

## **Interplay between Non-coding RNAs and NRF2 in Different Cancers: Spotlight on miRNAs and Long non-coding RNAs**

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## List of abbreviations

5-FU: 5-Fluorouracil	RASSF1A: Ras association domain family member 1A
AATBC: Apoptosis-associated transcript in bladder cancer	ROS: Reactive oxygen species
AKT: Protein kinase B	SLC7A11: Solute carrier family 7 member 11
AML: Acute Myeloid Leukemia	SNHG14: Small Nucleolar RNA Host Gene 14
CCND2: Cyclin D2	TE-1: Human squamous cell carcinoma cell line of the esophagus
CircRNAs: circular RNAs	TUG1: Taurine upregulated gene 1
DNMT: DNA methyltransferases	VEGF: Vascular endothelial growth factor
EMT: Epithelial-mesenchymal transition	VEGF-A: Vascular endothelial growth factor A
H3K27: Histone H3-lysine 27	VEGFR2: Vascular endothelial growth factor receptor 2
HIN1: Hairpin inducing protein-1	VPF: Vascular permeability factor
HO-1: Heme Oxygenase-1	ZO-1: Zonula occludens 1
JNK: c-Jun N-terminal Kinase	$\beta$ -TRCP1: $\beta$ -Transducin repeat-containing protein
KEAP1: Kelch-like ECH-associated protein 1	
KRAL: Keap1 regulation-associated lncRNA	
lncRNAs: long non-coding RNAs	
Loc344887: NmrA-like family domain containing 1 pseudogene	
miRNAs: microRNAs	
NLUCAT1: Nuclear Lung Cancer Associated Transcript 1	
NQO1: NAD(P)H Quinone Dehydrogenase 1	
PABPC1: Poly(A) Binding Protein Cytoplasmic	
p-AKT: Phospho- protein kinase B	
PCK1: Phosphoenolpyruvate carboxykinase 1	

## **Abstract:**

Cancer is a multifactorial disease and wealth of information has enabled basic and clinical researchers to develop a better conceptual knowledge of highly heterogeneous nature of cancer. Deregulations of spatio-temporally controlled transduction pathways played central role in cancer progression. NRF2-driven signaling has engrossed significant attention because of its fundamentally unique features to dualistically regulate cancer progression. Context dependent diametrically opposed roles of NRF2-induced signaling are exciting. More importantly non-coding RNA (ncRNA) mediated regulation of NRF2 and interplay between NRF2 and ncRNAs have added new layers of complexity to already intricate nature of NRF2 signaling. There is a gradual enrichment in the existing pool of knowledge related to interplay between microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) in different cancers. However, surprisingly, there are no clues about interplay between circular RNAs and NRF2 in various cancers. Therefore, future studies must converge on the functional characterization of additional important lncRNAs and circular RNAs, which regulated NRF2-driven signaling or conversely NRF2 transcriptionally controlled their expression to regulate various stages of cancer.

## **Significance Statement**

Recently, many researchers have focused on the NRF2-driven signaling in cancer progression. Excitingly, discovery of non-coding RNAs has added new layers of intricacy to already complicated nature of KEAP1/NRF2 signaling in different cancers. These interactions are shaping the NRF2-driven signaling landscape and better knowledge of these pathways will be advantageous in pharmacological modulation of non-coding RNA-mediated NRF2 signaling in various cancers.

## **Introduction:**

Nuclear factor erythroid 2-related factor 2(NRF2) is a master transcriptional regulator. NRF2 contextually integrates cellular stress signals and responds by regulation of myriad of transcriptional networks. NRF2 sequestration is regulated by an inhibitory molecule, Kelch-like ECH-associated protein-1 (KEAP1), until inducers undergo an interaction with cysteine thiol residues of KEAP1. These chemical modifications are

necessary to conformationally change the KEAP1-NRF2 complexes and release of NRF2, enabling NRF2 to regulate transcriptional network. NRF2 and its negative regulator, KEAP1, have stimulated many high-impact publications and have become the topic of an important controversy. The controversy is centered on whether NRF2 behaves as a tumor suppressor or conversely, oncogenic, leading to a critical question of whether NRF2 should be pharmaceutically targeted for cancer inhibition and prevention. There are direct pieces of compelling evidence which highlight deregulation of KEAP1/NRF2 pathway because of mutations (Liu et al, 2020; Matić et al, 2022). It has previously been experimentally verified that NRF2 stimulated the expression of antioxidant enzymes (Kwak et al, 2001). RTA404, an NRF2 activator induced apoptotic death in malignant glioma (Tsai et al, 2021). NRF2 signaling has a linchpin role in the regulation of cancer onset and progression (Panda et al, 2022; Han et al, 2022; Zhang et al, 2021; Cykowiak and Krajka-Kuźniak, 2021; Sánchez-Ortega et al, 2021; Renaud et al, 2019; Wang et al, 2020). Tumorigenesis in SCID mice was noted to be reduced in mice injected with irradiated NRF2-knockdown cancer cells (Kamble et al, 2021). There was an evident increase in apoptotic death in NRF2-silenced-MDA-MB-468 and MCF-7 cancer cells (Bovilla et al, 2021). Intraperitoneally administered brusatol (pharmacological NRF2 inhibitor) slowed down the growth of tumor xenografts. Brusatol increased trafficking of the lymphocytes towards xenografted tumor tissues in mice (Bovilla et al, 2021). NRF2 activation induced radio-resistance in colorectal cancer cells. NRF2 signaling contributed to a radio-resistant phenotype in patients of rectal cancer (O'Cathail et al, 2021). NRF2 stimulated the expression of ALDH3A1 (aldehyde dehydrogenase 3a1) and enhanced gemcitabine refractoriness in pancreatic cancer cells (Matsumoto et al, 2021). ABC transporter proteins (ABCB1, ABCG2 and ABCC1) critically regulate drug resistance. There was a significant reduction in the levels of ABCB1, ABCG2 and ABCC1 in NRF2-silenced-sorafenib-resistant Huh7 cells. NRF2 inhibition led to restoration of sorafenib sensitivity in HCC cells (Gao et al, 2021). Bioactive chemicals from natural sources have been shown to inhibit NRF2-driven pathway in different cancers (Bhattacharjee and Dashwood, 2020; Wang et al, 2022). Xanthohumol and phenethyl isothiocyanate inhibited nuclear accumulation of NRF2 in various cancers (Cykowiak et al, 2021). Importantly, in the absence of NRF2, Jurkat T cells were found to

be more sensitive to hydrogen peroxide-induced oxidative stress, further strengthening the view that NRF2 promoted survival of tumor cells (Zagorski et al, 2017). NRF2 activation by CDDO-Im (synthetic triterpenoid) suppressed IL-2 secretions after T cell stimulation which clearly demonstrated that NRF2 tactfully regulated early steps following activation of Jurkat T cells (Zagorski et al, 2017). Modern high-throughput sequencing analysis have enabled researchers in unraveling the cornerstone role of non-coding RNAs in different cancers and unprecedentedly identified a very large number of non-coding transcripts. Non-coding RNAs are broadly characterized into microRNAs (miRNAs), long non-coding RNAs (lncRNAs) and circular RNAs (CircRNAs). Prominently, miRNAs have attracted considerable attention and reportedly involved in regulation of different cancers (Volinia et al, 2006; Lytle et al, 2007; Khraiwesh et al, 2010). LncRNAs are untranslated transcripts >200 nucleotides in length having 3-Dimensional structures that enable intricate interactions with proteins, DNA and RNA (Cabili et al, 2015; Iyer et al, 2015; Guttman et al, 2009; Chiu et al, 2018).

In this review, we highlight recent advancements and emerging concepts for interplay between miRNAs and lncRNAs in different cancers.

### **Positive regulation of NRF2 by miRNAs: Tumor Suppressive role of NRF2**

There is reported evidence of tumor suppressive roles of NRF2 in different cancers. In accordance with this concept, miRNA mediated activation of NRF2-driven signaling is also an exciting avenue.

miR-1225-5p markedly reduced migratory and invasive features of HT29 cells (Yang et al, 2020). miR-1225-5p directly targeted KEAP1 and caused an increment in NRF2 levels. NRF2 shuttled into the nucleus and transcriptionally upregulated Heme Oxygenase-1 in colorectal cancer cells. Rosmarinic acid exerted inhibitory effects on EMT-associated proteins particularly N-cadherin and matrix metalloproteinases. Rosmarinic acid promoted miR-1225-5p mediated targeting of KEAP1 and facilitated NRF2 nuclear accumulation (Yang et al, 2020).

### **Positive regulation of NRF2 by miRNAs: Oncogenic role of NRF2**

Apart from tumor suppressor activities of NRF2, the darker side of NRF2 has also been investigated. miRNA-328-3p directly targeted KEAP1 and relieved the inhibitory effects of KEAP1 on NRF2. miR-328-3p mimics reduced, but miR-328-3p inhibitors induced an increase in apoptotic death in gastric cancer cells (Xiao et al, 2021). Phosphoenolpyruvate carboxykinase 1 (PCK1) has been shown to interfere with NRF2-driven signaling (Shao et al, 2020). miR-584-5p loaded extracellular vesicles derived from hepatocellular carcinoma cells facilitated angiogenesis through targeting of PCK1. PCK1 overexpression inhibited the activation of NRF2 pathway. However, miR-584-5p-mediated targeting of PCK1 and enhanced NRF2-driven signaling. Growth of tumor xenografts was significantly reduced in mice inoculated with miR-584-5p antagomirs and Hep3B cells. Immunohistochemical findings revealed that miR-584-5p overexpression facilitated micro-vascularization (high expression of CD31) in tumor tissues. Vascular endothelial growth factor/Vascular endothelial growth factor receptor (VEGF/VEGFR) has contributory role in carcinogenesis. Additionally, miR-584-5p overexpression increased the expression levels of Vascular endothelial growth factor A (VEGF-A), VEGF, VEGFR2 and matrix metalloproteinases (Shao et al, 2020).

CircKEAP1 inhibited miR-141-3p-induced targeting of KEAP1 (Wang et al, 2021). KEAP1 levels were found to be increased whereas, NRF2 and HDAC4 levels were found to be substantially reduced in circKEAP1-overexpressing-A549 cancer cells. circKEAP1 overexpression drastically suppressed the growth rate of tumor xenografts derived from PC9 and A549 cancer cells. The tumor weights were reduced remarkably by circKEAP1 overexpression (Wang et al, 2021). miR-141-3p increased the stability of NRF2 by direct targeting of KEAP1 to fuel carcinogenesis. OGT (O-Linked N-Acetylglucosamine (GlcNAc) transferase) induced O-glycosylation modifications in KEAP1 at Serine-104, which promoted the ubiquitination and degradation of NRF2 (Huang et al, 2021). OGT overexpression in A2780 and drug-resistant A2780 cells caused an increase in KEAP1 glycosylation, accompanied by significant increase in ubiquitination of NRF2. miR-181d acted as an oncogenic miRNA and efficiently promoted cisplatin

resistance in cancer cells. miR-181d directly targeted OGT and consequently inhibited KEAP1 glycosylation and reduced NRF2 ubiquitination. Cisplatin treatments inhibited tumor growth but use of miR-181d mimics led to growth of the tumors (Huang et al, 2021). Collectively, these findings provided convincing evidence that miR-181d promoted the stability and activity of NRF2 and enhanced tumorigenesis.

### **Negative Regulation of NRF2 by Tumor Suppressor miRNAs:**

miR-140-5p has been shown to directly target NRF2 in breast cancer cells (Mahajan et al, 2021). miR-140-5p overexpression efficiently suppressed tumor growth by direct targeting of NRF2 pathway. Moreover, tumor volume and weights were higher in mice injected with miR-140-5p-knockdown MDA-MB-231 cancer cells (Mahajan et al, 2021). NRF2 phosphorylation is a necessary step to promote its nuclear translocation and transcriptional regulation of NRF2-target genes (De Blasio et al, 2020). NRF2 transcriptionally downregulated by miR-29b-1-5p in MDA-MB-231 cells. Overexpression of miR-29b-1-5p in cancer cells caused a considerable reduction in p-AKT levels. Consequently, NRF2 phosphorylation by p-AKT greatly promoted its nuclear accumulation in MDA-MB-231 cells. Therefore, miR-29b-1-5p overexpression not only reduced the levels of p-AKT but also interfered with p-AKT mediated phosphorylation and activation of NRF2. miR-29b-1-5p also negatively regulated DNA methyltransferases (DNMT1, DNMT3A, DNMT3B) and stimulated the expression of tumor suppressor genes HIN1, CCND2 and RASSF1A. The sesquiterpene lactone parthenolide stimulated the expression of miR-29b-1-5p and simultaneously reduced p-AKT and nuclear accumulation of NRF2 (De Blasio et al, 2020). In esophageal squamous cell carcinoma, different miRNAs have been noted to negatively regulate NRF2 expression (Zuo et al, 2020). miR-153-3p inhibited cell proliferation and restored cisplatin sensitivity by downregulation of NRF2 in Eca-109 cells (Zuo et al, 2020). miR-27b-3p directly targeted NRF2 and also reduced N-cadherin levels in EC9706 cells (Han et al, 2020). Additionally, miR-27b-3p overexpression increased Zonula occludens-1 (ZO-1) and E-cadherin levels (Han et al, 2020).

NEDD4 significantly enhanced K63-linked polyubiquitination of KLF8 (Mao, 2022). NEDD4 enhanced the binding of KLF8 to promoter regions of miRNA-132 and transcriptionally downregulated miR-132. KLF8 enhanced migration and invasion of bladder cancer cells by repression of miR-132. miR-132 suppressed migratory capacity of the bladder cancer cells by targeting NRF2. Tumor weight and volume were also noted to be palpably increased upon overexpression of NEDD4 but were regressed upon the knockdown of NRF2. Lung metastases were noted following tail vein injections and NEDD4 + sh-Ctrl groups demonstrated an increase in the number of pulmonary metastatic nodules. Whereas, fewer metastatic nodules were reported on the surface of lungs in NEDD4 + sh-NRF2 groups (Mao, 2022).

BYSL overexpression enhanced NRF2-driven downstream signaling in osteosarcoma cells (Zhang et al, 2022). There was a notable reduction in the levels of BYSL and NRF2 in osteosarcoma cells transfected with miR-378a-3p mimics. However, BYSL overexpression caused an increase in NRF2 levels. Importantly, there was significantly regression of tumor growth in mice inoculated with miR-378a-3p-overexpressing-MG63 osteosarcoma cells (Zhang et al, 2022). MAFG (v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog G) is a bZIP (basic leucine zipper) transcription factor (Shan et al, 2021). MAFG forms heterodimers with NRF2. Importantly, MAFG-NRF2 heterodimers bind and transcriptionally stimulate many genes involved in antioxidant defense. MAFG has been shown to be directly targeted by miR-4660. miR-4660 overexpression inhibited progression of osteosarcoma. Intratumoral injections of MAFG-shRNA lentivirus or pre-miR-4660 lentivirus led to regression of the tumors in mice subcutaneously injected with pOS-1 cells (Shan et al, 2021).

