoptosis, and other cancer hallmarks [35]. Some studies indicate that m^{6}A modifications can significantly impact the metastatic potential of cancer cells by regulating key genes involved in cell adhesion, migration, and invasion, thus contributing to tumor spreading. Conversely, m^{6}A modifications can ensure the stability of tumor suppressor mRNAs by preventing their degradation, maintaining their inhibitory effect on cancer cells [36, 37].

m^{6}A pathway in BCa. m^{6}A modifications modulate expression of genes associated with cell proliferation, apoptosis, and metastasis [15]. m^{6}A methylation also plays a critical role in the progression of BCa [38]. Alterations in the m^{6}A methylation patterns in BCa

can lead to the upregulation of oncogenes or downregulation of tumor suppressor genes, thus contributing to tumorigenesis [38]. Expression of m^{6}A-pathway factors in BCa and healthy tissues have been investigated in several transcriptomic studies. For instance, Chen et al. [39] studied the levels of key m^{6}A regulators and analyzed correlation of their expression with clinicopathological variables. Clinicopathological information for 408 BCa patients and healthy donors was obtained from the TCGA (The Cancer Genome Atlas) database, allowing for the comparison between the non-tumor tissue group and BCa samples of varying grades and stages. It was shown that KIAA1429 (one of the major m^{6}A methyltransferases) and YTHDF (m^{6}A reader protein) were highly expressed in high-grade BCa. Expression of ALKBH5 (m^{6}A demethylase) positively correlated with the tumor grade and M1 stage. Although METTL3 (another major m^{6}A methyltransferase) was highly expressed in cancer tissues, its expression decreased as the grade increased and was low in high-grade tumors. FTO (an important m^{6}A demethylase) was expressed poorly in BCa [39]. Bioinformatic analysis revealed differential patterns in the expression of m^{6}A-related genes across tumor tissues. Specifically, expression of FTO, ZC3H13, YTHDF3, YTHDC1, WTAP, METTL16, and METTL14 was downregulated in cancerous tissues compared to normal tissues. Conversely, HNRNPA2B1, IGF2BP1, IGF2BP3, METTL3, YTHDF2, and YTHDF1 demonstrated high expression levels. Moreover, expression of HNRNPA2B1, HNRNPC, IGF2BP2, RBM15, YTHDF1, and YTHDF2 was associated with the advanced clinical stages of BCa [40]. Alterations in the expression of m^{6}A factors indicate a critical role of m^{6}A methylation in BCa development. Therefore, both expression of m^{6}A-related factors and m^{6}A-methylation patterns could potentially serve as prognostic markers to help clinicians define the clinical stage of cancer, differentiate cancer subtypes, predict disease progression and outcome, evaluate the risk of recurrence, and design the treatment plans accordingly. For instance, abnormal m^{6}A levels in transcripts associated with tumor suppression may correlate with the advanced clinical stages, while changes in the methylation levels of oncogenic transcripts can indicate aggressive cancer subtypes or higher risk of disease progression. By analyzing the m^{6}A methylation status of key transcripts, clinicians can more accurately define the clinical stage of BCa, differentiate between the luminal and basal subtypes, predict the likelihood of disease progression, and estimate the outcomes. Specific m^{6}A methylation signatures could help evaluate the risk of recurrence and guide personalized treatment plans, e.g., help in selecting patients who may benefit from immunotherapy or targeted therapy. While this approach is still in the realm of clinical theory, advancements in RNA epigenomics and single-molecule sequencing technologies are paving the way for its practical application in clinical practice in the foreseeable future.

m^{6}A factors with the oncogenic role in BCa. In recent years, the m^{6}A demethylase FTO has gained significant attention due to its potential role in various cancers, including BCa. Several targets of FTO in BCa have been identified, including PTPN6 (tyrosine protein phosphatase non-receptor type 6), a non-receptor tyrosine phosphatase that dephosphorylates and regulates the activity of numerous proteins involved in signal transduction, cell growth, differentiation, cell cycle, and oncogenic transformation [41]. PTPN6 is aberrantly expressed in various cancers, such as hepatocellular carcinoma, renal cell carcinoma, gastric cancer, and BCa [41]. Wu et al. [42] discovered that FTO induces expression of *PTPN6* and stabilizes it, thus promoting proliferation and metastatic capabilities of BCa cells. The authors analyzed human BCa and adjacent normal tissues collected from 20 patients diagnosed with BCa (sex and age of the patients were not taken into account), as well as investigated human BCa cell lines (T24, 5637, RT4, J82, and HTT1378) and normal bladder epithelial cell line SV-HUC-1 in *in vitro* experiments The samples were not stratified into groups based on the disease stage or tumor grade, limiting the generalizability of the study. While m^{6}A-modified *PTPN6* mRNA was detected, no specific m^{6}A sites were mapped, which hinders the understanding of how FTO regulates this transcript. Despite the limitations, the findings of this study suggest that the FTO-PTPN6 axis may serve as a potential prognostic marker in BCa.

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), an lncRNA involved in the regulation of gene expression through epigenetic mechanisms, has been associated with the tumorigenesis in most cancer types, including BCa [43]. It was found that in BCa, FTO acts as an oncogene by reducing the m^{6}A methylation of the 5′-UTR of *MALAT1*, leading to the increased stability and upregulated expression of this ncRNA. Elevated *MALAT1* then functions as a sponge for *miR-384*, reducing its availability, which increases MAL2 expression and promotes cancer cell viability and tumor growth. This study was conducted in human BCa paired tissue samples (tumor and adjacent noncancerous) collected from 25 patients; 144 cancer tissue microarrays were used for the validation. Human BCa cell lines (5637, J82, 253J, T24, SCABER) and SV-HUC-1 cells were investigated in the *in vitro* experiments. The authors did not stratify patients by a specific BCa type or distinct m^{6}A modification profile; tissue samples were analyzed primarily for the overall pathologic stage (pTa-T1 vs. pT2-T4). Upregulated FTO expression was found to be associated

with more advanced pathologic stages (pT2-T4), while no statistically significant association between the FTO or MAL2 levels and patients' sex or age was observed.

FTO has been shown to modify the maturation of *pri-miR-576* in an m^{6}A-dependent manner and to promote proliferation, migration, and invasion of BCa cells by regulating the miR-576-CDK6 pathway [44]. The authors examined BCa and adjacent normal tissues from 20 patients who underwent radical cystectomy. BCa cell lines (T24, 5637, UM-UC-3) and SV-HUC cells were used in *in vitro* experiments. Tissue microarrays from 67 BCa patients were analyzed. It was found that FTO expression did not differ significantly between males and females or different age groups (p = 0.755 for sex, p = 0.995 for age), but was associated with a higher TNM stage (p = 0.035). The authors provided detailed insights into the m^{6}A modification-based mechanisms by which FTO regulates the miR-576-CDK6 axis and contributes to BCa progression. By identifying FTO as a potential prognostic biomarker, the study facilitates future diagnostic or therapeutic advancements in BCa. The correlation between the FTO expression and higher TNM stages suggests a potential use of FTO for predicting cancer progression.

