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Drug Screening Based on Network Pharmacology and Molecular Docking Targeting the Brg1/iNOS/HO-1 Signalling Pathway

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ABSTRACT

Objective: To investigate the role of Brg1, iNOS, and HO-1 in HSE through network pharmacology, and then targeted the pathway for screening of drugs.

Methods: Firstly, the disease targets of "Herpes Simplex Virus Encephalitis" were collected through TTD, OMIM, Drugbank, Genecards and PharmGKB, and the core targets of Brg1, iNOS, and HO-1 were collected through String database. The core targets of Brg1, iNOS, and HO-1 were collected from String database, and Venn diagrams were created to obtain the intersection of the targets. Then, the literature was reviewed to explore the expression of intersecting targets in diseases and the reverse drug screening was performed by Connectivity Map. After uploading the two intersections to the David database, GO bio enrichment analysis was performed. Finally, the screened drugs were molecularly docked with Brg1, iNOS, and HO-1 to explore the interactions.

Results: Using "Herpes Simplex Virus Encephalitis" as the keyword, 1004 disease targets were screened, and 33 core targets of Brg1, iNOS, and HO-1 and 21 intersecting targets were obtained from the String database. Dipyridamole as a potential drug for the treatment of HSE obtained by reverse drug screening with Connectivity Map. The results of GO suggest that positive regulation of pri-miRNA transcription from RNA polymerase II promoter, response to xenobiotic stimulus, response to drug. Molecular docking showed that dipyridamole binds strongly to Brg1, iNOS, and HO-1.

Conclusion: Dipyridamole can play a role in alleviating HSE by regulating Brg1, iNOS, and HO-1 pathways. This study provides a new idea for the mechanism of HSE occurrence, a new drug for the treatment of HSE, and broadens the clinical application of dipyridamole.

INTRODUCTION

Herpes simplex virus encephalitis (HSE) is an acute infection of the central nervous system caused by Herpes simplex virus type 1 (HSV-1), with the incidence in children accounting for 1/3 of the disease Zhang et al. (2022). HSE is the most common cause of acute childhood sporadic encephalitis, with complications such as convulsions, lethargy, and brain herniation, with high rates of disability and impairment and mortality of up to 90% Li et al (2022). The mechanism and treatment of HSE is still a worldwide problem and a research hotspot. Therefore, it is very important to explore novel drugs and targets for clinical practice in HSE treatment. It has been found that HSV-1 infection increases neuronal inflammation and oxidative stress, with hippocampal neurons being the main target Lindsberg et al. (2002). After infection, reactive oxygen species (ROS) increase dramatically and the level of redox is disturbed. Apoptosis increases and aggravates the inflammatory response, leading to neurological impairment and brain tissue damage, with a very poor prognosis Chen et al. (2015). Brahma-related gene 1 (Brg1), inducible nitric oxide synthase (iNOS), and heme oxygenase-1 (HO-1) occupy an important position in the development of oxidative stress, inflammation level increase, and neuronal apoptosis in neurons closely. Therefore,we hypothesize that Brg1 could maintain neuronal structure and function by down-regulating iNOS expression, decreasing Nitrogen monoxide (NO) release, reducing neuronal oxidative stress and inflammation levels, and in turn up-regulating HO-1 levels, enhancing neuronal antioxidant capacity. In recent years, with the development of technology, network pharmacology has taken an important position in the development of pharmaceutical business. Network pharmacology is an emerging, developing, and rapidly accessible new subject that integrates proteomics, systems biology, and

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and multivariate pharmacology Li et al. (2013). Currently, many drugs can be used to treat diseases by precisely modulating a single target, thus reducing the side effects of the drug, but once the "off-target" phenomenon occurs, the efficacy of the drug will be reduced or even ineffective. The key principle of network pharmacology is to apply a network-based approach to construct multi-level networks, which allows predicting and exploring the therapeutic effects and mechanisms of action of drugs at the tissue and molecular levels in the treatment of complex diseases. This also reflects the multi-target, multi-mechanism and multi-faceted therapeutic effects.

