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MAP1a associated light chain 3 increases microtubule stability by suppressing microtubule dynamics

Elliott M. Faller *, Tania S. Villeneuve, David L. Brown

University of Ottawa, Department of Biology, Gendron Hall, 30 Marie Curie, Ottawa, Ontario, Canada K1N 6N5

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ABSTRACT

Microtubule associated proteins (MAP) have been shown to play a role in microtubule stability in axons and dendrites, in determining neuronal shape and in regulating the balance between rigidity and plasticity in neuronal processes. MAP1a is the most abundant MAP in the adult brain, localized in axons and dendrites of neurons. MAP1a associates with three light chain molecules (LC1, LC2, LC3) that have been shown to bind microtubules independent of heavy chain molecules. In the present study we investigate the role of MAP1a associated light chain molecules in stabilizing microtubules and altering microtubule dynamics *in vivo*. All three light chain molecules co-localized with microtubules by fluorescence microscopy and bound taxol stabilized microtubules in an *in vitro* binding assay. LC-microtubule binding was associated with increased microtubule stability as shown by co localization of LC molecules with detyrosinated microtubules and increased amounts of detyrosinated tubulin in whole cell extracts. Both LC1 and LC2 binding to microtubules reorganized microtubules into wavy bundles that were resistant to nocodazole induced drug depolymerization. In contrast, LC3 bound microtubules were not resistant to nocodazole and the microtubule network of LC3 expressing cells was similar to media controls. Although LC3 bound microtubules were not resistant to drug induced depolymerization, *in vivo* measurement of microtubule dynamics shows that LC3 stabilizes microtubule networks by decreasing microtubule dynamicity and promoting growth over shortening events.

Introduction

The cytoskeleton is a three-dimensional network formed inside the cell by microfilaments, intermediate filaments and microtubules. Microtubules play an important role in maintaining and generating cell shape, organelle organization, and chromosome segregation during mitosis and meiosis. Microtubules are also essential components of extremely stable structures such as ciliary and flagellar axonemes as well as axons and dendrites in neurons (Chapin and Bulinski, 1992; Downing and Nogales, 1998; Laferriere et al., 1997; Wade and Hyman, 1997). Microtubules are composed of 50 kDa α and β-tubulin subunits. αβ tubulin heterodimers (100 kDa) associate in a head to tail fashion to form protofilaments. When thirteen protofilaments align together laterally, they form the 24 nm diameter microtubules. Microtubules are nucleated within the microtubule organizing centre (MTOC) located near the nucleus. The microtubule minus ends, or slow growing ends, are proximal and usually attached to the MTOC, while the microtubule plus ends, or fast growing ends, are distal to the MTOC and grow towards the periphery (Downing and Nogales, 1998; Joshi and Cleveland, 1989; Laferriere et al., 1997; Oakley, 1994; Wade and Hyman, 1997). A particular characteristic of microtubules is their ability to have different dynamic states, first proposed by Mitchison and Kirschner (1984). This model proposed that microtubules are labile polymers existing in either an elongation phase by the addition of tubulin molecules or in a shortening phase by the loss of tubulin molecules from the microtubule ends. The transition from the elongation phase to a shortening phase (catastrophe) and the transition from a shortening phase to an elongation phase (rescue) allow microtubules to participate in many cellular processes (Margolis and Job, 1994). Catastrophe and rescue transitions are modulated by a variety of factors. Expression of multiple α and β tubulin isotypes, encoded by distinct genes, and post-translational modifications of these tubulin subunits can influence microtubule dynamics.

Post-translational modifications of tubulin include phosphorylation, acetylation, tyrosination/detyrosination, polyglutamylation and polyglycylation. Microtubule associated proteins (MAPs) are considered to be the main modulator of microtubule dynamics (Chapin and Bulinski, 1992; Hirokawa, 1994; Laferriere et al., 1997). Tubulin purified from brain through repeated cycles of temperature-dependant microtubule assembly and disassembly always co-purifies with MAPs (Ludueña et al., 1992). Structural MAPs are particularly

* Corresponding author. University of Ottawa, General Campus, 501 Smyth Road, Ottawa, Ontario, Canada K1H 8L6. Fax: +1 513 737 8164.
E-mail address: efaller@ohri.ca (E.M. Faller).

