DNA Methylation in Tauopathies

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Together with histone variants and modifications, alterations in nucleosome positioning, non-coding RNAs, and DNA methylation constitute the epigenetic toolkit. DNA methylation describes the chemical modification of the DNA itself by the addition of methyl groups mostly on cytosines, but also on adenines via DNA methyltransferases (DNMTs), with DNMT1 and DNMT3A being the major DNMTs in the CNS. DNA methylation effects, i.a. transcriptional control when occurring at enhancer and promoter sites, alternative promoter choice and alternative splicing. At the level of transcriptional regulation, methylated motifs of transcription factor (TF) binding sites physically impede the binding of methyl-sensitive TFs, leading to transcriptional suppression. Furthermore, the interaction of the methyl-CpG-binding domain proteins (MBDs) with methylated DNA prevents binding of TFs and promotes inactive heterochromatin formation by recruiting other chromatin and nucleosome remodeling factors.

Alzheimer tauopathy TAU MAPT epigenetics neurodegeneration neurogenetic disease DNA methylation

1. Introduction

Neurodegenerative Diseases (NDDs) involve the irreversible loss of neurons or neuronal functions. The term NDD is often used to describe conditions of the central nervous system (CNS) characterized by neuronal dysfunction, neuronal loss and brain atrophy. The overwhelming majority of NDD-patients suffer from Alzheimer's Disease (AD), the most common form of tauopathy. Tauopathies are a heterogenous group of diseases, all characterized by abnormal accumulations and aggregations of the neuronal protein TAU [1][2][3].

TAU is a microtubule-associated protein (MAP) widely expressed in neuronal tissues and diverse cell types, in particular neurons and oligodendrocytes, but usually not in astrocytes. Roughly 80% of patients suffering from dementia are affected by a tauopathy [4]. In the clinic, patients are diagnosed with cognitive/dementia syndromes, movement disorders, motor neuron disease, either with isolated disease manifestation or in various assemblage [5], caused by the loss of neuronal function of the brain parts affected by TAU pathology. There is no such thing as a clinically well-defined single entity "tauopathy". Tauopathies can be classified as (i) primary tauopathies, with TAU being either the predominant or the causative pathology, and (ii) secondary tauopathies, where TAU pathology is either secondary to or appears in combination with other brain pathologies or insults (see below). Other classifications are e.g., the syndromic classification by Höglinger et al., that separates cognitive syndromes from motor syndromes (see e.g., [5]). Primary tauopathies may show an aggregation or pathology only of 3-repeat (3R) or 4-repeat (4R) TAU isoforms, while most secondary tauopathies present with an aggregation of all isoforms

(3R+4R/mixed). Hence, tauopathies can also be classified into 3R, 4R or mixed tauopathies, and can be based on structural features (i.e., the filament folds) of the aggregates [6][7][8].

2. Implication of DNA Methylation in AD and Tauopathies

2.1. Age-Dependent Changes of DNA Methylation Marks and the Relevance for AD and Tauopathies

Genomic instability, aberrant gene expression, and the loss in chromatin structure are features of both aging and multifactorial diseases such as AD [9][10]. These alterations are intimately associated to epigenomic changes [11], and can be responsive to environmental influence [12]. Aging represents the main risk factor for AD and most tauopathies, hence, age-associated epigenetic alterations likely contribute to the structural and functional changes of the brain that lead to progressive cognitive deficits and possibly derived augmented susceptibility to neurodegenerative disorders such as AD and tauopathies [13][14].

A common hallmark of both healthy aging and AD/tauopathies is the decline in memory function. Changes in the gene expression of chromatin remodeling enzymes, such as DNMTs and histone modifying proteins, are associated with alterations in synaptic plasticity, learning and memory [15][16][17][18][19][20]. Moreover, the expression or activity of epigenetic modifiers is altered in the aging brain [21]. Together, this underlines the relevance of epigenetic modifications in the context of aging and AD-related symptoms, which will be discussed as follows.

