Inflammasome-independent NLRP3 is required for epithelial-mesenchymal transition in colon cancer cells

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A B S T R A C T

Inflammasome NLRP3 plays a crucial role in the process of colitis and colitis-associated colon cancer. Even though much is known regarding the NLRP3 inflammasome that regulates pro-inflammatory cytokine release in innate immune cells, the role of NLRP3 in non-immune cells is still unclear. In this study, we showed that NLRP3 was highly expressed in mesenchymal-like colon cancer cells (SW620), and was upregulated by tumor necrosis factors-α (TNF-α) and transforming growth factor-β1 (TGF-β1) respectively, during EMT in colon cancer epithelial cells HCT116 and HT29. Knockdown of NLRP3 retained epithelial spindle-like morphology of HCT116 and HT29 cells and reversed the mesenchymal characteristic of SW620 cells, indicated by the decreased expression of vimentin and MMP9 and increased expression of E-cadherin. In addition, knockdown of NLRP3 in colorectal carcinoma cells displayed diminished cell migration and invasion. Interestingly, during the EMT process induced by TNF-α or TGF-β1, the cleaved caspase-1 and ASC speck were not detected, indicating that NLRP3 functions in an inflammasome-independent way. Further studies demonstrated that NLRP3 protein expression was regulated by NF-κB signaling in TNF-α or TGF-β1-induced EMT, as verified by the NF-κB inhibitor Bay 11-7082. Moreover, NLRP3 knockdown reduced the expression of Snail1, indicating that NLRP3 may promote EMT through regulating Snail1. In summary, our results showed that the NLRP3 expression, not the inflammasome activation, was required for EMT in colorectal cancer cells.

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1. Introduction

The epithelial-mesenchymal transition (EMT) is considered as one of pivotal steps in colorectal cancer cell invasion and metastasis, by which epithelial cancer cells lose their polarity and become motile mesenchymal cells [1,2]. EMT is typified by the loss of epithelial markers (e.g., E-cadherin, ZO-1), the gain of mesenchymal markers (e.g., vimentin, N-cadherin), and the increased production of extracellular matrix metalloproteinases (MMPs) [3]. Many autocrine or paracrine cytokines and oncogenic pathways, including transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), Nuclear factor-kappa B (NF-κB), Wnt, Notch, and growth factor receptor signaling cascades, are implicated in the induction of EMT [4]. Emerging studies indicate that TGF-β1 and TNF-α, pleiotropic cytokines that regulate the growth, differentiation and migration of various types of cells [5,6], have been generally recognized as potent inducers of EMT through NF-κB signaling pathway in human colon cancer cells [7,8]. Moreover, several transcriptional regulators such as Snail1, Twist, Zeb1 and Zeb2, which are downstream of the aforementioned signaling pathways, function in EMT of colon cancer cells [9]. For example, Snail family factors are indeed involved in EMT and proposed to be inducers of the invasion process [10].

The NLRP3 inflammasome, which consists of the NLRP3 scaffold, the ASC (PYCARD) adaptor and caspase-1, is by far the most fully characterized inflammasome [11]. Although initially thought to be restricted to immune cells, NLRP3 can also be functional in non-immune cells, including normal epithelial cells [12,13] and cancer cells [14,15]. NLRP3 expressed in colon cancer cells has been found to induce pyroptosis of human and murine colon cancer cells by liver X receptor activation [14]. NLRP3 inflammasome is activated upon exposure to whole pathogens, as well as diverse PAMPs, DAMPs and environmental irritants [11]. Once activated, assembly of the amino-terminal PYD of NLRP3 engages in homotypic interactions with the PYD of ASC.

