HepG2 Cell Line

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HepG2 is a hepatoblastoma cell line. It is used in a wide range of studies: a model of hepatoblastoma (HB) and hepatocellular carcinoma (HCC), cytotoxicity testing and the identification of drug metabolic pathways on the liver.

HepG2 cell line hepatoblastoma hepatocellular carcinoma mutations

1. Origin

Among hepatic cell lines, HepG2 cells were the first to exhibit the key characteristics of hepatocytes. The line was isolated in 1975 and described as hepatocellular carcinoma. The Wistar Institute filed a patent for the HepG2 cell line, like “a human hepatoma-derived cell line”. Since then, HepG2 cells have been entered into the ATCC (American Type Culture Collection, Rockville, MD, USA) repository as a human cell line (HB 8065), “derived from the liver tissue of a 15-year-old white male with a well-differentiated hepatocellular carcinoma” [1].

The HepG2 cell line was mistakenly labeled as hepatocellular carcinoma instead of hepatoblastoma, which caused considerable confusion for 40 years. This was discovered after an investigation by Lopez-Terrada D. et al.[1] on the nature of HepG2.

2. Cytological characteristics

The average diameter of the HepG2 cell is about 10–20 µm. Cells have large nuclei and contain 3–7 nucleoli. HepG2 accommodates low mitochondrial content and poorly developed smooth endoplasmic reticulum. The cell line contains translocations between the short arms of chromosomes 1 and 21[2], trisomies of chromosomes 2, 16, and 17, and tetrasomy of chromosome 20[3]. It has loses in the chromosome 4q3 region, which are associated with t(1; 4) translocation—a common occurrence with HB[4], as well as with other characteristic HB chromosomal abnormalities, including trisomies 2 and 20[1]. The number of chromosomes varies from 50 to 60[5], corresponding to the hyperdiploid karyotype. More than 100 of the chromosomes are observed in some cases, characterized by tetraploid enlargement. The HepG2 cell contains about 7.5 pg of DNA, 15% more than in a normal somatic cell[6].

The HepG2 cell line carries a mutation in the TERT promoter — C228T[7]. The mutation in TERT promoter occurs in both HCC and HB. This mutation contributes to immortalization, protecting telomeres in cancer cells.

Wild-type TP53[8] is observed in the HepG2 cell line, as in HCC and HB[9]. The TP53 gene is critical in suppressing cancer in humans, as it plays a role in cell cycle arrest, apoptosis, and ageing. Thus, this mutation in the gene can

promote cell proliferation.

HepG2 includes parameters of several pathways, including Wnt/β-catenin, dysregulation of cell growth, and survival pathways such as fetal and embryonal HB. The cell line hosts a characteristic deletion of the third exon of the CTNNB1 gene, which is identical to that described in epithelial type HB.

Regarding the differences between the HepG2 cell line and normal hepatocytes, the crucial point is the weak or absent expression of the cytochrome P450 (CYP) superfamily, CYP3A4, CYP2C9, CYP2C19, CYP2A6, CYP2D6, etc.[10], which are involved in phase 1 of xenobiotic oxidation in the liver.

3. Use in research

The HepG2 cell line was used to study the toxic effects of heavy metals, nanoparticles, and drugs in vitro. The validity of HepG2 cells as a model of hepatocytes is controversial because crucial proteins involved in the metabolism of substances (the liver's primary function) are poorly expressed. Furthermore, the shortage of uptake transporters and phase I enzymes is observed in HepG2 cells, which indicates the need for careful use of this cell line to predict the metabolism and elimination of xenobiotics in hepatocytes. At the same time, the use of HepG2 cells to study the metabolism of anticancer drugs is acceptable because there is a similarity in the expression of phase I, II, and III drug metabolism/transport proteins in cells with HCC and HB. In addition, due to the low basal activity of CYP proteins (CYP1A2, CYP2B6, and CYP3A4), the cell line can be used in studies of CYP inducers[11].

Work is in progress to modify the HepG2 cell line to increase the expression of cytochromes for the correct use of HepG2 cells as a model of hepatocytes in the study of drug metabolism[12]. Another approach is to derive three-dimensional spheroid cell cultures[13], presentingmore physiologically relevant system. As a result, metabolic activity, including cytochromes, is higher in 3D spheroidal HepG2 models than in 2D cells [14], bringing it closer to normal hepatocytes.

According to molecular profiling data, the HepG2 cell line can serve as an appropriate model for hepatoblastoma. However, the use of HepG2 cells as an HCC model is incorrect, and as shown in the study by Choi et al. (2015) [15], because there is no expression of the marker of HCC—hGSTP1.

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