DOI: 10.24996/ijs.2025.66.5.8

ISSN: 0067-2904

The Study of Histone Deacetylases Immunoexpression in Relation to Regulating Vascular Endothelial Growth Factor (VEGF) Implicated in Malignant Progression of Colorectal Cancer

Yasir B. Qaddoori[1,2*], Ahmed S.K. AL-Khafaji[1,2], Basim M. Khashman[2], Kifah H. Abdul Ghafour[3]

[1]*Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq*
[2] *Iraqi National Cancer Research Centre, University of Baghdad, Baghdad, Iraq*
[3]*College of Medicine, University of Baghdad, Baghdad, Iraq*

Received: 12/12/2023 Accepted: 20/6/2024 Published: 30/5/2025

Abstract

Colorectal cancer is a malignant condition that can arise from multiple causative factors. It ranks second, behind lung cancer, as a leading cause of cancer-related deaths worldwide. Extensive research has been conducted to unravel the genetic underpinnings and molecular mechanisms underlying the development of colorectal cancer (CRC). However, epigenetic modifications of histones at the DNA level have become significantly involved in several malignant diseases such as CRC. Hence, this research sought to assess, for the first time locally, the immunoexpression of HDAC-1 and 3 in a group of colorectal patients. Additionally, we explored potential correlations between the expression of HDAC-1, 3 and VEGF. This retrospective study encompassed the analysis of 95 paraffin-embedded tissue samples from CRC cases. Participants in the research varied in age from 22 to 79 years, consisting of 60 males and 35 females. The study findings revealed a noteworthy correlation between VEGF expression and the patients' sex (p = 0.005, rho = 0.289). Intriguingly, the analysed data demonstrated a significant correlation between VEGF expression and the cytoplasmic localization of HDAC3 in colorectal cancer tissues (p < 0.001, rho = 0.476). However, the expression of VEGF showed a negative and statistically significant correlation with both HDAC3 expression (p = 0.02, rho = -0.243) and the cytoplasmic localization of HDAC1 (p = 0.02, rho = -0.305). The demonstrated negative regulatory relationship between HDAC3 and VEGF suggests this correlation could potentially be leveraged in both disease prognosis and treatment. Targeting the negative regulatory interaction between HDAC3 and VEGF may provide promising opportunities in both prognostic assessment and therapeutic strategies. This highlights the potential for developing targeted strategies that capitalize on the interplay between angiogenesis and epigenetic regulation.

Keywords: Colorectal cancer, Immunoexpression, HDACs, VEGF

دراسة التعبير المناعي لـ Histone Deacetylases وعلاقته بتنظيم عامل نمو بطانة الأوعية الدموية (VEGF) الذي يسهم في تطور سرطان القولون والمستقيم

* Email: yasir.basim@sc.uobaghdad.edu.iq

ياسر باسم قدوري, [2,1]احمد سالم كاظم الخفاجي, [2]باسم محمد خشمان, [3]كفاح حمدان عبدالغفور [2,1]

[1]قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق

[2]المركز الوطني الريادي لبحوث السرطان، جامعة بغداد، بغداد، العراق

[3]كلية الطب، جامعة بغداد، بغداد، العراق

الخلاصة:

سرطان القولون والمستقيم هو حالة خبيثة يمكن ان تنشأ عن عوامل متعددة، و هو يحتل المرتبة الثانية بعد سرطان الرئة كمسبب رئيسي للوفيات المرتبطة بالسرطان على مستوى العالم. لقد تم إجراء أبحاث واسعة النطاق لكشف الأسس الوراثية والآليات الجزيئية التي تقف وراء تطور سرطان القولون والمستقيم (CRC). مع ذلك، أصبحت التحورات الفوق جينية على مستوى الحمض النووي والهيستونات ذات دور مهم في العديد من الأمراض الخبيثة مثل سرطان القولون والمستقيم. لذلك، سعت هذه الدراسة و لأول مرة على المستوى المحلي لتقييم التعبيرالنسجي المناعي لإنزيمات هيستون ديسيتيلاز HDAC−1 و HDAC−3 في مجموعة من مرضى سرطان القولون والمستقيم. بالإضافة إلى ذلك، تمت دراسة العلاقة المحتملة بين تعبير HDAC−1،3−HDAC و عامل النمو البطاني الوعائي VEGF . شملت هذه الدراسة 95 عينة من انسجة مرضى سرطان القولون والمستقيم. تراوحت الفئة العمرية لعينات الدراسة من 22 إلى 79 سنة، وتضمنت هذه العينات 60 ذكرًا و 35 أنثى. أظهرت نتائج الدراسة ارتباطًا معنويا ملحوظًا بين تعبير VEGF وجنس المرضى ($p = 0.005$, rho = 0.289)، كما أظهرت النتائج ارتباطًا معنويا هامًا بين تعبير VEGF و تعبيرHDAC3 ضمن سايتوبلازم الخلايا في أنسجة سرطان القولون والمستقيم ($p < 0.001$, rho = 0.476). من ناحية اخرى، أظهر تعبير VEGF ارتباطًا معنويا سالبًا مع كل من تعبيرHDAC3 = p) (0.02, rho = −0.243و تموضعHDAC1 في السايتوبلازم ($p = 0.02$, rho = −0.305) . من الممكن ان تمثل هذه العلاقة التنظيمية السالبة المثبتة بين HDAC3 و VEGF مجالا مهما يمكن الاستفادة منه في تقييم مرض سرطان القولون والمستقيم فضلا عن علاجه. كذلك تسلط هذه العلاقة الضوء على امكانية تطوير انظمة علاجية جديدة تركز وبشكل نوعي على التفاعل بين عملية تولد الأوعية الدموية Angiogenesis خلال تطور مرض سرطان القولون والمستقيم و الية تنظيم ذلك على مستوى ما فوق الجين.

