EXPERT REVIEWS

Phosphatase Wip1 as a new therapeutic target for intestinal ischemia-reperfusion injury

Expert Rev. Clin. Immunol. 10(12), 1591–1595 (2014)

Xiaofei Shen[1‡], Junfeng Du[2‡], Yong Zhao*[3] and Wenxian Guan[1]

[1]*Department of General Surgery, Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China*
[2]*Department of General Surgery, General Hospital of Beijing Military Command, Beijing, China*
[3]*State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China*
**Author for correspondence: Tel.: +86 106 480 7302 Fax: +86 106 480 7313 zhaoy@ioz.ac.cn*

[‡]*Authors contributed equally*

Intestinal ischemia/reperfusion (I/R) injury is a pathophysiology involving local tissue injury and organ dysfunction. Accumulating evidence has confirmed that the infiltration of neutrophils is of central importance in mediating intestinal I/R injury. On the other hand, adequate neutrophils in the intestine could also benefit the antibacterial translocation and tissue repair. Consequently, regulation of neutrophil immunity after intestinal I/R might be a promising therapy for controlling intestinal injury. Wip1 is a serine/threonine protein phosphatase that acts as the master regulator of tumorigenesis. However, emerging evidence highlights the importance of Wip1 in regulating neutrophil development, maturation, migration and neutrophil pro-inflammatory cytokine productions. Our recent studies showed that Wip1 negatively regulates neutrophil inflammatory responses and plays a protective role in intestinal I/R injury. In light of this discovery, we believe that Wip1 might be a new therapeutic target for treating intestinal I/R injury.

KEYWORDS: intestinal ischemia/reperfusion injury • neutrophils • Wip1

Intestinal ischemia reperfusion (I/R) injury is a pathological condition that occurs after aortic surgery and liver and intestinal transplantation and is characterized by an initial restriction of blood supply to the intestine and the following restoration of perfusion and re-oxygenation [1]. The restoration of blood supply and re-oxygenation is frequently associated with an exacerbation of intestinal tissue injury and gut barrier dysfunction with increased bacterial translocation, local inflammatory response and infiltration of innate immune cells especially during the early phase of reperfusion [2]. To date, although numerous therapeutic approaches have been implicated in treating I/R injury, no definite treatment options are available.

Neutrophils, which are one of the earliest innate immune cells recruited to the sites of infection and inflammatory response, are crucial for the pathophysiology of I/R injury [3]. High number of neutrophils may promote uncontrolled inflammation and intestinal injury, and the depletion of redundant neutrophils ameliorated tissue injury in various I/R models including liver and heart I/R models [4–6]. It is also noteworthy that decreased number of neutrophils may not allow for adequate tissue repair [2,7,8], and possibly for sufficient immune responses against intestinal bacterial translocation [9]. Thus, the appropriate control of neutrophil immunity after intestinal I/R might have therapeutic potential.

Wild-type p53-induced phosphatase 1 (Wip1, also called PP2Cδ) is a serine/threonine protein phosphatase belonging to the type 2Cδ protein phosphatases [10]. It is activated by various stresses and involved in various cellular processes such as tumorigenesis and aging [11,12]. Previously, Liu *et al.* [13] showed that phosphatase Wip1 negatively regulates granulocyte development and maturation. Furthermore, the antibacterial and inflammatory function of neutrophils is also critically regulated by Wip1 as its deficiency in neutrophils contributed to the resistance to CXCL1-induced CXCR2 internalization and desensitization, and subsequently the enhanced migratory ability [14]. Since intestinal I/R

 10.1586/1744666X.2014.975211 ISSN 1744-666X

injury is a pathophysiological process that includes oxidative stress and neutrophil-driven pathologies, we speculate that phosphatase Wip1 might be a new therapeutic target for treating intestinal I/R injury.

