European Journal of Cell Biology 91 (2012) 171–179

Contents lists available at SciVerse ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.de/ejcb

Formation of atypical podosomes in extravillous trophoblasts regulates extracellular matrix degradation

Anand Patel, Philip R. Dash*

School of Biological Sciences, Hopkins Building, University of Reading, Whiteknights Campus, Reading, Berkshire RG6 6UB, UK

ARTICLE INFO

Article history:
Received 1 September 2011
Received in revised form
23 November 2011
Accepted 23 November 2011

Keywords:
Trophoblasts
Invadosome
Invasion
Extracellular matrix
Podosome
Pre-eclampsia

ABSTRACT

Throughout pregnancy the cytotrophoblast, the stem cell of the placenta, gives rise to the differentiated forms of trophoblasts. The two main cell lineages are the syncytiotrophoblast and the invading extravillous trophoblast. A successful pregnancy requires extravillous trophoblasts to migrate and invade through the decidua and then remodel the maternal spiral arteries. Many invasive cells use specialised cellular structures called invadopodia or podosomes in order to degrade extracellular matrix. Despite being highly invasive cells, the presence of invadapodia or podosomes has not previously been investigated in trophoblasts. In this study these structures have been identified and characterised in extravillous trophoblasts. The role of specialised invasive structures in trophoblasts in the degradation of the extracellular matrix was compared with well characterised podosomes and invadopodia in other invasive cells and the trophoblast specific structures were characterised by using a sensitive matrix degradation assay which enabled visualisation of the structures and their dynamics. We show trophoblasts form actin rich protrusive structures which have the ability to degrade the extracellular matrix during invasion. The degradation ability and dynamics of the structures closely resemble podosomes, but have unique characteristics that have not previously been described in other cell types. The composition of these structures does not conform to the classic podosome structure, with no distinct ring of plaque proteins such as paxillin or vinculin. In addition, trophoblast podosomes protrude more deeply into the extracellular matrix than established podosomes, resembling invadopodia in this regard. We also show several significant pathways such as Src kinase, MAPK kinase and PKC along with MMP-2 and 9 as key regulators of extracellular matrix degradation activity in trophoblasts, while podosome activity was regulated by the rigidity of the extracellular matrix.

Introduction

Trophoblasts are cells that differentiate from the blastocyst during early pregnancy and migrate into the uterus, anchoring the developing embryo and connecting with the maternal blood supply. The migration of trophoblast cells is an essential part of early pregnancy and placental development, yet the molecular mechanisms and signalling pathways which regulate this process are largely unknown. An invasive sub-population of trophoblasts – the extravillous trophoblasts – migrate as far as the myometrium, remodelling the maternal blood vessels and adapting them for the higher blood flow requirement of the fetus later in pregnancy. The importance of trophoblast migration is highlighted by the fact that complications of pregnancy such as pre-eclampsia are commonly associated with poor trophoblast invasion (Goldman-Wohl and Yagel, 2002; Kaufmann et al., 2003). Pre-eclampsia affects 5–8% of pregnancies, is characterised by hypertension and proteinuria and is a major cause of maternal death (HMSO, 1994). Offspring from pregnancies complicated by pre-eclampsia may also suffer long-term health problems, including an increased incidence of hypertension, heart disease and diabetes and mothers whose pregnancies are complicated by pre-eclampsia have a significantly increased risk of cardiovascular disease later in life including hypertension, ischaemic heart disease and stroke (Barker, 1997).

Many studies have investigated the regulation of trophoblast invasion and subsequent spiral artery remodelling (Cartwright et al., 2002; Cartwright and Wareing, 2006) however no attempt has been made to determine if trophoblasts employ specialised invasive structures such as invadopodia or podosomes, which are collectively known as invadosomes. Specialised invasive structures have been reported in cancer and monocytic cells in 2-D and more recently 3-D studies have demonstrated the importance of these structures in tissue invasion (Buccione et al., 2009; Linder, 2007). Invadosomes are actin rich membrane protrusions, similar in principle to filopodia, that protrude into and degrade the extracellular matrix (ECM). The two types of invadosome, invadopodia

* Corresponding author. Tel.: +44 118 378 7400.
E-mail address: p.r.dash@reading.ac.uk (P.R. Dash).

