Ubiquilin-1 Augments Recovery from Cerebral Ischemia

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Abstract
Excess production and actions of reactive oxygen species (ROS) is a major trigger for the progression of molecular abnormalities that accompany many diseases. For example, cerebral ischemia mediated surge of ROS induce misfolding and microaggregation of proteins resulting in protein dysfunction and toxicity in the affected areas. Timely removal of the ROS inducers and dysfunctional proteins may be an ideal strategy to prevent the complications caused by the ROS. A recent article published by Liu and colleagues demonstrated that ubiquilin-1 (Ubqln), an ubiquitin-like protein, prevents ROS mediated complications in cerebral ischemia. Ubqln mediated timely degradation of dysfunctional proteins is sought to mediate this protective effect.

Keywords: ubiquilin, cerebral ischemia, proteasome, ROS

Cerebral ischemia is one of the major causes of stroke- a fatal condition that proffers high rate of morbidity and disability. The high production of reactive oxygen species (ROS) in cerebral ischemia often worsens the underlying complications, for example, toxic byproducts generated from the interaction of ROS with other reactive species facilitate aberrant gene expression, protein misfolding and microaggregation resulting in neuronal cell death and motor impairment [Broughton et al., 2009; Luo et al., 2013]. Thrombolytic therapy within hours of ischemic insult is highly promising in reversing the complications associated with cerebral ischemia, however, as the time prolongs to initiate the therapy, the survival rate of neurons may decline radically. Thrombolytic therapy combined with removal of the undesirable proteins- for example, ROS inducers and misfolded proteins- from the ischemic milieu may provide better opportunities in enhancing the survival rate of neurons. A recent work by Liu and colleagues [2014] demonstrated that Ubiquilin-1, generally referred as Ubqln, protects neurons from ROS mediated complications in cerebral ischemia. Ubqln mediated degradation of dysfunctional proteins has been shown to be the mechanism by which ubqln renders the protective effect.

Ubqln comes under the family of ubiquitin (Ub) like proteins, consists of an Ub domain, an Ub associated domain (UBA) and a central region that contains several asparagine-proline repeats [Mah et al., 2000]. The N-terminal Ub domain binds with proteasome subunits whereas the C-terminal UBA domain binds with poly-Ub substrates, thus Ubqln serves as an adaptor between ubiquitinated substrates and the proteasome and facilitates Ub-Proteasome System (UPS) mediated degradation of proteins [Ko et al., 2004]. Additionally, Ubqln interacts with non-ubiquitinated substrates as well [Mah et al., 2000; Ko et al., 2002].

A previous work by Ko et al [2002] demonstrated a protective role for Ubqln

in neuroblastoma cells *in vitro*. Liu and colleagues [2014] confirmed and validated these findings *in vivo* using transgenic animals that over express (Tg) or lack (cKO) Ubqln. Both Tg and cKO mice were completely healthy and devoid of any developmental or functional defects indicating that the major alterations in Ubqln levels are not detrimental. Liu and colleagues [2014] used menadione (150mg/kg) injection intraperitoneally (IP) to induce oxidative stress in the mice. Further, transcardial injection of oxidative stress reagent CellRox was used to measure ROS production in the liver. Post 6h menadione administration the liver cells from the Tg mice showed lesser ROS production, reduced vacuolization, cell disruption and karyolysis compared to the WT. Even after 2 months post menadione administration the Tg mice has comparatively higher ATP levels- a marker for metabolic activity- and reduced apoptotic changes - evidenced by decreased caspase activity and TUNEL positivity - indicating a protective role for Ubqln in the liver.

Liu and colleagues [2014] then asked whether Ubqln protects brain cells from oxidative stress induced damage. In this experiment the menadione administration was done for continuous 3 days in order to achieve pathological levels of ROS in the mice brain. As observed in the liver, the brain cells from the Tg mice generated lesser ROS levels. The neuron degeneration profile- as evidenced by FJB staining- and apoptotic changes were also remarkably lower in these mice. These results recapitulate the finding that Ubqln, in general, protects cells from oxidative stress induced tissue damage.

