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<sup>[3]</sup>▶ Aberrant expression of miR-143/miR-223/miR4478 and miR145 as prognostic factor  
for colorectal cancer patients

Abstract

Colorectal cancer (CRC) is a type of malignancy that develops in the colon or rectal region.<sup>[8]</sup>▶ It develops due to abnormal growth and proliferation of cells in the lining of the colon, forming a tumor. This study included tissue samples (56 malignant and 56 matched normal samples). miRNA expression levels varied. Only miR223 revealed greater expression than normal matched tissue. MiR143, miR4478, and miR145 exhibited lower expression than matched normal tissue.<sup>[3]</sup>▶ Only miR143 and miR145 showed considerable variations in the expression among groups while miR4478 did not demonstrate statistically substantial difference.<sup>[3]</sup>▶ In conclusion, this study highlights that upregulation of P53 and miR223 and downregulation of miR145 and miR143 were associated with cancer advancement and unfavorable prognosis in Saudi CRC patients, indicating P53, miR223, miR145 and miR143 to be novel and valuable signatures for predicting the outcomes for patients with CRC.

Keywords: Colon, Cancer, p53, miR, Gene Expression.

1. Introduction

Globally, cancer is the persistent and burgeoning health issue. It constitutes an enormous group of diseases in which cells continue to divide randomly and incessantly (Parkin et al. 2005; Alhadheq et al., 2023). The cells contain genetic materials which control structure and function of cells including its development, survival, and longevity. In the early period of life, cells divide and differentiate rapidly. However, later in life, cells follow organized regulation systems to maintain the human life (Doubeni et al. 2012). Cells contain genetic material that includes coding and non-coding genes. Coding genes control the production of proteins which have specific roles in the cells. Dysfunction genes may lead to abnormality as a consequence uncontrolled cell division and cancer occurred. Also, some of gene mutations lead to cancer diseases, abnormality and inhibition of translation (Adjiri, 2017). In addition to genetic factors, there are environmental factors such as; smoking, radiation, drinking alcohol and others that cause cancer diseases (Anand et al. 2008). Globally, colorectal cancer (CRC) stands as a prominent global health challenge, representing a significant cause of disease prevalence and death. According to recent statistics, CRC ranks as the 3<sup>rd</sup> frequently diagnosed malignancy and 2<sup>nd</sup> principal cause for cancer-linked mortality globally (Hadjipetrou et al. 2017). Its incidence varies

geographically, with developed countries bearing a higher burden, possibly due to lifestyle factors such as diet, obesity, and sedentary habits. Annually, around 1.24 million cases have been identified related to CRC while around 40,000 deaths occur worldwide (Ferlay et al. 2015). According to scientific report (2021), incidence of CRC was documented at a rate of 29 cases per 100,000 individuals in nations with elevated human development indexes. While prevalence of CRC has gradually risen since the 1960s, advancements in treatments have led to decrease death rate over recent decades. In Saudi Arabia, CRC ranks second constituting approximately nine percent of the recent cases, standing first in the male and third among the female population, respectively (Alsanea et al. 2015). The likelihood of developing CRC over one's lifetime is around 5%, and this risk tends to rise with advancing age. Roughly 1 out of every 23 men and 1 out of every 25 women are expected to receive a diagnosis of CRC (Miller et al. 2019).<sup>[3]</sup> CRC is uncommon in individuals under the age of 50. Although men generally experience higher incidence rates, the lifetime risk is comparable between genders, mainly owing to women's longer life expectancy. Beyond gender, age and race/ethnicity markedly increase the odds of disease susceptibility (Hossain et al. 2022). Despite advancements in diagnosis and treatment modalities, the prognosis for CRC patients remains guarded, necessitating continued efforts to unravel novel prognostic markers and therapeutic targets. MicroRNAs (miRNAs) have emerged as pivotal regulators of gene expression, orchestrating various cellular processes including proliferation, apoptosis, differentiation, and metabolism (Divisato et al., 2020).<sup>[2]</sup> Dysregulation of miRNAs has been involved in the progression of numerous malignancies, contributing to initiation of tumor, development, and metastasis (Hussen et al., 2021). Aberrant miRNA expression profiles have been extensively documented in CRC, underscoring their potential as diagnostic, prognostic, and therapeutic biomarkers. Among the myriad of dysregulated miRNAs in CRC, miR-143, miR-223, miR4478, and miR145 have garnered considerable attention for their multifaceted roles in tumorigenesis and cancer suppression across diverse cancer types.<sup>[28]</sup> miR-143, situated on chromosome 5q32, has been implicated in CRC as a tumor suppressor, modulating key signaling pathways involved in cell proliferation, migration, and invasion (Abd El Fattah et al., 2023). Similarly, miR-223, located on chromosome Xq12, has been shown to exert tumor-suppressive effects in CRC by targeting various oncogenic signaling cascades (Liu et al., 2021; Wang et al., 2021).<sup>[28]</sup> Furthermore, miR4478, although less characterized, has been implicated in CRC pathogenesis through its regulatory effects on critical cancer-related genes (Wang et al., 2020). Conversely, miR145, located on chromosome 5q32, is frequently

