Bioorganic & Medicinal Chemistry 19 (2011) 3616–3624

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

After the grape rush: Sirtuins as epigenetic drug targets in neurodegenerative disorders

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ARTICLE INFO

Article history:
Received 5 November 2010
Revised 5 January 2011
Accepted 11 January 2011
Available online 15 January 2011

Keywords:
Sirtuins
Histone deacetylases
Neurodegenerative disorders
Alzheimer
Parkinson's disease
Huntington's disease
Resveratrol

ABSTRACT

Class III histone deacetylases (sirtuins) are becoming increasingly recognized as important epigenetic drug targets in cancer and metabolic disorders. As key regulators involved in numerous cellular signalling pathways, sirtuins are also emerging as potential targets in various neurodegenerative diseases such as Alzheimer, Parkinson's disease and others, thus suggesting modulation of sirtuin activity could provide an interesting and novel therapeutic option. In particular, much attention has been raised by neuroprotective effects attributed to SIRT1 activation due to genetically induced sirtuin overexpression or administration of resveratrol, a natural compound found in the skin of red grapes and also in wine. Similarly, also sirtuin inhibitors display benefits in various neuropathologic disease models. In light of the growing interest in sirtuin modulation and with regard to the lack of conclusive data on small molecule activators of sirtuins this review recapitulates the known facts about sirtuins and their relevance in neurodegenerative diseases.

1. Introduction

Epigenetic regulation of gene expression depicts a hallmark of cellular adaptation in evolution and is highly conserved among species. Complementary to posttranscriptional mechanisms such as alternative splicing, epigenetically controlled gene activation or suppression by chromatin modifying enzymes allows cells to respond to external stimuli in a fast and inheritable, yet non-mutation-based manner. Common modifications include methylation and acetylation of nucleosomal histone tails which alter chromatin plasticity and hence access of transcription factors and DNA or RNA polymerases to gene promoter sites. This of course has a major impact on fundamental cellular processes like proliferation, metabolism and apoptosis. Among all tissues particularly neurons are also required to adapt quickly to allow such complex processes like learning and memorizing. It is therefore not surprising that epigenetic defects can elicit various neurodegenerative disorders. Sirtuins are a family of histone deacetylases (HDACs) which cleave acetyl groups off histone lysine residues. Aberrant sirtuin activity has been linked with various malignancies such as cancer but consequently, there is also growing evidence that sirtuins might play a crucial role in Alzheimer's and Parkinson's disease, for example. We will summarize some general facts about sirtuins and then focus on their reported physiological relevance to neurodegenerative disorders. Finally, selected modulators of sirtuin activity with potential benefit for treatment of neuronal degradation will be presented.

1.1. Sirtuin classes and their biological functions

Sirtuins are a family of histone deacetylases (HDACs) which catalyze the hydrolysis of acetylated histone tails. In co-operation with their functional counterparts, the histone acetyltransferases (HATs), they regulate the transcription of genes and thus cellular protein expression. The Sir2 gene was originally identified in yeast and its name, silent information regulator type 2, relates to its function as an epigenetic silencer of chromatin. In total there are four classes of HDACs of which the seven human orthologue sirtuins SIRT1-7 so far identified depict class III (Table 1). Based on domain sequence homology, the sirtuins can be further distinguished into four sub-classes. Of those, SIRT1 depicts the only member of sub-class Ia, whereas SIRT2 and SIRT3 both belong to sub-class Ib. Sub-classes II and III comprise SIRT4 and SIRT5, respectively. Finally, sub-class IV includes both SIRT6 and SIRT7.

Except for sirtuins, all other HDACs classes require zinc as a cofactor for catalysis whereas SIRT1-7 are dependent on NAD^+. During reaction, sirtuins remove an acetyl group of ε-*N*-acetylated lysines which is transferred to the ribose moiety of NAD^+, thus yielding the deacetylated substrate and *O*-acetyl-ADP-ribose (OAADPR). The third reaction product, nicotinamide (**2**, Chart 1), also operates as a physiological inhibitor of sirtuins by means of negative feedback.[1,2] As OAADPR itself may act as a second

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doi:10.1016/j.bmc.2011.01.018

Table 1
Classification of human HDACs

Type	Zn^{2+}-dependent	Zn^{2+}-dependent		NAD^{+}-dependent					Zn^{2+}-dependent
Class	I	II		III					IV
Sub-class		IIa	IIb	Ia	Ib	II	III	IV	
Members	HDAC1	HDAC4							
	HDAC2	HDAC5	HDAC6	SIRT1	SIRT2	SIRT4	SIRT5	SIRT6	HDAC11
	HDAC3	HDAC7	HDAC10		SIRT3			SIRT7	
	HDAC8	HDAC9							

