Increased Expression of Histone Proteins during Estrogen-Mediated Cell Proliferation

Zheying Zhu, Robert J. Edwards, and Alan R. Boobis

Department of Experimental Medicine and Toxicology, Imperial College London, London, United Kingdom

BACKGROUND: There is concern about the potential risk posed by compounds with estrogen-like activity present in the environment. As previous studies have shown that combined exposure to such compounds results in dose additivity, it should be possible to assess estrogen exposure with suitable biomarkers of effect.

OBJECTIVES: Our goal was to identify candidate protein biomarkers of effect for estrogenic compounds.

METHODS: In the search for biomarkers, we assessed the effect of several estrogenic compounds on the expression profile of proteins in breast-derived cell lines varying in their estrogen receptor (ER) phenotype using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. We identified responsive proteins, after separating them by SDS-polyacrylamide gel electrophoresis, and analyzing the trypsin-digested proteins by tandem mass spectrometry.

RESULTS: The estrogenic compounds 17β-estradiol, genistein, bisphenol A, and endosulfan produced similar protein profile changes in MCF-7 cells (phenotype: ERα/ERβ2), but had no effect on MDA-MB-231 (ERα/ERβ²), MCF-10F (ERα/ERβ²), or MCF-10A (ERα/ERβ²) cells. The most responsive proteins in MCF-7 cells were identified as histones H2A, H2B, H3, and H4. Histone levels were not increased in cell lines that showed no proliferative response to estrogens despite their rapid intrinsic growth rate in culture.

CONCLUSION: Our results indicate that ER-mediated cell proliferation results in up-regulation of core histone proteins.

KEY WORDS: biomarkers, breast cancer cell lines, estrogenic compounds, histones, protein profiles, SELDI-TOF MS. Environ Health Perspect 117:928–934 (2009). doi:10.1289/ehp.0800109 available via http://dx.doi.org/ [Online 7 February 2009]

A rising incidence of endocrine-related diseases in recent decades (Damstra et al. 2002) has led to the suggestion that exposure to endocrine-modulating chemicals (so called endocrine disruptors) is the cause (Colborn et al. 1993; Waring and Harris 2005). There are many such chemicals in the environment that have estrogenic activity, and they are derived from a number of different potential sources. Phytoestrogens, such as genistein (GEN, occur naturally in food as components of dietary plants such as legumes, lentils, chickpeas, soybean, cereals, fruits, and vegetables (Jordan et al. 1985). Industrial contaminants, such as bisphenol A (BPA) and polychlorinated biphenyls, may be present in the atmosphere or as by-products of industrially produced materials such as plastics used as containers for drinks or food (Sheehan 2000). Several organochlorine pesticides, such as endosulfan (EDS), also have estrogenic activity; exposure may occur from household use or from residues in treated food (Safe and Zacharewski 1997). Although EDS is no longer approved for use in many countries, exposure may still be possible through environmental contamination.

To determine the risk to human health, attempts are being made to determine levels of exposure, by measuring either specific compounds or classes of compounds (Dekant and Volkel 2008; Wolff et al. 2007) or by the use of bioassays (Fernandez et al. 2007; Rasmussen et al. 2003) to measure total estrogenic activity present in blood, urine, or adipose tissue (Soto et al. 1997). Although this is useful, it is also informative to determine the biological consequences of exposure to such levels. Consequently, there is much effort to identify suitable biomarkers of effect that can be used for this purpose. To this end, a number of in vitro assays have been designed that measure the proliferation of MCF-7 cells (E-screen) (Soto et al. 1995) or binding of estrogenic compounds to the ER through competitive binding assays, or reporter gene assays such as the yeast estrogen screening assay (Gutendorf and Westendorf 2001). Several in vivo mammalian assays have also been designed. These are based mostly on measurements of female rodent reproductive tissue development. Although it can be argued that some of these tests have physiologic relevance, they appear to perform relatively poorly in terms of quantitation and sensitivity (Ashby 2003).

These approaches may be limited by the complexity of the effects of estrogenic compounds, which appear to have multiple mechanisms of action (Jefferson et al. 2002) that vary for different compounds (Frigo et al. 2002; Naicif et al. 2002). For example, estrogens may act on either or both of the estrogen receptors (ERα and ERβ) as agonists or antagonists and in addition can elicit nonestrogenic effects (Fertuck et al. 2001). Certainly, gene expression studies of both uterine tissue and MCF-7 cells indicate that there is a diverse response to estrogen exposure at the mRNA level (Buterin et al. 2006; Coset et al. 2003; Dip et al. 2008; Frasor et al. 2003). Similarly directed studies have led to a variety of suggested biomarkers of effect by measuring the expression of such genes in cell-based assays (Choi and Jeung 2003). Although changes at the mRNA level are indicative of functional changes, it is more pertinent to study changes in the expression of proteins. Here, to this end, we have employed the use of surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) to determine proteomic changes. This technique has been applied to identify potential biomarkers for prognosis, diagnosis, and treatment of human diseases. It has the advantage of being rapid, reproducible, and quantifiable, allows direct sample comparison, and can analyze a wide range of proteins (Petricoin and Liotta 2004). Its use has been explored in classification and evaluation of treatment of breast cancer in patients (Carter et al. 2002; Laronga et al. 2003; Wulfkule et al. 2001).

