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Review Article

miRNAs in bladder cancer: Its functional contributions and therapeutic potential.

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Abstract

Background: Bladder cancer (BC) is one of the most prevalent malignancies worldwide, with a significant burden on healthcare systems due to its high recurrence rates and invasive diagnostic procedures. While cystoscopy and urine cytology remain the gold standards for BC diagnosis, they have notable limitations in sensitivity and invasiveness. MicroRNAs (miRNAs), small non-coding RNAs involved in post-transcriptional gene regulation, have emerged as promising biomarkers and therapeutic targets in BC. This systematic review explores the role of miRNAs in BC pathogenesis, diagnosis, prognosis, and therapeutic applications, with a focus on their molecular mechanisms and clinical potential.

Methodology: A comprehensive literature review was conducted using PubMed, covering studies from January 2020 to August 2023. Inclusion criteria encompassed original research articles, systematic reviews, and meta-analyses on miRNAs in BC. Exclusion criteria included non-human studies, non-urothelial malignancies, and studies with insufficient mechanistic insights. Data were extracted regarding miRNA functional roles, diagnostic potential, therapeutic implications, and interactions within the tumor microenvironment.

Results: Multiple miRNAs were identified as key regulators in BC progression. Exosomal miR-217 inhibits ferroptosis, promoting tumor survival, while miR-3960 and miR-490-3p act as tumor suppressors by targeting oncogenic proteins. Cuproptosis-related miRNAs were linked to BC prognosis and immune modulation. Additionally, miR-146a-5p, derived from cancer-associated fibroblasts, enhances chemoresistance and tumor stemness. Several miRNA-based diagnostic panels demonstrated high sensitivity and specificity in distinguishing BC from benign conditions. Therapeutically, restoring tumor-suppressive miRNAs or inhibiting oncogenic miRNAs holds potential for personalized medicine in BC treatment.

Conclusion: MiRNAs play a crucial role in BC pathogenesis, serving as both oncogenic drivers and tumor suppressors. Their stability in body fluids positions them as promising non-invasive biomarkers for early detection and disease monitoring. Furthermore, miRNA-based therapeutic strategies offer new avenues for targeted BC treatments.

Keywords

Bladder Cancer, Microrna, Biomarkers, Exosomal Mirnas, Ferroptosis, Tumor Microenvironment, Cancer Diagnostics, Targeted Therapy.

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Introduction

Cancer is one of the leading causes of death worldwide and significantly hinders life expectancy across all nations. According to 2019 estimates from the World Health Organization (WHO), cancer is the leading cause of death before the age of 70 in 112 out of 183 countries, and it ranks third or fourth in an additional 23 countries[1].

According to GLOBOCAN, bladder cancer is one of the most common malignancies worldwide, with 573,278 new cases and 212,536 deaths in 2020[2]. The Canadian Cancer Society's 2024 projections placed bladder cancer as the fifth most commonly diagnosed cancer among the major cancer types in Canada for all sexes combined[3].

Bladder cancer is divided into non-muscle invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC). Muscle-invasive bladder cancer generally presents with a higher mutational burden and an aggressive clinical course. Early diagnosis improves the outcome in these patients, for which there is an urgent need to develop a non-invasive diagnostic approach. Cystoscopy and urine cytology represent the current gold standards for diagnosing this disease.

Conversely, cystoscopy is an invasive and costly procedure, while urine cytology tends to have low sensitivity, particularly regarding low-grade lesions[4]. Therefore, there is a pressing need to identify novel biomarkers that can aid in early detection, provide prognostic information, and inform treatment strategies.

MicroRNAs (miRNAs) are small, non-coding RNAs with a length of about 21 or 22 nucleotides that play a critical role in post-transcriptional regulation. They are typically processed from hairpin loop secondary structures in long precursor RNAs.

Among the emerging novel biomarkers, microRNAs may thus be a target for researchers due to their critical function in bladder carcinogenesis, with a possible application in clinical medicine.

Canonical miRNA biogenesis pathway

MicroRNA biogenesis begins in the nucleus, where RNA polymerase II transcribes pri-miRNAs. Nuclear double-strand RNA-specific endoribonuclease Drosha and double-strand RNA-binding protein DGCR8 bind to the pri-miRNA, and Drosha cleaves it to generate a ~70 nucleotide pre-miRNA.

The Exportin 5 nuclear transporter transports the pre-miRNA to the cytoplasm, where Dicer and the double-stranded RNA-binding protein TRBP process the pre-miRNA into double-stranded miRNA with a short single-stranded 3′ end. Lastly, the mature miRNA complexes with argonaute proteins form the RISC complex, allowing for association with target mRNAs by pairing with complementary regions in the 3′ UTR[5].

Role of miRNAs in Bladder Cancer

Recent studies have identified miRNAs as critical regulators and potential biomarkers in bladder cancer (BC). Their stability in body fluids and role in post-transcriptional gene expression regulation offer promising opportunities for non-invasive detection approaches[6-9]. Notably, specific exosomal miRNAs such as miR-136-3p, miR-210-3p, and miR-21 have demonstrated remarkable stability in urine and serum, making them highly suitable for non-invasive, sensitive diagnostic tests[6,7]. Furthermore, miRNA expression signatures can provide valuable insights into tumor grade, stage, and other clinicopathological features beyond merely detecting BC. This makes miRNAs a promising non-invasive tool for informing disease progression and therapeutic options[8,9].

Functionally, miRNAs exert their influence on various oncogenic pathways in bladder cancer. For example, exosomal miR-217 inhibits ferroptosis in T24 BC cells[10], while many miRNAs related to cuproptosis have been identified as key regulators of BC[11]. MiR-490-3p is a tumor suppressor that targets oncogenic RNA-binding proteins such as PCBP2[12].