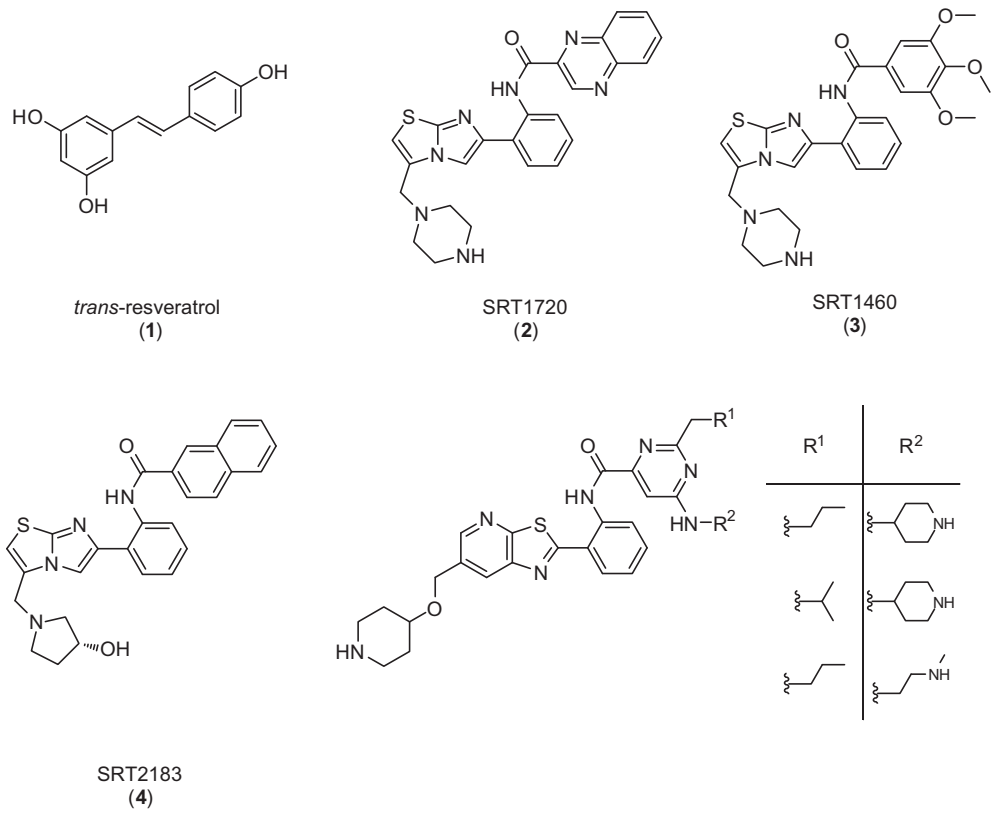

Chart 1. Structures of putative sirtuin activators.

messenger in cell signalling,[3–5] initial studies assumed that sirtuins might play an equally important role as ADP-ribosyltransferases.[6] Furthermore, for some isoforms such as SIRT4 a deacetylation substrate could not be identified until now.[7] However, there is agreement that sirtuins predominantly exert their catalytic activity by means of deacetylation[8] as kinetic studies have revealed that ADP-ribosylation mediated by sirtuins is probably of minor physiological relevance.[9]

Structurally, sirtuin proteins comprise two main domains represented by a large Rossman fold and a comparably smaller zinc binding domain. At the interface between those two domains, a large groove provides access for binding of NAD^{+}. Based on the localization of the molecular subunits of NAD^{+}, the active site can be subdivided into three sections: pocket A which accommodates the adenine moiety, followed by the ribose binding pocket B and the nicotinamide binding pocket C. The acetylated substrate peptide enters a cleft comprised of the two domains.[10,11]

The seven human sirtuin proteins localize to different subcellular compartments which correlate with their distinct functional roles. The most extensively studied isoform, SIRT1, is a predominantly nuclear protein and has been shown to deacetylate H4K16[12] as well as a number of non-histone targets such as p53,[13–16] Ku70,[17] PPARγ,[18] PGC-1α,[19,20] NF-κB,[21] HIF-2α,[22] XPA[23] and several FOXO isoforms.[24,25] Hence SIRT1 is involved in the regulation of various important cellular processes including proliferation, DNA repair mechanisms and apoptosis and is thought to play an important role in cancer[26] and glucose homeostasis, thus diabetes.[20,27] Furthermore, upregulation of SIRT1 is discussed to mediate the life-extending effects of calorie restriction in lower organisms[28,29] although some studies suggest that longevity is not exclusively promoted by SIRT1 in this setting.[30] Physiologically, SIRT1 activity is regulated by DBC1 (deleted in breast cancer 1),[31,32] HIC1 (hypermethylated in cancer 1)[33] and AROS (active regulator of SIRT1).[34] The second human orthologue SIRT2 resides in the cytoplasm and acts as a tubulin deacetylase[35] but it was also found in the nucleus during G2/M transition and was shown to interact with histones[12] as well as transcription factors from the FOXO class.[25,36] Despite reports suggesting SIRT2 as a potential tumour suppressor,[37] a recent study indicated that inhibition of both SIRT1 and SIRT2 is essential for p53-mediated induction of apoptosis in cancer cells.[38] SIRT3, SIRT4 and SIRT5 are mostly mitochondrial proteins and, in line with this localization, have been associated with numerous processes which regulate cellular metabolism.[39] For example, SIRT3 deacetylates and thus activates the mitochondrial complex I,[40] acetyl-CoA synthase 2 (AceCS2)[41,42] as well as glutamate dehydrogenase (GDH)[43] which triggers production of

