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MINIREVIEW ARTICLE

Spermine and gene methylation: a mechanism of lifespan extension induced by polyamine-rich diet

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Abstract

The polyamines spermidine and spermine are synthesized in almost all organisms and are also contained in food. Polyamine synthesis decreases with aging, but no significant decrease in polyamine concentrations were found in organs, tissues, and blood of adult animals and humans. We found that healthy dietary patterns were associated with a preference for polyamine-rich foods, and first reported that increased polyamine intake extended the lifespan of mice and decreased the incidence of colon cancer induced by repeated administration of moderate amounts of a carcinogen. Recent investigations have revealed that changes in DNA methylation status play an important role in lifespan and aging-associated pathologies. The methylation of DNA is regulated by DNA methyltransferases in the presence of *S*-adenosylmethionine. Decarboxylated *S*-adenosylmethionine, converted from *S*-adenosylmethionine by *S*-adenosylmethionine decarboxylase, provides an aminopropyl group to synthesize spermine and spermidine and acts to inhibit DNMT activity. Long-term increased polyamine intake were shown to elevate blood spermine levels in mice and humans. In vitro studies demonstrated that spermine reversed changes induced by the inhibition of ornithine decarboxylase (e.g., increased decarboxylated *S*-adenosylmethionine, decreased DNA methyltransferase activity, increased aberrant DNA methylation), whose activity decreases with aging. Further, aged mice fed high-polyamine chow demonstrated suppression of aberrant DNA methylation and a consequent increase in protein levels of lymphocyte function-associated antigen 1, which plays a pivotal role on inflammatory process. This review discusses the relation between polyamine metabolism and DNA methylation, as well as the biological mechanism of lifespan extension induced by increased polyamine intake.

Keywords Spermine · Spermidine · Gene methylation · Senescence · Lifespan · Aging-associated diseases

Abbreviations

LFA-1	Lymphocyte function-associated antigen 1
ODC	Ornithine decarboxylase
CVDs	Cardiovascular diseases
SAM	*S*-Adenosylmethionine
DNMT	DNA methyltransferase
dcSAM	Decarboxylated *S*-adenosylmethionine
ATP	Adenosine triphosphate
AdoMetDC	*S*-Adenosylmethionine decarboxylase
ODC-AZ	Ornithine decarboxylase antizyme
DFMO	α-D,L-Difluoromethylornithine hydrochloride

Introduction

Polyamines, as represented by spermine, spermidine, and their precursor putrescine, are indispensable for cell growth and differentiation. They have many biological activities, such as gene expression, transcription, cellular signaling, and protein synthesis, and they act to protect cells and genes from harmful stimuli. We found that polyamines suppress the synthesis of proinflammatory cytokines and decrease cell adhesion by selectively decreasing lymphocyte function-associated antigen 1 (LFA-1), which is involved in immune cell activation and inflammation. The anti-inflammatory properties of polyamines are not accompanied by a decreased cellular

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activity. Polyamines maintain biological activities and extend lifespan of immune cells taken from human blood and cultured in plastic plate (Soda et al. 2005). Chronic inflammation and the resulting increase in oxidative stress have been shown to contribute to most aging-associated chronic diseases. Moreover, aging itself is associated with a proinflammatory status, e.g., immune system dysregulation leading to chronic mild inflammation and sustained oxidative stress (Soda et al. 2005). Based on the beneficial functions of polyamines and the fact that they are derived primarily from food, we started to examine the possible role of dietary polyamines in maintaining a long, healthy life. We first found that chow with added synthetic polyamines extended the life span of mice (Soda et al. 2009a).

Substances contained in foods that inhibit or counteract the aging-associated proinflammatory status and decrease resulting increases in oxidative stress (i.e., chemicals that inhibit the transfer of electrons from a substance to an oxidizing agent) on lifespan extension have been attracted scientists' interest. Among these substances, antioxidant polyphenols and antioxidant vitamins are considered to be important candidates for extending healthy lifespans. Examples include isoflavones, found at high levels in soybeans, and resveratrol and vitamins, which are prevalent in the Mediterranean diet. The molecules have many biological activities that may counteract the pathogenesis of aging-associated pathologies. For example, they have antioxidant and anti-inflammatory properties, and they activate autophagy (Ferraresi et al. 2017; Sacks et al. 2006; Wang et al. 2018b; Zhang et al. 2018) and protect cells and genes from harmful stimuli (George et al. 2017; Guthrie et al. 2017). However, many studies have failed to show any effects on the prevention of aging-associated pathologies and the extension of lifespan (Burnett et al. 2011; Sacks et al. 2006; Staats et al. 2018; Strong et al. 2013).

Polyamines also have biological activities similar to those of antioxidant polyphenols and vitamins; for instance, they exert anti-inflammatory (Paul and Kang 2013; Soda et al. 2005) and antioxidant properties (Rider et al. 2007), protect cells and genes from harmful stimuli (Douki et al. 2000; Okumura et al. 2016; Pothipongsa et al. 2012), and promote autophagy (Sacitharan et al. 2018). However, in the case of antioxidant polyphenols and vitamins, these functions do not extend the lifespan in mammals, so they are unlikely to play the major role in the extension of mouse lifespans by polyamines. Here, I discuss the biological process whereby increased polyamine intake extends humans and animals lifespans, and refer to previously published studies to summarize the effects of aging on polyamine concentrations in the body and the effects of increased polyamine intake on polyamine concentrations.