الكلمات المفتاحية: سرطان القولون والمستقيم، التعبير المناعي، هيستون ديسيتيلاز ، عامل النمو البطاني الوعائي

Introduction

Colorectal cancer (CRC) is a complex disease with multiple potential causes. It ranks as the third most commonly diagnosed cancer, representing about 10% of all cancer cases, and is the second leading cause of fatalities related to cancer globally [1, 2]. Around two million new instances and 935.000 deaths from CRC were recorded during 2020 globally [3, 4]. The incidence of colorectal cancer in Iraq experienced a notable rise after 2007, marked by a shift in rates from 2.07 in 2007 to 3.74 per 100,000 people in 2016 [5]. Colorectal carcinoma has different etiological factors; these include self or family history of having cancer, diseases and inflammation within the digestive system like colon polyps or Crohn's disease, other medical conditions like diabetes mellitus or cholecystectomy [6, 7]. Individuals who engage in unhealthy lifestyles, such as smoking, heavy alcohol use, obesity, or following nutritionally inadequate diets, are at elevated risk of developing colorectal cancer due to their increased susceptibility [8, 9]. Furthermore, gut microbiome and demographic factors can also lead to the incidence of this malignancy [6, 10].

It is recognized that CRC has a strong genetic basis that is impacted by a number of variables. Progress in molecular sequencing technologies, coupled with the exploration of the

human exome, has enabled many studies to identify genes distinctively associate with various cancer forms, including CRC [11-14]. However, epigenetic modifications of histones at the DNA level have become significantly involved in several malignant diseases such as CRC [15]. Histone modifications are essential post-translational modifications (PTMs) processes which have a leading role in chromatin organizing, and greatly influence on the process of gene regulation. Any disturbance in these PTMs can affect gene expression leading to a number of malignancies [16, 17]. Furthermore, PTMs have been demonstrated to exert a crucial role in tumour formation and disease progression processes, alongside their potential to modulate gene expression patterns in various types of cancer. These findings highlight the significant influence of PTMs and other regulatory factors on cancer development and clinical outcomes[18, 19], including colorectal cancer[20]. As a result, a new field that may be promising in the research for cancer medicines that specifically target these modifications has been evolved[21].

The acetylation of histones on lysine residues, facilitated by enzymes known as histone acetyltransferase (HATs) constitutes a prevalent form of PTMs. This process is reversed by the activity of a class of enzymes called histone deacetylases (HDACs), these deacetylases have the capability to alter the structure of chromatin and the accessibility of DNA by adjusting the intensity of histone acetylation[22, 23]. Such mechanism has the potential to induce substantial alterations in essential cellular processes like proliferation, cycle progression, programmed death and the transition between two states which are the epithelial and mesenchymal [24, 25]. In humans, the HDACs encompass an array of 18 enzymes categorized into four distinctive classes: Class I includes the types 1-3, and HDAC-8; Class II consists of the HDACs types 4-9, and HDAC-10; Class III encompasses the SIRTs 1–7, and Class IV includes HDAC-11.The prominent expression of various members of the HDACs is seen in multiple types of cancer in humans [26].
Given that aberrant activity and overexpression of HDACs have been observed in various cancer subtypes, HDACs have been established as potentially efficacious therapeutic targets [27]. Since any imbalance between HDACs and HATs can result in down-regulation of gene regulators contributing to initial tumour formation, encouraging the build-up of acetylated histones by HDACs inhibitors indirectly increases the therapeutic impact against cancer[28], which in turn restores normal gene expressions within cancer cells and activates additional pathways, including the immune response [29]. Currently, the FDA has issued approvals for many HDACs inhibitors to be used in treating haematological cancers, such as vorinostat [30], belinostat, romidepsin [31], and panobinostat [32]. Although the efficiency of these inhibitors in treating solid cancers is still not up to par[33], their potent anti-proliferative impact as well as controlling a various biological functions, like protein breakdown and DNA repair was established [32].

