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Vitamin C in Stem Cell Reprogramming and Cancer

Luisa Cimmino, Ph.D.[1,2,#], **Benjamin G. Neel**[2], and **Iannis Aifantis, Ph.D.**[1,2,#]

[1]Department of Pathology, NYU School of Medicine, New York, NY, 10016, USA.

[2]Laura and Isaac Perlmutter Cancer Center and Helen L. and Martin S. Kimmel Center for Stem Cell Biology, NYU School of Medicine, New York, NY, 10016, USA.

Abstract

Vitamin C is an essential dietary requirement for humans. In addition to its known role as an anti-oxidant, vitamin C is a cofactor for Fe^{2+} and α-ketoglutarate-dependent dioxygenases, which comprise a large number of diverse enzymes, including collagen prolyl hydroxylases and epigenetic regulators of histone and DNA methylation. Vitamin C can modulate embryonic stem cell (ESC) function, enhance reprogramming of fibroblasts to induced pluripotent stem cells (iPSCs) and hinder the aberrant self-renewal of hematopoietic stem cells (HSCs) through its ability to enhance the activity of either Jumonji-C-domain containing histone demethylases or ten-eleven translocation (TET) DNA hydroxylases. Given that epigenetic dysregulation is a known driver of malignancy, vitamin C may play a novel role as an epigenetic anti-cancer agent.

Keywords

Vitamin C; anti-oxidant; epigenetic reprogramming; stem cells; histone demethylases; TET proteins; 5-hydroxmethylcytosine; DNA methylation; leukemia; cancer therapy

Main Text

Vitamin C – a novel epigenetic regulator

Vitamin C is an essential dietary requirement for humans, most well known for its role in protection against scurvy, a disease caused by severe vitamin C deficiency that if left untreated can be fatal and characterized by hemorrhages and poor wound healing, [1]. Vitamin C replenishment in the diet reverses the symptoms of scurvy, largely attributed to vitamin C-mediated restoration of the activity of collagen proly hydroxylases, a family of Fe^{2+} and α-ketoglutarate-dependent dioxygenases (α-KGDDs) that regulate collagen synthesis [2]. Many other α-KGDDs also depend on vitamin C as a cofactor to maintain their enzymatic activity, including key epigenetic regulators of histone demethylation and DNA hydroxymethylation that play pivotal roles in the epigenetic reprogramming of stem cells and cancer. Recent studies have shown that vitamin C can protect hematopoietic stem cells from epigenetic alterations that drive leukemia progression and, therefore, the ability of

[#]**To Whom Correspondence Should Be Addressed:** Luisa Cimmino, Ph.D. or Iannis Aifantis, Ph.D. Department of Pathology and Laura and Isaac Perlmutter Cancer Center, New York University School of Medicine 521 First Avenue, Smilow 1303 New York, NY 10016 luisa.cimmino@nyumc.org or iannis.aifantis@nyumc.org.

vitamin C to modulate the epigenome has re-ignited interest in the potential therapeutic benefits of vitamin C. Pharmacological doses of vitamin C have also been show to synergize with standard chemotherapy in the treatment of both solid and hematopoietic cancer cells. In this review we summarize the known biological functions of vitamin C, its homeostatic regulation in the body and role as a cofactor of α-KGDDs to modify the epigenome of stem cells. In addition, we highlight the potential benefits of high-dose vitamin C treatment as an anti-cancer therapy.

Vitamin C biosynthesis and uptake.

Most mammals are capable of synthesizing vitamin C *de novo* in the liver from glucose via the enzymatic action of L-gulono-γ-lactone oxidase (GULO). However, in humans and other primates, in addition to guinea pigs, bats and fish, the GULO gene is mutated, rendering its product inactive and making vitamin C an essential dietary requirement [1]. In order to maintain optimal physiological levels of vitamin C, the recommended daily intake is 200mg/day, which results in a plasma concentration of approximately ~70–80μM and can be readily sustained from the consumption of dietary supplements or a variety of fruits and vegetables [1, 3]. Prolonged periods of low dietary vitamin C intake (<10mg/day), leading to plasma levels below ~10μM, manifests in its most severe form as scurvy. However, milder vitamin C deficiency may be underreported owing to its non-specific symptoms such as fatigue, irritability, dull aching pains, and weight loss [4, 5]. In the United States, it is estimated that more than 7% of the population (>20 million people) are deficient in vitamin C [5].