ATP via the citric acid cycle. Lately, long-chain acyl coenzyme A dehydrogenase (LCAD) has been reported as a target of SIRT3 underscoring its role in fatty-acid oxidation which might also imply SIRT3 relevance for related disorders such as diabetes and cardiovascular diseases.[44] Moreover, knockout of SIRT3 boosts tumourigenesis and malignant transformation and those effects are even more pronounced when the SIRT3$^{-/-}$ genotype concurs with overexpression of oncogenes such as Myc or Ras, clearly demonstrating its role as a tumour suppressor which maintains mitochondrial integrity.[45] Interestingly, it appears that SIRT4, for which no deacetylation substrate has been identified so far, antagonizes the stimulatory effect of SIRT3 on GDH by ADP-ribosylation of GDH.[7] SIRT4$^{-/-}$ mice display increased levels of insulin upon glucose stimulation compared to control whereas overexpression of SIRT4 significantly diminishes insulin secretion.[7,46] Recent findings on SIRT4-dependent fatty-acid oxidation in liver and muscle cells also support the idea that inhibition of SIRT4 may elicit beneficial effects in the treatment of diabetes.[47] By deacetylating carbamoyl-phosphate synthetase 1 (CPS1) SIRT5 controls the initial step in the urea cycle and thus elimination of toxic ammonia.[48,49] In contrast, SIRT6 is a nuclear protein which deacetylates H3K9 modulating telomeric chromatin. Knockdown of SIRT6 results in chromatin defects which have been implicated with symptoms of premature ageing[50,51] as well as metabolic perturbations such as enhanced triglyceride synthesis and fatty liver formation.[52] SIRT6 also contributes to genome stability by regulating DNA repair mechanisms via deacetylation of C-terminal binding protein interacting protein (CtIP).[53] CtIP is essential for DNA end resection during homologous recombination evoked by DNA double-strand breaks. Finally, SIRT7 is thought to be involved in transcription by associating with RNA polymerase I (Pol I) in the nucleus and was shown to be important for cell viability.[54,55] A study by Vakhrusheva et al. proposed p53 as a direct substrate of SIRT7 and also showed evidence that SIRT7 might prevent ageing effects in the heart by ensuring tissue homeostasis.[56]

Aside from the discussed diverse effects mediated by sirtuins there are growing evidences for a role of these enzymes in neurodegeneration, for example, Alzheimer's, Parkinson's and Huntington's disease which will be in the focus of this review. Due to lack of evidence regarding a direct interaction between resveratrol (**1**) (Chart 1) and sirtuins (see Section 3.1) the studies presented in this review were selected on the premise that effects attributed to SIRT1 activation by resveratrol have also been validated by an alternative approach, for example, genetic overexpression.

2. The role of sirtuins in neurodegenerative disorders

2.1. Alzheimer's disease

Alzheimer's disease (AD) is one of the most prevalent and devastating neurodegenerative disorders especially in patients of 65 years age and above. As at the same time the demographic distribution is shifting towards an increasingly ageing population the treatment of AD with its social and economic implications is prone to become one of the major challenges in health care in the very near future.[57] Alzheimer patients commonly suffer from memory loss as well as cognitive and functional decline hence requiring constant caretaking. The average life expectancy is approximately nine years after diagnosis. Despite almost one century of intensive research after its initial discovery the pathogenic molecular mechanisms underlying AD are still not fully understood. One prevailing-yet not undisputed-[58] hypothesis relates neuronal decline in AD to both the formation of neurotoxic extracellular aggregates of β amyloid (Aβ) protein and intraneuronal filaments which consist of the microtubule-associated protein τ. These so-called senile plaques and neurofibrillary tangles of hyperphosphorylated τ protein are considered classical hallmarks of AD manifestation in the brain.[59,60] Studies have shown that the plaque assembly occurs due to sequential cleavage of the membrane-bound amyloid precursor protein (APP) by β- and γ-secretases generating the Aβ peptides 1–40 and 1–42 (Fig. 1).[61] Notably, if APP proteolysis is processed by α- secretases, it is associated with a neuroprotective effect which was observed in AD animal models.[62–64] There are various therapeutic approaches in the treatment of AD, for example, muscarinic receptor agonists and β- and γ-secretase inhibitors with numerous trials investigating their clinical efficacy. Yet unfortunately, the development of a break-through drug still seems a long way ahead.[65] Recently, Donmez et al. discovered that SIRT1 is able to direct APP processing by activating expression of the α-secretase ADAM10.[66] Deacetylation of retinoic acid receptor β (RARβ) by SIRT1 led to an increased transcription of the ADAM10 gene which correlated with a significant suppression of neurotoxic Aβ peptide production in cells. Furthermore, ADAM10 is also involved in Notch signalling by cleaving the activated Notch receptor, the remaining membrane-bound domain of which then undergoes further proteolysis by γ-secretase to yield Notch intracellular domain (NICD). The release of NICD initiates the Notch signalling and transcriptional cascade which has been implicated in neuronal development and repair mechanisms, hence suggesting SIRT1 activation could provide double benefits in AD therapy. This would depict a huge advantage in AD therapy as γ-secretase inhibitors abolish Notch signalling which leads to unwanted side effects such as intestinal goblet cell metaplasia.[67] SIRT1-dependent inhibition of Rho kinase ROCK1 expression was also reported to prevent AD pathogenesis by boosting α-secretase-mediated cleavage of APP,[68] yet a similar correlation could not be observed in the transgenic mouse model used by Donmez et al. Positive effects of SIRT1 activation in AD models either by gene overexpression, caloric restriction (CR) or administration of resveratrol (**1**) have also been described in several other studies. However, since CR induces upregulation of a multitude of genes and there is lack of evidence for a direct interaction of resveratrol and sirtuins (see Section 3.1), further investigations are required to clarify the significance of sirtuins in those model systems. For example, the temporal cortex of squirrel monkeys fed a CR diet displayed a significant reduction of Aβ peptides which correlated inversely with SIRT1 protein expression in this brain region.[69] In the context of chronic inflammatory processes driving AD progression it was shown that stimulation of microglia with Aβ peptide oligomers initiates gliosis by activation of NF-κB, finally culminating in neurodegenerative events. Overexpression of SIRT1, which is known to interfere with NF-κB signalling by deacetylating its subunit RelA/p65,[21] as well as administration of resveratrol, mitigated this neurotoxicity significantly. In both experiments lower acetylation levels of RelA/p65 at Lys310 were detected although it should be noted that no direct correlation between resveratrol dosage and SIRT1 expression levels was presented.[70]