We will use network pharmacology to find drug that can specifically regulate Brg1, iNOS and HO-1, and provide a novel strategy, methods and drugs to alleviate HSE symptoms in children.

MATERIALS AND METHODS

Collection of HSE-Related Genes

The targets of HSE were collected from the disease databases OMIM (https://omim.org/, accessed on March 10, 2023), Drugbank (www.drugbank.ca, accessed on march 10, 2023) Genecards (https://www.genecards.org, accessed on March 10, 2023) and PharmGKB (https://www.pharmgkb.org/, accessed on March 10, 2023) by using the keyword "Herpes Simplex Virus Encephalitis", and the duplicate values were removed from the target of HSE.

Acquisition of core proteins of Brg1/iNOS/HO-1 signaling pathway

In the String database, select 'multiple proteins', input Brg1, iNOS, HO-1 proteins, select 'Homo species', search for core proteins related to Brg1, iNOS, HO-1, export to 'tsv' format, use Cytoscape software for network analysis, and set the size and colour of the targets by Degree values.

Acquisition of Brg1/iNOS/HO-1 signaling pathway and HSE intersection targets

The disease targets of HSE and the core targets of Brg1, iNOS, HO-1 were uploaded to the Venn online website for obtaining the intersecting targets, which were the target proteins of Brg1, iNOS, HO-1 with HSE. Furthermore, the expression of the intersecting target proteins in HSE was reviewed through the literature.

Reverse drug derivation

Upregulated targets were uploaded to the Connectivity Map and using the plugin Clue Query to screen for new drugs. Based on positive scores suggestive of deterioration, negative scores suggestive of treatment and combined with literature research and clinical applications, drugs with therapeutic potential were chosen.

Bio enrichment analysis of intersectional targets

The 21 intersecting targets were uploaded to the David (https://david.ncifcrf.gov/, accessed on March 13, 2023) database for GO enrichment analysis, which consisted of three main categories, biological processes (BP), cellular composition (CC) and molecular function (MF).

Molecular docking

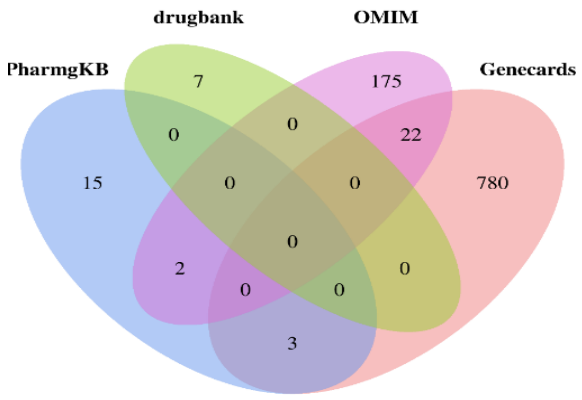
The 3D structures of Brg1, iNOS and HO-1 were downloaded from PDB database and target drug was downloaded from PubChem database, and using PyMOL and Auto Dock Tools 1.5.6 software for pre-processing, including removal of ligand proteins and water molecules. The molecular docking was run through Auto dock vina and the docking results were scored. The docking results were visualised using Discovery studio.

RESULTS

Target information for HSE

The HSE targets sequences were investigated through the OMIM, Drugbank, Genecards and PharmGKB databases and 233, 7, 805 and 20 HSE targets were obtained, respectively. After removing duplicate values, we obtained a total of 1004 disease targets (Figure 1).

Figure 1: Venn diagram of HSE disease targets



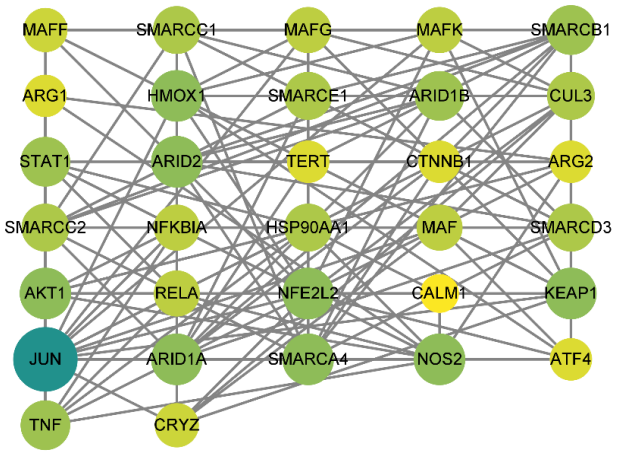
Core targets of the Brg1/iNOS/HO-1 pathway

