Ha, T-S., Nam, J. A., Seong, S-B., Saleem, M. A., Park, S. J., & Shin, J. I. (2017). Montelukast improves the changes of cytoskeletal and adaptor proteins of human podocytes by interleukin-13. *Inflammation Research*, *66*(9), 793-802. https://doi.org/10.1007/s00011-017-1058-y

Inflammation Research

Montelukast improves the changes of cytoskeletal and adaptor proteins of human podocytes by interleukin-13

--Manuscript Draft--

Manuscript Number:	INRE-D-16-00379R1
Full Title:	Montelukast improves the changes of cytoskeletal and adaptor proteins of human podocytes by interleukin-13
Article Type:	Original Article
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Funding Information:	Ministry of Education, Science and Technology (2011-0013789, 2013R1A1A1012112 and 2015R1C1A1A01052984) — Professor JAE IL SHIN
	Ministry of Education, Science and Technology (NRF-2013R1A1A4A03006207) — Prof Tae-Sun Ha
Abstract:	Objective and design Interleukin-13 (IL-13) has recently been reported to be a potential cytokine in the pathogenesis of minimal-change nephrotic syndrome (MCNS). However, the mechanistic insights associated with podocyte dysfunction mediated by IL-13-induced changes in various slit diaphragm (SD) and cytoskeletal molecules have not yet been shown in cultured human podocytes in vitro yet. Materials Human conditionally immortalized podocytes were used Treatment Podocytes were incubated with various concentrations of IL-13 during the indicated time periods (6, 12, and 24 h) and montelukast was administered with the dose of 0.1 μg. Results Treatment of IL-13 resulted in a progressive decrease in distinct processes or projections of the human podocytes and high dose of IL-13 increased podocyte permeability in vitro at 6 hours. IL-13 had a substantial impact on the redistribution and rearrangement of zonula occludens (ZO)-1, synaptopodin, α-actinin, CD2-associated protein (CD2AP) in podocytes and disrupted the cytoskeletal connections in a concentration-dependent manner on confocal microscopy. IL-13 also down-modulated ZO-1, synaptopodin, α-actinin, CD2AP and p130Cas at protein levels and up-regulated β-catenin and B7-1 in podocytes. Furthermore, we demonstrated that down-modulated changes in various SD and cytoskeletal structures of human podocytes induced by IL-13 was significantly restored after treatment with montelukast with upregulation of B7-

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Response to Reviewers:	COMMENTS FOR AUTHOR: Dr. Ha and colleagues presenting in their submitted manuscript their current findings, that the incubation of IL-13 induces structural and functional changes in cultured human podocytes. Their study is based on the clinical observations that minimal changes-GN seems to be a T-cell mediated disease and that relapses are often associated with viral, bacterial or even allergic reactions. After the incubation of human podocytes with Il-13 in various concentrations actin-cytoskeleton associated proteins (synapotopodin, alpha-actinin, ZO-1 and others) partially decreased and the co-incubation with montelukas suppressed these changes. Based on the fact that there is a growing body of evidence that podocytes may directly interact with the innate and required immune system, the new findings of this working group gain importance, even though some major changes to the manuscript have to be performed and additional studies included. 1) One major concern is that most of the studies included in this paper are only descriptive and lacking the functional explanation of the changes observed after IL-13 incubation. Can the authors at least theoretically link the various studied proteins to each other? -->Thank you for the comments! We provided the podocyte permeability in Supplementary Figure S2 and added the following sentences: These findings show that high dose of IL-13 mainly increases podocyte permeability at early stages (2-6 h), suggesting the functional explanation of the cytoskeletal changes shown by confocal microscopy after IL-13 incubation. 2) Negative and positive controls are missing in the in vitro studies. It would be helpful to distinguished between unspecific cellular reactions due to the incubation of IL-13 and specific responses of the podocytes to this chemokine. --> Thank you for the comments! We provided negative controls in Fig. 1, 2. Positive controls were described previously (Ref. 5) 3) I would highly recommend improving the quality of the IF-pictures. How did the authors quantify the documented changes of podocytes morphology? Please add this missing part. --> Thank you for the comments! We improved the quality of the IF-pictures and also quantified the documented changes of podocytes morphology by graphs using ImageJ. We also added the following sentence to methods section: The densitometry values of the fluorescent bands were analyzed using the Image J image processing and analysis software (National Institutes of Health, Bethesda, MD, USA). 4) Some of the western blots are not really convincing, such as Fig. 3, 4 and 7. Any chances to improve the quality of these pictures for the resubmission of the manuscript? --> Thank you for the comments! We improved the quality of the Figures. 5) Relapsing episodes of MCGN never causes renal apoptosis in humans. Therefore I would propose to delete the small apoptosis section. I would rather stick to the actin-associated proteins. If the authors want to keep this section, then they have to explain their findings and its inconsistency to the clinical outcome of the children with NS. --> Thank you for the comments! According to the comments, we deleted the small apoptosis section in a whole manuscript. 6) The values of B7-1 expression is currently highly debated, since many working groups could not repeat the initially findings. I would suggest to discuss with few sentences the contradictive results.

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Original Article

Montelukast improves the changes of cytoskeletal and adaptor proteins of human podocytes by interleukin-13

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Abstract

Objective and design Interleukin-13 (IL-13) has recently been reported to be a potential cytokine in the pathogenesis of minimal-change nephrotic syndrome (MCNS). However, the mechanistic insights associated with podocyte dysfunction mediated by IL-13-induced changes in various slit diaphragm (SD) and cytoskeletal molecules have not yet been shown in cultured human podocytes *in vitro* yet.

Materials Human conditionally immortalized podocytes were used

Treatment Podocytes were incubated with various concentrations of IL-13 during the indicated time periods (6, 12, and 24 h) and montelukast was administered with the dose of 0.1 μg.

Results Treatment of IL-13 resulted in a progressive decrease in distinct processes or projections of the human podocytes and high dose of IL-13 increased podocyte permeability *in vitro* at 6 hours. IL-13 had a substantial impact on the redistribution and rearrangement of zonula occludens (ZO)-1, synaptopodin, α-actinin, CD2-associated protein (CD2AP) in podocytes and disrupted the cytoskeletal connections in a concentration-dependent manner on confocal microscopy. IL-13 also down-modulated ZO-1, synaptopodin, α-actinin, CD2AP and p130Cas at protein levels and up-regulated β-catenin and B7-1 in podocytes. Furthermore, we demonstrated that down-modulated changes in various SD and cytoskeletal structures of human podocytes induced by IL-13 was significantly restored after treatment with montelukast with upregulation of B7-1.

Conclusion Our results suggest that targeting IL-13 may be one of the important cytokines in the pathogenesis of MCNS and targeting IL-13 could be one of the potential therapeutic strategies in MCNS.

