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REVIEW

# Roles of vitamins in stem cells

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## Abstract

Stem cells can differentiate to diverse cell types in our body, and they hold great promises in both basic research and clinical therapies. For specific stem cell types, distinctive nutritional and signaling components are required to maintain the proliferation capacity and differentiation potential in cell culture. Various vitamins play essential roles in stem cell culture to modulate cell survival, proliferation and differentiation. Besides their common nutritional functions, specific vitamins are recently shown to modulate signal transduction and epigenetics. In this article, we will first review classical vitamin functions in both somatic and stem cell cultures. We will then focus on how stem cells could be modulated by vitamins beyond their nutritional roles. We believe that a better understanding of vitamin functions will significantly benefit stem cell research, and help realize their potentials in regenerative medicine.

**Keywords** Vitamin · Cell culture · Vitamin A · Vitamin $B_3$ · Vitamin C · Vitamin E · Embryogenesis · Stem cells

## Introduction

Vitamins are natural organic compounds that play essential roles in normal physiological functions in minimum amounts, but the host either cannot synthesize them, or cannot produce an adequate amount to meet the normal physiological demands [1]. The word vitamin comes from the Latin word "*vita*" meaning "life", which reflects its essential roles in the survival and well-being of humans [2]. Vitamins are involved in diverse cellular functions, and their deficiency often leads to serious symptoms to people, sometimes even death [3]. Since the discovery of vitamin A in 1912, 13 vitamins have been identified based on their essential roles in human health [4]. Most vitamins can be obtained through balanced food intake, and vitamin supplements are also widely used in healthcare practices. In the 1950s, people found that vitamin supplements are also essential for in vitro cell culture due to their nutritional functions [5, 6]. Recently, various vitamins are shown to possess regulatory mechanisms on the cellular level, especially in stem cells [7].

Stem cells are a special group of cells that can proliferate extensively and have the potential to generate various cell types in the human body [8]. Embryonic stem cells (ESCs) are pluripotent and can differentiate to all cell types. ESCs only transiently exist during embryogenesis, and finally give rise to all the cells in an embryo. Adult stem cells possess limited potential to differentiate to specific cell types, and can be classified into multipotent and unipotent stem cells [9]. They are responsible for the daily maintenance and repair of tissues [10]. With somatic reprogramming technologies, stem cells can now be generated from somatic cells with defined factors [11]. Stem cells are widely used in basic research to understand embryogenesis and homeostasis, to model diseases, and are also important source materials for cell therapies in regenerative medicine [12]. Most stem cell-related studies and applications involve cell culture systems, which provide essential components for specific cell types to survive and properly exert their normal functions.

A typical cell culture system normally contains ten categories of components, including water, inorganic salts, growth factors, amino acids, buffering reagent, energetic substrates, extracellular matrix, vitamins, vitamin-like organic factors and the cell culture atmosphere. Functional stem cells require a culture system in which all components

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are suitably balanced. To realize the great potentials of stem cells in regenerative medicine, people often modulate and optimize cell culture components to improve stem cell functions. Regulation of signal transduction pathways with growth factors has traditionally been the main approach [13, 14]. However, nutritional regulation is emerging as a viable target for stem cell modulation, which could affect not only cell survival but also pluripotency and cell fates [15–17]. As an essential part of cell culture, the important roles of vitamins are manifested in our daily use of cell culture in basic research and clinical applications. This article will try to review how vitamins are utilized in stem cell applications. We will first introduce the general vitamin requirements in cell culture. Then we will focus on vitamin A, vitamin $B_3$, vitamin C and vitamin E, and discuss how they are utilized in stem cell applications [18–21].

## A brief background on vitamins in the human body

Human vitamins are generally categorized into two classes, nine water-soluble vitamins and four fat-soluble vitamins (Table 1) [22]. Water-soluble vitamins include 8 members of the B type vitamins and vitamin C, and fat-soluble vitamins include vitamins A, D, E and K. All the vitamins can be obtained from food to fulfill the nutritional needs (Table 1). Some vitamins can be synthesized in the human body, but at a very low rate (Table 2) [23, 24]. In this review, we will briefly summarize some key vitamin-dependent processes and the role these vitamins play in stem cell biology.

All the water-soluble vitamins are coenzymes for important metabolic enzymes that are essential for cellular functions. Their essential roles in metabolic pathways are illustrated in Fig. 1. Vitamins $B_1$, $B_3$, $B_6$ and $B_7$ are involved in glucose metabolism that includes glycolysis, pentose pathway, glycogenolysis and gluconeogenesis. Fatty acid synthesis and degradation require vitamins $B_2$, $B_3$ and $B_5$. Meanwhile, amino acid degradation requires vitamins $B_3$, $B_6$, $B_9$ and $B_{12}$. The TCA cycle and oxidative phosphorylation take place in mitochondria, and utilize vitamins $B_1$, $B_2$, $B_3$, $B_5$ and $B_7$ in specific steps. Often times, multiple vitamins are involved in the same metabolic process. For example, When acetyl-CoA is generated from pyruvate by pyruvate dehydrogenase, four of the five coenzymes involved in this step are vitamins, including vitamins $B_1$, $B_2$, $B_3$ and $B_5$ [25, 26]. Any deficiency in these vitamins could lead to malfunction of the TCA cycle.

Besides type B vitamins, other vitamins' functions are more diverse. Vitamin C is the only water-soluble vitamin that does not belong to the vitamin B family, and it is known to regulate collagen synthesis by acting as a cofactor for prolyl hydroxylases, reducing its iron center [27–29]. In addition, vitamin C is an antioxidant that suppresses the production of reactive oxygen species (ROS). It is well known for its role in the prevention of scurvy [30, 31]. Vitamin A family members have distinctive functions, including the prevention of night blindness. At the molecular level, vitamin A functions through antioxidation and transcriptional regulation [32, 33]. Vitamin D is a hormone that binds to nuclear receptors to regulate transcription, and it is best known for its role in calcium absorption [34]. Vitamin E is a potent fat-soluble antioxidant. Some vitamin E isoforms were also reported to modulate signal transduction [35]. Vitamin K is a cofactor for γ-glutamyl carboxylase that is essential for blood clotting [36, 37].

**Table 1** Vitamins and their functions [25, 26, 34, 36, 71, 92, 161, 166, 199, 207, 248, 249, 251, 253–259]

| Vitamin | Names | Daily dose | Cellular function |
|---|---|---|---|
| Vitamin $B_1$ | Thiamine | 1.2 mg | Glycolysis, non-oxidative phase of pentose pathway |
| Vitamin $B_2$ | Riboflavin | 1.2 mg | Coenzyme in carbohydrate and lipid metabolism; activation of $B_6$ and $B_9$; antioxidant |
| Vitamin $B_3$ | Niacin, nicotinamide, nicotinamide riboside | 15 mg | Coenzyme in carbohydrate, amino acid and lipid metabolism |
| Vitamin $B_5$ | Pantothenic acid | 5 mg | Coenzyme in carbohydrate and lipid metabolism, lipid biosynthesis |
| Vitamin $B_6$ | Pyridoxine, pyridoxamine, pyridoxal | 1.5 mg | Coenzyme in glycogenolysis and amino acid metabolism |
| Vitamin $B_7$ | Biotin | 30 µg | Lipid synthesis; leucine catabolism; conversion of amino acids and propionate to glucose in liver; gluconeogenesis |
| Vitamin $B_9$ | Folic Acid | 400 µg | Coenzyme in nucleotide synthesis, methylation of chromatin, DNA, RNA, histone and transcription factors, amino acid metabolism |
| Vitamin $B_{12}$ | Cobalamin | 2.4 µg | Coenzyme in folate and homocysteine metabolism |
| Vitamin C | Ascorbic acid | 85 mg | Antioxidant; coenzyme in collagen synthesis |
| Vitamin A | Retinoic acid, retinol, all-trans-RA | 800 µg | Vision, cell differentiation, reproduction |
| Vitamin D | Cholecalciferol, ergocalciferol | 15 µg | Mg, Ca and P absorption |
| Vitamin E | Tocopherols, tocotrienols | 15 mg | Antioxidant, cell membrane integrity |
| Vitamin K | Phylloquinones, menaquinones | 115 µg | Protein synthesis in blood coagulation |