downregulated in CRC and is recognized as a potent tumor suppressor, governing diverse cellular processes including epithelial-mesenchymal transition (EMT), angiogenesis, and drug resistance (Guzel and Ozturk, 2021; Sachdeva and Mo, 2010; Zou et al., 2012). While the individual roles of miR-143, miR-223, miR4478, and miR145 in CRC have been elucidated somewhat, their collective significance as prognostic markers still to be completely explained. Understanding the interplay between these miRNAs and their intricate regulatory networks in CRC pathogenesis holds promise for new prognostic models and targeted therapeutic strategies. The objective of current research study was to comprehensively review the aberrant expression patterns of miR-143, miR-223, miR4478, and miR145 in CRC and their prognostic implications for patient outcomes. By elucidating the molecular mechanisms primarily causing their dysregulation and exploring their potential as prognostic biomarkers, we endeavor to expand the knowledge aimed at improving the clinical management and outcomes of CRC patients.

## 2. Methods

### 2.1 Study population

We obtained 112 tissue samples: 56 CRC tissues and 56 corresponding normal colorectal tissue samples, sourced from King Khalid University Hospital in Riyadh, Saudi Arabia. Clinical data, including age, family history, gender, and tumor location, were collected. Participant ages ranged from 23 to 88 years. Each contributed tissue sample, preserved in RNA later solution. Ethical approval was granted by King Saud University's medical college ethics committee, ensuring compliance with ethical standards.

### 2.2 Total RNA isolation

Total RNA extraction was carried out for a total of 56 CRC tissues and 56 corresponding normal colorectal tissue samples, following the manufacturer's guidelines with a Qiagen RNA extraction Kit. 10 $\mu$ l  $\beta$ -ME ( $\beta$ -mercaptoethanol) and 350 $\mu$ l RLT (lysis buffer) were added to 20 mg of each tissue and then homogenized by using the Medic Tools Dispomix Drive. 350 $\mu$ l RLT lysis buffer was Added for each sample tube. Afterwords 1x of 70% ethanol 350 l was added. Mixed immediately by vortex. After mixing, 700 $\mu$ l was shifted to the Kit columns. 700 $\mu$ l of washing buffer and 500 $\mu$ l RPE (twice) were added in each loading with subsequent centrifugation at 8000 rpm for two minutes, respectively. 50 $\mu$ l of elution buffer were added to each sample. Centrifugation at 8000rpm for about 1min. The concentration and purity of the extracted RNA were evaluated by utilizing the NanoDrop8000 spectrophotometer (Thermo Scientific). The storage of extracted samples at -20 °C.

