Journal of Developmental Origins of Health and Disease (2011), **2**(1), 9–16.

doi:10.1017/S2040174411000043

REVIEW

Cancer as development gone awry: the case for bisphenol-A as a carcinogen

C. Sonnenschein, P. R. Wadia, B. S. Rubin and A. M. Soto*

Program in Cellular Molecular and Developmental Biology, Tufts University School of Medicine, Boston, MA, USA

The discovery of a rare clear cell carcinoma of the vagina in young women gestationally exposed to the estrogen diethylstilbestrol (DES) lent empirical support to the hypothesis that prenatal exposure to xenoestrogens might cause cancer. This fact contradicted two well-accepted notions: (i) mammalian development was merely the unfolding of a genetic program and (ii) only mutagenic agents could cause cancer. The ecological developmental biology (eco–devo) movement revitalized the concept of developmental plasticity through the occurrence of polyphenisms whereby a single genotype produces diverse phenotypes which are determined by environmental cues. Based on the principles of eco–devo and the tissue organization field theory of carcinogenesis, we hypothesized that developmental exposure to xenoestrogens increased the propensity to develop mammary cancer during adulthood. Bisphenol-A (BPA), a ubiquitous xenoestrogen, was chosen as a model for environmental estrogen exposure. In mice, perinatal exposure to environmentally relevant BPA levels induced alterations of the mammary gland architecture which manifested during fetal morphogenesis and throughout life, including the development of pre-neoplastic lesions. In rats, gestational exposure to BPA induced pre-neoplastic lesions and carcinoma *in situ* that manifested in adulthood in the absence of any additional treatment. Emerging epidemiological data reveal an increased incidence of breast cancer in women exposed to DES during gestation. Hence, both animal experiments and epidemiological data strengthen the hypothesis that fetal exposure to xenoestrogens may be an underlying cause of the increased incidence of breast cancer observed over the past 50 years.

Received 24 August 2010; Revised 16 December 2010; Accepted 12 January 2011

Key words: endocrine disruptors, fetal mammary gland development, fetal origins of adult disease, stroma–epithelium interactions, tissue organization field theory

Introduction

The classical view of mammalian development proposes that, other than to mediate aspects of androgenization of the male brain in some mammals, estrogens play an inconsequential role during organogenesis of the reproductive system and mammary glands. It was also assumed that, in the absence of male hormones, the default outcome would be feminine. Recent findings in the field of environmental endocrine disruption have revealed this view to be incorrect because developmental exposure to estrogenic chemicals has been shown to induce morphological, functional and behavioral anomalies associated with reproduction.[1–5] These and previous findings[6–8] are prompting a re-evaluation of the basic concepts that drive developmental and cancer research.[9]

The beginning of the 21st century brought a paradigm change in biology. The realization that reductionism has failed to bring about an understanding of complex phenomena has resulted in reappraisals of old research concepts in embryology and cancer research. We will briefly review the historical and theoretical underpinnings of this paradigmatic change because they are central to the study of fetal origins of adult disease in general and the fetal xenoestrogen exposure syndrome in particular.

*Address for correspondence: Dr A. M. Soto, Program in Cellular Molecular and Developmental Biology, Tufts University School of Medicine, Boston, MA 02111, USA.
(Emails ana.soto@tufts.edu, cheryl.michaelson@tufts.edu)

Origins of evo–devo

In the middle and late 19th century, the main interest of embryologists was the role of the environment in determining a phenotype.[10] Embryologists frequently observed that nutrition and temperature determined phenotype. From their perspective, the environment was not merely acting on selection of the existing variation (due to different genotypes); rather, the environment was an active determinant of specific adaptations needed to increase fitness. Scott Gilbert identified the development of laboratory animal models as the main circumstance that led embryologists to depart from this tradition in the 20th century. Uniform temperature, invariable light cycles and nutrition *ad libitum* obliterated the manifestation of environmental influences when animals were raised in the laboratory.[10] The philosopher and historian Lenny Moss also identified a change of perception in the 20th century that led to the 'phylogenetic turn' whereby '…the gene and the genetic program became understood to be the principal means by which adapted form is acquired; the *theater of adaptation* changed from that of individual life histories, that is ontogenies, to that of populations over multiple generations, that is phylogenies'.[11] Finally, the dis-

covery of the DNA structure in the 50s led to the perception that biology was reduced to chemistry, and to the notion of a genetic program, in which development was reduced to the automatic execution of a program encoded in the DNA.[12a]

This previously rigid view of development is rapidly changing, as epidemiological studies reveal the developmental plasticity of the human fetus.[14] Thus, we now witness the rebirth of ecological developmental biology. Several paths have been identified that could mediate environmental cues into the building of a phenotype, namely, (i) the neuroendocrine route, whereby the nervous system monitors the environment and transfers signals to the endocrine system, (ii) the epigenetic route, whereby environmental agents change the methylation pattern of genes, thereby altering their transcriptional capabilities and (iii) direct modulation of gene expression, particularly by hormonally active agents.[10] Although there is plenty of evidence that environmental cues affect these paths, the ways they produce changes in phenotype is yet unknown. During the last 50 years, the focus on genetics has mapped the involvement of genes in development by switching them on and off; however, this approach could not explain how form is generated. This realization prompted developmental biologists to return to their tradition, which explained morphogenesis in terms of mechanics and physics.[15–18]