Aging and polyamine concentration

The activities of enzymes involved in polyamine synthesis, especially ornithine decarboxylase (ODC), decrease with aging (Yoshinaga et al. 1993). And, age-dependent decreases in polyamine concentrations in tissues, organs, blood, and urine have been reported in animals and humans. In the older papers published before 1985, it was reported that spermidine and spermine concentrations in tissues and organs in rats decreased with aging, and their concentrations in brain and muscles of older rats were lower than those in adult animals (Das and Kanungo 1982; Jaenne et al. 1964). However, there was as significant decline in spermine and spermidine concentrations during young age, and this decline seemed to slow markedly by adulthood. In the recent articles, the descriptions in abstracts and titles indicated that aging-associated declines in polyamine concentrations occurs during early life. Nishimura et al. found that polyamine concentrations in various tissues and organs were significantly lower in 10- and 26-week old mice than in 3-week-old mice, but no differences in spermine and spermidine concentrations were observed between 10- and 26-week-old mice, except skin (Nishimura et al. 2006).

Polyamine concentrations in blood cells, especially erythrocytes and leukocytes, and urinary polyamine excretion are known to reflect polyamine levels in organs and tissues throughout the body. In human blood, no age-associated decline in spermine or spermidine concentrations were observed, but large inter-individual differences were found (Elworthy and Hitchcock 1989; Soda et al. 2005). Elworthy and Hitchcock measured red blood cell polyamine concentrations in 117 patients (ranging from 0 to 80 years old) who were largely in good health but had various neurological problems known not to affect polyamine levels. No statistically significant age-dependent changes in spermine or spermidine concentrations was observed (Elworthy and Hitchcock 1989). Our three separate analyses of aging-associated changes in blood polyamine concentrations in human volunteers [42 males whose age raged from 26 to 69 (Soda et al. 2005), 58 males ranging from 40 to 69 years old (Soda et al. unpublished), and 33 females from 61 to 83 years old (Soda et al. unpublished)] showed similar findings, namely no decrease in blood spermidine or spermine concentrations. The blood samples were examined by two different laboratories, and the results were the same. Madeo et al. stated (2018) that we reported an age-dependent decline in spermidine concentrations in human organs (Soda et al. 2009b), but our manuscript contained no text, tables, or figures indicating an age-dependent decline in spermidine concentrations in the organs of either humans or animals. I summarize

blood polyamine concentrations in three separate mouse experiments published previously (Soda et al. 2009a, b, 2013), and there was no aging-associated changes in blood polyamine concentrations in mice fed chow of which polyamine concentrations were not increased (Fig. 1).

Similarly, urinary polyamine excretion, which reflects blood polyamine concentrations, does not change with age during adulthood. van den Berg et al. measured urinary polyamine excretion in 51 healthy volunteers whose ages ranged from 4 days to 77 years, and found an age-dependent decrease in urinary excretion of spermidine in terms of creatinine excretion. However, they clearly indicated that the overall age-dependent decline was merely due to the rapid decrease during the first year of life, and it did not occur during adulthood (van den Berg et al. 1986). Yodfat also examined urinary polyamine concentrations in 171 male and 166 female healthy volunteers whose ages ranged from 14 days to 84 years (Yodfat et al. 1988). They demonstrated an age-dependent decrease in diamine levels in male, but no age-dependent decrease of polyamines (either spermidine or spermine) in either gender. These data indicate that findings of age-associated decrease in polyamine concentrations reflect their rapid decline during early life, and no decrease is observed in healthy adult animals and humans.

Dietary polyamine and epidemiological studies

Foods contain polyamines, though at widely varying levels (Cipolla et al. 2007; Nishibori et al. 2006; Nishimura et al. 2006; Soda et al. 2017). Therefore, personal food preferences and regional dietary patterns may greatly affect polyamine intake from food. We examined the relationship between polyamine content and dietary pattern using the food supply database of 49 Western countries from the Food and Agriculture Organization of the United Nations. The study was an ecological investigation, and the data used do not indicate the amount of foods actually consumed, however, the food supply must reflect the food demand, and thus, we examined the following relationships: the calories of specific supplied foods relative to the total calories of all supplied foods, and the amount of polyamines contained in specific supplied foods relative to the total calories in all supplied foods. Mediterranean diet preferred in Mediterranean area was associated with an increased amount of polyamines on a per calorie basis (Binh et al. 2011).

Epidemiological analyses and interventional trials have shown that differences in food preferences and dietary patterns are among the many life-style factors that may play a role in the inhibition of aging-associated diseases and senescence. For example, increased consumption of soybeans and their byproducts is associated with a decreased incidence of cardiovascular diseases (CVDs) (Nagata et al. 2017) and malignancies such as breast (Wu et al. 2008) and colon cancer (Yang et al. 2009). The Mediterranean diet and increased vegetable intake are also associated with a decreased incidence of lifestyle-related diseases, such as CVDs (Estruch et al. 2018) and breast and colon cancer (Couto et al. 2011). We found that healthy foods, such as germ and bran, legumes such as soybeans, vegetables, and shellfish, are rich in polyamine on a calorie basis (Soda et al. 2017), and the preference of polyamine-rich diet has a close association with the low incidence of CVDs and with long life span (Soda et al. 2012). And, a recent prospective study also showed that higher spermidine intake is linked to lower mortality (Kiechl et al. 2018).

