REVIEW

Is autophagy an elective strategy to protect neurons from dysregulated cholesterol metabolism?

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Abstract

The balance of autophagy, apoptosis and necroptosis is crucial to determine the outcome of the cellular response to cholesterol dysregulation. Cholesterol plays a major role in regulating the properties of cell membranes, especially as regards their fluidity, and the regulation of its biosynthesis influences the shape and functions of these membranes. Whilst dietary cholesterol can easily be distributed to most organs, the central nervous system, whose membranes are particularly rich in cholesterol, mainly relies on *de novo* synthesis. For this reason, defects in the biosynthesis of cholesterol can variably affect the development of central nervous system. Moreover, defective synthesis of cholesterol and its intermediates may reflect both on structural cell anomalies and on the response to inflammatory stimuli. Examples of such disorders include mevalonate kinase deficiency, and Smith-Lemli-Opitz syndrome, due to deficiency in biosynthetic enzymes, and type C Niemann-Pick syndrome, due to altered cholesterol trafficking across cell compartments. Autophagy, as a crucial pathway dedicated to the degradation of cytosolic proteins and organelles, plays an essential role in the maintenance of homeostasis and in the turnover of the cytoplasmic material especially in the presence of imbalances such as those resulting from alteration of cholesterol metabolism. Manipulating the process of autophagy can offer possible strategies for improving neuronal cell viability and function in these genetic disorders.

Key Words: *cholesterol; inflammation; apoptosis; autophagy; neurons; inherited disease; necroptosis; neuronal dysfunction*

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Introduction

Apoptosis and autophagy are both considered forms of programmed cell death. They actually represent the main cellular processes to respond to an external damage. Indeed, both mechanisms allow to eliminate damaged cells and organelles in a "physiological" way. Also necroptosis, that shares some typical features of apoptosis leads to the rapid demolition of cellular structures and organelles after activation of catabolic enzymes.

All these mechanisms of programmed cell death are fundamental to maintain the homeostasis of tissues. As an example, during developments, programmed cell death allows the cells to form the correct architecture of tissues and organs (Meier et al., 2000) and, in particular, autophagy, can predicts which parts of the cytoplasm and organelles should be blocked and directed to lysosomes for degradation.

The functional relationship between apoptosis and autophagy is complex because in many conditions, autophagy is a process of adaptation to stress that protects against cell death; other times, however, autophagy becomes an alternative way of cell death (Maiuri et al., 2007).

Recent evidences have identified the alterations in the balance between apoptosis and autophagy as the causes of the pathogenesis of various neurodegenerative diseases and in particular the interest is focused on the cellular responses caused by alterations in the metabolism of cholesterol as a cause triggering the disequilibrium (Marcuzzi et al., 2015; Miettinen and Bjorklund, 2016; Suárez-Rivero et al., 2018). Cholesterol is a lipidic macro-molecule fundamental to ensure the homeostasis of the organism. It plays an essential role in the construction of cell membranes, in the production of hormones and vitamin D and it is an essential component of the central nervous system and peripheral nervous system (Zhang and Liu, 2015). About 25% of all the human body's cholesterol is contained in the brain, even though it represents only 2% of body weight. Cholesterol is a fundamental constituent of cell membranes, in which together with the phospholipids it forms the lipid leaf that regulates the imbibition of the cell and the transport of the liposoluble molecules (Pfrieger and Ungerer, 2011). It is also present in the mitochondrial membrane and in the endoplasmic reticulum structures.

The supply of cholesterol depends on the balance between dietary intake and *de novo* synthesis. The liver distributes cholesterol to other organs by lipoproteins. However, blood-brain barrier does not allow the passage from the bloodstream to the brain of the cholesterol-containing lipoprotein complexes and thus the brain supply of cholesterol depends almost only on *de novo* synthesis: 99% of brain cholesterol is contained in non-esterified form (Lim et al., 2016; Moutinho et al., 2017). During embryogenesis and the first years of life, the cells responsible for the synthesis of cerebral cholesterol are neurons. Subsequently, in adulthood, when the processes of myelination and cerebral maturation are terminated, the

neurons become the main "users" of cholesterol, demanding its synthesis to glial cells, in particular to microglia, astrocytes and, to a lesser extent, oligodendrocytes.