In the present study, four compounds with estrogenic activity were assessed: 17β-estradiol (E2) (the natural ligand for ER); GEN (a phytoestrogen); BPA (an industrial contaminant); and EDS (an organochlorine pesticide). Their effect on the protein profiles in four related human breast cell lines that vary in their ER phenotypes was determined. These were human breast cancer cell lines MCF-7 (ERα/ERβ²) and MDA-MB-231 (ERα/ERβ²) and human breast epithelial cell lines MCF-10F (ERα/ERβ²) and MCF-10A (ERα/ERβ²), which are well documented for their differential expression of these receptors (Fuqua et al. 1999; Girdler et al. 2001; Hu et al. 1998; Jiang and Jordan 1992; Tong et al. 2002). The objectives of this work were to determine how the proteome of the cell
responds to estrogenic compounds, whether different estrogenic chemicals act similarly and, if so, to identify potential common biomarkers of effect.

Materials and Methods

Chemicals. We obtained E$_2$ (99% purity), GEN (≥ 98% purity), BPA (> 99% purity), and EDS (mixture of forms I and II; 99% purity) from Sigma-Aldrich Company Ltd (Gillingham, UK). Stock solutions of these four estrogenic compounds were prepared as previously described (Zhu et al. 2008b). Unless stated otherwise, we purchased all other analytical grade reagents used in this study from Sigma-Aldrich.

Cell culture. We obtained MCF-7 and MDA-MB-231 human breast cancer cell lines and MCF-10F and MCF-10A human breast epithelial cell lines from American Type Culture Collection (ATCC; East Greenwich, RI, USA). We cultured MCF-7 and MDA-MB-231 cell lines in RPMI-1640 medium (phenol red-free) supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, and 100 µg/mL streptomycin. MCF-10F and MCF-10A cell lines were cultured in DME/F12 medium, as appropriate, before treatment with the estrogenic compounds. Each treatment comprised six replicates. We performed curve fitting of each set of concentration–response data and calculations of the lowest effective concentration that produced maximal response (EC$_{50}$), the effective concentration that produced 50% of maximal response (EC$_{50}$), and the highest effective concentration that produced no measurable response (EC$_{min}$) values of each of the estrogenic compounds as described previously (Zhu et al. 2008b). In some instances, we assessed effects on cell viability by trypan blue exclusion, as described previously (Thatcher et al. 2000).

SELDI-TOF MS analysis of cells. Cells from each of four cell lines were seeded at a density of 1 × 10$^3$ cells/mL (2 mL) in 6-well plates (four replicate wells for each treatment) before being exposed to estrogenic compounds. We prepared cell lysates and determined total protein content using the bicinchoninic acid method, as previously described (Zhu et al. 2008b). Analysis by SELDI-TOF MS was performed using CM10 ProteinChip arrays (Bio-Rad) at pH 4.0 with sinapinic acid (Fluka, Steinheim, Germany) as an energy absorbance matrix in a 96-sample bioprocessor format. We analyzed ProteinChip arrays using a Protein Biology System IIc Reader (Bio-Rad), and spectra using Biomarker Wizard software (Bio-Rad). Details of these procedures have been described previously (Zhu et al. 2008b).

Liquid chromatography–MS/MS. We denatured, reduced, and alkylated the cell lysates and separated them by 1D-SDS PAGE (NuPAGE; Invitrogen, Paisley, UK), we stained the gel with Coomassie blue; excised regions of interest; and decolorized, washed, dehydrated, and digested the proteins with trypsin (sequencing grade, Promega, Southampton, UK) at 37°C for 18 hr, as described previously (Zhu et al. 2008a). Nanoflow LC-MS/MS was performed using an LTQ MS (Thermo-Fisher Scientific, Pittsburgh, PA, USA) to analyze parent ions and data-dependent MS/MS spectra simultaneously. We identified the proteins using Bioworks Browser software,
2 μM (Table 1). The growth rate of MCF-7 cells also increased 4-fold by treatment with GEN and BPA and 3-fold by EDS relative to control (Table 1). However, GEN and BPA were more than four orders of magnitude less potent and EDS was six orders of magnitude less potent than E2. Both GEN and BPA had EC_{50} values of 1 μM (Figure 1), although the maximum responses were similar to that found for E2. A full concentration–effect curve for EDS was not possible, as the compound was cytotoxic above 10 μM; this compound was similarly cytotoxic to MCF-10F cells but not the other two cell lines. GEN, BPA, and EDS did not cause MDA-MB-231, MCF-10F, or MCF-10A to increase their growth rate (Table 1).

