# NRF2 Pathway

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Contributor: Lucia Anna Muscarella

The NRF2 pathway represents one of the most intriguing pathways that promotes chemo- and radioresistance of neoplastic cells. Increasing findings suggest that the NRF2 signaling can be modulated by multiple epigenetic factors such as noncoding RNAs, which influence a large number of oncogenic mechanisms, both at transcriptional and at post-transcriptional levels. As a consequence, the identification and characterization of specific noncoding RNAs as biomarkers related to oxidative stress may help to clarify the relationship between them and NRF2 signaling in the tumor context, in terms of positive and negative modulation, also referring to their intersection with other NRF2 crosstalking pathways.

Keywords: NRF2 pathway ; Cancer ; NRF2 signaling

## 1. Introduction

The NRF2 protein, encoded by the Nuclear factor erythroid 2-related factor 2 (NFE2L2) gene, is a transcription factor endogenously expressed in eukaryotic cells and represents the main protector of the cellular antioxidant and cytoprotective response to harmful insults, as well as to xenobiotic damage and oxidative stress [1].

The activity of NRF2 is strictly dependent on a battery of transcriptional modulators that govern its physiologic activity under basal conditions as well as under internal and external stimuli arising from an oxidative stress condition [2]. In normal state, NRF2 orchestrates the maintenance of basal expression levels of more than 200 target antioxidant response element (ARE) genes through its direct link to this specific consensus sequences located at their promoter regions [3]. During the cellular adaptation to environmental modifications, the activation of NRF2 signaling is triggered by competitive interactions with the three ubiquitin ligase complexes—Cullin 3-RING-box protein-Kelch-like ECH-Associated Protein 1 (CUL3-RBX1-KEAP1), Skp1-cullin-F-box protein-transducin repeat-containing proteins (SCF/β-TrCP) and ERAD-associated E3 ubiquitin-protein ligase (HRD1). This complex interaction controls the ubiquitination and proteasomal degradation of NRF2 and, as a consequence, its abundance in specific subcellular compartments [4]. When NRF2 moves into the nucleus, it specifically binds to the ARE gene regions by heterodimerizing with small MAF (sMAF, avian musculo aponeurotic fibrosarcoma oncogene homolog) proteins and turning on the transcription of a large number of antioxidant and detoxification genes [5][6].

The main well-characterized mechanism of NRF2 regulation is tightly linked to its negative repressor, the KEAP1 protein. The KEAP1 is able to form an ubiquitin ligase complex with CUL3 and RBX1 to bind the NRF2 and enhancing its proteolitic degradation (in normal cell conditions) or detach from NRF2, because of KEAP1 cysteine modification (upon stress exposure) and favoring its nuclear translocation [7][8][9]. A KEAP1-independent modulation of the NRF2 signaling pathway may also occur both at transcriptional, post-transcriptional and at post-translational levels, with a consequent modification of its cellular localization, protein folding/stability and its DNA-binding ability [10][11].

Over the recent decades, the NRF2 transcription factor has been found to be overexpressed in various human disease, and a growing number of studies have identified abnormal NRF2 functions that go over the physiologic stress-regulating processes, including cancerous processes [12]. Fascinating scientific evidence indicates that the potential double role of NRF2 in cancer suppression and promotion [12][13] is under the control of genetic and epigenetic events, the latest ones strictly related to the activity of ncRNAs, such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) [14][15]. These small pieces of RNAs are noncoding nucleotides that modulate transcriptional and post-transcriptional variations of oncogene and tumor suppressor genes, also not in physiologic processes such as cell differentiation, proliferation, cell cycle regulation, self-renewal capacity of stem cells and apoptosis, but also in the chemo- and radioresistance ability of tumors [16][17].

## 2. NRF2 Deregulation in Cancer: Focus on the Epigenetic Modifications

Cancer development and progression depends on multiple genetic and epigenetic alterations. Specifically, the epigenetic processes are mechanisms that alter the expression level of the genes without modifying the sequence of DNA nucleotides. They include several changes in DNA methylation patterns, histone modifications and small noncoding microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) expression [18].

