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BRIEF COMMUNICATION

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Expression pattern of Dkk-3, a secreted Wnt pathway inhibitor, in mouse intestinal tissue and three-dimensional cultured Caco-2 spheroids

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We investigated the expression pattern of Dkk-3, a secreted Wnt pathway inhibitor, in mouse intestinal tissue and three-dimensional cultured Caco-2 spheroids. Dkk-3 was expressed at the bottom of crypts from the mouse small intestine. Human colon adenocarcinoma Caco-2 cells expressed Dkk-3 under a semi-confluent condition, but Dkk-3 expression was seen in only some of the Caco-2 cells when the cells were sparse. Caco-2 cells formed hollow spheroids in Matrigel, and the layer-forming cells in the hollow spheroids expressed Dkk-3. Our results demonstrated that Dkk-3 might affect intestinal cells when the fate of stem cells changes.

Key Words: Dkk-3, intestinal tissue, Caco-2 cells

Introduction

Dkk-3, a secreted Wnt pathway inhibitor, was down-regulated in a number of human cancer cell lines and clinical cancer tissues and thus Dkk-3 is known as REIC because of its reduced expression in immortalized cells[1]. Overexpression of Dkk-3 using an adenovirus vector induced apoptosis in a variety of cancer cells and this anti-tumor effect was triggered by ER stress caused by overexpression of Dkk-3[2]. Expression of Dkk-3 was observed in many tissues including the small intestine and colon[3]. Furthermore, we previously revealed that Dkk-3 expression was reduced in normal skin cells in inflammatory conditions and also in skin cancer cells[4]. However, the physiological function of Dkk-3 is still unclear. In the present study, we investigated the expression of Dkk-3 in mouse intestinal tissue and three-dimensional cultured Caco-2 spheroids.

Results

To screen for Dkk-3 expression in mouse normal tissues, we performed fluorescent immunohistochemistry of Dkk-3 protein using adult mouse tissues. The mouse small intestine showed strong and localized expression (Figure 1A). Interestingly, Dkk-3 expression was seen at the bottom of intestinal crypts (Figure 1B).

Next we analyzed the expression pattern of Dkk-3 in human colon adenocarcinoma Caco-2 cells in a monolayer culture condition. Almost all of the Caco-2 cells expressed Dkk-3 under a semi-confluent condition, and the cell membrane showed strong expression (Figure 2A). However, Dkk-3 expression was seen in only some of the Caco-2 cells when the cells were sparse (Figure 2B). Interestingly, Dkk-3 and an intestinal transcription factor, Caudal-related homeobox transcription factor 2 (CDX2), showed reciprocal expression patterns in sparse Caco-2 colonies.

We then cultured Caco-2 cells in a three-dimensional condition. Single-cell suspensions of Caco-2 cells were re-suspended in a basement membrane matrix (Matrigel) and cultured in 24-well plates. After cultivation for five days, Caco-2 cells had formed small solid spheres. Then their morphology changed to hollow spheroids after another five days (Figure 3A). Immunocytochemistry experiments demonstrated that Caco-2 cells in the hollow spheroids expressed Dkk-3.

Discussion

Based on our previous studies using skin tissue, Dkk-3 expression was localized at the stem cell niche and/or the neighboring cells[5]. Dkk-3 expression was detected in many epithelial tissues including small intestine and colon[3].

In this study, we analyzed Dkk-3 expression in the mouse small intestine and 3D culture Caco-2 spheroids. Expression of Dkk-3 in the small intestine was restricted to the bottom of crypts (Figure 1B). Stem cells of intestinal tissue are located at the bottom of crypts[6]. Dkk family proteins are known as Wnt pathway inhibitors, and Wnt5a signaling was reported to regulate crypt regeneration after tissue injury[7]. The expression of Dkk-3 in the crypts was not in the precise position where the intestinal stem cells have located. However the Dkk-3 expressing cells reside next to the intestinal stem cell niche therefore the results indicate a possible role of Dkk-3 in stem cell function. Interestingly, Dkk-3 was shown to be expressed at the stem cell niche not only in intestinal tissue but also in the skin hair bulge region[5].

