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A brief introduction and the clinical meaning of Nrf2-Keap1 pathway

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Abstract. In a normal physiological state, Nrf2-Keap1 pathway is associated with antioxidation progress. Mostly, Nrf2 interacts with Keap1 and shows a low cellular abundance. When the intracellular oxidative stress level increases, Nrf2 uncouples with Keap1 then performs nuclear translocation to regulate the RNA levels of its downstream targets, control the redox-related balance in the cell and exert cytoprotective effect. The mutation of related factors in the signal pathway will lead to the abnormality of oxidative damage, which may lead to lung cancer. Therefore, the remission treatment of lung cancer can be achieved through the inhibition of Nrf2-Keap1pathway. We will concentrate on Nrf2 and Keap1's structural and functional domains in the review. Then we summary the mutation sites and signal pathway of Nrf2-Keap1. Most importantly, we discuss the potential clinical application of Nrf2-Keap1 and further adopt the method of molecular docking to screen the small molecules acting on Nrf2-Keap1 to provide the potential improvement to current lung cancer treatment.

Keywords: Nrf2-Keap1 pathway, oxidative damage, drug resistance, tumor therapy.

1. Introduction

Until 2020, lung cancer has been the cancer with the highest incidence and mortality rate in the world, and GLOBOCAN2020 data showed that worldwide, more than 2 million new cases and 1 million deaths from lung cancer annually in average. Among them, the cases happened in China take up more than 30% of the global in the new or dead cases. It is the malignant tumor with the highest incidence and mortality rate among all cancers. Based on this current situation and the study of proteins, this report centers on the research and study of the effect of Keap1-Nrf2 pathway inhibition on lung cancer.

Keap1-Nrf2, a classic two-component system, shows critical roles in anti-oxidation and responses to pro-electrophilic stress. Because oxidative damage is ubiquitous in carcinogenesis, and the Nrf2-Keap1 pathway shows potential clinical application as a therapeutic target. Today, although many studies have demonstrated the effectiveness of the Nrf2-Keap1 pathway as a potential therapy of many kinds of cancers, the counterproductive effects of overexpression cannot be ignored. Not only does Nrf2 have an important role as a chemopreventive agent in normal and pre-cancerous tissues, but at the same time, it has been found to be associated with the growth of tumor cells and the survival of malignant cells as well. It has been shown in several papers that increased expression of detoxifying enzymes,

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cytoprotective proteins and transporter proteins are associated with Nrf2. It gives a lot of convenience to cancer cells through the promotion of cell proliferation and may lead to the chemotherapy resistant, allowing cancer cells to become more viable and gain the opposite effect compared to before. It has been that Keap1-Nrf2 can be associated with lung cancer, breast cancer and other diseases, and the further research is need to provide more potential clinical application of this pathway.

2. The brief introduction of Nrf2-Keap1

The Nrf2-Keap1 pathway acts as a genetic defense system that provides protection against a variety of stressors, in which both oxidative and chemical stresses are included. The Nrf2- Keap1 pathway also associated with the alternation of gene expression for a variety of cytoprotective and detoxifying enzymes, which are indispensable for maintaining intracellular redox homeostasis [1].

2.1. The structure of Nrf2

Nrf2 is an important protective transcriptional regulation molecule, distributing in all organs of the body. The amino acid residues of Nrf2 constitute six functional domains. N-terminal Neh1 forms dimers with DNA and other substances and is required in the activation of transcribed genes. The DLG and ETGE sequences on the functional domain of Neh2 are very important, and bind to the Kelch functional domain of Keap1 protein. The ability to bind to the Kelch functional domain of Keap1 protein makes this functional domain a focus of research. It has been shown that the ETGE and DLG motifs bind to Keap1 with different binding affinities, although in a very similar manner.. Both motifs have the same interaction surface with the Keap1-DC structural domain of the Keap1 homodimer. The C-terminal positive Neh3 regulates the trans-activation of ARE-related genes; Neh4 and Neh5 regulate the transcriptional activity of Nrf2; Neh6 is serine-rich and controls the stability of Nrf2 [2] (Figure 1).