The age-related decline in *Dnmt3a2* expression seems to be linked to diminished cognitive abilities, as these were restored upon the rescue of decreased *Dnmt3a2* levels in mice [22]. In line with the decline in DNMT expression upon aging, global hypomethylation with local sites of hypermethylation were observed in aging brains across species, affecting the expression of genes related to synapse function, cellular homeostasis but also neuronal development [23][24]. Such age-associated DNA methylation changes are proposed to contribute to transcriptional alterations of AD-related genes, possibly predisposing for the disease [25][26]. Indeed, the expression levels of key genes associated with AD and taupathypathophysiology may be regulated by DNA methylation in an age-dependent fashion. This is true e.g., for the membrane protein APP (Amyloid-Precursor Protein), concentrated in the synapses. As indicated above, mutations in the *APP* lead to EOAD, due to an augmented or aberrant generation of the Aβ protein. The APP coding gene, which is frequently methylated, displays an age-related demethylation of cytosines in the promoter region (those at −207 to approximately −182), suggested to be linked to the Aβ deposition in the aged brain [27][28]. In contrast, the promoter regions of the neprilysin (NEP) gene, known to inhibit AD occurrence by clearing Aβ in the brain, turned out to be highly methylated and down-regulated in AD and aged healthy brains [29][30]. The elevated methylation of the *NEP* gene results in decreased expression, negatively impacting Aβ clearance, possibly causative for the elevated Aβ plaque burden in AD [29].

Also, methylation status of cytosines in the promoter region of the *MAPT* gene changes with age to reduce *MAPT* transcription in the cerebral cortex in humans: While in the binding sites of the transcriptional activator SP1 a

significant age-related increase in 5mC was observed on autopsy, a decrease with age of 5mC in the binding sites for GCF, and a repressor of GC-rich promoters was revealed [27].

Global DNA methylation changes in the brain, but also in peripheral tissues including the blood [31][32], have been identified to correlate well with aging. This epigenetic clock has even been used to predict the chronological (actual) age [32], hence serving as a measure of age-acceleration when comparing the biological (estimated) with the chronological age of an individuum. Age-acceleration has been associated with diminished physical and cognitive fitness [33], and an increase in all-cause mortality [34], but also with a range of age-related diseases, such as AD [35]. Due to this, the epigenetic clock is discussed as a biomarker of aging and age-related disorders, such as AD [36][37][38], as well as of disease progression [39].

2.2. Evidence for the Implication of Altered DNA Methylation Signatures in AD and Tauopathies

Similar to the aging brain, global DNA hypomethylation was reported for AD, supported by decreased immunoreactivity for 5mc in cortical neurons of postmortem AD brains (hippocampus, entorhinal and prefrontal cortex, cerebellum) compared to controls [38][40][41], in line with diminished staining with antibodies directed against DNA methylation maintenance factors in the hippocampal tissue of AD patients [40]. Monozygotic twin studies collecting twin pairs discordant for AD found reduced levels of DNA methylation in neuronal nuclei of the AD twin in the temporal neocortex [42].

Neuronal and glia cell-type specific differential methylation dynamics associated with AD Braak stage progression were observed for genes such as *ANK1, MCF2L, STK32C, LRRC8B, MAP2* and *S100B*, and methylation changes at the key AD risk genes *APP* and *ADAM17* were identified in a meta-analysis [43]. The increased risk of dementia and AD was further correlated with elevated DNA methylation levels in the promoter region of *APOE* [44]. Genetic variation in the *APOE* gene is related to AD risk and Aβ burden, with the *APOE4* variant being the most consistent (see above) genetic risk factor [45][46]. The DNA methylation-dependent effect was, however, independent of the *APOE* genotype [44]. This points to an independence of allelic and methylation variation of *APOE* for the risk to develop dementia.

2.2.1. DNA Methylation Changes Lead to Pathological Phosphorylation of TAU

Disturbed methylation levels in the promoter regions of genes related to TAU phosphorylation, which plays a critical role in tauopathies, were revealed by diverse clinical and basic research studies in the context of AD [47]. GSK3β is the kinase most commonly implicated in hyperphosphorylation of the TAU protein, which in turn is believed to be a prerequisite for the aggregation and formation of NFTs [48]. During early AD development, low DNA methylation levels were found in the promoter region of the GSK3β gene (*GSK3B)* in the prefrontal cortex tissue of AD patients, and consequently GSK3β expression was increased in patients with initial AD [49]. While at Braak stages I-II, a decrease of the inactive GSK3β was found in the cortex from AD patients, a considerable increase was observed in AD patients at stages V-VI compared to control subjects. The authors propose that GSK3β hyperactivity, and then NFTs formation, could be initiated at an early stage of the disease and turned off at the final stages [49].