Caco-2 cells were initially established from human colon adenocarcinoma tissue and they differentiate in appropriate culture to express intestinal epithelium markers. Since the cell-cell junction of well-differentiated Caco-2 cells is similar to that of small intestine

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tissue, Caco-2 cells have been used for drug metabolism assays[8]. In a monolayer culture condition, semi-confluent Caco-2 cells expressed Dkk-3 and the cell membrane showed strong expression (Figure 2A). Conversely, only some of the Caco-2 cells in sparse small colonies expressed Dkk-3 (Figure 3). Our results also showed that Dkk-3 and CDX2 have reciprocal expression patterns in these sparse colonies. Additionally, we have compared the number of CDX2-positive cells in sparse small colonies and calculated the percentage of co-expression. CDX2 positive rate in Dkk3-positive cells was 28% and that in Dkk3-negative cells was 64% (Supplementary Figure 1). Localization of Dkk-3 was changed during Caco-2 status, however further analysis will be needed. The homeobox gene CDX2 is a widely used marker of intestinal differentiation[9]. These data demonstrated that Dkk-3 might affect stem cell fate during intestinal differentiation.

It was reported that Caco-2 cells could form spheroids in several types of hydrogel. Elamin and co-workers reported that Caco-2 cells formed hollow spheroids consisting of a single cell layer in Matrigel and that the cells forming a layer of the spheroids were differentiated [10]. In our experiments, Caco-2 cells formed hollow spheroids after 5-11 days in Matrigel (Figure 3A). Immunocytochemistry of the spheroids demonstrated that the layer-forming Caco-2 cells in the hollow spheroids expressed Dkk-3 (Figure 3B). Thus, differentiated Caco-2 cells expressed Dkk-3 in a three-dimensional culture system.

The actual functions of Dkk-3 in intestinal tissue and Caco-2 spheroids were still unclear, but our data indicated that Dkk-3 might affect intestinal cells when the fate of stem cells changes. Further analysis of the stem cell biology of intestinal tissues is needed.

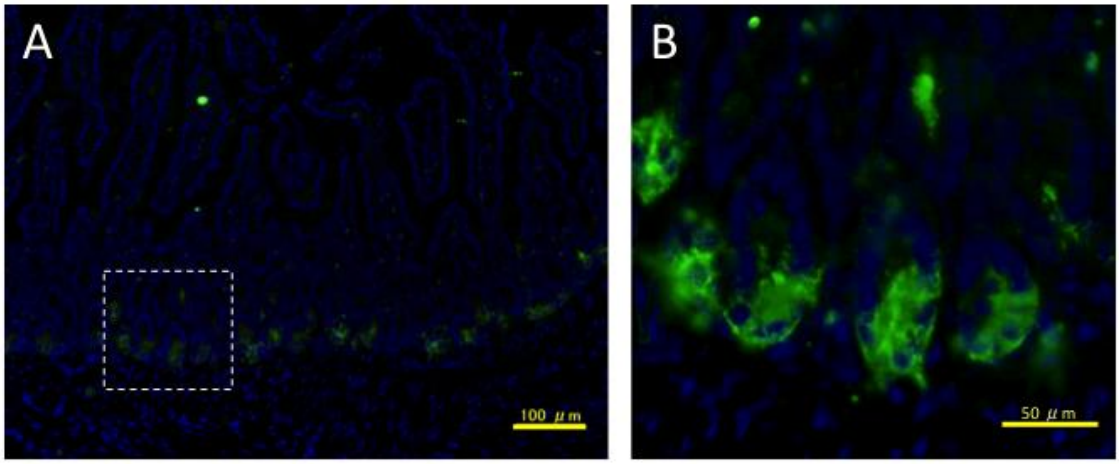

Figure 1. Expression of Dkk-3 in the mouse small intestine*. Sections of small intestine tissue were stained for Dkk-3 (in green). Nuclei were stained with DAPI (in blue). Stained sections were observed by a fluorescent microscope at a low magnification (A) or a high magnification (B). The dashed square in A delineates the magnified area in B. Scale bars: 100 μm (A), 50 μm (B).*