Neutrophils & I/R injury

I/R injury is a common challenge during organ transplantation, vascular and general surgery. An imbalance in metabolic supply and demand results in tissue hypoxia along with microvascular dysfunction, and the following reperfusion in local tissues and/or organs further enhances the activation of innate and adaptive immune responses and leads to cell death programs [2]. While I/R injury often refers to a sterile inflammation, it is a completely different story for intestinal I/R injury. As is known to all, the intestine contains over 100 trillion commensal bacterial [15]. It is noteworthy that germ-free rodents subjected to mesenteric I/R showed higher survival rates than conventionally raised animals with less intestinal and lung histopathology [16]. Thus, the enteric bacteria also play a critical role in the pathogenesis of intestinal I/R-induced local and remote dysfunction [9]. Since neutrophils are responsible for microbiota clearance and augment recovery of transepithelial electrical resistance in ischemia-injured mucosa via the IL-1β-dependent upregulation of COX-2 [17], the infiltration of an appropriate number of neutrophils after intestinal reperfusion may be beneficial for the recovery of injured local tissues and for the protection from bacterial translocation. Thus, fine-tuning the infiltration of neutrophils in local tissues has been of central importance in I/R-induced immune responses [3,18,19]. Too many neutrophils lead to uncontrolled inflammation and subsequent tissue injury, whereas too few may not allow for adequate tissue repair and innate immune responses [2]. Although animal studies and clinical trials on the depletion of neutrophils before I/R have been linked to reduce I/R injury [4,20–22], depletion of neutrophils is not an ideal treatment for I/R injury. Therefore, therapies based on the regulation of local and circulating neutrophils still need to be elucidated.

Several factors have been studied for the regulation of local and systemic neutrophils. The complement protein C5a, which is locally produced and activated, acts as a pathological factor in amplifying sterile inflammation in numerous I/R models [19]. Mechanism studies showed that the interaction of C5a with its receptors C5aR leads to the release of cytokines and chemokines and finally promotes tissue recruitment of neutrophils [23]. Another complement protein C3a, which is the upstream anaphylatoxin of C5a, also plays a role in intestinal I/R injury and functions through different mechanisms from C5a [24]. The use of C3aR agonists successfully reduced neutrophil mobilization in circulation to ameliorate intestinal I/R pathology [24]. It is noteworthy that therapies based on the inhibition of the complement system to reduce the number of neutrophils after I/R have been examined in animal models with partial effectiveness, and results from clinical trials have largely been disappointing due to the limitation of the inhibitors used [25–27]. It is also notable that the complexity of the complement system and incomplete mechanistic insight into the functional cascade may lead to difficulties in the therapeutic targeting of complement pathways [2].

Evidence from the studies on IL-17A, one of the most important cytokines in the intestine [28], has also suggested a crucial role for neutrophils in various I/R models. IL-17A can be greatly produced by various subsets of cells including γδT cells [29], Paneth cells [30] and even neutrophils themselves after I/R [4]. The elevated level of IL-17A promotes cell apoptosis and the expression of chemokines and adhesion molecules on local resident cells to recruit granulocytes to target tissues and/or organs. The resistance to intestinal I/R injury in IL-17A-depleted mice and IL-17A knock-out mice [30] indicated that targeting IL-17A to eliminate the higher number of infiltrating neutrophils into local tissues may be a promising therapy for I/R injury. However, as mentioned above, IL-17A is crucial for effective immune responses against commensal bacteria and pathogens in the intestine [31,32]. The simple depletion of IL-17A may induce severe side effects such as infection and bacterial translocation [31,33] and limit clinical trials.

Phosphatase wip1 & neutrophils

Phosphatase Wip1 has long been recognized as an oncogene in tumorigenesis because of its overexpression in human tumors, as well as the phenomenon of tumor-resistant Wip1-deficient mice [34]. Emerging evidence also highlights the importance of Wip1 in regulating stress-induced and DNA-damage-induced pathways [35]. Our group did a series of work on the roles of Wip1 in immune and inflammatory responses and identified an unknown role of Wip1 in regulating neutrophil immunity [13]. We found that Wip1 was preferentially expressed in neutrophils among immune cells, and the Wip1 expression was gradually upregulated during the differentiation of myeloid precursors into mature neutrophils. Furthermore, the Wip1 expression level was negatively correlated with the inflammatory cytokine production by neutrophils in sepsis patients. Through establishing Wip1-deficient mice and chimera mice with Wip1 knock-out hematopoietic cells, we discovered an expanded pool of neutrophils with hypermature phenotypes in the periphery. Further *in vivo* and *in vitro* studies showed that Wip1 intrinsically regulates neutrophil development and maturation through the p38-MAPK pathway [13]. Thus, phosphatase Wip1 also plays a critical role in the generation and homeostasis of neutrophils.

In an acute lung injury model in mice, our group further demonstrated that the migration of neutrophils, as well as their proinflammatory cytokine production, was also tightly controlled by phosphatase Wip1 [14]. Wip1-deficient mice displayed increased bactericidal activities to *Staphylococcus aureus* and were hypersensitive to Lipopolysaccharide-induced acute lung damage with increased neutrophil infiltration and inflammation. Mechanism studies showed that the increased migration ability of Wip1 knock-out neutrophils was mediated by

decreased CXCR2 internalization and desensitization through the p38 MAPK pathway [14].

