IJBPAS, July, 2019, 8(7): 1290-1299

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

BIOINFORMATICS ANALYSIS TO EXPLORE ISCHEMIC STROKE

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Received 7th Jan. 2019; Revised 29th Jan. 2019; Accepted 7th Feb. 2019; Available online 1st July 2019

https://doi.org/10.31032/IJBPAS/2019/8.7.4755

ABSTRACT

Stroke continues to be a major health threat around the world. Ischemia-reperfusion events negatively affect cerebrovascular tissue thus leading to impaired functionality of the brain and other organs. Dysfunctionality and degeneration of tissues as a result of ischemia-reperfusion injury leads to chronic diseases and death. With improving bioinformatics technology, researchers in the field of stroke biology are generating informative studies that have widespread application. Previous bioinformatics research has provided insight into the role of neuregulin-1 in suppressing neuroinflammatory pathways following focal cerebral ischemia. Functional studies involving knockout mice and short-interfering RNA (siRNA) knockdown experiments will be beneficial to determine the therapeutic significance of genetic and protein targets identified from large-scale bioinformaticsdata.

Keywords: Bioinformatics, Ischemia, Stroke, Neuregulin-1, Neuroprotection

INTRODUCTION

Stroke is currently one of the major causes of death in the United States according to the American Heart Association (Benjamin et al., 2019). According to the American Heart Association's Heart Disease and Stroke Statistics, it is estimated that approximately 2,300 people die each day due to cardiovascular diseases in the United States. Moreover, the AHA reports that billions are expended each year to deal with stroke and other cardiovascular diseases. Thus, there exists a critical need to develop and

investigate novel targeted molecular strategies for the prevention and treatment of stroke. Ischemic stroke is caused by acute reduction in blood flow to brain tissue triggered by an obstruction of cerebral and non-cerebral blood vessels (Kalogeris, Baines, Krenz, & Korthuis, 2012). Previous studies suggest that neuroinflammation mediated by neutrophils and microglia also play important roles in the pathogenesis of ischemic stroke.

Focal cerebral ischemia activates the cellular inflammatory response in the central nervous system resulting in the production of proinflammatory cytokines, apoptotic factors, reactive free radicals, complement, chemokines, prostaglandins, and cellular adhesion molecules. Activation of microglial cells result in the release of cytokines, recruitment of immune cells, activation of proinflammatory neutrophils, and the release of tissue-damaging substances which exacerbatebrain damage and neurodegeneration (Kalogeris, Baines, Krenz, & Korthuis, 2012; Weinstein, Koerner, & Möller, 2010).

Stroke involves complex interactions between neurons, brain tissue, and vascular tissue.

These interactions result in the concerted expression and repression of many genes that contribute to the progression of ischemic brain damage. An excellent study using Expression Analysis Systematic Explorer (EASE) analysis demonstrated that similar and distinct gene expression patterns result from transient (tMCAO) and permanent middle cerebral artery occlusion (pMCAO) stroke models (Ford, Xu, Gates, Jiang, & Ford, 2006). Genes associated with middle cerebral artery occlusion include inflammation, growth factors, cytokine receptors, apoptosis, cytokines, chemokines, adhesion molecules, transcription factors, and genes associated with immune cell activation. Microarray technology is useful in the study of ischemic stroke because it allows for the examination of the expression of thousands of transcripts in a cell in order to elucidate the molecular mechanisms associated with stroke. Microarray experiments and bioinformatics analysis using analytic software allow investigators to: a) identify and comparegene expression patterns in response to chemical treatments in ischemic mammalian models, 2) classify signaling pathways associated with specific treatments, and 3) generate relational information which can be used to compare the effects of different stroke treatments. This article provides an examination of experimentally-derived information from

bioinformatics analyses after administration of neuregulin-1 and other potentially beneficial chemotherapeutics and stroke treatments. The author contends that thorough examination of bioinformatics data will uncover new molecules and pathways that could provide the foundation for stroke prevention and treatment strategies.

Neuregulins

Neuregulins are an important family of growth factors mediated by the ErbB family of receptor tyrosine kinases and play essential roles in the regulation and proliferation of neuronal cell and non-neuronal cell types as well as cardiac developmental processes (Buonanno & Fischbach, 2001; Odiete, Hill, & Sawyer, 2012; Rupert & Coulombe, 2015). Currently, there are four identified proteins that comprise the neuregulin family: NRG-1, NRG-2, NRG-3, and NRG-4. While very little is known about the biological functions of NRG-2-NRG-4, NRG-1 has been shown to be involved in the development of the nervous system and circulatory system. NRG-1 has also been demonstrated to exert a neuroprotective action following ischemic stroke although the molecular mechanisms underlying the neuroprotective effects are not completely understood (Rupert & Coulombe, 2015; Xu, Croslan, Harris, Ford, & Ford, 2006). The current review briefly highlights the effects of NRG-1 on inflammation-associated gene expression, cytokine production, and apoptotic events in cerebrovascular tissue, immune cells, and mammalian models of stroke.