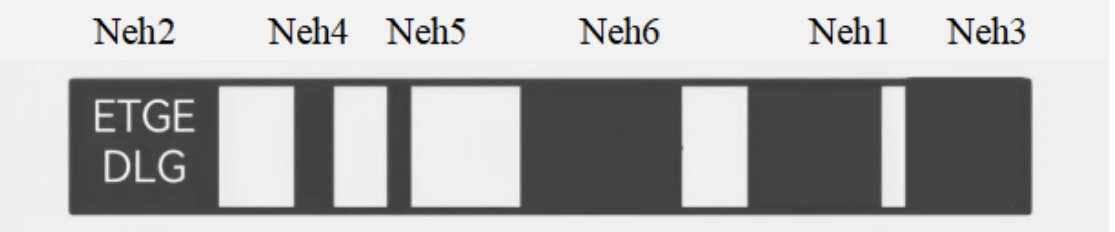

Figure 1. Structural domains of Nrf2.

2.2. The structure of Keap1

The expression distribution of Kelch-like ECH-associated protein-1 (Keap1, also known as KLHL19), a substrate receptor protein of the cullin 3 (CUL3) E3 ligase complex, differs somewhat from other substrate receptor proteins, such as CRBN, VHL, RNF4 and RNF114, and it is highly expressed in tumor tissues, higher than CRBN and comparable to VHL. The five functional domains of Keap1 are the N-terminal region (NTR), the intermediate region (IVR), the BTB (Broad complex, Tramtrack, and Bric-à-Brac) region, and the double glycine repeat region (DGR).The IVR contains nuclear exocytosis signal (NES), which regulates the cytoplasmic localization of Keap1 [3].The IVR region is rich in cysteine residues with highly reactive reactive sulfhydryl groups on their side chains that readily covalently bind to other molecules, making the DGR and IVR regions the focus of Keap1 binding to Nrf2.The BTB structural domains are responsible for the dimerization of Keap1 and its binding to the Cullin3 (Cul3) E3 ligase, and their activation is dependent on the presence of a charged group and reactive oxygen species (ROS). During this process of normal ubiquitination of Nrf2, the DGR region of Keap1 binds Nrf2, allowing Nrf2 with to be able to ligate to the E3 complex, while at the same time allowing the ubiquitination process by E3 ligase on the lysine residues of Nrf2 (Figure 2).

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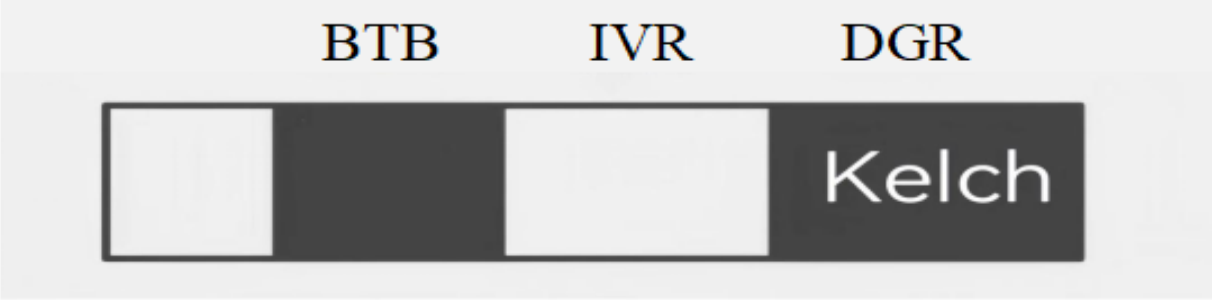

Figure 2. Structural domains of Keap1.

