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Vitamin C: An Epigenetic Regulator

Fadime Eryilmaz Pehlivan

Abstract

Vitamin C is an essential micronutrient, a free radical scavenger; while it has functions such as blocking oncogenic transformation induced by carcinogens. A different view of the potential action of vitamin C in cancer came from the discovery of its importance for activation of ten-eleven translocation (TET) enzymes that are involved in demethylation of DNA and histones. Aberrant DNA and histone methylation are hallmarks of all cancers and may result from altered expression or point mutations in the genes encoding these regulatory enzymes. Recent studies have shown that vitamin C potentiates the effects of DNA methyltransferase inhibitors. Epigenetic alterations, along with genetic mutations, are known to contribute to onset of cancer. Vitamin C is found to be a key mediator of the interface between genome and environment, regulating DNA demethylation as a cofactor for TET dioxygenases. It is shown that vitamin C drives active removal of DNA methylation by enhancing TET enzymes, which helps to erase DNA methylation and epigenetic memory encoded by it to improve reprogramming of differentiated cells to an embryonic-like state. Here, an overview of the role of vitamin C as an essential factor for epigenetic regulation and its potential in epigenetic therapy in cancer patients is provided.

Keywords: vitamin C, epigenesis, DNA modulation, TET enzymes

1. Vitamin C

1.1 Vitamin C, free radicals and antioxidant mechanism

Vitamin C (L-ascorbic acid) is a multifunctional water-soluble antioxidant substance in both plants and animals, having vital and ubiquitous roles in the life processes. It was first isolated and characterized by Szent-Gyorgyi back in 1928 [1]. In plants especially in green leaves, this metabolite is one of the most abundant, representing 10% of the total soluble carbohydrate pool [2]. In both plants and animals, it has important roles as a cofactor for a large number of key enzymes, influencing mitosis and cell growth by modulating the expression of specific genes involved in defense and hormonal signaling pathways. It is also required for iron utilization, connective tissue, cardiovascular functions, and immune cell development [3]. The disease, scurvy, which is defined as a medical situation caused by lack of L-ascorbic acid has considerable historical significance in the discovery of this amazing substance. In nineteenth century sailors and others who did not have access to natural sources of vitamin C (fresh vegetables and fruits) suffered from bleeding under the skin, severe ulcers, depression, loss of teeth and joint weakness [1].

In an effort to find a cure, scientists discovered that by consuming certain vegetables and fruits, this condition can be treated and prevented from reoccurring [1]. Plants and most animals synthesize vitamin C on their own, but humans lack this ability due to a deficiency in an enzyme called “L-gulono-gamma-lactone oxidase” [1–3] that catalyzes the final step in vitamin C biosynthesis, representing that humans must obtain this vital compound from exogenous sources such as natural diet and supplements.

Electrons prefer to pair up with each other so that they can remain energetically stable. However, due to biotic and abiotic influences such as infection, wounding, UV radiation, air pollutants, smoking, and natural oxygen metabolism (aerobic respiration), this balance is disrupted. Consequently various molecules can end up with a single electron in their outermost orbital, making them highly unstable and reactive towards their surroundings [4]. The newly formed molecule is now desperately looking for another electron to balance and stabilize itself with. This is what is known as a “free radical,” that are urgently searching for electrons and stealing them from other molecules. When a molecule loses an electron to a free radical molecule, it becomes a free radical itself and should now find an electron from another molecule to energetically stabilize itself [4, 5]. By this way, a single free radical can initiate a chain reaction that can result in severe damage to cell membranes, organelles, and structural and genetic coding (DNA and RNA) of body [4, 5]. These destructive mechanisms result in chronic free radical assault (oxidative stress). On the contrary, living organisms have a large arsenal of active substances such as antioxidants, to scavenge them and fight back [4, 5]. Vitamin C is a highly potent (reducing agent) antioxidant capable of neutralizing reactive oxygen species (ROS) and nitrogen derived free radical species [4, 5], achieving this by donating an electron to free radicals (**Figure 1**). As being a small, water soluble, reductone sugar acid with antioxidant properties, vitamin C acts as a primary substrate for detoxification of a number of ROS such as H_2O_2 , and neutralize it to superoxide radicals ($\text{O}_2^{\cdot-}$), singlet oxygen ($\text{O}^{\cdot-}$) or hydroxyl radical ($\text{OH}^{\cdot-}$) by acting as a secondary antioxidant during reductive recycling of the oxidized form of α -tocopherol [2, 4] (**Figure 1**).