### 2.3 RNA Quality

The quantification of RNA isolated from tissue specimens was carried out through measuring optical density at 260nm (OD260) and 280nm (OD280) using a NanoDrop 1000 spectrophotometer (Peqlab Biotechnologie GmbH, Erlangen, Germany). Examination of RNA integrity (RIN) was conducted by means of Agilent 2100 Bioanalyzer (Agilent Technologies GmbH, Waldbronn, Germany) as well as with RNA 6000 series II Nano LabChip analysis kit. The 2100 Bioanalyzer generated numerical RIN values, which span from 0 – 10, where higher values indicate superior RNA integrity. Notably, the RIN values obtained for the total RNA extracted from both CRC and adjacent normal tissues demonstrated high integrity, with values exceeding 8.0.

#### 2.4 Synthesis of cDNA

The process of converting RNA into cDNA was executed using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Warrington, USA) as per manufacturer's provided instructions. Specifically, 10µl of the master mix from the cDNA reverse transcription kit was thoroughly combined with 1µg of pristine RNA sourced from the isolated samples. The resulting mixtures were then exposed to cDNA synthesis within a master cycler gradient Eppendorf. The procedural steps encompassed an initial incubation of 10 mins at temperature 25°C, accompanied by 2 hrs at temperature 37°C, and a final phase of 5 minutes at 85°C to ensure optimal cDNA generation. The resultant cDNA was appropriately preserved at -20°C for subsequent use.

#### 2.5 P53 mRNA quantification

Gene expression analysis was conducted using quantitative RT-PCR in triplicate, employing TaqMan gene expression assay Hs01034249 from Applied Biosystems. The GAPDH gene (Hs02758991\_g1) served as the internal control.<sup>[49]</sup> Each qPCR reaction (20µl) consisted of 2 µl of 1µg cDNA template, Universal TaqMan MasterMix (2x concentrated, Life Technologies), TaqMan assay (20x concentrated, Life Technologies), and H<sub>2</sub>O.<sup>[18]</sup> The reactions were conducted using the 7500 Fast Real-Time PCR System (Life Technologies).<sup>[30]</sup> The qPCR reactions, performed thrice, involved initial incubation at 50°C for 2 mins, accompanied by denaturation for 10 mins at 95°C. Subsequently, 40 rounds of denaturation at similar temperature for 15 sec and annealing/extension for one minute at 60°C were executed.<sup>[54]</sup> Non-template controls were systematically incorporated as controls (negative) in each experimental run. Relative RNA quantities were determined utilizing  $\Delta\Delta CT$ .<sup>[3]</sup> To normalize gene expression data, the GAPDH gene was employed, and mean expression in the tumor and normal samples was matched, expressed as an n-fold ratio.

#### 2.6 Characterization of miRNAs regulated by p53

In present research, we sought to determine microRNAs (miRNAs) that are moderated by p53 gene by leveraging the principle of sequence complementarity. The known miRNA mature sequences were retrieved from miRBase database. The interaction between P53 and miRNAs is crucial for understanding the regulatory network in cancer and other bioprocesses. The seed region typically consists of nucleotides 2-7 or 2-8 of the miRNA, which are imperative for target recognition. We developed an in-house Perl program to identify miRNAs bound by P53, aimed at seed region's complete matching criteria to enhance the accuracy of predictions.

### 2.7 Statistical analysis

The target sample size was determined utilizing PS software (Power and Sample Size Calculation Software Package, Vanderbilt University, Nashville, TN). P <sup>[6]</sup>0.05 was considered statistically significant. <sup>[28]</sup>Mann-Whitney U test was performed to analyze the association between gene expressions. <sup>[28]</sup>All the statistical analysis was performed using SPSS version 22 (Allen et al., 2014).

