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EPIGENETIC REGULATION OF THE NEURODEGENERATION BY THE METHYLTRANSFERASE G9A

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Abstract

Current studies suggest that changes in epigenetic modifications are associated with aging and neurodegeneration. Chromatin alterations can contribute to the development of neurodegenerative diseases, as has been reported in cases of Alzheimer's disease. As our understanding of this pathology has advanced, epigenetic regulation by methyltransferase G9a has been suggested as an important mechanism involved in learning and memory formation. In the cell, G9a forms a complex with G9a-like protein (GLP), which is responsible for depositing methyl groups to the lysine-9 position of histone H3 (H3K9) in euchromatin. This H3K9 methylation is an indicator of silence transcription, which has been associated with autophagy and synaptic plasticity deficiencies.

Keywords: epigenetic, neurodegeneration, methyltransferase G9a, autophagy, plasticity synaptic.

Resumen

Actualmente, diferentes estudios sugieren que los cambios en las modificaciones epigenéticas están asociados con el envejecimiento y la neurodegeneración. Las alteraciones de la cromatina pueden contribuir al desarrollo de enfermedades neurodegenerativas, como se ha descrito en la enfermedad de Alzheimer. A causa de que el conocimiento para esta patología ha avanzado, la regulación epigenética de la metiltransferasa G9a se ha sugerido como un mecanismo crucial involucrado en el aprendizaje y en la formación de la memoria. En la célula, G9a forma un complejo con su proteína homóloga GLP, la cual resulta ser responsable de metilar la posición de la lisina 9 de la histona H3 (H3K9). Esta metilación de H3K9 es un indicador del silenciamiento de la expresión y transcripción génica, que se ha relacionado con deficiencias en la autofagia y la plasticidad sináptica.

Palabras clave: epigenética, neurodegeneración, metiltransferasa G9a, autofagia, plasticidad sináptica.

Resum

Actualment, diferents estudis suggereixen que els canvis en les modificacions epigenètiques estan associats amb l'envelliment i la neurodegeneració. Les alteracions de la cromatina poden contribuir al desenvolupament de malalties neurodegeneratives, com s'ha descrit en la malaltia d'Alzheimer. Com que el coneixement d'aquesta patologia ha avançat, la regulació epigenètica de la metiltransferasa G9a ha estat suggerida com un mecanisme crucial implicat en l'aprenentatge i la formació de la memòria. A la cèl·lula, la G9a forma un complex amb la proteïna anàloga, la GLP, el qual és responsable de metilar la posició de la lisina 9 de la histona H3 (H3K9) en l'eucromatina. Aquesta metilació de la H3K9 és un indicador del silenciament de l'expressió i la transcripció de gens, que s'ha associat amb deficiències en l'autofàgia i la plasticitat sinàptica.

Paraules clau: epigenètica, neurodegeneració, metiltransferasa G9a, autofàgia, plasticitat sinàptica.

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1. Introduction

1.1. Epigenetics

In 1939, Conrad Hal Waddington established the term epigenetic as the field of biology related to causal interactions between genes. The complete definition for this term is: "an epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence". Thus, it depends on chemical modifications in DNA, RNA, and proteins (Waddington, 2012; Guillaumet-Adkins *et al.*, 2017).

1.1.1. Introduction to epigenetic mechanisms

After Waddington's publication about the term epigenetic, scientists focused on explaining why gene expression and function might not be related to changes in the DNA sequence (Song and Johnson, 2018). In general, there are three main mechanisms: DNA methylation, histone modifications, and noncoding RNA (ncRNA) (Figure 1) (Berson *et al.*, 2018).

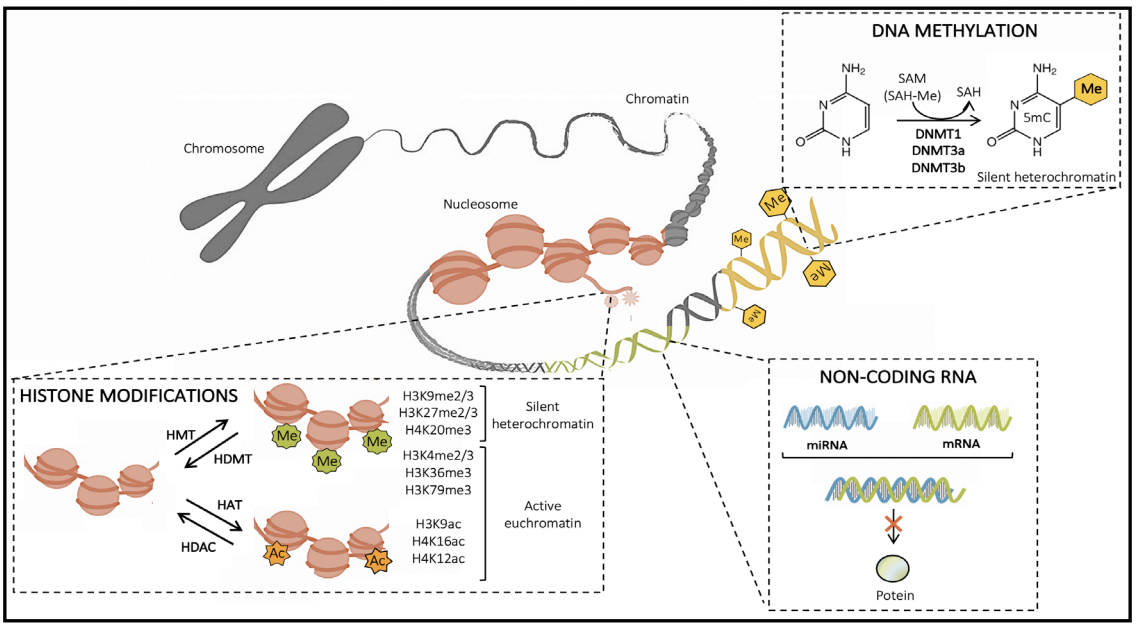

Figure 1. Diagram of the main mechanisms of epigenetic regulation.

