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[Review]

Potential roles of reactive oxygen species derived from chemical substances involved in cancer development in the female reproductive system

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Abstract

Reactive oxygen species (ROS) are major sources of cellular oxidative stress. Specifically, cancer cells harbor genetic alterations that promote a continuous and elevated production of ROS. While such oxidative stress conditions could be harmful to normal cells, they facilitate cancer cell growth in multiple ways by causing DNA damage and genomic instability, and ultimately, by reprogramming cancer cell metabolism. This review provides up to date findings regarding the role of ROS generation induced by diverse biological molecules and chemicals in representative women's' cancer. Specifically, we describe the cellular signaling pathways that regulate direct or indirect interactions between ROS homeostasis and metabolism within female genital cancer cells.

1. Introduction

Oxidative stress is caused by an imbalance in reactive oxygen species (ROS). The regulation of ROS homeostasis plays a major role in cellular growth, metabolism, and survival (1). When present at low levels, ROS is an important signaling molecule that can maintain cellular functions such as viability, migration and apoptosis (2). However, the excessive occurrence of ROS causes biological systems to incompletely detoxify the reactive intermediates and to block the normal functions of biomolecules (3). Both ROS and oxidative stress are considered to be involved in aging (4), inflammation (5), and many diseases, including cancer because ROS play a significant role in the post-transcriptional adjustment of genes and repercussion effects on cellular development, differentiation, proliferation, and apoptosis and the development and progression of cancer (6, 7).

Furthermore, excessive ROS may affect women's diseases occurring in reproductive organs (8). Endometriosis has been found to be correlated with pro-inflammatory mediators and ROS, which can lead to cellular proliferation and activation of ERK1/2 (9). In addition, the toxic effects of ROS have been shown to induce malignancy of ovarian cancer (OC) through reduced expression of antioxidant enzymes (10).

This review describes the adaptive mechanisms that cancer cells in women's reproductive organs take to face oxidative stress conditions. We will discuss the role of ROS induced by diverse biological molecules and chemicals in regulating the related signaling pathways and consequential oxidative stress-mediated responses in female reproductive cancers occurring in organs such as the placenta, ovary, and endometrium.

2. Relationship between ROS and cancer

Oxidative stress has been reported to affect all phases of the oncogenic process including initiation, promotion, and progression (11, 12). Under the impact of ROS in the cancer development stage **(Figure 1)**, ROS can induce altered expression of several transcription factors associated with regulating pathways such as genetic mutations, proliferation, suppression, differentiation, and senescence (8, 13).

A variety of chemical substances and naturally occurring chemicals can mediate carcinogenesis, malignant behavior, and treatment response in cancer development via regulation of ROS imbalance. For instance, several studies have shown that cancer cells induce apoptosis or necrosis of damaged cells to maintain homeostasis of ROS and prevent ROS-induced toxicity. This process is thought to be the initiation of cancer development (13). Moreover, when the oxidative stress state of cells persists from the imbalance of ROS, DNA damage and induction of mutations affect cellular signaling pathways (14, 15), leading to the activation of a variety of protein kinases that regulate diverse cellular functions including the cell cycle, survival, migration, angiogenesis, apoptosis and cell death (16). The infinite cell proliferation capacity of cancer cells has an effect on angiogenesis related mechanisms (17), which can induce cancer metastasis by promoting cancer progression-associated processes such as proliferation, migration, and tube formation (18).

Generally, there are a variety of ways to treat cancer, including surgery, chemotherapy, radiation, immunotherapy, and other targeted therapies. Among them, chemotherapy and radiation therapy lead to the generation of ROS with strong toxicity to cancer cells (19). Specifically, it has been reported that the production of ROS also affects mitogen-activated protein kinases (MAPK) (20) that act as a switch to block or

transmit signals because of the phosphorylation of surrounding proteins. Moreover, activation of the MAPK pathway can result in apoptosis via death signals including the JNK pathway and caspase family (21). The cancer cells that continue to proliferate tend to induce DNA damage and to elicit cell cycle arrest (22, 23). Therefore, excessive production of ROS can provoke cell cycle arrest, apoptosis, and senescence. Additionally, ROS are known to be correlated with malignant progression of cancer cells by increasing invasion and metastatic potentials via MAPK signals (13). ROS-stimulated growth factors and Ras (renin-angiotensin system) in cancer cells play a role in inducing activation of the MAPK pathway, and the activated Ras-MAPK pathway has been shown to lead to cell proliferation (24, 25) and extracellular matrix (ECM) alteration via the upregulation of specific matrix metalloproteinases (MMPs) (26). The upregulated MMP then leads to the invasion via degradation of ECM (27). Successively, the decomposed ECM collapses the layer structures and then enables cancer cells to migrate. Therefore, ROS has been shown to activate the MAPK pathway leading to cancer progression and dissemination (24, 28).