## 3. Results

### 3.1 Influence of p53 on the growth and advancement of human CRC

To explore the effect of p53 gene expression alterations in human CRC, we conducted a comparative analysis between healthy individuals and those with tumors. To assess differences in p53 gene expression, we employed GAPDH (Glyceraldehyde-3-PhosphateDehydrogenase) as a reference gene in both groups to estimate the fold-change in p53 gene expression. In the healthy group, the mean value of expression for p53 gene was  $31.66 \pm 1.51$ <sup>[21]</sup>, and it was  $20.07 \pm 0.82$  for GAPDH gene. Conversely, in the cancer group, the mean expression value for the p53 gene was  $30.62 \pm 1.97$ , and for the GAPDH gene, it was  $19.27 \pm 0.88$ . To mitigate bias in the fold change values, we normalized each gene's value by dividing it by the average group fold change value. The normalized fold change value for the normal group ranged from 0.06 to 8.54. However, the range was significantly higher in tumor group which was 0.04 to 52.66. Our initial observation reveals that the expression bias in p53 potentially responsible for growth and development of human CRC. This is underscored by the apparent higher p53 gene expression pattern among the groups, showing nearly a 2-fold induction (P value; 0.02), as displayed in (figure 1).

### 3.6 Detection and analysis of p53-regulated microRNAs (miRNAs)

In our quest to unveil miRNAs showcasing altered expression patterns triggered by

p53 activation within CRC, we embarked on a comprehensive genome-wide miRNA expression analysis utilizing the GEO dataset GSE67181. The dataset's contributor utilized doxycycline as an inducer to prompt p53 expression in CRC. Leveraging the robust capabilities of edgeR, we meticulously identified miRNAs exhibiting differential expression concerning p53 activation in this cellular context. Our investigation unveiled 400 miRNAs displaying significantly differential expression. Notably, eight miRNAs stood out with remarkable statistical significance, as underscored by their p-values, while also exhibiting logFC values exceeding 2 or falling below -2, as outlined in (table 1).<sup>[89]</sup> These findings shed light on potential miRNA candidates intricately associated with the regulatory network modulated by activation of p53 in CRC, offering valuable comprehension into the molecular mechanisms driving cancer growth and development. To evaluate the binding affinity of differentially expressed miRNAs with p53, we retrieved the gene sequence along with its 500 base pairs upstream (5' UTR) and 500 base pairs downstream (3' UTR) sequences. Each mature miRNA sequence was individually aligned with these genomic sequences. Our alignment analysis uncovered specific mismatch patterns between the miRNAs and the p53 gene sequence. Specifically, has-miR-143 exhibited 7 mismatches, hsa-miR-145 had 6 mismatches, hsa-miR-200b displayed 8 mismatches, hsa-miR-200c showed 9 mismatches, hsa-miR-205 presented 9 mismatches, hsa-miR-223 demonstrated 8 mismatches, hsa-miR-34a indicated 8 mismatches, and hsa-miR-4478 featured 8 mismatches with the p53 gene sequence (figure 2). These mismatch patterns shed light on the possible interactions between miRNAs and the p53 gene, elucidating their regulatory roles in colorectal cancer pathogenesis.

### <sup>[25]</sup> 3.7 Expression levels of mir143, mir4478, mir223, and mir145 in CRC with p53 mutations

Our study conducted a comprehensive analysis of miRNA expression levels in CRC with p53 mutations, focusing particularly on four miRNAs: miR143, miR4478, miR223, and miR145. We compared these miRNAs expression between two distinct groups: one representing normal tissue while another representing tumor tissue. To derive meaningful insights, we utilized the small noncoding RNA RNU6B as a reference gene to estimate the values of fold change in all miRNA for each sample in both groups. Upon analysis, we noticed that average expression of miR145, after normalization with the normal group, was 0.52 in test group, indicating that miR145 exhibited greater expression in control group than tumor group.<sup>[25]</sup> Further examination of the normalized fold-change values revealed a range of 0.14 to 9.86 for control and 0.07 to 6.70 for test group.<sup>[63]</sup> To analyze the