Another key m^{6}A-related regulator is the reader protein IGF2BP3 (insulin like growth factor 2 mRNA binding protein 3) that may play an oncogenic role in BCa progression. Expression of IGF2BP3 was higher in BCa cells compared to adjacent healthy tissues [45] and was associated with several factors indicating more advanced stages of BCa: (1) advanced tumor characteristics and (2) more aggressive disease progression [45]. BCa samples were collected from 95 patients who underwent resection; SV-HUC-1 cells and BCa cell lines (5637, J82, UMUC3, and T24) were used for *in vitro* analysis. The study included the data for 414 BCa patients from the TCGA, categorized by the IGF2BP3 expression levels (high and low). The patients were classified as ≤70 years vs. >70 years old, but no significant correlation between the IGF2BP3 expression and age was found (p = 0.372). The elevated IGF2BP3 expression levels correlated with poor overall survival (p = 0.015) and were associated with more advanced tumor stages (T3/T4) and high tumor histologic grade, reinforcing the role of IGF2BP3 as a marker of BCa progression. However, no correlation between the IGF2BP3 expression and nodal (N) or distant metastasis (M) status was observed, which was unexpected given the role of IGF2BP3 in cell migration and invasion. This discrepancy suggests that IGF2BP3 may be more involved in the local tumor progression rather than in metastasis. The study highlighted a strong correlation between the IGF2BP3 expression and immune cell infiltration in BCa. High IGF2BP3 levels were associated with an increased infiltration of macrophages, neutrophils, and CD8$^+$ T cells, suggesting a potential immunomodulatory role of IGF2BP3. Moreover, the elevated IGF2BP3 levels have been linked to poorer outcomes for BCa patients, suggesting that IGF2BP3 may participate in determining the severity of BCa and influence patients' survival. In a recent study [33], it was suggested that IGF2BP family proteins can recognize m^{6}A in the transcripts and stabilize such transcripts, thus increasing their half-life time and duration of protein expression. For example, stabilization of the neuropilin 1 (NRP1) mRNA led to the M2 macrophage polarization associated with BCa progression [46].

High-mobility group protein B1 (HMGB1) was identified as a target of IGF2BP3. HMGB1 is a nuclear DNA-binding protein integral to numerous cellular processes, including inflammation, cell differentiation, and apoptosis. Interestingly, the levels of IGF2BP3 were notably elevated in the individuals who showed positive response to immunotherapy in contrast to those who did not respond. Moreover, increased expression of IGF2BP3 and its m^{6}A-mediated interaction with *HMGB1* mRNA correlated with the improved overall survival, suggesting that IGF2BP3 may impact the immune microenvironment of BCa, thus influencing the efficacy of immunotherapy [47]. Using bioinformatic methods, the authors identified multiple m^{6}A motifs in *HMGB1* mRNA that could serve as IGF2BP3-binding sites. IGF2BP3 expression and function was investigated in BCa cell lines RT112/84 and BFTC905.

Another gene of significant interest is *ITGA6*, which encodes integrin alpha-6 subunit. ITGA6 is a cell surface receptor involved in the cell–cell and cell–substrate interaction, cell migration, differentiation, tissue repair, and regulation of cell growth [48, 49]. The association between the ITGA6 expression and cancer progression is rather complex. The upregulation of ITGA6 has been linked to the enhanced invasion, metastasis, and poor prognosis in various cancers [50-52]. Thus, ITGA6 was found to maintain cell adhesion-mediated drug resistance in ovarian cancer and leukemia [53, 54].

Recently, a mechanism of ITGA6 regulation by the m^{6}A modification has been proposed [55]. Tissue samples from of 186 BCa patients who underwent radical cystectomy and bladder biopsies were investigated for m^{6}A methylation. It was confirmed that the 3′-UTR of *ITGA6* mRNA contains four m^{6}A motifs methylated by METTL3. Methylation promoted translation of the *ITGA6* transcript through binding of the YTHDF1 and YTHDF3 reader proteins *in vitro* and *in vivo*. Interestingly, mutation of a single m^{6}A site did not decrease the translation rate of the transcript, whereas introduction of several mutations into multiple m^{6}A sites noticeably slowed it down [55]. In another study,

removal of m^{6}A from several positions in the *ITGA6* transcript by the designed multisite editor dCasRx-m^{6}A led to the reduction in the transcript translation and inhibition of BCa progression *in vitro* and *in vivo*, suggesting a significance of cooperative transcript regulation by multiple m^{6}A sites. The off-target activity of the dCasRx–m^{6}A editor has been assessed and proven to be limited [56]. These findings highlight a potential importance of m^{6}A methylation of the *ITGA6* mRNA in tumorigenesis and suggest that further research in this area could lead to the identification of novel therapeutic targets for BCa.

The CUB domain-containing protein 1 (CDCP1) is a transmembrane protein that acts as a protein-protein interaction hub for proteins regulating cell–cell and cell–substrate adhesion through proteolytic processing and tyrosine phosphorylation [57]. Dysregulation of CDCP1 expression in cancer cells has been observed in colon [58, 59], lung [60], and kidney [61] cancers. CDCP1 was found to induce cell detachment and promote metastasis in breast cancer [57]. In general, an elevated expression of CDCP1 is associated with the enhanced migratory capabilities of cancer cells, which facilitates tumor dissemination and metastasis and leads to poor disease outcome [62]. The oncogenic role of CDCP1 in BCa, including promotion of cancer development and progression, has been demonstrated in several studies.

The METTL3-m^{6}A-CDCP1 axis was also found to be implicated in BCa oncogenesis, suggesting a regulatory role of m^{6}A modification in the CDCP1 expression and translation in aberrant uroepithelial cells [63]. The authors utilized 114 BCa and 30 cystitis samples from patients who underwent radical cystectomy and bladder biopsies and identified specific m^{6}A modification sites in the 3′-UTR and coding region of the *CDCP1* mRNA, which were observed in both control and malignant BCa cells, whereas CDCP1 expression itself was barely detectable in cystitis tissue. Methyltransferase METTL3 facilitated methylation at these sites, which served as a signal for the YTHDF1 reader protein to bind to the *CDCP1* transcript, thus stabilizing it and promoting its translation. Conversely, depletion of METTL3 moderately inhibited proliferation, invasion, and migration of BCa malignant cells [63]. Another research confirmed these data by demonstrating that the targeted RNA methylation system RCas9-METTL3 introduced m^{6}A modification to the 3′-UTR of the *CDCP1* transcript, leading to its enhanced translation and promotion of tumor growth *in vitro* and *in vivo*. The system used exhibited satisfactory on-target activity [64].

