

List of Ten Best Papers with Summary of Important Discoveries

1. Arunabh Sarkar; Lalit Kumar; Rohil H; Shamsuzzama Ansari; **Aamir Nazir*** (2022) Multiple checkpoints of protein clearance machinery are modulated by a common microRNA, miR-4813-3p, through its putative target genes: Studies employing transgenic *C. elegans* model. **Biochim. Biophys. Acta - Molecular Cell Research** 1869(12):119342 (**Impact factor 5.01**).

Summary of Important Discovery: We endeavored to study microRNA molecules with potential role on regulating multiple checkpoints of protein quality control within cells. Carrying out global miRNA profiling in a transgenic *C. elegans* model that expresses human alpha synuclein, we identified novel miRNA, miR-4813-3p, as a significantly downregulated molecule. Further studying its putative downstream target genes, we were able to mechanistically characterize six genes *gbf-1*, *vha-5*, *cup-5*, *cpd-2*, *acs-1* and *C27A12.7*, which relate to endpoints associated with alpha synuclein expression, oxidative stress, locomotory behavior, autophagy and apoptotic pathways. Our study reveals the novel role of miR-4813-3p and provides potential functional characterization of its putative target genes, in regulating the various pathways associated with PQC network. miR-4813-3p modulates ER^{UPR} , MT^{UPR} , autophagosome-lysosomal-pathway and the ubiquitin-proteasomal-system, making this molecule an interesting target for further studies towards therapeutically addressing multifactorial aspect of Parkinson's disease.

2. Arunabh Sarkar , Hameed R, Mishra A, Bhatta RS, **Aamir Nazir*** (2022) Genetic modulators associated with regulatory surveillance of mitochondrial quality control, play a key role in regulating stress pathways and longevity in *C. elegans*. **Life Sciences** 2022 Feb 1;290:120226. (**Impact factor 6.70**).

Summary of Important Discovery: Our studies, employing transgenic *C. elegans* strain expressing human α -synuclein, led us to identification of mitochondrial genes nuo-5 (involved in oxidative phosphorylation), F25B4.7 (exhibits ATP transmembrane transporter activity) and C05D11.9 (having ribonuclease activity), which form predicted downstream targets of most elevated and down-regulated mi-RNA molecules. RNAi mediated silencing, gene ontology and functional genomics analysis studies demonstrated their role in modulating major MQC pathways. The attenuated MQC pathways mainly affected clearance of misfolded and aggregated proteins, redox homeostasis and longevity with compromised dopaminergic functions. Therefore, this study unveils the regulatory role of mitochondrial genes as critical modulators of stress control involved in effects associated with PD pathogenesis.

3. Ali R, Pal HA, Hameed R, **Nazir A***, Verma S*, (2019) Controlled release of hydrogen sulfide significantly reduces ROS stress and increases dopamine levels in transgenic *C. elegans*. (*Corresponding Authors) **Chemical Communications (Camb)**. 2019 Sep 4; 55(68):10142-10145. **(Impact factor 6.16)**.

Summary of Important Discovery: Hydrogen sulfide, an endogenous signalling molecule, is central to several pathophysiological processes in mammalian systems. It scavenges reactive oxygen species and is known to ameliorate dopaminergic neuronal degeneration in neurotoxin-induced Parkinson's disease models. The rapid volatilization of H₂S from spontaneously releasing sulfide salts being a challenge, we describe peptide conjugates which exhibit tris(2-carboxyethyl)phosphine mediated "slow and sustained" H₂S release. These conjugates reduced hydrogen peroxide-induced oxidative stress and significantly increased dopamine levels in transgenic *C. elegans* making this strategy innovative and beneficial towards therapeutic efficacy in case of Parkinson's disease.

4. Sashidhara KV, Modukuri RK, Jadiya P, Rao KB, Sharma T, Haque R, Singh DK, Banerjee D, Siddiqi MI, **Aamir Nazir***. (2014). Discovery of 3-Arylcoumarin-tetracyclic Tacrine Hybrids as Multifunctional Agents against Parkinson's Disease. **ACS Med Chem Lett.** 2014 Aug 20;5(10):1099-103. PMID:25313319 (**Impact factor 3.52**).

Summary of Important Discovery: A series of multifunctional directed 3-aryl coumarin-tetracyclic tacrine derivatives was designed and synthesized for the treatment of Parkinson's disease (PD). A number of derivatives (18, 19, 20, 21, and 24) demonstrated significant reduction of aggregation of "human" alpha-synuclein (α -synuclein) protein, expressing on transgenic *C. elegans* model NL5901. Moreover, compounds 16, 18, and 24 also exhibited good antioxidant properties and significantly increased the dopamine (DA) content in N2 and NL5901 strains of *C. elegans*. Interestingly, the protective efficacy of these hybrids seems to be mediated via activation of longevity promoting transcription factor DAF-16. In addition, molecular modeling studies have evidenced the exquisite interaction of most active compounds 18 and 24 with α -synuclein protein. Taken together, the data indicate that the derivatives may be useful leads against aging and age associated PD.

