TITLE

Development of a method of passaging and freezing human iPS cell-derived hepatocytes to improve their functions and application.

AUTHORS AND AFFILIATIONS

Jumpei Inui, Yukiko Ueyama-Toba, Seiji Mitani, Hiroyuki Mizuguchi

INVENTORY OF SUPPLEMENTAL INFORMATION

Supplemental Figures (Figures S1-S6)

Supplemental figures

Figure S1

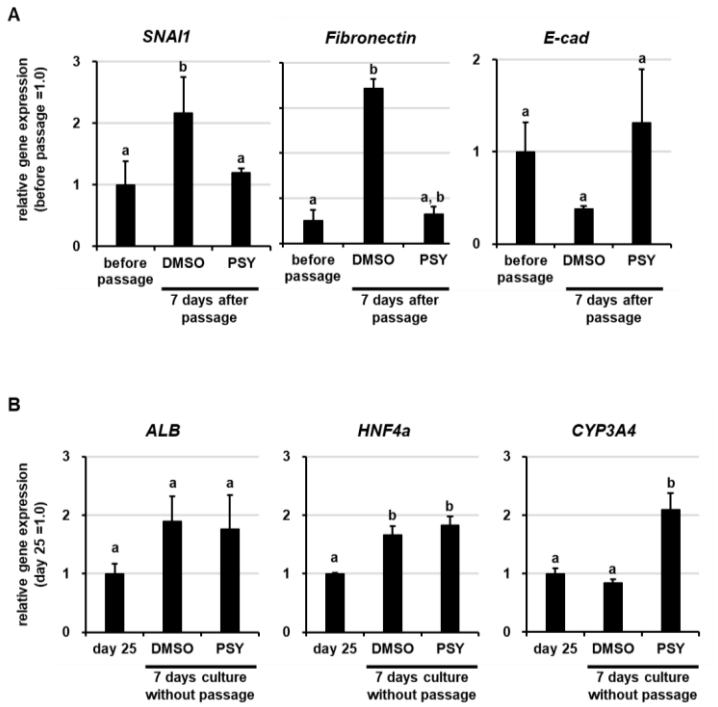

Figure S1. Effects of the EMT inhibitors on HLCs

(A) The gene expression levels of mesenchymal (*SNAI1* and *Fibronectin*) or epithelial (*E-cadherin, E-cad*) markers were examined in HLCs before passage, HLCs after passage cultured for 7 days with vehicle control (DMSO), and with the combination treatment of 0.5 μM PD0325901 (P), 2 μM SB43154 (S) and 10 μM Y-27632 (Y) (PSY) by real-time RT-PCR. The gene expression levels in HLCs before passage were taken as 1.0. Data represent the means ± SD (n=3). (B) The gene expression levels of hepatocyte markers (*ALB*, *HNF4α*, *CYP3A4*) were examined in HLCs on day 25 and HLCs after 7 days of extended cultured with vehicle control (DMSO), and with the combination treatment of P, S and Y (PSY) by real-time RT-PCR. The gene expression levels in HLCs on day 25 were taken as 1.0. Data represent the means ± SD (n=3). Statistical significance was evaluated by one-way ANOVA followed by Tukey's post-hoc tests to compare all groups. Groups that do not share the same letter are significantly different from each other ($p < .05$).

