Application of Proteomics in Optic Nerve Injury Diseases

Subjects: Pathology | Neurosciences

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Optic nerve damage is a common cause of blindness. Optic nerve injury is often accompanied by fundus vascular disease, retinal ganglion cell apoptosis, and changes in retinal thickness. These changes can cause alterations in protein expression within neurons in the retina. Proteomics analysis offers conclusive evidence to decode a biological system. Optic nerve damage can significantly reduce the vision of patients, thereby having a serious impact on their daily lives and their families. In clinical practice, optic nerve injury is mainly diagnosed by optical tomography (OCT) detection and by fundus angiography.

optic nerve injury | proteomics | bioinformatics | retinal ganglion cells

1. Application of Proteomics in Diabetic Retinopathy (DR) Research

Proteomics has been widely used in the research of optic nerve injury diseases. The number of differentially expressed proteins and their main roles were obtained from the literature. Targeted proteomic studies using only ELISA or other immunological assays were excluded. Weber et al. [1] used 23 vitreous samples of proliferative diabetic retinopathy (PDR) and divided them into three groups according to the severity of the disease for tandem mass spectrometry calibration analysis. They quantified 2400 proteins and three PDR differences. Bioinformatic analysis of the proteins found that the glycolysis and gluconeogenesis pathways were activated in the vitreous of PDR patients, while the expression of carbon metabolism-related molecules was upregulated. Gao [2] conducted proteomic analysis on the PDR vitreous of patients with diabetic retinopathy. Compared with the normal control group, there were 119 differentially expressed proteins in the PDR of patients with diabetic retinopathy. Among them, the level of CA-I was significantly increased. Further research found that this protein could increase the permeability of the retina and choroid. Increases in VEGF are also known to promote the formation of new blood vessels in the retina, thereby indicating it to be an important protein for aggravating the disease. Ranibizumab is often used in the treatment of DR in clinics. Zou [3] and others performed proteomic detection on the vitreous humor of eight DR patients, nine DR patients treated with leizumab, and nine nondiabetes patients. Compared with the control group, a total of 72 differential proteins were screened out; compared with the PDR group, there are 3 upregulated proteins and 16 downregulated proteins in the treatment group, which may be one of the important proteins to promote the improvement of PDR. After biological analysis, it was found that these proteins were related to pathways involving “the innate immune response”, “complement activation”, and “proteolysis”. The early detection of PDR is a challenge that has not yet been overcome in clinical work, and this is due to there being a

