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Research Paper

Expression Analysis of *PVT-1 lncRNA* and *TGF-β* in Colorectal Cancer and Non-cancerous Colorectal Polyps: A Case-control Study

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ABSTRACT

Introduction: Colorectal cancer ranks among the most prevalent types of cancer across the globe, particularly in developing countries. Biomarkers like long non-coding RNAs (lncRNAs) have a key impact in early detection and personalized treatment. This study aimed to compare the expression levels of *PVT-1 lncRNA* and *TGF-β* gene in colorectal cancer tissues and non-cancerous polyp tissues.

Materials & Methods: Fifty colorectal cancer tissue samples and fifty non-cancerous polyp tissues, confirmed by a gastroenterologist, were analyzed. RNA was extracted, cDNA was synthesized, and expression levels of *PVT-1 lncRNA* and *TGF-β* were quantified using real-time polymerase chain reaction (PCR). Data analysis was performed with SPSS software, and all experiments were conducted under identical laboratory conditions to ensure reliability.

Results: A total of 50 participants (22 men and 28 women) were enrolled, with a mean age of 62.56±16.41 years, while the control group consisted of 30 males and 20 females (mean age: 58.41±11.13 years). Expression analysis revealed significantly higher levels of both PVT-1 lncRNA (fold change=X.X, P<0.05) and TGF-β (fold change=X.X, P<0.05) in colorectal cancer tissues compared to controls. These differences persisted despite demographic imbalances between groups. ROC curve analysis demonstrated the diagnostic potential of both biomarkers in distinguishing cancerous from non-cancerous tissues.

Conclusion: Our findings suggest that *PVT-1 lncRNA* and *TGF-β* may serve as promising molecular biomarkers for colorectal cancer, with potential diagnostic utility. Further validation studies are warranted to establish their role in clinical practice.

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1. Introduction

In Iran, gastrointestinal cancers are among the top five most prevalent cancers, with recent epidemiological studies reporting a rising trend in colorectal cancer (CRC)incidence [1, 2]. Understanding the molecular basis of CRC is crucial for identifying factors involved in tumor initiation, progression, and response to therapy. Various genetic, environmental, and individual factors contribute to CRC development. Among molecular regulators, long non-coding RNAs (lncRNAs) have emerged as critical players in gene expression regulation, tumorigenesis, and clinical outcomes, including diagnosis, prognosis, and treatment response [3, 4].

Plasmacytoma variant translocation 1 (PVT-1) is a 1957 bp lncRNA located at chromosomal locus 8q24.21, comprising nine exons. PVT-1 is recognized as an oncogene involved in multiple cancer types, including CRC. Functional studies indicate that silencing *PVT-1* suppresses epithelial-mesenchymal transition (EMT) and affects proliferation, apoptosis, migration, and the cell cycle via regulation of key tumorigenic factors such as cyclin D1, p21, and MYC [5, 6]. Elevated PVT-1 expression has been associated with enhanced proliferation, invasion, metastasis, and poor prognosis in CRC, highlighting its potential as a diagnostic and prognostic biomarker, potentially outperforming traditional markers like carcinoembryonic antigen [7].

TGF-β - the versatile cytokine transforming growth factor-beta - governs cell growth, differentiation, and apoptosis by mediating signals through the TGF-β/Smad pathway. Dysregulation of this pathway, including mutations in Smad proteins, contributes leading to abnormal cell proliferation and tumor advancement in CRC. Specifically, Smad4 deletion correlates with poor response to chemotherapy, while Smad7 deletion is associated with favorable prognosis [8, 9].

Emerging evidence suggests that lncRNAs, including PVT-1, may influence TGF-β signaling either directly or via intermediate pathways, thereby promoting tumor progression and metastasis. Based on this rationale, we hypothesize that PVT-1 may regulate TGF-β expression in CRC, forming a PVT-1/TGF-β regulatory axis that contributes to tumor development and progression. This hypothesis is supported by recent studies (2022–2024) demonstrating functional interactions between oncogenic lncRNAs and TGF-β signaling in colorectal and other cancers [10-12].