The glycoprotein known as vascular endothelial growth factor (VEGF) has an approximate molecular weight of 45 kDa, existing as a homodimer, it serves as the primary mediator of angiogenesis that is pivotal for malignant cells supply of nutrients and oxygen to grow and metastasize[3, 34-36]. Beside its function in diseases, VEGF has a role in health as well, since it promotes angiogenesis during fetal growth [37] and contributes to the process of wound healing in adults[38]. The overexpression of VEGF influences several processes crucial in tumour development, including endothelial cell proliferation, promotion of neo-vascularization [3, 39], and immune system suppression[40]. As a result, the administration of drugs that inhibit VEGF has shown effectiveness when employed as a secondary treatment option for patients suffering from colorectal cancer [41]. This could result from the impact of these inhibitors, involving both angiogenesis and immunological aspects. The inhibitors may counteract the detrimental effects of VEGF on key immune cell functions like dendritic cells,

macrophages, and T cells, that play pivotal roles in cancer immunity[42]. Eventually, VEGFi can promote the antitumour immune response [43].

In the context of angiogenesis regulation on an epigenetic level, applications of HDACs inhibitors (HDACis) may indirectly impact angiogenesis by controlling the expression of genes involved during this process, including VEGF [44].

Therefore, this study aimed to assess the immunohistochemical expression of HDAC-1 and HDAC-3 for the first time in Iraq among a cohort of colorectal cancer patients. Furthermore, we studied potential associations between the expression of HDAC-1, 3 and VEGF as well as their relationship to the stages of disease progression.

Materials and methods

Study Samples

This retrospective investigation involved analysing 95 paraffin-embedded colorectal cancer (CRC) tissue samples. The study included patients aged 22 to 79 years (comprising 60 males and 35 females). These specimens were sourced from the records of the Gastrointestinal and Liver Teaching Hospital in Baghdad, covering the period from October 2021 to December 2022. Ethical approval was obtained from the Iraqi National Cancer Research Centre, Ref: NCRCEC/01/004 on 03/1/2022.The CRC grades and stages were determined by a pathologist using the tumour, node, and metastasis (TNM) internationally approved staging system [45].

The Immunohistochemistry Analysis

Tissue sections of 4 mm thickness were obtained from formalin-fixed, paraffin-embedded blocks by utilizing a microtome. For each block, a single section was placed on a standard microscope slide and subjected to haematoxylin and eosin (H&E) staining. Additionally, three other sections from the identical block of tissue were mounted on charged slides for immunohistochemistry analysis. In this experiment, rabbit anti-HDAC1 monoclonal antibodies were used (ab150399, abcam, UK), and anti-HDAC3 rabbit monoclonal antibodies (ab32369, abcam, UK). In addition, rabbit anti-P53 monoclonal antibodies were also employed in this test (ab32049, abcam, UK). The slides were deparaffinized using xylene and rehydrated through a range of graded alcohols, followed by distilled water. Blocking of endogenous peroxidase activity was achieved by applying 3% hydrogen peroxide for a duration of 10 min. The slides were then washed in phosphate-buffered saline (PBS), and then a protein blocker was added, this was followed by incubating at 37°C for 5 min, and subsequent washing with PBS. The slides were treated with the primary antibody solution and left for 1 hour for incubation in a chamber under humidified conditions at 37°C. A dilution of 1/50 was applied for the primary antibodies solution preparation. The slides were first gently rinsed with PBS. Then, the secondary antibody was applied for 10 minutes at room temperature, followed by the addition of streptavidin-HRP antibodies which were incubated for 10 more minutes at 37°C. Following a washing step, the specimens were stained with a diluted DAB for 20 min at room temperature. A 30-second counterstaining with hematoxylin was performed on the slides, followed by thorough washing in running tap water. Subsequently, the slides were dehydrated, mounted with a permanent-mounting medium (DPX), and ultimately observed under a light microscope[46].

The Scoring System

The slides of all study samples were examined by a qualified pathologist. A semi-quantitative scoring system was utilized for evaluation. Cells exhibiting a brown colour in the nucleus or cytoplasm were deemed positive, while uncoloured cells were classified as

negative. Following an outlined method [47], the scoring system was implemented, assigning the following scores: score 0 (0%), score 1 (< 10%), score 2 (10-50%), and score 3 (> 50%).

Statistical Analysis

The correlation coefficient (rho) was assessed statistically using the Spearman's test among clinicopathological characteristics of the examined colorectal cancer patients, including p53 and HDACs (1&3) proteins alongside their two tailed p-values. Besides, pertinent data from The Cancer Genome Atlas (TCGA) was input and analysed using tumour analysis platform at TNMplot.com to study the correlation of P53 & HDAC3 genes expression both in malignant and normal tissue. Overall survival of patients with colorectal cancer relevant to the studied parameters was estimated using Kaplan-Meier method. Tests with a p-value under 0.05 were deemed to be statistically significant.

Results and discussion

The study involved 95 participants who had been diagnosed with colorectal cancer. The cohort comprised 60 males and 35 females, with ages varying from 22 to 75 years. About 58% of the patients were above 50 years old, and males predominated in each age category, as illustrated in Figure 1.