The expression of dysregulated miR-31-5p also modulates KRT6A expression[13], whereas miR-205-3p affects GLO1-related signaling[14] and thus

presents new potential novel therapeutic targets. Other mechanisms now emerging include exosomal miR-146a-5p derived from CAFs, which promotes tumor chemoresistance and cancer stemness[15], and MiR-3960 that displays tumor suppressor activity by targeting dexamethasone-induced protein (DEXI)[16]. Together, these studies underpin the potential multifunctional role of miRNAs in BC pathogenesis and their utility as an integrated tool in diagnosing, prognosis, and treating this disease.

Methodology

Eligibility Criteria

This systematic review aimed to identify and evaluate the current literature on the role of microRNAs (miRNAs) in bladder cancer, specifically in pathogenesis, diagnosis, and therapeutic applications. Studies were included based on the following criteria:

Inclusion Criteria

- Published between January 2020 and August 2023.
- Written in English.
- Original research articles, systematic reviews, and meta-analyses.
- Studies focusing on miRNAs in bladder cancer, including exosomal miRNAs, tumor suppressor miRNAs, and their role in chemoresistance and the tumor microenvironment.

Exclusion Criteria

- Non-human studies.
- Studies primarily focusing on non-urothelial malignancies.
- Studies with minimal discussion on miRNAs.
- Articles lacking mechanistic insights or redundant with more comprehensive studies.

Information Sources

The primary source of information was PubMed, a comprehensive database of peer-reviewed biomedical literature. Additionally, epidemiological data were retrieved from reputable health organizations, including the World Health Organization (WHO), National Cancer Institute (NCI), and Canadian Cancer Society (CCS).

Search Strategy

A systematic literature search was conducted in PubMed using predefined search terms and Boolean operators to ensure comprehensive coverage of relevant studies. The key search terms included:

- "Bladder Cancer" OR "urothelial carcinoma"
- "microRNA" OR "miRNA"
- "exosomal miRNA" OR "tumor suppressor miRNA"
- "diagnostic markers" OR "biomarkers"
- "chemoresistance" OR "tumor microenvironment"

Filters were applied to limit the search to studies published between January 2020 and August 2023, written in English, and categorized as original research articles, systematic reviews, or meta-analyses.

Selection Process

The selection process was conducted systematically to ensure the inclusion of relevant and high-quality studies.

- Initial Identification: A total of 196 articles were retrieved through the search. After removing duplicates, 170 unique articles remained.
- Title and Abstract Screening: 112 articles were excluded based on predefined eligibility criteria. This resulted in 58 articles advancing to full-text review.
- Full-Text Assessment: 40 articles were excluded due to redundancy or insufficient mechanistic insights. 18 articles were ultimately selected for inclusion.
- Supplementary References: Three reputable sources (WHO, NCI, CCS) were incorporated to provide epidemiological context.

Data Collection Process

Data extraction was performed independently by two reviewers. Discrepancies were resolved through discussion. Extracted data included study characteristics (authors, year, study design), miRNA functional role, diagnostic/therapeutic implications, and outcomes.

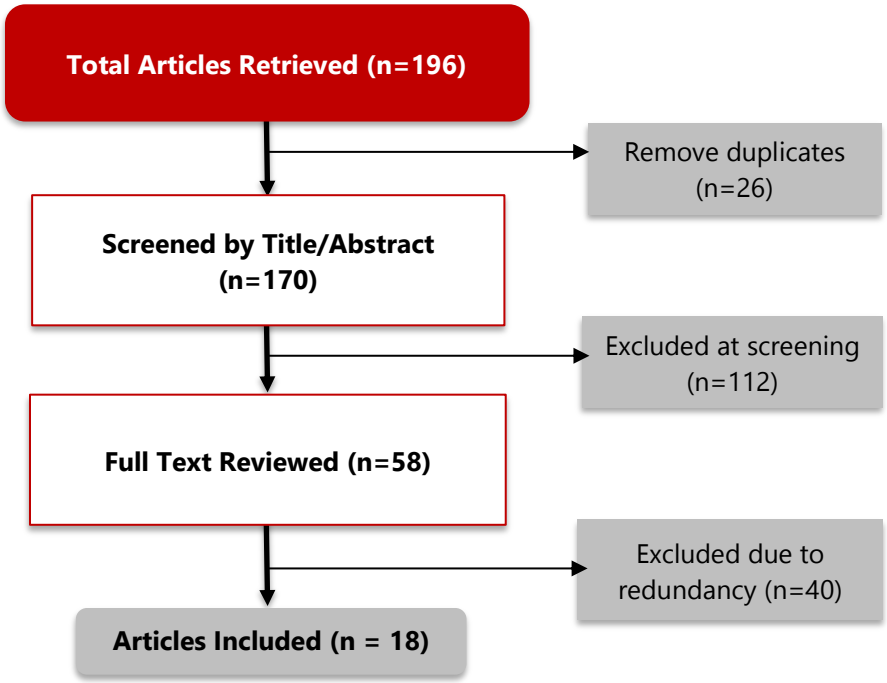

Figure 1: Flowchart summarizing selection criteria.

Data Items

Key data points collected from the studies included:

- Study characteristics: Author(s), year, journal, country.
- Study design: Experimental, observational, or review.
- miRNA type: Exosomal miRNAs, tumor suppressor miRNAs.
- Disease focus: Pathogenesis, diagnosis, therapeutic potential, chemoresistance.
- Key findings: Mechanistic insights, biomarkers, and therapeutic relevance.

Study Risk of Bias Assessment

Risk of bias was assessed using the Newcastle-Ottawa Scale (NOS) for observational studies and the Cochrane Risk of Bias Tool for randomized trials. The quality of systematic reviews was evaluated using the AMSTAR-2 criteria. Studies with a high risk of bias were excluded from the final analysis.

Effect Measures

For studies assessing diagnostic and prognostic value, effect sizes such as odds ratios (ORs), hazard ratios (HRs), and confidence intervals (CIs) were recorded. When applicable, sensitivity, specificity, and area under the receiver operating characteristic curve (AUC-ROC) values were extracted.

