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Regulation of Keap1–Nrf2 signaling: The role of epigenetics

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Abstract

Kelch-like ECH-associated protein 1 (Keap1)-nuclear factor erythroid 2-related factor 2 (NFE2L2/Nrf2) signaling pathway is a pivotal player in the antioxidant response to oxidative and electrophilic stress and can play a role in many human diseases. Activation or inhibition of Nrf2 has been an approach to treating many diseases such as cancer and regulation of this pathway has been thoroughly studied. Recently, epigenetics has emerged as another layer for regulating Keap1–Nrf2. Epigenetics modification is defined as heritable changes to gene expression without changing DNA sequence and various modifications have been found to be involved in regulating Keap1–Nrf2. Therefore, targeting these epigenetic changes on Keap1–Nrf2 provides a potential pathway for modulating Keap1–Nrf2 to treat disease. In this review, several important and recent findings on epigenetic regulation and perspectives on Keap1–Nrf2 are discussed and shared.

1. Introduction

Oxidative stress is an important factor in contributing to a wide-range of chronic diseases including cardiovascular, neurodegenerative and neoplastic. One way cells combat against excess oxidative stress, mediated by reactive oxygen species (ROS), is through activation of the transcription factor, nuclear factor erythroid 2-related factor 2 or Nrf2 [1]. A key negative regulator of Nrf2 is the Kelch-like ECH-associated protein 1 (Keap1), which in the cytoplasm and under basal conditions, binds to Nrf2 to promote its ubiquitination and proteasomal degradation. Presence of compounds, particularly electrophiles, or ROS allows Nrf2 to leave Keap1 and translocate and accumulate in the nucleus activating a wide battery of genes including antioxidant and drug metabolizing genes [2, 3]. Together, the Keap1–Nrf2 signaling pathway operate as the cells’ defense against oxidative stress.

Much research has been performed to investigate the influence of Keap1–Nrf2 in many different disease models. Current evidence shows that Keap1–Nrf2 can play both protective and harmful roles in different diseases [4]. Thus, regulation of this vital signaling pathway is of particular interest to better understand how the context and mechanisms of disease affect Keap1–Nrf2 function.

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Recent research has looked towards epigenetics as one important potential mechanism for regulating the Keap1-Nrf2 pathway. Epigenetics is defined as the study of heritable differences in gene expression without altering DNA sequence [5]. Various epigenetic mechanisms exist to influence and regulate gene expression including DNA methylation, histone modifications, non-coding RNAs, and chromatin remodeling. These epigenetic modifications to DNA and chromatin are critical in mammalian development and in human diseases such as cancer and obesity [6]. And accumulating evidence shows that epigenetic modifications can play a role in regulating Keap1-Nrf2 [1]. Several of these epigenetic modifications are represented and summarized in Fig. 1 and Table 1.

2. Epigenetic Modifications regulating Nrf2

Nrf2 is a transcription factor affecting the expression of many different genes involved in antioxidant stress and drug metabolism. Thus, regulation of Nrf2 expression is an important target for treating or preventing disease and this regulation has been reported to be influenced by epigenetics. Yu et al. found that expression of Nrf2 was regulated by differential DNA methylation patterns in TRAMP mice, TRAMP C1 and TRAMP C3 cells, all murine models of prostate cancer. Specifically, hypermethylation of the first 5 CpG sites in the Nrf2 promoter was associated with mouse prostate tumors but not in normal prostate tissue. This was further validated by observing the increased methylation in tumorigenic TRAMP C1 cells compared with TRAMP C3 cells and treatment of TRAMP C1 cells with DNMT inhibitor 5-aza and HDAC inhibitor TSA increased Nrf2 and NQO1 expression in TRAMP C1 cells [7]. Similar results on 3 CpG sites of the human Nrf2 promoter were reported in human prostate tissue and cells [8].

