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Cardiovascular Research 53 (2002) 577–588

Cardiovascular Research

www.elsevier.com/locate/cardiores

Review

Estrogen and homocysteine

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Received 13 June 2001; accepted 12 September 2001

Abstract

Cardiovascular diseases are the major causes of illness and death in women. Premenopausal women are relatively protected from coronary artery disease and atherosclerosis as compared to postmenopausal women, and this protection is attributed to the effects of the female sex hormone (estrogen). The vasculature, like the reproductive tissues, bone, liver, and brain, is now recognized as an important site of estrogen's action. Although estrogen's beneficial effects on the cardiovascular system are well described in many studies, the molecular basis of estrogen protective mechanisms are still quite vague. Both genomic mechanisms, mediated primarily through estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ), and non-genomic mechanisms, through nitric oxide (NO), of estrogen action are controversial and do not entirely explain the effects of estrogen on vascular preservation during conditions of oxidative stress. Until recently, the atheroprotective effects of estrogen were attributed principally to its effects on serum lipid concentrations and cholesterol levels. However, two recent reports that estrogen therapy has no effect on the progression of coronary atherosclerosis in women with established disease, despite the favorable changes in LDL and cholesterol levels, leads to questions about the lipid/cholesterol mechanism of estrogen-mediated effects on atherosclerosis. Alternatively, the high level of homocysteine, found to correlate with accelerated cardiovascular disease and identified as an independent risk factor for atherosclerosis, was recently described to be diminished by estrogen. Protection against disturbed sulfhydryl metabolism and higher homocysteine level could be the missing link in understanding how exactly estrogen affects vascular cells metabolism and responses to oxidative stress. This review focuses on estrogen/homocysteine interactions and their relevance to the cardiovascular system.

Keywords: Atherosclerosis; Gender; Hormones

1. Introduction

Women in Western societies generally live close to one half of their lives after the onset of menopause, during which they are at progressively increased risk for developing cardiovascular disease. While the death rate from coronary artery disease (CAD) is five to eight times greater in men than in women during ages 25–55 years [1], this difference in mortality rate narrows considerably after menopause and suggests that premenopausal women have vascular/endothelial protective factors lost after menopause. More than 40 years ago, Pick et al. [2] observed that estradiol benzoate reduced the incidence of atherosclerotic lesions in coronary arteries of cholesterol-fed chicks. An early epidemiological study showed that estrogen replacement therapy (ERT) in postmenopausal women leads to significant reduction of the risk of myocardial infarction [3]. Since then, ERT has been prescribed for treating perimenopausal symptoms and future prevention of postmenopausal cardiovascular disease.

This clinical practice was mainly based on widely accepted theory that the preventive effects of estrogen against atherosclerosis are mediated through its effects on cholesterol and lipid metabolism. However, estrogen mediated protection against atherosclerosis in LDL receptor-deficient mice was found to be independent of estrogen's effect on plasma cholesterol/lipid levels [4]. Furthermore, recent randomized studies (HERS) in women with established coronary artery disease demonstrated no benefit of estrogen replacement therapy, in spite of its favorable

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Time for primary review 28 days.

0008-6363/02/$ – see front matter
PII: S0008-6363(01)00462-X

effects on lipid profile and cholesterol level [5,6]. At the same time, many investigations confirm that estrogens have remarkable beneficial effects on endothelial integrity, inhibit smooth muscle cell proliferation and migration and activate the anti-oxidant system, etc. [7–12]. This discrepancy in the reports from basic science research and randomized clinical trials indicates that other mechanisms must have outweighed the beneficial effects of estrogens on vasculature, beyond their effects on plasma lipids. Alternatively, McCully has established the homocysteine theory of atherosclerosis in the 1970s and since then many reports confirm that homocysteine metabolism is an important mediator in development of atherosclerosis and cardiovascular disease. In previous studies, in vitro and in vivo, we observed that estrogen prevents vascular injury in conditions of experimentally induced hyperhomocysteinemia and decreases homocysteine level in both controls and animals on hyperhomocysteinemic diet [13,14]. Higher concentrations of homocysteine are a source of hydrogen peroxide, a harmful free radical, which in turn damages endothelium. In the same studies we found that estrogen, by enhancing glutathione levels, (GSH), leads to lower hydrogen peroxide concentrations and alleviation of the endothelial injury. However, the enhancement of GSH by estrogen can not directly explain why homocysteine level decreases after estrogen administration. This phenomenon may be a fundamental effect of estrogen/steroid action on cellular metabolism and the basis of many unclear pathological disturbances including atherosclerosis. In the present review we will focus on homocysteine biochemical metabolism and its interaction with estrogen's effects, particularly in vascular tissue.

2. Homocysteine

High level of homocysteine is recognized as an independent risk factor for atherosclerosis and cardiovascular disease [15]. Hyperhomocysteinemia is associated with myocardial infarction, coronary artery disease, strokes, genetic disorders, Alzheimer's disease, and loss of cognitive functions [16–23]. Elevated plasma homocysteine levels are also associated with an increased risk of neural-tube defect, placental infarcts, abruptio placenta, eclampsia, etc [24–28], and some reports suggested that hyperhomocysteinemia might be a manifestation of an underlying common biologic disorder which is directly associated with the process of DNA methylation [29,30].

