fact which was believed to enhance the cell attachment.[5,6] Hence, we are curious about whether this phenomenon of fibroblast-like cells is also observed in the HAC culture system.

Thirdly, the authors used 6 cases of mature human hepatocytes isolated from healthy and diseased donor livers. To our knowledge, we only isolated the mature hepatocytes from the normal parts of the diseased liver, which are termed non-tumor liver. Strictly speaking, we only use the hepatocytes either from the healthy liver or the non-tumor part of a diseased liver. This is a vital point to consider when interpreting this data, otherwise one may be misled into believing that the culture system can also reprogram the diseased hepatocytes (tumor cells *etc.*) into progenitor cells.

We applaud Kim and colleagues for providing the successful HAC culture system for reprogramming human liver progenitor cells with a rapid expansion rate. It not only overcomes the low conversion efficiency but also reported no other fibroblast-like cells in YAC culture system. Their important work also advances our understanding of the chemical reprogramming of human progenitor cells *in vitro*, offering a different view for the optimization of culture systems in the future.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

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Authors' contributions

Conception, design and writing: YH and SE. Review and revision of the manuscript: YH, YS, TM, TH, WG and SE. Study supervision: SE.

Supplementary data

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Reply to: "Role of HGF for reprogramming human liver progenitor cells: Non-essential but stimulative supplement"

To the Editor:

We thank Dr. Huang and colleagues for their interest and comments on our recent study, "Small molecule-mediated reprogramming of human hepatocytes into bipotent progenitor cells" published in the *Journal of Hepatology*.[1]

We learnt with great interest from the Dr. Huang's letter that the YAC cocktail (Y27632, A83-01, and CHIR99021) originally developed by Katsuda *et al.*[2] for reprogramming of rodent hepatocytes was also effective in human hepatocytes. The process of conversion of human hepatocytes into stemness state using the

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YAC system took a long time and was independent of HGF supplementation. Based on these data, Huang *et al.* propose an indirect role of HGF in the reprogramming of human hepatocytes.

In our work, we found that a combination of 2 small molecules (A83-01 and CHIR99021) with HGF (termed HAC system) was very effective in reprogramming human hepatocytes, whereas treatment with either HGF or AC alone induced neither expansion of small epithelial cells nor expression of hepatic progenitor marker genes.

Furthermore, pharmacologically selective inhibition of MET receptor and ERK1/2 signaling caused a complete suppression of hepatic progenitor cell generation. Therefore, we believe that combined HAC treatment played an essential role in reprogramming human hepatocytes into chemically derived hepatic progenitors (hCdHs).[1]

It is worth noting that Y27632, one of the components used by Huang and colleagues for reprogramming human hepatocytes, is an inhibitor of a Rho-associated protein kinase (ROCK) known to affect various cellular functions by modulating diverse signaling pathways.[3,4] More specifically, it was reported that ROCK inhibition promoted cancer stem cell characteristics by activating the phosphorylation of the MET receptor.[5] It seems possible that the YAC system utilizing ROCK inhibition could be associated with activation of MET receptor-dependent signaling. Further studies are needed to systematically address this issue.

In response to their question regarding the presence of fibroblast-like cells, we would like to point out that the process of reprogramming human hepatocytes in the HAC system was considerably more rapid (2 weeks compared to 4 weeks in the YAC system). We did not observe proliferation of fibroblast-like cells when hepatocytes were isolated from either healthy livers or non-tumorous healthy parts of diseased liver. However, when we applied the HAC method to hepatocytes isolated from donors with liver fibrosis or end-stage liver diseases, we found proliferation of fibroblast-like cells. Our current efforts are directed towards understanding the mechanisms underlying the molecular differences driving chemical reprogramming in healthy versus diseased hepatocytes.

Finally, in response to the concern regarding the type of cells used for chemical reprogramming in our published manuscript, we would like to clarify that hepatocytes were isolated either from donor liver samples of the patients suffering from non-cancerous diseases (gallbladder polyp and intrahepatic duct stone) or from non-tumorous parts of livers taken at least 3 cm apart from hepatocellular carcinoma (3 cases) and metastatic tumor of colon cancer (1 case) to minimize the possibility of contamination with tumor cells.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

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Authors' contributions

SBL and JJ wrote the letter. YK, KK and DS discussed the experiments. JHY, DC and VF revised the letter.

Supplementary data

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