

Molecular mechanisms of mammary gland remodeling: A review of the homeostatic versus bisphenol a disrupted microenvironment

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ABSTRACT

Mammary gland (MG) undergoes critical points of structural changes throughout a woman's life. During the perinatal and pubertal stages, MG develops through growth and differentiation to establish a pre-mature feature. If pregnancy and lactation occur, the epithelial compartment branches and differentiates to create a specialized structure for milk secretion and nurturing of the newborn. However, the ultimate MG modification consists of a regression process aiming to reestablish the smaller and less energy demanding structure until another production cycle happens. The unraveling of these fascinating physiologic cycles has helped the scientific community elucidate aspects of molecular regulation of proliferative and apoptotic events and remodeling of the stromal compartment. However, greater understanding of the hormonal pathways involved in MG developmental stages led to concern that endocrine disruptors such as bisphenol A (BPA), may influence these specific development/involution stages, called "windows of susceptibility". Since it is used in the manufacture of polycarbonate plastics and epoxy resins, BPA is a ubiquitous chemical present in human everyday life, exerting an estrogenic effect. Thus, descriptions of its deleterious effects on the MG, especially in terms of serum hormone concentrations, hormonal receptor expression, molecular pathways, and epigenetic alterations, have been widely published. Therefore, allied to a didactic description of the main physiological mechanisms involved in different critical points of MG development, the current review provides a summary of key mechanisms by which the endocrine disruptor BPA impacts MG homeostasis at different windows of susceptibility, causing short- and long-term effects.

1. Introduction

The mammary gland (MG) is one of the most dynamic organs in organisms of mammalian species. During life, the MG goes through several repeated cycles of growth and involution to allow the preparation of the tissue for milk production and nourishment. Although slight morphological alterations take place during the estrous/menstrual cycle as a response to ovarian hormones [1], profound modifications occur during gestation and lactation to allow full mammary development and its regression after weaning [2].

Indeed, an estrogenic environment dictates mammary fate to a well-developed gland in females [3]. This is the reason why so much interest has been given to the effects of endocrine-disrupting chemicals' on MG mechanisms of development and regression [4]. Endocrine disruptors (ED) are a class of chemicals that modify the normal functionality of the

endocrine system [5]. Several synthetic chemicals (including plasticizers, pesticides, and synthetic compounds used in drugs) and naturally occurring compounds (such as phytoestrogens) are classified as EDs. They disrupt the orchestrated action of endogenous hormones by altering their synthesis, transportation, metabolism, and excretion, or even by competing for hormone receptor binding in the target tissue [6]. Xenoestrogens are EDs that impact pivotal estrogenic pathways [7] and consequently disrupt the reproductive system in humans and other mammals [8]. The harmful effects are not limited to the exposed individual and may also extend to the progeny, causing changes in their postnatal endocrine function [9].

Research groups have focused on the context of xenoestrogens exposure during developmental "windows of susceptibility" in different tissues [10]. These windows are stages of development which involve cell proliferation, differentiation, and apoptosis, as well as a

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well-defined stroma/epithelium crosstalk process controlled by hormones [2,11]. Thus, in the MG not only fetal or infant exposure is cause for concern; adult exposure may also impact developmental windows of susceptibility and have adverse consequences [10]. In this aspect, since MG has the ability to grow and involute several times during the reproductive age in a homeostatic environment, xenoestrogen exposure and disruption during these tissue remodeling phases comprise a risk [7, 12]. Currently, the most commonly studied MG windows of susceptibility are the in utero and postnatal developmental phases, however, in addition to these, the periods of puberty, gestation/lactation, and involution during post-weaning and menopause are matters of concern, since the gland is undergoing deep tissue morphological and physiological remodeling [13].

This review brings together aspects of morphology, physiology, and molecular signaling of the mammary structure – which will be designated as breast, when talking about the humans' structure and MG when related to other mammals – differentiation phases considered windows of susceptibility for endocrine disruption, aiming to discuss in parallel the pathological alterations caused by endocrine disruptor bisphenol A (BPA) exposure (Table 1). The “Normal mammary development” topic addresses homeostatic physiological effectors and molecular pathways that present relevant alterations when disrupted by BPA.

