The potential of vitamin C in treatment of ovarian cancer

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Bachelor thesis Biomedical Sciences
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Abstract

This research evaluates the potential of vitamin C in treatment of ovarian cancer. Studies show that vitamin C might be able to reduce ovarian tumorigenesis through a variety of mechanisms. In these pathways, vitamin C, acting as an important electron donor, stimulates enzymatic activity. A key point of focus in (gynaecological) oncology is the epigenetic regulation of genes. Like many other cancer types, ovarian carcinogenesis is associated with aberrant DNA and histone methylation patterns. Vitamin C promotes the demethylation of DNA and histones by stimulation of the catalytic activity of ten-eleven translocation (TET) and Jumonji C-domain-containing histone demethylase (JHDM) enzymes, respectively. For ovarian tumours associated with hypermethylation of tumour suppressor gene promoter regions, resulting in downregulated expression of these genes, vitamin C administration might be an effective therapeutic strategy to inhibit tumorigenesis. Another epigenetic mechanism by which vitamin C could target ovarian tumour development, is by stimulation of demethylation of human endogenous retrovirus (HERV) promoter regions. The consequent increased HERV expression in ovarian tumours may elicit an immune response against these cells, resulting in tumour cell apoptosis. In addition to its role in epigenetic pathways, studies report that vitamin C also affect cancer cell metabolism, in context of the Warburg effect. This phenomenon describes the metabolic transition from mitochondrial oxidative phosphorylation to aerobic glycolysis as predominant energy generation process. Vitamin C activity targets the Warburg effect in a PKM2/GLUT1 dependent manner. Although several studies indicate that vitamin C administration may be an effective therapeutic strategy to treat ovarian cancer patients and *in vitro* results are promising, more *in vivo* research and subsequent clinical trials are needed in order to validate the efficacy of vitamin C treatment in different histologic subtypes of ovarian cancer.

Introduction

Epithelial ovarian cancer (EOC) is the eight most common cause of cancer related death in women worldwide (Sung et al., 2021). It is characterized by poor prognosis and little progress has been made regarding new therapeutic strategies (Natanzon et al., 2018). EOC consists of five different histologic subtypes, including endometrioid, mucinous, clear cell and low- and high-grade serous carcinoma (Matulonis et al., 2016). Platinum-based chemotherapy is the standard of treatment for all EOC subtypes. However, tumour chemoresistance reduces therapeutic efficacy in most patients (Christie et al., 2017). In order to improve therapeutic regimens, extensive research has been done to reveal the pathogenic mechanisms contributing to this disease. For some time, ovarian cancer development is associated with genetic aberrations. The most prevalent genetic mutation occurs in the well-known tumour suppressor gene *TP53*, involved in growth arrest, apoptosis and maintenance of genomic integrity, which is reported to be mutated in nearly 97 percent of all high-grade serous ovarian carcinoma cases (HGSOC), the most common subtype of EOC. (Ahmed et al., 2010). Also, mutations in homologous recombination repair genes have been observed in half of the HGSOC cases. (Kroeger et al., 2017). From these genes, especially *breast cancer type 1 and 2 susceptibility (BRCA1/2)* gene mutations are linked to EOC. In the last decade, increasing evidence implies that disturbed epigenetic regulation is also involved in ovarian carcinogenesis. In epigenetics, gene expression is regulated by heritable DNA and histone modifications without any change in DNA sequence, also referred to as changes in the epigenome. In ovarian cancer development two main epigenetic regulatory mechanisms have been described, including DNA methylation and histone modifications (Yang et al., 2018).

DNA methylation is an important epigenetic process regulating gene expression. Depending on the genomic site at which methylation takes place, it could result in both stimulation or repression of gene expression. For many different cancer types, including ovarian cancer, hypermethylation of promoter regions of tumour suppressor genes are observed. The extensive addition of methyl groups to these promoter sequences prevents essential transcription machinery to bind due to steric hindrance, resulting in less gene transcription and thus expression. As tumour suppressor genes inhibit tumorigenesis, hypermethylation of these genes promotes tumour formation. Regarding ovarian cancer, studies show that promoter regions of genes are differentially methylated in all histologic subtypes in comparison to healthy ovarian tissues (De Caceres et al., 2004). Interestingly, several of these genes are tumour suppressor genes; *BRCA1/2* is predominantly hypermethylated in HGSOC while hypermethylation of *Ras association domain family member 1A (RASSF1A)* occurs in all different ovarian cancer subtypes (Choi et al., 2006). These findings may implicate that also in ovarian cancer aberrant DNA methylation stimulates tumorigenesis.

