Revisiting therapeutic strategies for ovarian cancer by focusing on redox homeostasis (Review)

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Abstract. Recent advances in molecular genetics have expanded our understanding of ovarian cancer. High levels of reactive oxygen species (ROS) and upregulation of antioxidant genes are common characteristic features of human cancers. This review reconsiders novel therapeutic strategies for ovarian cancer by focusing on redox homeostasis. A literature search was performed for preclinical and clinical studies published between January 1998 and October 2021 in the PubMed database using a combination of specific terms. ROS serves a central role in tumor suppression and progression by inducing DNA damage and mutations, genomic instability, and aberrant anti- and pro-tumorigenic signaling. Cancer cells increase their antioxidant capacity to neutralize the extra ROS. Additionally, antioxidants, such as CD44 variant isoform 9 (CD44v9) and nuclear factor erythroid 2-related factor 2 (Nrf2), mediate redox homeostasis in ovarian cancer. Furthermore, studies conducted on different cancer types revealed the dual role of antioxidants in tumor progression and inhibition. However, in animal models, genetic loss of antioxidant capacity in the host cannot block cancer initiation and progression. Host-derived antioxidant systems are essential to suppress carcinogenesis, suggesting that antioxidants serve a pivotal role in suppressing cancer development. By contrast, antioxidant activation in cancer cells confers aggressive phenotypes. Antioxidant inhibitors can promote cancer cell death by enhancing ROS levels. Concurrent inhibition of CD44v9 and Nrf2 may trigger apoptosis induction, potentiate chemosensitivity and enhance antitumor activities through the ROS-activated p38/p21 pathway. Antioxidants may have tumor-promoting and -suppressive functions. Therefore, an improved understanding of the role of antioxidants in redox homeostasis and developing antioxidant-specific inhibitors is necessary for treating ovarian cancer.

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Contents

1. Introduction

Epithelial ovarian cancer (EOC) is a highly lethal gynecologic malignancy (1). Cancer cells often produce more endogenous reactive oxygen species (ROS) due to increased cell growth and metabolic demands for oxygen and nutrients (2). They are also persistently exposed to exogenous oxidative stress conditions (2). To survive oxidative stress and adapt to ROS exposure, cancer cells have evolved various defense mechanisms, including antioxidant enzymes, DNA-repair enzymes, and endoplasmic reticulum stress response (3). A balanced antioxidant system neutralizes excess endogenous and exogenous ROS through a defense system that consists of enzymatic and non-enzymatic antioxidants (2,4). However, increased defense against ROS is a leading cause of treatment resistance and poor prognosis (5). Furthermore, cancer stem cells contain lower intracellular ROS levels due to enhanced ROS defense than non-cancer stem cells (5). Cancer stem cells represent a small subtype of tumor cells with unlimited self-renewal, differentiation, and tumorigenesis capacity. They are a determining factor contributing to tumor metastasis, recurrence, therapeutic resistance, and poor prognosis (6). Additionally, the potential reason for treatment failure is primarily attributed to cancer stem cells (6). CD44v9 (a variant isoform of CD44 and a cell surface marker of cancer stem cells) (7) and nuclear factor erythroid 2-related factor 2 (Nrf2) genes (8,9) are major regulators of ROS defense in ovarian cancer. Cancer cells can escape oxidative injuries by producing high levels of

intracellular antioxidants via a unique protection system, such as CD44v9 and Nrf2 genes.

This review focuses on the molecular mechanisms of redox homeostasis in human cancers and discusses targeted therapies for ovarian cancer based on redox modifications.

2. Search strategy and selection criteria

A computerized literature search was performed to identify relevant studies reported in English. PubMed electronic databases published between January 1998 and October 2021 were searched, combining the following keywords: *Nrf2*, *CD44v9*, *antioxidant*, *cancer*, *ovarian cancer*, *treatment*, *inhibitor*, and *redox*. References of each article were searched to identify potentially relevant studies. In addition, publications of original studies and review papers were included. Given the heterogeneity in the research theme, data from the studies were synthesized using a descriptive review design with narrative methods. Fig. 1 shows that the first identification phase includes records identified through a database search. Terms in the titles and abstracts were focused on in the *first screening* stage. However, duplicates were removed during the second screening phase, and titles, abstracts, and full-text articles were read to remove inappropriate papers. The final eligibility phase included the full-text articles for analysis after excluding those for which detailed data cannot be extracted.

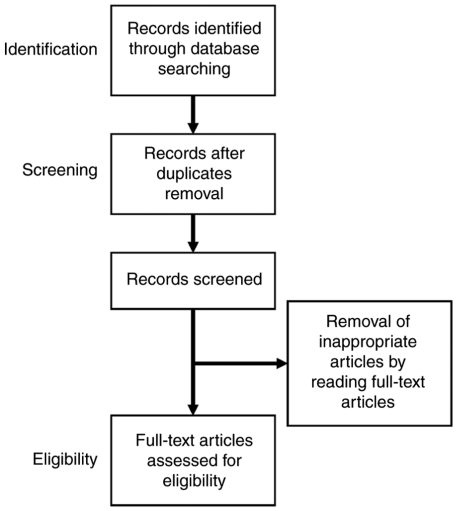
3. Unique redox homeostasis in cancer

Cancer cells utilize oxygen for adenosine triphosphate (ATP) production through metabolic reprogramming, supplying them with energy and fueling their proliferation. ROS, such as superoxide anion and hydroxyl radicals, are generated during ATP production through oxidative phosphorylation (OXPHOS) in mitochondria (10). ROS are produced by mitochondria, endoplasmic reticulum, and peroxisome; thus, cancer cells specifically accumulate high ROS levels. Furthermore, ROS cause oxidative damage to protein, lipid, and DNA and induces genomic instability, promoting tumor initiation and malignant progression (10). Also, ROS concentration with extremely high levels can induce cancer cell death, making it a promising cancer treatment (2). Therefore, ROS have a positive and negative impact on cancer evolution, leading to cancer progression (ROS levels below the threshold) or cell death (ROS levels beyond the threshold) (11). Therefore, modulating the unique redox homeostasis may be a promising strategy to eliminate cancer cells.

Cells encode pivotal defense systems to protect themselves against oxidative stress (3,10). Molecular targets as antioxidant defense systems are the mitochondrial electron transport chain and the OXPHOS system, the endoplasmic reticulum system, peroxisomal proteins, and the redox-sensitive signaling pathways (e.g., Nrf2, glutathione, and thioredoxin). Since the availability of the antioxidant system determines ROS concentration, this system contributes to conflicting biological activities, such as cell fate, i.e., survival or death. A review targeting redox imbalance for cancer treatment was published by Narayanan *et al* (12). Furthermore, researchers have developed novel therapies targeting oxidative vulnerabilities in various cancers. For example, gene silencing or pharmacological inhibition of ROS-scavenging, upregulation of ROS-generating enzymes, or pro-oxidant therapy can induce excessive oxidative stress, leading to cell death (12). A redox shift from an antioxidant condition toward a pro-oxidant state can inhibit tumor development and progression, improving treatment resistance. The redox balance is a critical molecular switch that controls cancer stimulation and suppression (2). Additionally, targeted therapy turns off this antioxidant switch to convert a mediator of tumor progression into an accelerator of cell death.

Therefore, this section discusses recent advances in cancer treatment strategies that control redox balance. We mainly summarize the following antioxidant defense systems: 1) redox cofactors [e.g., nicotinamide adenine dinucleotide phosphate (NADPH)], 2) antioxidant transcription factors (e.g., Nrf2 and CD44v9), and 3) detoxifying enzymes and molecular scavengers [e.g., glutathione-S-transferase (GST), superoxide dismutase (SOD), and glutathione] (Fig. 2A).