**Table 2** Vitamins in cell culture media [1, 39, 44, 45, 47, 50, 71, 79, 155, 157, 235, 260–265]

| Vitamin | Concentration in blood | Concentration in DMEM/F12 | Endogenous source | Application in stem cells |
|---|---|---|---|---|
| Vitamin $B_1$ | 66–200 nM | 6.44 μM | Gut bacteria | In all the base medium; used for somatic and stem cell culture |
| Vitamin $B_2$ | 174–471 nM | 0.58 μM | Colon bacteria | In all the base medium; used for somatic and stem cell culture |
| Vitamin $B_3$ | 81–213 nM | 16.6 μM | Biosynthesis from tryptophan<br>Colon bacteria | In all the base medium; used for somatic and stem cell culture |
| Vitamin $B_5$ | 0.5–1.9 μM | 4.7 μM | Colon bacteria | In all the base medium; used for somatic and stem cell culture |
| Vitamin $B_6$ | 15–73 nM | 9.8 μM | Colon bacteria | In all the base medium; used for somatic and stem cell culture |
| Vitamin $B_7$ | > 400 ng/L<br>1.64 nM | 14.3 nM | Hindgut bacteria | In most base medium, such as BME, α-MEM, Ham's F12 and DMEM/F12; Used for somatic and stem cell culture |
| Vitamin $B_9$ | > 3.0 nM | 6 μM | Gut bacteria | In all the base medium; used for somatic and stem cell culture |
| Vitamin $B_{12}$ | 118–716 pM | 50 nM | Gut bacteria; not clear whether $B_{12}$ can across colon | In most base medium, such as α-MEM, Ham's F12 and DMEM/F12; Used for somatic and stem cell culture |
| Vitamin A | 1.4–3.2 μM | – | – | Retinol promotes self-renewal and pluripotency; Retinoic acid mainly drives cell differentiation by modulating epigenetics |
| Vitamin C | 25–85 μM | – | In kidney and liver (except high primates) | Supports cell reprogramming, survival and collagen production; Reduces ROS |
| Vitamin D | 30–100 μg/L | – | Precursor synthesized in the sebaceous glands of the skin | Leukocyte production and differentiation |
| Vitamin E | 20–35 μM | – | – | Component of B-27 supplement and chemically defined lipid concentrate; Protects stem cells and progenitor cells against oxidative stress; Affects ESC differentiation through ROS levels |
| Vitamin K | 0.22–2.22 nM | – | Menaquinones synthesized by bacteria in the large intestine | Promotes the differentiation of dental pulp stem cells (DPSCs) to osteoblast in vitro |

## Essential vitamins in regular cell culture

Because of vitamins' important functions, they are essential not only for the whole organism but also for individual cells. However, the vitamin dependency of the human body is often different from cells in culture media. The importance of individual vitamins is gradually discovered through the years. In 1950, Morgan and colleagues first showed that cell survival was improved by a vitamin mixture in serum-free synthetic medium [38]. In 1955, Eagle systematically analyzed the impact of individual vitamins on the growth of both mouse fibroblasts and Hela cells [39]. Six vitamins were shown essential for cell proliferation of both cell lines. They all belong to the B vitamin complex, including $B_1$, $B_2$, $B_3$, $B_5$, $B_6$ and $B_9$. The medium was named Basal Medium Eagle (BME), which is the first synthetic medium with defined vitamin functions. In the following years, Minimum Essential Medium (MEM) and Dulbecco's Modified MEM (DMEM) were developed with increased amino acid or vitamin concentrations, but the vitamin composition still remained at six [40, 41].

Although the above basic media can support short-term proliferation of a few cell lines, their capacity is insufficient for many other lines in long-term culture. To support clonal growth and long-term culture of Chinese hamster cell lines, Ham developed the Ham's F-10 and F-12 media that contain additional $B_7$ and $B_{12}$ [42, 43]. $B_7$ was important for the cell growth and viability of a variety of cell types [44], while vitamin $B_{12}$ was found to be essential for lipid metabolism [45, 46]. People later found that cell growth is improved when DMEM and Ham's F12 are mixed in a 1:1 ratio, and this medium was named DMEM/F12 [47]. The eight B vitamins ($B_1$, $B_2$, $B_3$, $B_5$, $B_6$, $B_7$, $B_9$ and $B_{12}$) in DMEM/F12 are also present in a variety of other basic media such as RMPI, IMDM and α-MEM [48]. These vitamins are generally considered as essential vitamins for most cells cultured in vitro. DMEM/F12 is the most commonly used base medium for human embryonic stem cells [17, 49–51], so we will use

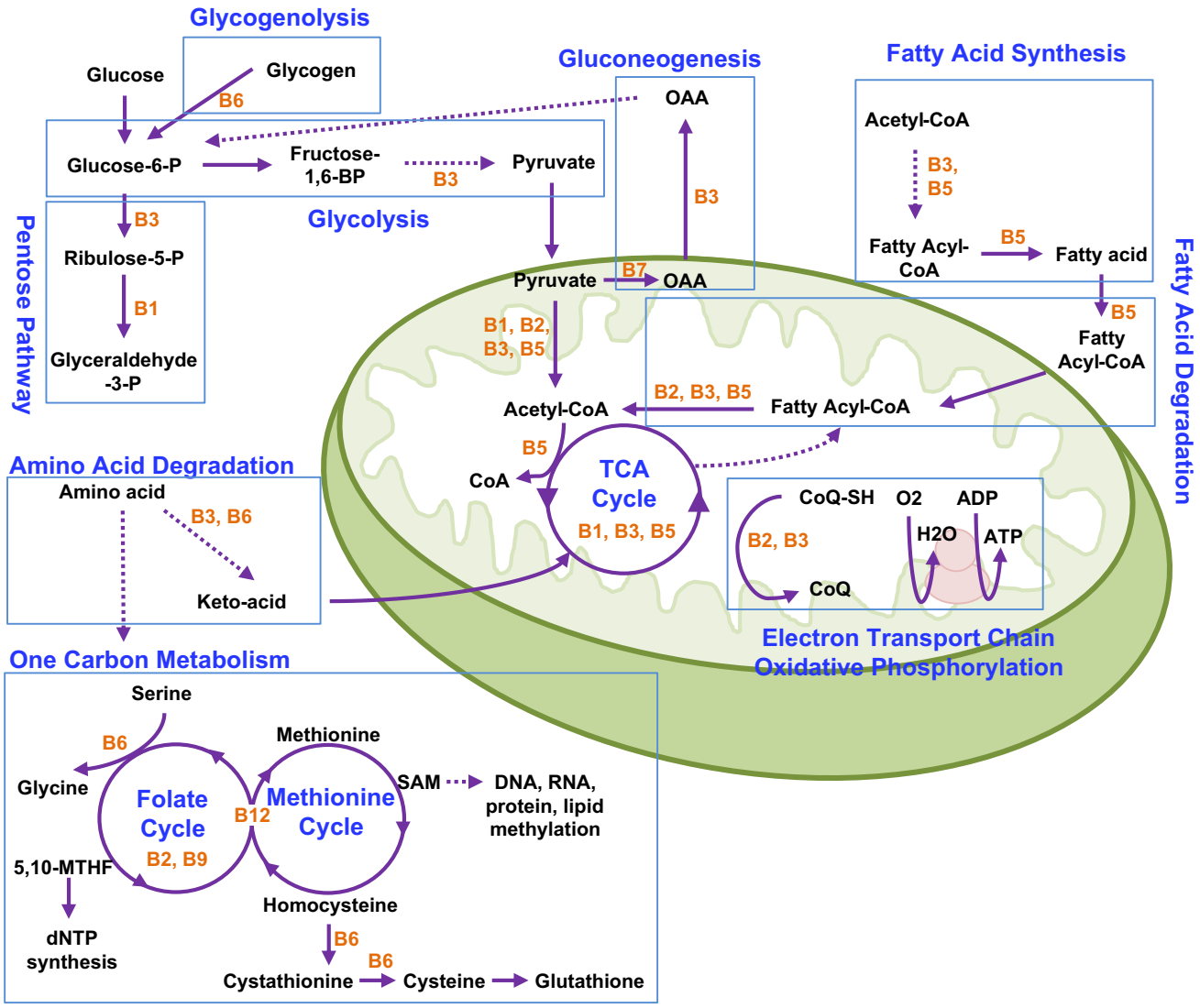


**Fig. 1** Vitamin B in metabolism. B vitamins are essential for the major metabolic pathways. Specific vitamins (orange fonts) are highlighted in multiple metabolic processes (blue fonts). *OAA*, oxaloacetic acid

DMEM/F12 as a reference to discuss vitamin formula and concentration effect in this review.

