Musashi-1 suppresses expression of Paneth cell–specific genes in human intestinal epithelial cells

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Background. Musashi-1 (Msi-1) is a RNA-binding protein, known as a putative marker of intestinal stem cells (ISCs). However, little is known about the function of Msi-1 within human intestinal epithelial cells (IECs). Thus, the present study aimed to clarify the role of Msi-1 in differentiation and proliferation of IECs.

Methods. A human intestinal epithelial cell line stably expressing Msi-1 was established. Proliferation of the established cell lines was measured by bromodeoxyuridine incorporation, whereas differentiation were assessed by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of lineage–specific genes. Activities of the Notch and Wnt pathways were examined either by reporter assays or expression of downstream target genes.

Results. Constitutive expression of Msi-1 in IECs had no significant effect on cell proliferation, but suppressed expression of Paneth cell–specific genes, including PLA2G2A. Msi-1 appeared to suppress expression of the PLA2G2A gene at the mRNA level. Analysis of Notch and Wnt pathway activity, however, revealed no significant change upon Msi-1 expression. The expression of Msi-1 and PLA2G2A in vivo was determined by immunohistochemistry. PLA2G2A expression in vivo was restricted to IECs residing at the lowest part of the human intestinal crypt, but was clearly separated to within basal columnar cells or mature Paneth cells, respectively. These findings suggest Msi-1 is a negative regulator of Paneth cell differentiation, an may contribute to maintain the undifferentiated phenotype of ISCs.

Conclusions. Msi-1 suppresses expression of Paneth cell–specific genes in IECs, presumably through a pathway independent from Notch or Wnt. These findings suggest Msi-1 is a negative regulator of Paneth cell differentiation, an may contribute to maintain the undifferentiated phenotype of ISCs.

Key words: Musashi-1, intestinal epithelial cells, Paneth cells, PLA2G2A

Introduction

The rapid and continuous renewal of the intestinal epithelium is maintained by the regulated supply of newborn cells that arise from a common progenitor cell called the intestinal stem cell (ISC). Such tissue–specific stem cells share common potentials to self renew and also to give rise to all cell lineages composing the residing tissue. Such properties of stem cells are maintained by a complex interaction of various cell–signaling pathways. Among such signaling pathways, Wnt and Notch represent the core molecular pathways that play crucial roles in maintaining stem cell properties. Indeed, both Wnt and Notch signaling have been shown to function in intestinal crypt epithelial cells, including the ISCs. A recent study identified Lgr-5, a direct target of the canonical Wnt pathway, as a definite marker of murine ISCs. This study further emphasized the dominant role of the canonical Wnt pathway in maintaining cell proliferation and multipotency of ISCs. It is, however, known that activation of the canonical Wnt pathway is present not only in ISCs but also in Paneth cells residing just adjacent to ISCs, where it promotes maturation and restricts the cell position of such cells. Thus, activation of the canonical Wnt pathway appears to have a completely different function in ISCs and Paneth cells, presumably depending on the cell context determined by other molecular factors. However, the molecular mechanism regulating such lineage–specific functions of canonical Wnt signaling in IECs remains largely unknown.

Musashi-1 (Msi-1) is an RNA-binding protein, and its gene was formerly reported as another candidate marker gene for ISCs. Its molecular function has been deter-
mined as translational repression of target genes, such as m-Numb, a negative regulator of Notch signaling. In both mice and humans, Msi-1 has been shown to be expressed in ISCs, but in sharp contrast, its expression is completely lacking in mature Paneth cells. Thus, Msi-1 might function within intestinal epithelial cells (IECs) to maintain their undifferentiated state. However, the molecular function of Msi-1 in IECs has never been described.

Herein, we show that expression of Msi-1 in IECs suppressed expression of Paneth cell-specific genes, such as PLA2G2A. Msi-1 appeared to downregulate expression of the PLA2G2A gene at the mRNA level, through a molecular pathway independent of both Notch and Wnt, the two major molecular pathways that are known to regulate Paneth cell differentiation. These results suggest that Msi-1 is a negative regulator of Paneth cell differentiation in IECs, and that it has a functional role in maintaining the undifferentiated state of ISCs.

Materials and methods

Cell culture

Human colon cancer-derived LS174T cells were cultured in minimal essential medium (GIBCO, Billings, MT, USA) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA, USA), at 37°C in a humidified incubator with 5% CO2.

Plasmids

For construction of the expression plasmid for Msi-1 (pcDNA3.0-Msi-1), the entire coding lesion of the mouse Msi-1 gene was polymerase chain reaction (PCR) amplified from pcDNA3.0-FLAG-Msi-1 (kindly provided by Dr. Hideyuki Okano), and inserted into pcDNA3.0 (Invitrogen). For construction of the enhanced green fluorescent protein (EGFP) expression plasmid (pCMV-FLAG-EGFP), the coding sequence of pEGFP-C1 (Stratagene, La Jolla, CA, USA) was PCR amplified and inserted into the pCMV-FLAG-Tag vector (Stratagene).

Establishment of cell lines stably expressing Msi-1 or EGFP

Expression plasmids for Msi-1 (pcDNA3.0-Msi-1) or EGFP (pCMV-FLAG-EGFP) were transfected into LS174T cells as previously described. After 2 days of culture, cells were selected by addition of G418 (1 μg/ml) to the culture medium. Subsequently, G418-resistant cells were cloned into sublines expressing Msi-1 or EGFP, designated as LS174T/Msi-1 cells or LS174T/EGFP cells, respectively.

Microscopic imaging and immunostaining of cultured cells

Microscopic images of cultured cells were collected using an epifluorescence microscope system (BZ-2000, Keyence, Osaka, Japan). Immunostaining of cultured cells were done as previously described. For detection of Musashi-1, primary antibody (14H1, kindly provided by Dr. Hideyuki Okano) was diluted to 1:1000 and detected by an Alexa-488-conjugated secondary antibody (Molecular Probes, Eugene, OR USA). Cells were counterstained by 4′,6-diamidino-2-phenylindole (DAPI), and mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA).

Cell proliferation assay

Incorporation of bromodeoxyuridine (Brd-U) was examined using a cell proliferation enzyme-linked immunosorbent assay (ELISA) kit (Roche Diagnostics, Mannheim, Germany), according to the manufacturer’s instructions. Briefly, cells were seeded onto a 96-well dish at various cell densities, cultured for 48 h, and labeled with Brd-U for 8 h at the end of culture. Each condition was measured in triplicate and the results analyzed by Student’s t test.

Reverse transcription-polymerase chain reaction

Total RNA was prepared using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. Reverse transcription (RT) was carried out as previously described. Forward and reverse primers used for the PCR reaction are summarized in Table 1. For semiquantitative PCR, 1 μl of cDNA was amplified with 0.25 U of LA Taq polymerase (Takara, Otsu, Japan), using the optimized amplification cycles determined for each primer set. Amplified products were separated by 1.8% agarose gel electrophoresis, stained with ethidium bromide, and visualized by the Lumi-Imager F1 system (Roche Diagnostics). For quantitative PCR, 1 μl of cDNA was amplified using SYBR green master mix (Qiagen, Valencia, CA, USA), and analyzed by a 7500 real-time PCR system (Applied Biosoftware, Foster City, CA, USA).

Immunoblotting

Immunoblot analysis was done as previously described. Briefly, 1 × 106 cells were seeded onto 6-cm culture dishes and collected for protein extraction after 48 h of...