Organicism and reductionism: the role of the cell within the organism

Toward the middle of the 19th century, the cell theory introduced the basic concept that the cell is the unit of life.[19] However, in multicellular organisms, single cells do not have an existence independent of the whole. Organisms and their cells are ontogenetically linked. From the very start of embryonic life, the levels of biological organization are entangled, meaning that a zygote is both a cell and an organism.[18] The reductionist perspective favors bottom-up causation, meaning that cells 'make' the organism by proliferating. In contrast, from a holistic view, the organism 'makes' cells by facilitating their division, indicating that causality is a top–down event. Finally, the organicist view considers the embryo as a dynamic open system whereby there are bottom–up, top–down, reciprocal and multiple causalities; meaning that there is no unique, exclusive type of developmental causality.[18,20]

The organism imposes global constraints, whereas locally biophysical and biochemical interactions among neighboring cells, tissues and cellular environment determine shape. Differential cell movement and differential cell adhesion are the products of variable physical forces within the developing organism. Morphogens, that is, chemicals created in various areas of a developing organism, form a concentration gradient as they disperse, causing cells that receive dissimilar local concentrations to enter distinct developmental pathways. Such a developing system is not a thing, but a process. It is this dynamic property of the organism that results in level entanglement, as exemplified by the dual nature of the zygote, which is a cell and an organism.[18]

How does cancer begin?

In the 19th century, cancer was viewed in the context of the relationship of cells within the organism, and carcinogenesis was viewed in this organicist context. Theories of carcinogenesis then centered on ontogenesis by viewing cancer as a problem of development. For example, Conheim saw cancer as a product of embryonic rests, while Ribbert viewed it as the product of a failure of the restraints exerted by the tissue upon its cells.[21] As recalled above, at the beginning of the 20th century, a change of stance, the 'phylogenetic turn', took place and the center of attention focused on the interior of the cell and the dominant view on carcinogenesis was the somatic mutation theory. From this perspective, cancer is a cell-based disease caused by mutations in the DNA of a single founder cell.[22] The research program emanating from this theory has yet to explain how cancer arises and has failed to provide successful therapies. The re-interpretation of evolutionary trends (*proliferation* as the default state of all cells) has enhanced interest in the earlier focus on the tissue level of organization and the updated tissue organization field theory of carcinogenesis and neoplasia has been gaining momentum.[23,24] A central motif in this theory is the persistence of morphogenic fields throughout adult life; these fields orchestrate histogenesis and organogenesis before birth as well as tissue maintenance and regeneration throughout postnatal life. The tissue organization field theory posits that neoplasms result from a flawed interaction among cells and tissues and that carcinogenesis is potentially reversible.[25]

The new emphasis on eco–devo and carcinogenesis as a problem akin to development gone awry has prompted scientists to hypothesize that fetal exposure to xenoestrogens may be an underlying cause of the increased incidence of uterine leiomyoma, testicular cancer and breast cancer observed in European and US populations over the past 50 years.[26,27]

The developmental xenoestrogen exposure syndrome

During the years between 1948 and 1971, the synthetic estrogen diethylstilbestrol (DES) was administered therapeutically to prevent spontaneous abortion.[6,28] The practice was stopped when rare pathologies like clear cell adenocarcinoma of the vagina and abnormalities in the uterus, oviduct and cervix were diagnosed in young women who had been exposed to DES *in utero*.[6,29] Experiments in rodent models following *in utero* exposure to DES were able to reproduce strikingly similar abnormalities,[6,7] and also revealed an increased incidence of

[a] This view was succinctly summarized by Erwin Schrodinger: 'In calling the structure of the chromosome fibers a code-script we mean that the all-penetrating mind, once conceived by Laplace, to which every causal connection lay immediately open, could tell from their structure whether the egg would develop, under suitable conditions, into a black cock or into a speckled hen, into a fly or a maize plant, a rhododendron, a beetle, a mouse or a woman'.[13]

https://doi.org/10.1017/S2040174411000043 Published online by Cambridge University Press

mammary cancer and an early onset of cessation of estrus cycles. Two decades later, the same outcomes (increased breast cancer incidence and early menopause) were revealed in women gestationally exposed to DES.[30,31]

The DES syndrome played a central role on the conceptualization of environmental endocrine disruptors and was used as a model to guide research on the effect of exposure to xenoestrogens. Developmental exposure to the xenoestrogen bisphenol-A (BPA) in mice caused a complex array of effects that resembled those observed after developmental exposure to DES. Exposure to environmentally relevant doses of BPA during pregnancy alone and during pregnancy through lactation induced both earlier vaginal opening and earlier first estrous and altered estrous cyclicity in offspring. Exposed animals also showed an increase in the number of blood-filled ovarian bursae at 6 months of age, which are thought to be indicative of advanced reproductive aging.[1] In the mammary gland, it induced preneoplastic lesions in mice[2] and carcinoma *in situ* in rats.[3] In addition, when BPA exposure was continued into lactation, it induced increased body weight (BW). Not surprisingly, neonatal exposure to DES also induces obesity.[4]