3. Mutations in Nrf2-Keap1

3.1. The mutation of Nrf2

3.1.1. The mutations site in Nrf2. Most of the mutation region of Nrf2 is located in the Neh2 region. More specifically, the mutations always occur in the DLG region and the ETGE region of Neh2, both of them take responsibility for binding to the Kelch (also called DGR) region in the Keap1. Figure 3 shows the mutation site in Nrf2 [4] (Figure 3).

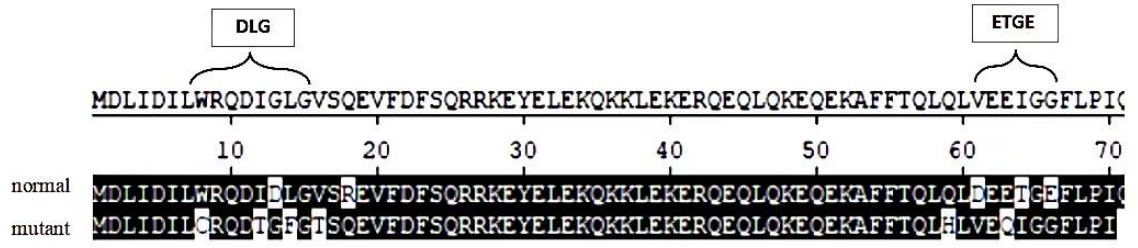

Figure3A

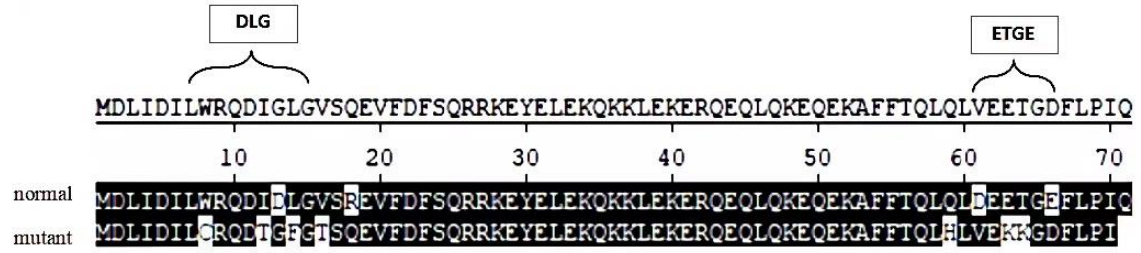

Figure3B

Figure 3. the figures A and B respectively indicate the mutations in different cases.

3.1.2. The mutations in Nrf2 damages two-site substrate recognition model. The DGR region and the C-terminal area of Keap1 can be recognized by the DLG and ETGE regions of Nrf2. . All mutations on the ETGE motif damage the association with Keap1-DC.

There are two simulated structures of E79Q and E82D mutant [4]. In the structure of the E79Q mutation (glutamic acid to glutamine), compared with the wild-type protein, The glutamine in ETGE lose the electrostatic interaction with the 483 site (Arginine) and the 508 site(serine) of Keap1, so the mutation weakens the binding force. Similarly, the E82D mutation (glutamic acid to aspartic acid) seems to weaken the contact with the 363 site (Serine), the 380 site(Arginine) and the 508 site(Serine) of

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Keap1, although both of them are acidic residues, probably due to the short side chains of Asp, which weakens contact with Keap1.

3.2. The mutation of Keap1

Keap1 contains 624 amino acids and contains more cysteine. Cysteine contains sulfur atoms. The existence of disulfide bond makes the amino acid have a certain catalytic effect. Part of the cysteine is modified and the conformation of Keap1 is changed, which makes it dissociate from Nrf2. The DGR region and IVR region of Keap1 are the main sites of mutation [5], meanwhile DGR region is an important binding site to Neh2 region in Nrf2, while IVR region is rich in cysteine residues and is the functional regulatory region of the whole Keap1 protein. The effect of these two mutant sites on the binding of Keap1 with Nrf2 is similar to the influence in 2.1.2 (Figure 4).