Liu and colleagues [2014] then induced cerebral ischemia in the animals, using median cerebral artery occlusion (MCAO) method, in order to assess the protective role of Ubqln in brain cells undergoing ischemic insult. A standard 1h occlusion and 24h reperfusion of the median cerebral artery generated only milder infarcts in the Tg mice. Moreover, the body weight and motor functions were recovered faster in these mice supporting a protective role for Ubqln. Additionally, MCAO in mice that were devoid of Ubqln in neurons (cKO); generated by cross breeding floxed-ubiquilin-1 mice with synapsin-1 promoter driven Cre mice- resulted in larger infracts and delayed functional recovery indicating that the lack of ubiquilin pace up the detrimental effects posed by cerebral ischemia.

In addition to facilitating protein degradation Ubqln has been shown to have protein stabilizing functions [Ko et al., 2004; Mah et al., 2000; Beverly et al., 2012]. Therefore, Liu and colleagues [2014] asked whether the lack of neuronal protection observed in the cKO mice-that are null for Ubqln - was due to excessive accumulation or destabilization of proteins. Noticeably, ubiquitinated proteins accumulated in the cKO mice indicating a critical role for Ubqln in mediating UPS dependent protein degradation. Further, Liu and colleagues [2014] generated another transgenic mouse in which a modified form of GFP (GFPu) was fused with a degradation signal under Ubqln null background. In these animals the levels of GFPu inversely correlates with the UPS function. As expected these animals accumulated GFPu substantiating a functional role for Ubqln in promoting UPS dependent protein degradation. Altogether Liu and colleagues [2014] concluded that Ubqln protects brain tissue from cerebral ischemia by targeting the accumulated proteins-

possibly the misfolded proteins generated from oxidative stress - for degradation.

Even though the study by Liu and colleagues [2014] suggest that the Ubqln targets abnormal or aberrantly expressed proteins, its substrate specificity is a critical question to be addressed. Ko et al [2004] showed that Ubqln preferentially recognizes poly-Ub chains but not mono-ub. However, the requirement of Ubqln in promoting mono-ubiquitination of proteins has also been demonstrated, for example, Ubqln enhances mono-ubiquitination and trafficking of Bclb protein [Beverly et al., 2012]. Proteins tagged with mono-ub or poly-ub chains have multiple fates and thus the critical role of Ubqln in facilitating the mono-ubiquitination of proteins is worth analyzing for its regulatory roles on protein functions. In what capacity Ubqln renders protein stabilizing functions, does the adaptor function of Ubqln a specific requirement for all the proteins that undergo UPS dependent degradation, are the some other important questions to be addressed.

Reperfusion following a cerebral ischemic insult often leads to upregulation of many survival proteins. Untimely removal of those protective proteins may delay the survival of cells in the affected areas. Liu and colleagues [2014] and others [Ko et al., 2002] have suggested that the surplus actions of Ubqln in oxidative stress is not detrimental but protective. In this context, Ubqln might also targets the free radical inducing proteins, however, the substrate specificity of Ubqln on free radical inducers needs to be validated. A direct interaction of Ubqln and 19S proteasome has been reported previously [Ko et al., 2004]. 19S proteasome has been reported to promote recovery of the native structure of proteins [Braun et al., 1999]. Thus a regulatory role for Ubqln in recovering the native structure of proteins- which may prevent microaggregation of proteins- cannot be ruled out. The critical role of Ubqln in regulating the autophagy process is also worth analyzing in the animal models that are generated by Liu and colleagues [2014].

Liu and colleagues [2014] showed, in their work, the critical role of ubqln in protecting brain cells. The facilitatory role of Ubqln in UPS dependent protein degradation is proposed to be the mechanism for the protective effect. Revealing the exact identity of its substrates would expose the real promise in supplementing Ubqln for the management of cerebral ischemia. Systematic docking analysis of Ubqln with potential targets, followed by validation of the targets using binding assays would be a promising start in this direction.

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Conflict of Interest

The author declares no conflict of interest.

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