In addition to DNA methylation, chromatin remodelling is also an important epigenetic regulatory mechanism associated with ovarian cancer. In this process, ATP-dependent chromatin remodelling complexes modify the compaction of the DNA through nucleosome sliding. Relatively uncompacted DNA, meaning that double stranded DNA is loosely wrapped around nucleosomes, is more accessible for transcription machinery than heavily compacted DNA, resulting in more expression of genes located in that genomic region. Importantly, histone modifications and DNA methylation are related and part of a highly interlinked system in which different epigenetic regulatory mechanisms work together to either stimulate or repress gene expression (Fuks et al., 2005). Chromatin remodelling is directed by the presence of specific marks on histone tails. A spatial pattern of methyl, acetyl and ubiquitin groups added to amino acid residues in histone tails determines the degree of compaction of DNA. To date, multiple genes have been identified that were differentially expressed in ovarian cancer cell lines compared to healthy tissues and in these cases a relation between gene expression and chromatin remodelling has been established (Caslini et al., 2006, Kwon et al., 2010). The same studies also implicate that in tumorigenesis of ovarian cancer not only chromatin-associated silencing of tumour suppressor genes takes place, but also activation of oncogenes.

As it has become clear that disturbed epigenetic regulation contributes to the pathogenic mechanisms underlying several types of cancer, including ovarian cancer, research has focused on the identification of factors that direct epigenetic regulation in tumours, in order to improve therapeutic strategies. The well-known antioxidant vitamin C has been identified as such a factor involved in epigenetic regulation (Gillberg et al., 2018). Interestingly, vitamin C is shown to induce DNA demethylation in promoter regions of tumour suppressor genes (Shenoy et al., 2019). These studies suggest that vitamin C, normally associated with functions in enzyme activation, oxidative stress reduction and immune system (Schlueter et al., 2011), may play a role in tumour formation.

The aim of this literature research is to evaluate the role of vitamin C in ovarian cancer oncogenesis and to assess potential therapeutic strategies targeting this relation. This study elaborates further on the mechanisms by which vitamin C directs differences in DNA methylation as well as histone modifications. Increased understanding of this process may enable us to improve current treatment regimens. In this research, novel treatment options regarding vitamin C function are not only discussed in context of epigenetics, but also in relation to the Warburg effect, a hallmark of many tumour types. The Warburg effect describes a well-known phenomenon in which the transition of normal cells to cancerous cells is characterized by a metabolic switch from primarily mitochondrial oxidative phosphorylation to mostly glycolysis in order to generate ATP (Liberti et al., 2016). This transition is accompanied by an increased glucose uptake and fermentation of glucose to lactate in

the presence of oxygen. The metabolic switch enables cancer cells to increase growth, proliferation and survival. In different types of EOC, the Warburg effect is shown to promote disease progression and chemoresistance (Tyagi et al., 2021). Attention has been drawn to vitamin C as a potential therapeutic to treat ovarian cancer patients, as it has become clear that pharmacological levels of vitamin C interfere with the Warburg effect (Aguilera et al., 2016). This paper gives an overview of the current understanding about the role of vitamin C in the development of cancer and it will evaluate its implications for potential therapeutic strategies to treat ovarian cancer.

Vitamin C, its role in human physiology

Vitamin C exerts a wide variety of functions in human physiology. Most of its functionality is performed by acting as an electron donor in several redox systems (Schlueter et al., 2011). As a reducing agent, it plays an important role in the detoxification of reactive oxygen and nitrogen species. Vitamin C, or ascorbic acid, refers to the reduced state of this compound. Upon oxidation, ascorbic acid is converted to dehydroascorbic acid (DHA) (Carr, A. C., & Maggini, S., 2017). The reduced and stable form of vitamin C (ascorbic acid) represents most of the vitamin C in tissues and is the active functional compound. In literature, the terms vitamin C and ascorbic acid are used in an interchangeable fashion. DHA is converted back to ascorbic acid through recycling processes, catalysed by a variety of enzymes (figure 1). As cofactor in many enzymatic reactions, vitamin C maintains enzyme activity through reduction of the metal ions in enzymes. Despite the ability of other electron donors to exert this function, this is not quite as effective. Consequently, vitamin C deficiency is associated with several disease states in humans (Levine et al., 1986), including scurvy and immune deficiency (Jafari et al., 2019), due to reduced enzymatic function. In a similar way, vitamin C is a key regulator in the epigenetic regulation of genes.

