Established Liver Cell Lines: Are You Sure to Have the Right Ones?

Ralf Weiskirchen

Abstract: In liver research, immortalized cell lines have assumed an important role in studying general physiological and pathological processes. However, misidentification and cross-contamination of cell lines is a widespread problem in biomedical sciences resulting in irreproducible results and false conclusions. Although the huge impact of working with wrong cell lines on life science research and publication has been well recognized, there are only limited efforts and strategies to prevent cell misidentification. This commentary provides a catalogue of the most important cell lines used in hepatology research, examples of misidentified cell lines, and short guidelines to be considered when working with continuous lines.

Keywords: cell line misidentification; cross-contamination; STR profiling; capillary electrophoresis; translational research; cell banking

The use of primary liver cells in research can be problematic due to unpredictable availability, limited supply and self-renewal, donor-dependent variability, and their general sensitivity during isolation and culturing that often require additional, sometimes expensive, nutrients not included in classical media [1]. Since they are not homogenous, prolonged culturing might lead to dedifferentiation, selection of specialized cell subsets, or growth promotion of contaminating cells [2]. Furthermore, their fragility and slow growth rate limit the efficient transfer of foreign nucleic acids, thereby hindering their application in gene and protein function studies.

In contrast, immortalized cells lines are cost effective, easy to handle, provide an unlimited supply of biological material, and bypass potential ethical concerns associated with the use of primary cells [3]. Moreover, a cell line is theoretically homogenous and genetically identical, ensuring establishment of consistent and reproducible results. As such, immortalized cell lines are valuable tools that have revolutionized scientific research. Consequently, scientists and biochemical companies working in the field of hepatology have developed a large number of immortalized hepatocytes (Table S1), hepatic stellate cells (Table S2), Kupffer cells (Table S3), liver sinusoidal endothelial cells (Table S4), and bile duct/cholangiocyte cell lines (Table S5).

In particular, continuous growing hepatocytes are of fundamental importance for research on hepatitis virus biology, hepatic drug uptake and metabolism, toxicity studies, and investigations on general hepatocyte function [4,5]. Similarly, several hepatic stellate cell lines are important experimental tools to understand the signaling processes and roles of individual extracellular matrix proteins in the pathogenesis of hepatic fibrosis [6].

Some of these cell lines developed spontaneously from a normal or diseased liver, while others were immortalized by biological, chemical, or physical methods. The most common is the transfection with the Simian virus 40 large T antigen (SV40T), which overrides the cell cycle by inactivating tumor suppressor genes and blocking cellular senescence [7]. Another possibility is the introduction of telomerase reverse transcriptase (TERT) that prevents age-dependent telomere shortening, thereby avoiding replicative
senescence [8]. There are also examples in which continuously growing liver cells were immortalized by transfection with oncogenes (e.g., HPV E6E7, c-myc) that interfere with the activity of cellular tumor suppressor genes or control of cell proliferation [9,10]. Moreover, there are examples in which continuous cell lines were spontaneously derived from liver outgrowths established from experimental models or patients suffering from liver disease.

In most cases, these manipulations are associated with gross gene expression alterations allowing the cells to acquire an indefinite replicative capacity and escape from cell growth crisis. Consequently, immortalized cells cannot be considered as “normal” because these gene patterns are not found in their primary counterpart. In addition, during prolonged culturing, cell lines can change their characteristics, develop abnormal traits, and obtain chromosomal abnormalities and complex mutations. Moreover, evidence has accumulated during the last decades that cell lines are frequently misidentified or contaminated. Apparent contamination was first reported in 1968 for the human cell line HeLa [11]. A list of cross-contaminated cell lines published in 2010 listed, in total, 360 cell lines that were either misidentified or affected by intraspecies or interspecies contaminations [12]. Currently, the latest register of the International Cell Line Authentication Committee (ICLAC, version 11, released on 8 June 2021) lists 576 cell lines known to be misidentified through cross-contamination or other mechanisms such as mislabeling [13]. The sources for cross-contamination and misidentification are manifold. Cross-contamination might result from spreading via aerosols, unplugged pipets, and sharing media and reagents among different cell lines, while misidentification might be the result of exchange during routine handling, mislabeling, poor freezer inventory control, or by acquiring a mislabeled cell line from another laboratory [12,14].