To better clarify the possible relationship between neutrophil and Wip1 in mediating intestinal I/R injury, we established a mesenteric I/R model and found that Wip1-deficient mice displayed much more severe intestinal pathology with increased neutrophil infiltration in local intestinal tissues compared to WT mice. Furthermore, we discovered that Wip1 negatively regulates the expression of IL-17A in neutrophils (Shen XF, Du JF, Zhao Y, *et al.*, Unpublished Data).

Therefore, phosphatase Wip1 acts as a master regulator of neutrophil homeostasis and immune function, and controlling of Wip1 may be an alternative to regulating neutrophil immunity after intestinal I/R.

Expert commentary & five-year view: weighing the benefits of Wip1-based therapy for intestinal I/R injury

Oxidative stress, especially for the burst of oxygen-free radicals, is generated after I/R and is known to prime inflammatory cells for subsequent stimuli [2]. During numerous downstream pathways of oxidative stress, Toll-like receptor 4 and the following NF-κB pathway play important roles in mediating intestinal I/R injury [2]. Since Wip1 has been implicated to be responsible for oxidative stress [36] and a downregulator for NF-κB signaling [37], therapeutic target of Wip1 may be beneficial in treating intestinal I/R injury. Oshima et al. found that in a model of colonic epithelial barrier dysfunction induced by hydrogen peroxide, Wip1 was upregulated by H_2O_2 in a p53-dependent manner. *In vivo* and *in vitro* studies further indicated that the upregulated expression of Wip1 functioned to protect the colonic mucosal permeability and maintain the function of tight junctions by selectively dephosphorylating and inactivating p38 MAP kinase [38].

Except for local intestinal injury, remote organ and/or tissue injury, especially lung injury after intestinal I/R, is also mediated by the increased infiltration of neutrophils [39–41]. Our recent studies have shown that Wip1-deficient mice were more hypersensitive to Lipopolysaccharide-induced acute lung damage with increased neutrophil infiltration and inflammation [14]. Furthermore, through establishing an intestinal I/R injury model, we found that Wip1-deficient mice displayed much more severe lung injury after intestinal reperfusion with higher number of infiltrated neutrophils (Shen XF, Du JF, Zhao Y, *et al.*, Unpublished Data). Thus, modulating the expression of Wip1 in neutrophils might also help to regulate the infiltration of neutrophils and its inflammatory activities to ameliorate lung injury after intestinal I/R.

In conclusion, phosphatase Wip1 might act as a master regulator for the control of local and remote infiltration of neutrophils after intestinal I/R, and epigenetic regulation of phosphatase Wip1 expression may be a critical mechanism underlying neutrophil immunity after intestinal I/R, as well as oxidative stress. Agents or other means to increase Wip1 activity could be a new therapeutic strategy for treating intestinal I/R injury.

Acknowledgements

We thank Mr. Steven Shao for his careful reviewing our manuscript.

Financial & competing interests disclosure

This work was supported by grants from the National Basic Research Program of China (2010CB945301, 2011CB710903, Y Zhao), the National Natural Science Foundation for General and Key Programs (C81130055, C81072396, Y Zhao; 81372364, 81000189, W Guan), and Knowledge Innovation Program of Chinese Academy of Sciences (XDA04020202-19, Y Zhao), the CAS/SAFEA International Partnership Program for Creative Research Teams (Y Zhao), the China Postdoctoral Science Foundation (2014M552695, X Shen). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Intestinal ischemia/reperfusion (I/R) injury is a major challenge during intestine transplantation, superior mesenteric artery embolus and general surgery. Currently, there are no ideal therapies for treating intestinal I/R injury.
- Intestinal I/R injury is often characterized as a neutrophil-driven pathology, and better control of neutrophil infiltration in local and remote tissues, as well as their pro-inflammatory cytokine production, is crucial to treating intestinal I/R injury.
- Phosphatase Wip1 acts as a master regulator for neutrophil immunity, which influences the development and maturation of neutrophils and their antimicrobial and inflammatory activities.
- The Wip1 expression level was negatively correlated with inflammatory cytokine productions of neutrophils in sepsis patients. Wip1-deficient mice were also hypersensitive to Lipopolysaccharide-induced acute lung injury.
- Neutrophil immunity is tightly controlled by Wip1, and targeting Wip1 may be a promising approach for treating I/R injury.

