EUROPEAN JOURNAL OF NEURODEGENERATIVE DISEASES Vol. 3, no. 1, 13-24 (2014)

# NF-κB UNBALANCE AND DYSFUNCTION IN ACUTE AND AGE-RELATED NEURODEGENERATIVE DISEASE

A. LANZILLOTTA[1], V. PORRINI[1,2], A. BELLUCCI[1], M. BENARESE[1],
C. BRANCA[1], E. PARRELLA[1], PF. SPANO[1,2] and M. PIZZI[1,2]

[1]*Department of Molecular and Translational Medicine and National Institute of Neuroscience, University of Brescia, Italy;* [2]*IRCCS, San Camillo Hospital, Venice, Italy*

**The mechanisms underlying the progressive loss of neurons in age-related neurodegenerative diseases remain unknown to date. NF-κB factors are cardinal transcriptional regulators of inflammation and apoptosis and have been involved in the brain programming of systemic aging as well as in the pathogenesis of brain ischemia. Studies focusing on the complexity of NF-κB transcriptional activity in neuronal cell death showed that the composition of NF-κB active dimers and epigenetic mechanisms modulating histone acetylation finely condition neuronal vulnerability to brain ischemia. The atypical activation of NF-κB RelA acetylated on lysine 310 (K310) residue can trigger the expression of apoptotic genes but also constitutes a target for a neuroprotective combination of epigenetic drugs. Conversely, activation of NF-κB/c-Rel promotes neuroprotective effects through the transcription of specific anti-apoptotic genes. In addition, the absence of c-Rel shatters the resilience of nigral dopaminergic (DA) neurons to aging and induces parkinsonian features in mice. Indeed, we found that c-Rel-deficient mice show an increased RelA activation in the basal ganglia, and develop an L-DOPA-responsive parkinsonism associated with loss of DA neurons in the substantia nigra, neuroinflammation, accumulation of alpha-synuclein and iron during aging. Here, we discuss the effect of unbalanced activation of RelA and c-Rel during aging and propose novel challenges for the development of potential therapeutic strategies for neurodegenerative diseases.**

According to a recent World Health Organization report, the number of people aged 65 and over is expected to double in size within the next 30 years, thus becoming 25% of the entire population. It can be foreseen that in the next 20 years the diagnostic and therapeutic management of patients affected by age-related neurodegenerative disorders, such as brain ischemia, Alzheimer's disease (AD) and Parkinson's disease (PD), will represent a high priority health challenge for the world population. Hence, the clarification of the biological basis of these devastating disorders could facilitate the development of novel therapeutic strategies to limit, delay or halt brain damage and thus ameliorate patients' clinical cares and lifestyle.

Several studies have shown that, in the nervous system, NF-κB transcription factor acts as a pleiotropic regulator of target genes controlling physiological function (Crampton and O'Keeffe, 2013) as well as pathological processes associated with neurodegeneration (Pizzi and Spano, 2006; Camandola and Mattson, 2007).

Different members of the NF-κB family of transcription factors have been identified in mammalian cells; these include p65 (RelA), RelB, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2).

*Key words: NF-κB, c-Rel, Ac-RelA(K310), epigenetic drugs, BDNF,Parkinson's disease, stroke*

*Corresponding author:* Marina Pizzi,
Division of Pharmacology,
Department of Molecular & Translational Medicine,
School of Medicine, University of Brescia,
V.le Europa, 11 25123 Brescia, Italy
Tel.: +39 030 3717501 Fax: +39 030 3717529
e-mail: pizzi@med.unibs.it

2279-5855 (2014)

**DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.**

In the absence of stimuli, these factors are present as homo- and hetero-dimers bound to the inhibitor IκB family proteins. However, the transcriptional activity of NF-κB is highly complex during neuronal apoptosis with the composition of active dimers finely tuning the vulnerability of neurons to brain insults. Indeed, the RelA subunit, composing the activated p50/RelA dimer, and its post transcriptional modifications play a pivotal role in the onset of neurodegenerative processes triggered by ischemic insults as well as glutamate or beta-amyloid toxicity (Pizzi et al., 2002, 2005a,b; Inta et al., 2006; Lanzillotta et al., 2010). Conversely, the c-Rel subunit within activated NF-κB dimers counteracts the ischemic injury (Sarnico et al., 2009) and is responsible for neuroprotection. The c-Rel factor is reduced in ischemic neurons, and its overexpression can limit cell loss during ischemia. Moreover, the deficiency of c-Rel induces an age-related behavioral parkinsonism in mice, with degeneration of nigral (DA) neurons and development of a PD-like neuropathology (Baiguera et al., 2012).

Recent evidence has shown that activation of NF-κB/RelA drives the systemic and brain aging process in mice, while prevention of NF-κB/RelA activation extends lifespan (Zhang et al., 2013).

We thus postulate that while RelA activation accompanies normal brain aging, a misbalance between RelA and c-Rel might drive pathological aging by affecting the survival of substantia nigra (SN) DA neurons and turning old mice toward a parkinsonian phenotype.

### *Rel-A and c-Rel: two opposing regulators of neuronal resilience to brain ischemia*

In the central nervous system, NF-κB factors are key players of a number of physiological processes such as neurogenesis (Koo et al., 2010), neuritogenesis (Rolls et al., 2007), synaptic plasticity, learning and memory (Levenson et al., 2004; O'Riordan et al., 2006; Ahn et al., 2008). In recent years, a body of data has shown that NF-κB dysregulation participates in neurodegenerative mechanisms that occur in brains exposed to trauma or ischaemia (Bethea et al., 1998; Schneider et al., 1999), as well as in the brains of patients affected by PD (Hunot et al., 1997; Ghosh et al., 2007) and AD (Kaltschmidt et al., 1997).

