PERIODICUM BIOLOGORUM
VOL. 126, No 3–4, 133–143, 2024
DOI: 10.18054/pb.v126i3-4.35608

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Review article

Targeting ovarian cancer using high-dose vitamin C therapy

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Keywords: ovarian cancer; therapy; vitamin C; pharmacological ascorbate; oxidative stress; reactive oxygen species

Abbreviations

αKG – α-ketoglutarate
αKGDDs – αKG-dependent dioxygenases
ADP – adenosine diphosphate
BER – base-excision repair
CRC – colorectal cancer
DDR – DNA damage repair
DHA – dehydroascorbic acid
DSB – double strand break
FIH-1 – factor-inhibiting HIF
GAPDH – glyceraldehyde 3-phosphate dehydrogenase
GLUT – glucose transporter
HGSOC – high-grade serous ovarian carcinoma
HR – homologous recombination
HRD – homologous recombination deficiency
HRR – homologous recombination repair
JHDM – Jumonji-C domain-containing histone demethylase
LGSC – low-grade serous carcinoma
MDR1 – multidrug resistance protein 1
NHEJ – non-homologous end joining
OS – overall survival
PARP – poly (ADP-ribose) polymerase
PARPi – poly (ADP-ribose) polymerase inhibitor
PD-L1 – programmed cell death ligand 1
PHD1-3 – proline hydroxylase domain proteins 1-3
PROC – platinum-resistant ovarian cancers
ROS – reactive oxygen species
SOD – superoxide dismutase
SSB – single strand break
TET – ten-eleven translocation
VEGF – vascular endothelial growth factor
VHL – von Hippel-Lindau

Received March 17, 2025
Revised April 1, 2025
Accepted April 2, 2025

Abstract

Ovarian cancer is one of the most common and deadliest tumors among women. Despite recent clinical advances, the combination of platinum-based chemotherapy and surgery remains the first-line therapeutic option. Due to frequent detection at an advanced stage and development of therapy resistance, the disease prognosis is often poor, and mortality rates remain high. Therefore, it is necessary to investigate new therapeutic approaches that would contribute to increased survival and higher life quality of ovarian cancer patients. One of these approaches is the use of the pro-oxidant effect of certain drugs that can disrupt the redox balance in tumor cells and lead to their death. Vitamin C (ascorbic acid or ascorbate) used in high concentrations suppresses cancer cell growth and metastasis. There are several mechanisms by which vitamin C exerts its antitumor function including pro-oxidant activity, epigenetic reprogramming and tumor environment oxygen sensing. The potential use of vitamin C in combinatorial therapies (as a support to standard therapies) should be reconsidered through more detailed mechanistic and clinical studies. Finding new therapeutic approaches would augment the available mechanisms in the fight against cancer leading to the increase in patient welfare and overall survival of cancer patients.

INTRODUCTION

Ovarian cancer is one of the most severe malignancies in women and the deadliest among the gynecological tumors. In contrast to some other cancer types, 5-year survival rates for ovarian cancer patients still have not improved significantly, remaining below 30 % *(1)*. Main factors

that contribute to high mortality of ovarian cancer are lack of effective early detection strategies, usual detection at an advanced stage, tumor heterogeneity and development of resistance to therapy *(2)*. Most ovarian cancers originate from epithelial cells; however, they show high heterogeneity regarding the histological and molecular characteristics. Among ovarian cancers the most frequent and lethal is the high-grade serous ovarian carcinoma (HGSOC) causing the death outcome in more than 70 % of women with this diagnosis *(3)*.

First-line therapy for advanced ovarian cancer comprises cytoreductive surgery and adjuvant platinum- and taxane-based chemotherapy (e.g., carboplatin/paclitaxel) *(4)*. As HGSOCs often show deficiency in DNA damage repair (DDR) mainly due to *BRCA1*, *BRCA2* and *RAD51* gene alterations, those tumors are usually sensitive to initial chemotherapy *(5)*. However, very often, the tumors relapse after certain period post-therapy showing usually even more aggressive and resistant phenotype. In spite of emerging new therapies, like poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi), antiangiogenic drugs and immune checkpoint inhibitors, the improvement of the overall survival (OS) of ovarian cancer patients is still modest and unsatisfactory. Therefore, the investigation and potential implementation of additional therapeutic approaches that could improve patient welfare and disease outcome is of major healthcare interest.