As key factors in cell adhesion, ITGA6 and CDCP1 are linked to cancer progression, drug resistance, and poor prognosis in BCa [55, 63]. Both are regulated by the METTL3-mediated m^{6}A modification, which suggests a common regulatory axis for potential therapeutic targeting. While these findings are promising, several challenges related to the specificity, resistance, and clinical implications must be addressed. Although the analyzed samples were from post-radical cystectomy BCa patients, the data on the comorbidities were lacking and no stratification based on the tumor stage, grade, or molecular subtype was performed, raising concerns about generalizability and interpretation of the study results. The possibility of using ITGA6-CDCP1 and related m^{6}A factors (e.g., METTL3) as therapeutic targets is questionable, since their inhibition may disrupt the downstream pathways, potentially, even in healthy cells. Utilizing m^{6}A sites as direct prognostic markers is a novel approach [65-67]. The use of advanced molecular tools has demonstrated a potential for precise RNA editing and its therapeutic applications (for instance, removal of aberrant modifications to nullify the negative effects of said modifications). However, challenges remain, such as predicting long-term effects of RNA editing and ensuring safe and efficient delivery of editing systems via nanoparticles, exosomes, or viral vectors, [68-70]. The cooperative regulation of ITGA6 by multiple m^{6}A sites complicates the development of the targeted therapy. Aberrant m^{6}A profiles correlating with the tumor stage and grade, could serve as prognostic markers for disease progression, help stratify patients into high- and low-risk groups, and guide the treatment decisions. The studies have provided mechanistic insights into the regulation of ITGA6 and CDCP1 by m^{6}A modifications, linking RNA methylation to cancer progression. Development of targeted tools, e.g., molecular editors, offers exciting possibilities for precision medicine, but further research is needed to validate these findings and translate them into effective therapies for BCa patients.

m^{6}A factors acting as tumor suppressors in BCa. The effects of m^{6}A modifications are diverse and may include inhibition of tumorigenesis. NOTCH1 (Notch receptor 1), a component of the Notch pathway, is responsible for the self-renewal of tumor-inducing cells (TICs) and oncogenesis [71]. Primary BCa samples (ranging from early non-invasive to advanced invasive BCa) from 6 patients have been studied in order to confirm the role of the NOTCH1-m^{6}A axis in tumorigenesis. It was found that the m^{6}A modification of the *NOTCH1* transcript by METTL14 attenuated its expression [71], but the exact mechanism of this process remains largely unknown and requires further research. These findings revealed a novel METTL14-m^{6}A-Notch1 regulatory axis in BCa, highlighting a possible implementation of the m^{6}A modification as a therapeutic target and biomarker of BCa progression. However, the study used only six BCa samples for the initial analysis of the m^{6}A content. Such small

sample size limited the generalizability of the findings; moreover, the samples varied significantly in terms of age, sex, tumor size, stage, and metastasis status. Also, no tissues from healthy controls were analyzed, which could potentially affect the results of m^{6}A mapping.

m^{6}A may also act as an mRNA decay signal, as it was shown for the tumor-suppressor factors SETD7 (SET domain containing 7), KLF4 (Krüppel-like factor 4), and SYTL1 (synaptotagmin-like protein 1). The roles of SETD7 and KLF4 in cancer still remain contradictory. Downregulation of SETD7 (lysine methyltransferase involved in histone and non-histone protein methylation) has been linked to the enhanced migration and invasion of lung cancer cells [72] and to correlate with a poor outcome in patients with gastric cancer [73]. A large body of evidence suggests that SETD7 acts as an oncogene, based on the positive correlation between its expression and cancer stage in hepatocellular adenocarcinoma [74]. Conversely, SETD7 has been associated with the prevention of the epithelial-to-mesenchymal transition in various cancer types, indicating its potential involvement in the inhibition of metastasis [75]. KLF4 was found to act as a tumor suppressor by preventing metastasis in colorectal cancer [76]), but also as an oncogene in osteosarcoma by promoting tumorigenesis *in vivo* [77]. KLF4 is significantly downregulated in urothelial BCa cells, indicating poor overall survival and risk for recurrence [78, 79]. KLF4 overexpression induced by the CRISPR-ON transcriptional activation system inhibited cell proliferation and promoted cell cycle arrest in G1 phase via regulation of the AKT-p21 signaling pathway [79]. The data obtained suggest that *SETD7* and *KLF4* mRNAs are the downstream targets of METTL3, which installs m^{6}A in their transcripts. The modified transcripts are recognized by the YTHDF2 reader protein, resulting in mRNA degradation. Repression of METTL3 correlated with the cell cycle arrest in G1 phase and reduction in cell proliferation and migration *in vitro* [80].

KLF4-m^{6}A and SETD7-m^{6}A axes represent promising but complex targets for cancer therapy. While the contradictory roles of SETD7 and KLF4 as both tumor suppressors and oncogenes in different cancers pose serious challenges, advancements in understanding molecular mechanisms underlying their activity could lead to novel therapeutic and prognostic strategies. Future research should focus on resolving these contradictions, identifying context-specific factors, and developing targeted interventions with minimal toxic effects. However, the implication of the aforementioned axes yields a prognostic value, since aberrant m^{6}A profiles, as well as SETD7 and KLF4 expression levels, may correspond to specific tumor stages and grades.

SYTL1 plays a critical role in enhancing the anti-tumor immune response. Thus, the overexpression of SYTL1 was found to activate natural killer (NK) cells, promoting the effect of the anti-tumor immunity both *in vitro* and *in vivo*. [81] The m^{6}A methyltransferase WTAP downregulates SYTL1 expression via m^{6}A methylation of the *SYTL1* mRNA, which marks it for degradation after recognition by the YTHDF2 reader protein. The authors analyzed 35 BCa and adjacent tissue samples collected from patients who underwent radical cystectomy. BCa cell lines (5637, T24, SW780, J82), SV-HUC-1 cells, and NK-92 cells were used in the *in vitro* experiments, and the results of these experiments were validated *in vivo*. No sex, age, or disease stage were taken into account in this work, as the study was primarily focused on BCa samples and cell lines. The authors did not investigate the SYTL1 expression in healthy individuals and patients with other diseases or comorbidities. However, analysis of bioinformatics databases allowed them to identify broader expression patterns, in particular, reduction in the SYTL1 expression in BCa cells compared to adjacent normal tissues. No changes in the SYTL1 expression over time or in response to treatment were studied. Although the authors suggested that modulation of SYTL1 expression could enhance the tumor suppression mediated by NK cells, they did not provide therapeutic strategies for clinical use.

Ferroptosis and m^{6}A modifications in BCa. Ferroptosis, a form of regulated cell death characterized by iron-dependent lipid peroxidation, has been increasingly recognized for its role in various pathological conditions. Ferroptosis is driven by peroxidation of phospholipids in cellular membranes, resulting in oxidative stress and accumulation of lipid peroxides followed by membrane damage and cell lysis [82]. Cell death occurring exclusively by ferroptosis correlates with the accumulation of lipid peroxidation markers and can be suppressed by iron chelators, lipophilic antioxidants, inhibitors of lipid peroxidation, and depletion of polyunsaturated fatty acids (PUFAs).

Ferroptosis play a particularly important role in BCa due to the unique metabolic and oxidative environment of tumor cells. Several ferroptosis inducers, such as erastin, artemisinin, conjugated polymer nanoparticles, and quinazolinyl-arylurea derivatives, have been shown to sensitize BCa cells to anti-cancer treatment [83]. A combination of these compounds with standard anticancer drugs, along with the ferroptosis-related m^{6}A factors and genes, could overcome the resistance to therapy by targeting ferroptosis-related vulnerabilities of BCa cells. This approach, which leverages both conventional and ferroptosis-inducing agents, presents a promising direction in improving the efficacy of treatment, especially in patients with therapy-resistant BCa.