lack of a simple, rapid, and specific molecular markers to detect the progression of PDR. In the plasma of PDR patients, a total of 23 upregulated and 13 downregulated proteins were found, with FCGR3A, DPEP2, and ADGRF5 identified as potential protein markers in the plasma [4]. Wang et al. [5] used RP-HPLC and ESI-MS/MS to detect 96 differentially expressed proteins, 37 upregulated proteins, and 59 downregulated proteins in the vitreous of PDR patients. The key identified differentially expressed proteins were angiopoietin-related protein 6, apolipoprotein AI, estrogen receptor alpha, and tubulin. Zhang et al. [6] used DB mice to build a model of diabetic retinopathy, treated the diabetic retina with phlorizin, and then analyzed the retinal proteomics of the mice in each group by LC-MS/MS to attempt to find effective treatments. The results of the study found a total of 1636 proteins in the mouse retina; 348 differentially expressed proteins were detected in the DR group compared with the blank control group, while only 60 differentially expressed proteins were detected in the treatment group compared with the DR group (including 27 upregulated proteins and 33 downregulated proteins). Bioinformatic analysis conducted on these differentially expressed proteins found that most were involved in processes pertaining to oxidative stress, apoptosis, and energy metabolism, among other pathways. The authors suggested that the ability of phloridzin to inhibit retinal cell apoptosis and slow down the development of DR was associated with the upregulation of γ-crystallin and Glrx-3 in DR mice. The authors believed that the pathogenesis and development of DR are related to the downregulation of Glrx-3 and that γ-crystallin may be an important therapeutic site for improving the clinical symptoms of DR. Winiarczyk [7] used two-dimensional electrophoresis to analyze the tear film of 15 diabetic dogs and 13 normal dogs by proteomic analysis to identify one upregulated protein and eight downregulated proteins in the diabetic group compared to the controls. The authors noted that the only upregulated protein in DR dogs (SRCIN1) is an important mediator of the VEGF pathway, and the observed increased expression of SRCIN1 functions by activating the VEGF/SRC kinase signaling axis, thereby promoting retinal barrier dissolution and retinal neovascularization. Prelamin-A/C, Flotillin-1, Pro-MCH, PI4KIIα, PARP12, PARP/GRIP, TPR36, and Serpin B3 were found to be downregulated in DR dogs, all of which are involved in cellular immunity, inflammation, apoptosis-related pathways, and the Src kinase pathway. In order to observe the changes in lens-related proteins that occur in diabetic retinopathy, Nagai et al. [8] used a STZ-induced diabetic retinopathy model in rats to identify a total of 229 proteins in the lens of the normal control group and 235 proteins in the lens of diabetic retinopathy rats, which included a total of 52 differentially expressed proteins. Among these 52 differentially expressed proteins, superoxide dismutase was significantly downregulated, while phosphorylated p38 was significantly increased. The antioxidant capacity of the eyes of patients with diabetes is known to be weakened, and this is because elevated blood sugar increases the load of ocular oxidants and oxidative free radicals in the eyes. This occurrence changes the overall sensitivity of the lens such that the aggravation caused by glycosylation and the inactivation of antioxidant enzymes can exacerbate the lens opacity velocity. The downregulation of superoxide dismutase is an important factor for activating the P38 pathway, and inhibiting the activation of the P38 signaling pathway may therefore be an important target for the treatment of diabetic cataracts.

2. Application of Proteomics in the Study of Traumatic Optic Nerve Injury

Traumatic optic nerve injury is one of the more common forms of optic nerve injury. It is characterized by either direct or indirect damage to the optic nerve that occurs when the face or head is damaged by an external force and involves the degeneration or death of retinal ganglion cells (RGCs). Yan [9] established a rat optic nerve transection (ON) model and used iTRAQ proteomics to analyze the retina to detect a total of 4717 proteins. Compared with the blank control group, 708 differentially expressed genes were detected in the ON group. In this study, differentially expressed proteins between different time points were also compared. The findings indicated that potential diagnostic targets in the early stage of the disease were different from those in the middle and late stages of the disease. Pathway analysis of these differentially expressed genes found that these were related to “carbon metabolism” and “the ribosome”. To examine the effect of therapeutic drugs on rats after optic nerve transection, Hollander et al. [10] injected Hepatoma-derived growth factor (HDGF) into the eyes of rats following ON and observed postoperative changes in retinal proteins at 7, 14, and 21 days. A total of 52 upregulated proteins and 19 downregulated proteins were found in the retina of the ON group. Compared with the treatment group, HDGF was able to activate both the MAP and PI3 kinases while also activating the AKT phosphorylation pathway.

To study the changes in RGCs after optic nerve transection in rats, Kwong et al. [11] established a rat unilateral ON model, obtained the retinas of rats 2 weeks after the operation, and analyzed them by quadrupole time-of-flight mass spectrometry (QTOF-MS). Murine retinas were evaluated for their protein contents. A total of 3641 proteins were found, of which 25 were downregulated and 37 were upregulated in the time quadrant, while 5 downregulated proteins and 20 upregulated proteins were found in the nasal quadrant. Differentially expressed proteins differed between different times of injury, which was consistent with previous findings. Among them, CLU, GFAP, GNG5, IRF2BPL, L1CAM, and CPLX1 are associated with the degeneration of RGCs. Lam et al. [12] conducted differential gel electrophoresis analysis on the superior, temporal, inferior, and nasal quadrants of rat optic nerve transection. The results revealed 24 differentially expressed proteins between the different quadrants. In summary, proteomic analysis on the ON model retina showed differentially expressed proteins according to the differences in modeling time, proteomic technology, and sampling sites. The signaling pathways acted on by these differentially expressed proteins are relatively few and different.