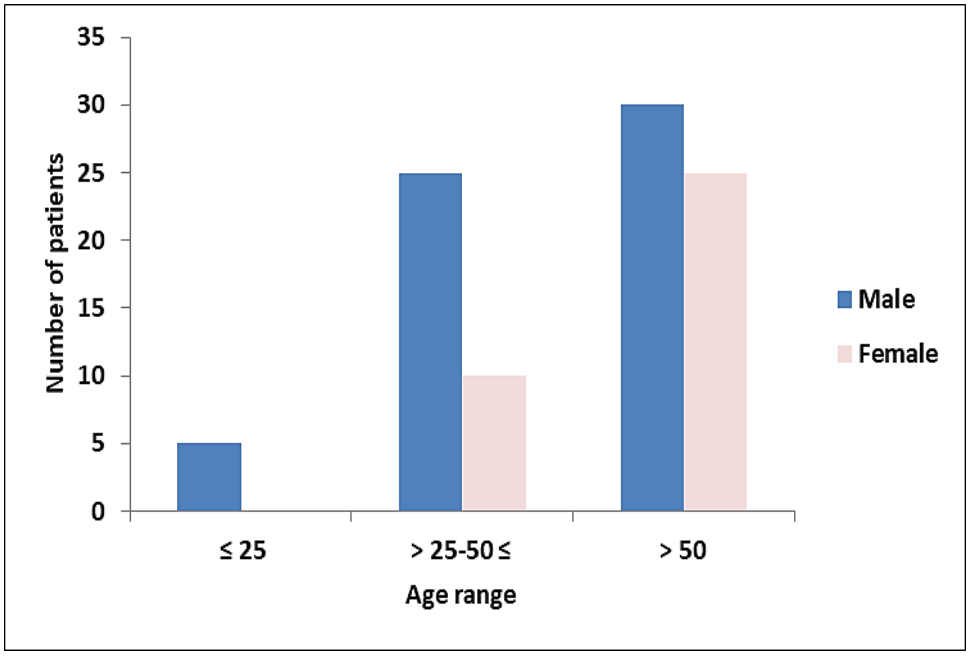


Figure1: Age and gender distribution of the study sample showing that most of the examined patients are above age 50 years old followed by those between 25-50 years old, while under 26 years old are the lowest.

The immunohistochemical staining results (represented in the images shown in Fig. 2) were statistically analysed to study the relationship between various clinicopathological features of the colorectal cancer patients, including VEGF and HDAC proteins (types 1 & 3) expression levels and localization patterns.

