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Centrosomal aggregates and Golgi fragmentation disrupt vesicular trafficking of DAT

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Abstract

Lewy bodies containing the centrosomal protein γ-tubulin and fragmentation of Golgi apparatus (GA) have been described in nigral neurons of Parkinson's disease (PD) patients. However, the relevance of these features in PD pathophysiology remains unknown. We analyzed the impact of proteasome inhibition in the formation of γ-tubulin-containing aggregates as well as on GA structure. SH-SY5Y cells were treated with the proteasome inhibitor Z-Leu-Leu-Leu-al (MG132) to induce centrosomal-protein aggregates. Then, microtubules (MTs) and Golgi dynamics, as well as the vesicular transport of dopamine transporter (DAT) were evaluated both in vitro and in living cells. MG132 treatment induced γ-tubulin aggregates which altered microtubule nucleation. MG132-treated cells containing γ-tubulin aggregates showed fragmentation of GA and perturbation of the *trans*-Golgi network. Under these conditions, the DAT accumulated at the centrosomal-Golgi region indicating that the vesicular transport of DAT was disrupted. Thus, centrosomal aggregates and fragmentation of GA are 2 closely related processes that could result in the disruption of the vesicular transport of DAT toward the plasma membrane in a model of dopaminergic neuronal degeneration.

Keywords: Aggresome; Centrosome; Dopamine transporter; γ-Tubulin; Golgi apparatus; Inclusion bodies; Lewy bodies; Microtubules; Neurodegeneration; Parkinson's disease; Proteasome; Protein aggregation

1. Introduction

Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder after Alzheimer's disease (de Lau and Breteler, 2006). Neuropathological lesions are characterized by severe loss of pigmented-dopaminergic neurons mainly from the sustantia nigra pars compacta (SNc). Nevertheless, other brain stem structures, spinal cord, or cortical regions are also affected (Lees et al., 2009; Levy et al., 2009). Degeneration of dopaminergic neurons leads to a progressive loss of dopaminergic terminals in the striatum and a decrease on dopamine (DA) levels with the consequent appearance of the main motor symptoms (Levy et al., 2009). Simultaneously to neuronal cell loss, protein aggregates appear forming intracytoplasmic inclusions known as Lewy bodies (LBs), pale bodies, and Lewy neuritis (Lees et al., 2009; Shults, 2006).

LBs are usually 1 or more eosinophilic spherical structures with a dense core surrounded by a halo and mainly located in the cytoplasm of remaining neurons (Shults, 2006; Wakabayashi et al., 2007). LBs are considered as the hallmark of PD pathology, although they may be present in other neurodegenerative diseases. The main component of LBs is an aggregated form of α-synuclein (Spillantini et al.,

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1997); but a large number of other proteins have also been identified into these inclusion bodies, including centrosomal proteins such as γ-tubulin and pericentrin (McNaught et al., 2002b; Shults, 2006). Thus, although α-synuclein is the main component of LBs, the consequences of protein aggregation in LBs of other key proteins for cell functioning should be studied in the context of PD pathophysiology.

Neuronal loss in neurodegenerative diseases including PD has been associated with potential common pathogenic mechanism such as oxidative stress, mitochondrial dysfunction, neuroinflammatory response, etc. (Jellinger, 2009; Levy et al., 2009). Growing evidence based on postmortem, genetic, and experimental research have suggested that ubiquitin-proteasome system (UPS) failure and protein aggregation could also play a key role in the etiopathogenesis of both familial and sporadic forms of PD (Jellinger, 2009; Levy et al., 2009; McNaught et al., 2002a). The UPS is the main nonlysosomal system responsible for the degradation of short-lived and unwanted proteins (Betarbet et al., 2005). It has been hypothesized, based on morphological and biochemical data, that LBs in PD are formed through an aggresome-related process (McNaught et al., 2002b; Olanow et al., 2004).

