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The Levels of microRNA-141 in Hepatocellular Carcinoma Cell Lines

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Abstract

Hepatocellular carcinoma is considered one of the most threats to human health and is considered a fatal threat globally. Hepatocellular carcinoma is still a health challenge, and its incidence is growing worldwide. The role of non-coding miR-141 RNA in Hepatocellular carcinoma needs more investigations. Micro-RNA consists of 18-22 nucleotides. The role of miR-141 in hepatocellular carcinoma needs many investigations. The expression of miR-141 was elucidated in different cell lines including HepG2, HuH7, and the normal cell lines. The cell survival rate was detected in the case of untreated cells, control cells, and transfection by overexpression vector for miR-141, and anti-miR-141 transfection in the HepG2 cell line. The survival rate was at its highest level in the case of overexpressed miR-141 while it showed the least survival rate at anti-miR-141 transfection. Additionally, the effect of miR-141 was tested on both the IL-6 as an inflammatory cytokine and on TNF-α as well. In conclusion, miR-141 plays a pivotal role in HCC carcinogenesis.

Keywords: human health, Hepatocellular carcinoma, miR-141, liver cancer.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is considered one of the most highest mortality in cancer types and it represents about 80% of cases of liver cancer (Llovet *et al*., 2021) (Jemal, 2011). The ages of 55- 59 are more exposed for HCC in China while in Europe and North America lies between 63 to 65. (Jemal *et al*., 2011; El-Serag, 2012).

On the other hand, HCC numbers may reach>1 million individuals will be affected by liver cancer annually by 2025 (Yang *et al*., 2019). Hepatocellular carcinoma (HCC) is the most common form of liver cancer and accounts for ~90% of cases as about 782,000 new cases and 745,000 deaths of liver cancer in 2012 worldwide. Consequently, HCC is known to be the second cause of death among cancer types (Ferlay *et al*., 2015). The HCC development may be a combination of both genetics and environmental factors as well. The main cause of HCC is infection by viruses like hepatitis C virus (HCV), and hepatitis B virus (HBV). Besides, alcohol consumption and exposure to vast amount of some toxins like aflatoxin B1, and nonalcoholic steatohepatitis are important risk factors for HCC development as well (Pimenta *et al*., 2010; Gomes *et al*., 2013).

The miRNAs is considered as non-coding RNAs which varied from 18-22 nucleotides (Sarkar and Kumar 2021). The role of miRNAs is in the regulation of gene expression. Each miRNA may affect one or more than one gene expression. This effect may be in different mechanisms like deletion of miRNA genes, dysregulation of transcription machinery, or causing epigenetic alteration on the targeted mRNA (Morishita *et al*., 2021). Additionally, MiRNAs may act as oncogenes which play a role in carcinogenesis or it may act as tumor suppressors in some cases which may hinder carcinogenesis under certain conditions (Peng and Croce 2016). MicroRNAs may reduce their specific target genes by binding specifically to the 3 '(3'-UTR) untranslated region of target mRNAs. The miRNAs can affect the expression of different genes via different mechanisms on the cell itself like autophagy, apoptosis, metastasis, inflammation, and DNA repair machinery. MicroRNAs also may contribute to carcinogenesis,

cancer diagnosis, cancer treatment and even to differentiate between cancer subtypes. Also, miRNAs can be used as diagnostics tools for tumors of unidentified origin and can be used as biomarkers due to their stability. Besides, miRNAs may be considered as a tool of treatment to overcome radiation resistance (Wang *et al*., 2018).As one important member of the miR-200 family, miR-141 is aberrantly expressed in many human malignant tumors, participating in various cellular processes including epithelial-mesenchymal transition (EMT), proliferation, migration, invasion, and drug resistance. Interestingly, miR-141 is overexpressed in ovarian tissues, nasopharyngeal carcinoma, prostate cancer, classic papillary thyroid carcinoma, bladder cancer and colorectal cancers, while down-regulated in gastric cancer, pancreatic ductal adenocarcinoma, pancreatic cancer, osteosarcoma, prostate cancer hepatocellular, primary peritoneal carcinoma, choriocarcinoma, esophageal cancer, breast cancer and renal cell carcinoma, raising a controversial issue about the role of miR-141 in cancer progression. Additionally, dysregulation of miR-141 depends on the type of cancer; in other words, miR-141 plays a dual role in tumorigenicity and can modulate cellular motility and control "stemness". The miR-141 may be considered as an oncogene or as a tumor suppressor gene therefore can be used as a cancer therapeutic agent. (Gao *et al.*, 2016). Therefore, the expression levels of miR-141 in HCC need more investigation. In this study, we will try to determine the expression level of miR-141 in HCC which may be proposed in the treatment for HCC.