The consequences of cell misidentification and cross-contamination are fatal. Horbach and Halfman identified 32,755 articles that reported research results with misidentified cells that in turn were cited by approximately half a million other papers [15]. Moreover, the number of studies that used problematic cell lines was found to be about 8.7%, corresponding to 16.1% of published papers [16]. To improve these unscientific conditions, several journals have introduced the Research Resource Identifiers that should alert researchers to problematic cell lines [16]. Similarly, a growing number of funding agencies insist that researchers prove the authenticity of their cell lines by accepted consensus methods. In regard to liver research, there are several cell lines that are listed by the ICLAC as misidentified cell lines (Table 1).

| Misidentified Cell Line | ICLAC ID | Claimed Liver Cell Type | Contaminating Cell Line | Actual Species | Actual Cell Type | Ref. |
|-------------------------|----------|-------------------------|-------------------------|----------------|-----------------|-----|
| BEL-7402                | 00549    | HCC                     | HeLa/HCT8               | Human          | CCA/colon carcinoma | [17] |
| BEL-7404                | 00550    | HCC                     | HeLa                    | Human          | CCA              | [18] |
| Chang liver             | 00002    | normal hepatic cells    | HeLa                    | Human          | CCA              | [19] |
| GREF-X                  | 00123    | myofibroblast           | unknown                 | Rat            | unknown          | [20] |
| H7D7A                   | 00203    | normal cell (SV40T)     | HepG2                   | Human          | Liver hepatoblastoma | [21] |
| H7D7B                   | 00204    | normal cell (SV40T)     | HepG2                   | Human          | Liver hepatoblastoma | [21] |
| H7D7BD5                 | 00560    | normal cell (SV40T)     | HepG2                   | Human          | Liver hepatoblastoma | [21] |
| H7D7C                   | 00205    | normal cell (SV40T)     | HepG2                   | Human          | Liver hepatoblastoma | [21] |
| H7D7D                   | 00206    | normal cell (SV40T)     | HepG2                   | Human          | Liver hepatoblastoma | [21] |
| HuL-1                   | 00318    | HCC                     | HeLa                    | Human          | CCA              | [12] |
| L02 (L-02)              | 00575    | normal hepatic cells    | HeLa                    | Human          | CCA              | [18] |
| QGY-7701                | 00551    | HCC                     | HeLa                    | Human          | CCA              | [22] |
| QGY-7703                | 00552    | HCC                     | HeLa                    | Human          | CCA              | [22] |
| QSG-7701                | 00553    | normal hepatic cells    | HeLa                    | Human          | CCA              | [17] |
| RBHF-1                  | 00155    | hepatoma                | unknown                 | Non-human      | unknown          | [20] |
| SMMC-7721               | 00554    | HCC                     | HeLa                    | Human          | CCA              | [17] |
| WRL 68                  | 00351    | embryonic cells         | HeLa                    | Human          | CCA              | [12] |

* All data were taken from the ICLAC register, version 11 [12]. Abbreviations used are: CCA, Cervical adenocarcinoma; HCC, hepatocellular carcinoma; SV40T, transformed by simian virus large T antigen.
The most prominent misidentified liver cell line is the ‘Chang liver cell’. This cell line was originally established from a normal human liver biopsy of a patient during an exploratory laparotomy by continuous subcultivation of epithelial-like cells [23]. The cells were then commercially distributed by the American Type Culture Collection (ATCC) as an immortalized human ‘normal hepatocyte model’ [19,24]. However, isoenzymes analysis and the identification of complex chromosomal rearrangement that were identical to those found in the HeLa cervical carcinoma cell line suggested that the ‘Chang liver cells’ are de facto HeLa or a derivative thereof [19]. Later, this assumption was confirmed by the definition of a short tandem repeat (STR) profile that matched that of parenteral HeLa cells [25]. Nevertheless, several scientists still ignore these facts and imperturbably use the ‘Chang liver cells’ as a normal liver or liver cancer cell line.

Similarly, the L-02 cell line, also known as HL-7702, is used mistakenly in many studies as a normal human fetal hepatocyte line. It was proposed as a cell line that exhibits good liver functions in vitro and as a suitable cellular source for liver support systems [26]. However, later on, it was unquestionably proven that this cell line is also identical with HeLa [17]. Based on the uncertainties associated with both ‘Chang liver’ and L-02 cells, these misidentified cell lines should not be used under any circumstances for research purposes.