Key words: interleukin-13, slit diaphragm, cytoskeletal molecules, B7-1, podocytes, leukotriene receptor antagonists

Introduction

Minimal-change nephrotic syndrome (MCNS) is the most common cause of nephrotic syndrome in children, but frequent relapses are often observed, requiring long-term use of corticosteroids [1]. However, the pathophysiologic mechanisms of MCNS are not fully understood and still elusive. Some studies have suggested that MCNS is a T helper (Th)2-dominated disease, because higher levels of serum interleukin-13 (IL-13) were observed in patients with MCNS [2, 3] and overexpression of IL-13 led to podocyte injury and induced a minimal-change-like nephropathy in rats [4]. Currently, however, there has been scarce report on the effect of IL-13 on cultured human podocytes *in vitro*. Only our previous report showed that IL-13 could induce alterations in the content and localization of zonula occludens-1 (ZO-1), one of the modified adherens junction (AJ) proteins [5].

The podocyte is a highly differentiated cell with a unique cytoskeletal architecture, and forms the final glomerular filtration barrier by the formation and maintenance of foot processes (FPs) and the interposed slit diaphragms (SDs) [6-13]. The extracellular SD is linked to the intracellular actin-based cytoskeleton through adaptor proteins, such as CD2-associated protein (CD2AP), zonula occludens (ZO)-1, β-catenin, and p130Cas, located at the intracellular SD insertion area near lipid rafts (6-13). Neighboring FPs are connected by a contractile apparatus consisting of F-actin, α-actinin-4 and synaptopodin [6-13].

Recently, CD80 expression on podocytes, also known as B7-1, has been proposed as a key player in the induction of proteinuria in MCNS [14-16]. However, the mechanistic insights associated with podocyte dysfunction mediated by IL-13-induced SD and cytoskeletal changes and upregulation of B7-1 have not yet been shown *in vitro* yet. The present study

shows that IL-13 down-modulates various SD and cytoskeletal structures at protein levels in human podocytes *in vitro*. Furthermore, we demonstrated that down-modulated changes in various adaptor proteins and cytoskeletal structures of human podocytes induced by IL-13 was significantly restored after treatment with a leukotriene receptor antagonist (montelukast), which has been used to treat allergic diseases, in conjunction with the upregulation of B7-1, suggesting that targeting IL-13 in MCNS could be one of the potential therapeutic strategies by modulating B7-1.

Materials and methods

Cell culture of human podocytes

Human conditionally immortalized podocytes (AB8/23), primarily cloned from human glomerular cultures, were characterized and generously provided by Dr. Moin A. Saleem (University of Bristol, Bristol, UK). Human podocytes were maintained in RPMI 1640 (WelGENE, Daegu, Korea) supplemented with 10% heat-inactivated fetal bovine serum (FBS), Insulin-Transferrin-Selenium-Pyruvate Supplement (ITSP; WelGENE), and antibiotics. Fresh media was supplied once every two days.

To stimulate human podocyte proliferation, cells were cultivated at 33℃ (permissive conditions) in a culture medium supplemented with human recombinant ITSP to induce expression of temperature-sensitive large T antigens. To induce differentiation, podocytes were maintained at 37℃ without ITPS (non-permissive conditions) for at least two weeks and for subculture, 0.05% trypsin was used to detach cells from the culture dishes [12].

IL-13 and montelukast treatment conditions

To imitate MCNS-like conditions, cells were incubated with various concentrations of IL-13 (Peprotech Inc., Rocky Hill, NJ, USA) during the indicated time periods (6, 12, and 24 h). IL-13 was administered with 1, 3, 5, 10, 20, 30, and 100 μg doses, respectively, into 0.5% RPMI at 37℃. Montelukast (Sigma-Aldrich Inc., St. Louis, MO, USA) was administered with the dose of 0.1 μg.

Monolayer permeability assay

Podocytes were seeded and grown to confluence in a monolayer pattern on the surface of 0.45 μm cellulose semi-permeable membranes (Millicell-HA, Millipore Corp., Bedford, MA, USA) in 10% RPMI. Hydrostatic pressure was applied continuously from lower to apical to basolateral aspect. Treated Cells were incubated with various concentrations of IL-13 during the indicated 24-h period in 0.5% RPMI. Then, 1 mg/mL FITC-tagged anionic dextran (Invitrogen, Eugene, OR, USA) was added to the apical media, and the filtered amounts of dextran at each incubation time (2, 4, 6, 8, 16, and 24 h) were measured by spectrophotometry at 492 nm.

Scanning Electron Microscopy

A Hitachi S-570 scanning electron microscopy (SEM; Hitachi, Tokyo, Japan) was used to view samples for SEM. The collagen-coated and differentiated cells were fixed in 5% glutaraldehyde in distilled water, incubated at 4°C for 1 h, rinsed three times in phosphate buffered saline (PBS), and then dehydrated in a graded series of ethanol solutions (60%, 70%, 80%, 95%, and 100% ethanol) for 7 min in each solution. The same process was performed

once more with the exception of fixation in 1% osmium tetroxide in distilled water. The cells were dried before coating with gold and observed by a SEM.

Immunofluorescence staining and confocal image analysis

Human podocytes that were grown on type I collagen-coated glass cover slips were incubated at 37°C for 2 h and fixed in 4% paraformaldehyde for 20 min. The cells were then permeabilized in 0.1% tritonX-100 for 10 min, blocked with 10% FBS for 30 min, washed three times for 5 min in phosphate buffered saline (PBS), and labeled with monoclonal rabbit anti-ZO-1 antibody (Invitrogen, Eugene, OR, USA), polyclonal goat anti-rat synaptopodin (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). polyclonal goat antimouse α-actinin antibody (Santa Cruz Biotechnology), polyclonal rabbit anti-rat CD2AP antibody (Santa Cruz Biotechnology). Primary antibody-bound specimens were incubated with 1:1000 (v/v) Alexa 594 for red conjugates and Alexa 488 for green (Invitrogen), respective of secondary anti-rabbit IgG, at room temperature for 40 min and at 37°C for 20 min without CO_2. Nuclei were stained with 4'-6-diamidino-2-phenylindole (DAPI) (1:1000) for 20 min in PBS. A mounting medium is used to adhere coverslips to the slide and viewed with a fluorescence microscope (Leica TCS SP2 AOBS, Mannheim, Germany). The densitometry values of the fluorescent bands were analyzed using the Image J image processing and analysis software (National Institutes of Health, Bethesda, MD, USA).

Western blotting

Confluent cell layers were incubated with additives for various time durations, and proteins were extracted using a protein extraction solution PRO-PREP (Intron Biotechnology,

Seongnam, Gyeonggi, South Korea) containing phenylmethylsulfonyl fluoride, ethylenediamine tetraacetic acid, pepstatin A, leupeptin, and aprotinin; protein concentrations were then determined. To perform western blotting for ZO-1, synaptopodin, α-actinin, CD2AP, β-catenin, p130Cas and B7-1, 30 μg of boiled extracts were resolved on 10% SDS-PAGE gels and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore Corp., Medford, MA, USA).