NADPH. Cancer cells mainly rely on aerobic glycolysis rather than OXPHOS, generating NADPH by activating the pentose phosphate pathway (PPP), adenosine monophosphate-activated protein kinase, and reductive glutamine and folate metabolism to prevent a rapid ROS generation (11,13,14). NADPH is a high-energy essential electron donor for antioxidants, such as glutathione and thioredoxin, playing a role in protecting against redox stress (14) (Fig. 2A). Furthermore, NADP is recycled to NADPH by three main enzymes: malic enzyme 1 (ME1), isocitrate dehydrogenase 1 (IDH1), and oxidative PPP [glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (PGD)] (14,15). These enzymes are ubiquitous in all mammals. The first two enzymes are tricarboxylic acid cycle-associated. ME1 is a multifunctional enzyme that decarboxylates malate to form pyruvate (16). This enzyme is also essential for NADPH production, glutamine metabolism, and lactate fermentation (16). IDH1 is a key metabolic enzyme involved in the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG), ultimately producing NADPH under cellular stress (17). The PPP is a major source of NADPH and plays a critical role in protecting cells from ROS (14). However, cancer cells actively produce NADPH for antioxidant defense, promoting tumor progression and survival in many cancer types (18). Defective NADPH production can cause cancer cell death (18). Intracellular NADPH levels are affected by nicotinamide phosphoribosyltransferase (NAMPT), an enzyme in the NAD salvage synthesis pathway. Also, NAMPT is overexpressed in various cancers, such as ovarian, colorectal, breast, prostate, gastric cancer, osteosarcoma, melanoma, and myeloma (19). Additionally, this enzyme is involved in cancer cell metabolism, survival, angiogenesis, and chemoresistance (19). A preclinical study demonstrated that suppressing NADPH production by targeting NAMPT enhanced cisplatin-treated cell death in ovarian cancer (19). NAMPT inhibitors, such as FK866, have become promising targets for platinum-resistant ovarian cancer (19). Therefore, interfering with NADPH production and modulating unique NADPH homeostasis may be an effective strategy for treating cancer. Preclinical studies targeting NADHP in cancer have been reviewed in reference 19.



Key words	1 + 3 + 4 + 6 + 7	1 + 3 + 5 + 6 + 7	2 + 3	2 + 3 + 4	2 + 3 + 5	3 + 4 + 6 + 7 + 8
Identification	477	15	8	7	2	472
Records after duplicates removal	31	1	8	7	2	149
Records screened	446	14	8	7	2	323
Removal of inappropriate articles	253	3	1	1	2	241
Eligibility	193	11	7	6	11	82

Figure 1. Number of articles identified by searching for keyword combinations. This figure shows the number of articles identified by key word combinations and the number of records identified through database search, records after duplicate removal, records screened, removal of inappropriate articles by reading full-text articles and full-text articles assessed for eligibility. Key words: 1, nuclear factor erythroid 2-related factor 2; 2, CD44 variant isoform 9; 3, antioxidant; 4, cancer; 5, ovarian cancer; 6, treatment; 7, inhibitor; and 8, redox.

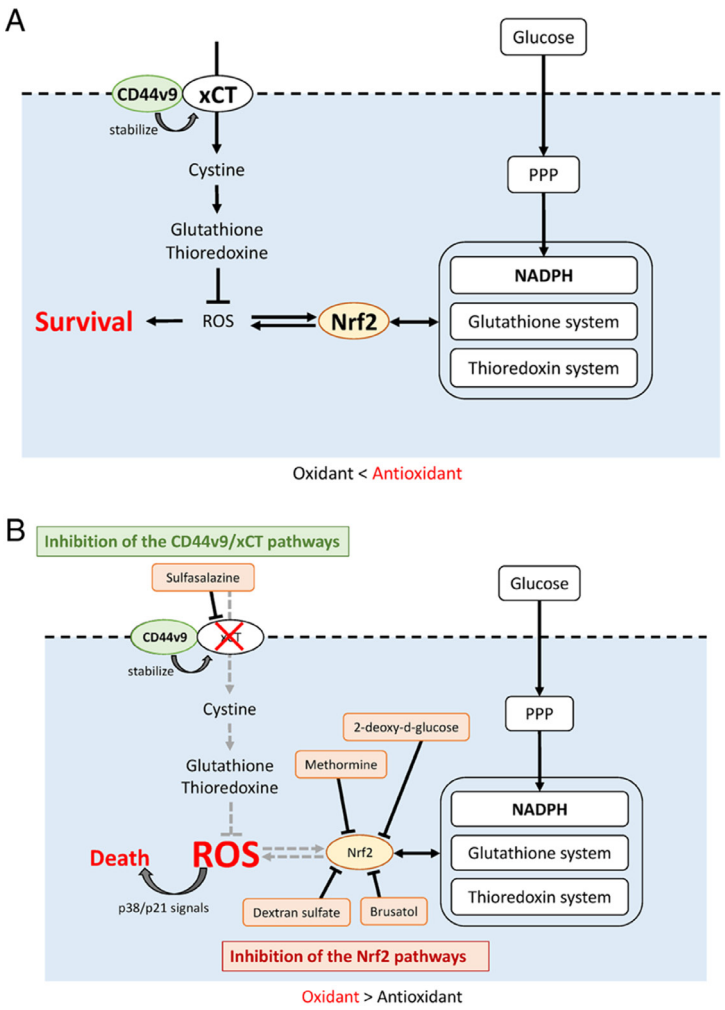


Figure 2. Antioxidant defense mechanisms against oxidative stress in ovarian cancer. (A) Role of Nrf2 and CD44v9 in antioxidant defense systems for ovarian cancer. (B) Pharmacological inhibition of Nrf2 and CD44v9. Ovarian cancer cells upregulate cellular antioxidant capacity to maintain ROS levels below a toxic threshold. The CD44v9/xCT and Nrf2 pathways are important antioxidant systems. Sulfasalazine is an inhibitor of the CD44v9/xCT pathway. Natural products, synthetic compounds or small interfering RNA that suppresses Nrf2 function have been used in preclinical studies to prevent cancer progression. However, concurrent inhibition of both pathways can induce ROS-dependent lethality in cancer cells. In addition, the CD44v9 and Nrf2 pathways serve the role of oncoproteins in certain cancer types. CD44v9, CD44 variant isoform 9; Nrf2, nuclear factor erythroid 2-related factor 2; PPP, pentose phosphate pathway; ROS, reactive oxygen species; xCT, cystine/glutamate antiporter SLC7A11.

Table I. Summary of the preclinical evidence of Nrf2 in cancer.

