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Role of Cadmium and Nickel in Estrogen Receptor Signaling and Breast Cancer: Metalloestrogens or Not?

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Abstract

During the last half-century, incidences of breast cancer have increased globally. Various factors—genetic and environmental—have been implicated in the initiation and progression of this disease. One potential environmental risk factor that has not received a lot of attention is the exposure to heavy metals. While several mechanisms have been put forth describing how high concentrations of heavy metals play a role in carcinogenesis, it is unclear whether chronic, low-level exposure to certain heavy metals (i.e. cadmium and nickel), can directly result in the development and progression of cancer. Cadmium and nickel have been hypothesized to play a role in breast cancer development by acting as metalloestrogens—metals that bind to estrogen receptors and mimic the actions of estrogen. Since the lifetime exposure to estrogen is a well-established risk factor for breast cancer, anything that mimics its activity will likely contribute to the etiology of the disease. However, heavy metals, depending on their concentration, are capable of binding to a variety of proteins and may exert their toxicities by disrupting multiple cellular functions, complicating the analysis of whether heavy metal-induced carcinogenesis is mediated by the estrogen receptor. The purpose of this review is to discuss the various epidemiological, *in vivo*, and *in vitro* studies that show a link between the heavy metals, cadmium and nickel, and breast cancer development. We will particularly focus on the studies that test whether or not these two metals act as metalloestrogens in order to assess the strength of the data supporting this hypothesis.

Keywords

Breast cancer; metalloestrogen; estrogen receptor; cadmium; nickel

Introduction

Breast cancer is one of the most common malignancies in the United States. Approximately 1 in 8 women in the U.S. will develop invasive breast cancer (1), underscoring the importance of understanding the factors that may contribute to the development of this disease. Multiple studies suggest that both genetic and environmental factors contribute to breast cancer development (2–4). Some of the environmental factors that have been recognized as suspected risk factors for breast cancer include ionizing radiation, hormone disruptors such as organohalogens, pesticides and environmental pollutants such as heavy metals. Regarding the latter, increasing epidemiological evidence suggests a strong

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association between exposure to heavy metals and the initiation, promotion and progression of breast cancer (5–8).

Heavy metals— which include arsenic, lead, mercury, cadmium, and nickel— are present naturally in the environment in minute concentrations; however with the increased usage in certain industrial processes such as smelting and electroplating, heavy metals have emerged as an environmental contaminant of growing concern. These heavy metals tend to accumulate in the body— a phenomenon called bioaccumulation (9). Bioaccumulation of heavy metals in soft tissues interferes with normal physiological functions. Generally, these heavy metals exert their toxic effects by forming complexes with organic compounds. When heavy metals bind to nitrogen-, oxygen- or sulfur-containing groups on enzymes, for example, they disrupt proper protein folding and thus can inactivate enzymes that function in key metabolic processes (10). Increased exposure to heavy metals is associated with impaired mitochondrial function, oxidative stress, DNA damage, deregulated cell growth and cell death (11, 12).

Recent studies have suggested that certain heavy metals such as cadmium and nickel can function as endocrine disruptors by mimicking the action of estrogens. As a result, these metals are often referred to as metalloestrogens (11–13). Since estrogen itself plays an important role in the development and progression of the disease, the ability of metalloestrogens to bind to and activate the estrogen receptors suggests that these compounds may also contribute to the development of breast cancer (5, 13). It is hypothesized that metal-induced estrogen receptor activation is a crucial step in the carcinogenic process (14). Therefore, the goal of this review is to examine the association between cadmium and nickel exposure and the development and progression of breast cancer. Specifically, we wish to determine (1) the carcinogenic potential of chronic, low-level exposure to these heavy metals and (2) whether or not the carcinogenic potential of cadmium and nickel is due, at least in part, to their ability to bind to estrogen receptors and act as metalloestrogens.

The role of estrogen receptor α , β , and GPER in breast cancer progression

Estrogens, produced by the female ovaries, play major roles in regulating the developmental processes of both normal and neoplastic breast epithelium. Estrogens are primarily synthesized during a female's reproductive years to promote the growth and differentiation of sex tissues and organs in the reproductive system. They also play a role in brain function, bone maintenance and the accumulation of adipose tissue (15). The effects of estrogen are mediated by two types of estrogen receptor (ER)-regulated pathways – (1) the nuclear estrogen receptors (nER), which when activated translocate to the nucleus to function as transcription factors, and (2) the membrane estrogen receptors (mER), which are located on the plasma membrane or within the membrane of the endoplasmic reticulum (16). The deregulation of estrogenic pathways can lead to elevated transcriptional activity that may contribute to the development of cancer. Removal of both ovaries has been shown to reduce breast cancer risk, underscoring the importance of estrogen in the development of breast cancers, especially in cancers that express the estrogen receptor (ER+). The factors that contribute to increased cell proliferation of ER+ breast cancer include elevated levels of endogenous estrogen, increased exposure to pharmaceutical and environmental estrogens (17), and deregulated expression of ERs in the cell, which can lead to abnormal expression of genes associated with cell growth. Therefore, overexposure to estrogen and the overexpression of ER can both contribute to the etiology of breast cancer (18).

More than 70% of primary breast cancers in women are nER α + and show estrogen receptor-dependent growth (19–21). ER α and ER β are nuclear receptors that display considerable homology in the DNA-binding and ligand-binding domains but vary greatly in the NH₂-terminal transactivation AF-1(activation function-1) domains (Fig. 1) (22, 23). Both isoforms regulate transcription through classical and nonclassical pathways. Through the classical mode of ER signaling, AF-1 functions in a cell- and promoter-specific manner to enhance the overall transcriptional response of ER (24). ER α and/or ER β then are activated by their ligand, estradiol, with similar affinities and bind directly to the the same consensus estrogen response element (ERE) (Fig 2) in promoter or enhancer regions of target genes, which is followed by recruitment of the coregulators (25). Target genes regulated by this classical pathway include breast cancer marker gene trefoil factor 1 (*TFF1* or *pS2*), cathepsin D (*CTD*), cyclin D1, insulin-like growth factor-binding protein 1 (*IGFBP1*), lactoferrin (*LTF*) and prolactin (*PRL*). Additionally, ER can also function through a nonclassical pathway by cross-talking with other transcription factors (26), such as activator protein-1 (AP-1), Sp1 and NF- κ B. This cross-talk allows estrogen receptor to regulate genes that do not contain an ERE, thereby increasing the number of genes that ER modulates (23, 27–33). Many of these transcription factors regulate expression of genes crucial for cell cycle progression, migration, proliferation and apoptosis; and therefore all can contribute to breast carcinogenesis. Examples of target genes studied extensively in breast cancer research include cyclin D1, c-myc and IGF-1.

