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Microtubule acetylation regulates dynamics of KIF1C-powered vesicles and contact of microtubule plus ends with podosomes

Ridhirama Bhuwania[a], Antonio Castro-Castro[b,c], Stefan Linder[a,*]

[a] Institut für medizinische Mikrobiologie, Virologie und Hygiene, Universitätsklinikum Eppendorf, Martinistr. 52, 20246 Hamburg, Germany
[b] Institut Curie, Research Center, 75005 Paris, France
[c] Membrane and Cytoskeleton Dynamics, Centre National de la Recherche Scientifique UMR144, 75005 Paris, France

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ABSTRACT

Microtubule dynamics are important for a variety of key cellular functions such as intracellular trafficking, adjustment of the cell surface proteome, or adhesion structure turnover. In the current study, we investigate the effects of altered microtubule acetylation levels on the subcellular distribution of kinesins and actin cytoskeletal architecture in primary human macrophages. Microtubule acetylation was altered by overexpression or siRNA-induced depletion of the acetylase MEC-17, or by blocking α-tubulin deacetylation by addition of the inhibitor tubacin. We show that microtubule acetylation influences the subcellular distribution of vesicles associated with the kinesin KIF1C, as well as their directionality, velocity and run length. Moreover, tubulin acetylation alters the targeting frequency of microtubule plus ends on podosomes and influences the number of podosomes per cell and thus the matrix-degrading capacity of macrophages. Collectively, our results point to α-tubulin acetylation as an important modification that impacts on kinesin vesicle dynamics, actin cytoskeletal architecture and cellular function of macrophages.

Introduction

Microtubules enable long-distance transport of cargo molecules that are destined for distinct subcellular sites (Etienne-Manneville, 2013), and also for endo- and exocytic processes (Benado et al., 2009). Directionality of these transport processes is achieved by (i) the intrinsic polarity of microtubules, with most microtubule plus ends of interphase cells located at the cell periphery (Manneville et al., 2010) and (ii) the directionality of microtubule-associated motor proteins, with most kinesin motors moving toward microtubule plus ends and dynein moving toward the microtubule minus ends (Franker and Hoogenraad, 2013; Schliwa and Woehlke, 2003).

Microtubule-dependent transport is important for many processes that regulate cell adhesion and migration, and especially for the formation and turnover of cell adhesions such as focal adhesions (Kaverina et al., 1998, 1999) and podosomes (Kopp et al., 2006; Linder et al., 2000b). Both structures are enriched in transmembrane proteins such as integrins (Block et al., 2008) that bind to components of the extracellular matrix and thus establish molecular links between the cell and the underlying substratum. Microtubules apparently influence all stages of adhesion structure development, including formation, maturation and dissolution (Etienne-Manneville, 2013; Stehbens and Wittmann, 2012). Maturation of focal adhesions (Kaverina et al., 1998) and podosomes (Linder et al., 2000b) is probably influenced by delivery of integrins to sites of adhesion structure formation (Gu et al., 2011), and the transport of components and regulators such as Src (Wu et al., 2008) to nascent adhesion sites (Kaverina et al., 1998; Wickström and Fässler, 2011). In turn, enhanced recycling of integrins from adhesions may in part be responsible for microtubule-dependent dissolution of focal adhesions (Kaverina et al., 1999) and podosomes (Kopp et al., 2006). Indeed, repeated targeting of microtubule plus ends at focal adhesions (Krylyshkina et al., 2002; Rid et al., 2005) and podosomes (Kopp et al., 2006) is associated with their subsequent dissolution. Also, kinesin-dependent delivery of a currently unidentified "relaxation factor" has been discussed for both focal adhesions (Krylyshkina et al., 2002) and podosomes (Kopp et al., 2006).