Most of the tumorigenic activities of ROS are associated with regulation of transcription factors such as activator protein 1 (AP-1), nuclear factor-κB (NF-κB), nuclear factor erythroid 2–related factor 2 (Nrf2), and hypoxia-inducible factor-1α (HIF-1α), as well as intercellular adhesion protein-1 (ICAM-1) and p53 (29). Moreover, diverse in-depth molecular regulatory mechanisms of ROS-induced cellular reactions under pathological conditions, including cancer, have been reported in previous reviews (13, 30). In this review, we will focus on cellular modulations induced by chemicals or natural substances-induced formation of ROS in women's cancers.

3. Roles of ROS in female reproductive cancers

3-1. Choriocarcinoma

Human placenta related to pregnancy can develop and lead to specialized fetal trophoblasts; therefore, it plays an important role in implantation and development of the maternal-fetal interface (31, 32). Choriocarcinoma (CC) is a rare cancer occurring in the trophoblastic cells and cytotrophoblast, which form a chronic membrane of the placenta (33). The form of CC is known to be a malignant trophoblastic tumor that quickly spreads to the organs from the uterus. The metastasis of CC occurs via hematogenous routes to the liver, brain, etc., although the most common site is the lungs (34).

Commonly, chemotherapy has a significant curative influence on CC. However, drugs generally used in cancer therapy have barriers such as drug resistance and side effects. Therefore, patients with refractory gestational CC do not have an optimistic outlook when being treated with chemotherapy (35). Chemotherapy and radiation therapy to remove cancer cells usually increase intracellular ROS and damage many other biomolecules (36, 37). Moreover, the cellular ROS concentrations may have been implicated in the selective activation of transcription factors, and either cell death or cell proliferation may result from exposure to oxidative stress (38). Here, we provide representative examples that show cellular adjustment to oxidative stress induced by chemical agents in CC.

Huovinen *et al.* evaluated the effects of diuron as an endocrine disruptor, which produced adverse development and reproductive effects in BeWo cells (a human CC model). Moreover, diuron appeared to produce ROS and to inhibit cell proliferation of BeWo cells because the protein expression of p53 as a biomarker for cell stress and p21

as a cell cycle arrest gene were increased by oxidative stress (39, 40). A study by Ham et al. revealed that the effects of silibinin, a flavonolignan with anti-cancer effects extracted from seeds of milk thistles, significantly inhibited proliferation and induced apoptosis in both JAR and JEG3 CC cells by increasing ROS production and lipid peroxidation. Moreover, silibinin interrupted mitochondrial function by inducing mitochondrial membrane potential and permeabilization of calcium ion efflux in these cancer models (41).

As another phytochemical, coumestrol was shown to induce cell death by regulating ERK1/2 MAPK and JNK MAPK signaling pathways and through disruption of Ca^{2+} and ROS homeostasis. Specifically, coumestrol suppressed proliferation and increased apoptosis in JAR and JEG3 cells by inducing the pro-apoptotic proteins, Bax and Bak, via ROS production and lipid peroxidation. Coumestrol also induced depolarization of MMP and increased cytosolic and mitochondrial Ca^{2+} levels in JAR and JEG3 cells, leading to apoptosis of CC cells through regulation of cell signaling and mitochondrial-mediated functions with a potential to impair progression of the cancer (42). A similar study evaluated the effects of chrysophanol, an anthraquinone compound, on JAR and JEG-3 cells. These results showed that chrysophanol decreased cell viability and induced apoptosis, while increasing oxidative stress in JEG-3 cells by inducing ROS generation followed by mitochondrial dysfunction, including depolarization of the mitochondrial inner membrane potential. In this experiment, the ERK1/2 and AKT signaling pathways were significantly activated in JEG-3 cells by ROS (42).

In another study, benzo(a)pyrene decreased cell viability and induced cell cycle arrest by increasing the ROS level in CC cells. The increased ROS levels enabled

induction of apoptosis and simultaneous activation of endoplasmic reticulum (ER) stress (43). These studies confirm that ROS induced by diverse stimuli play an important role in the induction of apoptosis in CC cells.

However, another study showed that formaldehyde and benzene increased the proliferation and migration of JEG-3 cells and epithelial mesenchymal transition (EMT) during ROS production (43). In this case, the increased level of ROS promoted the cancer progression of CC, unlike in previous studies. Taken together, these findings suggest that ROS-related mechanisms in CC are associated with cancer progression as well as cell death of CC.