A, Tumor-suppressing effects

First author/s, year	*In vitro*/*in vivo*	Human samples	Methods	Summary	(Refs.)
Moon and Giaccia, 2015			Review article	Discussing the dual role of Nrf2 in cancer prevention and progression depending on the cellular context and environment	(29)
Menegon *et al*, 2016			Review article	A tumor suppressor due to its cytoprotective functions against exogenous and endogenous insults, including oxidative stress	(28)
Cho *et al*, 2017		Human epithelial ovarian cancer samples	Immunohistochemistry	Patients with high Nrf2 expression displayed better overall survival and disease-free survival, but the association was not statistically significant	(31)
Czogalla *et al*, 2018		Human ovarian cancer samples	*In vitro* and gene expression analysis	Cytoplasmic Nrf2 expression in the serous ovarian cancer subtype was associated with longer overall survival (median 50.6 vs. 29.3 months; P=0.04)	(32)
Jaganjac *et al*, 2020			Review article	A tumor suppressor due to its role in reducing ROS and environmental carcinogens. The thioredoxin and glutathione systems play a protective role against carcinogenesis	(2)

B, Tumor-promoting effects

First author/s, year	*In vitro*/*in vivo*	Human samples	Methods	Summary	(Refs.)
Moon and Giaccia, 2015			Review article	Discussing the dual role of Nrf2 in cancer prevention and progression depending on the cellular context and environment	(29)
Harris *et al*, 2015	Isolated primary mammary epithelial cells		*In vitro*, *in vivo* murine tumor and xenograft models, and human tissue samples	While glutathione is required for cancer initiation, thioredoxin is a key driver in cancer progression in already established neoplasm. Inhibition of both GSH and thioredoxin pathways causes synergistic cancer cell death	(33)
Liew *et al*, 2015		Human ovarian cancer samples	Immunohistochemistry	Nrf2 expression was associated with poorer overall survival and disease-free survival in human ovarian cancer	(34)

Table I. Continued.

B, Tumor-promoting effects					
First author/s, year	*In vitro*/*in vivo*	Human samples	Methods	Summary	(Refs.)
Menegon *et al*, 2016			Review article	Hyperactivation of the Nrf2 pathway creates an environment that favors the survival of malignant cells, protecting them against oxidative stress, chemotherapeutic agents and radiotherapy	(28)
Kitamura and Motohashi, 2018			Review article	Persistently high levels of Nrf2 activity enhance therapeutic resistance of cancer cells and show malignant phenotypes leading to poor prognoses in patients with cancer. Nrf2 also drives metabolic reprogramming to establish cellular metabolic processes that are advantageous for cell proliferation	(21)
Jaganjac *et al*, 2020			Review article	Constitutive activation of Nrf2 contributes not only to the progression in the already-established tumor cells but also to the tumor development, revealing its novel role as an oncogene. Thioredoxin and glutathione systems support carcinogenesis	(2)
Li *et al*, 2021			Review article	Nrf2 protects cells by fighting oxidative stress and defending against harmful substances, such as chemotherapeutics	(30)

Nrf2, nuclear factor erythroid 2-related factor 2.

Transcription factor Nrf2. Nrf2 plays a central role in cellular defense against oxidative insults, tightly regulating the activation of specific downstream targets, including glutathione and thioredoxin systems, detoxification system, NADPH regeneration, and heme and iron metabolism (20). The Nrf2 pathway is often activated in various types of cancer. Under unstressed conditions, trapping Nrf2 by Kelch-like ECH-associated protein 1 (Keap1), degrading Nrf2 by ubiquitination (21). Oxidative stress disrupts Keap1 and Nrf2 binding, leading to constitutive activation of the Nrf2 transcription factor (22). Various antioxidant genes, e.g., glutathione and thioredoxin systems and NADPH production are activated by the Nrf2 gene (2,23,24) (Fig. 2A). In addition, cancer cells promote metabolic reprogramming from mitochondrial OXPHOS to aerobic glycolysis, thus, fueling the PPP (7,21). Nrf2 triggers G6PD activation, contributing to the activation of the PPP, NADPH synthesis, and metabolic reprogramming of cancer cells (25). The primary function of Nrf2 is stabilizing intracellular redox potential against physical and chemical insults involving oxidative stress (8,9). Nrf2 is a master regulator of cellular antioxidant response. Furthermore, the Nrf2 pathway induces many genes, regulating redox homeostasis, detoxification, autophagy, and DNA repair (26). Furthermore, Nrf2 is a pivotal regulator of stem cell self-renewal and unlimited proliferation (27).

Table I summarizes the role of Nrf2 in cancer (2,21,28-34). Nrf2 has been considered a tumor suppressor because the defense mechanism against endogenous and exogenous oxidative damage protects normal cells from neoplastic transformation (2,28). Nrf2-deficient mice are associated with increased susceptibility to redox-mediated spontaneous, chemical, and radiation carcinogenesis (29). However, Nrf2-knockout mice cannot eliminate cancer cells, suggesting that host-derived Nrf2 is essential to suppress cancer initiation (29). Therefore, Nrf2 activation in cancer cells upregulates target antioxidant genes; these alterations confer many advantages to cancer cells, including malignant phenotypes leading to tumor growth advantage, progression, aggressiveness, and poor prognosis (2,29). Ovarian cancer stem-like cells have increased Nrf2-induced antioxidant scavengers, protecting cancer cells from oxidative damage (35). Additionally, Nrf2 activation in cancer cells protects cells against harmful substances, such as chemotherapeutic agents and radiotherapy, conferring therapeutic resistance (21,30). The Nrf2-Keap1 system is responsible for platinum chemotherapy resistance in ovarian cancer (36).

Nrf2 pathway inhibition represents an attractive target for developing anticancer drugs (37-46) (Table II). The core results in Table II are summarized in illustrative Fig. 2B. Nrf2 inhibitors include brusatol (37,43), all-trans retinoic acid (ATRA) (38), ARE expression modulator 1 (AEM1) (40), ML385 (41), clobetasol propionate (CP) (42), dextran sulfate (45), 1-(2-cyclohexylethoxy)aniline (IM3829) (2), and malabaricone-A (MAL-A) (2). Dextran sulfate suppresses angiogenesis by inhibiting the Nrf2 signaling pathway and reducing the expression of hypoxia-inducible factor-1alpha (HIF-1α) in gastric cancer (45). Brusatol extracted from a family of natural products known as quassinoids inhibited Nrf2-related cell cycle transition from G2 to M phase, which depends on cyclin B-cyclin-dependent kinase 1 (CDK1) complex (43). Furthermore, brusatol downregulates c-MYC expression, leading to cell death (37). Thus, other natural products (low molecular weight organic compounds produced by plants) and natural product-derived synthetic compounds may be potential drug candidates for cancer therapy.

Additionally, a key role for Nrf2 in treating ovarian cancer has been validated by siRNA studies (39,47). Nrf2 downregulation enhanced sensitivity to oxidative stress by increasing intracellular ROS levels, which maintains an antitumor effect, and decreases cell viability in ovarian cancer cells (39). Nrf2 inhibitors specifically blocked the progression of Nrf2-positive cancers (39). Pharmacological inhibition and siRNA-induced downregulation of Nrf2 signaling in breast cancer cells resulted in sensitization of cancer cells to cisplatin (46). Nrf2 small-interfering RNA induces apoptosis and inhibits proliferation in various cancer cells (39,44-46). Nrf2 inhibitors also increased the sensitivity of cancer cells to ionizing radiation and chemotherapeutic drugs. Further, concurrent inhibition of the thioredoxin and glutathione systems, downstream targets of Nrf2, promoted cancer cell death in mouse models (48).

Interestingly, depending on the cellular context and environment, Nrf2 plays a dynamic tumor-suppressive or -promoting role. Although *in vivo* experiments in knockout mice demonstrate that Nrf2 protected mice from cancer development, the xenograft animal model showed that Nrf2 promoted cancer growth (29). Host- and cancer cell-derived Nrf2 may act as tumor suppressors and tumor promoters, respectively. Therefore, the Nrf2 pathway is often activated in various cancer but is thought to play a dual role in cancer initiation and progression. Preclinical studies demonstrated that antioxidant pathways might be a promising target for cancer therapy. However, some clinical studies on the impact of Nrf2 expression on the prognosis of cancer patients have yielded inconsistent findings (2,29,31,32,34). Further studies are required to determine whether Nrf2 is associated with tumor aggressivity and poor prognosis in various cancers, including HGSC.

