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Elucidating the Role of Nrf2 Factor Interactions in Various Disorders of Human System and It's Proteomic Approaches: A Novel Study

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ABSTRACT

Nuclear factor erythroid 2-related factor 2 (Nrf2), which is also known as nuclear factor erythroid-derived-like-2, is a transcription factor which is encoded by the NFE2L2 gene. It is a basic leucine zipper (bZIP) protein which coordinates the basal and stress-inducible activation of a vast array of cytoprotective genes. It modulates a cellular antioxidant response program and plays a major role in the protection against oxidants and electrophiles; extracellular and intracellular oxidant/electrophiles have great contributions to the damages in cellular macromolecules such as proteins, lipids or DNA. Keap1 protein which is a regulator of Nrf2, is a highly redox-sensitive member of BTB-Kelch family assembling with Cul3 protein to form a Cullin-RING E3 ligase complex for Nrf2 degradation. Thus, this factor is a regulator of many processes of life and it's signalling system (Nrf2-KEAP-1-ARE pathway) has been found to participate in various ocular or eye diseases and even other systemic diseases such as respiratory disease, chronic diseases or cancer. In microbial infections, the host oxidative stress response may lead to the production of cytoprotective molecules, which in turn induces the activation of cellular Nrf2 factor. The crystallins or eye lens proteins, (αB-crystallin being one of them) may possibly interact with Nrf2 factor and regulate oxidative stress, but it is yet to be deciphered. Proteomic studies may provide valuable information, regarding such detailed protein interactions and their pathways especially in case of diseases or infections in the upcoming days.

Keywords- Nrf2 factor, Nrf2-KEAP-ARE pathway, diseases, microbial infections, oxidative stress, protein - protein interactions, proteomic studies.

I. INTRODUCTION

Nrf2 factor, which is encoded by the gene NFE2L2 gene, basically belongs to the Cap 'n' Collar (CNC) subfamily of basic leucine zipper (bZIP) transcription factors and has become the best-known member for it's cytoprotective role in responses to oxidative and electrophilic stress. Generally, under normal conditions, the levels of Nrf2 protein are kept low by E3 ubiquitin ligase Keap1(Kelch ECH-associating protein 1) which ubiquinates the factor in cytoplasm and targets it for degradation by 26S proteasome. In cases of increased oxidative stress, or in presence of electrophilic stress, Keap1 activity diminishes leading to the accumulation of Nrf2 in the nucleus where it induces a high expression of it's target genes. The presence of two cysteine residues, Cys273 and Cys288 are presumed to be essential for Keap1 to control Nrf2 factor under both basal and stress conditions, whereas other residues such as Cys226, Cys434 and Cys613 seem important for the sensing of specific toxins which may be secreted by microbes. The Nrf2 protein in humans is 605 amino acids long with seven conserved regions known as Nrf2-ECH homology (Neh) domains. The Neh2 domains possesses DLG and ETGE motifs after their sequence conservation in the single amino acid code, two further redox-independent degrons which are not recognised by Keap1but instead targeted for degradation by E3 ubiquitin ligase β-TrCP. Such an alternative pathway, which is enhanced by the phosphorylation of Nrf2 by glycogen synthase-3β, provides another mechanism for the cellular control of Nrf2 activity.

The oxidative stress (OS) usually arises due to an imbalance between reactive oxygen species (ROS) production and the elimination due to the biological system defense mechanisms. Damages caused by such ROS target cellular macromolecules such as DNA, proteins, lipids in case of corneal, glaucoma, retinopathies, glaucoma, cataract etc.[1,2]

The small heat-shock protein αB-crystallin (CRYAB) is believed to play a key role in the cytoprotective and antioxidant response; it's upregulation leads to the activation of redox sensitive transcription factors Nrf2 and the AP component c-Jun but the exact mechanism about the pathway behind it is yet to be unleashed.[16]

The eye is an organ which is subject to constant physical and chemical oxidation, especially visible light, ultraviolet light, smog, fine particles, ionising radiation in the atmosphere; the cornea, eyes, retina may be affected particularly.

Many eye diseases such as cataract, glaucoma etc and even microbial infections in the eye caused by bacteria, parasites are associated with OS and regulation by Nrf2 factors.

Recent trends in proteomic studies and interactomics may help to unleash the possible interactions in Nrf2-Keap-ARE pathways with microbial proteins, proteins of the eye lens, cornea, retina; they might hold a possible therapeutic potential for the treatment of ocular, systemic diseases in near future.

