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Characterization of POTE-2 Expression in Hepatocellular Carcinoma Cell Lines

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CHARACTERIZATION OF POTE-2 EXPRESSION IN HEPATOCELLULAR CARCINOMA CELL LINES

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Abstract

Hepatocellular carcinoma is the most common cancer of the liver and is the third leading cause of cancer deaths globally. Due to the limited serum biomarkers, detection of hepatocellular carcinoma usually occurs in the later, metastasized stages of the disease where the 5-year survival rate falls to around 3%. The identification of early diagnostic biomarkers for hepatocellular carcinoma is necessary to provide both improved prognostic outcomes and make treatment options dependent on liver dysfunction, such as resection and liver transplantation, available to patients. This study performed real time polymerase chain reaction and western blot analysis for four hepatocellular carcinoma cell lines (SKHEP1, HEP3B, C3A, and HEPG2) to observe the expression of POTE-2 mRNA and protein. The data demonstrates significant overexpression of POTE-2 in HCC cell lines, indicating these HCC cell lines to be an ideal *in-vitro* model to study POTE-2 function in hepatocellular carcinoma.

Introduction

Hepatocellular carcinoma (HCC), the most common cancer of the liver, was the sixth leading cause of new cancer cases and the third leading cause of cancer mortality in 2020 [3]. According to the Cancer Statistics Center, the United States is estimated to have approximately 41,260 new liver cancer cases and 30,520 deaths in 2022 from liver and intrahepatic bile duct cancers [1]. Additionally, the 5-year survival rate changes drastically when liver cancer progresses from localized to distant. While there is a 35% 5-year relative survival rate for localized liver cancer, the chances of survival fall to 3% once cancer metastasizes to distant locations [1]. Diagnosis of HCC often does not occur until the advanced stages of the disease due to the absence of symptoms and the lack of surveillance for high-risk patients [5]. Thus, the identification and analysis of novel diagnostic biomarkers is essential to provide earlier detection of hepatocellular carcinoma and to improve its prognosis.

The POTE genes (prostate, ovary, testis, and placenta expressed) are a gene family located on various chromosomes, including chromosomes 2, 8, 13, 14, 15, 18, 21, and 22) [2]. Increased expression of POTE genes has been found to be associated with prostate and ovarian cancer. POTEE, one of POTE's paralogs, is located on chromosome 2 on 2q21.1 and has a gene size of approximately 47,212 base pairs. POTEE is an ideal candidate as a diagnostic biomarker to study as it is highly expressed in various cancers, including colorectal, prostate, lung, breast, and ovarian cancer, and has been shown to possess oncogenic function [7]. Analysis of POTE-2 mRNA and protein expression and its role in liver cancer can lead to the determination of POTE-2 as a novel diagnostic marker and potential therapeutic target for hepatocellular carcinoma.

Materials & Methods

Cell culture

Human HCC cell lines SKHep1, Hep3B, C3A, and HEPG2 were obtained from American Type Culture Collection (ATCC). The hepatocellular carcinoma cells were cultured in Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum (FBS) and antibiotics (Pen/Strp). All cells were cultured at 37° C containing 5% CO_2 under normoxia.

RT-qPCR

Total RNA was isolated from HCC cell lines SKHep1, Hep3B, C3A, and HEPG2 cells using TRIzol Reagent. The RNA was then used to make complementary DNA (cDNA) by reverse transcription, after the DNase treatment. The collected cDNA was further used to perform RT-qPCR using the Bio-Rad CFX-96 Thermal Cycler. The primers used were 5'-GCC TCA CAC CAC TGT TAC TT-3' (sense) and 5'-CAA CCT CTC TAT CAC CAT CCT TAT T-3' (anti-sense) for POTE-2.

Western blot

Total protein from the whole cell extract was fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer onto a polyvinylidene fluoride (PVDF) membrane. After being blocked (milk 10%), the membranes were incubated with primary antibodies against POTE-2 (Abcam) and later with secondary antibodies, and the signal was developed using chemiluminescence.

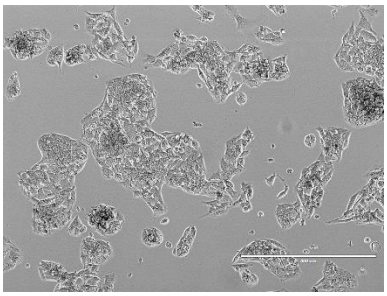
Statistical Analysis

Statistical analysis was performed using GraphPad prism. * indicate $p<0.05$, ** indicate $p<0.01$, *** indicate $p<0.001$, **** indicate $p<0.0001$. A two-way t-test was performed, and values were quantified relative to SKHEP1.

Results

HCC cell lines from ATCC

Hepatocellular carcinoma cell lines procured from ATCC are shown in Figure 1, indicating the morphology. The cells were maintained in EMEM (FBS 10%). The phase contrast images of the four hepatocellular carcinoma cell lines are shown in Figure 1.

