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Role of hypoxia-mediated cellular prion protein functional change in stem cells and potential application in angiogenesis (Review)

SEUNG PIL YUN[1], YONG-SEOK HAN[2], JUN HEE LEE[3], YEO MIN YOON[2], CHUL WON YUN[2], PETER RHEE[1] and SANG HUN LEE[2]

[1]Department of Neurology, Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA; [2]Department of Biochemistry, Medical Science Research Institute, Soonchunhyang University College of Medicine, Cheonan, South Chungcheong 31151, Republic of Korea; [3]Department of Pharmacology and Toxicology, University of Alabama at Birmingham School of Medicine, Birmingham, AL 35294, USA

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Abstract. Cellular prion protein (PrP^C) can replace other pivotal molecules due to its interaction with several partners in performing a variety of important biological functions that may differ between embryonic and mature stem cells. Recent studies have revealed major advances in elucidating the putative role of PrP^C in the regulation of stem cells and its application in stem cell therapy. What is special about PrP^C is that its expression may be regulated by hypoxia-inducible factor (HIF)-1α, which is the transcriptional factor of cellular response to hypoxia. Hypoxic conditions have been known to drive cellular responses that can enhance cell survival, differentiation and angiogenesis through adaptive processes. Our group recently reported hypoxia-enhanced vascular repair of endothelial colony-forming cells on ischemic injury. Hypoxia-induced AKT/signal transducer and activator of transcription 3 phosphorylation eventually increases neovasculogenesis. In stem cell biology, hypoxia promotes the expression of growth factors. According to other studies, aspects of tissue regeneration and cell function are influenced by hypoxia, which serves an essential role in stem cell HIF-1α signaling. All these data suggest the possibility that hypoxia-mediated PrP^C serves an important role in angiogenesis. Therefore, the present review summarizes the characteristics of PrP^C, which is produced by HIF-1α in hypoxia, as it relates to angiogenesis.

Correspondence to: Professor Sang Hun Lee, Department of Biochemistry, Medical Science Research Institute, Soonchunhyang University College of Medicine, 44 Suncheonhyang 4-gil, Cheonan, South Chungcheong 31151, Republic of Korea
E-mail: jhlee0407@sch.ac.kr

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Contents

1. Introduction

Prions are neuro-degenerative disease-causing agents, that are responsible for changing cellular prion protein (PrP^C) to the infectious β-structure-rich insoluble conformer (PrP^{SC}) in the neurons of the brain and spinal cord, as in Creutzfeldt-Jakob disease in humans and Bovine Spongiform Encephalopathy in animals (1). PrP^C is known for its involvement in regenerative processes including adhesion, proliferation, differentiation and angiogenesis. According to Stella *et al* (2), muscles with low PrP^C grow slowly compared with wild-type muscles, suggesting that PrP^C serves a role in tissue recovery and/or regeneration. For these reasons, recent research has focused on obtaining more conclusive information about the functional role of PrP^C in tissue regeneration. Additionally, regulating PrP^C expression by hypoxia has become an important topic (3). Hypoxia occurs when blood oxygen concentrations are insufficient and long periods of hypoxia can induce cell death. However, temporary or short periods of exposure to hypoxic conditions actually enhances cell survival by increasing hypoxia-inducible factor-1 (HIF-1), composed of α- and β-subunits, in addition to other transcription factors (4-6). During hypoxia, an alteration in HIF-1 expression is essential for metabolic adaptation (7,8), as HIF-1α is associated with angiogenesis and growth factors, glucose uptake, and metabolism (8). Therefore, the present review focuses on the association between HIF-1α and PrP^C in stem cells. It will also examine how HIF-1α-mediated PrP^C expression can serve a role in angiogenesis.