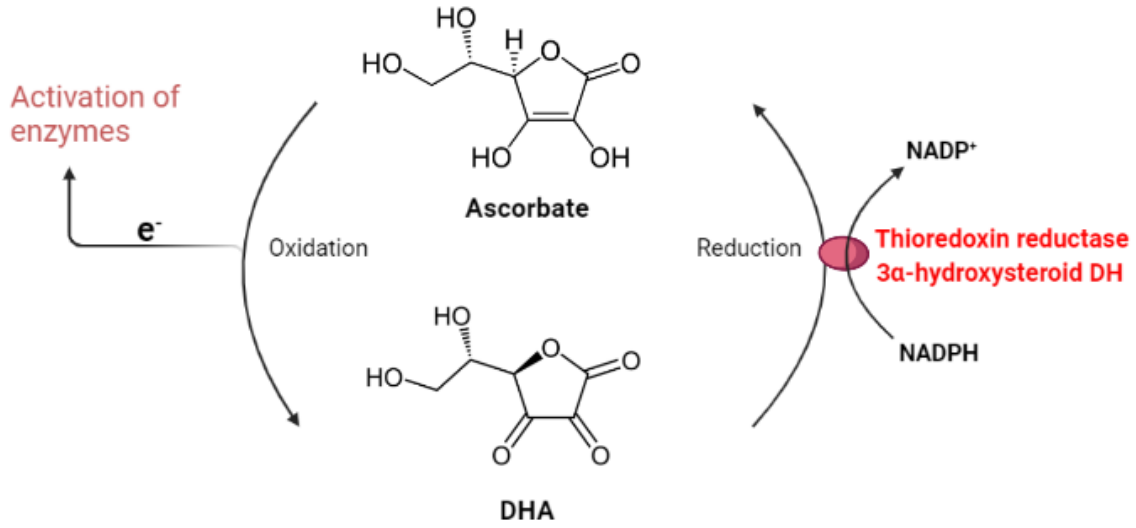

Figure 1. The oxidation of ascorbate to DHA yields an electron, which can reduce metal ions in the catalytic site of enzymes, resulting in enzyme activation. Ascorbate is recycled through reduction of DHA, catalysed by a variety of enzymes, like thioredoxin reductase and 3α-hydroxysteroid DH.

The role of vitamin C in DNA methylation

After decades of research into the vitamin C function, it has become clear that vitamin C is also involved in the epigenetic regulation of genes. Its role in DNA methylation is related to its interaction with ten-eleven translocation (TET) enzymes (Mikkelsen et al., 2021). TET is a member of the 2-oxoglutarate-dependent dioxygenase (2-OGDD) family and it consists of three different isotypes. Despite distinct structural features and differential expression between tissues, the isoenzymes show similar enzymatic activity (Ito et al., 2010). Although TET enzymes were already discovered in studies focusing on the pathogenesis of leukaemia (Ono et al., 2002), their specific functions remained unclear until experimental data showed that TET1 overexpression results in decreased DNA methylation (Koivunen et al., 2018). So, as opposed to DNA methyltransferase activity, in which methyl groups are added to DNA, TET enzymes are able to actively remove methyl groups from DNA. TET enzymatic activity requires molecular oxygen, Fe^{2+}, α-ketoglutarate and vitamin C as a reducing agent. The catalytic core of the TET dioxygenase enzyme is situated at its C-terminal and consists of a double-stranded β-helix (DSBH) and a cysteine-rich domain (Yin et al., 2016). The DSBH is essential to bring the iron ion, α-ketoglutarate and substrate in close proximity to each other. The cysteine-rich domain provides general enzyme stability. All α-ketoglutarate/ Fe^{2+} dependent dioxygenases, including TET, share the same reaction mechanism, in which the iron ion reduces molecular oxygen, forming a superoxide radical which targets α-ketoglutarate, ultimately resulting in the hydroxylation of the substrate. Enzymes of the 2-OGDD family differ in substrate specificity. The substrate of TET is a methylated cytosine residue, also referred to as 5-methylcytosine (5mC). By-products formed during TET catalytic activity are succinate and carbon dioxide.

The demethylation mechanism is initiated by the TET-dependent oxidation of 5mC. (Gillberg et al., 2018). Subsequently, the reaction product, 5-hydroxymethylcytosine (5hmC), can either be lost passively during a new replication cycle, or further oxidized by TET. Normally, during replication, 5mC residues are maintained in complementary newly synthesized DNA strands via maintenance DNA methyltransferase activity (DNMT1). However, DNMT1 does not recognize the oxidated 5hmC residue, resulting in its removal during subsequent replication cycles. If 5hmC is further oxidized by TET, the products 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) form. Both residues can be removed and replaced by unmethylated cytosine by either base-excision repair mechanisms or in a passive replication-dependent manner (Gillberg et al., 2018).

The presence of vitamin C at the site of reaction is an important prerequisite. It activates TET through reduction of the iron ion which is embedded in the catalytic site of TET. The reduction of Fe^{3+} to active Fe^{2+} results in the restoration of enzymatic activity. Remarkably, replacing vitamin C with an alternative electron donor does not initiate a catalysed reaction. This implies that vitamin C is an indispensable factor in the demethylation process by TET. A study by Hore et al. demonstrated that TET activity increases upon vitamin C addition. A schematical representation of the mechanism in which vitamin C affects the TET-dependent removal of methyl groups from genomic DNA, is shown in figure 2.