TAU hyperphosphorylation is further driven by up-regulated *Cdk5* expression, causing diminished long-term synaptic potentiation and culminating in impairments of spatial learning and memory. Low levels of cytosine methylation were detected in the promoter region of *Cdk5* in the hippocampal CA1 region in a rat model with Aβ-induced memory deficiency [50].

2.2.2. Altered DNA Methylation Signatures as a Consequence of Disease Pathophysiology, Such as Aβ Burden and TAU-Phosphorylation

Changes in DNA methylation could be caused by the altered neuronal physiology in AD and tauopathies, such as the accumulation of Aβ peptides [51][52]. Hence, altered epigenetic signatures could be a bystander of disease progression, leading to the devastating dysregulation of genes and driving the further progression of neurodegeneration in AD and other tauopathies. Furthermore, distinct mutations associated with these diseases could elicit “secondary” changes in the DNA methylation pattern. It is well-known that changes in DNA sequence trigger alterations in DNA methylation signatures [53][54].

2.2.3. Aβ Peptide and TAU-Phosphorylation-Driven Changes in the Expression and Localization of DNA Repair Related Proteins

Disruption of the maintenance of genomic integrity emerges to play a central role in AD and related tauopathies [55]. Early intraneuronal accumulation of Aβ peptides promotes global DNA hypomethylation and thereby an increased expression of genes involved in DNA repair, i.a. *BRCA1*, in a mouse model of AD [56]. *BRCA1* was up-regulated in response to Aβ stimulation, in both cellular in vitro and in vivo mouse models, acting neuroprotectively against Aβ-induced DNA double-strand breaks. Up-regulated expression of *BRCA1* was further observed in postmortem brain samples from AD patients [57]. However, in the hippocampal CA1 region and entorhinal cortex of the AD brain, BRCA1 protein was mislocalized to the cytoplasm and insoluble [58]. In line with the cytosolic mislocalization, the nuclear BRCA1 protein, but not other members of Defective DNA Repair (DDR) mechanisms, were found to be reduced in AD brains [59]. The cytoplasmic BRCA1 mislocalization may represent a consequence of TAU deposition, in line with the observation that brain regions without TAU pathology, namely the occipital lobe and the cerebellum, are free of cytoplasmic accumulation of BRCA1 despite decreased methylation of the coding gene.

2.2.4. Aβ-Associated Changes in DNA Methylation of Cell Cycle-Related Genes

In addition to compromised genomic integrity, dysregulated cell cycle control is an integral part of AD. While in a healthy neuron, abnormal cell cycle reentry leads to apoptosis, abnormal reentry in neurons of aged subjects with AD triggers a cycle of oxidative damage and mitogen production facilitating TAU hyperphosphorylation, Aβ deposition, and CI [60].

For genes promoting the activation of cell cycle reentry (i.e., via CDK5), hypomethylation was observed in AD or in AD disease paradigms [61]. Exposure of differentiated human neurons to Aβ results in DNA methylation abnormalities of cell-fate genes controlling neuronal differentiation and apoptosis, hinting at a downstream Aβ effect [61].

In this context, a recent study described a potential mechanism for DNA methylation-mediated Aβ overproduction, which then triggers Aβ driven hypomethylation of cell cycle-associated genes [62]. The same group (Li et al. (2019)) found that AD neurons display significant hypomethylation in the enhancer of the *DSCAML1* gene that targets *BACE1*. *BACE1* encodes the β-secretase, which cleaves APP thereby acting on Aβ production. Hence, the *DSCAML1* enhancer hypomethylation may activate *BACE1* transcription, putatively leading to an increased production of Aβ peptides, resulting in plaques typically preceding the spread of neurofibrillary tangles and neurodegeneration [63][64]. In agreement with this, changes of DNA methylation signatures in enhancer regulatory elements are frequently observed in AD brains [65][66]. Together, this indicates that epigenetic impairment of enhancer function is implicated in AD.