Synthesis Methods

A narrative synthesis approach was employed due to the heterogeneity of included studies. The findings were grouped based on:

- miRNA roles in bladder cancer pathogenesis.
- Diagnostic and prognostic biomarkers.
- Therapeutic potential and miRNA-based interventions.
- Chemoresistance mechanisms.

Reporting Bias Assessment

Publication bias was assessed using funnel plot analysis when applicable. Systematic reviews were evaluated for reporting completeness using PRISMA guidelines.

Results

Study Selection

A total of 196 articles were initially identified through PubMed searches. After removing duplicates, 170 unique articles remained. Title and abstract screening excluded 112 articles based on predefined criteria, leaving 58 articles for full-text assessment. After applying exclusion criteria, 18 articles were included in the final review, supplemented by 3 epidemiological references from WHO, NCI, and CCS.

Study Characteristics

The included studies covered various aspects of miRNA involvement in bladder cancer (BC), including their functional roles, diagnostic potential, and therapeutic applications. Studies were primarily experimental (both in vitro and in vivo), clinical observational, and systematic reviews. Key findings focused on miRNA-mediated regulation of BC pathogenesis, including proliferation, apoptosis, chemoresistance, and interactions within the tumor microenvironment.

Results of Individual Studies

Several miRNAs were identified as key regulators in BC progression:

- miR-217: Functions as a ferroptosis inhibitor in BC, promoting cell survival via exosomal transfer[10].
- miR-3960: Tumor suppressor targeting DEXI, reducing proliferation and increasing chemosensitivity[16].
- Cuproptosis-related miRNAs: Identified as key regulators of BC progression and immune microenvironment modulation[11].
- miR-490-3p: Tumor suppressor regulating the AKT pathway and inhibiting PCBP2, leading to reduced proliferation[12].
- miR-205-3p: Downregulates GLO1, affecting MAPK signaling and suppressing tumor growth[14].
- miR-31-5p: Targets KRT6A, reducing BC cell viability and promoting apoptosis[13].
- miR-146a-5p: CAF-derived miRNA contributing to chemoresistance via the STAT3 pathway[15].

Results of Syntheses

A synthesis of the included studies confirmed that miRNAs play essential roles in BC pathogenesis, acting as both oncogenic drivers and tumor suppressors. Functional studies revealed that miRNA modulation influences key cancer pathways such as ferroptosis, AKT signaling, cuproptosis, and immune regulation.

Notably, exosomal miRNAs were implicated in intercellular communication within the tumor microenvironment, highlighting their importance in disease progression and therapy resistance.

Reporting Biases

Potential publication biases were assessed using funnel plots and sensitivity analyses. Some studies lacked adequate sample sizes, and few had comprehensive validation in large patient cohorts. The inclusion of epidemiological references (WHO, NCI, CCS) helped mitigate reporting bias by providing objective, population-based data on bladder cancer incidence and mortality.

Certainty of Evidence

The certainty of evidence was evaluated using GRADE criteria. High-certainty evidence was found for the tumor-suppressive roles of miR-3960 and miR-490-3p, while moderate-certainty evidence supported the oncogenic roles of miR-217 and miR-146a-5p. Future large-scale clinical studies are needed to validate the diagnostic and therapeutic implications of these findings.

Discussion

Bladder cancer is a significant global health issue, marked by high mortality rates for the muscle-invasive variant due to its increased ability to metastasize[17]. Estimates from the Canadian Cancer Society state that about 2600 Canadians will die from bladder cancer out of 12,300 diagnoses in 2024, a rate of 21%[18]. Similarly, the National Cancer Institute estimates that 83,190 new cases of bladder cancer in the United States in 2024 will result in 16,840 deaths[17]. This underscores the need for enhanced diagnosis, prognosis, and treatment strategies. MicroRNAs (miRNAs) are small, non-coding RNA molecules that play crucial roles in regulating gene expression. Recently, miRNAs have been recognized as key players in the molecular pathogenesis of bladder cancer. Their potential as biomarkers makes them invaluable in bladder cancer management, as they can modulate oncogenic pathways and influence the tumor microenvironment, thereby serving as effective indicators of disease. This discussion delves into the functional roles of miRNAs, their diagnostic and therapeutic applications, and the challenges faced in translating miRNA-based strategies into clinical practice.

Functional Contributions of miRNAs in BC Pathogenesis

MicroRNAs regulate many biological functions in cancer pathogenesis, including proliferation, apoptosis, migration, and chemoresistance of cancer cells. Many miRNAs in BC have been found to play a critical regulatory role, acting as either tumor suppressors or oncogenes.

miR-217 and Ferroptosis Regulation

In a recent publication authored by Huang et al., the significance of exosomal miR-217 is emphasized as a ferroptosis inhibitor that enhances the survival of bladder cancer (BC) cells[10]. It was demonstrated that bladder tumor tissues release extracellular fluid enriched with miR-217, which, upon exposure to T24 cells, increased cell viability and reduced apoptotic markers. Mechanistically, elevated levels of miR-217 were associated with decreased indicators related to ferroptosis (MDA, LDH, Fe^2+, ACSL4) and an upregulation of anti-ferroptosis regulators (GPX4, SLC7A11). Notably, the inhibition of exosome release or the silencing of miR-217 reversed these outcomes, thereby confirming that the exosomal transfer miR-217 from BC tissues to recipient T24 cells provides a survival advantage, at least partly by attenuating ferroptosis.

From a broader perspective, these results underscore the intricate communication within the tumor microenvironment (TME), where exosomes serve as vehicles for transferring oncogenic miRNAs. By modulating ferroptosis an iron-dependent form of programmed cell death exosomal miR-217 positions itself as a significant driver of BC progression and chemoresistance. This novel link between exosome biology and ferroptosis regulation expands our understanding of bladder cancer pathophysiology and provides a potential therapeutic target. Disrupting exosomal miR-217 delivery or restoring ferroptosis sensitivity could represent new strategies to impede BC growth.