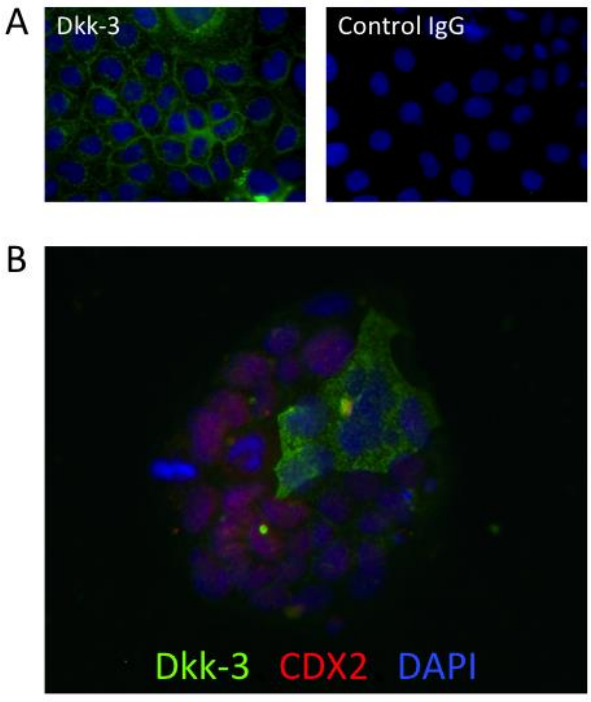

Figure 2. Expression of Dkk-3 in monolayer cultured Caco-2 cells. *Semi-confluent (A) and sparse colony-forming (B) Caco-2 cells were stained for Dkk-3 (in green) and CDX2 (in red). Nuclei were stained with DAPI (in blue). Control IgG was used as a negative control.*

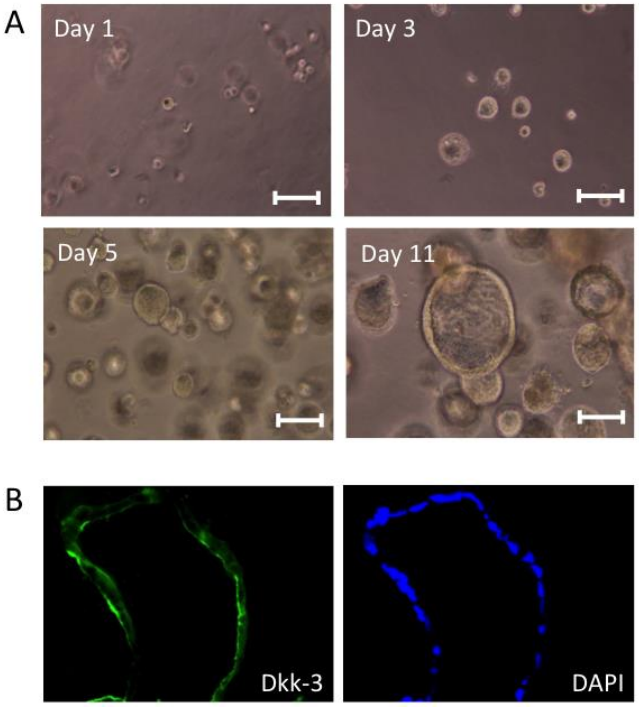

Figure 3. Formation of Caco-2 spheroids and their Dkk-3 expression. *(A) Spheroids formed by Caco-2 cells in Matrigel were observed by a bright field. (B) Sections of the spheroids were stained for Dkk-3 (in green). Nuclei were stained with DAPI (in blue). Scale bars: 100 μm.*

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Abbreviations

Dkk : Dickkopf
REIC : Reduced expression in immortalized cells
CDX : Caudal-related homeobox transcription factor
DAPI : 4',6-diamidino-2-phenylindole
EDTA : Ethylenediaminetetraacetic acid

Potential Conflicts of Interests

None

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Supplementary data

Supplementary Materials & Methods and Supplementary Figure associated with this article can be found, in the online version, at http://www.pubstemcell.com/epub/011020400003EPA091215_supplement.htm

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