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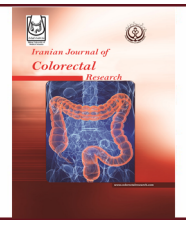

Expression Levels of the *YAP*, *TAZ*, and *TEAD1* Hippo Pathway Genes in Colorectal Villous Adenoma Polyps

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Abstract

Background: Villous adenoma polyps are associated with a high risk of developing Colorectal Cancer (CRC). The Hippo signaling pathway is related to CRC as it regulates cell proliferation and apoptosis; however, the role of this pathway in different adenoma polyps and its relationship to CRC development is not yet fully discovered.
Methods: Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was used to examine the mRNA expression levels of *YAP*, *TAZ*, and *TEAD1* in 30 pairs of villous adenomas polyps' tissues and adjacent normal tissues. In addition, the correlation between clinicopathologic features and the *YAP*, *TAZ*, and *TEAD1* expression in villous adenoma polyps patients and the related Receiver Operating Characteristic (ROC) curves were evaluated.
Results: The mRNA expression levels of *YAP* (P<0.01), *TAZ* (P<0.0001), and *TEAD1* (P<0.0001) were higher in villous adenoma polyps' tissues compared with adjacent normal tissues. Statistical analysis showed that the expression levels of *YAP*, *TAZ*, and *TEAD1* were associated with lymph node metastasis, while only *TAZ* was associated with the TNM stage (I-II). Moreover, *YAP*, *TAZ*, and *TEAD1* may have the potential to distinguish villous adenoma polyps from the adjacent normal tissues, given the large area under the ROC curve values (0.74, 0.70, and 0.68, respectively).
Conclusion: Our study highlights that the upregulation of *YAP*, *TAZ*, and *TEAD1* genes in the Hippo pathway can affect the progression of villous adenoma polyps to CRC and can be a potential target for further investigation, representing a novel biomarker for patients with villous adenoma polyps.

Keywords: Cancer, Hippo signaling pathway, Colorectal polyps

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Introduction

Colorectal Cancer (CRC) is the third cause of cancer-related death and the most common type of gastrointestinal cancer, with more than two million diagnosed patients in 2020 worldwide (1). Based on the heterogeneous nature of CRC, several factors can affect its initiation and proliferation, including family history, age, diet, drinking alcohol, cigarette smoking, and genetic or epigenetic alterations (2, 3). Most CRCs are formed by lesions known as polyps, which develop from the inner lining of the colon tissue into the lumen (4). Polyps are mostly considered benign (non-cancerous) growths, but

in some cases, they are categorized as pre-cancer polyps as they can accelerate CRC formation (4-6). Colon polyps are commonly found on routine CRC screening and their prevalence increase with age. Adenoma polyps are the primary type of colon polyps, including different growth patterns that can be seen under the microscope by the pathologist. Adenomatous polyps are conventionally classified as tubular, villous, or tubulovillous (7). The most well-known patterns are tubular and villous; most small adenomas (less than ½ inches) have a tubular growth pattern, while larger adenomas may have a villous growth pattern.

Adenomas with a villous growth pattern are also more likely to be associated with developing more advanced neoplasia. In addition, a mixture of both growth patterns has been reported in some polyps, referred to as tubule-villous adenomas (4, 7, 8). Despite the recent discoveries in carcinogenesis pathways and epigenetic changes in CRC, there is not much information available about the role of different types of adenoma polyps in CRC initiation and proliferation (9). The Hippo signaling pathway, also known as the Salvador-Warts-Hippo (SWH) pathway, with downstream transcription coactivators (*TAZ* and *YAP*), is a signaling pathway that plays a critical role in tissue homeostasis, angiogenesis, and organ size through modifying tissue-specific stem cells. This pathway takes its name from one of the Hippo protein kinases, which is a key element (10-12). The Hippo pathway is also important in stem cells, self-renewal, and tissue-specific progenitor cells (13, 14). This signaling pathway has received significant attention in the study of human cancer since many cancers are marked by unchecked cell division. The dysregulation of the Hippo pathway plays an essential role in cell growth, proliferation, apoptosis, and tumorigenesis, with its suppression reported in many cancers, such as colorectal and lung cancer (15-17).