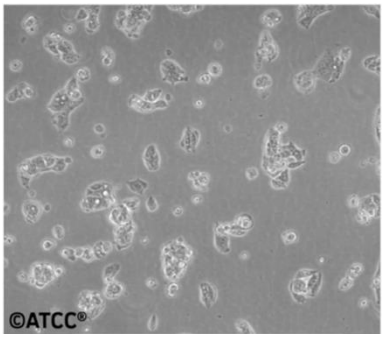


HepG2

Derivation: 15 yo white male

Differentiation and Stemness: Differentiated HCC

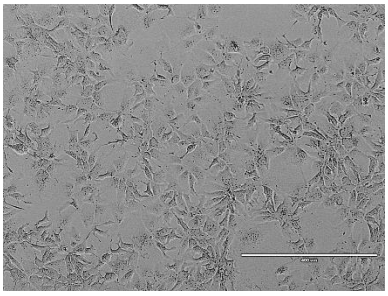
Expression markers: Insulin**,** IGF II



C3A

Derivation: HEPG2 clonal derivative selected for strong contact inhibition of growth, high albumin production, high AFP production and ability to grow in glucose deficient medium

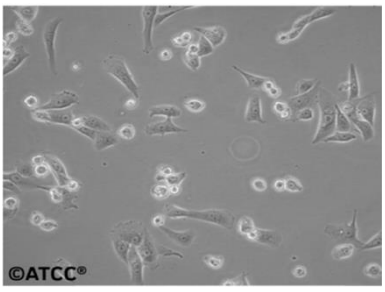
Differentiation: Differentiated HCC



HEP3B

Derivation: 8 yo black male

Comments: Contains Hepatitis B virus genome



SKHEP1

Derivation: 52 yo white male

Differentiation: Adenocarcinoma

RT-qPCR Analysis

RT-qPCR analysis (Fig. 2) reveals increased expression of POTE-2 in HCC cell lines. Each cell line demonstrates the mRNA expression of POTE-2. HEP3B, C3A, and HEPG2 cells show statistically significant changes in POTE-2 mRNA expression with a P value of less than 0.0001.

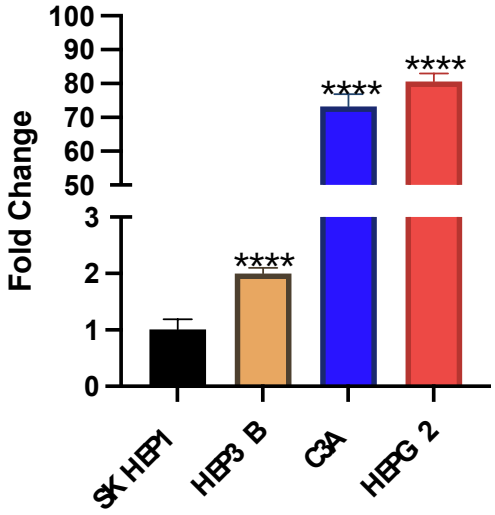


Figure 2. RT-PCR analysis of POTE-2 mRNA from HCC cell lines from ATCC.

Western Blot Analysis

Western blot analysis (Fig. 3) demonstrates the expression of POTE-2 protein in all four HCC cell lines. SKHEP1, although lacking a significant change in POTE-2 mRNA expression, demonstrates the highest protein expression, followed by HEPG2, C3A, and HEP3B, respectively. The difference in mRNA expression and protein expression in SKHEP1 cells demonstrate that lower mRNA expression may not directly reflect the amount of protein expressed.

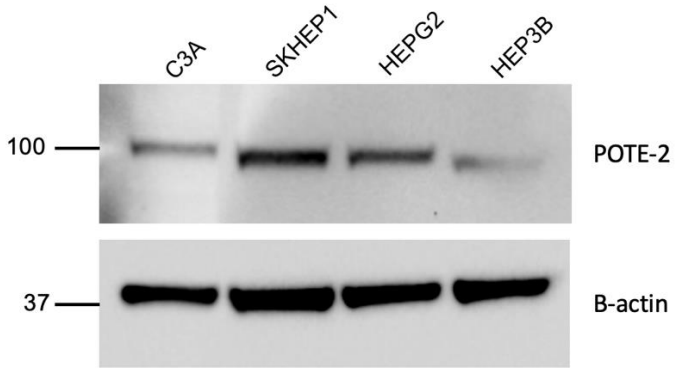


Figure 3. Western Blot showing POTE-2 Protein in HCC cell lines from ATCC.

Discussion

Higher POTE-2 expression has been shown to play a role in the regulation of cell invasion and cell migration and is believed to be associated with poor prognostic outcomes in diseased states [6]. Additionally, POTE-2 has demonstrated its ability to perform oncogenic functions in cancer [4-5]. RT-qPCR of HEP3B, C3A, and HEPG2 demonstrate statistically significant changes in POTE-2 expression. This result suggests a differential expression of the POTE-2 gene in hepatocellular carcinoma, which might help in in-vitro studies *via* gene modulation (overexpression and knockdown). Western blot analysis displays POTE-2 expression in all four cell lines, with increased expression in SKHEP1 cells. It can be observed that the amount of protein expression cannot always be determined by the quantification of RT-PCR. These results are consistent with The Cancer Genome Atlas (TCGA) database analysis that showed increased POTE-2 expression in hepatocellular carcinoma and provide a basis for the use of POTE-2 as an early detection biomarker due to its presence in all stages of hepatocellular carcinoma. Further work analyzing POTE-2's mechanisms in cell signaling, cell invasion, and cell migration in POTE-2 overexpressed cells can lead to a better understanding of its role in hepatocellular carcinoma progression.

References

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