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Complex molecular mechanisms involved in the oncogenesis of bladder tumors

- SUMMARY OF THE DOCTORAL DISSERTATION –

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LIST OF PUBLISHED SCIENTIFIC PAPERS

1. **Pavalean MI**, Lambrescu IM, Gaina G, Madan VL, Hinescu ME, Ceafalan LC. CD36 rs1761667 Polymorphism and Its Impact on Molecular Signatures in Bladder Cancer. Diseases. 2026;14:44. doi:10.3390/diseases14020044; https://www.mdpi.com/2079-9721/14/2/44

2. **Pavalean MI**, Dobre M, Pelisenco IA, Madan VL, Milanesi E, Hinescu ME. Association of miRNA-17-92 Cluster with Muscle Invasion in Bladder Cancer. Int J Mol Sci. 2025;26:7546. doi:10.3390/ijms26157546; https://www.mdpi.com/1422-0067/26/15/7546

3. **Pavalean MI**, Madan VL, Pavalean MC, Ceafalan LC, Hinescu ME. The role of CD36 in renal and bladder cancer. Discoveries. 2026;14:e224. doi:10.15190/d.2026.4; https://www.researchgate.net/publication/403549559_The_role_of_CD36_in_renal_and_bladder_cancer

LIST OF PRESENTED PAPERS

- **Association Between CD36 rs1761667 Polymorphism and Gene Expression Profiles in Bladder Cancer,** Pavalean MI, Lambrescu I, Gaina G, Ceafalan LC, Hinescu ME - CONGRESUL UNIVERSITĂȚII DE MEDICINĂ ȘI FARMACIE "CAROL DAVILA" DIN BUCUREȘTI, 23-25 OCTOMBRIE, 2025

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1. INTRODUCTION

1.1 Introductory Concepts in Bladder Cancer

Bladder cancer represents a major global public health concern, being one of the most common malignancies of the urinary tract and associated with significant morbidity and mortality. Despite advances in diagnostic and therapeutic strategies, this disease remains characterized by high recurrence rates, biological heterogeneity, and a limited number of predictive biomarkers, underscoring the need for a deeper understanding of the underlying molecular mechanisms [1–3].

From a clinical perspective, bladder cancer is classified into two main entities: non–muscle-invasive bladder cancer (NMIBC), characterized by a high rate of recurrence, and muscle-invasive bladder cancer (MIBC), associated with a poorer prognosis and an increased risk of metastasis. Current classification systems do not allow for optimal prediction of disease progression nor for effective personalization of treatment.

Metabolic reprogramming, particularly lipid metabolism, plays a critical role in cancer biology. The CD36 receptor, involved in fatty acid uptake as well as inflammatory and immune processes, has emerged as a molecule of interest; however, its specific role in bladder cancer remains insufficiently elucidated.

The originality of this research lies in the integrative evaluation of CD36 by correlating clinical, molecular, and metabolic data. The study aligns with current trends in personalized medicine and aims to identify relevant biomarkers and potential therapeutic targets.

The central hypothesis of this study is that CD36 plays a significant role in the pathogenesis and progression of bladder cancer, with its expression being associated with clinicopathological parameters such as tumor stage, degree of differentiation, and invasiveness.

1.2 Scientific Objectives

Genetic variability of the CD36 gene

- Analysis of the distribution of the rs1761667 polymorphism between patients and controls
- Evaluation of the association between genotypes and susceptibility to bladder cancer
- Determination of the impact of genotype on CD36 expression

Expression of genes involved in lipid metabolism

- Assessment of the expression of CD36, SLC27A1, SLC27A4, SLC27A5, ACSL1, ACSL3, and FASN
- Identification of expression changes associated with fatty acid transport and activation
- Correlation of gene expression with clinicopathological parameters
- Investigation of their prognostic value

miRNA expression profile

- Analysis of miRNA expression in tumor versus peritumoral tissue
- Identification of miRNAs involved in proliferation and invasion
- Analysis of differences between tumor subtypes and risk groups
- Evaluation of their prognostic value

This study employs a multidisciplinary approach, integrating clinical data with molecular biology techniques (PCR, RT-qPCR), alongside statistical analyses to identify relevant correlations.