ER α and ER β seem to display similar ligand-dependent transcriptional functions and yet appear to play different roles in breast cancer progression. Although ER α has long been established as an important player in promoting the development and progression of breast cancer (34, 35), the link between ER β and breast cancer is unclear due to conflicting data and the complexity of the ER β isoforms. While some studies have suggested that the expression of ER β is indicative of more advanced tumors (36–38), data from other studies have indicated that ER β may play a protective role in breast cancer by increasing apoptosis in the presence of antiestrogens (39–41). Thus, much of what we know about ER-related breast cancer has focused largely on ER α . In a study performed by Holst et al., 20.6% of 2,000 clinical breast cancer samples showed an amplification of the ER α gene (*ESR1*) (42). Of those tumor samples, 99% showed an overexpression of the ER α protein, suggesting the importance of ER α in breast cancer development. Consequently, reducing estrogen levels or estrogen receptor activity through the administration of anti-estrogen compounds has been used to treat breast cancer patients.

In addition to the genomic actions mediated by nuclear estrogen receptors, estrogen also stimulates nongenomic signaling through the activation of one membrane form of estrogen receptor, GPR30 (GPER). With mERs, estrogens can act without directly binding onto DNA or altering gene expression. Filardo et al. was the first to show a function of GPR30 in estrogen signaling (43); and in 2005, GPR30 was finally identified as a bona fide estrogen receptor (44, 45). GPR30 is a seven-transmembrane-spanning receptor reported to be located in the plasma membrane (45–47) and the endoplasmic reticulum (44). GPR30 functions by specifically binding to estrogen and causing rapid intracellular signaling and an activation of a downstream cascade, which includes epidermal growth factor release (EGFR) activation and increased intracellular cyclic AMP, leading to activation of transcriptional activities necessary for proliferation (Fig 2) (43, 47–49). Thus, EGFR plays a critical role in regulating normal cell growth and physiology (50). Other cellular responses mediated by EGFR include activation of mitogen-activate protein kinsases MAPKs (such as extracellular signal-regulated kinase Erk-1 and Erk-2) which in turn phosphorylate numerous proteins that alter cell structure and regulate cell cycle checkpoints and gene transcription (51).

In 2009, Liu et al. showed that GPR30 was expressed in 37 of 74 cases of invasive ductal breast carcinomas. The binding of estrogen to GPR30 has also been shown to induce breast cancer cell proliferation and migration in vitro (52, 53). This increased cell proliferation has been attributed to the rapid activation of Erk-1/2, a downstream effector of growth factor signaling (54, 55). The dysregulation of the EGFR to MAPK pathway may have particular significance to breast carcinogenesis. Both Filardo et al. and Sivaraman et al. hypothesized that MAPKs may provide a mechanism whereby hyperactive growth factor signaling may activate estrogen-dependent breast tumor growth (56, 57). However, other researchers have contradicted this hypothesis by implicating GPR30 in apoptosis and cell cycle arrest (58–60). Although the role of GPR30 in breast cancer remains unclear, the combined effects of nER and mER activation can possibly lead to increases in cell cycle progression and cell proliferation that are associated with breast carcinogenesis (Fig 2) (61).

Heavy metals— cadmium and nickel

Cadmium exists naturally in the earth's crust. However, the most common forms of cadmium found in the environment are salts, comprised of sulfides, chlorides or oxides. These compounds are found in soil, water, rock sediments and the atmosphere at varying concentrations. Cadmium is found in ocean waters at 0.1µg/liter or less, in river water between <1 and 13.5ng/liter, and in the soil of non-polluted areas ranging from 0.2 to 0.4 mg/kg (62, 63). Atmospheric levels of cadmium range up to 5ng/m³ in rural areas, from 0.005 to 0.015 µg/m³ in urban areas and up to 0.06µg/m³ in industrial areas (62, 63). Cadmium is released as a byproduct of various industrial activities, including the mining, galvanizing, and smelting of other metals like zinc, lead and copper. Cadmium is used to produce batteries, fertilizers, and paint pigments. Roughly 15,000 tons of cadmium are produced worldwide (64). Human exposure to cadmium is generally due to consumption of contaminated water or food or inhalation of cigarette smoke or fumes from smoldering metal. In 1989 and 1993, the World Health Organization (WHO) set the safe intake limit to 7µg cadmium/week/kg body weight.

Nickel is also a heavy metal and is widely distributed in the environment. It is found in agricultural soil at concentrations ranging from 3 to 1000 mg/kg and at concentrations of 1.5 to 8.5 mg/kg in forest floor samples collected from the northeastern United States (65). A small amount of nickel occurs naturally in water at levels of 0.228–0.693 µg/liter in ocean water and generally less than 2 µg/liter in fresh water (65). Nickel enters the human body through inhalation, ingestion and absorption. For the general population, the most common exposure to nickel is through the consumption of certain foods such as cacao products and nuts, which contain 10 and 3 mg nickel/kg, respectively (66). According to the WHO, the safe dietary intake of nickel is 4.2 µg Ni/kg/day (67).

Epidemiological studies linking cadmium and nickel exposure to breast cancer development

The International Agency for Research on Cancer (IARC) and the United States National Toxicology Program (NTP) designate cadmium as a human carcinogen (68–72). Cadmium has been associated with cancers of the pulmonary system, prostate, liver, hemopoietic system, urinary bladder and stomach and is also a multi-tissue animal carcinogen (23, 73–75). Particularly, cadmium exposure has also been associated with increased incidences of breast cancer development (13, 76). In a recent study carried out by Julin et al., dietary cadmium exposure was positively linked to breast cancer development in postmenopausal women (8). Antila et al. found high concentrations of cadmium (ranging from 3.2 to 86.9 µg/g) in breast tissue from breast cancer patients (6). Additionally, a case study published by

McElroy and colleagues recorded the cadmium levels in urine samples of 246 women diagnosed with breast cancer and of 254 controls (7). The study demonstrated a positive correlation between cadmium urine levels and breast cancer risk. Another case study carried by Strumylaite and colleagues found significantly higher levels of cadmium in malignant breast tumor tissue (0.053 $\mu\text{g/g}$) than in normal breast tissue (0.02 $\mu\text{g/g}$) (77). Other studies have confirmed this finding (78, 79). Although these data demonstrate a correlation between cadmium levels and breast cancer, they fall short at implicating cadmium as the etiological agent.