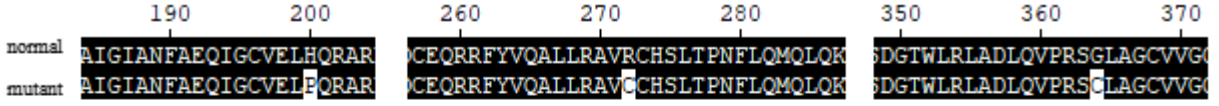

Figure4A

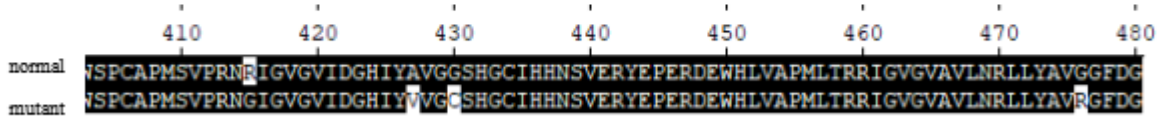

Figure4B

Figure 4. Figure A and B show the location of amino acid mutations in different cases.

4. The Nrf2-Keap1 pathway correlation with tumorigenesis

4.1. Normal expression of Nrf protects cells from oxidative damage

4.1.1. Nrf2-Keap1 signaling pathway. In a healthy physiological state, Nrf2 is the transcription factors, and which is also one of the basic leucine zipper family. In the cytoplasm, Keap1 is ubiquitinated and changed by Keap1 and also interacts to Nrf2, and is then degraded by the proteasome system, thus controlling its levels in the body and keeping it in a steady state. While the amount of oxidative stress occurring in the cell has increased, Nrf2 uncouples from Keap1 and enters the nucleus, regulating the expression of downstream target genes [6], regulated target genes are associated with intracellular redox homeostasis and exert cytoprotective role.

4.1.2. Nrf2-Keap1 in Oxidative stress inducing cellular carcinogenesis. In the normal state, Nrf 2 in the cytoplasm binds to Keap 1 and is in an active relative inhibitory state. When normal cells are treated with some exogenous substances, Nrf 2 is uncoupled with Keap 1 to activate the signal transduction pathway, and begin the transcription and expression of target genes such as superoxide dismutase (SOD) and quinone oxidoreductase 1 (NQO 1), improving the oxidative stress and repair function of cells, and avoiding the occurrence of tumor, similar target genes are GSH-Px and HO-l [7].

Oxidative stress usually represents the occurrence of cancer. It is find that patients with various chronic inflammatory diseases are more likely to catch cancer. The phenomenon lends credence to the idea that oxidative stress-induced lesions trigger the development of cancer. Cause oxidative stress increases the level of oxidative damage to DNA, this raises the possibility of mutation and may therefore have an impact on the development of cancer.

The Nrf2-Keap1 pathway shows a possible therapeutic application for chemoprevention due to the predominance of oxidative damage in carcinogenesis.

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4.1.3. Reduce the apoptosis of tumor cells. The overexpression of Nrf2 protein in tumor cells may reduce tumor cell apoptosis by reducing the oxidative damage of tumor cells, which has the opposite effect: the rapid growth and division of cells in tumor tissue will accelerate the function of mitochondria and produce extra ROS. Excessive ROS will lead to oxidative damage of DNA, lipids and proteins in tumor cells. Nrf2 can reduce ROS-mediated apoptosis of tumor cells by reducing oxidative stress [6].

4.2. Tumor cells are protected by the aberrant expression of Nrf2

Although Nrf2 has the potential of chemoprophylaxis in normal and precancerous tissues, it has been proved to work in the growth of tumor cells and the survival of malignant cells, too. People found highly expressed Nrf2 in different types of cancer cells, which may be caused by up-regulation of constitutive expression of genes due to Keap1 or Nrf2 mutations. Overexpression of Nrf2 seems to protect the normal cells and cancer cells. Increased Nrf2 levels have been linked to enhanced production of detoxifying enzymes, cytoprotective proteins, and transporters, according to studies. By rejecting chemotherapy, this gives cancer cells an edge and may promote increased cell growth [8].

