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Epithelial-Mesenchymal Transition: A Special Focus on Phthalates and Bisphenol A

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ABSTRACT: Epithelial-mesenchymal transition (EMT) is a process during which epithelial cells lose their polarity and ability to adhere. Instead, they gain properties to move, migrate through the extracellular matrix, become invasive, and finally become mesenchymal stem cells. This trans-differentiation is critical for embryo development, wound healing, and stem cell behavior. However, this same phenomenon is also observed in cancer progression. Phthalates and bisphenol A (BPA) are endocrine-disrupting chemicals (EDCs) that are linked to complex human diseases. These chemicals are suggested to disrupt normal hormonal balance (usually by existing estrogenic/antiandrogenic properties) and stimulate the development of reproductive tumors and steroid hormone-dependent cancers, such as breast cancer. Di(2-ethylhexyl) phthalate (DEHP), the most abundant phthalate, was shown to induce DNA damage in human cells via multiple molecular signals that include altered apoptosis and mitotic rate, increased cell proliferation, tumor mobility, and invasiveness of tumor cells. DEHP was also shown to inhibit gap junction intercellular communication and tight junctions and promote EMT. Phthalates may also cause the proliferation and metastasis of cancer cells and tumor progression via up-regulating histone deacetylase 6 (HDAC6). Phthalates can activate peroxisome proliferator activated receptors (PPARs) that may eventually lead to high proliferation of cancer cells. However, in ovarian cells the expression of Snail, Slug, and vimentin was enhanced by the treatment of BPA, whereas E-cadherin was decreased. Mechanistic studies are needed to show the underlying mechanisms of EMT caused by different EDCs.

KEY WORDS: epithelial-mesenchymal transition, phthalate, bisphenol A, E-cadherin, N-cadherin, vimentin

I. INTRODUCTION

The epithelial-mesenchymal transition (EMT) is a process during which epithelial cells lose their cell polarity and cell–cell adhesion and gain migratory and invasive properties to become mesenchymal stem cells. These stem cells are multipotent stromal cells that can differentiate into a variety of cell types.[1] Epithelial cells acquire a mesenchymal phenotype that improves their ability to migrate in an extracellular environment.[2] Mesenchymal-epithelial transition (MET) can be regarded as the opposite process of EMT, during which mesenchymal cells lose their motile migratory properties, acquire cell polarity, and gain ability of adhesion.[3] The trans-differentiation of epithelial cells into motile mesenchymal cells or vice versa is critical for development of embryo tissues and organs, wound healing, stem cell behavior, and cancer progression. Thus, EMT's a crucial role in the transformation from benign cells to invasive cells may also contribute to the pathophysiology of fibrosis.[4,5]

II. MOLECULAR HALLMARKS IN CELLS UNDERGOING EPITHELIAL-MESENCHYMAL TRANSITION

During EMT, epithelial cells reconstitute their cytoskeleton and have alterations in their signaling programs that describe cell shape. They also undergo reprogrammed gene expression that enhances their motility and creates an invasive phenotype.[6] The protein contents and cell specificities of epithelial and mesenchymal cells are shown in Figure 1.

The molecular hallmarks occurring in cells undergoing EMT include[2]

1. Detachment from neighboring cells and migration in adjacent tissue

2. E-cadherin relocalization (and finally loss of cell–cell adhesion)
3. Up-regulation of matrix-degrading proteases and mesenchymal cell–related proteins such as vimentin and N-cadherin
4. Actin cytoskeleton reorganization mediated by Rho small guanine triphosphatases (GTPases) to activate motility machinery
5. Up-regulation and/or nuclear translocation of transcription factors underlying the specific gene program of EMT, such as β-catenin and members of the Snail, ZEB, and basic helix-loop-helix families.

Factors acting at the tumor–stroma interface, including growth factors and their receptors (i.e., tyrosine or serine–threonine kinase receptors), extracellular matrix (ECM) proteins and related molecules (collagens, integrins, matrix-degrading proteases), and oncogenic signal transduction pathways (Ras, Src, β-catenin), seem to have important roles in accomplishment of EMT. Matrix-degrading protease-mediated breakdown of the basement membrane results in direct contact between carcinoma cells and the stromal microenvironment. Exposure to stromal-type collagens (epithelial cells would never come into contact with these collagens under normal conditions) as well as to stromal growth factors could initiate EMT.[7,8]

III. SUBTYPES OF EPITHELIAL-MESENCHYMAL TRANSITION

EMT has three different suptypes.[5,9]

1. Type 1 EMT is associated with implantation, gastrulation, and tissue–organ development in embryonic course.
2. Type 2 covers wound healing, tissue regeneration, and organ fibrosis.
3. Type 3 is related to cancer progression, invasiveness, metastasis, and resistance to radiochemotherapy and apoptosis.

