Review

Cellular Modulators of the NRF2/KEAP1 Signaling Pathway in Prostate Cancer

Giovanni Tossetta[1],*, Sonia Fantone[1], Daniela Marzioni[1], Roberta Mazzucchelli[2]

[1]Department of Experimental and Clinical Medicine, Università Politecnica delle Marche, 60126 Ancona, Italy
[2]Department of Biomedical Sciences and Public Health, Section of Pathological Anatomy, Università Politecnica delle Marche, 60126 Ancona, Italy
*Correspondence: g.tossetta@univpm.it (Giovanni Tossetta)

Abstract

Prostate cancer is the second most common malignancy in men worldwide. Prostate cancer can be treated by surgery, radiotherapy and hormone therapy. The latter, in the form of androgen-deprivation therapy is needed to reduce prostate cancer progression at an advanced stage. Several studies demonstrated that oxidative stress is involved in cancer occurrence, development and progression and the Nuclear factor erythroid 2-related factor 2 (NRF2)/Kelch Like ECH Associated Protein 1 (KEAP1) pathway is affected by reactive oxygen species (ROS). Furthermore, the NRF2/KEAP1 signaling pathway has been investigated by several studies related to anti-androgen therapy, biochemical recurrence and radiotherapy. In this review we analysed the current literature regarding the indirect modulators involved in NRF2/KEAP1 pathway regulation and their role as possible therapeutic targets in prostate cancer cells.

Keywords: NRF2; chemotherapy; prostate cancer; signaling; KEAP; antioxidants; modulator

1. Introduction

Prostate cancer is the second most common malignancy in men [1]. Currently, the treatment options for prostate cancer include surgery, radiotherapy, hormone therapy and chemotherapy. Surgery and radiotherapy are the main treatment for localized prostate cancer, while androgen deprivation therapy (ADT) is the mainstay treatment for patients with advanced prostate cancer [2]. Although ADT initially shows good efficiency, the therapy often fails because patients become insensitive to the androgen blockade, and eventually develop castration-resistant prostate cancer (CRPC) [3–5]. Therefore, understanding the molecular mechanisms characterizing prostate cancer onset and progression may significantly improve patient treatment and outcomes.
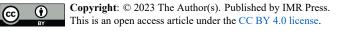
The Nuclear Factor Erythroid 2-Related Factor 2 (NRF2 or NFE2L2)/Kelch-like ECH-Associated Protein 1 (KEAP1) pathway plays a key role in protecting cells from oxidative stress damage. In fact, it is affected by reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydroxyl radical ($^\bullet OH$) and hydrogen peroxide (H_2O_2), which can damage cellular components such as DNA, proteins and lipids [6,7]. In normal condition, the NRF2 is bound to its repressor KEAP1 which is part of the CULLIN 3 (CUL3)/RING-box protein 1 (RBX1)/E3-ubiquitin ligase complex, targeting NRF2 for proteasomal degradation [8]. Under oxidative stress, ROS induce KEAP1 conformational changes that inhibit NRF2 ubiquitination and degradation, allowing NRF2 translocation into the nucleus and its binding to the antioxidant response elements (AREs) regions in the promoter of antioxidant genes, thereby promoting their expression. Therefore, ROS activate the NFR2/KEAP1 signaling pathway which is the main regulator of antioxidant enzymes involved in ROS neutralization [9,10] in order to balance oxidative mediators and maintain cellular redox homeostasis [11,12]. Although ROS are normally produced in cells, high ROS levels cause oxidative stress which is involved in several processes including endothelial dysfunction [13–17], inflammation [6,14,18,19] and cancer [6,20–22].

Since NRF2/KEAP1 signaling pathway promotes the survival and proliferation of cancer cells, it is emerging as a potential therapeutic target in many neoplasms including ovarian, cervical, endometrial and prostate cancer [11,21,23]. In fact, NRF2 expression is significantly higher in chemo- and radio-resistant cancer tissues protecting cancer cells from the oxidative damage caused by chemotherapy and radiotherapy [11,23–25]. Furthermore, NRF2 induces the expression of ATP-binding cassette (ABC) transporters to protect cancer cells from oxidative damage mediated by efflux chemotherapeutics [24]. NRF2 also plays a key role in regulating the self-renewal of cancer stem cells (CSCs), promoting the tumorigenicity, high rate of metastases and chemoresistance capacity of these cells [11].

The NRF2/KEAP1 signaling pathway plays an important role in modulating androgen receptor (AR) expression under ADT treatment. NRF2 can decrease AR expression in prostate cancer cells improving ADT therapy response and reducing cancer cells growth [26,27].

Thus, therapeutic drugs targeting NRF2/KEAP1 signaling pathway in prostate cancer patients may significantly improve the therapy efficiency, improving the outcome of this disease.

Funes and colleagues [28] reported that NRF2 mRNA expression significantly decreased in prostate cancer tissues compared to normal tissues and was accompanied by a decreased expression of the NRF2-dependent genes Glutamate-Cysteine Ligase Modifier Subunit (*GCLM*), Glutamate-Cysteine Ligase Catalytic Subunit (*GCLC*) and NAD(P)H Quinone Dehydrogenase 1 (*NQO1*). Moreover, decreased NRF2 mRNA expression in prostate cancer tissue was associated with biochemical cancer recurrence (BCR) [28]. This study is in disagreement with two studies evaluating NRF2 protein expression. Kawata *et al.* [29] showed that cytoplasmic NRF2 and NQO1 protein expression were significantly upregulated after androgen-deprivation therapy (ADT) in both human prostate gland and carcinoma specimens suggesting that ADT induces NRF2 activation in prostate tissues and that NRF2 is a candidate molecular target for the prevention of castration-resistance progression of prostate cancer. Moreover, Raatikainen and colleagues [30] reported that cytoplasmic NRF2 expression was more abundant in prostate cancer tissues compared to normal tissues. Cytoplasmic NRF2 expression was associated with the elevation in Prostate-Specific Antigen (PSA) levels, which is the first sign of BCR in the prostate. Furthermore, in the multivariate analysis, increased cytoplasmic NRF2 expression predicted shortened BCR and worse overall survival [30].

The different results reported by Funes and colleagues [28] may be due to the only evaluation of NRF2 mRNA expression. The expression of mRNA can be posttranscriptionally modulated and stabilized by non-coding RNAs, thereby promoting its expression [31].

