Induction of heat shock proteins in cerebral cortical cultures by celastrol

Ari M. Chow · Derek W. F. Tang · Asad Hanif · Ian R. Brown

Received: 17 June 2012 / Revised: 23 July 2012 / Accepted: 24 July 2012 / Published online: 3 August 2012
© Cell Stress Society International 2012

Abstract Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (ALS) are 'protein misfolding disorders' of the mature nervous system that are characterized by the accumulation of protein aggregates and selective cell loss. Different brain regions are impacted, with Alzheimer's affecting cells in the cerebral cortex, Parkinson's targeting dopaminergic cells in the substantia nigra and ALS causing degeneration of cells in the spinal cord. These diseases differ widely in frequency in the human population. Alzheimer's is more frequent than Parkinson's and ALS. Heat shock proteins (Hsps) are 'protein repair agents' that provide a line of defense against misfolded, aggregation-prone proteins. We have suggested that differing levels of constitutively expressed Hsps (Hsc70 and Hsp27) in neural cell populations confer a variable buffering capacity against 'protein misfolding disorders' that correlates with the relative frequencies of these neurodegenerative diseases. The high relative frequency of Alzheimer's may due to low levels of Hsc70 and Hsp27 in affected cell populations that results in a reduced defense capacity against protein misfolding. Here, we demonstrate that celastrol, but not classical heat shock treatment, is effective in inducing a set of neuroprotective Hsps in cultures derived from cerebral cortices, including Hsp70, Hsp27 and Hsp32. This set of Hsps is induced by celastrol at 'days in vitro' (DIV) 13 when cultured cortical cells reached maturity. The inducibility of a set of neuroprotective Hsps in mature cortical cultures at DIV13 suggests that celastrol is a potential agent to counter Alzheimer's disease, a neurodegenerative 'protein misfolding disorder' of the adult brain that targets cells in the cerebral cortex.

Keywords Hsp70 · Hsp32 · Hsp27 · Alzheimer's disease · Celastrol

Abbreviations

ALS Amyotrophic lateral sclerosis
DIV Days in vitro

Introduction

Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (ALS) are 'protein misfolding disorders' that exhibit common features of misfolded, aggregation-prone proteins and selective cell loss in the mature nervous system (Muchowski and Wacker 2005; Haass and Selkoe 2007; Asea and Brown 2008; Brown 2008; Neef et al. 2011). These neurodegenerative diseases impact different regions of the brain. Alzheimer's disease affects the cerebral cortex triggering memory loss and dementia (Martin 1999; Duyckaerts et al. 2009; Stranahan and Mattson 2010). Parkinson's disease is characterized by a loss of dopaminergic cells in the substantia nigra and is accompanied by muscle rigidity, bradykinesia and resting tremor (Schapira and Olanow 2004; Parent and Parent 2010). Cells in the spinal cord are selectively lost in ALS, resulting in progressive muscle wasting and weakness, culminating in paralysis and respiratory failure (Bruijn et al. 2004; Barber and Shaw 2010).

These neurodegenerative 'protein misfolding disorders' differ widely in frequency in the human population. Alzheimer's disease is 133-fold more frequent than ALS and 4-fold more frequent than Parkinson's disease (Martin 1999; Bruijn et al. 2004; Schapira and Olanow 2004; Chen and Brown 2007). Heat shock proteins provide a line of defense against misfolded, aggregation-prone proteins (Muchowski and Wacker 2005; Brown 2007, 2008; Asea and Brown 2008; Neef et al. 2011). We have suggested that differing

A. M. Chow · D. W. F. Tang · A. Hanif · I. R. Brown (✉)
Centre for the Neurobiology of Stress, Department of Biological Sciences, University of Toronto Scarborough,
1265 Military Trail,
Toronto, ON M1C 1A4, Canada
e-mail: ibrown@utsc.utoronto.ca

levels of constitutively expressed heat shock proteins Hsc70 (product of the HSPA8 gene) and Hsp27 (product of the HSPB1 gene) in neural cell populations confer a variable buffering capacity against protein misfolding disorders that correlates with the relative frequencies of these neurodegenerative diseases (Chen and Brown 2007). The defense capacity against protein misfolding disorders is higher in the spinal cord cells because of their elevated levels of constitutively expressed Hsc70 and Hsp27, compared to dopaminergic cells in the substantia nigra, which in turn have a higher defense capacity compared to cells in the cerebral cortex of the brain. The levels of these constitutively expressed Hsps correlate with the lower frequency of ALS, a disease that affects cells in the spinal cord, followed by a higher disease frequency for Parkinson's that affects cells in the substantia nigra and an even higher frequency for Alzheimer's disease that impacts cells in the cerebral cortex.