II. NRF2-KEAP INTERACTIONS AND DISEASES

Nrf2 is a basic leucine zipper (bZIP) transcription factor which consists of seven conserved Nrf2-ECH homology (Neh) domains. The Neh1 domain contains the bZIP motif which enables the binding of Nrf2 to ARE sequence, and can also interact with UbcM2, E2 ubiquitin conjugating enzyme to regulate the stability of Nrf2 protein. The Neh2 domain is located in the most N-terminal region and binds to Keap1 protein, thereby acting as a negative regulatory domain. The Neh3 domain is located in the most C-terminal region and assists Nrf2 transactivation by interacting with chromatin remodeling protein CHD6 whereas the tandem Neh4 and Neh5 domains are responsible for Nrf2-mediated ARE transactivation by interaction with a transcriptional coactivator e.g. CBP. The Neh6 domain is redox-sensitive possessing DSGIS and DSAPGS motifs and provides a phosphorylation-dependent binding platform for β- TrCP. The novel domain Neh7is known to interact with retinoic acid receptor α(RARα) which represses Nrf2 target gene expression. Keap1 protein possesses 5 domains: an amino-terminal region (NTR), a broad complex, Tramtrack and Bric domain (BTB), an intervening region (IVR), six Kelch/double glycine repeats (DGR) and a C-terminal region (CTR). Keap1 employs DGR regions to recognise two primary sequences in Neh2 domain of Nrf2 i.e. the ETGE and DLG motifs and forms a six-bladed propeller. Two interesting features regarding Nrf2 and Keap1 are: firstly, Keap1 can dimerize with each other, using BTB domain to interact with Cullin-3. Secondly, two Keap1 proteins bind to a single Nrf2 protein at a ratio of 2:1, where the overlapping ETGE and DLG motifs in Nrf2 can bind to Keap1 with a differential affinity; a single Keap1 interacts with ETGE motif (Ka= $20*10^7$ M^{-1}) and another Keap1 molecule binds to DLG motif with a weaker affinity (Ka=$0.1*10^7$ M^{-1}). The binding may be explained on the basis of 'hinge and latch' hypothesis where the 'hinge' mediates a high-affinity interaction between ETGE motif of Nrf2 and Keap1 which is unaffected by stress inducers, and the 'latch' mediates the displacement of DLG motif of Nrf2 from Keap1 in response to the treatment of Nrf2 inducers.([2,3]).

The ocular surface and especially cornea are constantly exposed to high oxygen tension, chemical burns, UV radiation, pathogenic microorganisms which are sources of reactive oxygen species (ROS). Under disease conditions, oxidative balance and mitochondrial function are abnormally altered. Nrf-2 mediated responses upregulate the expression of antioxidant proteins and playing a key role in cell protection.

In a disorder termed Fuchs' Endothelial Corneal Dystrophy(FECD) which is a leading cause of blindness with symptoms such as poor vision, blurry cornea, poor night vision, painful blinking etc, the genes related to FECD include TCF4, COL8A2, ZEB1, AGBL1, SLC4A11, DMPK, LOXHD1,LAMC1, ATP1B1, KANK4. UVA exposure leads to gene mutations, channelopathy, endoplasmic reticulum stress (ERS), mitochondrial dysfunction and even collagen deposition in Descemet membrane, cell morphological changes, apoptosis, endothelial cell loss. A stabilizer of Nrf2, DJ-1 is decreased dramatically in FECD corneal endothelial cells (CECs), accompanied by decreases in Nrf2 levels and HO-1 and impaired Nrf2 nuclear translocation. Such changes were attenuated to some extent by SFN, an activator of Nrf2. This activator enhanced the nuclear transmigration of Nrf2, followed by increasing expression of NQO1 and HO-1 and decreasing expression of p53 and a reduction in cell apoptosis.

Thus, Nrf2 -mediated antioxidant response plays a key role in regulation of FECD OS-induced apoptosis.([1,2,3])

In intracellular microbial infections, the host oxidative response stimulates cytoprotective molecule production which is responsible for the activation of Nrf2 factor. The factor in turn binds to consensus sequence of atioxidant response element (ARE) and results in the expression of protective antioxidant genes, including heme oxygenase-1 (HO-1). Thus, the upregulation of ARE/Nrf2 signalling pathway, in case of cutaneous leishmaniasis may play a significant role influencing the infection outcome. ([5,6])

Cataract, the opacification of eye lens, are one of the foremost causes of blindness worldwide. It primarily occurs in aged people; UV and oxidative damage are considered predominant factors for cataract formation. Human lens composed primarily of α, β, γ crystallins and oxidative stress leads to accumulation of aggregation of these proteins, developing clumps and loss of transparency with increasing age. Increased reactive oxygen species (ROS) which are the primary cause of damage to proteins, lipids (e.g. lipid peroxidation), DNA (e.g. methylation) may lead to aging as well. Nrf2, a major regulator of system defense against oxidative stress is found in lower levels, in older rats thereby indicative it's protective role in aging. Nrf2 also mediates cancer protection which is induced by calorie restriction. Tomatidine abundant in tomatoes, protandim which are Nrf2 activators are found to increase longevity via the Nrf2 pathway. Nrf2 activation, thus confers stress and extend lifespan of individuals and promotes further research into delaying prevention of aging.([14])

