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High-Efficiency Genetic Engineering of Stem Cell Organoids using Recombinant Adeno-Associated Virus

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Introduction: Three-dimensional (3D) organoids have been considered as potential tools to explore roles of stem cells in tissue genesis, to reconstruct disease development and develop novel therapeutics. Unfortunately, the wide use of these systems is limited largely due to the inefficiency of genetic manipulation.

Methods and Results: To overcome the current drawbacks, we used intestinal stem cell organoids and bile duct stem cell organoids. First, various recombinant adeno-associated virus (rAAV) serotype vectors containing either GFP or mCherry report genes were compared and the transgene expression was obtained using confocal microscopy. It was evident that rAAVDJ was the most significant one to high-efficiently transduce both organoids. Point mutation of surface further enhanced the viral-mediated transgene expression and led to more than 95% transduction at an MOI of 10,000 vgs/cell. The cell entry and intracellular trafficking of viral particles were then investigated by small molecule inhibitors. Moreover, both organoids showed more than 95% transduction efficiency at Day 3 post-viral infection, whilst only ~3% of GFP positive cells were remaining at Day 14 by flow cytometry analysis, compared to ~2% of mock treated cells. The viral genome numbers was further confirmed by quantitative PCR assays. The above data suggested that rAAVDJ vectors are extremely useful for exploring the tissue genesis of stem cell organoids. To this end, HNF4α and NICD was individually over-expressed in the bile duct stem cell organoids using rAAVDJ vectors. Our results clearly demonstrated that HNF4α is involved in the differentiation of bile duct stem cell to hepatocytes.

Conclusion: Taken together, the combination of organoid culture with rAAV vector will be a broadly applicable tool to study tissue homeostasis and disease, complementing classical conditional mouse models. As organoid transplantation has been employed to repair endodermal epithelia injury *in vivo*, our system also shows great therapeutic potential in regeneration medicine.