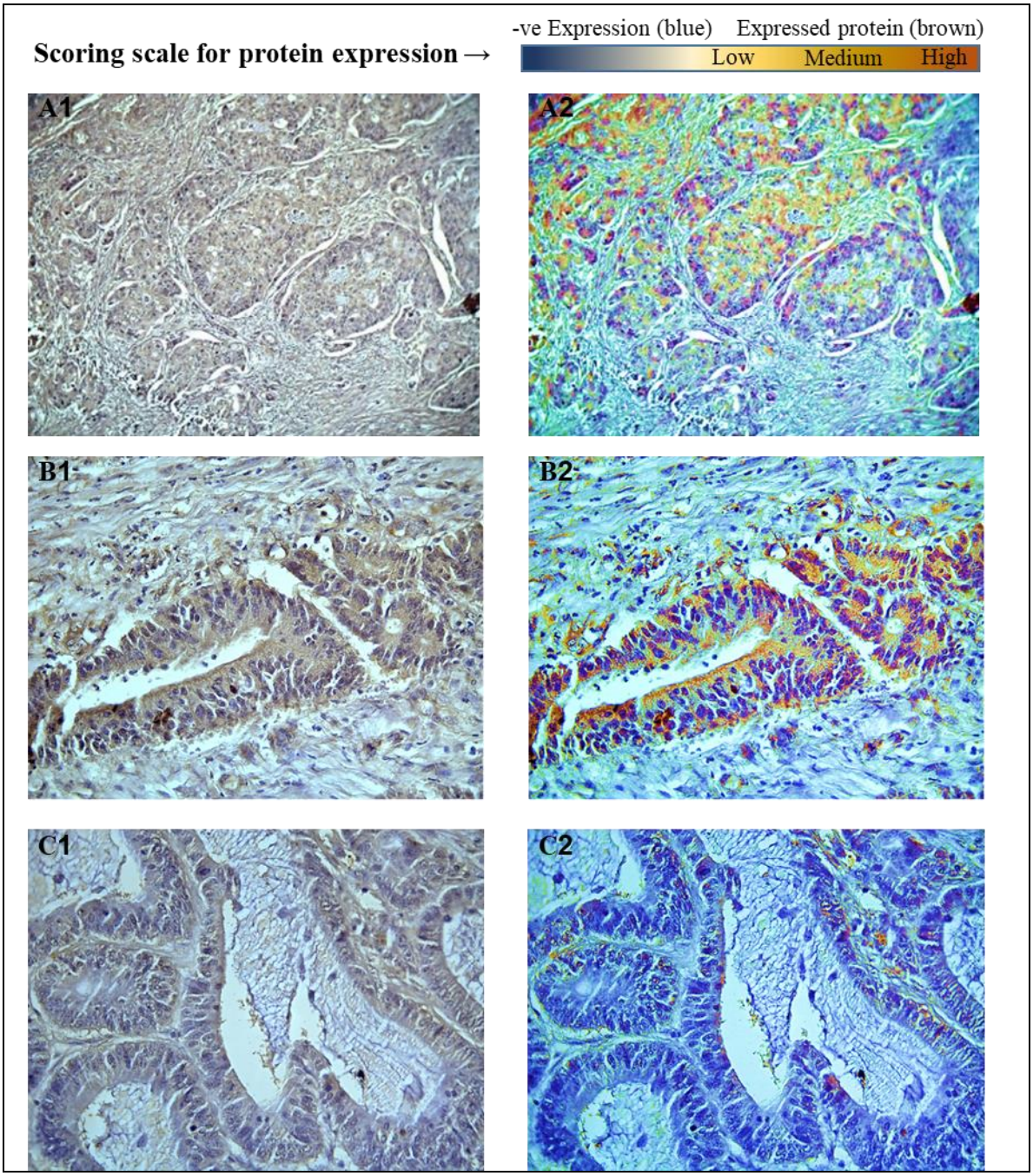


Figure 2 : Images showing the immunohistochemical staining for detecting the protein expression (Brownish) of **A:** VEGF **B**: HDAC1 **C:** HDAC3, in CRC patients. The scoring scale for increasing expression of **A2**: VEGF **B2**: HDAC1 **C2**: HDAC3 is depicted according to the brown colour intensity from low to high, while the blue demonstrates the non-expressed genes.

The study outcomes revealed that there is significant correlation between VEGF expression and patients' sex (p = 0.005, rho = 0.289).Interestingly, the analysed data illustrates that VEGF expression is significantly correlated with the cytoplasmic HDAC3 localisation of colorectal cancer cells (p = <0.001, rho = 0. 476). Nevertheless, VEGF expression is inversely correlated with HDAC3 expression (p = 0.02, rho = - 0.243) as well as HDAC1 localisation (p = 0.02, rho = - 0.305) in the cytoplasm. (i.e., it is associated with HDAC1 localised in the tumour cells nucleus). Table1.

Table 1: The correlation among clinicopathological features of the study sample**.**

Features	Spearman's test	TNM Stag.	HDAC1 Expr.	HDAC1 Loc.	HDAC3 Expr.	HDAC3 Local.	VEGF Expr.
Patients Sex	[1] rho (**ρ**)	.468**	-.332**	-.471**	0.17	.530**	.289**
	[2] p-value (***p***)	<0.001	0.001	<0.001	0.10	<0.001	0.005
	[3] Number	95	95	70	95	55	95
HDAC1 Localisation	[1] rho (**ρ**)				-.448**	.407**	-.305*
	[2] p-value (***p***)				<0.001	<0.001	0.01
	[3] Number				70	50	70
HDAC3 Expression	[1] rho (**ρ**)					-.468**	-.243*
	[2] p-value (***p***)					<0.001	0.02
	[3] Number					55	95
HDAC3 Localisation	[1] rho (**ρ**)						.476**
	[2] p-value (***p***)						<0.001
	[3] Number						55

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed).
[1]Correlation Coefficient (rho), [2]Two-tailed significant p value, [3]Sample size where the missing values of the handling samples number were excluded listwise.

In terms of the association between VEGF expression and gender, our data indicated that VEGF expression was elevated more in male patients compared to females. The difference was statistically significant (p = 0.005), Fig3. This result corroborates our findings presented in Table 2 and may suggest differences in the types or quantities of colorectal cancer risk factors that males and females were exposed to.

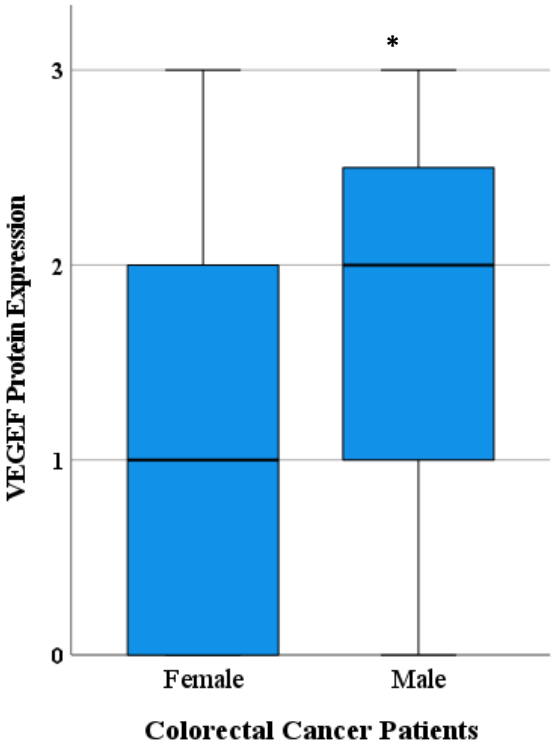


Figure 3 : Boxplots demonstrating a comparison between VEGF protein expression in female and male of colorectal cancer patients' biopsies. Expression was scored based on

chromogenic intensities and localisation of immunohistochemical data. Mann-Whitney (p = 0.005).

