Proceedings of Anticancer Research, 2024, Volume 8, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

Comprehensive Analysis of Estrogen Receptor 1 Dysregulation in Liver Hepatocellular Carcinoma: Implications for Prognosis and Therapeutic Targeting

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Abstract: The study investigates the expression pattern and regulatory mechanisms of estrogen receptor 1 (ESR1) in liver hepatocellular carcinoma (LIHC) through comprehensive bioinformatics analysis. Utilizing UALCAN and GEPIA2 databases, significant down-regulation of *ESR1* expression is observed in LIHC samples compared to normal controls, indicating its potential role in tumor progression. Further analysis reveals consistent down-regulation across different clinical variables including patient age, gender, race, and various stages of LIHC, affirming the regulatory role of *ESR1* in tumor development and progression. Additionally, promoter methylation analysis demonstrates hypermethylation of *ESR1* in LIHC samples, negatively correlating with its expression. This association persists across different clinical parameters, emphasizing the inverse relationship between *ESR1* methylation and expression levels. Survival analysis indicates that up-regulation of *ESR1* is associated with better overall survival, suggesting its potential as a prognostic biomarker in LIHC. Furthermore, genetic mutation analysis using cBioPortal reveals a spectrum of alterations in *ESR1*, including amplification, missense mutation, deep deletion, splice mutation, and truncating mutation, highlighting the genetic complexity of *ESR1* in LIHC. These findings collectively contribute to a deeper understanding of *ESR1* dysregulation in LIHC and its clinical implications as a potential therapeutic target and prognostic marker.

Keywords: Estrogen receptor 1; Liver hepatocellular carcinoma; Biomarker; Prognosis

Online publication: June 19, 2024

1. Introduction

Cancer is a serious medical and economic burden worldwide. Cancer is a major cause of death globally, with an estimated 20 million new cases and 9.7 million cancer deaths in 2020 alone. Cancer is characterized by uncontrolled cell division and metastasis in neighboring tissues. It is classified according to its origin in an organ or tissue [1-3]. Liver cancer is 6th most common type of malignant tumor and liver hepatocellular carcinoma (LIHC) constitutes 90% of cases of liver cancer, with an estimated 905600 new cases in 2020,

were considered 3rd major cause of death with an estimated 830200 deaths [4-6]. Major risk factor includes alcohol consumption, hepatitis B, and hepatitis C infection [7]. Because of high malignancy surgery with immunotherapy, target therapy, chemotherapy, and radiotherapy are recommended treatments [8,9]. However, due to drug resistance, low-effectiveness results are not satisfied [10,11]. Therefore, it is crucial to identify new diagnostic and prognostic markers to improve the survival rate.

Estrogen receptor 1 (*ESR1*) encodes protein and estrogen receptors (ER). This encoded protein plays a role in sexual development, growth, and metabolism, whereas ER is a transcriptional factor that plays a role in cell proliferation, osteoporosis, and cancers. Previous studies revealed that ER accounts for 70% of breast cancer and plays a role in breast cancer evolution. Receptor therapy is a major treatment in hormone receptor-positive cancers and specific *ESR1* mutation leads to hormonal therapy resistance with poor overall survival. Y537 and D538 are common mutations, while K3030R, E3800Q, and S432L, V534E lead to estrogen resistance and hormone-dependent activation respectively. *ESR1* mutations are more commonly found in metastatic cancers than in primary cancers. It has also been stated that ER has the ability as a therapeutic and prognostic target in lung cancer. As per studies, *ESR1* is the primary gene in liver cancer and is identified as a tumor suppressor gene. LIHC have increased G-protein coupled estrogen receptors as compared to normal cells and *ESR1* has an inverse correlation with LIHC tumor size [12-24]. Overall, it is evident that *ESR1* is associated with the progression of breast cancer, LIHC, and other cancers.

In this study, we aimed to perform a bioinformatics analysis of *ESR1* in LIHC. As per our knowledge, no such analysis has been administered to assess the role of *ESR1* in LIHC. We analyzed *ESR1* expression, mutation, overall survival, and function in LIHC using bioinformatics. We utilized UALCAN, Kaplan-Meier plotter, cBioPortal, and GEPIA for this analysis.