The involvement of the NRF2/KEAP1 signaling pathway in cancer progression was also evaluated in prostate cancer cell lines. It has been reported that ROS levels were significantly higher in PC3 cells than in DU145 cells. DU145 cells also showed higher levels of basal glutathione (GSH) content than PC3 cells. Moreover, DU145 cells had increased levels of NRF2 and NRF2-dependent genes such as *GCLM*, *GCLC*, Heme oxygenase 1 (*HO-1*) and Thioredoxin Reductase 1 (*TXNRD1*) leading to the radio-resistance of these cells. The sensitivity to radiation increased after NRF2 silencing [25]. Thus, *NRF2* plays a key role in the radio-resistance of prostate cancer cells.

In this review, we analyzed the current literature regarding the indirect modulators involved in NRF2/KEAP1 pathway regulation and their role in prostate cancer cells as possible therapeutic targets.

2. NRF2 Cellular Modulators

The polybromo-associated BAF (PBAF) chromatin remodeling complex belongs to the Switch/Sucrose Non-fermentable (SWI/SNF) family and is essential for mammalian gene transcription and development [32–34]. The PBAF complex includes BRG1, PBRM1, ARID2, and BRD7, and is involved in regulating cell differentiation and genomic integrity [32]. In addition, polybromo-1 (PBRM1) is involved in DNA damage-induced transcriptional repression and DNA repair regulating the expression of genes involved in the oxidative stress response and apoptosis [35–37]. Mucin 1 C subunit (MUC1-C) is an oncogenic protein that promotes the progression of CRPC to neuroendocrine prostate cancer (NEPC) promoting self-renewal capacity, epithelial–mesenchymal transition (EMT), cancer stem cell (CSC) state, and tumorigenicity [38–40].

SLC7A11/xCT is a cystine-glutamate antiporter involved in mediating the uptake of extracellular cystine which is reduced to cysteine, a precursor in GSH synthesis. SLC7A11 is widely expressed in human tissues and protects cells from oxidative stress, thereby promoting GSH production, an important peptide with antioxidant effects [41,42]. SLC7A11 plays also a key role in cancer favouring drug resistance [43].

Hagiwara and colleagues [44] reported that MUC1-C forms a complex with NRF2 and PBRM1 on the NRF2 target *SLC7A11* gene, which encodes the xCT cystine-glutamate antiporter, increasing chromatin accessibility and inducing SLC7A11/xCT expression. Silencing MUC1-C or PBRM1 in DU-145 and LNCaP prostate cancer cells led to a significant downregulation of NRF2 and GSH levels increasing oxidative stress. Moreover, the authors found that MUC1-C and PBRM1 are necessary for the induction of other NRF2 target genes such as *G6PD* and *PGD* that are involved in regulating the pentose phosphate pathway. Thus, MUC1-C/PBRM1 signaling interacts with NRF2 to promote the expression of NRF2-dependent genes [44].

Pannexin 2 (PANX2) is a member of the pannexin family of membrane channel proteins [45]. High PANX2 expression promotes cancer cells proliferation and is associated with poor survival in renal cell carcinoma (RCC) [46]. Ferroptosis is an iron-dependent form of cell death caused by an accumulation of ferric iron and lipid peroxidation [47–49]. the NRF2/KEAP1 signaling pathway plays a significant role in preventing ferroptosis through the activation of antioxidant enzymes that counteract ROS production during ferroptotic cell death [50,51].

PANX2 expression was significantly higher in prostate cancer cell lines (PC3, DU145, and LNCaP) compared to the normal immortalized human prostate epithelial cells (RWPE1). Moreover, PANX2 silencing significantly decreased cell proliferation, migration, and invasion, while increasing ferrous iron and malondialdehyde (MDA) levels in PC3 cells. However, these effects were rescued by oltipraz (an NRF2 activator), while PANX2 silencing significantly reduced NRF2, HO-1, and Ferritin heavy chain (FTH1) protein expression. Therefore, PANX2 may play a key role in prostate cancer pathogenesis since it regulates cancer cell proliferation, migration, invasion, and ferroptosis modulating the NRF2 signaling pathway [52].

The p62 protein, also known as sequestosome-1 (SQSTM-1), binds ubiquitinated proteins, targeting them for autophagy degradation [53]. Autophagy is a cellular mechanism involved in maintaining cellular homeostasis

through the degradation and recycling of organelles, proteins, lipids, and nucleic acids [53]. High levels of p62 are associated with several diseases including inflammation, metabolic dysfunction, and cancer [54,55]. An interesting study by Jiang and colleagues [56] found that p62 expression was significantly higher in prostate cancer cells compared to normal prostate tissues. Moreover, silencing p62 in DU145 cells significantly increased KEAP1 expression, while downregulating NRF2 expression and activation (nuclear expression). NRF2 silencing significantly decreased cell proliferation and invasion, whilst increasing apoptosis in DU145 cells. Furthermore, authors found that p62 activated the NRF2 pathway by binding KEAP1 and by inducing its proteasomal degradation, subsequently promoting NRF2 expression and the transcription of NRF2-dependent genes such as *HO-1*, *NQO1*, *GCLC* and *GLCM*, resulting in decreased ROS levels. This study showed that p62 promotes prostate cancer progression by activating the KEAP1/NRF2 pathway. Thus, p62 may be a therapeutic target to improve prostate cancer outcomes [56]. Another study investigated the role of Speckle-type BTB/POZ protein (SPOP) in regulating p62 and NRF2 activity in prostate cancer. SPOP is a substrate recognition receptor for the CUL3/RING ubiquitin ligase complex that leads to the ubiquitination of its target substrates. Point mutations have been reported in the MATH domain (the substrate binding domain) of SPOP in prostate cancers [57,58]. In addition, it has been reported that SPOP induces the non-degradative ubiquitination of p62, decreasing its ubiquitin-binding capacity, thereby suppressing p62-dependent autophagy in DU-145 cells. Moreover, SPOP attenuated p62-mediated KEAP1 sequestration facilitating NRF2 proteasomal degradation and decreasing the transcriptional activation of NRF2-dependent antioxidant genes. Furthermore, authors reported that SPOP mutants lose the capacity to ubiquitinate p62 by promoting autophagy and NRF2-induced antioxidant response in prostate cancer cells. Thus, SPOP plays a significant role in inhibiting autophagy through the p62/NRF2 signaling [59].

LncRNA taurine-upregulated gene 1 (*TUG1*) is a long non-coding RNA (lncRNA) located on chromosome 22q12.2. Non-coding RNAs (ncRNAs) represent approximately 98% of human genome transcripts which are classified as small ncRNAs (<200 nts) and long ncRNAs (lncRNAs; >200 nts). Small ncRNAs mainly include microRNA (miRNA), small interfering RNAs (siRNAs), piwi-interacting RNAs (piRNAs) while lncRNAs are mainly represented by enhancer RNAs (eRNAs), and circular RNAs (circRNAs) [60–63]. Alterations of ncRNA expression have been found in several diseases including pregnancy complications [64–66], inflammation [67–69] and cancer [70–72].