Carcinoma cells undergo type 3 EMT for invasion and metastasis. This process leads to life-threatening manifestations of cancer progression. However, it is still unclear how specific signals induce type 3 EMT in carcinoma cells. Some researchers suggest that such signals might originate from the tumor stroma; this may be associated with many primary carcinomas.[10]

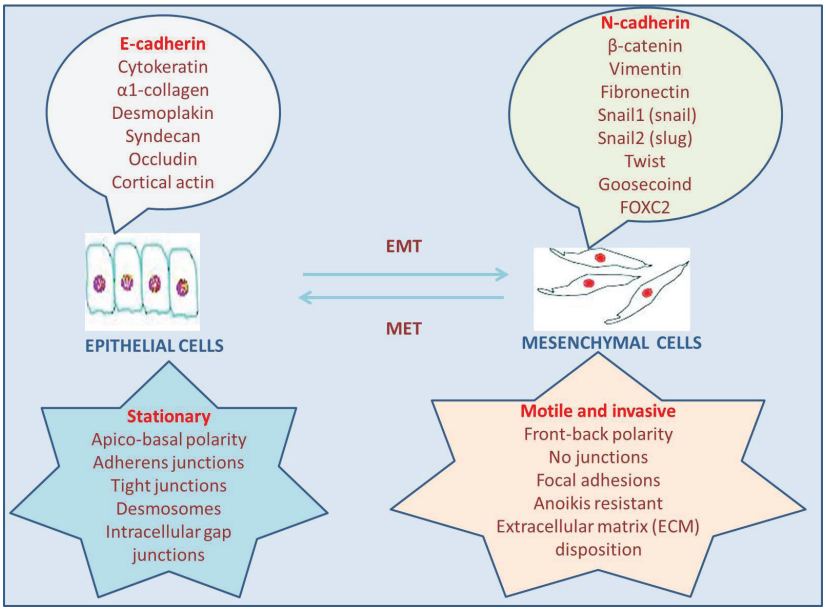

FIG. 1: Protein contents and cell specificities of epithelial and mesenchymal cells.

IV. MOLECULES AND PROTEINS RELATED TO EPITHELIAL-MESENCHYMAL TRANSITION

The research for understanding the essential roles, inducing factors, underlying mechanisms, and progression of both EMT and MET in cancer, has significantly increased in the last decade as the role of EMT in cancer progression is better understood. Both developmental studies in various organisms and tissue-culture studies have revealed a number of distinct signaling pathways that regulate EMT and MET.[11] Studies on the molecular basis of EMT showed that the signaling pathways involved in EMT have an important role in various developmental processes. Several extracellular activators can induce EMT. For example, collagen and hyaluronic acid, transforming growth factor β (TGF-β) and epidermal growth factor (EGF) family members, fibroblast growth factors (FGFs), hepatocyte growth factors (EGFs), and insulin-like growth factors are associated with the induction of EMT.[12]

A. Transforming Growth Factor β Superfamily

EMT is generally induced in epithelial cells by heterotypical signals, specifically those released by the mesenchymal cells that constitute the stroma of normal and neoplastic tissues.[5] TGF-β is a member of the cytokines family and the main inducer for EMT, especially during the course of embryonic development, wound healing, fibrotic diseases, and cancer pathogenesis.[11] Increased TGF-β signaling is the key effector of EMT in cancer progression and metastasis.[13] The important evidence that "EMT induced by oncogenic stimuli depends on TGF-β signaling" comes from the finding that TGF-β inhibitors are able to block EMT induced by oncogenes such as Ras or Raf in different carcinoma types.[14]

TGF-β has an important suppressor function at the early stage of tumorigenesis by inducing apoptosis and cell-cycle arrest and acting as a positive modulator of tumor progression in the late phase of tumorigenesis.[12] However, it can also be protective against excess apoptosis. In addition, it has immunosuppresive and proangiogenic roles and can therefore initiate cancer progression.[15] Immune system components in the tumor microenvironment, such as fibroblasts, immune cells, and the extracellular matrix, influence the ability of TGF-β to promote carcinoma progression and metastasis. The TGF-β superfamily contains ~40 secreted ligands that have regulatory roles in cell growth and differentiation via heteromeric signaling complexes of TGF-β type 1 and type 2 receptors (TGF-β R1 and TGF-β R2).[16] Normally, TGF-β binds onto TGF-β cell-surface receptors (types 1, 2, and 3) and sequentially activates complex formation of SMAD family transcription factors, which translocate to the nucleus and cooperate with transcription factors from the Snail and Twist families.[17]

B. SMAD Family

The SMAD family refers to "Sma and Mad"-related proteins, which are intracellular proteins that transduce extracellular signals from TGF-β ligands to the nucleus, where they activate downstream gene transcription. TGF-β may induce EMT through multiple prominent signaling mechanisms: direct phosphorylation through ligand-activated receptors of SMAD, transcription of certain cytoplasmic proteins, or regulation of cell polarity and tight-junction formation.[13,18]