1.2 Vitamin C and cancer

The carcinogenic effect of oxidative stress is focused on the genotoxicity of ROS. They can cause cancer through multiple mechanisms, and are known to play significant roles in the promotional stage of carcinogenesis [2, 4, 5]. Vitamin C is associated with its protective roles against ROS dependent oxidative stress, by stimulating immune function, inhibiting nitrosamine formation, and blocking the metabolic activation of carcinogens. It can also protect against oxidative DNA damage, that is implicated in tumor initiation [5, 6]. Although oxidative stress causes oxidative damage, moderate amounts of ROS are found to serve as a secondary messenger in the intracellular signaling cascades [5, 6]. Low oxidative stress is proved to be essential for cellular signal transduction that leads to the induction of detoxification or antioxidant enzyme systems. Limited amounts of ROS are needed for triggering the antioxidant signal transduction [5, 6]. Some phytochemicals are proved to induce phase II detoxification of antioxidant enzymes by triggering nuclear translocation of the transcription factors such as NF-E₂-related factor 2 (Nrf2) binding to antioxidant response element [7]. Many of the inducers are capable of activating these transcription factors mimic prooxidants; interestingly, most of them are antioxidants by nature [8]. Besides the antioxidant activity, prooxidant potential of vitamin C is contributed to its chemopreventive properties [8, 9]. Individuals

Figure 1.
Free radicals and antioxidant mechanism [2].

with cancer are reported to have significantly lower plasma vitamin C levels than healthy individuals [9]. Cancer-induced ROS formation and oxidative stress is one explanation for such a deficiency [10]. In this case, the prooxidant activity of vitamin C needs to be clarified while it is fighting, as an antioxidant, against oxidative stress. As a consequence, it is worthwhile to examine if vitamin C can induce the expression of phase II detoxification via its prooxidant potential, indicating that high intakes of vitamin C concentrations in serum are inversely associated with the risk of some cancers [10, 11].

Vitamin C at high doses has a very different (pro-oxidative) effect from vitamin C at physiological doses, that at high doses and under certain conditions (in the presence of redox-active metal ions) vitamin C can give rise to ROS, protein glycation, and DNA damage [11–13]. Increased levels of ROS and redox-active iron led to alterations in cancer cell oxidative metabolism resulting in selective sensitivity and pro-oxidative toxicity of high-dose vitamin C [11, 12]. It is reported that exposure to high-dose vitamin C sensitized cancer cells to ionizing radiation combined with chemotherapy [10–13].

1.3 Vitamin C and anti-inflammatory activity

It is evident that ROS are involved in chronic inflammation and cancer [14]. The generation of oxidative stress is an important part of the inflammatory response that is associated with tumor promotion. It is indicated that stomach cancer is a consequence of chronic inflammation [15]. This inflammatory process caused by the overproduction of ROS could be a target of vitamin C [15]. Vitamin C was proved to reduce inflammation caused by ROS [15], a recent study showed low vitamin C concentrations in gastric juice in the earlier stage of carcinogenesis [16]. Vitamin C protects gastric carcinogenesis by scavenging of the mucosal free radicals [15, 16] and by inhibiting the formation of carcinogenic nitrosamines [15, 16]. It is reported that, treatment of endothelial cells with vitamin C resulted

in the accumulation of a large amount of this substance inside the cells, which consequently decreased both the intracellular free radical status and inducible nitric oxide synthase induction [17]. During the inflammation process, vitamin C is shown to inactivate nuclear factor- κ B in endothelial cells besides of its antioxidant activity [18], indicating the anti-inflammatory activity of vitamin C with its intrinsic antioxidant activity [16–18].

