REVIEW ARTICLE

IMPAIRED AUTOPHAGY AS AN ETIOLOGICAL FACTOR OF VARIOUS DISEASE ENTITIES

Zaburzona autofagia jako czynnik etiologiczny zróżnicowanych jednostek chorobowych

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Abstract: Autophagy is a conservative process of lysosomal digestion of damaged cell organelles, pathogens and nonfunctional proteins, which determines the maintenance of cellular balance. This process is an alternative source of energy for the cell under stress conditions induced by starvation, chemical factors or hypoxia. In recent years, the interest in autophagy has increased, and its dysfunctionality is considered to be one of the factors contributing to the development of various disease entities. The likelihood of diseases such as cancer, cardiovascular diseases or neurodegenerative diseases increases with age, and the process of autophagy is inhibited in an ageing body, which further indicates the involvement of impaired autophagy in the pathogenesis of many diseases. Therefore, activities aimed at modifying the pathways related to autophagy are indicated as a potential therapeutic tool. In this review, we present selected diseases, the causes of which are believed to be disturbed autophagy, indicate potential therapeutic possibilities and emphasise the dichotomous role of autophagy, especially in the neoplastic process.

Streszczenie: Autofagia jest konserwatywnym procesem polegającym na lizosomalnym trawieniu uszkodzonych organelli komórkowych, patogenów i niefunkcjonalnych białek, co warunkuje utrzymanie równowagi komórkowej. Proces ten stanowi alternatywne źródło energii dla komórki w warunkach stresowych indukowanych głodzeniem, czynnikami chemicznymi czy niedotlenieniem. W ostatnich latach wzrosło zainteresowanie autofagią, a jej dysfunkcjonalność uznawana jest za jeden z czynników sprzyjających rozwojowi zróżnicowanych jednostek chorobowych. Prawdopodobieństwo występowania chorób, takich jak nowotwory, choroby układu sercowo-naczyniowego czy choroby neurodegeneracyjne wzrasta wraz z wiekiem, a proces autofagii ulega hamowaniu w starzejącym się organizmie, co dodatkowo wskazuje na udział upośledzonej autofagii w patogenezie wielu chorób. W związku z tym, działania ukierunkowane na modyfikację szlaków związanych z autofagią wskazywane są jako potencjalne narzędzie terapeutyczne. W niniejszym przeglądzie prezentujemy wybrane choroby, których przyczyn upatruje się w zaburzonej autofagii, wskazujemy także potencjalne możliwości terapeutyczne oraz podkreślamy dychotomiczną rolę autofagii, szczególnie w procesie nowotworzenia.

Key words: autophagy, autophagy-related diseases, impaired autophagy.

Słowa kluczowe: autofagia, choroby związane z autofagią, upośledzona autofagia.

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Introduction

Autophagy is a conservative process occurring in all eukaryotic cells: from yeasts, where it was first observed and described, to human cells [1, 2]. The name comes from Greek and it means “self-eating” [3]. Autophagy consists in lysosomal degradation of damaged and misfolded proteins, cellular organelles and pathogens [2]. In this process, cellular components are separated from the cytoplasm, surrounded by a membrane and bound in a vesicle called an autophagosome or autophagic vacuole [4]. As a result of the fusion between an autophagosome and a lysosome, its content is degraded by digestive enzymes to amino acids, sugars, fatty acids and nucleotides that can be used as alternative energy sources for the cell in stress conditions or provide building material for the synthesis

of new structures [5, 6]. Autophagy is activated in cellular stress conditions that may be induced by starvation, chemical stress or hypoxia (insufficient oxygen supply) [7]. Autophagy was first described in the 1960s, and further intensive studies on the process brought a Nobel Prize for Yoshinori Ohsumi in 2016 for research on the mechanism of autophagy. At present, autophagy, a process of cellular self-cleaning, is considered a determinant of health and longevity. Recently, an increasing number of scientific reports have demonstrated the relationships between impaired autophagy and various diseases, resulting in growing interest in the process itself and the methods of inducing it.