The SMAD family consists of eight members that form three subfamilies.[19]

1. The receptor-activated SMAD (R-SMAD) subfamily includes SMAD1 and SMAD2, which are phosphorylated by TGF-β and activin receptors, and SMAD5 and SMAD8, which are phosphorylated by bone morphogenetic protein (BMP) receptors.
2. The common-mediator (Co-SMAD) subfamily SMAD4 interacts with R-SMADs to participate in signaling.
3. The inhibitory SMAD (I-SMAD) family, SMAD6 and SMAD7, are induced by SMAD signaling, act as negative feedback control mechanisms, and block the activation of R-SMADs and Co-SMADs.

The following two signaling pathways can be identified as mediators of TGF-β–induced EMT.[5,10]

1. SMAD-mediated signaling via the AKL5 receptor: This phenomenon facilitates motility.
2. Inhibitory SMADs modulate differential effects of appropriate transcription factors and cytoplasmic kinases and induce the autocrine production of TGF-β, which is further able to reinforce and amplify EMT. For instance, TGF-β type 2 receptors can directly phosphorylate SMAD2, SMAD3, and the cell polarity protein PAR6A and cause loss of epithelial cell polarity and degradation of tight junctions and desmosomes between adjacent epithelial cells.

C. Cell Adhesion–Related Proteins

The functional units of cell adhesion are multiprotein complexes that include three general classes of proteins.[20,21]

1. Cell adhesion molecules (adhesion receptors) are generally transmembrane proteins and include superfamily members integrin, cadherin, immunoglobulin, selectin, and proteoglican.
2. Extracellular matrix proteins are the collagens, fibronectins, laminins, and proteoglycans.
3. Cytoplasmic plaque (peripheral membrane proteins) links adhesion systems to the cytoskeleton, regulates the functions of the adhesion molecules, and transduces signals that begin at the cell surface by adhesion molecules.

V. INCREASING AND DECREASING PROTEINS DURING EPITHELIAL-MESENCHYMAL TRANSITION

In recent studies performed to determine molecular alterations in EMT, several phenotypic markers (these usually cause increased capacity of migration, three-dimensional invasion, and resistance to apoptosis) were used.[22] The common markers can be divided into two main groups.[22]

1. Markers that increase proteins during EMT include N-cadherin, vimentin, fibronectin, Snail1 (Snail), Snail2 (Slug), Twist, Goosecoind, FOXC2, integrins, and NCAM.
2. Markers that decrease proteins during EMT include E-cadherin, desmoplakin, cytokeratin, occludin, and α1-collagen.

A. Cadherin Protein Family

The cadherin protein family mediates intercellular adhesiveness via hemophilic interactions and maintenance of intercellular connections and is involved in the control of cell movement.[21,23] Cadherins are generated from a calcium ion–dependent hemophilic transmembrane domain that binds to adjacent cells and an intracellular domain that binds to catenin proteins.[20] Cadherin's adhesive activity is dependent on catenins, which are cytosolic proteins that link the cadherin cytoplasmic domain to the actin cytoskeleton.[24]

B. N-Cadherin

Other than neurons, neural (N)-cadherin is also expressed in endothelial cells. A common feature of cancers of epithelial origin is increased de novo expression of N-cadherin and concomitant downregulation of E-cadherin, referred to as a "cadherin switch."[25] The dysregulation of N-cadherin's function may significantly contribute to the development of pathologic situations, including cancer. N-cadherin[26]

1. Has an essential role in the maturation and stabilization of normal vessels and tumor-associated angiogenic vessels.
2. Promotes tumor cell survival, migration, and invasion, and a high level of its expression is often associated with poor prognosis.
3. Maintains structural adhesive functions and cell-to-cell communications (signaling function), affects the cytoskeleton, interferes with other membrane receptors,

and intervenes in cell adhesion among cells of the same or different cell types.
4. Is involved in the establishment of functional synapses in neurons.
5. Has a role in the formation of a vascular wall that is essential for vascular stabilization.

C. E-cadherin

Epithelial (E)-cadherin (encoded by the *CDH1* gene) is major calcium-dependent cell–cell adhesion molecule expressed in most epithelial cells.[27] E-cadherin[28,29]

1. Establishes cell polarity and maintain normal tissue structure.
2. Inhibits invasion that might be related to invasiveness and progression of many human epithelial tumor types and can be defined as a "tumor-metastasis suppressor gene."