2. Normal mammary development

During fetal development, the origin of the MG is epidermal cells, which invaginate to form the ductal tree. From birth to puberty, the MG shows allometric growth and no significant differentiation takes place [2]. Once puberty arrives, the mature hypothalamic-pituitary-gonadal axis promotes the cyclic secretion of gonadotropins and sexual hormones, contributing to dramatic modifications in the gland (Fig. 1) [14].

The MG is under constant hormonal regulation, modifications in gene expression, cell metabolism, and plasticity [15]. These changes may impact physiological and metabolic homeostasis and make the individual prone to future disorders due to chemical exposure [16]. Three main windows of susceptibility were described and are responsive to these regulating factors, which orchestrate the following changes:

- (1) Postnatal and pubertal developmental phases: a massive architectural change takes place in response to estrogenic regulation, which mainly influences stromal-epithelial interactions [17] and induces intense epithelial cell proliferation [2]. During this window, xenoestrogens may increase the length of the period of ductal growth and alveologenesis throughout the menstrual cycle, by acting simultaneously to endogenous E2 [10].
- (2) Pregnancy and lactation (Figs. 2 and 3): the gland development is influenced by progesterone and prolactin receptor binding [18]. Impacts of gestational/lactational exposure to xenoestrogens have been described in the post-weaning gland and are related to changes in the expression of estrogen and progesterone receptors [19].
- (3) Post-weaning and menopause involution (Fig. 4): the remodeling MG mimics a tumorigenic malignant microenvironment in terms of proteinase expression [20] and inflammatory process [21]. In this aspect, not only epithelial cells but also stromal fibroblasts are targets of concern due to their role in the extracellular matrix (ECM) synthesis [22]. When exposed to disruptors during menopause, MG may be prone to an increase in ductal cell proliferation, with higher chances of DNA mutations and, consequently, breast cancer susceptibility [23].

Molecular signaling and physiological control act in the gland morphogenesis by different intra- and extracellular pathways. In the estrous/menstrual cycle, the MG undergoes a proliferation process followed by apoptosis of its ductal structures (Fig. 1B and F). In pregnancy, as detailed in Fig. 2, rapid proliferation of the duct and secretory

epithelium (Fig. 1C and G) occurs, stimulated by estrogen, and progesterone leads to the differentiation of specific epithelial cells for synthesis and secretion of milk (Fig. 2) [24]. This epithelial structure, called alveolus, presents luminal epithelial cells that synthesize milk components by prolactin stimulus to be released during lactation (Fig. 3) [11]. Bipotent mammary stem cells (MSC) proliferate and differentiate into luminal and myoepithelial progenitor cells [25]. These latter cells are located around the ductal and alveoli system and, when stimulated by oxytocin, contract to expel milk from the luminal cells to the nipples [11]. At weaning, the lack of suckling stimulus decreases the production of oxytocin and, later, lactating hormones, leading to the process of MG involution, with a drastic rearrangement of cell compartments mediated by apoptotic events (Fig. 4) [26].

Hormones, growth factors, cytokines, and several metabolites interact among complex pathways to trigger proliferation, differentiation, and/or apoptosis in epithelial and stromal cells and ECM. The role of physiological and molecular key elements to understand the mechanisms involved in MG processes, has been little studied [27–32]. Thus, the role of the following elements will be discussed in this review: hormones; growth factors; adipokines; epigenetic regulation; and microRNAs (miRNA).

2.1. Hormones

The steroid hormones estrogen and progesterone play a central role during the menstrual/estrous cycle and coordinate structural changes in mammary tissue. Post-natal activity of MG correlates to ovarian onset secretion of these hormones and their systemic release [33]. In MG, estrogen and progesterone interact with other physiological regulators through a paracrine mechanism in the epithelial compartment, and through paracrine/autocrine pathways in the stroma, mainly to promote proliferation during development, as well as secretion (lactation) and apoptosis (involution) [34,35].

