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The SH3 domain distinguishes the role of I-BAR proteins IRTKS and MIM in chemotactic response to serum

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ABSTRACT

The family of inverse BAR (I-BAR) domain proteins participates in a range of cellular processes associated with membrane dynamics and consists of five distinct members. Three of the I-BAR proteins, including insulin receptor tyrosine kinase substrate (IRTKS), contain an SH3 domain near their C-termini. Yet, the function of the SH3 domain of IRTKS remains uncharacterized. Here we report that in contrast to MIM, which is a prototype of I-BAR proteins and does not contain an SH3 domain, IRTKS promoted serum-induced cell migration along with enhanced phosphorylation of mitogen activated kinases Erk1/2 and p38, and activation of small GTPases Rac1 and Cdc42. In addition, cells overexpressing IRTKS exhibited an increased polarity characterized by elongated cytoplasm and extensive lamellipodia at leading edges. However, a mutant with deletion of the SH3 domain attenuated both cellular motility and p38 phosphorylation but had little effect on Erk1/2 phosphorylation. Also, a chimeric mutant in which the N-terminal portion of MIM is fused with the C-terminal IRTKS, including the SH3 domain, was able to promote chemotactic response to serum and cellular polarity. In contrast, a chimeric mutant in which the N-terminal IRTKS is fused with the C-terminal MIM failed to do so. Furthermore, treatment of cells with SB203580, a selective inhibitor of p38, also neutralized the effect of IRTKS on cell migration. These data indicate that the SH3 domain distinguishes the function of IRTKS in promoting cell migration and inducing signal transduction from those of SH3-less I-BAR proteins.

1. Introduction

Cell migration is fundamental to development, wound healing, cell-cell communication and immune surveillance, and is associated with extensive membrane dynamics, including extension at leading edges, generation of new adhesion sites, and detachment of adhesions at cell rear position [1]. Many of these cellular processes are subjected to the control by Rho-like small GTPases, such as Rac1 and Cdc42, which direct the process of cell polarization and directional migration through their effects on the assembly of the actin cytoskeleton [2]. Also, MAP kinases Erk1/2 and p38 have been implicated in the migration of various types of cells [3]. In addition to these well-characterized signaling molecules, There is emerging evidence for the important role of a protein family that shares a Bin-Amphiphysin-Rvs (BAR) domain in cell migration and cell shape changes [4]. The BAR domain is a dimeric motif that binds to phospholipid membranes through a curved interface. Depending on the shape of the interface, the BAR proteins can be divided into three subfamilies, classical BAR, F-BAR and I-BAR domain proteins. While the former two subfamilies induce membrane invagination, the I-BAR domain has a concave shape and induces membrane protrusion [5]. The mammalian genomes contain five I-BAR domain genes, encoding IRSp53, MIM, ABBA, IRTKS and PINKBAR, respectively [6]. Among them, IRTKS, IRSp53 and PINKBAR are distinguished from others by having an SH3 domain. The best characterized function of the I-BAR protein-associated SH3 domain is the one with IRSp53, which interacts with proline rich sequences of a wide range of actin cytoskeleton-associated proteins, including

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WAVE2 [7], Mena [8], Dia1 [9] and Eps8 [10]. As these proteins are implicated in the assembly of actin filaments in the cortex such as lamellipodia at cell leading edges, the SH3 domain provides presumably a functional link between IRSp53 and the actin dynamics.

In contrast to IRsp53, the function of the SH3 domain of IRTKS, which is also known as brain-specific angiogenesis inhibitor 1-associated protein 2-like 1 (BAIAP2L1), remains elusive. Although IRTKS is closely related to IRSp53, its SH3 domain is not apparently required for its recruitment to lamellipodia [11]. Nevertheless, IRTKS may use its SH3 domain to link to the actin cytoskeleton as evidenced by its ability to *Escherichia coli* secreted F-like protein encoded on prophage U, which triggers the actin assembly to facilitate bacterial invasion into host cells [12]. In addition, IRTKS is implicated in plasma membrane dynamics and actin bundling associated with cell shape changes, migration and proliferation [5]. Mice with depletion of the IRTKS gene showed insulin resistance symptoms such as glucose intolerance, hyperglycemia, insulin less-sensitivity, hyperinsulinemia, and excessive production of hepatic glucose [13]. Furthermore, IRTKS suppresses innate immune responses against RNA virus through the Rig-IMAVs signaling pathway, thereby down-regulating extravagant inflammation [14]. IRTKS is also involved in tumor progression and has been reported to inhibit the p53 induced apoptosis by direct regulating its transcriptional activity [15]. However, there is little known about the cellular function of IRTKS that is relevant to these reported phenomena.