CD44v9. CD44 is a transmembrane glycoprotein and surface receptor for hyaluronan involved in the mutual response between cells and their microenvironment (49) (Fig. 2A). The variant isoform of CD44 containing v8-v10 (CD44v9) is an ovarian cancer stem cell surface marker (50). CD44v9 interacts with xCT [also known as solute carrier family 7 member 11 (SLC7A11)], a cystine/glutamate transporter, for cystine uptake (50-52). A continual cystine supply is crucial for *de novo* synthesis of glutathione and thioredoxin antioxidant peptides (51). CD44v9 specifically stabilizes redox potential through antioxidant factors, such as glutathione and glutathione peroxidases (GPxs) (53). In addition, CD44v9 contributes to antioxidative response by reducing ROS levels. Various tumor cells, including ovarian cancer and normal cells, acquire protection and resistance against oxidative stress by activating CD44v9 (50). This section summarizes the relationship between the CD44v9 expression level and tumor progression in various patients with cancer (52,54-65) (Table III). Studies conducted on different cancers have shown the dual role of the CD44v9/xCT pathway in tumor progression and suppression.

Table II. Role of Nrf2 inhibitors during cancer development, progression and determining the therapeutic response in preclinical cancer models.

First author/s, year	Name	Means to suppress Nrf2 function	Cancer type	*In vitro*/*in vivo*	Summary	(Refs.)
Mata-Greenwood *et al*, 2002	Quassinoid brusatol	Natural product	Acute or chronic myeloid leukemia cell lines	*In vitro*	Brusatol downregulates c-MYC expression.	(37)
Wang *et al*, 2007	ATRA and RARalpha agonists		A human mammary MCF7-derived AREc32 reporter cell line	*In vitro*/*in vivo* mouse model	ATRA and RARalpha agonists reduce the ability of NRF2	(38)
van der Wijst *et al*, 2015	Small interfering RNA against NRF2	siRNA-induced downregulation of Nrf2 signaling	Ovarian cancer	*In vitro*	Downregulation of NRF2 enhances sensitivity to oxidative stress by increasing intracellular ROS levels, which promotes ovarian cancer cell death	(39)
Bollong *et al*, 2015	AEM1	A small molecule inhibitor	Lung adenocarcinoma cells	A high throughput screen identified small molecules which decrease NRF2 transcriptional activity at antioxidant response element sites	AEM1 sensitizes lung adenocarcinoma cells to various chemotherapeutic agents via downregulation of NRF2 controlled genes, inhibiting the growth of cancer cells *in vitro* and *in vivo*	(40)
Singh *et al*, 2016	ML385	A small molecule inhibitor	Non-small cell lung cancer cells	A quantitative high-throughput screen	Combination of ML385 and carboplatin is an efficient therapeutic approach in non-small cell lung cancer cells *in vitro*	(41)
Choi *et al*, 2017	CP	Clinical compound screening	Lung cancer	*In vitro*/*in vivo* mouse model	CP prevents nuclear accumulation and promotes degradation of NRF2 in a glucocorticoid receptor- and a glycogen synthase kinase 3-dependent manner. CP could be a repurposed therapeutic agent for cancers with high NRF2 activity	(42)

Table II. Continued.

First author/s, year	Name	Means to suppress Nrf2 function	Cancer type	*In vitro/in vivo*	Summary	(Refs.)
Lin *et al*, 2018	Brusatol	A natural product isolated from the seeds of Brucea	Early mouse embryo	*In vitro/in vivo*	Brusatol inhibits NRF2-related cell cycle transition from G_2 to M phase that is dependent on the cyclin B-CDK1 complex	(43)
Lee *et al*, 2020	Small interfering RNA against NRF2	siRNA-induced downregulation of Nrf2 signaling	Colorectal cancer	*In vitro* experiments and human colorectal cancer tissues	Small interfering RNA against NRF2 successfully inhibits tumor growth and markedly increases apoptosis	(44)
Xu *et al*, 2021	DS	Dextran Sulfate	Gastric cancer	*In vitro* and *in vivo* nude mouse intraperitoneal implantation metastasis model	DS reduces the angiogenic potential through suppressing Nrf2 expression in gastric cancer	(45)
Bovilla *et al*, 2021	siNrf2 and pharmacological inhibition	Pharmacological inhibition and siRNA-induced downregulation of Nrf2 signaling	Breast cancer	*In vitro/in vivo* mouse model/human breast cancer tissues	Pharmacological inhibition and siRNA-induced downregulation of NRF2 signaling results in reduced breast cancer proliferation and migration, cell cycle arrest, activation of apoptosis, and sensitization of cancer cells to cisplatin *in vitro*	(46)

AEM1, ARE expression modulator 1; ATRA, all-trans retinoic acid; CP, clobetasol propionate; DS, dextran sulfate; Nrf2, nuclear factor erythroid 2-related factor 2; RARalpha, retinoic acid receptor alpha; si/siRNA, small interfering RNA.

Table III. Summary of the preclinical evidence of CD44v9 in cancer.

A, Tumor-suppressing effects: An increase in CD44v9 expression suppresses cancer progression

First author/s, year	*In vitro*/*in vivo* or human samples	Methods	Summary	(Refs.)
Sato *et al*, 2004	Oral squamous cell carcinoma HSC-4 cells	Cell culture invasion assay and a three-dimensional culture invasion assay	Overexpression of CD44v9 resulted in downregulation of the invasive potential	(54)
Miwa *et al*, 2017	Gallbladder cancer NOZ cells	*In vitro* cell migration and invasion assays	CD44v9-positive cells exhibited decreased invasiveness compared with CD44v9-negative cells	(55)
Sato *et al*, 2000	Primary squamous cell carcinoma of the tongue	Immunohistochemical study. Biopsy specimens from primary squamous cell carcinoma of the tongue.	Downregulation of CD44v9 in squamous cell carcinoma of the tongue may relate to the detachment of tumor cells from primary lesions, establishment of lymph node metastasis and consequently the death of patients	(56)

B, Tumor-suppressing effects: A decrease in CD44v9 expression promotes cancer progression

First author/s, year	*In vitro*/*in vivo* or human samples	Methods	Summary	(Refs.)
Sato *et al*, 2004	Oral squamous cell carcinoma HSC-4 cells	Cell culture invasion assay and a three-dimensional culture invasion assay	Treatment with an anti-CD44v9 antibody enhanced the invasive potential of oral squamous cell carcinoma cell lines	(54)
Umeda *et al*, 2016	Invasive micropapillary breast carcinoma and ICNST	Immunohistochemistry. Twenty-one consecutive cases of mixed invasive micropapillary carcinoma of the breast.	Immunohistochemical scores of CD44v9 in the ICNST component of lymph node metastasis cases of breast cancer were lower compared with cases without lymph node metastasis	(57)

C, Tumor-promoting effects: An increase in CD44v9 expression promotes cancer progression

First author/s, year	*In vitro*/*in vivo* or human samples	Methods	Summary	(Refs.)
Yasui *et al*, 1998	Non-neoplastic mucosa, adenoma and adenocarcinoma of the stomach	Immunohistochemistry	Incidence of CD44v9 expression was higher in the cases of stages 3 and 4 in comparison with that in the stages 1 and 2 cases. The expression of CD44v9 may be associated with the development as well as progression of gastric cancer	(58)
Okano *et al*, 1999	Early colorectal cancer	Immunohistochemistry	Immunohistochemical expression of p53 and CD44v9 provides useful information for identifying those patients with early colorectal cancer who have a high risk of developing liver metastases	(59)

Table III. Continued.