LncRNA TUG1 showed significant oncogenic effects in several cancers including colorectal, gastric, and breast cancers [73–75]. An interesting study found that lncRNA TUG1 expression was significantly increased in prostate

cancer cells (DU145 and PC3 cell lines) compared to normal prostate epithelial RWPE1 cells. TUG1 silencing significantly decreased cell proliferation, migration, and invasion in PC3 cells but, these effects were reversed by using Oltipraz, a NRF2 activator. Authors also found that TUG1 silencing significantly decreased NRF2 expression, leading to a decreased expression of NRF2-dependent genes *HO-1*, *FTH1*, and *NQO1*. Thus, lncRNA TUG1 plays a significant oncogenic role in promoting cell proliferation and invasion through the modulation of NRF2 pathway in the PC3 cellular model [76]

Brain-type glycogen phosphorylase (PYGB), is the brain form of glycogen phosphorylase, a key enzyme involved in glycogen catabolism which converts glycogen into glucose-1-phosphate (G-1-P) providing energy to the body in an emergency state [77,78]. High PYGB expression has been reported in several cancer types including gastric [79], lung [77] and colorectal [80] cancer.

The expression of PYGB has been reported to be significantly upregulated in prostate cancer tissues and was associated with disease progression. Moreover, PYGB silencing in PC3 cells significantly inhibited cell viability, thereby inducing apoptosis. Interestingly, these authors found that PYGB silencing increased the ROS content, which inhibited NRF2 protein in PC3 cells. Thus, PYGB can modulate prostate cancer cell growth, altering the NRF2 signaling pathway and increasing ROS content [81].

BReast CAncer gene 1 (*BRCA1*) is a tumor suppressor gene involved in important cell functions such as DNA repair, cell-cycle regulation and maintenance of genomic integrity. *BRCA1* mutations confer an increased risk for breast, ovarian and prostate cancers [82,83]. The expression of BRCA1 has been shown to protect cells from oxidant agents, inducing antioxidant genes expression and subsequently activating/cooperating with important transcription factors such as NRF2 [11,84]. Labanca and colleagues [85] reported that BRCA1 binds to the HO-1 promoter increasing its expression in PC3 cells. BRCA1 overexpression significantly increased HO-1 expression while BRCA1 silencing had the opposite effect. These authors also reported that BRCA1 overexpression significantly decreased the expression of HO-1 suppressors such as MMP-9, uPA, and Cyclin D1 in PC3 cells. Moreover, HO-1 was more expressed when both BRCA1 and NRF2 were overexpressed compared to the single overexpression, indicating that BRCA1 cooperates with NRF2 to induce HO-1 expression in PC3 cells [85]. These data are in agreement with the study of Bae and colleagues [86] that found increased expression of important antioxidant genes such as glutathione S-transferases (*GSTs*), glutathione peroxidase 3 (*GPX3*), *NQO1* and alcohol dehydrogenase 5 (*ADH5*) in DU145 cells that overexpress BRCA1. Consistently, BRCA1 overexpression conferred resistance, while BRCA1 deficiency conferred sensitivity to oxidant agents such as hydrogen peroxide and Paraquat. BRCA1 also stimulated the NRF2 antioxidant response protecting cells
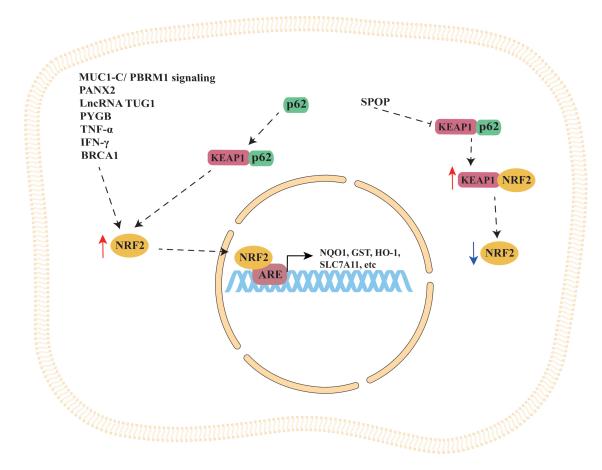
Fig. 1. Schematic representation of NRF2/KEAP1 signaling pathway regulation by cellular modulators. NRF2/KEAP1 signaling pathway can be indirectly upregulated by important cellular modulators such as MUC1-C/PBRM1 signaling, PANX2, p62, SPOP, lncRNA TUG1, PYGB, TNF-α, IFN-γ and BRCA1. Moreover, SPOP protein can inhibit NRF2 signaling. Red arrow, increase; Blue arrow, decrease; Dashed arrows, effect; Black solid arrows, activation of gene expression.

against oxidative stress (induced by H_2O_2). This protective effect was attenuated by NRF2 silencing demonstrating a cooperative effect of BRCA1 in promoting NRF2-dependent antioxidant response, subsequently preserving genomic integrity [86].

Inflammation plays a key role in the development and progression of several diseases including pregnancy complications [87–90], endothelial dysfunction [13,91,92], skeletal disorders [93] and cancer [94–96].

It has been reported that mesenchymal stem cells (MSCs) can be recruited to the tumor microenvironment due to local inflammation promoting angiogenesis and tumor growth [97]. Yang and colleagues showed that MSCs pre-treatment with Tumor necrosis factor alpha (TNF-α) and interferon-gamma (IFN-γ) mixed with murine RM-1 prostate cancer cells and subcutaneously injected in C57 mice significantly increased serum level of the pro-angiogenic factors platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), leading to increased RM-1 cell growth. Moreover, MSCs treatment with TNF-α and IFN-γ increased the expression of hypoxia-inducible factor-1alpha (HIF-1α) and NRF2. Authors also showed that TNF-α and IFN-γ pre-treatment

of NRF2-silenced MSCs significantly reduced HIF-1α, PDGF and VEGF expression demonstrating that HIF-1α signaling, which regulates PDGF and VEGF expression [98,99], is at downstream of NRF2 signaling. Thus, inflammatory cytokines activate the NRF2 pathway in MSCs which, in turn, induces HIF-1α pathways stimulating PDGF and VEGF expression, thereby accelerating prostate cancer growth [100].