We have suggested that Alzheimer's disease may exhibit a higher relative frequency due to low levels of Hsc70 and Hsp27 in cell populations in the cerebral cortex that results in a reduced defense capacity against protein misfolding compared to cell populations affected in ALS that exhibit higher levels of these potentially beneficial proteins (Chen and Brown 2007). Neural cells may rely on their constitutive levels of Hsc70 and Hsp27 as a 'preprotection' mechanism for defense against protein misfolding triggered by stressful stimuli or that associated with neurodegenerative diseases. This suggests that a therapeutic strategy to counter Alzheimer's disease, which is characterized by low constitutive Hsc70 and Hsp27 in impacted cell populations, is the enhancement of the buffer capacity of these cells against protein misfolding by induction of Hsp70 and Hsp27. Hsp70, an inducible protein of the Hsp70 family, shares close to 90 % amino acid homology with constitutively expressed Hsc70 and exhibits overlapping biochemical properties (Daugaard et al. 2007; Brocchieri et al. 2008; Kabani and Martineau 2008).

In the present report, we examine the ability of celastrol and classical heat shock treatment to induce Hsps in cultures derived from the cerebral cortices. Celastrol was selected because it has been identified as a potential neuroprotective candidate in a collaborative drug screen aimed at identifying therapeutic agents against neurodegenerative diseases (Abbott 2002; Heemskerk et al. 2002). Cortical cultures were employed for investigation as cells in the cerebral cortex of the brain are selectively affected during Alzheimer's disease (Martin 1999; Duyckaerts et al. 2009; Stranahan and Mattson 2010) and because previous work has suggested that agents that are effective in countering protein aggregation in established neural cell lines, such as PC12 cells, may not be effective on cortical cells in the nervous system that are impacted during Alzheimer's disease (Zhang et al. 2012). Cortical cultures have been used as a model system to investigate aging of the nervous system (Lesuisse and Martin 2002).

Materials and methods

Cortical cultures

Primary cortical cultures were prepared from cerebral cortices of 19-day-old embryonic rats (E19). Timed pregnant Sprague-Dawley rats were obtained from Charles River Laboratories (Saint-Constant, QC, Canada). E1 was considered as the day on which a vaginal plug was observed. Cerebral cortices were gently dissociated with a plastic pipette after digestion with trypsin (TripLE, Invitrogen, Burlington, ON, Canada) at 37 °C. Dissociated cells were resuspended in minimal essential medium (Gibco, Burlington, ON, Canada) supplemented with 10 % fetal calf serum, 5.56 mM glucose and 2 mM glutamine. Cell viability was assessed by trypan blue exclusion, and live cells were counted and plated at a final density of 1.8×10^5 cells/cm² on poly-ornithine (Sigma, Mississauga, ON, Canada) coated culture dishes (Sarstedt, Montreal, QC, Canada). Cultures were incubated at 37 °C in an atmosphere of 5 % CO₂ in Neurobasal media with B27 supplement (Gibco) for the specified 'days in vitro' (DIV) of culture before treatment with celastrol or heat shock. All procedures using animals were approved by the Animal Care Committee of the University of Toronto and were in accordance with the guidelines established by the Canadian Council on Animal Care.

Heat shock and celastrol treatments

For temperature elevation, cortical cultures were heat shocked at 43 °C for 10 min and subsequently incubated at 37 °C for 24 h prior to analysis by immunoblotting. Cultures were incubated in the presence of celastrol at the specified concentrations for 24 h prior to analysis by immunoblotting. Celastrol was obtained from Gaia Chemical Corporation (Gaylordsville, CT, USA) and dissolved in DMSO as the vehicle. Celastrol concentration up to 1.75 μM did not affect cell viability assayed using CellTrace calcein violet AM (Invitrogen, Burlington, ON, Canada) in combination with LIVE/DEAD fixable blue dead cell stain (Invitrogen).