The down-regulation of E-cadherin can be accompanied by increased expression of mesenchymal N-cadherin that promotes inappropriate signals through interaction with stromal cells. Down-regulation of E-cadherin expression can result in switching of cell morphology and therefore EMT induction.[30] On the other hand, the loss of or decrease in catenin's expression is important in tumor progression.[31,32] Functional loss of E-cadherin occurs in many carcinomas and is associated with a high tumor grade and invasiveness.[33] Inactivation or down-regulation of E-cadherin expression occurs through genetic (mutations) and epigenetic (such as promoter hypermethylation, transcriptional regulation, posttranslational modification) mechanisms and causes abnormal expression of E-cadherin and catenins. These events have been reported in various human malignancies.[34] Loss of E-cadherin expression together with the E-cadherin/β-catenin complex function is followed by up-regulation of vimentin expression, which finally leads to EMT.[22] E-cadherin repressors can be classified into two groups depending on their direct or indirect effects on the E-cadherin promoter. By binding and repressing, Snail, Zeb, E47, and KLF8 directly affect E-cadherin's activity, whereas Twist, Goosecoid, E2.2, and FoxC2 indirectly repress E-cadherin transcription.[35] TGF-β induces the expression of E-cadherin repressors including Twist 1 and 2, Snail, Slug, FOXC2, and Goosecoid. These transcriptional repressors bind to the E-box at the CDH1 promoter and repress E-cadherin expression. Later, the tumor cells sustain temporary hypermethylation and repression of E-cadherin, finally gaining a more invasive and metastatic phenotype.[36]

D. Catenins

Catenins are divided into two groups.[37,38]

1. Group 1 binds directly to the cadherin (β-catenin, γ-catenin, and p120ctn).
2. Group 2 is the vinculin-homology domain containing catenins (α E-catenin and α T-catenin).

Both α-catenin and β-catenin are known as plakoglobin and bind to the carboxy terminus; otherwise α-catenin binds to the cadherin-β-catenin or cadherin-plakoglobin complex and mediates linkage to the actin cytoskeleton.[38] α-Catenin, an actin-binding protein homologous to vinculin,[32] has roles in cadherin-mediated cell adhesion and links cadherins to the actin cytoskeleton.[39] β-Catenin or γ-catenin acts as an adaptor among cadherins, and α-catenin mediates binding of cadherins to α-catenin and thus the formation of zonula adherens. γ-Catenin is also involved in cadherin-mediated cell–cell contacts and in desmosomes.[40]

E. Twist Proteins

Twist proteins 1 and 2 are highly protected as principal helix-loop-helix transcription factors.[41,42] Twist 1 is a regulator of embryonic development and participates in EMT. If overexpressed, Twist 1 may be involved in tumor induction and metastasis.[11] Twist 2 is primarily expressed in a subset of mesodermal- and ectodermal-derived tissues and is a negative regulator of gene expression dur-

ing differentiation of a subset of mesenchymal cell lineages. Twist 2 increases the cell migration and colony-forming capability of epithelial cells.[42]

F. Snail Superfamily

The Snail superfamily of transcription factors originates from several proteins that have four to six zinc-finger domains.[45] Several studies have shown that Snail family members, including Snail and Slug, are able to repress the transcription of E-cadherin, enhance matrix metalloproteinase expression and activity, and mediate EMT.[43,44] Matrix metalloproteinases (MMPs), a family of zinc- and calcium-dependent enzymes, are central mediators of the increased proteolysis that occurs during tumor progression via their capability to degrade basement membrane and ECM components.[45] Another zinc-finger protein, Smad-interacting protein 1 (Sip1), is a DNA-binding transcriptional repressor and a member of the crystalline-enhancer binding factor 1 family.[46]

G. Vimentin

Vimentin is a type III intermediate filament protein normally found in mesenchymal cells with important roles in embryogenesis, organogenesis, wound healing, and tumor invasion. This protein can be expressed in migratory epithelial cells in necessary cases.[47] Studies showed that the vimentin promoter is a target of the β-catenin/T-cell factor pathway, and this functional regulation causes epithelial tumor cell invasion and/or migration.[48,49]

H. Histone Deacetylase 6

Histone deacetylase 6 (HDAC6) is a cytoplasmic enzyme and a microtubule-associated deacetylase that has been shown to regulate the cytoskeleton and cell migration, aid in immune synapse formation, and participate in the degradation of misfolded proteins.[50,51] HDC6A directly regulates HSP90, a significant regulator in cellular signal transduction.[52] This protein is also suggested to have an important role in EMT and cell motility.[53] Expression of HDAC6 is up-regulated by estrogens, which promote cell growth, EMT, and tumor development.[54] Estradiol can increase mRNA and protein levels of HDAC6 and thus provide cell motility, which can finally provoke EMT.[55] HDAC6 is one of the proteins responsible for breast cancer metastasis and efficient oncogenic transformation.[56,57]