Homocysteine is a key junctional metabolite in methionine metabolism (Fig. 1). It has two major metabolic fates: transmethylation catalyzed by methionine synthase or betaine homocysteine methyl transferase and transsulfuration catalyzed by cystathionine beta-synthase leading to cystathionine (Fig. 1). The latter is subsequently converted to cysteine, a precursor of glutathione (GSH). Previous studies reveal that approximately half of the intracellular glutathione pool in human liver cells is derived from homocysteine via the transsulfuration pathway [31]. The redox sensitivity of the transsulfuration pathway can be rationalized as an auto-corrective response that leads to an increased level of glutathione synthesis in cells challenged by oxidative stress. The importance of the homocysteine-dependent transsulfuration pathway is the maintenance of the intracellular glutathione pool, and the regulation of this pathway under oxidative stress conditions. Aberrations in this pathway could compromise the redox buffering capacity of cells, which may in turn may be related to the pathophysiology of the different homocysteine-related diseases. Whether homocysteine by itself is involved in the pathogenesis of these multi-organ system disorders is the topic of current investigations.

2.1. Homocysteine a basis for atherosclerosis?

Atherothrombotic vascular events were linked to raised total homocysteine levels in patients with homocysteinuria in 1969, but the relationship was not widely accepted until 1976, when a controlled study showed a clear association between moderately raised homocysteine and atherosclerotic disease [32]. Since then, a possible association between homocysteine and atherothrombotic vascular disease has been examined in more than 12 000 patients involving more than 100 cross-sectional, case-control, and prospective studies (see for review Ref. [33]). Cross-sectional and retrospective case-control studies and one large prospective observational study showed the strongest epidemiological evidence for a clear association between hyperhomocysteinemia and vascular risk [33–37]. However, other prospective studies, including those examining the common thermolabile methyltetrahydrofolate reductase (MTHFR) gene mutation associated with hyperhomocysteinemia, have not found a significant correlation between hyperhomocysteinemia and cardiovascular disease [38,39]. These inconsistent results may be due to the different methods used for serum homocysteine level measurements [40] and/or variability of homocysteine levels as a product of methionine transsulfuration/demethylation metabolism, depending on the individual diet and fasting state [41].

2.2. What do elevated levels of homocysteine actually mean?

A brief review of homocysteine metabolism associated with metabolic disorders resulting in homocysteine accumulation suggests several possible mechanisms for the observed effects. Either a genetic defect in one of the enzymes of homocysteine metabolism or a nutritional deficiency of one or more of the vitamins that participate in homocysteine metabolism can lead to metabolic disruption and potentially to hyperhomocysteinemia. In homozygous cystathionine β-synthase (CβS) metabolic defect