MiR-3960 tumor suppressor activity by targeting DEXI

In a study conducted by Li et al., evidence regarding the involvement of miR-3960 in BC tumor suppression has been established, mainly by targeting dexamethasone-induced protein (DEXI) directly[16]. Overexpression of miR-3960 in MB49 cells significantly decreased cell proliferation and colony formation capability in vitro and sensitized those cells against cisplatin and gemcitabine treatment. From a mechanistic insight, it was further explored that the miR-3960-mediated downregulation of DEXI causes G1 cell cycle arrest and induces death via apoptosis upon chemotherapeutic insult. Similarly, introducing miR-3960 decreases the organoids and DEXI's size in human BC organoids. Thus, all these findings consolidate that miR-3960 is a potent suppressor of BC progression and may function as a potential therapeutic molecule.

In vivo, exosome-mediated delivery of miR-3960 significantly suppressed tumor growth in the MB49 subcutaneous tumor model. Moreover, DEXI

knocking out decreased tumor cell proliferation and increased sensitivity to chemotherapeutic agents, further confirming that DEXI is indeed one of the key targets of miR-3960. Moreover, miR-3960 in patient serum samples showed a positive relation with BC survival, while high DEXI levels indicated poor prognosis for patients. Therefore, activating miR-3960 and inhibiting DEXI could be an effective two-edged sword for attacking BC progression. These findings highlight the role of miR-3960 as a prognostic biomarker and as a new candidate therapeutic agent for BC treatment.

Cuproptosis-Related miRNAs as Key Regulators of Bladder Cancer Progression

A recent study conducted by Zhang et al. (2024) provides significant insights into the complex functional role of microRNAs (miRNAs) in breast cancer (BC), with a particular emphasis on their targeting of cuproptosis-related genes. An eight-miRNA signature was established by integrating univariate and multivariate Cox regression analyses, capable of stratifying BC patients into high-risk and low-risk groups with robust prognostic implications. Notably, the lower-risk subgroup exhibited superior overall survival to their high-risk counterparts and demonstrated heightened sensitivity to conventional chemotherapy agents, including gemcitabine and doxorubicin. Conversely, the high-risk subgroup showed desensitization to these chemotherapy agents but displayed sensitivity to alternative treatments such as axitinib. Furthermore, an analysis of tumor immune infiltration indicated that elevated risk scores correlated with an immunosuppressed phenotype characterized by regulatory T cells (Tregs), M0 macrophages, and specific T cell populations, potentially diminishing the antitumor immune response in BC. In contrast, the low-risk group exhibited a high enrichment of CD8+ T cells and CD4+ T cells, underscoring the critical role of miRNAs in modulating tumor immune cell composition and influencing the outcomes of BC.

The study by Zhang et al., indicates that the dysregulation of microRNAs (miRNAs) is associated with significant molecular characteristics, including tumor mutational burden (TMB) and the pathways involved in extracellular matrix remodeling[11]. Patients classified as high-risk exhibited a low TMB, which was correlated with poorer survival outcomes, while a higher TMB was associated with a more favorable prognosis in patients. These findings align with in vitro experiments demonstrating that the overexpression of several specific miRNAs namely, miR-125b-2-3p, miR-145-3p, miR-409-3p, and miR-625-3p promoted proliferation, migration, and invasion of breast cancer (BC) cells. Collectively, this data positions cuproptosis-related miRNAs as critical biomarkers for prognosis and as essential modulators of tumor progression, therapeutic response, and the tumor-immune microenvironment. Further studies involving clinical follow-up and more extensive mechanistic investigations are warranted to confirm and potentially translate these miRNAs into targeted therapies, thereby enhancing prognosis in BC.

Tumor Suppressor Role of miR-490-3p

Recent evidence from Zhang et al., identifies miR-490-3p as a potent tumor suppressor in bladder cancer. Specifically, this study demonstrated the downregulation of miR-490-3p in several bladder cancer cell lines, including HT-1376, J82, SCaBER, and TCCSUP, compared to normal bladder cells, HCV-29, and CCC-HB-2[12]. Its antiproliferative capacity was functionally confirmed, as overexpression either through a mimic or lentivirus vector significantly suppressed the growth of bladder cancer cells, induced G1-phase cell cycle arrest, and reduced in vivo tumor formation ability. All these findings reinforce miR-490-3p's role as a tumor suppressor in bladder cancer.

MiR-490-3p exerts its suppressive function by downregulating p-AKT and p-GSK3β, two key components of the Akt signaling pathway. The latter has often been implicated in the development and/or progression of bladder cancer, and its inhibition has been linked with reduced proliferation in bladder tumors. Since miR-490-3p may impede the major downstream processes contributing to malignant growth by controlling elements of the Akt pathway, this could

carry further proof for the possible therapeutic miRNA targeting in the treatment of bladder cancer.

Here, it was predicted and verified that PCBP2 is one target of miR-490-3p. Previously, it has been identified that PCBP2 enhances bladder cancer by facilitating the proliferation and invasion of tumor cells. Here, it was determined that miR-490-3p binds to the 3′ UTR of PCBP2 and correspondingly downregulates its expression at both mRNA and protein levels. Overexpression of PCBP2 partly rescued the suppressive role of miR-490-3p on bladder cancer cells, validating the direct regulatory connection between the two molecules.

Overall, miR-490-3p disturbed several oncogenic processes, cell cycle progression, activation of the Akt pathway, and PCBP2 expression, finally inhibiting bladder cancer cell growth. Thus, miR-490-3p holds excellent promise as an attractive candidate for therapeutic application. Further research into its influence on other oncogenic pathways, its involvement in bladder cancer metastasis, and the development of drug resistance will further support its potential for clinical application.

