IJC
International Journal of Cancer

Critical depurinating DNA adducts: Estrogen adducts in the etiology and prevention of cancer and dopamine adducts in the etiology and prevention of Parkinson's disease

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Mini Review

Endogenous estrogens become carcinogens when dangerous metabolites, the catechol estrogen quinones, are formed. In particular, the catechol estrogen-3,4-quinones can react with DNA to produce an excess of specific depurinating estrogen-DNA adducts. Loss of these adducts leaves apurinic sites in the DNA, generating subsequent cancer-initiating mutations. Unbalanced estrogen metabolism yields excessive catechol estrogen-3,4-quinones, increasing formation of depurinating estrogen-DNA adducts and the risk of initiating cancer. Evidence for this mechanism of cancer initiation comes from various types of studies. High levels of depurinating estrogen-DNA adducts have been observed in women with breast, ovarian or thyroid cancer, as well as in men with prostate cancer or non-Hodgkin lymphoma. Observation of high levels of depurinating estrogen-DNA adducts in high risk women before the presence of breast cancer indicates that adduct formation is a critical factor in breast cancer initiation. Formation of analogous depurinating dopamine-DNA adducts is hypothesized to initiate Parkinson's disease by affecting dopaminergic neurons. Two dietary supplements, *N*-acetylcysteine and resveratrol complement each other in reducing formation of catechol estrogen-3,4-quinones and inhibiting formation of estrogen-DNA adducts in cultured human and mouse breast epithelial cells. They also inhibit malignant transformation of these cells. In addition, formation of adducts was reduced in women who followed a Healthy Breast Protocol that includes *N*-acetylcysteine and resveratrol. When initiation of cancer is blocked, promotion, progression and development of the disease cannot occur. These results suggest that reducing formation of depurinating estrogen-DNA adducts can reduce the risk of developing a variety of types of human cancer.

How Estrogens can Initiate Cancer

The organic chemistry of mammals is predominantly characterized by molecules belonging to aliphatic and heterocyclic chemistry. Molecules belonging to aromatic chemistry, which are chemical derivatives of the leukemogenic parent compound benzene, are rather limited. The aromatic compounds present in mammals include the hormones estrogens and the neurotransmitter dopamine.

Cancer is a problem of chemical carcinogenesis. This means that chemicals are involved in the processes leading to cancer. Knowledge of how certain molecules work is, therefore, essential for understanding how cancer begins. We think the chemicals that cause much of human cancer are the estrogens (see below), which become carcinogenic when an excess of dangerous estrogen metabolites, the catechol estrogen quinones, are produced.

Key words: catechol estrogen-3,4-quinones, dopamine 3,4-quinone, depurinating DNA adducts
DOI: 10.1002/ijc.30728
History: Received 22 Dec 2016; Accepted 17 Mar 2017; Online 7 Apr 2017
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Examination of how estrogen compounds interact with DNA has given insight into how cancer begins.[1–4]. The estrogens estrone (E_1) and estradiol (E_2) are important molecules in our bodies because they regulate how cells behave in both men and women. Estrogens are routinely metabolized to catechol estrogens. Sometimes the catechol estrogens are further metabolically converted to the electrophilic catechol estrogen quinones, which can chemically react with the Gua and Ade of DNA, forming depurinating estrogen-DNA adducts (99%).[5].

When $E_1(E_2)$-3,4-quinones (Q) react with DNA, they predominantly form the depurinating adducts 4-$OHE_1(E_2)$-1-N3Ade and 4-$OHE_1(E_2)$-1-N7Gua (97%) by 1,4-Michael addition (Fig. 1*a*).[1,5–7]. By error-prone base excision repair, apurinic sites in the DNA derived from the depurinating adducts lead to mutations[2,8] that can initiate cancer. A variety of error-prone DNA repair mechanisms continue to be studied, and error-prone base excision repair, has been implicated in cancer initiation.[9–11].

$E_1(E_2)$-2,3-Q form much less of the depurinating adduct 2-$OHE_1(E_2)$-6-N3Ade (2%) by 1,6-Michael addition (Fig. 1*b*),[5] following tautomerization of the quinone to the $E_1(E_2)$-2,3-quinone methide.[12] These results demonstrate the predominant effectiveness of $E_1(E_2)$-3,4-Q in reacting with DNA

