Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/11317
DOI URL: http://dx.doi.org/10.21474/IJAR01/11317

RESEARCH ARTICLE

MODULATION OF THENRF2 BINDING REGIONS THROUGH SYNTHETIC INHIBITORS

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Manuscript Info

Manuscript History
Received: 10 May 2020
Final Accepted: 15 June 2020
Published: July 2020

Key words:-
NRF2, KEAP1, Inhibitors, Cancer, and chemoresistance

Abstract

Importance: NFE2 related factor 2 (NRF2) is considered as a master molecule in transcription factors cascade, whereas the activation or suppression of the NRF2 leads to control of the physiological redox stress balance.

Recent Views: NRF2 has taken the attention of scientists and researchers when the abnormal structure and expression of the NRF2 appears in many pathological conditions; the majority of NRF2 abnormality is associated with cancer. Here, in the current review, we suggested using synthetic inhibitors to deregulate NRF2 in cancer because many studies have established that the NRF2 may have a role in chemotherapeutics resistance in some way.

Critical Concept: NRF2 could be considered as a key to access into the chemotherapeutic resistance world this accessing it may occur through Controlling of other molecules that act on suppression, binding, and activation of the NRF2 itself.

Future Prospect: NRF2's inhibitors are considering as an interesting and highly strong target candidate for cancer therapy strategy.

Introduction:-

NRF2 was discovered more than two decays ago, but recently it has taken the attention of researchers when the abnormal structure and expression of the NRF2 appeared in many pathological conditions such as multiple sclerosis, chronic kidney disease or cardiovascular diseases, Alzheimer and cancer. But researchers have observed that the majority of NRF2 abnormality is associated with cancer. Here in the current review, we shed a light on the use of inhibitors to downregulation of NRF2 in cancer because many studies have established that the NRF2 may have a role in chemotherapeutics resistance in some way.

NRF2 structural domains:

NFE2 related factor 2 (NRF2) is considered as a master molecule in transcription factors cascade, whereas the activation or suppression of the NRF2 leads to control of the physiological redox stress balance. Structurally the NRF2 is composed of many functional domains but the main regulatory domains are N-terminal domain, contains two motifs, called DLG and ETGE. These parts are responsible for interaction between Nrf2 and the Kelch domains of Keap1, due to this interaction occurs stability of Nrf2. The other domain is the Neh1 domain which contains the basic leucine zipper (bZIP) motif, which enables binding of Nrf2 with the Antioxidant Response Element (ARE). In addition to, Neh5 domain and Neh6 domain which are essential in a redox-sensitive nuclear export signaling and Keap1-independent degradation of Nrf2 respectively (1)(2)(3).

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Biological functions of NRF2:
Before NRF2 transforms to the abnormal molecule, obviously it has a significant vital intracellular roles which are representing in the regulation of cytoprotective defense system (GSH, TXN), heme and iron metabolism, signal transduction, regeneration of NADPH and xenobiotic detoxification (4). In cancer, the abnormality of NRF2 affecteddirectly on the detoxification andcytoprotective defense system which leads tooxidative stress imbalance. Oxidative stress is an imbalance between free radicals and antioxidants inside the body(5-7). The oxidative imbalance is thought to be it is one of the main reasons which cause chemotherapeutic resistance. So the NRF2 could be considered as a key to access into the chemotherapeutic resistance world. This accessing it may occur through Controlling of other molecules that act on suppression, and activation of NRF2 itself.