Classification of autophagy

Previously, three main types of autophagy were described: microautophagy, chaperone-mediated autophagy and macroautophagy [8]. This classification is based on the manner in which the elements intended for degradation are delivered to the lysosomes [9]. The simplest type of autophagy is microautophagy, which involves a direct absorption of the material to be digested by the lysozyme. Chaperone-mediated autophagy (CMA) is characterised by the presence of a specific amino acid sequence (KFERQ, Lys-Phe-Glu-Arg-Gln) in a substrate molecule [10]. This pentapeptide in damaged proteins interacts with chaperone proteins Hsc 70 (heat shock cognate 70 kDa) and only in the form of this complex is delivered to the lysosome, where it binds with a LAMP 2A (lysosome associated membrane protein type 2A) receptor, to be moved and hydrolysed [11]. Macroautophagy is the type of the process most frequently defined as autophagy [12]. It essentially involves four phases: initiation, elongation of the phagophore, maturation of the autophagosome, fusion between the autophagosome and lysosome and degradation of the content by proteolytic enzymes [11]. Autophagy is controlled antagonistically by AMPK (AMP-activated protein kinase) and mTOR (mammalian target of rapamycin), which act as cellular indicators of nutrients [13]. Ulk1 phosphorylation by AMPK activates autophagy, while mTOR inhibits the process [14]. The entire process is controlled by specific Atg proteins [15]. The key factors in the initiation of autophagy and creation of autophagosomes are beclin 1 (a homologue of the yeast Atg 6 protein) and PI3K class III (phosphoinositide 3-kinase class III). In subsequent phases, beclin 1 activates other Atg proteins, resulting in elongation of the vesicle [14]. One of the more important stages of autophagy is conversion of cytosolic LC-3 (LC3-I; microtubule-associated protein 1 light chain 3) to a form conjugated with the inner membrane of the autophagic vesicle, LC3-II, which is considered the principal marker of autophagy [14, 16].

Diseases associated with impaired autophagy

All diseases associated with impaired autophagy share a common characteristic: accumulation of damaged cellular organelle and/or dysfunctional proteins, which disturbs cellular homeostasis. These elements may accumulate as a consequence of impaired final phases of autophagy, observed in microscopic images as an accumulation of the structures specific for this process: autophagic vacuoles (AV), inclusion bodies (IB) or multi-vesicular bodies (MVB) [17, 18]. Progress in the research on autophagy was supported by genome-wide association studies (GWAS), which allowed for identification of genes associated with autophagy [19]. In recent years, an increasing number of diseases associated with autophagic dysfunction has been found. They include among others inflammatory bowel diseases, cardiovascular diseases, neurodegenerative conditions, diabetes, obesity and neoplasms [1, 20].

Inflammatory bowel diseases

Inflammatory bowel diseases (IBD) are chronic gastrointestinal conditions characterised by periods of exacerbation and remission [21]. The most common non-specific inflammatory bowel diseases are Crohn's disease (CD) and ulcerative colitis (UC) [22]. CD presents as non-specific inflammation of the gastrointestinal wall and may affect any section of the digestive tract[11], while UC affects the final fragment of the digestive tract [23]. Sometimes, when the inflammation affects the colon, differentiation and diagnosis is impossible; in such cases we talk about unclassified colitis (IBDU) [11]. Aetiology of inflammatory bowel diseases is varied, but the most commonly identified causes include environmental and genetic factors, impaired autophagy and dysbiosis [11, 24]. The development of GWAS contributed to the progress in IBD diagnostics. The genes associated with autophagy, whose mutations contribute to IBD, include *ATG16L*, *IRGM* and *LRRK2* [25]. Atg 16L protein plays a crucial role in the process of autophagy, as it participates in the creation of autophagosomes [26]. The best known modification associated with dysfunctional autophagy in the course of IBD is a single nucleotide polymorphism (SNP) in which threonine is substituted for alanine at position 300 (T300A), which doubles the risk of CD [27, 28]. This mutation disturbs the activity of Paneth cells and goblet cells, resulting in impaired autophagy dedicated to pathogen elimination, known as xenophagy [29, 30]. Apart from disturbed pathogen elimination, mutation in the *ATG16L* gene increase the secretion of proinflammatory cytokines by Paneth cells, and increase the secretion of interleukin-1β (Il-1β), interleukin-18 (IL-18) and reactive oxygen species (ROS) by macrophages due to the activity of lipopolysaccharide (LPS) [31]. Therefore, *ATG16L* mutations contribute to exacerbated inflammation. Another gene related to autophagy and the development of IBD is IRGM which codes the M protein (immunity-related GTPase family M protein, IRGM) [32]. This protein is responsible for the maturation of autophagosomes and participates in