Prolactin contributes with progesterone to stimulate development and establishment of the lobuloalveolar tree in late pregnancy [36–38]. Its highest levels are present with the post-parturition decrease in estrogen and progesterone (milk secretion-inhibiting hormones) due to removal of the placenta [39]. Suckling is the mechanical stimulation that promotes prolactin and oxytocin release during lactation [40]. Oxytocin is a hypothalamic hormone released in response to sucking stimulation by the newborn, transmitted through the neuronal system [41]. Its targets are myoepithelial cells acting to expel milk toward the nipple region [42]. Recently it was demonstrated that myoepithelial cell contraction is crucial not only for the ejection of milk secreted by luminal cells in alveoli but also for the extrusion of lipid droplets of these cells [43].

2.1.1. Estrogen

In puberty, estrogen is required for MG development each estrous/menstrual cycle [44–46]. Serum estrogens act on target cells, crossing the cell membrane and binding to estrogen receptors (ER). Targets are mainly epithelial cells, which develop a relevant proliferative signaling. Two major ER receptors have been described: ER-alpha (ER α) and ER-beta (ER β), which present different expression rates during pubertal, pregnancy, and lactation stages [47]. ER α and ER β may be co-expressed in cells and have been described during lactation, although estrogen levels are decreased for the action of lactating hormones, such as prolactin [45,48]. In rodents, both are expressed in mammary epithelial and stromal cells and develop an essential and independent role to coordinate the branching process of the ductal system [35,49]. ER α is fundamentally expressed in mammary duct epithelial cells, promoting its elongation during the developmental stage [35]. When expressed during pregnancy, ER α triggers proliferative and differentiation activity in epithelial cells [45,50]. For ER β , an antiproliferative role is assigned [49].

ER activation triggers the activity of many transcription factors and

Table 1

Aspects impacting mammary gland during different windows of susceptibility: normal versus BPA disruption.

	Normal physiology	BPA disruption*
Systemic changes	HPG axis regulates in puberty the estrous/menstrual cycles, promoting a follicular phase with estrogenic induction of MG growth and luteal phase with progesterone stimuli for establishment of structures for a possible pregnancy [1,2]	<i>Perinatal</i> - disruption of the hypothalamic-pituitary-gonadal axis [266]; - increase in estrous cycles length [181]; - reduced serum levels of E2 and progesterone during pregnancy [180]; <i>Puberty</i> - insulin and leptin resistance [183]; - alteration in adiponectin levels [267]; <i>Gestational</i> - gestational diabetes [268]; - damage in pancreatic cell cycle [185]; <i>Perinatal</i> - acceleration of epithelial morphogenesis [181]; - increase in estrogen sensitivity [208]; - changes in expression of pathways and growth factors that regulates proliferation (AKT/ERK and TGFβ) [216]; - MMP expression in stroma induced by ERR-γ activity – stromal organization [217]; - alteration in sensitivity to progesterone – triggers Wnt-4 and Nf-kB [219]; - increases RANKL expression, enhancing PR signaling [219,220]; - inflammation and autophagic activity induced by NF-kB and mTOR signaling [221]; - stromal changes by collagen fibers synthesis enhanced and in epithelial producing hyperplasia [209,255,269]; - tissue inflammation [256,257]; <i>Puberty</i> - adipocytes differentiation [270]; - increase in caspase-3 cascade for apoptosis [209]; - changes in expression of proliferative proteins – Ki-67, p16 and cyclin E [209,210,211] <i>Gestational</i> - overexpression of Areg [215]] <i>Perimenopausal</i> - increase in production of inflammatory and oxidative markers [237]; - increase in mammographic density [197] <i>General</i> - high affinity and boosted effect in ERβ+ signal; - act as agonist or antagonist in interactions with ERα; - long-lasting epigenetic alterations that impact gene expression [200]; <i>Perinatal</i> - DNA hypermethylation caused by oxidative stress [236]; - alpha-lactalbumin gene changes [239]; - increase the responsiveness of cells to estrogens by hypomethylation of Hoxa10 [232]; - increase miRNA-217 (endothelial cells damage) and miRNA-608 (cell cycle arrest) [250] <i>Gestational</i> - alterations in the β-casein gene's methylation [237]
Morphological and Molecular changes	Mammary gland develops during embryonic period from epidermal cells originating rudimentary bud structures [2]. Each estrous/menstrual cycle modifies the MG through hormonal signal and stimuli of many growth factors [29,30,31,32]. When pregnancy occurs, there is induction of proliferation through progesterone regulation for structural changes of secretory acini [66,68,69,70,71,72]. Luminal cells accumulate milk and secrete it after parturition, when lactation takes place [18]. Prolactin regulates the subcellular production of milk [36,37,38,39] and the mechanical-dependent hormone oxytocin controls the contraction of myoepithelial cells for milk ejection [11]. In post-weaning, apoptotic pathways are activated and immune cells are recruited for MG involution of epithelium and stroma [26].	
ER-related effects	At puberty, ER signal acts in MG development at each estrous/menstrual cycle [44,45,46]. Co-activators and co-repressors modulate the gene expression in MG [38,53,55]. ER-estrogen binding signal modulates activity of mitogens [61], regulates the stromal response [35,56], and differentiation activity in epithelial cells during pregnancy [45,49,50].	
Epigenetics and microRNA	Epithelial-luminal differentiation is regulated by several DNAm during MG development [115]. MicroRNAs regulate post-transcriptional gene expression for establishment of mature MG: - miRNA-101 (regulates differentiation) [151]; - miRNA-212 and miR-132 (MMP expression) [152]; - miRNA-378, miRNA-423–5p and miRNA-7 (milk synthesis) [154]; - miRNA-204 (Areg expression) [157]; - miRNA-152 (AKT pathway during the proliferation) [155]; - miRNA-126 family and miRNA-15a family (expression and secretion of milk proteins) [lange 1998]; - miRNA-101a (COX2 control) [151]; - miRNA-1, miRNA-152, and miRNA-155 (proliferative factors expression) [131];	

