Exportin-5 gene polymorphism and risk of HCC development in Hepatitis C Egyptian patients

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ABSTRACT

Background: MicroRNAs (miRNAs) are small non-coding RNAs that play a chief role in control of gene expression and contribute in a variety of biological functions. Because of the important role of nuclear export of pre-miRNAs in miRNA biogenesis by exportin-5 (XPO5), any alterations of XPO5 could affect miRNA expression and thus have profound effects on tumorigenesis. Objective: The aim of our work was to examine XPO5 gene polymorphism in HCV- HCC patients compared with HCV positive patients and healthy controls in Egyptian population. Material and methods: Forty nine HCV infected patients, Forty nine patients diagnosed with HCC on top of HCV and 47 healthy volunteers were included in the study. XPO5 gene polymorphism was detected by using RT-PCR (Real Time polymerase chain reaction) technique. Results: A/A, A/C and C/C frequencies in HCV patients were 5%, 22%, 22% respectively, furthermore the frequencies were 3%, 23%, 23% in HCC patients and 6%, 17%, 24% in the control group respectively. The frequencies of A and C alleles in HCV patients were 32 %, 66 % and in HCC patients were 29%, 69% while in the control group the frequencies were 29 %, 65 % respectively. There was no significant difference between the studied groups regarding A/A, A/C, C/C frequencies or A and C allele’s frequencies. Conclusion: XPO5 gene polymorphism is neither associated neither with HCV infection nor with development of Hepatocellular carcinoma (HCC) and its progression.

Keywords: miRNAs, exportin-5 gene polymorphism, HCV and HCC.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) considered the fifth common cancer worldwide. It causes more than half a million deaths each year; this makes it the third main cause of cancer deaths (Bartel, 2004). MicroRNAs (miRNAs), a group of small non-coding RNAs (about 22 nucleotides), which regulate post transcriptional gene expression by attaching to complementary sequences in the 3'- untranslated region (3'-UTR) of target messenger RNA (mRNA), and give rise to the silence of genes. Up to 30% of protein-coding genes could be regulated by miRNAs, although miRNAs constitute only 1%-3% of the entire human genome. Abnormal expression
of miRNAs is associated with many human diseases, such as hepatocellular carcinoma, lung cancer and endocrine pancreatic tumor (Bartel, 2009).

The miRNA biogenesis in mammals takes many steps. At first, miRNA genes are transcribed by RNA polymerase II, into primary transcripts (pri-miRNAs). Next, the pri-miRNAs are cleaved by Drosha/DGCR8 complex into the pre-miRNAs which composed of a 70-nt stem-loop structure. Following nuclear transport by GTP-binding nuclear protein Ran (RanGTP)/exportin-5 (XPO5) complex to the cytoplasm, the pre-miRNAs are cleaved by Dicer to produce the mature miRNAs. The mature miRNAs are incorporated into the RNA induced silencing complex (RISC) and organize gene expression through translational repression or mRNA degradation (Campayo et al., 2011).

Hence, XPO5-mediated nuclear export of pre-miRNAs could be a key element step through miRNA biogenesis. Nuclear export of pre-miRNAs is perfectly regulated in normal cells and its dysregulation could lead to abnormal expression of mature miRNAs in cancer cells. Remarkably more pre-miRNAs are found to be retained in the nucleus of both cancer cells and tumors, when compared with normal cells and normal tissues (Chiosea et al., 2007). Many miRNA-related SNPs are proved to have a significant role in hepatocellular carcinoma development such as: miR-DICER rs1057035, miR-XPO5 rs11077, pri-miR-34b/c rs4938723, miR-196a2 rs11614913, miR-106b-25 rs999885 and miR-146a rs2910164 (De Larrea et al., 2012).

The miR-SNP of rs11077 of XPO5 has been well-known to be associated with the risk of esophageal cancer as well as the outcome of non-small-cell lung cancer and multiple myeloma. This SNP located in 3' UTR of XPO5 may interfere with mRNA stability and associated with effective expression of XPO5. So far the mechanisms how this SNP modified the HCC survival remain unclear (Gomaa et al., 2008).

2. SUBJECTS AND METHODS
Our study was conducted in the Tropical department at Theodor Bilharz Research Institute (TBRI). The study duration was 12 months as it started at June 2019 and ends June 2020. 145 subjects were enrolled in the study and divided into 3 groups; group A contain 49 patients infected with HCV while group B composed of 49 patients diagnosed as HCC on top of HCV and The last group C involves 47 age and sex matched individuals as a control group. We exclude those with HBV infection, schistosomiasis, alcohol abuse or antiviral treatment from the work. An informed consent was taken from patients who participated in the study. Furthermore, the technical work was accepted by TBRI Ethics board according to Helsinki Declaration ethical approval number (FW00010609).

Serological markers
HCV antibodies and HBsAg were assessed using enzyme-linked immunosorbert assay (ELISA).

DNA extraction
Genomic DNA was extracted using the QIAMP DNA Mini Kit (Qiagen; catalog No.: 51104). 5ml venous blood was obtained in a sterile EDTA vacuum tube for genomic DNA extraction by means of standard protocol.