2. Material and methods

2.1. UALCAN

UALCAN is an online tool utilized to analyze TCGA gene expression. UALCAN is a public database used to analyze comparative expression in tumor and normal cells, as well as in different clinical attributes such as patients' gender, age, and cancer stages [25]. In the current study, we explored *ESR1* expression and methylation in LIHC using this tool.

2.2. GEPIA

Gene Expression Profiling Interactive Analysis (GEPIA) is a web-based tool that is applied to analyze cancer and normal gene expression [26]. In this study, we evaluated *ESR1* expression in LIHC compared to normal and at different stages.

2.3. Kaplan-Meier plotter

Kaplan-Meier (KM) plotter is a web-based tool that is used to investigate the correlation between gene expression and overall survival [27]. In the present study, we used the KM plotter to perform survival analysis to evaluate the prognostic value of *ESR1* in LIHC patients, where $P < 0.05$ is regarded as significant.

2.4. cBioPortal

cBioPortal is an online public database used to investigate cancer genetic data [28]. We utilized this tool to explore the genetic mutation of *ESR1* that is associated with LIHC.

3. Results

3.1. Expression analysis of ESR1 in LIHC and normal control samples

Firstly, expression analysis of the *ESR1* gene in LIHC samples and normal control samples is investigated utilizing the UALCAN database (**Figure 1**). We analyzed that *ESR1* was significantly down-regulated in LIHC samples as compared to normal samples. So *ESR1* is regulated in LIHC patients and it reveals its role in progression.

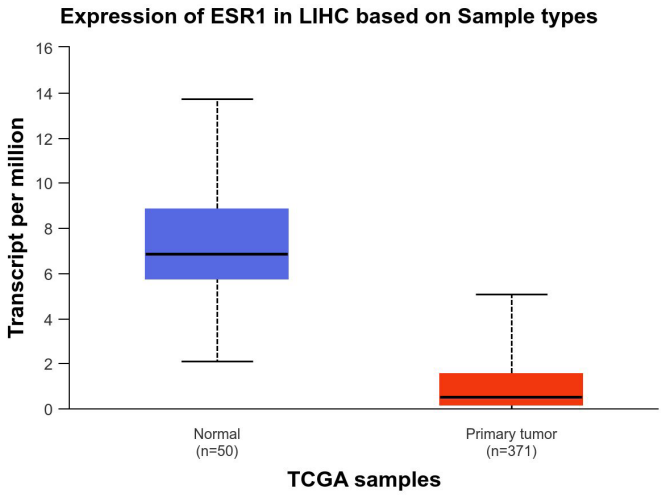

Figure 1. Expression model of *ESR1* in LIHC and normal control samples

3.2. Validation of expression analysis

ESR1 expression analysis in LIHC and normal samples is validated by utilizing GEPIA2. It was revealed that *ESR1* is significantly down-regulated in LIHC samples (**Figure 2**). This result supports the analysis that *ESR1* is regulated in LIHC and has a role in tumor progression.