Reactive oxygen species (ROS) are continuously generated through aerobic metabolism in eukaryotic cells and play important roles in different cellular processes *(6)*. Tumor cells produce higher levels of ROS due to their intensive metabolism, genomic instability and hypoxic environment, and in consequence they adopted more pronounced antioxidant mechanisms *(7)*. As exposure to higher levels of ROS induces excessive damage that can trigger cell death, pro-oxidant effect of different compounds is intensively investigated as potential anticancer therapy.

Vitamin C (ascorbic acid or ascorbate) was shown to exhibit antitumor properties when used in high pharmacological doses. In addition to impairing redox balance, vitamin C also targets epigenetic reprogramming and oxygen-sensing regulation in cancer cells *(8)*. In this review, we will summarize known mechanisms by which vitamin C exerts antitumor activity and discuss the potential of its implementation in combinatorial therapeutic approaches that could improve response of ovarian cancer patients to therapy.

HISTOLOGICAL AND MOLECULAR HETEROGENEITY OF EPITHELIAL OVARIAN CANCER

More than 85 % of ovarian cancer cases are of epithelial origin, however, epithelial ovarian cancer exhibits high heterogeneity based on histological properties, risk factors and response to therapy *(4, 9)*. Due to the existence of multiple histological subtypes, it was necessary to establish a more simplified classification also considering recent findings obtained by the molecular profiling of ovarian cancer. According to recent classification, epithelial ovarian cancers are divided into two groups: type I comprising endometrioid, mucinous, clear cell and low-grade serous carcinoma (LGSC), and type II comprising high-grade serous ovarian carcinoma *(9)*. Type I ovarian cancers generally have less severe clinical outcomes, whereas the type II tumors show more aggressive phenotypes and have worse prognosis. Type II tumors are considered to originate from the fallopian tube, which could be one of the reasons of its usual detection at an advanced stage *(10)*.

Most of the cancer subtypes from the type I group have activating mutations in *KRAS* or *BRAF* genes (except clear-cell carcinoma). Mutations in genes *PIK3CA*, *PTEN*, *CTNNB1*, and *ARID1A* are also frequent, whereas the pivotal tumor suppressor gene *TP53* is rarely mutated in type I cancers (not referring to the mucinous) *(11–15)*. In contrast, almost all tumors of type II group harbor *TP53* mutations. HGSOCs in general show higher genomic instability compared to type I tumors, including high frequency of germline and somatic mutations in *BRCA1* and *BRCA2* genes involved in homologous recombination (HR) and in general high frequency of copy number alterations in numerous genes *(5, 16)*. In addition to high heterogeneity between ovarian cancers from different patients, the data obtained by next-generation sequencing also revealed high intra- and inter-tumor diversity in the same patient. Latter are consequences of subclonal tumor evolution and response to specific anticancer therapy.

CURRENT THERAPEUTIC APPROACHES FOR OVARIAN CANCER

In spite of novel targeted tumor therapies and immunotherapies, combination of surgery and standard chemotherapy still remains first choice treatment for ovarian cancer *(1, 4, 17)*. Recent ICON8 clinical study revealed better progression-free survival (PFS) and OS for upfront cytoreductive surgery and subsequent chemotherapy compared to neoadjuvant chemotherapy *(18)*. Standard chemotherapy comprises carboplatin and paclitaxel administered intravenously. Most of the ovarian cancers initially respond efficiently to chemotherapy resulting in tumor remission. Disease recurrence occurs in around 75 % of advanced ovarian cancer and it is the main cause of cancer-related deaths. Platinum-sensitive tumors recur after 6 or more months post-therapy, whereas platinum-resistant ovarian cancers (PROCs) usually progress within 6 months of chemotherapy *(19)*. In such cases, extensive secondary cytoreductive surgery and further chemotherapy are considered as therapeutic options. However,

the efficacy and benefits of cytoreductive surgery in the context of PROC are still being investigated *(20)*. Lately, intraperitoneal administration of platinum-based chemotherapy has been shown as beneficial and more efficient than intravenous chemotherapy for advanced ovarian cancer, particularly for large residual tumors *(21, 22)*.