Human DNA is packed into chromatin within the nucleus. Overall, this supra-nucleoprotein complex is composed of DNA, histones, non-histone proteins, and interacting RNA molecules. Indeed, all these structures are related to mechanisms of epigenetic gene regulation, such as methylation of cytosines within DNA, and post-translational modification (PTM) of histones, among others (Song and Johnson, 2018).

DNA methylation was the first epigenetic modification that was described, and it is catalyzed by DNA methyltransferases (DNMTs) (Guillaumet-Adkins *et al.*, 2017). In general, this modification is accompanied by the addition of a methyl group, which is derived from S-5'-adenosyl-L-methionine (SAM), to promoter regions. It usually occurs on the C_5 position of cytosine (C) to form 5-methyl cytosine (5mC), in CpG islands (Guillaumet-Adkins *et al.*, 2017; Cui and Xu, 2018). These islands have C-G dinucleotide repeats that are usually found within promoter regions and transcription start sites of

genes. Thus, the effect of 5mC is to silence gene expression, as it interferes with transcriptional initiation. (Guillaumet-Adkins *et al.*, 2017, Sanchez-Mut and Graff, 2015).

As indicated previously, histones are the main protein components of chromatin structure (Füllgrabe *et al.*, 2014). Nevertheless, histone proteins do not only function as "DNA packaging". In reality, PTMs play a major role in the regulation of DNA (Sanchez-Mut and Graff, 2015). Mainly, there are two main modifications catalyzed by enzymes that are responsible for the specific addition or removal of PTMs and have functional consequences (Füllgrabe *et al.*, 2014). On the one hand, histone acetylation occurs at lysine (K) residues and is usually considered an active transcriptional mark. It occurs as a consequence of the antagonistic activity of histone acetyltransferases (HATs) and deacetylases (HDACs). On the other hand, histone methylation occurs at K or arginine (R) residues, which can act as a transcriptional activator or repressor. In this case, the equilibrium between histone methyltransferases (HMTs) and demethylases (HDMTs) regulate the methylation (Sanchez-Mut and Graff, 2015).

Moreover, other types of modification could also be possible. Hence, the histone code hypothesis has been suggested. It describes that the existence of different modifications within a cell can regulate the structure and activity of various chromatin regions (Sanchez-Mut and Graff, 2015).

Additionally, numerous studies have shown that ncRNA can also act as a regulator of gene expression to control cell differentiation (Wei *et al.*, 2017; Guillaumet-Adkins *et al.*, 2017; Song and Johnson, 2018). NcRNAs, which are not translated into proteins, are mainly divided into two groups based on size, as either long or short. In general, their mechanisms consist of recruiting different histone-modifying enzymes, recognizing, adding, removing, and replacing chromatin modifications (Guillaumet-Adkins *et al.*, 2017). Among other functions, ncRNAs, both short and long, play a significant role in neuronal epigenetic mechanisms. Especially, miRNAs are involved in interference RNA (iRNA) mechanisms, resulting in the degradation of the target messenger RNA (mRNA) or inhibition of translation (Wei *et al.*, 2017).

1.1.2. Impact of epigenetic effects in human diseases

1.1.2.1. The interplay between epigenetics and aging

Current studies accept that changes in epigenetic modifications are associated with aging. Aging is an inevitable outcome of life characterized by the progressive functional decline of organisms at molecular, cellular and physiological levels (Sen *et al.*, 2016). As a result, there is neuronal death, decline of memory and cognitive processes, and other functional impairments. This biological process is, therefore, one of the main factors in human diseases such as cancer, neurodegeneration, and cardiovascular disease (Delgado-Morales *et al.*, 2017).

In general, chromatin changes suggest a possible way to revert aging defects. For example, upregulation of activating marks, downregulation of repressive marks, and regulation of expression of longevity genes (Sen *et al.*, 2016). Regarding chromatin remodeling, there are altered levels of histone acetylation and methylation that occurs during aging. Accordingly, there is an increase in the acetylation of histones H3 and H4. Likewise, the global methylation of histones H3 and H4 gradually changes with age (Berson *et al.*, 2018; Pal and Tyler, 2016). Moreover, the correlation between aging, diseases, and changes in the expressions of that code for miRNAs is related to the regulation of several metabolic and senescence pathways (Pal and Tyler, 2016).

As chromatin alterations can act as modulators of gene expression, these are important targets to prevent neurodegenerative deterioration (Berson *et al.*, 2018). Furthermore, environmental factors can also act as direct modulators of disease development, or indirectly via genetic or epigenetic pathways (Lardenoije *et al.*, 2018).

In fact, aging has been established as the main risk factor for all types of dementia, which describes a group of symptoms associated with cognitive decline and a behavioral deficit. Some 60-80%

of cases of dementia are Alzheimer's disease (AD); hence, it is the most common form (Griñán-Ferré *et al.*, 2018; Maloney and Lahiri, 2016).

1.1.2.2. Epigenetic contribution to neurodegenerative diseases

Neurodegenerative diseases are characterized by a progressive loss of neuronal integrity and function, followed by neuronal death (Lardenoije *et al.*, 2018; Gangisetty *et al.*, 2018). As mentioned previously, the most common neurodegenerative disease is AD, which is associated with progressive and irreversible neurodegeneration (Plaza-Zabala *et al.*, 2017).