SIRT1 is an HDAC enzyme. It is involved in regulating estrogen receptor α (ERα) transcription in breast cancer.[58] Estrogen has been shown to increase the expression of SIRT1 in breast cancer cells. Furthermore, depletion of SIRT1 decreases growth of breast cancer cells.[59] SIRT1 expression is increased in human prostate cancer.[60] SIRT was also shown to play a part in EMT in prostate cancer.[61] SIRT1 overexpression induced EMT in an epithelial prostate model and knockdown of SIRT1 restored cell adhesion in prostate cancer cells. Similar to SET8, this was mediated by regulation of E-cadherin. In addition, SIRT1 decreased after exposure to genistein, a phytoestrogen, in human prostate cancer cells.[62] Recent studies indicate that both Set8 and Sirt1 have important roles in cancer and that their expression may be regulated by estrogenic compounds.[63]

I. SET8

SET8 is the only enzyme that monomethylates histone H4 lysine 20 (H4K20me1). In breast cancer, H4K20me1 has been shown to be an essential co-activator of ERα-mediated transcription, and it has a role regulating both E-cadherin and N-cadherin.[64,65] In prostate cancer, SET8 has been found to be enriched at the androgen receptor (AR) target gene prostate-specific antigen (PSA); loss of SET8 resulted in a loss of PSA expression. Additionally, SET8 was found to have an indispensable role for AR-induced proliferation of prostate cancer cells.[66]

J. microRNAs

The endogenously produced microRNAs (miRNAs or miR) are small, noncoding RNAs that modulate

gene expression posttranscriptionally. They are associated with the epithelial phenotype and play essential parts in many physiological and pathological processes during tumor development.[67,68] They are also components of the cellular signaling circuitry that regulates the EMT program. Studies that investigate the roles of miRNAs in EMT suggest that loss of expression of miRNA-200 family members may have a critical role in the repression of E-cadherin by Zeb1 and 2 during EMT and thereby can enhance migration and invasion during cancer progression.[10,69]

Recent studies showed that mi-R10b overexpression is associated with invasiveness in metastasis, and miRNAs can be up-regulated by the EMT transcription factor Twist. The miRNA family also has critical roles in regulating EMT via effecting EMT repressors Zeb1 and 2.[70] The c-myc oncogene binds to myc-binding sites located in the promoter regions of miR-200 genes on human chromosomes and activates the expression of all five members of the miRNA family. Expression of both c-myc and miR-200 members may lead to alterations in the expressions of vimentin, N-cadherin, and E- cadherin.[71]

VI. ENDOCRINE DISRUPTORS

Epigenetics is the study of heritable changes in gene expression occurring without changes in DNA sequence. The mechanisms are classified under several types; however, most need to be further elucidated. Mechanisms include DNA methylation, histone modifications (acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and ADP ribosylation), imprinting, and expression of noncoding RNAs (including miRNAs).[72] Several underlying mechanisms can cause alterations. Hormonal imbalance, primary or secondary oxidative stress, induction of apoptosis, and induction of peroxisome proliferator-activated receptors (PPAR; α, β, and γ) are the best known effective mechanisms. However, several other epigenetic mechanisms, including imprinting, might be involved in the mechanism of action of endocrine disrupting chemicals (EDCs).

The potential effect of environmental compounds on human health is a major concern because we are daily exposed to various harmful chemicals via pharmaceuticals, pesticides, air pollutants, industrial chemicals, heavy metals, and food. EDCs are human-made substances that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintenance, homeostasis, reproduction, development, and/or behavior.[73] EDCs, such as dioxins, heavy metals (cadmium, lead), parabens, bisphenol A (BPA), and phthalates, are suggested to induce different epigenetic modifications.[74] During recent years, several studies have been performed to understand the connection between environmental toxicants and human complex diseases such as cancer.

A. Phthalates and Bisphenol A

Phthalates and BPA are plasticizers that are widely connected with reproductive disorders, gynecomastia, and early onset of puberty in rodents and humans.[75–78] New research has connected these substances to obesity, autism, allergies, neurological disorders, and cancer.[74,79]

1. Phthalates

Phthalates are one of the most abundant synthetic chemical contaminants in the environment and they are produced in high volume. High- and intermediate-molecular-weight phthalates, such as di(2-ethylhexyl) phthalate (DEHP), di-*n*-butyl pthalate (DBP), benzyl butyl phthalate (BBP), and di-iso-nonyl phthalate (DINP), are used as plasticizers to impart flexibility and durability to plastics (PVC floors, car interiors, food packaging, medical devices, baby feeding tubes, nipples, and toys). Low-molecular-weight phthalates, such as diethyl phthalate (DEP) and dimethyl phthalate (DMP), are widely used in personal care products and cosmetics to preserve smell and color.[80,81]