Initial sensitivity to chemotherapy in around half of the HGSOC cases can be mainly explained by homologous recombination deficiency (HRD) as a result of genetic or epigenetic changes in genes that are important for this process. As already mentioned, the most frequent among them are germline and somatic mutations in *BRCA1* and *BRCA2* tumor suppressors *(23)*. Moreover, germline mutations in *BRCA1* or *BRCA2* genes increase the risk of developing certain types of cancer, including ovarian, as they contribute to genomic instability. BRCA2 protein has important role in DNA repair as it binds to and regulates RAD51, which is crucial factor in HR *(24)*. Additional modifications that result in HRD are epigenetic silencing of *BRCA1* and *RAD51C*, as well as modification of *BRCA1*, *BRCA2* and *RAD51* levels by increased expression of specific microRNAs *(19)*. The existence of *BRCA1/2* mutations in ovarian cancer can be considered as prognostic factor for the initial response to platinum-based therapy. Although *BRCA1/2*-deficient tumors show initial sensitivity to platinum compounds, eventually those tumors develop resistance to therapy. One of the mechanisms of the acquired therapy resistance are secondary intragenic mutations in *BRCA2* that result in rescuing its function in DNA damage repair, making the tumors resistant to therapy-induced DNA damage *(25)*. In addition, acquired resistance to chemotherapy in HGSOC is accompanied by inactivation of the *RB1*, *NF1*, *RAD51B* and *PTEN* tumor suppressor genes, and amplification of the *CCNE1* gene, a positive regulator of the cell cycle. In part of patients with the recurring disease increased expression of the *ABCB1* gene, which encodes for the multidrug resistance protein 1 (MDR1) was found *(26)*.

The implementation of PARPi in patients with tumors that have already deficient DDR makes these tumors more sensitive to DNA damage, being an example of synthetic lethality. The usage of PARPi in parallel with platinum-based chemotherapy leads to stronger synergistic response *(27)*. The mechanism of sensitivity of HR-deficient tumors to PARPi is still not completely elucidated. The initial model proposed that PARP inhibitors caused impairment of base-excision repair (BER) leading to accumulation of single strand breaks (SSBs) that are subsequently converted to replication-related double strand breaks (DSBs). This model has been challenged and complemented by the following discoveries: presence of the trapped PARP-1 on the SSB intermediate during BER, the role of PARP at stalled replication forks, inhibition of PARP-1-POLθ-mediated alternative-end-joining and hyperactivation of PARP in HR-defective cells *(23, 28)*. First PARPi therapy approved for ovarian cancer was olaparib in 2014, and since then several other inhibitors have been registered for the treatment of recurring ovarian cancer such as rucaparib and niraparib *(29)*. However, the development of resistance to PARPi occurs frequently. The mechanisms contributing to resistance to PARPi are mostly related to restoration of the functional homologous recombination repair (HRR) as a result of somatic restoration of the mutated *BRCA1*, *BRCA2* and *RAD51* or reversion of *BRCA1* and *RAD51* hypermethylation *(30–33)*. Novel therapeutic approaches should focus on inhibition of HRR to induce "BRCAness" in tumors with acquired resistance and those that gained resistance by restoration of the functional HRR.

Another targeted therapy approved for the treatment of advanced ovarian cancer previously treated by chemotherapy is antiangiogenic agent bevacizumab which is an anti-vascular endothelial growth factor (VEGF) antibody *(34)*. The heterogeneity of ovarian cancer reflects also on the response of different tumor types to immunotherapies. Platinum-sensitive and platinum-resistant recurrent ovarian cancer exhibit different immune microenvironment characteristics which results in different potential to respond effectively to immunotherapies. Although the platinum-sensitive cancers have more potential to benefit from immunotherapies due to higher mutational burden and higher expression of programmed cell death ligand 1 (PD-L1), combinations of different immunotherapies and other therapeutic approaches could contribute to more effective ovarian cancer treatment *(35–37)*.

Alterations in MAPK pathway are frequent in LGSCs and endometrioid ovarian cancers, which opens the possibility of implementation of the targeted MAPK inhibitor therapies. The most frequent mutations that impact the activity of the MAPK pathway in LGSC are those in *KRAS*, *NRAS*, *BRAF* and *NF1* genes. *KRAS*-mutated LGSCs show good response to MEK-inhibitor therapy *(38, 39)*. Mutations in the genes that are part of the PI3K/Akt/mTOR pathway (e.g., *PTEN*, *PIK3CA*) have been found in both type I and II ovarian cancer, therefore, therapies that target specific components of this pathway have been considered as a potential therapy for ovarian cancer *(12, 40)*.