Abbreviations: EMT, epithelial-to-mesenchymal transition; TNF-α, tumor necrosis factors-α; TGF-β, transforming growth factor-β; NF-κB, Nuclear factor kappa B; CRC, Colorectal cancer

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to recruit caspase-1, which mediates maturation of pro-inflammatory cytokines such as IL-1β and IL-18 [16]. NLRP3 inflammasome has been linked to many human diseases, including inflammatory bowel disease and colitis-associated cancer [17,18], but the crosstalk between NLRP3 inflammasome activation and colon cancer has not been clearly established. Inactivation of NLRP3 inflammasome by small molecule-driven mitophagy was responsible for the prevention of colitis-associated cancer [19]. However, deletion of NLRP3, the inflammasome component, exacerbated colorectal cancer metastatic growth [20]. Recent attention has highlighted that NLRP3 could function in an inflammasome-independent manner. NLRP3 has been reported to dramatically protect kidney epithelial cells from ischemia reperfusion injury independently of inflammasome [21]. In addition, NLRP3 deficiency has been certified to exacerbate hyperoxia-induced acute lung injury, which was independent of the production of IL-1β [22]. Importantly, inflammasome-independent NLRP3 has also been demonstrated to augment TGF-β-induced EMT in kidney epithelium [23] and promote cardiac fibrosis through regulating mitochondrial ROS production [24]. But the role of NLRP3 and the inflammasome component in EMT of colorectal cancer have not been elucidated.

2. Materials and methods

2.1. Reagents

Primary antibodies for Lamin A, β-actin and horseradish peroxidase (HRP) conjugated secondary antibodies were purchased from BioWorld Technology Inc. (BioWorld Technology Inc., CA). ASC antibody was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz Biotechnology Inc., CA). Antibodies against E-cadherin, vimentin, NLRP3, Snail1, NF-κB and Caspase-1 were purchased from CST Technology Inc. (CST Technology Inc., MA). MMP9 antibody was from Abcam Technology Inc. (Abcam Technology Inc, England). Fluorescein isothiocyanate (FITC)-phalloidin was from Sigma-Aldrich (Sigma-Aldrich, Vienna, USA). TGF-β1 and TNF-α were from Peprotech (Peprotech, USA). Caspase1 inhibitor VX-765 was obtained from Selleck (Selleck, USA). Bay 11-7082 was obtained from Peprotech (Peprotech, USA). Caspase1 inhibitor VX-765 was obtained from Selleck (Selleck, USA). Caspase1 inhibitor VX-765 was obtained from Selleck (Selleck, USA). Caspase1 inhibitor VX-765 was obtained from Selleck (Selleck, USA). Caspase1 inhibitor VX-765 was obtained from Selleck (Selleck, USA).

2.2. Cell culture

Human colon carcinoma HCT116, SW620 and HT29 cell lines were obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured with DMEM (HCT116 and SW620), RPMI-1640 (HT29) medium supplemented with 10% FBS and 1% Penicillin/Streptomycin, in a 37 °C humidified incubator with 5% CO2. To induce EMT, HCT116 and HT29 cells were seeded into 6-well plates and grown to 70–80% confluence in complete growth medium. Cells were then incubated in serum-free medium supplemented with TGF-β1 or TNF-α at the concentration of 0, 1, 5 or 10 ng/ml, and harvested at 24 h and 48 h after the indicated treatment. For NF-κB and Caspase-1 inhibitor treatment, Bay 11-7082 (10 μM) and VX-765 (10 μM) were added to the medium for 24 h.

2.3. Western blot

Proteins were extracted in lysis buffer (30 mM Tris, pH 7.5, 150 mM sodium chloride, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 1% Nonidet P-40, 10% glycerol, and phosphatase and protease inhibitors). Protein concentration in the supernatants was detected by BCA protein assay (Thermo, Waltham, MA). Then equal amount of protein was separated with 8% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA) using a semi-dry transfer system (Bio-rad, Hercules, CA). Proteins were detected using specific antibodies of E-cadherin, vimentin, MMP9, NLRP3, Caspase-1, NF-κB, β-actin and Lamin A overnight at 4 °C, followed by HRP-conjugated secondary antibodies for 1 h at 37 °C. All of the antibodies were diluted in PBS containing 1% BSA. Enhanced chemiluminescent reagents (Beyotime, Jiangsu, China) were used to detect the HRP on the immunoblots, and the visualized bands were captured by film. The band strengths were quantified by Quantity One software (Vision 4.62, Bio-rad, Hercules, CA), and represented the relative protein level normalized to β-actin.