Western blotting

Cortical cultures were solubilized in Laemmli buffer and boiled for 20 min. Lowry assays were performed for protein quantification (Peterson 1977). Equal loadings of 30 μg of protein per lane were separated by 12 % SDS-PAGE using the Mini-PROTEAN 3 Electrophoresis Module Assembly (Bio-Rad Laboratories, Hercules, CA) with a stacking gel of 4 %, using the standard buffer system of Laemmli before transfer to nitrocellulose membranes. Western blotting was performed with antibodies specific to Hsp70 (SPA-810, StressGen Biotechnologies, BC, Canada), Hsp32 (OSA-111, StressGen) and Hsp27 (SPA-801, StressGen) (Chow and

Brown 2007; Chow et al. 2010). Anti- β -tubulin antibody (MAB3408, Chemicon, Temecula, CA, USA) was employed to detect tubulin as loading control. Horseradish peroxidase-conjugated secondary antibodies (Sigma) were detected by enhanced chemiluminescence assay (Amersham, Piscataway, NJ, USA). Western blots representative of three experimental repeats are shown.

Results

Induction of Hsp70 in cortical cultures by celastrol

The ability of celastrol and classical heat shock treatment to induce Hsp70 in cortical cultures was investigated. Previous investigations using cortical cultures as a model system to study aging of the nervous system have employed cultures grown for variable DIV (Lesuisse and Martin 2002). The present study was therefore carried out at DIV6, DIV10 and DIV13. With increasing DIV, cortical cultures demonstrate progressive development, reaching maturity within 2 weeks of culture (Lesuisse and Martin 2002).

Induction of Hsp70 in cortical cultures was observed at 1.25 and 1.75 μ M celastrol, dosages that did not affect cell viability (see “Materials and methods”). Hsp70 induction was apparent at DIV13 but not at DIV6 and DIV10. In contrast, classical heat shock did not induce Hsp70 at any culture age that was examined.

Induction of Hsp27 in cortical cultures

As shown in Fig. 2, celastrol triggered a robust induction of Hsp27 in cortical cultures at DIV13, a weaker induction at DIV10 and no detectable signal at DIV6. Induction of Hsp27 at DIV13 was observed at a lower dosage of celastrol (0.75 μ M) compared to that required to induce Hsp70 (1.25 μ M) (compare Figs. 1 and 2). Interestingly, heat shock treatment induced Hsp27 at DIV13 but not DIV6 and DIV10, whereas Hsp70 was not induced by heat shock at any DIV examined.

Induction of Hsp32 in cortical cultures by celastrol and heat shock

Celastrol induced Hsp32 at all DIV stages that were examined, as did heat shock (Fig. 3), whereas induction of Hsp70 by celastrol was observed only at the later stage of DIV13 and Hsp27 at DIV10 and 13. Heat shock also induced Hsp32 at all three culture stages, whereas heat shock did not induce Hsp70 at any stage and induced Hsp27 only at DIV13. Basal levels of Hsp32 were apparent at DIV10 and DIV13 (NT and DMSO lanes).

Fig. 1 Induction of Hsp70 by celastrol varies with DIV of cortical cultures. Celastrol induced Hsp70 in mature cultures (DIV13) but not in immature cultures (DIV6 and DIV10). Heat shock failed to induce Hsp70 at any of the DIV examined. NT no treatment control, DMSO vehicle control

Discussion

Neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease and ALS, exhibit the common feature of accumulation of misfolded, aggregation-prone proteins, impact different regions of the nervous system and differ greatly in frequency in the human population (Selkoe 2003; Forman

Fig. 2 Induction of Hsp27 by celastrol and heat shock varies with DIV of cortical cultures. Celastrol induced Hsp27 at DIV10 and DIV13 but not at DIV6. Inducibility of Hsp27 by heat shock was detected only at mature cultures (DIV13)

Fig. 3 Celastrol and heat shock induce Hsp32 at an earlier DIV compared to Hsp27. Celastrol induced Hsp32 at DIV6, whereas it induced Hsp27 at DIV10. Heat shock also induced an earlier induction of Hsp32 (DIV6) compared to Hsp27 (DIV13)

et al. 2004; Muchowski and Wacker 2005; Haass and Selkoe 2007; Asea and Brown 2008; Neef et al. 2011). Alzheimer's disease is 133-fold more frequent than ALS and 4-fold more frequent than Parkinson's disease (Martin 1999; Buijn et al. 2004; Schapira and Olanow 2004; Chen and Brown 2007).