As already mentioned, almost all type II ovarian cancer cases harbor mutations in the *TP53* gene which is important regulator of various pathways involved in cellular response to therapy, such as cell cycle arrest, apoptosis, senescence and DNA repair *(41)*. Several therapeutic approaches aim to utilize the p53 status in ovarian cancer. Cells with mutant p53 have inefficient G1 cell cycle arrest after DNA damage, therefore, additional impairment of G2 arrest (e.g., by using Wee1 inhibitors), will make them more prone to cell death in consequence of chemotherapy *(42, 43)*. Another potential approach could be reactivation of p53 or removal of the mutated p53 by, for example,

inhibition of the Hsp90, which normally prevents its degradation *(44)*.

To conclude, although several molecular pathways have been thoroughly investigated and implemented as targets for therapies against advanced ovarian cancer, unsatisfactory outcomes still urge for opening novel potential therapeutic approaches.

TARGETING REDOX HOMEOSTASIS AS ANTICANCER THERAPY

During the aerobic metabolism ROS are produced including superoxide, hydroxyl radicals and hydrogen peroxide. Balance between oxidized and reduced cellular states (redox homeostasis) is maintained by oxidant and antioxidant processes. Cancer cells produce higher levels of ROS than normal cells due to their stronger metabolism and defective mitochondria *(7)*. In consequence, cancer cells have adapted to conditions of increased ROS by developing higher antioxidant capacity than normal cells. In spite that, due to extremely high ROS production, cancer cells are being close to their oxidative stress limit (Figure 1).

Endogenous ROS are mainly produced during oxidative phosphorylation in mitochondrial electron transport chain complexes 1 and III and NADPH oxidase complex *(45, 46)*. First, oxygen is reduced into superoxide, which is then converted to hydrogen peroxide by superoxide dismutase (SOD) in the intermembrane or mitochondrial matrix. Ultimately, hydrogen peroxide can be transformed into hydroxyl radicals in the presence of Fe^{2+} ions. In case of exogenously induced ROS, the cellular redox homeostasis can be perturbed leading to excessive cellular damage and possibly cell death (Figure 1). The role of oxidative stress in tumor progression is dual, depending on the level of the stress and tumor stage *(47)*. It was found that increased ROS promotes tumorigenesis through stimulation of cellular proliferation and genetic instability. Therefore, antioxidant agents have been examined as potential anticancer therapies. However, recent studies have not provided strong evidence of the efficacy of antioxidants in cancer prevention and suppression. In contrast, in some cancer types antioxidant treatment even promoted cancer progression *(48)*. On the other side, the potential of pro-oxidant agents came into focus as a possible therapeutic option by using specific vulnerability of redox imbalance in cancer cells. Oxidative stress-inducing