2. MATERIALS AND METHODS

2.1 Cell Lines and Its Propagation

Different cell lines were propagated in this study which first were checked for presence of mycoplasma. The normal cells, Huh7 and HepG2 cell lines were studies in this research.

2.2 Variation in the Levels of Mirna 141 in Different Cell Lines in This Study

To detect the levels of miR-141, the following experiments were performed. First, the total RNA was first extracted via TRIzol which then purified. This Purification was conducted by using RNeasy kit. Then cDNA was performed to estimate the miR-141 expression by qRT-PCR. The step for cDNA synthesis was done in a reaction duration of 30 minutes at 50˚C. Then, the next step was for 3 minutes at 95˚C and the number of cycles were 40 cycles. Then the next step was 95˚C for 30 seconds. Then 60˚C for 15 seconds. Then 72˚C for 15 seconds. Finally, this step was adjusted at 72˚C for 10 minutes.

2.3. Proliferation and Cytotoxic Effect on Mir-141 on Cell Line

Cells were grown in duplicate in a 6-well plate with 10X104 cells per well for the proliferation assay of transfected HepG2 cells with either an inhibitor or an overexpression vector for miR-141. An inverted microscope was used to keep an eye on cell morphology. Using a hemocytometer, the number of transfected cells that survived was counted. In a nutshell, the old media was discarded, and the cells were then given two PBS washes before being trypsinized for three minutes at 37 °C. The trypsinized cells were then given a reasonable volume of full RPMI medium, and the number of cells was manually counted (Hamouda *et al*., 2021). HepG2 cells were seeded in triplicate with 10x103 cells per well in 96-well plates to test the cytotoxicity of miR-141 transfection.

2.4. Elisa Test for Detection the Effect of Mir-141 on IL-6

To analyze the connection between miR-141 expression stage and the proinflammatory cytokines together with TNF SF15 and IL-6, the attention of TNF-α secreted IL-6 was monitored in transfected HepG2 cells in a time-path test. As proven in determine 3A, the amount of produced TNF-α elevated in cells transfected with miR-141 in a time-established way and reached 500pm/ml at forty-eight hrs put up-transfection. While the extent of TNF SF15 markedly reduced in cells transfected with a miR-141 inhibitor as compared with manage transfected cells. In contrast, the level of produced IL-6 was significantly reduced in miR-141 transduced cells while markedly growing up to 500pm/ml in cells transfected with the inhibitor antagonist miR-141 (Fig. 3B). This record shows that inhibition of miR-141 expression increases the production stage of TNF SF 15 and IL-6 in HepG2 cells.

3. RESULTS AND DISCUSSION

3.1. The Relative Gene Expression of Mir-141 in Different Cell Lines

The relative expression of miR-141 was quantified in different cell lines. This quantification was estimated in HuH7 cell line, HepG2 and normal cell lines. The results showed a variation in miR-141 expression as the highest expression was detected in HepG2 cell line while it showed its lowest expression in HuH7 cell line. Besides, the expression of miR-141 was also estimated in non-treated cells, Control cells, overexpressed miR-141, and Anti-miR141 HepG2 cell lines. The miR-141 is increased significantly in cells with overexpressed-miR-141 cells. On the contrary, miR-141 showed nearly no expression in case of transfection with anti-miR-141.

