Epigenetic effects of selenium and their implications for health

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Introduction

Epigenetics describes mitotically stable chromatin-based mechanisms that modulate gene expression without altering the genomic DNA sequence. These mechanisms include modifications to DNA [methylation of cytosine to 5-methylcytosine (5 mC), and 5mC oxidation products] and to histones (acetylation, methylation and many others), which interfere with chromosomal packaging and the binding of trans-acting factors. The recent advancements in DNA/RNA sequencing technology have made it possible to study epigenetic marks on a genome-wide level, gaining insight into the so-called epigenome. Multi-centered consortia, namely DEEP (Deutsches Epigenom-Programm, German Epigenome Program, www.deutsches-epigenom-programm.de) and IHEC (International Human Epigenome Consortium, www.ihec-epigenomes.org) are currently deciphering high-resolution epigenome maps of healthy and diseased tissues/cell types to obtain standard and disease-specific epigenome profiles. According to current knowledge, epigenomes show plasticity throughout an organism’s lifetime, during cellular differentiation and in response to manifold external stimuli. Changes to the epigenome are also associated with the onset and progression of cancer and other complex diseases like autoimmunity diseases, inflammatory bowel diseases, type 2 diabetes, and cardiovascular disease. Causality of most of these associations remains unknown, but in light of the principal alterability of epigenetic marks—in contrast to the largely stable DNA setup of a cell—targeting the epigenome may provide a promising strategy in disease therapy and prevention. Major determinants of risk and progression of the above-mentioned common diseases are—besides genetic predispositions—environmental factors like lifestyle and diet. Dietary patterns, specific (micro) nutrients and secondary plant compounds have been found to alter epigenetic marks, and evidence is increasing that the modulation of health outcomes by food components is (at least in part) mediated by their epigenetic effects. While also macronutrients (e.g., employed as experimental high-fat, high-protein, or calorie-restricted diets) have been shown to modify epigenetic marks, and mechanistic explanations for these observations are difficult to derive due to multiple confounding effects that are elicited by macronutrients and due to composition variability. Therefore, the majority of studies have assessed epigenetic effects in response to intervention with specific micronutrients and secondary plant compounds. In this regard, selenium (Se), an essential trace element that exists in a range of biologically active compounds, is a particularly interesting micronutrient. It has been found to modify epigenetic marks in studies employing cell systems and animals, and in a limited number of human studies, too. The importance of Se for maintaining optimal health is based on the biological functions that are exerted by 2 main groups of Se species: members of the selenoprotein family, which are encoded by 25 genes in humans and contain cotranslationally inserted selenocysteine, and, second, a non-selenoprotein pool of low-molecular-weight selenium compounds contained in the diet or derived from Se metabolism. Well-characterized selenoproteins, such as glutathione peroxidases (GPx), thioredoxin reductases (TrxR), and iodothyronine deiodinases (DIO) are oxidoreductases and act as antioxidant enzymes, regulators of redox-sensitive signaling pathways and of thyroid hormone metabolism. Other

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selenoproteins facilitate selenium transport (selenoprotein P), selenoprotein biosynthesis (selenophosphate synthetase 2), and are involved in maintaining endoplasmic reticulum homeostasis (e.g., selenoproteins S, K, and 15-kDa selenoprotein). The importance of selenoproteins for murine development and health has been demonstrated in transgenic mice with single or total selenoprotein depletion. Moreover, humans with genetically impaired selenoprotein biosynthesis suffer from a severe multisystem disorder. Biological outcomes of murine and human Se supplementation studies (e.g., disease incidences, effects on the transcriptome, epigenome, and on Se status, and references therein) are known to be affected not only by Se dosage, but also by its chemical form and the pre-supplemental Se status. We will therefore briefly present an overview of Se requirements and the metabolism of dietary Se compounds. Thereafter, we summarize the current knowledge of the effects of naturally occurring and synthetic Se compounds on epigenetic marks and editors and discuss their possible relevance for health and disease. An aim is to approach the biochemical basis of the Se–epigenome interaction, and to this end we focus on studies that have shown direct and indirect effects of Se compounds on the epigenetic machinery (e.g., DNA methyltransferases, histone modifying enzymes, one-carbon metabolism). These questions cannot fully be answered by the current knowledge, and we therefore indicate which studies may be useful to broaden our understanding of the Se-epigenome interaction in the future.