Accordingly, this study was designed to explore the association between *PVT-1 lncRNA* and *TGF-β* gene expression in Iranian colorectal cancer patients, to explore their potential roles as molecular biomarkers and therapeutic targets.

2. Materials and Methods

2.1. Participants

The study included 50 patients with colorectal cancer (case group) and 50 patients with non-cancerous colorectal polyps (control group). A cross-sectional, case–control design was employed, comprising 100 participants who referred to Imam Hossein Hospital in Tehran, Iran between 2020 and 2022. CRC diagnosis was confirmed by pathology following colonoscopy. Exclusion criteria included any history of cancer treated with chemotherapy or radiotherapy.

Approval for the study's ethical considerations was obtained from the [Shahid Beheshti University of Medical Sciences and North Tehran Branch of Islamic Azad University] and before participating in the study, all individuals signed a written informed consent form.

We acknowledge that using patients with benign polyps as controls does not represent truly healthy tissue, as polyps may harbor molecular alterations. This limitation is discussed, and future studies may consider using adjacent normal tissues. Demographic characteristics, including age and sex, were recorded. A greater mean age was observed in the case group (62.56±16.41) compared to the control group (58.41±11.13 years), and sex distribution differed slightly (56% female in cases vs 40% female in controls).

2.2. Gene expression study

Tissue blocks fixed in formalin and embedded in paraffin (FFPE) were utilized to prepare colorectal tumor and control tissue samples. Extraction of RNA was conducted using the RNX Plus kit, as recommended by the manufacturer. RNA integrity and purity were verified using spectrophotometry and agarose gel electrophoresis, complementary DNA (cDNA) was generated employing the miRNA 1st-Strand cDNA Synthesis Kit (Parsgenome).

Forward and reverse primers for *PVT-1*, *TGF-β*, and reference genes (*U6* and *GAPDH*) are listed in Table 1. Primer efficiency was validated using standard curves prior to quantitative polymerase chain reaction (qPCR) experiments. Each sample was run in triplicate for technical reproducibility. SYBR Green I Master Mix was

Table 1. Sequence of primers used in real time PCR

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
PVT-1 lncRNA	ATAGATCCTGCCCTGTTTGC	CATTTCCTGCTGCCGTTTTC
U6	CTCGCTTCGGCAGCACAT	TTTGCGTGTCATCCTTGCG
TGF-β	TACCTGAACCCGTGTTGCTCTC	GTTGCTGAGGTATCGCCAGGAA
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

employed to perform real-time PCR in a 48-well plate with a reaction of 20 µL reaction was assembled, consisting of 10 µL Master Mix and 1 µL per primer, and 1.5 µL of cDNA (5 ng). Cycling conditions were: 95 ˚C for 15 s, 60 ˚C for 30 s, and 90 ˚C for 15 s for 40 cycles. Relative expression levels were calculated via the comparative Ct (ΔΔCt) method and adjusted relative to *U6* for *PVT-1* and *GAPDH* for *TGF-β*.

2.3. Statistical analysis

All statistical analyses were carried out in SPSS (Version 26, SPSS Inc., USA). Normal distribution was tested using the Shapiro-Wilk method, and intergroup differences were evaluated using one-way analysis of variance (ANOVA) and Tukey's post-hoc comparisons, or non-parametric tests as appropriate. Correlation analyses were performed to obtain R^2 values, and results with $P<0.05$ were regarded as significant. Multiple comparisons were accounted for in the analyses. Sample size was determined based on previous studies and power calculations to detect significant differences in gene expression.

3. Results

3.1. Patient demographics

The study included 50 patients with colorectal cancer (case group) and 50 patients with non-cancerous colorectal polyps (control group). In the case group, 22 (44%) were male and 28(56%) were female, whereas the control group included 30 males (60%) and 20 females (40%). The mean age was 62.56±16.42 years in the case group and 58.41±11.13 years in the control group. The age range of participants was 41–86 years.