We have suggested that differing levels of the constitutively expressed heat shock proteins Hsc70 and Hsp27 in neural cell populations confer a variable buffering capacity against protein misfolding disorders that correlates with the relative frequencies of these neurodegenerative diseases (Chen and Brown 2007). The high frequency of Alzheimer's disease correlates with a low level of Hsc70 and Hsp27 in cells in the cerebral cortex that are affected in this disease. Whereas the low frequency of ALS correlates with a high level of Hsc70 and Hsp27 conferring enhanced buffering capacity against protein misfolding disorder in cells in the spinal cord that are impacted in ALS. This suggests that an approach to countering Alzheimer's disease is the enhancement of the buffering capacity of cerebral cortical cells against protein misfolding by induction of Hsps.

In this report, we demonstrate that celastrol, but not classical heat shock treatment, is effective in inducing a set of neuroprotective Hsps in cortical cultures, including Hsp70, Hsp27 and Hsp32. Cortical cultures were specifically selected because (1) cells in the cerebral cortex are targeted in Alzheimer's disease (Martin 1999; Duyckaerts et al. 2009; Stranahan and Mattson 2010), (2) cortical cells exhibit low levels of constitutively expressed Hsc70 and Hsp27 (Chen and Brown 2007) and (3) agents effective in countering protein aggregation in established neural cell lines, such as the rat

pheochromocytoma PC12 cells, may not be effective in cortical cells in the nervous system that are impacted in Alzheimer's disease (Zhang et al. 2012). Celastrol has been reported to induce Hsps by acting on HSF1 (Westerheide et al. 2004). Our previous studies have demonstrated that HSF1 is present in cerebral cortical neurons (Brown and Rush 1999).

Celastrol has been identified as a potential neuroprotective candidate in a collaborative drug screen aimed at identifying therapeutic agents against neurodegenerative diseases from a panel of existing drugs and natural products (Abbott 2002; Heemskerk et al. 2002). Candidates were scored on their ability to suppress aspects associated with neurodegenerative diseases such as protein aggregation. Celastrol, a quinine methide triterpene, is extracted from the vine *Tripterygium wilfordii* of the Celastraceae family (Zhou 1991). It has been used in traditional Chinese medicine for the treatment of various illnesses (Tao et al. 2002; Pinna et al. 2004). Celastrol is a proteasome inhibitor that targets the chymotrypsin-like activity of the proteasome (Yang et al. 2006). Other proteasome inhibitors, such as MG132 and lactacystin, have also been shown to induce Hsps (Choy et al. 2011; Kim et al. 2011).

Interestingly, our results indicate that the profile of Hsps induced by celastrol in cortical cultures is influenced by the time period ('days in vitro' or DIV) that the cortical cells were grown in culture. Hsp70, Hsp27 and Hsp32 are all inducible by celastrol at DIV13 when the cortical cultures have reached maturity, but this set of Hsps is not inducible by celastrol in immature cultures at DIV6 and DIV10 (summarized in Fig. 4). Alzheimer's disease is a 'protein misfolding disorder' of cells in the cerebral cortex of the mature brain (Selkoe 2004); hence, the inducibility of a set of neuroprotective Hsps in mature cortical cultures at DIV13 suggests that celastrol could be a potential agent to counter Alzheimer's, a neurodegenerative disease of the adult brain.

Fig. 4 Induction profiles of Hsp27, Hsp32 and Hsp70 vary with DIV of cortical cultures. The full set of Hsps is induced by celastrol, but not classical heat shock treatment, in mature cortical cultures (DIV13). The profile of Hsps induced by celastrol is influenced by the time period (DIV) that the cortical cells were grown in culture. Hsp70, Hsp27 and Hsp32 are all inducible by celastrol at DIV13 when the cortical cultures reached maturity, but this set of Hsps is not inducible by celastrol in immature cultures at DIV6 and DIV10

Our results demonstrate that celastrol induces Hsp27 and Hsp32 in addition to Hsp70 in mature cortical cultures. The neuroprotective features of these Hsps have received less attention compared to Hsp70 (Asea and Brown 2008). However, Hsp27 has been demonstrated to be involved in neuronal survival and neurite outgrowth (Hong et al. 2009; Williams and Mearow 2011). Neuroprotective effects of Hsp27 against ethanol toxicity have been reported (Toth et al. 2010), and Hsp27 protects cortical neurons from beta-amyloid mediated toxicity (King et al. 2009). Hsp27 exhibits neuroprotective effects in animal models of neurodegenerative diseases and following nerve injury (Read and Gorman 2009).