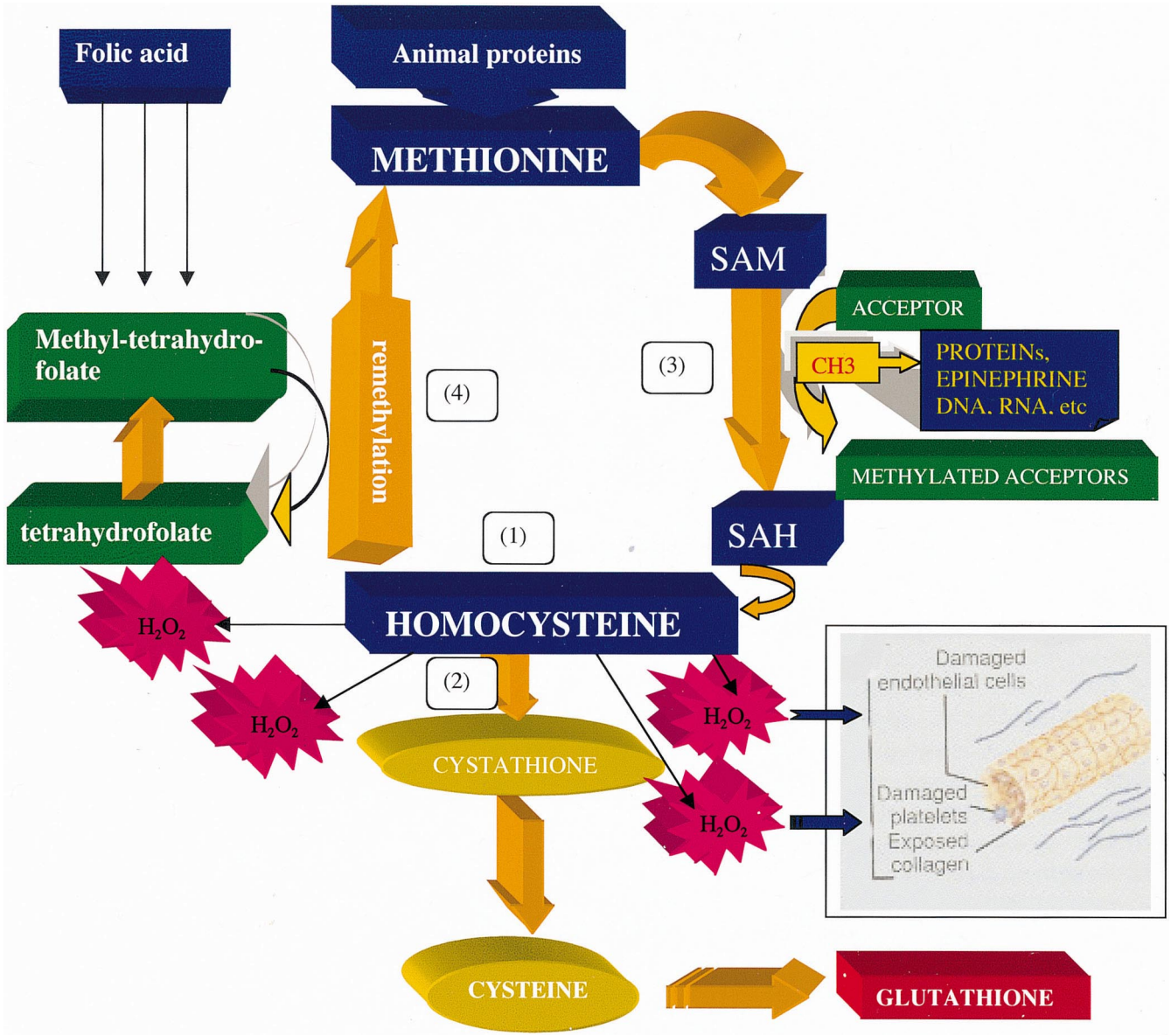

Fig. 1. Homocysteine metabolism in men and animals. (1) Higher concentrations of homocysteine lead to its auto-oxidation and hydrogen peroxide generation (H_2O_2). Homocysteine is a product of the reversible hydrolysis of S-adenosylhomocysteine (SAH). (2) Cystathionine beta-synthase (CβS, pyridoxal-5′-phosphate (PLP)-containing enzyme) is the enzyme catalyzing an irreversible transsulfuration of homocysteine to cystathionine. Cystathionine is hydrolyzed by a second PLP-containing enzyme, λ-cystathionase, to form cysteine and α-ketobutyrate. This transsulfuration pathway effectively catabolized excess homocysteine. Homozygous cystathionine β-synthase (CβS) metabolic defect leads to severely impaired homocysteine transsulfuration and an excess of homocysteine. (3) Methionine activated by ATP and methionine adenosyltransferase (MAT) forms S-adenosylmethionine (SAM). SAM serves primarily as a universal methyl donor to a variety of acceptors and is the main methyl donor to DNA and RNA. (4) Homocysteine acquires a methyl group from *N*-5-methyltatrahydrofolate or from betaine to form methionine, which is its remethylation pathway. Abbreviations: SAM, S-adenosylhomocysteine; CH3, methyl group; SAH, S-adenosylhomocysteine; and H_2O_2, hydrogen peroxide.

(see Fig. 1), severely impaired homocysteine transsulfuration and a diversion of the excess of homocysteine toward the remethylation pathway to methionine, has been observed [42]. The increased rate of methionine synthesis described in those patients leads to a temporal magnification of S-adenosylmethionine (SAM) concentrations and diminished levels of cysteine [43]. Consequently, lack of cysteine availability causes impaired formation of GSH, the principal antioxidant in cells [44]. In such hyperhomocysteinemic conditions, the combination of the intensified free radical generation and diminished GSH formation could be the main cause for the accelerated atherosclerosis. On the other hand, inborn defect of methyltetrahydrofolate reductase impairs homocysteine remethylation to methionine, leading to hyperhomocysteinemia and lower SAM levels [45]. The clinical progress of hyperhomocysteinemia, however, resulting from CβS defect, presents an interesting contrast to the clinical presentation

of patients with inborn methyltetrahydrofolate reductase deficiency. Although the highest levels of homocysteine are observed in people with CβS deficiency, in those patients the cardiovascular clinical events progress slowly compared to the severe and rapid development of fatal cardiovascular complications in patients with inborn defect of methyltetrahydrofolate reductase, a disorder accompanied by only moderately elevated homocysteine concentration [46–48]. Remarkably, severe dystrophic and atrophic multiple organ changes found postmortem in patients with methyltetrahydrofolate reductase defect, pointing to depressed DNA-synthesis, are very similar to those found in animals fed with methyl deficient diets. Moreover, experiments in rats treated with 5-azacytidine (inhibitor of DNA methylation, used as a tool for DNA demethylation) in different stages of development have shown the same results: severe impairment of the growth and dystrophic and atrophic changes almost in all examined tissues [49]. In the context of these findings, modified DNA methylation rather than hyperhomocysteinemia itself may be the actual underlying pathology responsible for hyperhomocysteinemia-associated pathologic cell proliferation, degeneration and transformation.

Hyperhomocysteinemia results in impaired balance between methionine, SAM and S-adenosylhomocysteine (SAH) [50,51]. This imbalance may lead to changes in methyl-donor substrate levels and consequently to a modulation of DNA methylation pattern [30]. SAM availability controls the binding capacity of DNA to methyltransferase and affects the velocity of methyltransferase reactions with DNA and RNA [52]. The presence of SAM leads to improved recognition and stronger binding of the DNA target by the methyltransferase, due to a conformational change of the enzyme to a more 'active' state [53] and SAM deficiency or its lower levels causes DNA hypo- and demethylation [54]. We and others have shown that homocysteine causes atherosclerotic changes in vivo and significantly reduces endothelial cell viability in vitro [13,14,55–57]. Although the concentrations utilized in our studies were significantly higher than those measured in the normal population, physiological concentrations of estrogen prevented homocysteine harmful effects and decreased its serum concentration in both controls and hyperhomocysteinemic animals. Estrogen, by affecting homocysteine/methionine metabolism, could impact on SAM levels and may relate to its action on DNA epigenetic modifications.

3. Estrogen and homocysteine

Estrogen impact on methionine/homocysteine metabolism may be a key aspect in elucidating its beneficial effects. Lower levels of homocysteine are observed in pregnant, premenopausal and postmenopausal women who are on ERT, compared to age-matched man and postmenopausal women who are not on ERT [58–61]. These finding were officially recognized with a recently published statement of the Third National Health and Nutrition Examination Survey that [62]: '' . . . higher estrogen status is associated with a decreased mean serum total homocysteine concentration, independently of nutritional status and muscle mass, and that estrogen may explain the previously reported male–female difference in total homocysteine concentration.''