2.4. Real-time PCR

Real-time PCR was performed as follows: RNA samples were reverse transcribed to cDNA and subjected to quantitative PCR, which was performed with the LightCycler® 96 Real-Time PCR System (Roche, Basel, Swiss) using AceQ qPCR SYBR Green Master Mix (Vazyme, Nanjing, China). The program for amplification was 1 cycle of 95 °C for 2 min followed by 40 cycles of 95 °C for 10 s, 60 °C for 30 s, and 95 °C for 10 s. The PCR results were normalized to Gapdh expression and were quantified by the ΔΔCT method. The primer sequences used in this study were as follows:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5′-3′)</th>
<th>Reverse (5′-3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLRP3</td>
<td>5′-AACATTCCGAGAGTTGTGGGACG-3′ (forward)</td>
<td>5′-GTGGTGAATTGTCCTGTCTGC-3′ (reverse)</td>
</tr>
<tr>
<td>Caspase-1</td>
<td>5′-TTACGACAAAGGTTGCTGAAACA-3′ (forward)</td>
<td>5′-TGACGATGGAAGAAAAAGCTGG-3′ (reverse)</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>5′-CTCCGACACTTCACCTCTC-3′ (reverse)</td>
<td>5′-AGACAATTCTTGCGTGAAGC-3′ (forward)</td>
</tr>
<tr>
<td>Vimentin</td>
<td>5′-ACGAAGTGCACGACCTAT-3′ (reverse)</td>
<td>5′-AACCCCTGACATCTGGAACG-3′ (forward)</td>
</tr>
</tbody>
</table>

2.5. Transwell migration and matrigel invasion assays

Transwell migration and matrigel invasion assays were performed using a transwell membrane (8-μm pore size, Corning, NY, USA) in a 24-well plate according to the manufacturer’s instructions. The difference was that the upper surface of the transwell membrane in the invasion assay was pre-coated with Matrigel (BD Biosciences, USA), while not in the migration assay. The lower chamber of the transwell plates was filled with 600 μl RPMI-1640 or DMEM medium containing 10% FBS. HCT116, HT29 and SW620 cells were detached from the culture plates and resuspended in RPMI1640 or DMEM medium containing 1% FBS and then loaded to the upper side of the chamber (200 μl/well). For the migration assay, HCT116 (1 × 105 cells/well), HT29 (1 × 105 cells/well) and SW620 (1 × 105 cells/well) were loaded to the upper side of the chamber (200 μl/well) for indicated time periods. For the invasion assay, HCT116 (2 × 105 cells/well), HT29 (2 × 105 cells/well) and SW620 (2 × 105 cells/well) were placed in incubators at 37 °C for different time periods according to preliminary experiments. Then cells on the upper surface of the filter were removed using cotton swabs. Those on the lower surface were fixed with ice-cold methanol, stained with 0.1% crystal violet and counted. Cells that migrated or invaded were counted in five random fields of each filter under a microscope (Olympus, Japan) at 100 × magnification.

2.6. Immunofluorescence

To allow direct fluorescence of actin cytoskeleton, the cells were stained with 0.25 mM FITC-conjugated phalloidin. Cells were incubated with E-cadherin, vimentin, NLRP3 and ASC primary
antibody in PBS containing 1% BSA (1:50) for 1 h at 37 °C and treated with Alexa Fluor® 488 goat anti-rabbit antibody or Alexa Fluor® 546 goat anti-rabbit antibody. Nuclei were visualized with DAPI. Images were acquired by confocal laser-scanning microscope (Olympus, Lake Success, NY).

2.7. NLRP3 Plasmid transfection

NLRP3 knockdown plasmid was obtained from YSY Biotech Company Ltd. (Nanjing, China) and transfected according to the manufacturer’s instructions of ExFect™ Transfection Reagent (Vazyme Biotech Ltd., Nanjing, China). The extent of gene knock-down was determined by western blot.

2.8. Statistical analysis

All experiments were repeated at least three times. Results were presented as the mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) and Student’s t-test using SPSS software (SPSS Inc. Chicago, IL, USA). P < 0.05 was considered to indicate a statistically significant result. See details of each statistical analysis used in figures and figure legends.