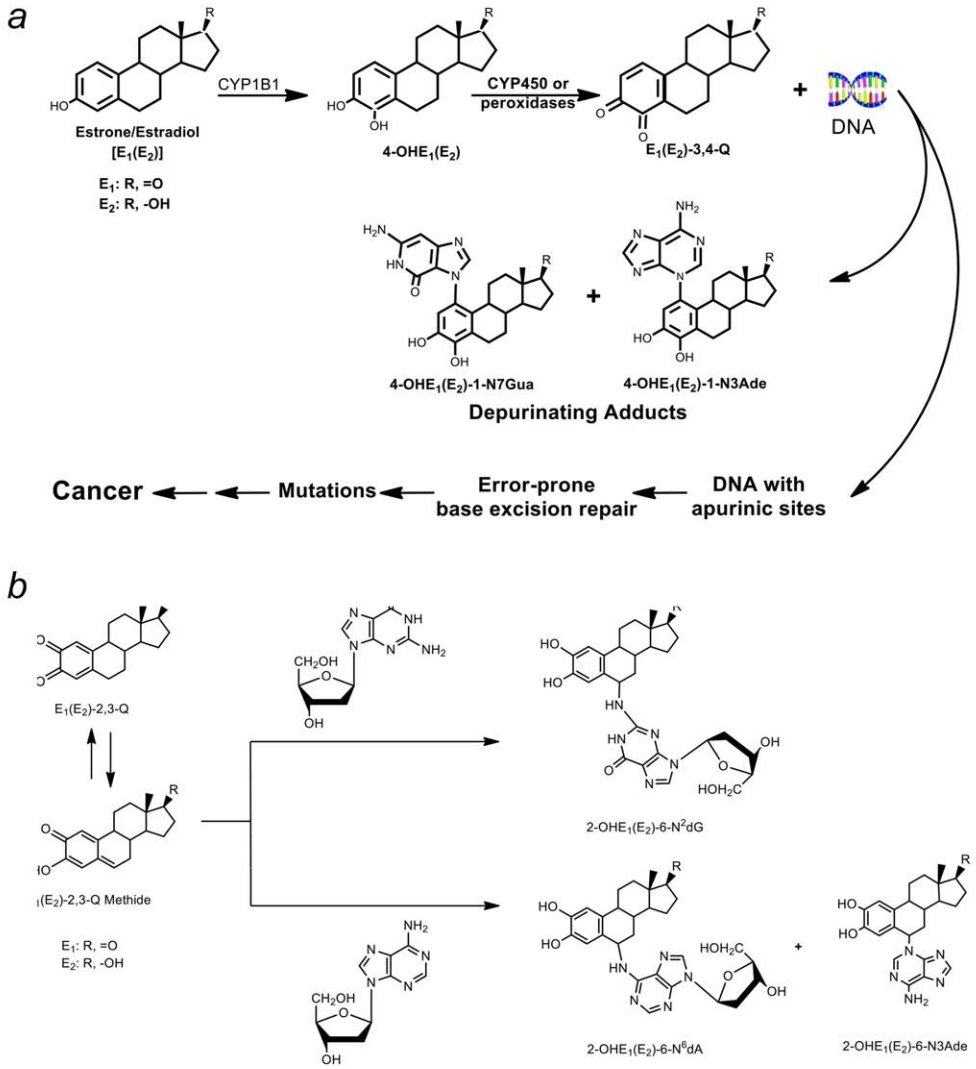

Figure 1. (*a*) Major metabolic pathway (97%) in cancer initiation. Reaction of $E_1(E_2)$-3,4-Q with DNA. (*b*) Reaction of $E_1(E_2)$-2,3-Q with dG or dA in DNA to form the two stable adducts 2-$OHE_1(E_2)$-6-N^2dG and 2-$OHE_1(E_2)$-6-N^6dA (<1%) and the depurinating adduct 2-$OHE_1(E_2)$-6-N3Ade (2%).

to form depurinating adducts. The $E_1(E_2)$-2,3-Q produce 10–50 times higher levels of stable DNA adducts than the $E_1(E_2)$-3,4-Q,[6,13] but the yield of stable adducts is <1% of total adducts.

The levels of depurinating DNA adducts produced by the $E_1(E_2)$-3,4-Q and $E_1(E_2)$-2,3-Q[5] are in agreement with the greater carcinogenic activity of 4-$OHE_1(E_2)$ compared to the borderline carcinogenic activity of 2-$OHE_1(E_2)$.[14–16]

The failure to demonstrate that estrogens induce mutations in bacterial and mammalian test systems[14,17–21] led to the classification of E_1 and E_2 as epigenetic carcinogens that function by stimulating abnormal cell proliferation via estrogen receptor (ER)-mediated processes.[18,22–26] The stimulated cell proliferation could result in increased accumulation of genetic damage that can lead to cancer initiation.[22,26,27] We do not think that ER-mediated processes play a significant role in cancer initiation for several reasons. These include the greater carcinogenic potency of 4-$OHE_1(E_2)$ compared to 2-$OHE_1(E_2)$[14–16] and the development of estrogen-induced mammary tumors in ERKO/wnt-1 mice, which have no functional ER-α.[28–30]

Compelling evidence has led to a new paradigm of cancer initiation by estrogens. Discovery that specific oxidative metabolites of estrogens can react with DNA[1,3,7,31] led to and supports the hypothesis that these metabolites can become endogenous chemical carcinogens. Some of the mutations generated by the specific DNA damage can result in the initiation of cancer in hormone-dependent and -independent tissues.[4,8,32,33]

Chemical carcinogens covalently bind to DNA to form two types of adducts: stable ones that remain in DNA, unless removed by repair, and depurinating adducts that are lost

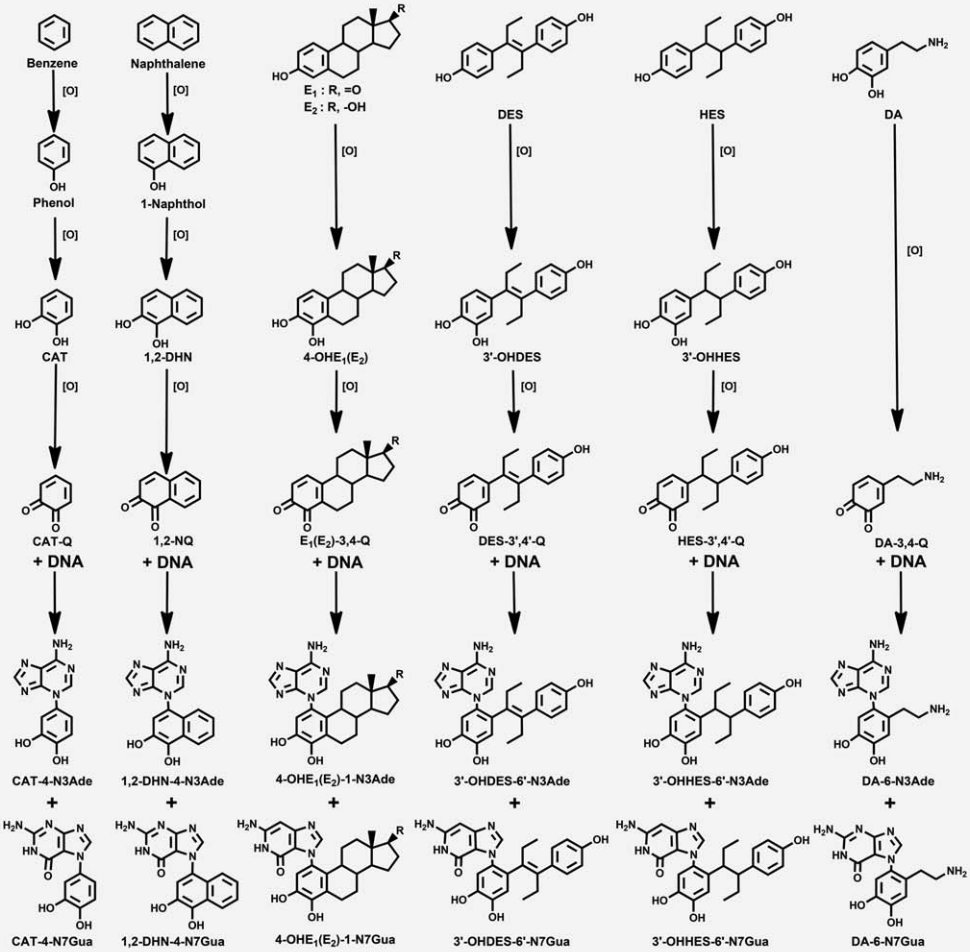

Figure 2. Mechanism of metabolic activation and reaction with DNA to form depurinating DNA adducts for benzene, naphthalene, estrone (E_1)/estradiol (E_2), diethylstilbestrol (DES), hexestrol (HES) and dopamine (DA).

from DNA by destabilization of the glycosyl bond.[6,7,34,35] Evidence that depurinating polycyclic aromatic hydrocarbon-DNA adducts and depurinating estrogen-DNA adducts play a major role in cancer initiation derives from a correlation between depurinating adducts that generate apurinic sites and oncogenic Harvey (H)-*ras* mutations in mouse skin papillomas, preneoplastic mouse skin and preneoplastic rat mammary gland.[2,8,32,36,37]

