

Modulation of miR-16 and HO-1 in Egyptian Liver Cancer Patients

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Abstract

Background: Hepatocellular carcinoma (HCC) poses a substantial global health burden characterized by high mortality rates and limited treatment options, particularly in regions with high prevalence rates such as Egypt. To investigate the role of miR-16 targeting HO-1 in Egyptian patients with liver cancer, elucidating the interplay between miRNAs and their target genes in hepatocarcinogenesis, and assessing their prognostic significance and therapeutic potential in this high-risk population. **Methods:** Fifty patients with HCC undergoing follow-up at the general hospital of Benha University, were included in this study. Tumoral and healthy marginal tissues were collected, and RNA was extracted and subjected to reverse transcription to synthesize complementary DNA (cDNA). The expression levels of miR-16 and HO-1 were determined using quantitative real-time PCR, and correlations with clinical parameters were assessed. **Results:** Analysis of demographic data revealed a higher proportion of patients over 60 years old, with a majority being female. Significant alterations in the expression levels of HO-1 and miR-16 were observed in tumor samples compared to controls, suggesting their involvement in HCC pathogenesis. Differential expression patterns between metastatic and non-metastatic samples indicate potential roles in metastasis initiation and progression. Correlation analysis revealed associations between genetic expression, demographic characteristics, and clinical parameters, underscoring the complex interplay between miR-16, HO-1, and disease progression. **Conclusion:** The findings highlight the potential of miR-16 targeting HO-1 as a therapeutic strategy and emphasize the importance of personalized treatment approaches in high-risk populations. Further research is warranted to elucidate the precise mechanisms and develop novel therapeutic interventions for liver cancer.

Keywords: HO-1, miR-16, HCC, therapeutic potential

1. Introduction

Liver cancer, primarily hepatocellular carcinoma (HCC), represents a significant global health burden with high mortality rates and limited treatment options, especially in regions like Egypt where it is prevalent [1], [2]. The dysregulation of microRNAs (miRNAs), small non-coding RNAs involved in post-transcriptional gene regulation, has been implicated in the pathogenesis and progression of various cancers, including liver cancer [3]. Among these, miR-16 has emerged as a critical regulator, with potential implications in hepatocellular carcinoma (HCC) development and progression [4].

Heme oxygenase-1 (HO-1), an enzyme involved in heme degradation and antioxidant defense, has been identified as a target of miR-16 in several cancers [5]. HO-1 overexpression has been associated with tumor growth, metastasis, and resistance to therapy in HCC, making it a promising therapeutic target. However, the precise role of miR-

16 targeting HO-1 in liver cancer, particularly in Egyptian patients, remains poorly understood.

Egypt has one of the highest incidences of HCC globally, attributed largely to factors such as endemic hepatitis C virus (HCV) infection, environmental toxins, and genetic predisposition [6]. Understanding the molecular mechanisms underlying HCC development and progression in Egyptian patients is crucial for the development of effective diagnostic and therapeutic strategies tailored to this population [7].

Profiling miR-16 targeting HO-1 in Egyptian patients with liver cancer offers a comprehensive approach to elucidating the intricate interplay between miRNAs and their target genes in hepatocarcinogenesis [8]. By investigating the expression levels of miR-16 and HO-1 in HCC blood from Egyptian patients, along with their clinical correlations, this study aims to shed light on their prognostic significance and therapeutic potential in this high-risk population.

Moreover, identifying specific molecular signatures associated with miR-16 targeting HO-1 in Egyptian patients may facilitate the development of novel biomarkers for early detection, prognosis, and personalized treatment strategies. Such advancements could potentially improve clinical outcomes and quality of life for HCC patients in Egypt and beyond [9].

In this context, this study presents a timely and pertinent exploration into the role of miR-16 targeting HO-1 in liver cancer among Egyptian patients, with implications for both basic research and clinical practice. By bridging the gap between molecular profiling and clinical outcomes, this research holds promise for advancing precision medicine approaches in the management of HCC, ultimately contributing to the global fight against liver cancer.

2. Materials and Methods

Patients and sampling

This study comprised fifty HCC patients who were referred for follow-up at the Egyptian Liver Hospital, Egypt. During the follow-up, the metastatic tumor patients and non-metastatic ones in addition to healthy control blood samples were collected from each individual. After collection, they were then promptly placed into RNAase inhibitor isolation (Qiagen, Germany) and kept at -80 until RNA isolation was completed. Every research participant completed an informed consent form, and the study's protocol was approved by the local ethical committee. The exclusion criteria included a history of radiation therapy and chemotherapy, co-occurrence of other cancers, and a family history of cancer.

RNA extraction and cDNA synthesis

Following the manufacturer's instructions, RNA was extracted using Tripura Isolation Reagent (Roche, Germany). Thermo Fisher Scientific, USA's Nanodrop spectrophotometer at 260/280 nm (ND-2000C) was used to measure the amount and quality of the RNA samples. Using the micrurgy LNA universal RT kit (Exaton, Verbeek, Denmark), reverse transcriptions of extracted RNA were

performed following the manufacturer's instructions to create complementary DNA (cDNA) [10].