Brain cholesterol is abundant in myelin, where it is involved in synaptic mechanisms. Moreover, this lipid is an essential component of neuronal cell membranes, involved in the maturation process of the central nervous system, in synaptogenesis, in signal transduction processes and in vesicular traffic.

As for monogenic disorders of the cholesterol (such as mevalonate kinase deficiency, Smith-Lemni-Opitz disease, Niemann-Pick disease), also in several neurodegenerative disease (such as Parkinson's disease, multiple sclerosis, Alzheimer's disease) recent evidences link the pathogenesis to alteration in autophagy (Moloudizargari et al., 2017; Cerri and Blandini, 2018; Obergasteiger et al., 2018). Different *in vitro* models of diseases reproduce the defective synthesis or regulation of cholesterol and the following reduction of oxysterols, which may be in part responsible for the neurodegeneration that characterizes these pathologies (Jira, 2013; Marcuzzi et al., 2015, 2018; Arenas et al., 2017).

Although clinically different, these disorders share the progressive accumulation of cellular materials as a pathogenic mechanism impairing tissue and cell homeostasis and possibly leading to cell loss by apoptosis, necroptosis and neuronal inflammation. Conversely, a proper clearance of damaged cell components and aggregated proteins by autophagy can preserve the viability and function of cells. Thus, both in monogenic that neurodegenerative diseases, pharmacological approaches promoting effective autophagy may represent a possible therapeutic strategy to prevent neuronal cell loss and improve neurological function in these disorders (Lim et al., 2016).

The articles used in this review were retrieved by replicating the following search terms. An electronic search of the Medline database for literature describing the role of deregulation of cholesterol associated to neuroinflammation from 2003 to 2018 was performed using the following conditions: cholesterol (MeSH Terms) AND neuroinflammatio (MeSH Terms) OR neurons (MeSH Terms) OR central nervous system (MeSH Terms). The results were further screened by title and abstract to only present monogenic disorders and chronic disease associated to deregulation of cholesterol pathaway. Other multifactorial disease and other genetic disease articles were also excluded.

In addition, an electronic search of the Medline database for programmed cell death linked to these diseases was completed. This included publications prior to May, 2018, with the following search criteria: autophagy, apoptosis, necroptosis, pyroptosis, mitochondria. Subsequent searches were completed that were specifically relevant to each programmed cell death type discussed in this article. The articles that did not correspond to human models of selected disease were excluded.

Different Strategy to Respond to a Cellular Damage

Autophagy

The word autophagy was coined by Christian de Duve in 1963, it derives from the Greek and means "eating himself" (Klionsky, 2008). Autophagy is a lysosomal catabolic process, it is ubiquitous and evolutionarily conserved. Autophagy is responsible for the degradation of damaged or aggregated proteins and aged organelles, with the aim of clearance of damaged cellular compartments and recycling cytoplasmic contents (Ward et al., 2016; Giampieri et al., 2018). The process is stimulated in response to various kinds of cellular stress such as nutrient deprivation, oxidative stress, hormonal signals, shortage of growth factors, and accumulation of damaged proteins (Rusmini et al., 2018). The mechanism of autophagy requires the interaction of two main pathways: the first, regulated by a molecular platform that include the ULK (Unc-51-like autophagy-activating kinase) complex and the second, connected to the pathway of mammalian target of rapamycin (mTOR) and phosphatidylinositol 3-kinase (Kim et al., 2011; Lazarus et al., 2015; Park et al., 2016; Singh et al., 2017). The entire process of autophagy involves many steps including formation and elongation of the isolation membrane (phagophore), cargo loading (inclusion of proteins or organelles, such as damaged mitochondria), formation of autophagosome and fusion with lysosome to form autolysosome (Shintani and Klionsky, 2004; Axe et al., 2008; Hayashi-Nishino et al., 2009; Tanida, 2011; Chan and Tang, 2013; Wu et al., 2014).