A large amount of scientific evidence suggested that NRF2 favors the survival of normal cells as well as of cancer cells, thus corroborating the idea that its activation might promote the neoplastic progression. The identification of a “dark side of NRF2” has been debated over the time, but it still appears quite uncertain, since NRF2 could act both as a tumor suppressor and oncogene [12][13]. As for many genes, the occurrence of genetic and epigenetic modifications affecting the NRF2 pathway promotes cancer-related molecular events such as tumor initiation, growth, invasion and metastasis. The main interesting effect of all reported genetic/epigenetic alterations of the NRF2 pathway is a potential translational impact in terms of patients’ survival and response to chemo-radio and targeted therapies, firstly reported in lung tumors [19][20][21][22][23]. The biallelic inactivation of the KEAP1 gene was reported to have a great impact on the upregulation of NRF2 and was firstly described in non small cell lung cancer (NSCLC) and then widely notified in many other solid tumors [24][25][26][27]. Point missense mutations of NFE2L2 gene were also reported to have a similar effect on the KEAP1/NRF2 binding affinity and frequently recurred not only in lung cancer [28],. but also in head and neck carcinoma [29], hepatocellular carcinoma (HCC) [30], papillary renal cell carcinoma (PRCC) [31] as well as esophageal and skin cancers [32]. In lung squamous carcinoma (LUSC) and head and neck cancers, an alternative splice variant of NFE2L2 gene lacking exon 2 was described to play a significant role in the loss of interaction of NRF2 with the Kelch domain of KEAP1. This in turn caused the stabilization of NRF2 and the induction of its transcriptional response [33]. Epigenetic modifications have been widely described to impact on the modulation of the KEAP1/NRF2 system in cancer. Aberrant methylation at the promoter island of the KEAP1 gene has been widely reported as one of the most important mechanisms of KEAP1 silencing in solid tumors, such as glioma, breast, prostate, colorectal, thyroid cancers, clear renal cell carcinoma and lung cancer and was linked to tumor development, chemoresistance and mortality risk [14]. More recently, in NSCLC Kirsten rat sarcoma viral oncogene homolog (KRAS) wild-type subpopulations, a novel and significant correlation was observed among promoter and in intragenic exon 3 cytosine-guanine dinucleotide island (CpG-I) methylation and the transcription levels of KEAP1, NFE2L2 and many ARE genes [34][35].

Given the real complexity and multiplicity of the cellular processes controlled by NRF2, one of the most recent and attractive pieces of evidence suggests that the regulation NRF2 can be also guided by the ncRNAs, such as miRNAs and lncRNAs [36]. The following two main sections will summarize the findings about ncRNAs related to oxidative stress and their relationship with the NRF2 network.

## 3. Conclusions and Perspectives

It is becoming increasingly clear that the NRF2 signaling pathway can be epigenetically modulated in different ways and that the identification of new sensitive and reliable biomarkers (miRNAs and lncRNAs) that guide this mechanism in solid tumors could be useful to better understand the link between oxidative stress and cancer. This review aimed to provide a concise summary of the most recent updates in this field, but further massive investigations are required to allow the translational utility of ncRNAs as potential biomarkers related to NRF2 activity.

The oxidative stress processes related to the NRF2 activity have become a main issue in cancer biology investigation over recent years, so the identification of ncRNA alterations related to these dynamic processes will surely provide new opportunities to understand cancer biology and treatment. Moreover, further characterization of the fundamental mechanisms by which altered processing of these specific ncRNAs contributes to tumor onset and progression will be crucial to ensure great effects of many promising cancer therapies that specifically target the altered processing of RNA, with minimal effects on normal cells. From a practical and more futuristic point of view, the advent of next-generation sequencing (NGS) and liquid biopsy techniques able to detect point mutations and ncRNA level variations in blood will also allow a better clarification of the existing relationship between ncRNAs and the NRF2 pathway in a dynamic and longitudinal monitoring context of cancer patients during the course of the disease.

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