1.4 Vitamin C and cell-to-cell communication

In multicellular organisms, cell-to-cell communication through gap junction channels is essential for maintaining homeostatic balance through modulation of cell proliferation and differentiation [19]. Inhibition of this communication is related to the carcinogenic process, especially to tumor promotion [20]. A well-known tumor promoter, hydrogen peroxide, is also found to inhibit gap junction intercellular communication (GJIC) [21]. It is found that the inhibition of GJIC is strongly linked to the biological phenomenon that involve the inflammatory and carcinogenic processes, suggesting that the inhibition of GJIC is involved in nongenotoxic cancer induction and tumor promotion [22]. It is reported that disruption of GJIC by hydrogen peroxide is protected by vitamin C [21], however, synthetic antioxidants such as Trolox has no ability to prevent hydrogen peroxide-mediated inhibition of GJIC [22], indicating the role of vitamin C on GJIC appears to be related to a different mechanism, such as the inhibition of signal transduction [22–24]. Therefore, it can be hypothesized that the chemopreventive effects of vitamin C in carcinogenesis may be associated with the protective effects of vitamin C against epigenetic mechanisms, such as inflammation and inhibition of GJIC, as well as to antioxidant activities [24] (Figure 2).

2. Epigenetics

Theory of epigenetics takes attention at processes involving in multistage carcinogenesis, which promotional phase of carcinogenesis is a consequence of epigenetic events involving inflammation and the inhibition of GJIC, mediating by ROS [25, 26].

Figure 2. Possible chemopreventive mechanisms of vitamin C in carcinogenesis. ROIs, reactive oxygen intermediates; GPx, glutathione peroxidase; GST, glutathione S-transferase; QR, quinone oxidoreductase; SOD, superoxide dismutase; CAT, catalase [24].

2.1 DNA demethylation and family of TET dioxygenases

It is known that cancers are caused by genetic alterations. These are genetic changes in oncogenes, such as mutations, deletions, amplifications, rearrangements and translocations, and changes in tumor suppressor or microRNA (miRNA) genes, that are involved in multistep carcinogenesis, and accumulate with tumor progression [27]. Nowadays, it is understood that classical genetics cannot explain all the properties of cancer alone, thus epigenetic abnormalities are also involved in tumorigenesis in addition to genetic alterations [27]. Thus, the epigenome means interface of environment and the genome. Epigenetic modifications catalyzed by certain enzymes and cofactors are the key to understanding molecular connections between the epigenome and the environment. Epigenetics is explained as heritable changes in gene expression that are not caused by DNA sequence alterations [28], including epigenetic alterations such as DNA methylation and histone modifications, that differ between cancer and normal cells [28]. The epigenome reflects the interface of a dynamic environment and the genome. Known epigenetic events include covalent modifications on nucleotides and histones, chromatin remodeling, and non-coding RNAs, which collectively constitute the epigenome [29].