statistical importance of variations in miR145 expression between these groups, we conducted a Student t-test. miR145 expression analysis showed that p-value less than 0.05, suggesting significant results, as demonstrated in (figure 3). These findings underscore the potential role of miR145 as molecular marker for distinguishing between non-cancer and cancer tissue in CRC with p53 mutations. Additionally, the average expression of miR223, after normalization with the normal group, was <sup>[63]</sup> 2.02 in the tumor group, indicating higher expression in the tumor group compared to the normal group. The normalized fold change values for miR223 ranged from 0.03 to 9.88 for the normal group and tumor group (0.01 to 12.84). Our Student t-test unveiled a significant result with p-value less than <sup>[56]</sup> 0.05, as illustrated in (figure 4). These investigations support that miR223 overexpression might pose important role play in colorectal carcinoma with p53 mutations. Similarly, for miR143, we observed an average expression of 0.6 in test group, signifying higher miR143 expression in control group. The normalized fold change values for miR143 noticed from 0.04 to 5.41 for normal group (control group) and 0.02 to 3.3 for tumor group (test group). The Student t-test demonstrated a statistically significant result (p 0.05) (figure 5). These results underscore the potential significance of miR143 expression differences between non-cancer and cancer tissues in CRC with p53 mutations. Finally, we investigated miR4478, which exhibited an average expression of 0.33 in controlgroup and 0.24 in the tumor group, implying greater expression in the normal group. The normalized fold change values for miR4478 ranged from 0.01 to 2.57 for the normal group and 0.02 to 0.81 for the tumor group. Unlike miR223, miR145, and miR143, the t-test results suggested no notable difference between two groups regarding miR4478 expression (figure 6). This result demonstrates that miR4478 may not be important biomarker in distinguishing between normal and tumor tissues in C R C with p53 mutations, as illustrated in the respective analysis. Altogether, our analysis offers valuable perspective about differential expression of these miRNAs in C R C individuals with p53 mutations. Notably, miR223, miR145, and miR143 exhibited statistically significant, it means there is difference between two groups was analyzed, while miR4478 did not show significant findings. These results underscore the crucial role of miR223, miR145, and miR143 in CRC development. Further research studies are needed to explore the involvement of miRNAs in the molecular mechanism responsible for causing colorectal cancer pathogenesis and this may discover new methods for diagnosis and treatment.

### <sup>[11]</sup> 3.8 Association between miRNA expression level and clinicopathological characteristics

The relationship between miRNA expression levels and clinicopathological characteristics displayed in (table 2).<sup>[11]</sup> Notably, advance age effect is significantly associated with higher miR-223 expression levels (p-value 0.04) and tumor location (p-value 0.01).<sup>[87]</sup> However, no significant association was observed between miRNA expression levels and other clinicopathological factors, including age (P = 0.12) and TNM stage (P = 0.19). Interestingly, miR-145 and miR-143 did not demonstrate any significant associations with clinicopathological characteristics. These findings highlight the potential role of miR-223 in CRC development and its association with certain clinicopathological features, providing valuable insights for further investigation into its clinical significance and potential biological marker in CRC.

### 3.9 Impact of miRNA expression on prognosis of CRC

As shown in (table 3) the results of univariate and multivariate analyses assessing various prognostic parameters in individuals with CRC. The multivariate analysis, conducted with a Cox proportional hazards model, confirmed that low levels of miR-145 expression (HR = 3.54, 95% CI: 1.95 – 9.86, P = 0.011) and miR-223 (HR = 2.91, 95% CI: 1.51 – 8.62, P = 0.0005) were significant independent predictors of poor survival in colorectal cancer.<sup>[3]</sup> These findings underscore the potential prognostic value of miR-145 and miR-223 expression levels in predicting outcomes for colorectal cancer patients.<sup>[2]</sup> Further research into the mechanisms underlying the influence of these miRNAs on cancer progression may yield valuable insights for the development of prognostic markers and therapeutic strategies in CRC management.