PCR

Using the pre-designed primers (Exaton, Verbeek, Denmark), the expression levels of miR-16 was determined using the mi Script SYBR Green PCR Kit (Qiagen, Germany) and Step One Plus Real-Time PCR (Applied Biosystems, Foster City, CA, USA). To match the expression levels of miRNAs, the housekeeping gene U6 was examined for expression. 8 µl of cDNA, 12 µl of SYBR Green Master mix, 1 µl of primers for each, and 25 µl of RNase-free water made up each reaction mixture. The quantitative Real-time PCR was conducted under the following thermocycling conditions: starting temperature of 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, 55°C for 30 seconds, and finally, 65°C for 60 seconds [11].

Statistical analysis

GraphPad Prism software version 9.2 (GraphPad Software Inc., San Diego, CA, USA) was used to plot data using graphs. The scale data across groups were compared using the Tukey one-way analysis of variance. The Spearman's rho test was employed to do a correlation study between the numerical variables. When appropriate, data were presented as numbers or as mean ± Standard error of the mean (SEM). It was established that less than <0.05 was the significant threshold in the statistical comparisons.

3. Results

Demographic characteristics

Based on the demographic data that appeared in (Table 1) of the clinical samples, we can observe that out of 50 samples, 15 individuals were below 60 years of age and 35 individuals were above 60 years of age. Among the samples, 19 individuals were male and 31 individuals were female. Out of 50 samples, 14 individuals were found to have liver metastasis and 36 individuals did not have liver metastasis. Similarly, 14 individuals showed portal invasion while the remaining 36 individuals did not show any portal invasion. These results can be useful in further analysis and study of the clinical samples.

Table (1) Demographic data of clinical samples.

Characteristics	Classification	N=50
Age	<60	15
	>60	35
Gander	Male	19
	Female	31
Liver Metastasis	Positive	14
	Negative	36
Portal invasion	Yes	14
	NO	36

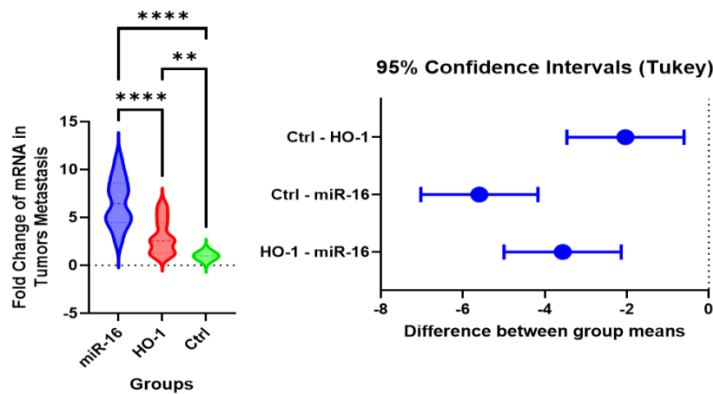


Fig. (1) Relative expression of mRNA in metastatic samples of HCC

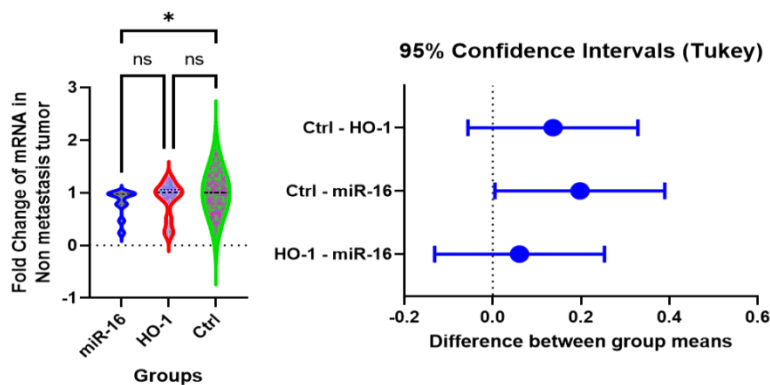


Fig. (2) Relative expression of mRNA in non-metastatic samples of HCC

Relative expression of HO-1 and miR-16 in tumor samples

There was a significant increase in mRNA expression of HO-1 in metastatic and non-metastatic HCC patients compared to control patients when genetic expression of HO-1 and miR-16 was compared. Similarly, miR16 expression was highest in the control sample compared to the ctrl sample (Figure 1). The results suggest that HO-1 and miR-16 are playing an important role in the progression of HCC. In order to understand the exact role these two molecules play in metastasizing HCC; further research is needed. The genetic expression of miR-16 and HO-1 is not upregulated in non-metastatic samples (Figure 2). As a result, miR-16 and HO-1 might be involved in the early stages of metastasis, but may not be important in later stages. The precise role of these two molecules in metastatic processes must be determined through further research. A significant difference was observed between metastatic samples and non-metastatic samples if we compare the two samples side by side. This is because metastatic samples had a significantly higher expression of certain genes that are not expressed in non-metastatic samples.

