CSSI workshop in Brazil highlights “Stress Responses in the Nervous System” in relation to neurodegenerative diseases and neuroprotection

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Abstract A review is provided of the two neuroscience sessions entitled “Stress Responses in the Nervous System” that were presented at the ninth Cell Stress Society International Workshop on the “Molecular Biology of the Stress Response” held in Port Alegre, Brazil, May 27–30, 2012. The sessions were organized and chaired by Ian R. Brown (Toronto, Canada) and Maria Estela Andrés (Santiago, Chile).

Keywords Neurodegenerative diseases · Heat shock proteins · Neuroprotection

Meeting review of neuroscience sessions

The Cell Stress Society International (CSSI) recently held an International Workshop on the “Molecular Biology of the Stress Response” in Porto Alegre, Brazil on the campus of the Pontifical Catholic University of Rio Grande do Sul (PUCRS) from May 27 to 30, 2012 with Professor Cristina Bonorino (PUCRS) as workshop chair. The meeting targeted researchers from Latin America and resulted in the formation of a Latin American chapter of the CSSI which will hold its first conference in Montevideo, Uruguay in 2 years. Maria Bausero (Montevideo) was elected chapter chairman and organizer. At the workshop in Porto Alegre, Brazil, Ian Brown (Toronto, Canada) and Mariá Estela Andrés (Santiago, Chile) organized two sessions that focused on current advances in “Stress Responses in the Nervous System” in relation to neurodegenerative diseases and neuroprotection.

Soledad Matus from Claudio Hetz’s group in Santiago, Chile discussed protein folding stress which is a salient feature of the most common neurodegenerative diseases, including Parkinson’s and amyotrophic lateral sclerosis (ALS). Although the accumulation of abnormally folded proteins is a well-characterized event underlying the pathology, the mechanism by which cells respond to this phenomenon is not well understood. Indications of endoplasmic reticulum stress and activation of the unfolded protein response (UPR) are common markers of neurodegeneration in many diseases (Matus et al. 2011).

The research group in Santiago, Chile has studied the contribution of X-box binding protein 1 (XBP1) in diverse diseases, a key UPR transcription factor that regulates genes involved in protein folding and quality control (Hetz 2012). Unexpectedly, the deletion of XBP1 in the nervous system in a mouse model of ALS protected against the disease by increasing autophagy and decreasing mutant SOD1 aggregation (Hetz et al. 2009). Similar results were obtained in Huntington’s disease models (Vidal et al. 2012). Overexpression of XBP1 using gene therapy in models of Parkinson’s disease (Sado et al. 2009), spinal cord injury (Valenzuela et al. 2012), and Huntington’s disease (Zuleta et al. 2012) protected against disease features. Thus, gain and loss of function of XBP1 may protect against degeneration through distinct mechanisms: (1) compensatory changes leading to upregulation of autophagy and (2) an enhanced of UPR transcriptional responses.

Data emerging from the literature point to secreted forms of heat shock proteins; however, the mechanisms responsible for their secretion and their roles in the extracellular space are under current debate (De Maio 2011). Vilma Martins (Sao Paulo, Brazil) described how stress-inducible protein 1 (STI1) secreted by glial cells interacts with a prion protein in neurons to trigger neuroprotection. STI1 is abundantly secreted in microvesicles by primary culture astrocytes, a type of glial cell; however, its secretion by neurons is not detected.

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Interestingly, STII secreted by astrocytes is a specific, high-affinity ligand for GPI-anchored prion protein (PrP^C) in neurons, and this complex modulates the activity of neuronal nicotinic acetylcholine receptors, inducing specific cellular signaling through calcium influx that results in neuroprotection. Thus, STII secretion by glial cells is proposed as a new neurotrophin that employs PrP^C as a receptor (Martins et al. 2010).

The inducible form of heat shock protein Hsp70 is rapidly synthesized in cells subjected to stressful stimuli. However, since the early 1990s, it has been known that Hsp70 is not induced by heat shock in cells exhibiting a neuronal phenotype (Brown 1994; Marcuccilli et al. 1996). Heat shock factor 1 (HSF1) is the master transcription factor inducing the expression of heat shock proteins (Hsps) during the stress response; thus, several hypotheses involving HSF1 have been raised explaining the lack of induction of Hsp70 in neuronal cells. Absence of HSF1 in neurons (Marcuccilli et al. 1996), impaired post-translational modification of HSF1 during stress (Batulan et al. 2003), and incapacity of HSF1 binding to heat shock response elements in DNA (Yang et al. 2008) have been proposed as possible explanations for the lack of Hsp70 induction in neuronal cells.

Mariá Estela Andrés (Santiago, Chile) reported that rat cortical neurons grown in culture show a significantly reduced induction of Hsp70 mRNA and protein levels following heat shock. HSF1 is transiently transported to the nuclei of cortical neurons after heat shock and HSF1 shows a lower migration in gels indicating that it is post-translationally modified. However, chromatin immunoprecipitation assays indicate that HSF1 does not bind to the *hsp70* gene promoter following heat shock in the cortical neurons.

Why is HSF1 impeded from binding to DNA in cortical neurons during heat shock stress? One explanation is that the chromatin environment on *hsp70* gene promoter presents features of closed chromatin. Indeed, treatment of cortical neurons with trichostatin A, an inhibitor of histone deacetylases, induces the maximum capacity of *hsp70* gene transcription. Mariá Estela Andrés reported that histone post-translational modification analysis of *hsp70* gene promoter appears to exclude an occluding effect of chromatin that might impair HSF1 binding. Correlation of histone post-translational modifications with *hsp70* gene expression revealed that a significant increment of histone acetylation is not enough to induce *hsp70* gene transcription in neurons. Hsp70 overexpression reduces neuronal impairment in several models of neurodegenerative diseases (Brown 2007; Asea and Brown 2008), thus elucidating that the mechanisms repressing *hsp70* expression in neurons are critical.

