Potential oncogenic role of human cytomegalovirus and histone deacetylase 3 expression in colorectal cancer

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

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

Yasir Basim Qaddoori
Department of Biology, College of Science, University of Baghdad, Iraq

Abstract---Aims: This study was conducted to investigate whether there is a link between HDAC3 expression and clinical characteristics in colorectal cancer (CRC) patients and to detect the oncopotential role of HCMV in colorectal cancer (CRC). Material and methods: 30 samples of formalin fixed-Paraffin embedded archival tissues were collected from patient suffering of CRC, In addition to 10 samples of free cancer tissue for determine of expression of HDAC3 and CMV by using immunohistochemistry. Results: HDAC3 expression by Immunohisto-chemical (IHC) staining was found in 19 samples (63.3%) of CRC. While there is no expression of CMV in all samples of CRC and control groups. Conclusion: This study proved HDAC3 expression pattern in CRC, and the findings support HDAC3 as a supplementary marker to known histopathological diagnostic components; it could potentially be used in prognosis and targeted therapy. We also found that there is no correlation of the CMV infection and CRC as a pro-tumorigenic factor.

Keywords---CMV, CRC, HDAC3, IHC, oncogenesis.
Introduction

The fourth most common cause of cancer death in both men and women, colorectal cancer is the third most common cancer in the world. Each year, more than 1.36 million new cases of the disease are identified worldwide, and more than 600,000 people pass away from it [1,2]. According to the latest WHO data published in 2018, the expected mortality in Iraq due to CRC cancer was 6.30%. While the death rate for colorectal cancer in some Arab countries recorded (13.92% Jordan, 14.88 %Syria, 4.23% Egypt, 5.64 %Oman, 7.14 %Saudi Arabia and 8.14% Qatar) [3]. Incidence of CRC are rising due to lifestyle and aging-related factors. However, a small number of CRC cases may be due to an underlying genetic disease, and the disease has a variable etiology that includes genetic and environmental factors as well as inflammatory bowel disease [4]. There are potential for early detection and prevention because the formation of CRC is a multi-step process that frequently takes place over a period of more than 10 years [5]. Histone acetylation is usually correlated with excess transcription, and regulatory Enzymes that catalyze the removal of acetyl groups from histones are known as histone deacetylases (HDACs).

The 11 HDACs can be classified according to their homology. I, II, and IV are the three classes. HDACs of Class I (1, 2, 3 and 8) play a significant role in the development of cancer and may be a candidate for therapy. Numerous cancer therapies have targets [6,7]. When compared to the nearby normal cells, Class I HDACs have an aberrant epigenetic status because they are overexpressed in malignancies and block certain tumor suppressor genes [8,9]. High HDAC3 expression and activity levels have been linked to cell epigenetic modifications linked to cancer according to research. [10,11]. However, the function of HDAC3 in CRC has yet to be determined. According to growing evidence, microorganisms may at least be extremely interesting cofactors in the oncogenesis and progression of colorectal cancer (CRC). Currently, at least 7 viruses have been recognized as human carcinogens; certain research have connected CMV with CRC.

Despite not being a fully acknowledged oncogenic virus. [12]. Although CMV is widespread in the community, it is more significant when there is immune suppression during pregnancy [13]. Asymptomatic hosts distribute CMV through a variety of bodily fluids, including saliva, urine and vaginal secretions [14]. Although there isn't as much epidemiological evidence linking CMV infection to CRC, There is epidemiologic and genetic proof that CMV causes ovarian, breast, and medulloblastoma tumors, among other malignancies [15]. CMV has however been discovered in CRC sections. Seven colon cancers from an early research were found to have CMV in 57 percent of them [16]. The goal of this study was to see if there was a link with HDAC3 expression and histopathologic traits in CRC patients, and determine expression of CMV in terms of developing latent infections, CMV can enhance oncogenesis.
Materials and Methods

Immunohistochemistry assay of HDAC3 and CMV expression

30 samples of formalin fixed-Paraffin embedded were collected from patient suffering of CRC (18 male and 12 female) randomly from the pathological records kept at the Gastrointestinal and liver teaching Hospital in Baghdad between January 2020 to October 2021. One pathologist evaluated the CRC samples to identify the grade of CRC. Each block was 4 mm thick when cut, and it was adhered to positively charged slides. To be used for hematoxylin and eosin staining, the first tissue section was mounted on a standard slide. Additionally, other sections were stained with anti-HDAC3 and CMV antibodies from the United Kingdom’s ABCAM firm on charged slides for immunohistochemistry, as per the manufacturer’s guidelines [17].