* The alterations mentioned in the third column are related to the specific periods of BPA exposure. HPG: hypothalamic-pituitary-gonadal; MG: mammary gland.

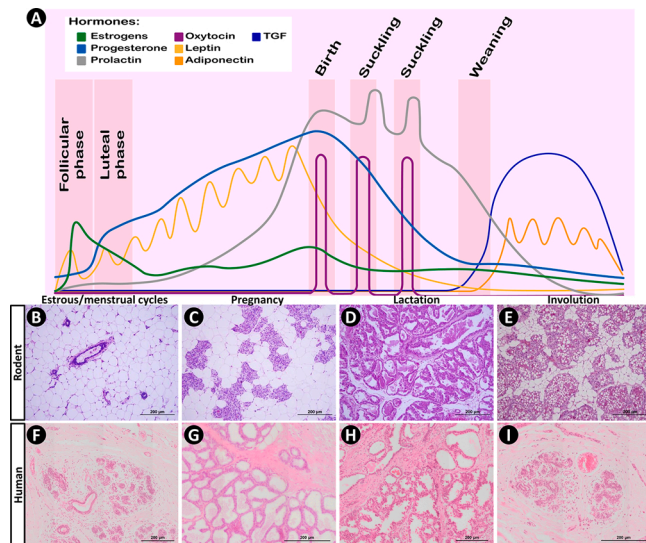


Fig. 1. The histological aspects of postpubertal rodent (*Meriones unguiculatus*) and human mammary gland in response to the main serum hormonal changes (A). During estrous or menstrual cycles (B and F, respectively) subtle morphological changes take place in response mainly to estrogens and progesterone. However, a drastic alteration in epithelial structures occurs in response to progesterone and leptin during gestation (C;G) and in response to prolactin and oxytocin during lactation (D;H). Weaning triggers involution of the epithelium, that responds to an increase in TGF- β and adiponectin and a reduction in other serum hormones (E;I).

nuclear estrogen response elements (ERs) in the DNA, which alters gene expression [51,52]. Co-regulators enhance the transcriptional activity promoted by estrogen-ER-binding, activating (co-activators) or repressing (co-repressors) the gene expressed [38,53,54].