Vitamin C transport and homeostasis.

Vitamin C is water soluble and absorbed in the diet through the intestinal lumen for whole body distribution via the bloodstream. In the gastro-intestinal tract, the ionized form of vitamin C, ASC, and its oxidized counterpart, DHA, are absorbed by luminal cells through different mechanisms, including passive diffusion, facilitated diffusion and active transport [3]. Passive diffusion is limited by the low hydrophobicity of ASC and DHA, and only contributes to a fraction of the overall regulation of vitamin C homeostasis in the body [6].

Transport of DHA, but not ASC, occurs by facilitated diffusion through a number of glucose transporters (GLUT1–4) with varying tissue distributions and affinities for vitamin C, and is competitively inhibited by glucose [3]. Facilitated diffusion of DHA through GLUT1 is the primary mode of vitamin C uptake by erythrocytes, Once inside the cell, DHA is reduced to ASC by glutathione (GSH), thus maintaining a concentration gradient that favors DHA uptake but only to the equivalent of plasma concentrations (See Box 1) [3]. In contrast to erythrocytes, the intracellular concentration of vitamin C in other cells can range from 1–4mM in white blood cells, up to as high as 10mM in brain cells [7]. The ability of cells to achieve such high (mM) intracellular concentrations of vitamin C from low (μM) plasma levels is attributed to the action of two sodium-dependent vitamin C transporters (SVCTs) that utilize the sodium gradient across plasma membranes to transport ASC but not DHA. SVCT1 is primarily expressed in epithelial cells and regulates gastrointestinal absorption and renal re-absorption, whereas SVCT2 is expressed in most tissues and is thought to be responsible for systemic cellular uptake [3, 7].

Plasma levels of vitamin C are tightly controlled by the two SVCTs, which in turn exhibit characteristics of substrate-dependent regulation. Following oral administration of very high doses of vitamin C (>500 mg/day), maximal plasma concentrations do not exceed 150μM, due to homeostatic down-regulation of SVCT1, which impedes intestinal absorption and kidney re-absorption, leading to urinary excretion of excess vitamin C [8]. Conversely, vitamin C deficiency can also promote the upregulation of both SVCT1 and SVCT2 mRNA expression in various tissues including the liver and intestine [3], highlighting the importance of these transporters in regulating vitamin C homeostasis.

The anti-oxidant role of vitamin C.

Vitamin C plays an important role in protecting cells from oxidative stress by maintaining intracellular redox balance. Acting as an electron donor, vitamin C can reduce reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals, singlet oxygen, and hypochlorous acid generated during normal metabolic respiration/mitochondrial oxidative phosphorylation (aerobic ATP generation). By quenching free radicals, vitamin C can therefore protect against mutations induced by oxidative DNA damage, lipid peroxidation, and the oxidation of amino acid residues to maintain protein integrity [1].

Vitamin C is a key cofactor for Fe^{2+} and α-KG-dependent dioxygenases.

In addition to the anti-oxidant role of vitamin C, its ability to act as an electron donor to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) is a key mechanism by which vitamin C acts as a cofactor for α-KGDDs. Diverse families of α-KGDDs are regulated by vitamin C, including prolyl hydroxylases [9], and epigenetic regulators such as the Jumonji-C (JmjC) domain-containing histone demethylases (JHDMs) [10], DNA and RNA demethylases of the AlkB homolog (ALKBH) family [11, 12] and the ten-eleven translocation (TET) family of DNA hydroxylases [13, 14] (see Box 2).

The α-KGDDs have relatively high K_ms for vitamin C (140–300uM), and for this reason may require above 1 mM intracellular levels for optimal activity [8]. Vitamin C is often essential for maximal α-KGDD activity and cannot be substituted in the reaction by other anti-oxidants such as spermidine, vitamin B1, vitamin E, glutathione, NADP, dithiothreitol or L-cysteine, indicating a specific need for vitamin C as a cofactor for these enzymes [2, 14, 15]. Furthermore, vitamin C has been shown to bind directly to certain α-KGDDs such as the TET proteins [14]. However, there is also evidence that vitamin C may act simply as a reducing agent to maintain iron in the ferrous state, independently of any direct cofactor activity given that, under certain reaction conditions, it has been shown that other anti-oxidants in addition to vitamin C can reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) to maintain the activity of the TET enzymes (new ref). Vitamin C may therefore play an indirect role in maintaining the activity of α-KGDDs through the regulation of redox active iron (see Box 3).