Methyltetrahydrofolic acid is an essential methyl group donor in homocysteine remethylation to methionine (Fig. 1), and its essential element is folic acid. Folate deficiency correlates with development of severe atherosclerosis and lower serum folate levels are associated with an increased risk of cerebrovascular and coronary artery disease [63]. Folate deficiency also is the most common vitamin deficiency among pregnant women due to estrogen induced increased turnover, suggesting that estrogen influences folate utilization as well [64]. The comparatively low levels of total homocysteine found in extra-embryonic coelomic fluid and amniotic fluid, and the concomitant high concentrations of methionine in these fluids is another finding suggesting that estrogen may interact with methionine/homocysteine metabolism during early human development [65].

Hypothetically, the biochemical interaction between S-nitrosothiols and homocysteine may be a central mechanistic feature in such diverse biological processes as infection, neoplasia, neurologic disease, and atherosclerosis [66–69]. The vasodilatory, antiplatelet, antioxidant, antiproliferative, and neuroregulatory actions of S-nitrosothiols oppose those attributed to homocysteine [70]. On the other hand, it is widely accepted that estrogen directly up-regulates nitric oxide synthetase (NOS) activity and increases NO availability [71–75]. However, peroxynitrite ($ONOO^-$) is a highly cytotoxic molecule and could be produced in conditions of high NO concentrations and superoxide availability (Fig. 2).

Estrogen dose-dependently increase myocardial and plasma GSH levels [7,13,14]. The presence of GSH prevents $ONOO^-$ formation by stabilizing the NO molecule in GSNO (s-nitrosoglutathione), which is a redox form of NO, and produces specific cyto-protective effect [76]. GSNO also increases GSH formation by up-regulation of GSH synthesis and promotes hexose monophosphate shunt (HPM shunt), thus reinforcing the GSH/GSSG system. Thus it seems that estrogen, by increasing GSH content, limits the formation of $ONOO^-$ and favors the production of GSNO [77]. Furthermore, estrogen by enhancing the activity of glucose-6 phosphate dehydrogenase (G6PDH) [13,78], the rate-limiting enzyme of the HMP shunt, increases the capacity of NADPH, which acts as a cofactor for NOS and replenishes GSH availability by donating hydrogen to its oxidative product GSSG.