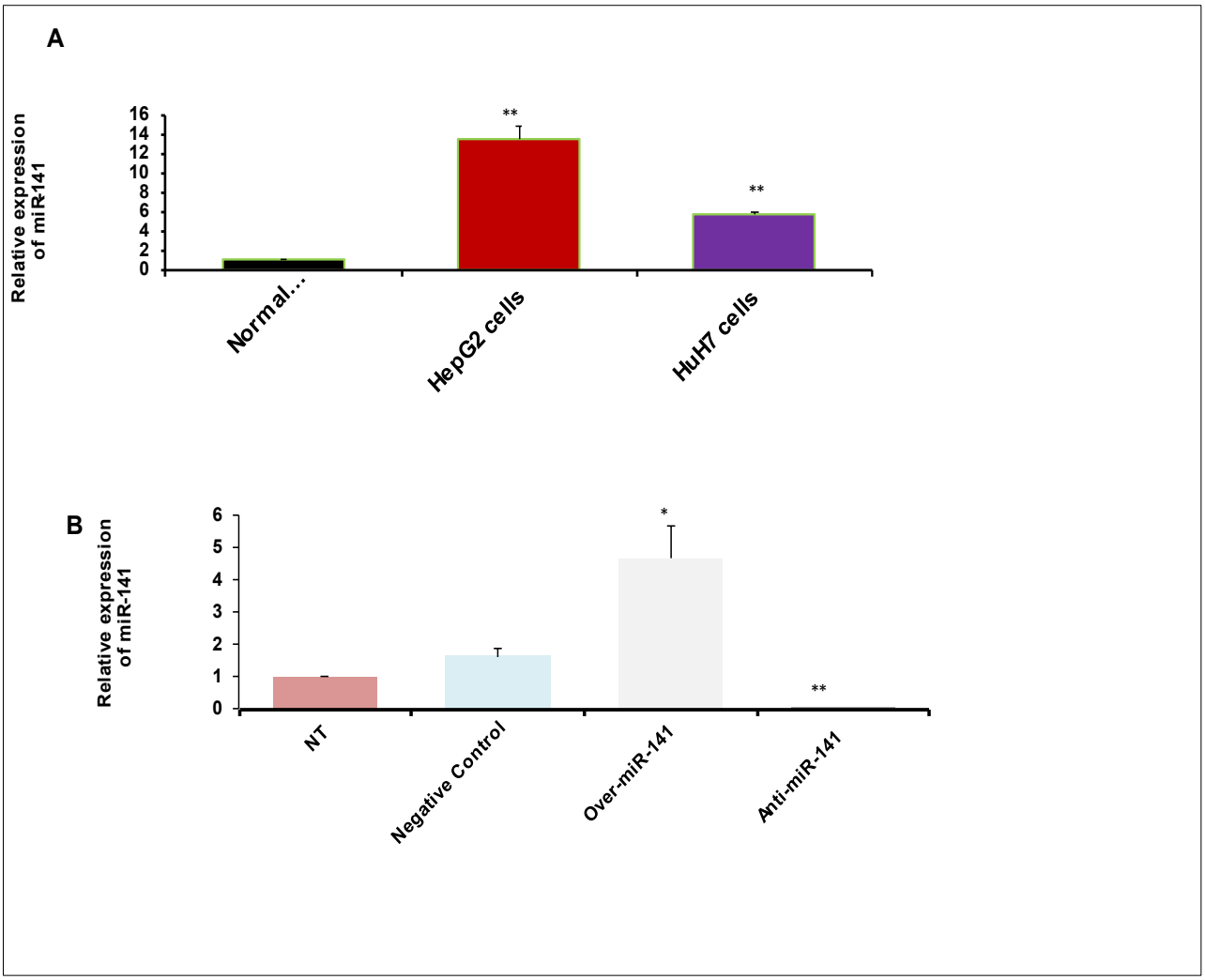

Fig. 1: The relative expression of miR-141 in different cell lines. The expression of miR-141 was detected in different cell lines a) the miR-141 expression level was the highest in HepG2 cell lines while it was the lowest in normal cells. B), the expression of miR-141 was compared in both non-treated cells, negative control, over-miR-141 transfected cells, and Anti-miR-141 transfected cells