Our studies also indicate that celastrol induces a particularly robust induction of Hsp32, a protein that has received comparatively little attention in neurodegenerative diseases compared to Hsp70 (Asea and Brown 2008). Hsp32 (also known as heme oxygenase-1) is involved in the removal of cytotoxic heme by converting it to carbon monoxide (CO), biliverdin and ferrous iron (Tenhunen et al. 1969). CO is a signalling molecule that has pro-survival and anti-inflammatory roles; biliverdin is cytoprotective and antioxidative; and ferrous iron has indirect cytoprotective effects by inducing expression of antioxidative ferritin (Grochot-Przeczek et al. 2012). In Alzheimer's, the level of cytotoxic heme increases and Hsp32 could aid in its conversion to beneficial antioxidant byproducts (Calabrese et al. 2010). Cytoprotective effects of Hsp32 have been implicated in conditions including inflammatory diseases and ischemic damage (Calabrese et al. 2010; Gozzelino et al. 2010). High concentration of heme has also been implicated as an injury factor in intracranial hemorrhage causing stroke (Lok et al. 2011). Hsp32 could be beneficial in alleviating this condition by converting heme to antioxidant byproducts. Hsp32 has been reported to have a protective role in cell death associated with neurodegeneration (Sato et al. 2003; Li et al. 2007; Guzman-Beltran et al. 2008; Scapagnini et al. 2011). This is of particular importance as an increase in heme in the cerebral cortex of the brains of Alzheimer's patients has been suggested to form heme-amyloid beta complexes leading to pathological symptoms (Pramanik and Dey 2011). Hence, the ability of celastrol to induce a set of Hsps in cortical cells, including a robust induction of Hsp32, could be beneficial to counter Alzheimer's disease and also ameliorate the effects of heme-mediated damage in stroke. Our results indicate that celastrol can induce Hsp32 in immature cortical cultures at DIV6 and DIV10, in addition to induction in mature cortical cultures at DIV13. This suggests that celastrol may be a potential agent to induce this neuroprotective Hsp in the cerebral cortex of the immature developing brain and neonatal brain after ischemic or hypoxic damage. Recently, in vivo administration of celastrol in a transgenic mouse model of Alzheimer's disease has been shown to reduce a key neuropathological hallmark of this degenerative disorder, namely beta-amyloid pathology in the brain (Paris et al. 2010).

Acknowledgements We thank Nasim Zamir for technical assistance with the maintenance of the cortical cultures. This work has been supported by grants from NSERC Canada to I.R.B who also holds a Canada Research Chair (Tier I).