3. Application of Proteomics in Retinal Ischemia/Reperfusion Injury Research

Retinal blood vessel blockage, deformation, and prolonged poor blood flow can each cause damage to the optic nerve. At least 70 proteins are known to play a key role in optic nerve ischemic diseases. Tian et al. [13] performed proteomic analysis on the retina of optic nerve ischemic mice and found 234 differentially expressed proteins. Among these proteins, the main cellular changes they represented were related to metabolism, followed by cell transcription. The study also found that when the optic nerve was deficient after blood injury, there was inhibition of the mTOR signaling pathway and synapse-related proteins were significantly downregulated, which again confirmed that optic neuron function gradually weakened after optic nerve injury and optic neuron function was aggravated by visual nerve injury. Proteomic analysis of the retina and optic nerve in N-methyl-d-aspartic acid-induced [14] excitotoxic ophthalmopathy in rats revealed 3532 proteins in the retina and 2593 in the optic nerve.

Differential protein analysis found that these proteins were associated with ferroptosis and autophagy. To further understand the protective mechanism of hydrogen sulfide (a commonly used neurotransmitter drug) on the optic nerve, Liu [15] established a rat optic nerve ischemia model and treated it with the chemical. Component analysis revealed a total of 1115 proteins, 18 of which were associated with hydrogen sulfide reduction/protection of the optic nerve from damage. Bioinformatic analysis of these differentially expressed proteins revealed that they were associated with mitochondrial dysfunction, iron homeostasis, and vasodilation activation. Zhao et al. [16] found 131 differentially expressed proteins in the rat retinal ischemia-reperfusion model, of which 24 proteins were related to histone translation modification. The authors believed that retinal ischemic injury and histone phosphorylation were associated. Vähätupa [17] used transgenic mice to establish a model of hypoxia-ischemia-induced retinopathy, used SWATH-MS technology to analyze the hypoxia-ischemia retinopathy mice, and correlated the proteomic results with human diabetic retinopathy. The results of patients with retinopathy of prematurity and retinal vein occlusion were compared and analyzed, and it was found that crystallin was one of the most abnormally secreted proteins in the early stage of retinal hypoxia-ischemia disease. The AR–Ras axis and filamin were significantly upregulated in the retina. These target proteins are known to be related to retinal neovascularization and vascular permeability.

Due to the difficulty in obtaining retina and optic nerve tissue in clinical practice, most experiments substitute this need by using animal models. Due to differences between animal species, retinal tissue preservation conditions, proteomic analysis methods, screening conditions, and experimental purposes, the same disease model study showed different results, including the number of differentially expressed proteins, the types of differentially expressed proteins, and the main action pathways, depending on these variables. Through analysis, it was found that most of the differentially expressed protein action pathways were enriched in the processes of neuronal damage, RGC degeneration and apoptosis, mitochondrial dysfunction, and carbon metabolism, among others.

4. Pathogenesis of Optic Nerve Injury Diseases

Diabetic retinopathy is a major microvascular complication of diabetes that can be divided into nonproliferative DR and proliferative DR in clinical practice. In diabetic retinopathy, the duration and degree of hyperglycemia maintenance in diabetic patients are closely related to the occurrence and progression of DR [18]. Retinal glial cells play an important role in maintaining the structural integrity of the retina and normal physiological functions [19]. After the body's blood sugar rises, the microglia are activated, and the secretion of the inflammatory factors TNF-α and IL-6 in the cells increase, thereby promoting the secretion of VEGF. Due to chronic increases in blood sugar, the retina exists in a highly inflammatory environment for an extended period, which further leads to the dysfunction of glial cells and endothelial cells, ultimately causing the retina to be damaged by blood vessels and nerves [20][21].