Selenium Requirements and Metabolism of Dietary Se Compounds

Overt Se deficiency is relatively rare in Western countries. A clinical condition that is associated with Se deficiency is Keshan Disease, which occurred in a Chinese province where affected people had total daily Se intakes of \( \leq 15 \, \mu g \). The tolerable upper intake level of Se has been defined by the World Health Organization as 400 \( \mu g \) per day, and the majority of health bodies recommends Se intake levels in the range from 55 to 70 \( \mu g \) per day. These recommendations are often based on total Se intake required to optimize activity/expression of GPx3 and SeP in plasma, 2 commonly used biomarkers of Se status. Optimization of plasma GPx3 activity is achieved at 40–47 \( \mu g \) Se/day, whereas SeP requires \( \sim 105 \, \mu g \) Se/day. The plasma Se concentration that coincides with SeP optimization (124 \( \mu g \) Se/l) is in a range that is associated with decreased mortality risk and prevention of several cancers. Supplementation beyond these Se levels appears to confer no additional benefit but may instead increase the risk of type 2 diabetes. Major dietary selenocompounds are the amino acids selenomethionine (SelMet), selenocysteine, Se-methylselenocysteine, and the anions selenite and selenate (for a listing of Se compounds and quantities in food see). Se compounds are metabolized through different routes (displayed in Fig. 1) to hydrogen selenide. Hydrogen selenide is methylated to excretory forms (dimethylselenide, trimethylselenonium, Se-methyl-N-acetylselenohexosamine) or phosphorylated to selenophosphate, used as precursor of the amino acid selenocysteine (Sec), which is cotranslationally inserted from a Sec-specific tRNA (Sec-tRNA\(^{\text{Sec}}\)) into selenoproteins. Selenite is reduced via glutathionylation to selenot(di)glutathione or via glutaredoxin directly to selenide. Metabolism of SelMet takes place via the transsulfuration pathway—catalyzed by the same enzymes that transform methionine to cysteine—to Sec, which is converted by selenocysteine β-lyase (SBL) to selenide and alanine. In a similar reaction, SBL generates methylselenol from Se-methylselenocysteine. The methylseleno-amino acids Se-methylselenocysteine and SelMet can be transaminated by glutamine transaminase K and by L-amino acid oxidase to β-methylselenopyruvate and to α-keto-γ-methylselenobutyrate, respectively (see for references referring to Se metabolism).

Influence of Selenium on Epigenetic Modifications of DNA and Histones

Influence of Se on DNA methylation

The methylation of cytosines in genomic DNA is the most common and probably most studied epigenetic modification in higher organisms. The methyl group is transferred in a reaction catalyzed by DNA methyltransferases (DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L) from the donor substrate S-adenosylmethionine (SAM) to the 5-carbon position of cytosine—often when it is bound to guanosine in 3′—, resulting in 5-methylcytosine (5 mC). DNA demethylation, on the other hand, is not catalyzed directly but results from either DNA replication-coupled dilution, wherein 5 mC or 5 mC oxidation products [5-hydroxymethylcytosine (5 hmC), 5-formylcytosine (5 fC) and 5-carboxycytosine (5 caC)] are not copied to the new DNA strand, or after replacement of 5 mC (or derivatives) together with short stretches of surrounding nucleotides via base excision repair (BER) or nucleotide excision repair (NER). Interference of nutrients with DNA methylation can therefore principally occur through modulation of (i) DNMT activity / interaction with accessory factors, (ii) SAM availability, and (iii) demethylation processes. A number of studies have reported effects of Se status or supplementation on global and gene-specific DNA methylation as well as on the expression or activity of DNMTs (listed in Table 1). Arai et al. incubated murine embryonic stem cells with a physiologic, non-toxic Se dose as found in maternal blood serum. Se caused a reversible alteration of the cell heterochromatin status and also changed the DNA methylation status of individual genes with roles in fetal development, including Hnf4α (hepatocyte nuclear factor 4α), Aebp2 (AE binding protein 2), Prickle2 (prickle homolog 2), and Rnd2 (Rho family GTPase 2), without compromising the cell potential to form embryonic bodies. These results imply an interesting link between Se and tissue-specific differentiation via effects on gene-specific methylation, as Se is well known to be required for hepatocyte differentiation in vitro, and the transcription factor HNF4α is a key regulator of this process. The change in chromatin structure observed by Arai et al. could be caused by global DNA methylation differences in Se-supplemented versus deficient cells. Studies employing rodents and cell lines have
Figure 1. Metabolism of dietary selenocompounds. The major organic and inorganic selenocompounds are metabolized by transsulfurations, transaminations and reductions by thioredoxin reductases, glutathione reductases and glutaredoxins. Participating enzymes marked with green background. For details see text. GTK = glutamine transaminase K; AAO = L-amino acid oxidase; GR = glutathione reductase; CGL = cystathionine γ-lyase; CBS = cystathionine β-synthase; SBL = selenocysteine β-lyase.

Table 1. Studies regarding effects of Se on DNA methylation and DNMT expression or activity