The neuronal response to external stimuli relies on a differential activation of NF-κB dimers. We found that targeting RelA or c-Rel expression by antisense oligonucleotides (Pizzi et al., 2002) or siRNAs (Pizzi et al., 2005b; Sarnico et al., 2009) produces opposite effects on neuronal survival. While over-activated p50/RelA dimers contribute to the apoptotic program, the c-Rel containing dimers increase the resilience of injured neuronal cells (Fig. 1). Neurotoxic stimuli, such as ischemia (Inta et al., 2006; Sarnico et al., 2009), glutamate (Pizzi et al., 2002), β-amyloid (Valerio et al., 2006; Pizzi et al., 2005b) or 1-methyl-4-phenylpyridinium (MPP+) (Sarnico et al. 2008; Yang et al., 2010), induce p50/RelA dimer activation and the transcription of a panel of pro-apoptotic genes (Inta et al., 2006). Conversely, c-Rel-containing dimers are responsible for anti-apoptotic gene expression by signals promoting neuroprotection in diverse neurotoxic settings, such as S100B in models of NMDA-mediated excitotoxicity (Kögel et al., 2004), agonists at mGlu5 receptors against β-amyloid (Pizzi et al., 2005b) and MPP+ toxicity (Sarnico et al., 2008) or adipocyte-derived hormone leptin in neurons exposed to oxygen glucose deprivation OGD (Valerio et al., 2009). Over-expression of c-Rel in cultured neurons promotes anti-apoptotic effects by inducing the transcription of manganese superoxide dismutase (MnSOD) and Bcl-xL (Chen et al., 2000; Bernard et al., 2001; Pizzi et al., 2005b). c-Rel overabundance also limits the generation of reactive oxygen species (ROS) by inducing transcription of the mitochondrial uncoupling proteins 4 (UCP4) (Ho et al., 2012), a brain-specific mitochondrial ion channel producing mild reduction of mitochondrial membrane potential and neuroprotection (Echtay, 2007).

The elucidation of the dual effects of NF-κB activation on neuron survival was more evident in studies of severe brain ischemia. The activation of p50/RelA rapidly occurs in neurons and glial cells and has been implicated in pathogenesis of post-ischemic injury (Crack et al., 2006; Herrmann et al., 2005; Nurmi et al., 2004). In brain ischemic tissue of mice subjected to permanent middle cerebral artery occlusion (MCAO) and in primary cortical neurons exposed to OGD, NF-κB followed a similar pattern of activation (Lanzillotta et al., 2010; Pizzi

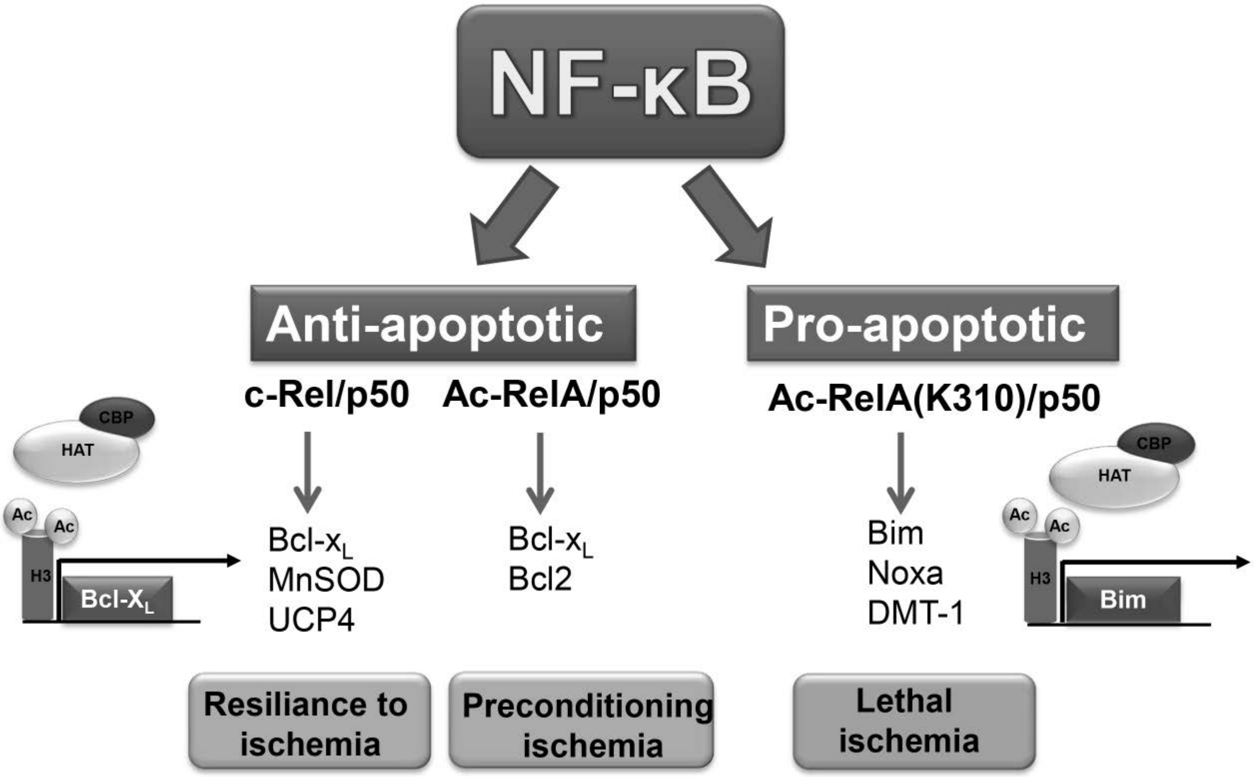

**Fig. 1.** *The p50/RelA and p50/c-Rel dimers regulate neuronal survival. Anti-apoptotic effects of NF-κB can be mediated by c-Rel containing dimers which enhance neuronal resilience to oxidative stress by inducing Bcl-xL, MnSOD, and UCP4 expression. NF-κB anti-apoptotic effects can also be mediated by p50/RelA during preconditioning ischemia through the transcription of Bcl-xL. The pro-apoptotic effect elicited by NF-κB p50/RelA dimer in lethal ischemia is dependent on changes in the RelA acetylation state. A lower grade of RelA general acetylation but a site-specific acetylation on K 310 residue addresses the NF-κB binding towards Bim, Noxa and DMT-1 promoters.*

et al., 2009) characterized by increased nuclear translocation of p50/RelA dimers (Crack et al., 2006; Inta et al., 2006) and decreased translocation of c-Rel-containing dimers (Sarnico et al., 2009). In these conditions, NF-κB activity was associated with an unbalanced expression of pro-apoptotic RelA target genes, i.e. an increased expression of the pro-apoptotic members of Bcl-2 family genes (Inta et al., 2006) and reduced level of the anti-apoptotic member Bcl-xL (Cao et al., 2002; Sarnico et al., 2009). During brain ischemia, NF-κB/RelA induced the expression of the 1B isoform of the divalent metal transporter-1 (1B/DMT1), the membrane carrier responsible for iron accumulation and brain damage after injury (Ingrassia et al., 2012). The RelA-induced 1B/DMT1 expression acted as an upstream mechanism responsible for iron accumulation and contributing to neuronal cell death. While the overexpression of RelA increased cell death, the overexpression of c-Rel prevented neuronal loss in cortical neurons exposed to OGD, by increasing the transcription of Bcl-xL gene (Pizzi et al., 2009; Sarnico et al., 2009). Knocking-down c-Rel expression exacerbated neuronal susceptibility to ischemic damage. Under brain ischemia, mice deficient for the c-Rel factor appeared insensitive to the neuroprotective activity of leptin, a c-Rel inducer able to limit cortical damage in wild-type mice (Valerio et al., 2009).

