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The cortactin-binding domain of WIP is essential for podosome formation and extracellular matrix degradation by murine dendritic cells

Inmaculada Bañón-Rodríguez[a], James Monypenny[b], Chiara Ragazzini[a], Ana Franco[a], Yolanda Calle[c], Gareth E. Jones[b], Inés M. Antón[a,*]

[a] Cellular and Molecular Department, Centro Nacional de Biotecnología – CSIC, Madrid 28049, Spain
[b] Randall Division of Cell & Molecular Biophysics, King's College London, London SE1 1UL, UK
[c] Haematology Dept., King's College London, London SE5 9NU, UK

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ABSTRACT

In immature dendritic cells (DCs) podosomes form and turn over behind the leading edge of migrating cells. The Arp2/3 complex activator Wiskott-Aldrich Syndrome Protein (WASP) localises to the actin core of forming podosomes together with WASP-Interacting Protein (WIP). A second weaker Arp2/3 activator, cortactin, is also found at podosomes where it has been proposed to participate in matrix metalloproteinase (MMP) secretion. We have previously shown that WIP$^{-/-}$ DCs are unable to make podosomes. WIP binds to cortactin and in this report we address whether WIP regulates cortactin-mediated MMP activity. Using DCs derived from splenic murine precursors, we found that wild-type cells were able to localise MMPs at podosomes where matrix degradation takes place. In contrast, WIP$^{-/-}$ DCs remain able to synthesise MMPs but do not degrade the extracellular matrix. Infection of WIP KO DCs with lentivirus expressing WIP restored both podosome formation and their ability to degrade the extracellular matrix, implicating WIP-induced podosomes as foci of functional MMP location. When WIP KO DCs were infected with a mutant form of WIP lacking the cortactin-binding domain (WIPΔ110-170) DCs were only able to elaborate disorganised podosomes that were unable to support MMP-mediated matrix degradation. Taken together, these results suggest a role for WIP not only in WASP-mediated actin polymerisation and podosome formation, but also in cortactin-mediated extracellular matrix degradation by MMPs.

Introduction

Normal cell behaviour relies on the capacity to respond to environmental cues including modifications in extracellular matrix (ECM) composition. Cell attachment to the ECM induces intracellular signalling that initiates at foci of cell-matrix contacts. Such contacts are fundamental aspects of cell and tissue organisation, and are mediated through specific adhesion receptors, normally integrins, that are linked to the cytoskeleton through defined signalling pathways (Adams, 2002). During cell migration, actin-rich dynamic protrusions known as filopodia and lamellipodia are both generated and retracted at the leading edge where cells make transient cell-substratum contacts (focal complexes) that may develop into more stable adhesions (focal adhesions) linked to the actomyosin-based contractile machinery. Although these are the most investigated adhesion structures, in the case of monocytes and lineage-related leukocytes, adhesion and migration on a planar matrix is achieved through formation of podosomes. These actin-rich structures can also be induced in endothelial cells, smooth muscle cells and in Src-transformed fibroblasts (Gimona et al., 2008). The dense, filamentous actin core of podosomes contain Wiskott-Aldrich syndrome protein (WASP) and Actin Related Protein 2/3 (Arp 2/3) complex proteins surrounded by a ring of adhesion related molecules including vinculin, paxillin, talin, fimbrin, gelsolin, vimentin, and numerous adaptor molecules associated with integrin signalling (Burns et al., 2001; Linder, 2007).

In migrating macrophages, osteoclasts and dendritic cells (DCs), podosomes localise behind the leading edge, playing a role in cell polarity, locomotion and ECM degradation (Brunton et al., 2004; Calle et al., 2004). In migrating immature DCs podosomes are highly dynamic structures with a half-life between 30 s and 10 min depending upon migratory status (Burns et al., 2004). Although the mechanisms of regulation of this cyclical turnover are still largely unknown, regulated proteolysis of the underlying matrix

Abbreviations: DCs, dendritic cells; Arp, actin related protein; WASP, Wiskott-Aldrich syndrome protein; WIP, WASP interacting protein; MMP, metalloproteinase; ECM, extracellular matrix; SH3, Src homology 3; N-WASP, neural WASP; PLL, poly-L-lysine; FN, fibronectin; MT1-MMP, membrane type 1-MMP; HS1, hematopoietic lineage cell-specific protein-1.

* Corresponding author at: CNB-CSIC, Darwin 3, Campus Cantoblanco, 28049 Madrid, Spain. Tel.: +34 91 585 5312; fax: +34 91 585 4506.
E-mail addresses: ibanon@cnb.csic.es (I. Bañón-Rodríguez), james.monypenny_of_pitmilly@kcl.ac.uk (J. Monypenny), cragazzini@cnb.csic.es (C. Ragazzini), afranco@cnb.csic.es (A. Franco), yolanda.calle@kcl.ac.uk (Y. Calle), gareth.jones@kcl.ac.uk (G.E. Jones), ianton@cnb.csic.es (I.M. Antón).

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(Adams, 2002), by secreted and membrane bound matrix metalloproteinases (MMPs), at sites of podosome formation have been implicated in their function (Linder, 2007).

MMPs are endopeptidases capable of cleaving a wide range of extracellular substrates, thus regulating the activity of such moieties (Gill and Parks, 2008). MMP activity favours the release of growth factors from the cell membrane or the ECM, the cleavage of growth factor receptors from the cell surface, the shedding of cell adhesion molecules and the activation of other MMPs. It is now well established that MMP-mediated degradation contributes to essential physiological functions related to cell migration such as tissue repair, immune response, regulation of chemokine activity, diapedesis and inflammation (Clark et al., 2008).