3-2. Ovarian cancer

Ovarian cancer is the fourth most common cause of cancer death in gynecological malignancies (44) and the second most diagnosed cancer among gynecologic malignant tumors (45). Ovarian cancer develops through the formation of a neoplasm in tissues of an ovary, and epithelial ovarian cancer is a major type of cancer (46, 47). Most advanced stage cancers originate from epithelial cells, although some originate from serous, mucinous, or endometrioid cells into the surface epithelium of the ovary or fallopian tube (48). The representative metastatic sites of OC are the endometrium, breast, colon, and stomach, and OC migrates through the body's blood stream and lymph fluid (49). Most cases of OC are difficult to treat because they are diagnosed at highly advanced stages (45, 50, 51). The advanced stage of OC is closely associated with high levels of ROS, which produce a large amount of hydrogen peroxide, whereas oxidative stress is induced in the oxidizing environment of the tumor (52).

Zet *et al.* previously sought to determine the effects of inhibiting intracellular ROS generation in epithelial ovarian cancer (EOC) cells. As a result, treatment with diphenyleneiodonium (DPI), a ROS inhibitor, significantly induced apoptosis in EOC cells by increasing caspase-3 activity. Moreover, DPI treatment resulted in reduced NADPH oxidase, SOD3 and HIF-1α levels in EOC cells (10), indicating that lowering oxidative stress, possibly through the inhibition of NADPH oxidase, induces apoptosis in OC cells.

3-3.Endometrial cancer

Endometrial cancer (EC) is a representative malignant gynecologic carcinoma that is the most common cancer in women except for breast cancer (53). The majority of endometrial malignancies (95%) occur in endometrial glands and are known as endometrial cancers. The remaining 5% occur in mesenchymal tumors and are known as carcinosarcoma (mixed epithelium/epilepsy tumor) (54).

Because of early symptoms such as abnormal uterine bleeding or pelvic pain, EC is often diagnosed at an early stage. However, this cancer, which is characterized by endometrial and lymphatic invasion, sometimes manifests as biologically aggressive mutations (55). Molecular alterations can induce cellular regulations, which play an important role in the development of EC (53).

Ellipticine, an alkaloid isolated from Apocyanaceae plants, has been shown to induce apoptosis in RL95-2 human EC cells via ROS formation. Ellipticine-induced apoptosis was found to be associated with the arrest of cells in the G2/M phase and accompanied by depolarization of the mitochondrial membrane potential, release of cytochrome c and apoptosis-inducing factor (AIF) from the mitochondrial membrane and activation of caspase. In this case, ROS accumulation was shown to activate the ERK and JNK pathway and finally to release AIF in the RL95-2 cells (56).

(−)-epigallocatechin-3-gallate (EGCG), the major polyphenol in green tea, has been shown to have anti-proliferative potential on human Ishikawa endometrial cancer cells. In this process, EGCG inhibited ERK and its downstream transcription factors fos and jun through marked enhancement of ROS and activation of p38 in Ishikawa cells. These results suggest that inhibition of ERK activation and induction of apoptosis through ROS generation and p38 activation may affect the pathway to inhibit

proliferation (57). Similar effects have been reported in the study using HEC-1A EC cells, in which ROS induced apoptosis and inhibition of cell growth (58).

The analysis of proteins under the ETV5-related proteome approach in the HEC-1A cell line reinforced the role of transcription factor in the regulation of metastatic and invasive tumor behavior in EC and showed a regulatory response to oxidative stress associated with endometrial invasion enhancement (59). Based on this research, it can be assumed that ROS production affects pathways involved in metastasis or invasion in EC cells.

4. Conclusion

Oxidative stress is known to relate to the pathogenesis of various malignant cancers, and this review specifically discussed the effects of biomolecules associated with ROS production in representative female cancers. We focused on ROS effects on apoptosis and cell proliferation in CC, OC, and EC.

First, we reviewed that the occurrence of ROS is crucial to the development of cancer (initiation, promotion, and progression) as shown in **Figure 1**. Initiation, the first step, was shown to maintain the homeostasis of ROS against imbalance of ROS and to regulate protein kinases, which have diverse cellular functions. Promotion, the second step, is related to DNA damage and induction of mutations appearing in cellular signaling pathway by ROS. Progression, the third step, appears to activate EMT-related genes and other intracellular signaling pathway markers by ROS.

According to a great deal of research data, the formation of ROS indicates various responses in gynecological cancers through activation of the signal transduction pathway as shown in **Table 1**. The current pathological evidence suggests that there is a correlation between the production of ROS and the progression of female cancers.