Phthalates leach out from the plastic matrix and generate extensive human exposures by vari-

ous means. These substances are peroxisome proliferators (PPs) and hepatocarcinogens in rodents, and they target fetal and pubertal testis and lead to alterations in endocrine and spermatogenic functions.[82,83] Epidemiological studies suggest that phthalates may play an important part in steroid hormone-dependent cancers (breast, uterine, testis, and prostate). DEP was suggested to induce breast cancer especially following exposure from the environment.[84] These substances are also suggested to increase the incidence of the development of reproductive tumors.[85] Moreover, both animal and human studies suggest that prenatal and/or postnatal exposure to phthalates could be the cause of infertility, decreased sperm count, cryptorchidism, reproductive tract malformations, hypospadias, reduction of testosterone levels, decreased anogenital distance, and later-life testicular tumors.[86] The term testicular dysgenesis syndrome (TDS) is used for this range of male reproductive defects.[87,88] Target organs and tissues for phthalates are given in Figure 2. Puberty is a critical period for exposure to phthalates because these substances are suggested to alter pubertal timing by interfering with normal hormonal synthesis and metabolic pathways.[89]

a. Phthalates and Epithelial-Mesenchymal Transition

Phthalates, suggested to be epigenetic carcinogens in rodents,[82,90,91] can induce cellular proliferation through a mechanism that involves the activation of PPARs. In rodent liver and human breast cells, phthalates were shown to induce peroxisome proliferation that sequentially caused increased cell size, growth, and division.[82,90–92] *In vitro* exposure of human cells or tissues to DEHP alters apoptosis and mitotic rate and increases cell proliferation. Phthalates were shown to affect cell proliferation and inhibit tamoxifen-induced apoptosis in ER-positive MCF-7 cells; however, this phenomenon was not observed in ER-MDA-MB-231 cells.[93] Stimulation of proliferation of MCF-7 cells by BBP and DBP can be completely suppressed by the ER antagonist ICI182780.[94]

Although for years phthalates have been known to be epigenetic carcinogens, our recent studies have shown that they can also have genotoxic effects.[95–97] Human cell and tissue-culture studies suggest that DEHP induces cancer via inducing DNA damage and altering multiple molecular signals.[95–98] Data in the literature show that EMT-related protein Twist overexpression prevents the up-regulation of

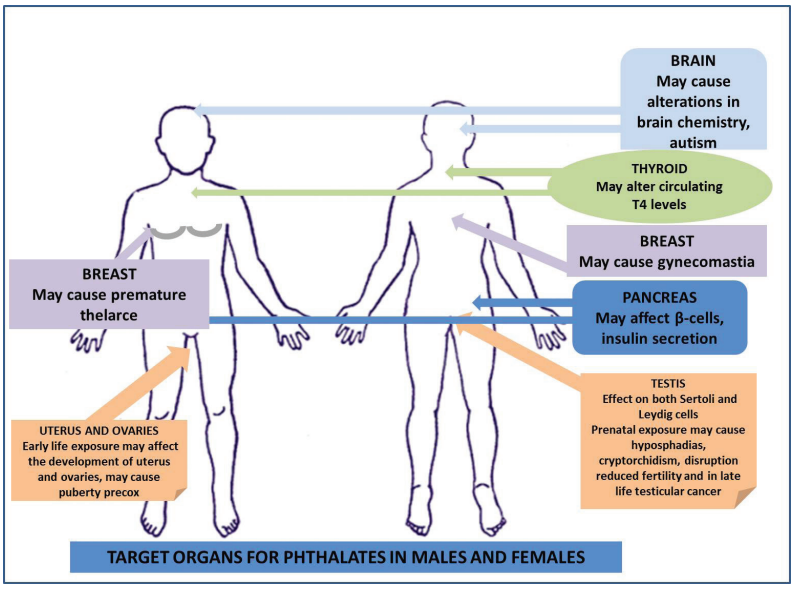

FIG. 2: Target organs and tissues for phthalates.

p21CIP1 and p53 upon genotoxic stress in several cell lines.[99–101] Moreover, Snail or Slug expression is also suggested to have a relationship with genotoxic stress and cell death, induced by the topoisomerase inhibitor adriamycin in MCF-7 cells. Induction of the expression of either Snail or Slug proteins totally prevented adriamycin-induced cell death in these cells. This phenomenon suggests that aberrant expression of these transcription factors in epithelial cells induced alterations both in cell morphology and the apoptotic response. In the context of cancer progression, alterations in the *in vitro* response to apoptotic stimuli can be correlated to tumor growth in animal models. The relationship among their genotoxicity, cell death, and proteins involved in EMT should also be enlightened with future studies.