C, Tumor-promoting effects: An increase in CD44v9 expression promotes cancer progression

First author/s, year	*In vitro*/*in vivo* or human samples	Methods	Summary	(Refs.)
Koyama *et al*, 1999	Primary gastric and esophageal carcinomas	Immunohistochemistry	Upregulation of the CD44v9 molecule in gastric cancer, especially metastatic adenocarcinoma, is associated with tumor growth and progression	(60)
Goi *et al*, 2002	Colorectal cancers	Immunohistochemistry	CD44v9 was expressed in the primary colorectal cancers in 42% of patients without pulmonary metastases and 88% of patients with pulmonary metastases	(61)
Bánkfalvi *et al*, 2002	Oral squamous cell carcinoma	Immunohistochemistry	In oral squamous cell carcinoma, an accumulation of CD44v9 was observed at the invasive tumor front. In metastases and recurrences, an increase of v9 was recorded. Changes of CD44v9 phenotype within the primary tumors were associated with poor prognosis	(62)
Kakehashi *et al*, 2016	Hepatocellular carcinoma	Immunohistochemistry	Patients with hepatocellular carcinoma with positive CD44v9 expression had poor overall and recurrence-free survival compared with those with negative expression	(63)
Miwa *et al*, 2017	Gallbladder cancer NOZ cells	*In vivo* animal model	CD44v9 cells exhibited increased tumorigenicity	(55)
Ogihara *et al*, 2019	*In vitro*/*in vivo* mouse metastasis model and bladder cancer	Immunohistochemistry	CD44v9 expression was associated with disease recurrence and death in muscle invasive bladder cancer	(52)
Go *et al*, 2019	Early gastric cancer	Immunohistochemistry	Both positive CD44v9 and high Ki67 expression are associated with poor prognosis in early gastric cancer	(64)

D, Tumor-promoting effects: A decrease in CD44v9 expression suppresses cancer progression

First author/s, year	*In vitro*/*in vivo* or human samples	Methods	Summary	(Refs.)
Suwannakul *et al*, 2020	Cholangiocarcinoma cells	CD44v9 silencing using siRNA transfection. *In vitro*/*in vivo* mouse xenografts.	CD44v9 silencing regulates redox system by reducing the expression levels of cysteine transporter xCT. CD44v9 silencing suppresses cell proliferation, migration and invasion by induction of apoptosis and cell cycle arrest. CD44v9 downregulation inhibited tumor growth in mouse xenografts	(65)

CD44v9, CD44 variant isoform 9; ICNST, invasive carcinoma of no special type.

An increase in CD44v9 expression suppresses cancer progression. i) Oral squamous cell carcinoma. CD44v9 overexpression downregulated the invasive potential of oral squamous cell carcinoma HSC-4 cells due to enhanced cell-cell adhesion (54).

ii) Gallbladder cancer. In gallbladder cancer, CD44v9-positive cells exhibited decreased invasiveness than CD44v9-negative cells in *in vitro* cell invasion assays (55).

iii) Tongue squamous cell carcinoma. CD44v9 expression was negatively correlated with lymphatic metastasis and unfavorable outcome in patients with tongue squamous cell carcinoma (56). Also, increased CD44v9 expression is associated with suppressing some cancer cell invasion and metastasis, suggesting that targeted CD44v9 activation is a novel approach to preventing cancer initiation.

A decrease in CD44v9 expression promotes cancer progression. i) Breast cancer. Immunohistochemical analysis showed that downregulating CD44v9 expression in the invasive breast carcinoma of no special type was a risk factor for lymph node metastasis (57).

ii) Oral squamous cell carcinoma. Reduced CD44v9 expression was correlated with increased invasive potential in oral squamous cell carcinoma cells (54). However, decreased CD44v9 expression may be associated with the progression of certain cancer types.

An increase in CD44v9 expression promotes cancer progression. i) Gastric cancer. CD44v9 expression is identified in normal gastric epithelium, *H. pylori*-infected pyloric gland cells, and primary gastric carcinoma cells (58,60). CD44v9 is involved in the wound-healing process of the gastric epithelium after injury (66). Positive CD44v9 expression is associated with gastric cancer progression (58,60), correlating with a poor prognosis in early gastric cancer (64).

ii) Colorectal cancer. CD44v9 significantly impacts the survival of early colorectal cancer (59) and may be a biomarker for cancer progression, particularly for predicting pulmonary metastasis, in patients with colorectal cancer (61).

iii) Oral squamous cell carcinoma. Immunohistochemistry showed that CD44v9-positive cancer cells were located at the tip of the invasive front of oral squamous cell carcinoma (62). Also, a significant increase in CD44v9 expression was identified in metastatic and recurrent lesions. CD44v9 is a predictive indicator of poor prognosis in oral squamous cell carcinoma and may play a role in patient risk assessment (62).

iv) Hepatocellular carcinoma. Positive CD44v9 expression was significantly associated with poor overall and recurrence-free survival in patients with hepatocellular carcinoma than those with CD44v9-negative expression (63). CD44v9 expression was negatively associated with Ki67 expression, proposing the importance of CD44v9 in maintaining the stemness of cancer stem-like cells.

v) Bladder cancer. CD44v9 may be a clinical biomarker for predicting poor outcomes in patients with muscle-invasive bladder cancer (52). Furthermore, CD44v9-positive cells enhanced tumorigenicity in xenotransplantation models (55).

A decrease in CD44v9 expression suppresses cancer progression. i) Cholangiocarcinoma. CD44v9 silencing inhibits proliferation and invasion, thus, promoting apoptosis and cell cycle arrest by downregulating xCT expression levels (65).

We reviewed several preclinical studies that suggest a causal effect between CD44v9 expression and increased or decreased risk of tumor formation. CD44v9 had divergent effects on cancer initiation and progression in different contexts. Different antioxidant-signaling pathways may be activated in different cancers. Furthermore, several documents reported that CD44v9 expression in cancer tissue is predictive of a poor prognosis (52,55,58-64). Therefore, drugs targeting CD44v9 and xCT may be effective in patients with cancer. The CD44v9/xCT pathway inhibitor may provide a therapeutic option for treating certain cancers (Table IV). Sulfasalazine is an oral pharmacological inhibitor of xCT (52,67-69). Sulfasalazine suppressed cell proliferation in lymphoma cells (67) and induced cell death in cholangiocarcinoma cells *in vitro*, possibly by enhancing ROS levels (68). Sulfasalazine also enhances chemosensitivity, promotes apoptosis, and suppresses the growth of various cancer cells (52,67-69). Combining CDDP treatment with sulfasalazine represents a novel therapeutic strategy for overcoming CDDP resistance in various cancers (52,68,69). Sulfasalazine showed favorable therapeutic effects in various malignant tumors. Therefore, inhibiting the CD44v9/xCT pathway can be of therapeutic value.