Currently, there is a need for further investigation to understand the biological and pathological features of ROS in female cancers because ROS-related effects are not uniform, and are instead associated with cancer progression and cancer cell death depending on the cancer types and ROS formation conditions. Therefore, understanding how the imbalance of ROS regulation affects the developmental tendency of cancer can help develop strategies that interfere with cancer development. Further information regarding this content will provide useful predictive factors and potential therapeutic targets for female cancer patients undergoing chemotherapy related to ROS intervention.

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Conflict of Interest Statement

The authors do not have any conflicts of interest to declare.

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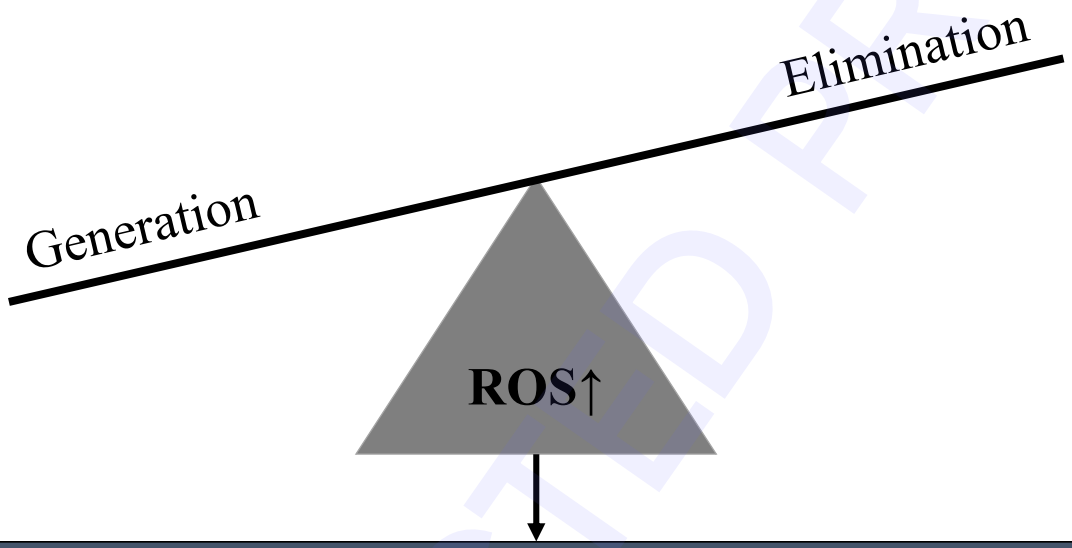
Table 1. Outcomes of various biomolecular pathways resulting from the production of ROS in gynecological cancers

Cancer	Biomolecular process related to ROS	Reference
Choriocarcinoma cancer	· Inhibition of cell proliferation via cell cycle arrest and mitochondrial dysfunction	(39-41)
	· EMT through controlling the cell cycle and migration ability	(43)
Ovarian cancer	· Apoptosis through inhibition of NADPH oxidase	(10)
Endometrial cancer	· Apoptosis via activation of the ERK and JNK pathways	(56, 58)
	· Inhibition of cell proliferation via activation of p38	(57)
	· Metastasis or invasion via EMT-related transcription factors	(59)

Figure Legend

Figure 1. ***Relationship between ROS production and cancer cell development.*** Initiation, the first step among three stages in cancer, maintains the homeostasis of ROS against imbalance of ROS and regulates protein kinases, which have diverse cellular functions. Promotion, the second step, is related to DNA damage and induction of mutations appearing in the cellular signaling pathway. Progression, the third step, appears to activate EMT-related genes and other intracellular signaling pathway markers. Therefore, the generation of ROS is crucial to the three developmental stages of cancer; initiation, promotion, and progression.



Cancer development		
Initiation	**Promotion**	**Progression**
Low level of ROS	High level of ROS	Persistent high level of ROS
Maintenance of cell signaling via homeostasis of ROS	DNA damage and mutations by ROS modification	Cellular proliferation due to promoting ability of ROS

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[Review]

Potential roles of reactive oxygen species derived from chemical substances ~~in the~~involved in cancer development ~~of~~ in the female reproductive system

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Abstract

~~The r~~Reactive oxygen species (ROS) are major sources of cellular oxidative stress. Specifically, cancer cells harbor genetic alterations that promote a continuous and elevated production of ROS. ~~Whereas~~ While such oxidative stress conditions ~~would~~ could be harmful to normal cells, they facilitate cancer cell growth in multiple ways by causing DNA damage and genomic instability, and ultimately, by reprogramming cancer cell metabolism. This review provides ~~the reader with~~ up to date findings ~~on~~ regarding the role of ROS generation induced by diverse biological molecules and chemicals in representative women's' ~~representative~~ cancer. Specifically, we describe the cellular signaling pathways that regulate direct or indirect interactions between ROS homeostasis and metabolism within female genital cancer cells.