In recent years, scientists have suggested that epigenetic mechanisms play an important role in the development of neurodegeneration. These mechanisms are involved in the functioning of the nervous system and participate in neurological disorders as well as in cognition, learning, and memory formation (Griñán-Ferré *et al.*, 2018). PTMs, DNA methylation, and nucleosome remodeling are three of the mechanisms that regulate the expression of genes associated to memory and learning processes (Campbell and Wood, 2019).

Interestingly, numerous studies show growing evidence that epigenetic dysregulations are involved in AD. The following table compiles some modifications that occur in this neurodegeneration:

TABLE 1. EPIGENETIC MODIFICATIONS IN ALZHEIMER'S DISEASE.

Alzheimer's Disease	References
Reduction in DNA methylation	(Griñán-Ferré *et al.*, 2018)
Reduced expression of genes associated with synaptic plasticity	(Gangisetty *et al.*, 2018)
Increase of inflammatory and immune response genes	(Griñán-Ferré *et al.*, 2018)
Changes in the methylation status of transcription factor binding sites of Tau promoter	(Berson *et al.*, 2018)
Hyperacetylation of histone H3 and H4	(Griñán-Ferré *et al.*, 2018)
Hypermethylation of H3K9 and H3K14	(Lardenoije *et al.*, 2018)
Hyperphosphorylation of global H3	(Griñán-Ferré *et al.*, 2018)
Dysregulation of several miRNAs	(Gangisetty *et al.*, 2018)

1.2. G9a histone methyltransferase

As described previously, one epigenetic way to control gene expression is by chromatin remodeling through methyltransferase activity. These enzymes, called HMTs, catalyze the addition of a methyl group from SAM. If the methylation occurs in K residues, it is related to either activation or repression of gene transcription and expression. However, if the methylation occurs in R residues, it is mainly related to transcriptional activation. Moreover, HMTs are also involved in the regulation of cellular signal transduction pathways (Milite *et al.*, 2019; Griñán-Ferré *et al.*, 2018).

Over the last decade, studies have suggested the epigenetic regulation by the methyltransferase G9a (also known as EHMT2) as a crucial mechanism involved in learning and memory formation (Rowe *et al.*, 2019). Mainly, this enzyme can catalyze mono- or dimethylation reactions on H3K9

of euchromatin. However, G9a is also involved in H3K9 trimethylation and H3K27 methylation in vivo (Shinkai and Tachibana, 2011).

In the cell, G9a forms a complex with a highly homologous methyltransferase named G9a-like protein (GLP, also known as EHMT1) (Rowe *et al.*, 2019; Füllgrabe *et al.*, 2014). Several studies have demonstrated that GLP and G9a have the same substrate specificities on histones and methyltransferase activity. Although both HMTs independently can exert this function, G9a generally cannot compensate for the loss of GLP, and vice versa (Shinkai and Tachibana, 2011).

The binding between G9a and GLP is through their SET domains, which contain ankyrin repeats involved in their methyltransferase activities and protein-protein interaction (Rowe *et al.*, 2019; Füllgrabe *et al.*, 2014). Indeed, both enzymes share approximately 80% sequence identity in these domains (Milite *et al.*, 2019). The physiological role of the G9a/GLP complex is typically involved in a large number of processes, including embryonic germ cell development, immune response, and brain function (Rowe *et al.*, 2019; Zhang *et al.*, 2015).

At the molecular level, G9a is essential for the repression of gene transcription. Especially, the G9a/GLP complex is responsible for the methylation of H3K9 in mammalian euchromatin. In fact, this epigenetic mark has been described as an indicator of silenced transcription (Hyun *et al.*, 2017; Zhang *et al.*, 2015). Nonetheless, the heterodimeric complex can also methylate non-histone proteins, such as SIRT1, transcription factors, and itself (Zhang *et al.*, 2015). Besides, the complex can recruit DNMT to promoters through direct interactions with several DNA-binding proteins and increase the methylation of CpG islands and gene repression (Hyun *et al.*, 2017).

As a consequence, the repression of gene expression becomes crucial in many biological processes, including memory formation, immune responses, and differentiation, among others. Therefore, G9a/GLP dysregulation has been associated with numerous human diseases (Hyun *et al.*, 2017).

2. Methodology and methods

The bibliographic research for this paper has been conducted through primary and secondary bibliographical sources found in the PubMed and Scopus databases. This research included articles dated from 1988 to 2019, with no restriction of language.

3. Results

3.1. Therapeutic targets for inhibition of neurodegeneration

3.1.1. Relevance of autophagy to the accrual of atypical proteins

Autophagy is emerging as a key regulator of the central nervous system (CNS) in aging and neurodegeneration (Plaza-Zabala *et al.*, 2017). There are three main forms according to the process and mechanism of cargo delivery to the lysosome. Macroautophagy, which is referred to as autophagy, is an adaptive catabolic process that consists of the segregation of intrinsic cytoplasmic material into autophagosomes with subsequent fusion with lysosomes for degrading, recycling, and generating molecules (Giampieri *et al.*, 2019). In fact, it is a highly regulated process that is essential to maintaining energy homeostasis, and protects the cell against stress (Hansen *et al.*, 2018). However, if autophagy is overstimulated it can also lead to cell death (Thorburn, 2018).