Apurinic sites are formed spontaneously in DNA.[38] Using the levels of depurinating adducts detected in mouse skin, one can conservatively estimate that carcinogenic aromatic hydrocarbons generate ca. 15–120 times more,[35,39,40] and E_2-3,4-Q[2] ca. 145 times more apurinic sites than the number of apurinic sites generated spontaneously in the cells. These extremely high levels of apurinic sites presumably overwhelm the ability of cells to repair them correctly, generating mutations. These findings contributed to discovering the estrogen metabolites that form depurinating DNA adducts, the precursors to cancer initiation.[1,3,31]

Experiments on estrogen metabolism,[41–43] formation of DNA adducts,[1,3,7,31] mutagenicity,[2,8,23,33] carcinogenicity[14–16] and cell transformation[44–47] have led to and support the realization that reaction of specific estrogen metabolites, mainly $E_1(E_2)$-3,4-Q, with DNA can generate critical mutations to initiate breast and other prevalent cancers.[31,33]

Unified Mechanism of Initiation for Benzene, Naphthalene, Natural Estrogens, Synthetic Estrogens and Dopamine

A unified mechanism of metabolic activation that produces weak ultimate carcinogens includes benzene, naphthalene, and natural and synthetic estrogens. In this mechanism the benzene ring of the compounds is enzymatically oxidized to yield a phenol (Fig. 2). A second hydroxylation produces a catechol, followed by a third oxidation to afford the electrophilic ultimate *ortho*-quinone metabolite. The reaction of the quinone with DNA by 1,4-Michael addition forms predominantly depurinating DNA adducts at the N3 of Ade and N7 of Gua (Fig. 2). These adducts detach from DNA, leaving behind apurinic sites (Fig. 1*a*). Erroneous repair of the apurinic sites gives rise to mutations that can initiate cancer. Evidence shows that this unified mechanism of activation occurs

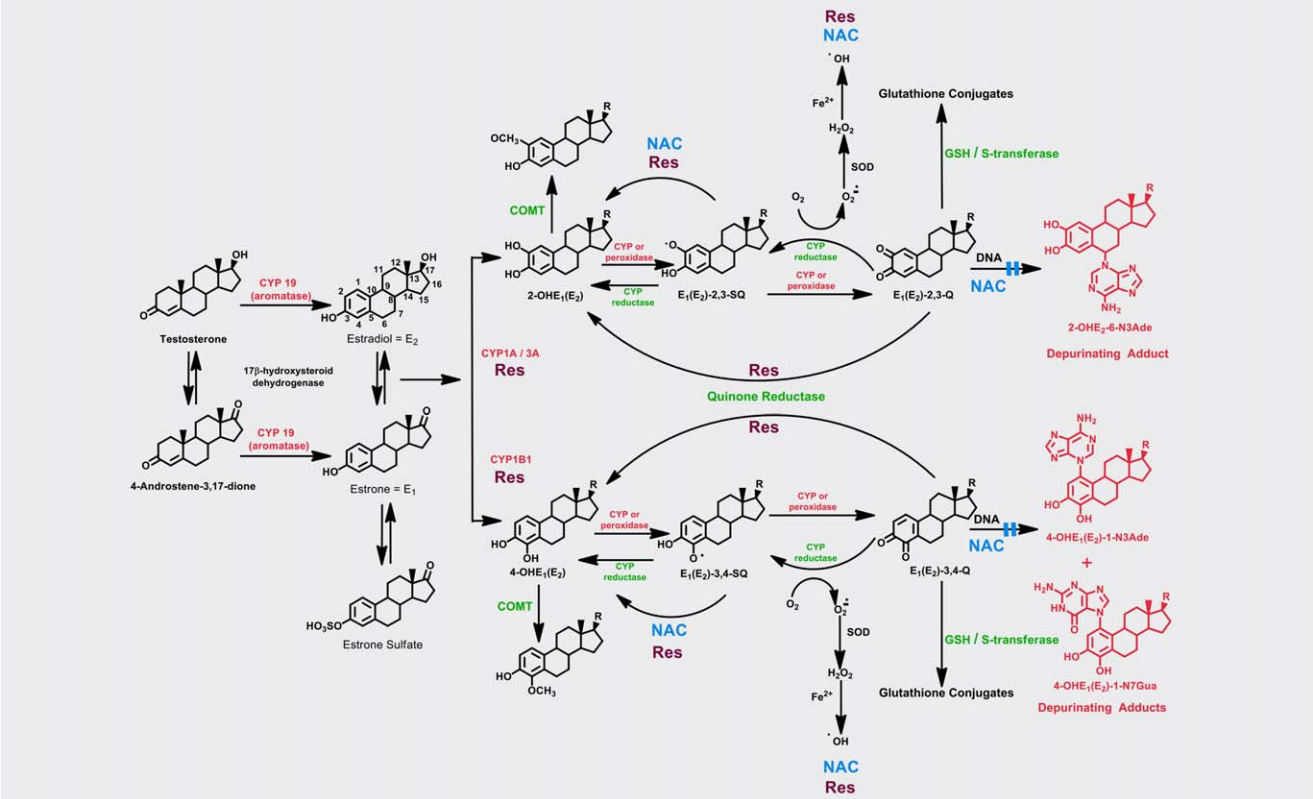

Figure 3. Formation of estrogens, catechol estrogen metabolic pathway of estrogens and depurinating DNA adducts of estrogens. Activating enzymes and depurinating DNA adducts are in red, and protective enzymes are in green. *N*-Acetylcysteine (NAC, shown in blue) and resveratrol (Res, shown in burgundy) indicate various steps where NAC and Res can ameliorate unbalanced estrogen metabolism and reduce formation of depurinating estrogen-DNA adducts.

with benzene,[48,49] naphthalene,[50,51] the natural estrogens E_1 and E_2,[1,5–7,13,52,53] and the synthetic estrogens diethylstilbestrol (DES)[54,55] and hexestrol (HES).[52,56,57]

The neurotransmitter dopamine (DA) follows the same mechanism of activation to form depurinating DA-DNA adducts (Fig. 2).[48,49,58] These adducts can generate mutations leading to Parkinson's disease, as described below (Formation of Dopamine-DNA Adducts and their Role in the Etiology of Parkinson's Disease Section).

Formation, Metabolism and Depurinating DNA Adducts of Estrogens

Metabolic formation of the estrogens E_1 and E_2 derives from aromatization of androstenedione and testosterone, respectively, catalyzed by cytochrome P450 (CYP) 19 (aromatase, Fig. 3). The estrogens E_1 and E_2 are interconverted by 17β-hydroxysteroid dehydrogenase. When an excess of estrogens is produced, it is stored as E_1-sulfate.