When comparing vitamin composition in blood and in DMEM/F12, there are two obvious discrepancies (Table 2). First, all B vitamins are present in DMEM/F12, but not other vitamins including vitamins A, C, D, E and K. Considering all B vitamins are coenzymes for essential metabolic processes, it is understandable that they are required for cell culture. It also implies that the non-B vitamins are probably not required for most cell types. It is also possible that those vitamins could be provided through serum or medium supplements such as B27. Second, all individual vitamins are provided at significantly higher concentrations in DMEM/F12 than in blood (Table 2). It indicates that cells in culture have differential reliance on vitamins.

## Vitamin-like nutrients for cell culture

In addition to essential vitamins, DMEM/F12 and many other basic media also contain minute amount of some organic compounds that are required to be supplemented to the organism from food sources (Table 3). We will brief some of them here.

Choline can be biosynthesized from serine [52], and it was first demonstrated as essential for cell survival and proliferation in Eagle's original vitamin study [39]. Choline is essential for the generation of phosphatidylcholine (PC) that is crucial for lipid transport and plasma membrane integrity. Choline is also used to generate acetylcholine that is important for neurotransmission [39, 53]. Choline can serve as methyl donor in one-carbon transfer pathways, and

**Table 3** Vitamin-like factors [1, 266–268]

| Names | Solubility | Function | Endogenous source |
|---|---|---|---|
| Choline | Hydrophilic | Lipid transport and metabolism, neurotransmission, methyl group donor | Choline de novo synthesized through S-adenosylmethionine (SAM)-dependent methylation of phosphatidylethanolamine by phosphatidylethanolamine N-methyltransferase (PEMT). This process occurs mostly in liver |
| *myo*-inositol, Inositol | Hydrophilic | Signal transduction and osmoregulation | Inositol can be de novo synthesized from glucose, and the biosynthesis occurs in brain, liver, and kidney |

contribute to DNA modulation and histone epigenetic modification with the help of vitamins $B_9$ and $B_{12}$ [54].

Inositol can be naturally synthesized by the human body from glucose in many tissues [55, 56], and Myo-inositol was also identified by Eagle as an essential factor for cell survival and proliferation in a wide variety of human cells, both malignant and nonmalignant [57]. Myo-inositol is the main source of phosphatidylinositol that mediates cell signal transduction, neurotransmission and osmoregulation [58, 59].

Besides choline and inositol, essential fatty acids, such as omega (ω)-3 and 6, are often found in culture media. They are metabolized to form eicosanoids that affect lipid homeostatic processes as well as the inflammatory response [60–63]. These lipids usually bind to albumin, and can be supplemented to cells through albumin without notice.

## Vitamin dependence in cell culture

Cells in culture, including somatic and stem cells, have different vitamin dependency in comparison with the human body as a whole. We believe that such difference is caused by the inherent difference between the human body and artificial cell culture systems. First, not all vitamins that are needed for the human body will be essential for cell culture. The deficiency of some vitamins often affects just one or a few specific organs in the human body. For example, vitamin K deficiency usually affects blood clotting but no other physiological functions [37, 64]. When it comes to cell culture, a vitamin may not be required for general cell culture if it is needed for the survival and proliferation of a specific cell type. Second, the human body usually has specific organs to produce and store vitamins, which allows people to tolerate temporary vitamin deficiency. However, there is no endogenous backup mechanisms to complement vitamin needs in cell culture, and all essential vitamins have to be provided. If an essential vitamin is not provided in culture, severe symptoms often emerge quickly in cells. For this reason, cell culture platforms have led to novel discoveries of vitamin functions in recent years. Third, some nutrients are not essential for the body, because specific organs can produce sufficient amounts for all the cells in the body. However, in cell culture, these nutrients are considered vitamin-like for cell culture, because they have to be provided for normal cellular functions in the medium. Fourth, cell culture is an artificial system, and nutrient concentrations in cell culture can be modulated as needed. Often times, nutrients can be tested and studied at concentrations that do not exist in physiological conditions. Some novel vitamin-dependent phenomena could only be identified in cell culture, in artificial conditions.

Differential vitamin dependence exists not only between individual cells and the whole organism, but also among different cell types. Vitamins affect metabolism similarly in both somatic and stem cells, but they could have additional impacts on stem cells. In somatic cells, modulation of specific vitamins will not change the cell identity. However, such changes might lead to loss of stemness or cell fate changes in stem cells. A few vitamins have gathered intensive interest in stem cell applications, and we will discuss them in more details here.

## Vitamin A

Vitamin A was the first vitamin discovered, and is actually a group of compounds also known as retinoids, including retinol, retinal and retinoic acid (RA) (Fig. 2). Vitamin A compounds are usually found in food of animal origin, while their precursor, carotenoid, is present in plants. Humans can synthesize vitamin A from carotenoids such as β-carotenes, a lipid-soluble pigment responsible for the vivid colors in plants. β-Carotenes can be converted into two retinals by β-carotene 15,15′-deoxygenase [65]. Retinal is then reduced to retinol by retinaldehyde reductase, using NAPDH (vitamin $B_3$) as a cofactor. Retinol either is esterified by acyltransferases LRAT (lecithin-retinol acyltransferase) and ARAT (retinol acetyltransferase) into retinyl palmitate for storage, or is oxidized into retinoic acid by aldehyde dehydrogenase (ALDH) [66]. In human cells, retinal and retinol are interconvertible; however, the conversion to retinoic acid is irreversible [67].