Cortactin is an actin assembly protein that functions in both the activation and stabilisation phases of branched actin assembly by the Arp2/3 complex (Tehrani et al., 2007; Uruno et al., 2001), and it is present at sites of dynamic F-actin assembly in cellular protrusions such as lamellipodia, invadopodia (Yamaguchi and Condeelis, 2007) and podosomes (Tehrani et al., 2006; Webb et al., 2006). It has been previously shown that the inhibition of invadopodia structure assembly by cortactin depletion results in the inhibition of matrix degradation due to a failure of invadopodia formation (Artym et al., 2006). More recently it has been proposed that cortactin has a critical function promoting the secretion of MMPs in invadopodia (Clark and Weaver, 2008). Cortactin has a Src homology 3 (SH3) domain that interacts with WASP interacting protein (WIP) and this interaction increases the efficiency of cortactin-mediated Arp 2/3 complex activation of actin polymerisation in a concentration-dependent manner (Kinley et al., 2003). Apart from cortactin, WIP interacts with other actin-linked proteins including WASP, N-WASP, Nck and myosin, as well as with actin itself (Anton et al., 2007; Krzewski et al., 2006). WIP localization at podosomes was first described in aortic endothelial cells (Moreau et al., 2003) and later confirmed in monocytes, DCs and osteoclasts (Chabadel et al., 2007; Chou et al., 2006; Tsuboi, 2006). WIP is essential for podosome formation, more specifically for the formation of actin cores containing WASP and cortactin (Chou et al., 2006). However, to date no information on the potential role of WIP in protease activity and ECM degradation is available.

In the present work, we investigate the possible role of WIP in regulated proMMP2/MMP2 and proMMP9/MMP9 secretion, where cortactin seems to have an essential role, and the function of the WIP–cortactin interaction in podosome formation and function in DCs. Our results demonstrate that in contrast to WT DCs, DCs derived from WIP KO mouse spleen are able to synthesise MMPs but do not secrete these MMPs. The observed defects in podosome formation and MMP activity are restored by infection of WIP KO DCs with lentivirus encoding WIP but not by virus encoding a mutant form of WIP lacking the cortactin-binding domain. We conclude that WIP is necessary for podosome formation and its interaction with cortactin is essential for ECM degradation.

Materials and methods

Cell culture

DCs were generated from mouse spleens as previously described (West et al., 1999). Briefly, spleens from 6- to 12-week-old WT and WIP KO SV129/BL6 mice (Anton et al., 2002) 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 DC medium (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, 7 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 (data not shown). Cell viability before experimental assays was tested by Trypan Blue exclusion. The work was carried out in accordance with EC Directive 86/609/EEC for animal experiments. Murine DCs, unlike murine platelets that express both cortactin and HS1 (Thomas et al., 2007), only express cortactin.

The 293T cells used for lentivirus production were cultured using DMEM (Sigma, UK) supplemented with 10% FBS and 2 mM glutamine at 37 °C in a 5% CO_2 atmosphere. The THP-1 human monocytic leukaemia cell line was cultured using RPMI (Sigma, UK) supplemented with 10% FBS and 2 mM glutamine at 37 °C in a 5% CO_2 atmosphere. This cell line expresses the variant cortactin HS1 (hematopoietic lineage cell-specific protein-1) but does not express cortactin (data not shown).

Reagents and antibodies

Monoclonal antibody to cortactin (clone 4F11) was purchased from Millipore and monoclonal antibody to human HS1 (clone 9/HS1) from BD Transduction Laboratories. Anti-vinculin (hVIN-1) was purchased from Sigma. Cy5-conjugated anti-mouse IgG antibody and Alexa Fluor 568 phalloidin and Alexa Fluor 488 phalloidin were obtained from Molecular Probes. GAPDH monoclonal antibody was purchased from AbD Serotec. GFP antibody (11814460001) was purchased from Roche. MMP2 rabbit antibody (MMP2/2C1) was from Abcam, MMP9 (GE-213) was from Genetex and MT1-MMP (M5808) from Sigma. Horseradish peroxidase (HRP)-labelled anti-mouse and anti-rabbit antibodies were purchased from Dako. Rhodamine B-isothiocyanate (R-1755) and gelatin (type A from porcine skin, G-2500) were purchased from Sigma and rhodamine fibronectin (FN) from Cytoskeleton.

Zymography

In vitro differentiated murine DCs were plated in 24-well plates (5×10^4 per well) previously coated with 10 μg/ml poly-L-lysine (PLL) or 10 μg/ml FN. After 2 h, growth medium was substituted for serum-free medium. Cells were maintained overnight (ON) at 37 °C in a 5% CO_2 atmosphere. After 24 h, medium was collected and boiled with non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) buffer. SDS-PAGE fractionation of protein was carried out on 0.75 mm, 10% (w/v) acrylamide gels containing 0.075% (w/v) gelatin. Gels were soaked in 2.5% (v/v) Triton X-100 with gentle shaking at room temperature (RT) for 30 min to remove the SDS and to allow protein renaturation. The gel was rinsed once with substrate buffer (0.05 M Tris–HCl, pH 8.0; 1 mM $CaCl_2$; 0.02% (w/v) NaN_3) and incubated in fresh substrate buffer at 37 °C ON. After incubation the gel was stained in Coomassie blue stain with gentle shaking for 30 min, followed by destaining in distilled water until suitable visualisation of digested gelatin bands was achieved.

Quantification of pixels intensity in zymographies was performed using Image J Software.

Western blotting

DCs plated onto PLL or FN were lysed in lysis buffer containing 0.1% Triton X-100, 150 mM NaCl, 50 mM Tris–HCl pH 7.4, 1 mM EDTA, 1 mM EGTA, with protease (Complete from Merck) and