Apoptosis

Apoptosis is well known as a process of programmed cell death, also identified as a "suicide" of the cell. It differs from the "passive" mechanism of death, the necrosis, since apoptosis involves a complex and controlled series of molecular events requiring energy. Apoptosis is triggered by different stimuli, which can initiate the intrinsic, extrinsic or perforin/granzyme pathway that, anyway, drive the cell to death *via* caspase-3 (Kerr et al., 1972; Riedl and Shi, 2004). The extrinsic pathway is activated by the binding between death ligand and receptors (such as tumor necrosis factor receptor 1 and tumor necrosis factor-α); this binding activates the signalling that brings to the activation of caspase-8 (initiator caspase), which, in turn, cleaves and activates caspase-3 (executioner caspase) (Beaudouin et al., 2013). Once activated, caspase-3 leads to the typical phenomena of apoptosis, including cell shrinking and condensation of chromatin, blebbing and formation of apoptotic bodies which allow a removal of the dead content without onset of inflammation (Mills et al., 1998; Croft et al., 2005; Iwasaki et al., 2013).

Necroptosis

Apoptosis and necrosis are the better clarified mechanisms involved in cell death, but recently other subclasses of these

mechanisms have been identified (Davidovich et al., 2014). Among these, necroptosis has been recently associated to neurodegenerative disorders as a key mechanism worthy of being considered as a potential therapeutic target (Funakoshi et al., 2016; Zhang et al., 2017).

As the term itself suggests, necroptosis shares some features of the necrosis associated to a highly regulated process seen in apoptosis. When apoptosis failed to be carried forward, necroptosis will be engaged. Tumor necrosis factor-α is the main signal for necroptosis and it drives the pathways, shared with apoptosis and nuclear factor-kappa B signalling, which include the trimerization of the tumor necrosis factor receptor and the formation of the intracellular complex-I that involves TRADD (tumor necrosis factor receptor associated death domain protein) and the kinase receptor-interacting protein (RIP)1. Complex-I recruits other factors, such as caspase-8, which initiates the apoptotic cascade. When caspase-8 resulted incompletely activated or blocked, the kinase RIP3 is recruited to form the necrosome, leading to the necroptotic cell death (Newton et al., 2014). The recruitment of RIP3 causes the engagement and subsequent phosphorylation of the pseudokinase MLKL (mixed lineage kinase domain-like). Although the molecular mechanisms of RIP1, RIP3 and MLKL is not completely depicted, it is clear that the pathway of necrosome leads to some characteristic features of this programmed cell death: cell and organelles swelling with membrane rupture that results in the release of intracellular content and DAMPs (damage associated molecular patterns) (Moriwaki and Chan, 2016). This implies the activation of the immune system, as occur in necrosis.

Inherited Diseases Related to Cholesterol Metabolism

Genetic disorders with dysregulation of cholesterol metabolism provide valuable models to study therapeutic approaches aimed at preventing neuronal dysfunction. Impaired cholesterol metabolism can be caused either by enzymatic defects of the mevalonate pathway, as in the mevalonate kinase deficiency and Smith-Lemli-Opitz syndrome, or by defects in the lysosome trafficking and function, as occurs in Niemann-Pick disease (Jira, 2013). These diseases show a very heterogeneous phenotypic spectrum but share various features of neuronal dysfunction (**Figure 1**).

