Estrogen, estrogen receptors, and hepatocellular carcinoma: Are we there yet?

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Estrogen, estrogen receptors, and hepatocellular carcinoma (HCC) was suggested a few decades ago according to clinical data showing higher HCC morbidity and mortality among males. Several recent studies further confirmed the anti-cancer effects of estrogen in the liver. However, it remains to be identified how to exploit estrogen signalling within clinical settings for HCC treatment. There are several unresolved issues related to the estrogen pathway in liver cells. The main problems include the absence of a clear understanding of which estrogen receptor (ER) isoform is predominantly expressed in normal and malignant liver cells, the ER isoform expression difference between males and females, and which ER isoform should be targeted when designing HCC therapy. Some of those questions were recently addressed by Iyer and co-authors. The current editorial review critically analyses the study by Iyer et al. (WJG, 2017) that investigated the expression of ER subtypes in liver samples collected from patients with a healthy liver, hepatitis C virus cirrhosis, and HCC. ER presence was evaluated in association with gender, intracellular localization, inflammation marker NF-κB, and proliferation-related effector cyclin D1. The study limitations and advantages are discussed in light of recent advances in the HCC and estrogen signalling areas.

Key words: Hepatocellular carcinoma; Hepatitis C virus; Hepatitis; Estrogen receptors; Cirrhosis

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Core tip: Recent discoveries confirmed that the female sex hormone estrogen protects against the development and progression of hepatocellular carcinoma (HCC). However, the mechanism of estrogen's anti-oncogenic effects and the specific impact of estrogen receptor (ER) signalling in HCC are unclear and controversial. It is essential to determine how to exploit the estrogen signalling pathway within a clinical setting for HCC.
treatment. The current editorial review critically analyses the Iyer et al (WJG, 2017) study that investigated the expression of ER subtypes in liver samples collected from patients with a healthy liver, hepatitis C virus cirrhosis, and HCC.

**INTRODUCTION**

Despite considerable advances in the treatment of various malignancies, there are still limited cure options for hepatocellular carcinomas (HCC), a high-lethality malignancy. Determined as a possible outcome of the chronic liver diseases with cirrhosis, HCC was strongly linked to hepatitis B and C, alcoholic liver disease, and non-alcoholic steatohepatitis\(^1\). As a preventive factor, the role of steroid hormones in the regulation of hepatic malignant transformation was suggested after a consistent gender disproportion was observed in the incidence of HCC worldwide. Females, at the premenopausal age when circulating estrogen is high, are protected from HCC and get better recovery after HCC treatment\(^{[2,3]}\). However, the mechanism of the hormone anti-oncogenic signaling and the specific impact of estrogen receptor (ER) isoforms in HCC development and progression are unclear and controversial.

The precedent is based on the high variability of the tested samples and low significance of the established association between ER levels and disease-specific outcome shown in several studies\(^{[4,5]}\). Consequently, clinicians are in understandable disagreement about the potential benefits of hormonal therapy in HCC patients. Nevertheless, the hypothesis about the protective impact of estrogen signaling pathway against HCC was confirmed by several groups\(^{[6,5]}\). The worldwide epidemiological data demonstrate the strong and consistent prominence of HCC among men, indicating estrogen-dependent protection against liver cancer that deserves serious consideration. Indeed, there is an urgent need for a detailed investigation of ER expression and signaling in normal liver and HCC, accenting the fact that HCC is a leading cause of cancer-related death with growing incidence worldwide. It is pleasing to see that Iyer et al\(^6\) addressed the problem using a few novel approaches.