| Species; tissue/cell type | Se dose and form | Main outcome | Ref |
|--------------------------|-----------------|--------------|-----|
| Human colon tissue lysate | Various doses (0–12 μM); selenite, BSC, p-XSC | Inhibition of DNMT activity by selenite (IC50 = 3.8 μM), BSC (IC50 = 8.4 μM), p-XSC (IC50 = 5.2 μM) | 45 |
| Human rectal mucosa | No intervention | Plasma Se concentration associated with methylation of WIF1 in both sexes. Sex-specific associations for LINE1, PCA1, N33, SFRP1/2 and APC methylation | 39 |
| Human leukocytes | No intervention | Inverse association of plasma Se and leukocyte DNA methylation | 32 |
| Human peripheral blood | No intervention | Differential methylation and expression of TLR2 and ICAM1 in Keshan disease vs healthy controls | 40 |
| Rat liver and colon | 0, 0.15, 4 mg/kg; SeMet | Less global methylation in high Se, but more at p53 | 27 |
| Rat liver and colon | 0.003, 0.2 mg/kg; selenite | Less global methylation in Se deficiency in liver but not in colon. Non-significant decrease in DNMT activity (liver + colon) in Se deficiency | 28 |
| Rat liver and colon | 0, 0.1, 2 mg/kg; selenite | Less global methylation in liver and colon under Se deficiency | 29 |
| Rat myocardium and cardiomyocytes | 0, 0.1, 2 mg/kg; selenite and SelMet | Selenite triggers methylation and silencing of TLR2 and ICAM1, inhibition of DNMT1 expression. Increased Gadd45α expression in Se deficiency | 40 |
| Rat liver extract | Various doses; selenite | Inhibition of DNMT activity by selenite (Ki = 6.7 μM) | 46 |
| Human LNCaP cells | 0.5, 1.5 μM; selenite | Demethylation and reexpression of GSTP1 and APC. Inhibition of DNMT1 expression, reduction of total 5-mC content | 31 |
| Human MCF-7 cells | 2 μM; MSA, 8 μM; selenite | Decreased expression of DNMT1 | 47 |
| Human Caco-2 cells | 250 nM; SeMSC | Demethylation and re-expression of VHL tumor suppressor by SeMSC | 36 |
| Rat mucosa | 0, 2 mg/kg; SeMSC | Induction of VHL by SeMSC | 36 |
| Mouse ES cells | 100 ppm; selenite | Heterochromatin formation reversibly affected. Decreased methylation at Aebp2, Prickle2, HNF4 and RND2 loci. | 26 |
indeed shown that dietary Se intake levels affect global DNA methylation. The rodent studies gave inconsistent results regarding an increase or decrease of global DNA methylation in response to supplemental Se, although comparable Se diets were used (Table 1): Se deficiency resulted in less DNA methylation in rat liver\textsuperscript{28,29} and colon,\textsuperscript{29} in contrast to a study by Zeng et al., wherein DNA methylation levels in liver and colon were higher in rats fed supranutritional vs. adequate and deficient Se diets. Zeng et al. pointed to differences in the employed animal strains and contents of the basal diets as possible modifiers of Se effects.\textsuperscript{27} Additionally, different techniques were applied for the assessment of global DNA methylation: an in vitro methyl group acceptance assay with [\textsuperscript{3}H-methyl]-SAM/SsSI methylase and isolated DNA,\textsuperscript{28,29} a 5 mC ELISA,\textsuperscript{27} and by HPLC detection of 5 mC monophosphate in enzymatically digested DNA.\textsuperscript{30} Corresponding data from in vitro studies is limited to one paper, showing that LNCaP prostate tumor cells treated with 1.5 \(\mu\text{M}\) selenite for 7 d had a \(\approx\)50\% decrease in 5 mC immunoreactivity.\textsuperscript{31} A conclusion that can be drawn is that impacts of selenite and SelMet on global DNA methylation are possibly masked by strain-specific effects and that they are also influenced by the nutritional context (e.g., high fat diet). This topic has been elucidated in a human study (\(N = 287\)) that found a significant inverse association of plasma Se and global DNA methylation in leukocytes.\textsuperscript{32} In addition to effects on global methylation, Se has also been shown to induce differential methylation at regions and specific CpG sites of individual genes. The study by Xiang et al. found that the genes encoding the phase II detoxification enzyme GSTP1 (\(\pi\)-class glutathione S-transferase) and the tumor suppressors APC (adenomatous polyposis coli) and CSR1 (cellular stress response 1), which are frequently silenced in prostate tumors due to hypermethylation of their promoters, were demethylated and re-expressed in LNCaP cells after selenite-treatment. Similarly, a different source of Se (100 \(\mu\text{M}\) SelMet) caused promoter demethylation and re-expression of GSTP1.\textsuperscript{31} Se compounds are commonly applied in in vitro studies at concentrations ranging from 10 nM up to 100 \(\mu\text{M}\). Growth inhibitory and toxic effects of Se are dependent on its chemical form and on the cell type. While Se has low toxicity when it is bound to proteins (as in plasma) or amino acids (e.g., SelMet), many cell lines do not tolerate selenite or methylseleninic acid at doses \(\geq 1 \mu\text{M}\).\textsuperscript{33} In consideration of the physiological concentration range of Se in human plasma (\(\sim 0.4–2.5 \mu\text{M}\)), Se doses of \(> 5 \mu\text{M}\) are supraphysiological and not applicable for supplementation trials. The use of Se for cancer prevention is particularly promising for prostate cancer, based on the observation of an inverse association of prostate cancer risk and Se status,\textsuperscript{34} as well as on supporting findings of accelerated prostate carcinogenesis in selenoprotein-deficient mice.\textsuperscript{35} In this context, a concept considering Se as an epigenetic drug against cancer progression through targeting of tumor suppressor genes, as implied by Xiang et al., may arise, but certainly in vivo studies need to be performed to strengthen and broaden it to other relevant genes that might also be targeted by Se in different forms and at different stages of carcinogenesis. Methylation of the von-Hippel-Lindau (VHL) gene promoter was found to respond to Se (250 nM Se-methylselenocysteine (SeMSC)) in Caco-2 cells; VHL promoter methylation was decreased by SeMSC in vitro and this was associated with increased VHL expression levels in Caco-2 and in rats fed 2 \(\mu\text{g}\) Se as SeMSC.\textsuperscript{36} VHL is frequently downregulated and mutated in renal cell carcinomas and also found to be deregulated during colorectal carcinogenesis,\textsuperscript{37} wherein Se has been attributed protective functions based on epidemiological and animal studies.\textsuperscript{18,38} A study with humans assessed the methylation status of colorectal cancer-related genes in healthy rectal mucosa specimens (84 males, 101 females) in relation to Se status.\textsuperscript{39} An association was found for WIF1 (wnt inhibitory factor 1) methylation and plasma Se concentration. Interestingly, Se status was also associated with methylation of other genes and retrotransposons, including LINE1 (long interspersed nucleotide element 1), PCA1 (cation-transporting P-type ATPase), SFRP1/2 (secreted frizzled-related protein 1/2), and APC, and this occurred in a gender-specific manner. It was not analyzed whether differential methylation of these genes was associated with differences in their expression levels. The EPIC (European prospective investigation of cancer and nutrition cohort) study revealed an inverse association of Se status and colorectal cancer risk with the association being stronger in women than men.\textsuperscript{38} Gender-specific effects of Se are also apparent from different responses of selenoprotein expression levels and biomarkers of Se status to Se supplementation in men and women (summarized in\textsuperscript{38}); the study by Tapp et al. suggests that this gender-specificity of Se-effects extends to epigenetic marks of cancer-related genes, but the relevance of these findings for disease etiology warrants further investigations. A recent study identified the inflammatory-related genes TLR2 (toll-like receptor 2) and ICAM1 (intercellular adhesion molecule 1) as novel targets of Se-dependent epigenetic regulation and proposed a mechanism whereby Se changes the expression of GADD45 (growth arrest and DNA-damage-inducible, \(\alpha\)) and DNMT1, leading to epigenetic silencing of TLR2 and ICAM1, and links it to a well-known condition that arises from prolonged periods of severely deficient Se intake: Keshan disease (\(K_D\)).\textsuperscript{40} \(K_D\) is a viral myocarditis with necrotic lesions of the myocardium that was first found in the Keshan County of north-eastern China in 1935 and that is endemic in selenium-deficient areas of China. It was found out that Se deficiency is a causal factor of \(K_D\), and the incidence rates lowered dramatically after Se was given as a supplement.\textsuperscript{41} The mode of action of Se in the etiology of \(K_D\) is not understood completely, but it is assumed to result from an impaired immune response to viral (Coxsackie virus B) infections in Se-deficiency. Yang et al. compared the DNA methylome in peripheral blood from \(K_D\) patients and healthy controls using methylated DNA-IP and subsequent analysis of the enriched DNA by a Roche-Nimblegen HG18 CpG Promoter array. The methylome profiles showed differences at several thousand differentially methylated regions (DMRs), which were confirmed for the TLR2 and ICAM1 promoters by methylisation-specific PCR. Furthermore, expression levels of both genes were inversely correlated with degrees of promoter methylation and with serum Se concentration. Similar results were obtained with rats fed diets containing 0, 0.1 and 2.0 mg/kg sodium selenite, whereas in contrast to the human...
subjects methylation/expression of Tlr2 and Icam1 genes were measured in myocardial tissue and in isolated neonatal cardiomyocytes. While a significant reduction of Dnmt1 protein expression level was observed in cardiomyocytes treated with 1.5 \( \mu M \) selenite compared to Se-deficient cells, this was unlikely and against anticipation to be the cause of increased Tlr2 and Icam1 promoter methylation. Instead, the authors suggested that this was due to diminished Gadd45a mRNA and protein expression levels in Se-treated cardiomyocytes. GADD45A has been linked to demethylation of specific genomic loci, e.g., in the genes for RARB2 (retinoic acid receptor β), via interaction with BER- and NER-executing proteins.\textsuperscript{25} But a role for GADD45A as mediator of Se-dependent modulation of (site-specific) DNA methylation, as implied by Yang et al.,\textsuperscript{40} remains speculation until substantiated by additional studies employing, for example, GADD45A gene silencing and chromatin immunoprecipitation. Also, numerous studies have reported that Se enhances DNA damage repair capacity (summarized in\textsuperscript{42}) via enhanced p53 binding activity\textsuperscript{29} and partly, in contrast to the results obtained with rat cardiomyocytes,\textsuperscript{40} associated with increased expression of GADD45—inc MCF7 cells via GPx-1\textsuperscript{43}—or interaction thereof with DNA repair enzymes, such as AP endonuclease 1.\textsuperscript{14} Whether these effects of Se on DNA repair enzymes do promote DNA demethylation is however not certain.