At both high and low concentrations, phthalate exposure was also shown to induce the growth of MCF-7 breast cancer cells due to estrogenic activity and their effects on the P13K/AKT signaling pathway.[84] Phthalates were shown to promote invasion and metastasis of SK-N-SH human neuroblastoma cells as well by inducing cell motility mediated by matrix metalloproteinase-2 and -9 expressions, again through the PI3K/AKT pathway.[102] Cell motility is an important feature in tumor progression following EMT,[103–105] which may be regulated by estrogen[103,106] and TGF-β.[107]

A short amount of exposure to DEHP was shown to inhibit gap junction intercellular communication (GJIC) in Syrian hamster embryo cells.[108] DEHP exposure also induced the inhibition of GJIC in hepatic carcinomas and adenomas in rodents.[109] In Chinese hamster V79 cells exposed to DEHP and MEHP, inhibition of GJIC was also reported.[110]

HDAC6 has a particularly important role in tumor development (growth, invasion, migration) and EMT induced by several endogenous and exogenous factors (i.e., TGF-α, estrogen, and phthalates). A recent report directly focused on the effects of phthalates on EMT. After immortalizing the parent cell line M13SV1R2 (a normal human breast epithelial cell type that has stem cell characteristics) via expression of SV40 large T antigen and subsequent transformation by X radiation, the new cell line (referred to as R2d cells) was exposed to phthalates (DBP and BBP).[111] The researchers focused on the role of HDAC6 particularly and results showed that phthalates stimulated EMT by activating the epigenetic factor HDAC6. Activation of HDAC6 led to activation of upstream and downstream pathways and finally to EMT. The researchers also found that ERs were responsible for phthalate-induced EMT in breast stem cells. Therefore, similar to estrogen, BBP and DBP have the ability to promote tumor growth and metastasis. The researchers also proposed a hypothetical mechanism of phthalate-induced HDAC6 expression mediated by the ER/EGFR/PKA/AP-2a pathway and leading to vimentin expression that involves Akt, GSK3β, and β-catenin subsequent to HDAC6 activation.[111] In another study performed by the same researchers, the effect of phthalates (both DBP and BBP) on HDAC6 gene expression was found to be through the nongenomic function of the cell-surface aryl hydrocarbon receptor (AhR) in ER-negative breast cancer cells (MDA-MB-231). The researchers indicated that phthalates stimulated the cell-surface AhR and triggered the downstream cyclic AMP (cAMP)-PKA-CREB1 signaling cascade.[112]

Results of another study supported the fact that HDAC6 is required for TGF-α–induced transition of the epithelial-like phenotype into a mesenchymal phenotype via the SMAD3 signaling pathway in various lung carcinoma cell lines.[113] Moreover, the results of this study were in agreement with a previous report showing that BBP enhances cell migration in a different breast epithelial cell line, MCF-10F.[53]

b. Bisphenol A

BPA is used to harden plastics and manufacture polycarbonate plastics and epoxy resins. Metal food and beverage cans have a thin coating of BPA on the interior that prevents corrosion of the can and contamination of the food.[114]

BPA is suggested to be an epigenetic carcinogen.[115] Fetuses and young infants are commonly exposed to BPA by trans-placental transfer of ma-

ternal BPA and through ingestion of maternal milk or formula in BPA-containing plastic bottles.[116,117] Perinatal exposure to environmentally relevant BPA doses results in morphological and functional alterations of the male and female genital tract and mammary glands. This may predispose the tissue to earlier onset of disease, reductive fertility, and mammary and prostate cancers.[118]

c. Bisphenol A and Ephitelial-Mesenchymal Transition

Epigenetic changes as a result of exposure to EDCs such diethylstilbestrol (DES) and BPA may predispose to malignancies in adulthood. Low doses of BPA and/or phthalates (DEHP, MEHP, BBP, and DBP) may cause DNA hypermethylation/hypomethylation at CpG islands near gene promoter regions and thus effect the expression of significant proteins that have a role in cancer progression.[79] In women, in utero exposure to DES is associated with an increased incidence of adult breast cancer.[119,120] Rodent studies have shown that in utero BPA exposure causes molecular changes in mammary tissue by altering estrogen sensitivity. This effect may lead to mammary ductal hyperplasia and cause an increase in carcinoma in situ of the breast.[121,122] It was previously shown that BPA exposure causes epigenetic changes in HOXA10 expression in the reproductive tract by altering DNA methylation.[123,124] BPA acts as a xenoestrogen, rather than an antiandrogen. Estrogenic effects of BPA have been studied, and it was reported that BPA activates ERα and ERβ and stimulates growth of MCF-7 breast cancer cells. After activating ERs, BPA affects tissue organization and causes gene induction.[118]