These data, strongly suggest that inhibition of c-Rel-containing dimers and activation of p50/RelA are key events in the pathogenesis of post-ischemic brain injury.

In spite of these premises, p50/RelA activation per se appeared to be insufficient to drive pro-apoptotic transcription during brain ischemia. A similar pattern of p50/RelA nuclear translocation was found in mice exposed to a brief preconditioning ischemia (Lanzillotta et al., 2010), generating brain tolerance to a subsequent lethal ischemic injury (Blondeau et al., 2001). In neuronal cells, as well as in tumor cells, gene targeting by p50/RelA is finely regulated by post-transcriptional modifications of RelA subunit, such as phosphorylation and acetylation (Chen and Greene, 2004). These modifications shape the strength and specificity of the NF-κB-DNA binding and final transcriptional responses. On this line, we showed that the activation of the p50/RelA dimer, in preconditioning or in lethal ischemia, differs in the RelA acetylation state (Lanzillotta et al., 2013).

### *RelA acetylation is a dynamic process tuning NF-κB-mediated pro-apoptotic transcription in brain ischemia and can be modulated by epigenetic drugs*

Acetylation is the key post-translational modification of histones that controls the accessibility of chromatin to the transcriptional machinery and plays an essential role in gene activation (Sweatt, 2009). Lysine acetylation is reversible and controlled by the opposing activities of histone acetyltransferase (HAT) and histone deacetylase (HDAC).

Besides histones, diverse non-histone proteins, including NF-κB transcription factors, are modified by HAT co-activators and HDACs (Haberland et al., 2009). Acetylation of NF-κB RelA is a dynamic process, indeed the acetylation status of specific lysine residues (K122, 123, 218, 221, and 310) differently affecting the interaction with IκBα, the DNA-binding ability and transcriptional activity of the protein (Chen and Greene, 2004; Kiernan et al., 2003).

Members of class I HDACs, particularly HDAC1, HDAC2, and HDAC3, inhibited by vorinostat and entinostat (MS-275), are the most responsible for the general deacetylation of NF-κB/RelA (Ashburner et al., 2001; Chen and Green, 2004). Besides, sirtuin 1 (SIRT1), an atypical class III HDAC that requires nicotinamide adenosine dinucleotide (NAD+) rather than zinc as a cofactor (Buck et al., 2004), and is activated by resveratrol, selectively deacetylates RelA at K310 (Lanzillotta et al., 2010; Yeung et al., 2004).

Our previous studies have shown that mechanisms affecting the acetylation state of RelA might discriminate between preconditioning and neurotoxic activation of NF-κB during ischemia (Lanzillotta et al., 2010). Protective ischemic preconditioning and harmful ischemia induced similar levels of p50/RelA activation, but only the ischemic injury induced the atypical RelA acetylation. Indeed, RelA activated in mouse cortex during preconditioning ischemia appeared specifically deacetylated on K310 residue, but conserved its general acetylation. Conversely, the activated p50/RelA dimer in ischemic cortices of mice subjected to prolonged MCAO displayed a reduction of RelA general acetylation and a specific increase of RelA K310 acetylation (Lanzillotta et al., 2010).

By undergoing such aberrant acetylation, RelA detached from the anti-apoptotic Bcl-xL promoter to bind the pro-apoptotic Bim promoter (Lanzillotta et al., 2013). Lethal ischemic insult also induces a significant reduction of H3 histones acetylation, in line with previous evidence (Faraco et al., 2006; Lanzillotta et al., 2013). Prompted by these findings and in order to correct altered acetylation of RelA and histones after brain ischemia, we studied the effects of the association of the specific class I HDAC inhibitor, MS-275, (Simonini et al., 2006) with resveratrol (Baur and Sinclair, 2006).

MS-275 is a synthetic benzamide derivative that is currently under clinical evaluation for cancer therapy (Tan et al., 2010). MS-275 has been shown to inhibit HDAC 1-3 with excellent pharmacokinetic properties (Simonini et al., 2006).

Resveratrol is a polyphenol endowed with a multiple activities, including anti-oxidant, anti-tumorigenic and neuroprotective (Yu et al., 2012; Baur and Sinclair, 2006). In various models of brain ischemia, resveratrol delayed axonal degeneration after injury and mitigated the formation of free radical species as well as mitochondria-mediated apoptosis (Agrawal et al., 2011; Li et al., 2011; Morris et al., 2011; Ren et al., 2011). Widely known mechanisms of resveratrol action include the activation of the longevity factors sirtuin 1 (Howitz et al., 2003) and AMP-activated kinase (AMPK), a serine-threonine kinase that acts as a key metabolic and stress sensor/effector (Ruderman et al., 2010).

We found that treatments with either MS-275 or resveratrol displayed a significant neuroprotective activity in cortical neurons exposed to OGD (Lanzillotta et al., 2013). Furthermore, we showed that the combination of MS-275 and resveratrol at sub-threshold doses, elicited a synergistic effect leading to maximal neuroprotection an *in-vitro* model of brain ischemia. MS-275 at the highest concentration tested of 1 μM increased acetylation of H3 histones on K9/18 residues in neurons exposed to OGD. Resveratrol, unable to modify per se the H3 acetylation, produced a synergistic increase of K9/18 H3 acetylation when used in combination with MS-275.

Notably, the synergistic effect produced by co-administration of low doses of MS-275 (0.1 μM) and resveratrol (3 μM) was sustained by AMP-activated kinase (AMPK) activation by resveratrol. This could be ascribed to the fact that AMPK can activate many catabolic pathways that produce ATP and induce acetyl-CoA (Turnley et al., 1999) generation, the fundamental co-factor for HAT activity. AMPK has also been found to indirectly support the resveratrol-dependent sirtuin 1 activation by inducing NAD+ generation (Ruderman et al., 2010). Following the AMPK-mediated enhancement of HAT and sirtuin 1 activity, the combination of MS-275 and resveratrol modulated the RelA acetylation state in neurons exposed to OGD by respectively enhancing the RelA general acetylation and reducing the acetylation at K310 residue. The neuroprotective effect and the transcription of anti-apoptotic factors observed following the treatment with the drug combination appeared to be closely related to the restoration of the optimal RelA acetylation state, a phenomenon that thus pharmacologically reproduces what occurs after a preconditioning ischemia (Lanzillotta et al., 2010; Raval et al., 2006). Treatments with MS-275 and resveratrol after OGD significantly reduced both RelA binding and H3 acetylation at the Bim promoter of neurons. The protective and transcriptional effects produced by resveratrol and MS-275 in cortical neurons were entirely reproduced in the mouse MCAO model. The combination of sub-threshold doses of the drugs, administered during the reperfusion period, elicited a synergistic effect that limited the cerebral infarct volume and the subsequent neurological deficits. MS-275 and resveratrol in combination showed a long-lasting efficacy as the beneficial effects were still evident 72 hours after the injury. Moreover, they displayed a wide therapeutic window as their efficacy was evident when administered up to 7 hours after the ischemic onset. Indeed, the treatment induced a transcriptional switch from pro- to anti-apoptotic genes, as RelA binding shifted from the Bim to the Bcl-xL promoter. Consequently, a decrease of the acetylation of histone H3 associated with the Bim promoter, and an increase in the acetylation of histone H3 at the Bcl-xL promoter was observed.

