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学位論文の題名	Gastric mesenchymal myofibroblasts maintain stem cell activity and proliferation of murine gastric epithelium in vitro （胃間質筋線維芽細胞は胃の組織幹細胞機能と上皮細胞の増殖能の維持に関与する） The American Journal of Pathology. 2015 Mar;185(3):798-807
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Introduction: The homeostasis of the gastrointestinal mucosa is maintained by a variety of mechanisms. Myofibroblasts in the scant mesenchyme between gland units are proposed to regulate stem cell activity (Gastroenterology. 2011; 140: 412-424). Stem cells are influenced by a microenvironmental niche that includes mesenchymal cells.

Materials and Methods: We established a novel long-term method for primary mouse glandular stomach culture with mesenchymal myofibroblasts to investigate gastric epithelial-mesenchymal interactions. A gastric mesenchymal myofibroblast (GMF) cell line was established from mouse glandular stomach. Glandular stomach cells from postnatal day 2 C57BL/6J mice and GMF cells were co-cultured in a collagen gel.

Results: Cultured stomach cells showed outer spindle cells and yielded expanding sphere structures. In the GMF co-culture system, the number and size of gastrospheres were increased compared to control cultures (P =0.009 and 0.008, respectively). Immunohistochemistry showed cells positive for human gastric mucin, HIK1083, and chromogranin A, indicating differentiation into surface mucous cells, mucous neck cells, and enteroendocrine cells, respectively. RNA *in situ* hybridization for Lgr5 revealed $Lgr5^+$ stem cells in the cultured gastrospheres. $Lgr5^+$ cells were observed persistently in the epithelium of gastrospheres in the GMF co-culture system for 2 months. GMFs allowed the cultured gastric epithelium to maintain active proliferation similar to that seen *in vivo*. Real-time quantitative reverse transcription-PCR showed that Gas1 expression was higher in GMFs (P=0.0445), and Hoxc8, Notch1, and Sox10 expression was higher in intestinal mesenchymal myofibroblasts (P=0.0003, 0.0143, and 0.0488, respectively).

Conclusions: We show the potential role of GMFs in sustaining $Lgr5^+$ stem cell activity and affecting normal gastric epithelial differentiation and proliferation.