Together, this provides evidence supporting the theory

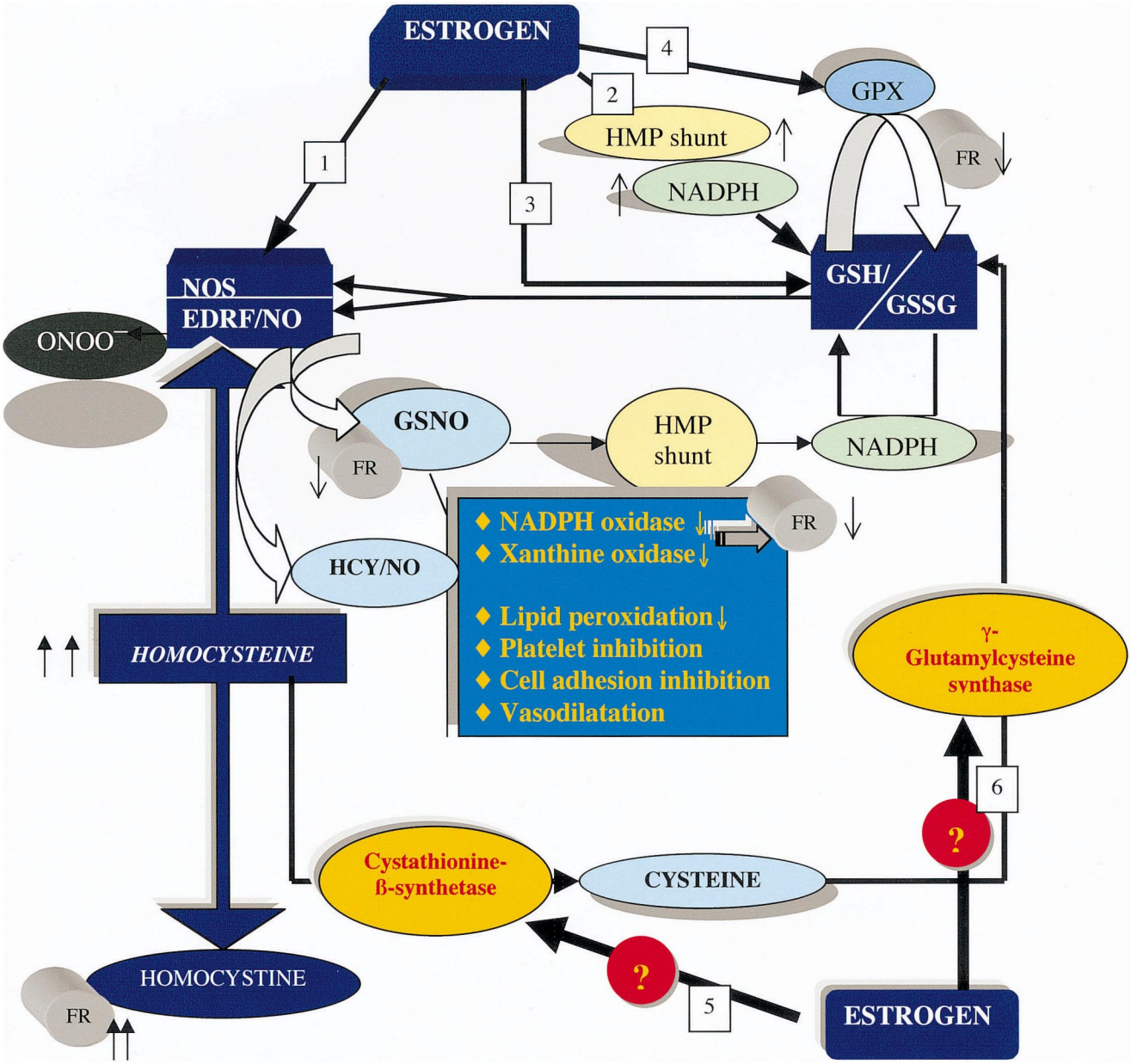

Fig. 2. Estrogen and homocyseine interactions. (1) Estrogen up-regulates NOS (Nitric Oxide Synthetase) activity and increases EDRF/NO (endothelium derived relaxing factor/nitric oxide) concentration. (2) Estrogen enhances the activity of glucose-6 phosphate dehydrogenase (G6PDH), the rate-limiting enzyme of the hexose monophosphate shunt (HMP shunt). Thus estrogen increases the capacity of NADPH, which acts as a co-factor for NOS and reduces GSSG to GSH. (3) Estrogen increases directly the intracellular GSH content. Estrogens increase NADPH/GSH availability and stimulate NOS activity (GSH and NADPH are co-factors for NO synthesis). Estrogen limits the formation of $ONOO^-$ by favoring the production of GSNO (s-nitrosoglutathione), which is a redox form of NO and deliver specific cyto-protective effects. Peroxynitrite ($ONOO^-$) is a highly cytotoxic product from NO and superoxide. The presence of GSH prevents $ONOO^-$ formation. GSNO increases GSH formation by up-regulation of GSH synthesis and promote HPM shunt, thus reinforcing GSH/GSSG system. (4) Estrogen increases glutathione peroxidase (GPX) activity, the enzyme catalyzed the scavenging of oxygen free radicals (FR) by GSH and its oxidation to GSSG. NADPH replenishes GSH availability by donating hydrogen to its oxidative product GSSG. (5) Estrogen could enhance cystathionine-β-synthase activity and directed homocysteine metabolism to forming cysteine and glutathione. (6) Estrogen probably influences on rate-limiting enzyme of glutathione synthesis.

that estrogen modulates thiol amino acid metabolism, particularly methionine metabolism. By interfering with the transsulfuration pathway, diminishing homocysteine and preventing its accumulation, estrogen may enhance the net production of GSH and stabilizes NO, which contributes to its beneficial effects on the vasculature.

4. DNA methylation and estrogens

Methylation of DNA is an important modification that plays a global role in genome management and in the regulation of gene expression. This part of the review will briefly highlight the major findings in the methylation field

over the past 20 years then summarize and juxtapose the most important and interesting relationships between estrogen effects and this modification of DNA.

The processes of methylation and demethylation of DNA are now generally accepted to have an epigenetic (alteration in gene expression without a change in nucleotide sequence) and a genetic control on cellular development, differentiation and transformation [79]. Mammalian development is accompanied by two major waves of genome-wide demethylation and remethylation, one during germ-cell development and the other after fertilization [80]. Methionine is the general methyl donor in eukaryotic cells and its concentrations directly influence methyl donor reactions with DNA, RNA, and other compounds [81]. DNA methylation, or the covalent addition of a methyl group to cytosine within the context of the CpG (cytosine–guanine dinucleotide), has profound effects on the mammalian genome. These effects include transcriptional repression via inhibition of transcription factor (TF) binding (Fig. 3) or the recruitment of methyl-binding proteins and their associated chromatin remodeling factors. Other effects include X chromosome inactivation, imprinting (a unique process, in which a gene on one chromosome is silenced, whereas its allele on the other chromosome is expressed), and the suppression of viral DNA sequences. In eukaryotic organisms, DNA methylation is catalyzed by a SAM dependent DNA cytosine-5-methyltransferase, which catalyzes the transfer of a methyl group from SAM to cytosine residues to form 5-methylcytosine, a modified base that is found at CpG islands (short sequences rich of the cytosine–guanine dinucleotide sites in the genome). This catalysis involves nucleophilic attack by the thiol of the enzyme active site cysteine at the C6 position of cytosine. In the absence of the cofactor-SAM this leads to exchange of the C5 hydrogen (Fig. 4), while the addition of SAM suppresses this exchange reaction and at its saturating concentrations, cytosine receives a methyl group and loses a proton from carbon 5, which stabilizes its structure. Methylation of cytosine within 5′CpG islands is associated with loss of gene expression. Cytosine is the most unstable base in DNA. Any mCpG sites are mutation hot spots. About 100 cytosines are spontaneously deaminated to thymidine (T).