Recently, we evaluated the acetylation of histone residues at the Brain-derived neurotrophic factor (BDNF) IV promoter in primary mouse cortical neurons exposed to OGD and treated with the synergistic MS-275 and resveratrol combination. In particular, we studied the H3K9/18 and H4K12 acetylation, that are considered a part of a “backbone” of histone modifications that are associated with active promoters (Wang et al., 2008).

Transcription of BDNF is controlled by multiple promoters, driving the expression of multiple transcripts encoding for the same protein. We focused on promoter IV, that is known to be important for synaptic plasticity, both during neuronal development and in the adult brain (Hong et al., 2008). The regulation mechanisms of this promoter have been thoroughly studied and exon IV containing transcripts are highly expressed in neurons (Lyons and West, 2011; Timmusk et al., 1993). In the cortex, promoter IV-dependent BDNF transcription accounts for the majority of the neuronal activity-induced BDNF expression (Tao et al., 2012; Timmusk et al., 1993). Furthermore, several studies have posed BDNF as possible mediators of the beneficial effects of HDAC inhibitors in nervous system disorders (Chen et al., 2006; Chiu et al., 2011; Zeng et al., 2011;Yasuda et al., 2009). The ChIP analysis showed that BDNF promoter IV activity was repressed by OGD exposure, while treatment in the post-OGD period with the combination of MS-275 and resveratrol, significantly increased acetylation at H3 and H4 histones at BDNF promoter IV (Fig. 2). These histone modifications may act cooperatively and possibly in parallel to other histone modifications,

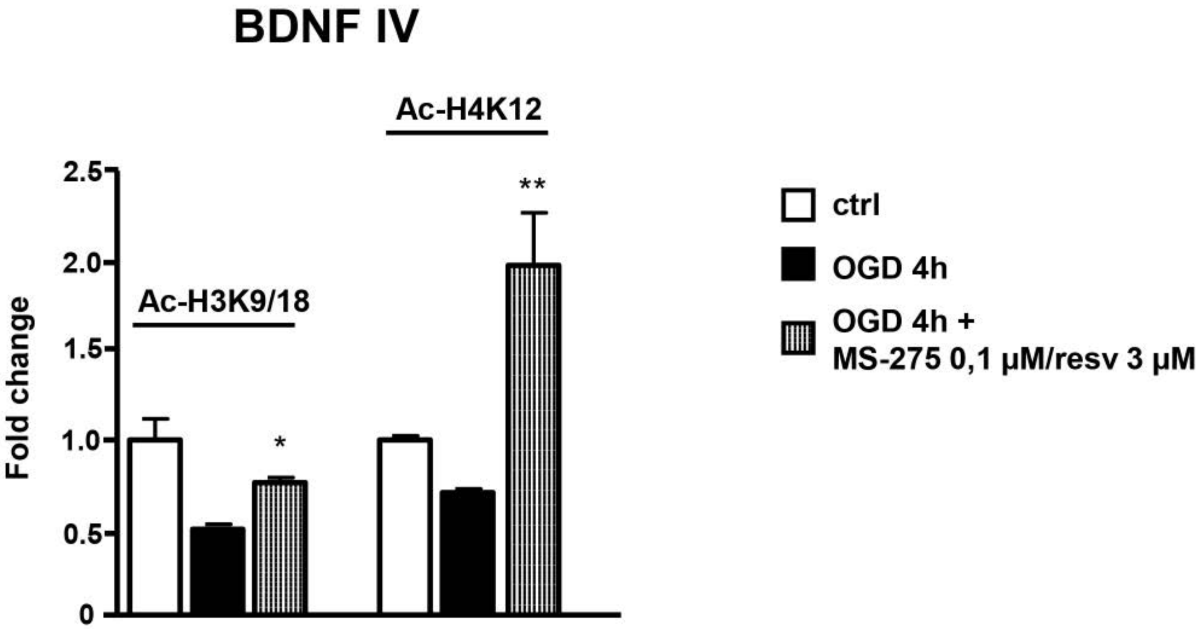

**Fig. 2.** *ChIP analysis of histone H3 acetylation (K9/18) and histone H4 acetylation (K12) on the BDNF IV promoter. Results are obtained by qRT-PCR analysis of BDNF IV promoter in immunoprecipitated DNA of cortical neurons exposed to OGD and treated with a combination of MS-275 (0.1 µM) and resveratrol (3 µM) during the 2 h-reoxygenation period. Treatment with drug combination significantly increases acetylation at both H3 and H4 histones associated with the BDNF IV promoter. Data are expressed as fold changes over values obtained in cells maintained in normal oxygen-glucose condition. Bars depict the mean±s.e.m. of three separate experiments run in triplicate, *p<0.05 or**p<0.01 versus the corresponding OGD value.*

to increase BDNF expression. Thus, we propose that neuroprotection elicited by MS-275 and resveratrol treatment, is also closely related to modulation of BDNF expression and may thus improve neurologic function by enhancing neuronal plasticity.

All together, these data provide the clear cut evidence that a pharmacological intervention targeting the epigenetic machinery that regulate gene expression represents an optimal strategy to limit post-ischemic injury with an extended therapeutic window.