The studies discussed in this section are summarized in Table 1 (Ref. [44,52,56,59,76,81,85,86,100]), while a schematic representation of NRF2/KEAP1 signaling pathway regulation by cellular modulators is shown in Fig. 1.

3. Effect of KEAP1 Mutations and Hypermethylation on NRF2/KEAP1 Pathway Signaling

Somatic mutations of NRF2 can impair NRF2 recognition by KEAP1 leading to an upregulation of NRF2 expression in some [101–103], but not in prostate cancer [103,104]. Somatic mutations of KEAP1 have also been reported in lung and gallbladder cancers and were associated with increased NRF2 expression and chemotherapy resistance [105–107].

Table 1. Cellular modulator of NRF2/KEAP1 pathway in prostate cancer.

Modulator	Model studied	Effect	Ref.
MUC1-C/PBRM1 signaling	DU-145 and LNCaP cells	MUC1-C forms a complex with NRF2 and PBRM1 on the NRF2 target *SLC7A11* gene increasing chromatin accessibility and inducing SLC7A11/xCT expression. MUC1-C or PBRM1 silencing led to the downregulation of NRF2 and GSH levels increasing oxidative stress.	[44]
PANX2	PC3, DU145, LNCaP and RWPE1 cells	Higher PANX2 expression in PC3, DU145, and LNCaP cells compared to the normal prostate epithelial RWPE1 cells. PANX2 silencing in PC3 cells reduced NRF2, HO-1, and FTH1 expression decreasing cell proliferation, migration, and invasion while increasing ferrous iron and MDA levels.	[52]
p62	DU145 cells	Higher p62 expression in prostate cancer cells compared to normal cells. p62 silencing increased KEAP1 expression, decreasing NRF2 expression and activation. NRF2 silencing decreased cell proliferation and invasion while increasing apoptosis. p62 binds KEAP1 inducing its proteasomal degradation and promoting NRF2 expression and the transcription of NRF2-dependent genes *HO-1*, *NQO-1*, *GCLC* and *GLCM*, subsequently decreasing ROS.	[56]
SPOP	DU-145 cells	SPOP binds and induces p62 decreasing p62-dependent autophagy. SPOP attenuated p62-mediated KEAP1 sequestration facilitating NRF2 proteasomal degradation and decreasing NRF2-dependent antioxidant genes expression. SPOP mutants lose the capacity to ubiquitinate p62, thereby promoting autophagy and NRF2-induced antioxidant response in prostate cancer cells.	[59]
LncRNA TUG1	DU145, PC3 and RWPE1 cells	Increased lncRNA TUG1 expression in DU145 and PC3 cell lines compared to normal prostate epithelial RWPE1 cells. TUG1 silencing decreased cell proliferation, migration, invasion, and NRF2, HO-1, FTH1, and NQO1 expression.	[76]
PYGB	PC3 cells	PYGB silencing inhibited NRF2 protein expression increasing ROS content and inducing apoptosis.	[81]
BRCA1	PC3 cells	HO-1 was more expressed when both BRCA1 and NRF2 were overexpressed compared to the single overexpression. BRCA1 facilitated HO-1 expression interacting with NRF2.	[85]
BRCA1	DU145 cells	BRCA1-overexpression increased GPX3, NQO1 and ADH5 expression. BRCA1 overexpression conferred resistance while BRCA1 deficiency conferred sensitivity to oxidant agents. BRCA1 stimulated the NRF2 antioxidant response, protecting cells against oxidative stress (which was attenuated by NRF2 silencing).	[86]
TNF-α and IFN-γ	Mesenchymal stem cells (MSCs) and murine RM-1 prostate cancer cells	MSCs treatment with TNF-α and IFN-γ increased HIF-1α and NRF2 expression. TNF-α and IFN-γ pre-treatment of NRF2-silenced MSCs reduced HIF-1α, PDGF and VEGF expression. Inflammatory cytokines activate the NRF2 pathway in MSCs which, in turn, induces HIF-1α pathways stimulating PDGF and VEGF expression, then accelerating prostate cancer growth.	[100]

MUC-1C, Mucin 1 C subunit; PBRM1, Polybromo-1; PANX2, Pannexin 2; SPOP, Speckle-type BTB/POZ protein BRCA1, BReast CAncer gene 1; PYGB, Brain-type glycogen phosphorylase; TNF-α, Tumor necrosis factor-alpha; IFN-γ, interferon-gamma.

Yoo and colleagues investigated KEAP1 mutations in prostate cancer and found that 1/75 (1.3%) of prostate cancer tissues analyzed processed a G1069A mutation that leads to a D357N amino acid substitution [104]. Another study by Zhang and colleagues [108] sequenced the *KEAP1* gene in six prostate cancer cell lines (DU-145, PC3, LNCaP, C42B, LAPC4, and CWR22RV1) and in normal prostate epithelial cells (PrEC). The study identified a C/T transition in CWR22Rv1 cells which leads to a threonine to methionine substitution at the 314th amino acid (T314M) in KEAP1 protein. Moreover, they found a T/C transition in LNCaP and C42B cells that causes a tyrosine to histidine substitution at the 255th amino acid position (Y255H). No mutation was indentified in *KEAP1* in DU-145, LAPC4, PC3, and PrEC cell lines. Furthermore, the authors demonstrated that both T314M and Y255H mutations significantly inhibited NRF2 degradation, causing a significant increase in NRF2 activity that leads to an overexpression of *NQO1* and *HO-1* genes. Additionally, the study reported an hypermethylation of CpG islands in the KEAP1 promoter and an aberrant splicing of KEAP1 in DU-145 cells which leads to low levels of KEAP1 mRNA and an increased NRF2 activity with consequent expression of antioxidant genes. NRF2 silencing decreased GSH levels in DU-145 cells making them sensitive to chemotherapeutic drugs (paclitaxel, cisplatin, and etoposide) and radiation [108]. Thus, the authors demonstrated that in addition to KEAP1 mutations, hypermethylation of CpG island and aberrant splicing of KEAP1 also play a key role in the antioxidant response of prostate cancer cells influencing their sensitivity to chemotherapy and radiotherapy.

Nevertheless, although quite rare (1.3%), mutations have been reported in the NRF2 repressor, *KEAP1* gene, in prostate cancer tissues. KEAP1 mutations have also been indentified in three prostate cancer lines: CWR22Rv1, LNCaP, and C42B cell lines. Finally, hypermethylation of the KEAP1 promoter and an aberrant splicing of KEAP1 have been reported in the DU-145 prostate cancer cell line [108].