2. CURRENT STATE OF KNOWLEDGE

2.1 Epidemiological Data

Bladder tumors represent the most common malignancies of the urinary tract. Approximately 96% are malignant, and their global incidence ranks them 10th overall and 7th among men, with a prevalence about four times higher in men compared to women [1].

Five-year survival varies significantly, ranging from less than 40% in developing countries to approximately 80% in the United States [4]. Mortality rates are higher in men (3.1 vs. 0.8 per 100,000/year) [5].

Approximately 75% of patients present with non-invasive disease (Ta, CIS, T1), which is associated with a more favorable prognosis compared to invasive forms [6,7].

Bladder cancer is a complex disease characterized by marked clinical and molecular heterogeneity, in which genetic, metabolic, and environmental factors interact to influence disease progression. Despite therapeutic advances, the lack of robustly validated biomarkers remains a major limitation.

The integration of molecular mechanisms—particularly lipid metabolism and the role of CD36—represents a promising direction for the development of personalized diagnostic and therapeutic strategies.

3. MOLECULAR MECHANISMS INVOLVED IN THE ONCOGENESIS OF BLADDER TUMORS

Bladder cancer involves distinct molecular mechanisms that reflect the clinical heterogeneity of the disease. Non–muscle-invasive bladder cancer (NMIBC) is frequently associated with activating mutations in the FGFR3 gene and dysregulation of the RAS–MAPK signalling pathway, thereby promoting cellular proliferation in the context of relative genomic stability [8]. In contrast, muscle-invasive bladder cancer (MIBC) is characterized by marked genomic instability and mutations in tumor suppressor genes such as *TP53* and *RB1*, leading to loss of cell cycle control and increased tumor aggressiveness [9].

Epigenetic mechanisms, including DNA methylation and histone modifications, contribute to carcinogenesis by regulating gene expression without altering the DNA sequence. Hypermethylation of tumor suppressor gene promoters leads to their silencing and facilitates tumor progression. In parallel, microRNAs (miRNAs) regulate gene expression at the post-transcriptional level, influencing processes such as proliferation, apoptosis, and metastasis. Metabolic reprogramming, particularly alterations in lipid metabolism, represents a hallmark of bladder cancer. Proteins such as CD36, involved in fatty acid uptake, contribute to tumor progression and interactions with the tumor microenvironment. Dysregulation of major signaling pathways, including PI3K/AKT/mTOR and Wnt/β-catenin, supports cell proliferation, survival, and therapeutic resistance [8,10].

3.1 Discovery, Structure, and Expression of CD36

Scavenger receptors are classified into ten classes (A–J) based on structural features and sequence similarity; however, inter-class similarity remains limited (154,155). CD36, also known as SR-B2, is the prototypical class B receptor and was initially identified as a receptor for thrombospondin (TSP) [11].

CD36 is capable of binding a wide range of ligands, including fatty acids, oxidized low-density lipoproteins (oxLDL), advanced glycation end products (AGEs), collagen, phospholipids, and thrombospondin, positioning it as a central regulator of lipid metabolism and inflammation [12–15].

CD36 is expressed in various cell types, including microvascular endothelial cells, stromal cells, and immune cells [16,17], with expression levels varying depending on the biological context (18).

Numerous studies have demonstrated a correlation between CD36 overexpression and unfavorable clinical outcomes across multiple cancer types, including oral carcinoma, melanoma, breast, gastric, prostate, and colorectal cancers [10,18–22]. Inhibition of CD36 reduces proliferation and metastatic potential, highlighting its oncogenic role.

CD36 plays a key role in metastasis through its involvement in lipid metabolism regulation [23]. Its overexpression significantly enhances metastatic capacity, including lymph node dissemination [10]. Furthermore, increased expression of lipid transporters such as CD36, FATP4, and ACSL1 has been associated with tumor progression [24].

Although these mechanisms are well documented in other malignancies, the role of CD36 in treatment resistance in bladder cancer remains insufficiently elucidated and warrants further investigation.