The core autophagy mechanism is regulated by autophagy genes (ATG genes), which encode essential proteins that participate in the regulation of this cellular process, such as autophagosome formation and maturation (Figure 2) (Thorburn, 2018; Hyun *et al.*, 2017).

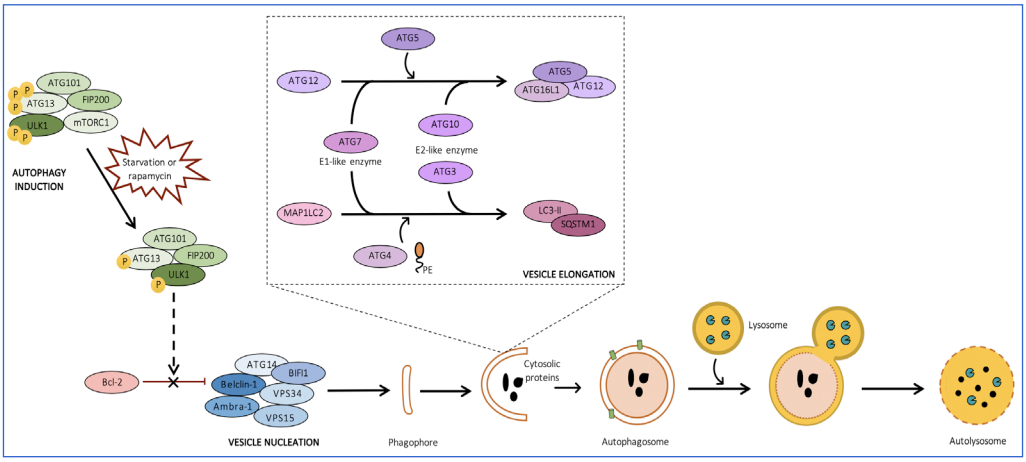

Figure 2. Autophagy mechanism (adapted from Füllgrabe *et al.*, 2014).

Numerous studies suggest that autophagy is closely related to aging. Due to autophagy activity declining during aging, it has been observed that Beclin-1, ATG5, ATG7, and light chain 3 (LC3) are down-regulated in humans (Escobar *et al.*, 2019). This is why upregulation of the autophagic function could be suggested as one possible approach to delaying aging (Plaza-Zabala *et al.*, 2017). In addition, there are indicative signals linked to the regulation of aging associated with autophagy, like starvation, the mechanistic target of rapamycin complex (mTORC1), AMP-activated protein kinase (AMPK), and p53 pathway, among others. Besides, some aging regulators also are necessary to induce autophagy under conditions of nutrient deprivation, such as SIRT1 and Forkhead box O's family (FOXO) (Giampieri *et al.*, 2019).

Neurodegenerative diseases are characterized by the progressive loss of neuronal structure and function, as well as the accumulation of misfolded proteins. In fact, these defects are often considered histological hallmarks of neurodegeneration. That is the reason why one of the pathological mechanisms for various neurodegenerative disorders consists of autophagic dysfunction (Condello *et al.*, 2019; Kim *et al.*, 2017).

In AD brains, numerous autophagosomes have been observed; hence, the late stages of autophagy pathway are suggested as a possible therapeutic strategy (Blennow and Zetterberg, 2018). Autophagosome membranes are rich in Presenilin 1 (PS1), which is required for lysosomal acidification (Metaxakis *et al.*, 2018). If the reduction of acidification alters the lysosome functionality, fusions between the autophagosome will not be possible. Therefore, the mutation of PS1 relates to the induction of AD (Condello *et al.*, 2019). Moreover, the loss of expression of Beclin-1 is correlated with early AD (Metaxakis *et al.*, 2018). Beclin-1 plays a role in the formation of the autophagosome, but it is disrupted by caspase 3. This protein is an important apoptosis activation component, which binds the phosphatidylinositol (PI) and arrests the progression of autophagy pathway (Condello *et al.*, 2019). Also, cytosolic levels of sequestosome 1 (p62) are altered in AD brains. The p62 is related to the accumulation of hyperphosphorylated Tau and regulates Aβ levels (Deng *et al.*, 2017). On top of that, some biochemical and genetic defects can induce AD through oxidative stress (OS) and chronic inflammation. Specifically, a nuclear receptor binding factor 2 (NRBF2), which is activated in response to the OS, has been observed to decrease in the hippocampus of AD mouse models (Condello *et al.*, 2019; Metaxakis *et al.*, 2018). Indeed, the activation of NRBF2 is involved in the induction of autophagy pathway and eliminates aggregated Tau proteins and Aβ oligomers through the autophagic receptor NDP52 (Metaxakis *et al.*, 2018).

3.1.2. The role of brain-derived neurotrophic factor (BDNF) in synaptic plasticity

Neurotrophins are a family of secreted proteins that regulate important neurological processes, such as synaptic plasticity, synaptogenesis, and neuronal survival (Buhusi *et al.*, 2017). BDNF is one of the

most important molecule mediators for learning and memory formation. Owing to its roles, this trophic factor is expressed in many brain regions, including the hippocampus, cortex, and hypothalamus (Angelucci *et al.*, 2019).

BDNF is synthesized by a precursor, which is known as pro-BDNF. Each form, mature BDNF and pro-BDNF, has a specific role that depends on the receptor which it activates (Buhusi *et al.*, 2017). Mature BDNF mediates survival and supports glutamatergic neurons by binding and activating the tropomyosin-related kinase receptor B (TrkB) (Angelucci *et al.*, 2019; Wang and Holsinger, 2018). For that reason, BDNF and TrkB are key regulators for structural and functional plasticity. Otherwise, pro-BDNF activates the p75 neurotrophin receptor (p75NTR) and promotes the induction of cell death (Figure 3) (Buhusi *et al.*, 2017).