The results are shown in the table1 and Figure 2. Brg1, iNOS, and HO-1 were each derived from 11 core proteins, the 11 core proteins were derived from Brg1, iNOS, and HO-1 respectively, and the JUN protein was a protein that intersected with iNOS and HO-1. Then the network was analysed by the 'tools' in Cytoscape and ranked according to the Degree value. the results suggested that JUN, SMARCA4, HMOX1, NOS2 and KEAP1 proteins occupied important positions in the PPI.

Table 1: The core proteins corresponding to Brg1, iNOS, HO-1

Brg1	iNOS	HO-1
SMARCE1	NOS2	BLVRB
SMARCC1	AKT1	BLVRA
SMARCC2	RELA	FECH
SMARCD3	HSP90AA1	HEPH
SMARCB1	NFKBIA	HMOX1
SMARCA4	JUN	NFE2L2
ARID2	TNF	FOS
ARID1A	STAT1	JUN
ARID1B	ARG1	IL-4
CTNNB1	ARG2	CREB1
TERT	CALM1	CAV1

Figure 2: Figure 2. PPI network results for Brg1, iNOS, HO-1 core targets.



Intersectional targets of Brg1/iNOS/HO-1 core targets and HSE

The intersection of the Brg1/iNOS/HO-1 core protein with the 1004 targets of HSE by Venn mapping showed a total of 21 intersecting targets: STAT1, TNF, RELA, HSP90AA1, JUN, TERT, CTNNB1, NFKBIA, AKT1, NOS2, CREB1, NFE2L2, FOS , SMARCB1, CAV1, HMOX1, SMARCA4, ARG1, SMARCC2, SMARCE1 and SMARCC1.By searching the relevant literature, we concluded that the target proteins upregulated in HSE or other diseases are tert Smith et al.(2013), Haberichter et al.(2015), smarce1 Huang et al.(2020), ctnnb1 Barilet al.(2013), fos Liu et al.(2019), jun Gober et al.(2005), creb1 Zhu et al.(2022), cav1 Mao et al. (2019), smarcc1 Cai et al. (2021), smarca4 Huang et al. (2020), smarcb1 Li et al.(2022), cul3 Chen et al.(2023).

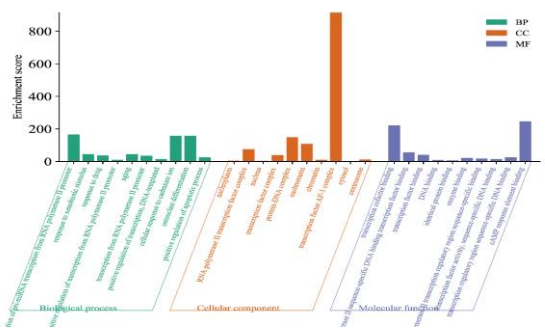
Reverse drug derivation results

The above upregulated genes were uploaded, and a total of 2239 drugs were predicted. Based on the scoring and various factors such as literature research and clinical application, dipyridamole was finally selected as the target drug in this study.

Results of core target bio enrichment

The intersection targets of Brg1/iNOS/HO-1 and HSE targets were uploaded to the David database with the GO results showing that the main biological processes involved include positive regulation of pri-miRNA transcription from RNA polymerase II promoter, response to xenobiotic stimulus, response to drug ; cell composition involved nucleoplasm, RNA polymerase II transcription factor complex, nucleus; molecular functions involved transcription cofactor binding, RNA polymerase II sequence-specific DNA binding transcription factor binding, transcription factor binding.