In conclusion, the molecular mechanisms underlying bladder cancer oncogenesis involve complex interactions among genetic, epigenetic, and metabolic alterations. CD36 emerges as a central regulator of lipid metabolism and tumor progression, being implicated in proliferation, metastasis, angiogenesis, and immunomodulation.

4. CD36 rs1761667 POLYMORPHISM AND ITS IMPACT ON MOLECULAR SIGNATURES IN BLADDER CANCER

4.1 Introduction

The *CD36* gene, located on chromosome 7q11.2 and comprising 15 exons, encodes a scavenger receptor involved in fatty acid uptake, lipid metabolism, and inflammatory signaling. The rs1761667 polymorphism (G>A), located in a regulatory 5′ intronic region, has been identified as a potential expression Quantitative Trait Locus (eQTL), suggesting a direct impact on CD36 mRNA expression levels [25]. This genetic variant has been extensively studied in cardiometabolic diseases, being associated with cardiovascular disorders, type 2 diabetes mellitus, obesity, and dietary lipid preferences [26–30], as well as with prognostic value in obese diabetic patients [31].

Recent evidence suggests the involvement of rs1761667 in oncology, including susceptibility to colorectal cancer and phenotypic variability in familial adenomatous polyposis [32,33]. In bladder cancer, this variant has been associated with increased risk and higher circulating CD36 levels in metastatic disease [34]. Given CD36's role in lipid metabolism, inflammation, and tumor microenvironment interactions, genetic variations

affecting its expression may contribute to the biological and clinical heterogeneity of bladder cancer.

Analysis of CD36 expression in peripheral blood may reveal host-dependent mechanisms complementary to tumor related processes and provide insights into the interplay between metabolism, inflammation, and tumor progression [35].

4.2 Materials and Methods

The case–control study included 49 participants (30 patients with bladder cancer and 19 healthy controls), recruited from a single hospital. The objective was to simultaneously evaluate the rs1761667 genotype and CD36 mRNA expression in peripheral blood using standardized molecular biology techniques.

Genotyping was performed using the PCR-RFLP method, which involves amplifying a specific DNA region and enzymatically digesting the amplicon to differentiate alleles based on fragment patterns.

Gene expression was quantified by RT-qPCR using the $2^{\Delta\Delta Ct}$ method, with normalization to the reference gene *GAPDH*.

Statistical analysis included non-parametric tests (Kruskal–Wallis, Mann–Whitney), parametric tests (ANOVA), assessment of Hardy–Weinberg equilibrium, and categorical associations (Pearson's χ^2 test).

This integrative approach correlates germline genetic variation with functional gene expression levels, providing insights into the molecular mechanisms underlying bladder cancer.

4.3 Results

A total of 49 participants were included, comprising 30 patients with bladder cancer and 19 healthy controls.

The distribution of rs1761667 genotypes did not differ significantly between patients and controls ($p = 0.053$), although a trend toward association was observed. The heterozygous GA genotype predominated in patients (50%), whereas it was much lower in controls (15.8%), while the GG genotype was more frequent in controls (57.9%).

This distribution suggests a potential differential effect of genotypes on disease susceptibility; however, the lack of statistical significance highlights the need for larger cohorts. Deviation from Hardy–Weinberg equilibrium in the control group ($p = 0.018$) underscores limitations related to sample size.

The main finding of the study was the identification of a significant association between rs1761667 genotype and CD36 mRNA expression in bladder cancer patients. A clear expression gradient was observed (AA < GA < GG), with statistically significant differences (Kruskal–Wallis, $p = 0.02$), and significantly lower expression in the AA genotype compared to GG ($p = 0.008$).

No significant differences in expression between genotypes were observed in the control group (ANOVA, $p = 0.137$), suggesting that the effect of rs1761667 on CD36 expression is context-dependent. These findings support the hypothesis that disease-specific factors, including the tumor microenvironment, systemic inflammation, and metabolic status, may amplify the functional effects of genetic variation.

From a biological perspective, CD36 lies at the intersection of lipid metabolism and tumor-related processes, including invasion, metastasis, and metabolic adaptation. Thus, genotype-dependent expression differences may reflect distinct metabolic phenotypes relevant to disease progression.

