The possible role of high risk HPV infection and RPD3 overexpression in Iraqi patients with colorectal cancer

Hujaz Ismail Abdulrazzaq Alqirbi  
Department of Microbiology, Al-Kindy College of Medicine, Baghdad University, Iraq

Heba F. Hassan  
Department of Basic Sciences, College of Dentistry, University of Baghdad, Iraq

Basim Mohammed Khashman  
National cancer research center (NCRC), University of Baghdad, Iraq  
*Corresponding author email: basim@bccru.uobaghdad.edu.iq

Abstract---Aims: the aim of this study is RPD3 expression in clinicopathological characteristics in CRC patients and to study the existence of a relationship between CRC and HPV in samples of Iraqi patients. Material and methods: 30 samples of formalin-fixed, paraffin-embedded archival tissues from CRC patients were obtained, together with 10 samples of normal tissue (free cancer), for immunohistochemical analysis of RPD3 and HPV expression. Results: Immunohistochemical (IHC) staining revealed only four cases (15%) with HPV infections while PRD3 was expressed in 19 CRC samples (63.3 percent). The PRD3 expression ratio. It was found in only four (15%) of the CRC samples. At ≤ P 0. 05, there is no significant difference. Conclusion: The results of this study support RPD3 as a supplementary marker to identified histologic screening components; it could possibly be used for the prognosis and targeted therapy. In addition, we discovered a relation with both HPV infection and CRC as oncovirus and tumorigenic factor.

Keywords---HPV, CRC, RPD3IHC, oncogenesis.

Introduction  
The third most prevalent form of tumor is colorectal cancer (CRC) diagnosed in (6.1%) and the second leading cause of death (9.2 %). It is predicted that by 2035, the total number of deaths from CRC will increase from (60% -71.5%)[1]. It is
widely recognized that disease is a sign of a country’s socio-economic status development [2]. Lifestyle, body obesity, and food trends all have a role in the rise in morbidity [3]. Physical activity appears to have a protective impact, according to the data. As the frequency of red color and its treatment rises, so does the danger of acquiring the condition, meat and alcoholic beverages are two of the most popular foods in the world [2,4]. According to the most recent WHO data from 2018, the predicted mortality rate from CRC cancer in Iraq was 6.30 percent.

Despite the fact that the death rate for colorectal cancer has increased in various Arab nations (5.64% Oman, 7.14% Saudi Arabia 13.92% Jordan, 14.88% Syria, 8.14% Qatar and 4.23% Egypt) [5]. CRC is a multi-step process that frequently takes more than ten years to develop, This implies that opportunities for early detection and prevention exist [6]. RPD3 complex catalytic component. RPD3 enzymes are important regulators of basic biological activities as (cell cycle, differentiation & apoptosis) [7]. RPD3 is a member of the subfamily that is dysregulated in CRC and can be used as a supplemental genomic identifier for histological detection as well as a prognostic biomarker for the disease [8]. RPD3 a player in the evolution and spread of CRC [9]. Overexpression PRD3 has been linked to CRC proliferation and invasion [10]. According to numerous studies, elevated of PRD3 expression and activity are linked to cancer-related cell epigenetic changes [11,12] Infections with (HPV) human papillomavirus are the most prevalent sexual transmission viruses. and epidemiological data between HPV with CRC is controversial and CRC’s pathogenesis is still unknown, despite the fact that number of studies have mentioned HPV may have a consequence on the progression of CRC [13]. In a review article published in 2011, HPV occurrence in CRC tissues was confirmed at 41.7 %, compared to 32.0 percent in neighboring normal tissues [14]. While HPV DNA was not found in CRC tissues in several studies [15,16]. Therefore, the aim of this study is RPD3 expression in clinicopathological characteristics in CRC patients and to study the existence of a relationship between CRC and HPV in samples of Iraqi patients.

Materials and Methods

Immunohistochemistry assay of RPD3 and HPV expression

Thirty samples of formalin-fixed, paraffin-embedded archival tissues from CRC patients (12 female &18 male) were obtained from the Liver and Digestive System Technical Hospital in Baghdad city between the months of February 2020 and September 2021. A pathologist examined the CRC samples to determine the grade of CRC. When cut, each block was 4 mm thick and was applied to positively charged slides. To be stained with hematoxylin and eosin. The first tissue section was put on a regular slide. Other sections were immunohistochemically staining using anti- RPD3 and HPV antibody from (ABCAM company/ United Kingdom) according to manufacturer’s protocol [17].

Determine of Immunohistochemistry

Absence of immunostaining on CRC tissue indicates a negative reading, whereas cells with brown cytoplasmic coloration reveal a positive reading. Using a light
microscope and a scoring system that considered both antibody strength and the frequency of positive cells [17].

**Statistical Analysis**

To determine the impact of various components, the Statistical Analysis System-application was used in research parameter to make a significant comparison between means, the Least Significant Difference –LSD test (ANOVA) was performed. The Chi-square test was used to analyze percentages in this study (0.05 and 0.01 probability) [18].