Ferroptosis is regulated by various pathways and factors, including NRF2 (nuclear factor erythroid 2-related factor 2), a transcription factor that plays an essential role in cellular defense against oxidative stress [84]. NRF2 regulates expression of antioxidant proteins, such as solute carrier family 7 member 11 (SLC7A11) [85] and glutathione peroxidase 4 (GPX4) [86], that can prevent ferroptosis. Conversely, NRF2 has been shown to sensitize cancer cells to ferroptosis by upregulating genes involved in iron metabolism and redox homeostasis, e.g., the gene for the multidrug resistance-associated protein 1 (ABCC1-MRP1) [87]. Ferroptosis can be a mechanism of cell death in tumors. Tumor cells, which often contain high levels of reactive oxygen species (ROS), can undergo ferroptosis due to the iron reaction with excessive hydrogen peroxide, leading to the production of hydroxyl radicals [88]. For example, activation of the p62-Keap1-NRF2 pathway promoted resistance to ferroptosis in hepatocellular carcinoma cells, highlighting a complex regulatory mechanism involved in the cell death pathways in cancer [89]. In BCa, the NRF2-dependent antioxidant response promotes tumor growth through the p62-KEAP1-NRF2 pathway [90]. A number of m^{6}A-associated ferroptosis-related genes (FRGs) linked to BCa have been identified. Thus, myosin binding protein H (MYBPH), sclerostin (SOST), small proline-rich protein 2A (SPRR2A), and cornulin (CRNN) were suggested as potential oncogenes, while CYP4F8 (cytochrome P450 family 4 subfamily F member 8), PDZD3 (PDZ domain containing 3), CRTAC1 (cartilage acidic protein 1), and LRTM1 (leucine rich repeats and transmembrane domains 1) were proposed as tumor suppressors. The mechanisms of interaction between these proteins and NRF2 remain largely unknown [91]. An aberrant expression of NRF2 in BCa is mediated in an m^{6}A-related manner, resulting in resistance to ferroptosis. WTAP introduces m^{6}A modifications in the 3′-UTR of the *NRF2* mRNA, making it a target for YTHDF1, which stabilizes this transcript and promotes its translation [92]. This process is associated with the accelerated cell proliferation and repression of the erastin-induced ferroptosis, thus ensuring poor disease prognosis. The authors used BCa and adjacent non-cancerous tissues from 45 paired samples obtained from patients who underwent nephrectomy, as well BCa cell lines (J82, UM-UC-3) and SV-HUC-1 cells (control). Neither sex or age of patients, nor the expression of WTAP or NRF2 in healthy individuals were taken into account in this study The authors focused on the mechanistic aspects of m^{6}A modification and did not explore potential therapeutic interventions targeting WTAP or NRF2 *in vivo.*

As reported in [93], WTAP is upregulated in BCa cells and its high expression correlates with a poor disease prognosis. The study included 48 males and 14 females in the BCa group and 14 males and 6 females in the control group. The average age in the control group was 57 ± 19 years vs. 52 ± 13 years in the BCa group. Statistical comparisons indicated no significant differences in sex and age between the groups ($p > 0.05$), suggesting that sex and age were not confounding factors in the analysis. Normal bladder mucosa tissue was used as a control. The authors did not differentiate between the BCa molecular subtypes, which made it unclear whether the expression of WTAP varied between them, neither did they consider potential confounding variables, such as smoking status, comorbidities, or genetic predisposition, all of which could influence WTAP expression. The downstream targets modified by WTAP and the potential of specific m^{6}A sites to serve as independent prognostic markers were not discussed as well. Complementary evidence comes from study [92], which elucidates the WTAP-induced m^{6}A-modification of NRF2 and links this axis to ferroptosis resistance and adverse outcome.

m^{6}A methylation in BCa-related ncRNAs. Various ncRNAs can also undergo m^{6}A methylation. Only around a third of transcribed genes in the human genome encode proteins, while the remaining two-thirds are non-coding genes. Some genes are transcribed into lncRNAs that regulate transcriptional and post-transcriptional processes by modulating gene expression via affecting chromatin remodeling. lncRNAs can serve as signal, decoy, guide, or scaffold molecules [94]. lncRNAs and circRNAs (covalently closed continuous loops) interact with miRNAs, resulting in the so-called ‘sponge’ effect, a specific mechanism of RNA interference in the regulation of gene expression [95]. Both lncRNAs and circRNAs have been implicated in the development and progression of various diseases due to their ability to act as miRNA sponges, which emphasizes their significance in biological processes and as potential therapeutic targets. Because of their abnormal expression, lncRNAs are of great significance in the early diagnostics of cancer. For example, lncRNA *CASC11* (cancer susceptibility 11) has been identified as an oncogenic lncRNA at the early stage of BCa development. It was found to activate cell proliferation through *miRNA-150* [96] and to promote tumor cell growth and metastasis in colorectal cancer by activating the WNT-β-catenin signaling pathway [97].

Huang et al. [98] identified 50 m^{6}A-lncRNAs with a potential prognostic value that were methylated mostly by METTL3 and RBM15 methyltransferases. A risk score model has been developed based on 11 lncRNAs with the prognostic significance. The knockdown of *METTL3* and *RBM15* in BCa cells inhibited proliferation, invasion, and migration of tumor cells *in vitro* and *in vivo*. The study did not fully address

the heterogeneity of BCa, as the authors did not differentiate between the tumor molecular subtypes, which may impact the generalizability of the developed prognostic model. Also, no specific m^{6}A sites have been identified in the lncRNAs.

miRNA-221 and *miRNA-222* play the oncogenic role in BCa by binding to the 3′-UTR of the tumor suppressor *PTEN* (phosphatase and tensin homolog) mRNA, inhibiting its expression and promoting cell proliferation *in vitro* and *in vivo* [99]. METTL3 was found to induce *miRNA221/222* maturation in an m^{6}A-dependent manner, which correlated with cancer progression [99]. The authors used BCa and paired normal tissues from patients who had been diagnosed with BCa and underwent surgery (180 cases), two BCa cell lines, and normal urinary epithelial cell line (control) for *in vitro* experiments; the results if these experiments were validated *in vivo*. The patients were categorized based on age and sex (<65 and ≥65 years old; male and female), however, the relation of the obtained data to those factors was not analyzed. The study was focused exclusively on BCa and did not detail whether the patients had any non-cancerous condition; METTL3 expression and *miR221/222* maturation were studied exclusively in the context of BCa development. Moreover, no multivariate analysis was performed to determine whether the METTL3-miR221/222 axis could be used as an independent prognostic factor when adjusted for confounding variables, such as tumor stage, grade, or molecular subtype. While technically challenging, targeting ncRNAs has become increasingly feasible with the CRISPR RNA-targeting systems like Cas13. However, low delivery efficiency, required specificity, and small size of miRNA targets remain significant obstacles.

BCa-associated lncRNA *BLACAT3* (BLCa-associated transcript 3) is implicated in the promotion of angiogenesis and hematogenous metastasis by activating the downstream NF-κB signaling in an m^{6}A-dependent manner [100]. The authors assessed clinical samples of tumor and adjacent normal tissues from 107 patients with MIBC who underwent radical cystectomy, although no stratification by sex or age was performed. *In vitro* experiments were carried in RT4 cells (NMIBC cell line), MIBC-derived cell lines (UM-UC-3, 5637, T24, J82, SW780), and SV-HUC-1 cells (control). The study provided experimental evidence linking epitranscriptomic modifications (m^{6}A) to cancer progression via lncRNAs (an emerging field in oncology). The authors demonstrated the following mechanistic pathway: m^{6}A-modified *BLACAT3* recruits YBX3 (DNA- and RNA-binding protein involved in the transcription regulation, RNA stabilization, and translation), leading to the upregulation of NCF2 (a subunit of the NADPH oxidase complex), which promotes angiogenesis and metastasis. The study suggested that *BLACAT3* might be specific to MIBC, as no significant differences were observed between the RT4 cells (NMIBC) and normal urothelial cells.