Neuregulin is clearly established as a dynamic regulatory protein in both normal and pathologic conditions in humans (Odiete, Hill, & Sawyer, 2012; Wang, Li, Paudyal, Ford, & Zhang, 2018). Li et al. (2007) demonstrated that NRG-1 can reduce brain infarction when administered before permanent MCAO ischemia in rat models. Several reports have also shown that neuregulins displayanti - inflammatory properties in the CNS both in vivo and in vitro (Croslan et al., 2008; Xu et al., 2005). Further,it was shown that NRG-1 blocks apoptotic neuronal death and prevents mononuclear infiltration, astrocyte activation, and cytokine production (Xu, Jiang, Ford, & Ford, 2004). Moreover, experimentation and bioinformatics analysis recently linked neuregulin-induced neuroprotective effects to the expression of signal transduction proteins such as STAT3, NF-κappa B, JNK, and Cdk5. It was shown that neuregulin-1 beta attenuated apoptosis in neuronal cells by increasing STAT3 levels as ischemia progressed (Li, Zhang, Guo, &Mei, 2009).

Neuregulin-1 was also shown using bioinformatics analysis to inhibit pro-inflammatory gene expression as well as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) in microglial cells in brain ischemia by disrupting the NF-κappa B signaling pathway that mediates their expression (Shahriary, Kataria, & Karimi-Abdolrezaee, 2019; Simmons et al., 2016). Specifically, Simmons et al. (2016) demonstrated using the Conserved Transcription Factor-Binding Site Finder (CONFAC) bioinformatics software package that modification of inflammatory gene expression in microglial cells following NRG-1 and middle cerebral artery occlusion is mediated by the NF-κappa B signaling pathway. Independent researchers also implicated the JNK signaling pathway in neuregulin-1-mediated ameliorative effects following ischemia-reperfusion. Western blot analysis confirmed that expression and activation levels of phospho-JNK, phospho-MKK4, and phospho-c-Jun were significantly reduced after neureulin-1 treatment. Neuregulin-1 also exerted anti-apoptotic effects and limited brain infarct sizes as demonstrated in other studies (Ji, Teng, Zhang, Sun, & Guo, 2017; Li, Zhang, Guo, & Mei, 2009).

Bioinformatics Analysis

Bioinformatics analysis of gene expression profile studies of brains from rats treated with

NRG-1 prior to ischemic-reperfusion events show that a large array of genes are reduced, compared to controls, that may be associated with ischemic insult (Xu et al., 2005). Briefly, Xu and other investigators used the Gene Expression Omnibus to explore differentially expressed genes (DEGs) from brain tissues ofsham controls and animals that underwent the middle cerebral artery occlusion procedure. Data was collected for three different time points post-reperfusion (e.g., 2h, 8h, and 24h). Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was performed on the DEGs for the three time points. There was an increase in the number of DEGs for each time point (e.g., 32 DEGs at 2h, 39 DEGs at 8h, and 91 DEGs at 24h) suggesting that the deleterious biological outcomes observed

post-reperfusion involves time-dependent genetic mechanisms. Not surprisingly, many of the DEGs were involved in the inflammatory response (Shao, Bao, Hong, Jiang, & Yu, 2018). Further investigation into the precise signaling pathways derived from the DEGs will elucidate novel

molecular suppression strategies that may attenuate tissue damage.

Qu et al. (2019) utilized established datasets to identify differentially expressed microRNAs (miRNAs) following treatment of middle cerebral artery occlusion rats with human umbilical cord mesenchymal stem cells. The investigators identified differentially expressed microRNAs that were involved in neuroinflammation and cessation of neutrophil migration suggesting that certain miRNAs may play a role in facilitating ischemic brain infarction. Moreover, using the Gene Expression Omnibus and bioinformatics analysis, researchers were able to identify microRNAs that may serve as helpful biomarkers to distinguish between embolic stroke and thrombotic stroke. The four miRNAs that were identified were: miR-15a-5p,miR-17-5p,miR-19b-3p, and miR-20a-5p (Chen & Jiang, 2018).

Proteomic bioinformatics analysis was utilized to compare the neuroprotective and neurogenic properties of one current stroke treatment, tissue plasminogen activator (TPA) and a common Chinese prescription for stroke, buyanghuanwu decoction (BHD) (Chen et al., 2015). Using a mouse model of brain ischemia-reperfusion it was shown that both TPA and BHD treatments were able to decrease the number of differentially expressed proteins after ischemic stroke.BHD treatments showed a slightly better reduction of differentially expressed proteins compared to TPA treatments. Correlation of proteomic analysis and functional responses were also investigated. Briefly, high-resolution positron emission tomography revealed significant restoration of brain function via glucose metabolic pathways in mice treated with BHD following ischemic stroke. TPA also demonstrated restoration in glucose metabolism, however its effect was not as pronounced as the BHD-treated mice.S100 calcium binding protein A9 and transthyretin were the only two proteins regulated by three different treatments (ischemic, BHD, TPA) suggesting that these proteins play a role in ischemic injury and that either treatment is able to modulate the expression of these proteins. The blood-brain-barrier which is extremely critical for the homeostasis of the brain was maintained following BHD treatment but not for the TPA-treated mice.