Aggresomes are intracellular accumulations of misfolded, aggregated proteins which form when the capacity of the UPS is overwhelmed either by excessive formation of unwanted proteins or by impaired protein degradation (Johnston et al., 1998; Olanow et al., 2004). In response to this proteolytic unbalance, unwanted proteins are retrograde transported through microtubules (MTs) from the cell periphery toward the centrosome where they fuse into an aggregate surrounded by intermediate filaments known as aggresome (Johnston et al., 1998). Therefore, the presence of γ-tubulin and pericentrin into LBs suggest that they could form through an aggresome-related process, and in this case the abnormally folded proteins could accumulate at the neuronal centrosome.

The centrosome is a nonmembrane-bound organelle composed of 2 orthogonally arranged centrioles surrounded by the pericentriolar matrix (Nigg and Raff, 2009) that is responsible for MT nucleation. This function is accomplished through the γ-tubulin ring complex, a protein complex that contains γ-tubulin (Raynaud-Messina and Merdes, 2007). Accumulation of centrosomal proteins such as γ-tubulin or pericentrin and impairment of MT nucleation have been described in osteosarcoma cells treated with proteasome inhibitors (Didier et al., 2008). Thus, centrosomal structure and functioning seem to be a target of proteasome inhibition, suggesting that this organelle could be affected during the neurodegenerative process induced by proteasome inhibitors.

In nonpolarized mammalian cells, the centrosome exhibits a close structural and functional relationship with the Golgi apparatus (GA). It is well-known that both the integrity and the pericentrosomal position of the Golgi ribbon depends on MTs (Thyberg and Moskalewski, 1999). In the absence of MTs, the GA fragments into many elements that appear dispersed throughout the cytoplasm. Interestingly, fragmentation of GA has been widely described in several neurodegenerative diseases, which are also characterized by the presence of abnormal protein aggregates (Fan et al., 2008; Gonatas et al., 2006). Indeed, fragmentation of GA has been observed in SNc of PD brain samples into the same neurons in which LBs and pale bodies were also identified (Fujita et al., 2006). In addition, it has been reported that α-synuclein aggregation induces fragmentation of GA in cell culture systems (Gosavi et al., 2002). This evidence might suggest that protein aggregation and fragmentation of GA could be common and related features of degenerative neuronal cells. However, the exact mechanisms that trigger or contribute to the fragmentation of GA in neurodegenerative diseases including PD as well as the relationship of centrosomal-protein aggregates with fragmentation of GA and the consequences that might arise from such morphological disturbances are still unknown.

In mammalian cells, the GA is a single organelle composed of hundreds of stacks of cisternae connected by tubular bridges forming a reticular network that is known as the Golgi ribbon. The GA is a polarized structure in which proteins arriving from the endoplasmic reticulum (ER) enter the organelle by the *cis*-face, go through the medial cisternae, where they are modified, and leave the organelle by the *trans*-face. Finally, the proteins within transport vesicles are either inserted into membrane organelles, including the plasma membrane, as transmembrane proteins are secreted to the extracellular space. The dopamine transporter (DAT) is a transmembrane protein exclusively expressed in dopaminergic neurons. DAT represents the most important mechanism to regulate DA levels in the synaptic cleft (Storch et al., 2004; Zahniser and Sorkin, 2009). DAT is post-translationally *N*-glycosylated at the second extracellular loop in the GA, and this modification seems to be important for both the integration of DAT into the cell membrane and DA uptake (Li et al., 2004; Zahniser and Sorkin, 2009). Therefore, changes in GA structure might disrupt vesicular transport of DAT, thus affecting the synaptic transmission of dopaminergic neurons.

In this work, the dynamics of centrosomal aggresome formation together with the GA fragmentation process were analyzed by live imaging in a dopaminergic neuroblastoma cell line treated with Z-Leu-Leu-Leu-al (MG132), which is a reversible proteasome inhibitor widely used to mimic parkinsonian features in vitro and in vivo experimental models of PD (Sun et al., 2006; Xie et al., 2010). We also studied the effect of such fragmentation on the vesicular transport of DAT. In MG132-treated cells, large centrosomal-protein aggregates and fragmentation of GA were observed. In addition, cells with centrosomal aggregates showed abnormal MT nucleation. The vesicular transport of DAT was disrupted on those cells, leading to an accumula-