Figure S2

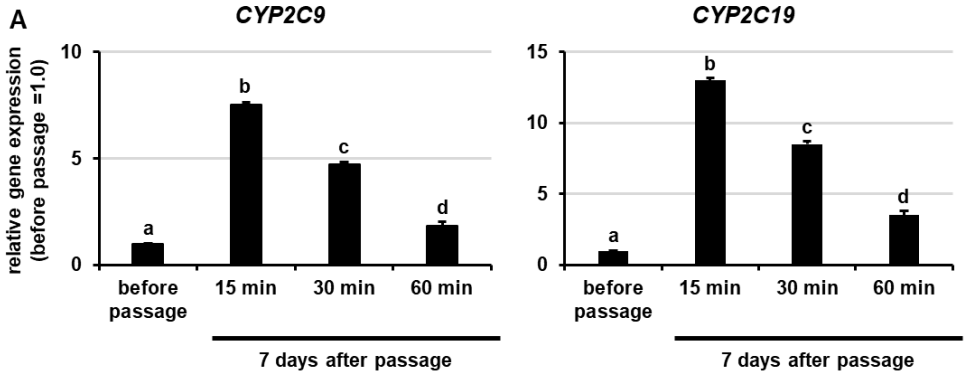

Figure S2. Gene expression levels of cytochrome P450 2C9 and 2C19

(A) The gene expression levels of hepatocyte markers (*cytochrome P450 2C9, CYP2C9* and *cytochrome P450 2C19, CYP2C19*) were examined in hepatocyte-like cells (HLCs) before passage and HLCs cultured for 7 days after passage under different dissociation times by real-time RT-PCR. The gene expression levels in HLCs before passage were taken as 1.0. Data represent the means ± SD (n=3). Statistical significance was evaluated by one-way ANOVA followed by Tukey's post-hoc tests to compare all groups. Groups that do not share the same letter are significantly different from each other ($p < .05$).

Figure S3

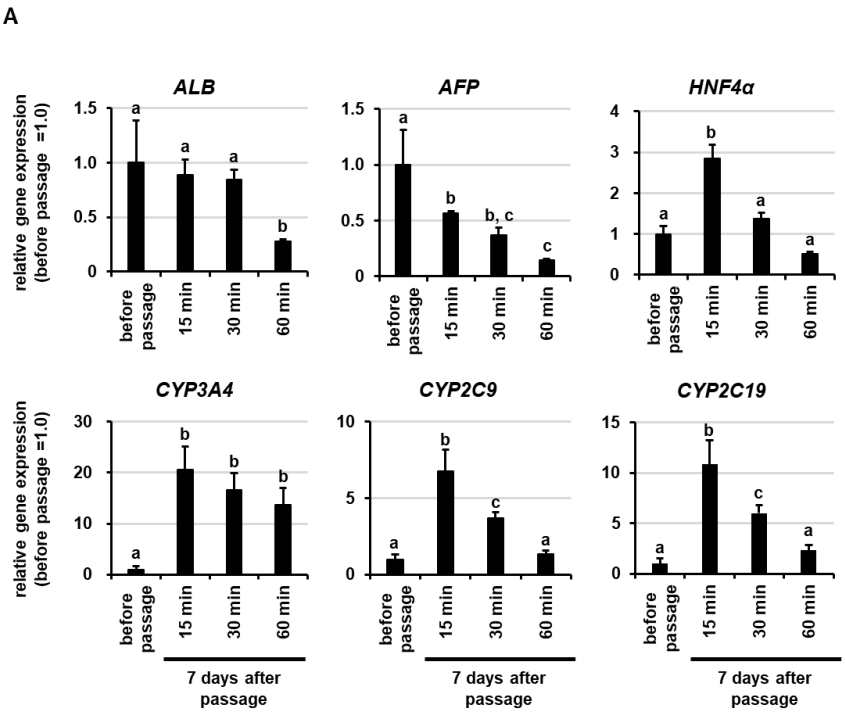

Figure S3. Effects of the optimum passage procedures on the hepatic functions in different iPS cell line-derived HLCs

Human iPS cells (DOO) were differentiated into hepatocyte-like cells (HLCs) as described in the Materials and Methods section. (A) The gene expression levels of hepatocyte markers (*ALB, AFP, HNF4α, CYP3A4, CYP2C9, CYP2C19*) were examined in HLCs before passage and HLCs cultured for 7 days after passage under different dissociation times by real-time RT-PCR. The gene expression levels in HLCs before passage were taken as 1.0. Data represent the means ± SD (n=3). Statistical significance was evaluated by one-way ANOVA followed by Tukey's post-hoc tests to compare all groups. Groups that do not share the same letter are significantly different from each other ($p < .05$).