Considerable effort has been invested in the elucidation

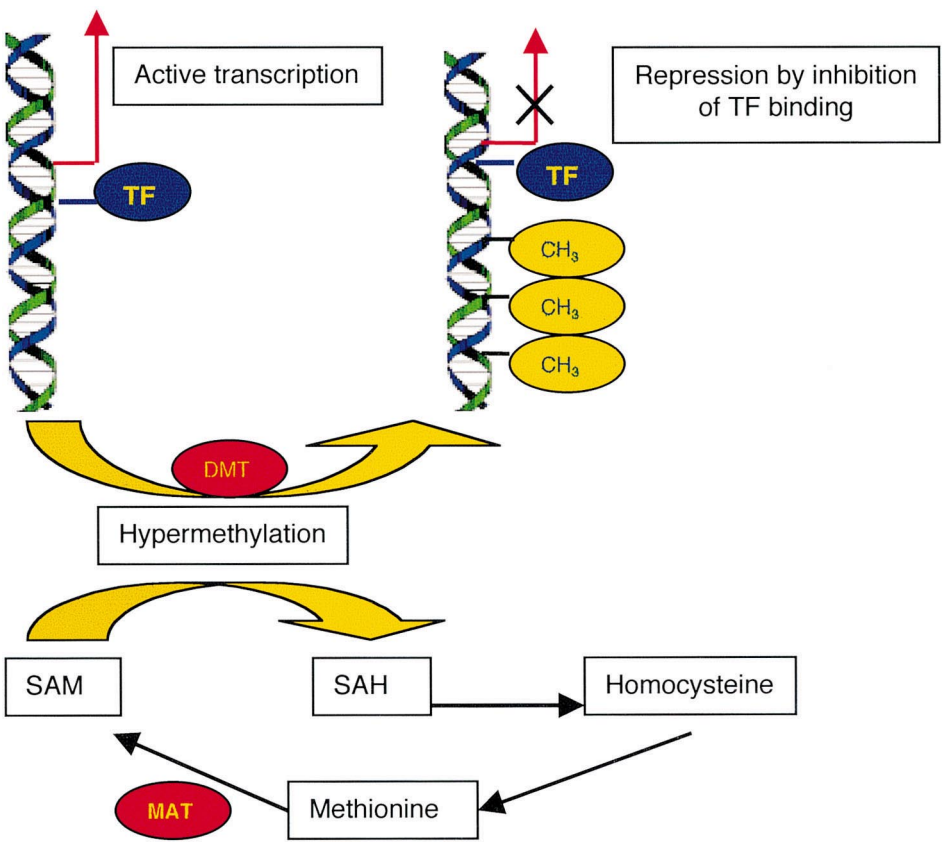

Fig. 3. DNA methylation. DNA methylation is a covalent addition of a methyl group to cytosine within the context of the CpG (cytosine–guanidine) dinucleotide and consequent transcriptional repression via inhibition of transcription factor (TF) binding In eukaryotic organisms DNA methylation is catalyzed by a SAM dependent DNA cytosine-5-methyltransferase, which catalyzes the transfer of a methyl group from SAM to cytosine residues to form 5-methylcytosine, a modified base that is found at CpG islands, short sequences rich of the cytosine–guanine dinucleotide sites in the genome. Methylation of cytosine within 5′CpG islands is associated with loss of gene expression. Abbreviations: DMT, DNA methyltransferase; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; MAT, methionine adenosyltransferase, TF, transcription factor; and CH_3, methyl group.