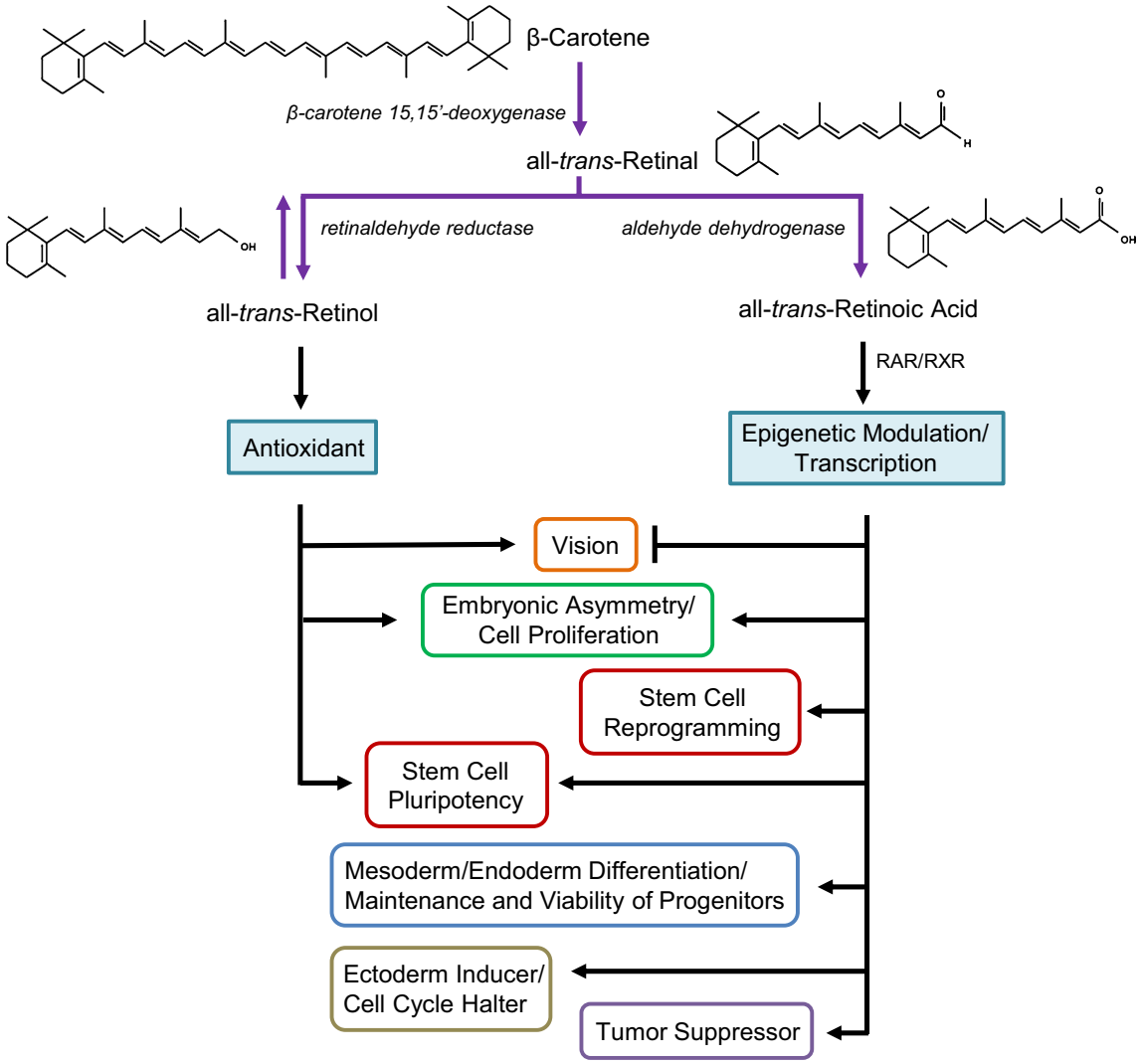


**Fig. 2** Vitamin A metabolism and function. Vitamin A is a group of compounds derived from β-carotene. In humans, its alcohol isoform has been reported to be beneficial for visual health. It also acts as an antioxidant and promotes the pluripotency of stem cells. The acid form, all-trans-retinoic acid, can interfere with the effect of retinol in vision. Retinoic acid is a determinant for embryonic development, and promotes cell proliferation. It can also promote reprogramming of stem cells and differentiation of progenitors and act as a tumor suppressor

Although belonging to the same family, retinal, retinol and retinoic acid play quite different roles in the human body. For example, retinal is incorporated into the light sensitive receptor rhodopsin in the retina, and prevents night blindness. In contrast, retinoic acid can cause night blindness by suppressing retinal production through the transcriptional inhibition of ocular retinol dehydrogenases [33].

Vitamin A family members also play distinctive roles in embryogenesis and stem cells in culture. Retinol and retinal are readily oxidized in culture, so they can act as antioxidants to promote cell survival and growth [32, 68]. Retinol has been reported to help maintain the pluripotency and self-renewal of hESCs [69], mESCs [70] and other progenitor cells [71–73]. In contrast, retinoic acid is a strong cell fate modulator, which will be discussed in more detail below.

As a lipid-soluble compound, retinoic acid can diffuse into the cytoplasm, bind to its nuclear receptor, and initiate nuclear translocation and downstream regulation. Retinoic acid initiates the dimerization of retinoic acid receptor (RAR) and retinoid X receptor (RXR). The heterodimer then either directly regulates gene expression through a DNA response element, or indirectly modulates transcription through intermediate transcription factors [74]. Over 500 genes are influenced by the action of retinoic acid, and many of the genes are involved in stem cell differentiation and metabolism [74]. It was shown to be an inducer that

initiates differentiation in ESCs, and also a modulator in lineage specific differentiation [75].

Retinoic acid modulates stem cell pluripotency and differentiation through the expression of mRNA and microRNA [21, 76]. It alters the expression of genes involved in DNA methylation, histone acetylation and histone methylation. In hESCs, the average level of DNA methylation is increased by RA, promoting stem cell differentiation [77]. RA also affect histone modifications, including acetylation of H3, H4 and H3K in hESCs and mESCs, which leads to stem cell differentiation [78, 79]. RA suppresses methylation in H3K27 while promoting methylation in H3K4 in mESCs and neuroblastoma, both stimulating cell differentiation [78]. At the same time, retinoic acid targets genes in metabolism, cell proliferation and pluripotency. It usually suppresses pluripotency gene expression, and promotes ectodermal differentiation in ESCs upon the exit of self-renewal [21, 80, 81]. Retinoic acid is used to promote neural differentiation through MAPK and integrin pathways [82].

Retinoic acid's roles during embryogenesis has been well documented. Retinoic acid promotes the expression of genes involved in the development of central nervous system, embryonal circulatory as well as heart asymmetry [83]. Vitamin A-deficient embryos presented various congenital malformations, such as absence of eyes as well as deficiencies in the central nervous system, skin, lungs and heart [84–86].

Retinoic acid also plays critical roles in cell fate determination in later stage of embryogenesis. For example, in heart development, retinoic acid is involved in cardiac differentiation. It modulates vascularization by suppressing the gene expression of N-cadherin, Msx1 and TGFβ pathways; It affects heart asymmetry through the inhibition of Nodal, Snail and Pitx2 genes; It also promotes cell proliferation and enhances BMP2 pathway by affecting the cardiogenesis transcription factor GATA4 [87–94]. Based on retinoic acid's function in embryogenesis, it has been used to generate atrial cardiomyocytes [20]. In hematopoiesis, retinoic acid enhances the ex vivo maintenance and viability of transplantable hematopoietic stem cells [95]. Retinoic acid suppresses the proliferation of dormant hematopoietic stem cells (HSCs), and prevents HSC differentiation to downstream cell types [96, 97]. As a result, retinoic acid helps maintain the multipotency of HSCs, being enriched in these cells compared to other multipotent progenitors [97–99]. Furthermore, retinoic acid is also involved in germline differentiation. Due to its interaction with BMP and NOTCH pathways, retinoic acid's targets are involved in four main developmental stages of fetal germ cell development [82, 93, 100]. Retinoic acid increases the expression of germline markers VASA, SCP3, TEKT1 and GDF9 [101], and promotes the generation of tailed male gamete-like cells that could generate offspring in mice [102].

Enzymes involved in retinoid acid production play essential roles in embryogenesis. The oxidation of retinol to retinal is the rate-limiting step in RA production, and the enzymes RDH10 (short-chain dehydrogenase in charge of the second oxidation of retinol) and DHRS3 (short-chain dehydrogenase reductase in charge of reducing retinal to retinol) are key in this process. Knockouts of these enzymes result in developmental defects in craniofacial, heart and limb patterning. RDH10-K.O. is lethal between E10.5 and E14.5, and DHRS3-K.O. is lethal between E17.5 and E18.5 [103–105]. Retinaldehyde dehydrogenase, which facilitates the generation of retinoic acid from all-trans retinal, is a key enzyme involved in cell fate determination [20, 66].

