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Genetic Variations of Estrogen Receptor (ESR) Associated with Hepatitis C Virus-Induced Hepatocellular Carcinoma: Scientific Ramifications

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Abstract---Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and a major public health concern in Egypt. HCC is a tough condition to cure, and genetic diversity has been linked to the disease's progression. HCC is a complicated condition in which 95% of patients have chronic liver disorders, the majority of which are caused by viruses. One of the causes of HCC is the hepatitis C virus (HCV). Sex hormones, such as estrogen, have an effect on the liver. Although estrogen (ER) is known to play a function in a range of biological processes, its role in the development of HCC is controversial, with evidence pointing to both a carcinogenic and a preventative effect on the liver. Estrogen receptor (ESR) was shown to be strongly expressed in HCV-infected people. This study reveals that ESR and its variants play a role in hepatocarcinogenesis development.

Some single nucleotide polymorphisms (SNPs) may have a functional influence on the end product of a gene, which may be assessed, and may play a role in pathogenic alterations.

Keywords---estrogen receptor, hepatitis C virus, hepatocellular carcinoma, single nucleotide polymorphisms.

Introduction

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the fifth major cause of cancer globally and a significant medical concern in Egypt, accounting for the fourth and second main causes of cancer death in males and females, respectively (Rashed et al., 2020). HCC is the most common primary liver tumor, and its prevalence is rising globally (Ozakyol, 2017). HCC is a complex illness, and genetic variability has been identified as a risk factor for its progression. HCC is a multifactorial illness in which 95 % of patients have underlying chronic liver disorders, most of which are viral in origin (Dragani, 2010) (Jeng et al., 2009).

Hepatitis C virus (HCV), hepatitis B virus (HBV), and alcohol use are among the etiological agents of HCC. Nonalcoholic steatohepatitis (NASH) (Anstee et al., 2019), hemochromatosis, B1 aflatoxin exposure, autoimmune hepatitis, primary biliary cirrhosis, and other hepatic metabolic illnesses are some of the other reasons that have a limited worldwide effect (Sagnelli et al., 2020),(Kamal, 2021). Because of changes in the timing and degree of exposure to environmental and viral risk factors, there are significant worldwide variations in the incidence and mortality of HCC (Yang et al., 2019). Nearly 85% of HCC cases are thought to occur in low- and middle-income countries, especially in Eastern Asia and Sub-Saharan Africa. In individuals with cirrhosis caused by persistent HBV or HCV infection, the yearly incidence of HCC is 2–5%¹⁵. HBV-associated HCC, on the other hand, usually develops in the absence of cirrhosis, accounting for 30–50% of HCC in HBV-endemic areas like Eastern Asia and most African nations (Konyn et al., 2021).

Genomic DNA variations, particularly single nucleotide polymorphisms (SNPs), might be host genetic variables that promote HCC risk. Numerous SNPs and other variants have been discovered as correlates with increased HCC risk in single gene or biological hypothesis-based research, as well as an unbiased survey via genome-wide association study (GWAS). Since many individuals who are exposed to established environmental risk factors never develop cirrhosis or HCC, and a considerable proportion of instances of the illness acquire HCC without any risk factors (Deng et al., 2017).

Males have a greater incidence of HCC than women, and their risk is 2–7 times higher in men, according to epidemiological research, albeit this ratio varies by country (Petruzziello, 2018). This is primarily due to three factors. Firstly, men are more likely to be exposed to hepatic carcinogens (tobacco or alcohol) and hepatitis C virus infections (Siddiqui et al., 2018); secondly, Estrogen may

suppress the inflammatory process mediated by interleukin-6 (IL-6) in women, reducing hepatic injury and compensatory hepatocyte proliferation (Hu et al., 2021); and thirdly, testosterone may increase signaling androgen receptor in men, promoting hepatocyte proliferation (Zhang et al., 2021). Given this gender-specific, essential trait, more research into the role of sex steroids and their link with HCC is critical, as the mechanisms of action of these hormones in the development of HCC are currently poorly known (Xia et al., 2021).

Estrogen receptors

The liver is one of the organs that is influenced by sex hormones. Sex hormones control a variety of liver functions in mammals, as well as sexual organs like the breast (Shen & Shi, 2015). Both males and females have androgen receptors, as well as high-affinity, low-capacity oestrogen receptors in their livers. Several lines of evidence suggest that sex hormones and their receptors have a role in liver carcinogenesis (Wang et al., 2006). Explicitly, the ESR variation was initially discovered in human primary HCC tissues by Villa et al. (Villa et al., 1995) furthermore, the male sex was likewise linked to the variation ER (vER). Iavarone et al. recently published a study that linked vER- to liver illness (Iavarone et al., 2003).