P25 mice represent an established AD model system with inducible expression of hyperphosphorylated τ protein and neurofilament. Lentiviral transfection with SIRT1 or treatment with resveratrol saved p25 transgenic mice from neurodegeneration in comparison to controls.[71] Cell culture experiments demonstrated that SIRT1's neuroprotective effects were dependent on its deacetylase activity.[72] Analogously, accumulation of paired helical τ protein was reported to coincide with a reduction of SIRT1 expression in the cerebral cortex of AD patients.[73] Cognitive deficits in 3xTg-AD mice, a transgenic mouse model of AD in which both Aβ peptide and τ protein pathologies are expressed, could be restored by oral administration of nicotinamide (**2**), a competitive physiological inhibitor of sirtuins.[74] In this case, amelioration of AD symptoms was ascribed to diminished levels of the Thr231-phosphorylated

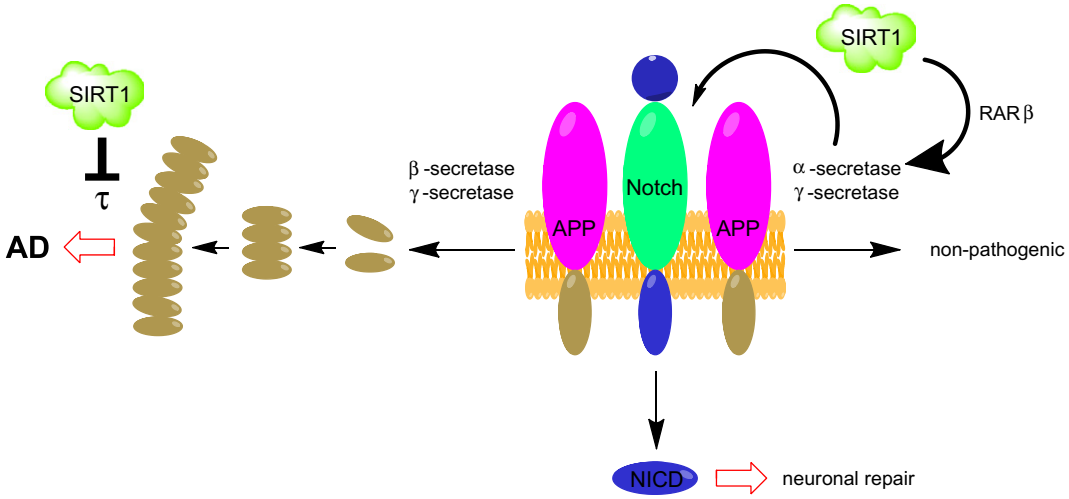

Figure 1. *Pathogenic mechanisms in Alzheimer's disease (AD).* Proteolytic cleavage of β amyloid precursor protein (APP) by β- and γ-secretases yields Aβ peptide monomers which are assembled to neurotoxic oligomers and ultimately form the characteristic plaques. Both Aβ peptide polymers and tauopathies are considered hallmarks of AD progression. SIRT1 can support non-pathogenic cleavage of APP by activating RARβ and thus α-secretase transcription. α-Secretase also induces neuronal repair mechanisms via positive activation of Notch signalling.

τ protein and increased expression of p25, whereas Aβ pathology was not affected. Possibly due to SIRT2 inhibition by nicotinamide (**2**), the authors also found elevated levels of acetylated α-tubulin which stabilizes microtubules thus also contributing to neuroprotection.[75] It is worth noting that p25 may have opposing roles in neuronal development depending on whether the protein is expressed transiently or in a constant manner.[76] Furthermore, recent findings revealed that τ protein is acetylated possibly regulating τ protein turnover. As SIRT1 gene silencing mediated by siRNA or downregulation of its activity by small molecule inhibitors elevated levels of acetylated τ protein, activation of SIRT1 may in contrast support proteosomal degradation and ease τ protein-associated pathology.[77] The relevance of the other human sirtuins, especially SIRT3–SIRT7, in AD remains elusive as experimental data is scarce. So far a single study by Pfister et al. has addressed this issue concluding that SIRT2, SIRT3 and SIRT6 actually induce apoptosis in healthy cerebellar granule neurons whereas SIRT1 protected neurons challenged with low potassium treatment. Interestingly, the beneficial effects of SIRT1 were not dependent on its deacetylase activity as demonstrated by catalytically inactive mutants or by using the sirtuin inhibitors nicotinamide and sirtinol. Results on SIRT5 were inconsistent and also SIRT6 displayed antiapoptotic effects in HT-22 neuroblastoma cells treated with homocysteic acid.[78] As secretase inhibition keeps providing a challenge in AD therapy due to interference with other non-disease-related physiological pathways causing unwanted side-effects, strategies involving modulation of sirtuin activity such as stimulation of α-secretase expression might offer new and alternative opportunities to antagonize AD progression.

