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Insights on supporting the aging brain microvascular endothelium

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Abstract

Blood brain barrier hyperpermeability has been associated with age-related affective disorders, including depression, mania, anxiety, Alzheimer's and Parkinson's disease. Our recent efforts suggest that a promising therapeutic approach may arise due to the activation of the unfolded protein response (UPR) element in the affected tissues. Growth hormone releasing hormone antagonists and heat shock protein 90 inhibitors have been shown to induce UPR. This mechanism (UPR) has been associated with tissue repairing processes.

Keywords

P53; Reactive oxygen species; Unfolded protein response; Growth hormone releasing hormone; Blood brain barrier

The microvascular endothelial cells of the neurovascular unit comprise the blood–brain barrier (BBB). This structure forms the crucial interface separating the brain from blood circulation, it exhibits high transendothelial resistance; and prevents neurotoxic components, blood cells and pathogens from entering the brain [1]. Increased BBB permeability has been associated with aging and a variety of psychiatric pathologies, including schizophrenia, depression, autism spectrum and mood disorders [2]. Thus, therapeutic strategies towards the restoration of BBB integrity will most probably benefit those affected by severe neurological and neurodegenerative abnormalities related to aging. Those conditions include Huntington's, Alzheimer's and Parkinson's disease, amyotrophic lateral sclerosis, traumatic brain injury and epilepsy [3], as well as COVID-19 [4,5].

Our lab is interested on the elucidation of the molecular pathways involved in the maintenance and integrity of the brain microvascular endothelial cells, as well as on delivering novel approaches to recover the normal physiologic function of that structure, after its breakdown. To that end, we have reported the beneficial role of heat shock protein 90 (hsp90) inhibitors and growth hormone releasing hormone (GHRH) antagonists against

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Conflicts of Interests

None declared.

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the hydrogen peroxide-induced disruption of the commercial available brain microvascular endothelium cells hCMEC/D3. Those immortalized cells were derived from human temporal lobe microvessels, and were enriched in cerebral endothelial cells [6].

GHRH antagonists are anti-cancer peptides which oppose the growth factor activities of GHRH in human cancers, have been associated with anti-inflammatory activities, and cross the BBB. Although GHRH is a hypothalamic neuropeptide which regulates the secretion of the Growth Hormone from the anterior pituitary gland, a solid body of evidence revealed that affects several extra-hypothalamic tissues via its binding to the widely expressed splice variants of the pituitary-type GHRH receptor (pGHRH-R) [7]. We investigated the effects of GHRH antagonist MIA-602, GHRH and GHRH agonist MR-409 in hCMEC/D3. Those cells express the splice variant 1 of the pGHRH-R.

Our observations revealed that MIA-602 deactivated the actin-severing activity of cofilin, suppressed the inflammatory RhoA, and induced the endothelial barrier enhancer P53. Cofilin disassembles the actin cytoskeleton, and RhoA induces the formation of the filamentous actin. Thus, both cofilin and RhoA favor hyper-permeability responses. GHRH and MR-409 violated the BBB function, and decreased the transendothelial resistance (TEER) in hCMEC/D3 cells. Furthermore, the GHRH antagonist MIA-602 opposed the hydrogen peroxide (H_2O_2)–triggered P53 and pCofilin suppression. Moreover, it enhanced the transendothelial resistance (TEER) values (decreased permeability) of cells exposed to this reactive oxygen species (ROS) inducer (H_2O_2) [7].

Hsp90 is a molecular chaperone, which assists towards the maturation of a plethora of intracellular proteins, essential components for cellular survival and defense. Hsp90 is employed by cancer cells to accelerate their growth and potentiate their metastatic potential. Hsp90 inhibitors are anti-cancer compounds, subjected to phase III clinical trials. Moreover, their affinity to Hsp90 is exponentially higher towards the activated (inflamed) Hsp90, as compared to its non-activated form. Our latest findings indicate that treatment of the hCMEC/D3 cells with Hsp90 inhibitors reduce the generation of the hydrogen peroxide – induced reactive oxygen species and support the integrity of the brain endothelium [8].

Both GHRH antagonists and Hsp90 inhibitors share similar properties, including their capacity to induce P53, which directly affects the redox status of the hCMEC/D3 cells. To be more specific, the pharmacologic suppression of P53 by Pifithrin, or small interfering (si) RNA for P53, or lipopolysaccharides (LPS) resulted in the dose-dependent increase of the malondialdehyde (MDA) expression, which is a marker of lipid peroxidation, and a predictive biomarker for post-stroke depression [9].