Epigenetic reprogramming of stem cells by vitamin C.

In 2010, two independent studies showed that vitamin C could promote DNA demethylation in ESCs [16] and enhance the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) [17]. Vitamin C is necessary to maintain ESC proliferation *in vitro* [16] and it

was originally added to the media of somatic cells to counteract the ROS generated during reprogramming in an attempt to improve the efficiency or quality of iPSCs generated [17]. However, vitamin C proved to be substantially more efficient at enhancing iPSC generation than other anti-oxidants. Moreover, inhibitors of α-KGDDs impaired reprogramming [10], implicating these enzymes in the mechanism of vitamin C-mediated reprogramming.

Jumonji histone demethylases in vitamin C-mediated reprogramming.— JHDMs were initially proposed to be key effectors of iPSC reprogramming downstream of vitamin C. Vitamin C has been shown to enhance the activity of JHDM1a/1b (KDM2a/2b) to promote H3K36me2/3 demethylation in mouse embryonic fibroblasts in culture and during reprogramming [10]. Consistent with these observations, overexpression of *Jhdm1a/1b* potently enhanced reprogramming, whereas knockdown impaired iPSC generation [10].

H3K9 methylation has been shown to be a barrier during somatic cell reprogramming into iPSCs [18]. Vitamin C, acting via JMJDa/1b (KDM3a/3b) induces a specific loss of H3K9me2 in ESCs [19], and can drive pre-iPSC-to-iPSC transition by enhancing both JMJD1 (KDM3) and JMJD3 (KDM4)-mediated H3K9me2/3 demethylation at core pluripotency gene loci [18]. Interestingly, vitamin C also enhances iPSC generation at least in part by delaying cell senescence [17], through JHDM1b-mediated removal of H3K36me2/3, which leads to silencing of the senescence-inducing *Cdkn2a* (*Ink4/Arf*) locus [10].

TET proteins in vitamin C-mediated reprogramming.—Recent studies have shown that enhanced iPSC reprogramming and DNA demethylation induced by vitamin C is also TET-dependent. *Tet1* or *Tet2*-depleted MEFs are unable to generate iPSC colonies, whereas overexpression of TET proteins enhances reprogramming [20–22]. Vitamin C can dramatically increase 5hmC production in ESCs and during the reprogramming of mouse and human fibroblasts to iPSCs [13, 15, 23]. The rapid increase in 5hmC observed in ESCs treated with vitamin C accumulates at transcriptional start sites (TSSs), and is followed by DNA demethylation at the promoters of germ line genes normally expressed during formation of a blastocyst-like state [13, 14]. Notably, 100μM vitamin C is sufficient to increase 5hmC by up to ~4-fold above basal levels in ESCs within 24hrs of treatment. Even larger effects are seen on the levels of 5fC (10-fold increase) and 5caC (20-fold increase), the successive oxidation products of 5hmC catalyzed by TET proteins that trigger active DNA demethylation [14]. The effects of vitamin C on 5hmC, gene expression and DNA demethylation are lost in $Tet1^{-/-}Tet2^{-/-}$ ESCs [13, 14] highlighting the importance of TET proteins as downstream targets of vitamin C-mediated DNA demethylation. Vitamin C supplementation also increases reprogramming efficiency by activating the expression of several microRNAs [10] and by preventing the aberrant DNA hypermethylation and silencing of imprinted genes at the *Dlk1-Dio3* gene cluster [24] that could be mediated by a combination of increased JHDM and TET activity.

Vitamin C-mediated epigenetic regulation of hematopoietic stem cells.

A role for vitamin C in maintaining physiological levels of 5hmC and fully functional TET enzymatic activity in hematopoietic stem cells (HSCs) has recently gained attention. TET

proteins are known tumor suppressors of the hematopoietic lineage (reviewed in Guillamot et al., 2016) [25]. Of the three *TET* genes, *TET2*-inactivating mutations are the most prevalent, occurring in up to 30% of patients with myelodysplasia (MDS), acute myeloid leukemia (AML) and clonal hematopoiesis of indeterminate potential (CHIP), a pre-malignant state seen in approximately 10% of healthy elderly individuals that increases their risk of progression to AML [26, 27].