Cytosine hydroxymethylation (5hmC) means of demethylating DNA and activating genes, which is a DNA modification associated with transcriptional silencing [30]. Methylation at the C5 position of cytosine (5-methylcytosine, 5mC) is the major modification of DNA and plays important roles in regulating transcription and cellular identity [30]. Methylation at the C5 position of cytosine (5-methylcytosine, 5mC) is the major and best-characterized epigenetic mark of mammalian DNA. However, 5hmC was generally regarded as oxidatively damaged cytosine in genome and may be replaced by DNA-repair mechanism [30, 31]. Methylation of DNA can change the functional state of regulatory regions, but it does not change the base pairing of cytosine, presenting epigenetic mark and is functionally involved in many forms of stable epigenetic repression [30, 31]. In honeybees DNA methylation patterns on cell and organismal fate is exemplified, in which differential DNA methylation determines whether the bee will be a worker or a queen [31, 32]. DNA methylation has also fundamental choices, such as gene silencing that leads to genomic imprinting, suppression of transposable elements, and the establishment of stable cellular identities [30–32], demonstrating that cellular reprogramming by nuclear transfer, cell fusion, and induced pluripotency can radically alter differentiated cellular states [33]. Epigenetic regulation by DNA methylation provides exciting insights into why reprogramming of cell fates is possible, showing that cytosines in mammalian cells can be hydroxymethylated to 5hmC (5-hydroxymethylcytosine). DNA methylation patterns are frequently observed in disease, particularly in cancer, including methylation of CGI promoters for tumor suppressor genes [34], supporting the recurring mutations in tumors, and thus providing insight into many aspects of biology and medicine. Factors that regulate methylation have been linked to human disease and contribute to malignances still remains largely unknown. The breakthrough in understanding the presence of 5hmC in the genome came from studies on a gene family that is known as ten-eleven translocation (TET) [35].

A group of enzymes termed methylcytosine dioxygenase ten-eleven translocation (TET, including TET1, TET2 and TET3) are identified to catalyze the hydroxylation of 5mC to 5-hydroxymethylcytosine (5hmC) [35, 36]. TET1 is located on human chromosome 10q21.3, TET2 on chromosome 4q24, and TET3 on chromosome 2p13.1. TETs further oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [37]. TETs belong to the Fe^{2+} and 2OG-dependent dioxygenase superfamily. All TET proteins contain a catalytic domain which binds

to Fe^{2+} and 2-oxoglutarate (2OG) to mediate oxidation of 5mC to 5hmC in DNA [37]. Subsequent experiments confirmed that TETs are Fe^{2+} and 2OG-dependent dioxygenases, and it is demonstrated that the catalytic activity of TETs is dependent on Fe^{2+} and 2OG. 2OG is also a critical intermediate metabolite of the Krebs cycle, and like collagen P4H, the catalytic activity of TET dioxygenases is indeed dependent on Fe^{2+} and 2OG [37]. All TET proteins contain a C-terminal catalytic domain that consists of a cysteine-rich region and a double-stranded β -helix fold characteristic of the Fe(II)- and 2-oxoglutarate (2-OG)-dependent dioxygenase superfamily [38]. These enzymes require Fe(II) as a cofactor metal and 2-OG as a cosubstrate to catalyze their reactions [38]. Demethylation of 5-methylcytosine (5mC) to 5-hydroxymethyl cytosine (5hmC) is shown to be mediated by TET proteins [38]. TET1 is identified as a Fe^{2+} and 2OG-dependent enzyme that converts 5mC to 5hmC. Thus, 5hmC, 5fC and 5caC have been proposed as demethylation intermediates. So far, our knowledge of TETs-mediated DNA demethylation is the following: the Fe^{2+} and 2OG-dependent TETs consecutively oxidize 5mC to 5hmC, then to 5fC and 5caC which can eventually be removed from the genome and substituted by unmodified C, thus completing the process of DNA demethylation. Although it involves multiple steps, the TET-mediated oxidation in combination with base excision repair constitutes the most important and consistent pathway responsible for the active demethylation of DNA. As being a DNA demethylation intermediate, 5hmC also serves as an epigenetic mark with unique regulatory functions [38–40].

2.2 Epigenetic alterations in cancer

It is demonstrated that tumor cells undergo various epigenetic modifications, DNA hypermethylation, that could lead to an imbalance in regulation of apoptotic genes, that is attributed as one of the important factors in the progression and treatment of cancer [38–40]. There are also small non-coding miRNAs are reported as epigenetic regulators, recently, regulating gene expression through posttranscriptional silencing of target genes.

