

Isolation of Bile Duct Progenitor Cells from Human Liver Samples

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INTRODUCTION

Samples of human liver tissue were obtained from the Liver Tissue Distribution System (LTCDS), a National Institutes of Health (NIH) service contract to provide human liver tissue from regional centers for distribution to scientific investigators throughout the United States. Each piece of human primary liver tissue obtained from LTCDS was minced well and digested with a buffer consisting of 1.67 mg/ml Collagenase Type II (Worthington), 1.33 mg/ml hyaluronidase (Sigma Aldrich) and 1.34 U/ml Dispase (BD Biosciences), dissolved in DMEM/ F12 (Gibco, CA), and supplemented with 0.5 mM EGTA, 5 mg/ml glucose, 5 mM KCl, and 10 mM HEPES, pH 7.4. The suspension was then incubated in a 37°C water bath for about 45 min with intermittent shaking. The large hepatocytes were sediment by centrifuging at 10 g for 3 min. The supernatant was then spun at 100 g for 5 min. followed by washing of the pellet with medium containing 10% serum followed by another spin at 100 g for 5 min. Non Parenchymal Cells (NPCs) including bile duct progenitor cells, were included in the pellets. Culture and propagation of bile duct progenitor cells in suspension and attachment cultures The bile duct progenitor cells expressing SOX9 were then plated in ultra-low attachment plates (Corning) in DMEM/F12 (Invitrogen) media containing 3% heat inactivated FBS, supplemented with 1X NEAA, and 20 ng/ml EGF (R&D Systems) for suspension culture. These cells formed small clusters of cells in suspension culture which were then placed in 6 well plates after 7 days and expanded in adherent culture in the same medium for 2-3 weeks with occasional splitting. Transduction of bile duct progenitor (SOX9+) cells with AdPNM An adeno viral vector (Ad-PNM), carrying mouse Pdx1, Ngn3 and MafA in a single polycistronic unit, separated by 2A sequences, was prepared as described previously. The SOX9+ cell clusters were trypsinised

to make

a single cell suspension. They were then transduced with Ad-PNM at a 100 Multiplicity of Infection (MOI) in the ultra-low attachment plate. The cells were then incubated in a 37°C incubator for 48-72 hrs. The infection was done in absence of serum in DMEM/ F12 medium. The medium was changed after two days and the cells were cultured in suspension culture for another 7-10 days in the culture medium previously described. They were next plated onto 6 well plates with or without hydrogel (ESIBio) coating according to manufacturer's instructions and grown for 2-3 weeks in adherent culture for maturation. Preliminary trials showed that a MOI of 25-100 gave maximum insulin+ cells with minimum toxicity, so this range was adopted as the standard for the study. The Ad-PNM infection efficiency was obtained by counting all the cells in three fields of vision and taking an average percentage of the cell population showing nuclear PDX1 immunostaining. Transplantation of bile duct progenitor cell derived insulin+ beta like cells under the kidney capsule of diabetic mice The NOD-SCID (Prkdcscid) mice from Jackson laboratories (three experimental and two controls) were initially made diabetic by injecting 120 mg/Kg of STP to Zotocin (STZ) intraperitoneal for approximately 2 weeks. The mice were monitored until they reached a blood glucose level of 400 mg/dl. The clusters of insulin+ expressing cells were slightly trypsin zed to detach from the plate without making them into a single cell suspension. Approximately 3-4 insulin+ clusters from each 6 well plate were injected into each of the three mice The cells (after centrifugation) were mixed with fibrinogen thrombin in 1:2 ratio in order to make a graft, each of which was slid under the kidney capsules of diabetic mice. The blood glucose level in these mice was monitored every other day following transplantation of the reprogrammed SOX9+ cells.

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