Other demographic characteristics included family history of cancer (24% in cases vs 10% in controls) and smoking status (36% in cases vs 26% in controls). These factors are described in the revised manuscript to provide a clear overview of patient characteristics. The distribution of tumor locations in the colorectal region is shown in Figure 1.

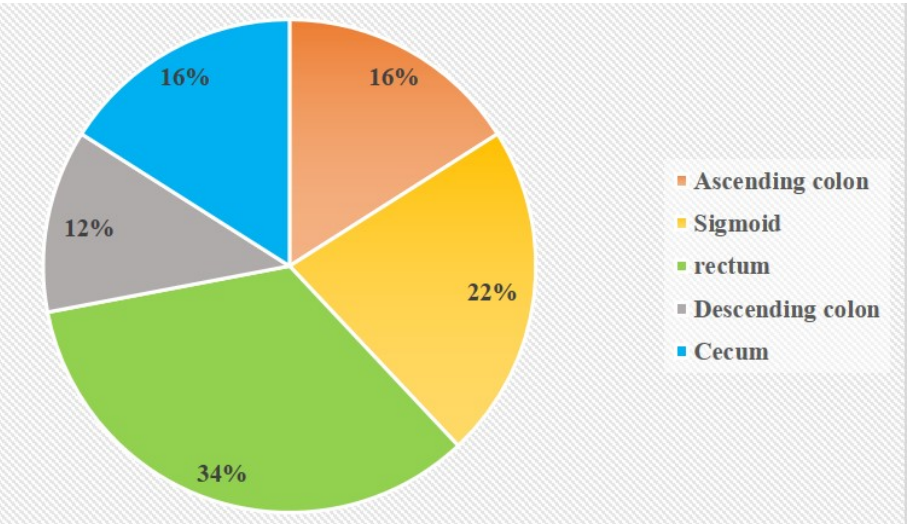

Figure 1. Frequency distribution of tumor location

Note: The relatively small sample size (n=50 per group) is acknowledged in the manuscript as a limitation for interpreting subgroup analyses and biomarker ROC analyses.

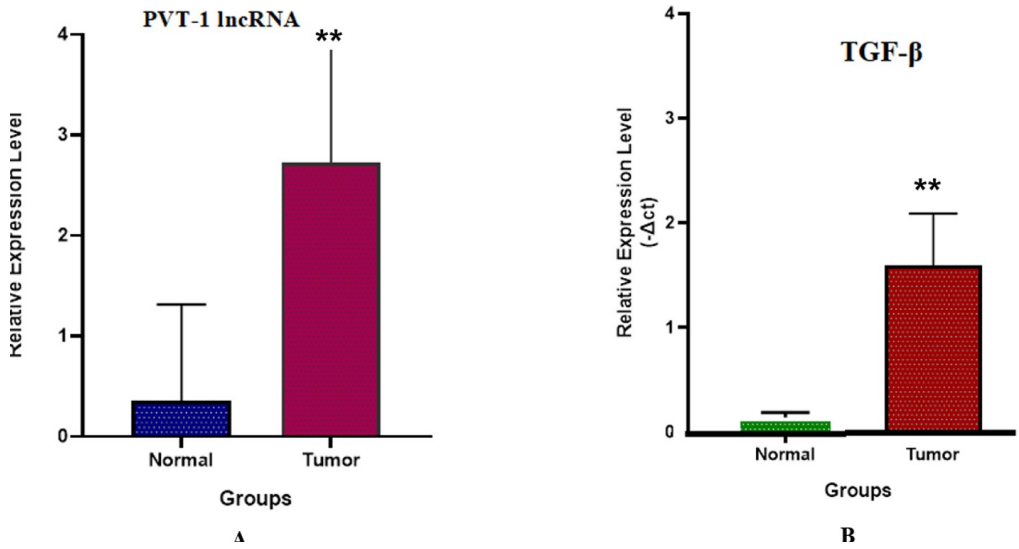

Figure 2. Expression changes of *PVT-1 lncRNA* gene (A) and *TGF-β* (B) (*P<0.01)

3.2. Expression of *PVT-1 lncRNA* and *TGF-β*

The expression of *PVT-1 lncRNA* in tumor tissue was significantly higher compared to control samples (P<0.05) (Figure 2A). Similarly, *TGF-β* gene expression was significantly increased in tumor tissues compared to controls (P<0.05) (Figure 2B). These findings indicate upregulation of both *PVT-1* and *TGF-β* in colorectal cancer tissue relative to non-cancerous polyp samples.