Minute amounts of nickel are considered nontoxic. In general, the average nickel concentration in urine ranges from 1 to 3 $\mu\text{g/g}$ creatinine (80). However, increased exposure to nickel compounds due to its increased usage in industrial processes has been shown to negatively impact the development of mammalian cells, leading to increased incidences of breast cancer development (13, 81). In fact, certain nickel compounds have been deemed carcinogenic by the IARC since the 1970s (82, 83). A recent study comparing 20 breast cancer patients with 8 healthy individuals showed higher levels of cadmium, chromium, lead, and nickel in the 20 breast cancer tissue biopsies than in the 8 healthy biopsies (78). These data supported an earlier study by Sherif and colleagues that reported a significant (albeit small) increase in nickel concentration in breast tumor tissue compared to normal breast tissue (84) indicating a positive correlation between increased nickel levels and breast cancer development.

Evidence of cadmium and nickel acting as metalloestrogens during breast cancer development

As mentioned previously, metalloestrogens are defined as a group of heavy metals that mimic the physiological actions of estrogen. The precise mechanism behind this mimicry is uncertain. Recent studies have suggested that metalloestrogens may function as endocrine disruptors, perturbing the normal hormonal cycle and altering the development of the mammary gland through both the classical and nonclassical ER binding pathways aforementioned (14, 85). However, most cadmium studies have largely focused on acute cadmium exposures, and little is known about the effects of chronic, low-level cadmium exposure on human breast cancer development and/or progression (Table 1). Because the half-life of cadmium ranges from 12 to 30 years (86–88) and the body does not possess an active mechanism for cadmium elimination, it remains in the body. Benbrahim-Tallaa and colleagues have shown that more than 40 weeks of exposure to 2.5 μM cadmium transformed normal human breast epithelial cells MCF-10A into cells displaying a basal-like phenotype. The cells showed an increase in colony formation and invasive potential, and a loss of contact inhibition (89). Animal studies (Table 1) have shown that acute cadmium exposure increases uterine weight, induces changes in the uterine lining, and increases the density of epithelial cells in the mammary glands, all of which are early signs of breast tumorigenesis (85, 90–92). Additionally, *in vitro* experiments have shown that cadmium can promote ER+ breast cancer cell growth, and this is found to be dependent on ER α (14, 93–96). According to Stoica et al. cadmium binds to ER α — with a K_D of 4.5×10^{-10} M—and blocks the binding of 17 β -estradiol (14). While the presence of cadmium does not alter the estrogen receptors' K_D for estrogen ($\sim 2.9 \times 10^{-10}$ M), it does decrease the total number of available estrogen binding sites. Thus, since cadmium does not alter the binding affinity of estradiol to the receptor, it is thought to interfere with estradiol binding in a noncompetitive manner. In a study carried out by our lab, concentrations as low as 1 μM Cd induced significant cell proliferation in three ER α -positive breast cancer cell lines (MCF-7, T-47D and ZR-75-1) after 2, 4 and 6 days of exposure (96). In this same study, we also deduced

that the nonclassical ER target genes— CycD1, c-myc and CTD— were up-regulated by Cd. Subsequent silencing of ER α or blocking the receptor with anti-estrogens mitigated the stimulatory affect of cadmium on ER+ breast cancer cells, thus showing requirement of ER α in mediating the cellular effects of cadmium (96). Similarly, several other studies have demonstrated that cadmium-induced gene expression is also dependent on the estrogen recpetor (14, 93, 97). Recent evidence also suggests that cadmium can initiate mitogenic actions through the binding of the membrane-bound form of estrogen receptor, GPR30, via the Erk-1/-2 cascading pathway (Fig. 2) (98, 99). Yu et al. showed that cadmium induces a proliferative response at concentrations ranging from 50 to 500nM in ER-negative/GPR30-positive SKBR3 breast cancer cells, but no such response was observed in a GPR30-mutant cell line. The lowest exposure of 50nM is comparable to the blood Cd level (140nM) reported by Fell et al. in occupationally-exposed people (100). These studies provide another mechanism for how cadmium may promote mammary gland carcinogenesis via an estrogen receptor.

Although animal studies have revealed that over-exposure to nickel compounds can lead to tumor formation in multiple tissue sites such studies have failed to link nickel with breast cancer development in experimental animals (Table 2) (101–103). Inhalation of metallic nickel (at a concentration of 15 mg/m³ for six hours/day, four to five days per week for 21 months) revealed neoplastic growth within the lungs of Wistar and Behesda black rats (104). Additionally, Ivankovic et al. observed fibrosarcomas, mesotheliomas, and/or rhabdomyosarcomas in 10% of Syrian golden hamsters exposed to a single high dose of nickel powder (105). No tumors were found in the negative control group. Such studies provide evidence that nickel exposure increases the incidence of tumors in several species and at multiple tissue sites, but not specifically breast tissue.

In spite of the lack of evidence from animal studies, other studies have suggested that nickel (like cadmium) may function as a metalloestrogen and alter estrogen receptor activity (Table 2) (13, 106–108). In vitro studies have shown the ability of nickel to mimic the effects of estradiol on cell proliferation and block estradiol binding to ER α (13). MCF-7 cells treated with either 10⁻⁹ M estradiol or 10⁻⁶ M Ni shows 2–5 fold increase in cell growth. Additionally, as with cadmium, the presence of nickel does not affect the receptor's K_D yet does decrease the number of estradiol binding sites, suggesting that nickel also interferes with estradiol binding in a noncompetitive manner (13). As with cadmium, the binding of nickel to the estrogen receptor appears to induce the expression of genes associated with cell growth (13). Although few studies have analyzed the effects of nickel on gene expression in mammary cells (Table 2), the effects of nickel has been widely studied in other cell types (109–113). Data from microarray analysis of nickel-transformed mouse fibroblasts revealed an overexpression of cyclin D1 (110), a gene that has been shown to play an important role in breast cancer cell growth (114–116). Furthermore, studies with human pulmonary cells suggest that nickel has the ability to increase cyclin D1, cyclin E and cyclin B1 expression (111). Despite the increased expression of cyclin D1 and cyclin E, which has been shown to promote the G1/S transition, the induction of cyclin B1 resulted in the induction of M-phase arrest and inhibited cell growth (111). Similarly, Ouyang et al. also demonstrated that nickel inhibits cell growth, this time by *decreasing* the expression of cyclin D1. The discrepancy in these studies may reflect both the different model systems (breast and fibroblast vs. lung) and concentrations of nickel used in these studies.

