The International Journal of Biochemistry & Cell Biology 50 (2014) 47–54

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Integrin linked kinase (ILK) regulates podosome maturation and stability in dendritic cells☆

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ARTICLE INFO

Article history:
Received 14 March 2013
Received in revised form
30 December 2013
Accepted 28 January 2014
Available online 6 February 2014

Keywords:
ILK
Dendritic cell
Podosome
WASP
PI3K

ABSTRACT

Podosomes are integrin-based adhesions fundamental for stabilisation of the leading lamellae in migrating dendritic cells (DCs) and for extracellular matrix (ECM) degradation. We have previously shown that soluble factors and chemokines such as SDF 1-α trigger podosome initiation whereas integrin ligands promote podosome maturation and stability in DCs. The exact intracellular signalling pathways that regulate the sequential organisation of podosomal components in response to extracellular cues remain largely undetermined. The Wiskott Aldrich Syndrome Protein (WASP) mediates actin polymerisation and the initial recruitment of integrins and associated proteins in a circular configuration surrounding the core of filamentous actin (F-actin) during podosome initiation. We have now identified integrin linked kinase (ILK) surrounding the podosomal actin core. We report that DC polarisation in response to chemokines and the assembly of actin cores during podosome initiation require PI3K-dependent clustering of the Wiskott Aldrich Syndrome Protein (WASP) in puncta independently of ILK. ILK is essential for the clustering of integrins and associated proteins leading to podosome maturation and stability that are required for degradation of the subjacent extracellular matrix and the invasive motility of DCs across connective tissue barriers.

We conclude that WASP regulates DCs polarisation for migration and initiation of actin polymerisation downstream of PI3K in nascent podosomes. Subsequently, ILK mediates the accumulation of integrin-associated proteins during podosome maturation and stability for efficient degradation of the subjacent ECM during the invasive migration of DCs.

1. Introduction

The directed migration of dendritic cells (DCs) through peripheral tissues is crucial for their function as T and B cell activators during the immune response and failure of appropriate migration can result in immunodeficiency, autoimmune responses or chronic inflammation (Angeli and Randolph, 2006). Many of the chemokines regulating DC chemotaxis are known but there is an incomplete understanding of the regulation of the cytoskeletal and adhesion remodelling that drives DC motility. We and others have shown an absolute requirement of integrin and actin-based adhesive structures called podosomes for normal migration and chemotactic responses of immature DCs (Calle et al., 2006a, 2008; Linder, 2009). Podosomes are highly dynamic adhesions involved in migration of cells that have to cross and invade boundaries (Calle et al., 2006a, 2008). They are characterised by a distinctive organisation, consisting of a core of F-actin surrounded by a circular array of integrins and integrin associated proteins (Calle et al., 2006a, 2008; Linder, 2009). We have previously shown that chemotactic factors such as SDF 1-α trigger podosome initiation whereas integrin ligands including fibronectin and ICAM-1 promote podosome maturation and stability behind the leading edge of motile DCs (Chou et al., 2006; Monypenny et al., 2011). In the absence of

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1357-2725/$ – see front matter.
http://dx.doi.org/10.1016/j.biocel.2014.01.021

stimulation with chemotactic factors DCs remain stationary and attach on integrin ligands through focal contacts (Monypenny et al., 2011). The intracellular signalling that allows the remodelling of adhesions from focal contacts to podosomes leading to the transition from a stationary to a motile phenotype in DCs remains largely unknown (Linder, 2009; Murphy and Courtneidge, 2011).

The first step for podosome initiation involves a burst of actin polymerisation leading to the assembly of a conical core of F-actin (Luxenburg et al., 2012). This is followed by the initial organisation of integrins and integrin-associated proteins in a circular array surrounding the core of F-actin (Monypenny et al., 2011). Subsequent binding of integrins to their ligands increases podosome size by further accumulation of integrins and F-actin leading to podosome maturation and increased adhesion stability. Failure to accumulate integrins and associated proteins forming a ring around in the nascent actin cores of podosomes results in rapid podosome turnover (Macpherson et al., 2012) and abnormal degradation of the subjacent extracellular matrix (Banon-Rodriguez et al., 2011; Dovas et al., 2009).

The Wiskott Aldrich Syndrome Protein (WASP) and the WASP Interacting Protein (WIP) comprise a functional unit that regulates actin polymerisation and integrin remodelling leading to polarisation and podosome initiation in DCs (Calle et al., 2008; Chou et al., 2006; Monypenny et al., 2011) and other myeloid cells (Calle et al., 2008; Cammer et al., 2009; Chabadel et al., 2007; Jones et al., 2002; Linder, 2009). Mutations in the gene coding for WASP result in abnormal adhesion and cytoskeletal organisation of leukocytes (including lack of podosomes in myeloid cells) that largely contribute to the phenotype of clinical diseases including the Wiskott Aldrich Syndrome (WAS) and X-linked thrombocytopenia (Calle et al., 2008). In the absence of WASP or WIP DCs fail to polarise and adhere to integrin ligands by assembling focal contacts, which are unable to degrade the subjacent extracellular matrix (Banon-Rodriguez et al., 2011; Chou et al., 2006; Monypenny et al., 2011). The exact signalling pathways during podosome maturation that may sustain clustering of integrin and integrin-associated proteins around the nascent F-actin core driven by WASP/WIP in podosomes has not yet been determined.