Nrf2 has been reported to disrupt the oxidative stress which activates nuclear factor ƙB(NFƙB) and proinflammatory cytokines , one of them being matrix metalloproteinase 9(MMP-9) which participates in the decomposition of lens epithelial cells(LECs) extracellular matrix and leads to cataract development. The association of Nrf2 and MMP-9 may be implicated in the progression of the disorder; polyphenol compounds such as flavonoids may lead to Nrf2

activation and MMP inhibition. Thus their protective role may act as potential therapeutic targets in cataract prevention.[11]

The upregulation of small heat shock protein αB- crystallin (CRYAB) in stress related cellular processes such as differentiation, apoptosis, redox homeostasis may play a key role in cytoprotective and antioxidant response, but the molecular pathway driving it's expression in muscle cells is yet to be deciphered. The simultaneous upregulation of Nrf2 and AP-1 component c-Jun which are transcription factors may support their involvement in CRYAB regulation; these changes have been correlated with specific phosphorylation of JNK and p38 MAPK kinases, the well-known mediators of signalling pathways.[12,16]

The role of Nrf2 in case of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease has been identified. Increased ROS have been implicated in case of such conditions; the expression of Nrf2 in brain thus is an important defense against toxic insults in glial cells and neurons. The endogenous antioxidant response pathway increases the expression of cytoprotective enzymes which is regulated by Nrf2. It exerts anti-inflammatory effects and modulates the mitochondrial function and biogenesis, two prominent features of many neurodegenerative diseases.[15]

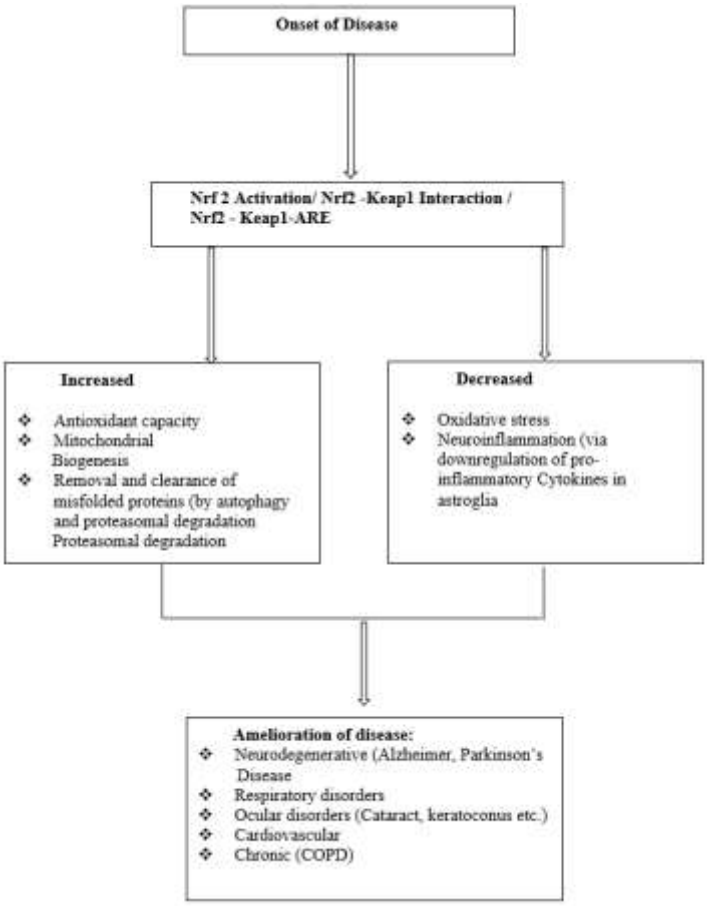

Figure 1: disease progression and role of Nrf2 in amelioration (*Ming-xuan Wang et. al,* 2020, *Mikah S. Brandes and Nora E. Gray,* 2020, *Cuadrado, A., Rojo, A.I., Wells, G. et. al,* 2019, *Qiang Ma,* 2013*)*

In respiratory diseases, Nrf2 associates with it's endogenous inhibitor Keap1 protein to inhibit oxidative stress in the lung which may result in the pathogenesis of various lung diseases including asthma, acute lung injury, chronic obstructive pulmonary disease (COPD) etc. Mutations in lung cancer in Keap1 disrupt the Keap1-Nrf2 complex formation, resulting in ubiquitination and degradation of Keap1 and constitutive Nrf2 activation; such alterations lead to an expression of genes that contribute to cell proliferation. The elucidation of mechanisms by which Nrf2 modulates initiation and progression of pulmonary diseases may lead to therapeutic development targeting specific pathways. The activation of Nrf2 and it's association with it's negative regulator Keap1 are critical in the maintenance of redox, metabolic and protein homeostasis, and inflammation regulation. Further research in target specificity, pharmacodynamic properties, efficacy are yet to be unleashed.[10,11]