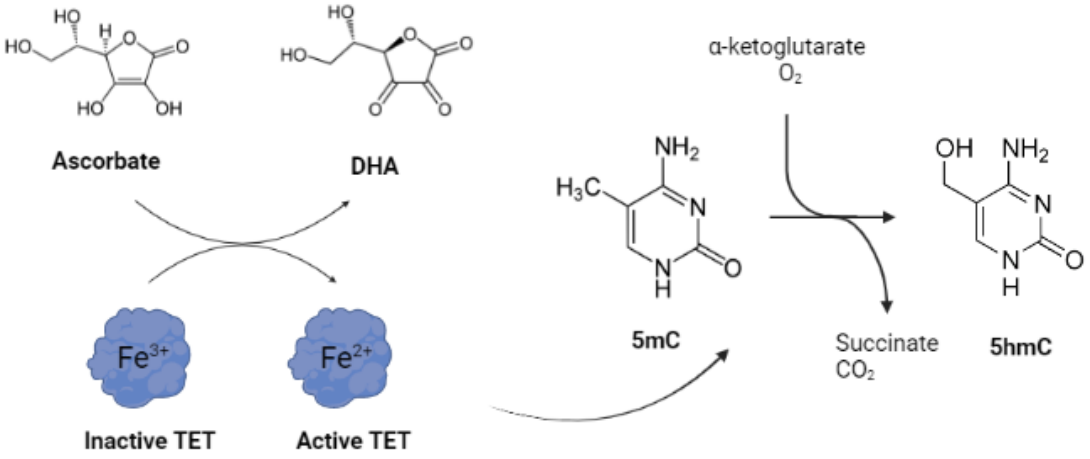

Figure 2. The role of vitamin C (ascorbate) in the first step of the TET-dependent demethylation of DNA. The oxidation of ascorbate to DHA yields an electron, which subsequently reduces the iron ion inside the catalytic site of TET, resulting in the activation of this enzyme. TET catalyses the conversion of 5mC to 5hmC.

The role of vitamin C in histone modification

In addition to its role in DNA methylation, it has become clear that vitamin C is also involved in histone methylation, another important epigenetic regulatory process. The reaction mechanism by which this occurs, is quite similar to the vitamin C dependent DNA demethylation by TET. Histone demethylation is catalysed by Jumonji C-domain-containing histone demethylases (JHDMs) via an oxidation reaction (Anand et al., 2007). Specifically, JHDM activity results in the removal of methyl groups from lysine and arginine residues in the tail of histones. The class of JHDM enzymes, consisting of different isoforms, is characterized by the presence of a Jumonji domain, a key domain for demethylation. A variety of histone demethylases exists, including the lysine-specific histone demethylase 1 (LSD1), but all differ in their specificity for amino acid residues and corresponding methylation status. JHDM is predominantly associated with the demethylation of the trimethylated lysine residues at position 9 and 36 in histone 3 (H3K9me3 and H3K36me3) (Whetstine et al., 2006). Remarkably, H3K9me3 is a repressive histone mark while H3K36me3 is associated with actively transcribed genes (Becker et al., 2016). Cell response to JHDM-dependent histone demethylation ultimately depends on the original distribution of histone marks in specific gene regions. JHDM demethylation activity could be of therapeutic interest in treatment of cancers which are associated with H3K9me3-related epigenetic repression of tumour suppressor genes. Demethylation of this repressive histone mark may release the epigenetic brake and induce tumour suppressor gene expression. On the other hand, in tumours with H3K36me3-associated upregulation of oncogenes, demethylation by JHDM may also inhibit tumorigenesis.

Similar to TET, JHDM contains an iron ion in its catalytic site and reduction of Fe^{3+} to Fe^{2+} by vitamin C is a prerequisite for catalytic function. Likewise, the α-ketoglutarate/ Fe^{2+} dependent reaction results through a hydroxylation step in the demethylation of lysine residues. The mechanism in which vitamin C promotes the JHDM-dependent demethylation of lysine residues is shown in figure 3.

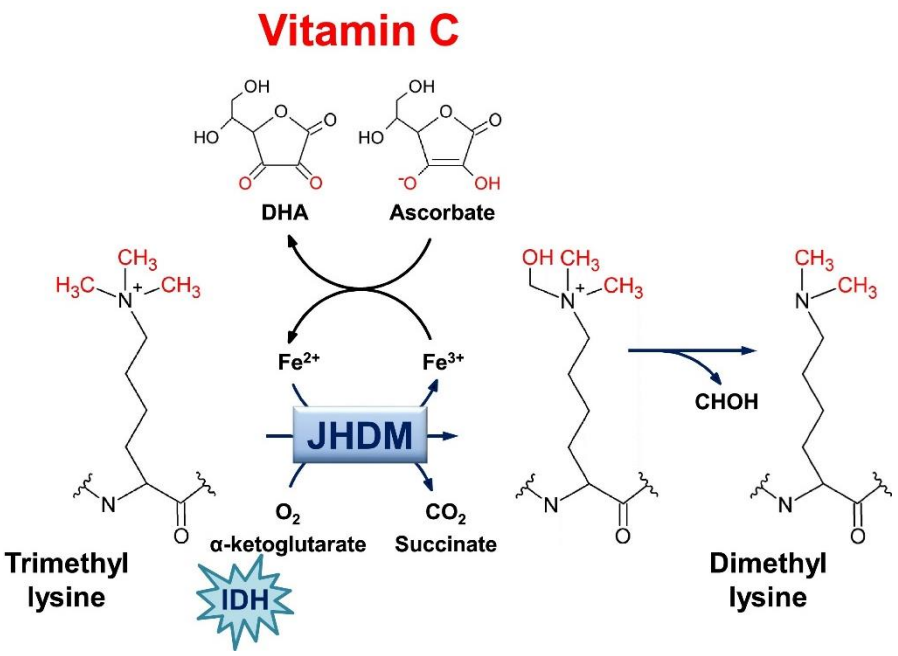

Figure 3. Adapted from Gillberg et al. (2018). The role of vitamin C in the first step of JHDM-dependent demethylation of lysine residues. The oxidation of ascorbate to DHA yields an electron, which subsequently reduces the iron ion inside the catalytic site of JHDM, resulting in the activation of this enzyme. JHDM catalyses the demethylation of trimethyl lysine to dimethyl lysine.