## 4 Discussion<sup>[3]</sup>

Tumor suppressor protein (p53) is vital for maintaining genomic integrity and preventing oncogenic transformation by regulating DNA repair, cell cycle, and cell development.<sup>[2]</sup> Inactivation of p53 is frequently observed in many cancers, including CRC, resulting in genome integrity losses (Zhou et al. 2014).<sup>[83]</sup> In our study, we conducted a comprehensive investigation into miRNAs expression profiles and its prognostic importance in CRC cases (Elsaid et al. 2019). Our findings elucidate several key aspects that enhance our understanding about molecular mechanisms driving CRC pathogenesis and progression. In human cancers, the p53 gene is commonly mutated (Kandoth et al. 2013).<sup>[2]</sup> It is assumed that mutations in p53 are crucial in the progression from adenoma to carcinoma during the pathological progression of tumors.<sup>[3]</sup> Prior research suggests that p53 expression serves as a reliable indicator of p53 gene mutation, detecting up to 75% of tumors

with confirmed gene mutations (Kapiteijn et al. 2001)<sup>[67]</sup>. Additionally, another study have reported a concordance rate of up to 94% between p53 expression and the mutation status of the p53 gene as determined by molecular studies (Servomaa et al. 2000)<sup>[2]</sup>. Therefore, our results indicate that the expression bias in p53 contributed to the advancement or escalation of human colorectal cancer by promoting substantial proliferation, forming metastases, and inducing drug resistance (Muller and Vousden 2014)<sup>[2]</sup>. Abnormal expression of the p53 protein, often due to mutations in the p53 gene, can lead to uncontrolled cell growth and the development of tumors (Kmet et al., 2003)<sup>[3]</sup>. The assessment of p53 expression patterns has both prognostic and therapeutic implications, guiding decisions on patient management and treatment strategies.<sup>[3]</sup> Several studies have investigated the prognostic significance of p53 expression in CRC (Lanza et al. 1996)<sup>[2]</sup>. The results of these studies have been mixed, but some have shown that patients with tumors that have high levels of p53 expression have a worse prognosis than patients with tumors that have low levels of p53 expression. This is likely due to the fact that mutant p53 proteins can promote tumor progression and metastasis.<sup>[2]</sup> In the context of our first objective, we evaluated the p53 gene expression pattern in both normal and tumor tissues.<sup>[17]</sup> We observed a slight downregulation of p53 expression in CRC tissues.<sup>[2]</sup> This observation aligns with findings of the existing literature, where the prevalence of p53 expression in colorectal cancer is reported to range from 27% to 76% (Tejpar et al. 2010)<sup>[45]</sup>. However, when normalized to account for potential bias in fold change values, the range of p53 expression was significantly higher in the tumor group compared to the normal group.<sup>[6]</sup> This observation implies that the expression bias in p53 could contribute to the development or progression of CRC. Likewise, research conducted by Aladhraei et al. in 2019 demonstrated the presence of positive p53 expression and its association with CRC in Yemeni patients.<sup>[6]</sup> However, our independent samples t-test comparing p53 gene expression between the normal and tumor group suggests that there was no statistically significant difference in the means of p53 gene expression between these two groups. Notably, research conducted by Horvath et al. revealed a noteworthy connection between p53 overexpression and the progression to neoplasia (Horvath et al. 2015). This finding warrants further exploration with larger sample sizes and more comprehensive analyses to conclusively determine the association between p53 expression levels and CRC prognosis in Saudi population.<sup>[72]</sup> Accumulating data discovered that both wild-type and mutant p53 are regulated by miRNAs, with specific targeting of mutated sites (Liu et al., 2017). The regulatory purpose of miRNAs on p53 was first exemplified with MiR-125b, which interacts to 3'-UTR mRNA of p53, leading to down-regulation and reduced activity (Liu et al., 2017). Subsequent studies have identified additional miRNAs targeting p53 through