2.2. Parkinson's disease

About 4–5% of people over 85 year's age suffer from Parkinson's disease (PD), a neurodegenerative disorder whose cardinal symptoms comprise tremor, rigidity, bradykinesia and postural instability. It depicts the second most common disease in the field of neurodegenerative diseases and although PD is less prevalent in young people, patients between 20 and 50 years account for 5–10% of all PD cases. Neuropathological characteristics of PD include loss of dopaminergic neurons in the *substantia nigra* and formation of Lewy bodies (LBs) which are insoluble protein aggregates mainly composed of α-synuclein (α-syn) (Table 2).[79] However, the biomolecular mechanisms enabling disease progression still require further investigation. For example, it is unclear to what extent LBs actually contribute to the phenotypic defects in PD or if α-syn deposits located at presynapses could induce neurotransmitter deprivation.[80] The main branch of available treatments focuses on the restoration of dopaminergic signalling by replacement of dopamine, most prominently applying L-DOPA. Unfortunately, L-DOPA therapy often encounters a decrease in efficacy and severe adverse effects.[81] Interestingly, gene expression studies in a *Caenorhabditis elegans* model identified sir-2.1, a SIRT1 orthologue, as a suppressor of α-syn inclusion formation. In a cytoplasmic hybrid (cybrid) approach mitochondria from PD patient samples that were transfected into NT2 cells exhibited decreased complex I activity as well as significantly lower maximum respiration capacity. Remarkably, those observations correlated with reduced levels of SIRT1 phosphorylation and PGC-1α expression yet increased NF-κB activity,[82] consistent with the findings that both PGC-1α and the NF-κB-subunit RelA/p65 are substrates of SIRT1, itself in turn known to be activated by phosphorylation.[83] More importantly, there is also evidence obtained in model systems for the relevance of sirtuins in PD pathogenesis. In a *Drosophila* model of PD, pharmacologic inhibition of SIRT2 protected transgenic flies against dopaminergic cell death induced by α-synuclein. Genetic knockdown of SIRT2 as well as inhibitor treatment also ameliorated α-synuclein toxicity in H4 neuroglioma cells and positively affected α-syn inclusion formation.[75] Obviously additional studies are needed to further explore the role of sirtuins in this setting but taken together, initial results seem promising and suggest that modulation of sirtuin activity could provide an alternative in PD therapy.

Table 2
Characteristics of Parkinson's disease

Neuropathological changes	Phenotypic symptoms
Loss of dopaminergic neurons	Resting tremor
α-syn deposits	Rigidity
Formation of Lewy bodies	Bradykinesia/akinesia
	Postural instability

2.3. Huntington's disease

Huntington's disease (HD), also known as Huntington's chorea, is a hereditary, late-onset progressive neurodegenerative disorder leading to disturbances in movement ('chorea' = Greek for 'dance') and cognitive impairment as well as premature death usually around 20 years after diagnosis. It is often accompanied by variable and diverse psychiatric disturbances such as apathy and irritability. The causative genetic defect, a dominant autosomal mutation in a gene encoding the huntingtin protein (Htt) on the short arm of chromosome 4, was discovered in 1993 as an unstable expansion of a trinucleotide CAG/polyglutamine (polyQ) repeat located in the 5′ terminal section of the gene.[84] The polyQ sequence found in mutant huntingtin has been linked to transcriptional dysregulation as nuclear mutant Htt aggregates interact with various transcription factors including CREB binding protein (CBP), TATA-box binding protein (TBP) and Sp1 (Fig. 2).[85] Furthermore, mutant huntingtin may interfere with cellular trafficking of several cell surface receptors such as EGF, transferrin and GPCRs.[86] Despite advances in understanding HD pathogenesis there is currently no effective disease-modifying treatment available. Preliminary evidence indicating that targeting sirtuins may provide a novel therapeutic option in HD arose from a genetic study in *C. elegans* where overexpression of sir2.1, orthologue of human SIRT1, rescued the transgenic worms from polyQ toxicity.[87] Notably, deletion of the forkhead transcription factor orthologue daf-16 abrogated SIRT1's neuroprotective effects in this model. This implies a functional connection between those two proteins that are also known to interact in mammals.[24,25] Resveratrol (**1**) also alleviated cytotoxicity in cells derived from the striatum of HdHQ111 knock-in mice which however, was reverted by the sirtuin inhibitors nicotinamide (**2**) or sirtinol (**3**) (Chart 1).[87] In a *Drosophila* model of HD, rescue of photoreceptor neurons could be achieved by treatment with resveratrol, yet strikingly, the observed neuroprotection was found to be independent of SIRT1 expression, emphasizing that this natural compound is very likely to mediate its neuroprotective actions also via other pathways. Still the same study reported that both reduced gene dosage or inhibition of either SIRT1 or SIRT2 by sirtinol (**3**) and nicotinamide (**2**) increased neuronal survival. It should be mentioned in this context that the authors also noted that simultaneous reduction of SIRT1 and Rpd3, a class I HDAC orthologue, exceeded the individual favourable effects.[88] Disease progression in HD also affects cellular metabolism perturbing glucose and sterol homeostasis. For example, abrogation of SIRT2 activity by isoform-selective inhibitors prevented neurotoxicity in *Drosophila* and *C. elegans* models of HD expressing mutant Htt fragments. The inhibitors which had previously also been shown to be effective in models of Parkinson's disease (see Section 2.2) reduced expression of genes associated with sterol biosynthesis, many of which are transcriptionally regulated by sterol responsive element binding protein 2 (SREBP-2). As protein expression levels and compartmental localisation of SREBP-2 were found to be dependent of SIRT2 expression the authors postulated inhibition of SIRT2 activity could reduce cerebral sterol levels thus restoring metabolic homeostasis and survival in HD.[89] However, as there are also studies indicating sterol content is rather decreased than raised in HD further investigations are required to fully elucidate the pathophysiological interplay of imbalanced sterol levels in HD.[90,91] Yet, another point of action for SIRT2 antagonists in HD could be the restitution of cellular transport of organelles by

