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Role of basigin in adaptor protein mediated signaling pathways

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Abstract. The potential role of basigin/EMMPRIN in activation of NF-i<B and AP-1signaling pathway was investigated. The MyD88- and TRIF-dependent activation of NF-i<B signaling pathways were inhibited by basigin specific antibody and siRNA. TRIF dependent AP-1 activation was also inhibited by basigin specific antibody and siRNA. Taken together, the current study suggests that basigin regulates the activation of MyD88- and TRIF-dependent signaling pathways, and warrants further investigation in these basigin mediated cellular processes.

Keywords: Basigin, Signal transduction, NF-i<B, MyD88, TRIF, AP-1

1 Introduction

Basigin is involved in a variety of pathphysiological functions including regulation of lymphocyte responsiveness and cellular proliferation. The interaction between basigin and cyclophilin A, for example, initiates activation of ERK 1/2 signaling and induces the expression of IFITM1 through ERK and PIK [1-2]. Basigin and syndecan association induces activation of MAPK, which promotes cell adhesion and chemotaxis [3]. Recently we reported basigin cascades a survival signaling through Wnt/β-catenin-dependant in CSC-like cells [4]. These evidences demonstrate that basigin plays a key regulatory role in cell signaling related with inflammatory diseases and cancers. Herein, we have investigated the potential role of basigin in MyD88 and TRIF adaptor protein dependent activation of signaling pathway. We have identified that basigin induces MyD88- or TRIF-dependent activation of NF-i<B and AP-1. To our knowledge, this is the first time to report the role of basigin in adaptor protein mediated activation of signal transduction, and provides additional insights of intracellular events of dynamic nature of basigin.

2 Materials and Methods

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2.1. Luciferase reporter assay

HEK293 cells were maintained in DMEM containing 10% FBS (Hyclone) and 100μg/mL streptomycin-penicillin (Gibco). Wild types of MyD88 and TRIF were obtained from Addgene (Cambridge, MA). Luciferase constructs containing NF-κB or AP-1 binding promoters and assay were conducted as we previously reported [5]. Briefly, subconfluent HEK293 was transfected with 0.75μg NF-κB-Luc or AP-1-Luc reporter gene with or without 0.75μg MyD88 or TRIF, as well as 3-galactosidase-Luc plasmid (0.15 μg). Cells were then treated with or without anti-basigin antibody (Genetex) for 8hr, and lysed with ice-cold 10mM KH_2PO_4/1mM EDTA. The luciferase activity was measured using the Luciferase Assay System (Promega) and VictorX4 luminometer (PerkinElmer). The experiments were carried out in quadruplet for each sample, and the luciferase activities were normalized to the 3-galactosidase activity of the internal control.

2.2. Knockdown of basigin protein expression by basigin directed siRNA

Method for gene silencing of basigin was essentially as same as the luciferase reporter assay described above, except for the following modifications. Cells were transfected with NF-κB or AP-1 luciferase reporter and plasmid harboring MyD88 or TRIF with and without 400nM chemically synthesized siRNA (Genolution) for basigin or negative control siRNA in complex with Lipofectamine according to the manufacturer's instructions. Cells were lysed and the luciferase activity was analyzed as described above.

3 Results

3.1. Basigin affects the activation of NF-κB signaling cascade through MyD88

Evaluation of endogenous basigin expression level by flow cytometry and western blot analysis showed that basigin was readily detectible in HEK293, but not in CHO cell line (data not shown). Hence, the subsequent studies reported herein were conducted using HEK293 cell line.

Activation of NF-κB driven luciferase activity was observed only for cells co-transfected with MyD88 (Figure 1A). And addition of anti-basigin antibody resulted in inhibition of MyD88 mediated NF-κB activation, suggesting that basigin may funnel through MyD88 to activate NF-κB signaling. To further investigate potential association between basigin and MyD88 leading canonical NF-κB activation, we analyzed the MyD88 mediated NF-κB luciferase activity following basigin specific siRNA treatment. About 85% inhibition of luciferase activity was observed by basigin gene knockdown while the control siRNA had no effect on the MyD88 mediated NF-κB activation (Figure 1B). Taken together, these results indicate that basigin regulates NF-κB activation through MyD88 signaling.