References

Papers of special note have been highlighted as
• of interest
•• of considerable interest

1. Hart ML, Jacobi B, Schittenhelm J, et al. Cutting Edge: A2B Adenosine receptor signaling provides potent protection during intestinal ischemia/reperfusion injury. J Immunol 2009;182(7):3965-8
2. Eltzschig HK, Eckle T. Ischemia and reperfusion–from mechanism to translation. Nat Med 2011;17(11):1391-401

•• **This review gave a systemic introduction of I/R injury.**

3. Kubes P, Hunter J, Granger DN. Ischemia/reperfusion-induced feline intestinal dysfunction: importance of granulocyte recruitment. Gastroenterology 1992;103(3):807-12
4. Tan Z, Jiang R, Wang X, et al. RORgammat+IL-17+ neutrophils play a critical role in hepatic ischemia-reperfusion injury. Journal of molecular cell biology 2013;5(2):143-6
5. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. Nature reviews Immunology 2011;11(8):519-31
6. Kohler D, Straub A, Weissmuller T, et al. Phosphorylation of vasodilator-stimulated phosphoprotein prevents platelet-neutrophil complex formation and dampens myocardial ischemia-reperfusion injury. Circulation 2011;123(22):2579-90
7. Williams IR, Parkos CA. Colonic neutrophils in inflammatory bowel disease: double-edged swords of the innate immune system with protective and destructive capacity. Gastroenterology 2007;133(6):2049-52

• **This short letter weighed the functions of neutrophils in IBD.**

8. Kuhl AA, Kakirman H, Janotta M, et al. Aggravation of different types of experimental colitis by depletion or adhesion blockade of neutrophils. Gastroenterology 2007;133(6):1882-92
9. Lu YZ, Wu CC, Huang YC, et al. Neutrophil priming by hypoxic preconditioning protects against epithelial barrier damage and enteric bacterial translocation in intestinal ischemia/reperfusion. Laboratory investigation; a journal of technical methods and pathology 2012;92(5):783-96

• **This article introduced the importance of neutrophils in promoting tissue repair after I/R.**

10. Fiscella M, Zhang H, Fan S, et al. Wip1, a novel human protein phosphatase that is induced in response to ionizing radiation in a p53-dependent manner. Proc Natl Acad Sci USA 1997;94(12):6048-53
11. Salminen A, Kaarniranta K. Control of p53 and NF-kappaB signaling by WIP1 and MIF: role in cellular senescence and organismal aging. Cell Signal 2011;23(5):747-52
12. Zhu Y, Demidov ON, Goh AM, et al. Phosphatase WIP1 regulates adult neurogenesis and WNT signaling during aging. J Clin Invest 2014;124(7):3263-73
13. Liu G, Hu X, Sun B, et al. Phosphatase Wip1 negatively regulates neutrophil development through p38 MAPK-STAT1. Blood 2013;121(3):519-29

•• **This article shed light on the importance of Wip1 in regulating neutrophil development.**

14. Sun B, Hu X, Liu G, et al. Phosphatase Wip1 negatively regulates neutrophil migration and inflammation. J Immunol 2014;192(3):1184-95

•• **This article placed emphasis on the role of Wip1 in regulating neutrophil inflammatory functions.**

15. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 2006;124(4):837-48
16. Souza DG, Vieira AT, Soares AC, et al. The essential role of the intestinal microbiota in facilitating acute inflammatory responses. J Immunol 2004;173(6):4137-46
17. Shifflett DE, Bottone FG Jr, Young KM, et al. Neutrophils augment recovery of porcine ischemia-injured ileal mucosa by an IL-1beta- and COX-2-dependent mechanism. Am J Physiol Gastrointest Liver Physiol 2004;287(1):G50-7
18. Kreisel D, Sugimoto S, Tietjens J, et al. Bcl3 prevents acute inflammatory lung injury in mice by restraining emergency granulopoiesis. J Clin Invest 2011;121(1):265-76
19. Farrar CA, Asgari E, Schwaeble WJ, Sacks SH. Which pathways trigger the role of complement in ischaemia/reperfusion injury? Frontiers in immunology 2012;3:341
20. Matsuo Y, Onodera H, Shiga Y, et al. Correlation between myeloperoxidase-quantified neutrophil accumulation and ischemic brain injury in the rat. Effects of neutrophil depletion. Stroke 1994;25(7):1469-75
21. Sisley AC, Desai T, Harig JM, Gewertz BL. Neutrophil depletion attenuates human intestinal reperfusion injury. J Surg Res 1994;57(1):192-6
22. Martinez-Mier G, Toledo-Pereyra LH, McDuffie JE, et al. Neutrophil depletion and chemokine response after liver ischemia and reperfusion. J Invest Surg 2001;14(2):99-107
23. Sarma JV, Ward PA. New developments in C5a receptor signaling. Cell health and cytoskeleton 2012;4:73-82
24. Wu MC, Brennan FH, Lynch JP, et al. The receptor for complement component C3a mediates protection from intestinal ischemia-reperfusion injuries by inhibiting neutrophil mobilization. Proc Natl Acad Sci U S A 2013;110(23):9439-44