experimental and in silico analyses (Hu et al., 2021), highlighting the intricate regulatory network between miRNAs and the p53 gene. To identify miRNAs exhibiting differential expression following p53 activation in CRC cells, we utilized the GSE67181 dataset (Huenten et al., 2015). Our analysis identified numerous miRNAs among significant differential expression profiles within p53-activated CRC cells, including hsa-miR-143, hsa-miR-145, hsa-miR-200b, hsa-miR-200c, hsa-miR-205, hsa-miR-223, hsa-miR-34a, and hsa-miR-4478. Previous research reports have demonstrated that as a result of DNA impairment, wild-type p53 protein has the ability to increase the post-transcriptional processing of various microRNAs, including miR-143 and miR-16-1, which possess growth-suppressive properties. These miRNAs have been identified to attack K-Ras, along with miR-145, which specifically attacks CDK6 (Suzuki et al., 2009). Furthermore, p53 can elevate the expression of the microRNA-200 family and also directly activates the oncosuppressive miR-205 (Chen et al., 2022, Piovan et al., 2012). Conversely, mutant p53 reduces miR-223 expression, contributing to chemoresistance (Masciarelli et al., 2014).

<sup>[2]</sup> Our findings align with these observations, showing a downregulation of miR-223 in tumor samples, consistent with its role in chemoresistance. Furthermore, we observed a downregulation of miR-4478 in CRC tissues, in alignment with previous study showing similar expression pattern in ovarian cancer (OC), suggesting that this process might conserve across different forms of carcinomas (Wang et al., 2021). These results suggest that activated p53 modulates the expression of these miRNAs, influencing downstream mRNA expression and regulating various processes within CRC. Overall, our study highlights the complex network of interactions between p53, miRNAs, and their downstream targets in CRC, underscoring the multifaceted regulatory mechanisms involved in CRC-related processes. Recent studies have increasingly highlighted the correlation between dysregulated miRNAs and CRC biology and clinical features (Ahadi, 2020; Niu et al., 2021). However, the ability of miRNAs as prognostic biological marker and treatment targets in CRC remains underexplored.

<sup>[88]</sup> The objective of the present research study is to evaluate the clinical significance of miR-145, miR-143, miR-4478, and miR-223 in CRC. Quantitative real-time PCR analysis revealed lower levels of miR-143 and miR-145 expression and higher expression levels of miR-223 in CRC tissues compared to matched adjacent normal tissues. While miR-4478 did not reveal a statistical significance among the groups, miR-223 overexpression was statistically related with gender and tumor location. Multivariate analysis confirmed that low miR-145 expression and high miR-223 expression were independent predictors of reduced viability in CRC. Previous literature

research reports have revealed that miR-145 is epigenetically silenced in various cancers and in relation with a more intense phenotype in CRC (Iorio et al., 2007;<sup>[69]</sup> Feng et al., 2014;<sup>[6]</sup> Li et al., 2016). Similarly, miR-143 and miR-145 downregulation disrupts cellular growth and apoptosis in CRC, particularly through the MDM2-p53 feedback loop (Zhang et al., 2013). Regarding miR-223, our findings of its upregulation in CRC align with previous reports, although the association with clinical stage observed in other studies was not evident in our study (Zhang et al., 2014; Wu et al., 2012). Further investigations into miR-223's regulatory role, including its impact on FOXO1 expression, could provide additional insights into its contribution to CRC pathogenesis.

## 5 Conclusion:<sup>[50]</sup>

In conclusion, our study sheds light on the intricate relationship between P53 gene expression, miRNA dysregulation, and CRC susceptibility and progression in the Saudi population.<sup>[50]</sup> Through comprehensive analysis, we identified key miRNAs, including miR-143, miR-145, miR-200b, miR-200c, miR-205, miR-223, miR-34a, and miR-4478, whose dysregulation is associated with p53 mutations and possibly dysfunction in CRC. Specifically, dysregulation of miR-145, miR-143, and miR-223 was linked to poor survival outcomes in CRC patients. These results emphasize the prominence of understanding the molecular mechanisms underlying CRC development and progression, particularly in diverse populations like the Saudi population.<sup>[50]</sup> Moving forward, further scientific work is warranted to establish precise roles of these miRNAs and their interactions with p53 gene polymorphisms in CRC pathogenesis, with eventual intention to develop targeted therapies and personalized interventions for CRC patients in Saudi Arabia and beyond.

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