Potential therapeutic strategies in ovarian cancer treatment in relation to vitamin C

Due to chemoresistance, current platinum-based chemotherapy is not effective as treatment of ovarian cancer. Although in early stages ovarian tumours are responsive for chemotherapy, in most patients recurrence occurs in the following 2 years (Schwarzenbach et al., 2019). Chemoresistance occurs in every ovarian cancer subtype, but the severity of this phenomenon differs between them (Cloven et al., 2004). Mucinous ovarian cancer showed most resistance to platinum-based chemotherapy. Interestingly, this chemoresistance is associated with epigenetic changes. Several genes were found to be differentially expressed between resistant and sensitive ovarian tumour cells and these observations were associated with differences in promoter methylation. Some of these genes were found to have effect on the apoptotic response upon introduction of platinum-containing compounds.

To date, the most promising novel treatment options to treat patients suffering from ovarian cancer are based on synthetic lethality. Synthetic lethality describes a relation between two genes in which a loss of function mutation in one gene will render the cell viable, while loss of function mutations in both genes will result in cell death. In different types of ovarian cancer, such a synthetic lethal relation has been found between *BRCA1/2* and poly ADP ribose polymerase (PARP). Experiments revealed that the survival of tumour cells with *BRCA1* or *BRCA2* mutations was significantly impaired upon PARP-inhibitor treatment (Ledermann et al., 2016). In clinical trials, therapeutic efficacy of PARP inhibitors has been validated in patients with *BRCA1/2* mutations. Currently, several PARP inhibitors are EMA-approved and used in the clinic. In ovarian clear cell carcinoma, a rare but very aggressive histologic subtype of EOC, another synthetic lethal relation has been found for the *AT-rich interaction domain 1A (ARID1A)* gene and *enhancer of zeste homolog 2 (EZH2)* gene (Yang et al., 2019). As ARID1A mutations are often found in ovarian clear cell carcinoma, inhibition of EZH2

expression may prove to be an effective therapeutic strategy for treatment of this ovarian cancer subtype.

In the past, quite some studies have assessed the relation between cancer risk and dietary vitamin C intake. Already in the 1950s studies reported that vitamin C deficiency promotes the spread of tumour cells. Furthermore, in small patient studies, vitamin C has been tried as treatment for different cancer types. Cameron and Pauling (1974) were first to assess the use of oral and intravenously administered vitamin C in the treatment of cancer. In a study with 100 terminal cancer patients with varying types of cancer and 1000 matched controls (same sex, age and tumour type), they concluded that pharmacological doses of intravenously administered vitamin C resulted in prolonged survival in advanced cancer patients with different tumour types in comparison to the control group which did not receive vitamin C treatment. However, the significance of these results were critically questioned by other scientists in this research field, as a clear dose-response relationship was not established. Although results remained inconclusive, there were several implications that vitamin C might be beneficial in cancer treatment. To date, the potential use of vitamin C in therapeutic regimens is still an ongoing topic. An *in vitro* study by Gregoraszcuk et al. (2021) concluded that vitamin C does have some anticancer properties in high-grade serous ovarian carcinoma cell lines (OVCAR-3).

As research has shed light on the mechanisms in which vitamin C affects epigenetic regulation, vitamin C and related pathways have become a point of focus in the development of new treatment options for cancer types associated with disturbed epigenetic regulation. In several solid and haematological tumours mutations are observed in genes important in epigenetic regulatory process, including the TET enzyme. Consequently, functional TET enzyme expression is reduced in these cancers. A study by Yin et al. (2011) reported that 5hmC levels, the reaction product after 5mC oxidation by TET, were decreased in squamous lung tumour cells in comparison to healthy lung tissue. Similar observations were done in brain tumour cells, but there 5hmC levels were reduced to even a greater extent. It is important to note that these findings could not be only attributed to the deficiency of functional TET enzyme, but also inadequate levels of the cofactors Fe^{3+}, α-ketoglutarate, molecular oxygen and vitamin C. As tumour environments are often hypoxic due to disturbed vascularization, molecular oxygen deficiency may have a significant effect on the rate of the TET-dependent oxidation reaction.