Another possible Se-target to induce differential DNA methylation is the class of DNMT enzymes; the modulation of DNMT expression or activity has been postulated to contribute to the modulation of methylation marks by Se seen in the studies mentioned above. Selenite and 2 synthetic selenocompounds, benzyl selenocyanate (BSC) and 1,4-phenylenebis (methylene) selenocyannate (p-XSC), have been shown to inhibit DNMT activity in nuclear extracts of human colon carcinomas.\textsuperscript{45} The IC\textsubscript{50} values of the 3 compounds were calculated as 3.8 \( \mu M \) (selenite), 8.4 \( \mu M \) (BSC) and 5.2 \( \mu M \) (p-XSC), and the setup of the experiments implies that the compounds acted in their non-methylated forms. DNMT prepared from rat liver was also inhibited by selenite in an in vitro assay at a \( K_\text{m} \) of 6.7 \( \mu M \), and the enzymatic activity was lower when isolated from Se-supplemented compared to control animals.\textsuperscript{26} In vivo, a non-significant decrease of DNMT activity was seen in the liver and colon of rats fed Se-adequate vs. -deficient diets (0.2 and 0 mg Se/kg diet as sodium selenite).\textsuperscript{28} Similarly, Dnmt1 protein expression was diminished by 1.5 \( \mu M \) sodium selenite in rat cardiomyocytes\textsuperscript{40} and in LNCaP cells\textsuperscript{35} and by 8 \( \mu M \) sodium selenite and 2 \( \mu M \) MSA in MCF-7 cells.\textsuperscript{47} The in vitro human and animal studies collectively indicate that Se is inversely associated with global DNA methylation and DNMT activity.