In addition, kinesin-dependent transport has emerged as a key mechanism influencing not only the turnover but also the functionality of adhesion structures, including focal adhesions (Stehbens and Wittmann, 2012), podosomes and invadopodia (Linder et al., 2011). Accordingly, kinesin-1-dependent transport is involved in dissolution of focal adhesions (Kaverina et al., 1999), while KIF1C-mediated transport regulates podosome dynamics (Kopp et al.,

* Corresponding author. Tel.: +49 40 7410 55175; fax: +49 40 7410 54881.
E-mail address: s.linder@uke.de (S. Linder).

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2006), and the kinesin KIF9 influences the matrix degrading ability of podosomes (Cornfine et al., 2011). A key event in this context is the localized delivery of the matrix metalloproteinase MT1-MMP to focal adhesions (Wang and McNiven, 2012), invadopodia (Steffen et al., 2008), and podosomes (Wiesner et al., 2010).

The variety of microtubule-dependent effects on adhesion structures points to the existence of a fine-tuned system that ensures the spatiotemporally correct delivery of components and regulators. This involves specific linkage of transport vesicles to motor proteins, often through adaptor proteins such as trafficking kinesin proteins (TRAKs) (Stephenson, 2013). However, the question remains how these cargo-motor complexes are sorted to their subcellular destination. This could, at least in part, be achieved by tubulin modifications acting as "roadsigns" for motor proteins (Verhey and Hammond, 2009; Wloga and Gaertig, 2010). For example, KIF5B kinesin moves preferentially on acetylated microtubules (Hammond et al., 2010; Reed et al., 2006) while Kif5c shows preferential movement on detyrosinated microtubules (Dunn et al., 2008; Verhey and Hammond, 2009). However, a recent in vitro study could not detect differences in kinesin-1 motility on acetylated vs. deacetylated microtubules (Walter et al., 2012), indicating that additional factors could play a role in this phenomenon. Still, a more global in vitro analysis using genetically engineered tubulins with specific posttranslational modifications demonstrated that tubulin modifications can indeed influence motor velocity and processivity (Sirajuddin et al., 2014).

Currently, several types of tubulin secondary modifications are known: acetylation, (poly-)glutamylation, (poly-)glycylation, and detyrosination (Janke and Bulinski, 2011). Glutamylation and glycylation can occur on both α- and β-tubulin at their C-termini. By contrast, acetylation and detyrosination are specific for α-tubulin, with acetylation occurring at the K40 residue, and detyrosination at the ultimate C-terminal residue. The latter modification can also be followed by removal of the penultimate glutamate residue, giving rise to Δ2-tubulin (Paturle-Lafanechère et al., 1991). All of these modifications, except deglutamylation, are reversible, with the forward reaction taking place on polymerized tubulin and the reverse reactions occurring on the soluble tubulin dimer (Janke and Bulinski, 2011). As the C-termini of microtubules are exposed to the cytoplasm, most of these modifications occur on the outside of microtubules, with α-tubulin acetylation being the only modification at the inner lumen of microtubules (Janke and Bulinski, 2011). The modifying reactions are catalyzed by specific enzymes, some of which are currently unknown. In particular, acetylation of α-tubulin is catalyzed by α-tubulin acetylase (αTAT), also known as MEC-17, (Akella et al., 2010; Kalebic et al., 2013; Shida et al., 2010), while the reverse reaction is catalyzed by histone deacetylase 6 (HDAC 6) (Hubbert et al., 2002) or sirtuin 2 (SIRT2) (North et al., 2003).

In the current study, we focus on the cellular effects caused by alterations in microtubule acetylation levels in primary human macrophages We show that tubulin acetylation influences the subcellular distribution of KIF1C-associated vesicles, their directionality, velocity and run length. Moreover, tubulin acetylation alters the targeting frequency of microtubule plus ends with podosomes and influences the number of podosomes per cell and thus the matrix-degrading capacity of macrophages.

Materials and methods

Cell isolation and cell culture

Human peripheral blood monocytes were isolated from buffy coats (kindly provided by F. Bentzien, University Medical Center Eppendorf, Hamburg, Germany) and differentiated into macrophages as described previously (Linder et al., 1999).

