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Effects of Phthalates on Epithelial Mesenchymal Transition

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Effects of Phthalates on Epithelial Mesenchymal Transition

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Abstract

Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells lose their polarity and ability to adhere. Instead, they gain properties to move, migrate through the extracellular matrix and become invasive. Finally, they become mesenchymal stem cells. This trans-differentiation is critical for the development of the embryo, wound healing and stem cell behavior. However, this phenomenon is also observed in cancer progression. Phthalates are endocrine disrupting chemicals (EDCs). Over the last years, several studies have been performed concerning the effects of EDCs on human complex diseases, such as different types of cancers. These chemicals are suggested to disrupt the normal hormonal balance (usually by existing estrogenic or anti-androgenic properties), stimulate the development of reproductive tumors and steroid hormone dependent cancers, such as breast cancer. Di(2-ethylhexyl) phthalate (DEHP) is the most abundant phthalate and has 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 the gap junction intercellular communication and promote EMT. Phthalates may also cause the proliferation and metastasis of cancer cells and tumor progression *via* upregulating histone deacetylase 6 (HDAC6), an enzyme that regulates different signaling pathways and EMT. Besides, phthalates can also activate peroxisome proliferator activated-receptors (PPARs), which might eventually lead to high proliferation of the cancer cells and EMT. Both human PPARα and PPARγ were shown to be activated by the major metabolite of DEHP, namely mono(2-ethylhexyl)phthalate (MEHP). More studies are needed to show the underlying mechanisms of EMT caused by different EDCs.

Keywords: Bisphenol A, E-cadherin, Epithelial-mesenchymal transition, N-cadherin, phthalate, vimentin

Introduction

Epithelial-Mesenchymal Transition

Epithelial cells are single cell layers with various functions. Epithelial cells have apical–basal polarity, adhere and communicate with each other through specialized intercellular junctions. Besides, these cells are positioned on a basement membrane that helps define their physiology. In this way, the epithelia function as permeability barriers that delineate tissues and organs. The transition of epithelial cells into mesenchymal cells (EMT), in development or pathologically, follows a common and conserved program with hallmarks. Therefore, EMT is a process in which epithelial cells lose their apical-basal polarity and ability to adhere. Instead, they gain properties to move, migrate through the extracellular matrix and become invasive (Hay, 1995).

The first steps of EMT are the disassociation of epithelial cell to cell contacts (i.e. the disruption of tight junctions, adherens junctions, desmosomes and gap junctions) and the loss of cell polarity (through the disruption of the Crumbs, partitioning defective (PAR) and Scribble (SCRIB) polarity complexes) (Lamouille et al., 2014). After EMT, epithelial cells acquire a mesenchymal phenotype. This phenotype has improved ability to move and migrate in an extracellular environment (Thiery et al., 2009). The trans-differentiation of epithelial cells into motile mesenchymal cells is critical for cancer progression. Thus, EMT has a crucial role for the transformation from benign cells to invasive carcinoma cells so that they can reach other parts of the body to cause metastasis (Lamouille et al., 2014).

Subtypes of Epithelial-Mesenchymal Transition

EMT has three different subtypes (Polyak and Weinberg, 2009): Type 1 EMT is associated with implantation, gastrulation and tissue-organ development in the embryo. Type 2 covers wound healing, tissue regeneration and organ fibrosis. Type 3 is related to cancer progression, invasiveness and metastasis. When cells undergo Type 3 EMT, they gain the resistance to radio-chemotherapy and apoptosis. Carcinoma cells undergo type 3 EMT for invasion and metastasis. This process leads to life-threatening manifestations of cancer progression (Kalluri and Weinberg, 2009).