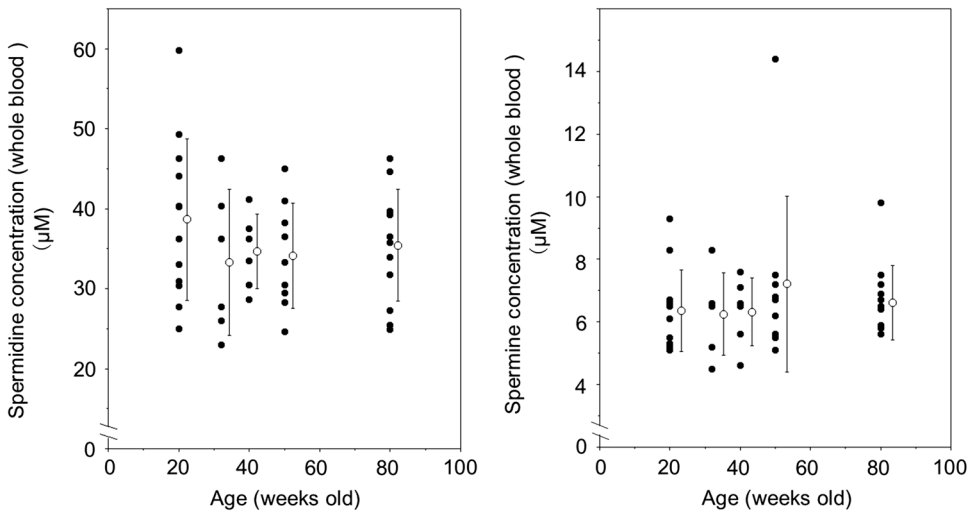

Fig. 1 Aging and polyamine levels in adult mice. Spermidine (left graph) and spermine (right graph) concentrations in whole blood in mice were measured by high performance liquid chromatography at each age. Each black dot indicates the concentration in one mouse, and each open circle indicates the mean concentration. There is significant inter-individual variation, however no aging-associated changes are observed

Polyamine intake and polyamine levels in the body

The ability of polyamine synthesis decrease with aging due to the decreased enzymatic activities for polyamine synthesis. However, the age-dependent decreases in polyamine concentrations were not found during adulthood. Instead, there is a large inter-individual differences in blood polyamine concentrations (Fig. 1). The loss of age-associated decline is considered because polyamine is supplied from intestinal lumen i.e. dietary polyamine and polyamine produced by microbiota and intestinal mucosa. The exact biological mechanisms underlying the large inter-individual differences in blood polyamine concentrations are not known, however, one factor is thought to be differences in the amount of polyamines supplied from the intestinal lumen and in the intestinal environment that are also likely to affect polyamine synthesis. In fact, suppression of the polyamine supply from both foods and the intestinal microbiota results in decreased blood polyamine concentrations (Cipolla et al. 2003; Nishimura et al. 2001).

Nishibori et al. analyzed polyamine concentrations in Japanese food and estimated that putrescine, spermidine, and spermine account for 45, 37, and 18% of polyamine intake by Japanese, respectively, and the ratio of spermidine and spermine intake was 2:1 (Nishibori et al. 2006). Bardocz et al. estimated that putrescine (57%) was also the most commonly consumed polyamine in Europeans, with spermidine and spermine accounting for 26 and 18%, respectively; the ratio of spermidine to spermine intake was about 1.5:1 (Bardocz et al. 1995). Spermine and spermidine in the intestinal tract are absorbed quickly, leading to a rapidly increase intestinal vein concentrations (Uda et al. 2003) and distribution to all organs and tissues (Bardocz et al. 1990, 1995). Considering these experimental results, it is logical that increased polyamine intake from foods increases spermidine supply more than spermine, and spermidine concentrations increase in response to increased polyamine intake. However, short-term increases in polyamine intake have thus far not been shown to elevate either spermidine or spermine concentrations (Brodal et al. 1999; Soda et al. 2009a, b). This indicates that blood polyamine concentrations rise for only a very short time, if any, after increased polyamine supply from the intestinal lumen. Further, rigorous mechanisms of polyamine homeostasis immediately degrade polyamines and maintain their intracellular concentrations.

We evaluated the effects of long-term increases in polyamine intake in mice and humans. First, we evaluated the effects of high polyamine chow, which had a polyamine content approximately three to four times higher than regular chow, on blood polyamine levels of mice. The high-polyamine chow was prepared by adding synthetic spermine, spermidine, and putrescine to regular animal chow, and contained spermidine concentrations about four times higher than that of spermine (Soda et al. 2009b). Increased polyamine intake for 26 weeks increased blood spermidine and spermine concentrations in mice, and the statistical significant difference was observed in both spermidine and spermine concentrations (Soda et al. 2009b) or only in spermidine concentrations (Soda et al. 2009a). However, we used only 6–9 animals for the evaluation of blood polyamine concentrations in each group. Later analysis with an increased number ($n = 12$) of mice fed high polyamine chow for 56 weeks showed that both spermine and spermidine concentrations were increased, as observed in our previous studies, but statistically significant increase was observed only in spermine levels (Soda et al. 2013). Since there is wide inter-individual variation in blood polyamine concentrations, it is likely that in the preliminary studies, animals with higher spermidine concentrations after consuming high-polyamine chow probably had higher concentrations before the dietary change (Fig. 1).