On the contrary, there are examples in which cells supposed to originate from other organs are contaminated by human hepatocytic cell lines (Table 2). However, the respective cell lines (i.e., F2-4E5, F2-5B6, P1-1A3, P1-4D6, and REPC) were fortunately never used much and, therefore, the resulting scientific damage is only minor.

### Table 2. Selection of cell lines contaminated with human liver cells *

| Misidentified Cell Line | ICLAC ID | Claimed Cell Type | Contaminating Cell Line | Actual Cell Type | Ref. |
|-------------------------|----------|-------------------|-------------------------|-----------------|------|
| F2-4E5                  | 00120    | Thymic epithelium | SK-HEP-1                | Liver carcinoma | [20] |
| F2-5B6                  | 00121    | Thymic epithelium | SK-HEP-1                | Liver carcinoma | [20] |
| P1-1A3                  | 00150    | Thymic epithelium | SK-HEP-1                | Liver carcinoma | [20] |
| P1-4D6                  | 00151    | Thymic epithelium | SK-HEP-1                | Liver carcinoma | [20] |
| REPC                    | 00487    | Kidney, normal renal cells | Hep3B | Liver, hepatocellular carcinoma | [27] |

* All data were taken from the ICLAC register, version 11 [11].

Furthermore, there are prominent examples of liver cells that were misclassified in regard to their origin. The human cell line HepG2 was originally classified as a hepatocellular carcinoma cell line derived from liver tissue of a 15-year-old white male with a well-differentiated hepatocellular carcinoma [28,29]. However, subsequent evaluation of the histopathologic background of the tumor, signaling pathway analysis, as well as the genetic profile as assessed by comparative genomic hybridization showed that the cells are more likely a hepatoblastoma-derived cell line than a hepatocellular carcinoma cell line [30]. It is obvious that proper information about the tissue or donor from which a cell line is derived increases the overall scientific value of a cell line and its particular purpose in biomedical research.

Based on the above-mentioned considerations, it is essential that scientists follow some general guidelines when acquiring a new cell line for their experiments. In principle, the implementation can be divided into five process phases, namely a theoretical (T), organizational (O), verifying (V), experimental (E), and confirmational (C) work step (Table 3).
Table 3. The ‘TOVEC’ concept for acquiring a new cell line.

| Phase      | Implementation | Key Questions to Be Answered                                                                 | Recommendations                                                                 |
|------------|----------------|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| 1 Theoretical (T) | - Which cell lines are available?  
- Which cell line is most suitable for my experiments?  
- Is there information available that allows authentication of the chosen cell line?  
- Are there reports available that successfully used the chosen cell line in similar experiments? | - Screen and read the literature  
- Clarify if your fellow researchers have good experience with a specific line  
- Clarify what the shortlisted cells were originally generated for  
- Check for potential hazards associated with shortlisted cells  
- Finally determine which cell line you would like to have |
| 2 Organizational (O) | - Where I can obtain the chosen cell line?  
- Is the cell line genetically modified?  
- Have I the institutional requirements to work with these cells in my lab? | - Clarify if the cell line is commercially available  
- Clarify if an MTA is necessary  
- Clarify if you can handle the cells in your lab  
- Organize required media and, if necessary, special supplements |
| 3 Verifying (V) | - Is the cell line I have obtained/bought the cell line that I have wanted?  
- Is the cell line free of contaminants?  
- Does the cell line show the desired properties? | - Document cell appearance  
- Prepare stocks from early passages of cells  
- Authenticate the cell line (morphology, potential marker gene expression, doubling rate, STR profile, etc.)  
- Screen for cross-contaminations and contaminating microorganisms |
| 4 Experimental (E) | - Can I reproduce experiments that were published before with this cell line?  
- Do the cell lines show the desired properties in my experiments?  
- Are there unknown changes not reported before?  
- Does the cell line help to obtain new findings and advance my research? | - Record passage numbers during each experiment  
- Reproduce experiments that led to the acquisition of the line (before starting with new experiments)  
- Make notes of undesired abnormalities (mutations, differences from described gene expressions, etc.) during experimentation |
| 5 Confirmational (C) | - Can I reproduce my key findings in primary cells or in any other suitable (established) in vivo model? | - Reproduce key findings in primary cells or, if possible, in a suitable in vivo model  
- Conclude if the cell line was helpful in your studies |

In each phase of this ‘TOVEC’ workflow, the scientist needs to answer key questions that will help him/her to find the most suitable cell line(s) for his/her work. It further allows for conclusions on whether the obtained results have any biological relevance apart from the cell line.