NRF2 and its molecules cascade:
These molecules are KEAP1-CLU3 complex, Small Maf Protein (SMF), and ARE. The communication between NRF2 and these cascade of molecules should occur through binding sides, so the changing on this binding attitude either through internal factor such as mutations or through external factor such as inhibitors may lead to deregulate the NRF2 activity and subsequently reduce the production of antioxidant or detoxifying enzymes genes, thereby could give a great space to chemotherapeutics agents to act effectively on cancer cell without interference with antioxidant complication. Antioxidants are molecules have the ability to neutralize free radicals or their actions(8).Free radicals are hydrogen peroxide and superoxide anion, which are generated either internally as byproducts of cellular metabolism such as mitochondrial respiration or externally such as antitumor therapies mediated by ROS(9)(10). ROS are not harmful molecules until reach to high level, then become toxic and can cause damage to macromolecules, including DNA, and/or cause permeabilization of the mitochondria, leading to the release of cytochrome c and apoptosis. The antitumor drugs are killingthe cancer cells through induced ROS toxicity mechanism (11).However, some of the tumor cells, especially in advanced stages have adapted to oxidative stress due to their antioxidant capacity. Moreover, it has been suggested that resistance to the agents that induce intracellular ROS production, such as paclitaxel, doxorubicin, or other drugs, is correlated to the increase of antioxidants (12, 13).

As mentioned earlier the inhibition of NRF2 may occur endogenously through mutation, but of course, the occurrence of mutation is a factor out of researchers control, because as it is known the mutation is an unpredictable process, No one could know when and where the mutation would happen (Only researchers could be able to study the type and location of mutation after the mutation has already existed).

NRF2 and its molecules cascade mutation:
Accumulating evidences have establishedthe mutations of NRF2, KEAP1 and ARE have a critical role in suppression or activation of NRF2 (14). Most of the mutations in NRF2 andKEAP1 are somatic mutations. For instance NRF2 mutations are observed in many human cancers due to the loss of NRF2-KEAP1 association. This has occurred through either mutation of NRF2 or KEAP1. Frequently NRF2 mutations are accumulated at the DLG orETGE motifsresult in diminished KEAP1 binding and ubiquitination,thus resulting a persistent localization of NRF2 in the nucleus. The common known mutation in NRF2 is Loss of exon 2 gene results in the synthesis of an NRF2 protein missing the KEAP1 interacting domain, thereby inducing NRF2 accumulation and transcriptional activation of its target genes. On the other hand, KEAP1 mutations are extended throughout the protein-coding region and result in loss of NRF2 degradation. All somatic mutations of NRF2 and KEAP1 are observed in human cancer such as lung, head neck, and gallbladder cancer cells especially are found to reside within DLG and ETGE motifs (15, 16)(17)(18)(19).However, it could say it depends on the mutation side because in case the mutation is located on region different than the binding region, then the mutation wouldn't effect on NRF2 binding with its receptors and subsequently, the mutant NRF2 still working to produce detoxifying enzymes and that is not good for the chemotherapeutic agent. However, some studies have demonstrated the correlation between NRF2 abnormality and chemoresistance.(20-23) . By accident in some cases, the mutation may locate at the binding region, thereby, it may reduce the NRF2 activity, So the mutation may be useful in the deregulation of NRF2 only if it is located at the binding side.

NRF2 and synthetic inhibitor:
The inhibition of NRF2 through external factor such as a pharmacological synthetic inhibitor, it could be controlled by scientists within the synthesis of inhibitory molecules to compete with NRF2 in the binding region. Inhibitors are quite small molecules. (24). But before working on designing inhibitors to NRF2 and its regulatory molecules, we would like to give a brief background on these molecules themselves.

NRF2 –KEAP1-CLU3 Cascade:
Some studies have identified that KEAP1 is a cytoplasmic protein that suppresses the NRF2 activity through adaptation for Cullin-3-based E3 ubiquitin ligase complex (25).KEAP1 is considered as the main intracellular regulator of Nrf2, it possesses five domains, that is three broad complex-tramtrack-bric a brac (BTB), one intervening region (IVR), and two glycine repeat domains (DGR), each one is important for inhibiting Nrf2 activity. The DGR domains of the Keap1 bind with different affinity to the DLG and the ETGEdomains in a single Nrf2 molecule. Under the oxidative situation, the DLG motif in Nrf2 is detached from the DGR domain in Keap1 thus blocking Nrf2 ubiquitination and degradation. The IVR domain, in addition to interacting with Cul3 protein which contains the E3 ligase complex also it is required for signaling exchange between nucleolus and cytoplasm for Keap1localization. In basal conditions, Nrf2 is sequestered by cytoplasmic Keap1 and targeted to proteasomal (26S proteasome) degradation(26)(27).