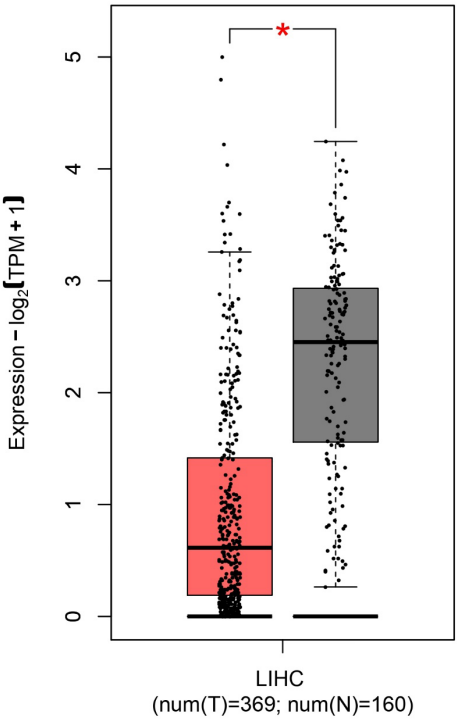

Figure 2. Down-regulated *ESR1* in LIHC as compared to normal samples

3.3. Expression analysis of *ESR1* in LIHC derived from distant parameters

Then we analyzed *ESR1* expression in LIHC selecting different parameters such as patients' age, gender, race, and different stages of LIHC. We utilized UALCAN and it revealed significant down-regulation at different stages of LIHC (**Figure 3A**). After that, we inspected *ESR1* expression in LIHC patients of different age groups and the result was down-regulation (**Figure 3B**). Similarly, we examined down-regulation in *ESR1* expression in LIHC patients' gender and different race (**Figure 3C, 3D**). These findings reveal *ESR1* regulation in LIHC and this explains the role of *ESR1* in the progression of cancers.

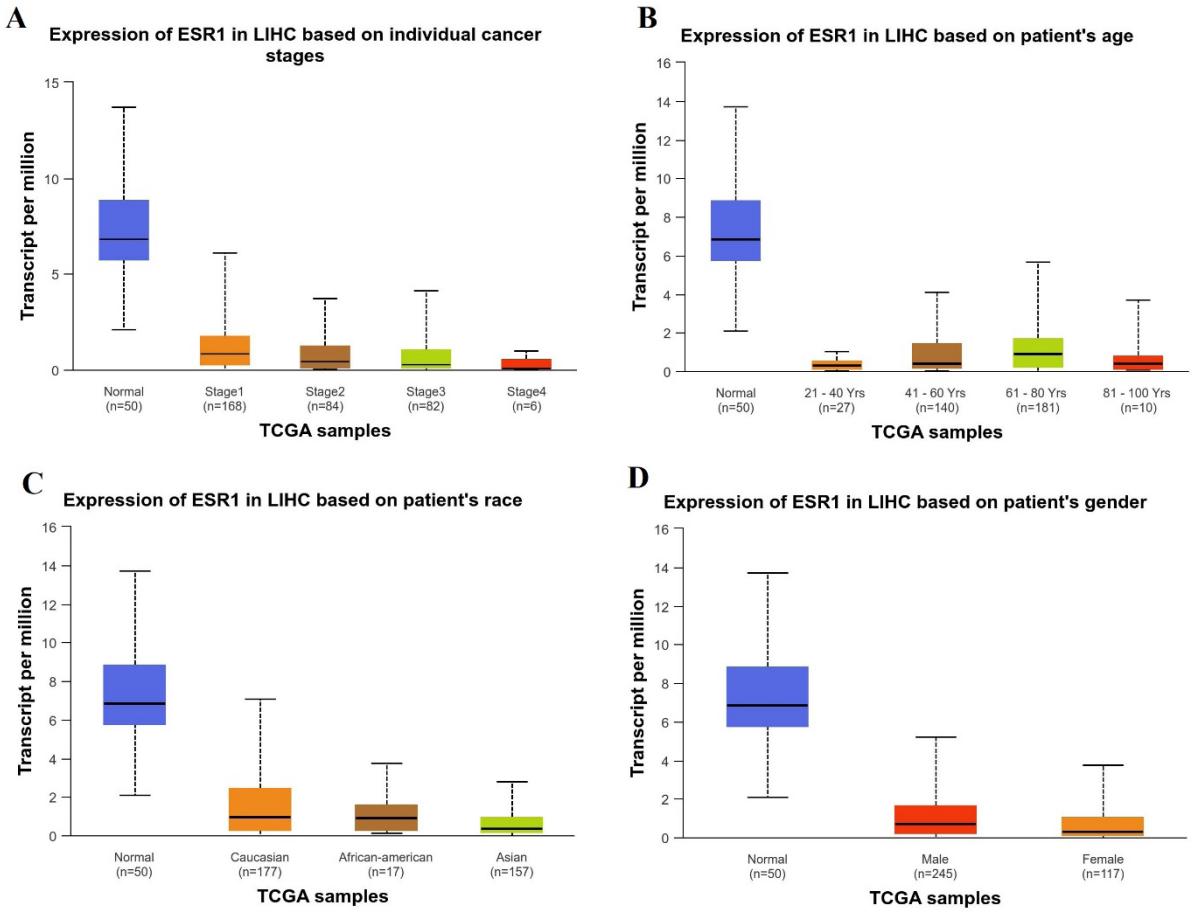

Figure 3. It shows expression analysis of *ESR1* in LIHC based on distinct clinical variables

