European Journal of Cell Biology 90 (2011) 172–180

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.de/ejcb

Dynamic membrane remodeling at invadopodia differentiates invadopodia from podosomes

Vira V. Artym[a,b,*], Kazue Matsumoto[b], Susette C. Mueller[a], Kenneth M. Yamada[b]

[a] Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University Medical School, Washington, DC 20057, USA
[b] Laboratory of Cell and Developmental Biology, NIDCR, NIH, Bethesda, MD 20892-4370, USA

ARTICLE INFO

Article history:
Received 30 March 2010
Received in revised form 1 June 2010
Accepted 22 June 2010

Keywords:
Invadopodia
Podosomes
Cortactin
Focal adhesions
Invasion

ABSTRACT

Invadopodia are specialized actin-rich protrusions of metastatic tumor and transformed cells with crucial functions in ECM degradation and invasion. Although early electron microscopy studies described invadopodia as long filament-like protrusions of the cell membrane adherent to the matrix, fluorescence microscopy studies have focused on invadopodia as actin–cortactin aggregates localized to areas of ECM degradation. The absence of a clear conceptual integration of these two descriptions of invadopodial structure has impeded understanding of the regulatory mechanisms that govern invadopodia. To determine the relationship between the membrane filaments identified by electron microscopy and the actin–cortactin aggregates of invadopodia, we applied rapid live-cell high-resolution TIRF microscopy to examine cell membrane dynamics at the cortactin core of the invadopodia of human carcinoma cells. We found that cortactin docking to the cell membrane adherent to 2D fibronectin matrix initiates invadopodium assembly associated with the formation of an invadopodial membrane process that extends from a ventral cell membrane lacuna toward the ECM. The tip of the invadopodial process flattens as it interacts with the 2D matrix, and it undergoes constant rapid ruffling and dynamic formation of filament-like protrusions as the invadopodium matures. To describe this newly discovered dynamic relationship between the actin–cortactin core and invadopodial membranes, we propose a model of the invadopodial complex. Using TIRF microscopy, we also established that – in striking contrast to the invadopodium – membrane at the podosome of a macrophage fails to form any process- or filament-like membrane protrusions. Thus, the undulation and ruffling of the invadopodial membrane together with the formation of dynamic filament-like extensions from the invadopodial cortactin core defines invadopodia as invasive superstructures that are distinct from the podosomes.

Introduction

Transformed and metastatic tumor cells use invadopodia – specialized proteolytically active, actin-rich cell protrusions – to degrade and invade surrounding extracellular matrix (ECM). Initially, invadopodia were identified as stable actin-rich protrusions emanating from the ventral cell membrane of transformed cells invading fluorescent two-dimensional (2D) matrix, which were shown to localize to the areas of matrix degradation (Chen, 1989). Culturing transformed cells on thin 2D layers of purified ECM proteins revealed that invadopodia degrade fibronectin, collagen type I, collagen type IV, and laminin (Kelly et al., 1994). Electron microscopy studies of transformed cells invading these thin 2D matrices described invadopodia as regions of the ventral membrane protruding toward the ECM substrate with a central electron-dense cytoplasmic core or with the central core extending into long, fine filament-like protrusions containing a meshwork of microfilaments (Kelly et al., 1994; Chen, 1989). Subsequent electron microscopy studies of invasive tumor cells grown on cross-linked gelatin beads revealed that cancer cells degrade the gelatin surface of the bead and insert invadopodia into the bead as long protrusions of the ventral cell membrane (Bowden et al., 1999). Measurements of the central electron-dense core in electron micrographs indicate that its diameter can range from 0.1 μm to 0.8 μm. The individual filament-like membrane extensions originating from the invadopodial core can be more than 2 μm in length. Recent ultrastructural analysis of the invadopodia of melanoma cells using a correlative confocal light and electron microscopy approach identified invadopodia as thin filament-like protrusions originating from a ventral cell membrane invagination averaging 8 μm wide and 2 μm

Abbreviations: ECM, extracellular matrix; TIRF microscopy, total internal reflection fluorescence microscopy; 2D, two-dimensional; 3D, three-dimensional; HFF, human foreskin fibroblast; HPFN, human plasma fibronectin; FN, fibronectin.