Epithelial-Mesenchymal Transition Process

During EMT, epithelial cells reconstitute their cytoskeleton and have alterations in their signaling programs that describe the cell shape. They also undergo reprogramed gene expression, which enhances their motility and creates an invasive phenotype (Lamouille et al., 2014). The molecular processes in the cells undergoing EMT are detachment from neighboring cells and migration in the adjacent tissue; E-cadherin down-regulation, up-regulation of vimentin and N-cadherin, actin cytoskeleton reorganization and up-regulation and/or nuclear translocation of transcription factors such as β-catenin and proteins like Snail (Thiery and Sleeman, 2006).

Molecules Related to Epithelial-Mesenchymal Transition

The research for understanding the essential roles, inducing factors, underlying mechanisms and progression of both EMT in cancer has significantly increased. Both developmental studies in various organisms and tissue culture studies describe a number of distinct signaling pathways that regulate EMT (Yang and Weinberg, 2008). Studies on the molecular basis of EMT show 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 beta family members (TGF-β), epidermal growth factor (EGF) family members, fibroblast growth factors (FGF), hepatocyte growth factor (EGF) and insulin–like growth factor are associated with the induction of EMT (Tsuji et al., 2009).

Increasing and Decreasing Proteins during EMT

To describe and prove the molecular changes during EMT, several phynotypic markers are being used (Lee et al., 2006). These markers usually show increased capacity of migration, the three-dimensional invasion and resistance to apoptosis.

The common markers can be divided in two main groups (Lee et al., 2006):

1. *Increasing proteins during EMT:* N-cadherin, vimentin, fibronectin, snail1 (snail), snail2 (slug), Twist, Goosecoind, FOXC2.
2. *Decreasing proteins during EMT:* E-cadherin, desmoplakin, cytokeratin, occludin.

Cadherin Protein Family

Cadherins are a group of transmembrane proteins that serve as the major adhesion molecules located within the adherens junctions. They can regulate cell-cell adhesion through their extracellular domain and their cytosolic domains connect to the actin cytoskeleton by binding to catenins. The cadherin protein family is also involved in the control of cell movement (Takeichi, 1991, Gumbiner and McCrea, 1993; Gumbiner, 1996).

N(neural)-cadherin (CDH2, Cadherin-2) is a calcium dependent cell-cell adhesion glycoprotein comprising five extracellular cadherin repeats, a transmembrane region and a highly conserved cytoplasmic tail. Other than in neurons, N-cadherin is also expressed in endothelial cells. The protein functions during gastrulation and is required for the establishment of left-right asymmetry. At certain central nervous system synapses, presynaptic to postsynaptic adhesion is mediated at least in part by this gene product (NCBI, 2014). A common feature of cancer of epithelial origin is the increased *de novo* expression of N-cadherin and the concomitant down-regulation of E-cadherin, which is called the "cadherin switch" (Hazan et al, 2004). Other than a switch from the expression of E-cadherin to the expression of N-cadherin, this term also includes situations in which E-cadherin expression levels do not change significantly but N-cadherin expression increases within the cells. Besides, it

includes examples in which other cadherins replace or are co-expressed with E-cadherin, including R-cadherin, cadherin 11, T-cadherin and even P-cadherin, and the expression of the `inappropriate cadherin' might alter the behavior of the tumor cells (Derycke and Bracke, 2004; Paredes et al., 2005; Riou et al., 2006; Stefansson et al., 2004; Taniuchi et al., 2005).

N-cadherin promotes tumor cell survival, migration and invasion, and a high level of its expression is often associated with poor prognosis. The dysregulation of the N-cadlerin's function may significantly contribute to the development of pathologic situations, including cancer. E-cadherin, on the other hand, is a major calcium dependent cell-cell adhesion molecule, which is expressed in most epithelial cells and functions to establish cell polarity and maintain normal tissue structure. It is an inhibitor of invasion (Liu et al., 2010). It has been suggested that E -cadherin might be related to invasiveness and the progression of many human epithelial tumor types and can be defined as a "tumor–metastasis suppressor gene" (Birchmeier, 1995). On the other hand, the loss of or decrease in catenin's expression is important in tumor progression (Takayama et al., 1996). E-cadherin downregulation can be accompanied by an increased expression of mesenchymal N-cadherin that promotes inappropriate signals through interaction with the stromal cells (Cavallaro and Christofori, 2004). A decrease in the E-cadherin expression can result in the switching of cell morphology and therefore cause EMT induction (Yao et al., 2011). Functional loss of E-cadherin occurs in many carcinomas and is associated with a high tumor grade and invasiveness (Peinado et al., 2007).