In this study, relationship mapping analysis was utilized to create a visual representation that depicted the interconnections among the measured values of multiple variables, specifically VEGF, HDAC1, and HDAC3. These relationships are illustrated in Figure 4. It indicates that the highly expressed VEGF protein (represented by muted violet coloured circle 2) is related to a decreased level of HDAC3 protein expression. This finding further confirms their inverse significant correlation (p = 0.02, rho = - 0.243) shown in Table 1. However, VEGF expression seems to be related to HDAC1 expression. These results may indicate that HDAC 1 and 3 expression play a reverse interchangeable role in expressing VEGF.

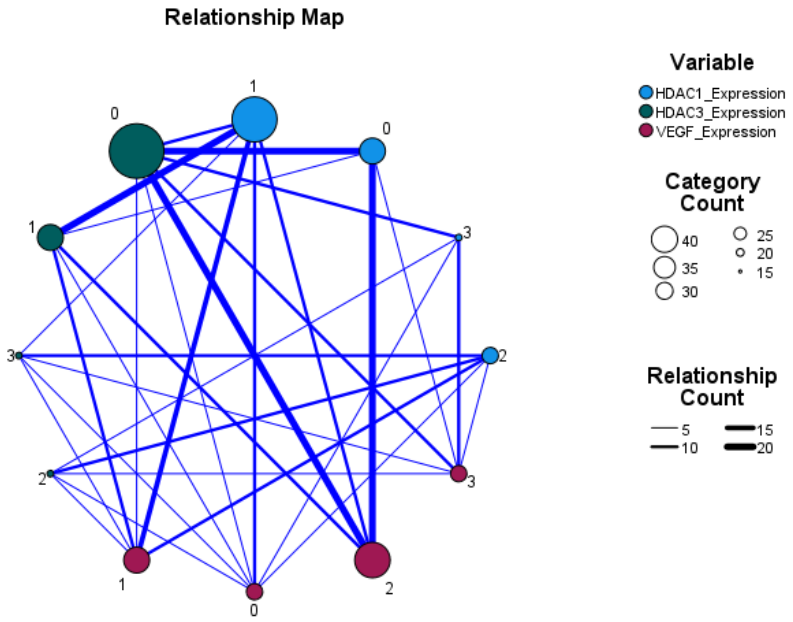


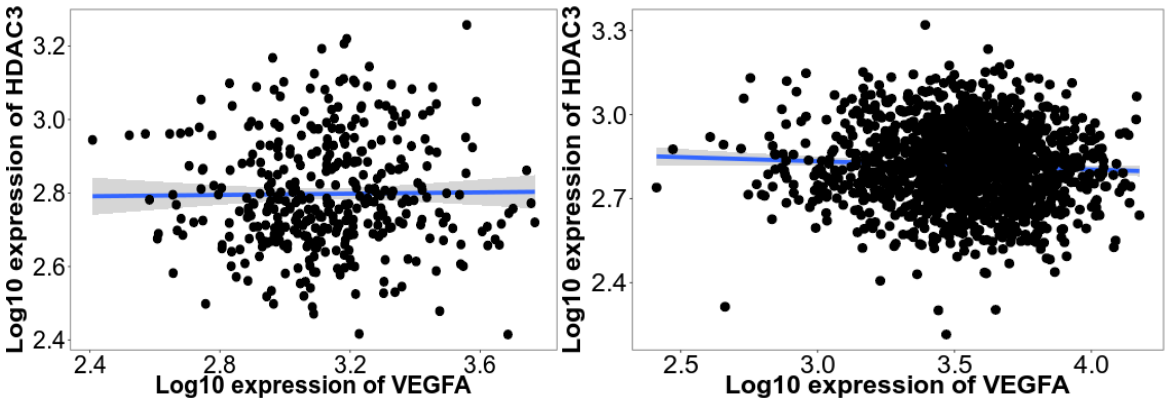
Figure 4 : A relationship map among the tested values of multiple variables of VEGF, HDAC1 and HDAC3 protein expression.

The map illustrates how the expressed proteins are related to each other by visually representing links and impacts through circles and interlinear connections. The circles demonstrate the expressed VEGF, HDAC1 and HDAC3 proteins while the interlinear connections demonstrate the impact degree between the expressed proteins. Bigger circles refer to the higher frequency of occurrence with thicker interconnecting link lines elucidating higher co-occurrence and vice versa.

We utilized gene expression data originally generated through RNA-sequencing and subsequent gene array analysis, which was obtained from The Cancer Genome Atlas (TCGA) at cancer.gov/ccg. The data has been extracted through unrestricted database mining from the Normal and Tumour analysis platform at TNMplot.com developed by Bartha and Gyorffy [48].