5. Sashidhara KV, Modukuri RK, Jadiya P, Dodda RP, Kumar M, Sridhar B, Kumar V, Haque R, Siddiqi MI, **Aamir Nazir***. (2014). Benzofuran-chalcone hybrids as potential multifunctional agents against Alzheimer's disease: synthesis and in vivo studies with transgenic *Caenorhabditis elegans*. **ChemMedChem.** 2014 Dec;9(12):2671-84. doi: 10.1002/cmdc.201402291. PMID: 25251917 (**Impact factor 4.60**).

Summary of Important Discovery: In the search for effective multifunctional agents for the treatment of Alzheimer's disease (AD), a series of novel hybrids incorporating benzofuran and chalcone fragments were designed and synthesized. These hybrids were screened by using a transgenic *Caenorhabditis elegans* model that expresses the human β -amyloid ($A\beta$) peptide. Among the hybrids investigated, (E)-3-(7-methyl-2-(4-

methylbenzoyl)benzofuran-5-yl)-1-phenylprop-2-en-1-one (4 f), (E)-3-(2-benzoyl-7-methylbenzofuran-5-yl)-1-phenylprop-2-en-1-one (4 i), and (E)-3-(2-benzoyl-7-methylbenzofuran-5-yl)-1-(thiophen-2-yl)prop-2-en-1-one (4 m) significantly decreased A β aggregation and increased acetylcholine (ACh) levels along with the overall availability of ACh at the synaptic junction. These compounds were also found to decrease acetylcholinesterase (AChE) levels, reduce oxidative stress in the worms, lower lipid content, and to provide protection against chemically induced cholinergic neurodegeneration. Overall, the multifunctional effects of these hybrids qualify them as potential drug leads for further development in AD therapy.

6. Haque R, **Aamir Nazir.*** (2016) Identification and functional characterization of a putative IDE, C28F5.4 (ceIDE-1), in *Caenorhabditis elegans*: Implications for Alzheimer's disease. **Biochim Biophys Acta**. 2016 Nov;1860(11 Pt A):2454-62. **(Impact factor 5.08).**

Summary of Important Discovery: Insulin-degrading enzyme (IDE) is a zinc metalloprotease, known to degrade insulin peptide and amyloid-beta (A β); the key protein involved in Alzheimer's disease (AD). Considering the important role played by IDE in disease progression of AD and type 2 diabetes mellitus (T2DM), we endeavored to identify the *Caenorhabditis elegans* (*C. elegans*) IDE orthologous genes and test them for their role in AD related outcomes. We employed bioinformatics, reverse genetics and molecular biology approaches towards identification and functional characterization of putative IDE candidates in *C. elegans*. Using in-silico analysis we have identified seven *C. elegans* genes that possess HXXEH motif, an identifying marker of IDE. We further carried out functional analysis of the identified genes in A β expressing *C. elegans* strain CL4176 [myo-3/A β 1-42 long 3'-UTR] via studying effect on A β induced toxicity, cholinergic neuroanatomy, content of acetylcholine/acetylcholine-esterase, extent of reactive oxygen species and expression of FOXO transcription factor DAF-16. Our findings reveal that

amongst the identified putative IDE orthologs, a functionally uncharacterized gene C28F5.4 had a profound effect on the tested endpoints. Knocking down C28F5.4 modulated the AD associated conditions by decreasing A β induced toxicity, severely compromising cholinergic neuroanatomy, reducing expression of acetylcholine-transporter, decreasing acetylcholine content, elevating ROS, with no effect on DAF-16 stress-response protein. These studies provide crucial insight into the structural/functional orthology of IDEs across human and nematode species and further our understanding of the involvement of these proteins and insulin pathway in AD.

7. Haque R, Kumar L, Sharma T, Fatima S, Jadiya P, Siddiqi MI and **Nazir A*** (2020) Human insulin modulates α -synuclein aggregation via DAF-2/DAF-16 signalling pathway by antagonising DAF-2 receptor in *C. elegans* model of Parkinson's disease **Oncotarget** 2020; 11:634-649. **(Impact factor 5.16).**