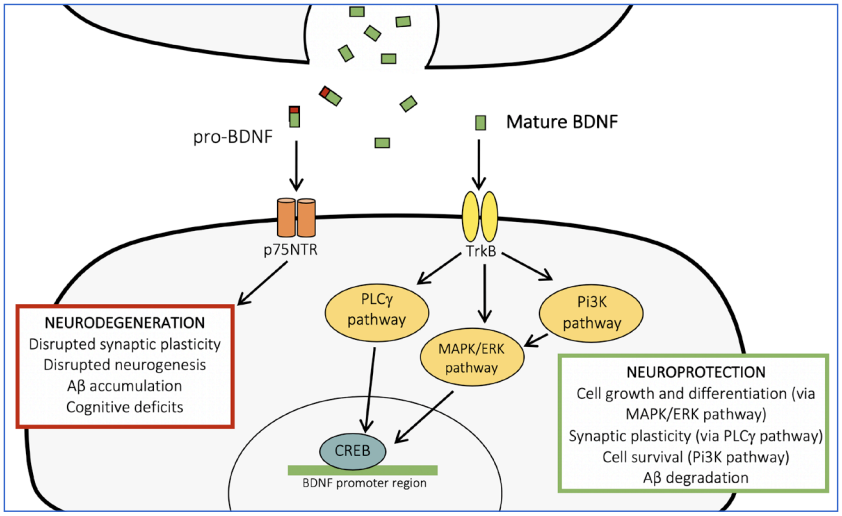

Figure 3. Role of BDNF and pro-BDNF (adapted from Angelucci *et al.*, 2019).

Several reports described that BDNF expression of its receptor are altered during aging (Sharma *et al.*, 2017b; Sharma *et al.*, 2017a; Jiao *et al.*, 2016; Angelucci *et al.*, 2019). In 2016, Buchman and collaborators demonstrated the correlation between higher BDNF levels and slower cognitive decline for dementia. Besides, it has been found that higher BDNF expression mitigates the detrimental effects of AD pathology in the brains of older adults; hence, BDNF has a neuroprotective role (Sharma *et al.*, 2017a; Buchman *et al.*, 2016).

In AD brains, it has been described that BDNF mRNA and protein levels are reduced in some brain regions, such as the hippocampus. This fact contributes to neuronal atrophy and death (Angelucci *et al.*, 2019). In the same way, the reduction of BDNF levels leaves neurons susceptible to OS caused by neurotoxic Aβ (Wang and Holsinger, 2018; Jiao *et al.*, 2016). Added to that, it has been observed that levels of BDNF are reduced in the nucleus basalis of Meynert, which is involved in the deterioration of cholinergic neurons. Besides, Braun and co-workers revealed that unilateral knockdown of hippocampal BDNF in 5xFAD mice models reduced microglial (Wang and Holsinger, 2018). In addition, it has been explained that the impact of BDNF Val66Met polymorphism could influence synaptic localization, activity-dependent secretion of neurothopic factor, and confer susceptibility to AD pathology (Lu *et al.*, 2013).

3.1.3. G9a as a target in neurodegenerative disorders

Growing evidence suggests that HMTs, which catalyze histone and non-histone proteins, act as a crucial regulator in human diseases. Especially, G9a and GLP are important enzymes for the methylation of H3K9, which is associated with transcriptional repression. When G9a and/or GLP is deleted, the

levels of H3K9me1 and H3K9me2 are reduced in euchromatin (Hyun *et al.*, 2017). Notably, this heterodimeric complex has been involved in learning and memory, addiction and disease (Sharma *et al.*, 2017b). In fact, G9a/GLP complex is mainly considered as an epigenetic suppressor that regulates synaptic activity and different signaling pathways, and, hence, modulates synaptic efficacy. Therefore, given G9a/GLP complex's role, its inhibition serves as a possible therapy in rescuing cognitive decline in pathological conditions related to epigenetic dysregulation, such as neurodegenerative diseases (Pang *et al.*, 2019).

Several reports describe that the inhibition of G9a can induce apoptosis or activate autophagic cell death. In 2012, G9a was suggested as an epigenetic regulator of autophagy that works like a repressor of autophagy-related gene expression. Thus, its inhibition is important during autophagy induction (Füllgrabe *et al.*, 2014; Baek and Kim, 2017). Artal-Martinez de Narvajas and collaborators showed that the methyltransferase G9a acts as a repressor of autophagy under normal growth conditions. However, the condition of nutrient deprivation leads to dissociation of G9a from the promoter with subsequent regulation of the chromatin landscape. As a result of this dissociation from the promoter, there is a decrease of H3K9me2, a repressive histone mark, and an increase of H3K9ac, an active histone mark. Therefore, transcriptional activation is induced (Baek and Kim, 2017; Artal-Martinez de Narvajas *et al.*, 2013; Sakamaki *et al.*, 2018). In other words, the inhibition of G9a activates autophagy genes, leading to an increase in the levels of LC3, WD-repeat domain phosphoinositide-interacting 1 (WIPI1), diabetes- and obesity-regulated (DOR) and p62, and subsequently feeding autophagic pathway (Park *et al.*, 2016).