### *c-Rel deficiency causes a progressive late-onset parkinsonism in mice*

After the evidence that RelA and c-Rel composing NF-κB dimers play opposing effects on neuron survival, and a misbalance of p50/RelA versus p50/c-Rel activation triggers apoptotic cell death in brain ischemia (Sarnico et al., 2009), we hypothesized that a constitutive defect in c-Rel protein expression might play a role in neurological conditions associated with age-related neurodegeneration. Hence, we studied whether c-Rel knockout mice may present symptomatological and neuropathological features of neurodegeneration. We investigated different animal groups of either c-Rel knockout or wt mice at 2, 12 and 18 months of age (Baiguera et al., 2012) and we found that c-rel-/- mice develop a PD-like pathology and substantia nigra pars compacta (SNc) revealed a loss of dopaminergic neurons that paralleled the total loss of Nissl-stained cells in c-Rel deficient animals at 18-months of age. No significant change in the estimated number of dopaminergic cells was evident in the SNc of 2- and 12-month-old c-rel-/- mice compared to age-matched controls. Notably, the loss of SNc dopaminergic neurons was associated with a decrease of TH-positive fibers as well as a reduction of dopamine transporter (DAT) and

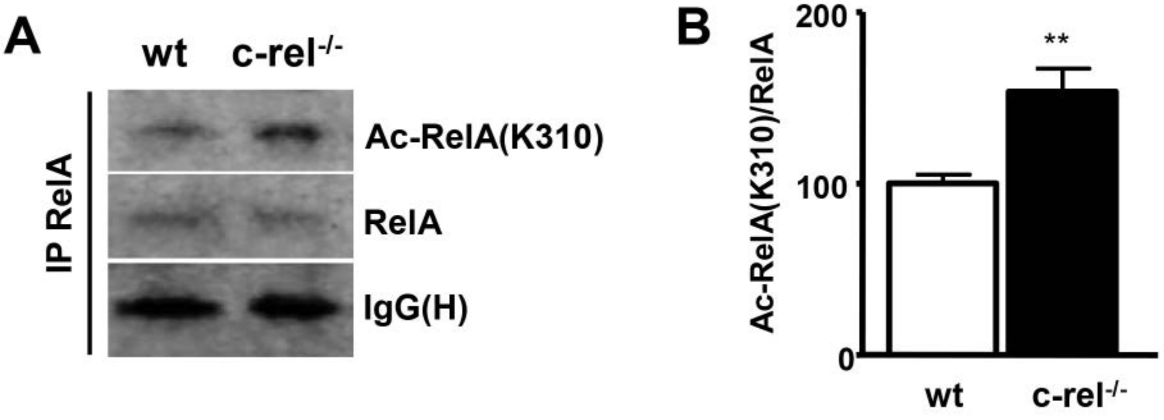

**Fig. 3.** *RelA acetylation at K310 residue in the striatum of c-rel-/- and wt mice.* ***A)*** *Representative picture of the immunoprecipitation analysis of RelA acetylation in total proteins of caudatus putamen. RelA acetylation at K310 residue increased in striatal total extracts of 18-month-old c-rel-/- mice. No significant change was detected in the total RelA content.* ***B)*** *Values from densitometry analysis of immunoblots are expressed as a percentage of the corresponding control value. The signal given by IgG(H) is used as a control for the quality of the immunoprecipitation. Bars depict the mean±s.e.m. of three separate experiments, **$p<0.01$ versus the corresponding wt value.*

dopamine content in the striatum. The 18-month-old c-rel-/- mice displayed no significant degeneration in other neuronal cell populations such as the ventral tegmental area, a region normally spared in PD, or in the nucleus basalis magnocellularis and medial septal area. Additional neuropathological examination of the aged c-rel-/- mouse brains showed the presence of a marked immunoreactivity for α-synuclein, the main protein constituent of Lewy bodies and Lewy neuritis, which represent the key pathological feature of PD (Spillantini et al., 1998), in the SNc. Of note, fibrillary aggregated α-synuclein, detected by Thiofalvin-S labelling, was present in the spared dopaminergic neurons of the SNc. Accumulation of insoluble α-synuclein in the mesencephalon was confirmed by the presence of a monomeric α-synuclein in the 8M urea/5% SDS extracts which solubilizes the insoluble fraction. In the SNc and striatum of aged c-rel-/- mice we also found increased iron levels and a significant augment of the iron transporter DMT-1 that is the above-mentioned transcriptional target of RelA acetylated on K310.

Chronic microglial activation is a main part of neuroinflammation in the post-mortem brain of PD patients (Kannarkat et al., 2013). Likewise in PD brain (Hirsch and Hunot 2009), SNc and striatum of aged c-rel-/- mice showed marked signs of microglia activation, as revealed by increased number as well as by swollen cell bodies and thickened processes of CD11b positive cells. A preliminary investigation of the state of RelA acetylation in striatal extracts of 18 month-old c-rel-/- mice showed higher levels of RelA acetylated on K310 residue, without a significant change in the total RelA (Fig. 3). Because inflammatory and neurotoxic activation of microglia also relies on K310 RelA acetylation (Chen et al., 2005), it may be plausible that microglia activation could participate in the neurodegenerative process in c-rel-/- mice. Extensive analysis of the neuroinflammatory profile of c-rel-/- mice along with disease progression will reveal the exact entity of this inflammatory process and the specific participation of innate and adaptive immunity.

The neurochemical changes observed in aged c-rel-/- mice were also accompanied by the onset of motor deficits. A significant impairment in spontaneous motor activity was evident in c-rel-/- mice at 18 months, but not in younger mice, as previously shown (O'Ryordan et al., 2006; Ahn et al., 2008). Indeed, either monitored for 1 hour or 6 consecutive days to avoid stress-related bias, 18 month-old c-rel-/- mice displayed a lower locomotor activity. Furthermore, the gaiting analysis supported the presence of a locomotor dysfunction related to bradykinesia and rigidity. Noteworthy, the treatment with L-DOPA plus benserazide, a cocktail that is considered the gold standard for PD therapy, totally reversed the locomotor deficits and normalized most of the gaiting parameters.

Despite these findings, how the constitutive c-Rel deficiency can specifically affect DA neurons of SNc, is still an open question (Baiguera et al., 2012). The selective vulnerability of SNc neurons in PD has been attributed to the peculiar "energy-demanding" physiology of these cells (Surmeier et al., 2011) which display enormous axonal field and impressive number of synapses for each axon (Arbuthnott and Wickens, 2007). Moreover, during their pacemaking activity, SNc dopaminergic neurons, but not the VTA neurons, generate autonomous action potentials by unusual engaging of L-type Ca2+ channels which require subsequent activation of ATP-dependent Ca2+ pumps to maintain Ca2+ homeostasis (Wilson and Callaway, 2000). The energy production by mitochondria and endoplasmic reticulum in SNc dopaminergic neurons associates with the generation of large amounts of ROS that are constantly neutralized by antioxidant systems, including SODs catalases, glutathione peroxidase (Halliwell, 2006), UCP4 and UCP5. It can be inferred that in the absence of c-Rel, a reduced expression of UCP4 (Ho et al., 2012) and MnSOD (Bernard et al., 2001; Pizzi et al., 2005b) might enhance ROS accumulation during aging in SNc neurons (Cardozo-Pelaez et al., 1999) and synergize with reduced expression of anti-apoptotic Bcl-xL (Chen et al., 2000; Sarnico et al., 2009) to affect neuronal resilience. It is also feasible that this oxidative stress may contribute to elevate intracellular levels of α-synuclein (Uversky et al., 2007), DMT-1 and iron (Salazar et al., 2008) and, in turn, may lead to α-synuclein aggregation (Uversky et al., 2007), microglia activation and neuronal damage (Zhang et al., 2005). In conclusion, these findings suggest that c-Rel factor might act as a regulator of SNc susceptibility to aging.