2.2.1 miRNAs

In some cases, the prognosis and the progression of cancer are associated with changes in the expression of miRNAs during tumorigenesis. miRNAs lead to cleave the mRNA or inhibit translation, depending on the sequence complementarity between the miRNA and its target [41]. DNA methylation is a well-defined epigenetic mark. In humans DNA methylation occurs at cytosine residues in cytosine-guanine (CpG) dinucleotides and is controlled by enzymes called DNA methyltransferases (DNMTs), including DNMT1, DNMT3A and DNMT3B [42]. DNA hypermethylation by CpG promoter inactivating transcriptional tumor suppressor genes is known as one of the alterations that contributes to tumorigenesis in cancer cells [42]. In addition, cancer cells also undergoes hypomethylation at tissue-specific repetitive sequences, while these regions are heavily hypermethylated in normal cells [43], contributing DNA hypomethylation to tumorigenesis by causing chromosomal instability or the reactivation of transposable elements [44, 45]. On the other hand, endogenous repeat element-driven activation of the oncogenic tyrosine kinase, CSF1R, is suggested that impaired epigenetic control and the subsequent transcriptional derepression of repeat elements may play roles in tumorigenesis. It is demonstrated that oncogenes can be activated by the derepression of endogenous repeats, in addition to genetic and epigenetic modifications, suggesting that activation of normally silenced genes by promoter DNA hypomethylation is involved in tumorigenesis (**Figure 2**) [45]. miRNAs are also thought to play a role in tumorigenesis by modulating tumor suppressor genes or

oncogenes, as epigenetic regulators of DNA methylation and histone modifications [46], contributing to tumorigenesis by controlling various biological processes, such as proliferation, differentiation, and apoptosis through regulation of or interactions with oncogenes or tumor suppressor genes, acting either as an oncogene or tumor suppressor gene depending on their target genes [47]. Up-regulation of miRNAs targeting tumor suppressor genes by overexpression, amplification, or epigenetic derepression might function as oncogenes inhibiting the activity of an anti-oncogenic pathway. By contrast, the genetic mutation, deletion or epigenetic silencing of a tumor suppressor miRNA that normally represses expression of oncogenes might result in derepression of oncogenes, thereby gain of oncogenic function. miRNAs have roles in the development of tumors, are also implicated in tumor progression by affecting migration, adhesion, and invasion of cancer cells [45–47].

2.2.2 Histone modifications

The roles of epigenetics in the development and progression of tumors is well established. A little is known about the epigenetic activation of cancer-associated genes, although it is focused on the epigenetic inactivation of tumor suppressor genes during tumorigenesis and DNA hypomethylation of some genes. Histone modifications are also important epigenetic marks that are involved in chromatin structure and gene expression, in addition to DNA methylation, including covalent modifications of histone tail residues acting in DNA packaging and regulating transcriptional machinery to coding sequences [48]. These histone modifications occur at histone residues, such as lysine, arginine and serine (methylated, acetylated and phosphorylated) [48, 49]. Gene expression may be regulated by interactions between multiple histone modifications [49], for example, in a growing body. In contrast, aberrant histone modifications, in addition to DNA methylation, are recognized as important epigenetic changes during tumorigenesis [50]. Tumor-suppressor genes are enriched with active histone marks in normal cells, while the transcriptional silencing of those genes in cancer cells is reported to be associated with a loss of active histone marks, and are shown to be common features of human cancer cells [50].

Recently, it is reported that overexpression of cancer-promoting genes in cancer is associated with the loss of repressive histone modifications, suggesting overexpression of oncogenes or cancer-promoting genes in tumors may contribute to tumorigenesis during epigenetic derepression (**Figure 3**). Epigenetically regulated genes may be promising therapeutic targets and biomarkers during tumor initiation. In fact, epigenetic mechanisms involved in the regulation of cancer-associated genes possible epigenetic therapies targeting epigenetically dysregulated genes are contributed to the improvement of patient outcomes [51, 52] (**Figure 3**).

