Isolation of human LGR5-positive cells from both colon adenoma or iPSCs derived organoids

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Background

The fast self-renewal rate of the intestinal epithelium from adult mammals is sustained by a small population of stem cells, located at the base of intestinal crypts[1,2]. LGR5 (Leucine-rich repeat containing G protein-couple receptor) is a well characterized marker for adult stem cells in the human and murine small intestine, colon, and hair follicles[3,4]. In the recent years, different studies have revealed that LGR5 is overexpressed in different types of human and mouse tumors[5], and has been also recognized as a marker for human colorectal cancer stem cells[6]. Recently developed methods for the long-term culture of human intestinal organoids allow for a reliable system to analyze the cellular heterogeneity of the intestinal epithelium[7,8]. However, studying human LGR5$^+$ intestinal stem cells (ISCs) can be challenging due to difficulties in obtaining cultures highly enriched in epithelial stem cells[9], and a lack of specific reagents to detect and isolate LGR5$^+$ ISCs[10]. Therefore, efficient dissociation of human intestinal organoids is crucial to isolate sufficient amounts of LGR5$^+$ ISCs as well as preserving LGR5 for subsequent analysis. This customer protocol describes a procedure used by Dame *et al.*[11] to isolate LGR5$^+$ cells from human intestinal organoids. Those organoids were derived from human colon adenomas comprised of epithelium only, or from induced pluripotent stem cells (iPSC), which contain both an epithelial and a mesenchymal component.

Materials and methods

Note: All plasticware used during the dissociation and cell isolation processes, including gentleMACS™ C Tubes, MACS® Columns, strainers, and pipette tips were coated for 20 minutes with DPBS buffer containing 0.1% bovine serum albumin (BSA).

Tissue dissociation

- Harvest of human intestinal organoids: Organoid cultures were established from patient-derived colon adenoma or human iPSC cells. Organoids were treated with 10 µM of the Rho-associated protein kinase (ROCK) inhibitor StemMACS Y27632 for 2.5 h prior harvest. Organoids were extracted from Matrigel by digestion for 30–45 min with cold DPBS buffer containing 2 mM EDTA, and washed three times with cold DPBS (100×g at 4°C) prior to resuspension in enzyme solution.
- Enzyme reconstitution: Enzymes H and R of the Tumor Dissociation Kit, human were reconstituted with warm HBSS medium containing 0.13 mM calcium and 0.9 mM magnesium (HBSS-CM buffer). Enzyme A of the Tumor Dissociation Kit, human was reconstituted in Buffer A supplied with the kit.
- Organoid dissociation: Harvested organoids were suspended in a 20 mL enzyme solution containing 18.7 mL of HBSS-CM buffer, 800 µL of Enzyme H, 400 µL of Enzyme R, and 100 µL of enzyme A. This enzyme solution contained a final concentration of 5 µM StemMACS Y27632. The 20 mL suspension was evenly distributed into two gentleMACS C Tubes and dissociated using a gentleMACS Octo Dissociator with Heaters using a customized program (*).

(*) This customized program is available upon request. For more information please contact **www.miltenyibiotec.com/support**

The obtained single cell suspension was poured over a succession of strainers (100 µm to 40 µm to 20 µm). Cells were washed three times in cold DPBS buffer containing 2 mM EDTA , 0.5% BSA, and 5 µM StemMACS™ Y27632 at 500×g for 5 min.