0171-9335/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ejcb.2011.11.006

and podosomes, share many similarities in structural components but there are key differences in several aspects. Firstly, podosomes are usually found in monocytic cells, endothelial cells and smooth muscle cells whereas invadopodia are found in invasive cancer cells (Buccione et al., 2004, 2009; Linder et al., 1999; Weaver, 2006). They can be distinguished by the size of the structure and their abundance. Podosomes are usually small shallow structures, 1 μm in diameter and 0.4 μm in height while invadopodia are much larger structures with dimensions as large as 8 μm by 5 μm. Podosomes are usually more abundant in cells than invadopodia, with cells typically forming a minimum of 20 podosomes (with over a hundred per cell reported in some cases), whereas the number of invadopodia per cell is usually between one and ten. The dynamics of each structure is another factor to distinguish between them. Invadopodia are more stable structures and can persist for over an hour whereas podosomes are highly dynamic and have a turnover of only a few minutes (Chan et al., 2009; Destaing et al., 2003; Linder, 2007).

Both invadopodia and podosomes are able to degrade the ECM, however the extent of degradation differs between the two structures (Bowden et al., 2006; Burgstaller and Gimona, 2005). Degradation by an invadopodium is much deeper into the matrix than the shallow degradation by podosomes. This is thought to be due to the different life spans of invadopodia and podosomes. The recruitment of proteolytic enzymes such as matrix metalloproteinases (MMPs) and serine proteinases to invadosomes are required for the ECM degradation. MMP-2, MMP-9 and MT1-MMP have been extensively implicated in degradation of ECM by invadopodia and podosomes as with trophoblast cell invasion (Nakahara et al., 1997; Redondo-Munoz et al., 2006; Sato et al., 1997; Whitley and Cartwright, 2010).

In this study we have identified and characterised the invasive structures that are formed by extravillous trophoblasts. With the use of confocal microscopy we have visualised these structures and the nature of ECM degradation and conclude that extravillous trophoblasts use atypical podosomes to degrade extracellular matrix and that these podosomes have properties that appear to be unique to trophoblasts. We have also identified key regulators of the structures and examined their ability to degrade the extracellular matrix.

Results

Trophoblasts exhibit matrix degrading actin rich protrusions

To investigate if trophoblast cells form invadosome-like structures that have been reported in other cell types, extravillous trophoblast derived HTR8-svneo (HTR8) cells were initially cultured on plastic and actin rich structures were stained using fluorescently labelled phalloidin and visualised (Fig. 1A) using confocal microscopy. Podosome like structures were observed on the ventral surface of these cells. The trophoblast cells were subsequently plated on AlexaFluor-546 fluorescently labelled 0.2% gelatin for 16 h to visualise if these invasive protrusions were capable of penetrating and degrading the matrix. Fig. 1B shows structures which were composed of cortactin that were capable of invading completely through the matrix . We also found trophoblast cells are able to completely degrade the matrix over longer time periods (48 h) (Fig. 1C) during which the cells migrate across the surface of the gelatin.