Estrogens are metabolized via two major pathways: formation of 16α-hydroxy$E_1(E_2)$ (not shown in Fig. 3) and formation of the catechol estrogens 2-$OHE_1(E_2)$ and 4-$OHE_1(E_2)$ (Fig. 3).[59] CYP1A1 hydroxylates E_1 and E_2 preferentially at the 2-position, whereas CYP1B1 hydroxylates E_1 and E_2 almost exclusively at the 4-position, forming 4-$OHE_1(E_2)$.[60–62] The most common path of conjugation of catechol estrogens in extrahepatic tissues is *O*-methylation catalyzed by catechol-*O*-methyltransferase (COMT).[63,64] When COMT activity is low, competitive oxidation of the catechol estrogens to semiquinones (SQ) and then quinones (Q), catalyzed by CYP or peroxidase, can occur (Fig. 3). Oxidation of semiquinones to quinones can also be accomplished by molecular oxygen (Fig. 3), generating lipid hydroperoxides (not shown in Fig. 3)[65] that act as unregulated cofactors for the oxidation of catechol estrogens by CYP.

Following the formation of $E_1(E_2)$-3,4-Q and $E_1(E_2)$-2,3-Q, they can be inactivated by glutathione (GSH) or by reduction to their respective catechols by quinone reductase,[66,67] a protective enzyme that can be induced by a variety of compounds.[68] If the catechol estrogen quinones are not eliminated, they can react with DNA to yield almost exclusively depurinating adducts.

Imbalance of Estrogen Metabolism and Generation of Mutations in Cancer Initiation

Metabolism of estrogens via the catechol estrogen pathway is characterized by homeostasis, a balanced set of activating and protective enzymes that minimizes formation of the catechol estrogen quinones. When homeostasis is disrupted, excessive

amounts of catechol estrogen quinones and depurinating estrogen-DNA adducts are formed and can lead to cancer initiation. Therefore, the estrogens become carcinogenic when homeostasis is disrupted in the catechol estrogen metabolic pathway.

Several factors can imbalance estrogen homeostasis. These include overexpression of CYP19 (aromatase, Fig. 3)[69–71] and unregulated sulfatase that converts excess stored E_1-sulfate into E_1.[72,73] Another factor is the formation of high levels of 4-$OHE_1(E_2)$, because of overexpression of CYP1B1.[60–62,74,75] In turn, higher levels of 4-$OHE_1(E_2)$ will produce higher levels of $E_1(E_2)$-3,4-Q, the strongest ultimate carcinogenic estrogen metabolites (Fig. 3).

Imbalanced estrogen metabolism can also be produced by low COMT activity because of polymorphic variation of the enzyme,[64,76] with low levels of methylation of 4-$OHE_1(E_2)$ and increased competitive oxidation of 4-$OHE_1(E_2)$ into $E_1(E_2)$-3,4-Q (Fig. 3). Higher levels of $E_1(E_2)$-3,4-Q can also derive from polymorphisms in quinone reductase, decreasing conversion of quinones into catechols (Fig. 3).[77] Furthermore, low cellular levels of GSH, which reacts efficiently with the quinones, can leave higher levels of $E_1(E_2)$-3,4-Q available to react with DNA.

Imbalance of estrogen metabolism has been seen in animal models for estrogen carcinogenicity: the kidney of male Syrian golden hamsters,[44] the prostate of Noble rats[42] and the mammary gland of ER-α knock-out mice.[29] Such imbalanced homeostasis has also been observed by comparing breast tissue from women with and without breast cancer.[43] In women with breast cancer, non-tumor breast tissue showed levels of 4-$OHE_1(E_2)$ nearly four-times higher than the levels in breast tissue from women without breast cancer.[43] A similar imbalance was also observed in women with breast cancer, who have greater expression of the activating enzymes CYP19 and CYP1B1, versus women without breast cancer, who have greater expression of the protective enzymes COMT and quinone reductase (Fig. 3).[78]

In summary, homeostasis in the metabolism of estrogens via the catechol estrogen pathway maintains the integrity of these molecules. Disruption of homeostasis with excessive formation of catechol estrogen quinones and depurinating estrogen-DNA adducts can lead to the initiation of cancer.

Imbalanced estrogen metabolism leading to excessive levels of depurinating estrogen-DNA adducts can generate oncogenic mutations. The ability to induce mutations was demonstrated by treating mouse skin[2] or rat mammary gland[8] with E_2-3,4-Q and analyzing both the estrogen-DNA adducts formed and the H-*ras* mutations generated. Additional demonstrations of estrogen mutagenicity were accomplished by using 4-OHE_2 to treat either transfected Big Blue® rat2 embryonic cells[33] or female Big Blue® rats.[4] The generation of mutations in cultured cells, mouse skin and rat mammary gland demonstrates that estrogens induce mutations that can generate the initiation of cancer.

Depurinating Estrogen-DNA Adducts: The Biomarkers of Cancer Risk and Initiation

The first evidence that depurinating estrogen-DNA adducts play a major role in cancer initiation was obtained from a correlation between the sites of formation of depurinating estrogen-DNA adducts and H-*ras* mutations in mouse skin and rat mammary gland treated with the ultimate carcinogenic metabolite E_2-3,4-Q.[2,8] Depurinating estrogen-DNA adducts have now been detected in human serum and urine samples.

Estrogen metabolites, estrogen-GSH conjugates and depurinating estrogen-DNA adducts are analyzed in human serum and urine by using ultraperformance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS). While absolute levels of these analytes differ among people, the ratio of the depurinating adducts, 4-$OHE_1(E_2)$-1-N3Ade, 4-$OHE_1(E_2)$-1-N7Gua and 2-$OHE_1(E_2)$-6-N3Ade, to the estrogen metabolites and conjugates provides a reliable measure of the balance or imbalance of estrogen metabolites in a person[79,80]:

$$\text{ratio} = \left(\frac{4\text{-OHE}_1(\text{E}_2)\text{-1-N3Ade} + 4\text{-OHE}_1(\text{E}_2)\text{-1-N7Gua}}{4\text{-catechol estrogens} + 4\text{-catechol estrogen conjugates}} + \frac{2\text{-OHE}_1(\text{E}_2)\text{-6-N3Ade}}{2\text{-catechol estrogens} + 2\text{-catechol estrogen conjugates}} \right) \times 1000$$

This ratio serves as a biomarker for risk of developing estrogen-initiated cancer.