Determine of Immunohistochemistry finding

Immunostaining on CRC tissue that is absent indicates a negative reading, while cells that have brown cytoplasmic pigmentation indicate a positive reading. By employing a light microscope and a scoring system that took into account both the strength of the antibody and the frequency of positive cells [17]. To determine the effect of different components in research parameters, the Statistical Analysis System (SAS) application was utilized. To make a significant comparison between means, the Least Significant Difference –LSD test (ANOVA) was performed. In this study, the Chi-square test was utilized to compare percentages (0.05 and 0.01 probability) [18].

Results and Discussions

30 samples (18 males and 12 females) 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. All of them had tumor adjacent normal (TAN) samples that matched. A method was used to determine expression of HDAC3 by using Immunohistochemistry. High levels of HDAC3 expression were discovered in this investigation. Immunohistochemical staining revealed a strong brown staining in the majority of cells with positive HDAC3 expression (IHC) was found in 19 samples (63.3%) of CRC were highly positive with a significant increase at $P < 0.05$ (Table 1, Fig. 1). While in control group there was no positive results. While the second part of this research is the investigation of CMV infection evidence of oncogenic activity. CMV expression was discovered in this study’s findings. There is no expression of CMV in all samples of CRC and control groups (Table 1, Fig. 1).

Table 1
Immunohistochemistry expression of HDAC3 and CMV

<table>
<thead>
<tr>
<th></th>
<th>HDAC3 No. (%)</th>
<th>CMV No. (%)</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>19 (63.3%)</td>
<td>0 (0.00%)</td>
<td>0 (0.00%)</td>
</tr>
</tbody>
</table>
Figure 1. Immunohistochemical results in Colorectal Carcinoma tissues. A (10x), B (40X): Negative expression of CMV in Colorectal carcinoma, C: positive expression of HDAC3 (100x)

CRC it is considered one of the most common causes of tumor and Patients with CRC the prognosis is greatly determined by their diagnosis stage. As a result, early detection is crucial for boosting survival time. The relevance of HDAC3 in CRC is illuminated by determining whether HDAC3 expression has clinical implications. The research suggests that HDAC3 is a key component of the HDAC family. Because of its potential to influence gene expression and function a HDAC3 is a well-known epigenetic factor that is required for a wide range of cellular functions and HDAC3 promotes cell proliferation while inhibiting apoptosis, suggesting it could be a target for chemotherapeutic HDAC3 inhibitors. [19]. In this study was found high expression of HDAC3 in CRC patients with (p < 0.05). This study agreement with other study discovered tumor samples had higher levels of HDAC3 protein expression than neighboring normal tissue and HDAC3 expression has been linked to poor differentiation, one of the advanced illness clinicopathological characteristics.

As a result, it can be utilized as a prognostic biomarker to predict a bad illness outcome [20]. HDAC inhibitors, on the other hand, restore the balance by encouraging tumor cell differentiation while reducing their proliferative potential [21]. HDAC3 can be utilized as a diagnostic marker to distinguish tumor borders from tumor margins. Using HDAC3 genetic data during surgery can help surgeons improve their rigor and reduce tumor removal surgical errors during
surgery can assist surgeons improve the rigor of their job and reduce tumor removal surgical mistake [22]. While the second field of this research is the investigation of CMV expression in CRC and the link between CMV infection and human cancers is becoming increasingly clear. We found that there is no relationship between CMV infection and CRC. This study in agreement with another study that proved there isn’t as much epidemiological evidence linking CMV infection to CRC.

Other cancers, such as breast, medulloblastoma, and ovarian cancer, have epidemiological and molecular evidence of CMV oncogenic activity [15]. This study agrees with another study that found no CMV in 65 colorectal adenomas and 65 colorectal adenocarcinomas using immunohistochemistry [23]. While according to meta-analysis, CMV infection is linked to a 6.6% chance of having CRC [24]. Possible explanations for this result include the fact that formalin processing can make it difficult to detect CMV within tissues, so PCR is a more sensitive method for determining prevalence [25]. CMV DNA was detected in 11% of the 56 formalin-fixed paraffin-embedded CRC by PCR [26]. A characteristic that implies the interaction between CMV and host cells derived from the intestine is still unknown, which could affect viral detection [12].

Conclusion

This study proved HDAC3 expression pattern in CRC, and the findings support HDAC3 as a supplementary marker to known histopathological diagnostic components; it could potentially be used in prognosis and targeted therapy. We also found that there is no link between CMV infection and CRC as a pro-tumorigenic factor.

References