Molecular interactions between estrogen receptors and the heavy metals, cadmium and nickel

Several researchers have attempted to map out the cadmium interaction domain within the estrogen receptor in order to better understand how cadmium functions as an estrogen receptor modulator. However, a consensus of where cadmium binds has not been determined. The two possibilities are that (1) cadmium binds to the ligand binding domain (LBD), and (2) cadmium may replace the zinc in the DNA binding domain. In one study, specific amino acids of the LBD, including C381, C447, E523, H524 and D538, were identified as possible cadmium interaction sites (14). However, further analysis using chemical modification and mass spectroscopy identified several other cysteine (C) residues as having high affinities for the divalent form of cadmium, and these amino acids were not the same as those previously identified (14, 117). Additionally, Glu (E), His (H) and Asp (D) residues were not protected from chemical modification when the ER was combined with cadmium, thus suggesting these residues actually have low affinity for Cd^{2+} , a finding that has been confirmed in studies of other metallo-proteins (117–119). Much less is known about the binding between nickel and the estrogen receptors. An analysis by Martin and colleagues proposed that nickel activates the estrogen receptor through the LBD of ER α (13). The LBD of ER α includes C381, C417, C447, and C530, and nickel is believed to interact with C381 and C447 to activate ER α (13).

Another prospective cadmium-binding site is the DNA binding domain, which is logical since the coordination chemistry of Cd^{2+} is similar to that Zn^{2+} . Cd^{2+} has the ability to replace Zn^{2+} because they have similar chemical properties (120). *In vitro* studies have shown that the replacement of Zn with Cd slightly increases the DNA binding affinity of ER α (95, 121–123). This may translate to changes in transcriptional activity. However, further studies are necessary to map the actual cadmium binding site and this will offer further insights into how cadmium functions as an estrogen receptor modulator. Similar *in vitro* studies have indicated that nickel can also replace Zn^{2+} in the DNA binding domains of the estrogen receptors (95, 123), but this replacement results in a decreased DNA binding affinity, likely due to the difference in the coordination chemistry of zinc (tetrahedral) and nickel (octahedral), which may in turn cause a conformational change that may decrease DNA binding. This suggests that the mechanisms of how nickel and cadmium function as metalloestrogens are likely different.

Other mechanisms of cadmium- or nickel-induced carcinogenesis

Chromatin modifications

Attempting to elucidate whether or not certain heavy metals contribute to breast cancer progression via estrogen receptor binding is complicated by the fact that heavy metals are capable of binding to a plethora of macromolecules and cellular structures. Above a certain concentration threshold, many heavy metals, such as copper, mercury, and silver, are very effective antimicrobial agents, as they bind to and inactivate various proteins and enzymes. Similarly, heavy metal-induced carcinogenesis can result from the binding of heavy metals to proteins other than the estrogen receptor. Nickel, for example, plays significant roles in chromatin remodeling, which is a proposed mechanism for nickel-induced carcinogenicity (124–130). Specifically, exposure of cells to nickel has been reported to alter the acetylation, methylation and ubiquitination of histone proteins (124, 125, 131), which in turn affects transcription. In general, histone acetylation is associated with gene activation and histone deacetylation results in gene repression (132–134). Methylation at histones H3K4, H3K36, and H3K79 has been linked to transcription activation, whereas methylation at H3K9,

H3K27, and H4K20 has been associated with gene repression (94, 134–136). Studies describing nickel-induced epigenetic changes have reported a global loss of both histone acetylation and H3K4 methylation and a global increase in H3K9 methylation, all of which are associated with gene silencing. These findings, however, contradict the observation that exposure to low concentrations of nickel results in not only gene repression but also gene activation (124–127, 134). Furthermore, pre-treatment of cells with trichostatin A, a histone deacetylase inhibitor, significantly reduces nickel-induced cell transformation, suggesting that histone deacetylation is an important step in nickel-mediated carcinogenesis.

In addition to nickel-induced histone modifications, nickel has been shown to preferentially bind to heterochromatin by replacing the Mg^{2+} ions that are naturally found in high concentrations and thus play an important role in the condensation of heterochromatin (126). It is surmised that nickel may lead to greater chromatin condensation, perhaps even promoting heterochromatin formation in critical parts of the DNA, such as those regions containing tumor suppressor genes (137). The nickel accumulated in the heterochromatin regions may also induce oxidative damage in the DNA via a Fenton-like reaction, further disrupting gene expression and perhaps contributing to carcinogenesis (138).

Nickel has also been shown to increase DNA methylation, which is also associated with gene silencing (126, 139). More specifically, nickel exposure has been shown to alter the methylation patterns of the p53 and p16 promoters (139). Silencing such key tumor suppressor genes likely serves as an important mechanism of nickel-induced carcinogenesis (139, 140). While many of these studies were carried out in other cancer types (i.e. lung), similar mechanisms of nickel-induced carcinogenesis are expected to be involved in breast cancer cells (140, 141). Furthermore, some of the mechanisms of nickel-induced chromatin modification described above (such as histone deacetylation) may actually involve the estrogen receptor and thus lend further support to the hypothesis that nickel does indeed act as a metalloestrogen in breast cancer development. Multiple histone acetylases (HATs) and deacetylases (HDACs) are known to interact with the estrogen receptor (CBP, p300, p/CAF, p160 SRC family of coactivators, and HDAC 1 and 6) (142–144), so it is possible that the interaction between nickel and ER α mediates the recruitment of chromatin-modifying proteins to the promoters of nickel-induced/repressed genes.

Unlike nickel, few studies have linked cadmium to epigenetic modifications, and such modifications are not likely a major mechanism of cadmium-induced carcinogenesis. Only a few studies have suggested that exposure to cadmium alters the global DNA methylation patterns (89, 145, 146). More specifically, acute cadmium exposure has been shown to induce hypomethylation, while chronic cadmium exposure results in hypermethylation (129). Changes in the methylation patterns of cells exposed to cadmium were mediated by altering the activity of DNA methyltransferase (145). In support of these observations, Benbrahim-Tallaa et al. showed that a 10-week exposure to 10 μ M cadmium was able to increase global DNA methylation and induce malignant transformation in prostate epithelial cells. Contrary to these observations, Huang et al. reported that chronic exposure of cadmium leads to global hypomethylation and cell proliferation in K562 leukemia cells (146). While these differences may be attributed to different model systems, it does suggest that further studies on cadmium's affect on DNA methylation are necessary, especially in breast cancer cells. Furthermore, there appear to be no studies linking cadmium exposure to histone modifications, underscoring the need for further studies in this area of metal-induced carcinogenesis.