3.2 The Effect of Mir-144 Expression Level on Cells and Cell Survival

The cell survival of HepG2 cell line was elucidated based on the expression of miR-141. The anti-miR-141showed a notable decrease in the cell survival in comparison between the transfection by miR-141 overexpressed vector in HepG2 cell lines. On the contrary, there is nearly no change in cell survival either in untreated cells or control cells. Ass the number of survived cells reached 100000 cells while it showed nearly 300000 cells in case of transfection with overexpressed miR-141 transfected cells. Notably, the cell survival rate did not differ both negative control and overexpressed miR-141 transfected cells.

3.3 The Regulation of miR-141 on IL-6

The IL-6 levels in HCC patients were significantly higher than in healthy controls and patients with hepatitis and cirrhosis. Also, IL-6 levels in patients with hepatitis and cirrhosis were significantly higher than in healthy controls. IL-6 is considered one of the most important cytokines involved in predicting survival of HCC. A high serum IL-6 level is an indicator of the tumor burden, i.e., tumor size, stage, and aggressiveness such as portal vein invasion and metastasis. IL-6 is a pleiotropic cytokine that has a central role in hematopoiesis and acute phase responses (Shakiba, Sadeghi, and Shakiba 2019). IL-6 is highly expressed in liver cancer tissue and loaded in serum, and overexpressed IL-6 is closely associated with the staging, severity, and prognosis of HCC (Xu *et al.*, 2021).