**SELDI-TOF MS protein profiles.** Protein profiling by SELDI-TOF MS was performed on MCF-7 cells treated separately with 20 pM E2, 1 μM GEN, 1 μM BPA, and 10 μM EDS. We used the concentrations that produced a maximal proliferative response in the cells. Each of the test compounds produced a change in the protein profile of the cells, and the changes were similar for all the test compounds, as described previously (Zhu et al. 2008b). In all, from approximately 100 peaks detected, we found 12 protein ions with m/z values of 4,128, 5,610, 6,160, 6,845, 7,010, 7,620, 11,260, 11,426, 11,680, 13,680, 14,020, and 15,260 that increased in intensity with treatment of each of the four estrogenic compounds (Figure 2). Other proteins detected by SELDI-TOF MS did not vary significantly between the untreated and treated groups. Among the 12 responsive ions, 8 appeared to be pairs of doubly charged and singly charged ions from the same proteins, that is, m/z 5,610 and 11,260; m/z 6,845 and 13,680; m/z 7,010 and 14,020; and m/z 7,620 and 15,260.

Subsequently, MCF-7 cells were treated with a series of different concentrations of E2, GEN, BPA, and EDS to examine levels of these protein ions. Changes in the levels of these eight ions were concentration dependent with each of the four estrogenic compounds examined (see Supplemental Material, Figure 1 [available online at http://www.ehponline.org/members/2009/0800109/suppl.pdf]). EC_{50} values determined from these data showed that the response to the compounds was similar to that assessed by measurement of cell proliferation (Table 2).

SELDI-TOF MS protein profiles were also obtained using the other three cell lines after treatment with 20 pM E2, 1 μM BPA, 1 μM GEN, 10 μM EDS, and vehicle. Although spectra containing numerous peaks were obtained with each of the cell lines, no changes in the protein profiles, or in any individual peak, were found after treatment with E2 (Figures 2C–2H) or any of the other compounds tested. Interestingly, although the spectra obtained for the different cell lines varied somewhat, ions with m/z 11,260, 13,680, 14,020, and 15,260 identified as potential biomarkers in treated MCF-7 cells were also present in these cell lines (Figure 2).

**Identification of responsive protein ions.** Whole-cell preparations of MCF-7 cells treated with either 20 pM E2 or vehicle were subjected to 1D SDS-PAGE. The stained gel shows that the overall protein compositions of the two preparations were similar except for an increase in the intensity of bands of approximately 12 and 17 kDa (Figure 3). The lane containing the E2-treated MCF-7 cell proteins was sliced into five sections over the region equivalent to 5–20 kDa corresponding to the likely masses of the proteins detected by SELDI-TOF MS and including bands with increased intensity. Each slice was subjected to trypsin digestion to hydrolyze the proteins into peptides, and then these were analyzed by LC-MS/MS.

Proteins identified on the basis of at least four detected peptides are listed in Figure 3 [for further details see Supplemental Material, Table 1 (available online at http://www.ehponline.org/members/2009/0800109/suppl.pdf)]. All of the core histone proteins

![Figure 2](http://www.ehponline.org/members/2009/0800109/suppl.pdf)
were represented (i.e., histones H2A, H2B, H3, and H4). Histone H4 was the only protein identified in slice 3 of the gel, equivalent to the 12 kDa band. Histone H2A occurred in slice 4 along with three other proteins. Both histones H2B and H3, as well as seven other proteins, were detected in slice 5. Protein masses determined by SELDI-TOF MS were accurate to within 0.3%. The masses of each of the core histones were within this error of measurement. None of the other proteins identified by LC-MS/MS had masses within 0.3% of the major peaks in the SELDI-TOF MS spectra (Figure 3; see Supplemental Material, Table 1 [available online at http://www.ehponline.org/members/2009/0800109/suppl.pdf]). Additional analyses were performed with the same samples on cation-exchange chips at pH 7 and 9 (data not shown). The intensities of the protein ions corresponding to the ions of interest were similar at pH 7 to those obtained at pH 4. They were also clearly detectable at pH 9, albeit with slightly reduced intensity, suggesting that the ions were from highly basic proteins. Histones were the most basic proteins detected in the SDS-PAGE gel [see Supplemental Material, Table 1 (available online at http://www.ehponline.org/members/2009/0800109/suppl.pdf)], further supporting their identification.