During the ‘T-phase’, the researcher defines which cell line is most suitable for his/her work by taking into account the accessible literature and recommendations of fellow researchers. In most cases, it is more advisable to use a good characterized cell line than an entirely novel cell line. This will simplify the work, since much information about the biology of this cell line is already accessible. It helps to save time by reducing the need to find optimal culture conditions or their response to exogenous stimuli. In this phase, it also makes sense to screen the literature for information showing that relevant signaling pathways or mediators to be analyzed are active or expressed in this cell line. Importantly, the usage of a well-established cell line further allows the correlation of novel results to previous findings established with this cell line and to increase the acceptance of your findings. In the ‘O-phase’, potential sources for the chosen cell line are evaluated, and
potential regulatory and institutional affairs are clarified. In addition, reliable sources for special culture reagents or supplements should be defined. If a large series of experiments with the chosen cell line are planned, one should ensure that necessary chemicals are accessible in the long term. At this step, it might be necessary to reserve batches of chemicals or biological compounds to guarantee reproducible work.

The following ‘V-phase’ is most important. After acquisition of the cell line, it is essential to verify that the cell line obtained is in fact the desired one and, further, that the introduced cell line is free of cross-contaminating cells or microbial contaminations. The most reliable test for authentication is short tandem repeat (STR) profiling [25]. STRs are short tandemly repeated DNA sequences that are highly variable among individuals [25]. In STR analysis, the number of repetitions at specific STR loci located on multiple chromosomes is determined by polymerase chain reaction. Subsequently, the resulting fragment sizes resulting from the amplification of the variant sites are determined by capillary electrophoreses or other electrophoresis methods [25]. If no reference STR profile is available for the chosen cell line, it is advisable to define one before starting experimentation. Cell authentications should then be completed by testing for marker gene expression or the presence of other already reported cellular characteristics (e.g., size, morphology, strength of attachment, doubling time, substrate and nutrient dependency, etc.). Potential biological contaminants with bacteria, yeast, and molds can be ruled out by microscopy, while the presence of mycoplasmas should be excluded by ELISA, PCR, immunostaining, Hoechst 33258 stain, or special microbiological assays. During this phase, cell stocks should be prepared at the lowest passage possible and kept in more than one storage vessels. Since cell lines can be markedly genetically instable as their passage number increases, working stocks should be replenished from these frozen seed stocks on a regular basis.

After ensuring possession of the correct, contamination-free cell line, the ‘E-phase’ can start. During this phase, it will become apparent whether the scientific questions raised could be answered with the aid of the chosen cell line. Unexpected results should be given special consideration. It must be clarified whether the experimental design was correct, measurements were correctly recorded, performed experiments were faulty, or the hypothesis that led to the execution of the experiments was wrong. Importantly, an unexpected finding does not necessarily mean that the result must be discarded. Identifying reasons for undesirable experimental outcomes might provide the basis for new discoveries.

During the ‘C-phase’, it is mandatory to critically set the obtained findings in relation to previous findings. Since a cell line is just a cell line and not more, it will be of fundamental importance to reproduce key findings in primary cells or in a suitable in vivo model. If the outcome is the same, the scientist can be sure that he/she has chosen the correct cell line for his/her work and that the obtained findings might have a true biological implication. However, when obtained findings cannot be accurately replicated in primary cells or in a suitable in vivo model, it should be kept in mind that cell lines are manipulated, which may alter their biological behavior, native functions, or their responsiveness to exogenic or endogenic stimuli. In such a case, it would be interesting to investigate whether observed anomalies are specific for this cell line or also reproducible in other lines. These examinations could then potentially clarify what is altered in the cell line and why the cell line reacts the way it does.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/livers2030015/s1, Table S1. Selection of immortalized hepatocyte cell lines; Table S2. Selection of immortalized hepatic stellate cell lines; Table S3. Selection of immortalized Kupffer cell lines; Table S4. Selection of immortalized liver sinusoidal endothelial cells; Table S5. Selection of immortalized bile duct/cholangiocyte and gallbladder cell lines.

