CHAPTER 40

MEDICAL SCIENCES
BIOMEDICAL RESEARCH

Doctoral Theses

01. ADHANA (Sujata)
Targeting the moonlight enzyme, glutamate racemase, to combat antibiotic resistance in Neisseria gonorrhoeae
Supervisor: Prof. Uma Chaudhry
Th 28389

Abstract

The emergence of drug-resistant Neisseria gonorrhoeae has led the World Health Organization (WHO) to classify it as a high-priority pathogen, demanding urgent advancements in diagnostics and therapeutics. This doctoral research presents a comprehensive, translational approach—spanning field diagnostics, computational drug discovery, and experimental validation—to combat this escalating public health threat. A low-cost diagnostic tool using the Foldscope—a paper-based microscope developed by Dr. Manu Prakash—was standardized for N. gonorrhoeae detection and deployed across aspirational districts in Rajasthan and Bihar. The tool achieved 95% sensitivity in male clinical samples, enabling early detection in resource-limited settings and revealing critical gaps in rural anti-microbial resistance (AMR) surveillance. Therapeutically, this study prioritized glutamate racemase of N. gonorrhoeae (NgGR), a multifunctional moonlight protein essential in bacterial peptidoglycan biosynthesis and predicted to interact with DNA gyrase. Virtual screening identified bisleucocurine A and nitrofurazone as promising inhibitors. Nitrofurazone, in particular, demonstrated dual-site binding to both the catalytic domain and the DNA-gyrase interaction interface of NgGR. Molecular dynamics simulations confirmed stable inhibitor–NgGR complexes. Epitope mapping further revealed conserved B-cell and T-cell epitopes, supporting NgGR's potential as a vaccine target as well. Experimental validation included successful cloning, expression, and purification of recombinant NgGR. Enzyme inhibition assays and isothermal titration calorimetry (ITC) confirmed nitrofurazone's specific, high-affinity binding, with ~50% enzymatic inhibition at 50 μM. The same concentration led to near-complete bacterial growth inhibition, indicating possible multitarget action. Supporting this, docking studies showed strong binding of nitrofurazone to HtpX, a stress-response protease, suggesting additional intracellular targets. This study highlights a laboratory pipeline to tackle gonococcal AMR, identifying NgGR as a novel druggable and immunogenic target. Nitrofurazone emerges as a multitarget antimicrobial agent, offering potential in combination therapies and guiding future vaccine strategies against drug-resistant N. gonorrhoeae. Collectively, our data points towards negative role of TLRs utilized by BCG in inhibiting protective defense responses by various mechanisms.

Contents

Neisseria gonorrhoeae for Novel Inhibitor Discovery 6. To evaluate Nitrofurazone as a Dual-site Inhibitor of Neisseria gonorrhoeae Glutamate Racemase (Ng-GR), and to assess its in vitro Efficacy in Inhibiting Bacterial Growth 7. Collateral Binding: Probing Nitrofurazone's Interaction with Ng-HtpX as a Potential Off-target in Neisseria gonorrhea 8. Thesis Summary. References.

02. ARORA (Ritu)
Discovery and Characterization of Mycobacteriophages and Endolysins as Potential Antimycobacterial Agents
Supervisor: Prof. Urmi Bajpai
Th 28390