Clinical characteristics analysis revealed a typical bladder cancer profile: male predominance, advanced age, and the presence of risk factors such as smoking and metabolic comorbidities. Patients with non–muscle-invasive tumors exhibited a higher metabolic burden (obesity, dyslipidemia), suggesting distinct biological mechanisms between tumor subtypes.

No significant association was identified between dyslipidemia and rs1761667 genotype ($p = 0.394$), although this finding should be interpreted cautiously given methodological limitations and the small sample size. Additionally, no clear relationship was observed between genotype or CD36 expression and smoking status.

An important finding was the significant association between tumor grade and 2-year mortality ($p = 0.024$), with a significant linear trend ($p = 0.013$), confirming the prognostic value of histological grade and the relevance of biological factors influencing tumor aggressiveness.

4.4 Discussion

The results suggest that rs1761667 is a functional determinant of CD36 expression, contributing to interindividual heterogeneity in bladder cancer. The observed expression gradient may reflect differences in tumor metabolic programming, with implications for invasiveness and treatment response.

From a clinical perspective, CD36 genotyping may aid in patient stratification and in identifying subgroups with distinct metabolic profiles. However, clinical applicability requires validation in larger studies and integration with additional molecular data.

4.5 Limitations

The main limitations of the study include the small sample size, the lack of tumor tissue expression analysis, and the absence of long-term clinical follow-up. The deviation from Hardy–Weinberg equilibrium in the control group further emphasizes the need for validation in larger cohorts.

4.6 Conclusions

In conclusion, this study demonstrates that the rs1761667 polymorphism of the *CD36* gene is not significantly associated with bladder cancer risk but significantly influences CD36 mRNA expression in a genotype-dependent manner, with an effect amplified in the disease context [36].

These findings support the role of rs1761667 as a functional determinant of CD36 expression and suggest its involvement in the metabolic heterogeneity of bladder cancer. Although promising, these observations require further validation before clinical implementation and provide a foundation for exploring novel therapeutic strategies targeting tumor lipid metabolism.

5. EXPRESSION OF GENES INVOLVED IN LONG-CHAIN FATTY ACID TRANSPORT IN TUMOR TISSUES

5.1 Introduction

Tumor cells exhibit significant metabolic plasticity, utilizing both exogenous fatty acid uptake and de novo lipid synthesis depending on microenvironmental conditions. This adaptability is regulated by a complex network of proteins involved in fatty acid transport, activation, and utilization, including the receptor CD36, members of the SLC27 (FATP) transporter family, fatty acid synthase (FASN), and acyl-CoA synthetases (ACSL1 and ACSL3).

CD36 plays a key role in long-chain fatty acid uptake and in integrating metabolic and inflammatory signals. In the oncologic context, CD36 has been associated with tumor progression and metastasis, contributing to tumor microenvironment adaptation and immune

response regulation (154,167). The SLC27 family is involved in both transport and activation of fatty acids, while FASN and ACSL enzymes regulate their intracellular synthesis and utilization.

In bladder cancer, the role of these genes remains incompletely understood, and characterization of their expression profile may provide important insights into the metabolic mechanisms underlying carcinogenesis and disease progression.

5.2 Materials and Methods

The study included 41 patients diagnosed with bladder cancer, all treatment-naïve. The expression of genes involved in lipid metabolism was analyzed in tumor tissue compared with peritumoral tissue. Gene expression was assessed using qRT-PCR, applying the $2^{\Delta\Delta Ct}$ method, with normalization to the reference genes RPS23 and TPT1.

The study design included a case–control analysis (tumor vs. peritumoral tissue) as well as stratified analyses based on tumor recurrence and risk. This approach allowed evaluation of metabolic alterations both globally and in relation to clinically relevant clinicopathological parameters.

5.3 Results

The study cohort displayed typical characteristics of bladder cancer patients: advanced mean age (~69 years), male predominance (≈78%), and a high prevalence of risk factors such as smoking and metabolic comorbidities. Most cases were non–muscle-invasive bladder cancer (NMIBC), although a relevant subgroup included muscle-invasive bladder cancer (MIBC), characterized by a more aggressive clinical profile.