pathogen elimination from mammalian cells. Previous studies demonstrated inconsistent results regarding the relationship between polymorphisms of this gene and CD phenotype [33, 34]. However, a correlation was found between mutations in the *IRGM* gene and incidence of UC [34]. Regarding the next gene, *LRRK2* (leucine-rich repeat kinase 2), it has been demonstrated that its polymorphism is related to the occurrence of CD [35]. An increased expression of this gene was observed in patients diagnosed with CD [36]. Interestingly, compared to other genes associated with autophagy, the expression of LRRK2 is observed in the leukocytes present in the lamina propria, not in the intestinal epithelial cells [37].

Cardiovascular diseases

The continuity of the cardiac function is maintained by the energy (ATP) synthesised in mitochondria. A selective type of autophagy, involving degradation of these structure, is known as mitophagy, and its impairment is associated with various disorders of the heart muscle function [38, 39]. A particular role of autophagy was found in heart failure, proteinopathy, ischaemia and reperfusion, in which proteins are damaged due to oxidative stress [14, 40]. Studies on mice revealed that deficits of the Atg 5 protein contribute to the accumulation of polyubiquitinated proteins, mitochondria, increased endoplasmic reticulum stress, changes in the structure of the sarcomere and apoptosis of the cardiomyocytes [39]. Accumulation of the p62/SQSTM1 protein and polyubiquitinated proteins have been correlated with atherosclerosis, and the findings were based on murine models, as well as on studies involving patients diagnosed with atherosclerosis [41]. Another study demonstrated a relationship between autophagy/mitophagy and heart failure or aortic stenosis. Initially, the processes were activated, but due to reduced effectiveness of mitochondrial function and progressive heart failure, they were inhibited [42]. A therapeutic role of autophagy in various cardiovascular diseases has also been established. For instance, cardiomyocyte hypertrophy in the course of cardiac hypertrophy was reduced following treatment with sophoricoside that activated the AMPK/mTORC1 pathway, inducing the autophagy process [43]. Another example is the use of metformin which, by blocking the pathway activating autophagy regulated by AMP-activated protein kinase, reduced the development of heart failure [44].

Neurodegenerative diseases

Probability of neurodegenerative diseases increases with age, due to changes such as oxidative stress, mitochondrial damage, energy deficits, hyperactivation of glutamate receptors and disturbed homeostasis [45]. The majority of neurodegenerative diseases is due to accumulation of specific proteins, characteristic for a particular disorder [46, 47]. This accumulation is caused by the impairment of the processes of protein degradation that progresses with age, including autophagy [48]. Protein accumulation may lead to disturbed transmission of neural impulses between synapses, and even to the death of nerve cells [46, 47]. The most frequently observed neurodegenerative diseases include Alzheimer's disease (AD) and Parkinson's disease (PD). In their course, accumulation of autophagosomes and damaged proteins is observed [49]. Alzheimer's disease involves a progressive loss of synapses in the cerebral cortex and in the hippocampus, resulting in memory disorders and impairment of the cognitive functions [50]. The aetiology of this condition is not fully understood. The most common explanations point to the accumulation amyloid-β (Aβ) and tau protein [51, 52]. Amyloid-β accumulates in the form of amyloid plaques, while tau builds up in the form of intraneural aggregates, creating neurofibrillary tangles [53]. Impaired autophagy is indicated as the principal cause of aggregation of these structures; therefore, strategies to activate autophagy are being explored as a therapeutic option in the treatment of Alzheimer's disease [54]. For instance, it has been demonstrated that using rapamycin (mTOR kinase inhibitor, activator of autophagy) blocked the aggregation of amyloid-β and tau protein, and improved cognitive functions; however, this effect was only observed in early stages of the disease [55, 56]. Parkinson's disease is caused by the accumulation of α-synuclein and degeneration of dopaminergic neurons [57]. The main symptoms include impaired motor function, sleep disorders, mood swings and reduced cognitive function [58]. Although the mechanisms behind the disease are not fully understood, GWAS made it possible to identify numerous genes associated with impaired autophagy and development of Parkinson's disease [59].