1. Introduction

Oxidative stress is caused by an imbalance in reactive oxygen species (ROS). The regulation of ROS homeostasis plays a major role in cellular growth, metabolism, and survival (1). When ~~exists~~ present ~~in a~~at low levels, ROS is an important signaling molecule that can maintain cellular functions such as viability, migration and apoptosis (2). However, the excessive occurrence of ROS ~~leads a~~causes biological systems to incompletely ~~detoxify~~ detoxify the reactive intermediates and to block the normal functions of biomolecules (3). Both ROS and oxidative stress are considered to be ~~incriminated~~ involved ~~for~~ in aging (4), inflammation (5), and many diseases, including cancer because ROS ~~has~~ play a significant role in the post-transcriptional adjustment of genes and repercussion effects on ~~the~~ cellular development, differentiation, proliferation, and apoptosis and the development and progression of cancer (6, 7).

Furthermore, excessive ROS may affect women's diseases occurring in reproductive organs (8). ~~The e~~Endometriosis ~~was~~ has been ~~revealed~~ found to be correlated with pro- inflammatory mediators and ROS, which ~~that~~ can lead to cellular proliferation and activation of ERK1/2 (9). In addition, the toxic effects of ROS have been ~~demonstrated~~ shown to induce malignancy of ovarian cancer (OC) through reduced expression of antioxidant enzymes (10).

This review describes the adaptive mechanisms that cancer cells in women's reproductive organs take to face oxidative stress conditions. We will discuss the role of ROS induced by diverse biological molecules and chemicals in regulating the related signaling pathways ~~related~~ and consequential oxidative stress-mediated responses in female reproductive cancers occurring in ~~the~~ organs such as the placenta, ovary, and endometrium.

2. Relationship between ROS and cancer

Oxidative stress ~~is~~ has been reported to affect all phases of the oncogenic process including initiation, promotion, and progression (11, 12). Under the impact of ROS in the cancer development stage **(Figure 1)**, ROS ~~could~~ can induce altered expression of several transcription factors associated with regulating pathways such as genetic mutations, proliferation, suppression, differentiation, and senescence (8, 13).

~~The multifarious kinds of~~A variety of chemical substances and naturally occurring chemicals can mediate carcinogenesis, malignant behavior, and treatment response in cancer development ~~stage~~ via ~~the~~ regulation of ROS imbalance. For instance, several studies have shown that ~~the~~ cancer cells induce apoptosis or necrosis of damaged cells to maintain ~~the~~ homeostasis of ROS and ~~to~~ prevent ROS-induced toxicity. This process is thought to be the initiation of cancer development (13). ~~And when the~~Moreover, when the oxidative stress state of ~~the~~ cells persists from the imbalance of ROS, DNA damage and induction of mutations affect cellular signaling pathways (14, 15), leading to the activation of a variety of protein kinases that regulate diverse cellular functions including the cell cycle, survival, migration, angiogenesis, apoptosis and cell death (16). The infinite cell proliferation capacity of cancer cells has relevance to [ED highlight – please rephrase this, I am not sure of your intended meaning. Do you mean, "impacts"?] angiogenesis related mechanisms (17), which can induce cancer metastasis by promoting cancer progression-associated processes such as proliferation, migration, and tube formation (18).

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Generally, there are a variety of ways to treat cancer, including surgery, chemotherapy, radiation, immunotherapy, and other targeted therapies. Among them, chemotherapy and radiation therapy lead to the generation of ROS with strong toxicity

