Origin and properties of hepatocellular carcinoma cell lines

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Abstract

The liver is one of the vital organs in human beings. It is responsible for several functions such as energy homeostasis, detoxification, regulating blood sugar levels, protein synthesis, bile acid secretion, cholesterol production and glycogen storage. Hepatocellular Carcinoma (HCC) alternately referred to as malignant hepatoma accounts to ~75% of all liver cancers and its resistance to therapeutics at progressed stages makes it lethal. Some of the main causes of HCC include HBV, HCV, aflatoxin, chronic alcoholism, excess nutrition, liver cirrhosis, iron over load, Wilson’s disease, and type 2 diabetes. It is essential to detect the underlying causes of HCC at the cellular and molecular levels to develop drugs and find a potential cure for the disease. Several ongoing studies on HCC cell lines help to understand the gene expressions, multiple signaling pathways and differential drug responses to HCC. This review article provides insights to the possible molecular pathways involved in the origin of commonly used HCC cell lines and the signals that are functionally active in them.

Keywords: hepatocellular carcinoma; malignant; cell lines; molecular pathways; Msignals.

Abbreviations: HCC: Hepatocellular Carcinoma; NAFLD: Nonalcoholic Fatty Liver Disease; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; CDK1: Cyclin-Dependent Kinase 1; C-Myc: Cellular Myelocytomatosis Oncogene; AEG-1: Astrocyte Elevated Gene-1; SND1: Staphylococcal Nuclease And Tudor Domain Containing 1; LSF: Late SV40 Factor; TGF-B: Transforming Growth Factor-B; ERK: Extracellular Signal-Regulated Kinase; MEK: Mitogen-Activated Protein Kinase; PI3K: Phosphoinositide 3-Kinases; Mtor: Mammalian Target Of Rapamycin; NF-Kb: Nuclear Factor Kappa-Light-Chain-Enhancer Of Activated B Cells; SHH: Sonic Hedgehog; TIMP: Tissue Inhibitor Of Metalloproteinase; PTEN: Phosphatase And Tensin Homolog; MMP: Matrix Metalloprotease; CD24: Cluster Of Differentiatation 24; Epcam: Epithelial Cellular Adhesion Molecule; BM-MSC: Bone Marrow Derived Mesenchymal Stem Cells; TNF: Tumor Necrosis Factor; IGF: Insulin-Like Growth Factor.
Introduction

Liver cancer or HCC is rapidly arising as a health priority accounting for a significant number of cancer-related deaths globally [1-5]. HCC is a common malignancy and can be fatal due to a lack of remedies for progressed stages of tumor [6]. To develop a potential curative for advanced HCC, it is essential to understand the underlying causes and features of HCC [7-9]. Although several reports associate the contribution of natural carcinogens such as aflatoxin to HCC, HBV and HCV plays a vital role [7,9,11]. At molecular level, the viral infection advances by associating with the p53 gene. This gene is tumor suppressor and obstructs cell cycle regulation. Oncogenes like aflatoxin promote mutation in tumor suppressor genes and evidence supports the role of proto-oncogenes in HCC [10]. Few principles oncogenes such as c-Myc (Cellular myelocytomatosis oncogene), AEG-1 (Astrocyte elevated gene-1), Staphylococcal Nuclease and Tudor Domain Containing 1 (p100, Tudor SN, SND1) and LSF (Late SV40 factor) are upregulated in HCC. Several signaling pathways like the TGF-β, ERK, MEK, PI3K/Akt/mTOR and NF-κB are active in HCC and activated signaling pathways differ based on cell lines [12,13,9,14-16]. It is required to carry out in vitro analysis to examine the individual signaling pathways in distinct cell lines. Some of the routinely analyzed cell lines are Hep3B, HepG2, Huh7, Sk-Hep1, QGY-7703, PLC/PRF/5 (Alexander) and immortalized THLE-2 and THLE-3 as listed in (Table 1). The establishment and characterization of cell lines from various sources has paved the way to the present understanding of HCC at the molecular level. However, it is essential to construct a systematic study of these cell lines based on their origin and molecular pathways to understand the drug-resistant mechanisms in HCC and develop novel medications.

Table 1: Origin and properties of commonly used HCC cell lines.