Mammary gland development: a lifelong process

Like other ectodermal appendages such as feathers, teeth, hair and salivary glands, the fetal mammary gland is formed by reciprocal interactions between multiple tissue compartments. The mammary epithelium, derived from the embryonic ectoderm, and the mammary mesenchyme, derived from the embryonic mesoderm, are first detected in the mouse between embryonic days (E)10 and E11. From E11 to E12.5, the epithelial placodes increase in size to form rounded buds. Of particular interest is the expression of estrogen receptor α and estrogen receptor β mRNA in the mesenchyme during this period (E12.5–14.5).[32] Between E13 and E15, the epithelial buds invaginate into the underlying mesenchymal tissue. At the same time, the mesenchyme abutting the mammary epithelium becomes denser than the surrounding mesenchyme with several concentric layers of fibroblasts aligning themselves around the epithelial compartment. At E15.5, the bud elongates to become a cord, the mammary sprout, which invades the underlying fat pad precursor. Branching of the epithelial cord starts at E16.[33] By E18, branching is apparent and the ductal lumen begins to form.[34] Ductal elongation and branching slow down soon after birth; that is, the gland grows isometrically until puberty.[33]

Estrogens drive massive ductal growth in the peripubertal mammary gland. The ductal ends develop into terminal end buds (TEBs); these are the structures that mediate ductal growth by invasion of the stroma. They become bulbous and show both high proliferative and apoptotic activity. Death of the body cells in the TEBs is essential for the formation of the lumen on the proximal side of the TEBs and for the growth of the subtending duct.[35] Thus, the ductal tree migrates into the stroma, led by a front of large TEBs. When the ductal tree reaches the edge of the fat pad, it eventually establishes a network of ducts, terminal ducts and a few alveolar buds. Once again, this morphology remains relatively quiescent; minor fluctuations occur with each estrous cycle, adding and removing alveolar buds, until pregnancy. During pregnancy, the entire epithelial compartment undergoes dramatic proliferation resulting in a plethora of alveolar buds and lobuloalveolar units in preparation for lactation. Once the period of lactation is over, the mammary gland undergoes rapid involution to return to its pre-pregnancy state, a process associated with widespread apoptosis.[36]

BPA and cancer

About 15 years ago, we performed experiments examining the effect of perinatal exposure to BPA on various endocrine organs and the reproductive tract of Sprague-Dawley rats.[5] Mammary tumors frequently develop in aged animals of this strain. We observed more tumors in the BPA-exposed animals than in their controls. This observation prompted us to collect mammary glands in all subsequent experiments both in the rat and mouse models. A significantly different phenotype was revealed by observing the entire mammary gland of adult animals exposed to BPA during fetal development when compared to vehicle-exposed controls. In addition, we examined mammary gland development from the fetal stage through 1.2 years of age. We hypothesized that in adult animals, the florid phenotype observed was due to (a) a direct action of BPA in the mammary gland anlagen, which altered the morphogenesis of the gland, and (b) an indirect effect induced by BPA on the hypothalamic-hypophyseal-ovarian axis that regulates mammotropic hormone secretion[37] (Fig. 1).

The road to cancer: BPA alters the development of the fetal mammary gland

Exposure to BPA from E8 to E18 has significant effects on the mesenchyme, the compartment where estrogen receptors are then expressed. It promotes maturation of the fat pad, an event necessary for ductal invasion and branching. BPA exposure also alters the localization of collagen fibers by increasing the density of collagen fibers directly abutting the epithelium in BPA-exposed mammary glands, while the density of collagen in the entire stromal compartment is significantly decreased in BPA-exposed females when compared with controls. Within the epithelium, BPA exposure leads to decreased cell size, delayed lumen formation, increased ductal area and ductal extension[34] (Fig. 2). Since mammary gland development is dependent on reciprocal interactions between these compartments, the advanced maturation of the fat pad and changes in the extracellular matrix may be responsible for the altered growth, cell size and inhibition of lumen formation observed in the epithelium. In summary, BPA does not uniformly accelerate the morphological

https://doi.org/10.1017/S2040174411000043 Published online by Cambridge University Press

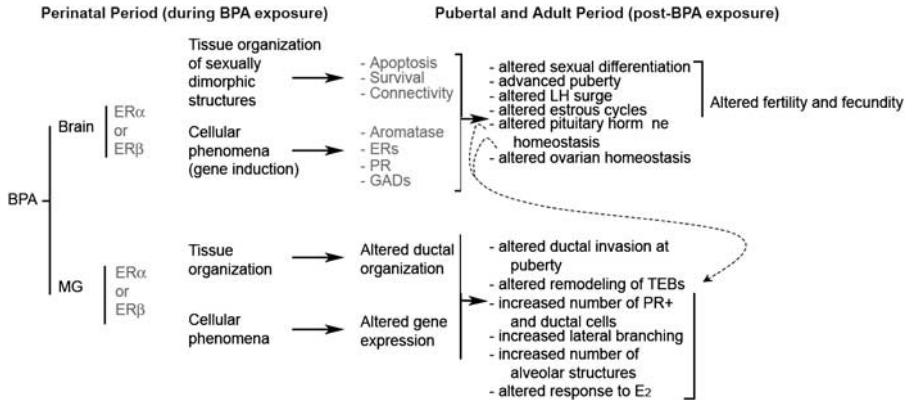

Fig. 1. Effects of bisphenol-A (BPA) exposure during development on female reproduction and mammary gland carcinogenesis. Solid arrows represent pathways whereby BPA affects directly the development of the mammary gland and of sexually dimorphic structures of the brain. Dashed arrows represent secondary effects in the mammary gland mediated by organizational changes in the hypothalamus. The proposed mediators of these outcomes are indicated in red.