However, context must be taken into account when considering the role Nrf2 plays in manifestation of a disease. Cheng et al observed that there was no significant difference in CpG methylation in the Nrf2 promoter between normal and gestational diabetic fetal umbilical vein endothelial cells (HUVECs) [9]. Nrf2 promoter TET-dependent demethylation was also assessed in human colorectal cancer cell line, SNUC5 and its 5-fluorouracil resistant counterpart, SNUC5/5-FUR. SNUC5/5-FUR exhibited higher expression levels of Nrf2 and its downstream gene HO-1 compared to the nonresistant SNUC5 and that drug resistance to 5-fluorouracil can be attributed to the decreased methylation in the Nrf2 promoter region and increased expression of the DNA demethylase ten-eleven translocation (TET) enzyme. This suggests that context is important in terms of what role DNA methylation plays in Nrf2 and that similar methylation patterns may have different meanings [4, 10].

Although DNA methylation is one of the more commonly studied epigenetic marks in human disease, other epigenetic modifications have been studied for their interactions with Nrf2. PcG enhancer of zester homolog 2 (Ezh2) is responsible for H3K27 trimethylation (H3K27me3), a histone mark associated with transcriptional gene silencing. Its interaction with Nrf2 was studied by Li and colleagues where in lung cancer, decreased Ezh2 lowered H3K27me3 at the promoter of Nrf2 and increased levels of Nrf2 protein with low Ezh2 expression being associated with poor survival outcomes [11]. However, increased Ezh2 expression is also a marker for advanced disease in many cancers and that context must be

considered with further investigation required for understanding the role Ezh2 plays in regulating the Keap1-Nrf2 pathway [1, 12]. In the bronchial epithelial cell line, BEAS2B, HDAC2 helps to stabilize Nrf2 and prevent its degradation and treatment with TSA, an HDAC inhibitor, decreased Nrf2 stability and activity [13]. HOTAIR (Hox transcript antisense intergenic RNA) is a known long noncoding RNA that is responsible for tumor development and progression but also involved in Nrf2 expression. Decreased HOTAIR expression was associated with decreased histone H4 acetylation at the Nrf2 promoter and subsequently reduced Nrf2 expression in patients with asthenozoospermia and oligoasthenozoospermia [14]. Another type of noncoding RNA, microRNA or miRNA, which is usually involved in “fine-tuning” gene expression, has also been investigated for its roles in regulating the Keap1-Nrf2 pathway including in the study of cancer [15, 16]. miR27a, miR153, miR142-5p, and miR144 were identified to have a potential role in regulating Nrf2 in neuronal cells [17]. In the MCF-7 breast cancer cell line, Yang et al. showed that miR28 is able to act on the 3' UTR region of Nrf2 mRNA to reduce Nrf2 mRNA and protein stability and influence disease progression [18]. miR93 was found to target and down-regulate Nrf2 in E2-induced breast cancer, highlighting miR93's oncogenic potential [19]. Interestingly, miRNAs can also be regulated by Nrf2 [20]. Systemic analysis of Nrf2 predicted that 85 miRNAs can bind to Nrf2 to decrease Nrf2 translation and that 63 out of 85 can be regulated by 35 transcription factors influenced by Nrf2 [21]. miR214 was recently found to have a conserved ARE promoter region in the miR199a/214 cluster and its expression is driven by Nrf2 in mouse erythroleukemia cells. Arsenic induced stress was observed to increase Nrf2 expression and subsequently reduce miR214 transcription which helped mediate regulation of ATF4 and Ezh2/Bim, demonstrating that miRNAs can also be downstream targets of Nrf2 [22].

Epigenetic modifying enzymes may also work not just on DNA or histones but directly on protein themselves through post-translational modifications. A recent study by He et al. examined SetD7 regulation of ROS signaling and the Nrf2 pathway and believe that because Nrf2 can be modified post-translation, that SetD7 may methylate lysine residues of the Nrf2 protein to directly inhibit Nrf2 protein. This adds further complexity to the epigenetic regulation of Keap1-Nrf2 [23].