MIBC cases were associated with higher recurrence rates, predominance of high-grade (G3) tumors, and advanced TNM stages, confirming their biological aggressiveness. Biochemical analysis also revealed marked anemia and altered immune status (lymphopenia) in MIBC, suggesting systemic implications of advanced disease.

Comparative analysis between tumor and peritumoral tissues revealed a distinct metabolic profile characterized by differential expression of genes involved in lipid metabolism.

Genes involved in fatty acid uptake, CD36 and SLC27A1, were significantly downregulated in tumor tissue. This decrease suggests reduced direct uptake of extracellular fatty acids or a metabolic shift toward alternative lipid sources. For SLC27A1, reduced expression was associated with tumor recurrence, indicating a potential prognostic role.

In contrast, ACSL1 and especially ACSL3 showed significant overexpression in tumor tissue. ACSL3 exhibited the most pronounced expression difference ($p < 0.001$), suggesting

a central role in fatty acid activation and in supporting metabolic processes required for tumor proliferation. This activation reflects enhanced conversion of fatty acids into acyl-CoA, a key step for β-oxidation, lipid synthesis, and oncogenic signaling.

Members of the SLC27 family showed a variable expression pattern:

- SLC27A4 and SLC27A5 were overexpressed in tumor tissue and significantly increased in high-risk tumor groups;
- SLC27A2 and SLC27A3 showed no significant differences.

These findings indicate selective activation of lipid transporters, potentially adapted to the metabolic demands of the tumor.

Although FASN is considered a classical marker of de novo fatty acid synthesis, no significant differences in its expression were observed in this study. This suggests that, in the analyzed bladder cancer cohort, fatty acid uptake and activation may play a more prominent role than endogenous lipid synthesis.

Stratified analysis revealed clinically relevant associations:

- SLC27A1 was significantly downregulated in recurrent cases, suggesting a role in disease progression;
- SLC27A4, SLC27A5, and ACSL1 were significantly overexpressed in high-risk tumors.

This pattern indicates that aggressive tumors exhibit enhanced lipid metabolic activity, supporting the concept of "lipid addiction" in cancer.

This metabolic reprogramming suggests that tumor cells do not rely exclusively on exogenous lipid supply but instead optimize lipid utilization through efficient metabolic activation. Selective activation of transporters may also reflect adaptations to specific tumor microenvironment conditions.

5.4 Discussion

The results support the central role of lipid metabolism in bladder carcinogenesis and highlight the potential of the analyzed genes as biomarkers and therapeutic targets. ACSL1 and ACSL3 emerge as key elements in sustaining tumor metabolism, and their overexpression may be associated with tumor aggressiveness and progression. In parallel, reduced SLC27A1 expression may have prognostic value for recurrence.

Overexpression of SLC27A4 and SLC27A5 in high-risk tumors suggests their involvement in the development of an aggressive tumor phenotype, possibly by facilitating fatty acid uptake and utilization from the tumor microenvironment.

The study demonstrates that lipid metabolic reprogramming represents a core mechanism in bladder cancer. The gene expression profile is characterized by a dynamic balance between fatty acid uptake, activation, and utilization, rather than reliance on de novo synthesis.

Overexpression of ACSL3 and ACSL1, together with selective activation of SLC27A4 and SLC27A5, indicates intensified lipid metabolism in aggressive tumors. Conversely, decreased expression of CD36 and SLC27A1 suggests complex, context-dependent metabolic adaptations.

5.5 Conclusions

These findings provide new insights into the metabolic biology of bladder cancer and support the potential of lipid metabolism–related genes as biomarkers and therapeutic targets, although further validation is required.

6. miRNA EXPRESSION PROFILE IN TUMOR TISSUE

6.1 Introduction

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression post-transcriptionally by binding to target mRNAs and inhibiting their translation or inducing their degradation. A single miRNA can target hundreds of mRNAs, while a single mRNA may be regulated by multiple miRNAs, forming a complex regulatory network [37]. This multilayered regulation enables miRNAs to simultaneously control numerous essential biological pathways.