Vitamin C on DNA demethylation is also reported [53]. It is found that vitamin C could directly enhance the catalytic activity of TET dioxygenases by uniquely interacting with the C-terminal catalytic domain of TETs, modulating the epigenetic control of genome activity [53]. Vitamin C acting as a cofactor in DNA demethylation catalyzed by TETs, deficiency of this vitamin, results in disruption of the methylation-demethylation dynamics of DNA and histone, which can contribute to phenotypic alterations or diseases [53].

3. Vitamin C as an epigenetic agent

Vitamin C is an water-soluble micronutrient that exists as ascorbate anion under physiological pH conditions. It is well established that ascorbate is an essential cofactor in various enzymatic reactions and, also and antioxidant and free radical

Figure 3.
Epigenetic regulation of cancer-associated genes. Modified from [45].

scavenger. It assists collagen P4H to complete the hydroxylation and thus prevents scurvy; overall, it is required to maintain a number of Fe^{2+} and 2OG-dependent dioxygenases in their fully active forms [53]. In recent years, it is identified that a number of novel Fe^{2+} and 2OG-dependent dioxygenases catalyze the hydroxylation of methylated nucleic acids (DNA and RNA) and methylated histones. Methylation of DNA and histone are the major epigenetic hallmarks in the mammalian genome [53, 54]. It has been shown that some of these dioxygenases require ascorbate as a cofactor to start DNA demethylation and histone demethylation processes [53, 54]. Vitamin C is suggested to effect the genome activity via regulating epigenomic processes [53, 54]. It serves as a cofactor TET dioxygenases that catalyze the oxidation of 5mC into 5hmC. Vitamin C also required for the JmjC domain-containing histone demethylases, acting as a cofactor for histone demethylation [53, 54]. Thus, by participating in the demethylation of both DNA and histones, vitamin C appears to be a mediator between the genome and environment. These findings demonstrate an unknown function of vitamin C in regulating the epigenome, which needs a re-evaluation of the functions of vitamin C in human health and diseases.

Life styles, such as smoking has effects on vitamin C availability. Smoking has also been shown to reduce vitamin C levels in the plasma, strongly [55]. Deficiency of vitamin C results in digestive diseases such as ulcerative colitis, Crohn's disease, chronic gastrointestinal and kidney diseases [55, 56]. Insufficient vitamin C intake during pregnancy, affect the embryonic development due to the changing in the catalytic activity of TETs, resulting in certain types of developmental defects, such as neural tube defect (NTD), and also increases the risk of gastroschisis which is a congenital defect of the abdominal wall [57].

Vitamin C has a long controversial history as a treatment for cancer. As a cofactor for TETs, it enhances the catalytic activity of TETs in cancer cells, and acts in the reprogramming of cancer cells by enhancing the activity of TETs. In cancerous cells, the TET-mediated DNA active demethylation appears to be downregulated [34–40], and a low level of 5hmC is identified as a novel epigenetic hallmark of cancer [34–40]. Mutations in TETs lead to the loss of 5hmC in cancer [34–40]. If vitamin C is deficient, enzymatic activity of TETs are adversely effected, resulting in 5hmC reduction. Studies have demonstrated higher

Figure 4.
Epigenetic therapy. Modified from [45].

incidence of scurvy in cancer patients [58, 59]. An association between ascorbate transporters and cancer is also indicated [58, 59]. These findings suggest that vitamin C plays a critical role in the demethylation of DNA and histone, serving as a cofactor for TET, thus, deficiency of this vital vitamin may disrupt the methylation-demethylation dynamics of DNA and histone, and may contribute to phenotypic alterations in different cells along the developmental stages and aging, cancer and other diseases [58, 59], (Figure 4).

3.1 Vitamin C and epigenetic regulation in cancer

Studies over the past decades has declared that normal epigenetic regulation is disrupted during tumorigenesis [60], indicating that DNA methylation is the most common event in carcinogenesis [60]. DNA methylation of CpGs in promoters can result in silencing of various genes, including tumor suppressors [61], as a consequence, disrupted gene expression in cancerous cells also can cause alterations in the methylation of lysine or arginine amino acids on histone tails [62].