**Funding:** The author is funded by the German Research Foundation (DFG) with grants WE2554/13-1, WE2554/15-1, and WE2554/17-1. The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Data Availability Statement:** Data is contained within the article or supplementary material.
Livers 2022, 2

Conflicts of Interest: The author declares no conflict of interest.

References

1. Richter, M.; Piwocka, O.; Musielak, M.; Piotrowski, I.; Suchorska, W.M.; Trzeciak, T. From donor to the lab: A fascinating journey of primary cell lines. Front. Cell Dev. Biol. 2021, 9, 711381. [CrossRef] [PubMed]

2. Bolleyen, J.; Fraczk, J.; Rogiers, V.; Vanhaecke, T. Epigenetic modifications as antidifferentiation strategy for primary hepatocytes in culture. Methods Mol. Biol. 2015, 1250, 203–211. [CrossRef] [PubMed]

3. Kaur, G.; Dufour, J.M. Cell lines: Valuable tools or useless artifacts. Spermatogenesis 2012, 2, 1–5. [CrossRef]

4. Serras, A.S.; Rodrigues, J.S.; Cipriano, M.; Rodrigues, A.V.; Oliveira, N.G.; Miranda, J.P. A critical perspective on 3D liver models for drug metabolism and toxicology studies. Front. Cell Dev. Biol. 2021, 9, 626805. [CrossRef] [PubMed]

5. Tabernilla, A.; Dos Santos Rodrigues, B.; Pieters, A.; Caufriez, A.; Leroy, K.; Van Campenhout, R.; Cooreman, A.; Gomes, A.R.; Arnesdotter, E.; Gijbels, E.; et al. In vitro liver toxicity testing of chemicals: A pragmatic approach. Int. J. Mol. Sci. 2021, 22, 5038. [CrossRef]

6. Herrmann, J.; Gressner, A.; Wessendorf, K. Immortal hepatocellular carcinoma cell lines: Useful tools to study hepatocellular carcinoma biology and function? J. Cell. Mol. Med. 2007, 11, 704–722. [CrossRef]

7. Kirchhoff, C.; Araki, Y.; Huhtaniemi, I.; Matusik, R.J.; Osterhoff, C.; Poutanen, M.; Samalecos, A.; Sipilä, P.; Suzuki, K.; Orgebin-Crist, M.C. Immortalization by large T-antigen of the adult epididymal duct epithelium. Mol. Cell. Endocrinol. 2004, 216, 83–94. [CrossRef] [PubMed]

8. Lundberg, A.S.; Hahn, W.C.; Gupta, P.; Weinberg, R.A. Genes involved in senescence and immortalization. Curr. Opin. Cell Biol. 2000, 12, 705–709. [CrossRef]

9. Faure-Dupuy, S.; Vegna, S.; Aillot, L.; Dimier, L.; Esser, K.; Broxtermann, M.; Bonnin, M.; Rivoire, M.; Passot, G.; et al. Characterization of pattern recognition receptor expression and functionality in liver primary cells and derived cell lines. J. Innuite Immun. 2018, 10, 339–348. [CrossRef]

10. Kitani, H.; Sakuma, C.; Takenouchi, T.; Sato, M.; Yoshioho, M.; Yamanaka, N. Establishment of c-src-immortalized Kupffer cell line from a C57BL/6 mouse strain. Results Immunol. 2014, 4, 68–74. [CrossRef]

11. Gartler, S.M. Apparent HeLa cell contamination of human heteroploid cell lines. Hepatology 1976, 29, 49–54. [CrossRef] [PubMed]

12. Capes-Davis, A.; Theodosopoulos, G.; Atkin, I.; Drexler, H.G.; Kohara, A.; Masters, J.R.; Nakamura, Y.; Reid, Y.A.; Reddel, R.R.; et al. Check your cultures! A list of cross-contaminated or misidentified cell lines. Int. J. Cancer 2010, 127, 1–8. [CrossRef] [PubMed]

13. ICLAC. Register of Misidentified Cell Lines. Available online: https://iclac.org/databases/cross-contaminations/ (accessed on 20 July 2022).