**STUDY ANALYSIS**

HCC is a disease with multifactorial causes and genetic variability. Although the liver is mostly considered an accessory organ of the digestive system, it is also a hormone-sensitive organ and therefore is influenced by gonadal hormones, such as estrogen. The hypothesis about the regulatory role of estrogen and ER in the development and progression of HCC inspired Iyer et al\(^6\) to test the level of ER expression in liver tissues from normal subjects and patients with chronic hepatitis C virus (HCV)-related cirrhosis and HCC. The authors analysed the respective association of ER isoforms with inflammatory and oncogenic markers of HCC pathogenesis. The expression of ER subtypes, ER\(\alpha\) and ER\(\beta\), was thoroughly evaluated at the mRNA and protein levels in relation to gender and type of disease\(^6\). The history of detection of ER variants expression requires special explanation. For several decades due to an absence of variant-specific antibodies and a lack of knowledge about the role of these variants in carcinogenesis, the level of ER expression was evaluated using non-specific antibodies that recognized either both ER\(\alpha\) and ER\(\beta\) variants unseparated, or identified only ER\(\alpha\) thus neglecting ER\(\beta\). This is not surprising, as the ER\(\beta\) isoform was only identified in 1996.

Obviously, the data received with the use of non-specific antibodies should be considered carefully and should not be used for generalization. Furthermore, the data received with the use of only one type of ER\(\alpha\)-specific antibodies that allows detection of one known ER\(\alpha\) subtype should be considered partial, as there are several variants of ER\(\alpha\) and ER\(\beta\) currently known with quite different tissue-specific expression patterns and functioning\(^{[7,8]}\). The wisely-designed and isoform-specific investigation of ER variant expression in liver samples becomes more complicated, time-consuming, and expensive, thus it is only affordable for large, well-financed, clinical laboratories. Unfortunately, this kind of comprehensive variant-specific analysis has not yet been conducted, while most of ER-HCC studies remain inconclusive, as they are based on a small sample size with the use of at most two types of ER isoform-specific antibodies.

Small sample size is one of the major problems of nearly all investigations aimed to determine the level of ERs in gender-specific settings for diseases of gastro-intestinal and accessory organs, including the liver and pancreas. As stated above, females are less susceptible to HCC and, as a result, women’s liver samples are often under-represented in small studies. Similarly, Iyer et al\(^6\) could not perform statistically relevant gender analyses due to the limited size of the patient cohort and the scarcity of female samples in the diseased groups. This problem could be overcome in a study conducted by two or more collaborating clinical laboratories that register a sufficient number of female patients with HCC.

The study determined significantly higher expression of ER\(\alpha\) and ER\(\alpha\):ER\(\beta\) expression ratio in normal (healthy) males as compared to females. The findings demonstrated by Iyer et al\(^6\) suggest some potential
predisposition of males to develop liver cancer as increased ER gene expression was previously shown in liver tumours from HCC patients\(^9\), and was linked to higher proliferation rate in other cancers. Iyer et al\(^6\) detected an increase in the liver mRNA expression of ER\(\alpha\) (ESR1) and ER\(\beta\) (ESR2) subtypes in chronic HCV and HCV-related HCC as compared to normal. However, in contrast to healthy liver, ER\(\alpha\) (ESR1) mRNA transcriptional levels were decreased in the male liver with chronic HCV and HCV-related HCC as compared to normal liver samples. The study included premenopausal female subjects (female controls age range 42-67) indicating that at least some of them had a reasonably high level of circulating estrogen. However, the HCV and HCC groups included only menopausal and post-menopausal females suggesting that the level of circulating estrogen in those females is comparable to men. Unfortunately, the real level of circulating estrogen was not measured in any of those groups.

Considering the applied methods, the authors (Iyer et al\(^6\)) evaluated the amount of ER\(\alpha\) and ER\(\beta\) proteins using the western blotting technique and immunohistochemistry (IHC) on paraffin-embedded tissue samples. The latter approach allows for the observation of intracellular localization of the receptor variants. Many studies addressed the mechanisms of the ER signaling pathways in cytoplasm and nuclear compartments, but those studies were mostly assessing breast carcinomas. The findings related to ER intracellular localization in breast tissues might be irrelevant to liver samples, although indicate the necessity of further investigations. To confirm the IHC data, ER isofrom localization was assessed using subcellular fractionation. The analysis indicated some favorable tendency towards nuclear translocation of ER\(\alpha\) and ER\(\beta\) proteins in HCV and HCC samples. The increased nuclear-to-cytoplasmic ratio and dominant presence of both ER variants in the nuclear space of cells from the diseased groups suggest ER-related gene activation.