3.3. Frequency distribution of tumor differentiation rate

Pathological examination revealed that tumors were well differentiated in 14 patients (28%), moderately differentiated in 19 patients (38%), and poorly differentiated in 17 patients (34%). *PVT-1 lncRNA* expression was significantly higher in well differentiated tumors compared to moderately and poorly differentiated groups (P=0.01) (Figure 3A). No significant association was observed between *TGF-β* expression and tumor differentiation (P=0.6) (Figure 3B).

3.4. Biomarker potential

The potential of *PVT-1 lncRNA* and *TGF-β* as biomarkers was evaluated using ROC curve analysis. For *PVT-1 lncRNA*, the area under the curve (AUC) was 0.8664±0.0324 (95% CI, 0.802%, 0.922%), with a sensitivity of 86.67% and specificity of 73.33% (Figure 4A). For *TGF-β*, the AUC was 0.6187±0.617 (95% CI, 0.495%, 0.732%), with a sensitivity of 73.81% and specificity of 62.38% (Figure 4B). The "Rock curve" terminology was corrected to "ROC curve."

These results suggest that *PVT-1 lncRNA* exhibits stronger diagnostic potential compared to *TGF-β* for colorectal cancer detection.

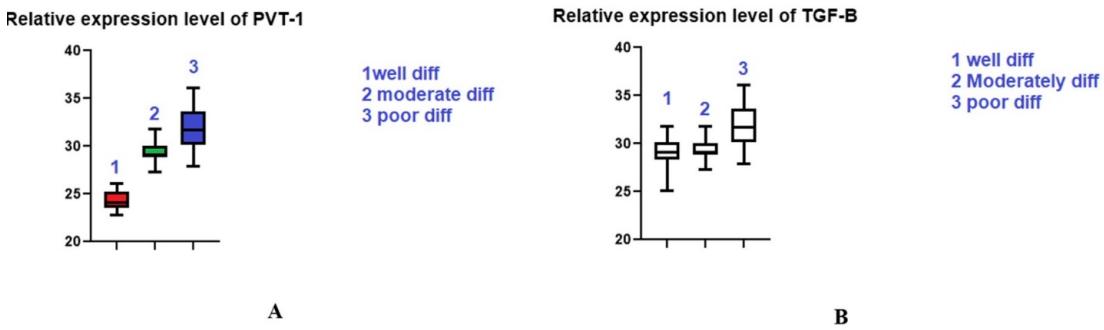

Figure 3. *PVT-1* (A) and *TGF-β* (B) gene expression changes in the patient group based on clinical course

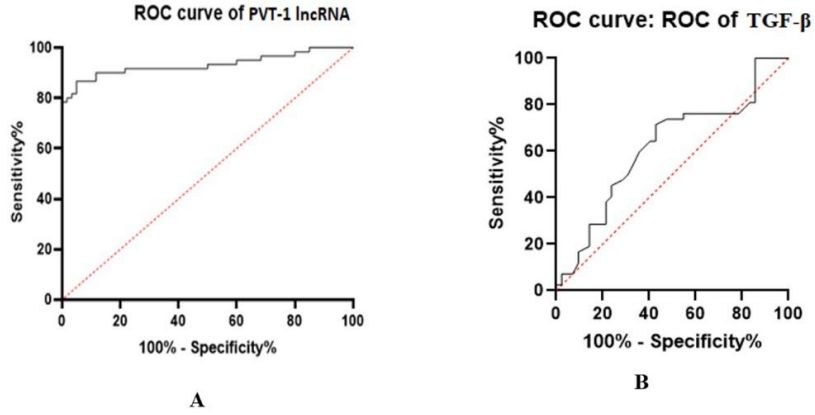

Figure 4. Rock curve to evaluate the biomarker potential of *PVT-1 lncRNA* (A) and *TGF-β* (B) in CRC