Aneuploidy

Aneuploidy—the phenomenon in which a cell possesses an abnormal number of chromosomes—is found in most cancerous lesions and is believed to play a significant role in cancer progression (147–150). In fact, some researchers have found evidence supporting the contention that aneuploidy is involved in the early stages— if not the actual initiation— of cancer cell development (151–153). Several studies have demonstrated the aneugenic potential of heavy metals (154–158). In one particular study carried out by Seoane and Dulout, cadmium and nickel salts such as CdCl₂ (1–4μM), CdSO₄ (0.033–0.134μM), NiCl₂ (13–54μM), and NiSO₄ (200–800μM) all induced aneuploidy in human fibroblast cells (159). The precise molecular mechanism behind this aneugenic effect, however, has not been determined.

Interestingly, estrogen itself has been shown to induce aneuploidy. In a study published in 2002 by Li et al., estrogen-induced mammary gland tumors (MGTs) in female ACT rats were found to have a significantly higher degree of aneuploidy than those MGTs induced by chemical carcinogens (153). Additionally, this same study revealed that these estrogen-induced MGTs greatly resembled invasive human ductal carcinoma in situ (DCIS) breast cancer in terms of degree of aneuploidy and increased amplification of the c-myc gene. An earlier *in vitro* study by Epe and colleagues demonstrated that peroxidative estrogen metabolites could directly interact with the tubulin protein and thus interfere with the assembly of microtubules, potentially affecting proper mitotic spindle formation (160). More relevant to this review, however, is a recent study by Hontz et al. which showed that estrogen (more specifically, estradiol-17β) increased expression of the mitotic kinases Aurora A and B via an ERα-dependent pathway (161). High levels of Aurora A and B lead to aneuploidy and consequently are believed to contribute to cancer progression (162–164). Specifically, Aurora A overexpression and aneuploidy have been found in primary invasive ductal breast cancer (161). Although more studies need to be done to determine exactly how cadmium and nickel lead to aneuploidy, the discovery that estrogen can induce aneuploidy through the estrogen receptor suggests that the aneugenic effect of cadmium and nickel could at least partially be due to their ability to bind to the estrogen receptor and act as metalloestrogens.

Conclusion

Breast cancer— like all cancers— is a complex disease, from its initiation to its progression. No two cases are alike, and the factors that contribute to its development can vary significantly between patients. Certain risk factors, such as increased exposure to estrogen, are well-established as integral to the development of most types of breast cancer. However, the rising incidence of breast cancer cases (currently 25% of all female cancers in the U.S. are breast cancers) has driven scientists to search for other factors that may explain this alarming increase.

Environmental contaminants such as heavy metals have emerged as a possible risk factor due to their increased usage in certain industrial processes, as mentioned previously. There is actually little debate as to whether or not heavy metals can cause cancer. Certain cadmium, nickel, and even arsenic compounds have been deemed carcinogenic by the IARC since the late 1970's and 1980's (68, 82, 165, 166). Of these heavy metals, however, we found that it was cadmium which has the strongest correlation with breast cancer development. Most of the epidemiological, *in vivo*, and *in vitro* studies we analyzed solidly supported the link between increased cadmium exposure and breast cancer development. For nickel, the evidence, though supportive, was much smaller. Epidemiological studies linking nickel to breast cancer were positive but few in number, and there were virtually no animal

studies testing the effect of increased nickel exposure to breast cancer development. There were some very compelling *in vitro* studies involving nickel, particularly those carried out by Martin and colleagues, which showed that nickel can bind to the estrogen receptor in breast cancer cells and induce the expression of genes associated with cell growth (13). However, such studies are also small in number (Table 2). Even sketchier is the evidence linking arsenic to breast cancer. Although an Australian case study conducted by Hinwood et al. reported that 40% of the cancers caused by arsenic-contaminated drinking water were breast cancers (167, 168), *in vivo* and *in vitro* studies indicate that arsenic disrupts ER function and actually suppresses estrogen signaling pathway (169, 170)— findings that, in our view, effectively argue against arsenic as a potential metalloestrogen.

The main goal of this review was to determine if there is enough evidence to support the hypothesis that the heavy metals, cadmium and nickel at chronic, low-level concentrations can induce breast cancer by mimicking estrogen in the estrogen receptor signaling pathway, thus acting as metalloestrogens. We found that most studies analyzed the effects of *acute* heavy metal exposure on breast cancer development and progression (Tables 1 and 2). There is a general consensus that exposure to cadmium or nickel at levels greatly exceeding the concentration limits dictated by the World Health Organization (WHO) is exceedingly dangerous due to the promiscuous protein-binding patterns of heavy metals when they are at high enough concentrations. Our contention, however, is that chronic exposure to cadmium and nickel at concentrations well below WHO limits is still dangerous due to bioaccumulation and the peculiarly high affinity for specific proteins like the estrogen receptors.

Evidence obtained from *in vivo* and *in vitro* studies strongly suggests that cadmium can behave as a metalloestrogen. Cadmium has been shown to bind to ER α (with a K_D nearly equivalent to that of estradiol), activate it, and induce expression of certain ER target genes. In addition, cadmium induces other estrogen-like effects which include: increased uterine weight; changes in uterine lining; increased epithelial cell density in mammary glands; increased cell proliferation; and increased aneuploidy. As strong as these data are, however, we feel that more experiments testing *chronic*, low-level cadmium exposure are needed to help confirm that cadmium-induced breast carcinogenesis is due, at least in part, to cadmium's estrogenic potential. Currently, there are only a few studies on the effects of prolonged exposure to low levels of cadmium on breast cancer development and progression (Table 1). An additional weakness in the literature is the fact that the cadmium-binding site on ER α has not truly been determined yet. Additional structural studies— perhaps involving NMR or X-ray crystallography combined with protein modeling and computational chemistry — could potentially aid in the future development of therapeutics that might counteract cadmium's effects.