4.2.1. Nrf2 help the formation of drug resistance in tumors. Recent studies have found that Nrf2 can serve as a protective role in tumor cell by reducing the chemotherapy sensitivity of tumor cells. For example, inducing resistance to the downstream genes of this signaling pathway such as HMOX-1, GPx and Prx1, are related to tumor cells pumping drugs. This signaling pathway takes part in the regulation of multidrug resistance proteins (MRPI-2), and MRPI-2 is directly associated with multidrug resistance in a variety of malignancies. In addition, Nrf2 can also interact with HO-1 to cause cellular drug resistance.

At present, the mechanism of Nrf2-Keap1 signaling pathway in tumor drug resistance is still in its infancy, but experiments have confirmed that downregulation of Nrf2 expression can improve the sensitivity of breast cancer cells, lung cancer cells and other malignant tumor cells to corresponding chemotherapeutic drugs [8].

Moreover, some experiments find that TRIB3 (tribbleshomolog3), as a stress and metabolic stress protein, plays a crucial function in upregulating the protein level of Nrf2. and then lead to the occurrence and development of tumors [9]. TRIB3 mainly interacts with Keap1 and inhibits the Nrf2-Keap1 interaction to enhance the stability of Nrf2 and up-regulate the expression of Nrf2. Figuring out the relationship between TRIB3 and Nrf2 will not only help to analyze the molecular mechanism of Nrf2 high expression in tumor cells under stress conditions, but also improve the mechanism of TRIB3 promoting the malignant progression of lung cancer.

In further studies, detecting the expression changes of Nrf2 can provide new drug sensitivity and tumor markers for clinical diagnosis and treatment and will lay the theoretical foundation for the development of drug resistance reversal agents for tumor drugs, improve the chemotherapy sensitivity of tumors, and improve the prognosis of malignant tumors.

4.2.2. Nrf2 may lead to enhancing cell proliferation. Nrf2 regulatory growth factors like fibroblast growth factor 13 (FGF-13) are overexpressed in many types of tumors, involved in tumor invasion and migration by maintaining homeostasis of tumor cell environment), transforming growth factorsβ1 andβ2 (TGF-1, TGF-2) as well as growth factor receptors such EphA2 and the Receptor of Advanced Glycation Endproducts (RAGE), and these growth factors or receptors are closely related to cell proliferation[6]. Moreover, FGF13 induces the platinum drug resistance through regulating the expression and distribution of hCTR1 and ATP7A.

5. The Nrf2-Keap1 in tumor therapy

5.1. Dual role of Nrf 2 in tumor cells

As mentioned earlier, Nrf2 is an important factor of intracellular antioxidant, which can reduce the cancer caused by oxidative mutations by activating the downstream pathways to improve the antioxidant

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capacity of the cells. Although Nrf2 has a positive impact on normal and precancerous cells, the growth and survival of tumor cells and malignant cells are also influenced. Elevated level of Nrf2 upregulate levels of detoxicating enzymes, cytoprotective proteins, and transport proteins. Many of these responses allow cancer cells to proliferate faster and better, and they also make diseased tissue resistant to chemotherapy, also known as drug resistance.

5.2. Relevant applications of Nrf 2 in tumor therapy

The discovery of the mechanism of action of the Nrf2-Keap1 pathway can help the cancer treatment to achieve better efficacy.