It is very difficult to observe temporal changes in blood polyamine concentrations in small animals. Therefore, to examine changes in blood polyamine concentrations in response to increased polyamine intake, we performed human interventional trials. Natto is a polyamine-rich food with a ratio spermidine and spermine of about 3:1. Our preliminary study showed increase in spermine levels after increased Natto intake, however spermidine levels did not increase (Soda et al. 2009b). Moreover, we further examined the effect of increased Natto intake on the changes in blood polyamine concentrations (30 male volunteers in intervention group and 27 male volunteers in control group) for 12 months. For the study, we developed new "Natto" by using polyamine rich soybeans and employing production methods suitable for polyamine-rich natto. All of the used soybeans and fungi were not transgenic. The selected "Natto" for the study contains 390 nmol/g of spermine and 1880 nmol/g of spermidine (Kobayashi et al. 2017). The increased amounts of spermine and spermidine intake estimated by meal records in the intervention group increased (22.00 ± 9.56 and 96.63 ± 47.70 μmol per day, respectively), while no changes were observed in control group. Because the amounts of spermine and spermidine intakes by Japanese were estimated to be 36 and 74 μmol per day (Nishibori et al. 2006), respectively, polyamine intake by volunteers was estimated to be almost doubled during the intervention. Spermine concentrations in whole blood gradually increased following Natto intake and was significantly higher than that in the control group by the end of the 12-month intervention, while blood spermidine concentration showed no change (Soda et al. unpublished).

These studies clearly showed that increasing the dietary polyamine supply for at least several months gradually increased blood spermine concentrations in humans and mice (Soda et al. 2013; Soda et al. unpublished), despite the fact that foods contain more spermidine than spermine. The mechanism whereby spermine concentrations are increased by elevated polyamine intake is not known. However, extracellular polyamine supply has a significant effect on intracellular polyamine concentrations, a phenomenon that is typically seen in cancer patients. Polyamine biosynthesis is up-regulated in cancer cells (Soda 2011), and therefore spermidine concentrations, which reflect the activity of cell growth, are increased in the blood and urine in cancer patients (Soda 2011). These facts indicate that spermine supply may be increased by increased polyamine (spermidine > spermine) intake via a still-unknown mechanism (Fig. 2).

Factors that may affect aging-associated pathologies

Several food components are considered to inhibit aging-associated pathologies and to help extend life span. Among these substances, antioxidant polyphenols and vitamins are considered to be important candidates for healthy lifespans. The molecules have many biological activities that are likely to inhibit aging-associated pathologies and help extend life span. They have antioxidant and anti-inflammatory properties, activate autophagy (Ferraresi et al. 2017; Sacks et al. 2006; Wang et al. 2018b; Zhang et al. 2018), and protect cells and genes from harmful stimuli (George et al. 2017; Guthrie et al. 2017). Early animal experiments and research performed under specific conditions or in particular animals demonstrated that the increased intake of polyphenols extended lifespans. However, many studies have failed to show any effects on the prevention of aging-associated pathologies and the extension of lifespan (Burnett et al. 2011; Sacks et al. 2006; Staats et al. 2018; Strong et al. 2013). In addition, vitamin E and β-carotene, two antioxidant vitamins with potent antioxidant properties, increased rather than decreased the incidence of CVDs and their related mortality (Cook et al. 2007; Miller et al. 2005; Vivekananthan et al. 2003). The study results obtained by numerous investigations indicate that these biological activities are not sufficient to inhibit aging-associated pathological changes or help extend lifespan. Spermine and spermidine have biological activities similar to the abovementioned substances, and therefore it is unlikely that polyamine intake extends lifespan via these activities.

A growing number of recent studies have shown a close relationship between aging and gene methylation (Kochmanski et al. 2018). Gene methylation is one of the mechanism for regulating gene expression without affecting gene sequence. A gene is comprised of combinations of four bases: adenine, guanine, thymine, and cytosine. Gene methylation is a change that involves only cytosine and creates gene information by adding a methyl group from *S*-adenosylmethionine (SAM) to cytosine residues at the C-5 position to yield 5-methylcytosine. Upstream of the gene, there is a direct repeat of cytosine and guanine called a CpG island. A CpG island is a site of transcription initiation, and in mammals, methylated cytosine within a CpG island can turn the gene off. Conversely, demethylation of cytosine initiates and