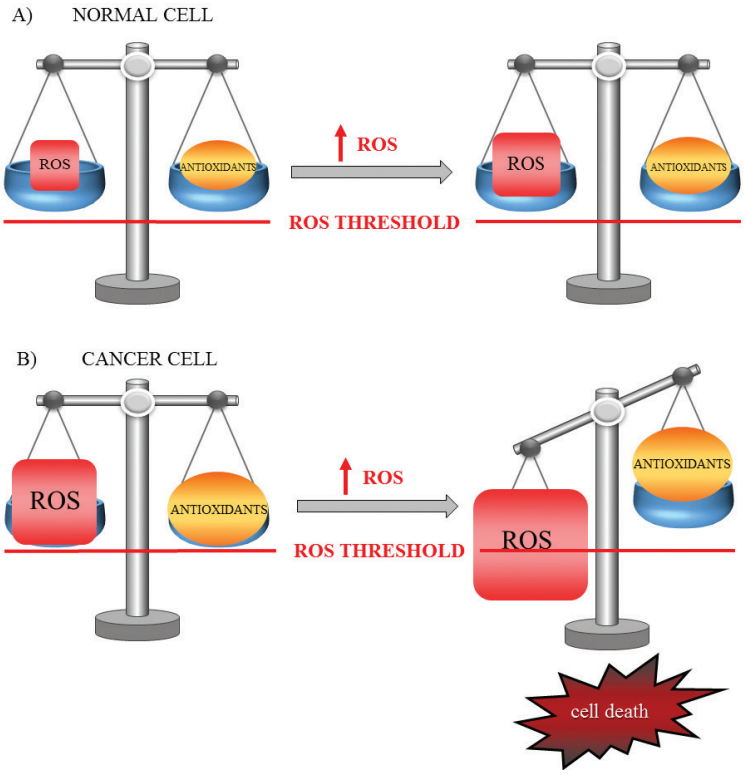

Figure 1. *Redox homeostasis and the effect of external pro-oxidant stimuli in normal and cancer cells. (**A**) In normal cells reactive oxygen species (ROS) are normally produced as byproducts of oxidative metabolism. Redox homeostasis is maintained by various antioxidant pathways, also under external stimuli resulting in increased ROS. (**B**) Cancer cells regularly produce higher level of ROS compared to normal cells due to intensive metabolism and mitochondrial and genomic defects. In spite of strong antioxidant activity, cancer cells are challenged with their almost maximal tolerable ROS levels. In case of exposure to higher levels of exogenously-induced ROS, the redox balance in cancer cells can be perturbed and cells are being pushed beyond their oxidative stress limit leading to excessive cellular damage and death.*

cancer therapies, such as radiation and certain chemotherapies further increase ROS accumulation in cancer cells impairing redox homeostasis and causing excessive cellular damage that can subsequently lead to cell death *(47, 49)*.

Recent studies suggest that oxidative stress could have an important role at different stages of ovarian cancer development. ROS can promote proliferation and metastasis of ovarian cancer having pro-oncogenic activities, whereas higher level of oxidative stress can have anti-oncogenic activity by promoting cancer cell death in consequence of excessive cellular damage *(50, 51)*. Anti-tumor chemotherapy and radiotherapy are shown to increase the level of oxidative stress, and their pro-oxidative activity is used for cancer treatment by induction of excessive ROS generation leading to ROS-induced cell death *(52)*. As current pro-oxidant agents used as anticancer therapy regularly have serious detrimental side effects, it would be of clinical benefit to explore additional less toxic pro-oxidants that could circumvent this problem.

BIOLOGICAL ACTIVITY OF VITAMIN C AND ITS USE AS POTENTIAL ANTICANCER THERAPY

Vitamin C, also known as ascorbate, is a natural compound that shows antioxidant properties crucial in defending normal cells from oxidative stress. Due to innate inactivation of *GULO* gene that encodes L-gulonolactone oxidase, an enzyme essential for vitamin C synthesis, primates and therefore humans need to supply vitamin C exogenously from diet. For humans, the recommended daily consumption ranges from 75-90 mg yielding plasma concentration of 30-80 μM. However, plasma levels under 10 μM can be an indication of reduced dietary intake of vitamin C or even malnutrition which can cause scurvy, the disease associated with vitamin C deficiency that leads to different serious symptoms which if left untreated can result in death *(53)*. Although vitamin C deficiency is uncommon in general population, it is frequently observed in patients with advanced cancer *(54)*. Interestingly, if plasma levels reach millimolar range, vitamin C can potentially be used as a strategy to fight cancer *(8)*.

The use of vitamin C as an anticancer therapy has a controversial history because of conflicting results of early clinical trials which were carried out around 50 years ago. In 1970s, several clinical studies and case reports described the influence of vitamin C on cancer patients when administered both intravenously and then orally. High doses of vitamin C led to tumor growth inhibition and cell proliferation impeding metastasis. In two clinical studies that enrolled mostly terminal cancer patients, Cameron and Pauling reported prolonged survival of ascorbate-treated patients compared to controls *(8, 55)*. Prolonged survival was also detected in independent clinical trial study reported few years later in Japan *(56)*. However, survival benefit was not detected in patients enrolled in the clinical study that was supervised by Creagan and Moertel at the Mayo Clinic which cast doubt on the antitumor effects of high-dose vitamin C *(57, 58)*. Although none of the studies measured plasma levels of vitamin C, the conflicting study results can potentially be explained by differences in pharmacokinetics that depends on the route of vitamin C administration *(59)*. The important property of vitamin C is that it acts as an electron donor, which defines its biological activities. When applied orally, as physiological ascorbate, vitamin C reaches peak plasma concentration in micromolar range while when applied intravenously, vitamin C can reach pharmacological concentrations that yield peak plasma concentrations in millimolar range. The anticancer capacity of vitamin C can be achieved by intravenous administration of vitamin C, known as pharmacological ascorbate, which exhibits pro-oxidant activity. Currently, several active clinical trials are using pharmacological ascorbate as anticancer therapy mostly applied as adjunctive therapy to chemo- or radiotherapy for treating different cancer types, such as pancreatic and lung cancer, glioblastoma, sarcoma, leukemia and lymphoma *(60–66)*. Understanding the molecular mechanisms of vitamin C anticancer activity is crucial for the identification of predictive biomarkers for the purpose of stratifying patients who will benefit the most from the use of pharmacological ascorbate, e.g., by prolonging their survival and causing cancer remission.