Paracrine signaling occurs when estrogen-ER binding promotes the proliferation of ER-negative epithelial and stromal cells during MG development [55,56]. The stromal paracrine induction pathway acts mainly by modulating the expression and effectiveness of mitogens [57, 58]. ER α expression in stromal cells is therefore mandatory for MG development through its interaction with and modulation of growth hormones and cognate receptors [59].

Although estrogen is not directly related to MG involution, its regulation during late lactation and the beginning of tissue remodeling is required. During involution, ER co-regulators are responsible for determining the apoptotic process of the cells, a drastic phenomenon to remodel the structure of the MG. The most important regulation of the involution process is the interaction of the transforming growth factor-beta (TGF- β) pathway and ER α [60]. Although ER α also modifies the TGF- β cascade, the high expression of TGF- β in mammary tissue restrains the ER pathway and activates apoptosis [61]. During the post-menopausal period, involution of the MG adipose tissue is the primary source of estrogen synthesis, assuring high local levels of 17 β -estradiol (E2) and ER α -positive epithelial cells despite low serum levels of steroids [62].

2.1.2. Progesterone

Progesterone is mainly secreted by the corpus luteum during the mid-term ovarian cycle, and the placenta during pregnancy [63,64]. Although progesterone and estrogen are interdependent hormones during mammary proliferative activity in the mouse model, the progesterone receptor (PR) requires transcriptional induction by ER α to be expressed in epithelial cells, while PR expression in stromal cells is estrogenic-independent [65,66]. This hormone has two receptor isoforms, PR-A and PR-B, in which PR-A presents high relevance in the uterus development, while PR-B is the main effector of the proliferative process in MG [67,68].