5.2.1. Nrf2 Inducer. Activators of Nrf2 are widely found in nature, including sulfamaniline (SF), curcumin, estrogen-3 gallant acid, resveratrol, alcohol, cafitol, cavil, cassia bark-based compounds, zero bone, garlic sulfur compounds, lycopene, and carnosol [10]By inducing Nrf2-mediated antioxidant responses and operating proteins, they protect cells and reduce the risk of cancer mutations. An isothiocyanate known as SF, which is found in cruciferous vegetables such as cauliflower, is a natural product that targets the Nrf2 signaling pathway, which is a widely [10]. Curcumin is a natural antioxidant that can prevent skin tumors from developing in mice with skin cancer in a mouse cancer model. Neutroph 3-dependent activation of Nrf2 is a well-studied form of activating Nrf2. The food administration of curcumin increased Nrf2's phrase and blocked the formation of DNA adducts, oxidative stress and inflammation in the liver and lungs of mice treated with benzene (A) [10].Diethyl fumarate (DMF), an Nrf2 activator, is a synthetic product that can react with ketone 1 to produce alkyl and cysteine residues, where cysteine residues are key factors in the activation reaction, preventing Nrf2Ubi stabilization, enhancing the Nrf2-stabilization and activation of the Nrf2 target gene in the Nrf2 target .

5.2.2. Nrf2 inhibitor. The activation Nrf2 pathway will not only affect cancer progression, but also change the drug resistance of cancer treatment. It can be seen that the control of Nrf2 signaling has become a new cancer treatment, especially for cancers caused by increased Nrf2 levels, Nrf2 will be better. It was found that a single distribution of Brussels alcohol was able to inhibit the Nrf2 pathway [11]. Brussels alcohol reduces Nrf2 protein levels in mammalian cells while decreasing the level of Nrf2 transcription cellular genes to an inhibitory effect. Thus, Brussels alcohol reduced the intracellular level of GSH by inhibiting GSH expression. A number of other small molecules were also found to inhibit the Nrf2 pathway, including ascorbic acid, lutein, gibberenin A, trigonomin, and whole molecule Transanic acid (TAR) [12]. ATa and most RA receptor A (α) agonists inhibit Nrf2 activity both intracand extracellular. As long as there is ATRA, then Nrf2 will form a complex with La-Laα, and because the Nrf2:αcomplex is unable to bind to the ARE, it reduces the activity of Nrf2, making it unable to activate the corresponding subsequent reaction. Interestingly, although these compounds were all shown to inhibit Nrf2 activities, in specific cases, they would similarly also stimulate Nrf2 activities, making it more active. Therefore, further studies are necessary to identify the specific features and mechanism for responding to the present Nrf2 inhibitors to better determine whether Nrf2 is applied or when Nrf2 is used in cancer therapy.

5.3. The Nrf2-Keap1 / ARE signaling pathway in tumor therapy

The Nrf2-Keap1/ARE signaling pathway not only prevents tumorigenesis, but also causes some tumor cells to undergo apoptosis. A novel compound, PBQC, was discovered and synthesized, and its high concentration was found through the enhancement of Nrf2 activity to inhibit the tumor cell growth. Moreover, PBQC can cause s-glutathione acylation of Keap-1 protein and promote Nrf2 shuttle into the cell nucleus and forcing the expression of proapoptotic genes, thus causing cancer cells to undergo apoptotic [13]. These results indicate that Nrf2-Keap1 / ARE signaling pathway has an inhibitory effect on some tumor cells, and its mechanism is mainly by enhancing the activity of Nrf2 and promoting its nuclear translocation to induce the ability of relevant protective proteins to resist malignant changes in

normal cells, while improving the expression of some proapoptotic proteins in tumor cells and then promoting their apoptosis.

6. Conclusion

Nrf2-Keap1 / ARE signaling pathway has a significant role in preventing oxidative damage, and a growing number of studies confirm that the abnormal Nrf2-Keap1 / ARE signaling pathway is closely related to the development of a variety of cancers. Nrf2 has great potential as a drug target in cancer therapy, and both Nrf2 inducers and inhibitors have possibilities as anticancer agents. Moreover, due to the two-sided effect of Nrf2-Keap1 pathway, it's better to combine an Nrf2 inducer or inhibitor with conventional anticancer drugs to increase the therapeutic efficacy. However, in view of the dual effects of Nrf2-Keap1 / ARE signaling pathway on tumor cells, there is no clear definition of whether to inhibit or induce pathway expression, and more in-depth research should be discussed in different cancers and different stages of development.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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