to cancer cells (19). ~~In detail,~~Specifically, it ~~was~~ has been reported that the production of ROS also affects mitogen-activated protein kinases (MAPK) (20) that act as a switch to block or transmit signals ~~due to~~because of the phosphorylation of surrounding proteins. Moreover, ~~Activation~~ activation of the MAPK pathway can result in apoptosis via death signals including the JNK pathway and caspase family (21). The cancer cells that continue to proliferate tend to induce DNA damage and to elicit cell cycle arrest (22, 23). Therefore, ~~the~~ excessive production of ROS can provoke cell cycle arrest, apoptosis, and senescence. Additionally, ROS are known to be correlated with malignant progression of cancer cells by increasing invasion and metastatic potentials via MAPK signals (13). ROS-stimulated growth factors and Ras (renin-angiotensin system) in cancer cells ~~have~~ play a role in inducing ~~the~~ activation of the MAPK pathway~~.~~, and the ~~Activated~~ activated Ras-MAPK pathway ~~was~~ has been shown ~~revealed~~ to lead to cell proliferation (24, 25) and extracellular matrix (ECM) alteration via the upregulation of specific matrix metalloproteinases (MMPs) (26). The upregulated MMP then leads to the invasion ~~process~~ via ~~by~~ degradation of ECM (27). Successively, the decomposed ECM collapses the layer structures and then enables cancer cells to migrate. Therefore, ROS has been shown to activate the MAPK pathway leading to cancer progression and dissemination (24, 28).

Most of the tumorigen~~s~~ic activities of ROS are associated with regulation of transcription factors such as activator protein 1 (AP-1), nuclear factor-κB (NF-κB), nuclear factor erythroid 2–related factor 2 (Nrf2), and hypoxia-inducible factor-1α (HIF-1α), as well as ~~and other factors,~~ intercellular adhesion protein-1 (ICAM-1) and p53 (29). ~~Besides~~Moreover, ~~, diverse indepth~~ diverse in-depth molecular regulatory mechanisms of ROS-induced cellular reactions under pathological conditions, including

cancer, have been reported in previous reviews (13, 30). In this review, we will focus on cellular modulations induced by chemicals or natural substances-induced formation of ROS in women's cancers.

3. Roles of ROS in female reproductive cancers

3-1. Choriocarcinoma

Human placenta related to pregnancy can develop and lead to specialized fetal trophoblasts; therefore, ~~they~~ it plays an important role in implantation and development of the maternal-fetal interface (31, 32). Choriocarcinoma (CC) is a rare cancer occurring in the trophoblastic cells and cytotrophoblast, which form ~~the~~ a chronic membrane of the placenta (33). The form of CC is known ~~as~~ to be a malignant trophoblastic tumor that quickly spreads to the organs from the uterus. The metastasis of CC occurs via hematogenous routes to the liver, brain, etc., although the most common site is the lungs (34). A case of metastasis CC manifested in a pregnancy period (35). [ED highlight – this does not flow well here, consider deleting this text or add more text to tie it to the rest of the paragraph.] Commonly, chemotherapy has a significant curative influence on CC. However, drugs generally used in cancer therapy have barriers such as drug resistance and side effects. Therefore, ~~the~~ patients with refractory gestational CC ~~are~~ do not have an optimistic outlook when being treated with ~~with~~ chemotherapy (36). Chemotherapy and radiation therapy to remove cancer cells usually increase intracellular ROS ~~as well as~~and damage many other biomolecules (37, 38). ~~Also~~Moreover, the cellular ROS concentrations may have been implicated in the selective activation of transcription factors, and either cell death or cell proliferation may result from exposure to oxidative stress (39). ~~In this part~~Here, we ~~will describe~~provide representative examples that show cellular adjustment to oxidative stress induced by chemical agents in CC.

~~A study by Marjo~~ Huovinen *et al.* evaluated the effects of diuron as an endocrine disruptor, which produced adverse development and reproductive effects in BeWo cells

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(a human CC model). ~~The~~ Moreover, diuron ~~has~~ appeared to produce ROS and to inhibit cell proliferation of BeWo cells~~,~~ because the protein expression of p53 as a biomarker for cell stress and p21 as a cell cycle arrest gene were increased by oxidative stress (40, 41). A study by Ham et al. ~~identified~~ revealed that the effects of silibinin, a flavonolignan with anti-cancer effects extracted from seeds of milk thistles, significantly inhibited proliferation and induced apoptosis in both JAR and JEG3 CC cells by increasing ROS production and lipid peroxidation. ~~In addition~~Moreover, silibinin interrupted mitochondrial function by inducing mitochondrial membrane potential and permeabilization of calcium ion efflux in these cancer models (42).