GST. There are two major antioxidant systems: highly complex antioxidant enzymatic systems, including SOD, catalase, GST, GPxs, glutathione S-reductase, and G6PD; and non-enzymatic antioxidant systems, such as vitamins, such as E, C, and A, tocopherol, glutathione, and bilirubin (70,71). GST is a key enzyme that maintains intracellular redox homeostasis and is often overexpressed in cancer cells (72). Glutathione and GST enzymes, such as GST P1-1 and GST A1-1, were overexpressed in various cancers, including ovarian cancer (73). Preclinical studies showed that GST overexpression had been commonly associated with high malignant phenotype, chemoresistance, and poor prognosis (72,74). Several clinical studies have also showed that GST expression was significantly correlated with drug resistance and poor prognosis in patients with ovarian cancer (75,76). For example, high GST-π mRNA expression correlated with lower three-year survival (76). GST is considered an effective marker for assessing the efficacy of chemotherapy and predicting prognosis in patients with ovarian cancer (76). However, we sometimes encounter conflicting information that GST activity in ovarian cancer tissue was positively associated with a better prognosis (77).

Researchers have developed several drugs that target unique redox-related enzymes in tumor tissue. One therapeutic strategy is to develop specific inhibitors of antioxidant enzymes, such as GST. The GST inhibitor, 6-(7-nitro-2, 1, 3-benzoxadiazol-4-ylthio) hexanol (NBDHEX), induced caspase activation, apoptosis, and cell death in human mesothelioma cell lines by activating c-Jun NH_2-terminal kinase and p38 mitogen-activated protein kinase (MAPK) pathways (78). TLK199 (Telintra; Ezatiostat®), a GST P1-1 inhibitor, has been proposed as a promising approach to treat patients with myelodysplastic syndrome (72). GST inhibitors are novel therapeutic candidates for human cancers in preclinical and clinical settings. Furthermore, auranofin, a thioredoxin reductase inhibitor, elicited cytotoxicity by perturbing the cellular redox

Table IV. Role of inhibiting the CD44v9/xCT pathway during cancer development, progression and determining the therapeutic response in preclinical cancer models.

First author/s, year	Name	Therapeutic uses	Cancer type	*In vitro*/*in vivo*	Summary	(Refs.)
Gout *et al*, 2001	Sulfasalazine	A medication used to treat rheumatoid arthritis, ulcerative colitis, and Crohn's disease	Lymphoma	*In vitro* rat Nb2 lymphoma cultures and *in vivo* animal model	Sulfasalazine (i.p.) markedly inhibited growth of rat Nb2 lymphoma transplants without apparent side-effects	(67)
Thanee *et al*, 2016	Sulfasalazine		Cholangiocarcinoma	*In vitro* and *in vivo* hamster model	Sulfasalazine inhibited cell growth and activated cell death. Sulfasalazine enhanced chemosensitivity to chemotherapeutic drugs (e.g., gemcitabine)	(68)
Wada *et al*, 2018	Sulfasalazine		Hepatocellular carcinoma	*In vitro* and *in vivo* nude mouse model and human samples/hepatocellular carcinoma tissues	High levels of xCT were expressed in poorly differentiated hepatocellular carcinoma tissues. Sulfasalazine is involved in enhancing CDDP chemosensitivity in xenograft tumor models	(69)
Ogihara *et al*, 2019	Sulfasalazine		Bladder cancer	Human samples/ immunohistochemistry, *in vitro* and *in vivo*	CD44v9 expression was independently associated with disease recurrence and death in muscle invasive bladder cancer. Sulfasalazine exerted cytotoxic effects against MBT-2V cells by inhibiting glutathione levels and inducing ROS production. Sulfasalazine in combination with CDDP exerts cytotoxic effects against MBT-2V cells by inhibiting CD44v9 expression and upregulating phospho-p38MAPK expression	(52)

CD44v9, CD44 variant isoform 9; i.p., intraperitoneal; xCT, cystine/glutamate antiporter SLC7A11; CDDP, cis-diamminedichloroplatinum.

balance, and increasing ROS production (79). Phase II study has been conducted to evaluate the efficacy and tolerability of auranofin in patients with recurrent EOC (79).

The second promising concept is creating redox-directed anticancer prodrugs that convert to active parent drugs *in vivo* by antioxidant enzymes that are specifically overexpressed in cancer. Prodrugs activated by GST (e.g., doxorubicin and etoposide) have been designed and synthesized as more effective and less toxic treatment regimens to overcome drug resistance (74,80). For example, Canfosfamide HCl for injection (TLK286, TELCYTA) is a prodrug cleaved and activated by GST to form a glutathione derivative and active metabolite phosphorodiamidate moiety as an anticancer agent (81). The phase II clinical trials showed that TLK286 had demonstrated a manageable safety profile as a single agent in patients with ovarian, non-small cell lung, breast, and colorectal cancers (82). TLK286 with a single agent or a combined chemotherapeutic regimen has shown safe antitumor activity and clinical benefit in phases II (81) and III (83) clinical trials for treating patients with ovarian and breast cancers (84). However, the phase III trial of TLK286 in non-small cell lung cancer could not reach a favorable efficacy, and further clinical trials are currently underway (82).

4. Targeted therapy for ovarian cancer based on redox modifications

Current status of ovarian cancer treatment. Surgical debulking and combining paclitaxel and carboplatin-based chemotherapy are the standard treatments for ovarian cancer (85). Clinical studies have shown the safety and efficacy of angiogenesis inhibitors (e.g., bevacizumab) and the poly(ADP-ribose) polymerase (PARP) inhibitors (e.g., olaparib and niraparib) (85). Additionally, several studies are evaluating PARP inhibitors in monotherapy and combined in a real-world cohort. Patients with ovarian cancer BRCA1/2 mutations or homologous recombination deficiency can benefit from PARP inhibitors. Unfortunately, even molecular targeted therapies cause treatment resistance and tumor recurrence. Therefore, additional alternative therapies are required. Mechanisms of drug resistance include epithelial-mesenchymal transition, DNA repair activation, reduced drug uptake, enhanced drug efflux, changes in pro-apoptotic and anti-apoptotic genes, changes in tumor cell microenvironment, and redox imbalance. However, oxidative stress and redox imbalance play an important role in the initiation, progression, drug resistance, and recurrence of ovarian cancer. This section discusses molecular mechanisms and therapeutic strategies for ovarian cancer, focusing on redox modification.

Mechanism of ovarian carcinogenesis. Steady progress has been made in the molecular understanding of the etiology of ovarian cancer, particularly HGSC (1). Accumulating evidence has shown that HGSC originates from the fimbriated end of the fallopian tube secretory epithelial cells (21,86,87). Also, human endometrial cells and fallopian tube epithelial cells are constantly exposed to menstrual blood or follicular fluid. Erythrocytes and follicular fluid induce intracellular ROS generation (88) and may induce oxidative injury to fallopian tube fimbria epithelial cells (89). Fallopian tube cells have evolved strategies to promote their survival by modulating genetic and epigenetic profiles, including antioxidative and proliferative changes (90). Fallopian tube fimbria epithelial cells acquire protective molecules and pathways, such as antioxidants, when exposed to oxidative stress environments. HGSC has been postulated to arise through a stepwise accumulation of (epi)genetic alterations from normal epithelium to secretory cell outgrowth, p53 signature, and serous tubal intraepithelial carcinoma to invasive HGSC (90). Some clones of fallopian tube fimbria epithelial cells can turn into precursor cells, allowing them to survive oxidative stress conditions, and eventually grow as ovarian cancer. Ovarian cancer is exposed to an oxidative stress environment from an early stage of carcinogenesis to the advanced stage.