The membranes were then washed with methanol and blocked in 5% fat-free milk before incubation with monoclonal rabbit anti-ZO-1 antibody (Invitrogen), polyclonal goat anti-rat synaptopodin (Santa Cruz Biotechnology). polyclonal goat anti-mouse α-actinin antibody (Santa Cruz Biotechnology), polyclonal rabbit anti-rat CD2AP antibody (Santa Cruz Biotechnology), polyclonal rabbit anti-rat p130Cas antibody (Santa Cruz Biotechnology), polyclonal rabbit anti-rat β-catenin (Santa Cruz Biotechnology) or anti-B7-1 antibody (Santa Cruz Biotechnology). Anti-β-tubulin antibody (Santa Cruz Biotechnology) was used as a loading control. After incubation with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology), protein bands were detected using the enhanced chemiluminescence (ECL) detection system (WEST-ZOL® plus; Amersham Biotech Ltd., Bucks, UK). Density values are expressed as percentages of the control. Data on densitometric analysis of the respective proteins/β-tubulin ratio were expressed as mean ± standard deviation (SD).

Statistical analysis

Results were described as the mean ± SD, as appropriate under different conditions. The significance of the data was assessed using Student *t*-test and nonparametric Kruskal-Wallis

statistical analysis using SPSS version 20.0 (SPSS Inc, Chicago, IL, U.S.A.). *P* values < 0.05 are considered significant. In the Figure, statistical significance is indicated by asterisks (*). $*P < 0.05$; $**P < 0.01$.

Results

Ultrastructural changes in human podocytes by IL-13

Human podocytes were treated with PAN and a high dose of IL-13 to induce an experimental nephrotic syndrome. Under scanning electron microscopy (SEM), there seemed to be podocytes with dense and tufted structures in the differentiated condition, but the cells displayed fewer distinct processes or projections in puromycin aminonucleoside (PAN)-induced podocyte injury (Supplementary Figure S1 online). As in PAN-induced podocyte injury, SEM ultrastructural analyses also revealed a progressive decrease in distinct processes or projections of the human podocytes after treatment with IL-13, particularly documented at 100 ng/mL of IL-13 (Supplementary Figure S1 online).

Changes in podocyte permeability by IL-13

High concentration (100 ng/mL) of IL-13 increased podocyte permeability *in vitro* to peak levels greater than 250% compared to the control (without IL-13) at 6 h, and then levels decreased to be similar to low concentrations of IL-13 by 8 hr (Supplementary Figure S2 online). IL-13 of the doses ≥ 3 ng/mL gradually increased the overall permeability by 24 h. These findings show that high dose of IL-13 mainly increases podocyte permeability at early

stages (2-6 h), suggesting the functional explanation of the cytoskeletal changes shown by confocal microscopy after IL-13 incubation. After 6 hours, the permeability continued to increase, possibly due to senescence of the podocytes.

Distribution of SD and cytoskeletal molecules of podocytes on confocal microscopy

Podocytes were double-stained for ZO-1 and synaptopodin and the cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). ZO-1 in human podocytes was highly expressed within the podocyte in the cytoplasmic aspect of the FP membrane, adjacent to the insertion of the SD, and synaptopodin is an actin-associated protein that may play a role in actin-based cell shape and motility (Fig. 1) [6, 7]. ZO-1 was distributed to the cell contact areas under physiologic conditions without IL-13 but was redistributed and internalized into the inner cytoplasm from the peripheral cell membrane as IL-13 concentrations increased (Fig. 1). Distribution of synaptopodin became more fused and conglomerated as IL-13 concentrations increased (Fig. 1).

α-Actinin-4 is located in the center of foot process and interacts with integrins and strengthens the podocyte-glomerular basement membrane (GBM) interaction and CD2AP is expressed primarily in podocytes at the cytoplasmic face of the SD and lipid rafts and serves as an adaptor anchoring nephrin and podocin to actin filaments of podocyte cytoskeleton [6, 7]. α-actinin and CD2AP were distributed to the cell surface areas under physiologic conditions without IL-13 but was redistributed and internalized into the cytoplasm and perinuclear areas from the cell surface areas as IL-13 concentrations increased (Fig. 2). These results suggest that IL-13 may have a substantial impact on the redistribution and

rearrangement of SD and cytoskeletal molecules in human podocytes and disrupts the cytoskeletal connections in a concentration-dependent manner.

Protein assays for adaptor and cytoskeletal molecules of podocytes by western blotting

In human podocytes, density values for ZO-1 (69 kD), synaptopodin (100 kD) and α-actinin (100 kD) proteins tended to decrease with IL-13 treatment in a dose-dependent manner at 12 h (Fig. 3). A dose of 20 ng/mL IL-13 significantly decreased the amount of ZO-1, synaptopodin and α-actinin protein in human podocytes at 12 h ($P < 0.05$) (Fig. 3). A dose of ≥ 10 ng/mL IL-13 significantly decreased the amount of CD2AP protein in human podocytes at 6 h ($P < 0.01$). The reduced CD2AP protein levels (90 kD) by 30 and 100 ng/mL IL-13 was restored by 0.1 μM montelukast ($P < 0.05$) (Fig. 4). A dose of ≥ 3 ng/mL IL-13 significantly increased the amount of β-catenin protein (90 kD) in human podocytes at 12 h ($P < 0.05$). The increased β-catenin protein levels by 30 and 100 ng/mL IL-13 was slightly decreased by 0.1 μM montelukast, although it was not statistically significant (Fig. 5). A dose of ≥ 3 ng/mL IL-13 significantly decreased the amount of p130Cas protein (130 kD) in human podocytes at 6 and 12 h ($P < 0.01$). The reduced p130Cas protein levels by 30 and 100 ng/mL IL-13 was significantly increased by 0.1 μM montelukast ($P < 0.01$) (Fig. 6). A dose of 10 and 30 ng/mL IL-13 significantly increased the amount of B7-1 protein (60 kD) in human podocytes at 6 and 12 h ($P < 0.05$). The increased B7-1 protein levels by 30 ng/mL IL-13 was significantly decreased by 0.1 μM montelukast ($P < 0.05$) (Fig. 7).

Discussion

The pathogenic mechanisms of proteinuria in MCNS have not been elucidated. Since Shalhoub proposed the hypothesis of MCNS is a disorder of T-cell dysfunction [17], several cytokines, such as IL-1, 2, 8, 12 and interferon-gamma, have been suggested to be involved in the pathogenesis of MCNS [18]. However, the results have not been consistent in subsequent studies [18]. Among various cytokines, IL-13 has recently been reported to be increased during relapse of MCNS [2, 3]. In addition, IL-13-transfected rats~~,~~ showed about 80% effacement of podocyte foot processes on electron microscopy in association with the downregulation of nephrin, podocin and dystroglycan [4]. Although our preliminary study have shown that IL-13 could induce the alterations in the content and localization of ZO-1 in human podocytes [5], there has been no comprehensive study to test whether IL-13 could affect the other various adaptor proteins and cytoskeletal changes in cultured human podocytes *in vitro* and their potential mechanisms, which would be important to understand the pathogenesis of MCNS.