* Corresponding author at: NIH/NIDCR, 30 Convent Dr., Room 408, Bethesda, MD 20892-4370, USA. Tel.: +1 301 451 6498; fax: +1 301 402 0897.
E-mail address: vartym@mail.nih.gov (V.V. Artym).

0171-9335/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ejcb.2010.06.006

deep (Baldassarre et al., 2003). Multiple filament-like invadopodia were shown to originate from a large ventral cell membrane invagination, indicating that invadopodia are part of an invasion superstructure.

In contrast, other research over the past two decades has characterized the invadopodium with fluorescence microscopy as a dot-like actin aggregate localized at the area of proteolytic degradation of a 2D fluorescent matrix. Functional studies identified the actin-binding protein cortactin as indispensable for invadopodial actin core formation and proposed to use cortactin as an invadopodial molecular marker in addition to actin (Artym et al., 2006). Using these invadopodia identification criteria, multiple proteins have been shown to co-localize to actin–cortactin cores of the invadopodia (Weaver, 2006; Poincloux et al., 2009; Buccione et al., 2009; Linder, 2007; Mueller et al., 2008). These invadopodia-associated proteins can be divided into four categories: (1) cell adhesion molecules, such as integrins; (2) actin and actin-associated proteins; (3) signaling proteins that include tyrosine kinases and small GTPases; and (4) soluble and membrane-bound proteases. The broad spectrum of proteins that localize to invadopodia and the function of invadopodia in ECM degradation involving directed targeting of proteases to invadopodia defines the invadopodium as a unique cellular structure characterized by coordinated interaction and interplay of cell adhesion, actin nucleation and polymerization, directed protease trafficking, endocytosis, and exocytosis. However, the identification and characterization of invadopodia structures as actin–cortactin aggregates has tended to oversimplify our understanding of invadopodia. In addition, comparisons of invadopodia to podosomes based on the similarity of their actin–cortactin cores can lead to the perception that invadopodia are the same as podosomes. Podosomes are dot-like adhesion structures of monocyte-derived cells, such as osteoclasts (Marchisio et al., 1984) and macrophages (Marchisio et al., 1987) that can also degrade ECM (Gil-Henn et al., 2007; Luxenburg et al., 2006). According to fluorescence microscopy studies, podosomes have an actin–cortactin-rich core 0.5–1 μm in diameter. This dense actin–cortactin core is surrounded by a ring of adhesion molecules and a cloud of unpolymerized actin (Gimona et al., 2008; Gimona and Buccione, 2006). Conventional electron microscopy studies of chemically fixed cells and ultrastructural studies of frozen and freeze-substituted cells have visualized the typical podosome to be densely packed with short, highly branched F-actin filaments that are surrounded by loose, long actin filaments radiating from the podosome F-actin core and connecting it with neighboring podosomes (Akisaka et al., 2008; Marchisio et al., 1984; Luxenburg et al., 2007). The entire podosome structure measures 1–2 μm in diameter. Electron microscopy of podosome sections cut perpendicularly to the ECM visualize podosomes as very short dot-like membrane protrusions; the ventral cell membrane between two podosomes is elevated above the ECM by 10–50 nm (Marchisio et al., 1984); alternatively, the ventral cell membrane can be in close apposition to the ECM separated by a uniform gap of 10 nm (Akisaka et al., 2008).