References

- Abbott A (2002) Neurologists strike gold in drug screen effort. *Nature* 417:109
- Asea AA, Brown IR (eds) (2008) Heat shock proteins and the brain: implications for neurodegenerative diseases and neuroprotection. Springer Science, New York
- Barber SC, Shaw PJ (2010) Oxidative stress in ALS: key role in motor neuron injury and therapeutic target. *Free Radic Biol Med* 48:629–641
- Brocchieri L, Conway de Macario E, Macario AJ (2008) Hsp70 genes in the human genome: conservation and differentiation patterns predict a wide array of overlapping and specialized functions. *BMC Evol Biol* 8:19
- Brown IR (2007) Heat shock proteins and protection of the nervous system. *Ann N Y Acad Sci* 1113:147–158
- Brown IR (2008) Heat shock proteins at the synapse: implications for functional protection of the nervous system. In: Asea AAA, Brown IR (eds) Heat shock proteins and the brain: implications for neurodegenerative diseases and neuroprotection. Springer Science, New York, pp 239–254
- Brown IR, Rush SJ (1999) Cellular localization of the heat shock transcription factors HSF1 and HSF2 in the rat brain during postnatal development and following hyperthermia. *Brain Res* 821:333–340
- Brujin LI, Miller TM, Cleveland DW (2004) Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annu Rev Neurosci* 27:723–749
- Calabrese V, Cornelius C, Mancuso C, Lentile R, Stella AM, Butterfield DA (2010) Redox homeostasis and cellular stress response in aging and neurodegeneration. *Meth Mol Biol* 610:285–308
- Chen S, Brown IR (2007) Neuronal expression of constitutive heat shock proteins: implications for neurodegenerative diseases. *Cell Stress Chaperon* 12:51–58
- Chow AM, Brown IR (2007) Induction of heat shock proteins in differentiated human and rodent neurons by celastrol. *Cell Stress Chaperon* 12:237–244
- Chow AM, Mok P, Xiao D, Khalouei S, Brown IR (2010) Heteromeric complexes of heat shock protein 70 (HSP70) family members, including Hsp70B', in differentiated human neuronal cells. *Cell Stress Chaperon* 15:545–553
- Choy MS, Chen MJ, Manikandan J, Peng ZF, Jenner AM, Melendez AJ, Cheung NS (2011) Up-regulation of endoplasmic reticulum stress-related genes during the early phase of treatment of cultured cortical neurons by the proteasomal inhibitor lactacystin. *J Cell Physiol* 226:494–510
- Daugaard M, Rohde M, Jaattela M (2007) The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. *FEBS Lett* 581:3702–3710
- Duyckaerts C, Delatour B, Potier MC (2009) Classification and basic pathology of Alzheimer disease. *Acta Neuropathol* 118:5–36
- Forman MS, Trojanowski JQ, Lee VM (2004) Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. *Nat Med* 10:1055–1063
- Gozzelino R, Jeney V, Soares MP (2010) Mechanisms of cell protection by heme oxygenase-1. *Annu Rev Pharmacol Toxicol* 50:323–354
- Grochot-Przeczek A, Dulak J, Jozkowicz A (2012) Haem oxygenase-1: non-canonical roles in physiology and pathology. *Clin Sci (Lond)* 122:93–103