Finally, our latest results (unpublished) suggest that, at a premotor phase (8-12 months), when there is still no loss of SNc DA neurons, c-rel-/- mice display gut dysfunctions, as shown by reduced stool frequency and stool water percentage, in addition to olfactory deficits. This disease progression mimics the pathological and clinical progression observed in PD patients, who usually show constipation and hyposmia (Simuni and Sethi, 2008), thus confirming that c-rel-/- mice represent an innovative disease model that may be useful both for studies aimed at dissecting the mechanisms of PD onset and to test novel therapeutic approaches for intervention at the premotor stages of the disorder.

## CONCLUSIONS

NF-κB factors are transcriptional regulators of inflammation and apoptosis, though their relevance in aging and neurodegeneration is still underestimated.

Aberrant activation of NF-κB RelA acetylated on K310 residue triggers pro-apoptotic gene expression and can be targeted by the synergistic association of HDAC inhibitors and resveratrol. Conversely, activation of NF-κB/c-Rel promotes neuroprotective effects through the transcription of specific anti-apoptotic genes: MnSOD, BcL-XL and UCP4.

Activation of NF-κB/RelA has been found to lead the systemic aging process in mice, being negligible in the hypothalamus of young mice and progressively increasing, earlier in microglia and later in neuronal cells, as the mice become older. Aging is delayed and lifespan is extended in mice by preventing aging-related NF-κB/RelA activation in the hypothalamus and in other brain regions (Zhang et al., 2013).

If RelA activation marks physiological elderly, a deficiency of c-Rel drives mice toward a parkinsonian phenotype during aging (Baiguera et al., 2012). The aberrant acetylation of RelA on K310 in the basal ganglia occurs in c-Rel-deficient mice during aging and is associated with the development of an L-DOPA-responsive parkinsonism accompanying the degeneration of DA neurons in the SNc, activation of microglia and α-synuclein pathology.

This body of evidence supports our hypothesis that the balance between c-Rel- and RelA-mediated transcription may be at the crossroad between normal and pathological aging of the brain. In the presence of higher RelA activation, a deficit of c-Rel activity reduces SN resilience to aging, thereby leading to a late-onset form of PD.

## ACKNOWLEDGEMENTS

This work was supported by Ex 60% 2008–2011; University of Brescia; NEDD Project, (CUP

H81J09002660007), Regione Lombardia, Italy; Ricerca Finalizzata Ministero della Salute RF-2010-2315142.

## REFERENCES

Agrawal M, Kumar V, Kashyap MP, Khanna VK, Randhawa GS, Pant AB (2011) Ischemic insult induced apoptotic changes in PC12 cells: protection by trans resveratrol. Eur J Pharmacol 666: 5-11.

Ahn, HJ, Hernandez CM, Levenson, JM, Lubin, FD, Liou, HC and Sweatt, JD (2008) c-Rel, an NF-kappaB family transcription factor, is required for hippocampal long-term synaptic plasticity and memory formation. Learn Mem 15: 539-549.

Arbuthnott GW, Wickens J (2007) Space, time and dopamine. Trends Neurosci 30: 62-9.

Ashburner BP, Westerheide SD, Baldwin AS Jr (2001) The p65 (RelA) subunit of NF-kappaB interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. Mol Cell Biol 21: 7065-77.

Baiguera C, Alghisi M, Pinna A, Bellucci A et al. and Pizzi M (2012) Late-onset Parkinsonism in NFκB/c-Rel-deficient mice. Brain 135: 2750-65.

Baur JA, Sinclair DA (2006) Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 5: 493-506.

Bernard D, Quatannens B, Begue A, Vandenbunder B, Abbadie C (2001) Antiproliferative and anti-apoptotic effects of crel may occur within the same cells via the up-regulation of manganese superoxide dismutase. Cancer Res 61: 2656-64.

Bethea JR, Castro M, Keane RW, Lee TT, Dietrich WD, Yezierski RP (1998) Traumatic spinal cord injury induces nuclear factor-kappaB activation. J Neurosci 18: 3251–3260.

Blondeau N, Widmann C, Lazdunski M and Heurteaux C (2001) Activation of the nuclear factor-kappaB is a key event in brain tolerance. J Neurosci 21: 4668–4677.

Buck SW, Gallo CM, Smith JS (2004) Diversity in the Sir2 family of protein deacetylases. J Leukoc Biol 75: 939-50.

Camandola S, Mattson MP (2007) NF-kappa B as a therapeutic target in neurodegenerative diseases. Expert Opin Ther Targets 11: 123-32.

Cao G, Pei W, Ge H, Liang Q et al. and Chen J (2002) In Vivo Delivery of a Bcl-xL Fusion Protein Containing the TAT Protein Transduction Domain Protects against Ischemic Brain Injury and Neuronal Apoptosis. J Neurosci 22: 5423-5431.

Cardozo-Pelaez F, Song S, Parthasarathy A, Hazzi C, Naidu K, Sanchez-Ramos J (1999) Oxidative DNA damage in the aging mouse brain. Mov Disord 14: 972-80.

Chen C, Edelstein LC, Gélinas C (2000) The Rel/NF-kappaB family directly activates expression of the apoptosis inhibitor Bcl-x(L). Mol Cell Biol 20: 2687-95.

Chen J, Zhou Y, Mueller-Steiner S, Chen LF et al. and Gan L (2005) SIRT1 protects against microglia-dependent amyloid-beta toxicity through inhibiting NF-kappaB signaling. J Biol Chem 280: 40364-74.

Chen LF, Greene WC (2004) Shaping the nuclear action of NF-kappaB Nat Rev Mol Cell Biol 5: 392-401.

Chen PS, Peng GS, Li G, Yang S et al. and Hong JS (2006) Valproate protects dopaminergic neurons in midbrain neuron/glia cultures by stimulating the release of neurotrophic factors from astrocytes. Mol Psychiatry 11: 1116-25.

Chiu CT, Liu G, Leeds P, Chuang DM (2011) Combined treatment with the mood stabilizers lithium and valproate produces multiple beneficial effects in transgenic mouse models of Huntington's disease. Neuropsychopharmacology 36: 2406-21.