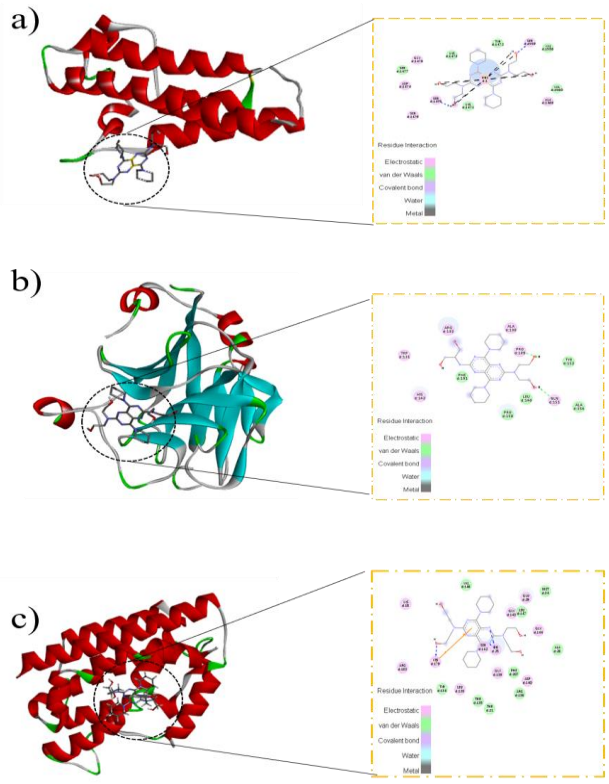
Figure 3: The core target GO Bio enrichment results



Molecular docking results

As the table shows, the docking results of Brg1, iNOS, HO-1 and dipyridamole were as follows. The binding energies of all were less than -5 kcal/mol, indicating that dipyridamole binds tightly with Brg1, iNOS and HO-1. Figure 4a illustrated that dipyridamole could bind to Brg1 by forming van der Waals forces with LYS d:1473, LYS d:1471,VAL d:1560, VAL d:1556 and electrostatic interactions with SER d:1559,SER d:1475, ALA d:1468; Figure 4b showed that dipyridamole could bind to iNOS by forming electrostatic interactions with amino acids GLN d:151,PRO d:189, ALA d:190 and van der Waals forces with ALA d:154,TYR d:152, LEU d:150; as shown in figure 4c, dipyridamole could bind to iNOS by forming Pi-pi conjugate bonds with LYS d:183, SER d:142,GLY d:144, GLU d:29 to form electrostatic interactions and van der Waals forces with MET d:34, VAL d:146, ALA d:28 to bind to HO-1.

Figure 4: Docking pattern of dipyridamole with Brg1, iNOS, HO-1. a) dipyridamole with Brg1. b) dipyridamole with iNOS. c) dipyridamole with HO-1