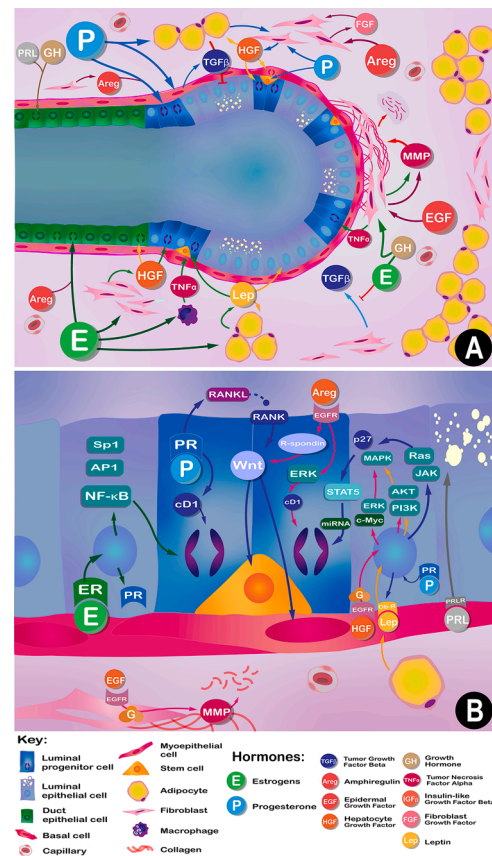


Fig. 2. Pregnancy. (A) The alveolar structure establishment during pregnancy occurs through stromal remodeling and epithelial proliferation. E and P act as the main hormones that control these changes. E-induced pathways: the proliferation of ductal epithelial cells is associated with Areg or induced by the synthesis and secretion of hormones by stromal cells – HGF by fibroblasts, Lep by adipocytes, TNF α by fibroblasts and inflammatory cells. Furthermore, E promotes stromal remodeling by stimulating the synthesis of MMPs by fibroblasts. P-induced pathways: in association with Areg promotes luminal epithelial proliferation, and stimulates HGF synthesis by fibroblasts for the establishment of myoepithelial cells, luminal cell proliferation, and basal cell differentiation. EGF acts primarily to stimulate the synthesis of MMPs to break down connective fibers. Areg acts on fibroblasts by increasing FGF synthesis for autocrine and paracrine proliferation signaling. (B) Molecular signaling in mammary development during pregnancy. E-receptor ER binding triggers the synthesis of progesterone receptor PR as well as proliferative signaling through NF- κ B, AP1, and Sp1. P-PR binding triggers a mitogenic response in luminal epithelial cells by increasing cD1 and RANKL-RANK/Wnt for paracrine signaling in basal and myoepithelial cells. Areg also acts via R-spondin to trigger a response via Wnt, in addition to activating ERK and cD1 for cell proliferation. In addition, P-PR signals leptin receptor synthase Ob-R. The proliferation of epithelial cells occurs through the expression of p27 and MAPK to trigger a signal via STAT5 and miRNA. The expression of p27/MAPK occurs through three main pathways: by progesterone activation, via the JAK/Ras pathway; by leptin stimulation through the PI3K/AKT pathway; or by HGF via ERK/c-Myc or leptin-like pathways.

The mechanism of action of progesterone is summarized in two steps: the first involves PR + cells and the performance of cyclins D1 (cyclin D1-dependent mechanism); while the second involves PR- cells and the paracrine function, dependent on receptor activator nuclear transcription factor-kappa B ligand (RANKL), a membrane receptor of the TNF superfamily (RANKL-dependent mechanism) [69]. The second step depends on binding of RANKL to its receptor, RANK, to promote alveolar development [67,70–73]. RANKL is a paracrine mediator expressed only in the presence of higher availability of progesterone and depends on the presence of estrogen, i.e., in PR + ER + cells [74]. PR + epithelial cells

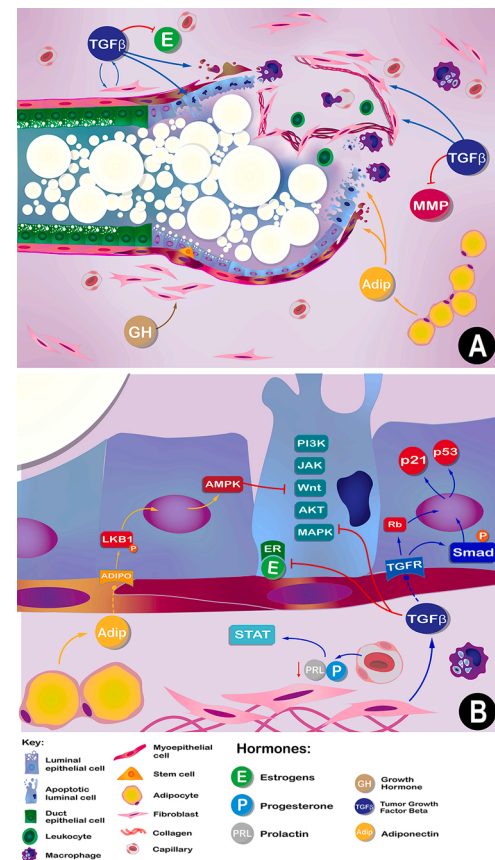
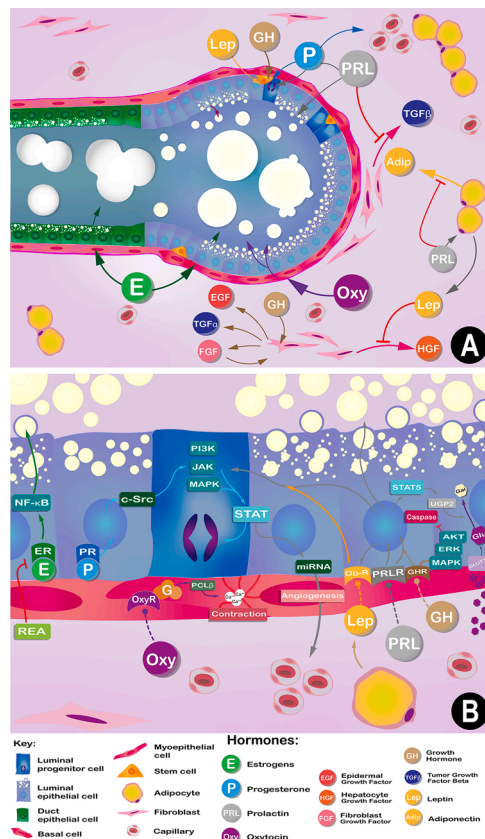