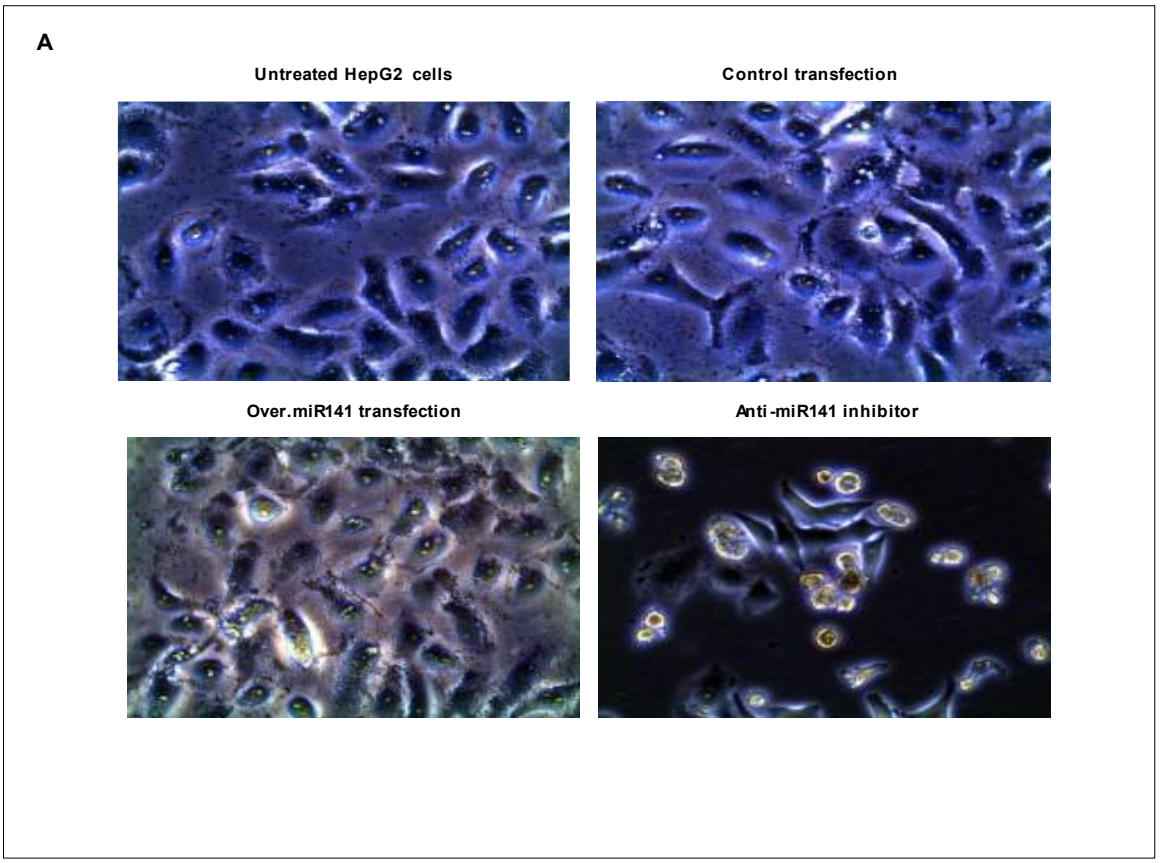

Fig. 2: The effect of miR-141 on the cell shape and confluency. The effect of miR-141 on HepG2 cell line. The HepG2 cells showed no alteration in the case of untreated cells and control transfection treatment. However, the hepG2 cell was vastly affected due to anti-miR141 inhibition transfection. While this effect was partially little in the case of overexpression vector of miR-141

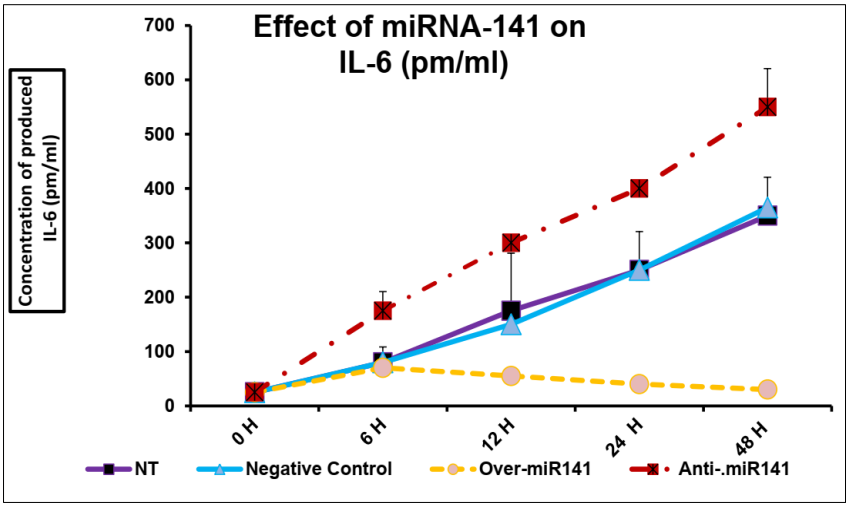

Fig. 3: The relation between miR-141 expression level and the IL-6 production in HepG2 cell line. The IL-6 showed no alteration in case of non-treated cell (NT) as well as the negative control. While it showed high production in case of inhibition of miR-141 via anti-miR-141 however, in case of overexpression of miR-141, the IL-6 production was reduced dramatically from 6 hours to 48 hours

CONCLUSION

HCC is considered as a global problem not only in treatment but also in early diagnosis and its pathogenesis pathways. The role of miRNA in HCC carcinogenesis is considered of great importance. The expression of miR-141 in HCC investigated as it showed its highest rate in HepG2 cell lines. Additionally, its expression showed its highest rate in overexpression of miR-141 while miR-141 expression decreased dramatically with the usage of antagonistic-miR-141. Also, miR-141 affected cell survival in a proportional way as with increase of its expression, cell survival was

high and whenever it decreases the cell survival depleted too nearly up to half. On the other hand, miR-141 affects negatively on both TNF- and IL-6. As the miR-141 expression decreases, the expression of TNF-α and IL-6 expression increased.

Conflicts of Interest: Authors declare no conflict of interest.

Authors' Contributions:

Ahmed Awad performed the experiments. Adel Guirgis, Mahmoud Nasr and Ghada Nasr helped in supervision. Hany Khalil designed the research plan, interpreted, and organized the results, and Ahmed Awad wrote the manuscript.

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