DISCUSSION

HSE remains a significant focus of research due to its severe impact on the central nervous system. Currently, studies are aimed at enhancing our understanding of HSE pathogenesis, diagnosis, treatment, and prevention. Research efforts have focused on elucidating the molecular mechanisms underlying viral entry, replication, and immune response modulation. The incidence is high in children, and untimely treatment can have serious and potentially fatal consequences. Our network pharmacology and previous studies in the literature suggest that Brg1, iNOS and HO-1 play an important role in the mechanism of HSE development, then we searched for the core targets related to Brg1, iNOS and HO-1 through PPI network. Furthermore, based on network pharmacology targeting Brg1, iNOS, and HO-1 for reverse drug screening, and finally selected dipyridamole as a potential drug for treatment. Through data collection of targets, we mined 1004 targets of HSE and obtained a total of 33 core targets of Brg1, iNOS and HO-1. Brg1 mediates apoptosis through its involvement in DNA damage Johnson et al. (2008), Fang et al. (2013). Studies have shown that in neuronal cells, Brg1 can enhance neuronal viability, reduce neuronal apoptosis and exert neuroprotective effects by promoting the formation of Z-DNA and recruiting the aggregation of RNA polymerase, thereby activating the expression of the antioxidant protein HO-1 in the downstream Fang et al. (2013), Li et al. (2018), Stocker et al. (1987), Zhang et al. (2006). It was also shown that in liver and cardiac myocytes, increased expression of Brg1 enhanced cellular antioxidant capacity, reduced cellular damage and protected the liver and heart from ischemia/reperfusion injury Ge et al. (2017), Ge et al. (2017), Gao et al. (2016), Li et al. (2015). In addition, Brg1 can modulate immune function by modifying the sensitivity of regulatory T cells to inflammatory signals, thereby suppressing auto-inflammatory responses rapidly and effectively Chaiyachati et al. (2013), Qi et al. (2021). Viruses and oxidative stress are closely related. Oxidative stress is defined as a state of balance between pro-oxidant and antioxidant systems, and when the equilibrium is disturbed then it means that physiological functions will be altered as a result. iNOS is found in neurons, and the sustained production of large amounts of NO plays an important role in oxidative stress, inflammation and apoptosis. Studies have shown that increased levels of intracranial oxidative stress led to accelerated degeneration of hippocampal neurons, and that pro-inflammatory factors induce iNOS production, leading to increased levels of ROS, causing increased levels of mitochondrial dysfunction and accelerated apoptosis of hippocampal neurons Liu et al. (2010), Nam et al. (2012). Zhang et al. established a model of pneumococcal pneumonia by intracranially injecting pneumococci into baby rats and administered edaravone (a free radical scavenger with powerful antioxidant, anti-inflammatory and neuroprotective effects), which was found to reduce the production of iNOS and ROS in hippocampal neurons, which then upregulated the expression of HO-1 in hippocampal neurons, restored oxidation-reduction homeostasis, adjusted the function of hippocampal neurons, produced neuroprotective effects and improved the physiological function of the brain Li et al.(2016). Kim

et al. (2017). In PPI network by Drgee value for target analysis, JUN occupies an important position in this network with the darkest color. It was shown that JUN is intimately related to HSE, and c-JUN accelerates the process of HSE Song et al. (2014). The level of c-JUN was significantly increased in HSV-1-infected hippocampal tissue, and c-JUN was involved in the apoptotic process induced by HSV-1 in hippocampal cells Perkins et al. (2003). In summary, Brg1, iNOS and HO-1 have been inextricably linked to HSE and play an important role in the progression of HSE.

A total of 8560 potential compounds were obtained by the reverse drug screening through the Connectivity Map and the Clue Query plug-in, and the compounds with negative scores were the potential drugs that modulate Brg1/iNOS/HO-1 to alleviate HSE, with a total of 4119 compounds. There were 739 compounds with scores below -90, with the more advanced compounds were canertinib and pirlindole. Canetinib is an irreversible EGFR inhibitor with potentially anti-tumor effects; poroxindole is a monoamine oxidase 1 inhibitor, and studies have shown that poroxindole inhibits both enterovirus D68 and coxsackievirus B3. However, in combination with the clinical pediatric drug safety examination index, both compounds were excluded as potential drugs for the treatment of pediatric HSE. Dipyridamole, with a score of -92.13, was selected as the subject of the research. Studies have shown that dipyridamole displays antiviral activity against various RNA and DNA viruses by inhibiting viral replication, reducing viral entry, and modulating host immune responses, such as also being effective against viral infections such as viral hepatitis, epidemic B encephalitis, dengue, mumps, and chickenpox. In addition, the anti-inflammatory and neuroprotective properties of dipyridamole make it a promising candidate for reducing neuroinflammation and neuronal damage associated with HSE, for restoring intestinal immune homeostasis and improving intestinal inflammation.

Moreover, we docked dipyridamole with Brg1, iNOS and HO-1 respectively, and the results showed that dipyridamole was strongly connected to Brg1, iNOS and HO-1 in space structure and bound tightly. It indicates that dipyridamole has an influence on Brg1, iNOS and HO-1.

In conclusion, this study was an original discovery of the important roles of Brg1, iNOS and HO-1 in HSE through the previous literature. We explored the core targets of Brg1, iNOS and HO-1 based on network pharmacology and reverse the drug screening to obtain dipyridamole with therapeutic HSE. The findings from this analysis shed light on the potential repurposing of dipyridamole as a therapeutic option for HSE and provide a foundation for future research in this field.

DECLARATIONS

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