A recent study by Liu et al. [101] unveiled a molecular mechanism of the interaction between *LINC01106* (long intergenic non-protein coding RNA 1106), *miR-3148*, and adaptor protein DAB1 (Disabled-1). It was found that *LINC01106* and *miR-3148* competitively bind to the *DAB1* mRNA. *LINC01106* stabilizes the transcript, leading to a better disease prognosis, while *miR-3148*, on the opposite, inhibits its translation, resulting in a poor overall outcome of BCa. Interestingly, CRISPR-mediated hypermethylation of *LINC01106* facilitated by dCas13b-METTL3-METTL14 increased its affinity to *DAB1*. The authors analyzed four BCa cell lines and SVHUC-1 cells (control), as well as 30 paired BCa tissue samples (malignant and adjacent normal tissues) obtained from BCa patients that underwent surgery.

circRNAs are generally known to play the oncogenic role. Thus, the IGF2BP3 reader protein was found to stabilize m^{6}A-methylated *circPSMA7*, resulting in the enhanced expression of mitogen-activated protein kinase 1 (MAPK1) and BCa progression and metastasis. *circPSMA7* acts a sponge for *miR-128-3p* that exhibits the tumor suppressor activity by targeting the *MAPK1* mRNA [102]. The study analyzed 33 paired samples of fresh BCa and adjacent normal tissues. BCa cell lines UM-UC3 and T24, SV-HUC-1 cells, and 293T human embryonic kidney cells were used in *in vitro* experiments. The authors predicted eight potential m^{6}A modification sites in *circPSMA7* and carried out methylated RNA immunoprecipitation (MeRIP) assay to confirm the presence of m^{6}A modifications in the transcript. However, no mutations were introduced in the identified sites to validate each site separately and to determine which sites contributed to the IGF2BP3-mediated stabilization of *circPSMA7*. It also remained unclear whether individual sites could be used independently as prognostic markers.

METTL14 plays a significant role in the advancement of BCa by promoting expression of *lncDBET* (D4Z4 binding element transcript lncRNA). Five m^{6}A sites in *lncDBET* methylated by METTL14 were predicted in [103]. The elevated levels of *lncDBET* trigger a cascade of events that ultimately contribute to the development of BCa, in particular, activation of the peroxisome proliferator-activated receptor (PPAR) signaling pathway through recruitment of fatty acids, which has a profound impact on the lipid metabolism in cancer cells. The mechanism involves a direct interaction between lncDBET and fatty acid-binding protein 5 (FABP5). The knockdowns of *METTL14*, *lncDBET*, or *FABP5* suppressed tumor growth *in vitro* and *in vivo*. The study was conducted in SVHUC-1 cells and BCa cell lines (UMUC3, 5637, T24, J82,

and EJ-M3), as well as in fresh BCa tumor tissues and adjacent non-tumor specimens collected from patients subjected to radical cystectomy or transurethral resection and were used to evaluate the levels of METTL14, lncDBET, and FABP5. The patients were not stratified based on the cancer stage or subtype. Because of cancer heterogeneity, it is unclear whether all BCa subtypes exhibited similar METTL14-lncDBET-FABP5 expression patterns. The authors proposed the METTL14-lncDBET-FABP5 axis as a potential therapeutic target, but did not address if such approach would be clinically feasible without disruption of essential pathways and unwanted off-target effects. The prognostic value of this axis still has to be elucidated, as m^{6}A patterns and expression profiles of m^{6}A factors may help stratification of patients into subgroups and prediction of disease progression.

Interestingly, an elevated expression of METTL14 was discovered to be characteristic of more 'aggressive' cell lines [104]. The authors analyzed 120 formalin-fixed paraffin-embedded bladder urothelial carcinoma samples (60 NMIBC and 60 MIBC), 40 normal urothelial tract samples from nephrectomy specimens, and 7 BCa cell lines. The knockdown of *METTL14* caused a significant decrease in the m^{6}A modification levels in the cells, leading to a substantial inhibition of cancer cell migration and invasion. Since METTL14 and METTL3 form a heterodimer that acts as a key m^{6}A 'writer' complex, downregulation of METTL14 led to the disruption of its catalytic activity [104]. However, others authors have reported that the METTL14 upregulation in BCa cells suppressed cell proliferation and migration of BCa cell lines [105, 106]. This discrepancy may arise from the tumor heterogeneity, as bladder tumors contain subpopulations of cells with different molecular profiles [107], i.e., different regions of the same tumor may have varying levels of METTL14. In [104], the samples were not stratified into subgroups based on the m^{6}A profiles, which makes it unclear whether METTL14 had a stronger impact in certain groups of patients. The authors did not explore potential downstream targets of METTL14, so it remains obscure how METTL14 contributes to the tumor progression or aggressiveness in BCa.

In summary, m^{6}A plays a multifaceted role in the regulation of both oncogenes and tumor-suppressors in BCa. The levels of m^{6}A methylation influence the transcriptome fate in a diverse and somewhat unpredictable way: either high or low methylation levels may either up- or downregulate transcript expression. m^{6}A is a highly dynamic modification, and methylation patterns may vary at different disease stages, which can be potentially used to precisely differentiate the stage of disease development and predict its progression, outcome, and possibility of recurrence. Figure 1 illustrates m^{6}A-related factors that play oncogenic role in the development of BCa, as well as the mechanisms of their action.

Factors associated with m^{6}A that exert tumor suppressive functions in the BCa development are illustrated in Fig. 2.

This novel approach has been extensively studied in a number of works that have proposed various targets and potential applications in cancer therapy. However, a deeper understanding of m^{6}A modification mechanisms and the role of this modification as a prognostic biomarker is still required, e.g., for the use of m^{6}A biomarkers for correcting existing management of patients. Many ongoing studies have limitations that need to be resolved. For example, no links have been established between the m^{6}A profiles or expression patterns of m^{6}A factors and tumor stage, grade, or other risk factors, even if it could help clinicians to stratify patients into groups. In most studies, no association between the expression of m^{6}A factors and patients' age and sex have been investigated (although many authors state that these parameters do not confound the analysis) [44, 93]. Often, the studies do not specify whether the patients had chronic diseases, comorbidities, or polymorbidities, which raises the possibility that the expression of m^{6}A factors could be associated with such concurrent conditions. Most studies are performed in BCa tissues and do not explore the possibility of m^{6}A factor assessment in the blood, urine, or other biological fluids, which would be more clinically relevant for non-invasive diagnostics.

m^{6}A-mediated drug resistance. Several studies have highlighted the role of m^{6}A modification in drug resistance, including resistance to chemotherapy, immunotherapy, and specific drugs (e.g., cisplatin and anthracyclines) in different cancers, including BCa [108-110].