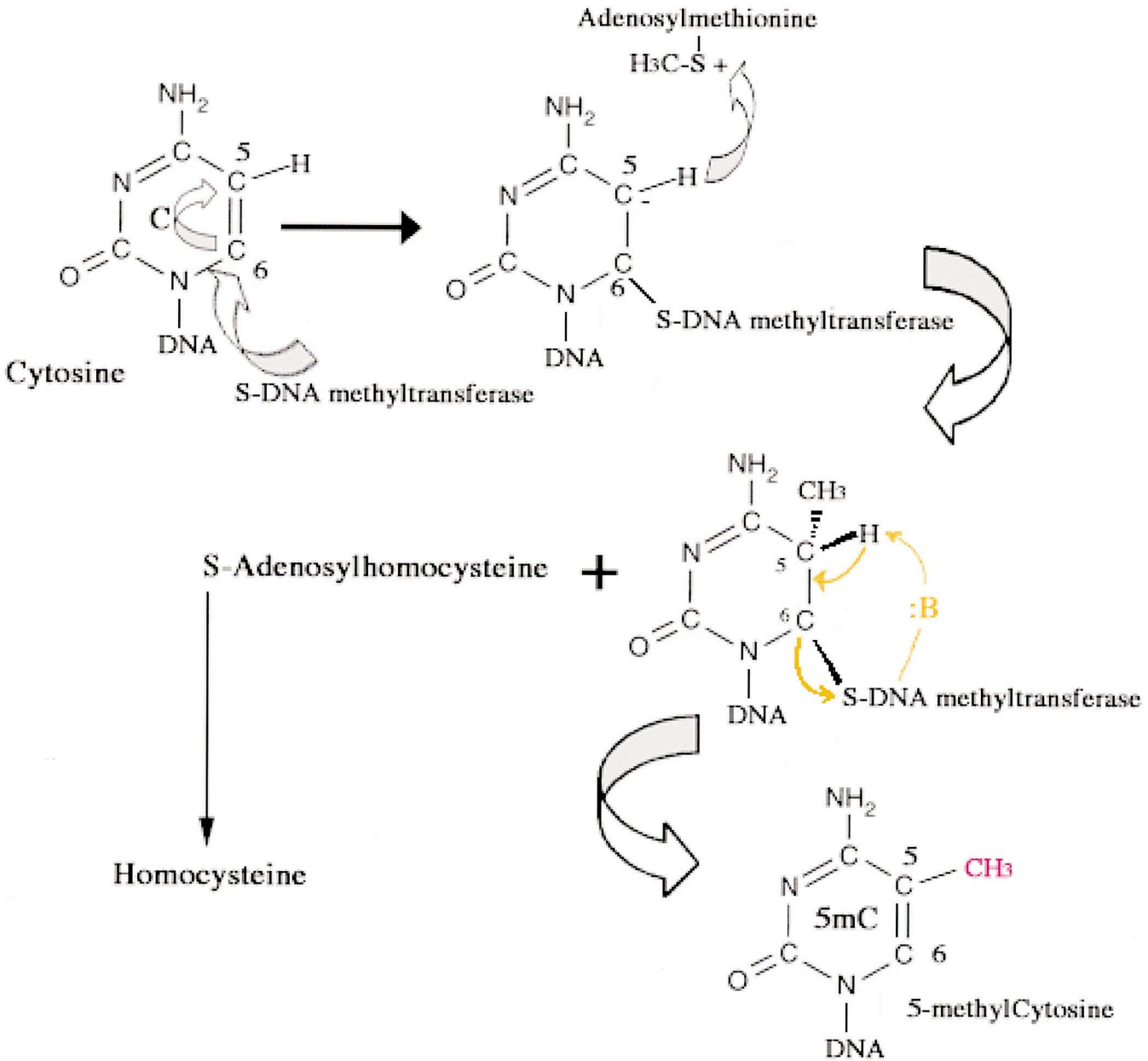

Fig. 4. Methylation of cytosine. Catalysis involves nucleophilic attack by the thiol of the enzyme active site cytosine at the C6 position of cytosine, which in the absence of the cofactor-SAM leads to exchange of the C5 hydrogen. The addition of SAM suppresses the exchange reaction and at its saturating concentrations cytosine receives methyl group and loses a proton from carbon 5, which stabilizes its structure.

of mechanisms that may contribute to the formation of methylated CpG sites and their demethylation in DNA. Under a condition of limited availability of SAM, overall DNA hypomethylation and deamination of the target cytosine to uracil, which codes from T during DNA replication, have been described [81,82].

Whether estrogen exerts its effects primarily via binding to the estrogen receptors (ER) has been subject of investigation. ER is a member of the steroid/thyroid superfamily of proteins that act as hormone-inducible transcription factors. Two ER gene products, i.e. ER alpha and ER beta, mediate the action of estrogens in target tissues, thus regulating estrogenic effects on vasculature, reproductive tissues, bone, liver and brain. The longer term effects of estrogen on atherosclerosis result from alterations in gene expression, and is believed to be mediated by ERs [112–114]. Ligands, e.g. estradiol (E2), bind to the ligand binding domain (LBD) of ER and induce conformational changes leading to high affinity ER homodimer binding to estrogen response elements (ERE) on genes [83]. Many observations have supported the existence of different functional roles for estrogen receptor alpha (ER alpha) and estrogen receptor beta (ER beta) in their cardioprotective effects. ER alpha and beta are homogenous in some DNA

binding domains, but other regions of the proteins share little homology. The two receptors show significant difference in their pattern of cell-specific expression, ligand-binding affinities and gene targets [112]. The precise mechanism of transcriptional activation, or repression, by the ER is unclear. The ER alpha promoter does not appear to be methylated in situ (normal aorta), but becomes methylated in proliferating aortic smooth muscle cells [86]. Alteration in methylation associated with the smooth muscle cell phenotypic switch does not seem to require heightened activity of the methyltransferase enzyme, and appears to be selective for the ER alpha and a limited pool of genes whose CpG island becomes either demethylated or de novo methylated. The genome of aortic smooth muscle cells is responsive to environmental conditions, and DNA methylation, in particular methylation of the ER alpha, could contribute to the switch in phenotype observed in these cells. Surprisingly, it is reported that estrogen administration inhibits the vascular injury response both in ovariectomized female ER alpha and ER beta knockout mice to the same extent as wild-type mice, bringing into question whether one of the ERs or another unidentified mechanism mediates the estrogen vascular protective effect [84,85]. Could this be due to estrogen induced modification of the epigenetic structure of DNA?

The first studies of estrogen mediated changes in DNA methylation pattern used genomic sequencing to examine the state of cytosine methylation in the upstream region of the avian vitellogenine gene [87]. Two methylated CpGs, present in the estrogen response element (ERE) become demethylated in liver and oviduct under the influence of estradiol, suggesting the existence of estrogen dependent DNA hypomethylation, which is not organ and/or tissue specific. In another studies the total genomic DNA methylation in kidney tissues is decreased 11–24% in response to estrogen treatment [88]. Demethylation of a CpG located in the estrogen-response element (ERE) was described in 1992 by Thakur and Kaur [89]. They observed that estrogen dose-dependently lowers the level of 5 methyl-cytosine (5mC) in uterus of young but showed no effect in DNA methylation of uterus in old mice treated with estradiol. Diethylstilbestrol (DES), a synthetic analog of estradiol, was recently found to produce an abnormal demethylation in the lactoferrin promoter [90]. Finally, Post et al. have proposed that the higher degree of methylation of ERs in a sub-population of cells of atherosclerotic tissue compared to normal aortas causes lack of ER gene expression, resulting in an inability to respond to estrogen's protective effects [91]. Based on such observations, it could be hypothesized that methylated and inactivated ER gene in diseased vessels may prevent the potential protective effects of estrogens in women with advanced atherosclerotic disease and therefore, account, at least in part, for the lack of benefit observed in HERS study.

Estrogens can modulate directly the phenotype of vascular cells [92]. Functional ERs are present in the arterial wall, endothelial cells, smooth muscle cells. The presence of functional ERs is associated with protection against coronary atherosclerosis [93,94]. The level of circulating estrogens directly influences ER expression, and ERs disappear shortly after menopause when the circulating level of estrogen is low. Proliferation of vascular smooth muscle cells is the most prominent hallmark of atherosclerosis, and a switching of the phenotype from contractile to de-differentiated phenotype is a key step in disease progression. The process of change in cellular behavior and phenotype during development of atherosclerosis is very similar to that observed in carcinogenesis and estrogen has been shown to regulate cell proliferation in both cancer and vascular cells. Although alterations in DNA methylation are well characterized in malignant tumors, the presence of methylation changes in atherosclerosis has not been studied, even though excessive cellular proliferation and alterations in gene expression characterize both diseases. In this context, the exact detection of the molecular mechanism(s) of estrogen action may lead to new approaches in the treatment of coronary artery disease, and a better understanding of the molecular mechanisms of steroid action in carcinogenesis.