3. The Potential and Limitations of DNA Methylation-Based Therapy Approaches

As described above, hypomethylation of AD risk genes (such as *APP*, *PSEN1*, and *PSEN2*) was described to be associated with defects in learning and memory. An increase in methyl donor S-adenosyl-L-methionine (SAM) was reported to reduce APP and PSEN1 expression by promoter hypermethylation [67][68]. In line with this, elevated levels of vitamin B12, folate and other methionine sources in the diet improve methionine bioavailability and were shown to reverse elevated expressions of APP and PSEN1 [69][70][71].

In addition to driving hypermethylation, there is ongoing screening for DNMT inhibitors capable of modulating the methylation of AD or tauopathy risk genes. DNMT inhibitors such as azacitidine and decitabine have already been approved by the FDA for cancer treatment such as leukemia [72][73][74]. The use of DNA demethylating agents has also been used in some other neurodegenerative diseases, such as Friedreich's ataxia [75], which however did not provide promising results in human cells.

Finally, due to gene locus-specific changes in DNA methylation signatures, sequence-specific DNA demethylating agents, such as the oligonucleotide antisense inhibitor MG98 [76][77][78], seem promising for future therapeutic approaches to reduce DNA methylation site specifically. Moreover, the hypomethylation of particular genes was described to be implicated in AD and tauopathy pathomechanisms. Hence, locus-specific editing technologies are required for altering or restoring DNA methylation. This can be achieved by clustered regulatory interspaced short palindromic repeats (CRISPR)-deactivated Cas9 (dCas9)-based editing systems that have been described as a specific and efficient method capable of manipulating site-specific DNA methylation [79]. This, in combination with improvements in cell type-specific application and blood-brain-barrier overcoming strategies, would open the way for targeted epigenetic therapies (see **Figure 1** for schematic depiction).