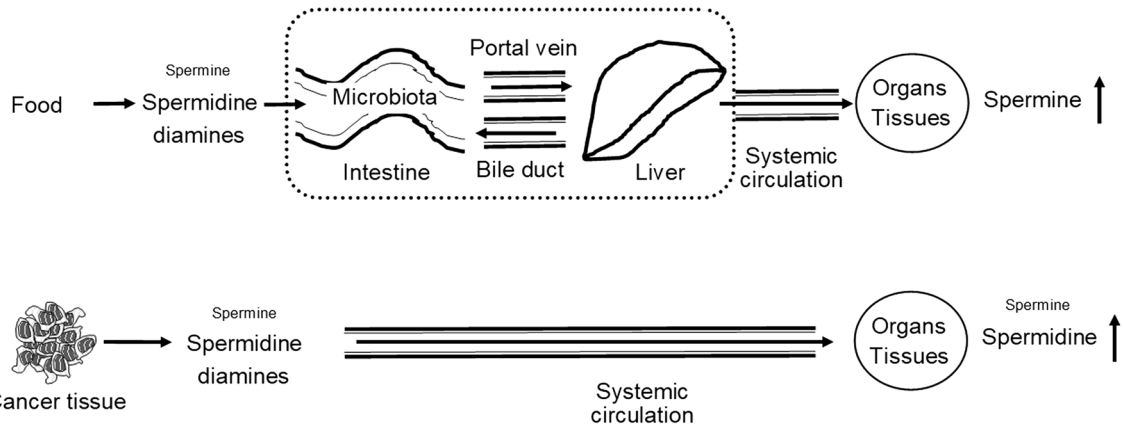

Fig. 2 Difference in polyamine supply from food and from cancer tissues. Despite the fact that food contains much higher concentrations of spermidine than spermine, increased polyamine intake increases spermine concentrations. Cancer tissues produce much greater amounts of spermidine than spermine. Spermidine concentrations in tissues, organs, and blood are increased in cancer patients, as is spermidine urinary excretion. One major difference between the two types of polyamine supply is that blood from the intestines drains into the liver and then enters the systemic circulation. However, blood from cancer tissues drains directly into the systemic circulation. Therefore, both the liver and the intestines may play a role in elevating spermine concentrations following increased polyamine intake

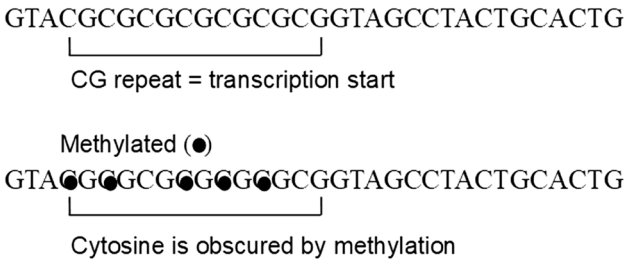

Fig. 3 Gene methylation and transcription. The genetic code of each gene is based on the arrangement of the four bases: adenine (A), guanine (G), thymine (T), and cytosine (C). (Upper row) the iterative array of C and G (called the CpG island) indicates the existence of gene information in the lower stream. (Lower berth) when a methyl group is supplied and cytosine is methylated, the iterative array of CG will become ambiguous. For this reason, the promoter region becomes ill defined, and it becomes difficult for transcription to occur

enhances transcription, resulting in the increased production of the protein encoded by the gene (Fig. 3).

Aging is associated with enhanced demethylation of DNA in various organs and tissues in several animals and humans (Avrahami et al. 2015; Nguyen et al. 2016). However, increased hypermethylation associated with age has also been reported in some genes (Khalil et al. 2016; Thalheim et al. 2018). The aging-associated changes in DNA methylation status, namely increased de-methylation in some areas and hyper-methylation in other areas, are considered to be among the most important mechanisms underlying aging-associated pathologies. When hypermethylation arises in the CpG islands encoding genes that suppress aging-associated disease(s) and/or when demethylation arises in the CpG islands encoding genes that cause aging-associated disease(s), the onset and the progression of aging-associated disease(s) are accelerated. Alteration of methylation status with aging changes chromatin accessibility, resulting in aberrant gene transcription, as well as genomic instability. These factors may be key regulators of the aging process and contributors to the development of aging-associated diseases (Cruickshanks et al. 2013; Lopez-Otin et al. 2013; Watson et al. 2016), including neoplastic growth (Kresovich et al. 2018; Meliso et al. 2017) and aging itself (Ianov et al. 2017; Spiers et al. 2016).

Various environmental factors have been reported to affect epigenetic alterations. For example, exposures to fine particulate air pollution, cigarette smoking, and alcohol consumption affect the DNA methylation status (de Lichtenfels et al. 2018; Gao et al. 2017; Liu et al. 2018; Wang et al. 2018a). In particular, cigarette smoking that has adverse effects on health that are associated with changes in epigenetic marks. Smoking-associated changes in methylation status are observed in genes related to the progression of CVDs (Zhang et al. 2016), malignant transformation (Vaz et al. 2017; Zhang et al. 2016), and age acceleration (Gao et al. 2017). Conversely, lifestyles that have favorable effects on health, such as moderate exercise alters epigenetic marks in human skeletal muscle and adipose tissue (Denham et al. 2015; Maejima et al. 2018), and nutritional habits change the methylation status of the promoter area (Barres et al. 2013). And these effect of exercise on improved cardiorespiratory fitness and running performance is accompanied by demethylation of several CpG islands, which is the opposite of the hypermethylation changes observed during aging (Denham et al. 2015; Maejima et al. 2018). However, the mechanism by which life style affects the status of DNA methylation is not known.

Polyamine metabolism and DNA methylation

The methylation of DNA is regulated by DNA methyltransferases (DNMTs) in the presence of SAM which is utilized as a methyl group donor. SAM is converted from methionine and adenosine triphosphate (ATP) by methionine adenosyltranferase. SAM and putrescine are substrates for polyamine synthesis. SAM is converted to decarboxylated *S*-adenosylmethionine (dcSAM) by the enzymatic activity of *S*-adenosylmethionine decarboxylase (AdoMetDC). For the synthesis of spermidine and spermine, an aminopropyl group is supplied by dcSAM (Fig. 4).