ARE:
Antioxidant response element (ARE), also termed as the electrophile response element is a cis-regulatory element or enhancer sequence, which is found in the promoter region of several genes encoding detoxifying enzymes and cytoprotective proteins. Mainly responds to oxidative stress situation whereas Nrf2 dissociates from Keap1, free and newly synthesized Nrf2 translocate into the nucleus forms a heterodimer with small Maf family of transcription factors, and binds to the ARE to transcriptionally activate antioxidant genes (28)(29)(30).

Small Maf Proteins:
MAFs are transcription factors were identified for first time in a viral oncogene, based on their size MAFs factors are classified into two groups; large MAFs and small MAF. The main difference between the two groups that the Large MAFs have the ability to form homodimers and activate the transcription of target genes, in contrast, the small MAFs form homodimers but lack the transactivation domain so they do not activate transcription of target genes. But here in this review, we focus on small MAFs because are essential inactivation of NRF2 through facilitating the binding of NRF2 to ARE(31).sMaf proteins, consist of Maf F, Maf G, and Maf K, have a basic leucine zipper (bZIP) domain that is necessary for homo-/hetero-dimerization with other bZIP transcription factors, whereas Nrf2 forms heterodimer with sMaf proteins due to of its specificity, thereby binding to high affinity ARE promoters of antioxidant and detoxifying genes(32)(33)(34).

So, It could understand that the process of cytoprotective proteins induction in response to oxidative stress is controlled primarily by three cellular molecules, which are included the antioxidant responsive element (ARE), the nuclear factor erythroid 2-related factor 2 (Nrf2), and the Kelch-like ECH-associated protein 1 (Keap1) in order to form what is called Keap1-Nrf2-ARE regulatory pathway. After its translocation into the nucleus, Nrf2 binds to antioxidant response elements (AREs) through the small protein transcription factor (Maf), consequently inducing the expression of cytoprotective proteins, all these processes to regulate detoxification and elimination of reactive oxidants and electrophilic molecules (35).

NF2 and chemotherapeutics resistance in cancer:
Chemotherapeutic drug resistance is a major challenge in cancer, according to the previously studied the resistance may occur sooner or later (36). It is thought that one of the main reasons toward chemotherapeutics resistance is attributed to overexpression of NRF2 which leads to increase the capacity of antioxidant level in cancer cells and thus enable them to overcome the chemotherapeutic killing process(37).See figure (1)

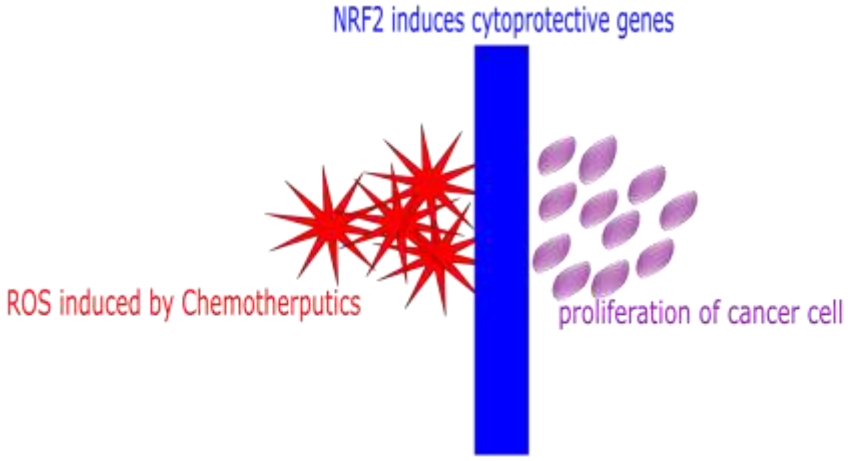

Figure 1:- NRF2 acts as a barrier for cancer cell from the toxicity of chemotherapeutics agent. The chemotherapeutic treatment kills cancer cell through increasing ROS to a toxic level which causes damage in large molecules of the cell and ultimately cell death, Nevertheless, the cancer cells have the ability to proliferate and escape from this toxicity through overexpression of NRF2 which leads to induce of cytoprotective genes.