2. The effect of hypoxia-preconditioning in cultured stem cells

According to previous studies, under hypoxic conditions, aged mesenchymal stem cells (MSCs) increase the secretion of angiogenic and anti-apoptotic related growth factors including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2, human growth factor (HGF) and insulin growth factor-1, resulting in enhanced angiogenic properties (9-12). To demonstrate the effect of growth factor secretion in MSCs under hypoxic conditions, a recent study transplanted hypoxia-conditioned stem cell media into rats with traumatic brain injury and demonstrated excellent rescue effects when compared to animals transplanted with normoxia-conditioned media (13). To observe the effect of restorative neurological function Chang *et al* (13) transplanted media from hypoxia-treated bone marrow (BM)-MSCs into rats with brain injury rat model and demonstrated that it was more efficient compared with normoxia conditioned medium. Chang *et al* (13) also demonstrated that the neuroprotective effect of hypoxia-conditioned media involved the generation of VEGF and HGF, which are associated with the inducement of endogenous neurogenesis. In another study, the therapeutic activity of MSCs under hypoxia or normoxia was compared in a massive hepatectomy rat model. *In vitro*, the levels of VEGF in MSCs under hypoxia were markedly higher than normoxia condition. *In vivo*, MSCs under hypoxia significantly elevated the expression of cyclin D1, proliferating cell nuclear antigen-positive hepatocytes, the liver weight/body weight ratio and survival when compared with normoxia. Notably, the therapeutic effect of hypoxia was negated by anti-VEGF antibody-induced blockade of VEGF *in vivo* (14). Increasing the activity of matrix metalloproteinase-2 also had a therapeutic effect that was associated with the protection of cardiomyocytes via the inhibition of caspase-3, transforming growth factor β1 and the upregulation of B-cell lymphoma 2 apoptosis regulator/Bcl-2 associated protein X apoptosis regulator ratio (15). According to Lee *et al* (16), the proliferation and migration of mouse embryonic stem (ES) cells increases upon activation of fibronectin-integrin β1 production through HIF-1α and phosphoinositide 3-kinase/Akt pathways under conditions of hypoxia. Additionally, mouse ES cells that have undergone hypoxic preconditioning exhibit HIF-1α-, mitogen-activated protein kinase- and nuclear factor κB-stimulated interleukin-6 production (17). Hypoxia preconditioning also facilitates the functional bioactivities of endothelial progenitor cells by mediating the regulation of the signal transducer and activator of transcription 3 (STAT3)-B-cell CLL/lymphoma 3 (BCL3) axis. Therefore, expansion and functional bioactivities of endothelial progenitor cells (EPCs) through modulation of the hypoxia-induced STAT3-BCL3 axis can be triggered by a hypoxic preconditioned *ex vivo* expansion protocol. It has been suggested that hypoxia preconditioning of EPCs may offer a therapeutic strategy for accelerated neovasculogenesis in ischemic diseases (18). In summary, the hypoxic conditioning of cultured stem cells can result in increased production and secretion of trophic factors, augmentation of angiogenic effects and enhanced anti-apoptotic activity from conditioned cells compared with normoxic conditioned culture.

3. PrP^C expression is increased under hypoxic conditions

Oxygen is an indispensable element required for biological energy (19). Thus, it is not surprising that a lack of oxygen causes cell damage (20). Oxygen concentrations within the vascular system that supplies mammals with oxygen vary: The heart and arteries have oxygen concentrations that range from 10-14% (21); however, the majority of tissues contain <5% oxygen, while bone marrow and the thymus contain <1% oxygen (22-24). At the cellular level, microenvironment changes are important for cell function. For example, EPC proliferation and cell functions have been demonstrated to be enhanced in hypoxic cultures (18). Jeong *et al* (25) revealed that hypoxia can protect neurons from PrP fragment-induced apoptosis and can increase PrP^C expression, suggesting that HIF-1α mediates PrP^C expression. PrP^C is generated in the early stages of embryogenesis (26,27) and exists in high levels in neurons of the brain and spinal cord (28). However, glial cells of the central nervous system, and a number of peripheral cell types in adults, possess lower levels of PrP^C (29,30). The majority of PrP^C molecules lie on the cell surface and are attached to the lipid bilayer through a C-terminal, glycosyl-phosphatidylinositol anchor (31).