TET loss of function has also been modeled genetically in mice. *Tet1*-deficiency leads to aberrant self-renewal and expansion of HSCs with a B-cell lineage bias [28, 29], whereas *Tet2*-deficiency causes aberrant self-renewal with a myeloid lineage bias [30–32]. In addition, the combined loss of *Tet1/2* restricts malignancy to the B cell lineage whereas *Tet2/3* deficiency causes an accelerated acute myeloid leukemia [28, 33]. In these mouse models, deficiency of TET proteins causes loss of 5hmC in the genome of HSCs and DNA hypermethylation that is associated with lineage-specific gene expression changes and genomic instability. Given the history of claims that vitamin C is important for maintaining a healthy immune system [34], the potential for vitamin C to modulate HSC function through the regulation of epigenetic factors had been surprisingly overlooked until recently.

Vitamin C deficiency and leukemia progression.—Recently, Agathocleous *et al.*, using a metabolomic screening approach, found that vitamin C levels are highest in human and mouse HSCs, compared with more differentiated hematopoietic cell types [35]. The vitamin C transporter *Svct2 (Slc23a2)* was also expressed most abundantly on HSCs, compared with lineage-restricted progenitors and mature immune cells. Using $Gulo^{-/-}$ mice [36], the authors showed that vitamin C deficiency led to increased HSC frequency and caused a loss of 5hmC in the genome. These effects could be reversed upon dietary vitamin C intake, implicating deficient TET activity as the cause for an aberrant HSC expansion [35]. In addition, vitamin C deficiency modeled systemically ($Gulo^{-/-}$) or using cell-intrinsic vitamin C transporter knockout mice ($Slc23a2^{-/-}$) [37] was shown to cooperate with the $Flt3^{ITD}$ oncogene to accelerate leukemogenesis in bone marrow transplantation studies. Vitamin C deficiency exacerbated 5hmC loss in HSCs with heterozygous or homozygous loss of *Tet2*, suggesting that a vitamin C-depleted micronutrient environment could globally impair the activity of TET proteins, including TET1 and/or TET3 [35].

Restoration of TET function by vitamin C treatment.—*TET2* mutations found in patients are almost exclusively heterozygous and affect the ability of the enzyme to bind Fe^{2+} or α-KG in the catalytic domain, leading to impaired hydroxylation of 5mC and DNA hypermethylation [38, 39]. We hypothesized that enhancing the activity of residual wild-type protein encoded by the non-mutated allele, or potentially increasing the activity of mutant TET2 proteins to restore normal levels of TET2 activity, could benefit patients with *TET2*-deficient diseases. Indeed, using genetic mouse models of reversible RNA interference, we found that restoration of endogenous *Tet2* expression levels in *Tet2*-knockdown cells is sufficient block aberrant HSC self-renewal, increase 5hmC, promote DNA demethylation and upregulate the expression of genes important for myeloid cell differentiation [40]. Vitamin C treatment of *Tet2*-deficient HSPCs could mimic *Tet2* restoration, causing increased 5hmC formation, a block in aberrant self-renewal of human or mouse HSPCs and

suppression of disease progression *in vivo* [40]. Similar to studies of ESCs and iPSCs, sensitivity to vitamin C and 5hmC induction was dependent on the amount of total TET expression. *Tet2* and *Tet3* account for >95% of *Tet* mRNA expressed in mouse HSPCs. Combined loss of *Tet2 and Tet3* rendered HSPCs resistant to vitamin C treatment and severely deficient in their ability to generate 5hmC [40], suggesting that a threshold of TET expression is required for vitamin C to modulate HSPC self-renewal.

High-dose vitamin C as an anticancer agent.