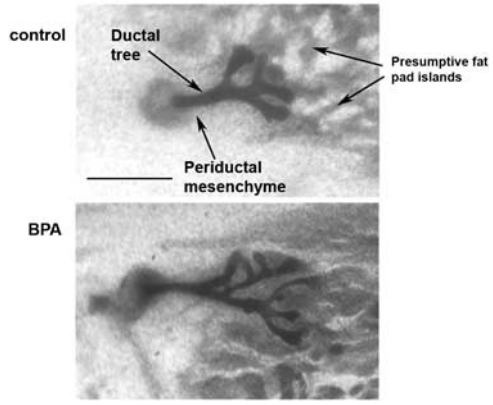

Fig. 2. Bisphenol-A (BPA) alters the organization of the mammary gland anlagen. Dams were exposed to vehicle or to BPA from gestational day 8 to birth. Upper panel: whole mount of the mammary gland at E18 from a vehicle-exposed female fetus. Lower panel, whole mount of the mammary gland from a female fetus exposed to 250 ng BPA/kg body weight/day. BPA-exposure significantly increased ductal area and ductal extension. Scale bar = 0.5 μm.

process; instead, it disrupts it by advancing some events (i.e. fat pad maturation) while delaying others (i.e. lumen formation).

Altered morphogenesis continues long beyond the end of the BPA exposure period

In animals exposed to BPA *in utero*, the discordance between events in the epithelium and stroma persists through postnatal life. For example, at postnatal day 10, the number of epithelial cells undergoing DNA synthesis is significantly reduced in the BPA-exposed animals, while at 30 days of age DNA synthesis in stromal cells is inhibited.[38] At puberty, animals exposed to BPA during fetal development show a decreased rate of ductal invasion of the stroma, probably due to a marked inhibition of apoptosis in the TEB epithelium, a process that results in lumen patency, and thus a decrease in growth of the subtending ducts. Concomitantly, there is an increase in the area and number of TEBs per ductal area, also indicating impaired ductal growth.[39] There is also an increase in progesterone-receptor positive cells lining the ducts, which are organized in clusters, an indication of presumptive branching points. This is followed by a significant increase in branching at 4 months of age.[39]

The morphological changes found in 30-day-old animals exposed perinatally to BPA could be attributed, at least in part, to an increased sensitivity of the mammary gland to circulating estrogens. Indeed, the magnitude of the response to E2 is significantly enhanced in their siblings that are ovariectomized and exposed to E2 for 10 days.[40] These results suggest that increased sensitivity to estrogens drives the induction of progesterone receptor expression in epithelial cells, leading to an increase in lateral branching. By 6 months of age, perinatally exposed virgin mice exhibit mammary glands that resemble those of a pregnant mouse (significant increase in the percentage of ducts, terminal ends, terminal ducts and alveolar buds).[38] These BPA-induced changes in mammary gland development are consistent with the notion that the untimely prenatal exposure to estrogens may predispose the tissue to cancer. Indeed, the persistence of epithelial structures, such as the TEBs and terminal ducts, has been associated with increased carcinogenesis in both rodents and humans.[41,42] Moreover, about 30% of animals that were exposed to similar low doses of BPA during gestation and until postnatal day 16 developed intraductal hyperplasias. The stroma surrounding the ducts bearing intraductal hyperplasias was abnormally collagen-rich.[2] This implies the persistence of altered stromal–epithelial interaction beyond the period of exposure, and suggests that understanding the mechanisms of these alterations during fetal life will also shed light on the development of the neoplastic phenotype observed in adulthood.

https://doi.org/10.1017/S2040174411000043 Published online by Cambridge University Press

From pre-neoplastic lesions to cancer

The identification of pre-neoplastic lesions in the mammary glands of mice prenatally exposed to BPA encouraged us to study a rat model because it more closely mimics the human disease with regard to hormone factors and histopathology. Wistar-Furth rats prenatally exposed to 2.5 μg BPA/kg BW/day administered via subcutaneous osmotic pumps to their mothers resulted in a significantly increased number of intraductal hyperplasias (pre-cancerous lesions) observed at 50 days of age and later, while higher doses induced the development of carcinomas *in situ* (25–33% incidence).[3] In addition, female Wistar rats exposed prenatally to 250 μg BPA/kg BW/day showed an increased proliferation/apoptosis ratio in the epithelial compartment at puberty. During adulthood, those BPA-exposed rats showed an increased number of hyperplastic ducts and the stroma associated with these ducts showed signs of desmoplasia and an increased number of mast cells, suggesting a heightened risk of neoplastic transformation.[43] In addition, animals prenatally exposed to BPA developed palpable tumors when injected at 50 days of age with the chemical carcinogen nitrosomethylurea while animals not exposed to BPA did not.[43] In another study, Sprague–Dawley rats were exposed to 250 μg BPA/kg BW/day via daily gavage at *post partum* days 2 to 20. These perinatally exposed pups showed signs of increased cell proliferation and decreased apoptosis in their mammary gland at puberty. Subsequent exposure to the carcinogen 7, 12, dimethylbenzanthracene at 50 days of age resulted in an increased tumor incidence per animal and a decreased latency period. Invasive cancer was apparent only after administration of a chemical carcinogen at puberty.[44] Even though these studies used different rat strains, exposure routes, exposure levels and end points, they all revealed an increased propensity for neoplastic development.