Figure S4

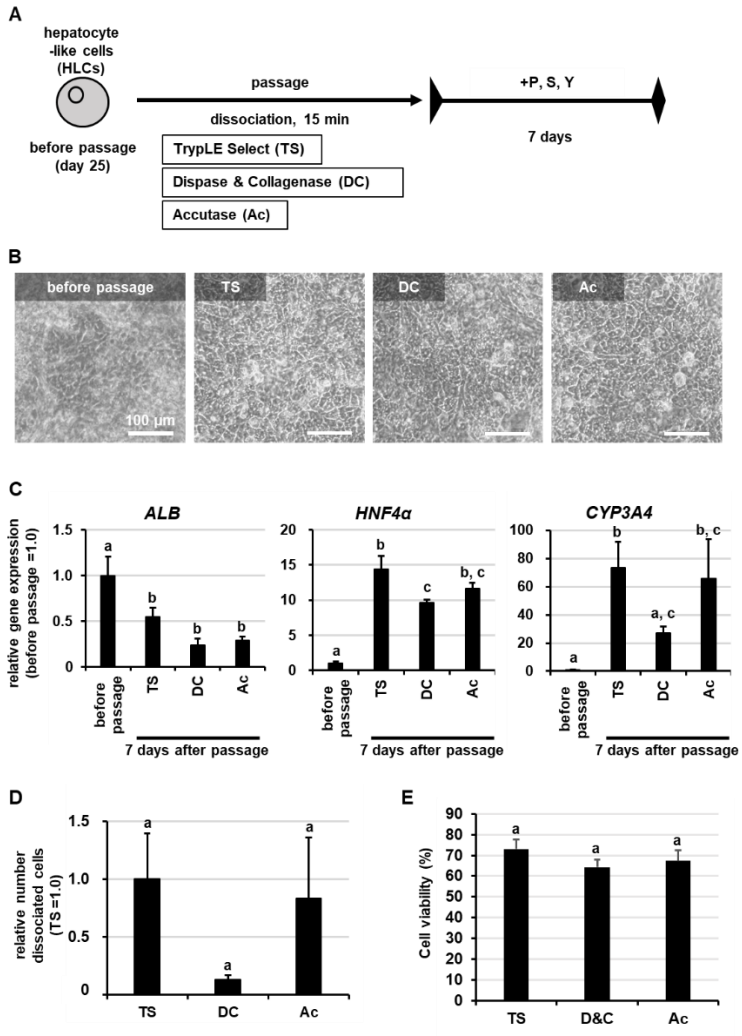

Figure S4. Effects of cell dissociation enzymes on HLCs

(A) The schematic overview shows the protocol for passage of hepatocyte-like cells (HLCs). (B) Phase-contrast micrographs of HLCs before passage and HLCs cultured for 7 days after passage by TrypLE select (TS), dispase & collagenase (DC), or accutase (Ac). (C) The gene expression levels of hepatocyte markers (*ALB*, *HNF4α*, *CYP3A4*) were examined in HLCs before passage (before passage) and HLCs cultured for 7 days after passage by real-time RT-PCR. The gene expression levels in HLCs before passage (before passage) were taken as 1.0. (D) The number of dissociated cells was counted under each cell enzyme condition. The relative values were calculated with the TS condition as 1.0. (E) Cell viability was calculated under each cell enzyme condition. Data represent the means ± SD (n=3). Statistical significance was evaluated by one-way ANOVA followed by Tukey's post-hoc tests to compare all groups. Groups that do not share the same letter are significantly different from each other ($p < .05$).