14. Geraghty, R.J.; Capes-Davis, A.; Davis, J.M.; Downward, J.; Freshney, R.I.; Knezevic, I.; Lovell-Badge, R.; Masters, J.R.; Meredith, J.; Stacey, G.N.; et al. Cancer Research UK. Guidelines for the use of cell lines in biomedical research. Br. J. Cancer 2014, 111, 1021–1046. [CrossRef] [PubMed]

15. Horbach, S.P.; Halfman, W. The ghosts of HeLa: How cell line misidentification contaminates the scientific literature. PLoS ONE 2017, 12, e0186281. [CrossRef]

16. Babic, Z.; Capes-Davis, A.; Martone, M.E.; Bairoch, A.; Ozyurt, I.B.; Gillespie, T.H.; Bandrowski, A.E. Incidences of problematic cell lines are lower in papers that use RRIDs to identify cell lines. Elife 2019, 8, e41676. [CrossRef]

17. Ye, F.; Chen, C.; Qin, J.; Liu, J.; Zheng, C. Genetic profiling reveals an alarming rate of cross-contamination among human cell lines used in China. FASEB J. 2015, 29, 4268–4272. [CrossRef]

18. Huang, Y.; Liu, Y.; Zheng, C.; Shen, C. Investigation of cross-contamination and misidentification of 278 widely used tumor cell lines. PLoS ONE 2017, 12, e0170384. [CrossRef]

19. Lavappa, K.S.; Macy, M.L.; Shannon, J.E. Examination of ATCC stocks for HeLa marker chromosomes in human cell lines. Nature 1976, 259, 211–213. [CrossRef]

20. MacLeod, R.A.; Dirks, W.G.; Matsuo, Y.; Kaufmann, M.; Milch, H.; Drexler, H.G. Widespread interspecies cross-contamination of human tumor cell lines arising at source. Int. J. Cancer 1999, 83, 555–563. [CrossRef]

21. Van Pelt, J.; Decorte, R.; Yap, P.S.; Feyver, J. Identification of HepG2 variant cell lines by short tandem repeat (STR) analysis. Mol. Cell. Biochem. 2003, 243, 49–54. [CrossRef]

22. Bian, X.; Yang, Z.; Feng, H.; Sun, H.; Liu, Y. A combination of species identification and STR profiling identifies cross-contaminated cells from 482 human tumor cell lines. Sci. Rep. 2017, 7, 9774. [CrossRef] [PubMed]

23. Chang, R.S. Continuous subcultivation of epithelial-like cells from normal human tissues. Proc. Soc. Exp. Biol. Med. 1954, 87, 440–443. [CrossRef] [PubMed]

24. Gao, Q.; Wang, X.Y.; Zhou, J.; Fan, J. Cell line misidentification: The case of the Chang liver cell line. Hepatology 2011, 54, 1894–1895. [CrossRef] [PubMed]

25. Masters, J.R.; Thomson, J.A.; Daly-Burns, B.; Reid, Y.A.; Dirks, W.G.; Packer, P.; Toji, I.H.; Ohno, T.; Tanabe, H.; Arlett, C.F.; et al. Short tandem repeat profiling provides an international reference standard for human cell lines. Proc. Natl. Acad. Sci. USA 2001, 98, 8012–8017. [CrossRef]

26. Hu, X.; Yang, T.; Li, C.; Zhang, L.; Li, M.; Huang, W.; Zhou, P. Human fetal hepatocyte line, L-02, exhibits good liver function in vitro and in an acute liver failure model. Transplant. Proc. 2013, 45, 695–700. [CrossRef]
27. Frede, S.; Freitag, P.; Geuting, L.; Konietzny, R.; Fandrey, J. Oxygen-regulated expression of the erythropoietin gene in the human renal cell line REPC. Blood 2011, 117, 4905–4914, Erratum in Blood 2014, 123, 3365. [CrossRef]

28. Aden, D.P.; Fogel, A.; Plotkin, S.; Damjanov, I.; Knowles, B.B. Controlled synthesis of HBsAg in a differentiated human liver carcinoma-derived cell line. Nature 1979, 282, 615–616. [CrossRef]

29. Knowles, B.B.; Howe, C.C.; Aden, D.P. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. Science 1980, 209, 497–499. [CrossRef]

30. López-Terrada, D.; Cheung, S.W.; Finegold, M.J.; Knowles, B.B. Hep G2 is a hepatoblastoma-derived cell line. Hum. Pathol. 2009, 40, 1512–1515. [CrossRef]