As an effort to understand the distinct function of I-BAR proteins, we compared IRTKS with MIM and found that while MIM inhibits cell migration in response to serum, IRTKS promotes chemotactic response and cell polarization. Importantly, we found that the IRTKS-mediated cell migration and polarization is dependent upon its SH3 domain. Thus, the SH3 domain is the primary motif that functionally distinguishes I-BAR domain proteins.

2. Materials and methods

2.1. Cell culture and DNA transfection

HeLa cells were cultured under 5% CO_2, 37 °C in Dulbecco's modified Eagle's medium (DMEM) (Corning, NY) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT) and 100 unit/ml Penicillin and Streptomycin. DNA transfection was performed with cells at 90% confluence by using FuGene HD Transfection Reagent (Active Motif, Carlsbad, CA) with the protocol recommended by manufacturer's instruction. Stable transfected cells were selected in the medium containing 0.5 mg/ml G418 for two weeks with constant medium changes every three days. The selected cells were pooled together and used for all the analyses as described.

2.2. Plasmids

Plasmids encoding MIM-GFP and GFP were prepared as described previously [16]. pIRTKS-GFP was synthesized and cloned into the vector pEZ-M98 by Genecopoeia (Gaithersburg, MD). pIRTKSΔSH3, which encodes a protein with a deletion of the SH3 domain (aa 342–399), was prepared by using a QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA) using pIRTKS-GFP as the template. To prepare pIRTKS-I-BAR-MIM-CT-GFP, a DNA sequence that has an open reading frame corresponding to the human IRTKS protein from aa 1 to 231 and human MIM protein from aa 235 to 755 was synthesized by GeneScript (Nanjing, China) and cloned into pUC57 vector. This DNA sequence is also flanked by a HindIII site at the 5′ and a BamH1 site at the 3′, respectively. After digestion with HindIII and BamH1, the insert was subcloned into pEZ-M98. To prepare pMIM-I-BAR-IRTKS-CT-GFP, a DNA sequence that is flanked by HindIII and BamH1 sites and has an open reading frame corresponding to human MIM protein from aa 1 to 250 and human IRTKS protein from aa 250 to 511 was synthesized by GeneScript. This DNA fragment was also subcloned into pEZ-M98 as above. For all the mutants, the fidelity of the mutations was confirmed by DNA sequencing.

2.3. Analysis of phosphorylated p38 and Erk1/2

The level of phosphorylated MAP Kinases p38 and Erk1/2 was measured by Western blot assay using antibodies against specifically pp38 and pErk1/2, as described previously [3].

2.4. Rac1 and Cdc42 activation assay

To measure GTP-Rac1 and GTP-Cdc42, equal amount of cell lysates was precipitated with 20 μl 50% GST-PAK-CRIB agarose beads (Millipore, MA). Samples were separated by 15% SDS-PAGE and subjected to Western blot using antibodies against Rac1 or Cdc42 [3].

2.5. Cell migration assay

Cell migration was evaluated by Transwell assay as described previously [3].

2.6. Analysis of cell morphology

Images of cells were captured by digital camera QImaging MicroPublisher 5.0 RTV that is equipped on Nikon Eclipse TE2000-U microscope and controlled by MetaMorph software. Cell elongation factor was determined according to Frances [17]. Briefly, the long axis was defined as the longest length of the cell, and the length across the nucleus in a direction perpendicular to the long axis was recognized as the short axis. The elongation factor was defined as the ratio of two axes. Both axes were measured by ImageJ software.

2.7. Statistical analysis

All the data were subjected to two tailed Student's t-test by using GraphPad Prism software. A difference with a P value < 0.01 was considered as statistically significant.

3. Results

3.1. IRTKS promoted cell migration in response to serum

To evaluate the effect of IRTKS on cell motility, cells expressing IRTKS-GFP and cells expressing GFP only were subjected to Transwell assay for chemotactic response to serum at different concentrations for various times. In the absence of serum, less than 2% of both cell types was migrated in 16 h (Fig. 1A). In the presence of serum, both control cells and IRTKS expressing cells showed significant motility responses in a dose dependent manner, which reached to a maximal level at 15% serum (Fig. 1A). However, the number of migrated IRTKS-GFP cells was nearly 2 and half of that of control cells at the maximal level. When cell migration towards 15% serum was analyzed for different times, IRTKS-GFP cells also showed a greater migration rate than did control cells during the period from 8 to 24 h (Fig. 1B). As a comparison, we also analyzed cells expressing GFP-tagged MIM protein. As shown in Fig. 1C and D, MIM-GFP cells showed a lower response to serum under the same condition compared with cells expressing GFP only. This

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