A few studies have discussed Nrf2–Keap1 - Inhibitors (38, 39).Through this review we suggest that the working on the modulation of NFR2 binding regions either binding with its suppressor (KEAP1) or its activator (ARE), may play a role in the curb of NRF2 overexpression, this modulation may occur through competitive or uncompetitive inhibitors mechanism or coenzymes molecules. The Competitive mechanism means when a certain molecule has a similar structure to target substance but is not reactive both of them compete on the same binding side (40) . On the other hand, the uncompetitive or also called anti-competitive occurs when the inhibitor bind with Enzyme Substrate (ES) and subsequent reduce catalytic action with no binding to free enzyme so that makes more difficult for target Substrate to dissociate or be converted to product(41). Also, there are coenzymes molecules, coenzymes act as carriers of several kinds of chemical groups, they work beside enzymes to enhance reaction rates. The most known of coenzymes are vitamins and NAD(nicotinamide adenine dinucleotide (NAD^+), which works as a carrier of electrons in oxidation-reduction reactions)(42).

It is known that the biological molecules should be bound properly with their receptors in order to give a desire action. So if the same concept has been taken in the Nrf2 binding situation, suppose there is a capability to make a synthetic inhibitors to compete with NRF2 binding side and subsequently downregulation its activity, thereby the chemotherapeutic agents could destroy the cancer cell more effectively in the absence of antioxidant obstacle which was produced due to NRF activation. Regarding using the synthetic inhibitors, the best side for them to compete with NRF2 is thought to be small MAF protein and KEAP1 for activation and suppression respectively.

NRF2-KEAP1 and uncompetitive inhibitors:

The regulation of NRF2 mainly occurs through controlled maintenance of NRF2 protein levels, for the response to electrophilic/oxidative stress situation, the main protein activated is CUL3-KEAP1 complex (43).KelchECH-associated protein 1 (KEAP1) is a substrate adaptor protein for a cullin 3 (CUL3)- containing E3 ubiquitin ligase. KEAP1 binds NRF2 as a dimer, interacting through its C-terminal Kelch domain with the DLG and ETGE motifs located in the Neh2 domain of NRF2. On the other hand N termini, KEAP1 dimers interact with CUL3E3 ligase RBX1 to act as a target for ubiquitin proteasomal degradation (44). So the interaction between KEAP1 and NRF2 is considered essential for efficient ubiquitination (degradation) of NRF2, but suppose this degradation doesn't occur normally then it would be better to use synthetic uncompetitive inhibitor in order to keep the NRF2 binds with its suppressor (keap1) that at least Guarantee doesn't release of NRF2 because if the KEAP1thiols are modified by electrophiles this is a signal to induce conformational alterations in the overall structure of the CUL3-KEAP1-NRF2 complex and to suppress the ubiquitination of NRF2 (45).Alternatively, also it could use the synthetic coenzyme as an enhancer to increase the process of NRF2 – KEAP1 – ligase complex ubiquitin degradation through protostome, as it is well known the coenzymes are small non-organic compounds are required in enzymatic reaction to accelerate the reaction. Both mechanisms would reduce free NRF2 availability and that is what we want at the time of using chemotherapeutic agents.For the binding of the uncompetitive inhibitors and coenzymes with NRF2, we suggested a certain side for that binding,see figure (2).