Caucasian women diagnosed with breast cancer, or at normal or high risk for developing the disease, have been investigated in three case-control studies.[79,81,82] The women at high risk were identified by 5-year Gail model scores $>1.66\%$.[83] In the first two studies, an aliquot of urine was analyzed by UPLC-MS/MS and the ratio of adducts to metabolites and conjugates (see above) was calculated for each subject.[79,81] The ratios in the high-risk women and those diagnosed with breast cancer were significantly higher than the ratios in the normal-risk women ($p < 0.001$ in both studies).[79,81] The third study was larger and used serum instead of urine.[82] Similar results were obtained, with even greater differences between the normal-risk women and the high-risk women or those with breast cancer ($p < 0.0001$, Fig. 4*a*).[82] No differences in the results were observed when the subjects were separated into pre- and peri/postmenopausal groups.[82] These results, especially the high ratios observed in high-risk women, indicate that formation of estrogen-DNA adducts plays a critical role in the etiology of breast cancer.

The ratio of estrogen-DNA adducts to metabolites and conjugates was also investigated in women with and without ovarian cancer (Fig. 4*b*).[84] The women diagnosed with ovarian cancer demonstrated higher ratios than the controls ($p < 0.0001$, Fig. 4*b*). DNA from saliva samples was also purified and single nucleotide polymorphisms (SNPs) analyzed in the genes for two estrogen-metabolizing enzymes, the

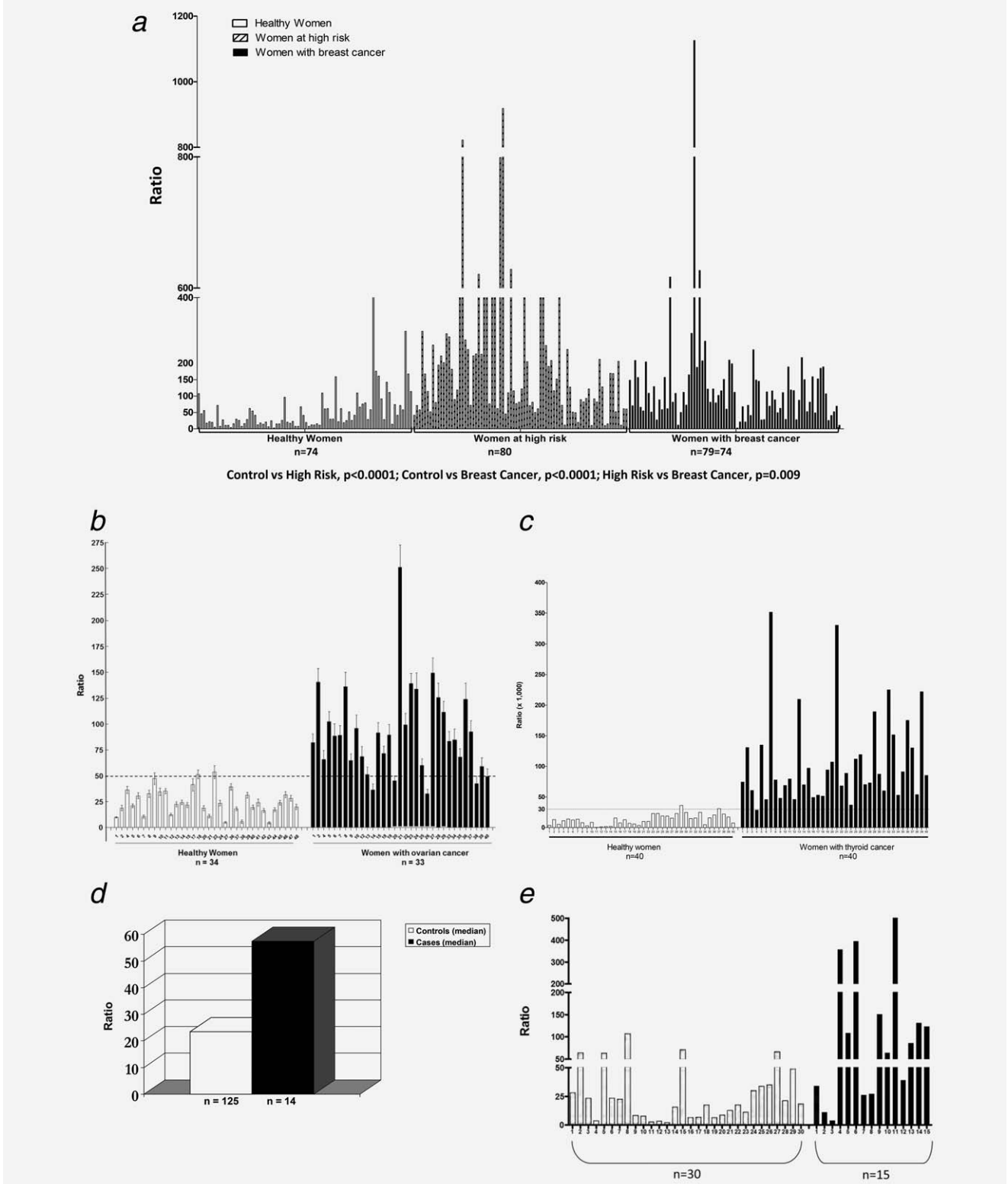

Figure 4. Ratios of depurinating estrogen-DNA adducts to estrogen metabolites and estrogen conjugates in (*a*) serum samples of healthy women, high-risk women and women with breast cancer;[82] (*b*) urine samples from women with and without ovarian cancer ($p < 0.0001$);[84] (*c*) urine samples from women with and without thyroid cancer ($p < 0.0001$). The dotted line at a ratio of 50 is the cut-point for sensitivity and specificity of the ratio;[86] (*d*) urine samples from men with and without prostate cancer (mean levels, $p < 0.001$)[88] and (*e*) urine samples from men with and without NHL ($p < 0.007$).[89]

activating enzyme CYP1B1 (V432L) and the protective enzyme COMT (V158M).[84] The women with two copies of the low-activity COMT allele plus the high-activity CYP1B1 allele demonstrated much higher values of the DNA adduct ratio.[84] With two copies of both the high-activity CYP1B1 allele and the low-activity COMT allele, the odds ratio for ovarian cancer was 6-fold higher compared to women with normal-activity alleles. These combined results suggest that initiation of ovarian cancer is strongly associated with unbalanced estrogen metabolism leading to formation of estrogen-DNA adducts.

Exposure to estrogens has been identified as a risk factor for thyroid cancer.[85] When estrogen metabolites, conjugates and depurinating DNA adducts were analyzed in a small study of urine samples from women with thyroid cancer plus healthy controls, the women with thyroid cancer had much higher estrogen-DNA adduct ratios ($p < 0.0001$, Fig. 4*c*).[86] Thus, formation of estrogen-DNA adducts has been associated with breast, ovarian and thyroid cancer in women.