The appearance of these lesions at puberty is reminiscent of the timing of appearance of DES-induced clear cell carcinoma of the vagina in humans, which manifested with a peak incidence at 19 years of age, suggesting that exposure to ovarian hormones contributes to the development of these pathologies. In the mammary gland, the peripubertal period is characterized by intense ductal morphogenesis encompassing tissue remodeling, epithelial invasion of the stroma and increased rates of cell proliferation and cell death, making the pubertal mammary gland particularly prone to neoplastic development. Indeed, the rat mammary gland is especially vulnerable to chemical carcinogenesis during the peripubertal period[45,46] and the human mammary gland is known to be especially sensitive to irradiation at this time.[47]

Toward an explanation linking altered development and neoplasia

Supporters of the novel theory of fetal origins of adult disease propose that epigenetic changes such as DNA methylation and chromatin remodeling play a central role in transducing perturbations of the fetal environment into the disease outcomes. For example, permanent alterations in DNA methylation patterns of multiple cell signaling genes identified in the BPA-exposed prostates have been postulated to be the underlying cause of neoplastic development later in life.[48] Like the somatic mutation theory, epigenetic theories of carcinogenesis imply that cancer originates in a single founder cell that undergoes genetic and/or epigenetic changes, which ultimately result in dysregulated cell proliferation.[49] As mentioned above, the tissue organization field theory postulates, instead, that carcinogenesis represents a problem of tissue organization comparable to organogenesis gone awry.[19,50] Accordingly, carcinogens and teratogens would disrupt normal dynamic interactions of neighboring cells and tissues during early development and/or adulthood.[25,51] As a result of this disruption in tissue organization, cells would regain their constitutive ability to proliferate and promote neoplastic development.

DES-induced clear cell carcinoma of the vagina also provides an example of tumor development that is likely due to the disruption of tissue organization. This carcinoma originates in areas of cervico–vaginal adenosis, which are regions of simple columnar epithelium that develop within the stratified squamous epithelium of the vagina.[52,53] Cervico–vaginal adenosis has been linked to aberrant cell-fate determination whereby vaginal epithelial cells acquire a uterine fate and become a simple columnar epithelium rather than a stratified squamous one.[54] The mesenchyme plays a major role in this epithelial fate determination process.[55] DES may also act directly on the vaginal epithelial cells by blocking the expression of p63, a protein that plays a major role in the fate determination of the vaginal and other stratified squamous epithelia.[54] More recently, msx2, which plays a critical role in cell-fate determination in the vaginal epithelium, was shown to be repressed by DES.[56] This homeobox-domain transcription factor is required for the correct expression of wnt7a, and the absence of msx2 would result in a complete failure of stratification of the vaginal epithelium.[56]

In contrast with the genital tract, knowledge about how estrogens and other endocrine disruptors affect mammary gland morphogenesis is limited. During the fetal period of exposure to BPA, estrogen receptors are only expressed in the mesenchymal cells. Hence, it is likely that the changes observed in the epithelium are entirely mediated by BPA action on the stroma. Since maturation of the fat pad is the driving event for ductal growth and branching, the increased ductal area in BPA-exposed mice might be due to the acceleration of this process (Fig. 3). Similar to the female genital tract, several members of the wnt signaling cascade and msx2 are expressed during fetal mammary gland development.[33] The expression of wnt4, wnt5b and msx2 is regulated by estrogens in the adult mammary gland.[57,58] Hence, it is plausible that fetal xenoestrogen exposure results in the extemporaneous expression of this set of genes that, in turn,

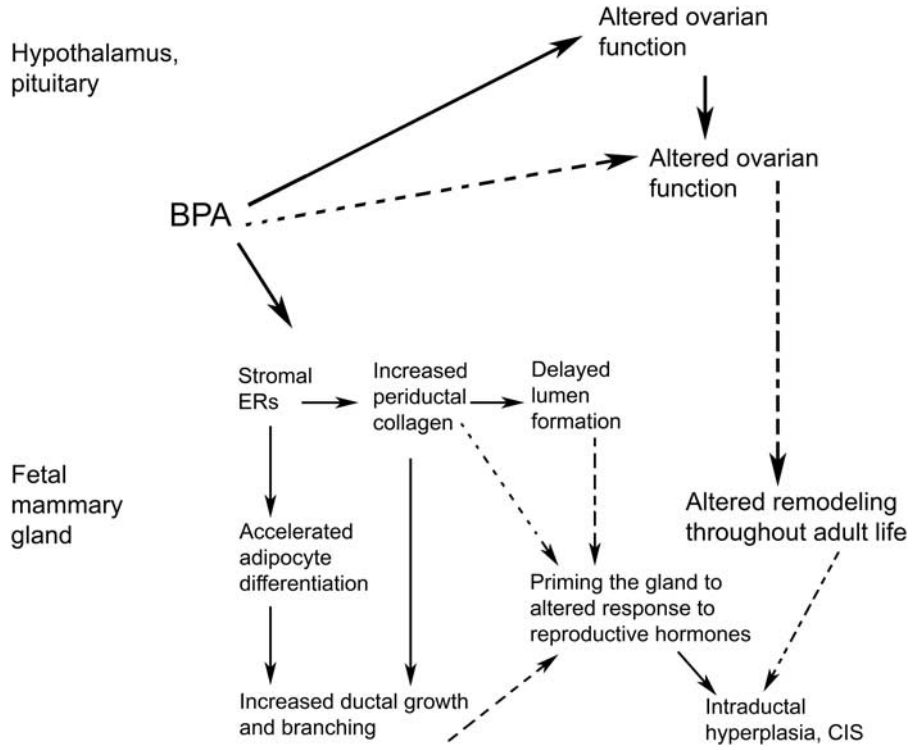

Fig. 3. Postulated causal links connecting perinatal exposure to bisphenol-A (BPA) with the development of pre-neoplastic lesions that manifest in adulthood. The full arrows link observations at embryonic day l8 with postulated causal links based on experimental observations from the literature. Dashed arrows link the observed effects at E18 with effects of prenatal BPA exposure observed during puberty and adulthood.

may indirectly cause altered morphogenesis and neoplastic development as is the case for clear cell carcinoma of the vagina.