3. Epigenetic Modifications regulating Keap1

Keap1 is an important negative regulator of Nrf2 and in studies involving DNA methylation in the Keap1-Nrf2 signaling axis, Keap1 DNA methylation modifications have received considerable interest in regulating the Nrf2 antioxidant response. DNA methylation of the Keap1 promoter has been found to play a role in a number of diseases including cancers of the breast [24], colon [25], prostate [26], cervical [27], and lung [28]. The influence of environmental toxicants on Keap1 and cancer was further illustrated by Wang et al who showed that long-term exposure to arsenic in HaCaT cells (human skin keratinocytes) increased hypermethylation of the Keap1 promoter and increased Nrf2 accumulation suggesting Keap1-Nrf2 plays a role in skin cell transformation by arsenic [29]. In the context of cancer, Keap1 hypermethylation lowers Keap1 expression thereby increasing Nrf2 expression, a mark of drug resistance and tumor progression. This is in contrast to previous reports where Nrf2 hypermethylation is also associated with tumor progression; further

investigations into the epigenetic modifications of these genes and signaling cross-talk can help elucidate how Keap1-Nrf2 is involved in cancer progression. Interestingly, Liu et al. compared the effect, an isoflavone, has on the Keap1 DNA methylation levels in the non-small cell lung cancer (NSCLC) cell line, A549, to normal lung fibroblast MRC-5 cells. Using pyrosequencing, higher DNA methylation was observed in A549 cells compared with MRC-5 cells and that treatment with genistein was comparable to 5-aza and decreased methylation of Keap1 in A549 but not in MRC-5 cells. The demethylation of Keap1 induced by genistein sensitized A549 cells but not MRC-5 cells to radiation treatment and apoptosis [30].

As opposed to Keap1 hypermethylation being involved in carcinogenesis, Keap1 hypomethylation has been shown to be involved in diabetic complications. In human diabetic cataractous lenses, CpG demethylation in the Keap1 promoter increased levels of Keap1 protein which increases Nrf2 degradation. This reduced Nrf2 activity dampens the expression of many antioxidant genes and shifts the redox state towards oxidation [31]. Similar findings were reported by Liu et al. in the context of diabetic cardiomyopathy and by Gao et al. in the context of age-related cataracts [32]. Liu et al. found that antioxidant protein levels such as SOD, HO-1, and NQO1 were reduced in patients with diabetic cardiomyopathy compared to those without diabetes and this could be partly attributed to the demethylation of Keap1 in the promoter region, which decreased Nrf2 activity and suppressed Nrf2 mediated activation of antioxidant genes [33].

However, epigenetic modifications surrounding Keap1 are not limited to just DNA methylation. In diabetic retinopathy, histone methyltransferase enzyme Set7/9 (SetD7) helps mediate Sp1 binding to Keap1 and enrich H3K4me1, a histone mark of gene activation, at the Keap1 promoter. Like in the previously mentioned studies involving Keap1 and diabetic complications, activation of Keap1 by SetD7 restricts Nrf2 activity. More interesting is that after returning to normal glucose levels, SetD7 remains active after a period of hyperglycemia. Reversing hyperglycemia did not reverse epigenetic changes at the Keap1 promoter but rather persisted, providing one possible explanation for the metabolic memory phenomenon that is associated with diabetes and its complications [34]. miRNAs are also involved in regulation of Keap1 expression. In breast cancer cells, miR200a is silenced and re-expression of miR200a targets the 3'-untranslated region (or 3'-UTR) of Keap1. This re-expression leads to Keap1 mRNA degradation and to activation of Nrf2 and its downstream gene NQO1. Activation of Nrf2 helped to impair cell growth in soft agar and supports the protective role of Nrf2 in disrupting tumor cell growth [35]. Inhibition of histone deacetylases (HDACs), enzymes that remove acetyl groups from histones leading to gene silencing, is also implicated in Keap1-Nrf2 where Wang et al. found trichostatin A (TSA) can lower Keap1 expression and increase Nrf2 activity to protect against cerebral ischemia [36]. This finding on TSA's effects on Nrf2 is somewhat contradictory to the findings by Mercado et al.

Epigenetic regulators are also found to be involved in Keap1-Nrf2 regulation. Recently, ubiquitin-like containing PHD and RING finger domains 1 or UHRF1 was found to influence levels of Keap1 protein in pancreatic cancer. UHRF1 is involved in maintaining DNA methylation by attracting DNMT1 to hemimethylated DNA and UHRF1

overexpression is associated with suppression of Keap1 protein and subsequent overexpression of Nrf2 in pancreatic cancer. However, by using pyrosequencing, the methylation of the Keap1 promoter by UHRF1 was found to be mediated significantly only in Suit-2 cells and less so in MiaPaca-2 and CFPac-1 cells. Although UHRF1 plays a role, other mechanisms are believed to exist [37].