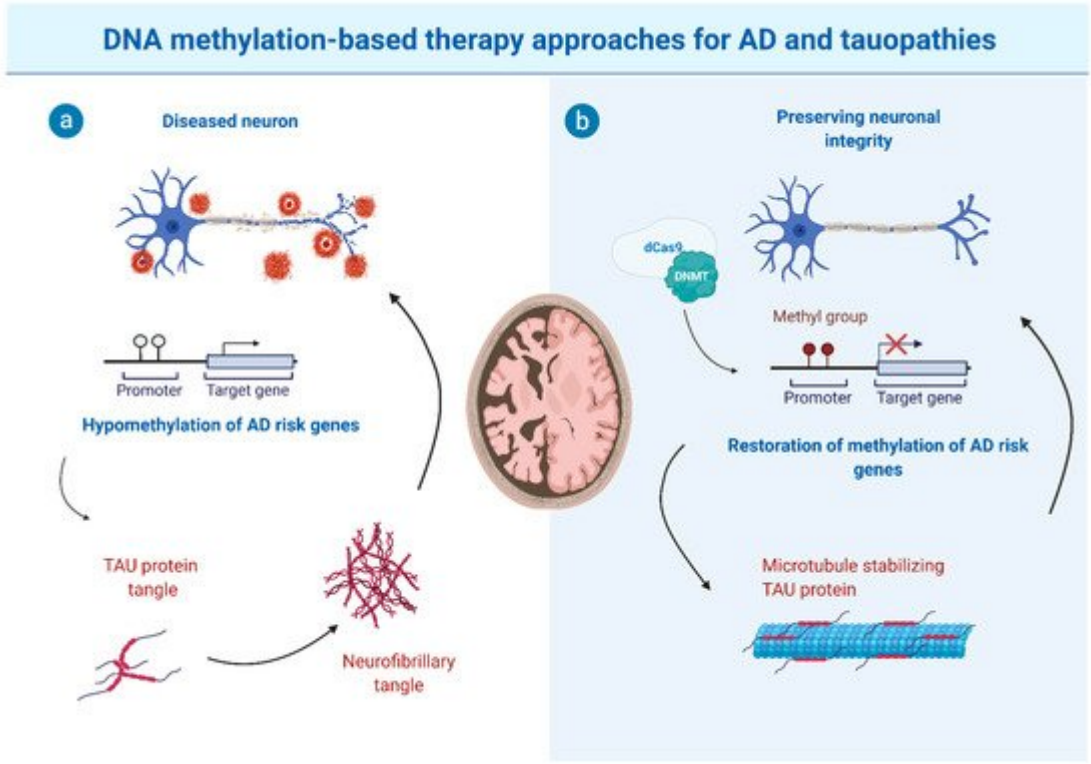

Figure 1. Putative potential of CRISPR/dCas9 editing-based therapeutic approaches for tauopathies that display impaired methylation patterns of selected genes/key regulator elements. (**a**) In disease paradigms, impaired DNA methylation (e.g., hypomethylation of risk genes associated with Alzheimer's Disease (AD) and related/other tauopathies) results in increased TAU expression, decreased TAU clearance, or mislocalization, all of which lead to the accumulation of TAU and eventually to the formation of TAU protein aggregates. Neurons affected by this TAU pathology become dysfunctional and decay, eventually leading to impaired cognitive function and neurodegeneration. (**b**) CRISPR/dCas9 editing approaches may restore methylation patterns of AD and tauopathy risk genes, preventing abnormal production or modification of TAU protein and NFT formation, preserving the physiological function of TAU (i.a. microtubule stabilization) and preventing or partially reverse brain damage and disease progression. Possible genetic and non-genetic interventions could be (i) drug-induced modulation of methylation patterns, (ii) gene-replacement or RNAi-based gene therapy, or (iii) site/gene-specific modulation of methylation, e.g., as depicted, site-specific methylation via dCas9-directed DNMT targeting. This figure produced by using BioRender.com with a respective publication licence, provided by the Biology department of the RWTH Aachen University.

4. Altered DNA Methylation Signatures as Potential Biomarkers for AD/Tauopathies Disease and Disease Progression?

Disease-specific reliable biomarkers for difficult-to-diagnose diseases that might require early intervention (such as in current treatment approaches for AD and tauopathies) are essential for early diagnosis, monitoring disease progression, and eventually the response towards potential therapies. Currently used biomarkers, e.g.,

neurofilaments, are often unspecific and respond proportionally to the degree of axonal damage in a variety of neurological disorders, including inflammatory, neurodegenerative, traumatic, and cerebrovascular diseases, and is thus implicated in diseases reaching from stroke and TBI over ALS to prionopathies and many other sorts of neurological disorders. For some tauopathies, especially AD, biomarkers (mainly from the CNS) are established and aid in the diagnosis, e.g., lower Aβ and higher pTAU and tTAU levels. Yet, for most other tauopathies biomarkers are not established and are understudied. So, may DNA methylation signatures be useful to serve as biomarkers for AD and other tauopathies in blood cells, thereby complementing currently applied biomarkers?

In leukocytes, the intron 1 of the *TREM2* gene (triggering receptor expression on myeloid cells 2) displays reduced methylation, associated with elevated expression at the mRNA level in AD subjects [57][59]. Moreover, increased levels of peripheral *BDNF* promoter methylation was proposed to be an epigenetic biomarker indicating the transformation of MCI to AD [80]. Similarly, increased DNA methylation levels were detected in promoter regions of the *COASY* and *SPINT* genes in plasma samples of AD and MCI subjects compared to controls [81]. Methylation of the *PICALM* gene in blood cells was found to be related to the cognitive decline of AD patients [82]. Interestingly, global DNA methylation levels also were increased in peripheral blood (mononuclear cells) of LOAD patients, paralleled with an increase in the DNMT1 gene and protein expression, hinting towards global DNA methylation as a promising biomarker for AD, AD progression and AD conversion [83].

In sum, monitoring global and site-specific DNA methylation in peripheral samples may be useful for individualized AD risk assessment. However, more detailed research and correlations are required that strengthen the use of DNA methylation as biomarkers for AD risk, diagnosis and progression, which might be expected in the near future.

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