Vitamin C levels are tightly regulated in human plasma to maintain physiological concentrations of 50–70μM, limiting the ability to achieve high cellular uptake by oral consumption alone [3, 8]. The concentration of vitamin C used *in vitro* (250μM) in our study exceeded normal plasma levels in mice or maximum achievable levels following dietary administration (~150μM) [5, 41]. Pharmacokinetic studies in humans have shown that intravenous (IV) administration of sodium L-ascorbate can generate up to 30mM peak plasma levels, 100-fold higher than the levels produced by high-dose oral administration, with minimal toxicity [42–44]. The first documented studies using high-dose IV vitamin C in cancer therapy were published by Linus Pauling and Ewan Cameron in the 1970s with reports of some efficacy [45]. Subsequent clinical trials of high-dose vitamin C, however, failed to show any benefit, most likely because these studies used oral administration only [46, 47]. Recent clinical trials and case studies have shown efficacy of vitamin C as an anticancer agent when administered at high-dose IV to treat patients with a variety of solid tumors, including breast, ovarian, prostate, kidney, lung and liver cancer [42–44, 48]. Exactly why only some tumors respond remains unclear, although the Cantley group has implicated RAS-pathway induced upregulation of GLUT1, followed by increased uptake of DHA and consequent redox stress, as a potential mechanism.

The ability of vitamin C to suppress leukemia progression by enhancing TET enzymatic activity [35, 40] suggests that TET deficiency might be a response biomarker for vitamin C therapy. Future clinical trials could target diseases of TET2 deficiency, including CHIP, MDS and AML, and lymphoid malignancies such as Diffuse Large B-cell Lymphoma (DLBCL) and Angioimmunoblastic T-cell Lymphoma (AITL), where *TET2* loss-of-function mutations are also prevalent [25].

Vitamin C as a hypomethylating agent.

Similar to the initial studies in ESCs that showed widespread DNA hypomethylation upon vitamin C treatment [16], we have also shown that vitamin C drives DNA hypomethylation and expression of a TET2-dependent gene signature in human leukemia cell lines [40]. DNA hypermethylation is a hallmark of aberrant HSCs from MDS and AML patients with *TET2* mutation [49, 50], and DNA hypomethylating agents, such as 5-azacytidine (5-aza) and 5-aza-2-deoxycytidine (decitabine), elicit a higher response rate in patients with *TET2* mutations [51]. Vitamin C has been shown to synergize with decitabine in a TET2-dependent manner to increase 5hmC and enhance DNA hypomethylation and upregulate the expression of endogenous retroviral genes, triggering an innate viral mimicry response that promotes apoptosis of several human cancer cells lines [52]. In the latter study, physiological concentrations of vitamin C were used that are achievable by oral administration (57μM

daily doses) suggesting that vitamin C supplementation in the diet could enhance therapeutic responses in patients treated with DNA hypomethylating agents.

Vitamin C as an adjuvant for cancer therapy.

Patients with hematopoietic malignancy or other cancers are often markedly vitamin C-deficient [52–54], and restoring or maintaining physiological levels has been shown to slow malignant cell growth in multiple settings, including leukemia [35, 52, 55]. However, pharmacological doses of vitamin C, administered IV, are receiving increasing attention in cancer research due to the ability to exploit multiple therapeutic mechanisms of action. At low doses, vitamin C acts as an anti-oxidant and maintains sufficient levels of iron in the ferrous state to promote the activity of dioxygenases. However, at higher doses, vitamin C can behave as a pro-oxidant causing oxidative stress and depletion of GSH that leads to the accumulation of reactive oxygen species (ROS) (see Box 4). Recently, high-dose vitamin C was shown to be selectively toxic to KRAS or BRAF mutant colorectal cancer cells [56]. In that study, increased cellular uptake of oxidized vitamin C (DHA) via upregulated GLUT transporters led to GSH depletion and lethal levels of ROS [56]. High-dose vitamin C also enhances the sensitivity of multiple hematopoietic malignancies to arsenic trioxide [54, 57] and increases chemosensitivity and radiosensitivity of various cancer cells including ovarian [58], pancreatic [59] glioblastoma and non-small cell lung carcinoma cells [60]. Increased labile iron and GSH depletion are hallmark effectors of ferroptosis, a form of non-apoptotic cell death caused by lethal lipid peroxidation [61]. Given that high-dose vitamin C can promote increased redox-active iron mobilization and GSH depletion [56, 60], the ability to induce ferroptosis could be an additional mechanism by which vitamin C can exert its function as an anti-cancer therapy.

