Abstract

Leucine-rich repeat containing G-protein-couple receptor 4 and related LGR5 and LGR6 proteins represents a B subgroup of transmembrane proteins belonging to the G-protein-coupled receptors (GPCRs) family. LGR4 is expressed in the broad spectrum of embryonic and adult tissue and at certain backgrounds its deficiency is connected with embryonal/perinatal lethality. The function of LGR4 is mainly characterised in relation with promotion of Wnt signalling upon binding its ligands R-spondins. To obtain a tool for classification of LGR4 specific populations and for characterization LGR4 interaction partners, we have generated epitope-tagged LGR4 mouse with tripleinfluenza hemagglutinin tag (3HA) inserted into N terminal part of LGR4 protein ($Lgr4^{3HA/3HA}$). $Lgr4^{3HA/3HA}$ mouse is viable and fertile. Anti-HA antibody based immunohistochemistry revealed similar expression pattern in the small intestine and in the colon, which was previously detected with anti-LGR4 antibodies.In the small intestine, a strong signal was observed in Paneth cells, transit amplifying cells and in stem cells. Conversely, in the colon the strongest signal was noticed at the upper part of colonic crypts and it diminished towards crypt base. Besides that, we have followed *Lgr4* expression at the mRNA level. While in the small intestine, *Lgr4* mRNA was presented mostly at the crypt bottom; in the colon, the signal was more dispersed in the central part of the colonic crypt. Using flow cytometry, we could characterize gene expression profile LGR4 positive cells from the small intestine. Finally, by anti-HA magnetic beads, we were able to immunoprecipitate LGR4 protein for mass spectrometry, which can be employed for identifying its binding partners.

Key words:

Leucine-rich repeat containing G-protein-coupled receptor 4 (*Lgr4*), small intestine, colon, immunohistochemistry (IHC), western blot (WB), immunoprecipitation (IP), Fluorescence-activated cell sorting (FACS), quantitative real-time polymerase chain reaction (qRT-PCR), gene modified mouse model, hemagglutinin (HA) tag, TALEN