<table>
<thead>
<tr>
<th>Name</th>
<th>Origin and ethnicity</th>
<th>Year</th>
<th>Morphology</th>
<th>Growth properties</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hep3B</td>
<td>8-year-old black male</td>
<td>1976</td>
<td>Epithelial</td>
<td>Adherent</td>
<td>Presence of HBV genome</td>
</tr>
<tr>
<td>HepG2</td>
<td>15-year-old Caucasian Argentinean male</td>
<td>1975</td>
<td>Epithelial</td>
<td>Adherent</td>
<td>No evidence of HBV genome</td>
</tr>
<tr>
<td>Huh-7</td>
<td>57-year-old Japanese male</td>
<td>1982</td>
<td>Epithelial</td>
<td>Adherent</td>
<td>Negative for HCV</td>
</tr>
<tr>
<td>THLE-2 &amp; THLE-3</td>
<td>Primary normal liver cells</td>
<td>1990</td>
<td>Epithelial</td>
<td>Adherent</td>
<td>Cells are infected with SV40 large T antigen</td>
</tr>
<tr>
<td>Sk-Hep-1</td>
<td>52-year-old Caucasian male</td>
<td>1971</td>
<td>Endothelial</td>
<td>Adherent</td>
<td>Absence of endothelial markers</td>
</tr>
<tr>
<td>QGY-7703</td>
<td>35-year-old Chinese female</td>
<td>1981</td>
<td>Epithelial-Mesenchymal transition</td>
<td>Adherent</td>
<td>Aflatoxin induced HCC</td>
</tr>
<tr>
<td>PLC/PRF/5 (Alexander)</td>
<td>24-year-old Shangaan male (Africa)</td>
<td>1975</td>
<td>Epithelial</td>
<td>Adherent</td>
<td>Presence of HBsAg</td>
</tr>
<tr>
<td>SNU-423</td>
<td>40-year-old Korean male</td>
<td>1990</td>
<td>Epithelial</td>
<td>Adherent</td>
<td>HBV DNA detected</td>
</tr>
<tr>
<td>FOCUS</td>
<td>60-year-old male</td>
<td>1985</td>
<td>Fibroblast</td>
<td>Adherent</td>
<td>Positive for HBV</td>
</tr>
</tbody>
</table>

HCC cell lines

Hep3B

Hep3B cell lines were initially established by Aden et al. [17]. It was isolated at Barbara B. Knowles lab, Wistar Institute, Philadelphia, USA. The cell line was sourced from hepatic biopsies of a young black male. Aged 8, he was a victim of primary liver carcinoma (Figure 1A). The cell line contains an integrated HBV genome and produces two primary polypeptides of HBV surface antigen (HBsAg) [18]. Several genes like MMP-9 (matrix metalloproteinase) [19], Survivin [20], TGF-β (transforming growth factor) [21] and SHH (sonic hedgehog) [22] are expressed in Hep3B. Genes like plasminogen [23], TIMP-1 and TIMP-3 (tissue inhibitors of metalloproteinases) are suppressed. PTEN tumor suppressor protein in expressed and pathways like ERK and NF-κB is active. Studies established the absence of tumor suppressor gene p53 [24] and the expression of transcription factor NF-κB is detected [25]. The doubling time of Hep3B is ~36 hours.

HepG2

The HepG2 cell line was also isolated at Barbara B. Knowles lab, Wistar Institute, Philadelphia, USA (Figure 1B). It is a human hepatoma obtained from liver tumor biopsies of a 15-year-old Caucasian Argentinean male [17]. These cell lines are epithelial like and non-tumorigenic with large multiplication rate [26]. Studies have reported that HepG2 is p53 wild type. Expression of genes like Cyclin D1, TGF-β type I receptor, TIMP-1 and TIMP-3 is up regulated, while there is a decline in the expression of TGF-β, SHH and MPP-9 [27]. A proteome profiling revealed that HepG2 retained hepatocytes like features [28]. A significant number of plasma proteins like albumin, transferrin, plasminogen, characteristic of hepatocytes are present in the cell line and pathways like ERK and NF-κB is active as well. The doubling time of HepG2 is ~48 hours [29].

Huh7

Huh 7 and its derivatives have its origin from the human hepatoma. This cell line was established in 1982 by Nakabayashi et al., [30]. It was sourced from a Japanese man, aged 57. Huh7 is an excellent substitute for primary hepatocytes and a suitable host for in vitro propagation of HCV. It is immortal, epithelial like and tumorigenic (Figure 1C). Huh-7 produces the following host for HBV and HCV etc. Besides it also secretes anti-carcinoembryonic antigen reactive proteins. Flow cytometry study of Huh-7 cells
ascertained the expression of CD24 (cluster of differentiation 24), prominin-1 and EpCAM (epithelial cellular adhesion molecule) genes. The p53 gene in Huh-7 cells with mutations at codon 249 and 220 respectively, has a prolonged half-life thus, accumulating in the nuclei [24]. ERK and NF-κB pathways are active. Doubling time of Huh-7 is ~24 hours.

**THLE-2 and THLE-3**

The immortalized cell lines THLE-2 and THLE-3 are human hepatocytes. They are derived by introducing SV-40 large T-antigen gene (Figure 2A). A retroviral vector containing the Bgl I-Hpa I segment of the gene is packed into PA317 (packing cells) to develop the virus [31]. Both THLE-2 and THLE-3 have features of typical mature hepatic epithelial cells. These cell lines are non-tumorigenic with a doubling time of ~40 hours. Almost all signaling pathways are active in these cell lines and they expressed cytokeratin 18, albumin and cytokeratin 19 [31]. Carcinogenic metabolites are produced through functional cytochrome p450 pathway. Enzymes like glutathione S-transferases and glutathione peroxidase are held on to by the cell lines to metabolize chemical carcinogens [32].