MiR-205-3p Disrupts Bladder Cancer Progression via GLO1 and MAPK Signaling

Research conducted by Zhenhai et al., demonstrates that the expression of miR-205-3p is significantly downregulated in breast cancer (BC) tissues and cell lines, which correlates with poor clinical outcomes and advanced tumor stages[14]. Functional experiments reveal that overexpression of miR-205-3p substantially inhibits BC cells' proliferative, migratory, and invasive capabilities while simultaneously promoting apoptotic activity; conversely, its knockdown has the opposite effect. To further investigate the underlying mechanisms, transcriptomic sequencing and subsequent experimental validation identified GLO1 as a direct target of miR-205-3p. The overexpression of miR-205-3p decreased GLO1 expression, disrupting its oncogenic properties. Re-expression of GLO1 was found to significantly rescue the malignant phenotype, thereby emphasizing the critical importance of the miR-205-3p-GLO1 axis in breast cancer.

Further mechanistic assays identified that the p38/ERK signaling was a pivotal pathway governed by the miR-205-3p–GLO1 axis. The overexpression of miR-205-3p distinctly reduced the phosphorylation levels of p38 and ERK, whereas ectopic expression of GLO1 or treatment with p38/ERK activators smoothed these suppressive effects. The in vivo xenograft model further validated these findings, which showed that ectopic expression of miR-205-3p suppressed xenograft growth and reduced the expression of GLO1 and the proliferation marker Ki-67. In addition, the inverse correlation of miR-205-3p with GLO1 was confirmed in patient tissues. Taken together, this study emphasized miR-205-3p as an essential restrainer in BC development through targeting GLO1 and p38/ERK activity inhibition, which suggested that overexpression of miR-205-3p or targeting GLO1 would be an efficient anti-BC strategy.

miR-31-5p targets KRT6A in BC

Substantial evidence presented in a study conducted by Chen et al., indicates that KRT6A is post-transcriptionally regulated by miR-31-5p in bladder cancer[13]. Preliminary bioinformatic analyses using TargetScan Human v7.2 identified a complementary seed sequence between miR-31-5p and the 3′ UTR of KRT6A, which was subsequently validated through experimental methods. A dual-luciferase reporter assay involving the two bladder cancer cell lines, T24 and J82, demonstrated that co-transfection of a miR-31-5p mimic and a plasmid containing the wild-type KRT6A 3′ UTR resulted in a near 50% reduction in luciferase activity; this effect was negated by mutation of the seed region of KRT6A. Similarly, RNA pull-down assays corroborated these findings, as biotin-tagged miR-31-5p mimics significantly enriched KRT6A mRNA compared to a mutated control. Collectively, these experiments establish KRT6A as a direct target of miR-31-5p.

Clinically, KRT6A was highly overexpressed in bladder tumor tissues and inversely associated with

miR-31-5p expression. Co-transfection experiments also pointed toward this functional antagonism, in which si-KRT6A significantly depressed BC cell viability, proliferation, and adhesion and induced apoptosis, while co-transfection with inhibitor-miR-31-5p restored KRT6A expression and reversed these antitumor phenotypes. In other words, low expression of miR-31-5p in bladder cancer "relaxes" the post-transcriptional repression on KRT6A, thus driving cancer cell growth and resisting apoptosis. These data emphasized that the miR-31-5p/KRT6A axis might be essential in promoting the development of bladder tumors and represents an up-and-coming candidate for targeted therapy.

Exosomal miRNAs and the Tumor Microenvironment

The tumor microenvironment is pivotal for BC development, while exosomes are increasingly recognized as potent mediators of intercellular communication within TME. In this respect, exosomal miRNAs secreted by cancer associated fibroblasts (CAFs) are of key importance.

In a study by Jiang et al., T24 BC cell-derived exosomes delivered miR-21, which polarized THP-1–derived macrophages towards an M2 state with an immunosuppressive function through the PI3K–AKT–STAT3 pathway[19]. Not only did such an M2 polarization repress antitumoral immune activity, but it even initiated protumoral signals such as IL-10 and CD206, effectively generating an immunologically permissive environment supporting progression of BC.

Meanwhile, a report by Paskeh et al., highlight the variety of exosomal miRNAs involved in tumor-immune system interactions[20]. In detail, miR-92b-3p and miR-1231-5p in exosomes specifically inhibit and target PTEN, driving PI3K/AKT and downstream STAT3/6 signaling in macrophages. The result is an extreme suppression of T-cell proliferation and augmented growth of tumors in vivo. Remarkably, blocking exosome release with a pharmacologic inhibitor, such as GW4869, effectively truncated the differentiation of macrophages towards an M2 state and lessened the burden of a tumor, underlining a role for exosomal trafficking in modulating immune function. Together, these studies reveal how miRNAs in exosomes allow effective communication between BC cells and macrophages, enhancing an immunosuppressive and protumoral TME. Thus, inhibition of the biogenesis of exosomes or selectively targeting their oncogenic miRNA cargo could represent a therapeutic opportunity in enhancing prognosis in bladder cancer.

Cancer Associated Fibroblast-Derived miR-146a-5p

CAF-derived miR-146a-5p has been identified as a crucial chemoresistance driver in BC, as shown by Zhuang et al[15]. It is well expressed in CAFs compared with NFs and BC cells, and this difference is further pronounced upon GemCis treatment. This striking upregulation in CAFs allows miR-146a-5p to exert a tumor-promoting effect in multiple intertwined ways. One of its main mechanisms involves enhancing cell-cell adhesion through upregulating the SVEP1 protein, a transcriptional effect of miR-146a-5p's competence in recruiting the YY1 transcription factor on the SVEP1 enhancer area. The resultant SVEP1–integrin β1 interaction in CAFs forms an adhesive niche that enhances CSC maintenance and protects bladder cancer cells from chemotherapy-induced cytotoxicity. In other words, CAF-derived miR-146a-5p could help establish CSC "safe havens" that permit tumor cells to survive and proliferate despite any drug pressures.