Abstract

Antimicrobial resistance (AMR) is one of the major threats to global health today. The World Health Organisation (WHO) has recognised AMR as one of the top ten public health threats humanity is facing globally. Mycobacteria cause a wide spectrum of diseases affecting both humans and animals. Mycobacterium tuberculosis is among the most significant human pathogens. The recent surge in drug-resistant TB in 2022, coupled with an increase in non-tuberculous (NTM) infections, is a cause of concern, which mandates discovering novel drugs and exploring alternative non-traditional therapeutic approaches. Mycobacteriophages, that specifically infect mycobacterial species are genetically diverse and are a promising source of novel antimycobacterial agents, including lytic enzymes (endolysins), especially in light of multi-drug resistanceTB. In contrast to Gram-positive or Gram-negative bacteria, mycobacteria cell membrane is more complex. This includes an outer membrane consisting of a lipid layer and mycolic acids, linked to arabinogalactan, which is in turn attached to the peptidoglycan. To overcome this additional barrier, most mycobacteriophages encode two lytic enzymes: LysinA And LysinB. LysinA targets peptidoglycan and LysinB cleaves the linkage between the mycolic acids and the arabinogalactan-peptidoglycan complex. Due to their targeted bacteriolytic activity, endolysins are promising alternatives/complements to antibiotics. In this study, mycobacteriophages were isolated using Mycobacterium smegmatis Mc2 155 as the host. A total of 18 mycobacteriophages were purified, of which five were selected for detailed characterization. From three selected phages, five endolysins were purified--three LysinA and two LysinB proteins. These lysins were characterized both in silico and in vitro. Both the isolated phages and lysins were tested against clinically relevant pathogenic mycobacterial species: M. fortuitum NIHJ1615, M. tuberculosis H37Rv and M. tuberculosis 6206, a double-mutant strain of H37Rv. Notably, two mycobacteriophages and one LysinB enzyme exhibited anti-Mycobacterium tuberculosis activity, demonstrating their potential as therapeutic agents.

Contents

03. BHARDWAJ (Sachin)
Analyzing MGMT (O6 -Methylguanine DNA methyltransferases) Regulatory Expression under the Influence of Splice Factor Proteins in Glioma cells.
Supervisor: Prof. Ajay Kumar Yadav
Th 28724

Abstract

Glioblastoma is the most aggressive type of malignancy that arises from glial cells, characterized with poor prognosis and a median overall survival of approximately 15 months. Chemotherapy resistance in Glioblastoma against first-line drug Temozolomide (TMZ) and TRAIL-based therapies poses a major clinical challenge. According to the WHO`s latest guidelines of Glioma classification, this study explores novel molecular mechanisms driving resistance and therapeutic response through splice factor protein hnRNPA1 and SF2/ASF1 regulation, deubiquitinating enzyme USP5 and USP8 regulation, and natural compounds of Withania somnifera. Increased expression of the splice factor SF2/ASF correlated with the higher isoform of hnRNPA1 (Var2) and MGMT upregulation in temozolomide-resistant cells, contributing to resistance. Knockdown of SF2/ASF1 reversed hnRNPA1 isoform switching from Var 2 to Var1. Knockdown or downregulation of hnRNPA1 Var2 results in downregulation of MGMT. Knockdown of hnRNPA1 Var 2 sensitizes cells to TMZ-induced apoptosis, especially when combined with PI3K inhibitor Wortmannin. In parallel, we investigated the role of deubiquitinating enzymes (DUBs), particularly USP5 and USP8, in regulating MGMT expression. TMZ-resistant U87MG and LN229 cells showed increased protein levels of USP5, USP8 MGMT, whereas MGMT was absent in LN229 TMZ glioma cells. siRNA-mediated knockdown of USP5 reduced MGMT expression and triggered apoptosis and autophagy, indicated by SMAC and LC3B-II upregulation. Immunofluorescence revealed cytoplasmic co-localization of USP5 and MGMT in U87 TMZ-resistant cells and patient samples. USP8 knockdown reduced MGMT independently of USP5, and its silencing, along with proteasome inhibition, further enhanced MGMT degradation, suggesting their distinct yet critical roles in resistance. Additionally, we explored the effect of Withania somnifera fruit extract (WS-FE) in TRAIL-resistant U87 and LN229 glioma cells. Ws-FE treatment increased USP5 expression, promoting cell survival. However, USP5 knockdown with Ws-FE enhanced DR5 and SMAC, Caspase 9, and Caspase 3 expression, restoring apoptosis in U87MG and LN229 cells. Collectively, our findings highlight splice variants of hnRNPA1 Var2 and DUBs USP5/USP8 as key modulators of resistance, with WS-FE offering a potential combinatorial strategy. Targeting these molecules may overcome resistance and improve therapeutic outcomes in glioma.