Intracellular concentrations of dcSAM rise in cells in which polyamine concentrations are decreased due to decreased polyamine synthesis: overexpression of ornithine decarboxylase antizyme (ODC-AZ), which help accelerate ODC degradation; or treatment with α-D,L-difluoromethylornithine hydrochloride (DFMO), which inhibits ODC activities (Pegg et al. 2011; Yamamoto et al. 2010). The increase in dcSAM may be due to the increased AdoMetDC activity caused by the reduced negative feedback exerted by spermine and spermidine and the decreased demand for aminopropyl group which is provided from dcSAM and used for spermidine and spermine synthesis. dcSAM is a strong inhibitor of DNMT, and therefore, increase in dcSAM has been shown to decrease DNMT activity (Tsuji et al. 2001; Yamamoto et al. 2010). Decreases in DNMT activity decrease donations of methyl groups to cytosine residues, and seem to enhance genome-wide demethylation; however, DNA methylation drift seems to be a non-directional change as it involves both hypermethylation and hypomethylation events (Kano et al. 2013; Perez et al. 2018; Soda et al. 2013; Zeng et al. 2018) (Fig. 5).

Our latest study showed that ODC inhibition by DFMO increased dcSAM concentrations and the dcSAM/SAM ratio, and decreased activities of DNMT 1, 3a, and 3b in Jurkat cells without affecting DNMT protein levels (Fukui et al. 2019). A methylation microarray analyses showed that

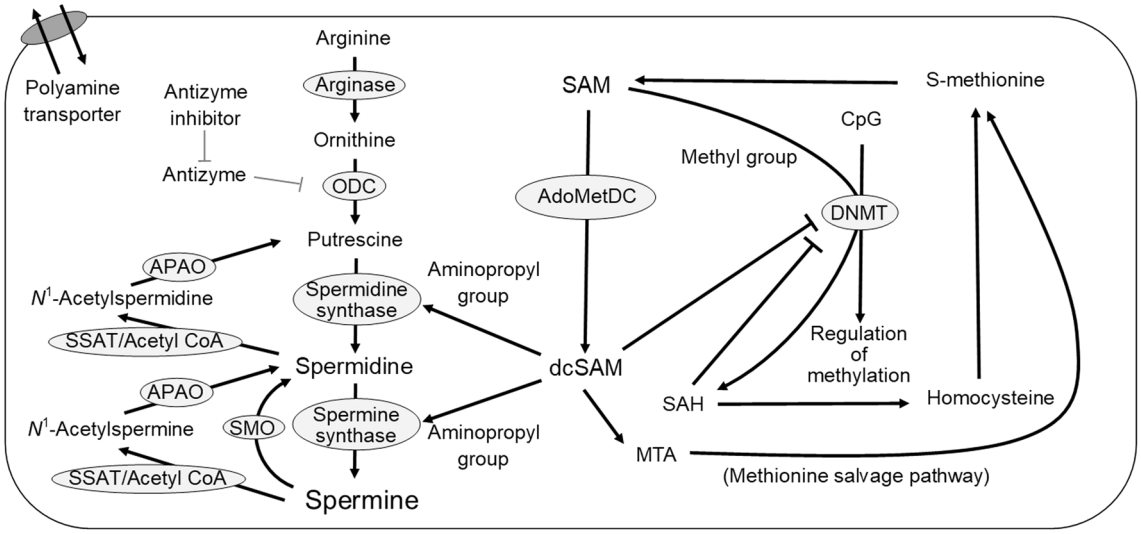

Fig. 4 Polyamine metabolism and gene methylation. Arrows indicate the metabolic pathway or flow of substances. T-bars indicate inhibitory activity. *AdoMetDC* *S*-denosylmethionine decarboxylase, *APAO* N^1-acetylpolyamine oxidase, *dcSAM* decarboxylated *S*-adenosylmethionine, *DNMT* DNA methyltransferase, *MTA* methylthioadenosine, *ODC* ornithine decarboxylase, *SAH* *S*-adenosylhomocysteine, *SAM* *S*-adenosylmethionine, *SMO* spermine oxidase, *SSAT* spermidine/spermine N^1-acetyltransferase

increased dcSAM caused by ODC inhibition were associated with aberrant methylation (increased demethylation in certain areas and increased hypermethylation in other areas) of the entire genome (Soda et al. 2013). Conversely, spermine supplementation inhibited AdoMetDC activity and decreased dcSAM concentrations with a decreased dcSAM/SAM ratio, as well as re-activated DNMT 3a and 3b in Jurkat cells treated with DFMO (Fukui et al. 2019). However, DNMT 1 was not re-activated by spermine supplementation. Similarly, the decreases in AdoMetDC activity have been shown to result in a decreased capability to convert SAM to dcSAM, resulting in increases in SAM and decreases in dcSAM concentrations (Pegg et al. 2011; Yamamoto et al. 2010), and changing the availability of methyl group donors has been shown to affect DNMT3a and 3b expressions (Poomipark et al. 2016). Decreases in AdoMetDC activity and dcSAM concentrations were also achieved when Jurkat cells were supplemented with spermine alone (Fukui et al. 2019). Decreases in dcSAM concentrations induced by spermine supplementation were associated with suppression of aberrant methylation induced by ODC inhibition (Soda et al. 2013) (Fig. 5).