In the same way, the G9a/GLP complex is involved in the regulation of plasticity-related products. So, the repression of transcription or translation of plasticity-related products, such as BDNF, have been associated with H3K9me2 marks (Pang *et al.*, 2019). Accordingly, Walker and collaborators observed that an increased level of H3K9 methylation was related by lower Bdnf gene expression in non-transgenic and 3xTg- AD mice. Specifically, G9a has been shown to directly bind to the Bdnf VI promoter within the mouse nucleus accumbens (Walker *et al.*, 2013). Indeed, lower levels of BDNF have been correlated with poor cognitive impairment and higher Aβ protein. Thus, the decreased expression of Bdnf mRNA suggests a high activity of the G9a/GLP complex during Aβ toxicity. In 2017, Sharma and co-workers showed the first evidence of the beneficial effects of inhibiting G9a/GLP complex activity to restore long-term plasticity (LTP). Moreover, another study by the same group observed that this inhibition reinforces early-LTP in protein synthesis and N-methyl-D-aspartate (NMDA) receptor-dependent manner. Furthermore, it has been proposed that the inhibition of the G9a/GLP complex could reestablish synaptic tagging/capture, which is described as one of the major mediators of associative plasticity (Sharma *et al.*, 2017a; Sharma *et al.*, 2017b).

3.2. Development of small-molecules that inhibit G9a/GLP

As mentioned before, chromatin structure is altered by PTMs, which have key roles in gene expression. Hence, small molecules have been proposed as promising targets for chromatin remodeling. G9a and GLP modulate transcriptional repression via methylating H3K9. Given its high homology, potent and selective inhibitors have been developed for both enzymes in the past decade (Table 2) (Herrera-Vázquez *et al.*, 2019; Yuan *et al.*, 2012).

Kubicek and co-workers identified BIX01294, the first potent which was discovered through a high-throughput screening (HTS) study. In the HTS, a minor inhibition of GLP was observed with respect to G9a and a competitive inhibitor with the peptide substrate. BIX01294 is active in cell-based assays, reduces the levels of H3K9 methylations at several G9a target genes, and has limited selectivity toward GLP. Nevertheless, the concentration values of the toxicity range and the latter activity were akin to one another, and this feature strongly limited its uses (Kubicek *et al.*, 2007).

To improve the therapeutic range, crystallographic studies based on BIX01294 allowed the identification of other inhibitor compounds. On the one hand, Liu *et al.* identified UNC0224 and

UNC0321 in 2009 and 2010, respectively (Liu *et al.*, 2009; Liu *et al.*, 2010). On the other hand, Chang *et al.* identified E72 in 2010. However, these three compounds are less potent than BIX0194 in cellular assays owing to reduced cell membrane permeability (Chang *et al.*, 2010). Consequently, new structures with favorable potency in cell lines were also identified in 2006: UNC0646 and UNC0631 (Herrera-Vázquez *et al.*, 2019). Nevertheless, these inhibitors were not effective in in vivo studies. Consequently, the optimization of their pharmacokinetic properties led to the identification of UNC0642 and UNC0638, which are two potent and selective G9a/GLP dual inhibitors. These efforts resulted in a reduction of H3K9me2 levels (Liu *et al.*, 2013; Liu *et al.*, 2011).

In 2012, Yuan *et al.* described a SAM-competitive inhibitor, known as BRD9539. Also, his group synthesized its prodrug, BRD4470. In cells, BRD4470 significantly reduced the levels of H3K9me2 and H3K9me3 and increased H3K9me1 levels. Also, this compound induced cellular apoptosis (Yuan *et al.*, 2012).

Sweis and collaborators reported another compound called A366 in 2014. This inhibitor, which has a novel spiro (cyclobutane-1,3′-indol)-2′-amine core, potently inhibited G9a and GLP. A366 was non-competitive with SAM and competitive toward the peptide substrate (Sweis *et al.*, 2014; Kaniskan *et al.*, 2018).

Given that G9a and GLP have different physiological and pathophysiological functions, the selective inhibitors have been important to describing their individual roles (Kaniskan *et al.*, 2018). Recently, Xion and co-workers suggested the synthesis of selective compounds, such as MS0124 and MS012. These compounds were more selective toward GLP than BIX01294 (Xiong *et al.*, 2017a).

TABLE 2. SMALL MOLECULES THAT INHIBIT G9A AND/OR GLP.

Name	Activity	References
BIX01294	G9a and GLP selective inhibitor	(Kubicek *et al.*, 2007)
UNC0646	G9a and GLP selective inhibitor	(Herrera-Vázquez *et al.*, 2019)
UNC0631	G9a and GLP selective inhibitor	(Herrera-Vázquez *et al.*, 2019)
UNC0224	G9a selective inhibitor	(Liu *et al.*, 2009)
UNC0321	Potent G9a selective inhibitor	(Liu *et al.*, 2010)
UNC0638	G9a and GLP selective inhibitor	(Liu *et al.*, 2011)
UNC0642	G9a and GLP selective inhibitor	(Liu *et al.*, 2013)
MS012	GLP selective inhibitor	(Xiong *et al.*, 2017a)
MS0124	GLP selective inhibitor	(Xiong *et al.*, 2017a)
E72	Potent GLP selective inhibitor	(Chang *et al.*, 2010)
BRD9539	G9a selective inhibitor	(Yuan *et al.*, 2012)
BRD4770	G9a selective inhibitor	(Yuan *et al.*, 2012)
A366	Potent G9a selective inhibitor	(Sweis *et al.*, 2014)

4. Discussion

In recent years, epigenetic regulation has been described as a molecular mechanism linking environmental influences during the course of life. Consequently, its dysregulation is closely related to cognitive decline in aging and neurodegenerative diseases (Harman and Martín, 2019). In fact, numer-

ous studies have described how genetic variants are involved in neurological disorders, such as the global methylation of histones H3 and H4 (Pal and Tyler, 2016). Notably, one of the most critical modifications is the methylation of H3K9. This methylation by methyltransferase G9a acts as a suppressor of gene expression and transcription. Therefore, epigenetic regulation by G9a is emerging as a promising mechanism to rescue the cognitive deficit in neurodegenerative disorders, such as AD (Pang *et al.*, 2019).

