Journal of Crohn's and Colitis (2012) 6, 425–434

Available online at www.sciencedirect.com

SciVerse ScienceDirect

Olfactomedin-4 is a glycoprotein secreted into mucus in active IBD☆

Michael Gersemann [a,b,*], Svetlana Becker [b], Sabine Nuding [b], Lena Antoni [b], German Ott [c], Peter Fritz [c], Naohide Oue [d], Wataru Yasui [d], Jan Wehkamp [a,b], Eduard F. Stange [a]

[a] *Department of Internal Medicine I, Robert Bosch Hospital, Stuttgart, Germany*
[b] *Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart and University of Tübingen, Germany*
[c] *Department of Pathology, Robert Bosch Hospital, Stuttgart, Germany*
[d] *Department of Molecular Pathology, Hiroshima University Graduate School of Biomedical Science, Hiroshima, Japan*

Received 12 August 2011; received in revised form 28 September 2011; accepted 28 September 2011

KEYWORDS
Inflammatory bowel disease;
Olfactomedin-4;
Intestinal stem cell marker;
Defensins;
Mucins;
Mucus

Abstract

Background: Olfactomedin-4 (OLFM4) is a glycoprotein characteristic of intestinal stem cells and apparently involved in mucosal defense of the stomach and colon. Here we studied its expression, regulation and function in IBD.
Methods: The expression of OLFM4, mucins Muc1 and Muc2, the goblet cell differentiation factor Hath1 and the proinflammatory cytokine IL-8 was measured in inflamed or noninflamed colon in IBD patients and controls. OLFM4 protein was located by immunohistochemistry, quantified by Dot Blot and its binding capacity to defensins HBD1-3 was investigated. The influence of bacteria with or without the Notch blocker dibenzazepine (DBZ) and of several cytokines on OLFM4 expression was determined in LS174T cells.
Results: OLFM4 mRNA and protein were significantly upregulated in inflamed CD (4.3 and 1.7-fold) and even more pronounced in UC (24.8 and 3.7-fold). OLFM4 expression was correlated to IL-8 but not to Hath1. In controls immunostaining was restricted to the lower crypts but in inflamed IBD it expanded up to the epithelial surface including the mucus. OLFM4 bound to HBD1-3 without profoundly inactivating these defensins. In LS174T-cells OLFM4 mRNA was significantly augmented after incubation with *Escherichia coli K12*, *Escherichia coli Nissle* and *Bacteroides vulgatus*. DBZ downregulated OLFM4 expression and blocked bacterial induction whereas IL-22 but not TNF-α was stimulatory.

☆ Conference presentation: DDW 2011, Chicago, USA (poster).

* Corresponding author at: Robert Bosch Hospital, Internal Medicine I, Auerbachstr. 110, D-70376 Stuttgart, Germany. Tel.: +49 711 81015912; fax: +49 711 81013793.
E-mail address: michael.gersemann@rbk.de (M. Gersemann).

1873-9946/$ - see front matter
doi:10.1016/j.crohns.2011.09.013

Conclusions: OLFM4 is overexpressed in active IBD and secreted into mucus. The induction is triggered by bacteria through the Notch pathway and also by the cytokine IL-22. OLFM4 seems to be of functional relevance in IBD as a mucus component, possibly by binding defensins.

1. Introduction

In both inflammatory bowel diseases (IBD) the chronic inflammation is mediated by an immune response directed against commensal bacteria, possibly triggered by a disproportionate immune response toward these microbes that damages the mucosa.[1] On the other hand, there is increasing evidence for a primary role of a defective mucosal barrier in Crohn's disease (CD) and ulcerative colitis (UC).[2–4] Bacteria from the lumen massively contaminate the mucus layer[5] which is normally sterile in the bottom stratum.[6] Some bacteria are epithelial adherent[5,7] or may even invade the subepithelial space[5,8] and thus trigger an immune response.