**Influence of Se on histone acetylation**

Histone proteins carry manifold posttranslational modifications (e.g., methylation and acetylation) added by histone methyltransferases, histone acetyltransferases (HATs), etc. at defined amino acid positions. Common histone marks that are studied by the IHEC consortium are methylation and acetylation at lysine residues 4, 9, 27, and 36 of histone 3. The highly diverse code of histone marks controls histone binding to DNA, interaction of DNA with trans-acting factors, and ultimately gene expression. Aberrant histone codes are found at the onset and progression of diseases and have therefore become therapeutic targets. Se and other micronutrients have been shown to induce or to be associated with changes to histone marks, thereby possibly affecting health outcomes. Interference of nutrients with histone marks can principally occur through modulation of histone modifying enzyme activity/expression and via interference with substrate availability. Given the large variety of marks and participating enzymes, the situation is even more complex than for DNA methylation; furthermore, crosstalks exist between DNA methylation and histone marks and together they form a complicated network of epigenetic regulation.\textsuperscript{48} From a clinical perspective, particular interest has been given to histone deacetylases (HDACs), as their abnormal function and/or expression is linked to cancers and some neurologic and immune disorders. Numerous synthetic HDAC inhibitors have been developed and are currently tested in clinical trials.\textsuperscript{49} Some naturally occurring dietary factors or metabolites like butyrate, polyphenols and Se have also been shown to act as HDAC inhibitors. Studies reporting the modulation of histone marks and editing enzymes (HDACs and HATs) by dietary and synthetic Se compounds are listed in Table 2 and discussed below.

Treatment of LNCaP cells with 1.5 \( \mu M \) selenite for 7 d significantly reduced HDAC activity, concomitant with slightly reduced HDAC3 and unchanged HDAC4 and HDAC5 protein levels.\textsuperscript{31} In line with reduced HDAC activity, the authors found both global and GSTP1 promoter-bound levels of H3K9ac—a repressive mark—to be increased and H3K9me3—an activating mark—to be decreased upon Se treatment. Other studies have confirmed that dietary and synthetic selenocompounds inhibit HDAC activity.\textsuperscript{50–53} Kassam et al. applied methylseleninic acid (MSA; 5–30 \( \mu M \)) to diffuse large B-cell lymphoma (DLBCL) cell lines and observed decreased HDAC activity and increased total levels of the HDAC targets acetylated histone H3 and acetylated α-tubulin.\textsuperscript{53} These effects required cellular metabolism of MSA, but the metabolite responsible for HDAC inactivation was not identified. Lee et al. discovered that seleno-α-keto acid metabolites derived from dietary selenocompounds act as inhibitors of HDACs.\textsuperscript{50} In their study, SeMSC and SelMet reacted in transaminations catalyzed by the enzymes glutamine transaminase K (GTK) and L-amino acid oxidase to β-methylselenopyruvate and to α-keto-γ-methylselenobutyrate, which are structurally similar to the well-known HDAC inhibitor butyrate (Fig. 2). Both seleno-α-keto acids inhibited HDAC activity in cell free assays, in contrast to SeMSC and SelMet, and led to rapid elevation of acetylated histone H3 levels in prostate cancer cells. The anti-cancer effects of high dose Se supplementation observed in studies of experimental carcinogenesis have been partly attributed to the inhibition of HDAC and stimulated the synthesis of more potent Se-containing HDAC inhibitors (HDACi). Based on the structure of suberoylanilide hydroxamic acid (SAHA, commercially available as Vorinostat), a HDACi that is used for the treatment of advanced T-cell lymphoma, 2 Se-based derivatives named SelSA-1 and SelSA-2 (Fig. 2) were synthesized.\textsuperscript{51} SelSA-2 showed higher inhibitory activity against
HDAC in HeLa nuclear cell extract (mainly isoforms HDAC1 and HDAC2) than SAHA and trichostatin A. Both selenocompounds were also more effective in inhibiting melanoma cell growth and melanocytic lesion development in skin reconstructs, but their use as drugs against melanoma or other cancers has not been tested yet. A recent paper has shown that histone H4 acetylation at its lysine residues 5, 8, 12, and 16 was decreased in macrophages treated with selenite (100–500 nM). This was concomitant with a decreased abundance of H4K12ac and H4K16ac at the promoters of the pro-inflammatory genes tumor necrosis factor- (TNF-α) and cyclooxygenase-2 (COX-2), whose expression had been shown before to be diminished in Se-treated RAW264.7 macrophage cells. HDAC activity in these cells was unaffected by selenite, and the authors proposed that the selenite-triggered decrease of H4 acetylation levels was rather due to inhibition of p300 HAT activity through a Se-dependent and haematopoietic prostaglandin D synthase (H-PGDS)- and COX-mediated production of anti-inflammatory Δ12-PGJ2 and 15d-PGJ2, which can covalently bind to p300 and thereby inhibit its activity. Importantly, selenite did not change H4 acetylation levels in macrophages that lacked Sec-tRNA[Ser]Sec, showing that selenoprotein biosynthesis was a prerequisite for selenite-induced modulation of H4 acetylation. The proposed cascade of events triggered by selenite (involving H-PGDS, COX-2, p300 HAT, and p65 and H4 acetylation) might trigger the switch from the M1 to the M2 phenotype of macrophages. It would thus also add to the understanding of the anti-inflammatory role of Se and selenoproteins in inflammatory bowel diseases and other chronic inflammatory diseases.