Gene polymorphism
XPO5 gene polymorphism (rs11077) was detected using Taq Man SNP genotyping assay. This assay consists of a single, ready to use tube that contains two sequences –specific primers for amplifying the polymorphism of interest. This PCR reaction was done in a thermal cycler with the following program shown in table (1).

<table>
<thead>
<tr>
<th>Table 1 PCR amplification run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Read Run</td>
</tr>
<tr>
<td>Cycle</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

Statistical analysis
The data were analysed using Microsoft Excel 2016 and statistical package for social science ‘IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA)’. Categorical variables were presented as frequencies and percentage; a p value < 0.05 was considered statistically significant.
3. RESULTS

Our results reported that there was no significant difference in allele frequencies between all studied groups (fig 1, table 2).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Frequency of XPO5 Allele and genotype among all the studies groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP</td>
<td>Group</td>
</tr>
<tr>
<td></td>
<td>Control N=47</td>
</tr>
<tr>
<td>A/A (wild)</td>
<td>6 (13%)</td>
</tr>
<tr>
<td>A/C (hetero)</td>
<td>17 (36%)</td>
</tr>
<tr>
<td>C/C (homo)</td>
<td>24 (51%)</td>
</tr>
<tr>
<td>AC+CC</td>
<td>41 (87%)</td>
</tr>
<tr>
<td>Allele</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>C</td>
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</table>

Fig 1 Frequency of XPO5 Allele and genotype among all the studies groups

4. DISCUSSION

XPO5 is a member of the karyopherin β family that organizes the nucleus export of pre-miRNAs. miRNAs are vital organizers in cellular processes such as cell development, proliferation, differentiation, and apoptosis by controlling the expression of specific genes. Therefore, the abnormal expression of miRNAs contributes to a wide range of human diseases and malignancy. miRNAs considered also as tumor suppressors or oncogenes (Hoshida et al., 2014). These mutations of XPO5 gene lead to isolation of the pre-miRNAs in the nucleus which leads to block the miRNA biogenesis and function. The XPO5 protein crosses the nuclear membrane, and mediates the nuclear export of Dicer mRNA which is responsible for the cleavage of pre-miRNA to the mature miRNA (Larrea et al., 2012).

XPO5 miR-SNP interacts with Dicer, decrease the expression of miRNAs, and thus interfere with miRNA biogenesis. rs2257082 and rs11077 are the main two XPO5 gene polymorphisms recognized by their importance in human diseases (Lee et al., 2008). These XPO5 miR-SNPs (rs11077) at the 3'UTR of the gene, can change miRNA and targeted genes expression so can affect cancer development, therapeutic efficacy and patient's prognosis (Li et al., 2016). XPO5 can transport certain tRNAs and proteins besides pre-miRNAs thus discovery of these other XPO5 substrates could be helpful to understand the role of XPO5 in different tumors (Liu et al., 2014). Among all types of malignant tumors, HCC is ranked as the second worldwide main cause of cancer death; it has been considered one of the most common types of primary malignant hepatic tumors that characteristically carry poor prognosis (Mansini et al., 2018).
Egypt has one of the highest global number of hepatitis C virus (HCV) infections; it is estimated that prevalence of HCV is around 4.5% to 6.7%. A national screening programme was started in Egypt in 2018 that aims to screen 62 million adults and 15 million adolescents by 2020. The hepatitis C screening programme is done with screening for diabetes, high blood pressure and obesity. People diagnosed with hepatitis C obtain a free 12-week course of treatment with generic versions of direct-acting antivirals (Melo et al., 2010). The pathogenesis of HCV-induced HCC is a multi-step process that typically develops within 20 to 40 years, these steps could be summarized in formation of chronic HCV infection simultaneously with hepatic inflammation, advanced liver fibrosis, induction of neoplastic clones together with permanent somatic genetic/epigenetic mutation and finally formation of carcinogenic tissue microenvironment with progression of these neoplastic clones (Ott et al., 2016).

The rs11077 was the most studied SNP, reported to be related to clinical outcome in several cancers. This SNP consists in an A to C transition, resulting in the loss of the miR-617 binding site to the 3'-UTR region of XPO5 mRNA and decrease XPO5 mRNA degradation via miR-617 which leads to increase of XPO5 expression in tissues (Patrao et al., 2018). Because of the harmful consequences of target mRNAs and cellular processes caused by small alteration of miRNAs activity or quantity, miR-SNPs of XPO5 gene were considered as a potential and valuable molecular biomarker in cancer prediction prognosis (Peng et al., 2016).

XPO5 expression is upregulated in colorectal cancer (CRCs) and prostate cancer, but downregulated in lung adenocarcinoma. It appears that XPO5 may exert its role dependent on tissue-specific expression of miRNAs. Therefore, whether XPO5 function as a tumor suppressor or oncoprotein in CRCs is still debatable and needs further investigation (Rah et al., 2013).