Antioxidant defense system in ovarian cancer. We have earlier summarized how ovarian cancer cells regulate their antioxidant defense system to cope with oxidative stress. Many antioxidants (e.g., NADPH, Nrf2, GST, GPxs, glutathione, peroxiredoxin, and CD44v9) exist in ovarian cancer cells to protect against oxidative stress (76,90-93). The association between these antioxidant-related gene expressions and clinical outcomes of ovarian cancer has been investigated. Glutathione, GPxs, and peroxiredoxin overexpressed in ovarian cancer are associated with an aggressive phenotype and poor prognosis (76,91-93). GPx3 upregulated in clear-cell ovarian cancer may promote chemotherapeutic resistance (94). Furthermore, a high GPx3 expression was significantly associated with poor overall survival in patients with HGSC (13). Peroxiredoxins have been implicated in tumorigenesis, therapeutic resistance, recurrence, and metastasis of ovarian cancer (95).

Various antioxidant genes, including glutathione, thioredoxin, and other antioxidants, are downstream targets of the Nrf2 gene. Aberrant expression and activation of Nrf2 are frequently observed in ovarian cancer because KEAP1 expression, as a negative regulator of Nrf2, is downregulated by DNA copy-number loss (47). Immunohistochemistry showed that positive staining for Nrf2 was observed in HGSC (36), showing that constitutive Nrf2 pathway activation often occurs in HGSC (39,47,96). Furthermore, altered expression of CD44v9 occurs in the early stage of HGSC carcinogenesis. Immunohistochemistry revealed that CD44v9 was expressed in normal fallopian tube fimbria epithelial cells (90). Additionally, a recent study demonstrated that CD44v9 loss followed by p53 mutation is the earliest and universal phase of ovarian neoplastic changes, which may confer positive clonal selection with growth and survival advantages (90). CD44v9 loss in fallopian tube fimbria epithelial cells leads to ROS increase. Furthermore, CD44v9 also reappeared in cancer stem-like cells of HGSC (90). Constant change by redox homeostasis may contribute to the high tumor heterogeneity of malignant cells, including plasticity of cancer stem-like traits and phenotypic diversity (2). A high ROS level and upregulation of antioxidant genes are characteristic features of ovarian cancer (47,97). HGSC has evolved antioxidant defense strategies to combat endogenous and exogenous oxidative stress. Therefore, CD44v9 and Nrf2 pathways may be key regulators of redox balance in ovarian cancer (92). Ovarian cancer cells promoted antioxidant defense by upregulating CD44v9 (7) and Nrf2 (39). The antioxidant system supports cancer progression by suppressing oxidative stress and increasing ROS-scavenging capacity.

Finally, BRCA1 is a key factor in DNA damage repair and upregulates, activates, and stabilizes the expression of multiple genes, including Nrf2, GST oxidoreductases, and other antioxidant genes involved in the cytoprotective antioxidant response (98). Genetic deletion of the BRCA gene significantly disrupted redox balance by downregulating Nrf2 expression, leading to apoptotic cell death triggered by excessive ROS production. In addition, BRCA1 deficiency enhanced auranofin (a thioredoxin reductase inhibitor) sensitivity of ovarian cancer cells (79). The BRCA1 gene plays a critical role in protecting ovarian cancer cells from oxidative stress by upregulating and activating the antioxidant defense system. Furthermore, silencing PARP reduces the expression of cystine/glutamate exchanger, xCT (SLC7A11), enhancing cell death by decreasing glutathione biosynthesis (99). PARP inhibitors offer a significant clinical benefit even in patients without BRCA1/2 mutations. This effect may be due to a decreased antioxidant capacity induced by PARP inhibitors. Thus, targeting the antioxidant defense system is considered a promising therapeutic strategy for ovarian cancer (39,47).

Potential therapeutic strategies targeting the antioxidant defense system. Treatments targeting antioxidant defense systems have conflicting results.

Suppressing tumor progression by inhibiting ROS generation. It has been proposed that oxidative stress inhibition may benefit cancer treatment. Polyphenols and flavonoids, such as resveratrol, genistein, curcumin, and quercetin, maintain the balance of antioxidant systems by scavenging ROS (100-103). Furthermore, preclinical studies showed that these antioxidant phytochemicals inhibit human cancers, including ovarian cancer, by anticancer activities, including anti-angiogenic, anti-proliferative, anti-inflammatory, and pro-apoptotic properties (100-103). The positive effects of antioxidant therapy have been reported in *in vitro* and *in vivo* animal studies. Additionally, preclinical studies have shown that inhibiting ROS production by pharmacological inhibitors, antagonists, or scavengers can inhibit the proliferation of ovarian cancer cells. Diphenylene iodinenium, a ROS inhibitor, promotes apoptosis in ovarian cancer cells by inhibiting NADPH oxidase (NOX) (91). Lysophosphatidic acid (LPA), a bioactive lipid mediator, induced cell proliferation by stimulating NOX-dependent ROS generation in ovarian cancer (104). A specific LPA receptor antagonist promoted cancer cell apoptosis by inhibiting LPA/NOX-dependent ROS production (104). Several antioxidants have exhibited therapeutic potential in preclinical studies. Furthermore, clinical trials have shown that the vitamin C-treated group improves survival efficacy in patients with ovarian cancer better than the non-treated group (105). In addition, vitamin C may improve tumor drug resistance via its antioxidant and anti-inflammatory mechanisms (105). These data provide a rationale to increase the efficacy of conventional chemotherapies combined with antioxidant therapies for ovarian cancer. However, few clinical studies have been conducted on cancer patients. Therefore, it is unclear whether antioxidants provide clinical benefits in patients with ovarian cancer.

Suppressing tumor progression by inhibiting the antioxidant defense system. Antioxidant inhibitions have beneficial effects on cancer therapy. Increased pro-oxidant or decreased antioxidant defense is considered a promising therapeutic option as excessive ROS production beyond a threshold value leads to cancer cell death. Such treatment strategies may benefit patients who already have ovarian cancer rather than cancer prevention. Here we focus on CD44v9 and Nrf2, antioxidants highly expressed in HGSC. Nrf2 (Tables I and II) and CD44v9/xCT inhibition (Tables III and IV) can promote cancer initiation or inhibit cancer progression in a context-dependent manner. Fig. 2B illustrates the therapeutic strategies based on Nrf2- and CD44v9/xCT-associated signaling pathways. Results showed that Nrf2 or CD44v9 inhibition delays tumor progression, but the treatment was insufficient to eradicate cancer cells. The endogenous antioxidant defense system, such as Nrf2 and CD44v9, may act coordinately to neutralize ROS, and protect cells against the biological damage of ROS-induced oxidative stress. Intracellular ROS elevated by concurrent inhibition of the Nrf2 and CD44v9 pathways promotes p38 MAPK activation and modulates the expression or activity of cell cycle regulators, including cyclin D1, the cyclin-dependent kinase inhibitors (CDKI; p16 and p21, checkpoint kinase 1 (CHK1), cell division cycle 25C (CDC25C) and p53 (106,107). p38 shows strong anticancer effects by inducing G2/M cell cycle arrest and apoptosis (108). However, this is still a hypothesis, not a clinically proven fact. Oxidative stress may be involved in the pathophysiology of HGSC, but it remains largely unknown how CD44v9 and Nrf2 play a regulatory role in HGSC prevention or development. Thus, further studies on the relevant mechanisms of Nrf2 and CD44v9 may help improve cancer therapy outcomes.