5. Estrogens, DNA methylation and development of cancer?

Although estrogens have many beneficial cardiovascular actions, concerns have been raised about their effects on the progression of breast and uterine neoplasms, and endometrial disease. The side effects of estradiol seem to be associated with extensive hypo- and de-methylation of DNA sequences responsible for the expression of some oncogenes (Table 1). Demethylation of these oncogenes activates their expression and is associated with consequent development of cancers in estrogen-sensitive tissues. On the other hand, the activation of other tumor suppressor genes can lead to reduced amplification of certain oncogenes. For example, the protective effect of estrogen against chemically induced murine colon carcinogenesis is associated with CpG island demethylation and increased mRNA and protein expression of the colonic vitamin D receptor [95]. Normal methylation pattern is frequently disrupted in tumor cells with global hypomethylation accompanying region-specific hypermethylation. When hypermethylation occurs within the promoter of a tumor suppressor gene, this will silence the gene and provide the cell with a growth advantage in a manner akin to deletions or mutations. Tumor development and lower threshold for chemical carcinogenesis have been observed in experimental animals fed with a diet deficient in methyl group providing substances, leading to decreased availability of SAM. Low availability of SAM, high level of homocysteine and reduced serum levels of folic acid (all

Table 1
High and low levels of estrogen effects on gene expression and DNA methylation

(A) High levels of estrogen Affected gene	Effect on gene	Effect on DNA methylation	Ref.
Vitellogenine gene	Amplified expression	Demethylation	[87]
Genomic	–	Total demethylation	[89]
Genomic	–	Total demethylation	[88]
Deiminases (PADs)	Amplified expression	–	[97]
c–ski	Amplified expression	–	[98]
c–fos	Amplified expression	–	[99]
GRx	Amplified expression	–	[100]
prolactine	Amplified expression	Demethylated promoter	[101]
Aromatase	Amplified expression	Demethylated promoter	[102]
VDR	Amplified expression (−) colonic cancer	Demethylated promoter	[94]
UPA	Amplified expression ↑ Invasive ability BC	Demethylated promoter	[103]
Cyclin D1	Amplified expression	Demethylated promoter	[104]
p53	Inactivation	Demethylated promoter of the suppressor gene	[105]
Bcl-2	Amplified expression	Demethylated promoter	[106]
SOD	Amplified expression	–	[107]
(B) Low level of estrogen Affected gene effect	Action	DNA	
ER alpha	Silenced	Methylated promoter	[91]
ER beta	Silenced	Methylated promoter	[108]
pS2	Activated Oncogene	Methylated promoter of the suppressor gene	[109]
RAR beta	Breast cancer	Methylated promoter	[110]
BRCA1	Tumor suppressor Gene silencing	Methylated promoter	[111]

these are cofactors in the remethylation pathway of homocysteine to methionine) are all found in conditions of rapid cellular growth [96].

Higher or lower levels of estrogen are associated with development or suppression of cancers in estrogen sensitive tissues and is related to the activation or inactivation of certain genes by their hypo- or hypermethylation (see Refs. in Table 1). This dual interaction between DNA methylation status and estrogen level could provide new molecular tools for better understanding and managing estrogen-dependent diseases including atherosclerosis.

6. Concluding remarks

Disease phenotypes arise from complex interactions of organisms with their environment. While many specific genes and gene defects have been associated with corresponding disease phenotypes, a growing body of data suggests that many disease phenotypes arise from the interactions of genes with their environments, including the genetic background in which the genes are expressed. Molecular mechanisms of some atherosclerosis-provoking factors seem to involve modifications of epigenetic switch, and some dietary factors also have the possibility to modify it. In the ancient times people believed that a meatless diet would help them against hypertension and heart disease (Hipocrates' teaching) Major religions prohibit eating of meat for certain periods of time in honor of holidays (fasting). Interestingly, the only major source for the essential amino acid methionine is the animal proteins. Homocysteine is a product of the transsulfuration and demethylation of methionine and its high plasma levels are described to correlate with variety of diseases and especially atherosclerosis. Conversion of methionine to homocysteine is associated with production of methyl group that is donated to DNA, RNA, proteins, hormones, etc. Methylation of DNA is the major epigenetic modulator of gene expression associated with the early human development and is described as a molecular basis of carcinogenesis, atherosclerosis, aging, infection diseases, genetic disorders and chromosomal imprinting. Estrogen has a long history as a potential beneficial drug against cardiovascular disease, osteoporosis, Alzheimer disease, etc. as a substance which can induce thrombo-embolic events and thrombotic disease, breast and uterine cancer.

Our review intended to provide a better understanding of the molecular basis of estrogen effects and its possible interaction with methionine/homocysteine metabolism. Reduction of the level of homocysteine by estrogen, either indirectly by its effect on gene expression, or directly by effects on homocysteine synthesis, might account for the

specific estradiol-induced enhancement of endothelium resistance to harmful action of homocysteine and/or for its general anti-atherosclerotic action. The results summarized here show that estrogens might influence the epigenetic blue print of methylation of certain genes by interacting with the main methyl donor biochemical pathways, e.g. methionine. For atherosclerosis, and probably for other effects of estrogen, methylation associated amplification/reduction of specific cardiovascular genes (CV genes) expression in vascular tissue may play a role in pathogenesis of the vascular system. This potentially reversible defect, apparently associated with the diet, may provide a new target for treatment of cardiovascular disease.

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