Formation of estrogen-DNA adducts has also been associated with cancer in men,[87–89] and the same estrogen-DNA adduct ratio can be used as a biomarker of risk. Urine samples from men diagnosed with prostate cancer or from healthy control men have been analyzed by UPLC-MS/MS.[87,88] In an initial study, diagnosis with prostate cancer was associated with significantly higher levels of 4-$OHE_1(E_2)$-1-N3Ade.[87] In a subsequent, larger study, the estrogen-DNA adduct ratio was significantly higher in men with prostate cancer than in controls ($p < 0.001$, Fig. 4*d*).[88] These results suggest that formation of estrogen-DNA adducts plays a critical role in the etiology of prostate cancer.

A similar small study of men diagnosed with non-Hodgkin lymphoma (NHL) plus healthy controls was conducted.[89] The estrogen-DNA adduct ratio was significantly higher in the men with NHL compared to the controls ($p < 0.0007$, Fig. 4*e*).[89] We think that investigation of other prevalent types of cancer will demonstrate that they, too, are initiated by formation of estrogen-DNA adducts. These cancers include brain, colon, endometrium, kidney, leukemia, lung of non-smokers, melanoma, myeloma, pancreas and testis.

In summary, the estrogen-DNA adduct ratios were significantly higher in cases compared to controls in all five types of cancer studied. In each study the adducts derived from 4-$OHE_1(E_2)$ predominated and the 2-$OHE_1(E_2)$-6-N3Ade adducts constituted a small portion (1–3%) of the total depurinating adducts.[79,81,82,84,86,89,98] The high adduct ratios in women at high risk for breast cancer ($p < 0.0001$ vs normal risk women) and the association of SNPs in CYP1B1 and COMT with 6-fold increased odds of ovarian cancer provide particularly strong evidence for a critical role of estrogen-DNA adducts in the etiology of these cancers.

Sensitivity and specificity curves for the ratio levels provide an initial cut-point of 77 for breast cancer,[82] 43 for ovarian cancer[84] and 30 for thyroid cancer.[86] This suggests that DNA adduct ratios above 77 indicate high risk for cancer, while ratios below 30 indicate low risk, while ratios of 30–77 are indeterminate. Additional studies with more subjects and other types of cancer will enable refinement of this potential biomarker of cancer risk.

Formation of Dopamine-DNA Adducts and their Role in the Etiology of Parkinson's Disease

The neurotransmitter dopamine (DA) is formed in the cell bodies of the dopaminergic neurons of the *substantia nigra*. Parkinson's disease (PD) is the result of the degeneration of nigrostriatal dopaminergic neurons and decreased production of DA.

Oxidation of DA affords its quinone (Fig. 5*a*), which at neutral pH undergoes intramolecular cyclization by 1,4-Michael addition to produce leukochrome. Further oxidation of leukochrome leads to aminochrome, which by polymerization yields neuromelanin (Fig. 5*a*). At acidic pH (5–6), the amino group of DA becomes partially protonated, slowing intramolecular cyclization of DA and leading to competitive intermolecular 1,4-Michael addition with nucleophiles, including those of DNA (Fig. 5*a*). Under these conditions, reaction of DA quinone with DNA leads to formation of the depurinating DA adducts DA-6-N3Ade and DA-6-N7Gua (Figs. 2, 5*a*, and 5*b*).[48,49,58] The mutations produced by this DNA damage may play the critical role in initiating the series of events leading to neurodegeneration and PD (Fig. 5*a*).

The reaction of DA quinone with DNA under slightly acidic conditions (pH 5–6) is analogous to the mechanism of metabolic activation of benzene, naphthalene, natural estrogens and synthetic estrogens, in which the quinones react with DNA by 1,4-Michael addition to form depurinating N3Ade and N7Gua adducts that generate the critical mutations leading to cancer initiation (Fig. 3).[2,8]

An excessive amount of glutamate, as occurs in excitotoxicity, can produce a stable pH of 5.5 during corelease of glutamate and DA from synaptic vesicles.[90] These data suggest that DA quinone could yield DNA adducts *in vivo* in dopaminergic neurons under these conditions. This proposed mechanism provides a solid foundation for the specific neurodegeneration of dopaminergic neurons observed in PD.

Thus, under slightly acidic conditions (pH 5–6) DA could be the initiator of PD by producing depurinating DA-DNA adducts (Fig. 5*a*) in dopaminergic neurons, analogously to the depurinating adducts of estrogens in the initiation of cancer (Fig. 3). The apurinic sites produced in the DNA could generate mutations that lead to degeneration of the dopaminergic neurons. Over time, in people prone to this drop in pH in these neurons, a significant number of dopaminergic neurons could degenerate, resulting in reduced production of DA and development of PD (Fig 5*a*).