Summary of Important Discovery: A strong association of insulin-signalling with Parkinson's disease (PD) has been proposed but the exact nature of molecular events and genetic associations are yet to be understood. We employed transgenic *C. elegans* strain harboring human α -synuclein::YFP transgene, towards studying the aggregation pattern of α -synuclein, a PD-associated endpoint, under human insulin (Huminsulin®) treatment and DAF-16/DAF-2 knockdown conditions, independently and in combination. The aggregation was increased when DAF-16 was knocked-down independently or alongwith a co-treatment of Human insulin (HumINS) and decreased when DAF-2 was knocked-down independently or alongwith a co-treatment of HumINS; whereas HumINS treatment per se, reduced the aggregation. Our results depicted that HumINS decreases α -synuclein aggregation via DAF-2/DAF-16 pathway by acting as an antagonist for DAF-2 receptor. Knockdown of reported DAF-2 agonist (INS-6) and antagonists (INS-17 and INS-18) also resulted in a similar effect on α -synuclein aggregation. Further by utilizing bioinformatics tools, we compared the differences between the binding sites

of probable agonists and antagonists on DAF-2 including HumINS. Our results suggest that HumINS treatment and DAF-16 expression play a protective role against α -synuclein aggregation and its associated effects.

8. Kumar L, Shamsuzzama, Jadiya P, Haque R, Shukla S, **Aamir Nazir***. (2018) Functional Characterization of Novel Circular RNA Molecule, circzip-2 and Its Synthesizing Gene zip-2 in *C. elegans* Model of Parkinson's Disease. **Mol Neurobiol.** 2018 Aug;55(8):6914-6926. **(Impact factor 6.19).**

Summary of Important Discovery: Considering the body of evidence that establishes critical functions of non-coding RNA molecules, we endeavored to study circRNAs in the context of Parkinson's disease (PD). Employing transgenic *C. elegans* model of PD, we used RNase R-mediated cleavage of linear RNA followed by divergent primer-based amplifications towards identifying circzip-2, a novel circRNA molecule. We went on to sequence circzip-2 which is synthesized from functionally important gene zip-2. Studying RNAi-induced knockdown conditions of zip-2, we observed a reduced aggregation of α -synuclein protein along with an enhanced lifespan of the worms. We further carried out transcriptome analysis of zip-2 silenced worms, which suggested that zip-2 might be functioning via Daf-16 pathway. Further interaction studies revealed that circzip-2 possibly sponges microRNA molecule miR-60 towards asserting an important role in various processes associated with PD.

9. Shamsuzzama, Kumar L, **Nazir A.*** (2017) Modulation of Alpha-synuclein Expression and Associated Effects by MicroRNA Let-7 in Transgenic *C. elegans*. **Front Mol Neurosci**; 10:328. **(Impact factor 5.07).**

Summary of Important Discovery: Continuing our studies on small RNA molecules and based on the fact that multiple studies have provided clues

toward the role of microRNAs (miRNAs) in various disease conditions. One of the crucial miRNA molecules, let-7, is highly conserved miRNA and is known to regulate important functions of development and viability; its altered expression has been reported in *C. elegans* model of PD. We carried out studies with let-7, employing transgenic *C. elegans* model expressing 'human' alpha-synuclein and developed a let-7 loss-of-function model toward studying the downstream effects related to PD. We observed that let-7 miRNA was upregulated in *C. elegans* model of PD and figured that loss of let-7 miRNA leads to decreased alpha-synuclein expression, increased autophagy, increased Daf-16 expression, increased oxidative stress and increased lipid content with no effect on dopaminergic/acetylcholinergic neurons. Our findings indicate that let-7 miRNA regulates PD-associated pathways. Our study provides insight toward the role of let-7 in regulating expression of genes associated with these pathways which might have implications on the multi-factorial nature of PD. Potential pharmacological agents modulating the expression of let-7 could be studied toward targeting the multi-factorial aspect of PD.

10. Jadiya P, Fatima S, Baghel T, Mir SS, **Nazir A.*** (2016) A Systematic RNAi Screen of Neuroprotective Genes Identifies Novel Modulators of Alpha-Synuclein-Associated Effects in Transgenic *Caenorhabditis elegans*. **Mol Neurobiol.** 2016 Nov;53(9):6288-6300. **(Impact factor 6.19).**

Summary of Important Discovery: We carried out the present studies towards identifying novel genetic modulators of PD-associated effects employing a transgenic *Caenorhabditis elegans* model expressing human alpha-synuclein. Employing a systematic RNA interference (RNAi)-based screening approach, we studied a set of neuroprotective genes of *C. elegans* with an aim of identifying genes that exhibit protective function under alpha-synuclein expression conditions. Our results reveal a novel set of alpha-synuclein effector genes that modulate alpha-synuclein aggregation and

associated effects. The identified genes include those from various gene families including histone demethylase, lactate dehydrogenase, small ribosomal subunit SA protein, cytoskeletal protein, collapsin response mediator protein, and choline kinase. The functional characterization of these genes reveals involvement of signaling mechanisms such as Daf-16 and acetylcholine signaling. Further elucidation of mechanistic pathways associated with these genes will yield additional insights into mediators of alpha-synuclein-induced cytotoxicity and cell death, thereby helping in the identification of potential therapeutic targets for PD.



(Aamir Nazir)