In the last decade, several *in vitro* studies, evaluating the effect of vitamin C on TET activity, have been performed. Research by Gerecke et al. (2020) showed that vitamin C increases 5hmC levels in a HCT116 cell line, which was obtained from patients with colon cancer and a clear dose-response relationship was found. This implies that in these cell lines vitamin C induces TET activity. Remarkably, this effect of vitamin C on 5hmC levels was significantly reduced in isocitrate dehydrogenase 1 (IDH1) knockout cell lines. This underscores the indispensability of the cofactor α-ketoglutarate, as IDH is an essential enzyme for the oxidative decarboxylation of isocitrate to α-ketoglutarate. Furthermore, some studies also assessed the effect of different concentrations of vitamin C on DNA methylation levels. One of these studies determined by bisulfite sequencing DNA methylation levels in clear cell renal cell carcinoma (ccRCC) cell lines in relation to vitamin C supplementation (Shenoy et al., 2019). Prior to vitamin C supplementation, in these cell lines aberrant DNA methylation was reported in genomic regions associated with tumour-suppressor genes, like *SMAD6*, a TGF-β regulator. The consequent silencing of these genes may contribute to ccRCC carcinogenesis. Furthermore, 5hmC levels were decreased in these cell lines, indicating that the TET-dependent oxidation of 5mC was inhibited. Strikingly, addition of vitamin C to these ccRCC

cell lines restored TET activity, resulting in increased 5hmC levels and simultaneous reduction of DNA methylation in the promoter regions of these tumour-suppressor genes.

The collection of studies validates that vitamin C stimulates the catalytic activity of the TET enzyme in different cancer types. In several tumours, including ovarian cancer, the extensive methylation of promoter regions of tumour suppressor genes and the subsequent silencing of these genes is believed to be an early event in tumorigenesis. On the other hand, many tumours have been described in which oncogene expression was upregulated through hypermethylation of the gene body, enhancing carcinogenesis. In both cases, stimulation of demethylation of those gene regions through activation of TET by vitamin C, might reduce tumour development. In addition to the demethylation of tumour suppressor and oncogenes, increased vitamin C-dependent TET activity might also inhibit cancer progression via different pathways.

Gillberg et al. (2018) introduced human endogenous retroviruses (HERVs) in this context. HERVs are remnants of past infections with retroviruses, in which their genome is implemented into the human genome. The viral elements are known to occupy around 8 percent of the human genome (Grandi et al., 2018). During human genome evolution mutations have accumulated in these viral remnants. However, their transcription products have remained functional and several of these products contribute to some disease states (Grandi et al., 2018). The same study argues that retroviral envelope proteins play a role in the pathogenicity of cancer and autoimmune diseases. In contrast, in ovarian cancer, HERV does seem to reduce tumorigenesis. Chiappinelli et al. (2015) concluded that upregulation of HERV in EOC may induce tumour cell apoptosis via a viral defence mechanism. When transcription of HERV occurs in a bi-directional manner, double stranded RNA (dsRNA) is formed (Gillberg et al., 2018). As normally the human transcriptome does not include dsRNA, the presence of dsRNA is interpreted by the immune system as foreign, eliciting an immune reaction. The detection of dsRNA by viral recognition receptors, results in an interferon gamma (IFN-γ) response. Through signalling pathways, this ultimately leads to cell apoptosis (Sistigu et al., 2014). The study by Chiappinelli et al. (2015) concludes that demethylation of hypermethylated regulatory sequences within the HERV gene region in ovarian cancer cells, results in more HERV expression and thus higher dsDNA levels. Subsequently, this leads to increased apoptosis of these ovarian cancer cells.

In conclusion, these studies show that increased vitamin C-dependent TET activity may not only result in inhibition of EOC tumorigenesis via demethylation of onco- and tumour suppressor genes, but also via the demethylation of HERV gene regions.

Vitamin C interferes with the Warburg effect

The therapeutic value of vitamin C is certainly not constrained to its role in epigenetic regulation. Specifically, the effect of vitamin C on cellular metabolic regulation in tumour cells has been of great interest. A wide variety of cancer types are characterized by a metabolic transition of mitochondrial oxidative phosphorylation to glycolysis during tumorigenesis, also referred to as the Warburg effect. In these tumours, high glucose uptake, high lactate production and low oxygen consumption is observed. In healthy tissue, primarily oxidative phosphorylation is the preferred mechanism by which energy, in the form of adenosine triphosphate (ATP), is generated. This could be explained by the observation that the net yield of ATP per glucose molecule is much greater via this pathway than with glycolysis. In aerobic conditions, pyruvate, the end product of glycolysis, can enter the citric acid cycle, in which its breakdown yields energy-rich electrons in the form of NADH and NADPH. Subsequently, these electrons are used to generate a mitochondrial membrane potential, ultimately

leading to the production of ATP via ATP synthase. In anaerobic conditions, oxidative phosphorylation is limited and a smaller amount of ATP is generated via glycolysis, after which pyruvate is broken down to lactate via anaerobic fermentation. Remarkably, although it seems beneficial for each cell to optimize their energy production, some cells, including tumour cells, limit their energy generation to glycolysis, even when oxygen is present. It has been shown that extensively proliferating and growing cells prioritize biomass production (eg. lipids, proteins and nucleic acids) over energy production (Spencer et al., 2019). In contrast, differentiated cells are strongly dependent on energy generation to perform their cell functions.