Diabetes

Diabetes, despite the differences between type I and type II, is characterised by a lack of glucose homeostasis, due to insufficiency of pancreatic β-cells [60]. Simplifying, the pancreas produces less insulin, which causes hyperglycaemia. The impaired function of β-cells is due to endoplasmic reticulum stress and oxidative stress [61]. Autophagy plays a key role in the function of pancreatic β-cells [62]. The correlation between inhibited autophagy and impaired β-cell function was demonstrated in a study using *Atg7* knockout mice [63]. Reduced insulin secretion and impaired glucose tolerance were observed in the cells of the knockout mice, which clearly points to the role of dysfunctional autophagy in the pathogenesis of diabetes. Another study, using obese diabetic mice, demonstrated impaired autophagy and death of pancreatic β-cells [64]. Following intermittent fasting, autophagy was activated and the course of obesity-induced diabetes was milder. Demonstration of the relationship between inhibited

autophagy and diabetes led to the development of methods aimed at induction of autophagy in the treatment of diabetes. One of effective methods of controlling the course of diabetes is diet modification. It was demonstrated that the fast-mimicking diet (FMD) supports regeneration of pancreatic β-cells in the murine model of type I and type II diabetes [65]. Highly effective in the treatment of diabetes is e.g. metformin, an insulin-mimetic agent. It was demonstrated to activate autophagy in β-cells and to inhibit apoptosis under lipotoxicity in an in vitro model [66]. What is interesting, in relation to diabetes, a varied effect of rapamycin was observed. On the one hand, it was demonstrated that this autophagy activator helps to reduce body weight growth and blood glucose levels in rodents receiving a high-fat diet [67, 68]; on the other hand, increased insulin resistance and impaired pancreatic function were observed, even when autophagy was activated [69].

Obesity

Obesity and its consequences, i.e. increased risk of diabetes, arterial hypertension, cardiovascular diseases and neoplasms, are a global problem [70]. Excessive fat content localised outside of the fatty tissue, e.g. in hepatic cells or skeletal muscles, may injure these tissues or induce systemic lipotoxicity due to a high concentration of free fatty acids in the blood serum [71, 72]. One of the factors activating autophagy is hunger, so excessive caloric intake, characteristic for the diet of obese patients, impairs this process. Studies demonstrated that excessive caloric intake may contribute to the inhibition of autophagy due to stimulation of its negative regulator, mTOR kinase [73]. Studies on mice demonstrated that in obese mice, autophagy is inhibited due to reduced expression of the genes related to autophagy: *ATG5* and *ATG7* [74]. On the other hand, there are numerous scientific reports demonstrating the accumulation of autophagosomes in the liver and adipocytes of obese mice and humans, which potentially points to the activation of autophagy [75]. Obesity is indicated as one of the factors inducing endoplasmic reticulum stress in the liver, and ER stress activates autophagy. Therefore, it is possible that autophagy is induced to restore the homeostasis disturbed by excessive body weight [74, 76]. However, effective autophagy should result in elimination of autophagic substrates, such as lipid droplets and protein aggregates, while many studies reveal accumulation of these elements in cells and tissues [75, 77]. Increased production and accumulation of autophagosomes points to the activation of autophagy, but also to its reduced efficiency [78]. The unclear problem of autophagy in the adipose tissue was addressed in studies on the function of lysosomes and proteases using *in vitro* and *in vivo* models [79]. The authors demonstrated that in the pathological adipose tissue, production of autophagosomes was accelerated, but at further stages the autophagic flow was inhibited, which resulted in accumulation of autophagosomes. The obtained results suggest an ineffective autophagy process in the adipose tissue.

Neoplasms

In the context of neoplasms, autophagy arouses the greatest controversy. On the one hand, it is a crucial process in cell growth and neoplastic transformation; on the other hand, it contributes to the death of neoplastic cells [80]. First reports regarding the role of autophagy in the neoplastic process date back to 1999, when the activity of beclin 1 was demonstrated to suppress tumour growth [81]. Studies revealed that deletion of the beclin1-coding gene was correlated with the development of neoplasms in the breasts, ovaries and prostate, while reduced expression of the gene was observed in neoplasms of the breasts, ovaries and brain [81, 82, 83]. Research demonstrated that mutation in the beclin-coding gene inhibited autophagy and increased susceptibility to neoplasms [84, 85]. Other studies showed that p62 protein (a selective autophagy protein) participated in the control of the neoplastic process [86]. In the murine model, aggregation of p62 was found to impair autophagy. Unclear is the role of autophagy in the development of colonic neoplasms. On the one hand, it was demonstrated that in advanced stages of the tumour, LC3-II (autophagy marker) is overexpressed, which indicates high activity of the process [86], but other studies showed reduced expression of *ATG 5*, a gene that activates autophagy [87]. A study on pancreatic tumours revealed that increased autophagy supports the development of cancer cells, not only providing the energy necessary for the progression of the neoplastic process, but also supplying substrates, such as proteins, nucleic acids and lipids that make increasing the biomass of the neoplastic cells possible [88]. It appears that the role of autophagy in the neoplastic process is determined by the stage of the disease. Initially, autophagy degrades the damaged organelle and proteins, preventing the development of a neoplasm, but in advanced stages autophagy enables the tumour to adapt to adverse conditions, such as hypoxia, and allows the disease to progress [89, 90].