We have shown that Tet2 restoration in murine HSPCs, or vitamin C treatment of human leukemia cell lines, induces a base excision repair (BER) gene expression signature including the upregulation of poly-ADP ribose polymerase (PARP) genes [40]. Increased TET activity, mediated by vitamin C treatment, can potentially mimic oxidative DNA damage by catalyzing the formation of 5fC and 5caC. These modifications are a trigger for BER and active DNA demethylation that is dependent on PARP proteins [62, 63]. PARP inhibition can increase tumor sensitivity to DNA damage induced by chemotherapy, and results in synthetic lethality in cells lacking intact BER or harboring homologous recombination (HR) defects [64]. We showed that the combination of vitamin C treatment with the PARP inhibitor, Olaparib, enhances the killing of human AML cells greater than either agent alone, in the absence of elevated ROS [40]. TET-mediated DNA oxidation induced by vitamin C can therefore potentially mimic a DNA damage response in AML cells, making them hypersensitive to PARP inhibition and providing a novel therapeutic strategy for combination therapy.

Closing remarks.

Vitamin C has recently emerged as an important regulator of stem cell biology and cancer progression through its ability to modulate the epigenome. Despite tight control in whole-body distribution upon dietary intake, pharmacological levels of vitamin C can be achieved parenterally, with minimal toxicity to patients, and have the potential for broad efficacy in

the treatment of cancer. Maintaining a diet proficient in vitamin C could help prevent or suppress cancer progression, and pharmacological doses might synergize with DNA hypomethylating or DNA damaging therapies to improve outcomes in cancer patients.

Whether the primary anti-cancer mechanism of high-dose vitamin C action is through its role as a pro-oxidant is unclear, given that the contribution of enhanced dioxygenase activity in sensitization to standard therapies upon vitamin C treatment has not been widely studied. Identifying biomarkers of sensitivity will be important to stratify patients that could benefit the most from vitamin C treatment. In addition, improving upon the bioavailability of vitamin C and understanding the structural basis by which vitamin C can act as a specific cofactor for α-KGDDs may help in developing targeted therapies to treat patients with deficiencies in epigenetic regulators such as the TET proteins (see Outstanding Questions). Loss of function in epigenetic regulators is a hallmark of cancer and in the case of hematopoietic malignancy, a driver of disease progression. The potential for vitamin C to modulate the epigenome as a natural, non-toxic epigenetic therapy for cancer prevention and treatment has further expanded the role of this essential vitamin in human biology.

Prolyl hydroxylation, peptide hormone and lipid biosynthesis.—In the absence of vitamin C, collagen prolyl-4-hydroxylase (C-P4H) cannot catalyze proline hydroxylation in collagen, leading to defective collagen production and the systemic tissue breakdown characteristic of scurvy [3]. Vitamin C is also required for prolyl hydroxylation of the hypoxia-inducible factor (HIF), which targets HIF-1 for degradation [66] and enhances the activity of the asparaginyl hydroxylase factor inhibiting HIF-1 (FIH-1), an important suppressor of the transcriptional activity of HIF [67]. Vitamin C is also required for the conversion of dopamine to noradrenaline, and to enhance the activity of enzymes involved in the α-amidation of numerous pro-hormones and L-carnitine biosynthesis [2].

Histone demethylation.—Vitamin C has been shown to be required for the optimal activity and demethylation capacity of several JHDMs [68], which include over 20 proteins in humans that hydroxylate and remove mono-, di- or trimethyl-lysines in histones [69]. Histone demethylation is catalyzed by the JmjC-domain to produce a highly reactive oxoferryl species that hydroxylates the methylated substrate, allowing spontaneous loss of the methyl group as formaldehyde [70]. JHDM1 (KDM2) specifically demethylates H3K36, JHDM2A (JMJD1A, KDM3A) demethylates H3K9 and JHDM3A (JMJD2A, KDM4) demethylates both trimethylated H3K9 and H3K36 to regulate chromatin state and gene expression [69].