In addition to Nrf2 and CD44v9, therapeutic strategies targeting antioxidant defense systems have been reported. SOD2 (Mn-SOD) localized in the mitochondrial matrix detoxifies superoxide radicals. Also, SOD2 is involved in tumorigenesis, proliferation, invasion, and metastasis in CCC (109). Reduced SOD2 expression effectively inhibited the malignant progression of CCC by upregulating ROS levels (109). Inhibition of thioredoxin reductase overcame cisplatin resistance and potentiated antitumor response via glutathione depletion and increased ROS generation (110). Also, diselenium nanoparticle encapsulating the cisplatin prodrug enhanced cisplatin-induced cytotoxicity by depleting glutathione and increasing ROS (111). Additionally, antioxidant defense systems also participate in regulating genes related to energy metabolism in drug-resistant cancer cells. Cisplatin-resistant ovarian cancer cells can avoid large amounts of ROS accumulation by stimulating the PPP and maintaining redox homeostasis (112). A recent study has reported that concurrent inhibition of endogenous antioxidant enzymes (e.g., glutathione biosynthesis) with enzymes involved in ROS production (e.g., NOX) may improve treatment outcomes (113). Altogether, pro-oxidant and antioxidant therapies may play contradictory roles in cancer treatment, depending on the concentration of intracellular ROS.

Lessons from animal studies. Redox homeostasis that controls the dynamic interaction between oxidative stress and antioxidants can differ between cancer prevention in cancer-free cases and treatment of cancer patients (114). Investigators created various antioxidant knockout mouse models (Fig. 3A). For example, Nrf2 knockout mice cannot eliminate cancer cells efficiently (29). Also, GPX-1 and -2 double-knockout

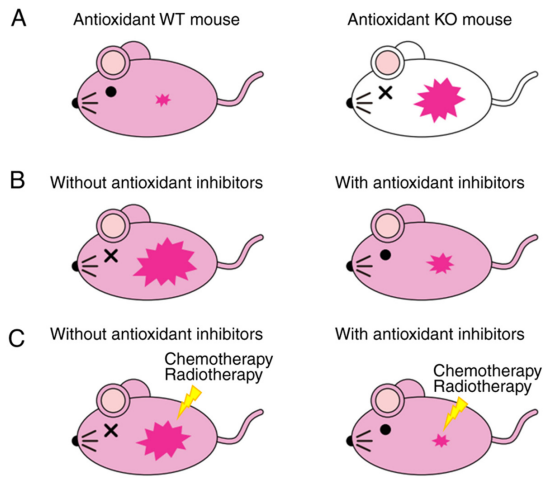


Figure 3. Dual role of antioxidant systems in cancer initiation and progression. (A) Antioxidant knockout mouse models. This figure shows an example of knockout mouse models generated by disrupting the Nrf2 gene sequence. Nrf2 WT and KO mice are shown in light pink and white, respectively. The dark, pinkish, zig-zag form indicates tumors derived from spontaneous carcinogenesis models or xenograft human cancer models. The size of the zig-zag form is proportional to cancer progression. Antioxidant KO mice cannot block redox-mediated carcinogenesis and cancer progression. Host-derived antioxidants are essential to suppress cancer development. (B) Human cancer xenograft models. Activating antioxidant systems in cancer cells upregulates target antioxidant genes, leading to tumor progression. Conversely, antioxidant inhibitors promote excessive ROS generation and suppress cancer progression. (C) Human cancer xenograft models. Chemotherapy or radiotherapy with antioxidant inhibitors. Activating antioxidant pathways in cancer cells protects cells against chemotherapeutic agents and radiotherapy. Antioxidant inhibitors block cancer progression by promoting chemotherapy and radiotherapy-induced excessive ROS generation. KO, knockout; Nrf2, nuclear factor erythroid 2-related factor 2; ROS, reactive oxygen species; WT, wild-type.

spontaneously develop intestinal cancer (115). Loss of GST zeta 1 (GSTZ1), a downstream target of Nrf2, promotes chemically induced hepatocellular carcinogenesis (116). Furthermore, antioxidant knockout mice are more prone to spontaneous or carcinogen-induced tumorigenesis due to increased endogenous ROS production. Host-derived antioxidants are essential to suppress carcinogenesis or cancer initiation. Decreased intracellular ROS-scavenging ability in the host significantly promotes cancer development (117). Therefore, antioxidants, such as CD44v9 and Nrf2, and their downstream targets protect the host from cancer development.

However, the antioxidant molecules, CD44v9 and Nrf2, are elevated in ovarian cancer cells, especially cancer stem cells. *In vitro* studies and cancer xenograft models demonstrated that antioxidants play an important oncogenic role in supporting cancer proliferation and growth (114). After cancer initiation, the CD44v9 and Nrf2 antioxidant pathways are required for cancer progression. In contrast, the elevation of ROS level above the cellular tolerability threshold leads to cell death, suggesting that high ROS levels can block cancer progression (118). Antioxidant inhibitors suppressed cancer proliferation in mouse xenograft models (Table II and Fig. 3B). Also, these inhibitors increase the sensitivity of cancer cells to chemotherapeutic drugs and ionizing radiation (21) (Fig. 3C). This suggests that antioxidant inhibitors prevent cancer progression. Nevertheless, the CD44v9/xCT pathway or Nrf2 inhibitors have limited therapeutic effects. Concurrent inhibition of both pathways can induce ROS-dependent lethality in cancer cells. Therefore, the depletion of antioxidant defense systems and increased ROS concentration can promote ovarian cancer cell death. Complete loss of antioxidant protein expression can cause cancer cell death by excessive elevation of ROS, whereas incomplete suppression may promote cancer progression. The role of antioxidant systems in inhibiting cancer progression remains controversial. However, insights into the opposite effects of antioxidants in initiating tumorigenesis and supporting cancer proliferation are essential to allow optimal exploitation of CD44v9 and Nrf2 as therapeutic targets.

5. Conclusion

This review focuses on the redox balance between ROS production and antioxidant defense systems, discussing proposed therapeutic strategies for the development and progression of human cancer, especially ovarian cancer. Cancer cells reprogram their oxidative metabolism to meet high-energy demand, generating ROS as undesirable side products. Excessive ROS generation has been reported in various cancers, including ovarian cancer. Cancer cells increase their antioxidant defense system to block the harmful effects of ROS. Antioxidant genes, such as Nrf2, GST, and CD44v9, and their downstream targets are overexpressed in many types of cancer, including ovarian cancer. They are involved in cancer initiation and progression, resulting in chemoresistance and unfavorable prognosis. Redox balance that controls the dynamic interaction between oxidative stress and antioxidants plays a key role in determining the fate of cancer cells. Furthermore, treatment strategies targeting redox balance can differ between taking preventive measures in cancer-free women, and treating patients receiving medical care who already have cancer. Preclinical studies assessing redox modulators have given conflicting results. In animal studies, inhibition of excessive ROS production by host antioxidants plays a vital role in suppressing carcinogenesis. In contrast, pharmacological inhibition of antioxidant defense systems may suppress cancer progression in cancer-bearing animals. Inhibition of antioxidant defense systems may benefit patients who already have cancer. Concurrent Nrf2 and CD44v9 inhibition might help develop targeted, personalized medicine in ovarian cancer. Furthermore, understanding the complex interplay between ROS and antioxidants in cancer initiation and progression may spur the development of natural or small-molecule modulators regulating redox balance. Thus, further research is needed to validate the therapeutic potential of regulating redox balance.

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Authors' contributions

HK and HS made substantial contributions to conception and design. SI and HS performed acquisition of data. HS performed analysis and interpretation of data. SI and HS confirm the authenticity of all the raw data. The first draft of the manuscript was written by HK. All authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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