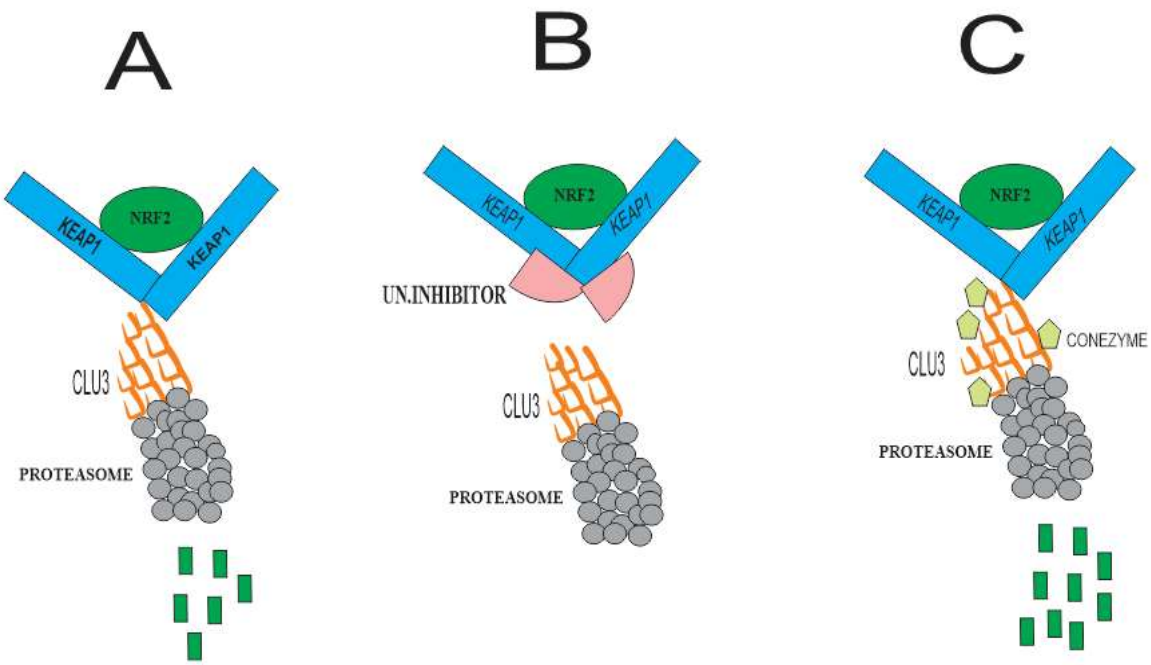

Figure 2:- Keeping the NRF2 in the binding state, during taking the chemotherapeutics drugs.

We need as much as possible to keep the NRF2 at down level (because overexpressed Nrf2 provides a growth advantage for cancer cells by protective them from oxidative stress which induced by anticancer agents, thus contributing to chemoresistance). One of the methods to achieve this downregulation is keeping the NRF2 in the binding state (not free) this takes place in the cytoplasm. **(A):** Under normal condition, NRF2 is captured by the Kelch-like ECH-associated protein 1 (KEAP1)–cullin 3 (CUL3) complex results in NRF2 being degraded by the proteasome; **(B):** The suggested binding region of synthetic uncompetitive inhibitor molecules to NRF2-KEAP1 complex to keep NRF2 in the binding state; **(C):** The suggested binding region of synthetic coenzyme with CUL3 ligase to accelerate the NRF2proteasomal degradation rate. The outcome of the three pathways is reduced availability of free NRF2, thereby gives a space to chemotherapeutic drugs to act efficacy against cancer cells.