TAZ is a transcriptional coactivator that interacts with multiple transcription factors and plays different functions by modifying the differentiation of stem cells and the development of multiple organs. Recently, *TAZ* has been identified as a major element of the Hippo pathway, playing its oncogenic role during tumorigenesis by accelerating proliferation, migration, and invasion (10, 18) YAP1 (Yes-Associated Protein 1) is a protein that plays a transcriptional regulatory function by activating the transcription of genes involved in cell proliferation and suppressing apoptotic genes. YAP1 is inhibited by the Hippo signaling pathway, allowing the cellular control of organ size and tumor suppression (14). Transcriptional Enhancer Activator Domain 1 (TEAD1) is a protein encoded by the *TEAD1* gene in humans and a member of the TEAD family, which affect proliferation and apoptosis (11). The TEAD family was first discovered through the purification and cloning of transcriptional factors in Simian Virus 40 (SV40) (17, 19). *YAP* and *TAZ* can promote the expression of target genes by combining with TEA Domain Family Member 1 (TEAD1) in the nucleus. In this study, we aimed to investigate the expression of Hippo pathway-related genes, including *YAP*, *TAZ*, and *TEAD1*, in Iranian villous adenoma polyps to provide new information about potential biomarkers for CRC detection.

Materials and Methods

Ethics, Consent, and Permissions

This study was approved by the Ethics Committee of Tehran University of Medical (IR.TUMS.VCR.REC.1400-220). Written consent was obtained from all patients who were informed that the data would be used for research purposes. Patients who had undergone chemotherapy and radiotherapy were eliminated from the study.

Patients and Tissue Specimens

Thirty villous adenoma polyps and 30 adjacent normal tissues were obtained from Iranian patients diagnosed with villous adenoma polyps during 2019-2020. The specimens were verified by a pathologist and were immediately frozen in liquid nitrogen and stored at −80 °C.

RNA Extraction, cDNA Synthesis, and qRT-PCR Reaction

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to evaluate the expression levels of selected Hippo pathway components. Total RNA was extracted from the specimens using Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol, and RNA quality was determined by OD26/OD280 measurements. *GAPDH* was used as the housekeeping gene as the control. The cDNA synthesis was carried out using TaKaRa Primer Script™ RT-PCR. The qRT-PCR was performed with SYBR® Premix Ex Taq™ II (TaKaRa) according to the manufacturer's protocol by Roche Light Cycle Real-time PCR (Roche, Basel, Switzerland). Reactions performed in a total volume of 20 µl (containing 2×SYBR II 10 µl, forward primer 1.5 µl, reverse primer 1.5 µl, ddH_2O 4.5 µl, and cDNA 2 µl) with the following amplification steps: 95 °C for 30 s followed by 40 cycles of denaturation at 95°C for 5 s and annealing at 60°C for 30 s. The relative expression levels (−ΔCt) were calculated. Primers for this PCR were designed with Primer3 software and are listed in Table 1.

Statistical Analysis

Statistical analysis was undertaken using GraphPad Prism 8. One-way analysis of variance (ANOVA) was used for comparisons between the experimental group and control; all results are expressed as mean±SD. Correlations were analyzed by Pearson's correlation test. The t-test was used to compare

Table 1: The sequence of primers used in the real-time polymerase chain reaction (RT-PCR) assays

Genes	Forward	Reverse	Amplicon size (bp)
YAP	5'TGAACAACGTCCACCAAGATAC3'	5'CAGGCCCCAAAATCAACAGTAG3'	150
TAZ	5'CTTGGATGTAGCCATGACTGG3'	5'TCAATCAAAACCAGGCAATG3'	165
TEAD1	5'AATCCCACCGCCAACATTGAGC3'	5'TAGGATACATTTGCCTTCGTC3'	220
GAPDH	5'AACGGCAAGCTTGTCATCAATGG AAA3'	5'GCATCAGCGAGGGGGCAGAG3'	180

bp: Bases pair

the means of two samples, and one-way analysis of variance (ANOVA) was used to compare two or more independent groups. The diagnostic values of our candidate genes were evaluated by Receiver Operating Characteristic (ROC) curves analysis, and the Area Under the ROC Curve (AUC) was considered a critical diagnostic value. The optimal cut-off value, sensitivity, and specificity were determined by calculating the Youden index. P<0.05 was considered statistically significant.

Results

The Relative Expression Levels of Hippo Pathway Molecules

In this study, the expression levels of candidate genes from the Hippo pathway were evaluated by RTqPCR. Our results showed that the mRNA expression levels of *TAZ*, *YAP*, and *TEAD1* increased in villous adenoma polyps relative to adjacent normal tissues (Figure 1).