Of note, the alteration of H3K9 methylation has been associated with autophagy dysregulation and BDNF downregulation (Artal-Martinez de Narvajas *et al.*, 2013; Sharma *et al.*, 2017a; Sharma *et al.*, 2017b). On one hand, autophagy plays a key role in the degradation of misfolded proteins and damaged cells. On the other, BDNF is one of the main molecule mediators of functional synaptic plasticity. Thus, an increase in each pathway could seemingly rescue cognitive deficit (Artal-Martinez de Narvajas *et al.*, 2013; Sharma *et al.*, 2017a; Sharma *et al.*, 2017b). In the same way, Mota and collaborators described the importance of the balance of homeostasis undergoing control through autophagy recycling and protein synthesis. They concluded that an impairment of neuronal autophagy leads to an accumulation of dendritic materials and as a consequence, there is a cognitive decline. Also, their results showed an increase of BDNF, a main inducer of dendritic growth, in older animals. However, this increase does not seem apparently functional. Hence, their group proposed a model where the equilibrium between autophagic activity and neurotrophins regulates dendritic growth to induce an improvement of cognitive function (Figure 4) (Mota *et al.*, 2019).

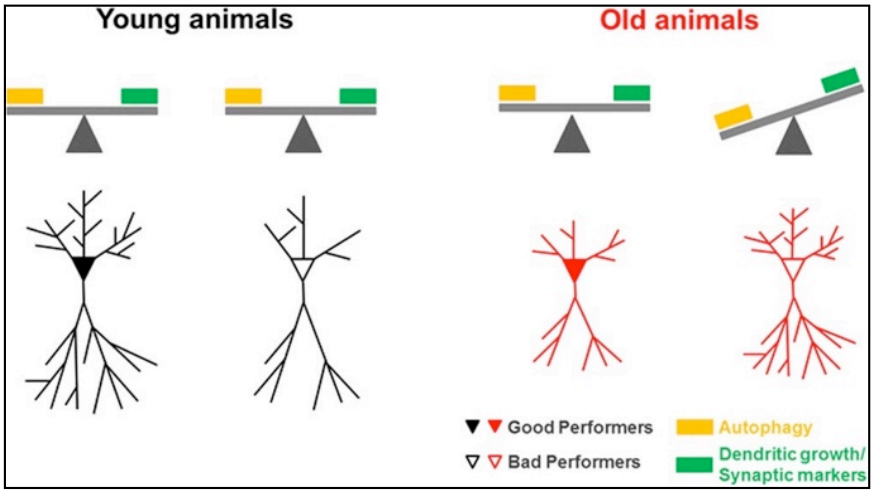

Figure 4. Schematic representation of the relations between cognitive performance, autophagy, and synaptic markers (Mota *et al.*, 2019).

Concerning the accumulation of aggregated proteins, autophagy plays an important role in their degradation. For that reason, one of the pathologic mechanisms of various neurodegenerative disorders consists of the dysfunction of this biological process. In 2013, Artal-Martinez de Narvajas and co-workers showed that dissociation of G9a from the promoter leads to a decrease of H3K9me2, a repressive histone mark (Figure 5) (Artal-Martinez de Narvajas *et al.*, 2013; Park *et al.*, 2016). Consequently they observed an increase in H3K9ac, which is an active histone mark, and proteins of autophagosome formation, including p62, WIPI1, DOR, and LC3. Added to that, a study released on Drosophila melanogaster suggests that G9a regulation of autophagy can be evolutionarily conserved (Füllgrabe *et al.*, 2014; Kramer *et al.*, 2011; Artal-Martinez de Narvajas *et al.*, 2013). In 2016, Ciechomska *et al.* showed how BIX01294 reduces H3K9me2 to induce autophagy in several tumor cell lines. In the same manner, Ke and collaborators described the effects of pharmacological inhibitors also reducing tumor growth in a mouse model (Ciechomska *et al.*, 2016; Ke *et al.*, 2014). Accordingly, it has

also been described as an increase of Beclin-1, after pharmacological inhibition of G9a/GLP complex in cancer cells (Park *et al.*, 2016). Likewise, another inhibitor, BRD4770, has been shown to increase levels of LC3-II and the numbers of autophagosomes in pancreatic cancer cells in vitro when in synergy with other inducers of autophagy (Yuan *et al.*, 2013).

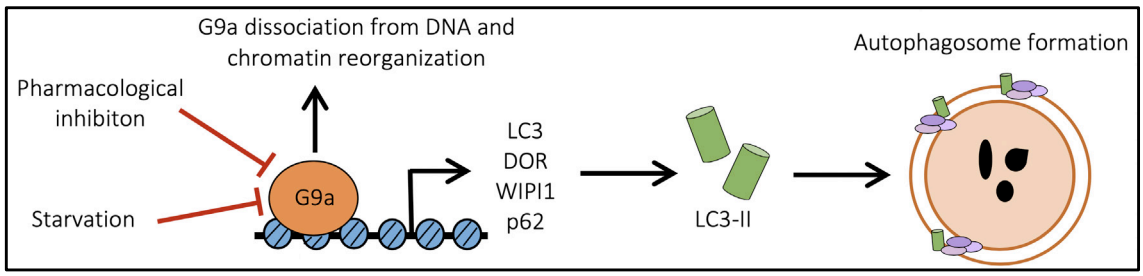

Figure 5. Inhibition of G9a inducing autophagosome formation (adapted from (Artal-Martinez de Narvajas *et al.*, 2013)).