Mevalonate kinase deficiency

Mevalonate kinase deficiency is a rare metabolic and autoinflammatory disorder caused by mutation of the *MVK* (mevalonate kinase) gene (Muller and Freed, 2017). Caus-

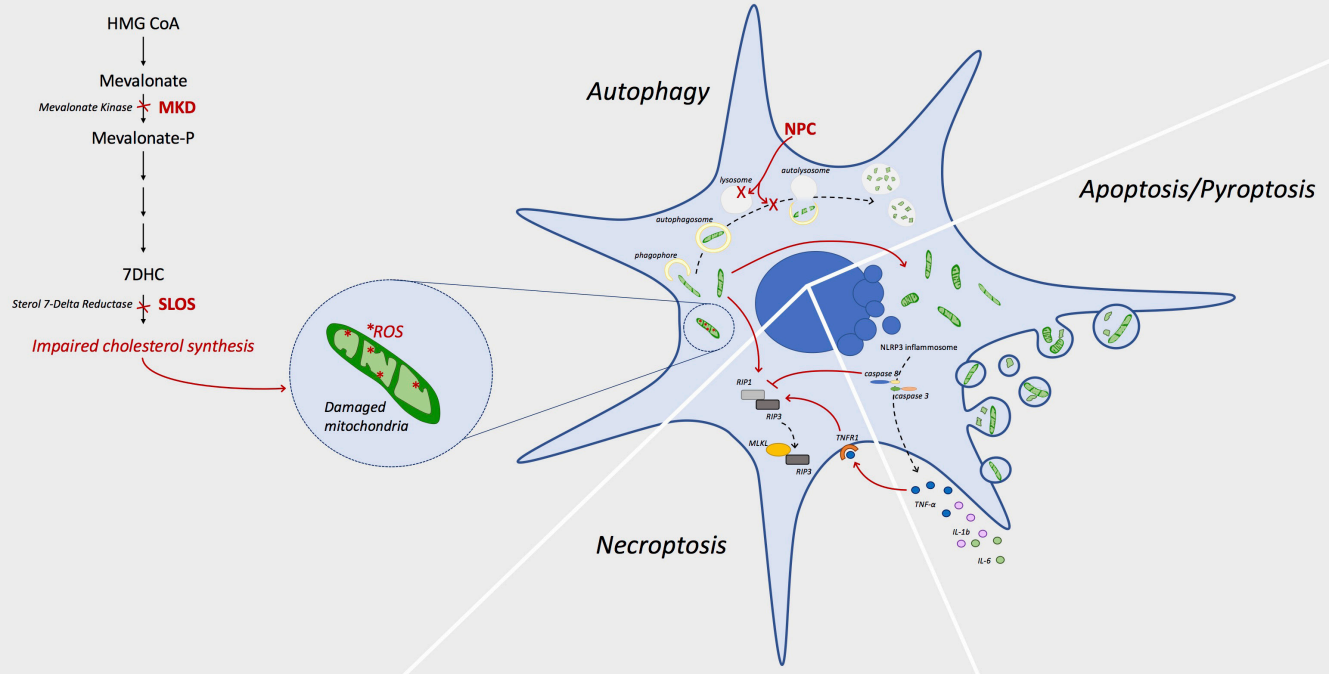

Figure 1 Link between cholesterol deregulation and programmed cell death mechanisms.
Chronic impaired cholesterol synthesis due to mutations in enzymes of the metabolic pathway, as occur in MKD or SLOS, causes a dysfunction of mitochondria. Organelles suffer from alterations of metabolism, mainly due to accumulation of ROS, that induce modifications of the morphology with shrunken shape and condensed cristae. Damaged mitochondria are usually removed by autophagy mechanisms, but accumulation of damaged organelles or mutations in such mechanism (as occur in NPC) cause a failed clearance with further accumulation of damaged mitochondria. As effect of this accumulation, the cell is driven toward programmed cell death. Caspase 8 is recognized as a modulator of the different fates of the cell: its activation leads to apoptosis or pyroptosis *via* NLRP3 inflammosome formation; when inactive or partially activated, it allows the formation of necroptosome consisting in RIP1, RIP3 and MLKL complex. 7DHC: 7-Dehydrocholesterol; HMG CoA: β-hydroxy β-methylglutaryl-coenzyme A (HMG-CoA); MKD: mevalonate kinase deficiency; MLKL: mixed lineage kinase domain like pseudokinase; NLRP3: NACHT, LRR and PYD domains-containing protein 3; NPC: Niemann-Pick disease type C; RIP: receptor-interacting serine/threonine-protein kinase; ROS: reactive oxygen species; SLOS: Smith-Lemli-Opitz syndrome; TNF-α: tumor necrosis factor α; TNFR1: tumor necrosis factor receptor 1; IL: interleukin.

ative mutations result in reduced enzymatic activity of mevalonate kinase, with accumulation of mevalonate, which can be found in plasma and urine during acute phases, and shortage of downstream compounds, including isoprenoid intermediates and sterols. The onset of the disease usually occurs in the first year of life and presents a continuum spectrum with different levels of severity, ranging from the milder form called hyperimmunoglobulinemia D (OMIM #260920) to the most severe form known as mevalonic aciduria (OMIM #610377) (van der Burgh et al., 2013). Common symptoms includ periodic attacks of fever associated with systemic inflammatory symptoms. Patients with hyperimmunoglobulinemia D present headaches, splenomegaly, adenopathy, pharyngitis, abdominal and musculoskeletal pain, while patients with mevalonic aciduria also present a significant psycho-motor and neurological involvement.