Contents

04. KRISHNA (Snigdha)
Investigating the Impact of Environmental Pollutants and Serum Metabolites on Transthyretin Amyloidosis.
Supervisors: Prof. Sunit K Singh and Prof. Laishram Rajendrakumar Singh
Th 28727

Abstract

Transthyretin (TTR) is an evolutionary conserved, homo-tetrameric, 55kDa transport protein synthesised mainly in the liver and choroid plexus with additional synthesis also detected in retinal epithelium, uterus, placenta and pancreatic islets. The main

function of TTR is transport of thyroxine (T4) and retinol-binding protein (RBP). The binding of thyroxine assists in maintaining stability of the native tetramer as dissociation of the tetramer into monomeric subunits and subsequent misfolding leads to formation of toxic amyloidogenic TTR species which are associated with myriads of diseases including cardiomyopathy, polyneuropathy, diabetes, preeclampsia, arthritis, dementia, cognitive impairment, depression, to name a few. Thus, maintenance of the TTR tetramer stability holds both physiological and pathophysiological importance. In the present study, we evaluated environmental pollutants and pH as destabilising agents and serum metabolites as stabilisers towards TTR. We found that Bisphenol-A (BPA), a microplastic, binds to TTR and acts strongly as a destabiliser of TTR structural-functional integrity and induces spontaneous formation of TTR amyloid fibrils leading to harmful cellular consequences. We also performed a systemic analysis of the role of pH on the structure-function paradigm and aggregation propensity of TTR and our findings define a pH-dependent threshold for TTR destabilization and aggregation, offering mechanistic insights into amyloid initiation and highlighting local pH as a determinant of disease-relevant aggregation pathways. In our study on serum metabolites, we identified taurine, a semi-essential amino acid, as a stabiliser of native tetramer which binds to TTR, enhances the T4 binding capacity and exhibits anti-aggregation potential towards TTR amyloidosis. Mechanistic insights revealed that taurine alters the internal dynamics or microstates of the native state of protein. This discovery of functional regulation of TTR by taurine holds clinical relevance in repurposing of taurine (an FDA-approved supplement) for TTR amyloidosis and TTR-induced hypothyroidism, especially in neuronal and gestational impairments.

Contents

05. NEELAM BHOLA

Investigating TIMP3 as A Novel Target of p73 and to Elucidate its Role in Angiogenesis, Metastasis Progression and Immune Dynamics in Colorectal Cancer.

Supervisor: Prof. Sunit K. Singh

Th 28725

Abstract

Colorectal cancer (CRC) is the third most common cancer worldwide and presents formidable challenges in both its diagnosis and treatment globally. The tumor suppressor p53`s structural and functional homologue, p73, has the ability to identify potential p53 binding sequences and trigger the transcription of target genes that are p53-responsive and engaged in various cellular functions. The exact mechanism by which p73 transforms cells remains unclear. We identified 70 key genes involved in CRC angiogenesis and prognosis, followed by the extraction of 20 hub genes. Various genes showed significant upregulation upon expression analysis, and several showed variation in expression among different tumor stages. The majority were also significant in the prognosis of CRC patients upon Kaplan–Meier analysis. Expression of MMP's was observed to be upregulated in HCT116 p53-/-p73kd cells. Parallelly, we identified tissue inhibitor of matrix metalloproteinase 3 (TIMP3), an endogenous inhibitor of matrix metalloproteinases, as a novel transcriptional target of p73, upregulated by DNA damage in a p73-dependent manner, confirmed by site-directed mutagenesis, ChIP, luciferase reporter assays, and bioinformatics analysis. Upregulation of various MMPs was validated using

GEO2R datasets where p53,p73 and TIMP3 were downregulated and MSigDB data of EMT genes. The genes were used to construct protein interaction networks using STRING, and hub genes were obtained via Cytoscape. Most EMT genes were associated with TIMP3 and p73. Collectively, we provided proof that p73 triggers TIMP3 expression in response to genotoxic stress, performing an anti-metastatic role. TIMP3 is a known inhibitor of MMPs and ADAMs; however, its influence on CRC prognosis and immune infiltration remains poorly explored. TIMP3 was significantly downregulated in CRC tissues, correlating with poor overall and relapse-free survival, and its expression was linked with increased infiltration of diverse immune cells, highlighting its pivotal role in CRC progression and tumor microenvironment regulation.