Magnetic cell isolation

- Prior to labeling, cells were resuspended and processed in cold DPBS buffer containing 2 mM EDTA, 0.5% BSA, and 5 µM StemMACS™ Y27632. Cells were labeled with anti-LGR5 MicroBeads, human according to the following modifications from the data sheet instructions. During incubations, tubes were gently mixed every 5 min by tapping. Cells were incubated in 70 µL of buffer with the addition of 10 µL FcR Blocking Reagent for 10 min prior to addition of 20 µL LGR5-Microbeads and incubation for another 15 min. Cells were washed by addition of 2 mL buffer and centrifuged at 500×g for 5 min. Supernatant was aspirated and cells were suspended in 90 µL of buffer with 10 µL Labeling Check Reagent (APC) followed by incubation for 10 min.
- After labeling, cells were washed twice in buffer and resuspended in HBSS-CM buffer containing 200 Kunitz units/mL DNAse, 0.5% BSA in DPBS, and 5 µM StemMACS Y27632. LS columns were pre-coated with this buffer for 45 min at 4°C prior use. To obtain a single-cell suspension before magnetic labeling, cells were applied through a 20 µm strainer onto LS Columns, according to instructions in the corresponding data sheet. Columns were washed three times with 2 mL DNAse-containing buffer.
- For elution of magnetically-labeled cells, the column was removed from the separator and 2 mL of the DNAse-containing buffer was applied onto the column. Cells were then flushed out using the plunger. Positive selected cells and flow through negative fractions were washed at 500×g for 5 min and resuspended in DPBS buffer containing 2 mM EDTA, 0.1% BSA, and 10 µM StemMACS Y27632. Prior to flow cytometric analysis, cells were stained with 1 µM DAPI for 1 min to exclude non-viable cells.

Results

Viable LGR5⁺ human intestinal cells were successfully enriched from human colon adenoma-derived organoids (fig.1 A) and iPSC-derived organoids (fig.1 B) after dissociation using the Tumor Dissociation Kit, human in combination with the gentleMACS™ Octo Dissociator with Heaters.