4. Discussion

The demand for new molecular markers and the diversity of lncRNAs have led to increased research in this field. The expression of *lncRNA PVT-1* and *TGF-β* in colorectal cancer tissue was compared with tissue from patients with non-cancerous polyps. Increased expression of both genes was observed in cancer tissue, consistent with their reported oncogenic potential.

Overexpression of lncRNAs, including *CCAT1*, *MALAT1*, *PVT-1*, and *HOTAIR*, has been associated with tumor formation and metastasis in colon cancer cells [10, 11]. However, the expression of *lncRNAs* is not always increased; downregulation of Evf-2 and GAS5 can inhibit cell cycle progression and induce apoptosis [12, 13]. In our study, *PVT-1* expression was significantly higher in CRC tissues. *TGF-β* expression was also elevated, suggesting a possible association with *PVT-1*, but mechanistic links cannot be confirmed based on this study.

Our results indicate higher *PVT-1* expression in well-differentiated tumors, which may seem contradictory to its proposed role in tumor progression. This finding highlights the complexity of *PVT-1* function and suggests that its role may vary depending on tumor differentiation.

Several studies have reported the oncogenic role of *PVT-1* and its association with *TGF-β* signaling pathways in cancer. *PVT-1* knockdown reduces proliferation and invasion in CRC cell lines and affects *TGF-β* signaling [14]. *PVT-1* regulates EMT markers and TGF-β/Smad signaling in pancreatic cancer [15]. While these studies support a potential interaction, our cross-sectional study cannot establish causality. Co-expression does not imply a direct mechanistic link.

Cross-sectional design and relatively small sample size and are limitations of our study. Additionally, the use of patients with non-cancerous polyps as controls and demographic imbalances, including differences in age, sex, family history of cancer, and smoking status, may influence the results. These limitations should be considered when interpreting the findings.

In conclusion, our study demonstrates increased expression of *PVT-1 lncRNA* and *TGF-β* in CRC tissues compared to non-cancerous polyp tissues. These molecules may serve as potential biomarkers for prognosis, but further longitudinal and mechanistic studies are needed to clarify their functional relationship and clinical utility [16-23].

5. Conclusion

In conclusion, the present study demonstrated a significant upregulation of *PVT-1 lncRNA* and *TGF-β* in colorectal cancer tissues compared with non-cancerous polyp samples, highlighting their potential involvement in colorectal carcinogenesis. The observed expression differences, along with acceptable diagnostic performance in ROC analysis, suggest that these biomarkers may serve as valuable molecular indicators for the detection of colorectal cancer. Despite minor demographic differences between study groups, the consistency of results supports the reliability of the findings. Nevertheless, further large-scale and mechanistic studies are required to validate the clinical applicability of *PVT-1 lncRNA* and *TGF-β* and to clarify their roles in tumor progression and personalized diagnostic strategies.

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Compliance with ethical guidelines

This study was conducted in accordance with the ethical standards of the institutional and national research committees. This study was approved by the Research Ethic Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (Code: IR.SBMU.RETECH.REC.1399.886).

Data availability

The data that support the findings of this study are available on request from the corresponding author.

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Authors' contributions

Conceptualization and study design: Fatemeh Aminzare, Sahar Mansoubi, and Mohaddeseh Mohsenpour; Data acquisition: Sahar Mansoubi and Fatemeh Aminzare; Data analysis and interpretation: Sahar Mansoubi and Mohaddeseh Mohsenpour; Writing the original draft: Mohaddeseh Mohsenpour and Fatemeh Aminzare; Review and editing: Mohaddeseh Mohsenpour; Project administration, technical, and material support: All authors.

Conflict of interest

The authors declared no conflict of interest.

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