When blood sugar rises, the glycolytic pathway is activated. Abnormal glucose metabolism leads to an increase in carbon metabolism, changes in glucose homeostasis in the vitreous, and changes in the vitreous environment to induce vitreous degeneration· After vitreous degeneration activates the proteinase A signaling pathway, the number of endothelia in retinal blood vessels increases, the vessel wall thickens, and the diameter of blood vessels decreases, which ultimately leads to retinal hyperproteinemia, reflecting the regulation of retinal angiogenesis after diabetic retinopathy [22]. Studies have shown that when DR occurs, the "semaphorin neuron rejection signaling

pathway" is inactivated, and the main regulators of the pathway (sema3A, sema3F, and sema6A) are significantly reduced. Both sema3A and sema3F can inhibit neovascularization. When the expression of sema3A in the retina is higher than that in the vitreous, sema3A induces retinal neovascularization to grow toward the vitreous, and the location of sema3A determines the growth direction of the neovascularization. The expression of sema3A in the vitreous is downregulated in DR, and sema3A can usually be injected into the lens to prevent neovascularization in the lens. Sema6A inhibits the formation of endothelial cells. Consequently, when the expression of sema6A in the retina or vitreous decreases, the production and number of new endothelial cells increases, thereby increasing their content in the vitreous and decreasing the clarity of the vitreous [23][24] (Nina et al., 2022; Guo et al., 2019).

In most cases, external forces applied to the optic nerve are indirect, causing oedema in the orbit of the optic nerve or bone degeneration, which leads to compression of the blood vessels in the retina, thickening of the spasm wall, and reduction of the diameter of the blood vessels, all of which can temporarily cause the capillaries of the arterioles to dilate. Under the stimulation of inflammatory cytokines, the vascular permeability increases, the blood flow slows down, and local edema increases, ultimately resulting in ischemia and hypoxia [13]. There is no effective method for the treatment of optic nerve injury in clinical practice. Treatments of optic nerve injury should aim to prolong the survival time of RGC, reduce the degeneration and apoptosis of RGCs, and prevent further progression to achieve the purpose of reversing the injury.

When the retinal surface blood vessels exhibit spasms, stenosis, and other symptoms, the retinal microvessels temporarily expand, the inflammatory secretions produced by the retinal cells increase, the vascular osmotic pressure increases, and the blood flow slows down, thereby resulting in retinal ischemia [25]. Retinal ganglion cells are composed of many unsaturated lipids. Following retinal ischemia, the secretion of superoxide free radicals and arachidonic acid in the retina increases, and optic ganglion cells are attacked by superoxide free radicals, which results in the production of lipids by optic ganglion cells [26].

Many studies have shown that when the internal retinal vein is occluded, the increased secretion of VEGF in the retina induces neovascularization, thereby resulting in either macular degeneration or vitreous opacity [27]. In the early stage of retinal ischemic disease, the thickness of the retina is normal, but the thickness of the optic nerve fiber layer is increased. In the middle stage of ischemia, the optic nerve begins to atrophy, and the peripheral optic nerve fibers show clear swelling, but their thickness is still less than in the early stage. In the later stage, atrophy, thinning, and visual field changes occur [27] to signify that the optic nerve has been severely damaged and many RGCs in the retina have died. Retinal ischemic diseases may be related to hereditary diseases. The principle of retinal ischemia-reperfusion disease treatment is to dilate the existing blood vessels and inhibit the formation of new blood vessels within the fundus. The main site of action was found through proteomic analysis, and it is expected that specific targeted drugs will henceforth be developed based on specific sites. These drugs can be designed to treat the disease by delaying the process of optic nerve damage and promoting the regeneration of RGCs.