The occurrence of aggregation-prone, misfolded proteins in neuronal cells in the brain is a feature of neurodegenerative disorders such as Alzheimer's disease, Parkinson's

disease, and ALS. Hsps are protein repair agents that provide a line of defense against misfolded, aggregation-prone proteins. Ian Brown (Toronto, Canada) reported that targeting of misfolded proteins in affected neurons by Hsps could be employed as a potential therapeutic strategy against neurodegenerative diseases (Asea and Brown 2008). His presentation focused on two stress-inducible members of the *HSP70* gene family and their localization in differentiated human neuronal cells. Stable cell lines of human SH-SY5Y neuronal cells were established that express YFP-tagged Hsp70B' and Hsp70, protein products of the human *HSPA6* and *HSPA1A* genes. Interestingly, the Hsp70B' (*HSPA6*) gene is found in the human genome but not in the genomes of rat and mouse. Hence current animal models of neurodegenerative diseases lack this potentially neuroprotective protein (Chow and Brown 2007; Chow et al. 2010).

Following thermal stress, the translocation of YFP-tagged Hsp70B' and Hsp70 was monitored to cytoplasmic and nuclear components of differentiated human neuronal cells. Ian Brown reported that both YFP-tagged proteins moved rapidly to centrioles in the cytoplasm with YFP-tagged Hsp70B' showing a more prolonged association. Centrioles play key roles in the organization of the cytoskeleton and cellular polarity that is required for the outgrowth of neuronal cellular processes that leads to synapse formation between neurons. Recently, defects in centrioles have been implicated in neurodegenerative diseases. Synaptic dysfunction is an early feature of these disorders of the nervous system (Brown 2008). The following sequence of nuclear events was observed in differentiated human neurons after thermal stress: (1) rapid movement of both YFP-tagged Hsp70B' and Hsp70 to nuclear speckles that are enriched in RNA splicing factors, (2) appearance of both Hsps at nucleolar sites engaged in ribosomal RNA processing and ribosomal subunit assembly, and (3) subsequent differential translocation of Hsp70B' to nuclear sites of active gene transcription at time points when transcription was recovering from stress-induced inhibition.

Ian Brown also reported on recent advances in his laboratory on the effects of thermal stress on the organization of chromosomes in the nuclei of differentiated human neurons. He stated that particular chromosomes occupy specific "domains" in the nucleus and that specific chromosomes are positioned next to each other by their centromeric regions fusing to form chromocenters. This "organizes" the nucleus and facilitates active genes on different chromosomes coming together to share common "transcription platforms". He reported that thermal stress changes the number and the position of chromocenters in the neuronal nucleus and that chromocenter dissociation could act as a mechanism to rapidly stall ongoing transcription in response to stressful stimuli.

Hugo Peluffo (Montevideo, Uruguay) described how acute brain injuries as a consequence of stroke, trauma, axotomy, or repeated seizures are major socioeconomic problems due to high mortality rates but also because the resultant lesions persist for life. They constitute an important alteration of central nervous system (CNS) homeostasis, which in turn reacts with a strong cell stress response. In the case of focal lesions, the initial reaction of the injured zone induces a propagating inflammatory response that increases the initial lesion volume if not limited. Classical anti-inflammatory drugs have failed in the limitation of this secondary pro-inflammatory damage, but cell-based strategies aimed to modulate this process show interesting potential (Peluffo et al. 2006, 2007). One of these strategies is gene therapy (Domingo-Espin et al. 2011; Peluffo 2011), which has recently shown important success in long-term clinical settings for CNS pathologies such as Parkinson's disease (Broadstock and Yanez-Munoz 2012).

Hugo Peluffo reported that application of gene therapy involving overexpression of immune/inflammatory modulating molecules has emerged as a promising strategy. In particular, new families of immune receptors capable of determining cell phenotype under stress conditions have gained attention. One family of these immune receptors termed CD300 shows both activating and inhibitory members, and its functions in the nervous system are largely unknown. However, two studies have shown that one of its members CD300f is neuroprotective in two different models of CNS injury: autoimmune experimental encephalomyelitis (EAE) and NMDA-induced excitotoxicity. In the EAE model of multiple sclerosis, the CD300f knock-out mice showed increased pathological features which correlated with increased pro-inflammatory mediators like iNOS and IL1 β (Xi et al. 2010). That study also showed that CD300f was not expressed in the normal non-injured CNS but is expressed in microglia/macrophages after the insult in demyelinating areas.

In the acute brain injury excitotoxic model, Hugo Peluffo reported that gene therapy mediated overexpression of the rat, or the human isoforms of CD300f induced an important reduction in the lesion volume (Peluffo et al. 2012). Interestingly, the study confirmed that CD300f was not expressed in the normal non-injured CNS, but it also showed that this receptor could be expressed by microglia, oligodendrocytes, and in neurons in culture. Moreover, although the endogenous ligand of this receptor is not known, the utilization of a fusion protein consisting of the extracellular domain of the receptor fused with an Fc region of an antibody evidenced the presence of the unknown ligand in the normal CNS. It was mainly present in the white matter, associated with oligodendrocytes, and after the acute excitotoxic injury, it was upregulated in the entire lesioned area.

Taken together, the data from Hugo Peluffo's research group suggest that both the CD300f receptor and its ligand

are in place after a CNS injury, and that this interaction modulates diverse components of the cellular stress response that determines the extent of the lesion.

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