As another phytochemical, coumestrol was ~~identified~~ shown to induce cell death by regulating ERK1/2 MAPK and JNK MAPK signaling pathways and through disruption of Ca^{2+} and ROS homeostasis. ~~In detail~~Specifically, coumestrol suppressed proliferation and increased apoptosis in JAR and JEG3 cells by inducing the pro-apoptotic proteins, Bax and Bak, via ROS production ~~as well as~~and lipid peroxidation. ~~Additionally, c~~Coumestrol also induced depolarization of MMP and increased cytosolic and mitochondrial Ca^{2+} levels in JAR and JEG3 cells, leading to apoptosis of CC cells ~~by~~ through ~~regulating~~ regulation of cell signaling and mitochondrial-mediated functions with a potential to impair progression of the cancer (43). ~~Similarly, a~~A similar study evaluated the effects of chrysophanol, an anthraquinone compound, on JAR and JEG-3 cells. These results showed ~~that~~ that ~~chrysophanol~~ chrysophanol decreased cell viability and ~~induced~~ induced apoptosis, while ~~and increased~~ increasing oxidative stress in JEG-3 cells by ~~inducing~~ inducing ~~ROS~~ ROS generation followed by mitochondrial dysfunction, including depolarization of the mitochondrial inner membrane potential. In this experiment, ~~it was confirmed that~~the ERK1~~-~~/~~-~~2 and AKT signaling pathways were

significantly activated in JEG-3 cells by ROS (43).

In another study, benzo(a)pyrene decreased ~~the~~ cell viability and induced cell cycle arrest by increasing the ROS level in CC cells. The increased ROS levels enabled ~~to induce~~induction of apoptosis and simultaneous~~ly to activate~~ activation of endoplasmic reticulum (ER) stress (44). These studies confirm that ROS induced by diverse stimuli play~~s~~ an important role in the induction of apoptosis in CC cells.

~~On the other hand~~However, ~~other~~ another study showed that formaldehyde and benzene increased the proliferation and ~~the~~ migration of JEG-3 cells and epithelial mesenchymal transition (EMT) ~~in the process of~~during ROS production (44). In this case, the increased level of ROS promoted the cancer progression of CC, unlike ~~the results of~~in previous studies. ~~Therefore, it is~~Taken together, these findings suggest~~ed~~ that ROS-related mechanisms in CC are associated with cancer progression as well as cell death of CC.

3-2. Ovarian cancer

~~OC~~ Ovarian cancer is the fourth most common cause of cancer death~~s~~ in gynecological malignancies (45) and ~~is~~ the second most diagnosed cancer among gynecologic malignant tumors (46). Ovarian cancer ~~OC is developed~~develops ~~by~~ through the formation of a neoplasm in tissues of an ovary, and epithelial ovarian cancer is a major type of cancer (47, 48). ~~Almost~~ Most advanced stage cancers ~~are origin of~~originate from epithelial cells, although some ~~and may~~ originate from serous, mucinous, or endometrioid cells into the surface epithelium of the ovary or fallopian tube (49). The representative metastatic sites of OC are the endometrium, breast, colon, and stomach, and OC migrates through the body's blood stream and lymph fluid (50). Most cases of OC are difficult to treat because they are diagnosed at highly advanced stages (46, 51, 52). The advanced stage of OC is closely associated with ~~the~~ high levels of ROS, which produce~~s~~ a large amount of hydrogen peroxide, ~~whereupon~~ whereas oxidative stress is induced in the oxidizing environment of the tumor (53).

~~Jiang~~ Zet *et al.* previously sought to determine the effects of inhibiting ~~the~~ intracellular ROS generation ~~on~~ in epithelial ovarian cancer (EOC) cells. As a result, treatment with diphenyleneiodonium (DPI), a ROS inhibitor, ~~treatment~~ significantly induced apoptosis in EOC cells by increasing caspase-3 activity. ~~Additionally~~Moreover, DPI treatment resulted in reduced NADPH oxidase, SOD3 and HIF-1α levels in EOC cells (10), indicating that lowering oxidative stress, possibly through the inhibition of NADPH oxidase, induces apoptosis in OC cells.

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3-3.Endometrial cancer

Endometrial cancer (EC) is a representative malignant gynecologic carcinoma ~~and~~ that is the most common cancer in women ~~occurs most commonly~~ except for breast cancer ~~in women~~ (54).[ED highlight – please ensure that my changes here are correct.] The ~~most~~ majority of endometrial malignancies (95%) occur in endometrial glands and are ~~called~~ known as endometrial cancers. The remaining 5% occur~~s~~ in mesenchymal tumors and ~~is~~ are ~~called~~ known as carcinosarcoma (mixed epithelium~~-~~/~~-~~epilepsy tumor) (55).

~~Due to early~~Because of early symptoms such as abnormal uterine bleeding or pelvic pain ~~in the pelvic area~~, EC is often diagnosed at an early stage. ~~But~~However, this cancer, which is characterized by endometrial and lymphatic invasion, ~~is~~ sometimes ~~manifested~~ manifests as biologically aggressive mutations (56). Molecular alterations can induce cellular regulations, which play an important role in the development of EC (54).