To investigate the identity of these proteins further, purified preparations of the core histones were obtained and analyzed by SELDI-TOF MS under the same conditions as those used to analyze the MCF-7 cell preparations (Figure 3). The histones used were from calf thymus; the sequences of human histones are identical or extremely similar to bovine histones (Marino-Ramirez et al. 2006). All of the core histones were readily detected and each was detected as both singly charged and doubly charged species, that is, [M+H]+ and [M+2H]2+ ions, respectively. In each case, the singly charged ion was more intense than the respective doubly charged ion. All of the ions corresponding to peaks detected using E2-treated MCF-7 cells (Figure 3).

Some additional peaks were detected in the spectra of each of the purified histones. These included slightly higher mass species, most evident as shoulders on the main [M+H]+ peaks, but also apparent as smaller [M+2H]2+ peaks. This was particularly noticeable in the spectra of histones H4 and H2B. For histone H4, ions with m/z 11,426 and

Table 2: Comparison of EC50 (mean ± SE) values for estrogenic compounds on MCF-7 cells determined by measurement of cell proliferation and SELDI-TOF MS protein ion intensity.

| Parameter                  | E2 (pM) | GEN (nM) | BPA (nM) | EDS (µM) |
|----------------------------|---------|----------|----------|----------|
| Cell proliferation         | 2.4 ± 0.7 | 40.7 ± 1.7 | 158 ± 51 | 2.5 ± 0.2 |
| m/z 2,5610 ion             | 2.6 ± 0.3 | 46.9 ± 7.0 | 155 ± 19 | 2.0 ± 0.1 |
| m/z 6,845 ion              | 2.2 ± 0.3 | 45.1 ± 7.7 | 143 ± 14 | 2.4 ± 0.4 |
| m/z 2,7,010 ion            | 2.3 ± 0.5 | 52.9 ± 7.8 | 149 ± 37 | 2.3 ± 0.4 |
| m/z 7,620 ion             | 2.6 ± 0.3 | 47.6 ± 7.0 | 139 ± 23 | 1.9 ± 0.4 |
| m/z 11,260 ion            | 2.3 ± 0.7 | 54.5 ± 15.3 | 144 ± 33 | 1.8 ± 0.4 |
| m/z 13,680 ion            | 2.2 ± 0.6 | 52.8 ± 14.6 | 155 ± 39 | 2.1 ± 0.6 |
| m/z 14,020 ion            | 2.6 ± 1.0 | 52.8 ± 3.0  | 176 ± 31 | 2.1 ± 0.2 |
| m/z 15,260 ion            | 2.5 ± 0.3 | 42.0 ± 5.0  | 176 ± 20 | 2.0 ± 0.2 |

EC50 values were determined from concentration–effect data by curve fitting using a four-parameter sigmoidal model by nonlinear regression. SEs were calculated from replicate experiments (n = 3). No significant differences between the values obtained by the cell proliferation method or the intensities of the ions determined by SELDI-TOF MS were found for any of the compounds.

*p < 0.05 in all cases by Student’s t-test.

Figure 3. Identification of responsive protein ions. (A) Coomassie blue–stained SDS-polyacrylamide gel of whole-cell protein extracts of MCF-7 cells treated with 20 pM E2 and vehicle (V). Molecular weight markers are shown on the left-hand side. The lane containing the E2-treated cells was divided as shown, and the proteins in each slice of the gel were digested with trypsin in preparation for analysis by LC-MS/MS. The identified proteins found in each slice of the gel are listed along with the predicted molecular weight of each protein. (B) Comparison of SELDI-TOF mass spectra of estrogen-responsive protein ions in MCF-7 cells and purified histones. (a) 20 pM E2-treated MCF-7 cells. The 12 estrogen-responsive protein ions identified are indicated. Purified preparations of calf thymus histones were analyzed under similar conditions with 0.1 µg each of histones (b) H3, (c) H2B, (d) H2A, and (e) H4. The major ions evident in each spectrum are indicated along with their likely charge state.
m/z 11,680 were present as overlapping peaks forming a shoulder of the main m/z 11,260 ion. Singly and doubly charged species of all of these ions appeared to be present in the spectra of MCF-7 cells. Also, a collection of small peaks was present in the spectrum of histone H4 with m/z 12,180–12,360. Ions with m/z 6,160 and m/z 4128 apparent in the MCF-7 cell spectra may represent doubly and triply charged species of this protein. A similar distribution of peaks was also seen in the spectra of histone H2B, in this case with an ion m/z 13,898 forming a shoulder of the main m/z 13,680 peak and several smaller peaks such as those with m/z 14,646. Although no distinct separate peaks were identified in the corresponding regions of the spectra of histones H2A and H3, the shapes of these peaks were distorted toward higher masses, suggesting that species with increased masses were also present in these preparations. These species probably represent post-translational modifications of the proteins, for example, by acetylation, methylation, phosphorylation, which occur commonly in histones, as well as variant forms of the histones. Such species were also apparent in the cell preparations, but were difficult to quantify as they did not form discrete peaks. Thus, the identity of all 12 of the responsive protein ions could be ascribed to histones or their posttranslationally modified forms.