It is also conceivable that fetal BPA exposure may alter the methylation pattern of genes involved in the reciprocal tissue interactions that mediate morphogenesis. In the prostate, developmental exposure to BPA causes changes in DNA methylation,[48] which also changes with age, suggesting that those changing patterns may instead represent a downstream event in a causal chain. It should be noted that DNA for methylation analysis is usually extracted from the whole tissue, which is morphologically heterogeneous and asymmetric. This may preclude the detection of truly significant changes occurring in structures like the presumptive fat pad, the epithelial leading edge, and the peri-epithelial mesenchyme. Alternatively, some relevant changes in gene expression may occur by means of induction and repression rather than silencing. In addition, important changes may not only be due to altered gene expression, but also to post-transcriptional and/or post-translational regulation as well. For example, breast interlobular and intralobular stroma had similar patterns of mRNA expression while showing significant differences in the expression levels of certain proteins and a very different matrix. In this latter regard, collagen I was more abundant in the interlobular stroma than in the intralobular stroma.[59] This is a relevant finding since mammographic density is considered to be a strong indicator of breast cancer risk, and this density correlates with stromal density due to collagen deposition.[60] Moreover, increased stromal collagen density significantly increased tumor formation and metastasis in a mouse transgenic model.[61]

Biomechanics and tissue organization

Bioengineers are studying the role of physical forces with the purpose of using these properties to build tissues for transplantation and reconstruction, while developmental biologists are interested in understanding how shapes are determined during histogenesis and organogenesis. Biophysical factors are powerful determinants of tissue organization; this has been known and used therapeutically, for instance, to reshape bones. The advent of tools that measure mechanical forces in soft tissue allows for the study of biomechanics in morphogenesis. Biochemical modulators such as morphogens interact and even transduce into mechanical force, while deformation caused by stretching, compression and shear are transduced into biochemical changes. So far, it is known that increased matrix rigidity results in altered organization of the mammary epithelium, resulting in solid structures that resemble carcinomas,[62,63] and that matrix rigidity inhibits lumen formation.[63] Our findings that prenatal exposure of mice to BPA resulted in changes in collagen deposition and organization suggests that

https://doi.org/10.1017/S2040174411000043 Published online by Cambridge University Press

matrix rigidity and fiber organization may be one of the early determinants of the altered tissue organization observed during fetal life[34] (Fig. 3).

Conclusions

Development and carcinogenesis are the result of reciprocal tissue interactions mediated by biophysical and biochemical modulators. These processes involve multiple and complex causality, which cannot be tackled by linear thought and reductionism. Systems biology approaches using mathematical modeling tools and computer simulations may help in the understanding of these complex phenomena. Finally, while research should continue to provide a better understanding of these biological phenomena, it is now clear that the weight of the evidence collected to date favors a swift and effective change in public health and environmental policies aimed at protecting the public at large, and the developing fetus and women of reproductive age, in particular. In this regard, The Endocrine Society Statement succinctly describes what should be done to reach those goals, namely, '…to increase understanding of the effects of endocrine disrupting chemicals, including enhancing increased basic and clinical research, invoking the precautionary principle, and advocating involvement of individual and scientific society stakeholders in communicating and implementing changes in public policy and awareness'.[64]

Acknowledgments

This work was supported by grants from the NIH (ES0150182, ES012301, ES08314 and ES018822) and the Parsemus Foundation. We are grateful to Cheryl Schaeberle for her excellent editorial assistance.