Contents

06. PRIYA

Role of Casein Kinase 2 (CK2) Signalling in Mesial Temporal Lobe Epilepsy (MTLE)

Supervisor: Dr. Aparna Dixit and Dr. Jyotirmoy Banerjee

Th 28822

Abstract

MTLE is associated with dysregulated excitatory-inhibitory balance in the brain. Casein Kinase 2 is a serine/threonine kinase constituting two catalytic (α and α′) and two regulatory β subunits. CK2 phosphorylates NMDAR subunit NR2B at Ser1480 altering receptor trafficking and localization. Although CK2α and CK2α′ share high sequence homology, they exhibit distinct substrate specificities and functional roles. Importantly, CK2 and Wnt/β-catenin signaling pathways converge on several processes which contribute to epilepsy. However, their precise roles in MTLE remain unclear. This study investigated CK2 expression, activity, and downstream signaling in hippocampus and ATL regions of tissues resected from MTLE -HS patients, acute and chronic TLE models. Gene and protein expression of CK2, NMDA receptor subunits (NR2A, NR2B), and Wnt/β-catenin signaling molecules were quantified in different regions. Functional roles of CK2α and α′ subunits were further investigated using siRNA mediated knockdown in acute TLE model. Significant increase of CK2 and NMDA receptor subunit levels was observed in MTLE patient and in the chronic TLE. Elevated CK2α expression correlated with enhanced NR2B phosphorylation and greater seizure severity, while CK2α knockdown reduced seizure frequency, diminished NR2B phosphorylation, and preserved neuronal morphology in different regions of siRNA mediated knockdown of acute TLE. In contrast, CK2α′ activity was also elevated in chronic TLE, but its knockdown led to increased pNR2B, increased seizure susceptibility, and dysregulation of Wnt/β-catenin target genes. CK2 and β-Catenin colocalization in the Chronic TLE, validated the crosstalk between the two. CK2 subunits demonstrate distinct roles in MTLE, with CK2α contributing in NR2B phosphorylation, therefore leading to hyperexcitability, while CK2α′ exerted a protective effect by restricting NR2B phosphorylation differentially in hippocampus and ATL. Also, CK2-mediated regulation of Wnt/β-catenin signaling, might contributes in chronic TLE. These results highlight the significance of subunit and regions specific CK2 regulation in MTLE and possible therapeutic targets for intervention

Contents

Introduction 2. Review of literature 3. Subunit specific altered expression and activity of casein kinase 2 in the brain tissues resected from mesial temporal lobe epilepsy with hippocampal sclerosis patients & rodent temporal lobe epilepsy mode4. Conclusion and Future Perspective 4. divergent functions of casein kinase 2 catalytic subunits ck2α and ck2α′ in mtle: differential regulation and modulation of nr2b excitotoxicity and wnt/β-catenin mediated network plasticity 5. Summary.

07. SAMRA (Monika)
To Study the Polymorphism, Differential Expression of Growth Differentiation Factor 15 in Coronary Artery Disease and Development of its Inhibitor using Insilico Approach.
Supervisors: Dr. Indu Arora and Dr. Kamna Srivastava
Th 28821

Abstract

Coronary artery disease is one of the major causes of death worldwide. Although there are many advancements in its management, traditional biomarkers lack in term of specificity and sensitivity and detect CAD in late stage of disease. The present study aims at exploring the role of Growth Differentiation Factor 15 (GDF15) and Neuregulin-1 (NRG1) as a predictive marker of coronary artery disease The present study is a case-control study in Asian Indian population, where the association of GDF15 rs4808793 (-3148G/C) promoter polymorphism with CAD was studied. The CG+GG genotype depicted significant association with CAD with an odd ration of 2.18, $p<0.0001$. The GDF15 levels significantly elevated in CAD patient with 5.78 folds at mRNA and 2.39 folds at protein level. Its levels also elevated with increase in CAD severity. NRG1(a co-expressing gene of GDF15) expression was found to be elevated with a 7.78-fold increase in mRNA and a 1.89-fold increase in protein levels. Its increased expression showed significant association with CAD. Both these markers depicted progressive elevation from Non coronary artery (NCA) to stable angina (SA) and most elevated in acute coronary syndrome (ACS) suggested strong ability for early detection and risk stratification. The risk assessment accuracy was found to be 83% and 87% for GDF15 and NRG1 respectively. The elevated expression of GDF15 in CAD suggested that it can be used as a therapeutic target for CAD treatment. In silico drug discovery approach was employed for identification of potential GDF15 inhibitors. After screening different compound libraries, few compounds with best binding affinity were chosen for further analysis. Out of the selected compounds, obacunone showed best pharmacokinetic and Pharmacodynamic properties. MD simulation of Obacunone-GDF15 complex at 100ns confirmed the stability of the complex. The consistent RMSD thus suggested obacunone to be a potential inhibitor for GDF15.