Our study firstly demonstrated that IL-13-treated cultured human podocytes showed a progressive decrease in distinct processes or projections similar to the PAN-induced podocyte changes, which is considered to be a traditional model of MCNS, on SEM analysis. Although PAN-induced podocyte injury is mediated by oxidative stress and direct insults [19, 20], we thought that the pathogenic mechanisms of IL-13-induced podocyte changes might be different from PAN-induced changes, because the pathogenesis of human MCNS might not be related to chemical podocyte injury, considering that there are no histological changes in glomeruli of MCNS.

We also showed that IL-13 increased podocyte permeability in addition to changes in morphology of processes of cultured human podocytes as in our previous studies such as

glucose- or PAN-induced podocytes [20-22]. Although the precise mechanisms of these deleterious effects have not been fully understood, derangement of the various SD and actin cytoskeleton molecules has been proposed as the common pathway leading to foot process effacement in podocytes [6, 13]. In the present study, we newly demonstrated that IL-13 had a substantial impact on the redistribution and rearrangement of ZO-1, synaptopodin, α-actinin, CD2AP in podocytes and disrupted the cytoskeletal connections in a concentration-dependent manner on confocal microscopy. IL-13 also down-modulated ZO-1, synaptopodin, α-actinin, CD2AP and p130Cas and up-regulated β-catenin at protein levels in cultured human podocytes. A well-developed SD, adaptor proteins, and actin cytoskeletal structures play an important role in the maintenance of podocyte foot processes architecture and filtration barrier function [6-13]. ZO-1, synaptopodin, α-actinin, CD2AP and p130Cas are all important SD-binding adaptor proteins or actin cytoskeletal molecules for maintaining podocyte integrity, and disruption of these molecules can cause podocyte permeability as shown in our previous and current studies [5, 20-26]. Recently, β-catenin has been shown to be involved in many pathological processes in podocytes [27]. Emerging evidence suggests that β-catenin is activated in podocytes in various proteinuric kidney diseases and genetic or pharmacologic activation of β-catenin is sufficient to impair podocyte integrity and causes proteinuria in healthy mice. Conversely, podocyte-specific ablation of β-catenin protects against proteinuria after kidney injury [27-29]. Our results also showed that IL-13 increased β-catenin levels in cultured human podocytes.

Recently, upregulation of B7-1 has been regarded to be one of the mechanisms involved in the development of MCNS [13-16]. This concept was firstly suggested by Reiser *et al.* in which lipopolysaccharide (LPS) was capable of up-regulation of B7-1, leading to nephrotic-

range proteinuria and reorganization of vital slit diaphragm proteins [30]. Conversely, proteinuria was not seen in LPS-treated B7-1 knockout mice, suggesting a pivotal role for this molecule in the development of proteinuria [30]. Ishimoto *et al.* also showed that sera from MCD in relapse, but not in remission, significantly increased B7-1 expression ($P < 0.004$) and B7-1 protein secretion by podocytes [16]. In addition, increased B7-1 urinary excretion was elevated in MCNS patients [14]. Also, overexpression of IL-13 caused upregulation of B7-1 in IL-13-transfected rats [4], but there has been no study to test whether IL-13 could increase B7-1 molecule in cultured human podocytes *in vitro*. Our study firstly showed that B7-1 was upregulated after IL-13 treatment in podocytes, which could be related to the disruption of the various SD and cytoskeletal changes in podocytes. However, the values of B7-1 expression is currently highly debated, since many working groups could not repeat the initially findings. Fiorina *et al.* showed that the immune-related molecule B7-1/CD80 is a critical mediator of podocyte injury in type 2 diabetic nephropathy (31), but Baye *et al.* demonstrated that the costimulatory receptor B7-1 was not induced in injured podocytes in several mouse models of podocyte injury including treatment with lipopolysaccharide or Adriamycin, a lupus prone model (NZB/W F1) and subtotal nephrectomy (32).

To test the hypothesis that targeting IL-13 with a leukotriene receptor antagonist which is widely used in the treatment of many allergic diseases [33] could be beneficial in IL-13-stimulated cultured human podocytes, we treated montelukast in IL-13-stimulated podocytes. Leukotriene metabolism in podocytes has not been reported previously. In experimental models of allergic diseases, it was well known that montelukast exerts its anti-inflammatory effect through the suppression of T helper type-2 (Th2) cytokines such as IL-4, IL-5 and IL-

13 [34]. We demonstrated that changes in various SD-binding adaptor proteins and cytoskeletal structures of human podocytes induced by IL-13 were significantly restored after the treatment with montelukast in conjunction with the upregulation of B7-1. In podocyte levels, we speculate that upregulation of cysteinyl leukotriene 1 receptor by IL-13 which is might be blocked by montelukast.

However, our study has some limitations. Firstly, it remains unclear whether the applied concentrations of IL-13 and montelukast reflect the serum concentrations in the animal model or in patients. Secondly, the dose of IL-13 used in in vitro study cannot be applied to the patients due to different conditions. Nevertheless, we firstly demonstrated that IL-13 could induce a progressive decrease in distinct processes or projections of the cultured human podocytes, which could be used as a new *in vitro* model of MCNS in addition to previous PAN and LPS-induced models. IL-13 also resulted in increased podocyte permeability, the redistribution and rearrangement or changes in protein contents of various SD-binding adaptor proteins and cytoskeletal molecules in conjunction with the upregulation of B7-1. Restoration of these changes in the cultured human podocytes after montelukast treatment suggests that IL-13 could have a direct impact on the cultured human podocytes, responsible for the development of proteinuria in MCNS.

Acknowledgement

The part of this article was presented as a poster presentation in the 16th IPNA congress in 2013, Shanghai, China (Interleukin-13 may increase podocyte permeability via modulation of zonula occludens-1). The authors would like to thank Keum Hwa Lee for her arranging references and proof-reading.

Author contributions

T. S. Ha, J. A. Nam, S. B. Seong, M.A. Saleem, S. J. Park and J. I. Shin designed study, coordinated data acquisition, statistically analyzed and interpreted the data, drafted and revised the manuscript. All authors read and approved the final manuscript.

Competing Financial Interests: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) and funded by the Ministry of Education, Science and Technology (2011-0013789, 2013R1A1A1012112 and 2015R1C1A1A01052984) to J.I. Shin and partly supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A4A03006207) to T.S. Ha.

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Figure legends

Fig. 1. Distributional changes in ZO-1 and synaptopodin by IL-13 in cultured human podocytes. Magnification: 1,000 x ; Scale bar = 20 μm.

Fig. 2. Distributional changes in α-actinin and CD2AP by IL-13 in cultured human podocytes. Magnification: 1,000 x ; Scale bar = 20 μm.

α-Actinin and CD2AP were redistributed and internalized into the cytoplasm and perinuclear areas from the cell surface areas as IL-13 concentrations increased (arrow heads)

Fig. 3. Effects of IL-13 on ZO-1, synaptopodin and α-actinin protein levels in cultured human podocytes as assayed by Western blotting.

Data on the densitometric analysis of the ZO-1, synaptopodin and α-actinin proteins/β-tubulin ratio are expressed as the mean ± SD. **P* < 0.05**.** Blots have been run under the same experimental conditions and data were summarized from 3 separated experiments.