References

1. Weber, S.R.; Zhao, Y.; Ma, J.; Gates, C.; Leprevost, F.D.V.; Basrur, V.; Nesvizhskii, A.I.; Gardner, T.W.; Sundstrom, J.M. A validated analysis pipeline for mass spectrometry-based vitreous proteomics: New insights into proliferative diabetic retinopathy. Clin. Proteom. 2021, 18, 28.
2. Gao, B.-B.; Clermont, A.; Rook, S.; Fonda, S.J.; Srinivasan, V.J.; Wojtkowski, M.; Fujimoto, J.G.; Avery, R.L.; Arrigg, P.G.; Bursell, S.-E.; et al. Extracellular carbonic anhydrase mediates hemorrhagic retinal and cerebral vascular permeability through prekallikrein activation. Nat. Med. 2007, 13, 181–188.
3. Zou, C.; Han, C.; Zhao, M.; Yu, J.; Bai, L.; Yao, Y.; Gao, S.; Cao, H.; Zheng, Z. Change of ranibizumab-induced human vitreous protein profile in patients with proliferative diabetic retinopathy based on proteomics analysis. Clin. Proteom. 2018, 15, 12.
4. Wu, H.; Wang, D.; Zheng, Q.; Xu, Z. Integrating SWATH-MS proteomics and transcriptome analysis to preliminarily identify three DEGs as biomarkers for proliferative diabetic retinopathy. Proteom. –Clin. Appl. 2021, 16, e2100016.
5. Wang, H.; Feng, L.; Hu, J.; Xie, C.; Wang, F. Differentiating vitreous proteomes in proliferative diabetic retinopathy using high-performance liquid chromatography coupled to tandem mass spectrometry. Exp. Eye Res. 2013, 108, 110–119.
6. Zhang, S.Y.; Li, B.Y.; Li, X.L.; Cheng, M.; Cai, Q.; Yu, F.; Wang, W.D.; Tan, M.; Yan, G.; Hu, S.L.; et al. Effects of phlorizin on diabetic retinopathy according to isobaric tags for relative and absolute quantification-based proteomics in db/db mice. Mol. Vis. 2013, 19, 812–821.
7. Winiarczyk, D.; Winiarczyk, M.; Winiarczyk, S.; Michalak, K.; Adaszek, Ł. Proteomic Analysis of Tear Film Obtained from Diabetic Dogs. Animals 2020, 10, 2416.
8. Nagai, N.; Yamamoto, T.; Mitamura, K.; Taga, A. Proteomic profile of the lens in a streptozotocin-induced diabetic rat model using shotgun proteomics. Biomed. Rep. 2017, 7, 445–450.
9. Yan, F.; Wang, X.; Jiang, X.; Chai, Y.; Zhang, J.; Liu, Q.; Wu, S.; Wang, Y.; Wang, N.; Li, S. Proteomic profiles of the retina in an experimental unilateral optic nerve transection: Roles of Müller cell activation. Clin. Transl. Med. 2022, 12, e631.
10. Hollander, A.; D'Onofrio, P.M.; Magharious, M.M.; Lysko, M.D.; Koeberle, P.D. Quantitative Retinal Protein Analysis after Optic Nerve Transection Reveals a Neuroprotective Role for Hepatoma-Derived Growth Factor on Injured Retinal Ganglion Cells. Investig. Ophthalmol. Vis. Sci. 2012, 53, 3973–3989.
11. Kwong, J.; Caprioli, J.; Sze, Y.; Yu, F.; Li, K.; To, C.; Lam, T. Differential Retinal Protein Expression in Primary and Secondary Retinal Ganglion Cell Degeneration Identified by Integrated SWATH and Target-Based Proteomics. Int. J. Mol. Sci. 2021, 22, 8592.