3.4. Ratifying expression analysis of *ESR1* in LIHC at different stages

We ratified *ESR1* expression analysis at individual cancer stages of LIHC using the GEPIA2 database. We examined that there is variation and down-regulation in the expression of *ESR1* in LIHC at different stages (**Figure 4**). Thus, this validates the expression analysis of *ESR1* in LIHC.

3.5. The promotor methylation level of *ESR1* in LIHC

Past studies revealed that cancer progression increased with erratic methylation in the promotor region of the gene [29]. Primarily, we analyzed the promotor methylation level of *ESR1* in the LIHC sample compared to the normal sample using the UALCAN database. We scrutinized that the promotor methylation level of *ESR1* was hypermethylated in LIHC samples (**Figure 5**). This illustrates a negative association with the expression of *ESR1*.

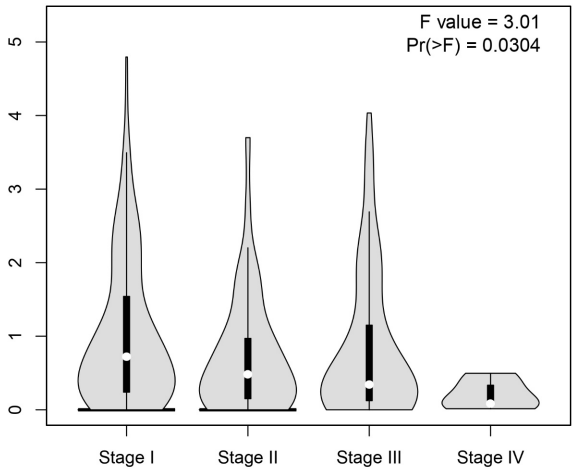

Figure 4. Expression pattern of *ESR1* in different stages of LIHC using GEPIA2

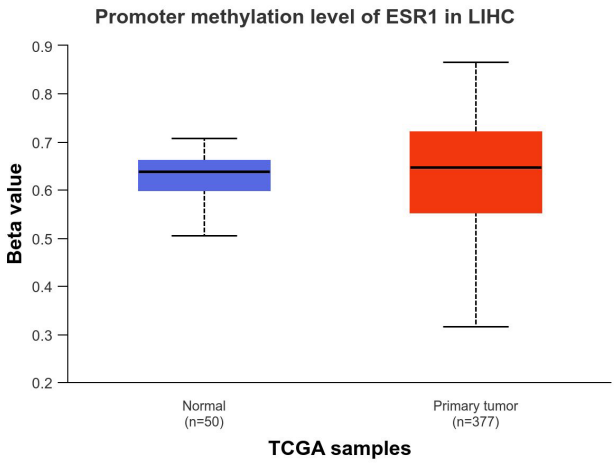

Figure 5. It shows the promotor methylation level of *ESR1* in LIHC and normal control samples

Later we also analyzed the promotor methylation level of *ESR1* in LIHC based on different parameters such as patients' age, gender, race, and different stages of LIHC (**Figure 6**). First, we investigated the hypermethylation of *ESR1* in LIHC individual cancer stages (**Figure 6A**). Correspondingly, *ESR1* was hypermethylated in LIHC in other entities as well (**Figure 6B–D**). So all these findings elaborate an inverse relation in *ESR1* methylation and expression.