Vimentin

Vimentin is a type III intermediate filament protein normally found in mesenchymal cells. It has important roles in embryogenesis, organogenesis, wound healing, and tumor invasion. This protein can be expressed in migratory epithelial cells in the necessary cases (Chaw et al., 2012). 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 (Gilles et al., 2003; Mandal et al., 2008).

Endocrine Disruptors

The potential effect of environmental compounds on human health is a major concern because humans are daily exposed to various harmful chemicals *via* pharmaceuticals, pesticides, air pollutants, industrial chemicals, heavy metals, and food. Endocrine disrupting chemicals (EDCs) are man-made substances that interfere with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that are responsible for the maintenance, homeostasis, reproduction, development and/or behavior (Kavlock, 1999). EDCs, like dioxins, heavy metals (cadmium, lead), parabens, bisphenol A (BPA), and phthalates, are suggested to induce the different epigenetic modifications (Auduze et al., 2010). Over the last years, multiple

lines of studies have been established to understand the connection between environmental toxicants and human complex diseases, such as cancer.

Phthalates

Phthalates or phthalate esters are esters of phthalic acid. They are mainly used as plasticizers to increase flexibility, transparency, durability, and longevity of plastic material. Phthalates are used in a large variety of products, including personal care products (perfume, eye shadow, moisturizer, nail polish, liquid soap, and hair spray), medical devices, detergents and surfactants, packaging, children's toys, paints, floorings, windows, printing inks and coatings and in the pharmaceutical industry (enteric coatings of pharmaceutical pills, lubricants, binders, emulsifying agents, and suspending agents) (Rudel and Perovich, 2008).

Phthalates have been widely connected with reproductive disorders, gynecomastia, and the early onset of puberty in rodents and humans (Durmaz et al, 2010; Frederiksen et al., 2007; Erkekoglu et al., 2010). These substances are also suggested to increase the incidence of the development of reproductive tumors (Frederiksen et al., 2007). Moreover, both animal and human studies suggest that prenatal and/or postnatal exposure to phthalates could be the cause of decreased fertility and later life testicular tumors (Lopez-Carrillo et al., 2010). The term testicular dysgenesis syndrome (TDS) is used for this range of male reproductive defects (Fisher et al., 2003; Yiee and Baskin, 2010). Besides, new research has connected these substances to obesity, autism, allergies, neurological disorders and cancers (Auduze et al., 2010, Singh and Li, 2012).

Some phthalates have been restricted in the European Union for use in children's toys since 1999. Di(2-ethylhexyl)phthalate (DEHP), benz butyl phthalate (BBP), and dibutyl phthalate (DBP) are restricted for all toys; diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), and di-n -octyl phthalate (DnOP) are restricted only in toys that can be inserted in the mouth. The restriction states that the amount of phthalates may not be greater than 0.1% mass percent of the plasticized part of the toy. These phthalates are allowed at any concentration in other products and other phthalates are not restricted (European Commission, 1999).

Epidemiological studies suggest that phthalates may play an important role in steroid hormone dependent cancers (breast, uterine, testis and prostate). Diethyl phthalate (DEP) was suggested to induce breast cancer especially following exposure from the environment (Chen and Chien, 2014).

Effects of Phthalates on Epithelial Mesenchymal Transition

DNA methylation, histone modification, and the expression of non-coding RNAs (including microRNAs) are the most important mechanisms underlying epigenetic alterations. The epigenetic effects on EDCs, such as phthalates, have expanded our understanding of the etiology of human complex diseases such as cancers and diabetes.