The aim of our study was to validate the association of XPO5 gene polymorphism with HCV infection and HCC incidence. This was done through assessing 49 HCV patients, 49 HCC patients against 47 healthy controls using RT-PCR. In our study, we found no significant difference in the frequency of A/A, A/C and C/C genotype carriers between HCV patients, HCC patients and the control group. Our results were concomitant with a study made by Liao et al., 2018 who found that after a multivariate analysis of XPO5 rs11077 genotypes in gastric cancer, there was no significant association between XPO5 rs11077 genotypes.

In contrast to our study other studies have reported that patients with AA genotype of rs11077 exhibit an increased risk of esophageal cancer and gastric cancer, and are associated with short overall survival in multiple myeloma (after autologous stem cell transplantation), Hodgkin’s lymphoma, and renal cell carcinoma compared to A/C or C/C genotypes which associated with better outcome and lower recurrence (WHO 2016). Ott et al. (2016) reported that melanoma cell lines and patient samples with the rs11077 CC genotype displayed a significantly higher XPO5 mRNA level than those with the rs11077 AA genotype (Wickens and Cox 2009). Since the Raf/MEK/ERK pathway is usually deregulated in melanoma, the authors investigated if alterations in this pathway can also have an impact in XPO5 and observed that, when treating melanoma cells with Raf/MEK/ERK inhibitors, a significant decrease of XPO5 was noted. The authors concluded that XPO5 upregulation in malignant melanoma was a result of upregulated MEK signaling and enhanced XPO5 mRNA stability induced by the XPO5 rs11077 SNP (Patrao et al., 2018).

Yet in another study, de Larrea and colleagues reported a trend of a 17.4% reduction of XPO5 protein in peripheral blood lymphocytes of healthy persons carrying the XPO5 rs11077 CC genotype. One possible explanation found by the authors for this protein reduction in the individuals with the XPO5 rs11077 CC genotype is the fact that the occurrence of the SNP could allow the binding of new miRNAs (miR-4763-5p) to the XPO5 mRNA sequence, producing a reduction of its protein levels (Xie et al., 2015). Based on the findings of these two studies we can conclude that the functional impact of the XPO5 rs11077 SNP varies according to the cell phenotype and molecular signature which may give the explanation on why in some cancers XPO5 has an oncogenic role and in others acts as a tumor suppressor and also why both variants of the same SNP can be associated with a worse disease outcome. In fact, when analyzing the results in the different studies regarding the impact of rs11077 in cancer outcome, we observed that both XPO5 rs11077 allele C and XPO5 rs11077 allele A carriers were associated with worse prognosis, depending on the type of cancer (Yuqian et al., 2018).

Our findings suggest that the A to C replacement of rs11077 was markedly associated with increased cancer risk this may be due to decreasing the mRNA levels of XPO5 which influence the expression of miRNAs, resulting in an aberrant expression of miRNA target gene at the post-transcriptional level. The relationship between polymorphisms in miRNA machinery genes and the outcome of cancer patients is controversial because the effects of miRNA-SNPs are modulated in a tissue specific manner; there are some other possible explanations. First, different ethnic populations were investigated in different studies, so data should be used to other ethnic groups cautiously. Second, stage of disease in enrolled patients as genotypes were significantly related to severity of disease so analysis of genetic polymorphisms in miRNA processing genes may help to identify patient subgroups with poor prognosis and may, accordingly, help to refine therapeutic decisions regarding HCC patients.

A main limitation of our results was the patients’ number, which interfere with drawing definitive conclusion. Also we could not perform a subgroup analysis with respect to the ethnicity, source of control groups and cancer type. Gene-environmental conditions which may affect cancer risk were not assessed due to the absence of related data. In view of all this, we suspect the potential role of...
the miRNAXPO5 (rs11077 A/C) SNPs in cancer risk which will be a new biomarkers of cancer. Further researches and functional evaluations are still needed to validate our findings due to the limitations mentioned above.

5. CONCLUSION

miRNAXPO5 (rs11077 A/C) SNPs might not be a useful determinant in predicting the outcome of HCV infection or HCC susceptibility in Egyptian population. Additionally, it would also be interesting to study SNPs in the other genes involved in the miRNA biogenesis and processing machinery to improve our knowledge in this research area.

**Abbreviations**

MicroRNAs: miRNAs.
Exportin-5: XPO5.
Hepatitis C virus: HCV.
Hepatocellular carcinoma: HCC.
Primary transcripts: pri-miRNAs.
GTP-binding nuclear protein Ran: RanGTP.
RNA induced silencing complex: RISC.
Single-nucleotide polymorphisms: SNPs.
Theodor Bilharz Research institute: TBRI.
Hepatitis B surface antigen: HBsAg.
Enzyme-linked immunosorbent assay: ELISA.
Deoxyribonucleic acid: DNA.
Real time polymerase chain reaction: RT-PCR.
Colorectal cancer: CRCs.
Overall survival: OS.

**Conflict of interest**
The authors declare that they have no conflict of interest.

**Author Contributions**

Rabab Fouad: Technical work and manuscript writing.
Marwa Wahdan: Sample collection and statistical work.

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**Ethical approval**

Ethical approval cleared by the ethic committee of Department of Hematology, Theodor Bilharz Research Institute, Cairo-Egypt.

**Data and materials availability**

All data associated with this study are present in the paper.

**REFERENCES AND NOTES**