In a study performed by Betancourt et al., pregnant rats were treated orally with 0, 25, or 250 μg BPA/kg body weight from days 10 to 21 postconception.[125] At the 21st or 50th day, female offspring were euthanized and mammary glands collected. Proteomic analysis led to the identification of 21 differentially abundant proteins including vimentin, SPARC, and 14–3–3. Western blot analysis of key downstream signaling proteins demonstrated increased phospho-AKT, c-Raf, phospho-ERKs-1 and 2, and vimentin but decreased TGF-β in mammary glands of 50-day-old rats exposed prenatally to BPA. The results of this study indicated that key proteins involved in signaling pathways such as cellular proliferation could be regulated at the protein level by BPA, perhaps influencing the susceptibility of the mammary gland to EMT in cancer transformation by decreasing TGF-β and increasing vimentin.[125]

Using two human prostate cancer cell lines (LNCaP and PC3 cells), the expression of Set8 and Sirt1 were examined after exposure to estradiol or BPA. These experiments were performed in the presence of natural hormones to understand the impact of additional exposure to estrogen or BPA on histone modifying enzyme (HME) expression. In LNCaP cells, estradiol did not affect relative Set8 expression in any of the doses applied (1, 5, and 10 nM), and BPA did not significantly affect relative Set8 and Sirt expression in any of the applied doses (10, 25, and 50 nM). In PC3 cells, estradiol decreased relative Set expression in all of the doses; however, this did not markedly affect relative Sirt expression. BPA was shown to decrease relative Set8 expression at 25 nM but did not affect relative Sirt expression. Furthermore, the changes in gene expression that occurred via ER signaling using the ER antagonist ICI 182,780 (fulvestrant) was determined. Interestingly, the researchers found that the combination of ICI with estrogen or BPA greatly affected the expression of Set8, even when the hormone alone had no effect. As expected, in LNCaP cells the expression of Sirt1 was reversed in the presence of ICI. In PC8 cells, treatment with ICI reversed the effect of estradiol on Set8 expression, clearly demonstrating that ER signaling is the mechanism by which estrogen regulates Set8 expression. The results of this study demonstrated that the effects of estradiol and BPA on HME expression vary and that the presence of both the estrogen receptor and AR may be important for therapeutic intervention.[63]

In a very recent work, BG-1 ovarian cancer cells were cultured in the presence of estradiol, BPA, and nonylphenol (NP). To confirm the effect of the applied substances, the alteration of EMT

markers such as vimentin was examined at mRNA levels using real-time and reverse-transcription polymerase chain reaction (RT-PCR). The expressions of Snail, Slug, and vimentin were enhanced by the treatment of estradiol, BPA, or NP versus the control (DMSO). Vimentin protein and Snail protein increased by estradiol and two EDCs, whereas E-cadherin decreased. Because EMT response in cancer cells can affect metastasis, the researchers also determined the migration ability caused by estradiol, BPA, or NP. Consequently, estradiol, BPA, and NP enhanced the migration capability of BG-1 cells and increased the expression of MMP-9 protein. Furthermore, to examine whether EMT and migration of BG-1 cancer cells were induced by BPA or NP via the ER dependent pathway, the cells were cotreated with the ER antagonist ICI 182,780 in the presence of estradiol, BPA, or NP. After this treatment, the expressions of E-cadherin, vimentin, Snail, and Slug were reversed. Moreover, ICI 182,780 cotreatment decreased the migration ability of BPA and NP to the control level. These results indicate that both BPA and NP may have the ability to influence ovarian cancer metastasis by regulating EMT markers and migration in ER-expressing BG-1 ovarian cancer cells.[126]

BPA and phthalates were also shown to exhibit similar toxicogenomics and adverse effects on human health due to their common interaction on 89 genes/proteins including ERα and ERβ, NR4A1 (nuclear receptor subfamily 4, group A), TNF-β, IGF1 (somatomedin C), IGF2 (somatomedin A), N-cadherin, PPARγ, and proliferating cell nuclear antigen (PCNA). It can be postulated that their combined effects can also be important in EMT, cancer progression, and invasion.

VII. CONCLUSION

In recent years, concern about roles of EDCs on cancer promotion and progression has been increasing. These chemicals, particularly phthalates and BPA, seem to have an impact on EMT, a very important but insufficiently investigated, mechanism that involves their effect on cancer progression. Studies on cultured breast cells clearly demonstrate that these chemicals can lead to EMT through different mechanisms. EDCs seem to affect prostate cancer progression by interfering with different genes/proteins. However, other mechanisms may need to be investigated. Because humans are exposed to these chemicals concomitantly, their combined effects on EMT must be clearly determined by future studies. Mechanistic *in vitro* and *in vivo* experiments will be useful for determining the effects of these chemicals on cancer and will help regulatory authorities and the entire community take strong precautions that limit their production and thereby exposure. Our future aim is to investigate the mechanism of EMT caused by both phthalates and BPA by *in vitro* and *in vivo* studies. In addition, more studies are needed to determine the relationship between EMT and other endocrine-disrupting substances.

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