Although retinoic acid leads to ESC differentiation, it is also paradoxically a potent promoter for somatic reprogramming. Somatic cells can be reprogrammed to induced pluripotent stem cells (iPSCs) by the overexpression of transcription factors, such as OCT4, KLF4, MYC and SOX2 [11, 106, 107]. The activation of retinoic acid pathway accelerates reprogramming, while its removal suppresses reprogramming efficiency [108, 109]. The activation of retinoic acid pathway is essential component in chemically induced reprogramming without overexpressing transcription factors [110, 111]. Short-term treatment with retinoic acid is reported to promote pluripotency of iPSCs by inhibiting the canonical Wnt pathway, while positively modulating AKT/mTOR signaling [112]. Additionally, retinol and RA promote the transcription of Ten-eleven translocation (Tet) proteins in naïve pluripotent stem cells, and the regulation of Tet proteins by vitamin A is independent of vitamin C, a known modulator of enzymatic activities (see more discussions in "Vitamin C" section) [113]. In addition, retinoic acid signaling is found to maintain the dormancy of HSCs through cell cycle regulation [97].

## Vitamin $B_3$

Similar to vitamin A, vitamin $B_3$ is also a family of compounds including niacin (nicotinic acid), nicotinamide (NAM) and nicotinamide riboside (NR). They are precursors of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) that serve as cofactors or substrates in a wide range of metabolic reactions [114, 115], so they are implicated in all metabolic processes that utilize NAD or NADP (Fig. 1). Because of NAD's importance in metabolism, there are both de novo and salvage pathways for NAD synthesis from niacin, nicotinamide and NR (Fig. 3). Nicotinamide is usually maintained at around 100–200 nM range in blood, while 16.6 μM nicotinamide is supplied in DMEM/F12, which is sufficient to sustain nutritional requirement of cells in vitro (Table 2).

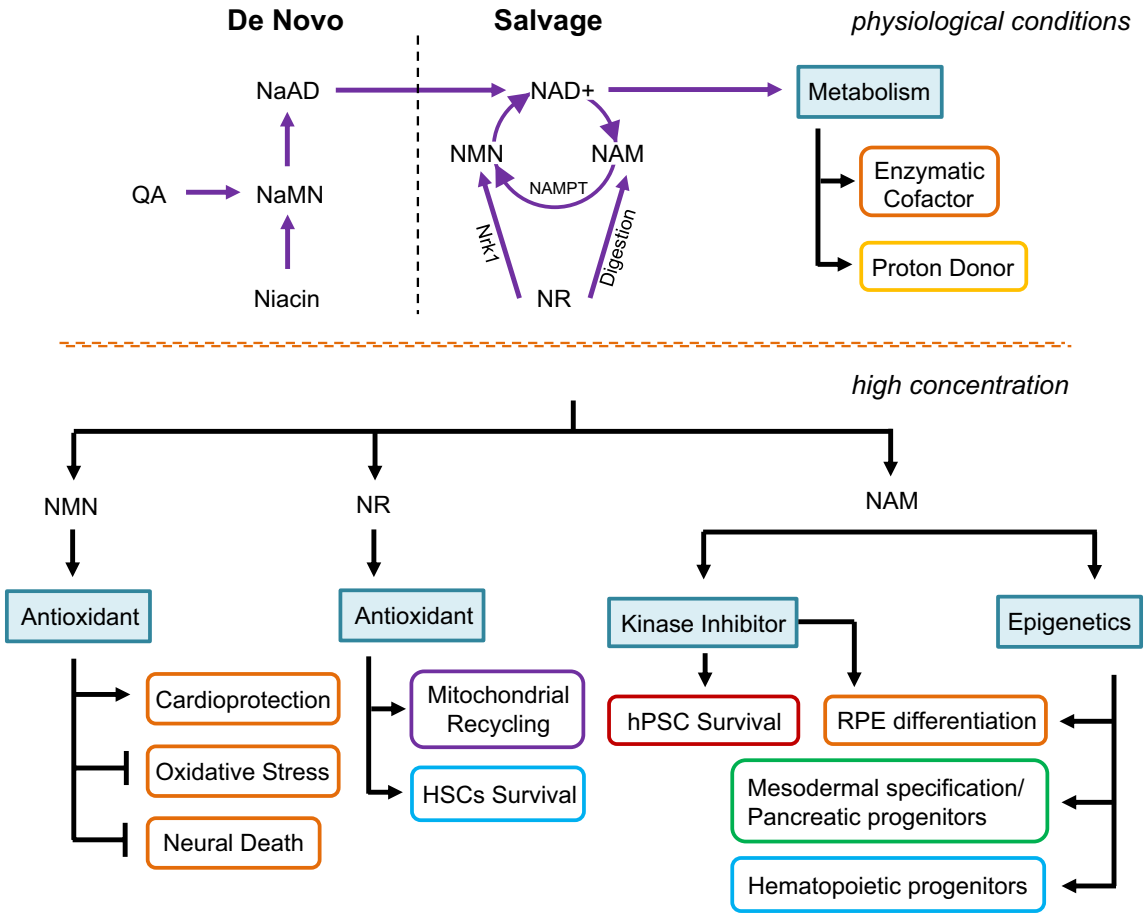


**Fig. 3** Vitamin $B_3$ function in metabolism and signal transduction. $NAD^+$ is synthesized in humans by de novo and salvage pathways. In the salvage pathway, the enzyme nicotinamide phosphoribosyltransferase (NAMPT) converts NAM (nicotinamide) to NMN (nicotinamide mononucleotide), which is then metabolized to $NAD^+$. NAMPT is the rate-limiting step in this process and is a crucial factor to maintain $NAD^+$ levels. Nrk1 can directly phosphorylate NR (nicotinamide riboside) to NMN, bypassing NAMPT, and NR can also be digested into NAM. In the de novo pathway, QA (quinolinic acid) and niacin are metabolized into NaMN (nicotinic acid mononucleotide) which can be further catalyzed into NaAD (nicotinic acid adenine dinucleotide). At high concentration, NAM functions as inhibitors in sirtuin, PARP and kinase pathways

**Table 4** Clinical applications of nicotinamide [269–275]

| Conditions | Dose of nicotinamide |
|---|---|
| Acne | 750 mg/day |
| Vitamin $B_3$ deficiency (Pellagra) | 300–500 mg/day |
| Diabetes | 1.2 g/mL/day |
| Hyperphosphatemia | 0.5–1.75 g/day |
| Larynx cancer | 60 mg/kg of niacinamide/h before inhaling carbogen |
| Skin cancer (other than melanoma) | 0.5 g niacinamide/day |
| Osteoarthritis | 3 g/day |

Nicotinamide has been utilized in clinical applications (Table 4). Nicotinamide ameliorates age-related macular degeneration phenotypes [116]. It prevents hepatosteatosis in obese mice while improving glucose metabolism and increasing health span in mice [117]. These therapeutic effects imply that nicotinamide could be involved in functions beyond nutritional regulation.

Compared to regular culture for somatic cells, a higher concentration of nicotinamide (5–10 mM) are often used in stem cell manipulations [19]. Nicotinamide in medium can easily cross plasma membrane and translocate into cytoplasm [19]. Nicotinamide was reported to promote cell survival of hESCs. In differentiation, it promotes cardiomyocyte differentiation, and facilitates the generation of endocrine pancreatic cells [118, 119]. Nicotinamide is also used in the maintenance of somatic stem cells [120], as well as organoid culture of different cell types [121–123]. It is used in the expansion of hematopoietic progenitors [124].

Nicotinamide is involved in various stem cell applications, but its exact molecular mechanism in each process is

still unclear. At high concentration, nicotinamide can inhibit the activities of sirtuins, a family of protein deacetylases that regulate epigenetic modification and potential cell fates [125]. Nicotinamide is used to enrich $CD34^+$ hematopoietic progenitors as a SIRT1 specific inhibitor [124]. At the same time, nicotinamide is also an inhibitor of poly(ADP-ribose) polymerase (PARP) that is involved in cell death [126, 127]. It is thought to improve cell survival by inhibiting apoptosis.