**Interrelation Between Selenium and One-carbon Metabolism**

The one-carbon metabolism pathway (depicted in Fig. 3) provides the methyl donor S-adenosylmethionine (SAM),

| Table 2. Modulation of histone modifications by dietary and synthetic selenocompounds |
|-------------------------------------------------|-----------------|-----------------|-------------------|
| Cell type                                        | Se dose and form | Se-based derivatives of SAHA: SelSA-1 and -2 | HDAC inhibition by SelSA-2 (IC50 = 8.9 nM) |
| Human Hela cell nuclear extract                  |                 | HDAC inhibition by SelSA-2 (IC50 = 8.9 nM) |
| Human LNCaP cells                                | 1.5 μM; selenite | Decreased HDAC activity, increased acetylated H3-Lys9 |
| Various normal and cancer cell lines             | SelSA-1 and -2  | HDAC inhibition by SelSA-1 and -2. Antiproliferative and proapoptotic in melanoma cells. Inhibition of melanocytic lesion development in vitro. |
| Murine RAW264.7 and primary macrophages          | 0–500 nM; selenite and SelMet | Decreased acetylated H4K5, H4K8, H4K12, H4K16. Less H4K12ac at the COX-2 promoter |
| Diffuse large B-cell lymphoma                    | 5–30 μM; MSA    | Decreased HDAC activity, increased acetylated H3 and α-tubulin |
| Human prostate cancer cells                      | 50, 200 μM; MSC and SelMet | Decreased HDAC activity, increased acetylated H3. HDAC inhibition by glutamine transaminase K and L-amino acid oxidase-derived seleno-α-keto acids |

**Figure 2.** Se-containing inhibitors of HDAC activity. Structural formulas of Se metabolites (β-Methylselenopyruvate and α-Keto-γ-methylselenobutyrate) and synthetic selenocompounds (B(PCP)-2Se [Bis(5-phenylcarbamoylpentyl)diselenide], SelSA-1 and PCP-SeCN (5-phenylcarbamoylpentyl selenocyanide), SelSA-2) with HDAC-inhibitory activity.
which is used as substrate by the DNMTs and other methylating enzymes for transfer of the methyl group to cytosines and target proteins. One-carbon metabolism is therefore tightly linked to DNA methylation, and conditions that have an impact on one-carbon metabolism, in particular the availability of the participating enzymes’ cofactors folate, choline/betaine and vitamins B2, B6, and B12, have been shown to result in differential DNA methylation.59 We briefly describe the reactions of the one-carbon metabolism and associated pathways and then assess the current literature regarding findings of Se-dependent modulations thereof. As depicted in Figure 3, methionine reacts to SAM, which is converted to S-adenosylhomocysteine (SAH) after the DNMT-catalyzed methylation of cytosine or proteins. Removal of the adenosyl group gives homocysteine (HCys). The concentration of HCys in blood or plasma is of clinical importance, as it has been associated with a multitude of complex diseases like cardiovascular and neurodegenerative diseases60 (and references therein). Clearance of HCys occurs through its remethylation to homocysteine (Hcy). The concentration of Hcy in blood or plasma is of clinical importance, as it has been associated with a multitude of complex diseases like cardiovascular and neurodegenerative diseases60 (and references therein). Clearance of Hcy occurs through its remethylation to methionine, in turn serving as a substrate for the formation of SAM, or otherwise via metabolism in the transsulfuration pathway. The first step of this pathway is the condensation reaction of Hcy with serine to cystathionine, catalyzed by cystathionine β-synthase (CBS) with pyridoxal phosphate (PLP, the active form of vitamin B6) as cofactor. A variant of the CBS gene (c.844ins68) was found to affect Hcy clearance and SAM/SAH ratio following methionine loading.61 The c.844ins68 was also associated with a significantly lower risk of coronary artery disease, baseline Hcy levels however appear to be unaffected by the CBS genotype in healthy individuals.61,62 Cystathionine is converted by the PLP-dependent enzyme cystathionine γ-lyase (CGL) to cysteine, which in turn condenses with glutamate to γ-glutamylcysteine, catalyzed by glutamate-cysteine ligase (GCL). Finally, fusion with glycine via glutathione synthetase (GS) results in the formation of glutathione. Remethylation of Hcy is facilitated by methionine synthase (MS) in an enzymatic reaction involving vitamin B12 and 5-methyl-THF. THF cycles back to 5-methyl THF via 2 enzymatic steps employing vitamin B6 and flavin adenine dinucleotide (FAD) as cofactors; the methyl group ultimately derives from the essential amino acid serine. A second route to methionine is the methylation of Hcy by betaine homocysteine methyltransferase (BHMT), which uses betaine as methyl donor.

Correlations between Se status and plasma homocysteine levels

Several human studies have shown an inverse correlation between plasma or serum Se and Hcy levels (Table 3). In an Inuit population with very high mean Se status (635.5 μg Se/l...
blood), plasma HCys was negatively predicted by Se.\textsuperscript{63} Klapcinska et al. similarly detected an inverse correlation between whole-blood Se and plasma HCys in a population with low mean Se status (62.5 \( \mu \)g Se/l blood).\textsuperscript{64} While both studies reported similar findings, possible confounders that are known to affect HCys levels, particularly folate and B vitamins, need to be taken into account. In a study where an inverse relationship between Se and HCys was reported for participants of the British National diet and Nutrition Survey aged \( \geq \) 65 years,\textsuperscript{65} this correlation became insignificant after adjustment for folate, PLP, and vitamin B\textsubscript{12}.\textsuperscript{60} In the study by Bekaert et al., however, an association of Se and HCys remained significant after adjustment for the same confounders, but only in males.\textsuperscript{66} Plasma Se was calculated to account for 1.8% of HCys variance by linear regression analyses. In line with this, serum Se predicted 5.8% variance in HCys in a Spanish population independently of folate and vitamin B\textsubscript{12}, and individuals within the highest versus lowest Se tertile had a 63% reduced risk of being in the top HCys tertile.\textsuperscript{66} Ischemic stroke patients aged <55 years that were assessed shortly after stroke onset (third day) were found to have lower Se status than healthy controls, which was inversely correlated with HCys and accounted for 15.4% of HCys variance independent of vitamin B\textsubscript{12} levels.\textsuperscript{67} In light of these baseline associations, trials have been conducted to determine whether Se supplementation affected plasma HCys. Se supplementation in form of daily doses of 100 or 300 \( \mu \)g Se as high-Se yeast for 6 months increased plasma Se but had no effect on HCys levels.\textsuperscript{66} This lack of effect supported an earlier intervention study where 200 \( \mu \)g Se (as SelMet) given daily for 20 weeks failed to change plasma HCys levels; yet a possible association of Se and HCys at baseline was not assessed in that study.\textsuperscript{68}