4. Conclusions and Perspectives

The Keap-Nrf2 signaling axis plays a pivotal role in both preventing and contributing to disease with the mechanisms for the dual role of Keap1-Nrf2 still being investigated. In the context of cancer, Nrf2 overexpression and impaired Keap1 expression mediated by Keap1 hypermethylation in the promoter region is often associated with carcinogenesis and resistance to chemotherapy. Although in certain cancers such as prostate, Nrf2 promoter hypermethylation is also associated with decreased Nrf2 expression and tumor aggressiveness. This suggests that even in different cancers, Keap1-Nrf2 may work in different ways. Likewise, in different disease states like in diabetes, Keap1-Nrf2 is found to be mostly protective with Keap1 promoter demethylation being associated with more diabetic complications. Further complicating the epigenetic landscape of Keap1-Nrf2 are the different layers of histone modifications, miRNAs, long non-coding RNAs, DNA methylation patterns, and epigenetic regulators that are involved in regulating Keap1-Nrf2. Future considerations into the epigenetic modifications of Keap1-Nrf2 should take into account disease context such as stage of progression or type of disease as similar epigenetic patterns may spell out different stories for different diseases. These differences in outcomes and roles of Keap1-Nrf2 in different diseases may be in part due to the increasingly sophisticated crosstalk between Keap1-Nrf2 and other signaling pathways and genes [15], all occurring in different cell types with different epigenomic profiles. And though much work on Keap1-Nrf2 has focused on cancer, further research into the epigenetic regulation of Keap1-Nrf2 in other diseases would be of great interest to better ascertain the mechanisms of how different epigenetic modifications assert their effects in different diseases.

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Highlights

1. Epigenetics is involved in regulating the activity of the Keap1-Nrf2 signaling pathway
2. Epigenetic modifications to Keap1 and Nrf2 have been found to have a role in human disease
3. Keap1 and Nrf2 have varying expression levels in different tissues and diseases
4. Keap1 and Nrf2 expression can be modulated by epigenetic modifications such as DNA methylation, histone modifications, and miRNAs

Fig. 1.
Diagram of various epigenetic modifications on Keap1-Nrf1 signaling.

Table 1
Brief summary of several epigenetic modifications regulating the Keap1-Nrf2 signaling pathway

Target	Modification type	Species	Tissue/cell lines	Effect on target	Effect
Nrf2	DNA methylation	Mouse & Human	Prostate, TRAMP-C1 cells	↓	Tumor progression[7, 8]
Nrf2	DNA demethylation	Human	Colorectal cancer cells	↑	Drug resistance[10]
Nrf2	H3K27me3 demethylation	Human	Lung cancer cells	↑	Pool survival outcome[11]
Nrf2	H4 deacetylation	Human	Spermatozoa	↓	Asthenozoospermia[14]
Nrf2	miRNA27a, 153, 142-5p, 144	human	Neuronal	↓	Alteration of Nrf2 dependent redox homeostasis[17]
Nrf2	miR-28, miRNA93	Human	Breast cancer cells	↓	Keap1-independent regulation[18, 19]
Nrf2	Lysine methylation by SetD7	Human	Bronchial epithelial cell	↓	Regulation on proinflammatory responses, mitochondrial function[23]
Keap1	DNA methylation	Human	Breast, colon, lung cancer	↑	Decrease Keap1 expression and increased tumor progression[24, 25, 28]
Keap1	DNA methylation	Human	Skin keratinocytes	↓	Skin cell transformation by arsenic[29]
Keap1	DNA demethylation	Human	Lung cancer	↑	Sensitize cells to radiation and apoptosis[30]
Keap1	DNA demethylation	Human	Diabetic cataractous lenses	↑	Reduced Nrf2 activity, oxidation[31]
Keap1	DNA demethylation	Human	Age-related cataracts	↓	Reduced Nrf2 expression, oxidation[32]
Keap1	H3K4me methylation	Human	Diabetic retinopathy	↑	Reduced Nrf2 activity, oxidation[34]
Keap1	miR-200a	Human	Breast cancer cells	↓	Activation of Nrf2 and NQO1[35]