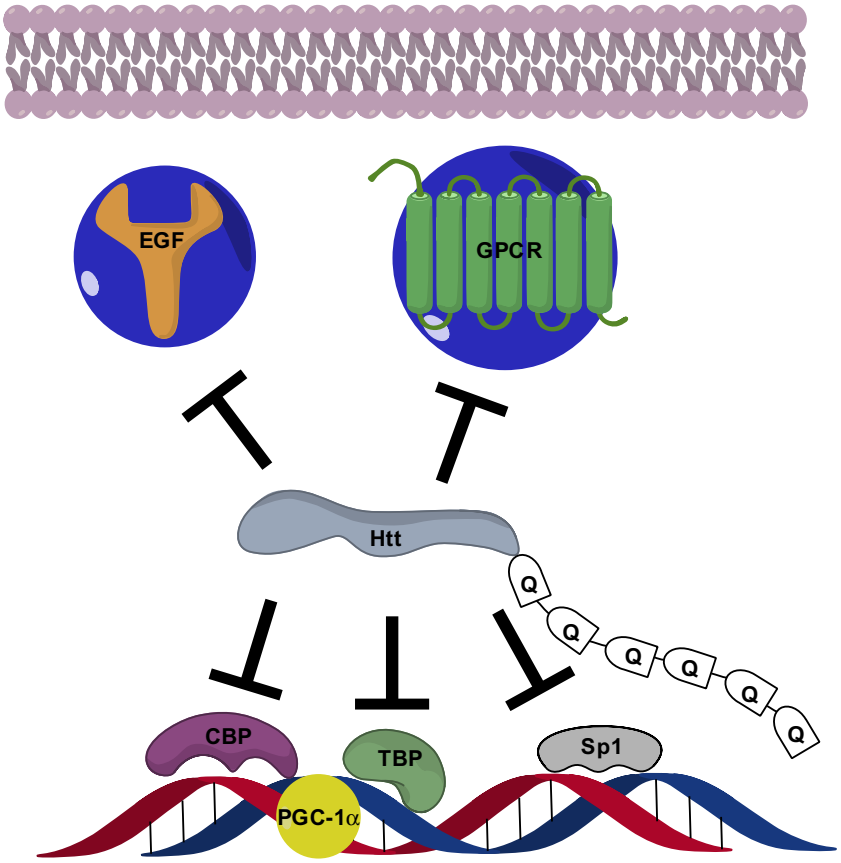

Figure 2. *Actions of mutant huntingtin (Htt) in Huntington's disease (HD).* Mutant Htt is characterised by a distinct polyQ extension and interferes with various cellular processes by binding transcription factors such as CBP, TBP or SP1 thus suppressing expression of, for example, PGC-1α. Impairment of cellular vesicle transport affects cell surface receptor trafficking.

stabilisation of α-tubulin, a mechanism which has already been proposed for HDAC6 inhibitors like trichostatin.[92] Finally, the SIRT1 target PGC-1α[19] is thought to play an important role in HD as PGC-1α deficient mice develop a phenotype comparable to HD whereas overexpression of PGC-1α demonstrated neuroprotective effects in a mouse model of HD. Results indicate mitochondrial biogenesis and respiration is impaired by mutant Htt by inhibition of PGC-1α expression thus driving neurodegeneration.[93,94] Unfortunately, this connection has not yet been examined exhaustively apart from one study which reported that administration of SRT501-M, a proprietary formulation of resveratrol, increased PGC-1α expression in brown adipose tissue but interestingly not in the striatum of HD transgenic mice.[95] Previous experiments by Lagouge et al. have established PGC-1α activation by resveratrol is mediated in a SIRT1-dependent manner.[96]

2.4. Other neurodegenerative disorders

Several neurodegenerative disorders share similarities in disease pathogenesis, exemplified by the common aggregation of misfolded proteins. For example, polyQ toxicity is not exclusive to HD but is also involved in spinocerebellar ataxia and spinobulbar muscular atrophy, suggesting sirtuins could also assist in the treatment of those and other disorders.[88] Moreover, the principle of microtubule stabilization by increase in tubulin acetylation could pave the way for SIRT2 inhibitors in the therapy of several neurodegenerative disorders as already indicated previously (see Section 2.3). Correspondingly, both knockdown of SIRT2 as well as treatment with nicotinamide (**2**) conferred resistance to axonal degeneration in mice, similar to the effect observed in slow Wallerian degeneration mice (Wld^S).[97] The latter transgenic mice express a chimeric protein composed of full-length nicotinamide mononucleotide adenylyltransferase 1 (Nmnat1) and a part of the ubiquitin assembly protein (Ufd2a). Transected neurons from Wld^S mice display a substantial delay in neurodegeneration in comparison to wildtype animals. Notably, animals also exhibited enhanced levels of microtubule acetylation.[97] Studies using Nmnat1 overexpression or administration of nicotinamide (**2**) have also directed attention to SIRT1 as a potential target to augment neuronal resistance.[98] However, its role in this context remains unclear as there are conflicting results on whether SIRT1 contributes positively to Wallerian degeneration.[98,99] Finally, increased expression of SIRT1 in a model of amyotrophic lateral sclerosis based on a superoxide dismutase 1 mutant (SOD1G93A) rescued neurons from toxicity. Resveratrol (**1**) was found to be comparably effective in a SIRT1-dependent manner as simultaneous expression of a catalytically inactive SIRT1 mutant deteriorated resveratrol's neuroprotective effects.[72]