3. Results

3.1. The expression of NLRP3 is upregulated in EMT of colorectal cancer cells

We firstly detected the endogenous expression level of NLRP3 and EMT markers in three colorectal cell lines SW620, HCT116 and HT29. As shown in Fig. 1A, the vimentin and MMP9 levels were much higher in SW620 cells when compared with that in HCT116 and HT29 cells. E-cadherin, a protein mainly associated with epithelial cells, showed an opposite pattern of expression with higher amounts in HCT116 and HT29 cell lines (Fig. 1A). The results were consistent with the findings that HCT116 and HT29 cells were epithelial-like cancer cells which were derived from one patient at an early point in tumor progression, whereas SW620 cells were mesenchymal-like cancer cells corresponded to a later metastatic stage [25]. Interestingly, NLRP3 in mesenchymal SW620 cells exhibited higher expression level than that in the other two epithelial cancer cells (Fig. 1A).

Previous reports have revealed that TGF-β1 could induce EMT in colon cancer cells [26]. As shown in Fig. 1B, TGF-β1 time- and dose-dependently enhanced the expression of NLRP3 in HT29 cells, as well as the expression of vimentin and MMP9, and concomitantly reduced the expression of E-cadherin. Real-time PCR assay showed a similar trend with increased mRNA expression of NLRP3 and the alteration of EMT-related markers (Fig. 1C).

Previously, Pino etc. has demonstrated that TGF-β1 can induce EMT in microsatellite stable (MSS) HT29 cells whereas HCT116 cells with microsatellite instability (MSI) and mutant TGFBR2 were unresponsive to TGF-β1 [27]. In agreement with that, here we found that TGF-β1 did not induce any significant change in expression levels of EMT markers and NLRP3 in HCT116 cells (Fig. 1D). TNF-α was therefore utilized to induce EMT in HCT116 cells. As shown in Fig. 2A, TNF-α successfully induced EMT in HCT116 cells, which was verified by decreased E-cadherin and enhanced vimentin and MMP9 at protein levels. Importantly, during this process, NLRP3 expression was also upregulated by TNF-α in a time- and dose-dependent manner. Similar trends in the transcription of NLRP3 and EMT-related markers were also observed in TNF-α induced EMT (Fig. 2B). These results suggested that in colon cancer cells, the expression of NLRP3 can be enhanced by different EMT inducers.

3.2. The role of NLRP3 in EMT is independent of the inflammasome activation

The effect of NLRP3 on EMT could be mediated by the production of cytokines such as IL-1β and IL-18 induced by NLRP3 inflammasome activation, which are known to contribute to
IL-1β could also play an indispensable role in the development of kidney and heart injury [21,30]. As shown in Fig. 3D and F, together, NLRP3 was involved in TNF-α-induced EMT, which was consistent with the real-time PCR assessments. caspase-1.

NLRP3 is essential for NLRP3 activation [31]. As seen in Fig. 3A and B, ASC speck formation was not found in HCT116 and HT29 cells treated with TNF-α or TGF-β1. As a positive control, NLRP3 in HT29 cells, and much less cleaved caspase-1 and IL-1β were detected at 0 h, 3 h, 6 h and 12 h prior to EMT of HCT116 and HT29 cells, despite the slight increase of pro-caspase-1 (Fig. 3B and C). As a control treatment, IL-converting Enzyme/Caspase-1 Inhibitor VX-765 (10 μM) did not abolish the changes of EMT-related markers, as determined by FITC-phalloidin staining, was significantly reduced in NLRP3-depleted HCT116 and HT29 cells (Fig. 4A and B). As shown in Fig. 4C, the depletion of NLRP3 markedly promoted mesenchymal-to-epithelial transition (MET)-like morphological changes in mesenchymal-like SW620 cells. Loss of vimentin and gain of E-cadherin were further confirmed by immunofluorescence, western blot, and real-time PCR assays (Figs. 4C and 5E and F). In addition, NLRP3 depletion also remarkably inhibited MMP9 expression at protein level (Fig. 5A, B and E).