Methods of autophagy activating

Effective autophagy is considered a determinant of good health. Elimination of the damaged cellular organelle and dysfunctional proteins supports homeostasis and prevents diseases, which helps also to delay the ageing process. General systemic effects promoting longevity were proven in mice, worms and flies [91], but in recent years there has been growing interest in methods of activating this process also in humans. One of the factors inducing autophagy is fasting stress, so various dietary models based on caloric restrictions are gaining popularity. Proautophagic effects of energy deficits consists in antagonistic activity of AMPK/mTOR, the cellular sensors of the availability of nutrients [13]. For

autophagy to be activated, the glycogen stored in the liver and muscles must be used, so the time necessary to induce the process is considered 72 hours [92]. However, three-day fasting is too challenging for consumers, so different fast-imitating nutritional models have been developed [93]. The most popular protocol is intermittent fasting (IF), involving periods of fasting and so called "eating windows". The most common IF schedule comprises 16 hours of fasting followed by an 8-hour period when eating is allowed [94]. Other nutritional strategies based on alternating fasting and eating include ADF (alternate days fasting), FMD (fast-mimicking diet), and TRD (time restriction diet) [95, 96]. An example of dietary activation of autophagy can be obtained by ketogenic diet (KD), in which daily carbohydrate intake is limited to approximately 5–10% of the total caloric intake or if their amount is less than 50 g a day [97]. With glucose deficits, fats are metabolised, resulting in ketosis, in which the main sources of energy are ketone bodies, used by the organism also during fasting [96]. The relationship between ketogenic diet and induction of autophagy was demonstrated e.g. in a study on mice in which an increased expression of LC3-II and beclin 1 was observed in animals on a high-fat diet, indicating that autophagy had been activated [98]. Due to the growing interest in autophagy, biologically active substances that can affect the process have been identified. They include among others curcumin and resveratrol. The role of curcumin in the activation of autophagic pathways was demonstrated e.g. in studies on human colon cancer cells (HCT116) and mouse embryonic fibroblasts (MEF) [99]. Resveratrol was shown to contribute to the degradation of amyloid plaques in mice, demonstrating a potentially therapeutic effect in Alzheimer's disease [100]. Physical activity also appears to stimulate autophagy. There are reports from studies assessing the effect of endurance training on the activation of autophagy in mice, depending on whether the training animals were fasting or after a meal. Based on the increased levels of autophagy markers, the study revealed that autophagy was activated in both cases. It should be emphasised that autophagy was more marked in the animals that were training in a fasted state [101].

Conclusion

Growing interest in autophagy in recent years results from its role in aetiopathogenesis of a broad spectrum of diseases, including among others inflammatory bowel diseases, cardiovascular diseases, neurodegenerative diseases, diabetes, obesity or neoplasms. Autophagy, as a process of cellular self-cleaning and recycling, maintains homeostasis in the organism, and elimination of harmful or defective components, such as pathogens and dysfunctional proteins, ensures normal function of cells and tissues. It reduces the risk of diseases associated with ageing. Since autophagy decreases with age, methods of activating the process are sought. Autophagy itself is considered to be a process that inhibits ageing and promotes longevity. The behavioural methods of inducing autophagy include nutritional restriction, such as reduction of the caloric intake, intermittent fasting and reduced carbohydrate supply. Autophagy is also promoted by physical activity, as well as by certain groups of products rich in biologically active compounds, such as curcumin and resveratrol. Although autophagy is believed to have a range of health-promoting properties, it should be emphasised that the process is dichotomous; therefore, both its impairment and excessive activation have an adverse impact on health.

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