Fig. 4. Effects of IL-13 with and without montelukast on CD2AP protein levels in cultured human podocytes as assayed by Western blotting.

Data on the densitometric analysis of the CD2AP/β-tubulin ratio are expressed as the mean ± SD. **P* < 0.05, ***P* < 0.01. Blots have been run under the same experimental conditions and data were summarized from 3 separated experiments.

Fig. 5. Effects of IL-13 with and without montelukast on β-catenin protein levels in cultured human podocytes as assayed by Western blotting.

Data on the densitometric analysis of the β-catenin/β-tubulin ratio are expressed as the mean ± SD. **P* < 0.05. Blots have been run under the same experimental conditions and data were summarized from 3 separated experiments.

Fig. 6. Effects of IL-13 with and without montelukast on p130Cas protein levels in cultured human podocytes as assayed by Western blotting.

Data on the densitometric analysis of the p130Cas/β-tubulin ratio are expressed as the mean ± SD. ***P* < 0.01. Blots have been run under the same experimental conditions and data were summarized from 3 separated experiments.

Fig. 7. Effects of IL-13 with and without montelukast on B7-1 protein levels in cultured human podocytes as assayed by Western blotting.

Data on the densitometric analysis of the B7-1/β-tubulin ratio are expressed as the mean ± SD. **P* < 0.05, ***P* < 0.01. Blots have been run under the same experimental conditions and data were summarized from 3 separated experiments.

Figure1

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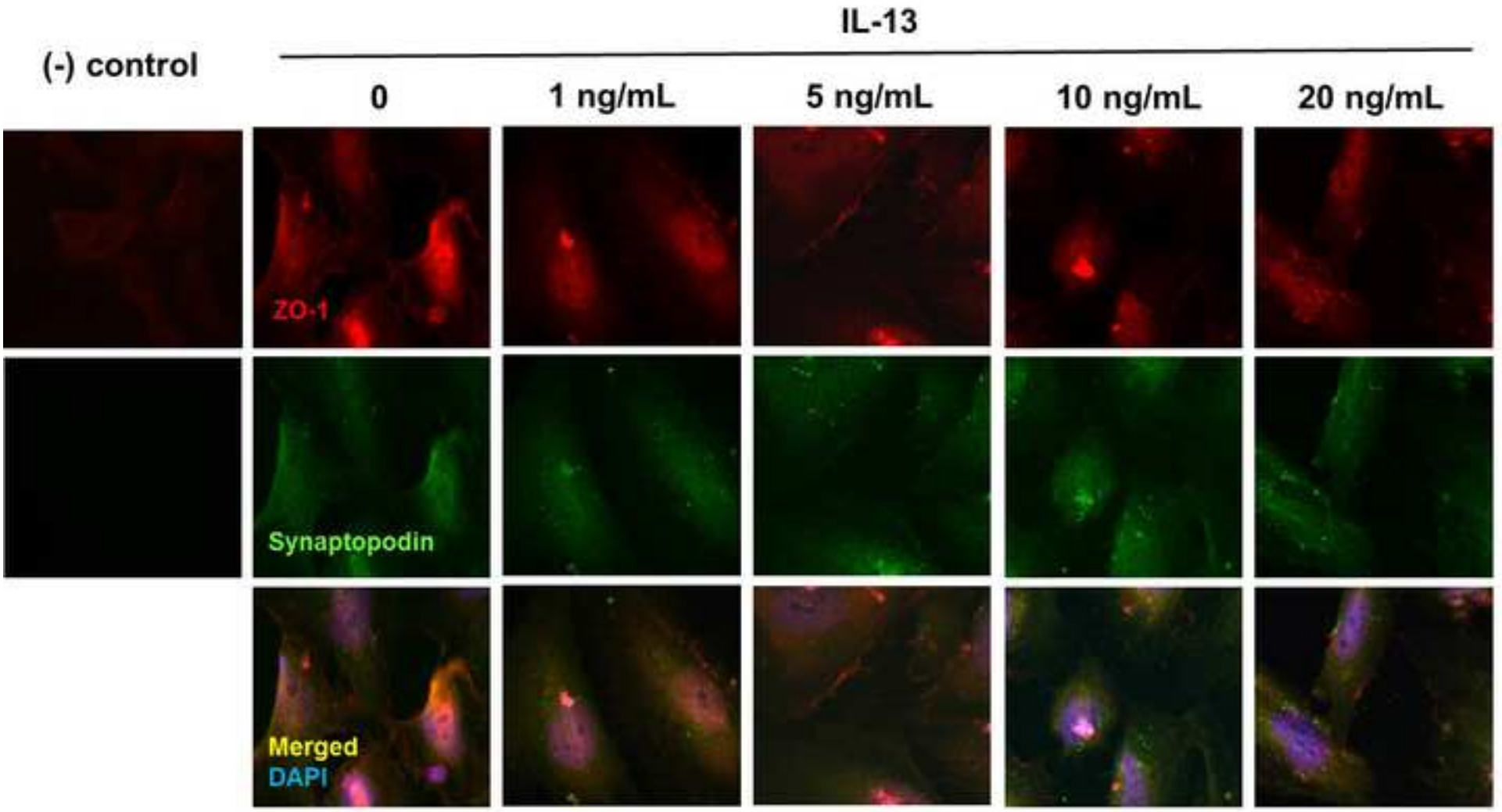