3. Modulating sirtuin activity in neurodegenerative disorders

3.1. Sirtuin activators

Not surprisingly, studies implying increased sirtuin activity with longevity in yeast, worms and flies[29,100–103] have put sirtuin activating compounds (STACs) in the very focus of public attention. Particularly resveratrol (**1**) (Chart 1), a natural polyphenolic *trans*-stilbene compound, was reported to be a potent activator of SIRT1[104] and consequently, a plethora of studies has used resveratrol *bona fide* to explore the pharmacologic effects of sirtuin activation. However, a considerable number of those investigations concluded that the beneficial effects of resveratrol treatment were not necessarily mediated by SIRT1. Furthermore resveratrol, which naturally occurs in red grape skins as well as red wine is subject to heavy metabolisation,[105–107] questioning modes of action previously suggested by in vitro experiments and possible in vivo efficacy. Consequently, those observations prompted efforts to re-examine the correlation between resveratrol and SIRT1 catalytic activity. To ensure physiologically active serum levels SRT501-M, a special proprietary formulation of resveratrol was developed and has been used in various pre-clinical and clinical models (also see Section 2.3). Intriguingly, mechanistic studies on the interaction between resveratrol and sirtuins revealed a strong dependency of enzymatic activity on the presence of a fluorescent tag on the substrate peptide used in the widely-applied 'Fluor-de-Lys' assay.[108,109] This has been confirmed by recent studies documenting resveratrol has no activating effect on SIRT1 when native peptides or natural full-length substrates (such as p53), lacking the fluorophore, are employed.[110–112] As a result, conclusions on sirtuin activation by resveratrol should be drawn carefully. Despite this caveats, there are still a reasonable amount of studies in which the positive effects attributed to resveratrol correlated with a significant upregulation of sirtuin expression. More importantly, resveratrol exerts a pleiotropic mode of action[113] involving a multitude of targets possibly including AMPK activation[30] and so it cannot be excluded that resveratrol might in fact induce activation of SIRT1 via other pathways which yet remain to be elucidated. Notably, during the preparation of this manuscript, GSK announced to halt all further development of SRT501 reportedly due to the formulation's minimal efficacy in a multiple myeloma study[114] whilst bearing the potential to exacerbate renal complications in some patients.

Various other STACs have been published apart from resveratrol. Some STACs are thought to exceed both the sirtuin activating potency as well as some of the therapeutic benefits ascribed to resveratrol.[103] Most prominently, SRT1720 (**2**), SRT1460 (**3**) and SRT2183 (**4**) have been suggested as promising drugs to target metabolic disorders such as diabetes, by restoring glucose homeostasis.[115] Although none of them have yet been evaluated in neurodegenerative disorders they will be discussed briefly. As in the case of resveratrol, SIRT1 activation by SRTs was again found to be highly dependent on the nature of the substrate suggesting initial studies were falsified by an assay artefact. Moreover, despite lack of detectable sirtuin activating activity, SRT1720, SRT1460 and SRT2183 were reported to interact with several off-targets including receptors, various enzymes, ion channels and transporters.[112] Recently, a few novel structures of compounds which did show some peptide-dependent sirtuin activation have been disclosed (Chart 1). However, in some cases, varied assay conditions also led to an increase in substrate acetylation. While an allosteric mode of action has been proposed,[111] clearly further investigations are needed to clarify this issue and there are still unanswered questions regarding the promiscuity of these putative sirtuin activators.

3.2. Sirtuin inhibitors

Advances in the identification and development of sirtuin inhibitors have been supported by both high-throughput screening efforts as well as the availability of sirtuin crystal structures. Several different structural classes of inhibitors have been presented ranging from synthetic peptides to natural compounds. This section will summarize inhibitors which have been used to study the role of sirtuins in neurodegenerative disorders (Chart 2), for more comprehensive reviews on sirtuin inhibitors we refer to existing literature.[116,117]

Nicotinamide (**5**), a side product of the sirtuins' catalytic reaction, conversely inhibits sirtuins in an NAD^+/substrate-non-competitive manner. An allosteric mechanism has been proposed[118] to account for this activity, yet other studies suggested that nicotinamide probably reverses the deacetylation process by intercepting a reactive intermediate.[119,120] Naturally, nicotinamide does not