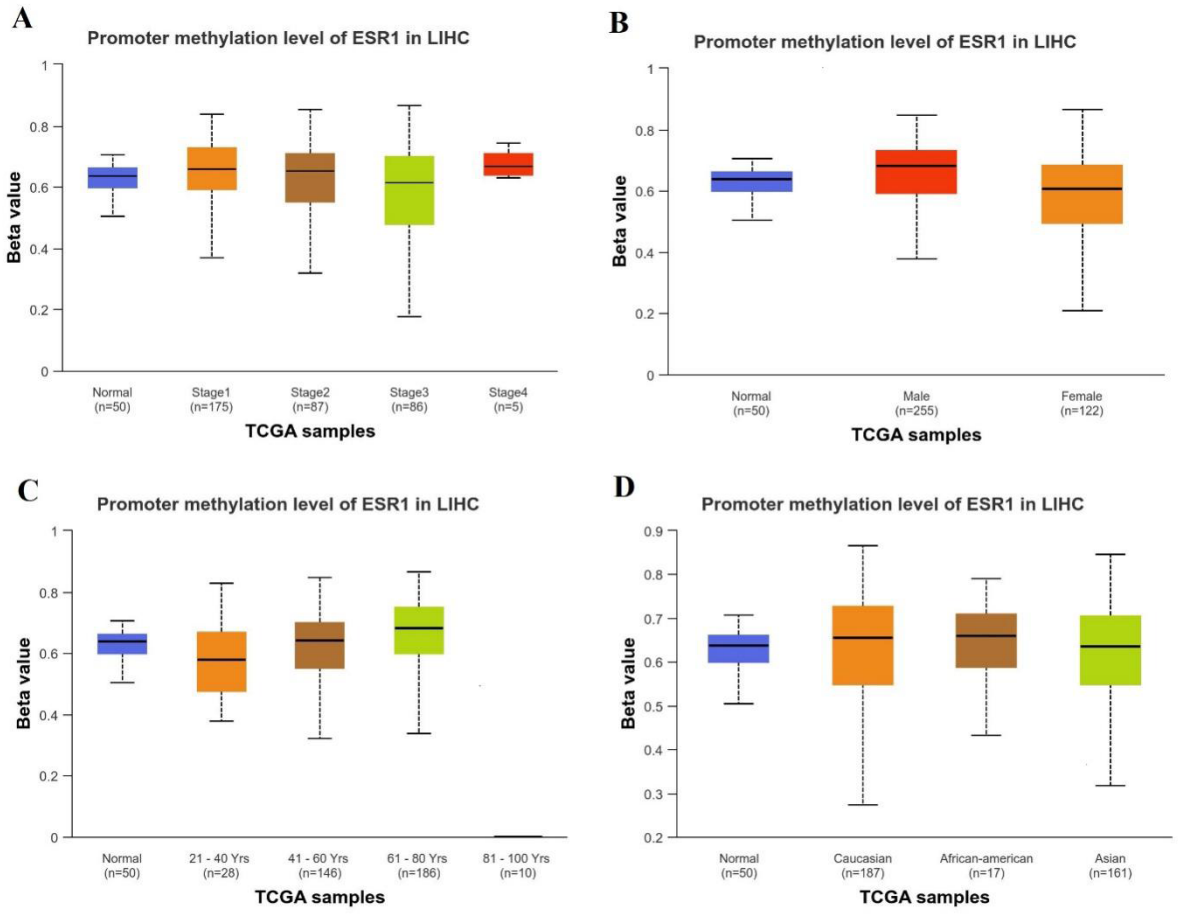

Figure 6. The promotor methylation level of *ESR1* in LIHC based on different parameters

3.6. Survival analysis of ESR1

To evaluate the prognostic value of *ESR1*, we performed a survival analysis using the KM plotter (**Figure 7**). We evaluated that *P* = 4.2e–0.5, HR = 0.49, this explains that up-regulation of *ESR1* relates to better overall survival value and down-regulation of *ESR1* is related to worse overall survival. That is why *ESR1* can be considered as a prognostic biomarker.