Phthalates are epigenetically toxic. However, they are also genotoxic and cause single strand DNA breaks (Erkekoglu and Kocer-Gumusel, 2014; Rusyn et al., 2006). In rodent liver, they were shown to induce peroxisome proliferation, which later caused an increase in cell size, cell growth and cell division. Phthalates can induce cellular proliferation through a mechanism that involves the activation peroxisome proliferator activated-receptors (PPARs) (Rusyn, 2006; Erkekoglu et al., 2014; Venkata et al., 2006).

Phthalates and Studies with MCF7 Breast Cancer Cells

In MCF7 breast cancer cells, treatment with BBP led to the demethylation of estrogen receptor α (ERα) promoter-associated CpG islands, suggesting that altered ER mRNA expression by BBP might be related to aberrant DNA methylation in the promoter region of the receptor (Kang and Lee, 2005). Exposure to DEHP during sexual differentiation of rats caused male reproductive tract malformations and the abnormal expression of insulin-like growth factor-(IGF-1), c-kit ligand (KITL), and leukemia inhibitory factor (LIF), genes that may contribute to the reproductive toxicity of phthalates (Lin et al., 2008).

In vitro exposure of human cells or tissues to DEHP alters apoptosis, mitotic rate and increases cell proliferation. Phthalates were shown to affect cell proliferation and the inhibition of tamoxifen-induced apoptosis in ER–positive MCF-7 cells; however this phenomenon was not observed in the ER–MDA-MB-231 cells (Kim et al., 2004). The stimulation of proliferation of MCF-7 cells by butyl benzyl phthalate (BBP) and di-n-butyl phthalate (DBP) can be completely suppressed by the ER antagonist, ICI182780 (Okubo et al., 2003). 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 (Chen and Chien, 2014).

Phthalates and Histone Deacetylase

A recent work (Hsieh R-H, 2012) 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 the expression of SV40 large Tantigen and subsequent transformation by X-radiation, the new cell line (referred to as R2d cells) was exposed to phthalates (DBP and BBP). Particularly, the researchers focused on the role of HDAC6 and the results showed that phthalates stimulated EMT through 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. A hypothetical mechanism of the 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 was also proposed (Hsieh et al., 2012).

Besides, the findings of another study supported that HDAC6 is required for TGF-α-induced transition of the epithelial-like phenotype into a mesenchymal phenotype *via* the SMAD3 signaling the pathway in various lung cancer cell lines (Shan et al., 2008). 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 (Fernandez and Russo, 2010).

Phthalates and Gap Junction Intercellular Communication

A short time of exposure to DEHP could inhibit the gap junction intercellular communication (GJIC) (Mikalsen and Sanner, 1993). It was shown that DEHP exposure induces the inhibition of GJIC in hepatic carcinomas and adenomas in rodents (Isenberg et al., 2000). Besides, in Chise Hamster V79 cells exposed DEHP and MEHP, inhibition of GJIC was also reported (Cruciani et al., 1997).

Phthalates and Other Mechanisms

Phthalates were also shown to promote the invasion and metastasis of SK-N-SH human neuroblastoma cells by inducing cell motility that is mediated by matrix metalloproteinase-2 and -9 expressions, again through the PI3K/AKT pathway (Zhu et al., 2010). Cell motility is an important feature in tumor progression following EMT (Thiery, 2002; Thompson et al., 2005; Xue et al., 2003), which may be regulated by estrogen (Ding et al., 2006) and thr transforming growth factor-beta (TGF-β) (Valcourt et al., 2005).