Crack P, Taylor J M, Ali U, Mansell A and Hertzog PJ (2006) Potential contribution of NF-kappaB in neuronal cell death in the glutathione peroxidase-1 knockout mouse in response to ischemia-reperfusion injury. Stroke 37: 1533-1538.

Crampton SJ, O'Keeffe GW (2013) NF-κB: emerging roles in hippocampal development and function. Int J Biochem Cell Biol 45: 1821-4.

Echtay KS (2007) Mitochondrial uncoupling proteins--what is their physiological role? Free Radic Biol Med 43: 1351-71.

Faraco G, Pancani T, Formentini L, Mascagni P et al. and Chiarugi A (2006) Pharmacological inhibition of histone deacetylases by suberoylanilide hydroxamic acid specifically alters gene expression and reduces ischemic injury in the mouse brain. Mol Pharmacol 70: 1876-84.

Ghosh A, Roy A, Liu X, Kordower JH et al. and Pahan K (2007) Selective inhibition of NF-kappaB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease. Proc Natl Acad Sci USA 104: 18754-9

Haberland M, Montgomery RL, Olson EN (2009) The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nat Rev Genet 10: 32-42.

Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now? J Neurochem 97: 1634-58.

Herrmann O, Baumann B, de Lorenzi R, Muhammad S et al. and Schwaninger M (2005) IKK mediates ischemia-induced neuronal death. Nat Med 11: 1322–1329.

Hirsch EC, Hunot S (2009) Neuroinflammation in Parkinson's disease: a target for neuroprotection? Lancet Neurol 8: 382–97.

Ho JW, Ho PW, Liu HF, So DH et al. and Ho SL (2012) UCP4 is a target effector of the NF-κB c-Rel prosurvival pathway against oxidative stress. Free Radic Biol MedJul 53: 383-94.

Hong EJ, McCord AE, Greenberg ME (2008) A biological function for the neuronal activity-dependent component of Bdnf transcription in the development of cortical inhibition. Neuron 60: 610-24.

Howitz KT, Bitterman KJ, Cohen HY, Lamming DW et al. and Sinclair DA (2003) Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature 425: 191-196.

Hunot S, Brugg B, Ricard D, Michel PP et al. and Hirsch EC (1997) Nuclear traslocation of NF-κB is increased in dopaminergic neurons of patients with Parkinson's disease. Proc Natl Acad Sci USA 94: 7531–7536.

Ingrassia R, Lanzillotta A, Sarnico I, Benarese M et al. and Pizzi M (2012) 1B/(-)IRE DMT1 expression during brain ischemia contributes to cell death mediated by NF-κB/RelA acetylation at Lys310. PLoS One 7: e38019.

Inta I, Paxian S, Maegele I, Zhang W et al. and Schwaninger M (2006) Bim and Noxa are candidates to mediate the deleterious effect of the NF-kappa B subunit RelA in cerebral ischemia. J Neurosci 26: 12896-903.

Kaltschmidt B, Uherek M, Volk B, Baeuerle PA and Kaltschmidt C (1997) Transcription factor NF-κB is activated in primary neurons by amyloid β peptides and in neurons surrounding early plaques from patients with Alzheimer's disease. Proc Natl Acad Sci USA 94: 2642–2647.

Kannarkat GT, Boss JM, Tansey MG (2013)The role of innate and adaptive immunity in Parkinson's disease. J Parkinsons Dis 3: 493-514.

Kiernan R, Brès V, Ng RW, Coudart MP et al. and Benkirane M (2003) Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65. J Biol Chem 278: 2758-66.

Kögel D, Peters M, Konig HG, Hashemi SM et al. and Prehn JH (2004) S100B potently activates RelA/c-Rel transcriptional complexes in hippocampal neurons: Clinical implications for the role of S100B in excitotoxic brain injury. Neuroscience 127: 913–920.

Koo JW, Russo SJ, Ferguson D, Nestler EJ, Duman RS (2010) Nuclear factor-kappaB is a critical mediator of stress-impaired neurogenesis and depressive behavior. Proc Natl Acad Sci U S A 107: 2669-74.

Lanzillotta A, Sarnico I, Ingrassia R, Boroni F et al. and Pizzi M (2010) The acetylation of RelA in Lys310 dictates the NF-κB-dependent response in post-ischemic injury. Cell Death Dis 1: e96.

Lanzillotta A, Pignataro G, Branca C, Cuomo O et al. and Pizzi M (2013) Targeted acetylation of NF-kappaB/RelA and histones by epigenetic drugs reduces post-ischemic brain injury in mice with an extended therapeutic window. Neurobiol Dis 49C: 177-189.

Levenson JM, Choi S, Lee SY, Cao YA et al. and Sweatt, JD (2004) A bioinformatics analysis of memory consolidation reveals involvement of the transcription factor c-rel. J Neurosci 24: 3933-3943.

Li H, Yan Z, Zhu J, Yang J, He J (2011) Neuroprotective effects of resveratrol on ischemic injury mediated by improving brain energy metabolism and alleviating oxidative stress in rats. Neuropharmacology 60: 252-8.

Lyons MR, West AE (2011) Mechanisms of specificity in neuronal activity-regulated gene transcription. Prog Neurobiol 94: 259-95.

Morris KC, Lin HW, Thompson JW, Perez-Pinzon MA (2011) Pathways for ischemic cytoprotection: role of sirtuins in caloric restriction, resveratrol, and ischemic preconditioning. J Cereb Blood Flow Metab 31: 1003-19.

Nurmi A, Lindsberg PJ, Koistinaho M, Zhang W et al. and Koistinaho, J (2004) Nuclear factor-kappaB contributes to infarction after permanent focal ischemia. Stroke 35: 987-991.

O'Riordan KJ, Huang IC, Pizzi M, Spano PF et al. and Levenson JM (2006) Regulation of nuclear factor kappaB in the hippocampus by group I metabotropic glutamate receptors. J Neurosci 26: 4870–79.

Pizzi M, Goffi F, Boroni F, Goffi F et al. and Spano P (2002) Opposing roles for NF-kappa B/Rel factors RelA and c-Rel in the modulation of neuron survival elicited by glutamate and interleukin-1beta. J Biol Chem 277: 20717–20723.

Pizzi M, Sarnico I, Boroni F, Benetti A, Benarese M, Spano PF (2005a) Inhibition of IkappaBalpha phosphorylation prevents glutamate-induced NF-kappaB activation and neuronal cell death. Acta Neurochirurgica Suppl 93: 59–63.