Though we did not find a single study arguing against nickel serving as a metalloestrogen in breast cancer development, the number of studies providing support for this hypothesis was scant (Table 2). As with cadmium, nickel has been shown to bind to ER α , promote cell proliferation, and induce aneuploidy. However, many more studies at the animal, cellular, and molecular levels need to be carried out to effectively determine if and how low-dose, chronic nickel exposure can lead to breast cancer. Since much less is known about the nickel-binding site than the cadmium-binding site on ER α , more structural studies are required as well to confirm nickel's role as a metalloestrogen.

Finally, it is also worth noting that both cadmium and nickel were found in various human samples including urine, hair, blood and breast tumor tissues (Table 3) (79, 84, 171–173). While healthy individuals had detectable levels of heavy metals, significantly higher levels

of cadmium and nickel were found in patients with breast cancer (Table 3). The studies presented in Table 3 further argue the need for additional studies on chronic exposures to these metals at low concentrations. Also lacking in the literature are any studies evaluating whether heavy metal exposure during a critical developmental window— e.g. prenatal, puberty, or postmenopausal— would lead to an increased risk of breast cancer. Many reports do indicate a positive correlation between certain childhood cancers and prenatal exposure to endocrine disruptors such as diethylstilbestrol (DES) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (174, 175). Animal studies have shown that rats exposed to tamoxifen during late gestation produce offspring with a greater sensitivity to DMBA-induced breast cancer (176). Epidemiological analyses have reported a positive correlation between prenatal exposure to elevated levels of natural estrogens and breast cancer (177); and strong evidence has implicated hormone replacement therapy (HRT) in increasing the breast cancer risk in peri- and postmenopausal women (178). Although a recently published study by Julin et al. indicated a correlation between dietary cadmium exposure and breast cancer risk in postmenopausal women, no other age group was analyzed (8). Thus, until more comprehensive analyses are carried out, it is not clear that exposure to cadmium or nickel during certain critical developmental periods increases breast cancer risk. However, despite the fact that more studies need to be done, there is sufficient evidence to warrant great concern over the increasing emission of heavy metals like cadmium and nickel into the environment. Acute exposures aside, the data suggest that even minimal levels of cadmium and nickel are potentially hazardous and could negatively impact the health of thousands of people.

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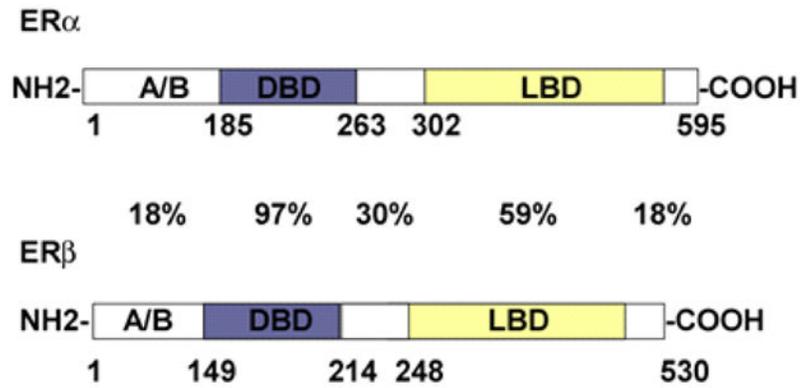


Figure 1. ER α and ER β are homologous in their functional domains

The two ER isoforms display a high degree of homology in the DNA binding domain (DBD) and ligand binding domain (LBD), but are highly variable in the NH₂-terminal transactivation AF-1 domain, also referred to as the A/B hypervariable domain. Percentages indicate percent identity between the two receptors. Figure is adapted from the review by Gustafsson (179, 180).

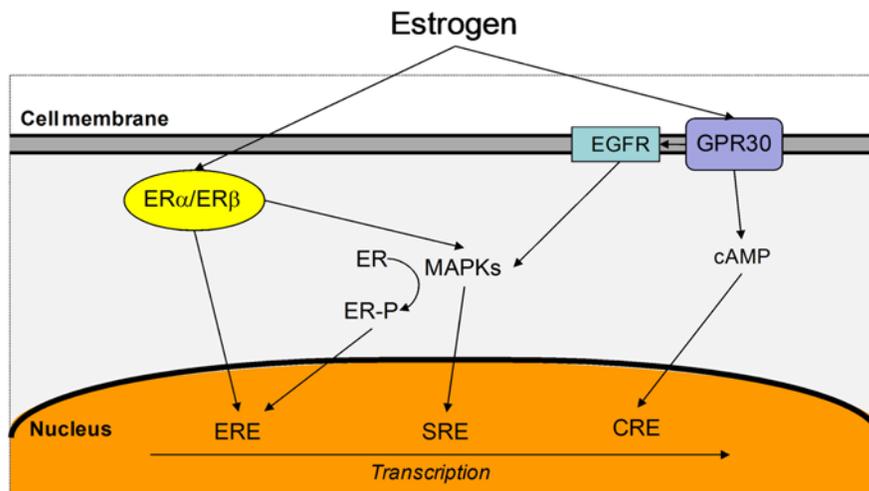


Figure 2. Cross-talking between ERα/ERβ and GPR30 signaling pathways
 Estrogen can activate both long-term genomic (left) and rapid nongenomic (right) pathways leading to the transcription of downstream genes necessary for cell growth and development. ERα/ERβ activation can lead to both direct transcription activation in the nucleus or via rapid signaling of mitogen-activated protein kinases (MAPKs). In contrast, GPR30 cannot directly activate transcription processes but can rapidly activate nongenomic signaling including the activation of MAPKs resulting in the expression of transcription factors such as c-fos. cAMP is also produced via GPR30 activation. ERα/ERβ and GPR30 signaling can induce both positive and negative effects on one another, depending upon the signaling components in the cell at a given time. Several other regulatory pathways are possible but not shown. Figure adapted from Prossnitz 2008 (181). ERE, estrogen response element; SRE, serum response element; CRE, cAMP response element.