Conclusions

Concerns on the roles of EDCs on cancer promotion and progression have increased in the last years. EDCs, particularly phthalates seem to have an impact on EMT, which is a very important mechanism, concerning their effect on cancer progression. Studies on cultured breast cells and prostate cells clearly demonstrate that these chemicals can induce EMT through affecting the expression of different proteins. New mechanistic studies are needed in determining the effects of these chemicals on cancer progression. The results of these studies will be very useful for scientists to understand the mode of action of these chemicals and to force the regulatory authorities for taking strong precautions concerning the use of these chemicals especially in baby products.

References

Audouze, K., Juncker, A.S., Roque, F.J., Krysiak-Baltyn, K., Weinhold, N., Taboureau, O, Jensen, T.S., Brunak, S. Deciphering diseases and biological targets for environmental chemicals using toxicogenomics networks. PLoS Comput. Biol. 6, 5 (May 2010), e1000788.

Birchmeier, W. E-cadherin as a tumor (invasion) suppressor gene. Bioessays. 17, 2 (Feb 1995), 97-99.

Cavallaro, U., Christofori, G. Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. Nat. Rev. Cancer. 4, 2 (Feb 2004), 118-132.

Chaw, S.Y., Majeed, A.A., Dalley, A.J., Chan, A., Stein, S., Farah, C.S. Epithelial to mesenchymal transition (EMT) biomarkers--E-cadherin, beta-catenin, APC and Vimentin--in oral squamous cell carcinogenesis and transformation. Oral Oncol. 48, 10 (Oct 2012), 997-1006.

Chen, F.P., Chien, M.H. Lower concentrations of phthalates induce proliferation in human breast cancer cells. Climacteric. 17, 4 (Aug 2014), 377-384.

Cruciani, V., Mikalsen, S.O., Vasseur, P., Sanner, T. Effects of peroxisome proliferators and 12-O-tetradecanoyl phorbol-13-acetate on intercellular communication and connexin43 in two hamster fibroblast systems. Int. J. Cancer. 73, 2 (Oct 1997), 240-248.

Derycke, L.D., Bracke, M.E. N-cadherin in the spotlight of cell-cell adhesion,differentiation, embryogenesis, invasion and signalling. Int. J. Dev. Biol. 48, 5-6 (2004), 463-476.

Ding, J.X., Feng, Y.J., Yao, L.Q., Yu, M., Jin, H.Y., Yin, L.H. The reinforcement of invasion in epithelial ovarian cancer cells by 17 beta-Estradiol is associated with up-regulation of Snail. Gynecol. Oncol. 103, 2 (Nov 2006), 623-630.

Durmaz, E., Ozmert, E.N., Erkekoglu, P., Giray, B., Derman, O., Hincal, F., Yurdakök K. Plasma phthalate levels in pubertal gynecomastia. Pediatrics. 125, 1 (Jan 2010), e122-e129.

Erkekoglu, P., Kocer-Gumusel, B. Genotoxicity of phthalates. Toxicol. Mech. Methods. 24, 9 (Dec 2014), 616-626.

Erkekoglu P, Rachidi W, Yuzugullu OG, Giray B, Favier A, Ozturk M, Hincal F. Evaluation of cytotoxicity and oxidative DNA damaging effects of di(2-ethylhexyl)-phthalate (DEHP) and mono(2-ethylhexyl)-phthalate (MEHP) on MA-10 Leydig cells and protection by selenium. Toxicol. Appl. Pharmacol. 248, 1 (Oct 2010), 52-62.

Erkekoglu, P., Zeybek, N.D., Giray, B.K., Rachidi, W., Kızılgün, M., Hininger-Favier, I., Favier, A., Asan, E,. Hincal, F. The effects of di(2-ethylhexyl)phthalate on rat liverin relation to selenium status. Int. J. Exp. Pathol. 95, 1 (Feb 2014), 64-77.

European Commission. Press Release Database. Ban of phthalates in childcare articles and toys IP/99/829 Brussels, (10 November 1999). Available at: http://europa.eu/rapid/press-release_IP-99-829_en.htm?locale=en.

Fernandez, S.V., Russo, J. Estrogen and xenoestrogens in breast cancer. Toxicol. Pathol. 38, 1 (Jan 2010), 110-122.