Interestingly, ER- was shown to be strongly expressed in HCV-infected people. This study reveals that ERs and their variants play a role in hepatocarcinogenesis development and are associated to the male preponderance of HCC as well as certain viral infections (Ma et al., 2014). Despite the fact that HCC accounts for 30-40% of all human malignancies in Egypt (Ozakyol, 2017), little is known about the expression patterns of AR and ERs in Egyptian HCC patients.

Estrogen action is mediated by two nuclear receptors called adrenoceptor alpha (ERa) and adrenoceptor beta (ERb), which are members of a family of nuclear receptors that regulate gene expression (Nilsson & Gustafsson, 2002). The estrogen receptor (ER) is a ligand-activated transcription factor with one estrogen binding domain and one DNA binding domain that has one estrogens binding domain and one DNA binding domain. ESR1 and ESR2, which encode ERa and ERb, are two distinct genes located on different chromosomes (Kyriakidis & Papaioannidou, 2016). According to several studies, ER subtypes play a variety of roles in various stages of liver disease and may even be involved in signal transduction. However, the many roles of ER subtypes in hepatic diseases, notably the ERb, are unclear and have long been the topic of investigation. ERa, which has different isoforms and expressions depending on whether the tissue is healthy, cirrhotic, or has HCC, is also known to mediate the majority of estrogen's biological activities in the liver (Shi et al., 2014). The mechanism by which ER subtype pattern expression, and its isoform pattern expression, changes in hepatocarcinogenesis is yet unknown.

Estrogen receptor isoforms

More than twenty ERa isoforms have been found in human malignancies. The most researched isoforms are ERa46 and ERa36. The ERa46 isoform appears to be linked to cell cycle arrest in the G0/G1 phase, as well as a refraction to E2-

driven growth during cell hyperconfluency (Campbell-Thompson et al., 2001). It also appears to play a role in the suppression of other cell cycle regulators. ERa36 appears to be associated to an increase in cell proliferation through activating MAPK-ERK (Omarjee, 2016). In human tissue, at least five isoforms of ERb have been identified, although their functional importance has yet to be elucidated. ERb2 is a well-studied isoform among the isoforms. Its role appears to be linked to the suppression of ERa. Because ERa is degraded by ERb2, the authors believe that ERa recruitment to estrogen-responsive promoters is reduced, resulting in the repression of genes that regulate ERa (Heldring et al., 2007).

Estrogen receptors and genetic polymorphisms

HCC-linked genetic variations are still a mystery. The accumulation of multiple genetic alterations appears to play a crucial role in HCC development, as it does in other malignancies. One of the most well-studied types is single nucleotide polymorphism (SNP), which occurs when a single nucleotide is substituted. Certain polymorphisms may have a functional impact on the end product of a gene, which can be measured, and may play a part in pathological alterations (Baldissera et al., 2016).

As a result, in some cases, a genetic variant may increase cancer risk. A genetic variation, such as an SNP, may or may not cause a change in receptor structure and, as a result, have an influence. Some studies found no link between SNPs and cancer risk, such as one that found no link between the rs9340799 polymorphism and the risk of HCC (Sun, Deng, et al., 2015) (Anghel et al., 2010), and another that found no link between the rs1801132 polymorphism and cancer risk in a pooled analysis (Anghel et al., 2010), (Sun, Hou, et al., 2015). The rs2077647 mutation, on the other hand, was connected to an elevated risk of HCC but not of other cancers such as colorectal, breast, or prostate cancer.

Assumption

ER polymorphisms may affect receptor function and expression by modifying transcription factor binding sequences or impacting alternative mRNA splicing. As a result, alternative splicing produces stable protein translation with varying Estrogen binding, nucleocytoplasmic translocation, and DNA interaction properties (Yaşar et al., 2017). As a basis, we believe that the presence of one or more SNPs in the ESR1 and ESR2 genes may be related to the increased risk of developing and the severity of HCC, as well as the response to various treatments. More solid research and data in this area are needed since ERa variant expression might serve as a possible prognostic indicator for HCC (Li et al., 2019), giving a novel target for HCC therapy and increasing patient response to currently available medications (Calderaro et al., 2019).