LPS is a prototypical endotoxin, a component of the outer membrane of Gram-negative bacteria, and exerts its effects via the toll-like receptor 4. P53 augmentation due to Nutlin or AUY-922 (Hsp90 inhibitor) resulted in the reduction of the MDA levels. The integrity of the brain endothelium was affected by P53 expression, since AUY-922 increased the transendothelial resistance of hCMEC/D3 cells, while Pifithrin weakened their barrier function [10]. AUY-922 represents the most advanced stage in the development of that class of Hsp90 inhibitors [11].

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The unfolded protein response (UPR) is a molecular machinery involved in processes of tissue repair and affect P53 in a positive manner. It is consisted of the protein kinase RNA-like ER kinase, the activating transcription factor 6, and the inositol-requiring enzyme-1α. Cells treated with the UPR inductors brefeldin A, dithiothreitol, and thapsigargin elevated their P53 expression levels, while UPR suppression by N-acetyl cysteine, kifunensine, and ATP-competitive IRE1α kinase-inhibiting RNase attenuator exerted the opposing results [12]. UPR was also shown to mediate the protective effects of Hsp90 inhibitors [13] and GHRH antagonists in lung cells [14]. Thus, it is highly probable that the targeted UPR induction might serve as a promising therapeutic strategy for disorders related to the aging brain endothelium. Fig. 1 delivers a graphic interpretation of the mechanisms involved in the regulation of brain microvascular endothelium integrity.

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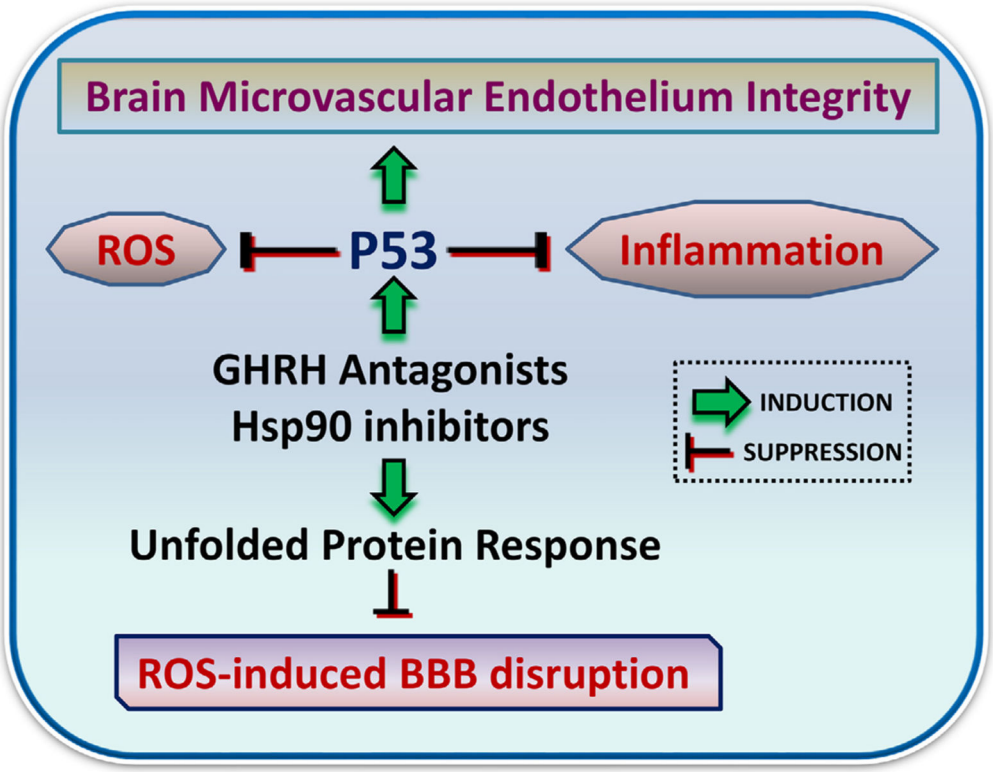

Fig. 1.

UPR in brain microvascular endothelium integrity. Mild UPR activation due to GHRH antagonists [14] and Hsp90 inhibitors [15,16] augment P53, which in turn suppresses the ROS generation and induces anti-inflammatory responses [17]. Those events enhance BBB function. UPR induction has also been associated with the suppression of the hydrogen peroxide – triggered brain microvascular endothelium disruption [8,14]. The exact molecular mechanisms involved in those events are to be elucidated.