4. Conclusions

The NRF2/KEAP1 signaling pathway plays a key role in prostate cancer onset and progression and thus is a promising target for the treatment of this disease. In this review, we discussed many studies highlighting the pleiotropic role of the NRF2/KEAP1 signaling pathway in modulating important cellular processes such as chemotherapy and radiotherapy response, cell proliferation, apoptosis, and autophagy. In particular, we highlighted that NRF2/KEAP1 signaling pathway can be indirectly regulated by several cellular modulators (see Table 1) including MUC1-C/PBRM1 signaling, PANX2, p62, SPOP, lncRNA TUG1, PYGB, and BRCA1. Moreover, it came up that NRF2/KEAP1 signaling can be modulated by TNF-α, IFN-γ and KEAP1 mutations. Interestingly, no mutations have been found in the *NRF2* gene in prostate cancer.

In conclusion, NRF2/KEAP1 signaling pathway modulation plays a pivotal function in protecting normal cells from oxidative stress and modulating cancer cell response to radiotherapy and chemotherapeutic agents. Although several studies are focused on directly modulation of the NRF2/KEAP1 signaling pathway, here we highlighted cellular modulators of the NRF2/KEAP1 signaling pathway that could be indirect therapeutic targets of this signaling in the treatment of prostate cancer.

Author Contributions

GT contributed to writing original draft. GT, SF, DM and RM participated in conceptualization and writing review & editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

Conflict of Interest

The authors declare no conflict of interest. Given the role as Guest Editor, Giovanni Tossetta had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Graham Pawelec.

References

[1] Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA: A Cancer Journal for Clinicians. 2022; 72: 7–33.

[2] Evans AJ. Treatment effects in prostate cancer. Modern Pathology. 2018; 31: S110–S121.

[3] Litwin MS, Tan HJ. The Diagnosis and Treatment of Prostate Cancer: A Review. The Journal of the American Medical Association. 2017; 317: 2532–2542.

[4] Sekhoacha M, Riet K, Motloung P, Gumenku L, Adegoke A, Mashele S. Prostate Cancer Review: Genetics, Diagnosis, Treatment Options, and Alternative Approaches. Molecules. 2022; 27: 5730.

[5] Achard V, Putora PM, Omlin A, Zilli T, Fischer S. Metastatic Prostate Cancer: Treatment Options. Oncology. 2022; 100: 48–59.

[6] Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radical Biology & Medicine. 2010; 49: 1603–1616.

[7] Kay J, Thadhani E, Samson L, Engelward B. Inflammation-induced DNA damage, mutations and cancer. DNA Repair. 2019; 83: 102673.

[8] Zhang DD. Mechanistic studies of the Nrf2-Keap1 signaling pathway. Drug Metabolism Reviews. 2006; 38: 769–789.

[9] Bellezza I, Giambanco I, Minelli A, Donato R. Nrf2-Keap1 signaling in oxidative and reductive stress. Biochimica et Biophysica Acta. Molecular Cell Research. 2018; 1865: 721–733.

[10] Ma Q. Role of nrf2 in oxidative stress and toxicity. Annual Review of Pharmacology and Toxicology. 2013; 53: 401–426.

[11] Tossetta G, Marzioni D. Natural and synthetic compounds in Ovarian Cancer: A focus on NRF2/KEAP1 pathway. Pharmacological Research. 2022; 183: 106365.

[12] Brieger K, Schiavone S, Miller FJ Jr, Krause KH. Reactive oxygen species: from health to disease. Swiss Medical Weekly. 2012; 142: w13659.

[13] Campagna R, Vignini A. NAD+ Homeostasis and NAD+-Consuming Enzymes: Implications for Vascular Health. Antioxidants. 2023; 12: 376.

[14] El Assar M, Angulo J, Rodríguez-Mañas L. Oxidative stress and vascular inflammation in aging. Free Radical Biology & Medicine. 2013; 65: 380–401.

[15] Delli Muti N, Salvio G, Ciarloni A, Perrone M, Tossetta G, Lazzarini R, et al. Can extremely low frequency magnetic field affect human sperm parameters and male fertility? Tissue & Cell. 2023; 82: 102045.

[16] Campagna R, Mateuszuk Ł, Wojnar-Lason K, Kaczara P, Tworzydło A, Kij A, et al. Nicotinamide N-methyltransferase in endothelium protects against oxidant stress-induced endothelial injury. Biochimica et Biophysica Acta. Molecular Cell Research. 2021; 1868: 119082.

[17] Zapotoczny B, Braet F, Kus E, Ginda-Mäkelä K, Klejevskaja B, Campagna R, et al. Actin-spectrin scaffold supports open fenestrae in liver sinusoidal endothelial cells. Traffic. 2019; 20: 932–942.

[18] Tossetta G. Metformin Improves Ovarian Cancer Sensitivity to Paclitaxel and Platinum-Based Drugs: A Review of In Vitro Findings. International Journal of Molecular Sciences. 2022; 23: 12893.

[19] Wang Y, Cheng H, Wang T, Zhang K, Zhang Y, Kang X. Oxidative stress in intervertebral disc degeneration: Molecular mechanisms, pathogenesis and treatment. Cell Proliferation. 2023; e13448.

[20] Marzioni D, Mazzucchelli R, Fantone S, Tossetta G. NRF2 modulation in TRAMP mice: an in vivo model of prostate cancer. Molecular Biology Reports. 2023; 50: 873–881.

[21] Tossetta G, Fantone S, Montanari E, Marzioni D, Goteri G. Role of NRF2 in Ovarian Cancer. Antioxidants. 2022; 11: 663.

[22] Emanuelli M, Sartini D, Molinelli E, Campagna R, Pozzi V, Salvolini E, et al. The Double-Edged Sword of Oxidative Stress in Skin Damage and Melanoma: From Physiopathology to Therapeutical Approaches. Antioxidants. 2022; 11: 612.

[23] Tossetta G, Marzioni D. Targeting the NRF2/KEAP1 pathway in cervical and endometrial cancers. European Journal of Pharmacology. 2023; 941: 175503.

[24] Mirzaei S, Mohammadi AT, Gholami MH, Hashemi F, Zarrabi A, Zabolian A, et al. Nrf2 signaling pathway in cisplatin chemotherapy: Potential involvement in organ protection and chemoresistance. Pharmacological Research. 2021; 167: 105575.

[25] Jayakumar S, Kunwar A, Sandur SK, Pandey BN, Chaubey RC. Differential response of DU145 and PC3 prostate cancer cells to ionizing radiation: role of reactive oxygen species, GSH and Nrf2 in radiosensitivity. Biochimica et Biophysica Acta. 2014; 1840: 485–494.