Increasing matrix density induces greater degradation

Trophoblasts were seeded on 0.2%, 0.5% and 1% concentrations of gelatin and the degrading activity compared. Degrading activity was assessed in untreated conditions and invasive podosomes were

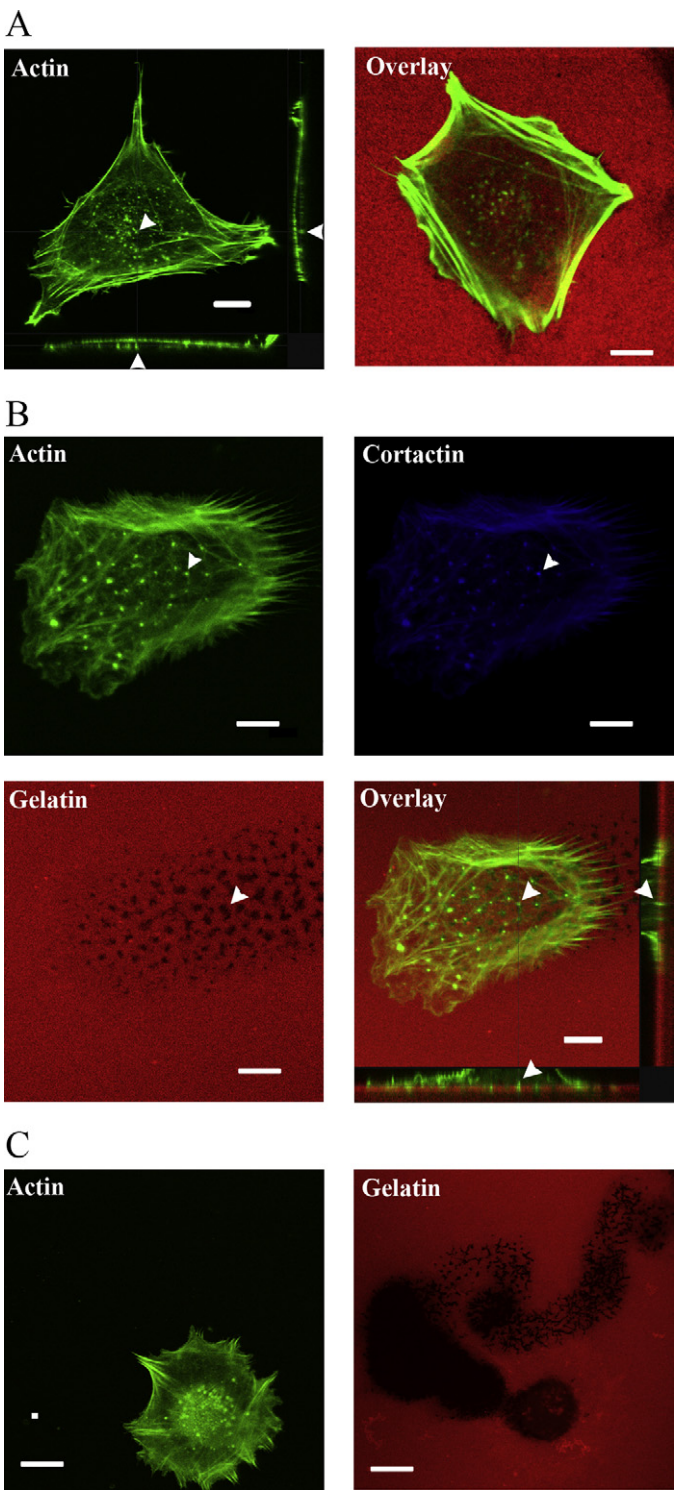

Fig. 1. *Trophoblasts form actin rich invasive protrusions which have the ability to degrade the extracellular matrix.* (**A**) Trophoblast cell stained with AlexaFluor-488-Phalloidin show actin rich structures on the ventral surface of the cell when plated on plastic. When plated on fluorescently labelled gelatin (0.2%, w/v), degradation of the extracellular matrix is observed. (**B**) Trophoblast cell displaying similar actin rich structures as in A which are also cortactin rich (blue), similar to the composition of invadosomes. Cells were also plated on fluorescently labelled gelatin and the overlaid image of actin (green) and gelatin (red) shows that these structures were able to invade and degrade the fluorescent matrix. (**C**) Trail of degradation by a migrating trophoblast cell through a fluorescently labelled matrix. Arrow heads indicate a single actin rich protrusion. All scale bars equal to 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)