The results of the plotted data below (Fig.5) do not show a significant difference between the expressed VEGF and HDAC3 genes in normal colon tissues. Nonetheless, the gene expressions of VEGF and HDAC3 in the malignant colon tissues were inversely correlated (p = 0.04, rho = - 0.05). The outcomes prove our results of the inverse relation between the

VEGF and HDAC3 proteins expressed in colorectal cancer tissues we analysed in Table 1 and Fig. 4.



HDAC3	VEGFA	rho	p	Analysis type
377	377	0.05	0.30	Spearman correlation
Normal colon samples				

HDAC3	VEGF	rho	p	Analysis type
1450	1450	-0.05	0.04	Spearman correlation
Tumour colon samples				

Figure 5 : Scatter plots illustrating the correlations between the tested VEGF and HDAC3 gene expression in normal and tumour CRC samples.

Data originally harboured by TCGA at cancer.gov/ccg was employed in this study. The harboured data has been presented through unrestricted RNA gene chip database from colon cancer analysis portals at kmplot.com developed by Lanczky & Gyorffy [49].
The Recurrence-Free Survival (RFS) curves depicted in Figure 6 illustrate that elevated VEGF expression is associated with poorer survival outcomes among CRC patients (A), whereas patients exhibiting robust HDAC3 expression tend to have a more favourable prognosis (B). These results provide further evidence for confirming our findings of the negative association between the genes VEGF and HDAC3 in affecting colorectal cancer patients' outcome.

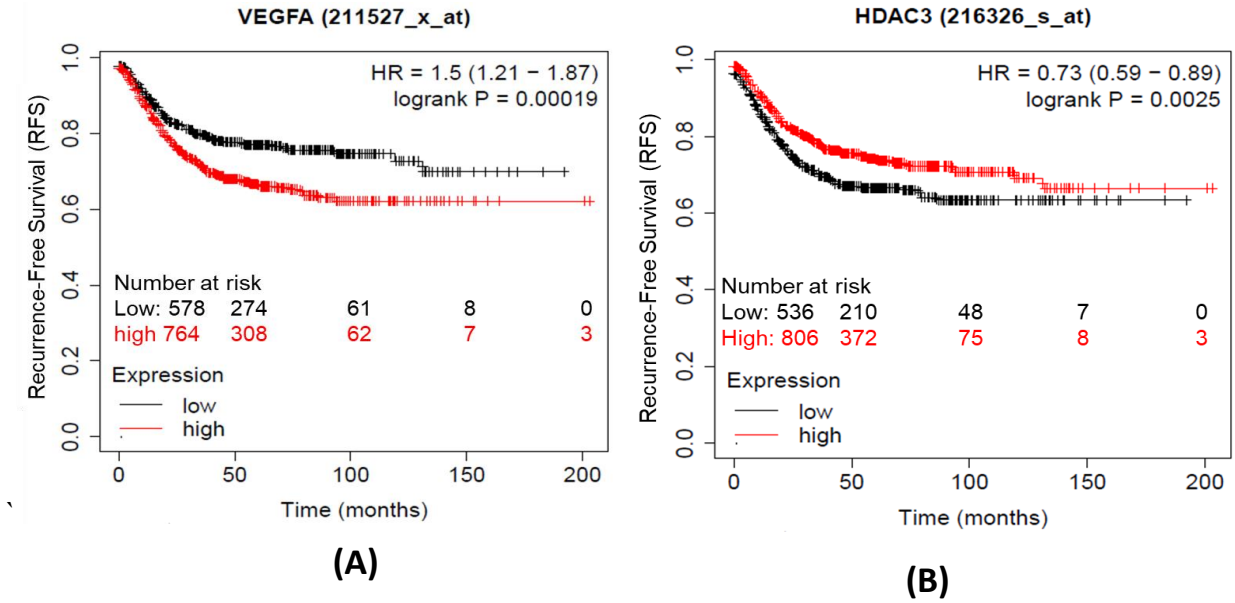


Figure 6: Kaplan-Meier plots demonstrating the Recurrence-Free Survival (RFS) of CRC cancer patients dichotomised by the median of VEGF and HDAC3 mRNA expression.

Colorectal cancer has been linked with advanced age [10]. In this context, our result was consistent with Ibrahem *et al.* who found that the bulk of CRC cases lies in the age category over 50 years among 20,880 patients; according to an Iraqi registry-based study [50]. In addition, Soliman and Mohamad also found that CRC rates increased with age, especially after 50 in a sample of Iraqis[51]. Numerous epidemiological studies conducted globally have consistently reported a similar pattern of higher CRC incidence rates among the elderly population [52-54]. In terms of causality, interaction between long exposure to risk factors by aging could trigger the commencement of cancer development and creates an environment that amplifies the mutations that drive the growth of cancer; epigenetic modification such as methylation of promoter CpG islands associated with age facilitates conditions conducive to oncogenic transformation [50, 55]. Moreover, suppression of the immune system with age weakens the immune surveillance of cancer in the elderly, represented by elevation in the quantity of regulatory T cells at the expense of the anti-cancer T helper and cytotoxic ones, this has been found to be related to the development of CRC [56]. However, a relative decrease in cases among the elderly was reported which was attributed to the increase and development of screening programs in developed countries that helped diagnose and treat the disease in the early stages [57, 58].On the other hand, the incidence of colon cancer is increasing at younger ages in recent years, which may due to differences in exposure pattern to risk factors in contemporary lifestyle [58].