Animal experiments allow greater flexibility in the design of supplementation studies, a better control for confounding factors, and more options for end point detections. Table 4 lists studies with rodents that were fed diets with different Se form and content, followed by subsequent assessments of metabolites and enzymes of the one-carbon metabolism. Some general trends, which are independent of the Se form and the species, can be derived from these studies: (I) an inverse correlation between HCys and Se intake/status appears to exist in the liver. This derives from a study with mice fed SelMet\textsuperscript{69} and a study with rats fed selenate.\textsuperscript{70} Both studies used diets with comparable Se contents that are alike the range of dietary intakes levels existing in human populations. Strikingly, slightly suboptimal Se intake levels (0.05/0.06 ppm Se) in comparison to adequate intake levels (0.15 ppm Se) caused in both studies a significant increase in liver HCys concentrations, by 30% in rats\textsuperscript{70} and 314% in mice.\textsuperscript{59} The latter study suggested that liver hyperhomocysteinemia was due to Se-dependent regulation of CBS. Other studies have also reported differential expression of enzymes belonging to the transsulfuration (GCL)\textsuperscript{71} and remethylation pathways (BHMT and glycine N-methyltransferase),\textsuperscript{28,71,72} but these effects appeared to be species-specific and were only seen under severe Se deficiency. (II) Plasma HCys concentrations were significantly reduced in those animals (mice and rats) that were fed diets considered as Se deficient (\( \leq 0.025 \) ppm),\textsuperscript{27,28,70-73} (III) A trend toward lower plasma HCys levels was seen in animals fed diets with supranutritional (>0.2 ppm) vs. adequate (0.1-0.2 ppm) Se content.\textsuperscript{27,29,70,71} Comparison of these 2 dietary groups is particularly interesting in consideration of the human studies showing an inverse correlation of plasma HCys and Se. Increased HCys concentrations result in accumulation of SAH as a consequence of the reversibility of the reaction catalyzed by ACHY (S-adenosyl-L-homocysteine hydrolase; see Fig. 3). SAH in turn is a competitive inhibitor of methyltransferases and, therefore, increased HCys concentrations would be associated with global DNA hypomethylation. This concept was supported by a study showing that women with normal (mean 7.2 \( \mu \)M; range 5.8-8.7 \( \mu \)M) HCys plasma levels had significantly higher plasma SAM/SAH ratio and lymphocyte DNA methylation levels than women with elevated (mean 12.3 \( \mu \)M; range 9.3-16.5 \( \mu \)M) HCys.\textsuperscript{74} Importantly, plasma SAH correlated with HCys and lymphocyte DNA methylation but SAM did not, meaning that a measurement of SAM is insufficient for predicting methylation potential. Tissue SAH concentrations were also found to correlate with DNA

| Type of study | Se dose and form | Measured sample | Se / HCys correlation | Ref |
|---------------|-----------------|-----------------|-----------------------|-----|
| Double-blinded placebo-controlled intervention (N = 249) | 0, 100, 200, 300 \( \mu \)g Se/day as Se-yeast for 6 months | Plasma | No effects of supplementation. Inverse corr. between Se and HCys in males at baseline. | 60 |
| Blinded placebo-controlled intervention (N = 167) | 0, 200 \( \mu \)g Se/day as SelMet for 20 weeks | Plasma | No effect of suppl on HCys | 68 |
| Prospective (N = 202) | — | Serum Se, plasma HCys | Inverse correlation between Se and HCys | 66 |
| Prospective (N = 99) | — | Whole-blood Se, plasma HCys | Inverse correlation between Se and HCys | 63 |
| Prospective (N = 204) | — | Whole-blood Se, plasma HCys | Inverse correlation between Se and HCys | 64 |
| Prospective (N = 906) | — | Plasma Se and HCys | Inverse correlation between Se and HCys, insignificant after correction for folate, PLP and vitamin B\textsubscript{12} | 60,65 |
| Prospective (N = 46 healthy controls; N = 39 cases with ischemic stroke) | — | Serum Se, plasma HCys | Inverse correlation between Se and HCys, independent of vitamin B\textsubscript{6} | 67 |