**Results and Discussions**

thirty specimens (12 females & 18 males) were collected from patients with CRC with grade G2 and their ages ranged from (22 to 79) year, with an average of 55.5 year. They all had tumor adjacent normal (TAN) samples that matched. A method was used to detect expression of RPD3 by using Immunohistochemistry. High levels of RPD3 expression were determine in this investigation showed strong brown staining was seen in most cells with positive RPD3 expression by Immunohistochemical (IHC) staining was found in 19 samples (63.3%) of CRC were strongly positive with a significant increased at $P < 0.05$ (Table 1, Fig. 1).

Table 1 Immunohistochemistry expression of PRD3 and HPV

<table>
<thead>
<tr>
<th></th>
<th>PRD3 No. (%)</th>
<th>HPV No. (%)</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>19 (63.3%)</td>
<td>4 (15%)</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>11 (36.7%)</td>
<td>25 (85%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Chi-Square ($x^2$)</td>
<td>(P≤0.01)</td>
<td>Non-Significant</td>
<td></td>
</tr>
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</table>
Figure 1. Immunohistochemical results in Colorectal Carcinoma tissues. A (40x), B (100X): Positive expression of HPV in Colerectal carcinoma, C: positive expression of RPD3 (10x)

Discussion

Colorectal cancer (CRC) was still the 3rd most frequent tumor in people all over the world. CRC is among the most prevalent generates of tumors, and the patients' prediction with CRC particularly dictated by their diagnosis step. As a result, early identification is critical for extending life expectancy. The relevance of PRD3 in CRC is illuminated by determining whether RPD3 expression has clinical implications. The research suggests that PRD3 is a key component of the PRD family. Because of its potential to influence gene expression and function a RPD3 is a well-known epigenetic factor that is essential for diverse range of cellular functions and PRD3 promotes cell proliferation while inhibiting apoptosis, suggesting it could be a target for chemotherapeutic PRD3 inhibitors [19].

In this study was found high expression of PRD3 in CRC patients with (p < 0.05). This research has found that tumors samples exhibited greater levels of PRD3 protein expression than nearby normal tissue, and PRD3 expression was linked to poor differentiation, one of the clinicopathological features of advanced sickness. As a result, it has the potential to be used as a potential factor. to forecast a poor prognosis [20]. another study reveals that PRD3 expression levels are elevated through qRT-PCR data are linked to depressing prediction in CRC patients and suggest that PRD3 is a predictive marker such as CRC and could be used as a prospect therapy in the future. [8]. Because acetylation-mediated epigenetic
alterations are convertible, inhibitors PRD3 have a lot of promise as chemotherapeutic drugs. Several PRD3 inhibitors have anticancer effects in vitro and in vivo in a range of cancers [21,22]. HPVs had been identified as causal organism of invasive cervical tumor, with high-risk HPVs being found in (96 %) of these malignancies [23].

HPV pathogen can cause a change in the infected cell’s biochemistry which is intriguing given that high-sugar diets, as well as metabolic problems that cause overweight as a whole, had been linked to an elevated risk of CRC [24,25]. Numerous researchers, one of which was conducted by our group, have found that elevated HPVs, notably types 16, 18, 31, 33& 35 were viewable in patients CRCs [26,27]. It’s interesting to note that high-risk HPV infection was insufficient to cause malignant transformation changes in person with normal epithelial tissues. Multiple genetic alterations must also occur in infected cells or/and be infected by some other oncovirus to complete their conversion and as a result form tumors. Based on this theory a study was conducted utilizing human normal epithelial (HNE) cells to investigate the influence of collaboration of the high HPVs and other of the high-danger HPVs and other human oncogenes, carcinogenesis was found that high-danger type 16 E6/E7 oncoproteins collaborate with receptor of ErbB-2 to cause cellular conversion in HNE cells.

This had been aided by the delocalization of -catenin from either the plasma membrane towards the nucleus, and it was later discovered that the E6,E7&ErbB-2 collaboration targets cyclin D1 via the transformation of -catenin's activity from a cell-cell adhesion molecule to a transcriptional regulator by -catenin delocalization from the plasma membrane to the nucleus [28]. And also found that in human HNE and mouse normal embryonic fibroblast (NEF) cells, D-type cyclins D1, D2& D3 were required for cell changes triggered such as E6,E7&ErbB-2 collaboration [28,29]. Finally, reveal that in human normal epithelium and tumor cells, the cooperative influence of E6 and E7 through ErbB-2 is induce by -catenin tyrosine phosphorylation and activation of pp60 (c-Src) kinase [30]. Thus, collaboration with both E6&E7 oncoproteins from high-risk HPVs and other oncogenes may aid in the development of colorectal cancer [31].

Conclusion

This study's findings RPD3 as a supplementary marker to identified histologic screening components; it could possibly be used for the prognosis and targeted therapy. In addition, we discovered a relation with both HPV infection and CRC as oncovirus and tumorigenic factor.

References

and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin., 68, 394–424. [CrossRef]