Statement of Interest

None

References

1. Markey CM, Coombs MA, Sonnenschein C, Soto AM. Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. *Evol Dev*. 2003; 5, 1–9.
2. Vandenberg LN, Maffini MV, Schaeberle CM, *et al*. Perinatal exposure to the xenoestrogen bisphenol-A induces mammary intraductal hyperplasias in adult CD-1 mice. *Reprod Toxicol*. 2008; 3-4, 210–219.
3. Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM. Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. *Reprod Toxicol*. 2007; 23, 383–390.
4. Newbold RR, Padilla-Banks E, Snyder RJ, Jefferson WN. Developmental exposure to estrogenic compounds and obesity. *Birth Defects Res A Clin Mol Teratol*. 2005; 73, 478–480.
5. Rubin BS, Murray MK, Damassa DA, King JC, Soto AM. Perinatal exposure to low doses of bisphenol-A affects body weight, patterns of estrous cyclicity and plasma LH levels. *Environ Health Perspect*. 2001; 109, 675–680.
6. Herbst AL, Bern HA. *Developmental Effects of Diethylstilbestrol (DES) in Pregnancy*, 1988. Thieme-Stratton, New York.
7. McLachlan JA, Newbold RR, Bullock BC. Long-term effects on the female mouse genital tract associated with prenatal exposure to diethylstilbestrol. *Cancer Res*. 1980; 40, 3988–3999.
8. Boylan ES, Calhoon RE. Transplacental action of diethylstilbestrol on mammary carcinogenesis in female rats given one or two doses of 7,12-dimethylbenz(a)anthracene. *Cancer Res*. 1983; 43, 4879–4884.
9. Soto AM, Sonnenschein C. Environmental causes of cancer: endocrine disruptors as carcinogens. *Nat Rev Endocrinol*. 2010; 6, 363–370.
10. Gilbert SF. Mechanisms for the environmental regulation of gene expression: ecological aspects of animal development. *J Biosci*. 2005; 30, 65–74.
11. Moss L. *What Genes Can't Do*, 2003. MIT Press, Cambridge, MA.
12. Soto AM, Sonnenschein C. Emergentism as a default: cancer as a problem of tissue organization. *J Biosci*. 2005; 30, 103–118.
13. Schrödinger E. *What is Life? The Physical Aspect of the Living Cell*, 1945. The University Press, Cambridge, England.
14. Barker DJP, Hanson MA. Altered regional blood flow in the fetus: the origins of cardiovascular disease? *Acta Paediatricia*. 2004; 93, 1559–1560.
15. Thompson DW. *On Growth and Form*, 1942. Cambridge University Press, Cambridge.
16. Ingber DE. Mechanical control of tissue morphogenesis during embryological development. *Int J Dev Biol*. 2006; 50, 255–266.
17. Patwari P, Lee RT. Mechanical control of tissue morphogenesis. *Circ Res*. 2008; 103, 234–243.
18. Soto AM, Sonnenschein C, Miquel P-A. On physicalism and downward causation in developmental and cancer biology. *Acta Biotheor*. 2008; 56, 257–274.
19. Sonnenschein C, Soto AM. *The Society of Cells: Cancer and Control of Cell Proliferation*, 1999. Springer Verlag, New York.
20. Noble D. *The Music of Life: Biology beyond the Genome*, 2006. Oxford University Press, Oxford.
21. Triolo VA. Nineteenth century foundations of cancer research advances in tumor pathology, nomenclature, and theories of oncogenesis. *Cancer Res*. 1965; 25, 76–98.
22. Hahn WC, Weinberg RA. Modelling the molecular circuitry of cancer. *Nat Rev Cancer*. 2002; 2, 331–342.
23. Soto AM, Sonnenschein C. The somatic mutation theory of cancer: growing problems with the paradigm? *BioEssays*. 2004; 26, 1097–1107.
24. Baker SG, Cappuccio A, Potter JD. Research on early-stage carcinogenesis: are we approaching paradigm instability? *J Clin Oncol*. 2010; 28, 3215–3218.
25. Maffini MV, Calabro JM, Soto AM, Sonnenschein C. Stromal regulation of neoplastic development: age-dependent normalization of neoplastic mammary cells by mammary stroma. *Am J Pathol*. 2005; 67, 1405–1410.
26. Skakkebaek NE, Meyts ER, Jorgensen N, *et al*. Germ cell cancer and disorders of spermatogenesis: an environmental connection? *APMIS*. 1998; 106, 3–12.
27. Soto AM, Sonnenschein C. Environmental causes of cancer: endocrine disruptors as carcinogens. *Nat Rev Endocrinol*. 2010; 6, 363–370.

