Innovation Session (cont.)

i6

GENERATION OF FUNCTIONAL INTESTINE FROM PATIENT DERIVED PLURIPOTENT STEM CELLS

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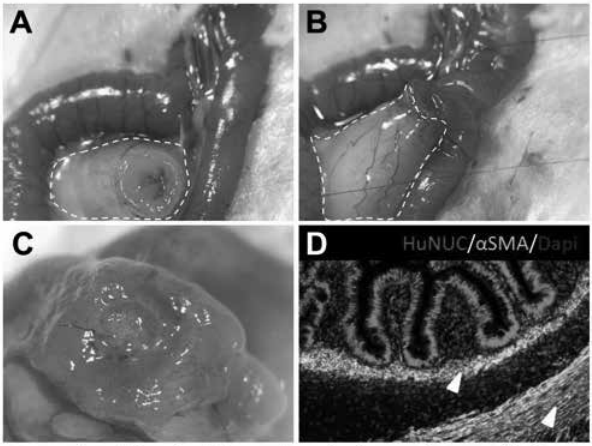
Purpose:
The differentiation of human pluripotent stem cells into organ-specific subtypes offers an exciting resource for therapeutic transplant. To date, limited tissue-engineering models exist for patient-derived human intestine. In this study, we developed a murine model utilizing intestinal organoid transplantation to generate patient derived specific intestinal tissue.

Methods:
Human intestinal organoids are generated *in vitro* from step-wise directed differentiation of patient induced pluripotent stem cells. After 28 days, intestinal organoids contain epithelial and mesenchymal cell types that are highly similar to their *in vivo* counterpart. Intestinal organoids cultured for 28-35 days are embedded into collagen and are transplanted in immunocompromised mice. Six weeks following transplantation, the engrafted organoids are processed for analysis or grafted in the mouse intestinal continuity (Fig. 1A-B).

Results:
We demonstrated that intestinal organoids engrafted *in vivo* to form mature human intestinal epithelium (Fig. 1C) with intestinal stem cells contributing to the crypt-villus architecture and a laminated human mesenchyme (Fig. 1D), both supported by the ingrowth of functional host vasculature. *In vivo* transplantation resulted in significant expansion and maturation of the epithelium and mesenchyme as demonstrated by differentiated intestinal cell lineages (enterocytes, goblet cells, Paneth cells, tuft and enteroendocrine cells), presence of functional brush-border enzymes (lactase, sucrase-isomaltase, dipeptidyl peptidase 4), and visible subepithelial and smooth muscle layers when compared with intestinal organoids grown *in vitro.* Furthermore, we demonstrated that engrafted intestinal organoids expressed active brush border enzymes and exhibited intact intestinal epithelial barrier and absorptive functions.

Conclusion:
We conclude that intestinal organoids from patient-derived induced pluripotent stem cells can be efficiently transplanted *in vivo* and generate a vascularized and fully functional human intestine. This system should pave the way for patient disease specific therapies and customized treatment approaches.



Generation of functional human intestine from patient specific iPSCs. **(A)** Intestinal organoid (dashed line) 6 weeks after transplantation in the mesentery of immunocompromised mouse. **(B)** Engrafted intestinal organoid (dashed line) sutured to the host intestinal continuity. **(C)** Intestinal mucosa of engrafted intestinal organoid (dashed line). **(D)** Section of engrafted intestinal organoid 6 weeks post-transplantation. Engraftment is almost entirely human with epithelium and majority of mesenchyme staining positive for human anti-nuclear antibody (HuNuc; red). Staining for smooth muscle actin (α-SMA; green) reveals contribution of supporting laminated smooth muscle (white arrowheads).

Notes: