Dysregulation of NRF2 in Cancer: from Molecular Mechanisms to Therapeutic Opportunities

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Abstract

Nuclear factor E2-related factor 2 (NRF2) plays an important role in redox metabolism and antioxidant defense. Under normal conditions, NRF2 proteins are maintained at very low levels because of their ubiquitination and proteasomal degradation via binding to the kelch-like ECH associated protein 1 (KEAP1)-E3 ubiquitin ligase complex. However, oxidative and/or electrophilic stresses disrupt the KEAP1-NRF2 interaction, which leads to the accumulation and transactivation of NRF2. During recent decades, a growing body of evidence suggests that NRF2 is frequently activated in many types of cancer by multiple mechanisms, including the genetic mutations in the KEAP1-NRF2 pathway. This suggested that NRF2 inhibition is a promising strategy for cancer therapy. Recently, several NRF2 inhibitors have been reported with anti-tumor efficacy. Here, we review the mechanisms whereby NRF2 is dysregulated in cancer and its contribution to the tumor development and radiochemoresistance. In addition, among the NRF2 inhibitors reported so far, we summarize and discuss repurposed NRF2 inhibitors with their potential mechanisms and provide new insights to develop selective NRF2 inhibitors.

Key Words: NRF2, KEAP1, NRF2 inhibitors, Cancer
homodimerization and interaction with the CUL3-based E3 ubiquitin ligase complex (Zipper and Mulcahy, 2002; Furukawa and Xiong, 2005). The IVR domain contains highly reactive cysteine residues, such as Cys273, Cys288, and Cys297, which are easily oxidized and are thus responsible for sensing oxidative stress (Dinkova-Kostova et al., 2002). The DGR domain contains six repetitive kelch structures that specifically bind to the Neh2 domain of NRF2 (Itoh et al., 1999).

In normal conditions, KEAP1 plays a major role in restraining NRF2 activity by binding to the DLG/ETGE motifs in the Neh2 domain and inducing ubiquitination and proteasomal degradation of NRF2 (Itoh et al., 2010) (Fig. 2). Upon oxidative and/or electrophilic stress, highly reactive cysteine residues in KEAP1 are oxidized, which prevents KEAP1 from binding to NRF2 for ubiquitination (Zhang et al., 2004). Consequently, NRF2 is accumulated and translocated into the nucleus where it heterodimerizes with sMAF via its Neh1 domain and binds to antioxidant response element (ARE), inducing the transactivation of its target genes (Taguchi et al., 2011) (Fig. 2). The majority of NRF2’s targets encode metabolic enzymes regulating redox homeostasis by detoxifying reactive oxygen species (ROS) or electrophiles, and repairing the oxidative damage. Thus, promoting anti-oxidant defense in normal cells by activating NRF2 has been considered an attractive and promising strategy to prevent cancer development (Kwak and Kensler, 2010).

However, importantly, recent studies have shown that NRF2 is frequently activated by multiple mechanisms with potent oncogenic effects in cancer (Leinonen et al., 2015; Megnegon et al., 2016; Taguchi and Yamamoto, 2017). Analysis in The Cancer Genome Atlas (TCGA) showed that genetic mutations leading to the activation of NRF2 were found in more than 20% of lung adenocarcinomas (LUAD) and 34% of lung squamous cell carcinomas (LUSC) (Cancer Genome Atlas Research Network, 2012, 2014). Accumulating evidence suggests that the activation of NRF2 is critical for tumor cell proliferation, growth, and survival (Ohta et al., 2008; DeNicola et al., 2011; Mitsuishi et al., 2012; Jia et al., 2016). Moreover, NRF2 activation is thought to be the main cause of resistance to chemotherapy and radiotherapy (Ramos-Gomez et al., 2001; Singh et al., 2006; Shibata et al., 2008a; Jiang et al., 2010; Zhang et al., 2010; Zhou et al., 2013; No et al., 2014; Choi and Kwak, 2016; Ryoo et al., 2016). Thus, these data strongly suggest that inhibition of NRF2, either alone or in combination, could be a promising therapeutic strategy for cancer. However, currently, NRF2 inhibitors are neither clinically available nor under clinical trial. Recently, several NRF2 inhibitors have been reported to have promising therapeutic efficacy (Zhu et al., 2016). In this review, we summarize the currently-known mechanisms of NRF2 dysregulation in cancer. We also summarize the NRF2 inhibitors particularly focused on the repurposed one reported so far and discuss their potential mechanisms and future directions to develop selective NRF2 inhibitors.

**MECHANISMS OF NRF2 ACTIVATION IN CANCER**

In normal cells, the KEAP1-CUL3-RBX1 complex plays a
central role in regulating NRF2 activity by inducing ubiquitination and proteasomal degradation of NRF2, keeping the protein levels very low (Fig. 2). However, in cancer, this tight regulation of the KEAP1-NRF2 pathway has been reported to be compromised by multiple mechanisms discussed below (Fig. 2).

**Genetic mutations**

Somatic mutations of the genes involved in the KEAP1-NRF2 pathway comprise the most well-known mechanism of NRF2 activation in cancer (Sporn and Liby, 2012; Menegon et al., 2016). Recently, large-scale cancer genome projects, such as TCGA, have provided comprehensive characterization of genomic alterations in the KEAP1-NRF2 pathway. In Lung Adenocarcinoma (LUAD), loss of function mutations in KEAP1 and CUL3 leading to the activation of NRF2 were found in 19% and less than 1%, respectively, while gain of function mutations in NRF2 were found in 3% of patients with cancer (Cancer Genome Atlas Research Network, 2014). By contrast, in lung squamous cell carcinoma (LUSC), loss of function mutations in KEAP1 and CUL3 leading to the activation of NRF2 were found in 12% and 7% respectively, while gain of function mutations in NRF2 were found in 19% of patients with cancer (Cancer Genome Atlas Research Network, 2012). In addition to lung cancer, mutations in KEAP1 or NRF2 have been found in diverse cancer types, such as breast cancer (Sjöblom et al., 2006; Nioi and Nguyen, 2007), gastric cancer, colorectal cancer, prostate cancer (Yoo et al., 2012), gall bladder cancer (Shibata et al., 2008a), ovarian cancer (Konstantinopoulos et al., 2011), liver cancer (Guichard et al., 2012; Cleary et al., 2013; Fujimoto et al., 2016), and esophageal carcinoma (Kim et al., 2010; Shibata et al., 2011). Notably, in contrast to the KEAP1 mutations, which occur throughout the gene and are either missense or nonsense mutations (Singh et al., 2006; Ohta et al., 2008), all the mutations in NRF2 are found exclusively within regions encoding the DLG/ETGE motifs, which prevent KEAP1 binding (Shibata et al., 2008b). Recently, recurrent loss of NRF2 exon 2 was reported as a novel mechanism for the activation of NRF2 in lung cancer and head and neck cancer (Goldstein et al., 2016). Loss of exon 2 from the NRF2 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. In addition, the loss of function mutations in CUL3 and RBX1 leading to the activation of NRF2 have been reported frequently in sporadic papillary renal cell carcinoma (PRCC)
Epigenetic modifications

Epigenetic modifications in KEAP1 and NRF2 promoter regions contribute to the activation of NRF2 in cancer. The promoter region of KEAP1 is hypermethylated in several cancers, including lung (Wang et al., 2008; Muscarella et al., 2011), colon (Hanada et al., 2012), and prostate cancers (Zhang et al., 2010), leading to the reduction of KEAP1 expression and the accumulation of NRF2. Importantly, methylation within the KEAP1 promoter region in patients with glioma is associated with poor prognosis. Recently, demethylation of NRF2 promoter regions resulting in the overexpression of NRF2 was also reported in drug-resistant colon cancer cells (Zhao et al., 2015). These observations suggest that reversal of KEAP1 methylation or NRF2 demethylation would inhibit NRF2 expression, which might contribute to a better outcome of chemotherapy.

KEAP1-NRF2 disruptors

Accumulation of KEAP1-NRF2 disrupting proteins and metabolites can activate NRF2 in cancer. p62, also known as sequestosome 1 (SQSTM1), is the most well-known disruptor, which competes with NRF2 for directly binding to KEAP1 through an STGE motif that is similar to the ETGE motif in NRF2 (Coppole et al., 2010; Jain et al., 2010; Komatsu et al., 2010; Lau et al., 2010). Once bound to KEAP1, p62 induces autophagic degradation of KEAP1 (Komatsu et al., 2010). Importantly, recent studies have shown that p62 is upregulated in hepatocellular carcinoma (HCC) and p62-induced activation of NRF2 is critical for HCC development (Inami et al., 2011; Umemura et al., 2016), which supports the physiological significance of the p62-NRF2 axis in cancer development. Similarly, dipeptidyl-peptidase 3 (DPP3) (Hast et al., 2013), encoded by a Wilms tumor gene on the X chromosome (WTX) (Camp et al., 2012), and partner and localizer of BRCA2 (PALB2) (Ma et al., 2012) have been shown to disrupt the KEAP1-NRF2 interaction by competing with NRF2 for binding to KEAP1. Importantly, a recent study showed that DPP3 is overexpressed in breast cancer and its expression correlates with NRF2 downstream gene expression and poor prognosis, particularly in estrogen receptor-positive cancer (Lu et al., 2017). Recently, cyclin-dependent kinase 20 (CDK20) was identified as a novel KEAP1-interacting protein, which competes with NRF2 for KEAP1 binding through its N-terminal ETGE motif (Wang et al., 2017). Importantly, CDK20 is overexpressed in lung cancer tissues and is critical for promoting cell proliferation and radiochemoresistance in lung cancer. In addition, p21 and breast cancer 1 (BRCA1) were shown to compete with KEAP1 for binding to the ETGE and/or DLG motifs of NRF2 (Chen et al., 2009; Gorrini et al., 2013).

In addition to proteins, oncometabolites fumarate can also activate NRF2 by interrupting the KEAP1-NRF2 interaction. Deficiency of the tricarboxylic acid cycle enzyme, fumarate hydratase (FH), in type 2 PRCC induces the accumulation of fumarate, which induces succinylation of cysteine residues in KEAP1, resulting in the accumulation of NRF2 (Adam et al., 2011; Ooi et al., 2011). This activation of NRF2 was shown to be critical for growth and survival of FH-deficient PRCC.

Oncogenic signaling

Oncogenic signaling pathways can drive NRF2 activation in cancer. Kirsten rat sarcoma viral oncogene homolog (K-Ras), one of the most activated oncogenes in cancer was shown to increase NRF2 transcription via activation of the B-Raf-MEK-ERK (V-Raf-1 murine leukemia viral oncogene homolog B–mitogen-activated protein kinase kinase) signaling pathway (DeNicola et al., 2011). Moreover, they showed that the activation of K-Ras and B-Raf stimulates the transcription of NRF2 via activation of transcription factors Jun and Myc. Recently, another group showed that K-Ras-ERK signaling pathway increases NRF2 transcription through TPA (12-O-Tetradecanoylphorbol-13-acetate) response element (TRE) reside in a regulator region in exon 1 of NRF2 (Tao et al., 2014). Importantly, this activation of NRF2 was shown to be critical for tumor growth and enhanced chemoresistance of K-Ras mutant cancer cells (DeNicola et al., 2011; Tao et al., 2014). In addition, the phosphatidylinositol-4,5-bisphosphate 3-Kinase (PI3K)-serine/threonine kinase (AKT) signaling pathway can also induce NRF2 accumulation, either through an increase in NRF2 transcription (Mitsuishi et al., 2012), nuclear accumulation (Maddumai Hewage et al., 2017), or inhibition of GSK3-β-TrCP-induced proteasomal degradation of NRF2 (Chowdhry et al., 2013).

Stress signaling

The tumor microenvironment can be characterized as a stressful condition, where tumor cells encounter inflammation, oxidative stress, and nutrient starvation (Koumenis et al., 2014). Oxidative stress is a well-known inducer of NRF2 activation through cysteine oxidation and inhibition of KEAP1. Interestingly, accumulating data suggest that inflammation and nutrient deficiency also activate NRF2 in tumor cells. It was shown that lipopolysaccharide (LPS) induced NRF2 transcription via activation of NF-κB, which directly binds to κB site within the promoter region of NRF2 (Rushworth et al., 2008, 2012; Liu et al., 2017). Moreover, NRF2 is constitutively active in human acute myeloid leukemia (AML) cells via activation of NF-κB and conferred chemoresistance in AML suggesting that inflammation can induce chemoresistance via activation of NRF2. In addition, glucose deprivation induced ER stress-dependent activation of PRKR-like endoplasmic reticulum kinase (PERK), which in turn phosphorylates and activates NRF2 (Cullinan et al., 2003; Cullinan and Diehl, 2004; Ding et al., 2016). In addition, 5′-AMP-activated protein kinase (AMPK), which is activated under energy stress conditions (Jeon, 2016), can phosphorylate and activate NRF2 by inducing its nuclear accumulation (Joo et al., 2016). Another group showed that AMPK can also indirectly activate NRF2 by reducing endoplasmic reticulum (ER) stress (Zimmermann et al., 2015), which is inconsistent with another study that showed positive effect of ER-stress on NRF2 activation via PERK, as mentioned above. Thus, further studies are required to understand the role of ER-stress on NRF2 regulation. Collectively, considering that oxidative stress, inflammation, and nutrient deficiency in tumor microenvironment are activators of NRF2 as well as AMPK in tumors (Jeon and Hay, 2012, 2015), hyperactivation of NRF2 would be a common phenomenon in most tumors in vivo, even in the absence of other alterations in the KEAP1-NRF2 pathway.

RNA processing

NRF2 activation can also occur at the post-transcriptional level in cancer through abnormal regulation of microRNA
(miRNA) or mRNA splicing. Among the downregulated miRNAs in esophageal squamous cell carcinoma (ESCC), four miRNAs, miR-507, miR-634, miR-450a, and miR-129-5p, directly target and inhibit the expression of NRF2 and are associated with poor prognosis (Yamamoto et al., 2014). MiR-141, which targets KEAP1 and induces NRF2 accumulation, is upregulated in cisplatin-resistant ovarian cancer and 5-fluorouracil (5-FU)-resistant HCC and contributes to chemoresistance (van Jaarsveld et al., 2013; Shi et al., 2015). In addition, abnormal splicing of KEAP1 mRNA, resulting in nonfunctional KEAP1 protein that is unable to restrain NRF2, was reported in colon cancer cells (Zhang et al., 2010). These observations suggest that NRF2 can be also activated during KEAP1 or NRF2 mRNA processing.

**Hormonal activation**

Lastly, hormonal activation of NRF2 has been reported in ovarian cancer. Compared with benign ovarian tumor, ovarian carcinoma overexpresses NRF2, which can be attributed to the effect of gonadotrophins and sex steroid hormones, such as follicle-stimulating hormone (FSH), estrogen (E2), and luteinizing hormone (LH) (Liao et al., 2012). These hormones can activate NRF2 by inducing ROS levels, which inhibits KEAP1 via oxidation of its multiple cysteine residues (Liao et al., 2012). Moreover, NRF2 activation is critical for FSH-induced activation of hypoxia-inducible factor 1 alpha (HIF-1) and vascular endothelial growth factor (VEGF) expression in ovarian cancer, which is critical for tumor angiogenesis (Zhang et al., 2013). Thus, these data suggest that NRF2 might also play a key role in the development and progression of hormone-related cancers, such as breast, prostate, and ovarian cancer.

**NRF2 INHIBITORS FOR CANCER THERAPY: A REPURPOSING APPROACH**

A growing body of evidence suggests hyperactivation of NRF2 in a variety of cancers and its critical role in tumorigenesis and radiochemo resistance; therefore, there is an increasing demand for the development of NRF2 inhibitors for clinical applications (Zhu et al., 2016). Although no inhibitors are currently clinically available or under clinical trial, some effective NRF2 inhibitors with potential antitumor efficacy have been reported (Zhu et al., 2016). These NRF2 inhibitors include natural compounds extracted from plants such as flavonoids and alkaloids, and novel synthetic compounds, such as ARE expression modulator 1 (AEM1) and ML385 (Bollong et al., 2015; Singh et al., 2016; Zhu et al., 2016). Moreover, some vitamins and commercial drugs developed for other indications have been identified as NRF2 inhibitors, including ascorbic acid (AA), all-trans-retinoic acid (ATRA), antitubercular agents, metformin, and glucocorticoids (GCs). Considering the high risk and time-consuming process of *de novo* anti-cancer drug development, a drug repurposing strategy to develop NRF2 inhibitors could be the first option in the current situation of unmet medical need. Thus, the reported repurposed NRF2 inhibitors are summarized and discussed below.

**Ascorbic acid**

AA, also known as vitamin C, is a powerful antioxidant and cofactor that participates in diverse enzymatic reactions (Mandl et al., 2009; Du et al., 2012). AA has been suggested to have anti-cancer properties without cytotoxicity in normal cells by selectively inducing ROS in cancer, but not in normal cells (Chen et al., 2005; Ranzato et al., 2011). However, the mechanisms of selective toxicity to cancer cells remain elusive. AA, a reduced form of vitamin C, is taken up by cells through sodium-dependent vitamin C cotransporters (SVCTs), while the oxidized form of vitamin C, dehydroascorbic acid (DHA), is taken up by cells through glucose transporters (GLUTs) (Mandl et al., 2009; Du et al., 2012). Once inside the cells, DHA is reduced to AA by consuming glutathione (GSH), thioredoxin (TRX), and nicotinamide adenine dinucleotide phosphate (NADPH). Recently, it has been shown that K-Ras and B-RAF proto-oncogene, serine/threonine kinase (BRAF) mutant colorectal cancer cells are selectively sensitive to AA by overexpressing glucose transporter type 1 (GLUT1), which is responsible for the uptake of DHA (Yun et al., 2015). The accumulation of DHA causes depletion of GSH and induction of oxidative stress in the cancer cells, suggesting that AA can be a selective prooxidant in cancer cells conferring cancer specific toxicity. In addition to this mechanism, considering that K-Ras and BRAF oncogenic signals were shown to activate NRF2 as discussed above (DeNicola et al., 2011), it would be also plausible to speculate that AA could selectively induce ROS and cytotoxicity in those cancer cells by inhibiting NRF2. Interestingly, a study published more than 10 years ago reported that AA can inhibit NRF2 signaling (Tarumoto et al., 2004). The authors showed that the imatinib resistant KCL22/SR leukemia cells have higher NRF2/ARE complex formation ability and NRF2 target expression than the parental imatinib-sensitive KCL22 cell line. AA treatment reduced the binding of NRF2 to ARE, possibly through the inhibition of nuclear translocation of NRF2, and restored imatinib sensitivity. Additionally, another study showed that AA induced the production of too high levels of hydrogen peroxide resulting in the inhibition rather than the activation of NRF2 and heme oxygenase 1 (HO-1) expression in Huh7 liver cancer cells (Wagner et al., 2011). Thus, further work is required to determine if NRF2 inhibition is the main mechanism of the anti-cancer effect of AA and if the application of AA could be a promising therapeutic strategy to treat cancer with high NRF2 activity.

**Retinoic acid (RA)**

Dietary vitamin A is metabolized into biologically active and functionally distinct metabolites called retinoids, which include retinol, retinal, and retinoic acid (RA). Among them, RA is considered the major form that exerts the anti-tumorigenic function of vitamin A, largely by inducing cell differentiation and inhibiting proliferation (Connolly et al., 2013). RA functions as a ligand of retinoic acid receptors (RARs) and retinoid X receptors (RXRs), which belong to the type II nuclear receptor family (Duong and Rochette-Egly, 2011). In the absence of RA, RARs and RXRs form heterodimers in the nucleus and recruit transcriptional co-repressors to promoter regions and inhibit transcription. Upon RA binding to the RAR-RXR heterodimer, the co-repressor is replaced with a co-activator in the promoter complex to promote transcriptional activation. Interestingly, RA, particularly all-trans-retinoic acid (ATRA) was shown to inhibit NRF2 through RARα (Wang et al., 2007). The RA-RARα complex can bind to NRF2 and interfere with ARE binding of NRF2, without affecting its nuclear translocation. Moreover, in acute myeloid leukemia (AML) and acute promyelocytic leukemia (APL) cells, ATRA was shown to sensitize arsenic trioxide
Antitubercular agents: Isoniazid (INH) and Ethionamide (ETH)

INH is the most reliable and commonly used medication for tuberculosis. ETH is a second line drug in tuberculosis therapy, used only in combination with other agents and for drug-resistance tuberculosis. INH and ETH have similar structures and mechanisms of action, and inhibit mycobacterial fatty acid synthesis (enoyl-ACP reductase), which is necessary for cell wall synthesis and repair (Vilcheze and Jacobs, 2014). Moreover, chronic treatment with these drugs induces severe liver injury, leading to acute liver failure as a major undesirable effect (Ramappa and Athal, 2013). Interestingly, it has been suggested recently that the hepatotoxicity caused by antitubercular drugs is attributed to inhibition of NRF2. In the Hep3B hepatoma cell line, INH prevented nuclear translocation of NRF2 by inhibition of extracellular signal-regulated kinase 1 (ERK1) phosphorylation, which leads to the oxidative stress and apoptosis (Verma et al., 2015). In addition, INH effectively inhibited the mRNA expression of NRF2-inducible genes in mouse preadipocyte 3T3-L1 cells (Chen et al., 2013). Importantly, via inhibition of NRF2, INH sensitized acute myeloid leukemia (AML) THP1 cells to cytotoxicity by arsenic trioxide (ATO) (Peng et al., 2016) suggesting that the inhibition of NRF2 by INH is a novel combination strategy to overcome chemoresistance.

Metformin

Interestingly, NRF2 signaling may also have clinical implications in diabetes management, given that diabetes carries an elevated risk of malignancy (Giovannucci et al., 2010) and some common anti-diabetic drugs have been suggested as potential NRF2 modulators. Metformin is widely used for the first-line treatment for type 2 diabetes mellitus (Rojas and Gomes, 2013). The anti-diabetic effects of metformin can be attributed, at least in part, to the activation of AMPK by inducing energetic stress caused by inhibition of mitochondrial metabolism. Interestingly, retrospective epidemiological analysis proposed that long-term administration of metformin reduced the incidence of cancer and mortality in diabetic patients (Evans et al., 2005; Decensi et al., 2010). Moreover, a growing body of evidence supports the anti-tumorigenic effects of metformin, either alone or in combination, in various types of cancer in vitro and in vivo (Morales and Morris, 2015). Although the involvement of the AMPK-mammalian target of rapamycin complex 1 (mTORC1) axis has been proposed, the mechanisms of metformin’s anti-tumor effect remain controversial (Kasznicki et al., 2014). Recently, NRF2 inhibition was proposed to mediate the anti-tumor effect of metformin. Metformin reduced NRF2 mRNA transcription by attenuating the RAF-ERK signaling pathway, but not by activating the AMPK signaling pathway in HepG2, HeLa, and A549 cancer cells (Do et al., 2013). A subsequent study by the same group found additional mechanisms by which metformin reduces NRF2 mRNA transcription through the induction of p53-dependent expression of miR-34a targeting SIRT1 (sirtuin-1) mRNA, and thereby inhibition of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)-mediated NRF2 transcription (Do et al., 2014). Consequently, metformin enhanced the susceptibility of cancer cells to oxidative stress and tumor necrosis factor superfamily member 10 (TRAIL)-induced apoptosis in a p53-dependent manner, suggesting that p53 status is a critical factor determining the efficacy of combinations comprising metformin. Currently, several clinical trials focusing on the therapeutic effects of metformin as an anti-cancer agent, either alone or in combination with chemotherapeutic drugs, are ongoing (Chae et al., 2016).

In contrast, a recent study showed that another class of anti-diabetic drug, dipeptidyl peptidase-4 (DPP-4) inhibitors, might potentially induce NRF2 activation, contributing to acceleration of cancer metastasis (Wang et al., 2016). DPP-4 inhibitors reduce blood glucose levels by increasing bioactive incretins, which promote glucose-dependent insulin secretion.
and inhibit glucagon secretion from the pancreas to maintain blood glucose homeostasis (Drucker, 2007). However, the mechanisms by which DPP-4 inhibitors induce NRF2 activity are largely unknown. Considering that they have the largest prescription volume among the new antidiabetic drug classes (Ahren, 2008; Phung et al., 2010; Noh et al., 2017), the recent findings regarding their potential NRF2-modulatory effects are of significant importance for patients with diabetes who are chronically exposed to the respective antidiabetic therapy and who are at increased risk of developing malignant complications because of underlying disease. Although the mechanisms of DPP-4 inhibitors’ effects on NRF2 activation remain elusive, it might be beneficial to use a DPP-4 inhibitor in combination with metformin in diabetes patients who also have cancer as a comorbidity.

**Glucocorticoids (GCs)**

Glucocorticoids (GCs), also known as stress hormones, are a class of corticosteroids that play a key role in the regulation of inflammation and metabolism (Kadmiel and Cidlowski, 2013). GCs are synthesized and released from the adrenal cortex upon activation of the hypothalamic-pituitary (HP) axis. The effects of GCs are mediated by binding to the glucocorticoid receptor (GCR), which belongs to the type I nuclear receptor subfamily 3. Upon binding to GCR, the GC-GCR complex translocates to the nucleus to regulate gene expression. Alternatively, the GC-GCR complex can elicit biological effects through direct protein-protein interactions in the cytosol.

The first link between GC and NRF2 came from a study investigating the effect of dexamethasone (DEX), a potent synthetic GC, on the expression of glutathione-S-transferase (GST), a well-known target of NRF2 (Ki et al., 2005). The promoter region of GST contains both glucocorticoid response element (GRE) and ARE sequences. In the present study, the DEX-GCR complex inhibited the expression of GST through binding to the GRE where it blocked ARE-bound NRF2 activity via silencing mediator of retinoic acid and thyroid hormone receptor (SMRT), suggesting that the inhibition of NRF2 by GCs is confined to certain promoter regions having both GRE and ARE sequences in a composite manner (Fig. 3A). However,
another group showed that cortisol inhibited NRF2 in a ARE-luciferase assay, suggesting that the inhibition of NRF2 by GCs does not require a GRE sequence in the promoter region (Kratschmar et al., 2012). Thus, the mechanism by which GCs inhibit NRF2 remains to be elucidated. In addition, the effects of NRF2 inhibition by GCs on cancer was not investigated.

Recently, using a cell-based ARE-luciferase assay, our group reported that unbiased drug repositioning screening identified clobetasol propionate (CP), a GC analog used for various skin disorders, as the most potent NRF2 inhibitor (Choi et al., 2017). CP induced both cytosolic accumulation and proteasomal degradation of NRF2 through GCR binding and in a GSK3b-TrCP dependent manner, suggesting that CP promotes protein-protein interaction between GCR and NRF2 (Fig. 3B). Importantly, CP potently and selectively inhibited anchorage-independent (AI) growth of KEAP1 mutant lung cancer cells, and the cytotoxicity of CP is dependent on the inhibition of NRF2. Notably, CP is 100 times more potent than DEX in the inhibition of NRF2, as well as in the AI growth of KEAP1 mutant lung cancer cells. Furthermore, CP, alone or in combination with the mTORC1 inhibitor rapamycin, strongly inhibited the in vitro and in vivo growth of tumors harboring mutations in KEAP1 or in both KEAP1 and liver kinase B1 (LKB1) that are frequently observed in lung cancer.

Consistently, a recent study supported the direct interaction between NRF2 and GCR as the mechanism of NRF2 inhibition by GCs (Alam et al., 2017). They showed that GCR was identified as the NRF2 binding protein and that the Neh4/5 transactivation domains of NRF2 interact with GCR. However, DEX inhibited NRF2 transcriptional activity by promoting GCR recruitment to ARE-bound NRF2 and blocked CBP’s interaction with NRF2, suggesting that GCs transrepress NRF2 by tethering GCR with NRF2, which is a similar mechanism to the inhibition of other transcription factors such as nuclear factor kappa B (NF-kB) and activator protein-1 (AP-1) by GCs (Kassel and Herrlich, 2007) (Fig. 3C).

FUTURE DIRECTIONS

A growing body of evidence has revealed frequent activation of NRF2 via diverse mechanisms in most cancers; therefore, inhibition of NRF2 should be a promising therapeutic strategy to treat cancer. No NRF2 inhibitors are currently available for clinical application; therefore, developing clinically relevant NRF2 inhibitors is highly demanded. Although several NRF2 inhibitors from natural and synthetic compounds, and existing drugs, have been reported, most of them suffer from low potency, non-specificity, and inconsistency in their effects on NRF2 (Meneon et al., 2016; Zhu et al., 2016). For example, AA, RA, and metformin among the inhibitors have also been reported to activate NRF2 in different setting (Zhu et al., 2016). In addition, high (millimolar) concentrations of INH and ETH were used to examine NRF2 inhibition and cytotoxicity in vitro (Verma et al., 2015; Peng et al., 2016). Moreover, the anti-tumorigenic effects of INH and ETH have not been tested in vivo. However, consistent results in the inhibitory effect of GCs on NRF2 have been reported (Choi et al., 2017). Moreover, GCs, particularly CP, potently inhibited NRF2 and tumor growth in vivo, suggesting that only CP is a valid candidate to be developed as an NRF2 inhibitor among clinical compounds (Choi et al., 2017). However, the potential limitations of using GCs for cancer therapy are their side effects such as hyperglycemia and immunosuppression. One approach to avoid such potential problems is to develop selective glucocorticoid receptor agonists and modulators (SEGRAM) (Sundahl et al., 2016). GCs inhibit NRF2 through the protein-protein interaction between GCR and NRF2, but not through the regulation of transcriptional activity of GCR which is responsible for the effects on metabolism and immune function; therefore, it would be possible to design a GC analog that binds to GCR only to induce the interaction with NRF2 and GSK3b, but not sufficient to induce its transcriptional activity (Fig. 4). SEGRAM would be an exciting strategy to develop selective and safe NRF2 inhibitors that warrant further intensive research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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