Fisher, J.S., Macpherson, S., Marchetti, N., Sharpe, R.M. Human 'testicular dysgenesis syndrome': a possible model using in-utero exposure of the rat to dibutylphthalate. Hum. Reprod. 18, 7 (Jul 2003), 1383-1394.

Frederiksen, H., Skakkebaek, N.E., Andersson, A.M. Metabolism of phthalates in humans. Mol. Nutr. Food Res. 51, 7 (Jul 2007), 899-911.

Gilles, C., Polette, M., Mestdagt, M., Nawrocki-Raby, B., Ruggeri, P., Birembaut, P., Foidart J.M. Transactivation of vimentin by beta-catenin in human breast cancer cells. Cancer Res. 63, 10 (May 2003), 2658-2664.

Gumbiner, B.M. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell. 84, 3 (Feb 1996): 345-357.

Gumbiner, B.M., McCrea, P.D. Catenins as mediators of the cytoplasmic functions of cadherins. J. Cell Sci. Suppl. 17, (1993), 155-158.

Hay, E.D. An overview of epithelio-mesenchymal transformation. Acta Anat (Basel). 154, (1995), 8-20.

Hazan, R.B., Qiao, R., Keren, R., Badano, I., Suyama, K. Cadherin switch in tumor progression. Ann. N. Y. Acad. Sci. 1014, (Apr 2004), 155-163.

Hsieh, T-H., Tsai, C.F., Hsu, C.Y., Kuo, P.L., Lee, J.N., Chai, C.Y., Hou, M.F., Chang, C.C., Long, C.Y., Ko YC, Tsai, E.M. Phthalates stimulate the epithelial to mesenchymal transition through an HDAC6-dependent mechanism in human breast epithelial stem cells. Toxicol. Sci. 128, 2 (Aug 2012), 365-376.

Isenberg, J.S., Kamendulis, L.M., Smith, J.H., Ackley, D.C., Pugh, G. Jr., Lington, A.W., Klaunig, J.E. Effects of Di-2-ethylhexyl phthalate (DEHP) on gap-junctional intercellular communication (GJIC), DNA synthesis, and peroxisomal beta oxidation (PBOX) in rat, mouse, and hamster liver. Toxicol. Sci. 56, 1 (Jul 2000), 73-85.

Kalluri, R., Weinberg, R.A. The basics of epithelial-mesenchymal transition. J. Clin. Invest. 119, 6 (Jun 2009),1420-1428.

Kang, S.C., Lee, B.M. DNA methylation of estrogen receptor alpha gene by phthalates. J Toxicol. Environ. Health. 68, (2005), 1995–2003.

Kavlock, R.J. Overview of endocrine disruptor research activity in the United States. Chemosphere. 39, 8 (Oct 1999), 1227-1236.

Kim, I.Y., Han, S.Y., Moon, A. Phthalates inhibit tamoxifen-induced apoptosis in MCF-7 human breast cancer cells. J. Toxicol. Environ. Health A. 67, 23-24 (Dec 2004), 2025-2035.

Lamouille, S., Xu, J., Derynck, R. Molecular mechanisms of epithelial-mesenchymal transition. Nat. Rev. Mol. Cell. Biol. 15, 3 (Mar 2014), 178-196.

Lee, J.M., Dedhar, S., Kalluri, R., Thompson, E.W. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. J. Cell Biol. 172, 7 (Mar 2006), 973-981.

Lin, H., Ge, R.S., Chen, G.R., Hu, G.X., Dong, L., Lian, Q.Q. Involvement of testicular growth factors in fetal Leydig cell aggregation after exposure to phthalate in utero. Proc. Natl. Acad. Sci. USA. 105, (2008), 7218–7222.