With the discovery of cancer immune checkpoints and checkpoint inhibitors, such as programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1), the possibilities for using novel treatment strategies have considerably expanded. For example, METTL3 and METTL14 were reported to suppress innate immune response to cancer cells in colorectal carcinoma and melanoma by introducing m^{6}A in the 3′-UTRs of transcripts of key factors involved in the immune response signaling pathways, namely *STAT1* (signal transducer and activator of transcription protein family) and *IRF1* (interferon regulatory factor 1). *METTL3-14* depletion caused a noticeable increase in the production of cytokines and chemokines, as well as activation of IFN-γ signaling, suggesting m^{6}A to be a key factor in the regulation of cell sensitivity to immunotherapy. Moreover, *METTL3-14*-depleted cells also exhibited profound sensitivity to anti-PD-1 treatment [111]. It was suggested that METTL3 expression is regulated by JNK1, a core component

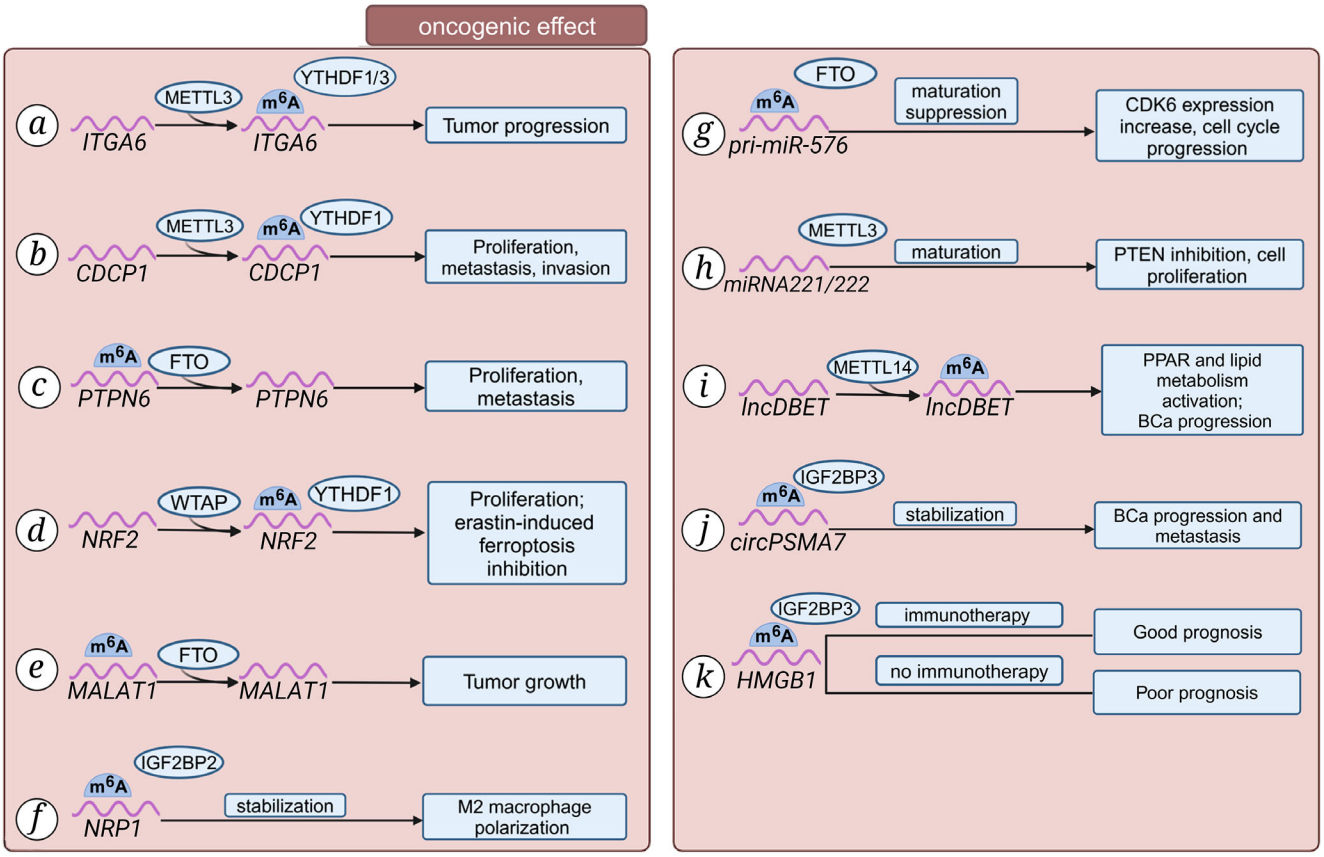

Fig. 1. Oncogenic effect of m^{6}A modification of RNA in bladder cancer biology. a) m^{6}A methylation of ITGA6 transcript mediated by METTL3 and YTHDF1/YTHDF3 reader proteins, promotes its translation and tumor progression [55]. b) METTL3-m^{6}A-CDCP1 axis regulates CDCP1 expression and translation and promotes tumor progression, cell migration, and metastasis [63]. c) m^{6}A-eraser FTO demethylase regulates expression and stability of PTPN6, a key phosphatase involved in cell signaling and cancer progression, suggesting its prognostic potential in BCa [42]. d) NRF2 promotes tumor growth and resistance to ferroptosis through the m^{6}A-mediated stabilization by WTAP and YTHDF1 [92]. e) FTO acts as an oncogene by reducing m^{6}A methylation of the MALAT1 lncRNA, enhancing its stability and expression, which promotes tumor growth, since MALAT1 sponges miR-384 leading to the increased MAL2 levels; higher FTO expression correlates with advanced cancer stages [43]. f) IGF2BP2 stabilizes m^{6}A-modified NRP1 transcript, leading to the M2 macrophage polarization and promotion of BCa progression [46]. g) FTO promotes BCa progression by modifying the maturation of pri-miR-576 through the m^{6}A-dependent mechanisms and regulates the miR-576-CDK6 pathway; higher FTO expression correlates with advanced TNM stages [44]. h) METTL3 promotes miRNA221/222 maturation through m^{6}A modification, facilitating cancer progression via inhibition of the tumor suppressor PTEN [99] i) METTL14 promotes BCa progression by increasing lncDBET expression, which activates the PPAR signaling pathway and alters lipid metabolism through direct interaction with FABP5 [103]. j) m^{6}A-methylated circPSMA7 is stabilized by IGF2BP3, which enhances MAPK1 stability and promotes tumor progression; miR-128-3p can potentially reverse this effect [102]. k) IGF2BP3 interacts with m^{6}A-methylated HMGB1 mRNA, influencing the immune microenvironment of BCa tumor and affecting immunotherapy outcome [47].

of the JNK signaling pathway, which results in the m^{6}A methylation of *PD-L1* mRNA, leading to its increased expression in BCa cells [112].

Casein kinase 2 (CK2) is a serine/threonine protein kinase that regulates glycolysis in cancer cells. Increased lactate production and glucose utilization contribute to cancer progression. Demethylase ALKBH5 decreases the stability of the *CK2* mRNA by removing m^{6}A from the transcript. ALKBH5 is downregulated in BCa cells, resulting in more pronounced cell proliferation and tumor growth. Overexpression of ALKBH5 increases the sensitivity of BCa cells to cisplatin via the CK2a-mediated glycolytic pathway [113, 114].