Bi-directional changes in methylation status were also observed in the promoter area of LFA-1 (called ITGAL) (Kano et al. 2013). Detailed base sequencing after treatment with bisulfite, which converts unmethylated, though not methylated, cytosine to uracil, showed that the site responsible for LFA-1 expression in immune cells (Zhang et al. 2002) was demethylated and associated with increased LFA-1 protein levels after ODC inhibition (Kano et al. 2013). However, other regions in CpG were either demethylated or hypermethylated in a site-specific manner. Spermine supplementation reversed spermine deficiency-induced demethylation of the CpG area responsible for LFA-1 expression and decreased spermine deficiency-induced increase of LFA-1 protein levels. Similarly, changes in the status of DFMO-induced methylation in most other areas were almost reversed by spermine supplementation (Kano et al. 2013). Defective functional activity of DNMT may fail to maintain appropriate methylation (Hatazawa et al. 2018).

Generally, aging is associated with decreases in ODC (Yoshinaga et al. 1993) and DNMT activities (Oliveira et al. 2012), increased aberrant methylation status (increases in demethylation and hypermethylation) of entire genome, and enhanced demethylation of the LFA-1 promoter area in association with increases in LFA-1 protein levels (Lu et al. 2002; Soda et al. 2005; Soda et al. unpublished). In a murine model involving chows with different polyamine concentrations, the methylation status of the entire genome in old mice fed regular chow showed an increase in aberrant methylation. However, lifelong intake of high-polyamine chow prepared by adding synthetic polyamines prevented aging-associated increase in aberrant methylation (Soda et al. 2013). The regulation of methylation status in old mice was very similar to that observed in our in vitro study in which DNMT suppression resulting from DFMO-induced ODC inhibition caused aberrant methylation, while spermine supplementation reversed this condition (Kano et al. 2013; Soda et al. 2013) (Fig. 5).

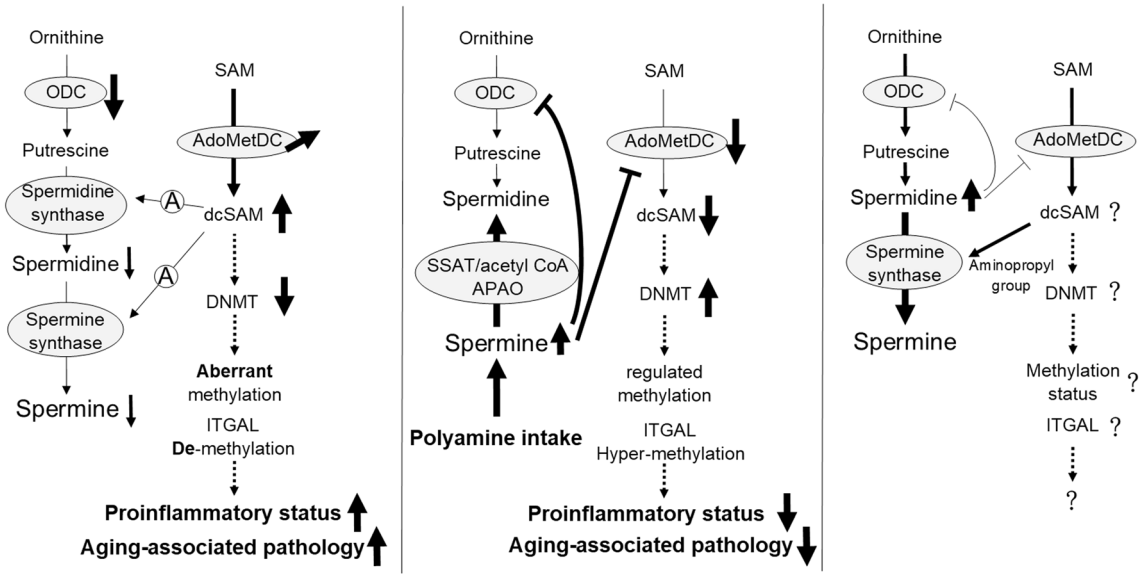

Fig. 5 Changes in polyamine metabolism and the consequence on aging-associated pathologies, including proinflammatory status. Arrows indicate the metabolic pathway or flow of substances. T-bars indicate inhibitory activity. Dashed arrows indicate the consequence (downstream) induced by the change (upstream). (Left) aging is associated with decreased ODC activity. Decreased ODC results in decreased supply of putrescine for polyamine synthesis. When ODC is suppressed, AdoMetDC activity is either activated or unchanged. The decreased supply of putrescine does not require an aminopropyl group from dcSAM. Therefore, surplus dcSAM inhibits DNMT activity, resulting in aberrant methylation of the entire genome and demethylation of ITGAL. Demethylation of ITGAL promotes a proinflammatory status by increasing protein levels of LFA-1. Aberrant DNA methylation promotes various aging-associated pathological changes. (Middle) when spermine is supplied from extracellular sources as a result of increased polyamine intake, spermidine is produced by the degradation of spermine via SSAT/Acetyl CoA and APAO and SMO, and therefore, no aminopropyl group is required for polyamine synthesis. This may result in strong inhibition of AdoMetDC, resulting in a decrease of dcSAM concentration. Decreased dcSAM re-activates DNMT, resulting in regulation of the methylation status of the entire genome as well as hypermethylation of ITGAL. Hypermethylation of ITGAL suppresses aging-associated enhancement of the proinflammatory status by decreasing protein levels of LFA-1. Inhibition of aberrant DNA methylation may result in inhibition of various aging-associated pathological changes. (Right) the potency of biological activity of spermine can be estimated by its effect on polyamine metabolism and gene methylation. When the spermidine concentration is increased as a result of supply from extracellular sources, spermine is synthesized by the enzymatic activity of spermine synthase. However, an aminopropyl group from dcSAM is required for spermine synthesis. This indicates that the ability of spermidine to inhibit AdoMetDC activity and the resultant decrease in dcSAM concentration are both weak (indicated by thin T-bar). A in circle: Aminopropyl group, *AdoMetDC* *S*-adenosylmethionine decarboxylase, *APAO* N^1-acetylpolyamine oxidase, *dcSAM* decarboxylated *S*-adenosylmethionine, *DNMT* DNA methyltranferase, *ODC* ornithine decarboxylase, *SAM* *S*-adenosylmethionine, *SMO* spermine oxidase, *SSAT* spermidine/spermine N^1-acetyltransferase