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Table 1: Impact of Nrf2 factor activation in various disease

Name of Disease	Impact of Nrf2 Factor Activation on System
1. Ocular diseases (cataract, FECD, keratoconus)	Positive
2. Respiratory (Asthma lung cancer, COPD, interstitial lung disease	Positive
3. Neurodegenerative (Alzheimer's, Parkinson's)	Positive
4. Cardiovascular (atherosclerosis, hypertension,	Positive
5. Cancer progression	Negative (*Ming-xuan Wang et. al, 2020)*

(*Ming-xuan Wang et.al*, 2020, *Mikah S. Brandes and Nora E. Gray*, 2020, *Cuadrado, A., Rojo, A.I., Wells, G. et. al*, 2019*)*

III. PROTEOMIC APPROACHES IN NRF2 INTERACTIONS

Proteomic analysis of Keap1 protein interaction network may be studied by affinity purification, shotgun mass spectrometry.[17] Thus analysis of Nrf2-Keap1 pathways by proteomic approaches may help to discover potential drugs or therapeutics targeting their pathways in disorders of the human system.

Mass spectrometry (MS/MS spectra approaches) are helpful for the identification of proteins involved in microbial and host species when the particular microbe invades the host cell. A proteomic study based on Multidimensional Protein Identification (MudPIT) approach with liquid chromatography tandem mass spectrometry (LC-MS/MS) is useful for the identification of protein signatures, including Nrf2 and characterise global protein expression which allows for the targeting of molecules in infected samples and uninfected ones.[6,7]

Future research in proteomics may demonstrate the potential of Nrf2 and other such proteins as possible biomarkers for future therapeutic interventions.

IV. CONCLUSION AND PROSPECTS

Nrf2 holds a negative role i.e. as the driver role of cancer progression. Cancer associated mutatins may activate Nrf2. When ROS exceeds the critical threshold, Nrf2 bids to ARE gene and leads to increased expression of Klf9. Klf9 inhibites Trx reductase two expression, amplifying ROS cascade and leads to cell death.

Thus, the positive and negative sides of Nrf2 activation must be kept in mind; further studies related to this particular factor are being performed.

LIST OF SYMBOLS/ ABBREVIATIONS

Nrf 2 - nuclear factor erythroid 2-related factor 2
NFE2L2 - Nuclear Factor Erythroid 2 Like 2
CNC - Cap 'n' Collar
bZIP - basic leucine zipper
Keap1 - Kelch-like ECH-asociated protein 1
ECH - Enoyl CoA hyydratase
Cys - cysteine
Neh - Nrf2-ECH homology
OS - oxygen species
ROS - reactive oxygen species
CRYAB -Crystallin Alpha B
β-Trcp - Beta-Transducin repeats-containing proteins
AP- activator protein
UbcM2 - Ubiquitin-Conjugating Enzyme
CBP -CREB- binding protein
ARE - antioxidant response element
CHD6 - Chromodomain Helicase DNA Binding Protein 6
RARα - retinoic acid receptor α
BTB- Tramtrack and Brick Domain
IVR -intervening region
DGR - Kelch/double glycine
NTR - N-terminal Region
CTR - C-terminal region
FECD -Fuchs' Endothelial Corneal Dystrophy
TRF4 - Transcription Factor 4
COL8A2 - Collagen alpha-2(VIII) chain
ZEB1- Zinc Finger E-Box Binding Homeobox 1
AGBL1- AGBL Carboxypeptidase 1
SLC4A11- Solute Carier Family 4 Member 11
DMPK - dystrophia myotonica protein kinase
LOXHD -1 -Lipoxygenase Homology PLAT Domains 1
LAMC1 - Laminin subunit gamma-1
ATP1B1 - ATPase Na+/K+ Transporting Subunit Beta 1
KANK4 - KN Motif and Ankyrin Repeat Domains 4
ERS -endoplasmic reticulum stress
CECs -corneal endothelial cellsl
HO-1 - heme oxygenase- 1
SFN - Sulforaphane
NQO1 - NAD(P) H dehydrogenase [quinone]1
UV - ultraviolet
MMP-9- matrix metallopeptidase 9
NFƙB- nuclear factor ƙB
JNK -c-Jun N-terminal kinase
MAPK -Mitogen-activated protein kinases
COPD -Chronic Obstructive Pulmonary Disease
MudPIT -Multidimensional Protein Identification
Klf9 - Kruppel-like family of transcription factor 9

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Not Applicable

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