Contents

1. Coronary Artery Disease: Risk Factors, Biomarkers, and Therapeutics 2. Role of Growth Differentiation Factor (Gdf15) and Coronary artery Disease 3. Role of Neuregulin 1 in Coronary Artery Disease 4. Identification of GDF15 Inhibitor Using Insilico Approach. Bibliography.

08. SHANKER (Ozasvi R)
Elucidating the Role of Protein Tyrosine Kinase 2 (Pyk2) in Temporal Lobe Epilepsy: Mechanistic Insights and Therapeutic Targeting.
Supervisor: Dr. Aparna Dixit and Dr. Jyotirmoy
Th 28396

Abstract

Temporal lobe epilepsy (TLE) is a widespread and challenging neurological disorder, often resistant to conventional treatments. A key player in TLE is protein tyrosine kinase 2 (Pyk2), a calcium-sensitive, non-receptor tyrosine kinase involved in neuronal activity and hyperexcitability. This study aimed to investigate Pyk2's expression, phosphorylation, and activity in different brain regions of TLE patients and a rat model, assessing both acute and chronic stages. Male Sprague Dawley rats were used in the lithium-pilocarpine model to simulate TLE. Methods such as western blotting, qRT-PCR, kinase assays, and FACS were employed to evaluate Pyk2's role, with pharmacological inhibition using PF-4618433 to explore its effect on seizure control. The results showed significant Pyk2 phosphorylation at Tyr402 in the hippocampus and anterior temporal lobe of MTLE patients, along with increased intracellular calcium. In rats, Pyk2 activation exhibited region- and stage-specific patterns, extending to cortical areas in chronic TLE model. Inhibition of Pyk2 using PF-4618433 reduced its activity, leading to a marked reduction in seizure severity. Continuous seizure monitoring confirmed lowered seizure frequency and intensity upon Pyk2 inhibition, supporting its role in regulating hyperexcitable neural networks. These findings underscore the temporal regulation of Pyk2 activation potentially regulated by calcium and its link to epileptogenic network progression. Targeting Pyk2 pathways offers a potential therapeutic approach for TLE, potentially reducing seizure severity and fostering network remodelling. Future research should focus on the long-term effects and optimization of Pyk2-targeting strategies to improve the clinical management of epilepsy.

Contents

09. SHARMA (Bhavna)
Therapeutic Potential of Demethoxycurcumin in Glioblastoma Treatment: Cytotoxic Effects on Primary Astrocytes and Modulation of Autophagy-Apoptosis-Migration Axis in Human Glioma Cells.
Supervisor: Prof. Sunit K Singh and Prof. Pratibha Mehta Luthra
Th 28726

Abstract

Glioblastoma multiforme (GBM), the most aggressive primary brain tumor, demonstrates poor prognosis due to inherent chemoresistance and rapid recurrence following standard temozolomide therapy. Therefore, development of newer drug molecules is still required. Natural compounds with multi-target potential offer promising alternatives for overcoming therapeutic resistance. Previously, it has been shown that curcumin exhibits strong potential to induce apoptosis of glioblastoma cells. To improve efficacy, it is crucial to investigate the effect of major curcumin components. This study evaluates demethoxycurcumin (DMC), a bioactive curcumin analog, as a potential anti-glioblastoma agent through systematic investigation of its isolation, cytotoxic selectivity, and mechanistic actions. DMC, along with curcumin and bisdemethoxycurcumin, was successfully isolated from turmeric oleoresin using optimized column chromatography with silica gel and gradient elution systems. Purification of DMC was confirmed by TLC separation and characterized using mass spectrometry, proton and carbon NMR spectroscopy, and IR analysis. Comparative cytotoxicity evaluation by sulforhodamine B assay revealed differential sensitivity across glioma cell lines (U87MG, T98G, LN229) and primary rat astrocytes, with DMC