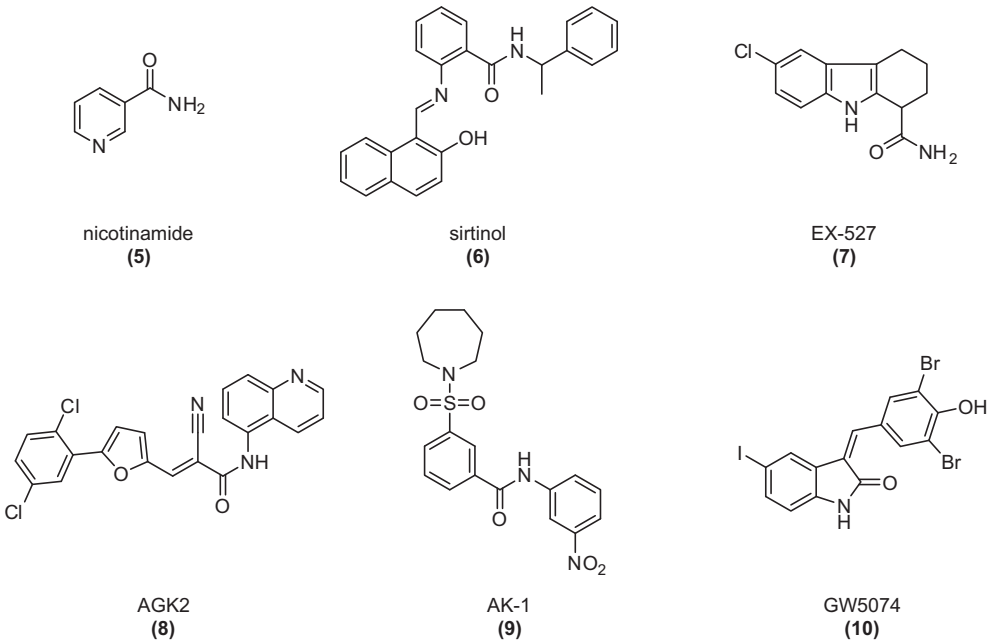

Chart 2. Structures of sirtuin inhibitors.

display significant selectivity for either isoform. A high-throughput phenotypic screening in yeast led to the discovery of the synthetic compound sirtinol (**6**) which inhibits SIRT1 and SIRT2 with IC_{50} values of 68 and 38 μM, respectively.[121] Subsequent SAR-based optimisation studies identified structural isomers of sirtinol which possess between 2- and 10-fold higher potency than the original compound.[122] However, it has been proposed that some of the observed effects of sirtinol may be due to in vitro precipitation, aggregation or even degradation.[117] The tetrahydrocarbazole compound EX-527 (**7**) is a potent SIRT1 inhibitor with high specificity (IC_{50} [SIRT1] = 38 nM, IC_{50}[SIRT2] = 19.6 μM).[123,124] Both activities refer to the racemate since the stereogenic centre at position 1 of the carbazole core induces chirality. Resolution of the racemic mixture revealed the (*S*)-enantiomer (also named EX-243) as the active isomer.[124] In 2007 Outeiro et al. reported the two potent though structurally diverse SIRT2 inhibitors AGK2 (**8**) and AK-1 (**9**) (IC_{50} = 3.5 μM and IC_{50} = 12.5 μM, respectively). Both compounds displayed good selectivity for SIRT2 over SIRT1 and SIRT3 and docking results indicated binding to pocket C which hosts the nicotinamide moiety during catalysis.[75] Studies on adenosine mimetics revealed that 3-arylidene-indolinones, a common kinase inhibitor scaffold, can exert potent activity against sirtuins.[125,126] Interestingly, GW5074 (**10**) and some structurally related compounds have also been shown to ameliorate the phenotype in a mouse model of Huntington's disease and to protect cerebellar granule and cortical neurons from various neurotoxic stimuli.[127,128] As sirtuins were not considered in those studies, further investigations are required to establish the relevance between those compounds, their inhibitory potential against sirtuins and neuroprotection.

4. Conclusion

Sirtuins have experienced an unprecedentedly fast and pervasive rise to fame due to their implication with ageing and its related disorders. In the field of neurodegenerative disorders evolving evidence of an important role of sirtuins raises hope that effective treatment of devastating, sometimes heritable and so far incurable diseases such as Alzheimer's and Huntington's disease may come into reach by modulating acetylation-dependent signalling pathways. Although the potential therapeutic benefits of validated sirtuin activators for these diseases still await confirmation under conditions more relevant to human pathophysiology, the results on sirtuin inhibitors in models of Parkinson's and Huntington's disease bear great promise. Unfortunately, the 'one-gene-one-enzyme' paradigm will very likely also not hold true in the various scenarios of neurodegeneration and thus a first critical step will be to gain further insights and a functional understanding of sirtuins and their potential interplay with other signal transducers. It has already been shown that simultaneous targeting of sirtuins and other HDAC classes can yield synergistic effects and the reported activity of kinase inhibitors in settings of neurotoxicity strongly suggests to further examine the possible crosstalk between acetylation and phosphorylation pathways (for a general review on HDAC inhibitors in neurodegenerative disorders see literature).[129] Hopefully the recent revelations on resveratrol and SIRT1 activators will ignite a salutary and equally sobering discussion of these classes of compounds as potent and selective modulators of sirtuin activity. One can envisage that more robust assays will become invaluable tools to decipher the acetylation code and also will provide a basis for the development of novel therapeutics. Furthermore, with termination of SRT501 development GSK has also proclaimed that two novel and more selective SIRT1 activator compounds called SRT2104 and SRT2379 which allegedly have no chemical relationship to SRT501 are currently undergoing exploratory clinical trials. Thus and to conclude, in case anyone wonders if the 'magnificent seven' targets discussed in this review will be of future importance in the treatment of neurodegenerative disorders, the tempting answer is 'almost SIRTainly'.

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