THE MECHANISMS OF ANTICANCER ACTIVITY OF VITAMIN C

Mechanisms related to sensitivity of cancer cells to vitamin C, while in the same time normal/healthy cells remain resistant to vitamin C, are still not completely understood. Since vitamin C can influence various cellular processes, there is a possibility that the activity of vitamin C depends on specific characteristics of cancer cell types. It is proposed that in high-doses vitamin C can target three main characteristics, i.e., redox imbalance, epigenetic regulators and HIF1 signaling thereby influencing cancer metabolism *(8, 67)*.

In the presence of intracellular ROS, vitamin C acts as an electron donor and shifts between different oxidation states that include ascorbate, a reduced state, and dehydroascorbic acid (DHA), an oxidized state *(68–71)*. The switch between ascorbate and DHA is facilitated by ferric ion, Fe^{3+} that is converted to ferrous iron, Fe^{2+} which in the presence of oxygen forms superoxide radical *(68)*. In the presence of superoxide dismutase (SOD), superoxide radical is converted to O_2 and H_2O_2 where the latter reacts with Fe^{2+} and forms reactive hydroxyl radical which is extremely dangerous and toxic to cells *(68)*. These Fen-

ton reactions are maintained by the switch of ascorbate to DHA that facilitates recycling of Fe^{3+} to Fe^{2+} eventually generating more ROS in the cells *(8, 68)*. Due to structural resemblance to glucose, DHA competes with glucose for uptake via glucose transporters (GLUTs) *(70, 72)* which have increased expression in highly glycolytic cancer cells *(73)*. Once inside the cells, DHA can switch to ascorbate and cause decrease in intracellular antioxidants by depleting the reduced glutathione (GSH) and NADPH ultimately generating oxidative stress *(70)*. Consequently, elevated intracellular ROS levels inhibit GAPDH (glyceraldehyde 3-phosphate dehydrogenase) causing energy crisis in very glycolytic cancer cells. Increased ROS levels also induce genotoxic stress by generating DNA damage that results in the activation of PARP1 which in turn consumes intracellular NAD^+ levels that are crucial for GAPDH activity ultimately leading to cancer cell death *(74)*. These effects are not only attributed to KRAS- or BRAF-mutated colorectal cancer (CRC) cells, since gastric cancer cells that exhibits high GLUT1 expression and are dependent on glycolysis have shown to be sensitive to high-dose vitamin C treatment *(75)*. Therefore, cancer cells with high GLUT1 expression are prone to oxidative stress upon high-dose ascorbate treatment.