Recently, nicotinamide was identified as a kinase inhibitor at high concentration (millimolar range) [19]. Nicotinamide targets multiple kinases that are involved in cell survival and pluripotency. It binds and inhibits ROCK kinases, and it suppresses cell death caused by ROCK activation after cell individualization. Nicotinamide is also an inhibitor of casein kinase 1 (CK1). The inhibition of CK1 leads to the exit of self-renewal, and also promotes differentiation towards retinal pigment epithelium [19]. It is foreseeable that nicotinamide could be involved in additional stem cell regulations as a modulator in sirtuin, PARP and kinase pathways.

The concentration-dependent phenomena also exist in some other nicotinamide derivatives, such as nicotinamide mononucleotide (NMN) and NR. Recent studies show that NMN and NR have functions beyond NAD synthesis. With elevated concentration, NMN reverses vascular dysfunction and oxidative stress, and promotes cardioprotection via glycolysis and acidic pH [128, 129]. NMN also protects against cognitive impairment and neuronal death induced by the inhibition of long-term potentiation (LTP) after Aβ1–42 oligomer treatment [130]. NR at elevated concentration increases mitochondrial recycling and cell survival in hematopoietic stem cells [131]. It also prevents aging, and extends life span [132]. It is intriguing why nicotinamide derivatives have such concentration-dependent effect. It would be interesting to explore potential connections in these biological processes.

## Vitamin C

Vitamin C, or L-ascorbic acid (AA/LAA), is soluble in water due to its sugar-like structure. Although ascorbic acid is found at equal amounts in both isomeric states, L and D-ascorbic acid, only LAA is chemically active. Ascorbic acid can be synthesized in plants and the majority of animals (Fig. 4, adapted from Linster's and Schaftingen's review) [133]. In vertebrates, the last step of ascorbic acid biosynthesis from glucose is the formation of 2-keto-gulonolactone which spontaneously enolizes into ascorbic acid. The enzyme for this step, L-gulonolactone oxidase, is found inactive in high primates, including humans, so human beings have to take vitamin C from food sources [133, 134]. LAA is not stable in nature due to its hydrogen ion, and acidic pH will increase its stability. When exposed to light, it gets oxidized to dehydroascorbic acid (DHA) [135]. In practice, more stable LAA derivatives are used in cell culture, such as magnesium ascorbyl phosphate (MAP) and ascorbyl 6 palmitate (AA6P) [136–138].

Vitamin C is a potent antioxidant and reduces reactive oxygen species (ROS), and it participates in various biological processes [139]. In addition, vitamin C acts as a kinase inhibitor. When it is oxidized into dehydroascorbic acid (DHA), it inhibits IκBα Kinase β and modulates NF-κB signaling [140, 141]. Vitamin C also reduces ferric to ferrous iron, and increases its absorption in the intestine [142].

High doses of vitamin C can actually promote an oxidative state in cancer cells, acting as a potential anti-cancer therapy [143–145]. It is proposed that the anti-cancer effect may be due to induction of ferroptosis, a form of programmed cell death related to vitamin E deficiency and lipid peroxidation [146–148]. High doses of ascorbic acid was reported to regress Charcot-Marie-Tooth disease in mice, a neuropathy with impairment in the myelination of peripheral nerves, due to the myelination effect of ascorbic acid [149–152]. The lack of Vitamin C is the trigger of a well-known avitaminosis called scurvy, which if prolonged in time can be fatal due to hemorrhages and impaired wound healing [31].

Vitamin C plays critical roles in promoting PSC survival and derivation. When hESCs are transitioned from mTeSR medium to albumin-free and more defined condition, cells die in the absence of vitamin C after a few days [50]. At the same time, vitamin C also regulates the homeostasis of the extracellular matrix [18]. It affects the folding and deposition of collagen proteins, which may have contributed to its effect on hESC attachment and survival [27, 50, 153]. During reprogramming, ascorbic acid promotes reprogramming in human and mouse cells [50, 154]. Vitamin C reduces cell senescence during reprogramming by suppressing p53 [155, 156]. It was shown to act through a mechanism independent from its antioxidant role, and accelerates transcriptional changes during reprogramming [154, 157]. Vitamin C also influences cell survival in reprogramming through epigenetic modulation. It is a cofactor for polyhydroxylates and demethylases [158], and promotes demethylase activity on shore CpG islands involved in tissue-specific DNA methylation and reprogramming [159, 160].

Besides its use for the maintenance of pluripotent stem cells, vitamin C also impacts the differentiation of multiple cell lineages. Vitamin C triggers mesoderm differentiation of mouse embryonic stem cells [161]. It promoted myogenesis and osteogenesis, and inhibited adipogenesis. Vitamin C inhibits neurogenesis to favor myogenesis through the activation of the p38 MAPK/CREB pathway and chromatin remodeling [161, 162]. It also promotes cardiac differentiation and increases the proliferation of cardiac progenitor cells by enhancing collagen synthesis [163].