DNA and RNA repair.—The mammalian ALKBHs are a family of nine dioxygenases (ALKBH1–8 and FTO) that oxidize the alkyl groups on alkylation-damaged nucleic acid bases, acting as DNA/RNA repair enzymes or direct demethylases that remove cytotoxic 1-methyladenine (1-meA), 3-methylcytosine (3-meC) and etheno-adenine (εA) [71]. The oxidized alkyl groups are subsequently released as aldehydes, regenerating the undamaged bases. Both FTO (fat mass and obesity associated) and ALKBH5 repair primarily N6-methyl-adenine (m6A) in RNA [71]. Interestingly, human variants of the FTO are associated with an increased body mass index and predisposition to diabetes [72, 73]. The potential for

vitamin C to modulate body weight through the activity of FTO however has not been explored.

DNA hydroxymethylation.—TET proteins (TET1–3) catalyze the hydroxylation of 5-methylcytosine (5mC) residues in DNA to 5-hydroxymethylcytosine (5hmC), which, in turn, can be oxidized further to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). The oxidative products of 5mC catalyzed by TET proteins can be stable modifications in the genome or transient modifications that provide a trigger for DNA demethylation [74–76]. Vitamin C directly enhances the catalytic activity of TETs leading to increased oxidation of 5mC to enhance 5hmC, 5fC and 5caC formation, and at physiological concentrations of Fe^{2+} (10μM) vitamin C treatment at concentrations ranging from 50–500μM increases TET activity in a dose-dependent manner and accelerates their reaction rate by up to 8-fold [14].

Glossary

Ascorbate (ASC).
The reduced form of vitamin C that participates as an electron donor and maintains the activity of Fe^{2+} and α-ketoglutarate dependent dioxygenases.

Dehydroascorbate (DHA).
The oxidized form of vitamin C that enters cells via glucose transporters and can be reduced back to ascorbate.

L-gulono-γ-lactone oxidase (GULO).
An enzyme that catalyzes the final step of vitamin C biosynthesis in the liver of mice but is non-functional in humans.

Sodium-dependent vitamin C transporters (SVCTs).
One of two molecules (SVCT1/2) that use a sodium gradient to actively transport ascorbate, generating high intracellular concentrations of vitamin C.

Glucose transporters (GLUTs).
Facilitate the transport of dehydroascorbate in competition with glucose.

α-ketoglutarate dependent dioxygenases (α-KGDDs).
A diverse family of enzymes that are also iron-dependent and utilize vitamin C as a cofactor.

Jumonji-C-domain containing histone demethylases (JHDM/KDM).
Vitamin C-dependent α-KGDDs catalyze lysine demethylation and regulate epigenetic reprogramming of fibroblasts into iPSCs.

Ten-eleven translocation (TET) proteins.
Vitamin C-dependent α-KGDDs that catalyze the oxidation of 5-methylcytosine in the genome to regulate DNA methylation, reprogramming, stem cell pluripotency and act as tumor suppressors of hematopoietic malignancy.

5-methylcytosine (5mC).

A methylated base modification generated by the activity of DNA methyltransferase enzymes to regulate gene expression and gene silencing.

5-hydroxymethylcytosine (5hmC).
A DNA modification generated by TET hydroxylase activity that protects cells from aberrant DNA hypermethylation and regulates gene expression.

Base Excision Repair (BER).
A mode of DNA repair involved in active DNA demethylation in response to TET activity, whereby a nucelotide is removed and replaced by a new base via a mechanism involving DNA glycosylases, endonucleases and ligases.

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Box 1.

Biologically active forms of vitamin C.

At physiological pH, vitamin C predominantly exists in its ionized form as ascorbate (ASC) [3]. ASC undergoes one-electron oxidation to form an ascorbyl radical that is relatively stable and can be enzymatically recycled back to ASC. However, two ascorbyl radicals can also dismutate to one ASC and one dehydroascorbate (DHA) molecule. DHA, which is structurally unstable, with a half-life of several minutes, irreversibly degrades if it is not rapidly reduced back to ASC by glutathione (GSH) and nicotinamide adenine diphosphate (NADP)-dependent enzymatic and non-enzymatic reactions [65]. Therefore, under physiological conditions, >95% of vitamin C is in the reduced ASC form in both intracellular and extracellular body fluids [1].

Box 2.

Dioxygenases regulated by vitamin C.

The ability of vitamin C to modulate the activity of numerous and diverse enzymes allows it to participate in a wide variety of biological processes.

Box 3.

Regulation of iron absorption and homeostasis by vitamin C.