• **This article discussed the complement system in mediating intestinal I/R injury.**

25. Diepenhorst GM, van Gulik TM, Hack CE . Complement-mediated ischemia-reperfusion injury: lessons learned from animal and clinical studies. Ann Surg 2009;249(6):889-99
26. Shernan SK, Fitch JC, Nussmeier NA, et al. Impact of pexelizumab, an anti-C5 complement antibody, on total mortality and adverse cardiovascular outcomes in cardiac surgical patients undergoing cardiopulmonary bypass. Ann Thorac Surg 2004;77(3):942-9.discussion 949-950
27. Verrier ED, Shernan SK, Taylor KM, et al. Terminal complement blockade with pexelizumab during coronary artery bypass graft surgery requiring cardiopulmonary bypass: a randomized trial. JAMA 2004;291(19):2319-27
28. Shen X, Du J, Guan W, Zhao Y. The balance of intestinal Foxp3(+) regulatory T cells and Th17 cells and its biological significance. Expert Rev Clin Immunol 2014;10(3):353-62
29. Shichita T, Sugiyama Y, Ooboshi H, et al. Pivotal role of cerebral interleukin-17-producing gammadeltaT cells in the delayed phase of ischemic brain injury. Nat Med 2009;15(8):46-950
30. Lee HT, Kim M, Kim JY, et al. Critical role of interleukin-17A in murine intestinal ischemia-reperfusion injury. Am J Physiol

Gastrointest Liver Physiol 2013;304(1): G12-25

31. Holtta V, Klemetti P, Salo HM, et al. Interleukin-17 immunity in pediatric Crohn disease and ulcerative colitis. J Pediatr Gastroenterol Nutr 2013;57(3):287-92
32. Garrido-Mesa N, Algieri F, Rodriguez Nogales A, Galvez J. Functional plasticity of Th17 cells: implications in gastrointestinal tract function. Int Rev Immunol 2013;32(5-6):493-510
33. Deshmukh HS, Liu Y, Menkiti OR, et al. The microbiota regulates neutrophil homeostasis and host resistance to Escherichia coli K1 sepsis in neonatal mice. Nat Med 2014;20(5):524-30

• **This article discovered the importance of IL-17A in neutrophil homeostasis.**

34. Buss MC, Remke M, Lee J, et al. The WIP1 oncogene promotes progression and invasion of aggressive medulloblastoma variants. Oncogene 2014;17. [Epub ahead of print]
35. Zhu YH, Bulavin DV. Wip1-dependent signaling pathways in health and diseases. Prog Mol Biol Transl Sci 2012;106:307-25
36. Lowe J, Cha H, Lee MO, et al. Regulation of the Wip1 phosphatase and its effects on the stress response. Front Biosci (Landmark Ed) 2012;17:1480-98
37. Echemendia-Blanco D, Van Driessche E, Ncube I, et al. Stability, subunit interactions and carbohydrate-binding of the seed lectin from Pterocarpus angolensis. Protein Pept Lett 2009;16(9):1120-34
38. Oshima T, Sasaki M, Kataoka H, et al. Wip1 protects hydrogen peroxide-induced colonic epithelial barrier dysfunction. Cell Mol Life Sci 2007;64(23):3139-47

•• **This article identified the relationship between Wip1 and intestinal oxidative stress.**

39. Altshuler AE, Richter MD, Modestino AE, et al. Removal of luminal content protects the small intestine during hemorrhagic shock but is not sufficient to prevent lung injury. Physiological reports 2013;1(5): e00109
40. Lapchak PH, Ioannou A, Rani P, et al. The role of platelet factor 4 in local and remote tissue damage in a mouse model of mesenteric ischemia/reperfusion injury. PLoS One 2012;7(7):e39934
41. Schmeling DJ, Caty MG, Oldham KT, et al. Evidence for neutrophil-related acute lung injury after intestinal ischemia-reperfusion. Surgery 1989;106(2): 195-201. discussion 201-192