Correlations between genetic expression and demographic data

Table 2 presents the correlation between genetic expression and demographic data. The table

includes four characteristics, namely Age, Gender, Liver Metastasis, and Portal Invasion, and their corresponding classifications. The table also shows the correlation coefficients of two genes, HO-1 and miR-16, with each of the characteristics. The correlation coefficients suggest that there is a weak positive correlation between the expression of HO-1 and Age (<60 years: 0.21; >60 years: 0.32), and between the expression of miR-16 and Age (<60 years: 0.25; >60 years: 0.29). In terms of Gender, the correlation coefficients indicate a weak positive correlation between the expression of HO-1 and Male (0.123), and a weak negative correlation between the expression of miR-16 and Female (0.021).

The expression of HO-1 shows a strong positive correlation with Liver Metastasis (Positive: 0.001), whereas miR-16 shows a weak positive correlation with Negative Liver Metastasis (0.011). Finally, for Portal Invasion, the correlation coefficients indicate a weak positive correlation between the expression of HO-1 and Yes (0.012) and a weak negative correlation between the expression of miR-16 and No (0.123). Overall, the correlation coefficients suggest that there is a weak association between the genetic expression of HO-1 and miR-16 and demographic data, with some variations depending on the characteristics and classifications.

Table (2) Correlation between genetic expression and demographic data.

Characteristics	Classification	HO-1	miR-16
Age	<60	0.21	0.25
	>60	0.32	0.29
Gender	Male	0.123	0.27
	Female	0.041	0.021
Liver Metastasis	Positive	0.001	0.001
	Negative	0.41	0.011
Portal invasion	Yes	0.012	0.004
	No	0.14	0.123

4. Discussion

Liver cancer, particularly HCC, presents a significant global health challenge, especially in regions like Egypt where its incidence is notably high [12], [13]. Understanding the molecular mechanisms underlying HCC development and progression is crucial for advancing diagnostic and therapeutic strategies tailored to specific patient populations [14]. The results obtained from the profiling of miR-16 targeting HO-1 in Egyptian patients with liver cancer provide valuable insights into the interplay between these molecules and their potential implications in HCC pathogenesis.

The demographic characteristics of the clinical samples shed light on important factors such as age, gender, presence of liver metastasis, and portal invasion. These parameters are essential for stratifying patients and understanding potential associations with disease progression and outcomes. In this study, a larger proportion of patients were over 60 years old, reflecting the higher incidence of liver cancer in older individuals. Additionally, the gender distribution revealed a higher representation of females, possibly influenced by factors such as hormonal and environmental differences. The presence of liver metastasis and portal invasion highlights the advanced stage of the disease in a subset of patients, underscoring the urgent need for effective therapeutic interventions [15].

The analysis of mRNA expression levels of HO-1 and miR-16 in tumor samples provides crucial insights into their potential roles in HCC progression [16]. The observed significant increase in HO-1 mRNA expression in both metastatic and non-metastatic HCC patients compared to control patients suggests its involvement in HCC pathogenesis. Similarly, the downregulation of miR-16 expression in tumor samples indicates its potential tumour-suppressive role, consistent with previous findings in various cancers [17].

Furthermore, the differential expression patterns observed between metastatic and non-metastatic samples suggest a potential role for miR-16 and HO-1 in metastasis initiation and progression. While the exact mechanisms underlying their involvement in metastasis warrant further investigation, these findings underscore the importance of exploring

miR-16 and HO-1 as potential therapeutic targets for inhibiting HCC metastasis [18].

The correlation analysis between genetic expression and demographic data provides insights into potential associations between miR-16, HO-1, and patient characteristics. The weak positive correlation between HO-1 expression and age suggests a potential age-related influence on HCC progression, possibly related to cumulative exposure to risk factors over time. Similarly, the association between HO-1 expression and male gender underscores the need for further exploration of gender-specific factors contributing to HCC pathogenesis.

Notably, the strong positive correlation between HO-1 expression and the presence of liver metastasis highlights its potential as a biomarker for metastatic disease [19]. Conversely, the weak positive correlation between miR-16 expression and negative liver metastasis suggests its potential role in inhibiting metastasis progression. These findings emphasize the importance of considering molecular markers in conjunction with clinical parameters for prognostic stratification and personalized treatment strategies in HCC [20]. The profiling of miR-16 targeting HO-1 in Egyptian patients with liver cancer provides valuable insights into the molecular mechanisms underlying HCC pathogenesis and progression. The observed associations between genetic expression, demographic characteristics, and clinical parameters underscore the complex interplay between miR-16, HO-1, and disease progression. Further elucidation of these mechanisms may lead to the development of novel therapeutic strategies aimed at improving outcomes for HCC patients, particularly in high-risk populations like Egypt.

5. Conclusion

Overall, the findings from this study contribute to the growing body of evidence implicating miR-16 and HO-1 in HCC pathogenesis and metastasis, particularly in high-risk populations like Egypt. Further research aimed at elucidating the precise mechanisms underlying their actions and exploring their therapeutic potential is warranted. Ultimately, the insights gained from this study may pave the way for the development of novel diagnostic biomarkers

and targeted therapies tailored to the unique molecular characteristics of HCC, with the potential to improve patient outcomes and reduce the global burden of liver cancer.

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