### **NRF2-mediated regulation of miRNAs:**

NRF2 induced upregulation of miR-125B and downregulated miR-29b in Acute Myeloid Leukemia (AML) (Shah et al, 2015). Primary AML samples transfected with miR-29b mimics and anti-miR-125b showed markedly higher apoptotic rate. miR-125b antagomiR and miR-29b mimics potentiated apoptotic cell death in AML cells and sensitivity to frontline chemotherapeutic agents (Shah et al, 2015). NRF2 transcriptionally upregulated by miR-129-3p (Sun et al, 2019). More importantly, miR-129-3p directly



targeted mTOR, induced autophagy and abrogated Trichostatin A-mediated killing effects. However, transfection of miR-129-3p inhibitors in MGC80-3 and Huh7 cells enhanced cytotoxic effects of Trichostatin A (Sun et al, 2019).

### **Interplay between long non-coding RNAs and NRF2 Contributes to Resistance against chemotherapeutic drugs:**

#### **Oncogenic role of LncRNAs:**

Taurine upregulated gene 1 (TUG1) promoted resistance against cisplatin in esophageal squamous cell carcinoma cells (Zhang et al, 2019). TUG1 interacted with NRF2 and enhanced its protein levels. However, NRF2 mRNA levels remained unchanged in TUG1-silenced cisplatin-resistant TE-1 cells. Use of NRF2-neutralizing antibody effectively abrogated TUG1-induced drug resistance in TE-1 cells (Zhang et al, 2019).

NRF2 overexpression increased the expression of TUG1 and enhanced adriamycin resistance in bladder cancer T24 and BIU-87 cell lines (Sun et al, 2019). Conversely, NRF2 knockdown markedly reduced expression levels of TUG1 in BIU-87 and T24 cells. Growth of the tumors was reduced significantly in experimental mice xenografted with either NRF2-silenced or TUG1-silenced T24 cells (Sun et al, 2019).

Co-localization of SLC7A11 Antisense RNA 1 (SLC7A11-AS1) with  $\beta$ -Transducin repeat-containing protein ( $\beta$ -TRCP1) has been experimentally verified in the nucleus (Yang et al, 2020). Exon 3 of SLC7A11-AS1 interacted with F-box motifs of  $\beta$ -TRCP1 and prevented ubiquitination and proteasomal degradation of NRF2. SLC7A11-AS1 overexpression in gemcitabine-resistant cancer cells reduced reactive oxygen species by blockade of  $\beta$ -TRCP-triggered ubiquitination and degradation of NRF2. SLC7A11-AS1 promoted chemoresistance by interfering with  $\beta$ -TRCP-regulated NRF2 degradation in pancreatic cancer cells (Yang et al, 2020). Small Nucleolar RNA Host Gene 14 (SNHG14) induced resistance against trastuzumab by stimulating the expression of Poly(A) Binding Protein Cytoplasmic 1 (PABPC1) through Histone H3-lysine 27 (H3K27) acetylation (Dong et al, 2018). Lysine 27 of Histone 3 was acetylated in SNHG14-expressing trastuzumab-resistant breast cancer cells. It was noted that NRF2

was reduced in SNHG14-silenced SKBR-3 cells, while ectopically expressed PABPC1 partially rescued these effects. On the contrary, NRF2 was increased in SNHG14-expressing breast cancer cells, but inhibition of PABPC1 partially impaired this increase in NRF2 expression (Dong et al, 2018). NRF2 regulation-associated lncRNA (NRAL) induced cisplatin resistance in HCC cells. NRAL interfered with miRNA-340-5p-mediated targeting of NRF2 and potentiated the expression of NRF2 in HCC cells. Cisplatin sensitivity was restored in NRAL-silenced cisplatin-resistant SMMC-7721 and HepG2 cells (Wu et al, 2019). MIR4435-2HG-knockdown restored drug sensitivity in cisplatin-resistant HCT116 cells. MIR4435-2HG induced an increase in NRF2 levels in HCT116 cells (Luo et al, 2020). Findings clearly indicated that MIR4435-2HG-mediated drug resistance through NRF2/HO-1 cascade.