As the Warburg effect, characterized by aerobic glycolysis, has shown to be essential for tumour cell proliferation and survival (Liberti et al., 2016), inhibition of associated pathways might be an effective strategy in cancer treatment. A study by Aguilera et al. (2016) proposed a mechanism in which vitamin C targets the Warburg effect. Immunohistochemistry (IHC) assays demonstrated that vitamin C supplementation results in reduced expression of glucose transporter-1 (GLUT-1) in colorectal cancer cells. GLUT-1, embedded in the cellular membrane, is essential for diffusion of glucose into cells. As tumour cells showing the Warburg effect display a very high demand for glucose, GLUT-1 activity is indispensable for these cells (Aguilera et al., 2016). Quantitative PCR experiments showed that vitamin C reduces GLUT-1 mRNA levels, which implies that vitamin C inhibits GLUT-1 production on a transcriptional level.

In the absence of vitamin C, membrane bound rat sarcoma virus (RAS) phosphorylates the ser37 residue in the pyruvate kinase isozyme M2 (PKM2) through phosphorylation of the downstream kinases Raf, MEK and ERK. This results in the translocation of PKM2 to the nucleus, in which it acts as cofactor for β-catenin. Subsequently, the activated complex induces expression of the oncogene c-Myc, which in turn stimulates GLUT-1 and Polypyrimidine Tract Binding Protein (PTB1) expression. PTB1, belonging to the family of heterogeneous nuclear ribonucleoproteins (hnRNPs), is known to regulate alternative splicing of PKM2 mRNA (David et al., 2010). PTB is an essential protein for the synthesis of functional PKM2 protein. PKM2 expression results in a positive feedback loop in which PKM2 promotes its own synthesis. Furthermore, PKM2, an isoenzyme of the pyruvate kinase enzyme, is able to catalyse the last step of glycolysis, in which phosphoenolpyruvate (PEP) is converted to pyruvate.

However, when vitamin C is present, PKM2 phosphorylation is inhibited through detachment of membrane-bound RAS by vitamin C activity. Consequently, PKM2 does not translocate to the nucleus, ultimately resulting in less GLUT1 and PTB transcription. Figure 4 shows the proposed mechanism by which vitamin C reduces GLUT-1 and PTB expression. Vitamin C-directed GLUT1 downregulation results in less capacity to take up glucose, thereby preventing the tumour cell to satisfy its high demand for glucose. This leads to less energy and biomass production. In addition, less PKM2 expression may lead to reduced rates of glycolysis in the tumour cell, depending on the availability of pyruvate kinase or other isotypes which stimulate the conversion of PEP to pyruvate. In conclusion, these results suggest that vitamin C treatment could inhibit the growth and proliferation of tumours by targeting their altered metabolism in a PKM2/GLUT-1 dependent manner. Importantly, also ovarian cancer cells depend on this altered metabolic state (Tyagi et al., 2021), indicating that vitamin C treatment might also be effective in this cancer type. To date, it has yet to be revealed whether the histologic subtypes of ovarian cancer differ in their dependency on the Warburg effect, which may give insights about the effectivity of vitamin C treatment in the distinct ovarian cancer types.

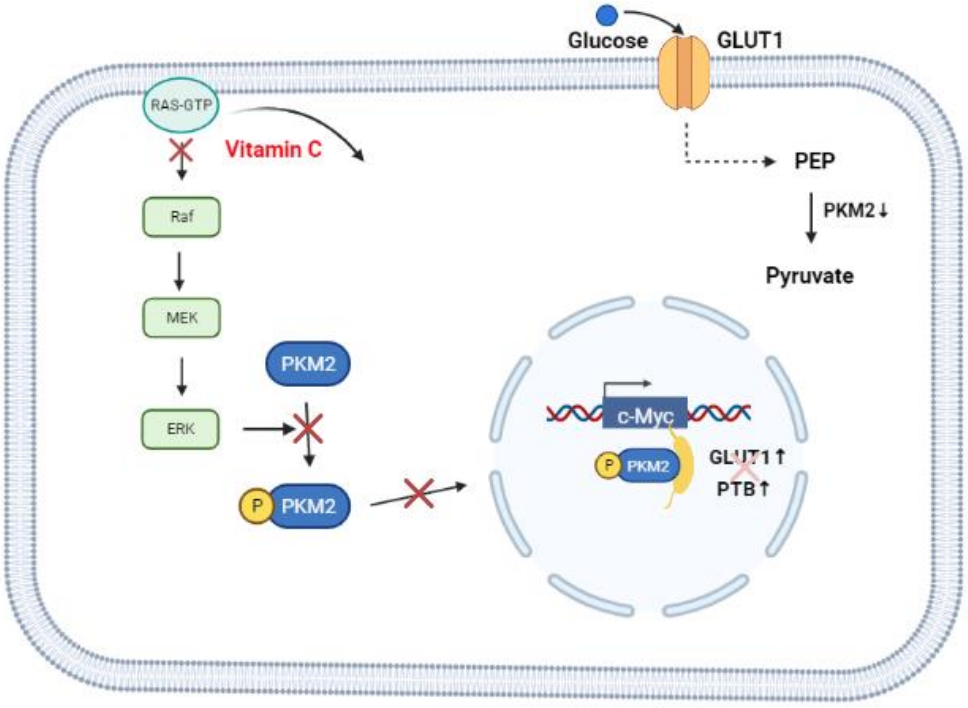

Figure 4. The mechanism by which vitamin C reduces GLUT1 and PKM2 expression. Vitamin C activity results in the translocation of membrane bound RAS, inhibiting the phosphorylation of PKM2. Consequently, PKM2 does not translocate to the nucleus and GLUT1 and PTB expression is downregulated. This mechanism contains a positive feedback loop, in which PKM2 stimulates its own synthesis.