Table 1

Summary of experiments examining the effects of acute and chronic cadmium exposure on gene expression in breast cancer models

Type of Exposure	Experimental System (ER status of cells)	Cd Concentration & Exposure Time	Response	Mechanism: Is it mediated through ER?	Gene Expression	References
Acute Cells	MCF-7 cells (ER α +/ER β +/GPR30+)	10 ⁻¹² M CdCl ₂ for 1 hr	Blocked estradiol binding in a noncompetitive manner	Yes	K _D of cadmium is 2.96 \times 10 ⁻¹⁰ M	Stoica (14)
		10 ⁻⁶ M CdCl ₂ for 6 days	Increased ER-regulated gene expression and cell growth	Yes	\uparrow PR and pS2	Martin (13)
		10 ⁻⁶ M CdCl ₂ for 24 and 48 hr	Increased ER-regulated gene expression and cell growth of MCF-7 and other ER α + cell lines: T47D and ZR-75-1	Yes	\uparrow cyclin D, CTD, c-myc, \uparrow p21	Siewit (96)
		10 ⁻⁶ M for 24 hr	Increased ER-regulated gene expression and cell growth	Yes	\uparrow PR and pS2	Garcia-Morales (93)
		20 or 50 μ M CdCl ₂ for 52.5 hr	Cadmium activated ER α mediated transcription; Hsp22 exhibited interaction with Hsp27	Yes	\uparrow Hsp22	Sun (97)
		10 μ M CdCl ₂ for 24 hr	Decreased levels of ER α and increased cell proliferation	Yes	\uparrow ERK1/2, Akt, PDGFR α , c-jun, c-fos	Brama (55)
		5 μ M for 1 to 48 hr	Induced transcription of Cholinephosphotransferase (CPT)	No	\uparrow CPT	Sinha Roy (194)
	T47D cells (ER α +/ER β +)	10 ⁻¹⁰ to 10 ⁻⁶ M	Activated mER signaling leading to ERK1/2 activation; activated ERE-dependent transcription	Yes	\uparrow ERK1/2 MAPK response	Zang (193)
	SKBR-3 cells (ER α -/ER β -/GPR30+)	5.0 \times 10 ⁻⁷ M CdCl ₂ for 24 hr	Rapidly increased mER gene expression; Increased cell growth	Yes GPR-30	\uparrow cAMP, Raf-1, MEK-1, Erk-1/2	Yu (98)
	COS-1 cells transfected with ER α	10 ⁻¹² , 10 ⁻⁶ M CdCl ₂ for 24 hr	Cadmium activates ER α mediated transcription (\uparrow CAT ^f)	Yes	\uparrow CAT	Stoica (14)
	MDA-MB231 cells (ER α -/GPR30-)	5.0 \times 10 ⁻⁶ M CdCl ₂ for 96hr	Decreased transcriptional activity and intracellular signaling of AEG-1 ² and NF-kB	No	\downarrow AEG-1, \downarrow c-fos, c-jun, p63	Luparello (182)
		5.0 \times 10 ⁻⁶ M CdCl ₂	Stimulates p38 transcriptional activity	No	\uparrow p38	Casano (183)
		5 or 50 μ M CdCl ₂ for 24 or 96 hr	Respiration activity increased after 96 hr exposure; increased reactive oxygen species; expression of hsp60, hsp70, Cytochrome oxidase II and IV	No	\uparrow hsc/hsp70 \downarrow Cytochrome oxidase II & IV	Cannino (184)

Type of Exposure	Experimental System (ER status of cells)	Cd Concentration & Exposure Time	Response	Mechanism: Is it mediated through ER?	Gene Expression	References
	Non-tumoral HB2 cells	5 µM for 96 hr	were dependent on duration of Cd exposure Cells exhibited protective role after exposure	No	↑ hsp70 and metallothioneins	Sirchia (185)
		50 µM for 24 hr	Induced cell death via non-apoptotic pathways	Not identified		Sirchia (186)
Acute Animals	Ovariectomized Rat	Single injection 5µg CdCl ₂ kg ⁻¹ b.w.	Increased uterine weight, thickness of uterine epithelium, and density of mammary gland	Not identified	↑ PgR & C2 in mammary gland	Höfer (90)
	Ovariectomized Rat	Single injection 0.05–2000 µg CdCl ₂ kg ⁻¹ b.w.	Increased uterine weight and thickness of uterine epithelium; increased pre-malignant characteristics	Not Identified		Johnson (85)
	Transgenic ERE-Luc reporter mouse	5 and 50µg CdCl ₂ kg ⁻¹ b.w. for 3 d	Increased uterine epithelium height	Not identified	↑ Erk1/2 in liver	Ali (91)
Chronic Cells	MCF-10A (ERα-)	2.5×10 ⁻⁶ M CdCl ₂ for 40 wk	Increased invasion and colony formation; decreased contact inhibition; displayed basal-like phenotype	Not identified	↑c-myc and k-ras	Benbrahim-Tallaa (89)
Chronic Animal	Mouse	2–3 mg CdCl ₂ kg ⁻¹ b.w. for 2 or 7 wk	Increased labuloalveolar development of mammary gland	Not identified		Alonso-Gonzales (92)

¹ Gray: Studies done with chronic cadmium exposure; White: Studies done with acute exposure

² CAT: chloramphenicol acetyltransferase

³ AEG-1: astrocyte-elevated gene 1

Table 2

Summary of experiments examining the effects of acute and chronic nickel exposure on gene expression in various cancer models