Table 3. Human studies of blood Se/homocysteine associations
Table 4. Murine studies on interference of Se with one-carbon metabolism

| Species | Se dose, form, duration | Organ/blood | SAMS/SAH ratio | Se / HCys correlation | enzyme expression/activity | SAM/SAH ratio |
|---------|-------------------------|-------------|----------------|-----------------------|---------------------------|---------------|
| Mouse   | 0.06 vs. 0.15 mg/kg; SelMet; 7 weeks | Liver | Lower in 0.2 ppm Se (liver and colon) | Lower in 0.2 ppm Se (liver and colon) | SAMS, CS, CTH lower in Se deficiency | Lower in 0.2 ppm Se (liver and colon) |
| Rat     | 0.015, 0.05, 0.15 mg/kg; selenate; 8 weeks | Liver, Plasma | Plasma: HCys lower in severe Se deficiency Lower in 0.2 ppm Se (liver and colon) | Lower in 0.2 ppm Se (liver and colon) | SAMS, CS, CTH lower in Se deficiency | Lower in 0.2 ppm Se (liver and colon) |
| Rat     | 0, 0.02, 0.05, 0.1 mg/kg; selenite; 6 months | Liver, Plasma | Plasma: HCys lower in severe Se deficiency Lower in 0.2 ppm Se (liver and colon) | Lower in 0.2 ppm Se (liver and colon) | SAMS, CS, CTH lower in Se deficiency | Lower in 0.2 ppm Se (liver and colon) |
| Rat     | 0.03 vs. 0.15 mg/kg; SelMet; 6 weeks | Plasma, liver, colon | Plasma: HCys lower in severe Se deficiency Lower in 0.2 ppm Se (liver and colon) | Lower in 0.2 ppm Se (liver and colon) | SAMS, CS, CTH lower in Se deficiency | Lower in 0.2 ppm Se (liver and colon) |
| Rat     | 0, 0.15, 0.025 mg/kg; selenite; 10 weeks | Plasma, liver, colon | Plasma: HCys lower in severe Se deficiency Lower in 0.2 ppm Se (liver and colon) | Lower in 0.2 ppm Se (liver and colon) | SAMS, CS, CTH lower in Se deficiency | Lower in 0.2 ppm Se (liver and colon) |
| Mouse   | 0.06 vs. 0.15 mg/kg; SelMet; 7 weeks | Plasma, liver, colon | Plasma: HCys lower in severe Se deficiency Lower in 0.2 ppm Se (liver and colon) | Lower in 0.2 ppm Se (liver and colon) | SAMS, CS, CTH lower in Se deficiency | Lower in 0.2 ppm Se (liver and colon) |

The regulation of gene expression by targeting of mRNA through non-coding RNA molecules such as microRNA (miRNA) is sometimes considered as an additional epigenetic mechanism. To date only one study has examined whether Se has an effect on microRNA (miRNA) expression. Microarray analysis (737 miRNAs in total) of the miRNA profiles of Caco-2 cells grown in Se-deficient or Se-supplemented medium revealed that the expression of 12 miRNAs was affected by Se supply. Expression levels of 50 miRNAs were also Se-responsive in the same study, and numerous of these miRNAs were predicted to be targeted by the Se-responsive miRNAs. One of these, miRNA-185, whose expression decreased under Se deficiency, was confirmed to regulate expression of glutathione peroxidase 2 (GPx-2) and selenophosphate synthetase 2 (SPS-2). As the enzymatic product of SPS-2 is part of the selenoprotein biosynthesis machinery, these findings indicate that Se availability affects the selenoproteome in part through epigenetic mechanisms involving miRNA-185 and possibly other miRNAs. miRNA-185 is a particularly interesting target of Se, as it has recently emerged as a tumor suppressor that is frequently downregulated in ovarian, breast, renal, prostate, and gastric cancers, and targets oncogenes, e.g., Six1, androgen receptor, and apoptosis repressor with caspase recruitment domain (ARC). Se has been shown to act anticarcinogenic in experimental settings and also in some human studies (18 for an overview); it will therefore be an interesting area of future work to uncover putative roles of miRNAs as mediators of Se-dependent tumor protection against malignant transformation.
Conclusions and Future Directions

Investigation of epigenetic effects elicited by Se species remains a relatively new field that has not been comprehensively studied. The current data from mainly murine and cell-based, but also some human studies show that Se regulation and status modify DNA methylation globally and at specific gene regions or loci. DNMT inhibition by Se and its interaction with one-carbon metabolism are possible routes through which this occurs. Additionally, histone modifications are altered by Se, and this has been shown to occur - at least in vitro - via inhibition of HDAC activity by the Se metabolism products seleno-α-keto acids. There is a need to systematically assess Se-related effects on epigenetic marks - DNA methylation and histone modifications - at a genome-wide level in murine and human intervention studies. These studies will ideally use different dietary forms of Se, subjects with low initial Se status, so that dose-response relationships can be observed, and include transcriptome assessments. The combined readouts will give important information about the role of Se in epigenetic and transcriptional regulation, and exploit interference of Se with epigenetic marks that are associated with or predict the risk of diseases. This is particularly relevant to better understand the role of Se in the prevention and progression of certain diseases (e.g., prostate cancer and type 2 diabetes), which has become unclear after publication of diverging results from large-scale Se intervention trials.18,84-86 Another research priority is to gain more insight into the inhibition of DNMT and HDAC enzymes by Se, to identify the active Se species, their possible DNMT/HDAC isoform specificity, mechanism of inhibition and the required doses for inhibition in vitro and in vivo. Se-responsive signaling pathways that influence nuclear proteins linked to epigenetic mechanisms, e.g., through nucleosomial remodeling, transcription or DNA repair, as exemplified for GADD45A, also need to be examined closer. We propose that a detailed genome-wide and mechanistic understanding of epigenetic processes elicited by Se is required to complete the picture of selenium’s systems biology and to clarify and predict its impact on health outcomes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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