Alterations in miRNA expression levels can dysregulate target gene expression and disrupt biological processes such as proliferation, differentiation, apoptosis, and immune response, thereby contributing to tumor initiation and progression [38]. Depending on their target genes, miRNAs may function either as oncogenes or tumor suppressors.

In bladder cancer, emerging evidence supports the role of miRNAs in tumor progression, metastasis, and treatment resistance, making them promising candidates as diagnostic and prognostic biomarkers [39]. A major advantage of miRNAs is their high stability relative to mRNA, owing to their small size, making them suitable for molecular analyses even after surgical processing [37,39].

The identification of differentially expressed miRNAs between tumor and non-tumor tissues may provide insights into the molecular mechanisms of carcinogenesis and open new avenues for therapeutic strategies [40].

6.2 Materials and Methods

The study included the same cohort of 41 bladder cancer patients previously analyzed, ensuring consistency between clinical and molecular data. Tumor and peritumoral tissue samples were collected, preserved under optimal conditions to prevent RNA degradation, and stored at −80°C.

Total RNA, including the miRNA fraction, was isolated using the miRNeasy Mini Kit, based on phenol–guanidine thiocyanate lysis and silica column purification. RNA quality and quantity were assessed by UV spectrophotometry, using A260/A280 and A260/A230 ratios as indicators of purity.

miRNA expression was analyzed using the miRCURY LNA PCR Array platform, which enables the simultaneous quantification of 84 miRNAs relevant to oncology. Locked Nucleic Acid (LNA) oligonucleotides provided high specificity and thermal stability of hybridization, facilitating the detection of short and highly similar sequences.

Expression analysis was performed using the $2^{\Delta\Delta Ct}$ method, with normalization to reference miRNAs (SNORD38B, SNORD49A). Inter-plate calibration was achieved using an interplate control (IPC)-based normalization factor. Statistical analysis was conducted using non-parametric tests (Mann–Whitney, Wilcoxon, Kruskal–Wallis), given the non-Gaussian distribution of the data.

6.3 Results

The case–control analysis (41 tumor vs. 28 peritumoral samples) identified 26 differentially expressed miRNAs, of which 25 were confirmed in paired analysis (24 tumor vs. 24 peritumoral samples), demonstrating the robustness of the findings.

Most identified miRNAs (15 out of 26) showed significant downregulation in tumor tissue, suggesting a predominant tumor-suppressive role. The most strongly downregulated miRNAs were:

- miR-133a-3p (FR = −13.49)
- miR-1 (FR = −9.20)
- miR-100-5p (FR = −8.12)
- miR-99a-5p (FR = −7.95)
- miR-125b-5p (FR = −7.15)

These miRNAs are involved in regulating proliferation, differentiation, and the cell cycle, and their loss may disrupt cellular homeostasis and promote tumor progression.

Additional tumor-suppressive miRNAs identified included miR-143-3p, miR-145-5p, miR-126-3p, and members of the let-7 family, which are known to inhibit invasion and metastasis.

External validation using the GSE40355 dataset confirmed 24 of the 26 identified miRNAs, with 22 showing consistent expression direction, further supporting the biological relevance of the results.

Comparative analysis between muscle-invasive (MIBC) and non–muscle-invasive bladder cancer (NMIBC) revealed overexpression of seven miRNAs in MIBC, including:

- miR-17-5p
- miR-18a-5p
- miR-19a-3p
- miR-19b-3p

These miRNAs belong to the miR-17-92 cluster, known for its oncogenic role across multiple cancer types. Overexpression of this cluster is associated with increased proliferation, inhibition of apoptosis, and tumor progression.

miR-17-5p was associated with tumor recurrence, suggesting its potential as a prognostic biomarker. Additionally, miR-18a-5p showed a significant association with tumor grade, being more highly expressed in high-grade (G3) tumors.

In NMIBC patients, four miRNAs were found to be overexpressed in the high-risk group:

- miR-7-5p
- miR-20b-5p (FR = 7.17)
- miR-195-5p
- miR-196a-5p (FR = 4.94)

These findings suggest their involvement in molecular mechanisms of tumor progression, including cell proliferation, angiogenesis, and tumor microenvironment remodeling.

Among these, miR-20b-5p and miR-196a-5p demonstrated the highest biological relevance and represent potential prognostic biomarker candidates.