The effect of NLRP3 depletion on EMT in colon cancer cells led us to examine its influence on cell migration and invasion. The ablation of NLRP3 expression in HCT116 and HT29 cells significantly inhibited cell migration and invasion, compared with untransfected cells (Fig. 6A-D). TNF-α or TGF-β1 treatment partially rescued the migration and invasion of NLRP3-depleted HCT116 and HT29 cells (Fig. 6A-D). Mesenchymal cells that underwent MET were characterized by reducing tumor cell migration and invasion [33]. Similar to HCT116 and HT29 cells, the depletion of NLRP3 markedly attenuated the migration and invasion of SW620 cells (Fig. 6E and F). These results strongly suggested that NLRP3 was required in EMT of colon cancer cells, as well as in cell migration and invasion.

3.4. NLRP3 upregulation in EMT is dependent on NF-κB signaling and NLRP3 regulates the expression of Snail1

Several studies have reported that NF-κB signaling is required for EMT, accompanied by cell migration and invasion [34,35]. Since TNF-α and TGF-β1 had been shown to upregulate NLRP3 expression, we assessed the role of NF-κB in this process. As shown in Fig. 7A and B, TNF-α treatment resulted in an obvious increase in the nuclear translocation of NF-κB and the subsequent EMT process, a specific NF-κB inhibitor Bay 11-7082 was used to suppress the nuclear translocation of NF-κB and its downstream gene transcription. The results showed that Bay 11-7082 suppressed the upregulation of NLRP3 in HCT116 and HT29 cells induced by TNF-α or TGF-β1 (Fig. 7C and D). Meanwhile, the depletion of NF-κB reduced EMT process induced by TNF-α or TGF-β1, as verified by the slight increase in E-cadherin expression and significant reduction in vimentin expression and chronic renal injury [28,29]. Alternatively, NLRP3 could also play an inflammasome-independent role as recently suggested in models of kidney and heart injury [21,30]. As shown in Fig. S1A, the endogenous expressions of pro-caspase-1 and pro-IL-1β were much higher in SW620 cells than that in HCT116 and HT29 cells, and much less cleaved caspase-1 and IL-1β were detected in these three colon cancer cells. To evaluate the role of NLRP3 in EMT, we measured the cleavage of pro-caspase-1, which was regulated by NLRP3 inflammasome activation, in HCT116 and HT29 cells upon TNF-α or TGF-β1 treatment. As shown in Fig. 3A and B, the expression of pro-caspase-1 was upregulated, but cleaved caspase-1 could not be detected in HCT116 and HT29 cells treated with TNF-α or TGF-β1 for 48 h. No cleaved caspase-1 and IL-1β were detected at 0 h, 3 h, 6 h and 12 h prior to EMT of HCT116 and HT29 cells, despite the slight increase of pro-caspase-1 (Fig. S1B and C). As a positive control, NLRP3 inflammasome could be activated in HCT116 and HT29 cells treated with LPS and ATC (Fig. S2). In addition to the cleavage of pro-caspase-1, another hallmark of inflammasome activation is the formation of ASC speck, which is essential for NLRP3 activation [31]. As seen in Fig. 3A and B, ASC speck formation was not found in HCT116 and HT29 cells treated with TNF-α or TGF-β1. To further exclude the possibility that the ASC-dependent inflammasome activation was involved in the process of EMT in our model, we have detected the role of ASC in EMT-related markers. As shown in Fig. 5, the EMT-related markers were not influenced by siASC in HCT116 and HT29 cells. In addition, IL-converting Enzyme/Caspase-1 Inhibitor VX-765, which has been reported to inhibit ICE/caspase-1-mediated IL-1 production [32], was utilized to analyze whether caspase-1 activity was required for the intermediate pathway of NLRP3 activation by extracellular TNF-α or TGF-β1. Inhibition of caspase-1 by VX-765 (10 μM) did not abolish the changes of EMT-related markers in HCT116 and HT29 cells in response to TNF-α or TGF-β1 (Fig. 3C and E), which is consistent with the real-time PCR assessments (Fig. 3D and F). Together, NLRP3 was involved in TNF-α or TGF-β1-induced EMT in an inflammasome-independent way which did not require the formation of ASC speck and the cleavage of pro-caspase-1.