4. HIF-1α regulates PrP^C

Under hypoxic conditions, HIF, a protein with a basic helix loop helix-Per/ARNT/Sim structure (32), regulates the expression of various target genes (33-35). HIF can be categorized into several types according to its subunits, which comprise an O_2-regulated α-subunit (i.e., HIF-1α, -2α or -3α) and a constitutively expressed β-subunit of the Aryl hydrocarbon nuclear translocator (ARNT) family (i.e., ARNT, ARNT2 or ARNT3). Under hypoxic conditions, HIF is inactivated by HIF-a degradation via von Hippel-Lindau E3 ubiquitin ligase (36); however, HIF can still function as a transcription factor by binding HIF-1α and ARNT, consequently increasing the expression of cell growth, proliferation and pro-angiogenesis factors. This is particularly the case for HIF-regulated pro-angiogenic genes including TEK receptor tyrosine kinase, monocyte chemoattractant protein-1, VEGF, basic FGF, angiopoietin (ANGPT)1, ANGPT2 and platelet-derived growth factor (37). HIF-regulated pro-angiogenic factors initiate the HIF-specific angiogenic program by increasing propagation, adhesion, tube formation, migration, vascular permeability and endothelial cell proliferation (38,39). HIF broadly targets pro-angiogenic genes and comprehensively regulates angiogenesis. Thus, HIF is often termed a 'master-regulator of angiogenesis'. As previously mentioned, PrP^C also is regulated by HIF, and PrP^C expression is increased under hypoxic conditions. Park *et al* (3) demonstrated that the effects of HIF-1α and PrP^C on neuronal cell death are prion peptide-induced. In hypoxic conditions, neurons are protected from PrP-induced cell death via the activation of p65 and HIF-1α and subsequent inactivation of p21 and p53 signals. Deferoxamine-elevated HIF-1α has similar effects to the hypoxia-mediated inhibition of neuronal cell death under normoxic conditions. Furthermore, knockdown of HIF-1α leads to the downregulation of PrP^C expression under hypoxic conditions.

5. Anti-oxidative effect of PrP^C

Numerous enzymes have copper or zinc as essential cofactors, as in the case of cytochrome *c* oxidase, tyrosinase, various metalloproteinases and Cu/Zn superoxide dismutase 1 (SOD1) (40,41). It has been demonstrated that PrP^C has an anti-oxidant effect relative to the level of copper, and that the level of this effect does not significantly vary between recombinant and tissue-purified PrP^C, although the molecular mechanism of the antioxidant properties exhibited by PrP^C remains to be elucidated (42,43). Nonetheless, it has been indicated that the decrease in oxidative stress is mediated by the interaction of copper and PrP^C (44). Therefore, it is suggested that PrP^C has a similar effect to the function of antioxidant enzymes including SOD1 (45). However, changes in expression levels of PrP^C do not induce changes in the activation levels of SOD1 (46). PrP^C knockout cells are more sensitive to copper toxicity by oxidative stress when compared with wild-type cells (47). Similarly, cerebellar cells obtained from PrP^C null mice are more vulnerable to oxidative stress than wild type cells (45,47). The deletion of octapeptide repeats within PrP^C inhibits the antioxidant properties of PrP^C (42). Indeed, PrP^C null mice are more sensitive to acute seizures (48). Therefore, it appears that the status of anti-oxidative defense in PrP^C null mice serves as an important factor in determining their lower thresholds of damage when reflecting the severity of injury and clinical pathology (49-51). Furthermore, in the skeletal muscles, heart and liver of PrP^C null mice, its absence greatly increases protein and lipid oxidation, leading to a lower catalase activity (50). Thus, being reduced in free Cu/Zn, via SOD and glutathione reductase in oxidative stress, PrP^C may have influence in the resistance against oxidative stress. Sauer *et al* (52) demonstrated that overexpression of PrP^C completely inhibits reactive oxygen species generation, even with increased activation treatment with adenosine triphosphate. This is in accordance with the hypothesis that PrP^C may have a function in protecting against oxidative stress as a free radical scavenger or a molecular sensor (52).