Western blotting of histones. Up-regulation of histones H2B, H3, and H4 in MCF-7 cells treated with E2 was demonstrated in a previous study by immunoblotting (Zhu et al. 2008a). These effects are compared here with those of GEN, BPA, and EDS (Table 3). All four compounds had similar effects on histone protein levels. Results were consistent with those obtained using SELDI-TOF MS (Table 3).

Comparative levels of histone H2B were determined in all four cell lines used here. Constitutive levels in MDA-MB-231 cells were similar to those in MCF-7 cells, whereas levels in MCF-10A and MCF-10F cells were only about 50% of those in MCF-7 cells (Table 4). Treatment with E2 affected H2B expression only in MCF-7 cells; levels were unchanged in the other three cell lines (Table 4).

Discussion
The ability of E2 to stimulate the proliferation of MCF-7 cells is well established (Katzendellenbogen et al. 1987). This is accompanied by a number of cellular changes that have been investigated most comprehensively at the level of gene expression (Buterin et al. 2006; Frasor et al. 2003), although a number of alterations at the protein level have also been demonstrated (Zhu et al. 2008a). Here, we investigated the effect of E2 on the whole-cell protein profile using SELDI-TOF MS. It was evident that treatment with E2 had a profound upregulatory effect on a number of protein ions detectable by this technique. The minimum concentration of E2 that produced a maximal response of the protein ions in MCF-7 cells was 20 pM, and this is similar to that determined using the E-Screen assay on these cells. This value for E-Screen is consistent with that found in several previous studies, which report similar EC50 values for E2 in the range of 10–20 pM (Soto et al. 1995; Suzuki et al. 2001; Villalobos et al. 1995), although a higher value of 80–100 pM was reported by Coser et al., and a value in the range of 50–200 pM can be deduced from the data shown in Rajapakse et al. (2004).

Table 3. Relative levels of histone proteins in MCF-7 cells after treatment with estrogenic compounds.

| Treatment | H2A (SOLDI) | H2A (IB) | H3 (SOLDI) | H3 (IB) | H4 (SOLDI) | H4 (IB) |
|-----------|-------------|----------|-------------|---------|-------------|---------|
| E2        | 2.3 ± 0.6   | 2.1 ± 0.3 | 2.3 ± 0.1   | 3.1 ± 0.3 | 2.9 ± 0.2   | 2.1 ± 0.4 |
| GEN       | 2.2 ± 0.3   | 2.2 ± 0.6 | 2.2 ± 0.2   | 2.5 ± 0.4 | 2.6 ± 0.3   | 2.1 ± 0.4 |
| BPA       | 2.1 ± 0.5   | 2.1 ± 0.1 | 2.2 ± 0.1   | 2.6 ± 0.3 | 2.2 ± 0.2   | 2.1 ± 0.2 |
| EDS       | 2.3 ± 0.2   | 2.0 ± 0.5 | 2.4 ± 0.1   | 2.4 ± 0.2 | 2.8 ± 0.2   | 2.2 ± 0.6 |

We treated cells with 20 pM E2, 1 μM GEN, 1 μM BPA, or 10 μM EDS for 6 days, and compared the levels of histones with those of vehicle-treated cells by SELDI-TOF MS (SOLDI) and immunoblotting (IB). Immunoblotting data was not obtained for histone H2A, as no suitable antibody was available. Both methods performed similarly; there were no statistically significant differences between levels of histones determined by SELDI-TOF MS or immunoblotting. p > 0.05 in all cases by Student’s t-test.

Table 4. Comparative levels of histone H2B in each of the four breast-derived cell lines.

| Cell line | Relative histone H2B level |
|-----------|---------------------------|
| Control cells | E2-treated cells |
| MCF-7 | 1.00 ± 0.06 | 2.30 ± 0.11** |
| MDA-MB-231 | 1.10 ± 0.10 | 1.22 ± 0.02 |
| MCF-10A | 0.52 ± 0.02** | 0.51 ± 0.03 |
| MCF-10F | 0.41 ± 0.05** | 0.43 ± 0.07 |

NS, not significant. The levels relative to total cell protein and normalized to those measured in vehicle-treated MCF-7 cells were determined by immunoblotting in each of control (vehicle-treated) cells and cells treated with 20 pM E2.