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prominent in the nervous system and have a particular developmental and expression pattern (Vallee, 1982). Most structural MAPs are able to stimulate assembly of microtubules and stabilize microtubules *in vitro*. MAPs are thought to stimulate microtubule assembly by interacting with the carboxy terminus region of tubulin. The carboxy terminus of tubulin is an endogenous inhibitor of microtubule assembly. Therefore, MAP binding to this region promotes microtubule assembly. It is thought that this MAP/microtubule interaction allows microtubules to determine neuronal cell shape (Chapin and Bulinski, 1992; Hirokawa, 1994; Laferriere et al., 1997; Matus, 1988).

The structural MAP, MAP1a is developmentally regulated and plays a morphological role in stabilizing the microtubules in mature axons and dendrites (Müller et al., 1994). The MAP1a heavy chain molecule is associated with three light chains (Kuznetsov and Gelfand, 1987; Kuznetsov et al., 1986; Vallee and Davis, 1983). The presence of MAP light chains was first discovered when immunoprecipitation of heavy chain MAPs coprecipitated with low molecular weight subunits (Schoenfeld et al., 1989). Further investigations showed that three light chain MAPs (LC1, LC2, LC3) were present in the MAP1A immunoprecipitates (Hammarback, 1997; Kuznetsov et al., 1986; Muller, 1994; Schoenfeld et al., 1989). LC1 (27 kDa) is encoded within the 3′ end of MAP1b (Hammarback et al., 1991). LC2 (24 kDa) is encoded within the 3′ end of MAP1a (Langkopf et al., 1992). Both LC1 and LC2 are post-translationally cleaved from their respective MAP1 heavy chains by selective proteolysis (Hammarback et al., 1991; Langkopf et al., 1992; Togel et al., 1998). LC 3 (19 kDa), on the other hand, is encoded in a distinct gene (Mann and Hammarback, 1994; Mann and Hammarback, 1996). Although not present in the same gene, LC3 is always co-expressed with either MAP1a or MAP1b (Mann and Hammarback, 1996).

Light chain molecules bind to the amino terminus of MAP1, near the microtubule binding domain, (Kuznetsov et al., 1986; Mann and Hammarback, 1994; Muller, 1994; Schoenfeld et al., 1989) and the phosphorylation state of MAPs does not appear to influence the ability of light chains to bind to heavy chain molecules (Pedrotti and Islam, 1995). LC3 appears to promote association of heavy chains to microtubules (Mann and Hammarback, 1994) and association of light chain molecules with MAP1a and MAP1b may alter their ability to bind microtubules and alter microtubule dynamics (Mann and Hammarback, 1996; Schoenfeld et al., 1989).

Light chain molecules have been detected at stoichiometries of 6–8 fold compared to their respective heavy chains. In addition, light chain molecules have been detected in non-neuronal cells and may be expressed in the absence of heavy chain molecules. This suggests that light chain molecules have *functions* in addition to their association with MAP1 heavy chains (Mei et al., 2000). LC1, LC2 and LC3 are capable of binding to microtubules in the absence of MAP1 molecules. In addition, light chain and heavy chain molecules co-localize on the same microtubules *in vivo*. The binding of LC1 and LC2 to microtubules reorganizes microtubules into wavy bundles and increases the resistance of microtubules to depolymerizing drugs (Noiges et al., 2002; Togel et al., 1998). Both LC1 and LC2 promote tubulin polymerization *in vitro*. Oligomerization of LC1 through association of its carboxy terminus domains may promote microtubule growth by recruiting tubulin to polymerized microtubules (Noiges et al., 2002).