3.3. Knockdown of NLRP3 inhibits EMT of colorectal cancer cells

To further verify the major roles of NLRP3 in TNF-α or TGF-β1-induced EMT, NLRP3 knockdown plasmid (NLRP3 KD) was used to reduce NLRP3 expression in HCT116, HT29 and SW620 cells (Figs. 4 and 5). HCT116 and HT29 cells underwent EMT when treated with TNF-α or TGF-β1, and acquired a complete mesenchymal-to-epithelial transition (MET)-like morphological changes in mesenchymal-like SW620 cells. Loss of vimentin and gain of E-cadherin were further confirmed by immunofluorescence, western blot, and real-time PCR assays (Figs. 4C and 5E and F). In addition, NLRP3 depletion also remarkably inhibited MMP9 expression at protein level (Fig. 5A, B and E).

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Furthermore, nuclear localization of NF-κB in mesenchymal-like SW620 cells was also blocked by Bay 11-7082, resulting in the decreased expression of NLRP3 and vimentin, and increased expression of E-cadherin (Fig. 7E). Then we examined the association between NLRP3 and Snail1 at protein and mRNA levels (Figs. 7F and S4). TNF-α or TGF-β1 treatment resulted in an obvious increase in Snail1 expression in HCT116 and HT29 cells. In addition, NLRP3 knockdown decreased the expression of Snail1 in HCT116 and HT29 cells treated with TNF-α or TGF-β1. Moreover, similar to HCT116 and HT29 cells, depletion of NLRP3 markedly attenuated the expression of Snail1 in SW620 cells. Taken together, these results demonstrated that the effect of NLRP3 protein on EMT was mediated by NF-κB signaling and the Snail1 expression was associated with NLRP3.

4. Discussion

EMT is accompanied by characteristics of malignancy, such as invasion and metastasis [5,36,37]. Metastasis is responsible for as much as 90% of cancer-associated mortality, including colorectal carcinoma [38]. In this study, we found that NLRP3 was required for the EMT process in colon cancer cells. NLRP3 is highly expressed in mesenchymal-like SW620 cells and upregulated during EMT in two epithelial-like cell lines HCT116 and HT29. Moreover, knockdown of NLRP3 resulted in the reversal of EMT in HCT116 and HT29 cells, and the mesenchymal characteristics of NLRP3-depleted SW620 cells were also partly changed. Interestingly, the effect of NLRP3 on EMT was independent of caspase-1 cleavage or ASC speck formation, indicating an inflammasome-independent function of NLRP3 in EMT of colon cancer cells.

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Fig. 4. The influence of NLRP3 deficiency on morphology in colon cancer cells. (A and B) HCT116 and HT29 cells were transfected with NLRP3 plasmid (described as NLRP3 KD) for 24 h, with or without the treatment of TNF-α or TGF-β1 (10 ng/ml) for 48 h. Cell morphological changes associated with EMT were shown in the phase contrast image. Immunofluorescence images of cells showed the localization of E-cadherin, vimentin and NLRP3. Fluorescence images of cells showing reorganization of actin cytoskeleton by staining with FITC-phalloidin. (C) The same detections were also performed in SW620 cells transfected with NLRP3 plasmid for 48 h. Scale bars: 20 mm.

Fig. 5. Knockdown of NLRP3 affects EMT-related markers. (A and B) HCT116 and HT29 cells were transfected with NLRP3 plasmid for 24 h, with or without the treatment of TNF-α or TGF-β1 (10 ng/ml) for 48 h. Western blot was performed to detect the expressions of E-cadherin, vimentin, MMP9 and NLRP3 using the corresponding antibodies in HCT116 and HT29 cells. (C and D) Real-time PCR analysis was performed to detect the expressions of E-cadherin, vimentin, and NLRP3 in HCT116 and HT29 cells. (E) The expressions of E-cadherin, vimentin, MMP9 and NLRP3 were determined by western blot in control and NLRP3 knockdown SW620 cells. (F) The expressions of E-cadherin, vimentin and NLRP3 were determined by real-time PCR in control and NLRP3-knockdown SW620 cells. Values are expressed as mean ± SD (n = 3). *P < 0.05, **P < 0.01; ***P < 0.001.