**p < 0.01 compared with control (vehicle-treated) MCF-7 cells by Student’s t-test.

Albeit less potent than E2, GEN, BPA, and EDS also increased the proliferation rate of MCF-7 cells, as found in previous studies (Soto et al. 1994; Wang et al. 1996; Zhu et al. 2008b) and resulted in protein profile changes similar to those produced by E2. GEN, BPA, and BPA have previously been reported to produce similar effects on the expression of a number of genes in both MCF-7 cells and the developing female rat reproductive system (Buterin et al. 2006; Dip et al. 2008; Naciff et al. 2002). All the other cell lines studied here lacked expression of ERα, and none of them showed changes in either proliferation rate or in protein profile after treatment with any of the estrogenic compounds. Thus, these data are consistent with the concept that ERα (or ERα/ERβ heterodimer) in MCF-7 cells plays a controlling role in the response to estrogenic compounds (Ter Veld et al. 2006). It was apparent that ERβ alone did not facilitate any response, at least for the compounds examined here. In other studies (Zhu et al. 2008b), we have shown that none of the compounds affected the response to others in MCF-7 cells, suggesting that ERβ response is not modified by any effect on ERβ. GEN and BPA appear to bind more strongly to ERβ than ERα (Kuiper et al. 1997) and so might exert a greater effect via ERβ. It is possible that compounds with a greater affinity for ERβ may produce effects other than those found here.

The principal feature of the SELDI-TOF MS protein profile changes in MCF-7 cells after estrogen treatment was an increase in the levels of core histone proteins. Histone proteins are a group of relatively small basic proteins with masses in the range of 11–15 kDa and isoelectric points (pI/s) > 10. The ready detection of such proteins by SELDI-TOF MS is consistent with the conditions used. Basic proteins would be expected to bind strongly to the cation exchange interactive surface at pH 4, and proteins in the range of 5–20 kDa are particularly well suited to detection by SELDI-TOF MS (Petricoin and Liotta 2004). Up-regulation of these core histones is also supported by immunodetection and a label-free quantitative LC-MS/MS approach used to examine the differentially expressed proteins in E2-treated MCF-7 cells (Zhu et al. 2008a).

Previously, microarray and RT-PCR analysis of MCF-7 cells treated with 100 pM E2 for 24 hr was reported to cause up-regulation of histone H2A genes (members X and Z), although in the same study a decrease in the expression of histone 1 H2ac and H2BE genes was also reported (Buterin et al. 2006). In contrast, no variation in mRNA levels of histones in the developing female reproductive system of rats treated with 17β-ethynyl estradiol (Naciff et al. 2002) was reported. Histone proteins are core components of nucleosomes. A nucleosome is composed of an octamer of histone proteins comprising two molecules each of histones

Table 2. Comparative levels of histone H2B in each of the four breast-derived cell lines.

| Cell line | Relative histone H2B level |
|-----------|---------------------------|
| Control cells | E2-treated cells |
| MCF-7 | 1.00 ± 0.06 | 2.30 ± 0.11** |
| MDA-MB-231 | 1.10 ± 0.10 | 1.22 ± 0.02 |
| MCF-10A | 0.52 ± 0.02** | 0.51 ± 0.03 |
| MCF-10F | 0.41 ± 0.05** | 0.43 ± 0.07 |

NS, not significant. The levels relative to total cell protein and normalized to those measured in vehicle-treated MCF-7 cells were determined by immunoblotting in each of control (vehicle-treated) cells and cells treated with 20 pM E2.

**p < 0.01 compared with control (vehicle-treated) MCF-7 cells by Student’s t-test.
Human histone modifications and nuclear architecture: a review. J Histochem Cytochem 58:580–591.

Bonisch C, Nieratscher SM, Orfano NK, Hake SB. 2008. Chromatin proteomics and epigenetic regulatory circuits. Expert Rev Proteomics 5:105–119.

Bosch FX, UVa, MA, Rosencrantz E, Harold-Mende C, Schuhmann A, Maier H, et al. 1993. Expression of the histone H3 gene in benign, semi-malignant and malignant lesions of the head and neck: a reliable proliferation marker. Eur J Oral Surg 1:235–245.

Buterin T, Koch C, Naegeli H. 2006. Convergent transcriptional profiles induced by endogenous estrogen and distinct xenestrogens in breast cancer cells. Carcinogenesis 27:1567–1578.

Carter D, Douglas JF, Cornellion CD, Better RM, Johnson JC, Benninga AA, et al. 2002. Purification and characterization of the mammaglobin/biphillin B complex, a promising diagnostic marker for breast cancer. Biochemistry 41:6716–6722.

Choi KC, Jeung EB. 2003. The biomarker and endocrine disruptors in mammals. J Reprod Dev 49:337–345.

Colborn T, vom Saal FS, Soto AM. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 101:278–284.

Coser KR, Chesnes J, Hur J, Ray S, Ilesbacher K, Shiota T. 2003. Global analysis of ligand sensitivity of estrogen inducible and suppressible genes in MCF-7 breast cancer cells by DNA microarray. PNAS 100:13994–13999.