**Sk-Hep-1**

Sk-Hep-1 is a continuing cell line. It was procured from the ascites fluids of a 52-year-old Caucasian male with hepatic carcinoma (Figure 2B). This cell line is tumorigenic with a doubling time of ~40 hours. Almost all signaling pathways are active in these cell lines and they expressed cytokeratin 18, albumin and cytokeratin 19 [31]. Carcinogenic metabolites are produced through functional cytochrome p450 pathway. Enzymes like glutathione S-transferases and glutathione peroxidase are held on to by the cell lines to metabolize chemical carcinogens [32].

**QGY-7703**

The QGY-7703 was isolated in Shanghai Institute of Cell Biology, China. This cell line was obtained from primary HCC of 35 years old female patient belonging to Qidong region which has the highest morbidity rate of HCC in China (Figure 2C). Studies have reported that aflatoxin exposure and HBV infection as interactive risk factors for primary liver cancer in this region [36]. QGY-7703 demonstrates characteristics and expresses IGF-II (insulin like growth factor) [37]. Two proteins acyl-protein thioesterase 2 and 17-beta-hydroxysteroid dehydrogenase 10 expressed in QGY-7703 are vital for tumor development. ERK and NF-κB pathways are active in QGY-7703. During apoptosis, TGF-beta, TNF, FAS, p38MAPK, and p53 signaling pathways are active in the cell line but there is no expression of E-cadherin. The doubling time for QGY-7703 is ~20 hours.

**PLC/PRF/5(Alexander)**

Dr. J. J. Alexander established the Alexander or PLC/PRF/5 cell line in Johannesburg, South Africa. It was sourced from a 24 years Shangaan male with HCC from Mozambique (Figure 3A). This cell line continuously produces the HBsAg, however there was no expression for other markers of HBV synthesis [38]. Some oncogenes like c-Myc, c-abl, c-ha-ras c-fes, c-fms, and c-sis oncogenes are indicated in the cell line along with HBsAg. The ERK, TGF-β, NF-κB, Akt/mTOR pathways are active and p53 gene mutation is indicated. Doubling time of this cell line is 30-40 hours.

**SNU-423**

The cell line SNU-423 (Seoul National University-423) was established by J.G. Park and et al. It was derived from primary hepatocellular carcinoma taken from a Korean male (Figure 3B). The cultured cells are multinucleated and HBV DNA was detected. SNU cells shows loss of normal functioning of p53 gene [39]. The cell lines are generally used to examine the expression of HBV and IGF. The ERK, TGF-β, NF-κB, Akt/mTOR pathways are active in this cell line. SNU-423 has a doubling time of 72 hours.

**FOCUS**

FOCUS (Friendship of China and United States) cell line originates from human HCC. It was obtained from a male patient aged 63 who had a history of yellow jaundice and chronic alcoholism (Figure 3C). Transcripts of p53 were not detected while there is expression of DLC-2 gene in FOCUS. This cell line is positive for HBV and expresses alpha-fetoprotein, fibrinogen, alpha 1-antitrypsin and CEA. Activities of aspartate aminotransferase and glucose-6-phosphatase are detected in FOCUS [40]. ERK, TGF-β, NF-κB and Akt/mTOR pathways are active. The cell line lacks Alpha-Fetoprotein and EMT markers. Doubling time of the cell line is 42 - 48 hours.
Conclusion

HCC is a complex health condition caused due to several factors like excess nutrition, exposure to toxins, alcoholism, HBV and HCV. Recent surge in the number of HCC cases due to diabetes, obesity and fatty liver diseases has increased the necessity for developing new drugs. Screening of various drugs in vivo along with in vitro studies has contributed to several drug discoveries. Laborious maintenance of animal models can be overcome by a cell line-based system to identify novel markers of HCC for improved diagnosis. Due to their immortality, HCC cell lines provide a provision for easy handling and they resemble the original tumor from which they were established. Over the last few decades various HCC cell lines were established from multiple ethnic groups all over the world and developed as stated in (Table 1). They are exploited for drugs screening, examining cell signaling pathways and to understand gene expression profiles associated with the HCC. The effects of drug like taxol and 5-Flourouracil on QGY-7703 have already been elucidated. Studies have also examined the response of HepG-2 and Huh-7 towards therapeutic agents like curcumin. Doxorubicin and Sorafenib are used to examine the properties of Hep3B, HepG2, PLC-PRF-5 and SK-Hep. In summary, this review helps in further understanding of various HCC cell lines, of Hep3B, HepG-2 and Huh-7 towards therapeutic agents like curcumin. In addition, the relevance of drug resistance can be overcome by a cell line-based system to identify novel markers of HCC for improved diagnosis.

Declarations

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