Transfection of cells

Cells were transiently transfected using the Neon Microporator (Peqlab, Erlangen, Germany). For transfection of primary human macrophages, the following parameters were used: 1000 V, 40 msec, 2 pulses, 0.5 μg DNA per 1×10^5 cells or 50 nm of siRNA.

Expression constructs

mMEC-17-YFP was a kind gift from C. Janke (Institut Curie, Paris, France). mMEC-17-pTagRFP was generated by cloning a PCR-generated insert, using mMEC-17-YFP as template, (F-primer: 5′AAAAGCTAGCATGGAGTTCCCG3′ R-primer: 5′AAAAGGATC CTTCCAAGGCCTG3′) into the *Nhe*I and *Bam*HI restriction sites of pTagRFP-N. GFP-KIF1C and pTag-RFP-KIF1C were generated as described in (Kopp et al., 2006). GFP-KIF9 and pTagRFP-KIF9 were generated as described in (Cornfine et al., 2011). Lifeact-RFP and Lifeact-GFP were kind gifts from M. Sixt (Institute of Science and Technology, Austria). KIF16B-YFP was a kind gift from M. Zerial (Max-Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany). GFP-CLIP-170 was a kind gift from N. Galjart (Erasmus Medical Center, Rotterdam, Netherlands). EGFP-MEC-17-^{D157N} was generated as described in (Castro-Castro et al., 2012). FLAG-cortactin, FLAG-cortactin 9KR and FLAG-cortactin 9KQ as described in (Zhang et al., 2007) were kind gifts from Edward Seto (H. Lee Moffitt Cancer Center & Research Institute, Tampa, USA).

siRNA-induced knockdown

For siRNA induced knockdown of MEC-17, the following siRNAs were used: ON-TARGETplus SMARTpool MEC-17 siRNAs (Dharmacon) 5′GUAGCUAGGU CCCGAUAUA-3′, 5′-GAGUAUAGCUAGAUCCCUU-3′, 5′-GGGAAACUCACCAGAA CGA-3′, 5′-CUUGUGAGAUUGUCGAGAU-3′ firefly luciferase negative control siRNA (Dharmacon): 5′-AGGUAGUGUAACCGCCUUGUU-3′.

Immunofluorescence and microscopy

Cells were fixed for 10 min in 3.7% formaldehyde and permeabilized for 5 min in 0.5% TritonX/PBS. Mouse monoclonal anti-vinculin (V9264), was purchased from (Sigma–Aldrich, Missouri, USA) mouse monoclonal anti-Arp2 (ab49674) was purchased from (Abcam, Cambridge, UK), 555-conjugated mouse monoclonal anti-cortactin was purchased from (Upstate Cell Signaling Solutions, Millipore, USA) and mouse-monoclonal anti-FLAG (F1804) was purchased from Sigma–Aldrich. F-actin was stained using phalloidin coupled to Alexa Fluor 568, Alexa Fluor 488 or Alexa Fluor 647 (Life Technologies, CA, USA). Cells were stained for tubulin by fixation in −20 °C methanol for 30 s. Mouse monoclonal anti-acetylated tubulin (clone 6-11B-1) was purchased from Sigma–Aldrich (Missouri, USA) and rat monoclonal anti tyrosinated tubulin (clone YL1/2) was purchased from Abcam (Cambridge, UK). Secondary antibodies were Alexa-Fluor-647-labeled donkey anti-mouse (Jackson ImmunoResearch, PA, USA) and Alexa-Fluor-568- labeled goat anti-rat (Molecular Probes, Eugene, USA). For staining acetylated cortactin, cells were fixed with 3.7% formaldehyde containing 0.1% TritonX for 10 min at room temperature. This was followed by fixing with 3.7% formaldehyde for additional 10 min at room temperature. Rabbit polyclonal anti-acetylated cortactin was purchased from Merck Millipore (MA, USA). Coverslips were mounted in Mowiol (Calbiochem, Darmstadt, Germany) containing *p*-phenylenediamine (Sigma) as anti-fading reagent and sealed with nail polish. Microscopy was performed as described