Since hypermethylation of promoters of tumor suppressor genes been identified as one of the important factors supporting cancer development, demethylation agents are become the main focus of molecular-targeted therapeutics. Vitamin C is shown to play a central role in the conversion of 5mC to 5hmC by enhancing the catalytic activity of TET dioxygenases [56, 57], indicating, vitamin C is an important factor in reducing the risk of promoter hypermethylation and supporting the maintenance of the 5hmC state that plays a major role in the epigenetic regulation [56–58].

3.1.1 Vitamin C and DNA demethylation

Requirement for vitamin C as an additional cofactor for dioxygenases indicated a potential role for this reducing vitamin in TET-mediated DNA demethylation,

suggesting that vitamin C has the capacity to modify DNA methylation in human cells [56], for instance, it is known that epigenetic reprogramming occurs during the embryonic development, which involves DNA demethylation and re-methylation. Vitamin C is found to cause DNA demethylation of nearly 2000 genes in embryonic stem cells [56]. These results indicate that vitamin C can be involved in the DNA demethylation process, however, it is unclear whether vitamin C participates directly in DNA demethylation or it is mediated by the enhanced catalytic activity of TETs by vitamin C. DNA hypermethylation is found at promoters of tumor suppressors and developmental genes, typically, whereas CpG sites in other gene regions are hypomethylated in cancerous cells. 5hmC is lower in a most of cancers, due to a loss of TET activity [56–58], as a result of inactivating mutations, down-regulation of TET gene expression, or insufficient supply of TET co-factors. In embryonic stem cells, in which vitamin C addition is shown to promote DNA demethylation through increased TET activity [63], supports the idea of sufficient vitamin C is important for maintenance of normal 5hmC levels. High-dose vitamin C is also shown to compensate for the loss of TET proteins [64]. Histone modifications and the expression of the genes that regulate these modifications are frequently disrupted in cancer by mutations, translocations/ amplifications, or deletions [65]. The loss of TET activity caused by DNA hypermethylation *in vitro* demonstrated increased methylation at tumor suppressor gene promoters [64, 65], suggesting that vitamin C is a cofactor for TET dioxygenases in the conversion of 5mC to 5hmC, thus modulating DNA demethylation [65] (Figure 5).

3.1.2 Vitamin C and histone demethylation

The basic unit of eukaryotic chromatin is composed of a short length of DNA wrapped around an octamer that consists of 2 copies of each histone (H2A, H2B, H3 and H4), called nucleosome. Histones are substrates for post-translational modifications (PTM). PTMs on histones include methylation, acetylation, phosphorylation, and others [66]. The dynamic PTMs in the histone regulate genome stability, gene transcription and chromatin structure. Methylation at lysine and arginine amino acids is epigenetic modification in histones. Histone methylation is a key component

Figure 5.
The role of TET proteins in DNA demethylation [65].

in the epigenome along with DNA methylation. The most studied methylation occurs on histone H3 at lysine (K) 4 (H3K4), H3K9, H3K27, H3K36, H3K79, H4K20 and on histone H3 at arginine (R) 2 (H3R2), H3R8, H3R17, H3R26, H4R3. The methyl donor in histone methylation is S-adenosylmethionine (SAM), the same donor for DNA methylation. JmjC domain histone demethylase 1 (JHDM1) was purified, which specifically demethylates H3K36 in the presence of Fe^{2+} and 2OG [67]. After a long time, 20 proteins that belong to the JmjC domain have been discovered to have the catalytic capacity to demethylate histones [67]. It is now known that the JmjC domain-containing histone demethylases, like TETs, also belong to the Fe^{2+} and 2OG dioxygenase superfamily, that demethylate mono-, di-, and trimethylated histone lysine residues [68], indicating that vitamin C is required for optimal catalytic activity of JHDM1 [68]. It appears to be important in the late phase of reprogramming from terminally differentiated cells, which is also involved in cell differentiation, such as T cell maturation, indicating the role of vitamin C in demethylation of DNA and histone in T cell maturation [69]. The role of vitamin C in histone demethylation is only examined in *in vitro* assays, deducing that vitamin C can be a cofactor for the JmjC domain-containing histone demethylase family, thus modulating histone demethylation in a similar way as it does on DNA demethylation [68–70].