Some circRNAs play a major role in facilitating chemoresistance in cancers (including BCa) in an m^{6}A-dependent manner. Xu et al. [115] demonstrated that the expression of *circ104797* in BCa tissues was elevated compared to adjacent healthy tissues, and the underlying mechanism of this upregulation is m^{6}A-dependent. The content of m^{6}A-methylated *circ104797* was increased in cisplatin-resistant cell lines, while targeted demethylation using the CRISPR-dCas13b-ALKBH5 system significantly reduced it. circ104797 is known to act as a sponge for *miR-103a* and *miR-660-3p* that modulate cell sensitivity to drugs by controlling the ability to expel chemotherapeutic

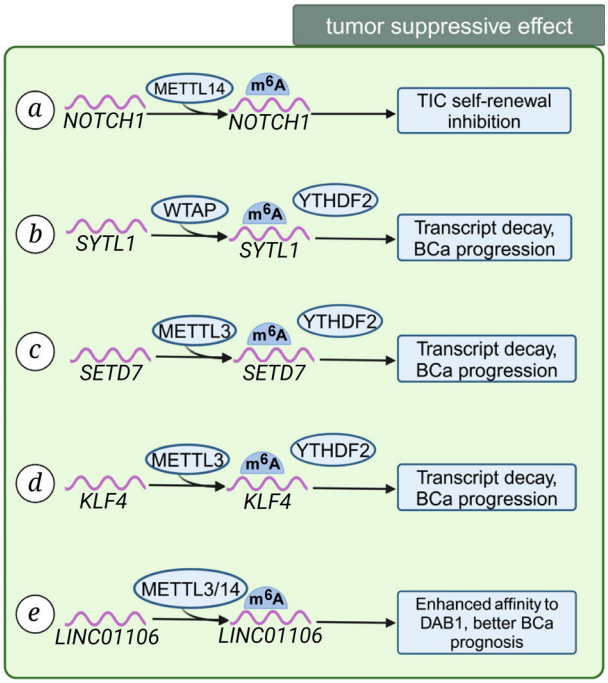

Fig. 2. Tumor suppressive effect of m^{6}A modification of RNA in bladder cancer biology. a) METTL14-m^{6}A-Notch1 axis inhibits self-renewal of tumor-inducing cells (TICs) [71]. b) SYTL1 enhances anti-tumor immune response by activating NK cells; m^{6}A methyltransferase WTAP downregulates SYTL1 expression through m^{6}A modification, leading to the transcript degradation by YTHDF2 [81]. c) METTL3-introduced m^{6}A modification is recognized by YTHDF2 and leads to the decay of mRNA for the tumor-suppressor factor SETD7 [80] d) KLF4 is a tumor suppressor downregulated in BCa cells and linked to poor survival and recurrence risk. KLF4 overexpression inhibits cell proliferation and induces cell cycle arrest in G1 phase. METTL3 m^{6}A-methylates the KLF mRNA, causing it recognition by the YTHDF2 reader protein and further degradation [80]. e) LINC01106 stabilizes DAB1 mRNA, improving BCa prognosis, while miR-3148 inhibits its translation, leading to poorer disease outcome. CRISPR-mediated hypermethylation of LINC01106 enhances its affinity to DAB1 [101].

agents and regulating genes involved in the DNA damage response and DNA repair, respectively. The authors suggested that *circ104797* can be used as a biomarker for predicting cell response to the anti-cancer treatment by measuring its expression levels or levels of its m^{6}A-methylation, or as a target of treatment aimed to disrupt *circ104797* interaction with miRNAs to improve the treatment outcome [115].

Another circRNA upregulated in BCa cells is *circ0008399*. It binds WTAP to promote formation of the WTAP-METTL3-METTL14 m^{6}A methyltransferase complex that induces m^{6}A modification in tumor cells. Moreover, it facilitates the posttranscriptional regulation of TNFAIP3 (tumor necrosis factor, alpha-induced protein 3), an anti-apoptotic protein that inhibits TNF-induced apoptosis [116], thus promoting cell resistance to chemotherapy [117].

Interestingly, key m^{6}A regulators may also act as tumor suppressors. Thus, lower levels of METTL16 were found to correlate with a poor outcome in BCa patients [118]. Furthermore, METTL16 exerted a potent anti-cancer effect in BCa cells both in *in vitro* and *in vivo* by reducing cell proliferation and increasing the sensitivity to drugs (cisplatin). These effects were achieved through a complex molecular pathway involving HIF-2α (hypoxia-inducible factor 2α), PMEPA1 (prostate transmembrane protein, androgen induced 1), and autophagy regulated by the m^{6}A methylation of the respective mRNAs. These findings open new prospects for developing targeted therapies against BCa and selecting prognostic biomarkers for prediction of patients' response to chemotherapeutics.

There has long been a demand for more efficient methods for predicting potential therapeutic resistance and evaluating the treatment outcomes. Abnormal expression of m^{6}A regulators is typically associated with various forms of resistance, offering a basis for predicting cancer response to treatment. First, there is a number of effective small-molecule inhibitors for almost all major groups of m^{6}A pathway factors [109], which can be used to overcome the drug resistance caused by the expression of these factors. Second, expression levels of the m^{6}A pathway factors and the levels of m^{6}A methylation of certain transcripts associated with the drug resistance can be used as diagnostic markers for predicting resistance to therapy.

Mutations in m^{6}A motifs and m^{6}A factors. m^{6}A modifications are prevalent in certain mRNA regions, such as stop codons, 3′-UTRs, and long inner

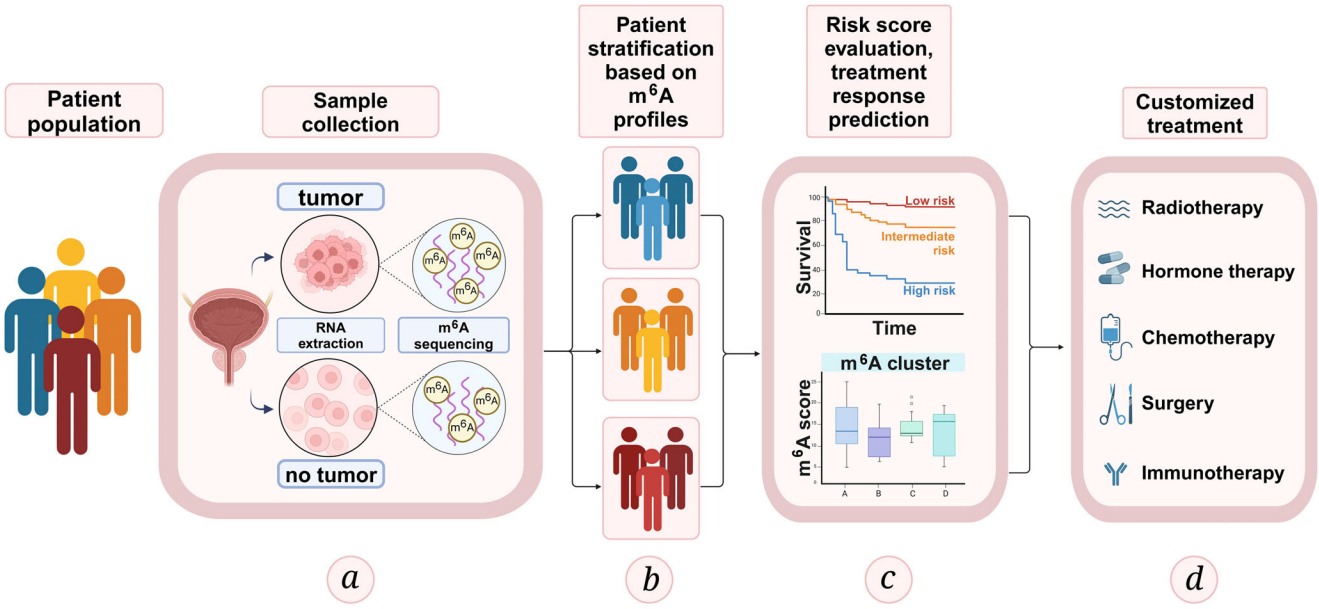

Fig. 3. The workflow for developing personalized treatment strategies based on the m^{6}A RNA modification profiling in a patient population. a) Tissue samples are collected from the patients, including those with different BCa stages. m^{6}A sequencing is performed to assess m^{6}A modification patterns in RNA, which may vary depending on the disease severity. b) Based on m^{6}A patterns, patients are classified into distinct subgroups. c) Patients' survival probability is assessed based on the m^{6}A profiles to provide insights into the correlation between the m^{6}A modification of RNA and clinical outcomes and to help predict patients' response to specific treatments. d) Personalized treatment strategy is designed based on the assessed risks.