The most reliable hypothesis regarding the pathogenesis of mevalonate kinase deficiency is that its typical inflammatory phenotype is caused by the lack of pre-squalene isoprenoid intermediates, with reduced prenylation of the small GTPases that would consequently lose their membrane localization (van der Burgh et al., 2014). The final events lead to the activation of NLRP3 (NACHT, LRR and PYD domains-containing protein 3)-inflammasome that triggers the process of pyroptosis with the secretion of the inflammatory cytokines (interleukin-1β, interleukin-6, tumor necrosis factor-α). Furthermore, the incorrect post-translational prenylation of the small GTPase (for example Ras, Rho and Rac), does not allow the formation of autophagosome, and therefore the mitophagy is damaged, with potential consequences on the neurological damage observed in the most severe forms of the disease (van der Burgh et al., 2013). Shortage of cholesterol in immune cells may also play a role in some features of the disease, such as IgA and IgD hypergammaglobulinemia, due to reduced conversion to 25 hydroxycholesterol, a molecule affecting membrane function and antiviral defense (Simon, 2014).

Smith-Lemli-Opitz syndrome

The Smith-Lemli-Opitz syndrome (OMIM #270400) described by Smith et al. (1964) is a congenital syndrome characterized by multiple anomaly and intellectual disability caused by an inborn error of cholesterol metabolism. It is caused by genetic deficiency of 7-dehydrocholesterol (7DHC) reductase, encoded by *DHCR7*, that leads to toxic effects that can depend both on reduced synthesis of cholesterol and total sterols and on the accumulation of 7DHC-derived compounds (Nowaczyk and Irons, 2012; Ramachandra Rao et al., 2018).

The deficiency of cholesterol synthesis can account for a wide spectrum of clinical manifestations involving the nervous system, which include prenatal and postnatal growth retardation, microcephaly, intellectual disability (Kelley and Hennekam, 2000).

The potential Smith-Lemli-Opitz syndrome therapy aims to prevent the formation or neutralization of the most toxic 7DHC-derived oxysterols (Korade et al., 2010).

