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Inhibition Of Autophagy Enhances The Characteristic Of Chemically Derived Hepatic Progenitors (CdHs)

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Background : Stem cell therapies have been suggested as a new therapeutic strategy as an alternative way to tissue transplantation in liver diseases. Regarding this, we established human chemically derived hepatocytes (hCdHs) that have the ability to proliferate into hepatocytes and cholangiocytes in previous research and confirmed the effective engraftment of hCdHs in the mouse. These results supported that CdHs provide a new approach to stem cell transplantation. However, the reprogramming mechanisms of CdHs remain unclear. Autophagy, a self-degradation process, is well-known as a key pathway in several cell reprogramming processes. Thus, we aimed to identify autophagic activity during CdHs generation.

Methods : Human and mouse chemically derived hepatic progenitors (hCdHs, mCdHs) are generated from human and mouse primary hepatocytes (hPHs, mPHs) by using reprogramming media including HGF, A83-01, and CHIR99021 (HAC) for 7 days. mPHs and hPHs maintained in basal media without HAC were used as a control. The generation of hCdHs and mCdHs was confirmed by qPCR. Then, expressions of major autophagy markers during CdHs generation were analyzed by western blotting and qPCR. mCdHs in the generation and the maintenance phase were treated with bafilomycin A1 (Sigma-Aldrich, B1793) to inhibit autophagy flux. Then, the specificity of mCdHs was confirmed by measuring mRNA levels of major progenitor markers by qPCR.

Results : Two major autophagy markers, p62 and LC3BII increased in protein level during the generation of mCdHs. This elevation diminished slightly on day 12, after the completion of mCdHs generation. Moreover, other autophagy-related markers, such as ATG5, Beclin1, and ULK1 reduced in mRNA levels compared to control during the generation. Notably, levels of these genes were 10-fold lower than the control on day 6. Beclin1, ATG12, and LAMP2a elevated in a time-dependent manner within a range lower than control. Lastly, BafilomycinA1 increased gene levels of hepatic progenitors, Sox9, and EpCAM in mCdHs not only when generation is completed, but as well as in the maintenance phase.

Conclusions : Overall, these results suggest that autophagy is downregulated during the generation of CdHs and recovered to the basal level as the generation is completed, and support that inhibition autophagy enhances the features of mCdHs. This research was supported by the National Research Foundation of Korea (2022R1A2C2004593), and the Korean Fund for Regenerative Medicine funded by Ministry of Science and ICT, and Ministry of Health and Welfare (21A0401L1).

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