In the present study we show that integrin linked kinase (ILK) is required for the accumulation of integrin-associated proteins in podosome rings downstream of WASP-mediated initiation of the actin core in podosomes. We report that ILK plays a key role in the regulation of the adhesive properties and the invasive motility of DCs across extracellular matrix-based barriers.

2. Materials and methods

2.1. Animals

Conditional inactivation of the ILK gene was accomplished as previously described (Herranz et al., 2012) by crossing mice carrying the floxed ILK allele (genotype: ILKfl/fl) with CreERT transgenic mice, which express Cre under the control of the cytomegalovirus promoter. Eight-week-old male mice were injected intraperitoneally with a tamoxifen solution once a day for 5 consecutive days. Animals were killed 20 days after the last injection. All animal procedures were approved by the institutional animal care and use committee from the University of Alcala, Madrid (Spain). The mouse colonies were established from mice obatined from S. Shoukat, Department of Integrative Oncology, BC Cancer Research Center, Vancouver, BC, Canada. Pathogen free C57Bl/6 mice purchased from Harlan and WASP-null mice on a C57Bl/6 background were bred in our own animal facility in pathogen free conditions. All animals were handled in strict accordance with good animal practice as defined by UK Home Office Animal Welfare Legislation, and all animal work was approved by the Institutional Research Ethics Committee (University College London, UK) and performed under project licence number 70/7024. Pathogen free WIP−/− mice from SV129/C57/BL6 mouse strain and SV129/C57/BL6 control (wild-type) mice were bred in the facilities of Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid (Spain). These mice were housed till 6–8 weeks old in a pathogen-free animal facility. Handling of mice and all manipulations were carried out in accordance with national and European Community guidelines, and were reviewed and approved by the institutional committee for animal welfare.

2.2. Cell culture

DCs were generated from mouse spleens and they were infected using lentiviral vectors as previously described (Chou et al., 2006). Briefly, spleens from 6- to 8-week-old SV129 mice were homogenised through a cell strainer to obtain a cell suspension. Cells were washed twice with RPMI (Sigma, UK) containing 1% heat-inactivated foetal bovine serum (FBS) and then resuspended in RPMI supplemented with 10% FBS, 1 mM pyruvate (Sigma, UK), 1× non-essential amino acids (Sigma, UK), 2 mM glutamine (Sigma, UK), 50 μM 2-ME (Gibco BRL), 20 ng/ml recombinant mouse GM-CSF (R&D Systems) and 1 ng/ml recombinant human TGF-β (R&D Systems) and plated at a density of 2×10^6 cells/ml in 75 cm^2 culture flasks at 37 °C in a 5% CO_2 atmosphere. After 5 days of culture, 5 ml fresh medium were added per flask and at day 8, the cells in suspension were collected, replated and kept in suspension in fresh medium. After a total of 17–18 days ex vivo, 80–90% of the cells in culture were DCs as determined by the expression of CD11c and DEC205 by FACS analysis. Cell viability before experimental assays was tested by Trypan Blue exclusion. The mouse microvascular endothelial immortalised cell line, SVEC 4-10 (O'Connell and Edidin, 1990) was obtained from the American Type Culture collection and cells were cultured using DMEM (Sigma, UK) supplemented with 10% FBS at 37 °C in a 5% CO_2 atmosphere.

2.3. Infections of DCs with lentiviral vectors

Lentiviral vector stocks were produced in 293 T cells by cotransfecting the transfer vector SFFVeGFP-WASP, the envelope plasmid pMD.G, and the packaging plasmid pCMVR8.91. 3×10^7 cells were seeded onto 150 cm^2 flasks and transfected with 10 μg DNA envelope, 30 μg DNA packaging, and 40 μg DNA transfer vector by precomplexing with 0.125 μM PEI (22 kDa) for 15 min at room temperature in Optimem. After 4 h at 37 °C, the medium was replaced with fresh DMEM 10% FCS and virus were harvested 48 and 72 h post-transfection. After filtering through a 0.45 μm-pore-size filter, the virus suspension was concentrated by centrifugation at 50,000 × *g* for 2 h at 4°C. The resulting pellet was resuspended in RPMI (Sigma) and stored at −80 °C until use. The desired number of DCs were plated on fibronectin coated coverslips (10 μg/ml) overnight in complete culture medium and then, lentivirus containing supernatant was added to the cells at an MOI between 100 and 150 and incubated for 24 h. Media was replaced for complete DC culture medium after 24 h, and cells were cultured for another 48 h to allow maximal expression of lentiviral vectors before being used in experiments.

2.4. Interference reflection microscopy (IRM) and analysis of adhesion turnover

DCs were plated on fibronectin (Sigma, UK) coated glass coverslips (10 μg/ml) in complete culture medium and incubated overnight at 37 °C in a 5% CO_2 atmosphere as previously described. Coverslips were mounted onto viewing chambers in culture medium. As previously described (Chou et al., 2006; Holt et al., 2008), interference reflection micrographs were collected