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Intestinal selenoprotein P in epithelial cells and in plasma cells

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ABSTRACT

The micronutrient selenium and selenium-containing selenoproteins are involved in prevention of inflammation and carcinogenesis in the gut. Selenoprotein P (Sepp1), the plasma selenium transport protein, is secreted primarily from hepatocytes, but Sepp1 mRNA is also abundant in the intestine. By immunofluorescence analysis, we show that Sepp1 levels in epithelial cells of the rat jejunum increase along the crypt-to-villus axis. A different Sepp1 distribution pattern was observed in the rat colon, where the epithelial cells located at the base and at the top of the crypts were similarly positive for Sepp1. In addition, we found pronounced Sepp1 immunoreactivity in CD138-positive plasma cells scattered within the lamina propria of the colon. This hitherto unrecognized presence in terminally differentiated B-cells was corroborated by detection of Sepp1 in plasma cells residing in the rat spleen. Following supplementation with dietary selenium compounds, polarized intestinal epithelial Caco-2 cells secreted Sepp1 into the culture medium across the basolateral membrane. Our data suggest that Sepp1 secreted from epithelial cells may support the intestinal immune system by providing immune cells (including plasma cells) with selenium for the biosynthesis of endogenous selenoproteins.

Introduction

The essential trace element selenium (Se) exerts most of its biological actions as an integral constituent of selenocysteine (Sec)-containing selenoproteins. Adequate dietary Se supply is a prerequisite for optimized biosynthesis of selenoproteins [1,2]. Sec and selenomethionine (SeMet) as well as sodium selenite and selenate represent organic and inorganic Se species, respectively, which are commonly contained in food and dietary multivitamin/mineral supplements [1,3]. Absorption of dietary Se takes place mainly in the small intestine [4]. The solute carrier amino acid transporters B^0 and $b^{0,+}$ rBAT mediate apical uptake of Sec and SeMet into enterocytes [5], while anion exchangers of the SLC26 family have been proposed as candidates for uptake of selenate [1]. The selenite transporter remains to be identified. Absorbed dietary Se is released from enterocytes into the portal circulation and, in addition, is used for biosynthesis of intestinal selenoproteins.

Key selenoproteins including glutathione peroxidases (GPx), thioredoxin reductases (TrxR) and selenoprotein P (Sepp1) have been detected in the gut of humans and rodents [6–10]. The distribution pattern of several selenoproteins depends on the cellular differentiation state, suggesting a role of selenoproteins in the continuous renewal of the intestinal epithelium [10]. Transcription of GPx2, TrxR2 and TrxR3 is stimulated by Wnt proteins; these three selenoenzymes are predominantly expressed in proliferating cells located in the crypts of the jejunum and at the crypt base of the colon, where Wnt signaling is active [10]. In contrast, GPx1 and GPx4 mRNA and/or protein levels have been reported to increase from proliferating to differentiated cells along the crypt-to-villus-axis in jejunum and/or from the base to the top of the crypts in colon [6,8]. As part of cellular and extracellular antioxidant defense systems, selenoproteins are involved in prevention of oxidative stress-related intestinal inflammation and carcinogenesis [11–13]. Aberrant intestinal expression of selenoproteins has been described in patients suffering from colorectal carcinoma (CRC)[1] [9,12,14]. Down-regulation of Sepp1 was particularly strict, with some CRC patients exhibiting complete loss of Sepp1 gene expression in tumor tissue [14].

Primarily, Sepp1 is known as a liver-derived plasma protein [2], but it is also abundant in the healthy human colon, both at the

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[1] *Abbreviations used:* CRC, colorectal carcinoma; FoxO1a, forkhead box class O1a; GPx, glutathione peroxidase; HNF-4α, hepatocyte nuclear factor 4α; IAP, intestinal alkaline phosphatase; LY, lucifer yellow; RNS, reactive nitrogen species; ROS, reactive oxygen species; Se, selenium; Sec, selenocysteine; SeMet, selenomethionine; Sepp1, selenoprotein P; TrxR, thioredoxin reductase.

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mRNA and protein level [9,14]. In the intestine of mice, Sepp1 exhibits the highest mRNA levels among the selenoproteins [15]. A clue to understand the function of Sepp1 in the intestinal epithelium may lie in its structural properties: the Sepp1 N-terminal domain contains one Sec residue with enzymatic antioxidant activity while the C-terminal domain with up to 9 Sec is required for its main physiological role as the Se transport and supply protein [2,16]. It is conceivable that Sepp1 secreted by colonocytes might participate in protecting their basolateral and/or luminal membranes against oxidative damage. Secreted Sepp1 could also supply other cells located in the epithelium or in the lamina propria with Se for the biosynthesis of selenoproteins. This is particularly important for maintenance of the local immune defense in the intestinal tract, as Se and selenoproteins are involved in activation and differentiation of immune cells [17].

Here, we applied immunofluorescence analysis to examine the distribution of Sepp1 within the rat small and large intestine. In addition to epithelial cells, particularly strong Sepp1 immunoreactivity was detected in terminally differentiated B-cells (plasma cells) scattered within the colon. By using differentiated human intestinal epithelial Caco-2 cells grown in filter chamber devices to a polarized cell monolayer, we provide evidence that cultured epithelial cells are capable of secreting Sepp1 to their basolateral side.

Material and methods

Animals

Wistar rats were kept in the animal facility of the University Hospital Duesseldorf according to German guidelines for animal care and fed a Se-adequate (0.3 mg Se/kg) standard diet (Ssniff; Soest, Germany).