Similarly, miR-146a-5p function is extended in parallel through exosome-mediated transfer, with CAFs secreting miR-146a-5p-rich vesicles, which fuse with bladder cancer cells and deliver their oncogenic cargo. Once internalized in the tumor cells, miR-146a-5p suppresses two crucial tumor suppressor genes, ARID1A and PRKAA2 (AMPKα2). ARID1A downregulation compromises SOCS1 transcription, thus relieving negative regulation of the STAT3 pathway, whereas reduced PRKAA2 activity unleashes a brake on the mTOR-STAT3 axis. Together, these changes feed into a robust STAT3 activation loop well known to support properties

such as cancer stemness, proliferation, and resistance to multiple drugs. In a clinical setting, higher serum exosomal miR-146a-5p levels are found to be associated with advanced disease in bladder cancer patients, chemotherapy failure, and the risk of recurrence. These findings, therefore, position the miR-146a-5p-SVEP1-STAT3 axis as a potential therapeutic target in the treatment of refractory bladder cancer, highlighting the importance of CAFs in reshaping the tumor environment toward the evasion of conventional therapy.

Diagnostic and Prognostic Applications of miRNAs

MiRNAs' stability in bodily fluids and their tissue-specific expression profiles make them ideal candidates for non-invasive diagnostic and prognostic tools. In a study by Piao et al., the authors used a ratio of two urinary miRNAs (miR-6124, miR-4511) to differentiate bladder cancer patients from benign hematuria cases with high accuracy[21]. Screening, training, and validating cohorts established the performance of the ratio: a single cut-off value beat standard cytology of the urine by providing greater than 90% sensitivity to diagnose both muscle-invasive BC (MIBC) and non-muscle invasive BC (NMIBC). The authors also optimized diagnostic performance with a scoring approach with a variable range of cut-off levels to present physicians with a flexible approach that can reduce unnecessary cystoscopies among individuals with hematuria who are malignancy-free.

Beyond detection, the research predicts that the use of miRNA-based tests might also reduce the number of follow-up cystoscopies for the recurring NMIBC that necessitate regular surveillance. With traditional markers (e.g., NMP22, BTA stat) being criticized for low sensitivity or excessive false positives, this ratio of miR-6124/miR-4511 demonstrated robust performance among patient subgroups, highlighting its potential to translate into the clinic. With further testing within larger or longitudinal cohorts, such miRNA signatures could potentially inform risk stratification, surveillance of recurrence, and therapy choice, representing a significant movement toward precision oncology in bladder cancer.

Diagnostic Panels

The primary focus of the study conducted by Güllü Amuran et al., was to develop a biomarker panel capable of effectively distinguishing between breast cancer (BC) patients and healthy controls, as well as follow-up patients, with high reliability[7]. By employing logistic regression on urine exosomal microRNAs and urinary proteins that exhibited significant differences among these groups, a comprehensive panel was developed. This panel integrated the presence or absence of miR-136-3p, miR-139-5p, and miR-19b1-5p along with the concentrations of Ape1/Ref1, BLCA4, and CRK. The quality assessment of the model, utilizing the Hosmer and Lemeshow test, indicated a satisfactory fit ($p = 0.9879$), and all variables made significant contributions to the diagnostic efficacy. The Receiver Operating Characteristic curve for the panel demonstrated an area under the curve (AUC) of 0.899, with a sensitivity of 80% and a specificity of 86.4% at a cutoff of 0.571, thereby allowing for the clear differentiation of BC patients from non-cancerous individuals (healthy controls and follow-ups). Focusing exclusively on healthy controls (with follow-ups excluded), the sensitivity and specificity achieved were 80% and 88.2%, respectively. Notably, the panel exhibited relatively high detection capabilities for low-risk BC patients, with an AUC of 0.976, a sensitivity of 93.33%, and a specificity of 97.06%.

These results also suggest that alterations in miRNA presence and protein levels can be observed early in the disease and are pronounced enough to distinguish low-risk BC from healthy samples. The presence or absence of specific miRNAs rather than their exact levels makes the diagnostic workflow easier because cumbersome normalization steps become superfluous. The panel's performance surpasses standard urine cytology regarding sensitivity and specificity, implying fewer superfluous cystoscopies, improving patient comfort, and reducing healthcare costs. More considerable cohort validation will be required, but this preliminary

evidence underlines the promise of simultaneous measurement of exosomal miRNAs and urinary proteins to provide clinically relevant early detection of bladder cancer.

Building upon a multi-stage screening strategy, Li et al., developed a diagnostic panel comprising four microRNAs miR-181b-5p, miR-183-5p, miR-199a-5p, and miR-221-3p that exhibited notable accuracy in differentiating bladder cancer (BC) patients from healthy controls[6]. Following the reduction of an initial list of 147 differentially expressed microRNAs to 15 candidates, quantitative polymerase chain reaction (PCR) was utilized in a detection cohort to identify six microRNAs that displayed significant differences in BC expression. Validation in a larger cohort confirmed that miR-181b-5p, miR-183-5p, and miR-199a-5p demonstrated moderate diagnostic power, with area under the curve (AUC) values within the range of 0.70 to 0.75, while the remaining microRNAs showed lower discriminatory capacity.

Further backward stepwise logistic regression reduced these to a four-miRNA panel: The resultant model showed a good AUC of 0.925 in the detection cohort (sensitivity = 82.14%; specificity = 92.86%) and 0.893 in the validation cohort (sensitivity = 82.14%; specificity = 83.33%). Noticeably, this combination outperformed every single miRNA, confirming the additive value of multi-marker approaches. Although only a few associations existed between clinicopathological parameters and miRNA expression, except for miR-183-5p and histological grading, this four-miRNA signature still held promise as a non-invasive diagnostic tool complementing conventional diagnosis methods such as cystoscopy and urine cytology but probably being more sensitive in an early detection setting.

Therapeutic Potential of miRNAs

Many studies support miRNAs as therapeutic agents and targets in BC, considering their complex involvement in tumorigenesis, chemoresistance, and metastasis. Replacing the functionally lost tumor-suppressive miRNAs or suppressing oncogenic miRNAs can reprogram malignant cells toward a much less aggressive phenotype. The following table summarizes the function of the miRNAs discussed in this review.