Vitamin C could play an indirect role in maintaining the activity of α-KGDDs by regulating iron homeostasis. The ability of vitamin C to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) is the mechanism by which it enhances non-heme Fe^{3+} absorption from the diet and increases cellular uptake of transferrin-bound Fe^{3+} transported in plasma (recently reviewed in Lane and Richardson, 2014). Reduction of Fe^{3+} to Fe^{2+} allows it to enter cells through a divalent metal ion transporter, which can increase the intracellular redox active labile iron pool. In addition, vitamin C can stimulate synthesis of the cellular iron storage protein, ferritin, inhibit lysosomal ferritin degradation and cellular iron efflux, and can induce iron uptake from low molecular weight iron-citrate complexes [77].

Box 4.

A pro-oxidant role for high-dose vitamin C.

Vitamin C, at low concentrations, functions as a mild reducing agent and anti-oxidant, protecting cells from oxidative stress. At higher concentrations, however, it can act as a pro-oxidant, increasing oxidative stress to promote cell death, which can be exploited to target tumor cells. At high doses, vitamin C is oxidized in extracellular fluid to an ascorbate radical ($AscH^-$), causing iron to be reduced to the ferrous form ($AscH^- + Fe^{3+}$ to $Fe^{2+} + AscH^- + H^+$). Ferrous iron can react with oxygen to produce a superoxide anion (O_2^-), via the so-called Fenton reaction to form toxic levels of H_2O_2 [78]. The enzyme catalase, under physiological conditions, can metabolize H_2O_2 into oxygen and water [79]. However, elevated basal levels of ROS, deficiency in catalase activity or increased uptake of vitamin C by tumors cells can render them selectively vulnerable to the pro-oxidant effect of high-dose vitamin C [56].

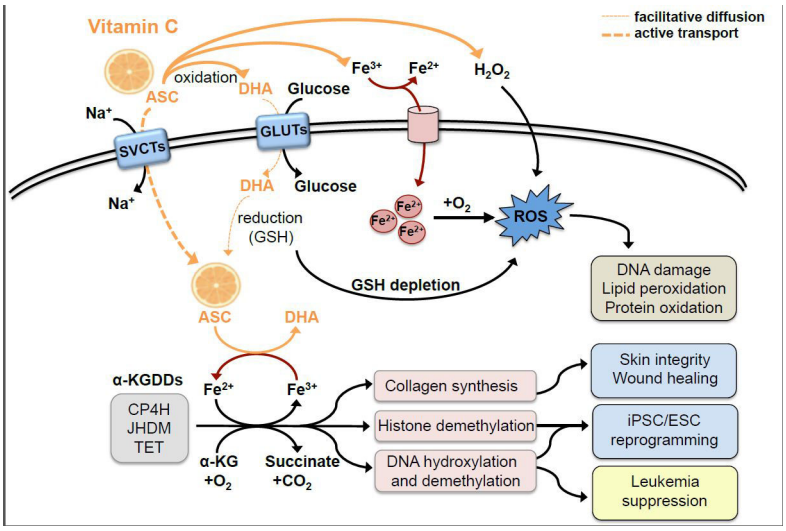

Figure 1. Vitamin C uptake and regulation of dioxygenases involved in epigenetic reprogramming and leukemia suppression.

Vitamin C is taken up from the plasma by sodium-dependent vitamin C transporters (SVCTs) in its reduced form (ascorbate, ASC) or can enter cells via glucose transporters (GLUTs) in its oxidized form (dehydroascorbate, DHA). Inside the cell, DHA is rapidly reduced back to ASC by glutathione (GSH). Vitamin C plays an important role in multiple biological processes by acting as a cofactor or Fe^{2+} and α-ketoglutarate dependent dioxygenases (α-KGDDs) including collagen prolyl hydroxylases (CP4H), JmjC-histone demethylases (JHDMs) and ten-eleven translocation (TET) DNA hydroxylases. These diverse enzymes regulate collagen synthesis, to maintain tissue integrity and for efficient wound healing, drive histone and DNA demethylation to enhance iPSC reprogramming and suppress leukemia progression. Vitamin C can also enhance the cellular uptake and intracellular mobilization of redox active iron (Fe^{2+}), and increase the production of reactive oxygen species (ROS) that can be exploited in cancer therapies to promote cell death.