Liu, L.K., Jiang, X.Y., Zhou, X.X., Wang, D.M., Song, X.L., Jiang, H.B. Upregulation of vimentin and aberrant expression of E-cadherin/beta-catenin complex in oral squamous cell carcinomas: correlation with the clinicopathological features and patient outcome. Mod. Pathol. 23, 2 (Feb 2010), 213-224.

Lopez-Carrillo, L., Hernandez-Ramirez, R.U., Calafat, A.M., Torres-Sanchez, L, Galván-Portillo, M., Needham, L.L., Ruiz-Ramos, R., Cebrián, M.E. Exposure to phthalates and breast cancer risk in northern Mexico. Environ. Health Perspect. 118, 4 (Apr 2010), 539-544.

Mandal, M., Myers, J.N., Lippman, S.M., Johnson, F.M., Williams, M.D., Rayala, S., Ohshiro, K., Rosenthal, D.I., Weber, R.S., Gallick, G.E., El-Naggar, A.K. Epithelial to mesenchymal transition in head and neck squamous carcinoma: association of Src activation with E-cadherin down-regulation, vimentin expression, and aggressive tumor features. Cancer. 112, 9 (2008), 2088-2100.

Mikalsen, S.O., Sanner ,T. Intercellular communication in colonies of Syrian hamster embryo cells and the susceptibility for morphological transformation. Carcinogenesis. 14, 2 (Feb 1993), 251-257.

NCBI. CDH2 cadherin 2, type 1, N-cadherin (neuronal) [Homo sapiens (human)]. 2014. Available at: http://www.ncbi.nlm.nih.gov/gene/1000.

Okubo, T., Suzuki, T., Yokoyama, Y., Kano, K., Kano, I. Estimation of estrogenic and anti-estrogenic activities of some phthalate diesters and monoesters by MCF-7 cell proliferation assay in vitro. Biol. Pharm. Bull. 26, 8 (Aug 2003), 1219-24.

Paredes, J., Albergaria, A., Oliveira, J.T., Jerónimo, C., Milanezi, F., Schmitt. F.C. P-cadherin overexpression is an indicator of clinical outcome in invasive breast carcinomas and is associated with CDH3 promoter hypomethylation. Clin. Cancer. Res. 11, 16 (Aug 2005), 5869-5877.

Peinado, H., Olmeda, D., Cano, A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat Rev Cancer. 7, 6 (Jun 2007), 415-428.

Polyak, K., Weinberg, R.A. Transitions between epithelial and mesenchymal states:acquisition of malignant and stem cell traits. Nat. Rev. Cancer. 9, 4, (Apr 2009), 265-273.

Riou, P., Saffroy, R., Chenailler, C., Franc, B., Gentile, C., Rubinstein, E., Resink, T., Debuire, B., Piatier-Tonneau, D., Lemoine, A. Expression of T-cadherin in tumor cells influences invasive potential of human hepatocellular carcinoma. FASEB J. 20, 13 (Nov 2006), 2291-2301.

Rudel, R., Perovich, L. Endocrine disrupting chemicals in indoor and outdoor air. Atmospheric. Environ. 43, 1 (January 2008), 170–81.

Rusyn, I., Peters, J.M., Cunningham, M.L. Modes of action and species-specific effects of di-(2-ethylhexyl)phthalate in the liver. Crit. Rev. Toxicol. 36, 5 (May 2006), 459-479.

Shan, B., Yao, T.P., Nguyen, H.T., Zhuo, Y., Levy, D.R., Klingsberg, R.C., Tao, H., Palmer, M.L., Holder, K.N., Lasky, J.A. Requirement of HDAC6 for transforming growth factor-beta1-induced epithelial-mesenchymal transition. J. Biol. Chem. 283, 30 (Jul 2008), 21065-21073.

Singh, S., Li, SS. Bisphenol A and phthalates exhibit similar toxicogenomics and health effects. Gene. 494, 1 (Feb 2012), 85-91.