Immunofluorescence analysis

Cryosections of intestinal tissue were prepared from three rats and treated as described by Olson et al. [18]. Sepp1 was detected using a custom-made rabbit anti-Sepp1 antibody (Eurogentec; Seraing, Belgium) [19] and an Alexa Fluor® 594-coupled anti-rabbit IgG antibody (Life Technologies; Darmstadt, Germany); F-actin and DNA were co-stained with Alexa Fluor® 488-phalloidin (Life Technologies) and ProLong Gold® antifade reagent with DAPI (Life Technologies). For co-staining of Sepp1 and immune cell markers, the Sepp1 antibody was applied together with an Alexa Fluor® 488-coupled anti-rabbit IgG secondary antibody (Life Technologies). For detection of plasma cells, a goat anti-CD138 antibody (Santa Cruz Biotechnology; Santa Cruz, CA) was used together with an Alexa Fluor® 546-coupled anti-goat IgG antibody (Life Technologies). For staining of leukocytes, a mouse anti-CD45 antibody (Acris; Herford, Germany) was used together with an Alexa Fluor® 546-coupled anti-mouse IgG antibody (Life Technologies). Digital images were produced using a Leica DM 2000 microscope equipped with a Leica DFC 425C camera (Leica; Bensheim, Germany). Excitation wavelengths for the applied fluorescent dyes were as follows: 340–380 nm (DAPI), 450–490 nm (Alexa Fluor® 488) and 515–560 nm (Alexa Fluor® 546).

Cell culture

Caco-2 cells (European Collection of Cell Cultures No. 86010202) were kindly provided by Dr. R. Schins (Leibniz-Institut für Umweltmedizinische Forschung; Düsseldorf, Germany) and maintained as described [8,20]. Cells were seeded onto ThinCert™ cell culture inserts (300,000 cells/ insert) with transparent membrane and 1 μm pore size (Greiner Bio-One; Frickenhausen, Germany) inside the chambers of 6-well-plates. To induce the formation of a monolayer of differentiated polarized cells [21], Caco-2 cells were cultivated for 21 days after reaching confluency, changing the culture medium every 2–3 days. For the experiments, the medium in the inserts (apical side) was replaced by FCS-free medium supplemented with 200 nM of sodium selenite, sodium selenate or L-selenomethionine, whereas the chambers (basolateral side) contained FCS-free medium. Apical and basolateral culture medium was collected after 48 h, and subjected to analysis of IAP activity and Sepp1 secretion.

The integrity of the Caco-2 cell monolayer was verified by testing the permeation of the fluorescent dye lucifer yellow (Sigma; Taufkirchen, Germany) as described by Yamamoto et al. [22]. Hanks balanced salt solution (HBSS) containing 100 μM lucifer yellow (LY) was added to the inserts, whereas the medium in the chambers was replaced by HBSS. After 2 h of incubation, LY fluorescence ($\lambda_{excitation}$: 440 nm, $\lambda_{emission}$: 520 nm) was measured in HBSS samples from the apical and the basolateral side using a FLUOstar OPTIMA plate reader (BMG Labtech; Jena, Germany).

Immunoblotting

Tissue lysates were prepared from the rat small and large intestine using M-PER mammalian protein extraction reagent (Thermo

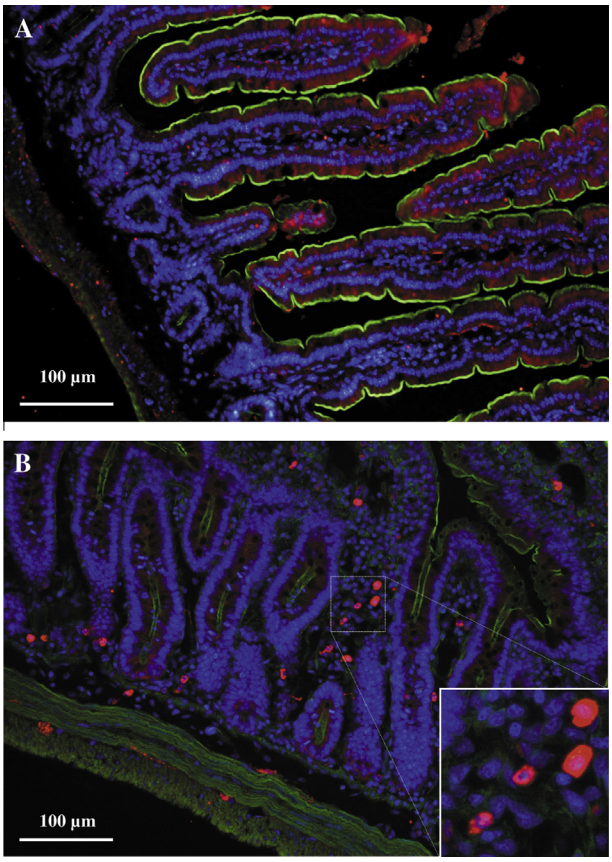

Fig. 1. Localization of selenoprotein P in the rat intestine. Sepp1 (red) was detected by immunofluorescence analysis of cryosections in jejunum (A) and colon (B) of rats fed a Se-adequate standard diet. F-actin (green) and nucleic DNA (blue) were co-stained with phalloidin and DAPI, respectively. In the insert of (B), individual Sepp1-positive cells within the lamina propria of the colon are shown in more detail. Digital images were produced using a Leica DM 2000 microscope equipped with a Leica DFC 425C camera (Leica; Bensheim, Germany). The images are representative pictures taken from three cryosections. (For interpretation of the reference to color in this figure legend, the reader is referred to the web version of this article.)