Isobaric tags for relative and absolute quantitation (iTRAQ) is a protein identification and quantification technique that utilizes radiolabeling and mass spectroscopy to determine the identity and relative amount of proteins in a sample

(Zieske, 2006). Lin et al. (2018) employed an iTRAQ proteomic analytical system to determine the effect of Rhubard on ischemic stroke outcomes in rats. Rhubarb-treated rats demonstrated reduced levels of brain infarction and better neurological deficit scores when compared to non-Rhubarb treated MCAO rats. Their study accurately identified 76 overlapping differently expressed proteins in which Rhubarb contributed to either upregulation or downregulation compared to other experimental groups. The researchers used Cytoscape to determine specific molecular networks and KEGG pathway analysis to generate potential signaling pathways that play a role in the neuroprotective outcomes of Rhubarb treatment. Bioinformatics analysis demonstrated that oxytocin signaling, synaptic vesicle cycling, and cGMP-PKG signaling circuits which have been previously shown to affect ischemic stroke and the central nervous system. Targeted perturbation of the aforementioned signaling pathways may lead to more significant ischemic-based therapies.

Recently, a meta-analysis was performed involving multiple genome-wide expression profiles for cebebral stroke (Moreno-Ramírez, Gutiérrez-Garzón, Barreto, & Forero, 2018). Meta-analysis studies consist of an aggregate of statistical measurements from several similar studies exploring a distinct biological question. These studies are informative because they facilitate the resolution of mixed results and elucidate trends regarding biological processes and genetic mechanisms that are not detectable by examination of only one bioinformatics study. Following statistical procedures, Moreno-Ramírez et al. (2018) identified 41 differently expressed genes with 28 appearing to be upregulated and 13 appearing to be down regulated. These results also indicated that inflammatory pathways indeed play a role in ischemic stroke as reported in other articles (Lambertsen, Finsen, & Clausen, 2019; Parrella, Porrini, Benarese, & Pizzi, 2019). These types of studies offer the best way to edify our knowledge regarding ischemic stroke biology and potential treatment strategies.

CONCLUSION

Stroke represents a significant health threat in the United States. It is now well understood that ischemic stroke leads to acute and chronic inflammatory responses and that unregulated inflammatory responses can lead to significant neuronal cell death and poor prognosis in stroke patients. Current

treatment strategies for stroke have a limited therapeutic window.

Although NRG-1 has shown to exert significant neuroprotective effects in rodent models of focal cerebral stroke, specific cellular and genetic mechanisms regarding positive neurological outcomes has yet to be elucidated. Microglial cells are the primary immune cells of the central nervous system. Microglial and neutrophil activation in the brain following cerebral ischemia-reperfusion injury results in serious complications for patients and even death (Lakhan, Kirchgessner, & Hofer, 2009; Yenari, Kauppinen, & Swanson, 2010). Identifying specific molecular mechanisms through bioinformatics analysis that regulate proinflammatory microglial cells and neutrophils is of great importance and may facilitate the design of specific therapeutics to improve cerebral stroke outcomes.

The literature is replete with seminal research articles detailing rudimentary and sophisticated disease-based bioinformatics research reports. Bioinformatics studies have been conducted with disease models at the level of cell culture, animal models, and clinical trials. Many reports on ischemic stroke offer encouraging results on the utility of bioinformatics to identify biomarkers and treatment options. In an effort to elucidate valuable epidemiologic and chemotherapeutic information regarding ischemic stroke future bioinformatics investigations could be designed to compare stroke drugs, ischemic-related medical treatments, and gender and racial differences of stroke patients. Moreover, stroke biologists could employ bioinformatics analysis to compare the efficacy of maintaining a specific diet and exercise regimen after stroke. In these cases, bioinformatics analysis could highlight neuroprotective or neurogenic protein profiles or alert scientists and physicians to lifestyle patterns that produce genetic and protein profiles that lead to negative pathophysiologic outcomes after stroke. Moreover, it would be interesting and potentially illuminating if bioinformatics studies were performed to examine patients with multiple diseases at the same time (e.g., stroke and cancer). These types of studies may offer novel information about both diseases that may prove useful. Further, it is important that researchers couple bioinformatics analysis with elegant molecular quantification experiments (e.g., qPCR, Western blotting) and functional assays (e.g., siRNA) that validate the correlation between genetic and proteomic

analysis with physiological events in humans.

ACKNOWLEDGEMENTS

This work was supported by a grant funded by the National Science Foundation (HRD-1533536). The opinions that are discussed in this review article are solely those of the author.

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