showing preferential toxicity toward malignant cells while sparing normal astrocytes. Mechanistic studies revealed that DMC induces dose-dependent glioma cell death through reactive oxygen species generation, triggering both apoptotic and autophagic pathways via AKT/mTOR signaling inhibition. U87MG cells were more sensitive to DMC-induced apoptosis and autophagy, whereas migration inhibition was stronger in T98G cells. To elucidate autophagy-apoptosis crosstalk, combination studies with 3-methyladenine (3-MA), a selective autophagy inhibitor, were conducted. LC3B immunofluorescence and Western blot analysis of autophagy markers provided molecular insights into pathway interactions. The results demonstrate the therapeutic potential of DMC through selective cytotoxicity sparing normal brain cells, dual engagement of cell death pathways, and effective migration inhibition. The differential responses across glioma cell lines highlight tumor heterogeneity considerations for personalized therapy. DMC may contribute to arrest tumor recurrence addressing the long-term control of glioblastoma. Overall, DMC shows promise as a candidate for further preclinical investigation in glioblastoma therapy.

Contents

10. SHARMA (Kajal)
Empirical study on the role of quinoline-based ionic liquids in combating antimicrobial resistance (AMR) in the fungal pathogen, Candida albicans.
Supervisor: Dr. Meenakshi Sharma
Th 28395

Abstract

The ever-growing menace of Antimicrobial Resistance (AMR) jeopardizes the potency of the prevailing antibiotics against the relentlessly sprouting infections. In the current situation, the study of antifungal resistance has veered off course due to the frivolity regarding fungal infections. Even though Candida albicans is one of the perilous human fungal pathogen, research on the protein-protein cross-talk that persists in its cell wall is yet to be fully accomplished. Proteins from C. albicans have been found to have characteristics similar to those of amyloids. Prevailing strategies are clearly inutile against the aggregation-induced biofilm formation of this lethal pathogen, entailing the development of potentially innovative anti-fungal arsenals. Strikingly, ionic liquids (ILs), have emerged as essential components of many antibiotic and non-antibiotic treatments. Due to their capacity to refold unfolded proteins, ILs have also been used as artificial chaperones. The impact of quinolinium-based ILs, an unexplored class of chemicals, as antibacterial agents and artificial chaperones must be explained in light of the vast applications of ILs in the biomedical sector. We have, therefore, synthesized of a series of quinoline-based ILs in the current work, specifically 1-Dodecylquinolin-1-ium bromide [C12quin]Br, 1-Hexadecylquinolin-1-ium bromide [C16quin]Br, and 1-Eicosylquinolin-1-ium bromide [C21quin]Br, respectively, using a one pot synthesis approach, advocating the significance of green chemistry. In order to establish pertinent evidence regarding the therapeutic effect of varying the alkyl-chain lengths of the desired compounds, a comparative study of the same was conducted against a number of microbial strains. The ILs were further examined for their ability to curtail amyloid-mediated biofilm formation in C. albicans, using various biological assays, and the explicit mechanism of their antifungal effect was delineated. The drug carrying

capacity and the invivo toxicity analysis of the synthesized ILs, further ratified their biomedical prospects. Moreover, investigation of the chaperone-like effect, of the synthesized ILs on the time-sensitive and self-induced amyloid fibrils that developed in bovine serum albumin (BSA) as a result of extended incubation, inflamed the applications of these synthetic game changers in the protein industry.

Contents

1. Identification, synthesis and characterization of aseries of bio-compatible, quinoline-based ILs as compounds of interest 2. Appraising the biological applications of a series of 1-alkylquinolin-1-ium bromide ILs against various pathogenic microbes, with a rapt attention on the prevailing AMR in C. albicans AMR, traversing through their mechanism of action and toxicity assessment. 3. Results.