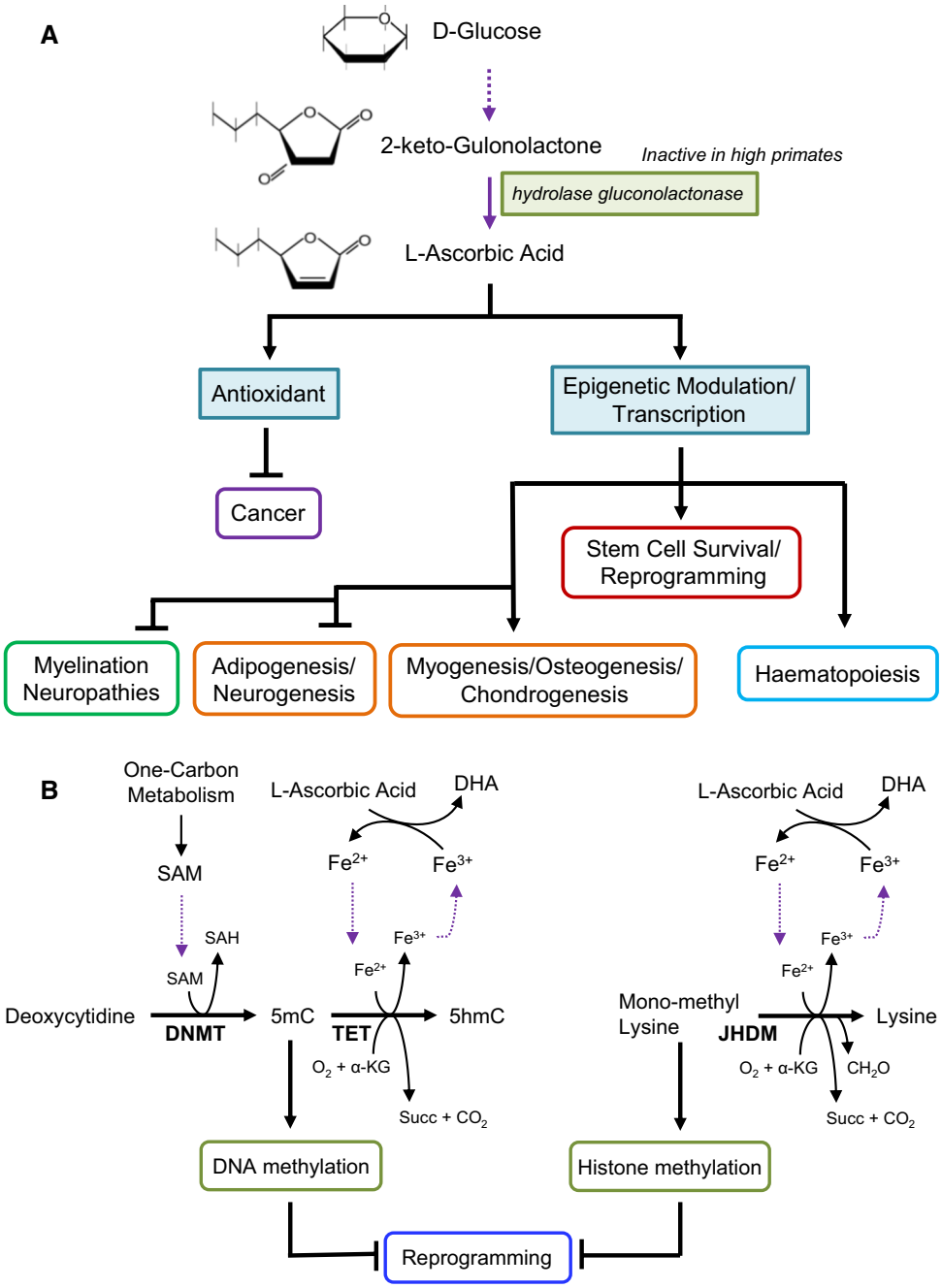


**Fig. 4** **a** Vitamin C regulation in stem cells. Vitamin C, commonly referring to as L-ascorbic acid, cannot be synthesized by humans due to the lack of the *hydrolase gluconolactonase* enzyme. Similar to vitamin A, it acts as an antioxidant and tumor suppressor, and increases the myelination of neurons. Its effect on chromatin remodeling and other epigenetics marks allows it to affect reprogramming of pluripotent stem cells, but it is also necessary for the culture of both embryonic and mesenchymal stem cells. It promotes hematopoiesis differentiation and promotes mesoderm lineages including cardiomyocytes, bone and cartilage. The effect of vitamin C on adipocyte differentiation depends on the platform and concentration. **b** Vitamin C regulation of reprogramming. L-Ascorbic acid facilitates the reaction that converts ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$). $Fe^{2+}$ is required for the activity of both Tet proteins and JHDM histone demethylases, and it is oxidated into $Fe^{3+}$ when α-KG and $O_2$ are converted into succinate and $CO_2$ during DNA and histone demethylation

In addition to ESCs, vitamin C also regulates mesenchymal stem cell growth and differentiation [164–166]. It suppresses hypoxia inducible factor 1 (HIF1α) activity through two parallel pathways. Vitamin C suppresses HIFα transcription, while activating HIF1α hydroxylase to breakdown HIF1α. Inhibition of HIF1α leads to mitochondrial activation, affecting cell proliferation and metabolism [167]. MSCs cultured with vitamin C show upregulation of Oct4 and Sox2, without affecting the expression of MSC markers such as CD105 and CD13 [168, 169]. Vitamin C in combination with TGFβ treatment was shown to promote MSC differentiation toward vascular smooth muscle cell types [170, 171]. Vitamin C also facilitates osteogenic differentiation by increasing collagen secretion, since it is used as a cofactor for enzymes that hydroxylate proline and lysine in pro-collagen [171–174]. Vitamin C also enhances chondrogenic differentiation [175], and protects chondrocytes from oxidative stress due to hydrogen peroxide ($H_2O_2$) [176].

Vitamin C is also beneficial to hematopoietic differentiation and it has been used to promote the maturation of T cells and NK cells from HSC-derived progenitors [177, 178]. Ascorbic acid is used to generate hematopoietic

stem cell progenitors (hemangioblasts) from hESCs [179]. Ascorbic acid concentration is high in human and mouse hematopoietic stem cells (HSCs), and declines upon differentiation. With the accumulation of intracellular ascorbic acid, HSC frequency is limited, while leukemogenesis is suppressed [180, 181].

Besides its antioxidant activity, vitamin C mainly acts as an enzyme cofactor for the demethylation of DNA and histone in stem cells (Fig. 4b). Changes in DNA and histone methylation are often associated with stem cell differentiation and reprogramming [182–184]. The methylation on the fifth position of the pyrimidine ring of cytosine (5mC) is the most common DNA modification, and its demethylation to 5-hydroxymethylcytosine (5hmC) is catalyzed by Tet proteins [185–187]. On the other hand, histone demethylation is carried out by histone demethylases such as the Jumonji-C domain-containing family (JHDMs) [184, 188, 189]. Both Tet and JHDM proteins are vitamin C-dependent, $Fe^{2+}$/alpha-ketoglutarate-dependent hydroxylases ($Fe^{2+}$/α-KGDDs). During demethylation, $Fe^{2+}$/α-KGDD catalyzes the reaction that converts α-ketoglutarate (α-KG) and $O_2$ into succinate and $CO_2$. $Fe^{2+}$/α-KGDD activity requires $Fe^{2+}$ that is oxidized to $Fe^{3+}$ in the process [148, 181, 190–192]. Vitamin C reduces $Fe^{3+}$ back to $Fe^{2+}$ which could then be utilized by Tet or JHDM in demethylation again, while vitamin C itself is oxidized into dehydroascorbic acid (DHA) [113, 193]. Vitamin C influences the biological outcome of Tet-mediated DNA demethylation, and promotes the demethylation of histones such as H3, H3K9, H3K36 and H3K27 [194]. Collectively, vitamin C enhances the efficiency of somatic programming [154]. In addition, vitamin C also impacts stem cell differentiation. Vitamin C improves HSC differentiation by modulating Tet activity [180, 181], and it also increases the expression of key genes in dopaminergic neurons in the fetal brain [195], as well as trophectoderm genes like Cdx2, Eomes and Elf2 in the differentiation of mouse embryonic stem cells [196].

## Vitamin E

Since the discovery of α-tocopherol in 1922 [197], vitamin E has been extensively studied and become one of the most commonly consumed vitamins. There are eight known natural isoforms of vitamin E, including four tocopherols and four tocotrienols, each designated as α, β, γ and δ based on the position of methyl groups on the chromanol ring [198–200]. Vitamin E exists in almost all the tissues in the human body, with highest levels in the adipose tissue and adrenal gland [200]. Early studies on vitamin E mostly focused on α-tocopherol, the most abundant vitamin E isoform [200]. Compared to the other isoforms, α-tocopherol has higher bioavailability and longer retention time, due to its preferential incorporation into lipoproteins by alpha-tocopherol transfer protein (α-TTP) in the liver [199, 201]. It is also the isoform commonly provided in dietary supplements [199]. In recent years, non-α-tocopherols have received increasing attention, and the tocotrienols are reported to be superior over tocopherols in many clinical applications [201–203]. Synthetic forms of vitamin E and its chemically modified analogs, such as trolox [204], tocoflexol [205] and esters of vitamin E [206–209] are also widely used for improved bioavailability and stability.

Vitamin E is a lipid soluble, chain-breaking antioxidant, capable of neutralizing free radicals and terminating chain reactions in the oxidation of polyunsaturated fatty acids. It is one of the major antioxidants in the human plasma [210]. Due to its lipid solubility, vitamin E effectively protects against oxidative damage from lipid peroxidation in the membrane as well as in lipid vesicles, but is less effective against damage from aqueous free peroxyl radicals [210, 211].

In addition to its antioxidant role, vitamin E also modulates cellular signal transduction through kinases, phosphatases, lipid mediators and transcription factors [35, 212]. α-Tocopherol inhibits protein kinase C (PKC), while other vitamin E isoforms were reported to have no influence or opposing effect [213–215]. Regulation of PKC by vitamin E leads to changes in cell proliferation, adhesion, gene expression and downstream signal transduction [35, 213, 216, 217]. Another important target of vitamin E is protein kinase B (PKB/AKT), which plays a key role in cell survival. Vitamin E may activate or inhibit PI3K/AKT pathway and cell survival in a cell type-specific manner [218–221]. Other signaling pathways regulated by vitamin E include ERK [219], p38 MAPK [222] and Wnt signaling [223]. Due to its influence on membrane composition, vitamin E can not only directly or indirectly activate/inhibit its targets, but also change specific structural features of the plasma membrane (such as lipid rafts), which may be involved in the membrane translocation or activation of signaling molecules [212].