Hippo Pathway Components Associated with the Clinicopathologic Factors

We investigated the relationship between expression levels of *TAZ*, *YAP*, and *TEAD1* and clinicopathological data. The results showed that higher expression levels of *TAZ*, *YAP*, and *TEAD1* were significantly associated with lymph node metastasis (*TAZ* and *YAP*, P<0.01; *TEAD1*, P<0.05), while only *TAZ* expression was significantly associated with the TNM stage (I-II) (P<0.01). In addition, there was no significant relationship with age, gender, and location of cancer (Table 2). We also evaluated the correlation between higher expression of *YAP* with *TAZ* and *TEAD1*. The results showed that higher expression of *YAP* was correlated with higher expression of *TAZ* (Pearson r correlation coefficient 0.690, P<0.001) and *TEAD1* (r: 0.822, P<0.001) in villous adenoma polyps of our samples.

The Potential Diagnostic Values of TAZ, YAP, and TEAD1

To analyze the potential diagnostic values of candidate genes (*TAZ*, *YAP*, *TEAD1*), ROC curve analysis was performed. A larger Area Under the ROC Curve (AUC) shows a higher diagnostic value. The AUC, sensitivity, and specificity of *TAZ*, *YAP*, and *TEAD1* are shown in Figure 2.

Discussion

Recent studies have discovered that CRC can develop from different adenoma polyps. A villous adenoma is associated with a higher risk of becoming malignant and turning into cancer, among other polyps, based on its dysplasia growth pattern over time (8, 20-22). The Hippo signaling pathway is a very conserved pathway with a tumor suppressor role, regulating diverse cellular processes. Dysregulation of the Hippo pathway and its components results in an overgrowth phenotype and has been frequently reported in different cancers, including gastric and colorectal cancer (14, 15). The Hippo pathway includes a kinase cascade component (Lats1/2 and Mst1/2) that can regulate nuclear *YAP*/*TAZ* activity; therefore, its inactivation can promote CRC development from villous adenoma polyps (17, 18).

The expression of *YAP*/*TAZ* has been upregulated in lung, prostate, and colorectal cancers, while their inhibition has significantly decreased growth and invasion and induced apoptosis. *YAP* and its paralog

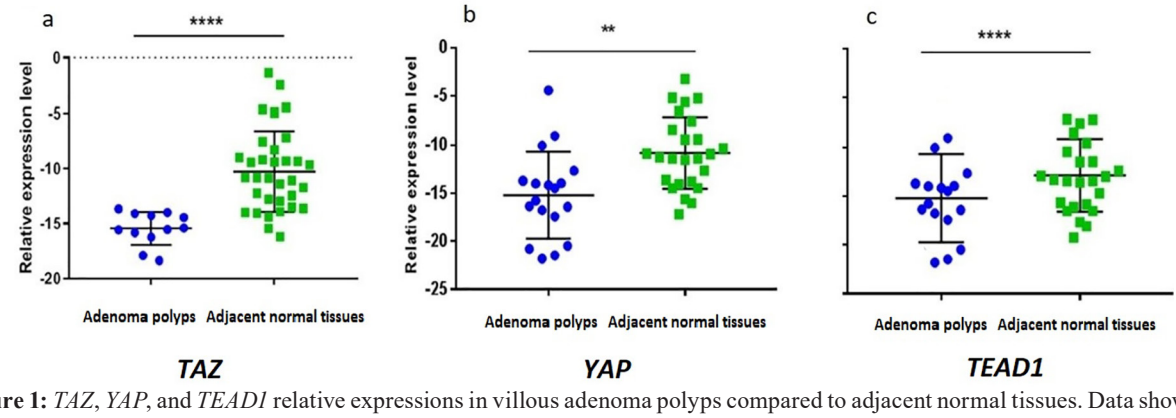

Figure 1: *TAZ*, *YAP*, and *TEAD1* relative expressions in villous adenoma polyps compared to adjacent normal tissues. Data show that *TAZ*, *YAP*, and *TEAD1* were upregulated significantly compared with adjacent normal tissues (**P<0.01, ****P<0.0001).

Table 2: The correlation of *TAZ*, *YAP*, and *TEAD1* expression levels (-ΔCt) with clinicopathological features of the patients