6.4 Discussion

The global miRNA expression profile reveals an imbalance between tumor-suppressive and oncogenic miRNAs, reflecting the complexity of regulatory networks in carcinogenesis.

Downregulation of tumor-suppressive miRNAs disrupts the control of proliferation and differentiation, while overexpression of oncogenic miRNAs activates oncogenic pathways and promotes tumor adaptation to stress conditions, including hypoxia.

The results of this study demonstrate a distinct miRNA expression profile in bladder cancer, characterized by predominant downregulation of tumor-suppressive miRNAs and upregulation of a subset of oncogenic miRNAs.

The identification of the miR-17-92 cluster as being associated with muscle invasion, recurrence, and tumor grade highlights its central role in disease progression. Furthermore, miR-20b-5p and miR-196a-5p emerge as potential biomarkers of progression risk [41].

6.5 Conclusions

These findings support the potential of miRNAs as valuable tools in the diagnosis, prognosis, and management of bladder cancer. However, further studies involving larger cohorts and functional analyses are required to validate these observations and facilitate their integration into clinical practice.

7. CONCLUSIONS AND ORIGINAL CONTRIBUTIONS

This doctoral thesis aligns with current trends in oncological research, which focus on elucidating the molecular mechanisms underlying carcinogenesis and identifying relevant biomarkers for the diagnosis and prognosis of bladder cancer. Through an integrative approach structured into three complementary studies, this research simultaneously investigated CD36 genetic variability, the expression of genes involved in fatty acid transport and activation, and miRNA expression in tumor and peritumoral tissues, thereby providing a comprehensive perspective on tumor biology.

The results validate the thesis's central hypothesis, demonstrating that both miRNA dysregulation and reprogramming of lipid metabolism significantly contribute to the development and progression of bladder cancer.

The first study, focused on the CD36 polymorphism (rs1761667), revealed differences in genotype distribution between patients and controls, suggesting a potential role for this polymorphism. Although statistical significance was limited, genotype-dependent expression analysis indicated a possible functional effect, highlighting the involvement of CD36 in tumor heterogeneity and metabolic adaptation.

The second study identified a distinct expression profile of genes involved in fatty acid metabolism within tumor tissue. Downregulation of CD36 and SLC27A1, associated with

recurrence, suggests alterations in lipid uptake mechanisms. Overexpression of SLC27A4, SLC27A5, and ACSL1 in high-risk groups indicates activation of alternative metabolic pathways involved in tumor progression. The marked upregulation of ACSL3 underscores the central role of fatty acid activation in sustaining cellular proliferation, while the absence of significant changes in FASN expression suggests a reduced reliance on de novo lipid synthesis in this tumor context.

The third study identified a specific miRNA expression profile in tumor tissue, characterized by predominant downregulation of tumor-suppressive miRNAs and upregulation of a subset of oncogenic miRNAs. The association of these alterations with muscle invasiveness, tumor grade, and progression risk supports their potential as biomarkers. The involvement of the miR-17-92 cluster in tumor aggressiveness highlights the importance of post-transcriptional regulation in disease evolution.

Integration of the results from the three studies reveals a complex interplay between genetic variability, miRNA regulation, and lipid metabolism, contributing to the definition of tumor phenotype and clinical outcome. This interdependence reflects the complexity of molecular networks involved in carcinogenesis and underscores the need for multidisciplinary approaches in oncological research.

Study limitations, including the relatively small sample size, lack of functional validation, and observational design, warrant cautious interpretation of the findings and highlight the need for further investigation. Future research directions include validation of these results in larger, independent cohorts, integration of multi-omics analyses, and elucidation of the mechanisms through which miRNAs and lipid metabolism–related genes interact within the tumor microenvironment.

In conclusion, this thesis demonstrates that lipid metabolism reprogramming and post-transcriptional regulation mediated by miRNAs are key determinants of the biological behavior of bladder cancer. The identification of specific molecular profiles and potential biomarkers provides valuable perspectives for the development of diagnostic, prognostic, and personalized therapeutic strategies, contributing to the advancement of knowledge in urological oncology.

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