Experimental data show that Smith-Lemli-Opitz syndrome cells display an elevated autophagy activity, likely in response to the toxic effect of 7DHC accumulation resulting in excessive mitochondrial oxidative stress and activation of mitophagy (Saffari et al., 2017). However, *in vitro* studies have demonstrated that the accumulation of dysfunctional mitochondria is concomitant with a defective autophagic system which would interfere with the role of mitophagy to clear the defective proteins and organelles (Chang et al., 2014). Thereby, the protective function of the autophagy is altered by the co-existence of dysfunctional mitochondria and impairment in the autophagy process (Chang et al., 2014).

Niemann-Pick disease

Niemann-Pick disease is a very severe rare genetic disorder, which belongs to the family of lysosomal storage diseases, a condition that affects many body systems. Patients with Niemann-Pick disease cannot metabolize cholesterol and other lipids properly, leading to abnormal accumulation of these substances in liver, spleen and other organs (Guo et al., 2016).

Niemann-Pick disease presents a broad clinical spectrum, depending on the degree of defect in lipid trafficking. The onset can be at birth with a fatal disorder, or in children or even adults, with milder phenotypes characterized by progressive psychomotor impairment, in addition to liver and spleen enlargement. The defect of cholesterol trafficking to mitochondria is associated to mitochondrial dysfunction and impairment in antioxidant defense strategies. Moreover, besides the neurodegenerative aspect of the disease, Niemann-Pick disease phenotype implies systemic features since non-esterified cholesterol accumulate also in liver and spleen (Vanier, 1999, 2010; Patterson et al., 2012). Different genetic forms of Niemann-Pick disease are known and, in particular, Niemann-Pick disease type C (NPC) is caused by mutations in *NPC1* (OMIM #257220, 95% of cases) (Carstea et al., 1993, 1997) and *NPC2* genes (OMIM #607625, 5% of cases) (Naureckiene et al., 2000) resulting in functional defects of proteins with lysosomal localization (Torres et al., 2017; Liu and Lieberman, 2018) that trigger an accumulation of non-degraded substrates that interferes with different cellular functions (Sarkar et al., 2013). These molecular mechanisms are not fully elucidated yet, and a deepen knowledge of these processes is of crucial importance because each step of the pathogenetic cascade in Niemann-Pick disease may be a potential target of therapy (Schultz et al., 2018; Wang et al., 2018). Moreover, given the role of autophagy in the clearance of damaged cellular components, the impairment of autophagy itself can contribute in a vicious circle to lipid accumulation and cell injury (Platt et al., 2012; Osellame

and Duchen, 2014). The neuronal manifestations of NPC are related to a selective damage of neurons that have a stronger spontaneous activation of autophagy, if compared to systemic compartments (*i.e.*, fibroblasts), and a block of autophagic progression leads to an exceptionally severe mitochondrial fragmentation. For this reason patients with *NPC1* may benefits from the treatment with autophagy inhibitors (such as 3-methyladenine) or with drugs that mobilize cholesterol from the lysosomal compartment (such as cyclodextrin) (Davidson et al., 2016).

Conclusions

Mevalonate kinase deficiency, Smith-Lemli-Opitz syndrome and Niemann-Pick disease are monogenic disorders, extremely various as regard pathogenesis and molecular mechanisms of onset, but they share some features that can be useful to unravel possible therapeutic targets. First, the onset of these disorders is related with a dysfunctional metabolism of cholesterol; second, recent insights suggest that the dysfunctional cholesterol metabolism, at the basis of disease onset, is related to altered autophagy and other programmed cell death processes; third, all the diseases show an important involvement of the central nervous system related to altered mechanisms of clearance because of impaired autophagy. Thus, autophagy could be a keystone in the treatment of this rare disorders. Indeed, nowadays, all these pathologies can benefit from only a small repertoire of therapeutics, and none of them are able to completely control the neuronal aspect of the disease. Therefore, it is essential to know the mechanisms that regulate neuronal loss, to evaluate the most suitable pharmacological treatments able to protect from neurodegeneration or prevent the effect of the extended activation of the inflammatory response.

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