Table 1: Summary of miRNA function.

miRNA	Therapeutic Potential
miR-217	Exosomal miR-217 suppresses ferroptosis, promoting BC cell survival. Blocking its exosomal release or inhibiting miR-217 could restore ferroptosis sensitivity and limit tumor progression.
miR-3960	Targets DEXI, stopping proliferation and sensitization for chemicals. Restoration of miR-3960 expression i.e., by exosome delivery one prevention strategy against the proliferation of BC.
Cuproptosis-Related (eight-miRNA signature)	Stratifies BC inflicted individuals into high-/low-risk groups and correlates with immunosuppression and chemo-response. These miRNAs can potentially be modified for enhanced customized treatment and stimulation of the immune system.
miR-490-3p	Downregulates PCBP2 and inhibits the Akt pathway, stopping the cell proliferation of BC. Upregulated miR-490-3p levels can counteract oncogenic signals and cell proliferation
miR-205-3p	Suppresses GLO1 and inhibits MAPK signal, attenuating the aggressiveness of BC. Upregulation of miR-205-3p can induce apoptosis and suppress tumor invasions.

miR-31-5p	Targets and suppresses KRT6A, inducing cell death and cell survival inhibition. Restorative treatment is able to suppress tumour formation and strengthen anti-tumour activities.
miR-146a-5p	CAF-derived miR-146a-5p drives chemoresistance by activating STAT3. Inhibiting its exosomal release or function could sensitize BC cells to chemotherapy and reduce tumor progression.

Restoring Tumor-Suppressive miRNAs

The expression of several miRNAs has been shown to exert anti-tumoral effects in BC. Examples include exogenous delivery of miR-3960, which results in reduced proliferation in MB49 cells, sensitization to cisplatin and gemcitabine, and tumor growth inhibition in vivo via DEXI targeting[16]. Re-expression of miR-490-3p results in reduced Akt/GSK3β signaling and downregulation of the oncogenic driver PCBP2, thus impairing the proliferation of BC cells and inducing G1-phase cell cycle arrest[12]. Another example is miR-205-3p, which downregulates GLO1 and blocks the p38/ERK pathway; thus, restoring one single miRNA can inactivate multiple oncogenic networks[14].

Although promising, this approach presents a significant challenge: the efficient and target-specific delivery of miRNA mimics into breast cancer (BC) cells. Recently, nanoparticle and exosome-based carriers have been investigated to overcome these challenges related to tissue specificity and systemic toxicity. This methodology assures stability in biological fluids, a prerequisite for translating such experimental strategies into safe and effective therapeutic options.

Inhibiting Oncogenic miRNAs

On the other hand, various miRNAs behave like oncogenes by promoting cell survival and resistance to drugs. For example, exosomal miR-217 confers ferroptosis resistance in BC cells and has thus been implicated in chemoresistance and disease progression[10]. Similarly, miR-146a-5p from cancer-associated fibroblast reinforced the STAT3 pathway and created a chemoprotective niche by SVEP1–integrin β1 interactions[15]. Blocking such oncogenic miRNAs using antisense oligonucleotides or sponges could potentiate chemotherapy and restrain cancer stem cell dynamics.

Targeting miRNAs & Cuproptosis

Cuproptosis-associated signatures are yet another avenue for therapy. Eight cuproptosis-related miRNAs were associated with tumor aggressiveness, drug responsiveness, and the immunosuppressive tumor microenvironment[11]. Targeted modulation may thus restore sensitivity to standard chemotherapeutics and change the immune-cell infiltrations in favor of a tumoricidal milieu.

Future Directions

Moving miRNA-based strategies into clinical practice will require addressing several challenges:

- Delivery & Stability: Designing vectors (e.g., lipid nanoparticles, targeted exosomes) that protect miRNAs from degradation while ensuring selective accumulation in tumor tissues remains a top priority.
- Dose & Toxicity: Overexpression or inhibition of miRNAs may have unforeseen consequences on non-tumor tissues, given that many miRNAs possess a broad range of downstream targets.
- Combination Therapy: As some miRNAs regulate multiple cancer pathways, combining miRNA-based interventions with existing immunotherapies or targeted drugs may yield synergistic outcomes, further improving response rates.
- Personalized Medicine: Patient-specific signatures, such as the four-miRNA panels[6] or expanded biomarker panels[7], could lead to individualized miRNA therapies tailored to each tumor's molecular profile.

These findings pinpoint the enormous potential of miRNAs as next-generation therapeutics in bladder cancer. They can be reintroduced to enhance tumor-suppressive functions or inhibited to neutralize oncogenic effects. However, further studies, larger-scale trials, and more sophisticated delivery systems will be required to attain full clinical potential.

Conclusion

The cumulative evidence substantiates microRNAs (miRNAs) as essential molecular regulators and promising clinical tools in bladder cancer. These molecules have the potential to modulate cell proliferation, metastatic behavior, chemoresistance, and immune evasion, thus underscoring the significant roles that both tumor-suppressive miRNAs (such as miR-3960, miR-490-3p, and miR-205-3p) and oncogenic miRNAs (including miR-217 and miR-146a-5p) play in disease progression and therapeutic outcomes. Investigations into the integration of miRNA signatures into diagnostic panels have yielded encouraging results, demonstrating that multimarker combinations can achieve high sensitivity and specificity, particularly for early-stage and low-risk BC cases. Furthermore, novel pathways, including those related to cuproptosis gene networks, reveal additional mechanistic layers through which miRNAs influence tumor cell metabolism and immune cell composition. These findings indicate that miRNAs are at the forefront of potentially transformative advancements in managing BC. These advancements encompass diagnostic assays that may diminish the necessity for invasive procedures such as cystoscopies and next-generation therapies that target miRNA-regulated pathways. The forthcoming challenge lies in translating these findings into widespread clinical practice. Addressing issues related to miRNA delivery, off-target effects, and therapeutic resistance will require multifaceted strategies. However, the expanding molecular landscape of BC supports the notion that miRNAs will play a pivotal role in future precision medicine approaches.