### **Tumor Suppressor role of LncRNAs:**

KRAL is a lncRNA reportedly involved in regulation of KEAP1/NRF2 pathway (Wu et al, 2018). KRAL upregulation was necessary to induce apoptosis in 5-fluorouracil-resistant HCC cells. KRAL restored 5-fluorouracil sensitivity in HCC cells by potentiating the expression of KEAP1. KRAL overexpression notably increased levels of KEAP1 (mRNA and protein). On the contrary, KRAL silencing in SMMC-7721 and HepG2 cells significantly suppressed KEAP1 levels. KEAP1 was negatively regulated by miR-141. KRAL sequestered miR-141 away and potentiated the expression of KEAP1. KRAL-silenced SMMC-7721 and HepG2 cells demonstrated markedly higher resistance against 5-FU (Wu et al, 2018).

LAMTOR5-AS1 is upregulated in chemo-sensitive osteosarcoma cells (Pu, 2021). However, cisplatin induced downregulation of LAMTOR5-AS1 in osteosarcoma cells. NRF2 overexpression led to significant increase in the resistance of G-292 osteosarcoma cells to cisplatin. NRF2 transcriptionally upregulated the expression of LAMTOR5-AS1. Excitingly, level of NRF2 was found to be upregulated in LAMTOR5-AS1 overexpressing cells but decreased upon the knockdown of LAMTOR5-AS1. NRF2 phosphorylation led to its ubiquitination and consequent degradation. LAMTOR5-AS1 considerably reduced the phosphorylation of NRF2. LAMTOR5-AS1 exerted inhibitory effects on NRF2 K48 type ubiquitination. LAMTOR5-AS1 not only inhibited the activity of NRF2 by promoting the association

between KEAP1 and NRF2. LAMTOR5-AS1 binds to NRF2 and prevents KEAP1-mediated ubiquitylation of NRF2. PI3K/AKT activation inhibited GSK3 $\beta$  functions by promoting its phosphorylation. Contrarily, GSK3 $\beta$  dephosphorylation promoted NRF2 phosphorylation, ubiquitination and consequent degradation. LAMTOR5-AS1 knockdown caused an increase in the phosphorylated levels of AKT3 and GSK3 $\beta$ , but overexpression of LAMTOR5-AS1 inhibited the phosphorylation of AKT3 and GSK3 $\beta$ . LAMTOR5-AS1 overexpression enhanced cisplatin effects and inhibited tumor growth, tumor volume and weight in xenografted mice (Pu, 2021).

### **NRF2 mediated regulation of LncRNAs:**

NRF2 stimulated the expression of NmrA-like family domain containing 1 pseudogene (Loc344887) in gallbladder cancer cells. Downregulation of Loc344887 inhibited EMT (epithelial-mesenchymal transition) in gallbladder cancer cells (Wu et al, 2017). However, there is a direct piece of evidence that highlights tumor suppressive role of Loc344887 in sulforaphane-treated colon cancer cells. Results suggested that sulforaphane induced NRF2-mediated upregulation of Loc344887 and it suppressed cell proliferation and migration (Johnson et al, 2017). NRF2 also transcriptionally upregulated Smoke and cancer-associated lncRNA-1 (SCAL1) mRNA level in cigarette smoke-treated cells (Thai et al, 2013). Knockdown of SCAL1 expression potentiated cigarette smoke-induced toxicological effects in Human bronchial epithelial-1 (HBE-1) cells. SCAL1 expression was found to be markedly higher in some lung cancer cell lines such as A549, CL1-5, HCC-827 and NCI-H1975. Series of experiments provided evidence of baseline activation of NRF2. It was noted that prevalence of KEAP1-inactivating mutations in A549 and HCC-827 cells also resulted in higher levels of basal NRF2 (Thai et al, 2013). The nuclear hypoxia-regulated NLUCAT1 lncRNA was shown to be transcriptionally upregulated by NF- $\kappa$ B and NRF2. NLUCAT1 inhibition potentiated ROS production and cisplatin-induced ROS-dependent apoptotic cell death (Moreno et al, 2019).