Ellipticine, an alkaloid isolated from Apocyanaceae plants, has been shown to induce apoptosis in RL95-2 human EC cells via ROS formation. Ellipticine-induced apoptosis was found to be associated with the arrest of cells in the G2/M phase and ~~was~~ accompanied by depolarization of the mitochondrial membrane potential, release of cytochrome c and apoptosis-inducing factor (AIF) from the mitochondrial membrane and activation of caspase. In this case, ROS accumulation was shown to activate the ERK and JNK pathway and finally to release AIF in the RL95-2 cells (57).

(−)-~~Epigallocatechin~~epigallocatechin-3-gallate (EGCG), the major polyphenol in green tea, ~~revealed~~ has been shown to have ~~the~~ anti-proliferative potential on human Ishikawa endometrial cancer cells. In this process, EGCG inhibited ERK and its

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downstream transcription factors fos and jun ~~with~~ through ~~a~~ marked enhancement of ROS and activation of p38 in Ishikawa cells. These results suggest that inhibition of ERK activation and induction of apoptosis through ROS generation and p38 activation may affect the pathway ~~for inhibiting cell~~to inhibit proliferation (58). Similar effects have been reported in the study using HEC-1A EC cells, in which ROS induced apoptosis and ~~the~~ inhibition of cell growth (59).

The analysis of proteins under the ETV5-related proteome approach in the HEC-1A cell line reinforced ~~a~~ the role of transcription factor in the regulation of metastatic and invasive tumor behavior in EC and showed a regulatory response to oxidative stress associated with endometrial invasion enhancement (60). Based on this research, it can be assumed that ROS production affects pathways involved in metastasis or invasion in EC cells.

4. Conclusion

Oxidative stress is known to relate to the pathogenesis of various malignant cancers, and this review specifically discussed ~~on~~ the effects of biomolecules associated with ROS production in representative female cancers. ~~Especially, w~~We focused on ROS effects on apoptosis and cell proliferation in CC, OC, and EC.

First, we reviewed that the occurrence of ROS is crucial to the development of cancer (initiation, promotion, and progression) as shown in **Figure 1**. Initiation, the first step, was shown to maintain the homeostasis of ROS against ~~from the~~ imbalance of ROS and to regulate protein kinases, which have diverse cellular functions. Promotion, the second step, is related to DNA damage and induction of mutations appearing in cellular signaling pathway by ROS. Progression, the third step, appears to activate EMT-related genes and other intracellular signaling pathway markers by ROS.

According to ~~many~~ a great deal of research data, the formation of ROS indicates various responses in gynecological cancers through activation of the signal transduction pathway as shown in **Table 1**. The current pathological evidence suggests that there is a correlation between the production of ROS and the progression of female cancers.

Currently, ~~we~~ there is a need for further investigation to understand the biological and pathological features of ROS ~~on~~ in female cancers because ROS-related effects are not uniform, ~~being~~ and are instead associated with cancer progression ~~as well as~~and cancer cell death depending on the cancer types and ROS formation conditions. Therefore, understanding how the imbalance of ROS regulation affects the developmental tendency of cancer can help ~~to~~ develop strategies that interfere with cancer development. Further information ~~on~~ regarding this content will provide useful predictive factors and potential therapeutic targets for female cancer patients undergoing

chemotherapy related ~~with~~ to ROS intervention.

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Conflict of Interest Statement

The authors do not have any conflicts of interest to declare.

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Table 1. Outcomes of various biomolecular pathways resulting from the production of ROS in gynecological cancers

Cancer	Biomolecular process related to ROS	Reference
Choriocarcinoma cancer	· Inhibition of cell proliferation via cell cycle arrest and mitochondrial dysfunction	(40-42)
	· EMT through controlling the cell cycle and migration ability	(44)
Ovarian cancer	· Apoptosis through ~~the~~ inhibition of NADPH oxidase	(10)
Endometrial cancer	· Apoptosis via activation of the ERK and JNK pathways	(57, 59)
	· Inhibition of cell proliferation via activation of p38	(58)
	· Metastasis or invasion via EMT-related transcription factors	(60)

Figure Legend

Figure 1. ***Relationship between ROS production and cancer cell development.*** Initiation, the first step among three stages in cancer, maintains the homeostasis of ROS against ~~from the~~ imbalance of ROS and regulates protein kinases, which have diverse cellular functions. Promotion, the second step, is related to DNA damage and induction of mutations appearing in the cellular signaling pathway. Progression, the third step, appears to activate EMT-related genes and other intracellular signaling pathway markers. Therefore, the generation of ROS is crucial to the three developmental stages of cancer; initiation, promotion, and progression.