Possible role of polyamines in inhibiting tumorigenesis

Many recent reports have shown that aging-associated aberrant DNA methylation are closely related to the occurrence of cancer (Johnson et al. 2017; Okuchi et al. 2016). Polyamines have many biological activities that may inhibit aging-associated pathological processes, including the aging-associated progression of aberrant DNA methylation. In addition, epidemiological studies have shown that a polyamine-rich diet (Soda et al. 2009a) was associated with a decreased incidence of breast and colon cancer (Couto et al. 2011; Trichopoulou et al. 2010). Therefore, it is reasonable to assume that increased polyamine intake may suppress neoplastic diseases. To test this hypothesis, animal models were employed in which dietary patterns and carcinogen exposure were similar to those in humans. The majority of humans are born without an increased risk of tumorigenesis and grow up with a regional dietary pattern. Under such circumstances, humans are continuously exposed to imperceptible carcinogenic stimuli throughout their lives. BALB/c mice were fed chows with different polyamine concentrations, and were then repeatedly administered moderate amounts of a carcinogen (20 mg/kgBW of 1,2-demethylhydrazine) once a week for 12 consecutive weeks. Mice fed high-polyamine chow had a lower incidence of colon cancer (Soda et al. 2013). A group at Josai University examined enhancement of tumorigenesis by increased polyamine intake. However, in rats administered low-dose of a carcinogen (85 mg/kgBW of 2-amino-1-methyl-6-phenylimidazole) for 8 days and chows containing three different polyamine concentrations, increased polyamine intake did not increase carcinogenesis and even seemed to suppress it (Wada et al. 2002). In a cohort study, increased polyamine intake from

food was associated with an increased number of colon polyps in patients who were already at high risk (Vargas et al. 2012). However, a study of subjects at low risk of neoplastic diseases showed that increased polyamine intake was associated with decreased tumorigenesis (Vargas et al. 2015).

ODC is a focus of therapies that aim to prevent carcinogenesis because it is a transcriptional target of a proto-oncogene (Bello-Fernandez et al. 1993), and many studies have shown that transfection of the ODC gene results in increased intracellular polyamine levels and malignant transformation. Most reports on the roles of ODC in malignant transformation have examined cells and animals that were already at risk of tumorigenesis. However, a recent study showed that ODC overexpression is sufficient to induce tumorigenesis (Shukla-Dave et al. 2016), and this result further emphasizes the importance of ODC in tumorigenesis. Moreover, the role of ODC in neoplastic growth in animals has also been examined using ODC inhibitors such as DFMO and ODC-AZ, an enzyme that which help accelerate ODC degradation (Shantz and Levin 2007). Reaffirming the importance of ODC inhibition in tumorigenesis, increased polyamine concentration reduces ODC translation in reticulocyte lysates (Kashiwagi et al. 1991) and in cell cultures (Lovkvist et al. 1993). In cells with normal homeostasis, the influx of polyamines from the extracellular space suppresses ODC activity, with spermine being the most effective polyamine in regulating ODC activity (Yuan et al. 2001) (Fig. 5). Significant suppression of ODC activity in the intestinal mucosa of rats fed chow with high polyamine concentrations was reported (Brodal et al. 1999). In addition, the potency of spermine's biological activities on gene methylation is reflected by the relation between polyamine concentrations and LFA-1 expression, the latter of which is controlled by methylation of ITGAL (LFA-1 promoter area). Among healthy volunteers, blood spermine levels inversely correlated with LFA-1 expression, while blood spermidine levels had no correlation with LFA-1 expression (Soda et al. 2005; Soda et al. unpublished).

Conclusion

At this point, despite extensive studies, there is no proof that changes in a specific signal transduction pathways or specific proteins can extend lifespans, especially in mammals (Lopez-Otin et al. 2013). Instead, there is an overwhelming scientific consensus supporting the important role of epigenetic changes in aging-associated pathologies and lifespan alteration (Chen et al. 2016; Maeda et al. 2017). Based on these perspectives, it is important to inhibit aging-associated aberrant DNA methylation by maintaining DNMT activity in a sustained manner.

This may be achieved by a long-term increase in polyamine intake, resulting in continuous elevated spermine levels that persistently boost its biological activities. A study reported that in volunteers over age 90, the proportion of spermine relative to total polyamines was significantly higher than in individuals from ages 60 to 80 (Pucciarelli et al. 2012), while the proportion generally decreases with aging (Elworthy and Hitchcock 1989; Soda et al. unpublished). The increased ratio of spermine to spermidine in very elderly people may be related to the increased polyamine intake or to an increased supply of spermine from the digestive system.

Compliance with ethical standards

Conflict of interest The author declares no conflict of interest.

Research involving human participants and/or animals The article is a review article. Therefore, there is no human participants or animals.

Informed consent The article is not a research article.

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