### **LncRNAs work synchronously with NRF2 during Carcinogenesis:**

Apoptosis-associated transcript in bladder cancer, LOC284837 (AATBC/LOC284837) is a long intergenic noncoding RNA (lincRNA) (Zhao et al, 2015). AATBC acted as an oncogenic lincRNA and data clearly indicated that tumor xenografts were considerably reduced in mice injected subcutaneously with AATBC-silenced hypertriploid human cell line UM-UC-3. AATBC knockdown induced activation of c-Jun N-terminal kinase (JNK)-driven cascade and NRF2 inhibition in bladder cancer cells. Apoptosis induced by knockdown of AATBC was impaired by JNK inhibition in bladder cancer cells. JNK inhibition also stimulated an increase in NRF2 levels (Zhao et al, 2015). Overall, AATBC induced cancer progression via NRF2-mediated reduction in oxidative stress in bladder cancer cells. NRF2 was downregulated in TUG1-silenced prostate cancer cells (Yang et al, 2020). Treatment of TUG1-silenced prostate cancer cells with NRF2 activator markedly reversed inhibitory effects of TUG1 knockdown on proliferation, invasion and metastasizing potential (Yang et al, 2020).

### **Concluding Remarks:**

Interplay between mRNAs and NRF2 has been extensively explored but, the interaction between lncRNAs and NRF2 has not been adequately studied in different cancers. LncRNAs have pleiotropic role in different cancers, but the current scientific evidences do not provide comprehensive and in-depth analysis of oncogenic and tumor suppressor lncRNAs in context of KEAP1/NRF2 signaling. Likewise, certain clues regarding circular RNAs and NRF2 have emerged in brain injuries, but these aspects need to be tested in preclinical studies to comprehensively interpret their role in cancer initiation and progression (Yang et al, 2018; Xhang et al, 2019; Xu et al, 2020). Therefore, futuristic studies must converge on the dissection of the pathway between NRF2 and circular RNAs. These findings will be helpful in the refinement of our understanding related to NRF2 signaling and circular RNAs.

### Authorship Contributions:

Participated in research design: Yaylim, Farooqi, and Saso

Wrote or contributed to the writing of the manuscript: Yaylim, Farooqi, Telkoparan-Akillilar, and Saso

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#### **FIGURE LEGENDS:**

**Figure 1.** Shows (A) KEAP1 mediated control of NRF2. (B-D) NRF2 transcriptionally downregulated by miR-29b-1-5p. Therefore, miR-29b-1-5p overexpression not only reduced the levels of p-AKT but also interfered with p-AKT mediated phosphorylation and activation of NRF2. miR-29b-1-5p negatively regulated DNMT1, DNMT3A and DNMT3B and stimulated the expression of tumor suppressor genes (HIN1, RASSF1A and CCND2). (E) PCK1 negatively regulated NRF2 and blocked its transcriptional activity. However, miR-584-5p targeted PCK1 and promoted the oncogenic activity of NRF2. (F) miR-1225-5p directly targeted KEAP1 and enhanced tumor suppressor activities of NRF2. Abbreviations: KEAP1, Kelch-like ECH-associated protein 1; NRF2, Nuclear factor E2-related factor 2 ; p-AKT, phospho-AKT (also known protein kinase B, PKB; DNMT, DNA Methyltransferase; HIN1, Hairpin inducing protein-1; RASSF1A, Ras-association domain family 1, isoform A; CCND2, cyclin D2; PCK1, Phosphoenolpyruvate carboxykinase 1.