Vitamin C treatment in the clinic

Increasing evidence shows that vitamin C could be a potent inhibitor of tumorigenesis through a variety of mechanisms. Although vitamin C has already been used by some health care practitioners in treatment of cancer, this remains quite controversial. Important to note, for such clinical purposes, intravenous administration of vitamin C (IVC) is used. Due to limited absorption in the gastrointestinal tract, oral intake achieves vitamin C plasma concentrations up to 0.25 mM while IVC can reach plasma concentrations of 30mM, which is more than a 100-fold higher than the oral maximum (Fritz et al., 2014). Vitamin C plasma concentrations after oral dosing are too low to achieve an antitumoral effect (Moertel et al., 1985). To date, a clear therapeutic rationale for cancer treatment with vitamin C has not yet been established. In order to introduce vitamin C as treatment for ovarian cancer types, its therapeutic efficacy has to be evaluated in large controlled clinical trials. Furthermore, it is yet unclear how vitamin C may optimally contribute to existing therapeutic regimens. It has to be determined whether vitamin C is most effective as sole treatment or in combination with other therapies. Vitamin C might be used as supplement to the conventional platinum-based chemotherapy or together with novel synthetic lethal strategies. Regarding ovarian cancer, there may be differences in tumour response to vitamin C treatment between different histologic subtypes of ovarian cancer.

Conclusion and discussion

In this literature research, the role of vitamin C in the epigenetic regulation of genes has been assessed. Furthermore, this study discussed the potential of vitamin C treatment for ovarian cancer patients. Epigenetics is a key point of focus in current oncology research as aberrations in epigenetic regulation have been associated with multiple stages of tumour development (Tsai et al., 2011). A collection of studies has shown that vitamin C promotes the demethylation of gene regions in a TET-dependent manner. Ovarian cancer is associated with hypermethylation of promoter regions of several tumour suppressor genes, like *BRCA1* and *RASSF1A.* This suggests that epigenetic demethylation by vitamin C could be an effective treatment in ovarian cancer patients. Furthermore, vitamin C stimulates the demethylation of primarily lysine residues in histone tails by enhancing the catalytic function of JHDM histone demethylases. However, no studies have yet been performed to validate a relation between specific histone methylation patterns and ovarian cancer tumorigenesis. Therefore, it remains unclear whether vitamin C induced histone demethylation could be beneficial in ovarian tumours. Not only the vitamin C-related demethylation of onco- and tumour suppressor genes, but also the demethylation of promoter gene regions of HERVs may show anti-cancer properties. This research demonstrated that vitamin C-dependent induction of HERV expression might promote an immune response against ovarian cancer cells.

In addition to epigenetic regulation, the therapeutic potential of vitamin C has also been evaluated in context of the Warburg effect. Aguilera et al. (2016) proposed a mechanism by which vitamin C can downregulate GLUT1 and PTB, thereby targeting the altered metabolic state of tumours. The dependence of ovarian cancer cells on aerobic glycolysis for growth and proliferation implies again a promising role for vitamin C in treatment of this cancer.

All these findings suggest that vitamin C might be an effective treatment option for ovarian cancer. However, the majority of results was obtained by *in vitro* studies, which show limitations in comparison to *in vivo* experiments. The reported *in vitro* studies all evaluated vitamin C activity under controlled conditions, while tumour microenvironments are highly dynamic (Han et al., 2018). It is yet unclear whether the vitamin C-induced TET activity can be reproduced in *in vivo* experiments. Oxygen availability varies between tumours (Little et al., 2018). Due to the dependence of TET catalytic activity on molecular oxygen, the extent to which TET dependent DNA demethylation takes place may differ between tumour types. This implies that vitamin C-dependent TET activation may not elicit the same effect in every tumour type. Also regarding the different subtypes of ovarian cancer, it is not fully understood whether the difference in genetic status and cell physiology may lead to a different response to vitamin C treatment between these distinct tumour types.

Research into the application of vitamin C in treatment of cancer has revived with the gain of new insights regarding epigenetic and metabolic regulation in cancer. Before vitamin C treatment can be included in standard therapeutic regimens for ovarian cancer treatment, more *in vivo* research and ultimately clinical trials are needed to evaluate the efficacy of vitamin C treatment in several histologic subtypes of ovarian cancer. In conclusion, despite some uncertainties, vitamin C administration is a promising therapeutic strategy for treatment of ovarian cancer patients.

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