[26] Khurana N, Kim H, Chandra PK, Talwar S, Sharma P, Abdel-Mageed AB, et al. Multimodal actions of the phytochemical sulforaphane suppress both AR and AR-V7 in 22Rv1 cells: Advocating a potent pharmaceutical combination against castration-resistant prostate cancer. Oncology Reports. 2017; 38: 2774–2786.

[27] Khurana N, Talwar S, Chandra PK, Sharma P, Abdel-Mageed AB, Mondal D, et al. Sulforaphane increases the efficacy of anti-androgens by rapidly decreasing androgen receptor levels in prostate cancer cells. International Journal of Oncology. 2016; 49: 1609–1619.

[28] Funes JM, Henderson S, Kaufman R, Flanagan JM, Robson M, Pedley B, et al. Oncogenic transformation of mesenchymal stem cells decreases Nrf2 expression favoring in vivo tumor growth and poorer survival. Molecular Cancer. 2014; 13: 20.

[29] Kawata H, Kamiakito T, Nakaya T, Komatsubara M, Komatsu K, Morita T, et al. Stimulation of cellular senescent processes, including secretory phenotypes and anti-oxidant responses, after androgen deprivation therapy in human prostate cancer. The Journal of Steroid Biochemistry and Molecular Biology. 2017; 165: 219–227.

[30] Raatikainen S, Aaltomaa S, Kärjä V, Soini Y. Increased nuclear factor erythroid 2-related factor 2 expression predicts worse prognosis of prostate cancer patients treated with radical prostatectomy. Human Pathology. 2014; 45: 2211–2217.

[31] Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. Nature Reviews. Molecular Cell Biology. 2021; 22: 96–118.

[32] He S, Wu Z, Tian Y, Yu Z, Yu J, Wang X, et al. Structure of nucleosome-bound human BAF complex. Science. 2020; 367: 875–881.

[33] Tossetta G, Fantone S, Gesuita R, Montironi R, Marzioni D, Mazzucchelli R. AT-rich interactive domain 1A (ARID1A) cannot be considered a morphological marker for prostate cancer progression: A pilot study. Acta Histochemica. 2022; 124: 151847.

[34] Fantone S, Mazzucchelli R, Giannubilo SR, Ciavattini A, Marzioni D, Tossetta G. AT-rich interactive domain 1A protein expression in normal and pathological pregnancies complicated by preeclampsia. Histochemistry and Cell Biology. 2020; 154: 339–346.

[35] Hopson S, Thompson MJ. BAF180: Its Roles in DNA Repair and Consequences in Cancer. ACS Chemical Biology. 2017; 12: 2482–2490.

[36] Porter EG, Dhiman A, Chowdhury B, Carter BC, Lin H, Stewart JC, et al. PBRM1 Regulates Stress Response in Epithelial Cells. IScience. 2019; 15: 196–210.

[37] Kakarougkas A, Ismail A, Chambers AL, Riballo E, Herbert AD, Künzel J, et al. Requirement for PBAF in transcriptional repression and repair at DNA breaks in actively transcribed regions of chromatin. Molecular Cell. 2014; 55: 723–732.

[38] Yasumizu Y, Rajabi H, Jin C, Hata T, Pitroda S, Long MD, et al. MUC1-C regulates lineage plasticity driving progression to neuroendocrine prostate cancer. Nature Communications. 2020; 11: 338.

[39] Rajabi H, Hiraki M, Kufe D. MUC1-C activates polycomb repressive complexes and downregulates tumor suppressor genes in human cancer cells. Oncogene. 2018; 37: 2079–2088.

[40] Kufe DW. MUC1-C in chronic inflammation and carcinogenesis; emergence as a target for cancer treatment. Carcinogenesis. 2020; 41: 1173–1183.

[41] Rodella U, Honisch C, Gatto C, Ruzza P, D'Amato Tóthová J. Antioxidant Nutraceutical Strategies in the Prevention of Oxidative Stress Related Eye Diseases. Nutrients. 2023; 15: 2283.

[42] Giustarini D, Milzani A, Dalle-Donne I, Rossi R. How to Increase Cellular Glutathione. Antioxidants. 2023; 12: 1094.

[43] Jyotsana N, Ta KT, DelGiorno KE. The Role of Cystine/Glutamate Antiporter SLC7A11/xCT in the Pathophysiology of Cancer. Frontiers in Oncology. 2022; 12: 858462.

[44] Hagiwara M, Fushimi A, Yamashita N, Bhattacharya A, Rajabi H, Long MD, et al. MUC1-C activates the PBAF chromatin remodeling complex in integrating redox balance with progression of human prostate cancer stem cells. Oncogene. 2021; 40: 4930–4940.

[45] Koval M, Schug WJ, Isakson BE. Pharmacology of pannexin channels. Current Opinion in Pharmacology. 2023; 69: 102359.

[46] Kim KM, Hussein UK, Bae JS, Park SH, Kwon KS, Ha SH, *et al*. The Expression Patterns of FAM83H and PANX2 Are Associated With Shorter Survival of Clear Cell Renal Cell Carcinoma Patients. Frontiers in Oncology. 2019; 9: 14.

[47] Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, *et al*. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. Cell. 2017; 171: 273–285.

[48] Yu Y, Xie Y, Cao L, Yang L, Yang M, Lotze MT, *et al*. The ferroptosis inducer erastin enhances sensitivity of acute myeloid leukemia cells to chemotherapeutic agents. Molecular & Cellular Oncology. 2015; 2: e1054549.

[49] Jiang L, Kon N, Li T, Wang SJ, Su T, Hibshoosh H, *et al*. Ferroptosis as a p53-mediated activity during tumour suppression. Nature. 2015; 520: 57–62.

[50] Abdalkader M, Lampinen R, Kanninen KM, Malm TM, Liddell JR. Targeting Nrf2 to Suppress Ferroptosis and Mitochondrial Dysfunction in Neurodegeneration. Frontiers in Neuroscience. 2018; 12: 466.

[51] Imai H, Matsuoka M, Kumagai T, Sakamoto T, Koumura T. Lipid Peroxidation-Dependent Cell Death Regulated by GPx4 and Ferroptosis. Current Topics in Microbiology and Immunology. 2017; 403: 143–170.

[52] Liao D, Yang G, Yang Y, Tang X, Huang H, Shao J, *et al*. Identification of Pannexin 2 as a Novel Marker Correlating with Ferroptosis and Malignant Phenotypes of Prostate Cancer Cells. OncoTargets and Therapy. 2020; 13: 4411–4421.