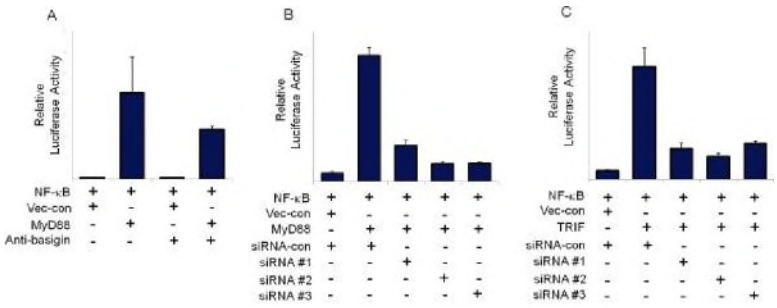

Fig. 1. Effect of basigin on MyD88 induced NF-idB activation by anti-basigin antibody (*A*) and by basign-specific siRNA (*B*), and on TRIF induced NF-idB activation by basigin specific siRNA (*C*). Data shown are representative of 3 independent experiments expressed as the mean+SEM. The empty parent vector corresponding to the MyD88 and TRIF was used as a negative control vector.

3.2. Basigin affects the activation of TRIF dependent signaling pathways

Since TRIF associates with TLR families, the involvement of basigin in the TRIF-mediated NF-idB signaling was also investigated. Cells co-transfected with TRIF showed a strong induction of the NF-idB driven luciferase activity comparable to the corresponding activation by MyD88 (Figure 1C). And TRIF induced NF-idB luciferase activity was also blocked by the basigin-specific siRNA, which suggests that basigin also mediates TRIF- dependent NF-idB activation.

Activation of AP-1 signaling in Rac-1 dependent manner by basigin was also reported [6]. Hence, we further sought to investigate if basigin mediates AP-1 activation in MyD88- and TRIF-dependant signal transduction. As shown in Figure 2, cells treated with the basigin-specific siRNA had ~30% reduced AP-1 activity mediated by MyD88, suggesting potential inhibition of intrinsic AP-1 activation by basigin knockout. However, a strong enhancement of AP-1 activation by TRIF was significantly reduced by basigin-specific siRNA. These observations suggest that basigin also regulates AP-1 activity in a TRIF-dependant manner.

4 Discussion

Evaluations of signaling pathways have demonstrated an association between basigin and the p38 MAPK and ERK signaling pathways [1]. Recently, we also demonstrated that the suppression of basigin expression results in activation of GSK3 and down-regulation of Wnt/β-catenin signaling pathway [4]. In this study we demonstrated the downstream signaling association between basigin and MyD88 on NF-idB activation by basigin-specific neutralizing antibody or siRNA. Our observation also suggests that association of basigin and TRIF leads to the canonical NF-idB activation as evidenced by reduction of TRIF mediated NF-idB activation by basigin-specific siRNA. In addition, AP-1 activation is triggered through basigin in a

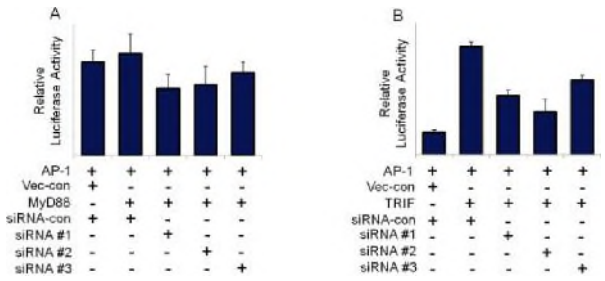

Fig. 2. Effect of basigin on AP-1 activation mediated by MyD88 (***A***) and TRIF (***B***). Data shown are representative of 3 independent experiments as described for Figure 1.

TRIF dependent, but independent of MyD88, as reported for TLR signaling pathway [7]. Current study provides the first evidence that basigin is coordinately associated with AP-1 activation in the TRIF-dependant manner.

In summary, our results show that basigin-mediated activation of signaling pathways is through either MyD88- or TRIF-dependent manner, and provides new insight into the basigin-mediated cellular functions.

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