12. Lam, C.; Li, K.K.; Do, C.W.; Chan, H.H.; To, C.H.; Kwong, J.M.K. Quantitative profiling of regional protein expression in rat retina after partial optic nerve transection using fluorescence difference two-dimensional gel electrophoresis. Mol. Med. Rep. 2019, 20, 2734–2742.
13. Tian, H.; Wang, L.; Cai, R.; Zheng, L.; Guo, L. Identification of Protein Network Alterations upon Retinal Ischemia-Reperfusion Injury by Quantitative Proteomics Using a Rattus norvegicus Model. PLoS ONE 2014, 9, e116453.
14. Suo, L.; Dai, W.; Chen, X.; Qin, X.; Li, G.; Song, S.; Zhang, D.; Zhang, C. Proteomics analysis of N-methyl-d-aspartate-induced cell death in retinal and optic nerves. J. Proteom. 2021, 252, 104427.
15. Liu, H.; Perumal, N.; Manicam, C.; Mercieca, K.; Prokosch, V. Proteomics Reveals the Potential Protective Mechanism of Hydrogen Sulfide on Retinal Ganglion Cells in an Ischemia/Reperfusion Injury Animal Model. Pharmaceuticals 2020, 13, 213.
16. Zhao, X.; Sidoli, S.; Wang, L.; Wang, W.; Guo, L.; Jensen, O.N.; Zheng, L. Comparative Proteomic Analysis of Histone Post-translational Modifications upon Ischemia/Reperfusion-Induced Retinal Injury. J. Proteome Res. 2014, 13, 2175–2186.
17. Vähätupa, M.; Järvinen, T.A.H.; Uusitalo-Järvinen, H. Exploration of Oxygen-Induced Retinopathy Model to Discover New Therapeutic Drug Targets in Retinopathies. Front. Pharmacol. 2020, 11, 873.
18. Romero-Aroca, P.; Baget-Bernaldiz, M.; Pareja-Rios, A.; Lopez-Galvez, M.; Navarro-Gil, R.; Verges, R. Diabetic macular edema pathophysiology: Vasogenic versus inflammatory. J. Diabetes Res. 2016, 2016, 2156273.
19. Ramirez, J.M.; Trivino, A.; Ramirez, A.I.; Salazar, J.J.; Garcia-Sanchez, J. Immunohistochemical study of human retinal astroglia. Vis. Res. 1994, 34, 1935–1946.
20. Miller, J.W.; Adamis, A.P.; Shima, D.T.; D'Amore, P.A.; Moulton, R.S.; O'Reilly, M.S.; Folkman, J.; Dvorak, H.F.; Brown, L.F.; Berse, B.; et al. Vascular endothelial growth factor/vascular permeability factor is temporally and spatially correlated with ocular angiogenesis in a primate model. Am. J. Pathol. 1994, 145, 574–584.
21. Serhan, C.N.; Brain, S.D.; Buckley, C.D.; Gilroy, D.W.; Haslett, C.; O'Neill, L.A.J.; Perretti, M.; Rossi, A.G.; Wallace, J.L. Resolution of in flammation: State of the art, definitions and terms. FASEB J. 2007, 21, 325–332.
22. Gariano, R.F.; Gardner, T.W. Retinal angiogenesis in development and disease. Nature 2005, 438, 960–966.
23. Zippel, N.; Kenny, C.H.; Wu, H.; Garneau, M.; Kroe-Barrett, R.; Gupta, P.; Low, S.; Bakker, R.A.; Thomas, L. Sema3A Antibody BI-X Prevents Cell Permeability and Cytoskeletal Collapse in

HRMECs and Increases Tip Cell Density in Mouse Oxygen-Induced Retinopathy. Trans. Vis. Sci. Technol. 2022, 11, 17.

24. Guo, S.; Ren, J.; Li, Z.; Fan, X.; Qin, L.; Li, J. Aqueous semaphorin 3A level correlates with retinal macular oedema and ganglion cell degeneration in patients with retinal vein occlusion. Acta Ophthalmol. 2019, 97, 273–278.
25. Oubaha, M.; Miloudi, K.; Dejda, A.; Guber, V.; Mawambo, G.; Germain, M.-A.; Bourdel, G.; Popovic, N.; Rezende, F.A.; Kaufman, R.J.; et al. Senescence-associated secretory phenotype contributes to pathological angiogenesis in retinopathy. Sci. Transl. Med. 2016, 8, 362ra144.
26. Weise, J.; Isenmann, S.; Bähr, M. Increased expression and activation of poly(ADP-ribose) polymerase (PARP) contribute to retinal ganglion cell death following rat optic nerve transection. Cell Death Differ. 2001, 8, 801–807.
27. Zlobin, I.V.; Zhukova, S.I.; Shchuko, A.G.; Iureva, T.N. Complex assessment of retinal ischemic damage in patients with retinal vein occlusion. Vestnik Oftal’mologii 2019, 135, 62–69.