- Guzman-Beltran S, Espada S, Orozco-Ibarra M, Pedraza-Chaverri J, Cuadrado A (2008) Nrdihydroguaiaretic acid activates the anti-oxidant pathway Nrf2/HO-1 and protects cerebellar granule neurons against oxidative stress. *Neurosci Lett* 447:167–171
- Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* 8:101–112
- Heemskerk J, Tobin AJ, Bain LJ (2002) Teaching old drugs new tricks. Meeting of the neurodegeneration drug screening consortium, 7–8 April 2002, Washington, DC, USA. *Trends Neurosci* 25:494–496
- Hong Z, Zhang QY, Liu J, Wang ZQ, Zhang Y, Xiao Q, Lu J, Zhou HY, Chen SD (2009) Phosphoproteome study reveals Hsp27 as a novel signaling molecule involved in GDNF-induced neurite outgrowth. *J Proteome Res* 8:2768–2787
- Kabani M, Martineau CN (2008) Multiple hsp70 isoforms in the eukaryotic cytosol: mere redundancy or functional specificity? *Curr Genom* 9:338–348
- Kim HJ, Joo HJ, Kim YH, Ahn S, Chang J, Hwang KB, Lee DH, Lee KJ (2011) Systemic analysis of heat shock response induced by heat shock and a proteasome inhibitor MG132. *PLoS One* 6: e20252
- King M, Nafar F, Clarke J, Mearow K (2009) The small heat shock protein Hsp27 protects cortical neurons against the toxic effects of beta-amyloid peptide. *J Neurosci Res* 87:3161–3175
- Lesuisse C, Martin LJ (2002) Long-term culture of mouse cortical neurons as a model for neuronal development, aging, and death. *J Neurobiol* 51:9–23
- Li Q, Li J, Zhang L, Wang B, Xiong L (2007) Preconditioning with hyperbaric oxygen induces tolerance against oxidative injury via increased expression of heme oxygenase-1 in primary cultured spinal cord neurons. *Life Sci* 80:1087–1093
- Lok J, Leung W, Murphy S, Butler W, Noviski N, Lo EH (2011) Intracranial hemorrhage: mechanisms of secondary brain injury. *Acta Neurochir Suppl* 111:63–69
- Martin JB (1999) Molecular basis of the neurodegenerative disorders. *N Engl J Med* 340:1970–1980
- Muchowski PJ, Wacker JL (2005) Modulation of neurodegeneration by molecular chaperones. *Nat Rev Neurosci* 6:11–22
- Neef DW, Jaeger AM, Thiele DJ (2011) Heat shock transcription factor 1 as a therapeutic target in neurodegenerative diseases. *Nat Rev Drug Discov* 10:930–944
- Parent M, Parent A (2010) Substantia nigra and Parkinson's disease: a brief history of their long and intimate relationship. *Can J Neurol Sci* 37:313–319
- Paris D, Ganey NJ, Laporte V, Patel NS, Beaulieu-Abdelahad D, Bachmeier C, March A, Ait-Ghezala G, Mullan MJ (2010) Reduction of beta-amyloid pathology by celastrol in a transgenic mouse model of Alzheimer's disease. *J Neuroinflammation* 7:17
- Peterson GL (1977) A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Anal Biochem* 83:346–356
- Pinna GF, Fiorucci M, Reimund JM, Taquet N, Arondel Y, Muller CD (2004) Celastrol inhibits pro-inflammatory cytokine secretion in Crohn's disease biopsies. *Biochem Biophys Res Commun* 322:778–786
- Pramanik D, Dey SG (2011) Active site environment of heme-bound amyloid beta peptide associated with Alzheimer's disease. *J Am Chem Soc* 133:81–87
- Read DE, Gorman AM (2009) Heat shock protein 27 in neuronal survival and neurite outgrowth. *Biochem Biophys Res Commun* 382:6–8
- Satoh T, Baba M, Nakatsuka D, Ishikawa Y, Aburatani H, Furuta K, Ishikawa T, Hatanaka H, Suzuki M, Watanabe Y (2003) Role of heme oxygenase-1 protein in the neuroprotective effects of cyclopentenone prostaglandin derivatives under oxidative stress. *Eur J Neurosci* 17:2249–2255
- Scapagnini G, Vasto S, Abraham NG, Caruso C, Zella D, Fabio G (2011) Modulation of Nrf2/ARE pathway by food polyphenols: a nutritional neuroprotective strategy for cognitive and neurodegenerative disorders. *Mol Neurobiol* 44:192–201
- Schapiro AH, Olanow CW (2004) Neuroprotection in Parkinson disease: mysteries, myths, and misconceptions. *JAMA* 291:358–364
- Selkoe DJ (2003) Folding proteins in fatal ways. *Nature* 426:900–904
- Selkoe DJ (2004) Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases. *Nat Cell Biol* 6:1054–1061
- Stranahan AM, Mattson MP (2010) Selective vulnerability of neurons in layer II of the entorhinal cortex during aging and Alzheimer's disease. *Neural Plast* 2010:108190
- Tao X, Younger J, Fan FZ, Wang B, Lipsky PE (2002) Benefit of an extract of *Tripterygium wilfordii* Hook F in patients with rheumatoid arthritis: a double-blind, placebo-controlled study. *Arthritis Rheum* 46:1735–1743
- Tenhunen R, Marver HS, Schmid R (1969) The enzymatic conversion of hemoglobin to bilirubin. *Trans Assoc Am Phys* 82:363–371
- Toth ME, Gonda S, Vigh L, Santha M (2010) Neuroprotective effect of small heat shock protein, Hsp27, after acute and chronic alcohol administration. *Cell Stress Chaperon* 15:807–817
- Westerheide SD, Bosman JD, Mbadugha BN, Kawahara TL, Matsumoto G, Kim S, Gu W, Devlin JP, Silverman RB, Morimoto RI (2004) Celastrols as inducers of the heat shock response and cytoprotection. *J Biol Chem* 279:56053–56060
- Williams KL, Mearow KM (2011) Phosphorylation status of heat shock protein 27 influences neurite growth in adult dorsal root ganglion sensory neurons in vitro. *J Neurosci Res* 89:1160–1172
- Yang H, Chen D, Cui QC, Yuan X, Dou QP (2006) Celastrol, a triterpene extracted from the Chinese “Thunder of God Vine,” is a potent proteasome inhibitor and suppresses human prostate cancer growth in nude mice. *Cancer Res* 66:4758–4765
- Zhang W, Benmohamed R, Arvanites AC, Morimoto RI, Ferrante RJ, Kirsch DR, Silverman RB (2012) Cyclohexane 1,3-diones and their inhibition of mutant SOD1-dependent protein aggregation and toxicity in PC12 cells. *Bioorg Med Chem* 20:1029–1045
- Zhou BN (1991) Some progress on the chemistry of natural bioactive terpenoids from Chinese medicinal plants. *Mem Inst Oswaldo Cruz* 86(Suppl 2):219–226