**Figure 2.** Shows (A) KRAL mediated increase in KEAP1 expression. KRAL blocked miR-141-mediated targeting of KEAP1. (B) NRAL-interfered with miR-340-5p-mediated targeting of NRF2 and allowed NRF2-mediated signaling and transcriptional regulation of target genes. (C) SLC7A11-AS1 also inhibited  $\beta$ -TRCP-mediated degradation of NRF2 and enhanced its oncogenic activities. (D) NRF2-mediated upregulation of Loc344887 has dualistic role in cancer progression. Loc344887 has a darker side as it promotes metastasis but Loc344887 also repressed cell proliferation. Loc344887 worked synchronously with NRF2 and triggered an increase in the expression of NQO1. Abbreviations: KRAL, Keap1 regulation-associated lincRNA; KEAP1, Kelch-like ECH-associated protein 1; NRAL, NRF2 regulation-associated lincRNA; SLC7A11-AS1, SLC7A11 Antisense RNA 1;  $\beta$ -TRCP,  $\beta$ -Transducin repeat-containing protein; Loc344887, NmrA-like family domain containing 1 pseudogene ; NRF2, Nuclear factor E2-related factor 2 ; NQO1, NAD(P)H Quinone Dehydrogenase 1.

Figure 1.

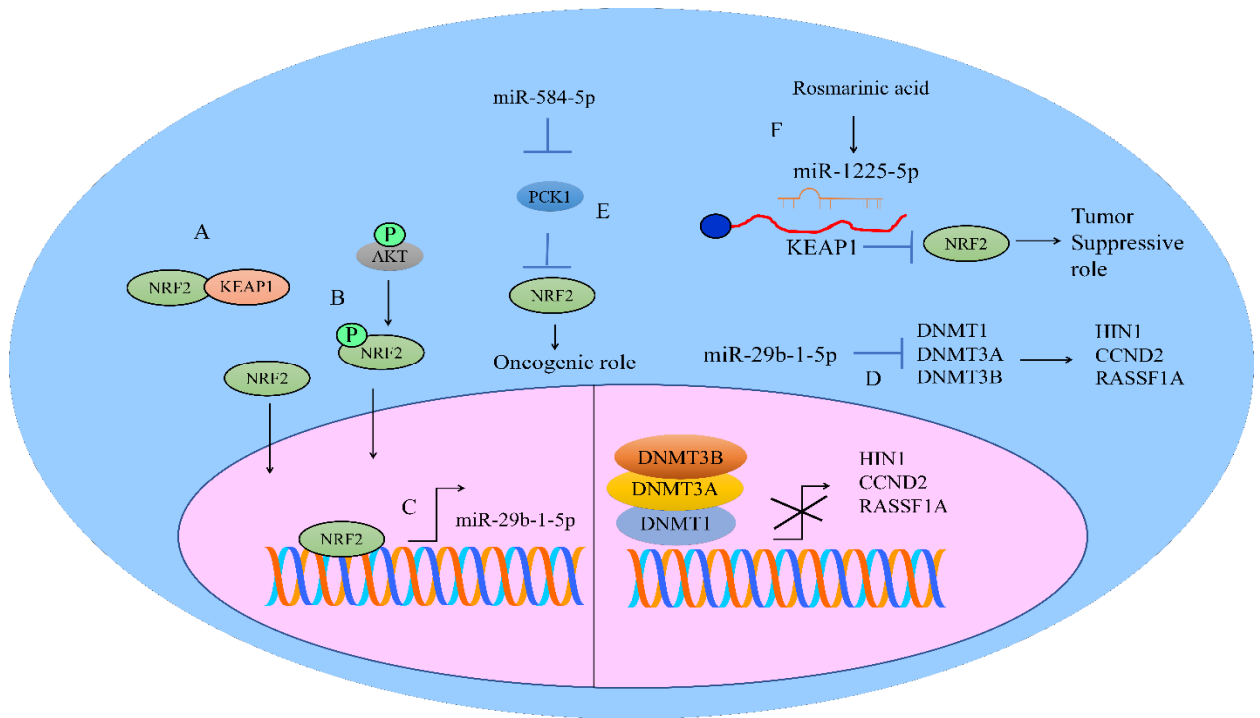


Figure 2.