The anticancer activity of vitamin C is also attributed to its function as cofactor for many enzymes such as Fe^{2+}-containing and α-ketoglutarate (αKG)-dependent dioxygenases (αKGDDs) *(68)*. By functioning as cofactor, vitamin C can bind to the catalytic domains of TET (ten-eleven translocation) enzymes and positively regulate enzyme activity thus impacting DNA methylation *(76)*. As an electron donor to Fe^{3+}, vitamin C can generate Fe^{2+} which is necessary for TET enzyme activity. TET enzymes belong to αKGDDs that through multiple oxidation reactions catalyze cytosine demethylation by first converting 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) which is further oxidized to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). Afterwards, both 5fC and 5caC are efficiently recognized and removed by thymine DNA glycosylase (TGD), the enzyme involved in BER machinery *(77)*. Cancer aggressiveness and increased metastatic potential of numerous cancers were shown to be associated with dysregulation of TET enzymes. Frequently, TET loss of function occurs either due to genetic mutations or epigenetic dysregulation which is recognized in hematological malignancies or different solid tumors *(78–80)*. Consequently, DNA hypermethylation of numerous tumor suppressor genes is detected. Interestingly, high-dose ascorbate treatment can increase TET activity, cause DNA demethylation and reactivation of tumor suppressor genes as well as enhance chemosensitivity in lymphoma cells *(81)*. In addition, ascorbate treatment enhances T-cell recruitment, promotes antigen presentation in TET-dependent manner and immunotherapy response of melanoma cells *(82, 83)*. Vitamin C can also remodel epigenetic reprograming by regulating Jumonji-C domain-containing histone demethylases (JHDMs) which belong to αKGDDs and catalyze histone demethylation by removing methyl groups from lysines in histones. Vitamin C is necessary for the optimal activity and demethylation capacity of diverse JHDMs thereby regulates chromatin state and gene expression *(84)*. Next to TET and JHDMs, HIF hydroxylases also belong to αKGDDs whose activity is also positively regulated via vitamin C *(68)*. HIF hydroxylases include proline hydroxylase domain proteins 1-3 (PHD1-3) and asparagine hydroxylase (factor-inhibiting HIF, FIH-1) that negatively regulate transcription factor HIF-1. HIF-1 activates the transcription of numerous genes involved in crucial aspects of cancer biology, including cell survival, angiogenesis, glucose metabolism and invasion. In hypoxic conditions, cancer cells overexpress HIF-1 subunit, HIF-1α which is associated with cancer progression and worse patient outcome. Under normoxic conditions, hydroxylation of specific proline residues in HIF-1α is dependent on PHD1-3 which facilitates the binding of prolyl-hydroxylated HIF-1α to von Hippel-Lindau (VHL) E3-ubiquitin ligase that targets HIF-1α for proteasomal degradation. On the other hand, asparagine hydroxylated HIF-1α is unable to interact with a coactivator p300 resulting in the inhibition of HIF-1 transcriptional activity *(85)*. By functioning as both cofactor and co-substrate, ascorbate can increase the levels of HIF hydroxylases leading to proteasomal degradation of HIF-1α *(86, 87)*.

THE ROS-INDUCING EFFECTS OF VITAMIN C IN OVARIAN CANCER

Several studies have shown different anticancer activities of vitamin C in ovarian cancer that can be attributed to increased ROS levels in the cells causing activation or inactivation of numerous proteins associated with genotoxic or metabolic stress (Figure 2). In the pharmacological dose, vitamin C can increase both cell membrane permeability and apoptosis in ovarian cancer cells. Cytotoxic effect of high-dose vitamin C treatment is evident via decreased cancer cell proliferation that can be influenced by reduced levels of CDK2 and increased levels of CDK inhibitor p21 and p53. At pharmacological dose of 1 mM, vitamin C can decrease the expression of PARP enzyme. These cytotoxic effects of vitamin C were observed in OVCAR-3 ovarian cancer cells and not in non-cancer cells *(88)*. In murine ID8 ovarian cancer model, vitamin C significantly reduces the number of tumor nodules and prevents spheroid formation compared to control mice. Reduced level of vimentin and increased level of E-cadherin was observed in ID8 tumor nodules isolated from mice treated with vitamin C compared to untreated control mice which is an indication of EMT inhibition upon vitamin C treatment in ovarian cancer cells. In vitro, vitamin C can prevent multicellular spher-