3.1.3 Vitamin C and the loss of 5hmC in cancer

In contrast to the high level of 5hmC in embryos, cancer cells have very low or undetectable 5hmC. It is reported that the loss of 5hmC is a novel epigenetic hallmark of most, especially in certain types of human cancer [56–58]. The loss of 5hmC is resulted in disruption in DNA methylation-demethylation processes leading to malignant transformation [56–58]. Mutations in TETs or a decreased expression of TETs are also attributed to the loss of 5hmC in cancerous cells [56–58], requiring further studies to determine whether there is a local vitamin C deficiency in cancer cells. Linus Pauling proposed the treatment of cancer patients with intravenous vitamin C, in 1970s [71], followed by other investigators, such as Mutlu Demiray in Turkey, nowadays [72]. Epigenetic modulation of vitamin C in the gene activity might shed a new light on this issue. As a cofactor for TETs, vitamin C is found to maximize the catalytic activity of the TETs in cancer cells. In the light of these findings, it can be suggested that the rebuilding the 5hmC content can offer a potential treatment for certain cancers.

3.1.4 Vitamin C in epigenetic treatment of cancer

Vitamin C is a safe and well-tolerated dietary supplement that is utilized in patient care and in treating cancer. Tumor cells are known to be resistant to programmed cell death. It is considered to induce E-cadherin expression in sensitizing tumor cells towards apoptosis [73], as increased expression of E-cadherin is declared to sensitize cancerous cells to cell death [74]. Thus, reactivation of E-cadherin seems to be an important target for epigenetic therapy in cancer. Researchers observed an increase in the expression of E-cadherin by a combination of 5-AZA + vitamin C [75]. They reported an increase in E-cadherin expression by treatment with vitamin C and highlighted the role of vitamin C as an epigenetic player, opening a window for vitamin C-enhanced, TET-dependent conversion of 5mC to 5hmC [75]. Vitamin C consumption may also increase the activity of other epigenetic regulators such as histone demethylases, for which new drugs are currently being developed. Based on the effects of vitamin C on the methylation of DNA and histones, epigenetic regulation has implications in all cancers.

4. Conclusion

Vitamin C is an essential compound with functions far beyond scurvy prevention. As an important mediator between genome and environment, it participates in the demethylation of DNA and histones, epigenome. Genetic and environmental factors that influence the synthesis, absorption, transportation and metabolism of vitamin C could have significant consequences for health and disease by regulating the epigenetic control of genome activity. Cancer is driven by epigenetic modifications along with genetic changes. Vitamin C activates the TET enzymes which are responsible for the removal of methyl groups for DNA and histones, regulating DNA demethylation as an essential cofactor for TET dioxygenases, and regulating histone demethylation as an essential cofactor for Jmjc domain-containing histone demethylases. Deficiency in vitamin C contributes to different diseases, resulting of failure to maintain the catalytic activity of TET dioxygenases and Jmjc domain-containing histone demethylases. Diet and lifestyle are known to affect the level of vitamin C in the human body, dramatically. Deficiency in vitamin C is seen in cancer patients frequently, thus, adequate dietary vitamin C in these patients is needed increasingly, who have mutations in epigenetic regulators. This novel epigenetic function of vitamin C needs to become recognized by the general public. Future studies needs be done for greater understanding of vitamin C impact upon TET and the epigenome which have medicinal relevance in cancer and other diseases.

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