11. SINGH (Ankit)
Investigation of A2AR antagonist induced activation of the astrocytic Nrf2/HO1 pathway in neuroprotection using the 6-OHDA-induced primary midbrain neuronal cells isolated from P0/P1 rat pups and SD rat model of Parkinson's disease.
Supervisor: Prof. Madhu Chopra and Prof. Pratibha Mehta Luthra
Th 28824

Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder marked by degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), causing motor and non-motor impairments. Standard dopaminergic therapies, such as levodopa, provide symptomatic relief but lead to adverse effects, including dyskinesia, hallucinations, and mood disturbances. To overcome these limitations, non-dopaminergic strategies are being investigated. Among them, adenosine A2A receptor (A2AR) antagonists show therapeutic promise. This study evaluates two novel A2AR antagonists: AW00032, identified through virtual screening, and TTP, synthesized in-house. The first chapter of the thesis included a decade of literature relevant to PD. In the second chapter, we used a pharmacophore-based selectivity model and molecular docking. AW00032 (N-(furan-2-ylmethyl)-5-methylthiazole-4-yl) thiophene-2-sulfonamide was screened from the MayBridge database. It exhibited strong binding affinity for A2AR (1.23nM, ΔG -10.49 kcal/mol) with favorable ADMET properties. In 6-OHDA-induced SH-SY5Y cells, AW00032 enhanced dopamine content, tyrosine hydroxylase (TH) expression, and mitochondrial function while reducing reactive oxygen species (ROS). Its activity was comparable to the standard antagonist ZM241385, and in haloperidol-induced C57BL/6 mice, AW00032 alleviated behavioral deficits. In the third chapter, we used our lab-synthesized compound, TTP [8-(2-Thioxo-7(3-allyl)-2-(2-furyl)thiazolo[4,3-e]1,2,4-triazolo[1.5-c]pyrimidine], which demonstrated potent binding affinity (Ki 0.016nM) and high selectivity (1260-fold) for A2AR. The synthesis and characterization of the potential A2AR antagonist TTP has been carried out to study its anti-Parkinsonian effect on the 6-OHDA-induced Primary midbrain neuronal (PMDN) cells and unilaterally lesioned Sprague Dawley (SD) rat model of PD. TTP enhanced cell survival, restored mitochondrial membrane potential, and reduced ROS, while restoring catalase, SOD, and dopamine levels. It further inhibited caspase-3 activation and enhanced Bcl-2 expression, effectively preventing apoptosis in 6-OHDA-induced primary midbrain neuronal (PMDN) cells and in the unilaterally lesioned Sprague Dawley (SD) rat model of Parkinson's disease. TTP induced mechanism of neuroprotection was examined via upregulation of the Nrf2/HO-1 pathway in astrocytes in the presence of 6-OHDA. The results demonstrated that TTP enhanced Nrf2 activation and upregulated downstream antioxidants HO-1 and NQO1, thereby suppressing ROS accumulation and protecting

neighboring neurons. Invitro results were also verified using 6-OHDA lesioned invivo SD rat model of PD. In conclusion, A2AR antagonist TTP mediated activation of the Nrf2/HO-1 pathway to protect dopaminergic neurons in 6-OHDA-induced PMDN cells, and the unilateral lesioned SD rat model of PD has been reported for the first time, and it may provide potential target in the therapy of PD.

Contents

1. Review of Literature 2. Discovery of Novel A2AR Antagonist via 3D-QSAR Pharmacophore Modeling: Neuroprotective Effects in 6- OHDA-Induced SH-SY5Y Cells and Haloperidol-Induced Parkinsonism in C57 bl/6 Mic 3. Neuroprotective Effects of TTP Against 6-OHDA-induced Neurotoxicity in Primary Midbrain Neuronal Cells and SD Rat Model of Parkinson's Disease via Modulation of Oxidative Stress, Apoptosis, and Astrocytic Nrf2/HO-1 Pathway 4. Conclusion and Future Perspective.

12. SONALI KUMAR

Comparative analysis of Histone Deacetylases (HDACs) 2, 4 and 6 mediated epileptogenesis in cortical dysplasia (CD) and non-cortical dysplasia (non-CD) pathologies.