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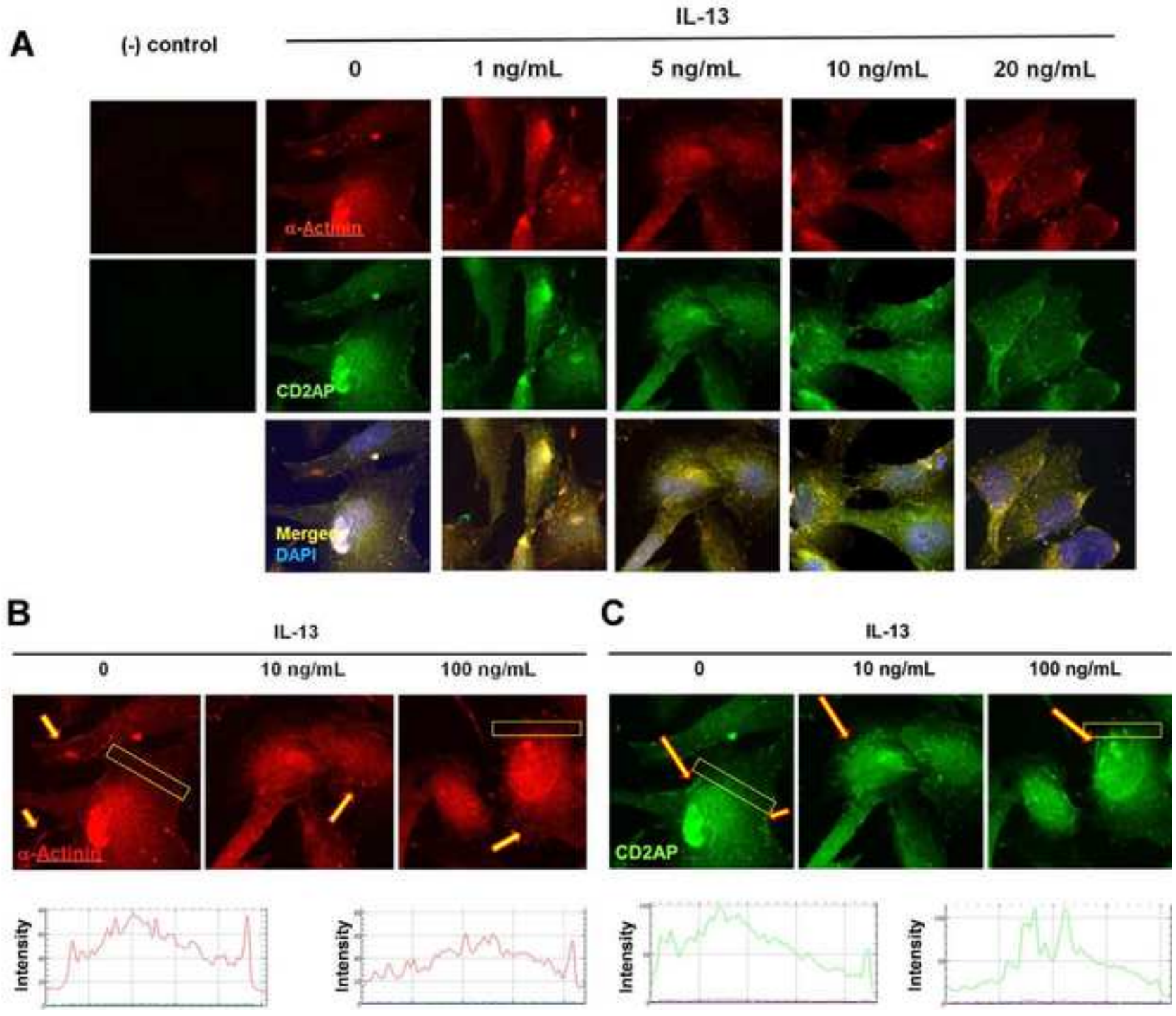

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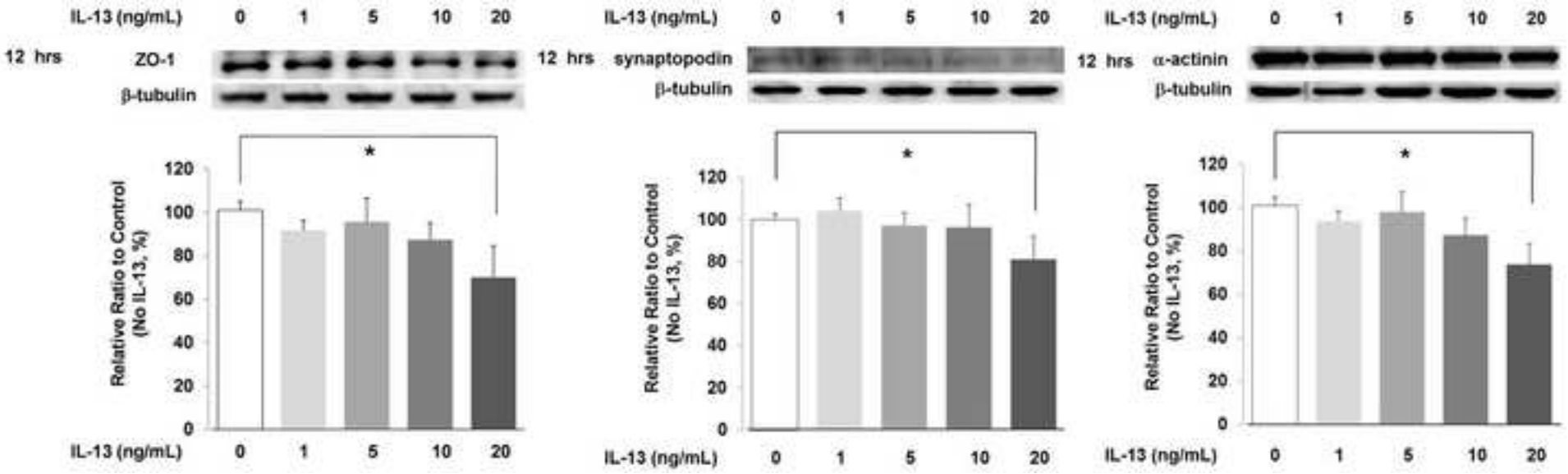

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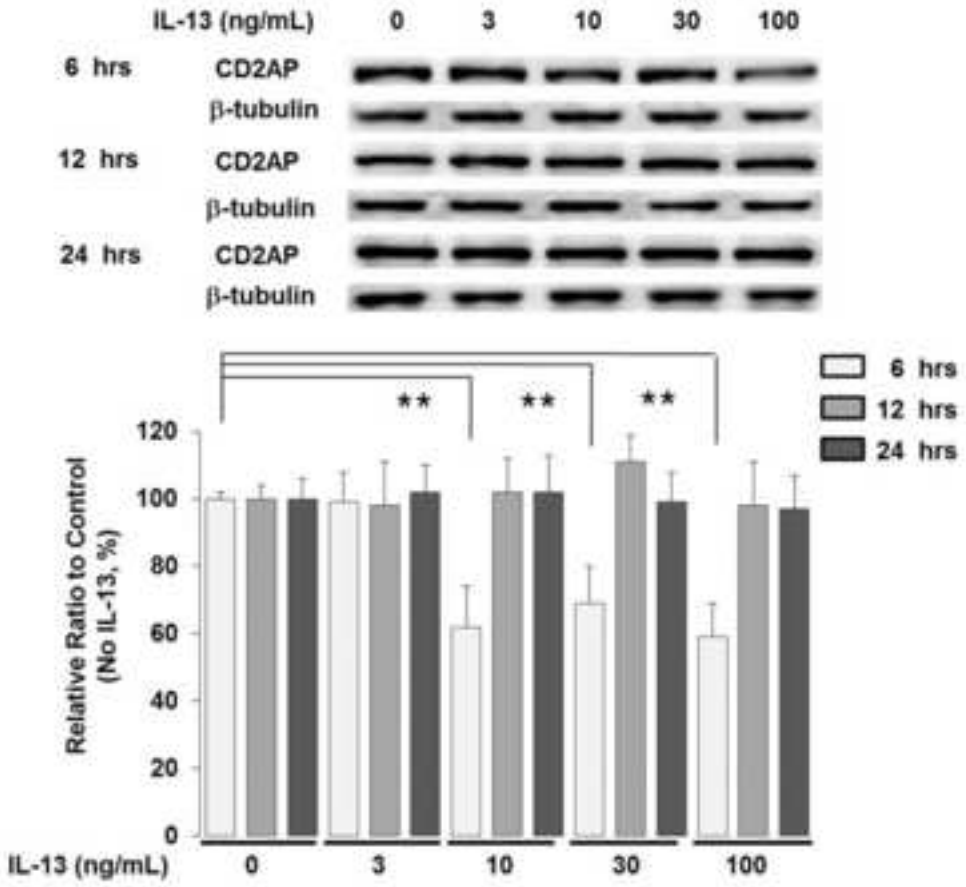