Vitamin E was frequently used in primary cell culture to prevent cell death and preserve cell function after exposure to stress conditions, and both antioxidant and signal transduction modulating mechanisms may be involved. For example, vitamin E treatment during enzymatic dissociation protected rat mammary epithelial cells against oxidative damage and improved survival [224]. γ-Tocotrienol was reported to enhance AKT phosphorylation in intestinal tissue following total body irradiation, thereby protecting the tissue against damage by radiation [221]. Low micromolar concentrations of α-tocopherol suppressed the rise of metalloproteinase 1 (MMP-1) expression in UVA-irradiated fibroblasts, suggesting a photoprotective effect [225]. In an endothelial cell model for type I diabetes, 20 mg/L α-tocopherol showed protective effects against endothelial dysfunction caused by

hyperglycemia [226]. In some studies, high concentrations (200–2500 µmol/L) of vitamin E were used for cell culture [227–229], far exceeding the reported plasma vitamin E levels ranging from 15 to 27 µmol/L [230–233].

Commercial cell culture supplements containing vitamin E are available. The B-27 supplement is widely used for neuronal cell culture [234, 235], and chemically defined lipid concentrate is used to support mammalian and insect cell culture in place of fetal bovine serum [236]. The isoform of vitamin E supplied in these supplements are α-tocopherol or α-tocopherol acetate in low micromolar concentrations.

As a potent antioxidant, vitamin E was reported to be protective for stem cells and progenitor cells which are sensitive to oxidative stress. Treatment with α-tocopherol protected mesenchymal stem cells (MSCs) against $H_2O_2$-induced apoptosis and promoted MSC survival via the AKT pathway [220, 237]. Similarly, trolox was reported to enhance the proliferation of human dental pulp stem cells under oxygen tension [238]. α-tocopherol also promoted the survival of cultured human neural progenitors, and the effect was abolished by inhibitors of PI3K/AKT and Src signaling [239]. This is consistent with in vivo studies using mouse models, in which vitamin E deficiency or impairment of its uptake resulted in neural tube defects [240, 241].

In addition to affecting cell survival, vitamin E was also reported to affect differentiation of stem cells as a free radical scavenger. Reactive oxygen species (ROS) were proposed to participate in cellular signaling and regulate embryonic stem cell (ESC) differentiation, and vitamin E typically antagonizes the ROS effects. Arachidonic acid, the precursor of prostaglandins and leukotrienes, was reported to promote the generation of vascular progenitor cells from mouse ESC embryoid bodies. ROS was elevated in the process, and trolox treatment from day 3 to day 10 abolished the effect of arachidonic acid on differentiation [242]. In another study, electrical field treatment stimulated endothelial differentiation of mouse ESCs through a mechanism involving ROS, and trolox treatment inhibited its effect [243]. In cardiac differentiation from mouse ESCs, treatment with valproic acid from day 3 to 7 was reported to inhibit embryoid body growth and suppress cardiomyocyte differentiation while increasing ROS. Co-administration of trolox antagonized the inhibitory effect and restored cardiomyocyte differentiation [244]. In contrast, icariin treatment from day 5 to 16 of cardiac differentiation, which elevated ROS and induced ERK/p38 phosphorylation, significantly enhanced cardiac differentiation, and vitamin E treatment decreased the promoting effect by half [245]. Similarly, elevated intracellular ROS by cardiotrophin-1 (CT-1, from day 7 on) is associated with improved cardiomyocyte differentiation and increased Ki-67 expression, suggesting better cardiomyocyte proliferation. Vitamin E abolished these effects as well through a mechanism involving Jak/Stat and ERK pathways [246]. Taken together, vitamin E can play regulatory roles during ESC differentiation toward multiple lineages, potentially through a mechanism involving ROS generation and activation of relevant signaling pathways. The exact effect may depend on the setting of differentiation and the timing of treatment.

The functions of vitamin E are summarized in Fig. 5.

## Coordinated vitamin actions in stem cell regulation

Each vitamin has its distinctive role in biochemical processes, and many of them work together to carry out critical cellular functions. For example, the generation of acetyl-CoA requires $B_1$, $B_2$, $B_3$ and $B_5$, which is essential for both somatic and stem cells. Many other biological processes also demand collaborative actions of multiple vitamins, and some of them are especially important to stem cells.

Epigenetic regulation is essential for self-renewal and cell fate determination [247]. DNA and histone methylation are a key modification, and it is responsive to nutrition and metabolic changes. Appropriate epigenetic regulation is essential for pregnancy and embryonic development. Vitamin $B_{12}$, $B_9$, and $B_6$ are key coenzymes in one carbon metabolism and can synergistically influence DNA and histone methylation [248, 249]. One carbon metabolism involves the donation of carbon units from amino acids for utilization in various biochemical reaction. In the folate ($B_9$) cycle, a carbon unit

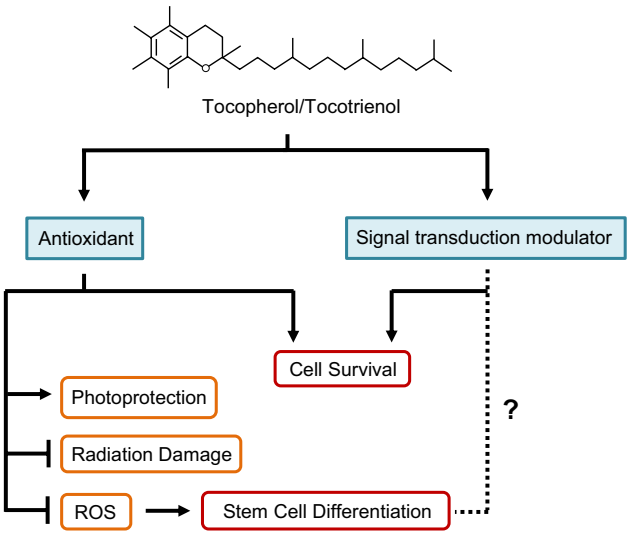


**Fig. 5** Vitamin E in stem cell culture. Vitamin E is a potent lipid-soluble antioxidant, and is capable of protecting stem cells and progenitor cells against oxidative damage. In addition, vitamin E can modulate signaling pathways, including the PI3K/AKT pathway, to promote survival and proliferation of cells in culture. The impact of vitamin E on ESC differentiation is mainly mediated through ROS levels. Whether vitamin E can directly modulate signal transduction events involved in cell fate determination has not been reported

produced from the conversion of serine to glycine is transferred to tetrahydrofolate (THF) by serine hydroxymethyltransferase (SHMT), a vitamin $B_6$-dependent enzyme. The resulting 5,10-methylene-THF is important for nucleotide synthesis. In the methionine cycle, vitamin $B_{12}$ serves as a coenzyme in the conversion of homocysteine to methionine by accepting a carbon unit from the folate cycle. Methionine is further converted to *S*-adenosylmethionine (SAM) [250, 251], which is the main methyl group donor for the methylation of proteins, DNA, RNA and lipids [185].

The combined actions of vitamins are also reflected in multiple stem cell media containing vitamin combinations (Table 2), and are utilized in some stem cell protocols [252].

## Concluding remarks

Vitamins are deeply involved in various basic metabolic and signaling processes, and many of them are required for normal functions in specific stem cells. Besides the conventional approach of stem cell modulation through growth factor signaling pathways, vitamin modulation could become a critical approach to improve stem cell maintenance and downstream differentiation. Studies on vitamins such as A, $B_3$ and C have shown that vitamin-dependent pathways are effective targets in stem cell manipulation. However, most vitamins have not been systematically explored in different stem cell studies. Considering that specific cell types rely on distinctive combinations of vitamins, it is possible that more stem cell applications could be developed using different vitamin formulations in media. At the same time, stem cell culture also provides a unique platform to study vitamin function in human embryogenesis. Following the recent discoveries of vitamin-related molecular mechanisms, more novel mechanisms could be identified in stem cell models. We believe that vitamin study in stem cell research will lead to new modulations to improve stem cell applications, and help realize their great potentials in basic research and regenerative medicine.

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### Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

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