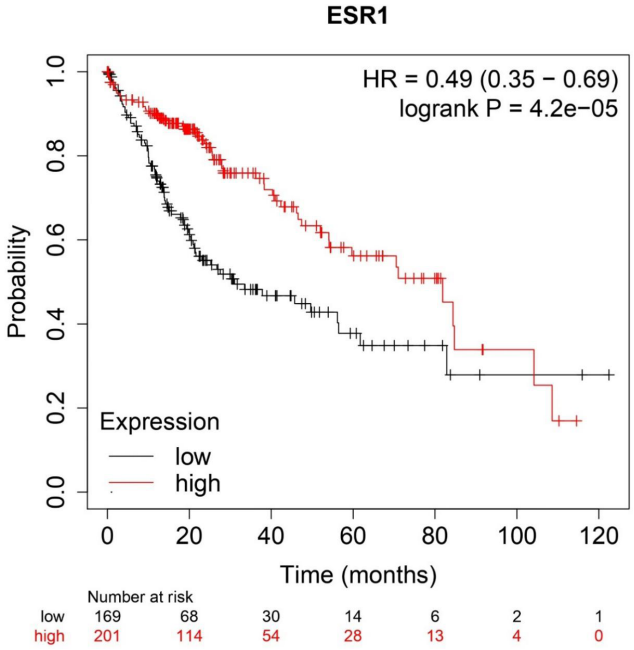

Figure 7. It shows survival analysis of *ESR1* in LIHC and normal control samples

3.7. Genetic mutation of *ESR1* in LIHC

Additionally, we used cBioPortal to analyze *ESR1* genetic mutation in LIHC patients. Comprehensively, 4% alterations were analyzed including amplification, missense mutation, deep deletion, splice mutation, and truncating mutation (**Figure 8**).

Figure 8. Mutational sequences of *ESR1* in LIHC using cBioPortal

4. Discussion

Liver hepatocellular carcinoma (LIHC) is the 6th most common type of cancer with 830,200 deaths, and the 3rd most common cause of cancer deaths in 2020 [30]. Where chemotherapy, surgery, and target therapy are some of the conventional methods of cure, but these methods have some solid side effects. So there is an urgent need for new therapeutic and diagnosis methods without complications. So in the present study, we analyzed *ESR1* expression in LIHC to evaluate its role as an efficient biomarker. We performed our systematic analysis using web tools like the UALCAN database, GEPIA2, KM plotter, and cBioPortal.

Primarily, we analyzed *ESR1* gene expression in normal samples and LIHC samples operating the UALCAN database. We inspected that *ESR1* was down-regulated in LIHC samples as compared to normal samples. This shows an abnormal aspect of *ESR1* in LIHC. Then we selected different entities like patients'

age, race, gender, and individual cancer stage to conduct expression analysis of *ESR1* in LIHC. We evaluated upregulation in *ESR1* expression at these parameters. Thereafter, we used the GEPIA2 database to validate *ESR1* expression, expression was downregulated in LIHC samples. Thus, it is evident that *ESR1* regulation in LIHC has an inverse correlation to LIHC. So it explains *ESR1* as a potential biomarker [31,32].

Promotor methylation, plays a key role in the regulation of gene expression. We analyzed the hypermethylated promoter methylation level of *ESR1* in LIHC. This reveals the reason for the downregulation of *ESR1* expression in LIHC. Subsequently, promotor methylation was analyzed based on different parameters and it revealed hypermethylation of *ESR1* in LIHC in different ages, genders, stages, and races. So based on findings, the promotor methylation downregulates *ESR1* expression and plays a role in LIHC progression [33-36].

Survival analysis of *ESR1* in LIHC was performed using the KM plotter to evaluate the OS rate. The analysis revealed downregulation of *ESR1* expression in LIHC is associated with a worse prognostic value and vice versa. This explains *ESR1* as a prognostic indicator in LIHC. We also conducted a mutational analysis using cBioPortal. Where 4% genetic mutation of *ESR1* was inspected, that explains genetic mutation has an insignificant effect on the regulation of *ESR1* [37,38].

Overall, we concluded that our results relate to other studies that explain the participation of *ESR1* in LIHC progression. This investigated downregulation, hypermethylation, and OS rates highlight the potential of *ESR1* as a prognostic and diagnostic biomarker.

5. Conclusion

LIHC is a medical and economic burden affecting people worldwide. In this study, we conducted expression analysis of *ESR1* in LIHC, elucidating promoter methylation level, expression design, and prognostic values. These findings highlight the *ESR1* effect on LIHC progression, which emphasizes the potential of *ESR1* as a therapeutic and prognostic biomarker. Further research is crucial for this perspective.

Disclosure statement

The authors declare no conflict of interest.

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