Supervisor: Dr. Aparna Dixit and Dr. Jyotirmoy

Th 28397

Abstract

Drug-resistant epilepsy (DRE) poses a significant clinical challenge, particularly in focal cortical dysplasia (FCD), a cortical dysplasia (CD) pathology , and temporal lobe epilepsy (TLE), a non-cortical dysplasia (non-CD) pathology. Histone deacetylases (HDACs) have emerged as crucial epigenetic regulators in epileptogenesis, yet their isoform- and pathology-specific roles remain inadequately defined. This study aimed to compare the expression and functional implications of HDAC2, HDAC4, and HDAC6 in FCD and TLE to uncover potential therapeutic targets. mRNA and protein expression profiles of HDACs were assessed in resected brain tissues from FCD and TLE patients and the animal models. Using quantitative real-time PCR, western blotting, immunofluorescence, co-immunoprecipitation, and biochemical assays for oxidative stress the results revealed that HDAC2 protein expression remained largely unchanged across models except for a mild increase in the TLE hippocampus. These findings suggest a limited and potentially transient role for HDAC2 in chronic epileptogenesis. HDAC4 expression was elevated in the hippocampus and ATL of TLE patients, as well as in FCD cortex, and corresponding rodent models. In TLE, HDAC4 showed cytoplasmic accumulation, disrupting its interaction with the non-histone substrate Serum Response Factor (SRF), leading to increased expression of immediate early genes that might drive neuronal hyperexcitability. HDAC6 was also significantly upregulated in both the pathologies. In FCD, HDAC6 deacetylated the antioxidant protein Prdx1, contributing to oxidative stress. Pharmacological inhibition with Tubastatin A restored Prdx1 function, reduced ROS, and significantly lowered seizure burden. These substrate-specific interactions underscore a broader role for HDACs in non-genomic regulation of epileptogenesis. The results demonstrated HDAC6 and HDAC4 as key modulators epileptogenesis in CD and non-CD pathologies respectively and highlighted the translational potential of developing isoform- and pathology-specific HDAC inhibitors as targeted anti-epileptogenic therapies. Future research should focus on characterizing the wider spectrum of non-histone substrates and downstream networks regulated by these HDACs.

Contents

13. WALECHA (Vaishali)
Study the effect of A2AR Antagonist mediated Mitochondrial Dysfunction via p38MAPK axis in Primary Neurons, Astrocytes and in-vivo Parkinson's Disease model & Investigate the Adult Rat Neural Stem Cells isolated from SVZ and DG zone for the differentiation into Astrocytes/Neurons.
Supervisor: Dr. Kamna Srivastava and Dr. Pratibha Mehta Luthra
Th 28823

Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder largely attributed to dopaminergic neuronal loss and alpha synuclein aggregation in Substantia Nigra. The primary pathogenic features include dysregulated calcium and mitochondrial homeostasis, oxidative stress, and neuroinflammation. Current treatments, such as L-DOPA offer symptomatic relief but fail to halt disease progression. The adenosine A2A receptor (A2AR) has emerged as a promising therapeutic target because of its enriched expression in basal ganglia and astrocytes, where it modulates dopaminergic signaling and neuroinflammation. In the present study, the neuroprotective mechanism of the selective A2AR antagonist, IDPU (Ki = 0.0038 nM), was explored in 6-OHDA-induced in-vitro and in-vivo PD models, focusing on mitochondrial homeostasis through the p38MAPK axis. The Primary midbrain dopaminergic neuronal cells (PMDNs) and primary astrocytes isolated from neonatal rat pups exposed to 6-OHDA to mimic PD pathology were employed for this study. Complementary in-vivo models were established through stereotaxic injection of 6-OHDA in rat substantia nigra. Our findings provide the first evidence that IDPU demonstrates neuroprotection in PD-like conditions via the attenuating the mitochondrial fission and mitophagy associated proteins such as DRPP1and Parkin respectively mediated through p38MAPK, suggesting a potential therapeutic mechanism for targeting mitochondrial dynamics through A2AR antagonism. The dual restoration of mitochondrial homeostasis in both primary neurons and astrocytes establishes IDPU as a promising candidate for disease-modifying therapy in PD. The third objective of this work is to investigate the differentiation potential of adult rat neural stem cells (NSCs) isolated from the subventricular zone (SVZ) and dentate gyrus (DG). These neurogenic niches harbor multipotent NSCs capable of generating both neurons and astrocytes, but adult NSCs are often biased toward gliogenesis, limiting their regenerative potential. Understanding the intrinsic and extrinsic factors that govern this differentiation balance is essential for devising strategies to cure neurodegenerative conditions such as Parkinson's disease.

Contents