6. Role of PrP^C in endothelial cells under angiogenesis

Endothelial cells express and present PrP^C on their surface (53). As resting vascular endothelial cells exhibit minimal or no PrP^C *in vivo*, normal resting endothelial cells of the umbilical cord and adult blood vessels (aorta, saphenous vein and normal transplant endothelial cells) did not produce detectable quantities of PrP^C (54). PrP^C is expressed in endothelial cells of the blood capillaries in the intestinal wall of the digestive tract and in renal capillaries (55). Another study noted a sudden increase in expression of PrP^C on the surface of endothelial cells, astrocytes and neurons in penumbra regions in a rat model of cerebral ischemia (56). Endothelial cells can express PrP^C and release it through the cell membrane, as a soluble protein and as a form bound to microparticles, while vascular endothelium may be an origin for PrP^C released within the blood (53,57,58). PrP^C has been demonstrated to be a component of caveolae, which are the lipid raft of flask-shaped membrane invaginations in endothelial cells that take part in signal transduction associated with cell survival, differentiation and angiogenesis (59). Another study suggested that caveolae have functions in angiogenesis, as implied by the involvement of caveolae in VEGF signaling in the endothelium (60). This signaling mechanism confirms a key function for caveolae, and possibly PrP^C, in the regulation of angiogenesis (59). Satoh *et al* (61) identified that disruption of the PrP^C gene results in abnormal regulation of genes important for cell proliferation, differentiation and survival, including Ras and Rac signaling pathways connected to angiogenesis. During development, neonatal brain endothelial cells temporarily express PrP^C transcripts, indicating a role in central nervous system angiogenesis and blood-brain barrier maturation (62,63). PrP^C expression may be regulated by various growth factors through protein-protein interactions with normal protease sensitive PrP^C (52,64,65).

7. The function of PrP^C in tissue regeneration

Muscle regeneration and its association with PrP^C has been investigated in a cardiotoxin-induced injury animal model (2). Adult stem cells have the ability to regenerate specific tissues, recapitulating mechanisms observed during morphogenesis (17). Experiments conducted by Stella *et al* (2) indicated that cardiotoxin-degenerated skeletal muscles release tumor necrosis factor-α (TNF-α), which is affected by PrP^C, a factor that is involved in both muscle differentiation and downstream signaling pathways. Thus, *in vivo* morphogenesis of adult injured muscle tissue can be influenced by PrP^C. Their data also support the possibility that the activity of TNF-α converting enzyme (TACE), which hydrolyzes TNF-α from its precursor, is modulated by PrP^C. Prospective *in vitro* studies may investigate this hypothesis and elucidate whether the two proteins (PrP^C and TACE) interact directly or indirectly. The current review has provided a number of examples of the interaction of PrP^C with extracellular proteins or neuronal membranes, e.g., its interaction with β-secretase 1, which is glycosaminoglycan-mediated, attenuates β-secretase cleavage of the amyloid precursor protein (66). Furthermore, the binding of PrP^C to the N-Methyl-D-aspartic acid receptor 2D subunit attenuates glutamate-induced Ca^{2+} influx (67). Lastly, the results also substantiated that in muscle tissue, the Akt signaling pathway and the regulation of p38 by PrP^C have specific physiological significance. This also suggested that PrP^C serves a significant role in the regeneration process, specifically in the proliferation and differentiation of myogenic precursor cells (2).

8. Conclusion

Recent studies have clearly established hypoxia and HIF-1α as master regulators of stem cell growth factors. In hypoxia-pretreated stem cells, HIF-1α mainly controls angiogenesis and tissue regeneration factors, including HGF, VEGF and FGF. Hypoxia has also been revealed to increase the expression of the prion protein and growth factors involved in the function of cells and PrP^C has been shown to be regulated by HIF-1α. Stella *et al* (2) have suggested that PrP^C is involved in muscle differentiation and that it influences the morphogenesis of adult injured tissue *in vivo*. Additionally, regulation of the p38 and Akt signaling pathways by PrP^C has clear physiologic importance in tissue *in vivo*, in addition to the promotion of tissue regeneration. In conclusion, the present

review summarized the essential roles of PrP^C and HIF-1α in the promotion of tissue regeneration and in the function of stem cells.

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