Conflicts of Interest

The author(s) have no conflicts of interest.

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References

1. World Health Organization. Global health estimates 2020: Deaths by cause, age, sex, by country and by region, 2000–2019 [Internet]. Geneva: WHO; 2020. Available at: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-leading-causes-of-death
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021;71(3):209-249. Doi: 10.3322/caac.21660.
3. Canadian Cancer Society. Projected cancer cases and deaths by sex, Canada, 2024. Data visualization. Available at: https://cancerstats.ca/Incidence/Sex
4. Dyrskjøt L, Hansel DE, Efstathiou JA, Knowles MA, Galsky MD, Teoh J, Theodorescu D. Bladder cancer. Nat Rev Dis Primers. 2023; 9(1):58. Doi: 10.1038/s41572-023-00468-9.
5. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front endocrinol. 2018;9:402. Doi: 10.3389/fendo.2018.00402.
6. Li X, Chen W, Li R, Chen X, Huang G, Lu C, Wen Z, Peng X, Liu K, Zhang C, Li H. Bladder cancer diagnosis with a four-miRNA panel in serum. Future Oncol. 2022;18(29):3311-3322. Doi: 10.2217/fon-2022-0448.
7. Güllü Amuran G, Tinay I, Filinte D, Ilgin C, Peker Eyüboğlu I, Akkiprik M. Urinary micro-RNA expressions and protein concentrations may differentiate bladder cancer patients from healthy controls. Int Urol Nephrol. 2020;52:461-468. Doi: 10.1007/s11255-019-02328-6.
8. Mamdouh S, Sherif H, Romeih M, Elesaily K. Urine micro-RNA signature as a potential non-invasive diagnostic biomarker in bladder cancer. APJCP. 2023;24(1): 121–131. Doi: 10.31557/APJCP.2023.24.1.12.

9. Yu Z, Lu C, Lai Y. A serum miRNAs signature for early diagnosis of bladder cancer. Ann Med. 2023; 55(1):736–745. Doi: 10.1080/07853890.2023.2172206.
10. Huang ZM, Wang H, Ji ZG. Bladder cancer tissue-derived exosomes suppress ferroptosis of T24 bladder cancer cells by transporting miR-217. Environ Mol Mutagen. 2023;64(1):39-49. Doi: 10.1002/em.22520.
11. Zhang Z, Liu F, Yu Y, Xie F, Zhu T. Prognosis and immune landscape of bladder cancer can be predicted using a novel miRNA signature associated with cuproptosis. PeerJ. 2024;12:e18530. Doi: 10.7717/peerj.18530.
12. Zhang CM, Song LD, Wang JW, Ye HB, Chen S. Role of miR-490-3p in blocking bladder cancer growth through targeting the RNA-binding protein PCBP2. Kaohsiung J Med Sci. 2022;38(1):30-37. Doi: 10.1002/kjm2.12457.
13. Chen Y, Ji S, Ying J, Sun Y, Liu J, Yin G. KRT6A expedites bladder cancer progression, regulated by miR-31-5p. Cell Cycle. 2022 Mar 20;21(14):1479–1490. Doi: 10.1080/15384101.2022.2054095.
14. Zhenhai Z, Qi C, Shuchao Z, Zhongqi W, Xue S, Zhijun G, Zhijie M, Jianmin L, Beibei L, Yuanyuan G. MiR-205-3p suppresses bladder cancer progression via GLO1 mediated P38/ERK activation. BMC cancer. 2023;23(1):956. Doi: 10.1186/s12885-023-11175-9.
15. Zhuang J, Shen L, Li M, Sun J, Hao J, Li J, Zhu Z, Ge S, Zhang D, Guo H, Huang R. Cancer-associated fibroblast–derived miR-146a-5p generates a niche that promotes bladder cancer stemness and chemoresistance. Cancer res. 2023;83(10):1611-1627. Doi: 10.1158/0008-5472.CAN-22-2213.
16. Li W, Wang Z, Jiang Z, Yan Y, Yao X, Pan Z, Chen L, Wang F, Wang M, Qin Z. MiR-3960 inhibits bladder cancer progression via targeting of DEXI. Biochem Biophys Res Commun. 2023;668:8-18. 872–886. Doi: 10.1016/j.omtn.2021.01.009.
17. National Cancer Institute. Bladder cancer treatment (PDQ®)–Health professional version. 2024 U.S. Department of Health and Human Services. [Updated: February 12, 2025]. Available at: https://www.cancer.gov/types/bladder/hp/bladder-treatment-pdq
18. Canadian Cancer Society Canadian Cancer Statistics. 2024. Available at: https://cancer.ca/en/research/cancer-statistics
19. Jiang Z, Zhang Y, Zhang Y, Jia Z, Zhang Z, Yang J. Cancer derived exosomes induce macrophages immunosuppressive polarization to promote bladder cancer progression. Cell Commun Signal. 2021;19:93. Doi: 10.1186/s12964-021-00768-1.
20. Paskeh MD, Entezari M, Mirzaei S, Zabolian A, Saleki H, Naghdi MJ, Sabet S, Khoshbakht MA, Hashemi M, Hushmandi K, Sethi G. Emerging role of exosomes in cancer progression and tumor microenvironment remodeling. J Hematol Oncol. 2022;15(1):83. Doi: 10.1186/s13045-022-01305-4.
21. Piao XM, Jeong P, Kim YH, Byun YJ, Xu Y, Kang HW, Ha YS, Kim WT, Lee JY, Woo SH, Kwon TG. Urinary cell-free microRNA biomarker could discriminate bladder cancer from benign hematuria. Int j cancer. 2019;144(2):380-388. Doi: 10.1002/ijc.31849.