Figure S5

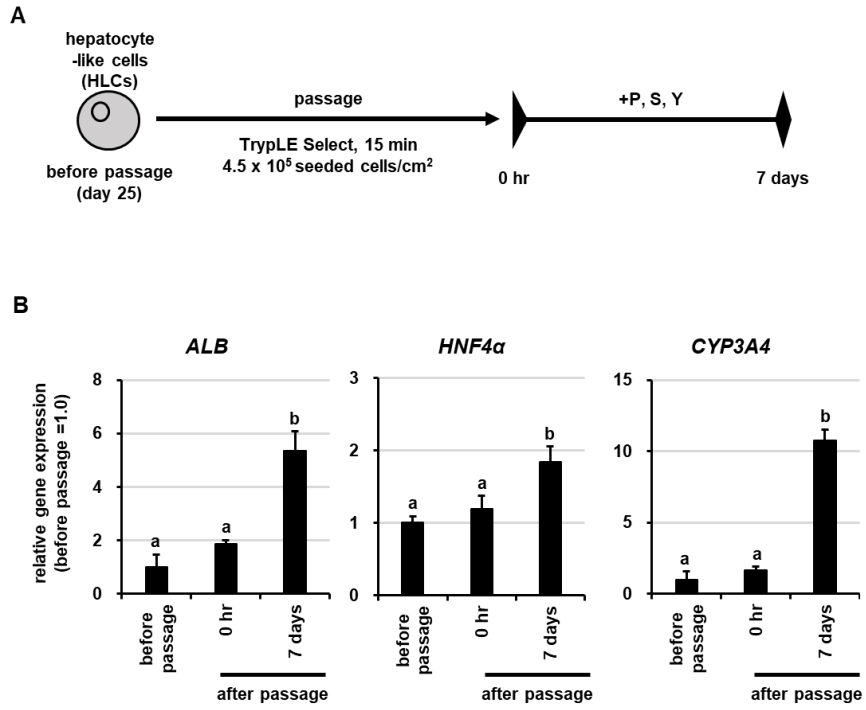

Figure S5. Culture of HLCs after passage was required for the enhanced hepatic functions

Human iPS cells (Tic) were differentiated into hepatocyte-like cells (HLCs) as described in the Materials and Methods section. (A) The schematic overview shows the protocol for passage of HLCs. HLCs before passage, HLCs immediately after passage, and HLCs cultured for 7 days after passage were collected. (B) The gene expression levels of hepatocyte markers were examined in HLCs before passage, HLCs immediately after passage, and HLCs cultured for 7 days after passage by real-time RT-PCR. The gene expression levels in HLCs before passage were taken as 1.0. Data represent the means ± SD (n=3). Statistical significance was evaluated by one-way ANOVA followed by Tukey's post-hoc tests to compare all groups. Groups that do not share the same letter are significantly different from each other ($p < .05$).

Figure S6

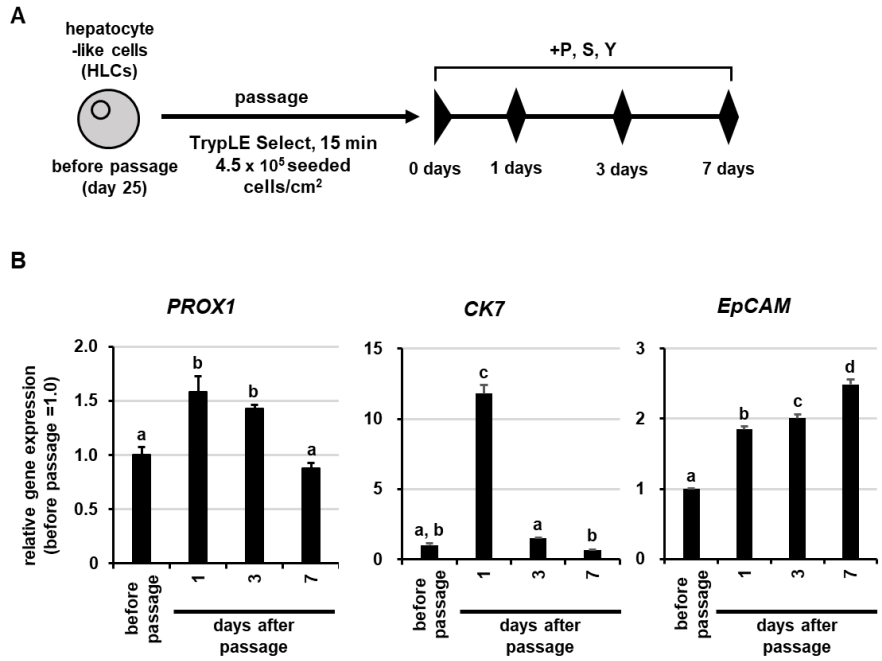

Figure S6. Transient dedifferentiation in HLCs after passage

Human iPS cells (Tic) were differentiated into hepatocyte-like cells (HLCs) as described in the Materials and Methods section. (A) The schematic overview shows the protocol for passage of HLCs. (B) The gene expression levels of hepatic progenitor markers (*PROX1, CK7, EpCAM*) were examined in HLCs before passage and HLCs cultured 1, 3 and 7 days after passage by real-time RT-PCR. The gene expression levels in HLCs before passage were taken as 1.0. Data represent the means ± SD (n=3). Statistical significance was evaluated by one-way ANOVA followed by Tukey's post-hoc tests to compare all groups. Groups that do not share the same letter are significantly different from each other ($p < .05$).