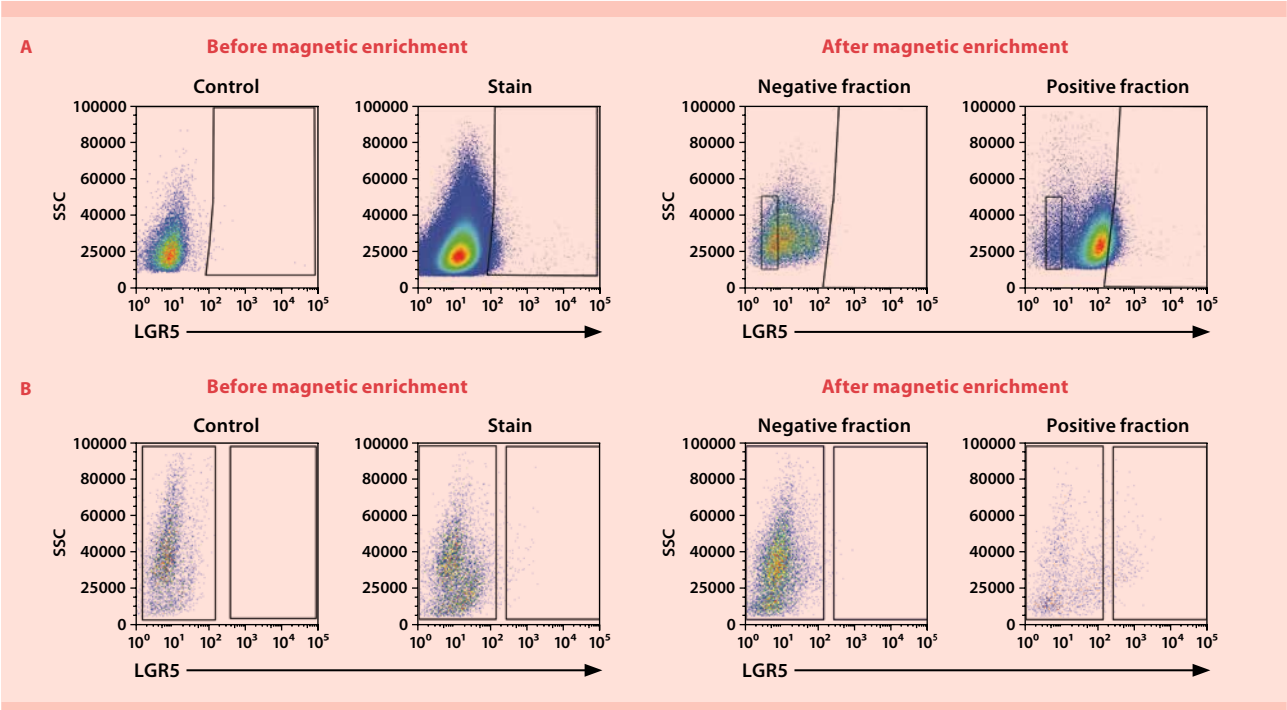

Figure 1: Enrichment of LGR5⁺ cells from human intestinal organoids. Representative flow cytometry data of LGR5 expression in human colon adenoma-derived organoids (A) and iPSC-derived organoids (B) before and after magnetic enrichment using Anti-LGR5 MicroBeads, human. Doublets and dead cells were excluded from the analysis based on scatter signals and DAPI staining. The marker EpCAM was used to positively select the epithelial component to interrogate for LGR5 (not shown). Before magnetic separation, control samples were set based on viable fluorescence-minus-one-APC control. Adapted with permission from Dame, M.K. *et al.*[11].

Conclusion

Human intestinal organoids, derived from both colon adenomas or iPSC cells, can be efficiently dissociated into single cell suspensions using the Tumor Dissociation Kit, human, in combination with the gentleMACS™ Octo Dissociator with Heaters. This method enables the preservation of LGR5, necessary for the study of ISCs. Furthermore, by using of Anti-LGR5 MicroBeads, human LGR5$^+$ ISCs can be specifically enriched, and used for further downstream analysis.

MACS Product	Order no.
StemMACS™ Y27632	130-103-922
gentleMACS Dissociator with Heaters	130-093-235
Tumor Dissociation Kit, human	130-095-929
C Tubes	130-096-334
FcR Blocking Reagent, human	130-059-901
Anti-LGR5 MicroBeads, human	130-104-072
QuadroMACS™ Starting Kit (LS)	130-091-051
MACS SmartStrainer (100 µm)	130-098-463
(optional) MACS SmartStrainer (70 µm)	130-098-462
(optional) MACS SmartStrainer (30 µm)	130-110-915
(optional) Pre-Separation Filters (20 µM)	130-101-812
(optional) Pre-Separation Filters (30 µM)	130-041-407
(optional) Pre-Separation Filters (70 µM)	130-095-823

References

1. Barker, N. *et al.* (2008) The intestinal stem cell. Genes Dev. 22: 1856–1864.
2. Kozar, S. *et al.* (2013) Continuous clonal labeling reveals small numbers of functional stem cells in intestinal crypts and adenomas. Cell Stem Cell 13: 626–33.
3. Barker, N. *et al.* (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449: 1003–1007
4. Jaks, V. *et al.* (2008) Lgr5 marks cycling, yet long-lived, hair follicle stem cells. Nat Genet. 40: 1291–1299.
5. Cao, H.Z. *et al.* (2017) LGR5 promotes cancer stem cell traits and chemoresistance in cervical cancer. Cell Death Dis. 8(9): e3039
6. Kemper, K. *et al.* (2012) Monoclonal antibodies against Lgr5 identify human colorectal cancer stem cells. Stem Cells 30: 2378–2386
7. Sato, T. *et al.* (2011) Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology 141: 1762–1772.
8. Spence, J. R. *et al.* (2011) Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro*. Nature 470: 105–109
9. Wang, X. *et al.* (2015) Cloning and variation of ground state intestinal stem cells. Nature 522: 173–178.
10. Barker, N. (2014) Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. Nat. Rev. Mol. Cell Biol. 15: 19–33.
11. Dame, M.K. *et al.* (2018) Identification, isolation and characterization of human LGR5-positive colon adenoma cells. Development 145(6), dev153049. doi :10.1242/dev.153049

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