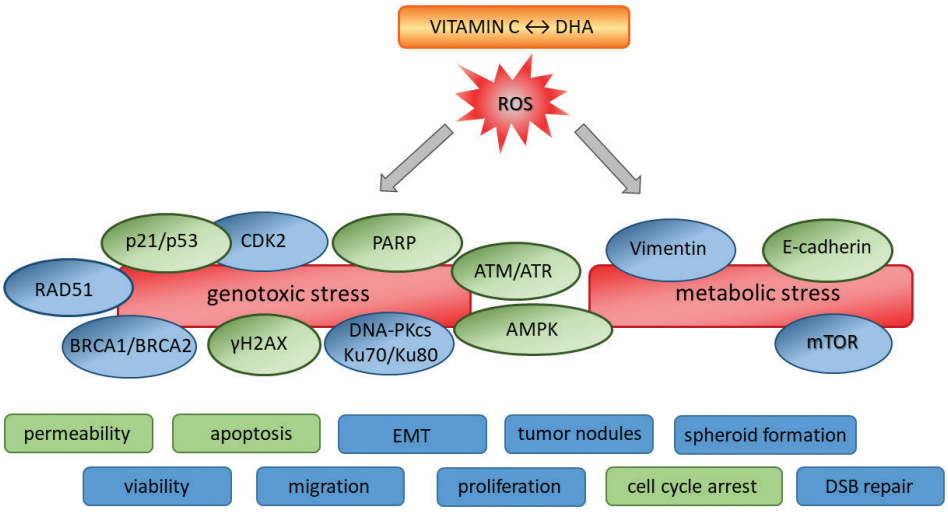

Figure 2. *Mechanism of action of pharmacological ascorbate in ovarian cancer cells. Switch of vitamin C to DHA results in generation of ROS which cause genotoxic and metabolic stress evident by increased expression/activation (green) or reduced expression/inactivation (blue) of numerous markers that either enhance (green) or decrease/inhibit (blue) different cellular processes (for details see the text).*

oid formation, reduce migration, inhibit proliferation and induce apoptosis as well as cell cycle arrest at S stage in ID8 cells *(89)*.

Ma and colleagues observed cytotoxic effect of the pharmacological ascorbate using larger panel of ovarian cancer cells (OVCAR10, SKVO3, OVCAR3, A2780, OVCAR5, OVCAR8 and SHIN3) which was reversed by the addition of catalase prior to vitamin C treatment in SHIN3 cell line. The authors have shown that in millimolar range, vitamin C induces DNA damage and metabolic stress which were evident by the activation of markers of DNA damage (γH2AX) and cellular response to DNA damage (ATM and ATR) as well as energy sensor AMPK most probably due to ROS production and/or intracellular ATP depletion. Vitamin C also reduced the mTOR pathway activity by inducing metabolic stress. Furthermore, the additive to synergistic effect for high-dose vitamin C and carboplatin was observed in OVCAR5, OVCAR8 and SHIN3 cell lines. Combined treatment of vitamin C and carboplatin/paclitaxel was more effective in reducing tumor burden in mice xenograft model compared to monotreatment. Authors also reported the results of clinical study conducted in patients diagnosed with stage III or IV ovarian cancer. Patients were randomized in two study groups where one group received conventional carboplatin/paclitaxel treatment while other group included high-dose vitamin C intravenously injected beside conventional chemotherapy. The reduction of chemotherapy-associated toxicities and trend toward overall survival improvement were observed in high-dose vitamin C treated patients compared to chemotherapy only treated patients *(90)*.

Few years later, the same group reported that the pharmacological ascorbate sensitizes BRCA1/2-wt ovarian cancer cell lines (SHIN3 and OVCAR5) to PARPi treatment. The combined treatment of pharmacological ascorbate with either olaparib or veliparib significantly decreased cell viability compared to monotreatment. The authors also observed the activation of PARP after vitamin C treatment, most probably due to ROS-induced DNA damage, which was inhibited upon PARPi treatment. In addition, the authors observed reduced levels of BRCA1 and BRCA2 as well as RAD51 upon high-dose vitamin C treatment which would suggest that vitamin C inhibits HR and causes "BRCAness" phenotype in BRCA1/2-wt ovarian cancer cells. Dependent on the cell line, the authors also observed reduced expression of DNA-PKcs, Ku70 and Ku80, proteins involved in non-homologous end joining (NHEJ) DNA repair mechanism. The above-mentioned results imply that vitamin C impedes with two main DNA DSB repair mechanisms, HR and NHEJ, promoting further DNA damage to occur. The authors also reported that combined treatment of pharmacological ascorbate and PARPi increases the level of DSBs in vitro and reduces tumor burden in mice xenograft model *(91)*.

In the study reported 20 years ago, vitamin C was used as an adjunctive therapy to conventional chemotherapy and was first applied orally then intravenously (60 g twice a week) which resulted in cancer remission and normalization of CA-125 three years and more in two ovarian cancer patients *(92)*.

CONCLUSION

In this article, we have summarized the latest therapeutic approaches in the treatment of ovarian cancer. Due to the unsatisfactory response of many tumors to available

therapies, it is necessary to investigate new adjunctive therapeutic approaches that could improve the response of ovarian cancer to standard therapies. One of these approaches is the application of high pharmacological doses of vitamin C, which has already shown promising effects in several in vitro and in vivo studies. Further investigation and elucidation of more precise molecular mechanisms of vitamin C anticancer activity could lead to the improvement of the ovarian cancer patient welfare and overall response to anticancer therapy.

Acknowledgements: *This work was supported by donation of the dm-drogerie markt d.o.o. through the contest "{TOGETHER} for a better tomorrow".*

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