Pizzi M, Sarnico I, Boroni F, Benarese M et al., and Spano PF (2005b) NF-kappaB factor c-Rel mediates neuroprotection elicited by mGlu5 receptor agonists against amyloid beta-peptide toxicity. Cell Death Differ 12: 761–772.Pizzi M, Spano P (2006) Distinct roles of diverse nuclear factor-kappaB complexes in neuropathological mechanisms. Eur J Pharmacol 545: 22-8.

Pizzi M, Sarnico I, Lanzillotta A, Battistin L, Spano P (2009) Post-ischemic brain damage: NF-kappaB dimer heterogeneity as a molecular determinant of neuron vulnerability. FEBS J 276: 27-35.

Raval AP, Dave KR, Pérez-Pinzón MA (2006) Resveratrol mimics ischemic preconditioning in the brain. J Cereb Blood Flow Metab 26: 1141-7.

Ren J, Fan C, Chen N, Huang J, Yang Q (2011) Resveratrol pretreatment attenuates cerebral ischemic injury by upregulating expression of transcription factor Nrf2 and HO-1 in rats. Neurochem Res 36: 2352-62.

Rolls A, Shechter R, London A, Ziv Y, Ronen A, Levy R, Schwartz M (2007) Toll-like receptors modulate adult hippocampal neurogenesis. Nat Cell Biol 9: 1081-8.

Ruderman NB, Xu XJ, Nelson L, Cacicedo JM, Saha AK, Lan F, Ido Y (2010) AMPK and SIRT1: a long-standing partnership? Am J Physiol Endocrinol Metab 298: E751-60.

Salazar J, Mena N, Hunot S, Prigent A et al. and Hirsch EC (2008) Divalent metal transporter 1 (DMT1) contributes to neurodegeneration in animal models of Parkinson's disease. Proc Natl Acad Sci USA 105: 18578-583.

Sarnico I, Boroni F, Benarese M, Sigala S et al. and Pizzi M (2008) Activation of NF-kappaB p65/c-Rel dimer is associated with neuroprotection elicited by mGlu5 receptor agonists against MPP(+) toxicity in SK-N-SH cells. J Neural Transm 115: 669–676.

Sarnico I, Lanzillotta A, Boroni F, Benarese M et al. and Pizzi M (2009) NF-kappaB p50/RelA and c-Rel-containing dimers: opposite regulators of neuron vulnerability to ischaemia. J Neurochem 108: 475-85.

Schneider A, Martin-Villalba A, Weih F, Vogel J, Wirth T, Schwaninger M (1999) NF-kappaB is activated and promotes cell death in focal cerebral ischemia. Nat Med 5: 554–559.

Simonini MV, Camargo LM, Dong E, Maloku E, Veldic M, Costa E, Guidotti A (2006) The benzamide MS-275 is a potent, long-lasting brain region-selective inhibitor of histone deacetylases. Proc Natl Acad Sci USA 103: 1587-92.

Simuni T and Sethi K (2008) Nonmotor Manifestations of Parkinson's Disease. Ann Neurol 64 Suppl 2: S65-80.

Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) Alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. Proc Natl Acad Sci U S A 95(11) : 6469-73

Surmeier DJ, Guzman JN, Sanchez-Padilla J, Schumacker PT (2011) The role of calcium and mitochondrial oxidant stress in the loss of substantia nigra pars compacta dopaminergic neurons in Parkinson's disease. Neuroscience 198: 221-31.

Sweatt JD (2009) Experience-dependent epigenetic modifications in the CNS. Biol. Psychiatry 65: 191-7.

Tan J, Cang S, Ma Y, Petrillo RL, Liu D (2010) Novel histone deacetylase inhibitors in clinical trials as anti-cancer agents. J Hematol Oncol 3: 5.

Tao J, Ji F, Liu B, Wang F, Dong F, Zhu Y (2012) Improvement of deficits by transplantation of lentiviral vector-modified human amniotic mesenchymal cells after cerebral ischemia in rats. Brain Res 1448: 1-10.

Timmusk T, Palm K, Metsis M, Reintam T, Paalme V, Saarma M, Persson H (1993) Multiple promoters direct tissue-specific expression of the rat BDNF gene. Neuron 10: 475-89.

Turnley AM, Stapleton D, Mann RJ, Witters LA, Kemp BE, Bartlett PF (1999) Cellular distribution and developmental expression of AMP-activated protein kinase isoforms in mouse central nervous system. J Neurochem 72: 1707-16.

Uversky VN (2007) Neuropathology, biochemistry, and biophysics of alpha-synuclein aggregation. J Neurochem 103: 17-37.

Valerio A, Boroni F, Benarese M, Sarnico I et al. and Pizzi M (2006) NF-κB pathway: a target for preventing β-amyloid (Aβ )-induced neuronal damage and Aβ42 production. Eur J Neurosci 23: 1711–1720.

Valerio A, Dossena M, Bertolotti P, Boroni F et al., and Nisoli E (2009) Leptin is induced in ischemic cerebral cortex and exerts neuroprotection via NF-κB/c-Rel-dependent transcription. Stroke 40: 610-7.

Wang Z, Zang C, Rosenfeld JA, Schones DE et al. and Zhao K (2008) Combinatorial patterns of histone acetylations and methylations in the human genome. Nat Genet 40: 897-903.

Wilson CJ, Callaway JC (2000) Coupled oscillator model of the dopaminergic neuron of the substantia nigra.

J Neurophysiol 83: 3084-3100.

Yang HJ, Wang L, Xia YY, Chang PN, Feng ZW (2010) NF-kappaB mediates MPP+-induced apoptotic cell death in neuroblastoma cells SH-EP1 through JNK and c-Jun/AP-1. Neurochem Int. 56: 128-34.

Yasuda S, Liang MH, Marinova Z, Yahyavi A, Chuang DM (2009) The mood stabilizers lithium and valproate selectively activate the promoter IV of brain-derived neurotrophic factor in neurons. Mol Psychiatry 14: 51-9.

Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW (2004) Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 23: 2369-80.

Yu W, Fu YC, Wang W (2012) Cellular and molecular effects of resveratrol in health and disease. J Cell Biochem 113: 752-9.

Zeng Y, Tan M, Kohyama J, Sneddon M, Watson JB, Sun YE, Xie CW (2011) Epigenetic enhancement of BDNF signaling rescues synaptic plasticity in aging. J Neurosci 31: 17800-10.

Zhang W, Wang T, Pei Z, Miller DS et al. and Zhang J (2005) Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson’s disease. FASEB J 19 :533-42.

Zhang G, Li J, Purkayastha S, Tang Y et al. and Cai D (2013) Hypothalamic programming of systemic ageing involving IKK-β, NF-κB and GnRH. Nature 497: 211-6.