Parameters	No. of patients (%)	*YAP* Mean±SD	P value	*TAZ* Mean±SD	P value	*TEAD1* Mean±SD	P value
Sex							
Male	12	2.816±0.245	0.31	3.411±0.244	0.27	3.344±0.159	0.51
Female	18	3.110±0.285		3.798±0.241		3.445±0.200	
Age							
< 45	10	2.787±0.232	0.42	3.440±0.194	0.33	3.343±0.134	0.51
> 45	20	3.019±0.312		3.753±0.251		3.453±0.215	
Polyp location							
Right	11	3.216±0.342	0.14	2.342±0.550	0.08	2.402±0.162	0.11
Left	10	2.716±0.221		3.452±0.258		3.446±0.157	
Rectum	9	2.097±0.252		4.357±0.115		3.921±0.106	
Polyp size							
<45 mm	9	1.551±0.155	0.15	4.18±0.178	0.11	2.190±0.193	0.21
>45 mm	21	1.369±0.172		3.1479±1.168		2.468±0.264	
Lymph node metastasis							
Positive	13	3.452±0.343 **	P<0.01	3.806±0.261**	P<0.01	3.451±0.146*	P<0.05
Negative	17	2.816±0.225		3.414±0.185		3.231±0.147	
TNM stage							
I-II	14	2.592±0.250	0.24	2.115±+0.20**	P<0.01	2.939±0.228	0.12
III-IV	16	3.327±0.249		2.587±0.264		3.282±0.221	

P value: probability value; P value was evaluated with the t-test between two groups and analysis of variance (ANOVA) for three groups (*P<0.05, **P<0.01).

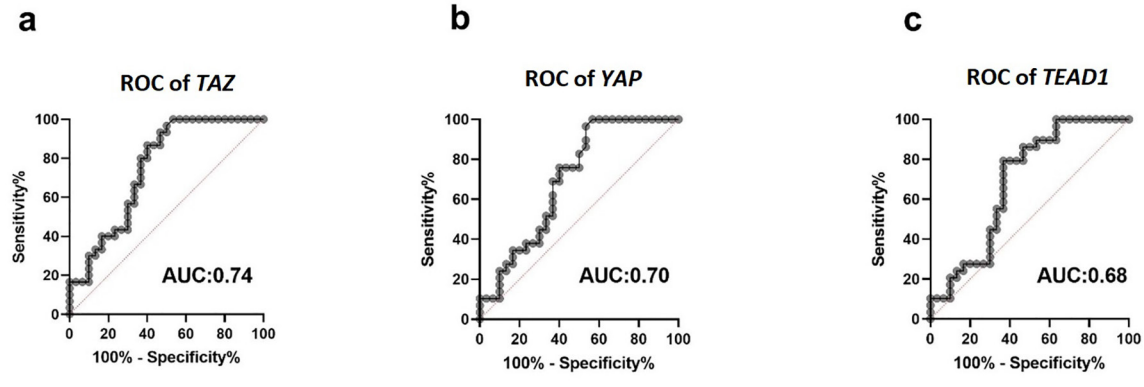

Figure 2: Receiver Operating Characteristic (ROC) curve analysis revealed the potential diagnostic values of *TAZ*, *YAP*, and *TEAD1* in villous adenoma polyps. The sensitivity and specificity were (a) 0.63 and 0.66 for *TAZ*, (b) 0.62 and 0.63 for *YAP*, and (c) 0.58 and 0.63 for *TEAD1*, respectively.

TAZ have been suggested to be core elements involved in the regulation of the Hippo signaling pathway in tumor cells, promoting initiation, transition, and development of CRC tumors after combining with *TEAD1* (23). *YAP* was also suggested as a prognostic biomarker for overall survival and associated with tumor differentiation in hepatocellular carcinoma patients (24). The overexpression of *YAP* in the intestine promotes tumorigenesis in CRC through the inactivation of MST1 and MST2 (10, 25). Therefore, the overexpression of *YAP* or *TAZ* might lead to excessive cell proliferation, adenoma formation, and CRC progression, representing potential targets for selective CRC tumor therapy (23).

In this study, we evaluated the expression level of selected genes from the Hippo signaling pathway in villous adenoma polyps and analyzed the correlation between the expression of candidate genes and their relation with other clinicopathological characteristics. We found that *YAP*, *TAZ*, and *TEAD1* overexpression positively correlated with lymph node metastasis, while only TAZ expression was correlated with the TNM stage (I-II). In addition, the ROC curve analysis showed the diagnostic potential for *TAZ*, *YAP*, and *TEAD1* based on their high AUC values (0.74, 0.70, and 0.68, respectively), suggesting them as novel diagnostic biomarkers for distinguishing patients with villous adenoma polyps.

Conclusion

Overexpression of *YAP*, *TAZ*, and *TEAD1* is related to lymph node metastasis of villous adenoma polyps and may increase the risk of CRC. Further studies should be done to uncover their roles in carcinogenesis and tumorigenesis.

Acknowledgments

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Data Availability

Data supporting the findings of this study are available on request from the corresponding author.

Ethics Approval and Consent to Participate

This research was approved by the Ethics Committee of Tehran University of Medical Sciences and followed the ethical guidelines published by the Ministry of Health.

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Conflicts of interest: None declared.

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