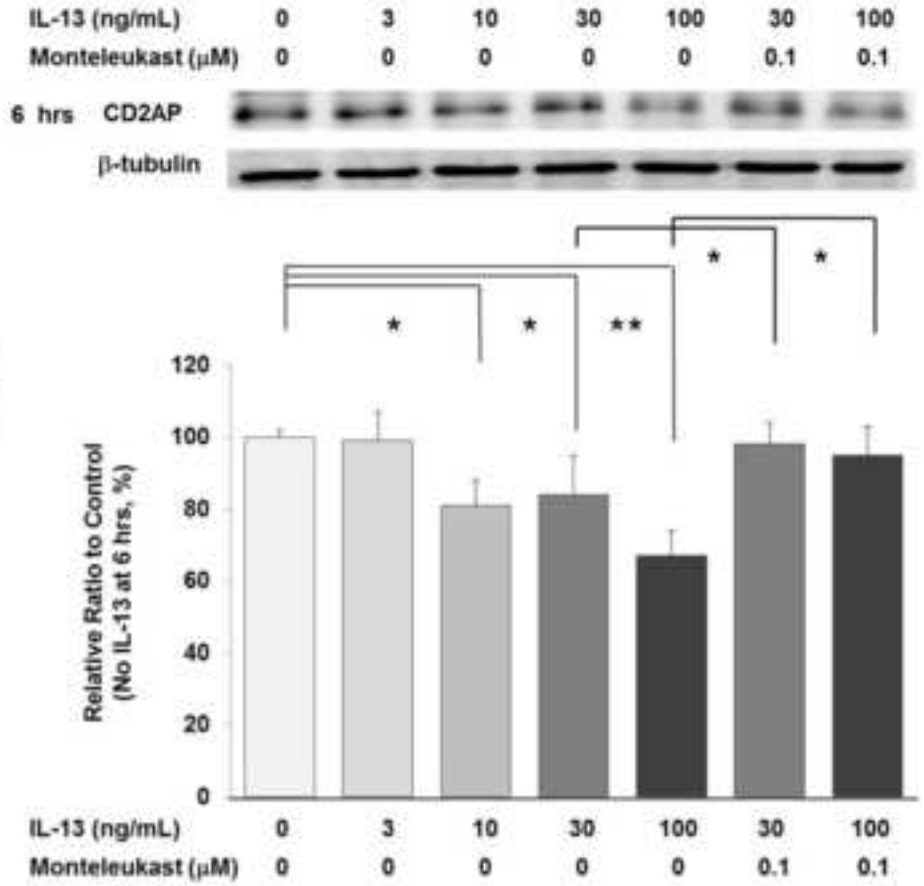

Figure5

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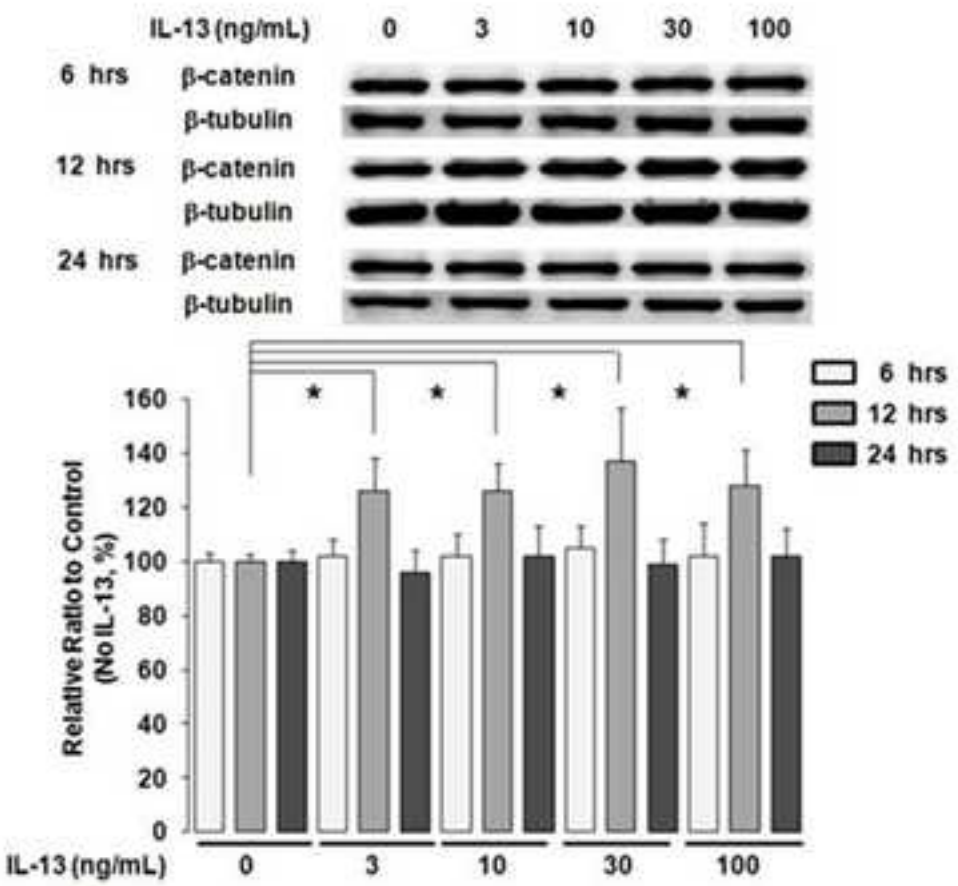

