NEW ANTIBODY REAGENTS TO STUDY STEM CELL-MEDIATED DEVELOPMENT AND REGENERATION OF THE MAMMALIAN INTESTINAL EPITHELIUM

Antony W. Wood, Richard Cotta, Katherine Crosby, Aparna Viswanathan, Christine Gagen and Jessica Simendinger

Cell Signaling Technology, Inc., Danvers MA 01923

Abstract #1374

ABSTRACT

Growth and regeneration of the mammalian intestinal epithelium is driven primarily by a discrete population of stem cells, known as LGR5+ crypt base columnar (CBC) cells, resident at the base of the intestinal crypt. Despite their fundamental importance for intestinal development and regeneration, there is a lack of validated reagents that can discretely identify LGR5+ CBC cells and their direct progeny. This is likely due to the low abundance and/or accessibility of the LGR5 epitope. To address this problem, Cell Signaling Technology, Inc. (CST) has developed highly specific rabbit antibodies targeting Olfactomedin-4 (OLFM4), a glycoprotein that exhibits distinct expression in LGR5+ CBC cells in both human and mouse intestine. These antibodies allow the detection of human OLFM4 and murine Olfm4, respectively, by Western blot in cell and tissue lysates, and by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded tissue samples. Antibody specificity was confirmed by validation on cell types and/or tissues in which the presence/absence or relative abundance of Olfactomedin-4 protein has been described in the published literature. These antibody reagents provide a means to visualize CBC cells with exquisite sensitivity and specificity without the need for transgenic mice models. The cell line allows for great insights into the role and regulation of CBC cells during development, regeneration, and oncogenic transformation of the intestinal epithelium.

METHODS

Antibody Validation

OLFM4 (D1E4M) XP® Rabbit mAb # 14369 and Olfm4 (D6Y5A) XP® Rabbit mAb (Mouse Specific) #39141 are recombinant rabbit monoclonal antibodies, generated at Cell Signaling Technology, Inc. using patented XMT® Technology.

Western Blot

Western blot analyses were performed using extracts from cell or tissue extracts, as described in the figure legends. Western blot protocol details can be found at http://www.cellsignal.com/common/content.jsp?id=western

Immunohistochemistry

Paraffin-embedded mouse intestine sections were deparaffinized and rehydrated then antigen retrieval in sodium citrate, pH 6.0. Primary antibodies were incubated overnight at 4°C. Detection was performed using SignalStain Boost® HRP Detection Reagent (HRP, Rabbit) #8114 and SignalStain DAB Substrate Kit #5000.

Immunofluorescence

Paraffin-fixed mouse small intestine was cryopreserved in 30% sucrose and embedded in OCT medium. 12 um sections were incubated in PBS prior to blocking and permeabilization with 0.5% goat serum/PBS containing 0.3% Triton X-100 (PBST). Primary antibodies were diluted in PBST containing 1% BSA and incubated overnight at 4°C. Detection was performed using SignalStain Boost® HRP Detection Reagent (HRP, Rabbit) #8114 and SignalStain DAB Substrate Kit #5000.

CONCLUSIONS

OLFM4 (D1E4M) XP® Rabbit mAb #14369 is a recombinant rabbit monoclonal antibody that detects human OLFM4 protein in western blot and immunohistochemistry with high specificity and sensitivity.

Olfm4 (D6Y5A) XP® Rabbit mAb (Mouse Specific) #39141 is a recombinant rabbit monoclonal antibody that detects mouse Olfm4 protein in western blot, immunoprecipitation, immunofluorescence and immunohistochemistry with exceptional specificity.

These antibodies may be used to detect or visualize OLFM4-expressing cells, including intestinal stem/progenitor cells and neutrophils, in normal tissues, intestinal adenomas, and tumors, and in organisms derived from normal or cancerous tissues [1, 2].

For stem cell research, these tools provide a means to identify intestinal stem/progenitor cells without the obligatory use of transgenic mice models [3].

References


Acknowledgements

Mouse spleen tissues (wild-type and Olfm4−/−) were a generous gift from Dr. Griffin P. Rodgers, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).