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NLRP3, a functional component of the inflammasome, together with ASC and caspase-1, regulates the maturation of IL-1β and IL-18 in immune cells [39]. Previous studies demonstrated that NLRP3 in non-immune cells also plays an important role in acute [21] and chronic kidney injury [40]. NLRP3 expressed in colon cancer cells has been found to be implicated in cell proliferation [14]. Our data showed that NLRP3 was required in EMT of HCT116 and HT29 cells. The ability of TNF-α or TGF-β1 to induce the expression of vimentin and MMP9 and to suppress E-cadherin was impaired in NLRP3-knockdown colon cancer cells. These findings agree with previous study that in epithelial cells NLRP3 was increased and found to play a role in TGF-β1-mediated EMT [23]. However, we should note that there are other factors that mediate the EMT in colon cancer cells in addition to NLRP3, and the NLRP3 inflammasome also regulates IL-1β release, which then induce EMT process [41,42].

Several reports have identified the inflammasome-independent functions of NLRP3. Previous studies showed that NLRP3 itself could suppress lupus-like autoimmunity by driving the immunosuppressive effects of TGF-β receptor signaling [43]. It was also suggested that NLRP3 could play a role in acute ischemia-reperfusion injury independent of cleaved caspase-1 [21]. Recently, NLRP3 in epithelial cells was described to target mitochondria in the development of cardiac fibrosis associated with EMT independently of NLRP3 inflammasome [24]. Consistent with these studies, the novel finding in our study was that NLRP3, independent of its ability to form a caspase-1-activating inflammasome with ASC, was required for the EMT process in colon cancer cells. Compared with macrophages, we detected lower expression of inflammasome components such as NLRP3 and caspase-1 in colon cancer cells, indicating a limited capacity to form functional inflammasome in the EMT process. In addition, inhibition of caspase-1/IL-1β signaling by VX-765 did not affect the changes of EMT-related genes such as E-cadherin and vimentin.

Nevertheless, the exact mechanism by which NLRP3 regulates EMT remains elusive. Our study suggested that the upregulation of NLRP3 in TGF-β1 or TNF-α-induced EMT was dependent on NF-κB signaling. TGF-β1 or TNF-α can induce EMT and invasion via the activation of transcription factor NF-κB. A correlation between the treatment of TGF-β1/TNF-α and activity of NF-κB has been seen in several tumor cell lines [44,45]. We should note that despite the lack of effect of TGF-β in HCT116 cells, TNF-α, as another common cytokine, could induce EMT of HCT116 cells through NF-κB signaling pathway. It has been demonstrated that NF-κB activation...
could increase the expression of inflammasome components such as NLRP3 [46]. These were the reasons why the mechanisms of NF-κB and NLRP3 in EMT were so similar. In our study, inhibition of the nuclear translocation of NF-κB by Bay 11-7082 could decrease NLRP3 expression and reverse the EMT process, suggesting that NF-κB plays a key role in TGF-β1 or TNF-α-induced EMT. Moreover, the effect of NLRP3 knockdown on EMT process resulted in the downregulation of transcription factor Snail1, which repressed the expression of E-cadherin, and indirectly increased the expression of mesenchymal markers [47]. It has been recently described that NLRP3 can act as a transcription factor to enable Th2 lymphocyte differentiation [48]. Then we performed real-time PCR in Ctrl or NLRP3KD cells to evaluate the effect of NLRP3 on Snail1 mRNA expression. NLRP3 Knockdown in three cells lines decreased the Snail1 mRNA expression, but the nuclear localization of NLRP3 was not detected in the process of EMT in HCT16 cells (Fig. S5), excluding the possibility of NLRP3 to be a transcription factor in our model. Previous findings demonstrated that NLRP3−/− mice exhibited diminished Stat3 expression in an inflammasome-independent way [22], and the overexpression of Stat3 could enhance Snail expression [49]. Hence, we speculated that NLRP3-Stat3-Snail axis might be involved in the EMT process.

In summary, our data provided critical insights into the role of NLRP3 in EMT. These findings identified a novel inflammasome-independent function of NLRP3 in TGF-β1 or TNF-α-induced EMT of HCT116 and HT29 cells, proposing a potential therapeutic target for the treatment of colon cancer metastasis.

Conflicts of interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.yexcr.2016.03.009.

References