Damstra T, Barlow S, Bergman A, Kavlock R, van der Kraak G. 2002. Global Assessment of the State-of-the-Science of Endocrine Disruptors. Geneva:International Programme on Chemical Safety.

Dekant W, Volkel W. 2008. Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental and health impacts. Toxicol Environ Chem 90(9):114–134.

Dip R, Lenz S, Antignac JP, Bizet B, Gmuender H, Naegeli H. 2008. Global gene expression profiles induced by phytoestrogens in human breast cancer cells. Endocr Relat Cancer 15:161–172.

Edwards RJ. 2005. Targeting antibody epitopes toward cytochrome P450 enzymes. Methods Mol Biol 320:173–182.

Fernandez MF, Santa-Maria L, Ibaruza JM, Exposito J, Aurrekoetxea JI, Torre P, et al. 2007. Analysis of population characteristics related to the total effective xenestrogen burden: a biomarker of xenestrogen exposure in breast cancer. Eur J Cancer 43:1290–1299.

Fertuck KC, Kumar S, Sikka HC, Matthews JB, Zacharewski TR. 2001. Interaction of PAN-related compounds with the alpha and beta isoforms of the estrogen receptor. Toxicol Lett 121:167–177.

Fraser J, Dannes JM, Komm B, Chang KC, Lyttle CR, Katenlenzenen BS. 2003. Profiling of estrogen up- and downstream-regulated gene expression in human breast cancer: insights into gene networks and pathways underlying estrogen-dependent transcription and cell phenotype. Endocrinology 144:4562–4574.

Frigo DE, Burov ME, Mitchell KA, Chiang TC, McLachlan JA. 2002. DDT and its metabolites alter gene expression in human uterine cell lines through estrogen receptor-independent mechanisms. Environ Health Perspect 110:1239–1245.

Fukua SA, Shiff R, Parra I, Friedichs WE, Su JL, McKee DD, et al. 1999. Expression of wild-type estrogen receptor beta and variant isoforms in human breast cancer cells. Cancer Res 59:5425–5428.

Girdler F, Bowrell DA, Cunifile WJ, Shenon BK, Hemning JD, Scorer P, et al. 2001. Use of the monoclonal antibody DACO-Erbeta (RDS-1) to measure oestrogen receptor beta in breast cancer. J Clin Pathol 54:527–533.

Gutendorf B, Westendorf J. 2001. Comparison of an array of in vitro assays for the estimation of the estrogenic potential of natural and synthetic estrogens, phyoestrogens and xenestrogens. Toxicology 166:79–89.

Hu YF, Lau KM, Ho SM, Russe J. 1998. Increased expression of estrogen receptor beta in chromatically transformed human breast epithelial cells. Int J Oncol 12:225–228.

Jefferson WN, Padilla-Banks E, Clark G. 2002. Assessing estrogenic activity of phytochemicals using transcriptional activation and immature mouse uterotropic responses. J Chromatogr B Analyt Technol Biomed Sci 776:187–198.

Jenuwein T, Allis CD. 2001. Translating the histone code. Science 290:1074–1080.

Ji SY, Jordan VC. 1992. Growth regulation of estrogen receptor-negative breast cancer cells transfected with complementary DNAs for estrogen receptor. J Natl Cancer Inst 84:580–591.

Jordan VC, Mittal S, Goods B, Koch R, Lieberman ME. 1985. Structure-activity relationships of estrogens. Environ Health Perspect 61:97–110.

Katenlenzenen BS, UVa, MA, Rosencrantz E, Harold-Mende C, Schuhmann A, Maier H, et al. 1993. Expression of the histone H3 gene in benign, semi-malignant and malignant lesions of the head and neck: a reliable proliferation marker. Eur J Oral Surg 1:235–245.

Kuiper GG, Carlson B, Grandiden K, Enmark E, Haggblad J, Nilsson S, et al. 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. J Steroid Biochem Mol Biol 61:797–807.

Laronga C, Becker S, Watson P, Gregory B, Cazares L, Lynch H, et al. 2003. SELDI-TOF serum profiling for prognostic and diagnostic classification of breast cancers. Dis Markers 22:98–226.

Marino-Ramirez L, Jordn IK, Landsman D. 2006. Multiple independent evolutionary solutions to core histone gene regulation. Genome Biol 7:122; doi:10.1186/gb-2006-7-12r122[Online 21 December 2006].

Muskhelishvili L, Latendresse JR, Kedel RL, Henderson EB. 2003. Evaluation of cell proliferation in rat tissues with BrdU, PCNA, Ki-67(MIB-1) immunohistochemistry and in situ hybridization for histone mRNA. J Histochem Cytochem 51:1681–1688.