Moreover, another characteristic of AD pathology is the loss of associative plasticity and memory. In neurons, synaptic plasticity regulates the secretion of BDNF, which is a mediator for learning and memory formation (Angelucci *et al.*, 2019). Therefore, it has been described that lower levels of BDNF have been correlated with cognitive decline and higher concentrations of Aβ protein (Sharma *et al.*, 2017a). In fact, the repression of BDNF expression was associated with H3K9me2 marks. Lubin and co-workers showed that a specific hypomethylation of promoter regions BDNF gene in the hippocampus could be involved in behavioral training (Harman and Martín, 2019). Along similar lines, Ma and collaborators showed that DNA demethylation at specific BDNF promoters leads to neurogenesis through electrical neuronal stimulation (Ma *et al.*, 2009). Hence, the repression of G9a/GLP activity has been proposed to upregulate BDNF levels. As indicated previously, Sharma and collaborators showed the first evidence of how the inhibition of G9a/GLP complex activity in the hippocampus results in facilitating LTP-maintenance and rescuing synaptic deficits (Sharma *et al.*, 2017a). Their findings are based on the regulation of G9a/GLP activity via pharmacological inhibitors, such as BIX01294 and UNC0638 (Figure 6) (Sharma *et al.*, 2017a; Sharma and Sajikumar, 2018; Sharma *et al.*, 2017b). Nevertheless, in other reports it is also described that this pharmacological inhibition has no effect on basal synaptic transmission in Schaffer collateral/commissural-CA1 synapses in the hippocampus (Sharma and Sajikumar, 2018).

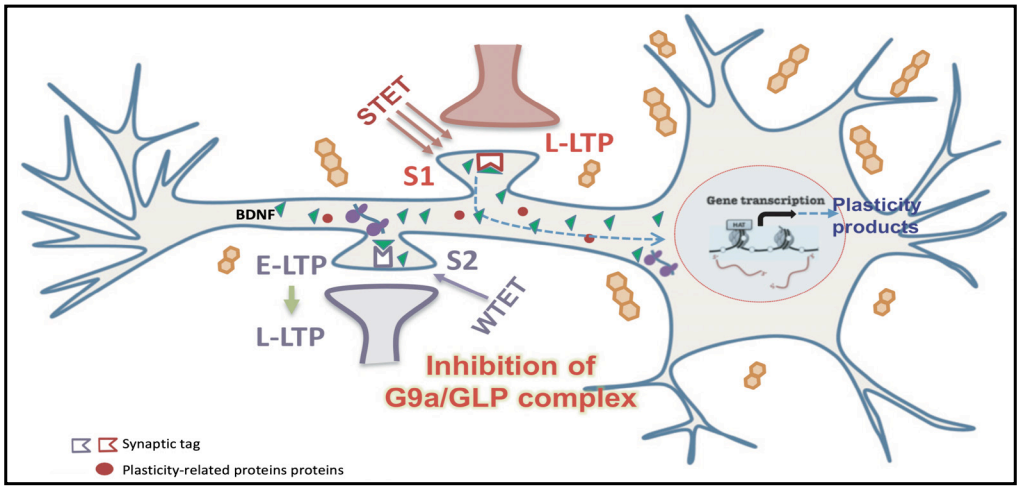

Figure 6. Inhibition of G9a/GLP in the synthesis of plasticity proteins (adapted from Sharma *et al.*, 2017a).

According to these findings, G9a and GLP have been proposed as promising targets for chromatin remodeling and small-molecule inhibitors have been developed. Regarding BIX01294, this was the first potent and selective dual inhibitor of GLP and G9a (Kubicek *et al.*, 2007). Nonetheless, there are reports that have described ineffective inhibition on its part. The results obtained by Scheweizer and collaborators did not show neuroprotection through BIX01294 in an in vitro model of cerebral ischemia. However, this discrepancy might be due to the concentrations of the pharmacological inhibitor used in primary neuronal culture (Schweizer *et al.*, 2015). To improve results, other small molecules have been developed during the last decade, such as UNC0642 and A366. In 2018, Wang and co-workers demonstrated reduced anxiety-like behaviors and H3K9 methylation in adult mice brains (Wang *et al.*, 2018). Additionally, several studies have reported interactions between HMTs and DNMTs. Hence, in recent reports the development of new therapeutic strategies has been described. López-López and co-workers identified dual selective inhibitors of epigenetic targets, such as G9a and DNMT1. They proposed that the new molecules should derive from 4-aminoquinoline with variations in the substituents of the residues 1 and 3. These ligands show interaction with the polar amino acids in their respective binding sites, favoring dual activity (López-López *et al.*, 2018).

5. Conclusion

In conclusion, to date, treatments of AD have been based on the clearance of Aβ accumulations, the reduction of Tau hyperphosphorylation, the inhibition of neuronal death, and the replacement of lost neurons. Nevertheless, several studies have described a potential role for histone methylation in AD pathology, as changes in chromatin have been related to a negative impact on cognitive function. This paper has described how the epigenetic modulation of the major HMT, which deposits methyl marks, could improve deficiencies during neurodegeneration. Selective inhibitors of this HTM seem to induce autophagy and revert BDNF downregulation. Thus, G9a inhibition may be one way to counter cognitive deficits thought to be associated with AD.

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