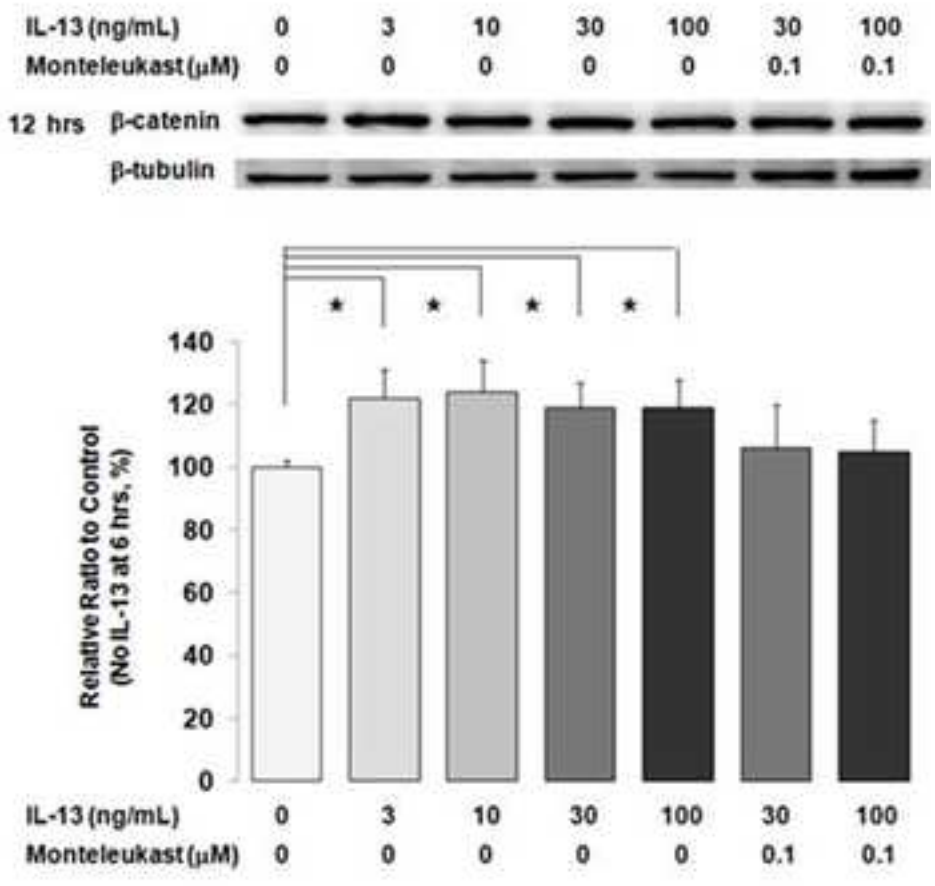

Figure6

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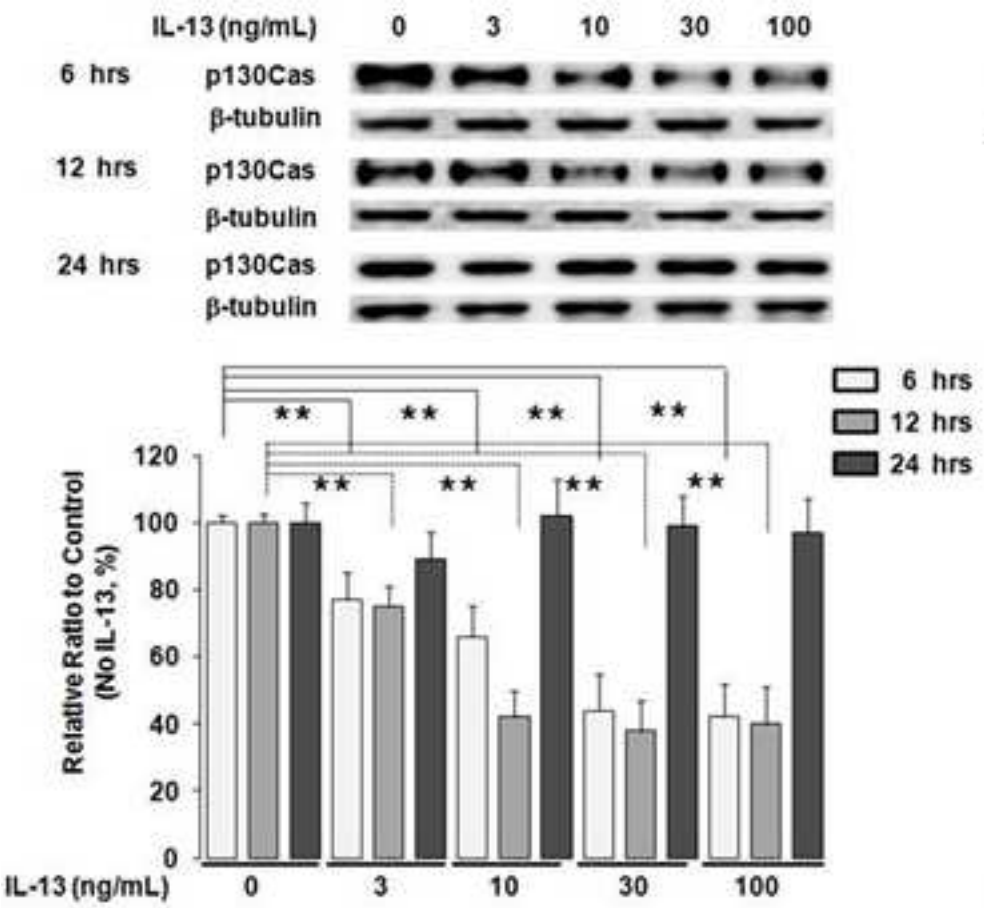

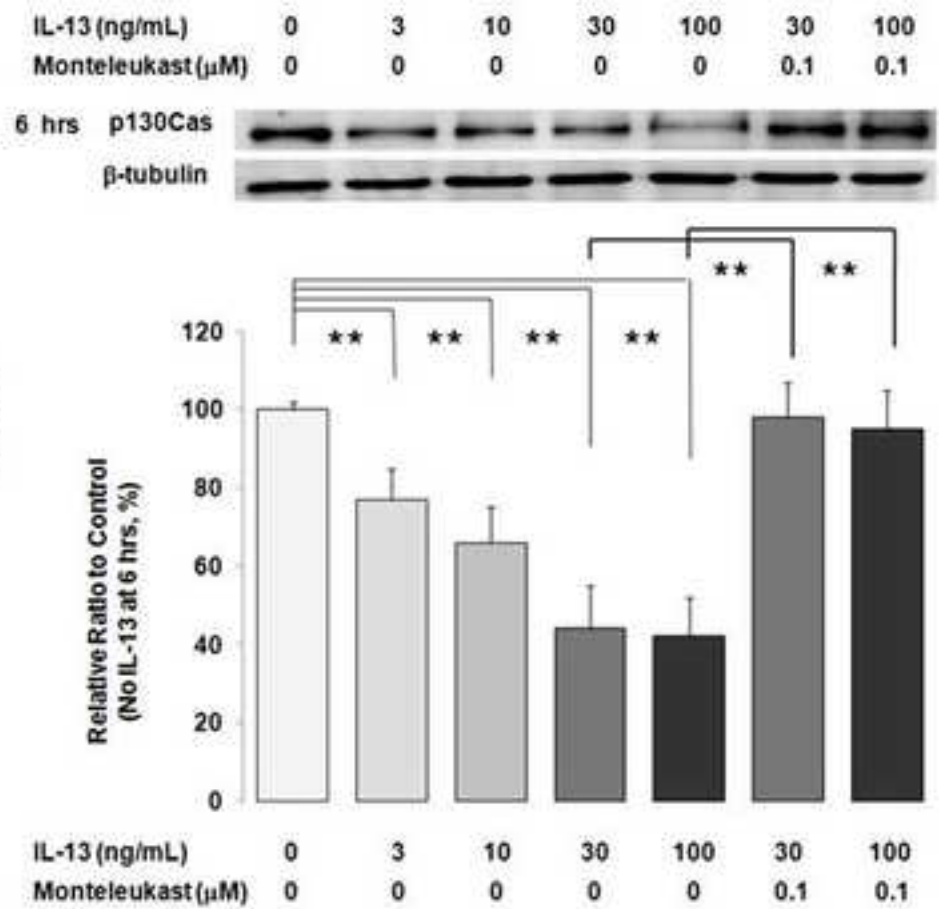

Figure7

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