The mucosal barrier is a multilayer structure composed of the mucus layer[6] and its origin, the epithelium. In the large intestine the key secretory cells are the goblet cells. They produce various mucins forming the mucus layer which is acting as a physical and chemical barrier against commensals and pathogens.[9] The colonic epithelium also produces antimicrobial peptides which are ultimately secreted into the mucus.[10,11] The more important colonic peptides, the ß-defensins, are characterized by a broad antimicrobial activity against a variety of gram-positive and gram-negative bacteria preventing luminal microbes to enter the lower mucus layer and attack the epithelium.[12]

This intestinal protective barrier mediated by mucus and defensins is disturbed in IBD. In CD, the expression of the antimicrobial peptides is compromised enabling the bacteria to invade the mucosa and thus trigger inflammation.[3,13] In case of colonic involvement, CD is linked to a diminished expression of HBD1 independent of inflammation.[14] In UC, the mucus layer is thinner than normal and may even be missing.[15,16] This is accompanied by a diminished mucin synthesis,[17–21] which is apparently related to a failure in the differentiation of intestinal stem cells toward goblet cells.[17] This differentiation is governed by the key transcription factor Hath1 which is correlated with mucin synthesis.[17]

Olfactomedin-4 (OLFM4) is an olfactomedin domain-containing protein which was found preferentially in the human gastrointestinal tract.[22–24] The function of OLFM4 in the digestive tract is probably complex. OLFM4 may be an important part of the gastrointestinal mucosal surface and therefore play a role in its defense.[25] For instance, OLFM4 is known to create large polymers stabilized by disulfide bonds,[25] similar to mucins.[9] Moreover, it was demonstrated to interact with cell surface cadherin and lectins facilitating cell adhesion.[26] A role of OLFM4 in epithelial defense was concluded from an upregulation in a mouse primary gastric epithelial cell line GSM06 incubated with *Helicobacter pylori*,[27] as well as in *H. pylori* infected patients in vivo.[28] Finally, in addition to LGR5[29] also OLFM4 was found to be a small and large intestinal marker for crypt stem cells in humans.[30]

However, little is known about its relevance in the colon. Shinozaki et al. found OLFM4 transcripts to be significantly upregulated in the epithelium in active vs. inactive UC but its precise function remained unclear.[31] In the present study we attempted to better define the role of OLFM4 in the pathogenesis of IBD and suggest that this peptide acts as an inflammation induced mucus component binding defensins.

2. Material and methods

2.1. Patients

The diagnosis of CD and UC was based on classical clinical, radiological and endoscopic findings.[32,33] Endoscopic biopsies were immediately snap-frozen in liquid nitrogen. All patients gave their written informed consent and the study was approved by the ethical committee of the University of Tübingen (Germany).

For real-time PCR analysis biopsies from the colonic sigma were obtained in a total of 160 individuals, who underwent routine colonoscopy for various indications, such as colon cancer screening, IBD, diarrhea or obstipation. Thirty-three of these biopsies were classified as healthy controls, 72 were from CD patients (36 noninflamed and 36 inflamed samples) and 55 had the diagnosis of UC (28 noninflamed and 27 inflamed samples). All samples were collected at the Robert Bosch Hospital (Stuttgart, Germany) and the intensity of the flare was clinically evaluated in these patients using the Colitis Activity Index (CAI) for UC and the Crohn's disease activity index (CDAI) for CD.

Immunostaining was performed in formalin-fixed or Carnoy-fixed paraffin-embedded colonic tissue. A total of 18 formalin-fixed colonic resections (6 controls, 6 inflamed CD and 6 inflamed UC samples) and 10 Carnoy-fixed rectal biopsies (5 inflamed CD and 5 inflamed UC samples) were investigated. For Dot Blot analysis, sigma biopsies of 4 controls, 4 inflamed CD and 4 inflamed UC patients, as well as mucus extracts obtained by colonoscopic brushings from 3 controls, 3 inflamed CD and 3 inflamed UC samples were collected. Brushings were performed by gently scrubbing the rectal mucosa with an endoscopic brush, removing the endoscope from the rectum and, outside the patient, the brush was cut with scissors and snap frozen in liquid nitrogen.

2.2. RNA isolation and reverse transcription

The frozen tissues were mechanically disrupted and total RNA was isolated using TRIzol reagent (Invitrogen, Karlsruhe, Germany). RNA quality was checked with the Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA, USA). 500 ng of total RNA was reverse transcribed with AMV reverse transcriptase according to the supplier's protocol