Of the three LC molecules, the effect of LC3 on microtubule stability and dynamics is the least characterized. In the present study we show that LC3 can co-localize with microtubules in live cells (independent of heavy chain MAPs) and bind to microtubules in an *in vitro* binding assay. In contrast to other MAP1a associated light chain molecules (LC1 and LC2), LC3 did not reorganize microtubules into wavy bundles and LC3 did not protect microtubules from depolymerization by

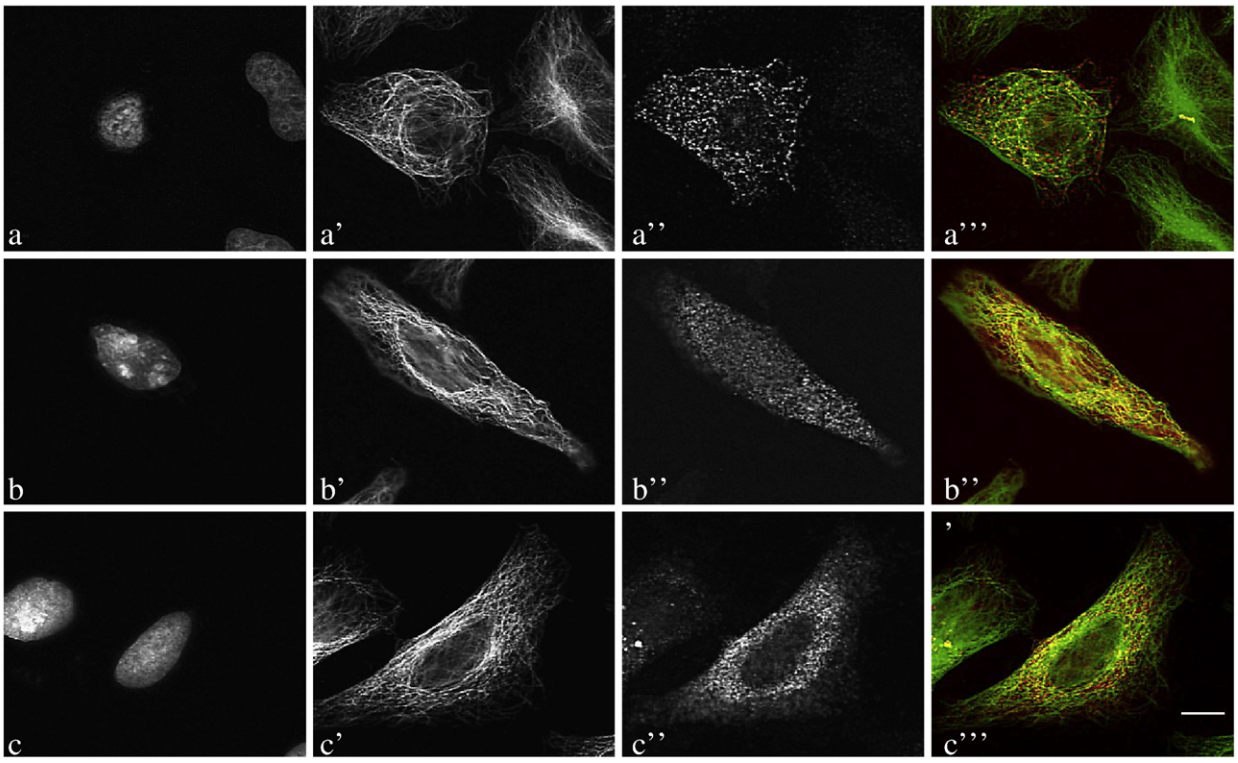

Fig. 1. Hoechst staining of DNA (a–c) and double immunofluorescence labelling in HeLa cells transfected with 6mycLC1 (a–a′′′), 6mycLC2 (b–b′′′) and 6mycLC3 (c–c′′′). Microtubules were visualized using a rat monoclonal antibody (clone YL1/2) specific against α-tubulin (a′–c′) whereas 6myc tagged LCs were visualized using a mouse monoclonal antibody (clone 9E10) specific for 6myc (a′′–c′′). All three LCs were found co-localized with microtubules (yellow in merged images) (a′′′–c′′′). 6mycLC1 and 6mycLC2 conferred to the microtubules a wavy pattern. Bar = 10 μM.