Stefansson, I.M., Salvesen, H.B., Akslen, L.A. Prognostic impact of alterations in P-cadherin expression and related cell adhesion markers in endometrial cancer. J. Clin. Oncol. 22, 7 (Apr 2004), 1242-1252.

Takayama T, Shiozaki H, Shibamoto S, Oka H, Kimura Y, Tamura S, Inoue M, Monden T, Ito F, Monden M. Beta-catenin expression in human cancers. Am. J. Pathol. 148, 1 (Jan 1996), 39-46.

Takeichi, M. Cadherin cell adhesion receptors as a morphogenetic regulator. Science. 1991 Mar 22;251(5000):1451-5.

Taniuchi, K., Nakagawa, H., Hosokawa, M., Nakamura, T., Eguchi, H., Ohigashi, H., Ishikawa, O., Katagiri, T., Nakamura, Y. Overexpressed P-cadherin/CDH3 promotes motility of pancreatic cancer cells by interacting with p120ctn and activating rho-family GTPases. Cancer Res. 65, 8 (Apr 2005), 3092-3099.

Thiery, J.P. Epithelial-mesenchymal transitions in tumour progression. Nat. Rev. Cancer. 2, 6 (Jun 2002), 442-454.

Thiery, J.P., Sleeman, J.P. Complex networks orchestrate epithelial-mesenchymal transitions. Nat. Rev. Mol. Cell Biol. 7,2 (Feb 2006), 131-142.

Thiery, J.P., Acloque, H., Huang, R.Y., Nieto, M.A. Epithelial-mesenchymal transitions in development and disease. Cell. 139, 5 (Nov 2009), 871-890.

Thompson, C.J., Ross, S.M., Hensley, J., Liu, K., Heinze, S.C., Young, S.S., Gaido, K.W. Differential steroidogenic gene expression in the fetal adrenal gland versus the testis and rapid and dynamic response of the fetal testis to di(n-butyl)phthalate. Biol. Reprod. 73, 5 (Nov 2005), 908-917.

Tsuji, T., Ibaragi, S., Hu, G.F. Epithelial-mesenchymal transition and cell cooperativity in metastasis. Cancer Res. 69, 18 (Sep 2009), 7135-7139.

Valcourt, U., Kowanetz, M., Niimi, H., Heldi,n C.H., Moustakas, A. TGF-beta and the Smad signaling pathway support transcriptomic reprogramming during epithelial-mesenchymal cell transition. Mol. Biol. Cell. 16, 4 (Apr 2005), 1987-2002.

Venkata, N.G., Robinson, J.A., Cabot, P.J., Davis, B., Monteith, G.R., Roberts-Thomson, S.J. Mono(2-ethylhexyl)phthalate and mono-n-butyl phthalate activation of peroxisome proliferator activated-receptors alpha and gamma in breast. Toxicol. Lett. 163, 3 (Jun 2006), 224-234.

Xue, C., Plieth, D., Venkov, C., Xu, C., Neilson, E.G. The gatekeeper effect of epithelial-mesenchymal transition regulates the frequency of breast cancer metastasis. Cancer Res. 63, 12 (Jun 2003), 3386-3394.

Yang, J., Weinberg, R.A. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev. Cell. 14, 6 (Jun 2008), 818-829.

Yao, D., Dai, C., Peng, S. Mechanism of the mesenchymal-epithelial transition and its relationship with metastatic tumor formation. Mol. Cancer Res. 9, 12 (Dec 2011), 1608-1620.

Yiee, J.H., Baskin, L.S. Environmental factors in genitourinary development. J. Urol. 184, 1 (Jul 2010), 34-41.

Zhu, X.B., Tay, T.W., Andriana, B.B., Alam, M.S., Choi, E.K., Tsunekawa, N., Kanai, Y., Kurohmaru, M. Effects of di-iso-butyl phthalate on testes of prepubertal rats and mice. Okajimas Folia Anat. Jpn. 86, 4 (Feb 2010), 129-136.