The hypothesis's ramifications and discussion

Although there is a strong link between genetic variability and HCC in the literature, there are very few investigations on the genetic variability of ERs and liver cancer. Some association studies have been conducted between SNPs and a variety of physiological and pathological situations, with contradicting findings,

with one variation acting as a protector and the other as a risk factor. On metastatic tumor and cirrhotic samples from retrospective cohorts and clinical trials, the first investigation reporting ESR1 mutations was conducted. Whole-genome sequencing revealed mutations, which were validated in our lab at the Egyptian Liver Research Institution and hospital by investigation of the originating malignancies. The cirrhotic samples revealed 14 documented genetic variations, 9 of which were shared by control samples with a frequency ratio of more than or equal to 50%. These samples revealed the following 5 genetic variations, one of which was identified in one control sample (frequency ratio 16.5 %) as shown in (Table 1).

Table 1
Variants detected within regions of interest in cirrhotic samples.

| Gene | c. variant | P. variant | Type | % | Avg Q | F/R test | Coverage | ROI | VOI | Review | Comment |
|-------|-----------------------|-------------|----------|--------|-------|----------|----------|-----|-----|--------|---------|
| ESR1 | c.1782G>A | | SNV | 46.92% | 37.98 | 1.00 | 373 | Yes | Yes | Valid | |
| EGFR | c.1856_1857delTGinsCA | p.Leu619Pro | MNV | 19.27% | 37.25 | 0.03 | 301 | Yes | No | Valid | |
| FGFR1 | c.396_398delTGA | p.Asp133del | Deletion | 4.13% | 29.40 | 0.93 | 121 | Yes | No | Valid | |
| FGFR2 | c.774C>G | | SNV | 11.79% | 34.55 | 0.01 | 263 | Yes | No | Valid | control |
| ERBB2 | c.1963A>G | p.Ile655Val | SNV | 52.31% | 36.24 | 0.92 | 130 | Yes | Yes | Valid | |

C. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations; P. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations; %: Detected variant frequency; Avg Q: Average quality score of the bases supporting the variant; F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: unbalanced); Coverage: The number of fragments covering the variant position; ROI: Number of Regions of Interest, i.e. reportable regions that overlap with the gene; VOI: Variant of interest, as specified for the analysis workflow; Review: Status of variant review.

According to HCC samples, they revealed 13 previously described genetic variations, as well as 10 genetic variants shared by control samples with a frequency ratio of greater than or equal to 50%. These samples revealed three distinct genetic variations, one of which was shared with one control sample (frequency ratio 16.5 %) as shown in (Table 2):

Table 2
Variants detected within regions of interest in HCC samples

| Gene | c. variant | P. variant | Type | % | Avg Q | F/R test | Coverage | ROI | VOI | Review | Comment |
|-------|-----------------------|-------------|------|--------|-------|----------|----------|-----|-----|--------|---------|
| ESR1 | c.1782G>A | | SNV | 55.61% | 40.78 | 1.00 | 205 | Yes | Yes | Valid | |
| EGFR | c.1856_1857delTGinsCA | p.Leu619Pro | MNV | 19.27% | 37.25 | 0.03 | 253 | Yes | No | Valid | |
| FGFR2 | c.774C>G | | SNV | 10.12% | 36.71 | 0.07 | 168 | Yes | No | Valid | control |

C. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations; P. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations; %: Detected variant frequency; Avg Q: Average quality score of the bases supporting

the variant; F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: unbalanced); Coverage: The number of fragments covering the variant position; ROI: Number of Regions of Interest, i.e. reportable regions that overlap with the gene; VOI: Variant of interest, as specified for the analysis workflow; Review: Status of variant review.

In this respect, more research into the numerous polymorphisms in the ESR1 and ESR2 genes, as well as their relationship with an increased risk of developing HCC, is needed to understand this difference. Because HCC is the world's fifth most prevalent malignancy and the second leading cause of cancer mortality, it has a substantial impact on global health. HCC is the fourth most deadly tumor in Egypt. As a result, research that can aid in the understanding of how HCC behaves and its factors, as well as genetic predisposition to HCC, are of considerable interest and relevance.

If polymorphisms associated with receptor isoforms are discovered, this approach of evaluating polymorphisms may become more straightforward. As a result, it might be developed into a prognostic tool that could cause behavioral changes in those who have a greater hereditary risk. The validation of our concept by scientific investigations might lead to the discovery of indicators that operate as prognostic variables for this illness, as well as new approaches to developing antitumor medications based on estrogen receptor antagonists and enhancing existing treatments.

Conclusion

On the whole, this validation study has shown that one or more SNPs in the ESR1 gene may be connected to an increased chance of developing and severity of HCC, as well as treatment response. Scientific study that backs up our hypothesis might lead to the identification of prognostic markers for HCC, as well as new techniques for generating antitumor drugs.

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Conflict of interest

There are no competing interests declared by the authors

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