[53] Vargas JNS, Hamasaki M, Kawabata T, Youle RJ, Yoshimori T. The mechanisms and roles of selective autophagy in mammals. Nature Reviews. Molecular Cell Biology. 2023; 24: 167–185.

[54] Dikic I, Elazar Z. Mechanism and medical implications of mammalian autophagy. Nature Reviews. Molecular Cell Biology. 2018; 19: 349–364.

[55] Moscat J, Karin M, Diaz-Meco MT. p62 in Cancer: Signaling Adaptor Beyond Autophagy. Cell. 2016; 167: 606–609.

[56] Jiang G, Liang X, Huang Y, Lan Z, Zhang Z, Su Z, *et al*. p62 promotes proliferation, apoptosis-resistance and invasion of prostate cancer cells through the Keap1/Nrf2/ARE axis. Oncology Reports. 2020; 43: 1547–1557.

[57] Cuneo MJ, Mittag T. The ubiquitin ligase adaptor SPOP in cancer. The FEBS Journal. 2019; 286: 3946–3958.

[58] Bernasocchi T, Theurillat JPP. SPOP-mutant prostate cancer: Translating fundamental biology into patient care. Cancer Letters. 2022; 529: 11–18.

[59] Shi Q, Jin X, Zhang P, Li Q, Lv Z, Ding Y, *et al*. SPOP mutations promote p62/SQSTM1-dependent autophagy and Nrf2 activation in prostate cancer. Cell Death and Differentiation. 2022; 29: 1228–1239.

[60] Mattick JS. The genetic signatures of noncoding RNAs. PLoS Genetics. 2009; 5: e1000459.

[61] Keller C, Kulasegaran-Shylini R, Shimada Y, Hotz HR, Bühler M. Noncoding RNAs prevent spreading of a repressive histone mark. Nature Structural & Molecular Biology. 2013; 20: 994–1000.

[62] Russo F, Fiscon G, Conte F, Rizzo M, Paci P, Pellegrini M. Interplay Between Long Noncoding RNAs and MicroRNAs in Cancer. Methods in Molecular Biology. 2018; 1819: 75–92.

[63] Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. Nature Reviews. Genetics. 2014; 15: 7–21.

[64] Licini C, Avellini C, Picchiassi E, Mensà E, Fantone S, Ramini D, *et al*. Pre-eclampsia predictive ability of maternal miR-125b: a clinical and experimental study. Translational Research. 2021; 228: 13–27.

[65] Sun N, Qin S, Zhang L, Liu S. Roles of noncoding RNAs in preeclampsia. Reproductive Biology and Endocrinology. 2021; 19: 100.

[66] Chen A, Yu R, Jiang S, Xia Y, Chen Y. Recent Advances of MicroRNAs, Long Non-coding RNAs, and Circular RNAs in Preeclampsia. Frontiers in Physiology. 2021; 12: 659638.

[67] Wen J, Liu J, Wan L, Wang F. Long noncoding RNA/circular RNA regulates competitive endogenous RNA networks in rheumatoid arthritis: molecular mechanisms and traditional Chinese medicine therapeutic significances. Annals of Medicine. 2023; 55: 973–989.

[68] Cai L, Lai D, Gao J, Wu H, Shi B, Ji H, *et al*. The role and mechanisms of miRNA in neonatal necrotizing enterocolitis. Frontiers in Pediatrics. 2022; 10: 1053965.

[69] Zeng M, Zhang T, Lin Y, Lin Y, Wu Z. The Common LncRNAs of Neuroinflammation-Related Diseases. Molecular Pharmacology. 2023; 103: 113–131.

[70] Avellini C, Licini C, Lazzarini R, Gesuita R, Guerra E, Tossetta G, *et al*. The trophoblast cell surface antigen 2 and miR-125b axis in urothelial bladder cancer. Oncotarget. 2017; 8: 58642–58653.

[71] Hao L, Wu W, Xu Y, Chen Y, Meng C, Yun J, *et al*. LncRNA-MALAT1: A Key Participant in the Occurrence and Development of Cancer. Molecules. 2023; 28: 2126.

[72] Kumar A, Girisa S, Alqahtani MS, Abbas M, Hegde M, Sethi G, *et al*. Targeting Autophagy Using Long Non-Coding RNAs (LncRNAs): New Landscapes in the Arena of Cancer Therapeutics. Cells. 2023; 12: 810.

[73] Zhang E, He X, Yin D, Han L, Qiu M, Xu T, *et al*. Increased expression of long noncoding RNA TUG1 predicts a poor prognosis of gastric cancer and regulates cell proliferation by epigenetically silencing of p57. Cell Death & Disease. 2016; 7: e2109.

[74] Li T, Liu Y, Xiao H, Xu G. Long non-coding RNA TUG1 promotes cell proliferation and metastasis in human breast cancer. Breast Cancer. 2017; 24: 535–543.

[75] Abushouk AI, Kattan SW, Ahmedah HT, Baothman E, Shaheen S, Toraih EA, *et al*. Expression of oncolong noncoding RNA taurine-upregulated gene-1 in colon cancer: A clinical study supported by in silico analysis. Journal of Cancer Research and Therapeutics. 2022; 18: S374–S382.

[76] Yang G, Yin H, Lin F, Gao S, Zhan K, Tong H, *et al*. Long noncoding RNA TUG1 regulates prostate cancer cell proliferation, invasion and migration via the Nrf2 signaling axis. Pathology Research and Practice. 2020; 216: 152851.

[77] Xiao L, Wang W, Huangfu Q, Tao H, Zhang J. PYGB facilitates cell proliferation and invasiveness in non-small cell lung cancer by activating the Wnt-β-catenin signaling pathway. Biochemistry and Cell Biology. 2020; 98: 565–574.

[78] Glaser T, Matthews KE, Hudson JW, Seth P, Housman DE, Crerar MM. Localization of the muscle, liver, and brain glycogen phosphorylase genes on linkage maps of mouse chromosomes 19, 12, and 2, respectively. Genomics. 1989; 5: 510–521.

[79] Xia B, Zhang K, Liu C. PYGB Promoted Tumor Progression by Regulating Wnt/β-Catenin Pathway in Gastric Cancer. Technology in Cancer Research & Treatment. 2020; 19: 1533033820926592.

[80] Tashima S, Shimada S, Yamaguchi K, Tsuruta J, Ogawa M. Expression of brain-type glycogen phosphorylase is a potentially novel early biomarker in the carcinogenesis of human colorectal carcinomas. The American Journal of Gastroenterology. 2000; 95: 255–263.