Type of Exposure	Experimental System	Ni Concentration and Exposure Time	Response	Mechanism: Is it mediated through ER?	Gene Expression	References
Acute Cell	Breast cancer cells (MCF-7)	1 μM NiCl ₂ for 6 days	Increased breast cancer cell proliferation	Yes	↑ PR and pS2	Martin (13)
		1mM NiCl ₂ for 6 and 24 hr	Increased HIF- α and p53 protein levels, accompanied by MDM-2 protein induction	No	↑ HIF- α and p53	Salmikow (106)
	Lung cancer cells (A549)	0.25 μM to 1 mM NiCl ₂	Inhibited cell growth through IKK α -dependent manner; increased cells in G1/G0 phase	No	↓ cyclin D1 ↑ p21	Ouyang (112)
		0.5 and 0.75 mM NiCl ₂ at 24 hr; 1mM NiCl ₂ for 12–72 hr	↑ H3K9 mono- and dimethylation, critical for long-term gene silencing	Epigenetic Modification: No	↓ H3K9 methyltransferase	Chen (127)
		1mM NiCl ₂ for 24 hr	↑ H3K4 trimethylation in promoter and coding regions for CA9 and NDRG1	Epigenetic Modification: No	↑ CA9 and NDRG1	Tchou-Wong (108)
		0.25 to 1.0 mM NiCl ₂ or Ni ₃ S ₂ for 24 hr	Induced histone modification; alters epigenetic homeostasis in cells	Epigenetic Modification: No		Ke (124, 125)
		1mM NiCl ₂ for 6 and 24 hr	Increased HIF- α and p53 protein levels	No	↑ HIF- α & p53	Salmikow (106)
	Lung epithelial cells (Beas-2B)	1 to 4 $\mu\text{g}/\text{cm}^2$ Ni ₃ S ₂ for 48 hr	Induced M phase arrest; inhibited cell growth	No	↑ cyclin B1, cyclin D, cyclin E	Ding (111)
		0.25 to 2 $\mu\text{g}/\text{cm}^2$ NiCl ₂ for 48 hr	Increased cell apoptosis, induced growth arrest through Akt/ASK1/p38 signaling pathway	No	↓ Bcl-2, Bcl-xL ↑ p38, MAPK	Pan (187)
		0.5 to 1.0 mM NiCl ₂	Increased COX-2 expression via NFAT and NF- κB	No	↑ COX-2	Cai (188)
Mouse fibroblast cells (L-929)	200 μM Ni ²⁺ for 24, 48 and 72 hr	Identified 20 up-regulated genes and 19 down-regulated genes	No		Liu (113)	
Mouse fibroblasts (HIF-1 α +)	1 mM NiCl ₂ for 20 hr	Activated hypoxia-inducible transcription factor-1 (HIF-1) and	No	↑ Nip3 ↑ p21, HSP70, p53, GADD45	Salmikow (107)	

Type of Exposure	Experimental System	Ni Concentration and Exposure Time	Response	Mechanism: Is it mediated through ER?	Gene Expression	References
			induced tumor marker Cap43 gene.			
	NER proficient human fibroblast (GM00637)	50 to 300 μ M NiCl ₂ for 24 hr	Enhanced BPDE-induced mutation frequency by inhibiting nucleotide excision repair (NER) pathway	No		Hu (189)
	Human osteoblast cells (HOS TE-85)	2–10 mg/mL NiS, for 24 hr	Decreased retinoblastoma (pRB) phosphorylation; acquired anchorage-independent growth	No	\downarrow pRb phosphorylation	Lin (190)
	Human hepatoma cells (HepG2)	150 μ M NiCl ₂ hexahydrate for 48 hr	Up-regulation of genes associated with cell cycle progression-similar to other carcinogenic chemicals	No	Induced gene expression	Kawata (109)
	Human keratinocytes	0.5mM to 2.0 mM NiCl ₂ for up to 48 hr	Decreased Δ Np63 activity; activation of NF- κ B	No	\downarrow Δ Np63, IRF3, IRF7, and \uparrow p21	Zhang (191)
	Human lymphocytes	15uM NiCH and NiO for 2hr	Exhibited higher DNA single-strand breaks	No		M'Bemba-Meka (192)
Acute Animal	Rats	0.9mg Ni/100g Ni(CO) ₄ i.v. at 2 and 4 wk intervals	Resulted in undifferentiated sarcomas, fibrosarcomas, carcinomas, hemangioendothelioma, leukemia, and lymphoma	Not identified		Lau (101)
Chronic Cells	Transformed mouse fibroblast cells	Cells resistant up to 200 μ M Ni, but not treated with Ni.	\uparrow GSH, GSTA4, GSTT ² suggest enforcement of antioxidant defenses	No	\uparrow β -catenin, c-myc, cyclin D1	Kowara (110)
Chronic Animal	Rat and Mice	0.15 to 1 mg/m ³ Ni ₃ S ₂ ; 1.25 to 2.5 mg/m ³ NiO for 2 yr	Increased alveolar/ bronchiolar and adrenal medulla neoplasms in male and female rats.	No		Dunnick (102)

¹ Gray: Studies done in breast cancer models; White: Studies done in other cancer models

² GSH: glutathione synthetase; GSTA4: glutathione-S-transferase A4; GSTT: glutathione-S-transferase theta

Table 3

Summary of experiments comparing the bioaccumulation of cadmium and nickel in biological samples from breast cancer patients and healthy individuals

Biological Sample	Metal	Sample Size	Experimental Findings	Reference
Breast Tissue	Cd	19 benign tissue samples and 21 malignant tissue samples	Cadmium (Cd) levels were higher in malignant than benign tissues ($p = 0.009$).	Strumylaite (77)
	Cd	32 healthy and 43 breast cancer subjects	A positive correlation was observed between cadmium concentration and smoking rate ($p < 0.05$).	Antila (6)
	Cd, Ni, Al	16 non-cancerous tissue samples, and 67 breast cancer tissue samples	Cd levels were higher in cancerous versus non-cancerous tissue ($p < 0.05$). No significant difference was observed in Ni levels ($p = 0.057$).	Romanowicz-Makowska (171)
	Fe, Ni, Cr, Cu, Pb, Zn, Hg, Ag, Sn, Au, Pd	20 breast cancer biopses and	Ni, Cr ($p < 0.00005$), Zn ($p < 0.00001$), Fe ($p < 0.0001$), and Cd, Hg ($p < 0.005$) levels were higher in breast cancer biopsies.	Ionescu (78)
Breast tissue, urine and blood	Ni, Ca, Cu, Zn, Fe, Cr, Mn, Se, Br, Rb, Sr, Hg, As, Pb, V, Se, Mo	Normal and neoplastic breast tissue from 25 patients	Levels of Ca, V, Cu, Zn, Se, Rb ($P < 0.001$) and Ni ($p < 0.05$) were higher in breast tumor tissue	Rizk (84)
	Cd	51 benign tumor patients and 57 breast cancer patients	Cd levels were significantly higher in malignant vs. benign tumor tissues $p < 0.01$. Additionally, higher levels of Cd were found in the urine samples of patients with breast cancer $p < 0.001$. A positive correlation exists between blood cadmium levels and tumorigenicity.	Strumylaite (173)
Urine	Cd	254 healthy subjects and 246 breast cancer subjects	Breast cancer risk increases with cadmium concentration ($p = 0.01$)	McElroy (7)
Hair Sample	36 elements including Cd and Ni	52 stage III breast cancer subjects and 52 healthy subjects	Higher levels of Ag, As, Au, Bo, Ba, Be, Ca, Cd, Ce, Co, Cs, Ga, Mn, Ni, Pb, Sb, Sc, Se and Zn were found in breast cancer patients in comparison to healthy subjects ($p < 0.05$).	Benderli Cihan (172)