Iyer et al\(^6\) assessed activation of an oncogenic marker cyclin D1 and found the increased expression of the protein in the nuclear and cytoplasmic fractions of diseased livers. Increased cyclin D1 level is supposed to stimulate hepatocyte proliferation during chronic HCV-infection. The expression of nuclear ER\(\alpha\) and ER\(\beta\) positively correlated with nuclear cyclin D1 in both HCV related cirrhosis and HCV-related HCC. The data suggests that ER-subtypes stimulate the activation of transcriptional activity during the progression of the disease towards malignancy. However, this effect and related ER gene activation require further confirmation on the level of established ER target genes including progesterone receptor and cathepsin D; although activation of ER target genes in the liver has not been well-studied.

Besides proliferation-related effector, the authors analysed activation of the inflammation-related signalling pathway of NF-\(\kappa\)B. Although NF-\(\kappa\)B activation was associated with ER signalling, the link is very controversial and NF-\(\kappa\)B also mediated anti-inflammatory effects of ER\(^6\). Considering evidence confirming the inflammation-related role of NF-\(\kappa\)B in the liver\(^11\), it is not surprising that Iyer et al\(^6\) detected a significantly higher expression of phosphorylated NF-\(\kappa\)B in chronically infected livers with HCV and in HCC tissue samples. The authors further tested the association between ER and NF-\(\kappa\)B. The regulatory involvement of ERs in inflammatory responses was previously shown in different cells, but was not clearly characterized in liver tissues. Interestingly, the authors (Iyer et al\(^6\)) demonstrate controversial data that reminds the effects which were detected in colon, gastric, and oesophageal cancers\(^12-14\). A weak negative correlation was found between nuclear ER\(\alpha\) and pNF-\(\kappa\)B in normal liver tissues, while a weak positive correlation between nuclear ER\(\alpha\) and pNF-\(\kappa\)B was detected in the HCV-related HCC group. The change of “polarity” for ER-related signalling confirms the previously suggested dependence of ER effects on different sets of co-factors\(^15\). The cancer-specific set of co-factors reverses or changes the tendency of ER-mediated biological effects. The kind of co-factors which mediate ER variant signalling in normal and diseased livers remains to be identified in future studies.

Cytoplasmic ER signalling is not well-defined in HCC, either. Iyer et al\(^6\) data demonstrated that ER\(\alpha\):ER\(\beta\) ratio was increased in the cytoplasmic compartment of the HCV-related cirrhosis and HCC groups compared to normal samples. The data indicates increased ER\(\alpha\) cytoplasmic presence. However, the analysis of NF-\(\kappa\)B activation pathway and cytoplasmic ER subtypes show a strong negative correlation in the HCC group. These findings open wide horizons for future investigations as the range of inflammation and proliferation-related cytoplasmic mediators of ER signalling includes MAPK, PI3K, and the Sphingolipid network. Besides ER-related signalling pathway in cytoplasm, membrane ER should not be neglected in future studies assessing HCC. G protein-coupled estrogen receptor (GPER) is a novel estrogen-binding receptor involved in many pathological conditions, including cancer. The role of the GPER in HCC was recently shown in estrogen-induced protection against HCV\(^14\), but this requires further testing. The availability, quality, and specificity of ER-isoform specific antibodies is being constantly improved, giving hope that the role and mechanism of estrogen/ER signalling in the liver will be clarified in near future.

**CONCLUSION**

The reviewed data\(^6\) indicated a favorable tendency towards nuclear translocation of ER\(\alpha\) and ER\(\beta\) proteins followed by NF-\(\kappa\)B transcriptional activation in HCV.
and HCC patient samples. According to this finding, testing ER modulators that target both ERα/ERβ isoforms would be a suggestive clinical strategy. However, ERα (ESR1) mRNA transcriptional levels were decreased in the HCC male liver compared to normal liver samples[6]. Since males are more susceptible to HCC development, the finding of decreased ERα in HCC male samples directs clinical design towards the use of ERβ modulators as a potential anti-HCC therapy. However, the rather low number of tested samples suggests the necessity for more and larger investigations. The change in ER isoform expression ratio and receptor localization indicates the complexity of ER signaling pathway and its transformation during oncogenesis, which we still do not entirely understand.

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