Gender disparity has also been reported to be associated with CRC [59]. Consequently, our data revealed that out of 95 CRC patients, around 68% of them were males. This outcome is consistent with the conclusions of a study performed by Ibrahem *et al*., where they found that men were more likely than women to develop colorectal cancer in 2019 and 2020[50]. In the same context, Hussein *et al*. found that there was a surge in the number of colon cancer cases after 2007 in Iraq, with a relative male predominance[5]. Furthermore, men with CRC tend to have a more unfavourable prognosis, facing an approximately 40% higher mortality rate than women [60]. The prevalence of colorectal cancer in males, as observed in our study, aligns with a global trend [10, 61]. Etiologically, factors that contribute to sex differences and CRC onset are not entirely known. However, it is thought that these gender-differences may be attributed to variations in the quality of patients' exposure to risk factors (e.g. consumption of alcoholic drinks and tobacco), dietary habits, and reproductive hormones[62].

The distinctive features of the immune system in males and females, variation in levels of hormones, and the expression of circulating hormone receptors, along with various genetic as well as epigenetic changes, are crucial biological elements that are likely to contribute to the diverse incidence rates and disease characteristics observed between different gender of patients with bladder cancer [63]. Consequently, the significant higher expression levels of VEGF in CRC male patients compared to females recorded within our study may reflect this aspect. In a like manner, Herichova *et al.* pointed out that expressing estrogen receptor β mRNA (ERβ) was correlated inversely with tumour staging in females but not males during colorectal cancer. Furthermore, a negative correlation was noticed between females' VEGF and mRNA ERβ expressions in the same study [64]. Such variations in sex-related hormones, including fluctuations in estrogen and androgen levels and their corresponding receptors which are linked to tumour development, coupled with disparities in lifestyle and exposure to risk factors, could elucidate the lower incidence of colorectal cancer in women compared to men[62].

Genetic and epigenetic changes work concertedly to control the course of cancer. HATs, HDACs, DNA methyltransferases, and chromatin shape modifiers are promising epigenetic

regulators. HDACs particularly function as modifiers of histones, controlling the expression of genes linked to metabolism, apoptosis, growth, and cell survival. Most HDACs are significantly overexpressed in cancer, whereas some have different roles and expressions as the disease progresses[65]. On a related level, our study sought to screen some of these epigenetic modifications in Iraqi colorectal cancer patients, namely HDAC1 and HDAC3, and their relationship to the biomarker that mediates the angiogenesis, VEGF. The prospective regulatory role of HDAC3 in the expression of VEGF was proved by immunoprecipitation, which highlighted the specific nature of the interaction between HDAC3 and the VEGF promoter in cancer cells [66]. The inverse relationship between HDAC3 and VEGF for both transcriptional and translational levels demonstrated in our study via three approaches comes in parallel with several previous researches. Park *et al.* discovered that heightened levels of HDAC3 expression are linked to a decline in VEGF expression in cancerous cell lines. This implies that HDAC3 functions as a negative controller for angiogenesis by exerting suppressive effects on angiogenic-related factors like VEGF and plasminogen activator inhibitor-1(PAI-1) [67]. Furthermore, another study revealed that hyaluronic acid mediated angiogenesis was also associated with decreased levels of HDAC3 [68]. The study found that cancer cells demonstrating resistance to anti-cancer drugs exhibited lower levels of HDAC3 expression along with higher levels of angiogenic markers such as PAI-1 and VEGF, indicating a relationship between HDAC3 expression and angiogenic marker levels in drug-resistant cancer cells [69]. In this context, the outcomes of Kaplan-Meier analysis that we conducted in this study supports this approach. Regarding the significant relationship between the expression of VEFG and HDAC1 concerning the localization of the latter in the nucleus, it was reported that anti-HDAC1treated prostate cancer cell LINE (pc3) majorly exhibited the presence of HDAC1 within the cell nucleus upon immunostaining. These findings underscore the efficacy of the novel HDAC ligand and suggest the possibility of revaluating mechanisms associated with the inhibition of nuclear HDAC-mediated deacetylation [70].

Conclusion

The association between cancer pathogenesis and epigenetic alterations is a topic of global research interest due to its significant prognostic implications and therapeutic potential. Our study is the first in Iraq to shed light on this important aspect in colorectal cancer patients. The results of this study regarding increased HDAC3 expression versus decreased VEGF expression represent a promising avenue to utilize this regulatory negative correlation for both prognostic and therapeutic aspects, as it highlights the potential for targeted strategies based on the crosstalk between angiogenesis and epigenetic control. However, additional research is required to validate and affirm the observed correlation in different cancer types for Iraqi patients.

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