Nacif JM, Jump ML, Torontali SM, Carr GJ, Tiesman JP, Oeverman GJ, et al. 2002. Gene expression profile induced by 17alpha-ethynyl estradiol, bisphenol A, and genistein in the developing female reproductive system of the rat. Toxicol Sci 68:184–199.

National Center for Biotechnology Information. 2009. Reference Sequence. Available [http://www.ncbi.nlm.nih.gov/RefSeq/]

Petricoin EJ, Liotta LA. 2004. SELDI-TOF-based serum protein pattern diagnostics for early detection of cancer. Curr Opin Biotechnol 15:30–40.

Rajapaksie N, Silva E, Schofield M, Kortenkamp A. 2004. Deviation from additivity with estrogenic mixtures containing 4-nonylphenol and 4-tert-octylphenol detected in the E-screen assay. Environ Sci Technol 38:6343–6352.

Rasmussen TN, Nielsen F, Andersen HR, Nielsen JB, Welte P, Grandjean P. 2003. Assessment of xenestrogenic exposure by a biomarker approach: application of the E-Screen bioassay to determine estrogen response of serum extracts. Environ Health 2(1):12; doi:10.1186/1476-069X-2-12 [Online 15 October 2003].

Safe SH, Zacharewski T. 1997. Organochlorine exposure and risk for breast cancer. Prog Clin Biol Res 396:133–145.

Sheehan DM. 2000. Activity of environmentally relevant low doses of endocrine disruptors and the bisphenol A controversy: initial results confirmed. Proc Soc Exp Biol Med 224:57–60.

Slowinski J, Mazurek U, Bierzyamska-Macyszyn G, Widel M, Latocha M, Glogowski-Ligus J, et al. 2005. Cell proliferative activity estimated by histone H2B mRNA level correlates with cytogetic damage induced by radiation in human glioblastoma cell lines. J Neurooncol 71:237–242.

Soto AM, Cheng KL, Sonnenschein C. 1994. The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. Environ Health Perspect 102:380–383.

Soto AM, Fernandez MF, Luzi MF, Oles Karasco AS, Sonnenschein C. 1997. Developing a marker of exposure to xenoestrogens in human tissues. Environ Health Perspect 105(suppl 3):647–654.

Soto AM, Fernandez MF, Oles Karasco AS, Sonnenschein C. 1997. Developing a marker of exposure to xenoestrogens in human tissues. Environ Health Perspect 105(suppl 3):647–654.

Soto AM, Fernandez MF, Oles Karasco AS, Sonnenschein C. 1997. Developing a marker of exposure to xenoestrogens in human tissues. Environ Health Perspect 105(suppl 3):647–654.

Sotolich N, Edward B, Moskowitz A, Doehmer J, Davies JD, Soto AM. 2000. The potential of acetaminophen as a prodrug in gene-directed enzyme prodrug therapy. Cancer Genet Cytogenet 127:519–521.

Tong D, Schuster E, Sarfert M, Czerwienka K, Leodolte S, Zeillinger F. 2002. Expression of estrogen receptor beta...
isoforms in human breast cancer tissues and cell lines. Breast Cancer Res Treat 71:249–255.
Villalobos M, Olea N, Brotons JA, Olea-Serrano MF, Ruiz de Almodovar JM, Pedraza V. 1995. The E-screen assay: a comparison of different MCF7 cell stocks. Environ Health Perspect 103:844–850.
Wang TT, Sathyamorthy N, Phang JM. 1996. Molecular effects of genistein on estrogen receptor mediated pathways. Carcinogenesis 17:271–275.
Waring RH, Harris RM. 2005. Endocrine disrupters: a human risk? Mol Cell Endocrinol 244:2–9.
Watson K, Edwards RJ, Shaunak S, Parmelee DC, Sarraf C, Gouderham N.J, et al. 1999. Extra-nuclear location of histones in activated human peripheral blood lymphocytes and cultured T-cells. Biochem Pharmacol 50:299–309.
Wolff MS, Teitelbaum SL, Windham G, Pinney SM, Britton JA, Chelimo C, et al. 2007. Pilot study of urinary biomarkers of phytoestrogens, phthalates, and phenols in girls. Environ Health Perspect 115:116–121.
Wulfkuhle JD, McLean KC, Paeoletz CP, Sgroi DC, Trock BJ, Steeg PS, et al. 2001. New approaches to proteomic analysis of breast cancer. Proteomics 1:1205–1215.
Zhu Z, Boobis AR, Edwards RJ. 2008a. Identification of estrogen-responsive proteins in MCF-7 human breast cancer cells using label-free quantitative proteomics. Proteomics 8:1987–2005.
Zhu Z, Edwards RJ, Boobis AR. 2008b. Proteomic analysis of human breast cell lines using SELDI-TOF MS shows that mixtures of estrogenic compounds exhibit simple similar action (concentration additivity). Toxicol Lett 181:93–103.