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Research Highlight

A small molecule cocktail breaks the bottleneck of human primary hepatocytes culture

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It is estimated that more than 300 million people in China are suffering from different liver diseases including viral hepatitis, fatty liver, alcoholic liver, liver fibrosis, cirrhosis and liver cancer, of which about 400,000 people die each year [1]. The clinical resolution of these diseases relies largely on the breakthroughs from basic research. Human primary hepatocytes (PHHs) have long been considered as the "gold standard" for in vitro liver disease research models. However, there are still a number of limitations, like high donor variability, short life spans and limited availability. We have systematically compared different in vitro hepatocyte culture models and found that PHHs rapidly dedifferentiate and lose their hepatocyte function under in vitro culture conditions [2]. Despite attempts to improve the culture conditions, how to maintain the maturation status of PHHs in vitro has been a challenge. In the recent issue of Science magazine, a paper titled "Long-term functional maintenance of primary human hepatocytes in vitro" presented a method that significantly extended the culture time of PHHs [3].

The authors first compared the gene expression profiles of fresh isolated PHHs and 24-hour cultured PHHs, and found that the expression of 15 genes involved in maintaining hepatocyte function was significantly down-regulated. At the meantime, several molecular pathways that cause hepatocyte dysfunction, including TGF-β signaling pathway, are activated. However, the inhibition of the TGF-β signaling pathway by small molecule inhibitors does not restore the expression of hepatocyte-specific genes. Next, the authors screened a series of small molecule compounds and found that the combination of at least 5 small molecule compounds (5C) can effectively prolong the culture time of PHHs in vitro, and support the function of hepatocytes including albumin secretion, urea synthesis and drug metabolism. These data are also supported by large dataset of PHH transcriptome with a focused analysis on genes involving drug metabolism.

Hepatitis B virus (HBV) basic and translational research have been hindered by the absence of relevant in vitro model that features the physiological condition of hepatocytes and permits efficient HBV replication and infection [4]. Human hepatoma cell lines, like Huh7 and HepG2, are widely used as a surrogate model for HBV replication or infection, even if they only partially mimic physiological hepatic functions. Transfect human hepatoma cells with HBV expressing plasmid has been used to investigate basic biology of HBV replication and pathogenesis [5]. Furthermore, stably HBV-transfected hepatoma cell lines including HepG2.2.15, HepG2-H1.3 and HepAD38 have been generated to produce high amounts of HBV virions [6–8]. Similarly, HBV-Met cell, an immortalized murine hepatocyte isolated from both c-Met and HBV transgenic mice, support HBV replication and virion secretion [9]. Nevertheless, these cell lines are not permissive for natural infection as they are unable to mediate early steps of virus infection including entry, uncoating and covalently closed circular DNA (cccDNA) formation. Alternative systems have been established through delivery of HBV genome by baculoviral or adenoviral vectors, resulting in sufficient HBV replication and viral particle production [10,11]. Normally, cultured PHHs rapidly lost the expression of HBV receptor-NTCP, but 5C-PHHs could still express high levels of NTCP at 4 weeks of culture. After infection with the virus, 5C-PHHs can maintain the HBV cccDNA at a high level and support viral replication within 5 weeks, and respond to existing antiviral drugs such as interferon and nucleoside analogs. Previous studies have found that HBV can effectively spread within the liver in infected animals, but the virus spreading in cell culture models was not observed. Until recently, we and others reported that the stem cell differentiated hepatocyte-like cells and a HepG2-NTCP cell clone can support the spreading of HBV [2,12]. Interestingly, the 5C-PHHs can also support HBV spreading at a certain degree. Next, by using this model, the author explores a long-standing controversial question in the field: Can HBV infection activate the innate immune signaling pathway in hepatocytes? Consistent with our previous published work on PHHs, HepG2-NTCP, and stem cell differentiated hepatocyte-like cells models [13], HBV infection does not activate interferon and its downstream pathways in hepatocytes.

It is expected that this 5C-PHHs model will play an important role in the field of HBV research in the future. The persistence of HBV cccDNA is the major obstacle in antiviral therapy. This model provides a new tool for both basic research and high-throughput drug screening platform for HBV cccDNA. This study also suggested that the combination of small molecular compounds may improve

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the maintenance condition of other hepatocytes cell models. For example, current differentiation methods do not allow fully maturation of stem cells derived hepatocyte-like cells. Dedifferentiation, which is similar to PHHs, occurs after differentiation process in vitro. Therefore, whether 5C conditions can be used to maintain in vitro culture of hepatocyte-like cells is worthy of further investigation. Based on their HBV and hepatitis C virus data, it is expected that the 5C-PHHs system could support other hepatotropic virus infection, like hepatitis D virus. Thus, this model can be used for dissecting the interaction of different viruses in the context of coinfections.

Conflict of interest

The author declares that he has no conflict of interest.

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