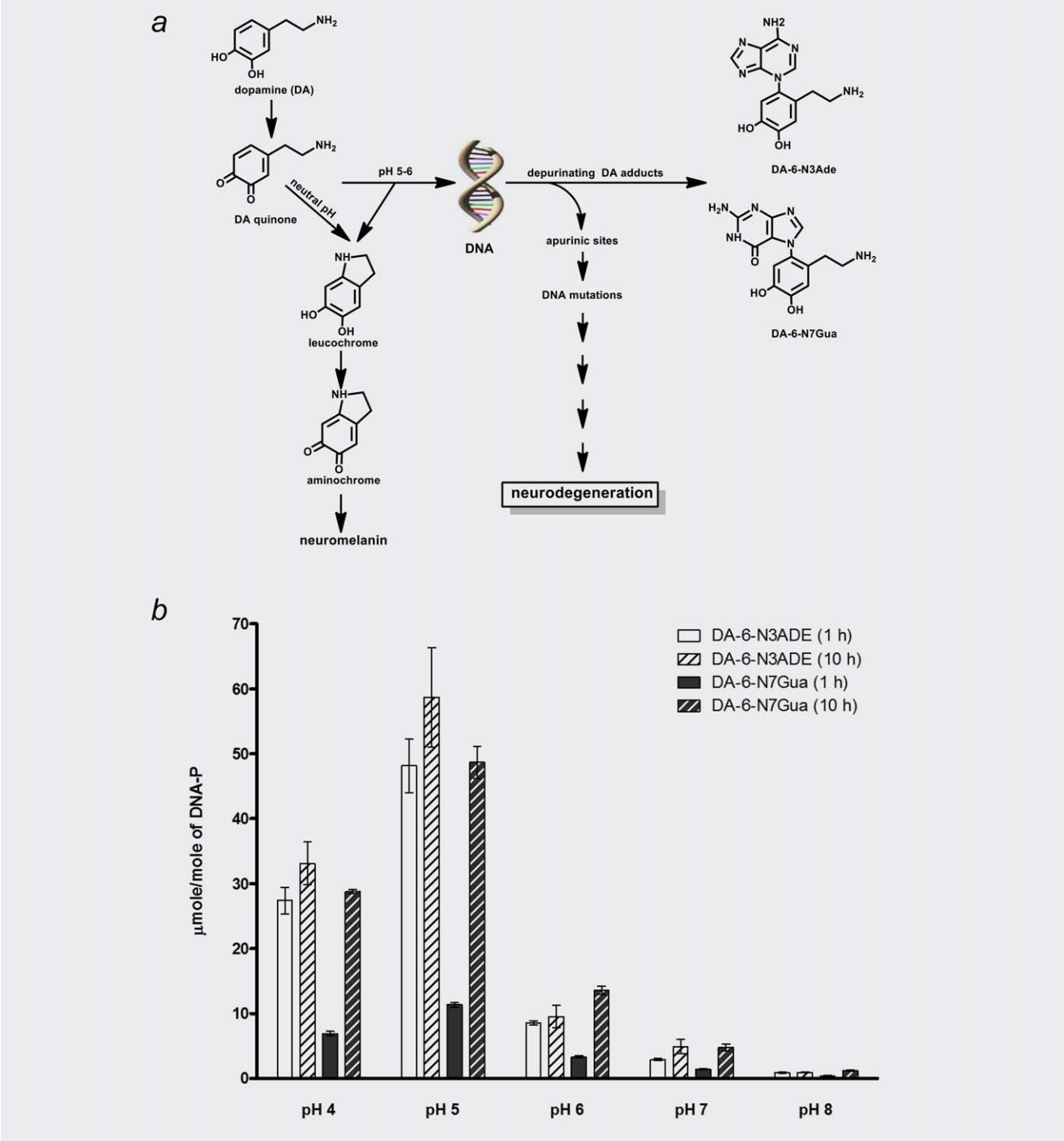

Figure 5. (*a*) Intramolecular 1,4-Michael addition of dopamine (DA) quinone at neutral pH to form leukochrome, followed by aminochrome and, then, neuromelanin. At pH 5–6, the competitive intermolecular 1,4-Michael addition to produce the depurinating adducts DA-6-N3Ade and DA-6-N7Gua and DNA with apurinic sites, then DNA mutations and neurodegeneration. (*b*) Effect of pH on the formation of depurinating adducts with reaction of tyrosinase-activated DA in the presence of DNA.[58]

Prevention of Cancer and Parkinson's Disease by *N*-Acetylcysteine and Resveratrol, Inhibitors of Depurinating Estrogen- and Dopamine-DNA Adducts

When estrogen homeostasis is disrupted, formation of catechol estrogen quinones increases and more depurinating estrogen-DNA adducts are formed. This can be inhibited by balancing or re-balancing estrogen metabolism through the use of specific dietary supplements such as *N*-acetylcysteine (NAC) and resveratrol (Res). These two compounds are particularly effective in preventing formation of estrogen-DNA adducts.[91] NAC (Fig. 6*a*) is the acetyl derivative of the amino acid cysteine. Res (Fig. 6*a*) is found in grapes, wine, peanuts and other plant

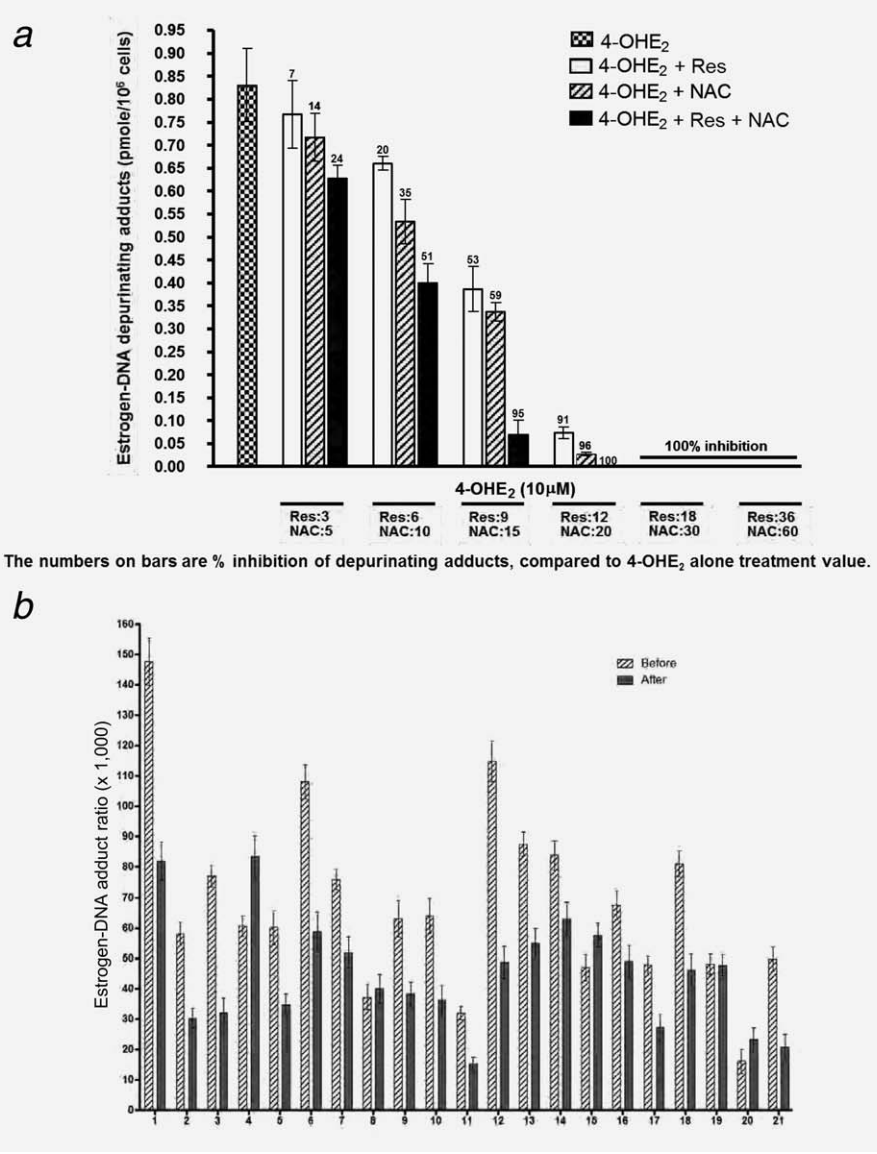

Figure 6. (*a*) Effects of NAC, Res, or NAC plus Res on the formation of depurinating estrogen-DNA adducts in MCF-10 F cells treated with 4-OHE_2. The number above each bar indicates the percent inhibition compared to treatment with only 4-OHE_2.[102] (*b*) Estrogen-DNA adduct ratios in women before and after following the Healthy Breast Protocol for three months.[103]

products. NAC and Res inhibit formation of catechol estrogen quinones and/or their reaction with DNA.[91]

NAC has multiple anticarcinogenic properties,[92,93] can generate the cellular scavenger GSH and has very low toxicity. NAC reacts efficiently with the electrophilic $E_1(E_2)$-3,4-Q,[91] thereby preventing them from forming adducts with DNA. By reducing catechol estrogen semiquinones to catechol estrogens (Fig. 3)[94] and/or reacting with $E_1(E_2)$-3,4-Q, NAC can prevent malignant transformation of the human MCF-10F cells[95] and mouse E6 mammary cells[96] treated with 4-OHE_2.