Small MafProtien and competitive inhibitor:

The other mechanism is a synthetic competitive inhibitor also it could use to compete withNRF2 in the binding region of small Maf proteins, because the binding of NRF2 with ARE in the DNA occurs in the presence of small Maf proteins, as demonstrated in the previous studies,Nrf2 has not been shown bind to DNA independently (46).As long as Nrf2-sMaf heterodimers form is considered as a critical regulator of antioxidant and xenobiotic metabolism, so any impairment of this heterodimers form through using of synthetic inhibitors may lead to reduce the level of NRF2 and subsequently enhance the cytotoxic efficacy of chemotherapeutic against cancer cells(47).For the binding of suggested side of competitive inhibitors with NRF2 see figure (3)

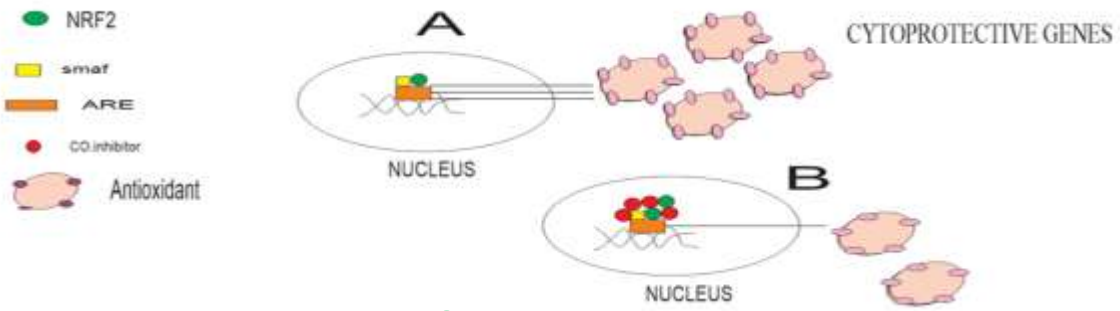

Figure 3:- The competition between NRF2 ≡ and Competitive inhibitor ≡ in the binding with smaf≡ ,

to induce cytoprotective genes and antioxidant molecules ≡ through ARE≡ .

(A): The binding of NRF2 with ARE is facilitated by smaf to enhance antioxidant response elements and initiates detoxification genes when exposed to oxidative stress. Cancer cell tends to overexpress of NRF2 and subsequently occurs high expression of antioxidant genes (to obstacle and reduce the efficacy of chemotherapeutics treatment). **(B):** The Competitive inhibitors compete with NRF2 on the smaf's binding region, thus the NRF2 has fewer opportunities in the binding and subsequently low level of induced antioxidant genes. Low level of the antioxidant molecule is considered a good environment for chemotherapeutics drugs to kill cancer cell more effectively.

Although this review focuses on the importance of using synthetic inhibitors (competitive, uncompetitive, or coenzymes) to reduce NRF2 to a low level, also there is a natural inhibitor, such as Brusatol. Brusatol is herbal medicine, it has recently been reported to act as an NRF2 inhibitor in many kinds of cancer cells (48) .

Generally, the NRF2 inhibitors could be applied as one of the approaches which follow in cancer treatment, Last but not least, the modulation of the Nrf2 pathway considers an interesting and highly explored strategy in the area of cancer science(49).

Conclusion:-

The majority of previous studies have already described using inhibitors to regulate the NRF2 expression in cancer, nevertheless, their concepts were general, In this regard here in this review we discuss in detail which type of inhibitor may be used (competitive, uncompetitive or coenzymes). Further, we suggest the exact binding region of inhibitors with NRF2cascade (binding with sMAF or KEAP1), in order to reduce the chemotherapeutic resistance which occurs due to up-regulation of NRF2. These are the reasons for considering this inhibitor pathway as a strong target candidate for cancer therapy or even cancer prevention.

Acknowledgment:-

We introduce great Thankful for Ms. **Najat Suliman** and Ms. **Hind Elseir** for their logistic support.

Authors' Contributions:

All Authors have met all of the authorship criteria which include contributions to the conception or design of the work, interpretation of data for the work, drafting the work or revising it critically for important intellectual content and final approval of the version to be published

Funding Support:

This work was funded by the authors themselves. No outside source funding obtained**.**

Conflict of interest disclosures:

The authors made no disclosures.

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