28. Mittendorf R. Teratogen update: carcinogenesis and teratogenesis associated with exposure to diethylstilbestrol (DES) *in utero*. *Teratology*. 1995; 51, 435–445.
29. Herbst AL, Anderson D. Clear cell adenocarcinoma of the vagina and cervix secondary to intrauterine exposure to diethylstilbestrol. *Semin Surg Oncol*. 1990; 6, 343–346.
30. Hatch EE, Troisi R, Wise LA, *et al*. Age at natural menopause in women exposed to diethylstilbestrol in utero. *Am J Epidemiol*. 2006; 164, 682–688.
31. Palmer JR, Wise LA, Hatch EE, *et al*. Prenatal diethylstilbestrol exposure and risk of breast cancer. *Cancer Epidem Biomar*. 2006; 15, 1509–1514.
32. Lemmen JG, Broekhof JLM, Kuiper GGJM, *et al*. Expression of estrogen receptor alpha and beta during mouse embryogenesis. *Mech Dev*. 1999; 81, 163–167.
33. Veltmaat JM, Mailleux AA, Thiery JP, Bellusci S. Mouse embryonic mammogenesis as a model for the molecular regulation of pattern formation. *Differentiation*. 2003; 71, 1–17.
34. Vandenberg LN, Maffini MV, Wadia PR, *et al*. Exposure to the xenoestrogen bisphenol-A alters development of the fetal mammary gland. *Endocrinology*. 2007; 148, 116–127.
35. Humphreys RC, Krajewska M, Krnacik S, *et al*. Apoptosis in the terminal end bud of the murine mammary gland: a mechanism of ductal morphogenesis. *Development*. 1996; 122, 4013–4022.
36. Daniel CW, Smith GH. The mammary gland: a model for development. *J Mammary Gland Biol Neoplasia*. 1999; 4, 3–8.
37. Soto AM, Sonnenschein C. Environmental causes of cancer: endocrine disruptors as carcinogens. *Nat Rev Endocrinol*. 2010; 6, 363–370.
38. Markey CM, Luque EH, Munoz de Toro MM, Sonnenschein C, Soto AM. *In utero* exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol Reprod*. 2001; 65, 1215–1223.
39. Munoz de Toro MM, Markey CM, Wadia PR, *et al*. Perinatal exposure to bisphenol A alters peripubertal mammary gland development in mice. *Endocrinology*. 2005; 146, 4138–4147.
40. Wadia PR, Vandenberg LN, Schaeberle CM, *et al*. Perinatal bisphenol-A exposure increases estrogen sensitivity of the mammary gland in diverse mouse strains. *Environ Health Perspect*. 2007; 115, 592–598.
41. Rothschild TC, Boylan ES, Calhoon RE, Vonderhaar BK. Transplacental effects of diethylstilbestrol on mammary development and tumorigenesis in female ACI rats. *Cancer Res*. 1987; 47, 4508–4516.
42. Schor SL, Schor AM, Howell A, Haggie J. The possible role of abnormal fibroblasts in the pathogenesis of breast cancer. In *Breast Cancer: Scientific and Chemical Progress* (eds. Rich MA, Hagar JC, Lopez DM), 1988; pp. 142–157. Kluwer Academic Publishers, Boston.
43. Durando M, Kass L, Piva J, *et al*. Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. *Environ Health Perspect*. 2007; 115, 80–86.
44. Jenkins S, Raghuraman N, Eltoum I, *et al*. Oral exposure to bisphenol A increases dimethylbenzanthracene-induced mammary cancer in rats. *Environ Health Perspect*. 2009; 117, 910–915.
45. Russo IH, Russo J. Mammary gland neoplasia in long-term rodent studies. *Environ Health Perspect*. 1996; 104, 938–967.
46. Grubbs CJ, Peckham JC, Cato KD. Mammary carcinogenesis in rats in relation to age at time of N-nitroso-N-methylurea administration. *J Nat Cancer Inst*. 1983; 70, 209–212.
47. Land CE, Tokunaga M, Koyama K, *et al*. Incidence of female breast cancer among atomic bomb survivors, Hiroshima and Nagasaki, 1950–1990. *Radiat Res*. 2003; 160, 707–717.
48. Ho S-M, Tang WY, Belmonte de Frausto J, Prins GS. Developmental exposure to estradiol and bisphenol a increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer Res*. 2006; 66, 5624–5632.
49. Weinberg RA. *The Biology of Cancer*, 2006. Taylor & Francis, New York.
50. Sonnenschein C, Soto AM. Theories of carcinogenesis: an emerging perspective. *Semin Cancer Biol*. 2008; 18, 372–377.
51. Maffini MV, Soto AM, Calabro JM, Ucci AA, Sonnenschein C. The stroma as a crucial target in rat mammary gland carcinogenesis. *J Cell Sci*. 2004; 117, 1495–1502.
52. Robboy SJ, Welch WR, Young RH, *et al*. Topographic relation of cervical ectropion and vaginal adenosis to clear cell adenocarcinoma. *Obstet Gynecol*. 1982; 60, 546–551.
53. Newbold RR, McLachlan JA. Vaginal adenosis and adenocarcinoma in mice exposed prenatally or neonatally to diethylstilbestrol. *Cancer Res*. 1982; 42, 2003–2011.
54. Kurita T, Mills A, Cunha GR. Roles of p63 in the diethylstilbestrol-induced cervicovaginal adenosis. *Development*. 2004; 131, 1639–1649.
55. Cunha GR. Stromal induction and specification of morphogenesis and cytodifferentiation of the epithelia of the Mullerian ducts and urogenital sinus during development of the uterus and vagina in mice. *J Exp Zool*. 1976; 196, 361–370.
56. Yin Y, Lin C, Ma L. MSX2 promotes vaginal epithelial differentiation and wolffian duct regression and dampens the vaginal response to diethylstilbestrol. *Mol Endocrinol*. 2006; 20, 1535–1546.
57. Weber-Hall SJ, Phippard DJ, Niemeyer CC, Dale TC. Developmental and hormonal regulation of Wnt gene expression in the mouse mammary gland. *Differentiation*. 1994; 57, 205–214.
58. Phippard DJ, Weber-Hall SJ, Sharpe PT, *et al*. Regulation of Msx-1, Msx-2, Bmp-2 and Bmp-4 during foetal and postnatal mammary gland development. *Development*. 1996; 122, 2729–2737.
59. Fleming JM, Long EL, Ginsburg E, *et al*. Interlobular and intralobular mammary stroma: genotype may not reflect phenotype. *BMC Cell Biol*. 2008; 9, 46.
60. Martin LJ, Boyd NF. Mammographic density. Potential mechanisms of breast cancer risk associated with mammographic density: hypotheses based on epidemiological evidence. *Breast Cancer Res*. 2008; 10, 201–214.
61. Provenzano PP, Inman DR, Eliceiri KW, *et al*. Collagen density promotes mammary tumor initiation and progression. *BMC Med*. 2008; 6, 11.
62. Paszek MJ, Weaver VM. The tension mounts: mechanics meets morphogenesis and malignancy. *J Mammary Gland Biol Neoplasia*. 2004; 9, 325–342.
63. Paszek MJ, Zahir N, Johnson KR, *et al*. Tensional homeostasis and the malignant phenotype. *Cancer Cell*. 2005; 8, 241–254.
64. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, *et al*. Endocrine-disrupting chemicals: an endocrine society scientific statement. *Endocr Rev*. 2009; 30, 293–342.