To reconcile the discrepancies between descriptions of the invadopodium as a filament-like protrusion as established by electron microscopy studies versus the invadopodium as an actin–cortactin aggregate visualized by fluorescence microscopy, we examined cell membrane dynamics at the invadopodial actin–cortactin core using total internal reflection fluorescence (TIRF) microscopy. TIRF microscopy is a technique that permits observation of an approximately 100–150 nm thick region of the ventral cell surface that comes into direct contact with the surface of a glass coverslip coated with a thin layer of 2D ECM. In this study, rapid, live-cell, high-resolution TIRF imaging of human breast carcinoma cells adherent to a 2D fibronectin (FN) matrix revealed that the cell membrane at the invadopodial actin–cortactin core undergoes rapid dynamic remodeling, and it defined the actin–cortactin core to be a part of an invasive superstructure. We suggest that for clarity, the term "invadopodial complex" be used for the invadopodial invasion superstructure that includes both the actin–cortactin core and dynamic membrane structures. We found that the initial stages of invadopodial superstructure assembly involve the formation of a primary invadopodial membrane process that extends from the ventral cell membrane lacuna toward the ECM. The tip of the invadopodial process flattens as it interacts with the 2D matrix, and it undergoes constant rapid ruffling. As the invadopodial complex matures, filament-like invadopodia form from the invadopodial process.

Our live-cell TIRF microscopy studies describe for the first time the relationship between the cortactin core and cell membrane at the invadopodia, visualize morphological changes of the cell membrane at invadopodial superstructures, and reveal the highly dynamic nature of invadopodia. In addition, parallel TIRF microscopy studies of macrophage podosomes compared to invadopodia established that, contrary to the invadopodium, the podosome membrane does not form any process- or filament-like membrane extensions or protrusions in cells on the 2D ECM. Thus, the relationship between the actin–cortactin core and the membrane in the invadopodial superstructure is different from that at the podosome. The undulation and ruffling of the invadopodial membrane together with this formation of dynamic filament-like invadopodia defines the invadopodial complex as a highly dynamic invasive superstructure that is distinct from podosomes.

Materials and methods

Materials

Human plasma fibronectin (HPFN) was prepared as described (Akiyama, 2001). Bovine serum albumin (BSA) was purchased from MP Biomedicals (Solon, OH). Phalloidin-AlexaFluor and AlexaFluor protein labeling dyes were from Invitrogen (Carlsbad, CA). Anti-vinculin antibody was from Sigma–Aldrich (St. Louis, MO), and secondary antibodies conjugated to Cy5 were from Jackson ImmunoResearch (West Grove, PA). Rat tail collagen type I was purchased from BD Biosciences (San Jose, CA).

Cell lines and transfections

A stable line of MDA-MB-231 cells transfected with wild-type c-Src (wt c-Src MDA-MB-231) was a generous gift from Dr. Toshiyuki Yoneda (Myoui et al., 2003). Cells were maintained in high glucose-DMEM (HyClone, Logan, UT) supplemented with 10% fetal bovine serum (HyClone), 2 mM L-glutamine, 10 U/ml penicillin, and 10 μg/ml streptomycin. The mouse macrophage IC-21 (TIB-186) cell line was obtained from the American Type Culture Collection (Rockville, MD). This macrophage line was cultured in RPMI-1640 (HyClone, Logan, UT) supplemented with 10% fetal bovine serum, 10 mM HEPES, 1.5 g/l sodium bicarbonate, and 4.5 g/l glucose. Human foreskin fibroblasts (HFF) were cultured in high glucose-DMEM supplemented with 10% fetal bovine serum, 10 U/ml penicillin, and 10 μg/ml streptomycin.

All cell lines were transiently co-transfected with GFP and mCherry cDNA vectors, and fluorescence of exogenous proteins was imaged at 24 or 48 h post-transfection. MDA-MB-231 cells were transfected with Lipofectamine 2000 (Invitrogen), IC-21 cells were transfected using AMAXA (Walkersville, MD), and HFF were transfected with PolyJet (SignaGen Laboratories, Ijamsville, MD) according to the manufacturers' instructions.