Both NAC and Res can cross the blood–brain barrier.[92,93,97,98] Res has shown chemopreventive effects in a variety of models.[97,98] It can modulate CYP1B1,[75,99] induce quinone reductase[75,100] and reduce catechol estrogen semiquinones to catechol estrogens.[75] Res inhibits formation of estrogen-DNA adducts in MCF-10F cells treated with 4-OHE_2.[75,101] NAC and Res showed additive effects in inhibiting formation of depurinating estrogen-DNA adducts in MCF-10F cells ($p < 0.0001$) (Fig. 6*a*).[102]

The effects of NAC and Res were studied in a Healthy Breast Protocol for women.[103] In this study, 21 healthy women (age 30–70), who had never been diagnosed with cancer, followed the Healthy Breast Protocol daily for three months and provided a spot urine sample immediately before and after the treatment; the urine samples were analyzed for estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts by using UPLC-MS/MS, and the ratio of adducts to metabolites and conjugates was calculated for each sample (Fig. 6*b*). Among the 21 participants, 16 showed lower adduct ratios after treatment, four showed no change and one had a higher

ratio. The average decrease in adduct ratio after treatment with the Healthy Breast Protocol was statistically significant ($p < 0.03$).[103] These results indicate that a treatment protocol that includes NAC and Res can reduce formation of depurinating estrogen-DNA adducts in people.

We hypothesize that NAC and Res could reduce formation of DA-DNA adducts in dopaminergic neurons in an analogous manner. Since both compounds can cross the blood-brain barrier, they could reduce formation of DA-DNA adducts by reacting with DA quinone and/or reducing DA quinone back to DA. Both compounds have been shown to reduce formation of DA-DNA adducts.[58]

In summary, both NAC and Res can reduce estrogen semiquinones to catechol estrogens (Fig. 3).[75,94] NAC can also help replenish GSH in cells, as well as reacting with $E_1(E_2)$-3,4-Q (Fig. 3). Res reduces formation of $E_1(E_2)$-3,4-Q by inducing quinone reductase and modulating CYP1B1 activity (Fig. 3). All of these effects can play a role in reducing formation of estrogen-DNA and DA-DNA adducts, thus reducing the risk of developing cancer or Parkinson's disease.

Conclusions

We have found a common origin for many prevalent types of cancer. In fact, compelling evidence from studies on estrogen metabolism,[41–43] formation of estrogen-DNA adducts,[1,3,7,31] mutagenicity,[2,4,8,32,33] transformation of mammalian cells[44–46] and carcinogenicity[14–16] has led to and supports the hypothesis that reaction of specific endogenously formed $E_1(E_2)$-3,4-Q with DNA can generate the critical mutations that initiate breast, ovarian, prostate and other prevalent types of human cancer.[31,33] This is a new paradigm of cancer initiation by estrogens, involving estrogen metabolism rather than ER-mediated events.

Metabolism of estrogens involves a balanced set of activating and protective pathways. This balance minimizes formation of the ultimate carcinogenic catechol estrogen quinones, as well as their reaction with DNA to form adducts. When estrogen metabolism becomes unbalanced, catechol estrogens are excessively oxidized to quinones, which can react with DNA to form mainly the depurinating adducts 4-$OHE_1(E_2)$-1-N3Ade and 4-$OHE_1(E_2)$-1-N7Gua (Fig. 3). The loss of these adducts from DNA generates apurinic sites, which can lead to mutations that initiate breast, prostate and other prevalent types of cancer.

The genotoxicity of endogenous estrogens was demonstrated in a variety of studies conducted *in vitro*, in cell culture and in animal models.[2,4,8,32,33] More recently, the role of estrogen metabolism and the resulting estrogen-DNA adducts in cancer etiology has been investigated in several human cancers: breast (Fig. 4*a*), ovarian (Fig. 4*b*) and thyroid (Fig. 4*c*) cancer in women[79,81,82,84,86] and prostate cancer (Fig. 4*d*) and non-Hodgkin lymphoma (Fig. 4*e*) in men[87–89] by using UPLC-MS/MS to analyze estrogen metabolites, estrogen conjugates and depurinating estrogen-DNA adducts in urine or serum samples. In all of these five types of cancer, people diagnosed with the disease have a higher ratio of DNA adducts to estrogen metabolites and conjugates, while healthy people without cancer exhibit a lower ratio of adducts to metabolites and conjugates because their estrogen metabolism is balanced.[80] It is particularly striking that women at high risk for breast cancer exhibit high adduct ratios.[79,81,82] These results indicate that these types of cancer have a common etiology, i.e., formation of depurinating estrogen-DNA adducts that can generate cancer-initiating mutations. We think that other prevalent types of human cancer, including brain, colon, endometrial, kidney, lung of non-smokers, pancreas, leukemia and melanoma may also be initiated by reaction of $E_1(E_2)$-3,4-Q with DNA to generate apurinic sites and the ensuing mutations that can initiate cancer.

An analogous mechanism of activation could occur in dopaminergic neurons to initiate Parkinson's disease (Fig. 5*a*). Under slightly acidic conditions (pH 5–6), dopamine quinone can react with DNA to form analogous depurinating DA-6-N3Ade and DA-6-N7Gua adducts, which could generate mutations that lead to the degeneration of the dopaminergic neurons and the development of Parkinson's disease.[49,58]

Prevention of estrogen-initiated cancers could be achieved by using compounds that can reduce formation of $E_1(E_2)$-3,4-Q and/or their reaction with DNA. In particular, NAC and Res reduce both formation of depurinating estrogen-DNA adducts and malignant transformation of cultured human or mouse mammary epithelial cells.[95,96] These two compounds have an additive response in reducing adduct formation in human cells.[102] Furthermore, the DNA adduct ratio was reduced in women who followed a Healthy Breast Protocol,[103] suggesting that these compounds could reduce the risk of developing breast cancer.

Promotion, progression and development of cancer cannot occur when initiation of the disease is blocked. This approach to cancer prevention can be followed without knowing the genes involved or the events that follow initiation. Therefore, a focus on the formation of depurinating estrogen-DNA adducts in the initiation of many prevalent types of cancer can provide powerful insights into the etiology and prevention of cancer.

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