[81] Wang Z, Han G, Liu Q, Zhang W, Wang J. Silencing of PYGB suppresses growth and promotes the apoptosis of prostate cancer cells via the NF-κB/Nrf2 signaling pathway. Molecular Medicine Reports. 2018; 18: 3800–3808.

[82] Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, *et al*. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science. 1994; 266: 66–71.

[83] Thompson D, Easton DF, Breast Cancer Linkage Consortium. Cancer Incidence in BRCA1 mutation carriers. Journal of the National Cancer Institute. 2002; 94: 1358–1365.

[84] Vurusaner B, Poli G, Basaga H. Tumor suppressor genes and ROS: complex networks of interactions. Free Radical Biology & Medicine. 2012; 52: 7–18.

[85] Labanca E, De Luca P, Gueron G, Paez A, Moiola CP, Massillo C, *et al*. Association of HO-1 and BRCA1 Is Critical for the Maintenance of Cellular Homeostasis in Prostate Cancer. Molecular Cancer Research. 2015; 13: 1455–1464.

[86] Bae I, Fan S, Meng Q, Rih JK, Kim HJ, Kang HJ, *et al*. BRCA1 induces antioxidant gene expression and resistance to oxidative stress. Cancer Research. 2004; 64: 7893–7909.

[87] Tossetta G, Fantone S, Gesuita R, Di Renzo GC, Meyyazhagan A, Tersigni C, *et al*. HtrA1 in Gestational Diabetes Mellitus: A Possible Biomarker? Diagnostics. 2022; 12: 2705.

[88] Tossetta G, Fantone S, Giannubilo SR, Marzioni D. The Multifaced Actions of Curcumin in Pregnancy Outcome. Antioxidants. 2021; 10: 126.

[89] Cecati M, Sartini D, Campagna R, Biagini A, Ciavattini A, Emanuelli M, *et al*. Molecular analysis of endometrial inflammation in preterm birth. Cellular and Molecular Biology. 2017; 63: 51–57.

[90] Licini C, Tossetta G, Avellini C, Ciarmela P, Lorenzi T, Toti P, *et al*. Analysis of cell-cell junctions in human amnion and chorionic plate affected by chorioamnionitis. Histology and Histopathology. 2016; 31: 759–767.

[91] Ju J, Liu Y, Liang H, Yang B. The role of pyroptosis in endothelial dysfunction induced by diseases. Frontiers in Immunology. 2023; 13: 1093985.

[92] Mateuszuk Ł, Campagna R, Kutryb-Zając B, Kuś K, Słominska EM, Smolenski RT, *et al*. Reversal of endothelial dysfunction by nicotinamide mononucleotide via extracellular conversion to nicotinamide riboside. Biochemical Pharmacology. 2020; 178: 114019.

[93] Tossetta G, Fantone S, Licini C, Marzioni D, Mattioli-Belmonte M. The multifaced role of HtrA1 in the development of joint and skeletal disorders. Bone. 2022; 157: 116350.

[94] Altobelli E, Latella G, Morroni M, Licini C, Tossetta G, Mazzucchelli R, *et al*. Low HtrA1 expression in patients with long-standing ulcerative colitis and colorectal cancer. Oncology Reports. 2017; 38: 418–426.

[95] Stromnes IM. IL18 at the Crossroads between Chronic Inflammation and T-cell Exhaustion in Pancreatic Cancer. Cancer Immunology Research. 2023; 11: 400.

[96] da Cruz NS, Pasquarelli-do-Nascimento G, E Oliveira ACP, Magalhães KG. Inflammasome-Mediated Cytokines: A Key Connection between Obesity-Associated NASH and Liver Cancer Progression. Biomedicines. 2022; 10: 2344.

[97] Cuiffo BG, Karnoub AE. Mesenchymal stem cells in tumor development: emerging roles and concepts. Cell Adhesion & Migration. 2012; 6: 220–230.

[98] Yoshida D, Kim K, Noha M, Teramoto A. Hypoxia inducible factor 1-alpha regulates of platelet derived growth factor-B in human glioblastoma cells. Journal of Neuro-Oncology. 2006; 76: 13–21.

[99] Chen X, Liu J, He B, Li Y, Liu S, Wu B, *et al*. Vascular endothelial growth factor (VEGF) regulation by hypoxia inducible factor-1 alpha (HIF1A) starts and peaks during endometrial breakdown, not repair, in a mouse menstrual-like model. Human Reproduction. 2015; 30: 2160–2170.

[100] Yang KQ, Liu Y, Huang QH, Mo N, Zhang QY, Meng QG, *et al*. Bone marrow-derived mesenchymal stem cells induced by inflammatory cytokines produce angiogenetic factors and promote prostate cancer growth. BMC Cancer. 2017; 17: 878.

[101] Shibata T, Ohta T, Tong KI, Kokubu A, Odogawa R, Tsuta K, *et al*. Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105: 13568–13573.

[102] Padmanabhan B, Tong KI, Ohta T, Nakamura Y, Scharlock M, Ohtsuji M, *et al*. Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. Molecular Cell. 2006; 21: 689–700.

[103] Kim YR, Oh JE, Kim MS, Kang MR, Park SW, Han JY, *et al*. Oncogenic NRF2 mutations in squamous cell carcinomas of oesophagus and skin. The Journal of Pathology. 2010; 220: 446–451.

[104] Yoo NJ, Kim HR, Kim YR, An CH, Lee SH. Somatic mutations of the KEAP1 gene in common solid cancers. Histopathology. 2012; 60: 943–952.

[105] Singh A, Misra V, Thimmulappa RK, Lee H, Ames S, Hoque MO, *et al*. Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. PLoS Medicine. 2006; 3: e420.

[106] Shibata T, Kokubu A, Gotoh M, Ojima H, Ohta T, Yamamoto M, *et al*. Genetic alteration of Keap1 confers constitutive Nrf2 activation and resistance to chemotherapy in gallbladder cancer. Gastroenterology. 2008; 135: 1358–1368.e4.

[107] Takahashi T, Sonobe M, Menju T, Nakayama E, Mino N, Iwakiri S, *et al*. Mutations in Keap1 are a potential prognostic factor in resected non-small cell lung cancer. Journal of Surgical Oncology. 2010; 101: 500–506.

[108] Zhang P, Singh A, Yegnasubramanian S, Esopi D, Kombairaju P, Bodas M, *et al*. Loss of Kelch-like ECH-associated protein 1 function in prostate cancer cells causes chemoresistance and radioresistance and promotes tumor growth. Molecular Cancer Therapeutics. 2010; 9: 336–346.