exon regions. They are typically located in the consensus motifs including UGACA, RRACH (R = G or A; H = A, C, or U), and DRACH (where D = A, G or U; R = A or G; H = A, C or U), as has been verified by high-throughput sequencing. m^{6}A modification is a major molecular mechanism regulating the fate of RNAs in response to various stimuli [119-121]. Functional m^{6}A sites are highly evolutionary conserved, whereas "accidental" nonfunctional m^{6}A modifications occurring as a result of methyltransferase off-site targeting, are typically purged by natural selection [122]. Mutations within the consensus motifs can be detrimental, as they could potentially prevent methylation of adenosines and attenuate or even abolish the enhanced translation of the corresponding transcripts. Cancer cells seem to be generally resistant to mutations within m^{6}A consensus motifs [123].

SNPs (single nucleotide polymorphisms) affecting m^{6}A-methylation can occur in two primary locations – near and directly at m^{6}A-modification sites – and significantly impact various biological processes and pathways. Some SNPs cause mutations, potentially interfering with the transcription and translation; others are located in the UTRs or near stop codons, affecting mRNA interaction with transcriptional regulators and RNA-binding proteins. These alterations can influence mRNA stability and efficiency of its nuclear transport [124]. As a result, gene expression and overall cellular functions can be affected. The effects of these m^{6}A-SNPs can vary depending on their exact location and genes involved [125]. Further research is needed to fully understand the implications of these genetic variations in cellular processes and disease mechanisms.

Identification of m^{6}A-related SNPs can be particularly important in molecular diagnostics. One of the examples of such m^{6}A-SNPs is rs3088107 in the *RNFT2* (ring finger protein, transmembrane 2) oncogene. Normally, expression of RNFT2 is elevated in BCa cells, which promotes proliferation and metastasis of cancer cells. rs3088107 SNP abolishes RNFT2 expression in an m^{6}A-mediated manner [125].

Mutations in the genes coding for m^{6}A-related factors may not only affect m^{6}A methylation and the fate of respective transcripts, but also result in the production of proteins with an altered substrate specificity. Indeed, the gain-of-function missense mutation R298P in METTL14, which is responsible for the recognition of the non-canonical sequence of GGAU instead of canonical GGAC, is frequently found in cancer patients. The effects of the R298P mutation are similar to those of the METTL3-METTL14 complex overexpression linked to cancer cell growth and proliferation [126].

In conclusion, since the m^{6}A methylation pathway plays a role in both tumorigenesis and tumor suppression at the levels of mRNA expression and ncRNAs, and because mutations in m^{6}A domains lead to changes in the expression of m^{6}A factors, m^{6}A methylation can serve as a diagnostic marker of disease

progression and development rate. Figure 3 presents a potential workflow for m^{6}A profiling as a novel prognostic biomarker for the stratification of BCa patients. RNAs or m^{6}A-methylated RNAs can be extracted from the patients' tissues or blood using specific m^{6}A-targeting antibodies, sequenced, and subjected to bioinformatic analysis, that would allow for the assessment of expression levels of mRNAs and ncRNAs, as well as the levels of m^{6}A methylation of target RNAs. Based on the results of analysis, predictions can be made regarding the rate of disease progression, likelihood of tumor recurrence, and therapy efficacy to help with the development of disease management strategy for individual patients.

CONCLUSION

BCa presents a significant challenge to healthcare because one of the highest prevalence among cancers, as well as high recurrence and mortality rates. Timely and accurate diagnostics and selection of appropriate treatment significantly affect the BCa prognosis in patients. While the m^{6}A-based molecular diagnostics is still at its inception, the usefulness of m^{6}A biomarkers have been demonstrated for many cancers. Numerous prospective m^{6}A biomarkers of BCa have been described, although in a limited number of patients. There is a current need for identification of new BCa biomarkers that could be conveniently analyzed, and not only in tumor cells, but also in patients' urine, blood cells, or circulation (e.g., exosomal biomarkers or free nucleic acids). The lack of valid and reliable molecular biomarkers for predicting BCa development, NMIBC-to-MIBC progression, and response to cisplatin and other drugs (including PD-1/PD-1L) negatively affects the prognostics in BCa patients.

The levels of m^{6}A methylation and SNPs in mRNAs and ncRNAs, as well as the expression levels of m^{6}A factors, may serve as valuable biomarkers to shed light on tumor behavior and patient prognosis and to define the optimal treatment options. Distinct m^{6}A patterns may act as early biomarkers for cancer detection and risk assessment. Identification of new m^{6}A-related genes and evaluation of their significance through the assessment of m^{6}A scores, clustering, enrichment analysis, and disease outcome prediction could help to pinpoint potential targets for precision therapy, evaluate the risk of cancer relapse, and diagnose BCa at the earliest possible stage to ensure high survival rates. m^{6}A profiling could be used for stratifying the patients according to cancer subtypes and clinicopathological characteristics, predicting their response to treatment, and evaluating the outcomes. The use of m^{6}A machinery for therapeutic and prognostic purposes is currently at its infancy. However, this topic has been studied extensively, and several prognostic models based on m^{6}A profiles have been already developed. A proposed prognostic m^{6}A-driven marker panel for renal cell carcinoma (RCC) based on the transcriptome-wide m^{6}A-seq data can identify dysregulated m^{6}A-modified target genes, such as *NDUFA4L2* (NDUFA4 mitochondrial complex associated like 2, 4-like), *NXPH4* (neurexophilin 4), and *UMOD* (uromodulin), associated with significantly poorer overall survival in RCC patients [127]. A novel m^{6}A classifier has recently been designed to predict the treatment efficacy and response to treatment in BCa patients [128]. All things considered, m^{6}A methylation may serve as a prospective non-invasive diagnostic biomarker in the diagnostics and prognostics of BCa.

Abbreviations. BCa, bladder cancer; METTL, methyltransferase like protein; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle-invasive bladder cancer.

Acknowledgments. The authors thank Priority 2030 Research Program (Sechenov University).

Contributions. T.S., D.K., and A.K. developed the study concept; T.S., Y.L., N.V-K., A.A., N.P., S.B., and I.G. wrote the original draft; T.S., A.D., A.V., D.K., and A.K. edited the manuscript; T.S. prepared the figures; V.C., A.D., A.V., and D.K. supervised the project; D.K. and A.K. provided project administration; A.K. acquired the funding. All authors have read and agreed to the published version of the manuscript.

Funding. The study was supported by the Russian Science Foundation (project no. 22-75-10032).

Ethics approval and consent to participate. This work does not contain any studies involving human and animal subjects.

Conflict of interest. The authors of this work declare that they have no conflicts of interest.

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