Fig. 3. Lactation. (A) During lactation, in addition to PRL, P and E aid the secretion of milk produced by luminal and ductal cells. P acts via induction of PRL in the secretion and proliferation of luminal cells, as well as Lep and GH, to maintain the alveolar structure. To expel milk from the alveoli, oxytocin acts on myoepithelial cells to promote their contraction. In the stroma, GH acts to stimulate the production of EGF and FGF by fibroblasts, while adipocytes are stimulated by prolactin to produce leptin, inhibiting the mitogenic action of HGF, and inhibiting the synthesis and activity of Adip and TGFβ. (B) The molecular pathways of synthesis and secretion, activated during lactation are co-ordinated by events stimulated by PRL. Through PRL-receptor (PRLR) binding there is a direct stimulation for the synthesis and secretion of milk and conversion of glycogen (Glu) into galactose (Gal) via STAT5/UGP2. In addition, during lactation, anti-apoptotic mechanisms are evoked, activated by GH, and triggered by the expression of MAPK/ERK/AKT, inhibiting the activity of Caspase, and proliferation for maintenance of the mammary gland epithelium through the expression of PI3K/JAK/MAPK for STAT establishment. Activation of angiogenic mechanisms occurs via miRNA. P-PR, via c-Src, activates cell mitogenic pathways similar to those produced by PRL and Lep stimulation. E helps to secrete milk into the lumen via NF-κB, a mechanism that may be inhibited by REA. Oxy acts on G protein-coupled receptors (OxyR) in myoepithelial cells to activate PCLβ, triggering the contraction of these cells.

express and secrete WNT4 protein that regulates progesterone paracrine activity through myoepithelial and basal cells [75–77].

The main role of progesterone role is stimulation of growth of terminal end buds (TEBs) and ductal elongation and branching [78]. Epithelial cells of the ductal tree show high sensitivity to progesterone related to its receptor since it increases the side branching process and alveologenesis in the MG and leads to the production of new epithelial PR sites [79]. In addition, progesterone promotes relevant activation of the lactation process, supporting the prolactin pathway.

In pregnancy, progesterone stimulates the synthesis of TGF-β and Wnt proteins which repress milk secretion [17]. With a perinatal decrease in progesterone levels, lipid and protein biosynthesis pathways, such as protein kinase B (AKT) and sterol regulatory element-binding proteins (SREBP), are upregulated [29]. Nonetheless,

Fig. 4. Involution. (A) During mammary involution, TGFβ, like Adip, acts as an apoptotic promoter in epithelial cells, inhibiting the proliferative action of E. TGFβ acts on the proliferation of stromal cells to increase this compartment, inhibiting the action of MMP and acting as a chemotactic factor for inflammatory cells. GH acts on the stromal compartment for fibroblast proliferation. (B) TGFβ and adiponectin act by inhibiting classical proliferative pathways in luminal cells, such as PI3K, JAK, Wnt, AKT, and MAPK. Adip acts specifically through a receptor-binding signal (ADIPO) to phosphorylate LKB1 to increase the synthesis of AMPK, a proliferative inhibitor. Apoptosis in epithelial cells is generated by the activation of Rb and via phosphorylation of the Smad protein, which activates the expression of p21 and p53, both pro-apoptotic proteins. Furthermore, the drastic reduction in PRL and P acts as a signal for the increase in STAT expression, providing a favorable environment for the gland regression process.

circulating progesterone serum levels decrease in late pregnancy and post-parturition, signaling for the secretion of milk, stored in luminal cells, in response to the increase in prolactin levels [69]. The upcoming increase in progesterone occurs only at the mid/end of the involution when there is a stimulus for STAT1 and STAT3 phosphorylation that triggers the cell death process [80,81].

2.2. Metabolic hormones and growth factors

2.2.1. Family of epidermal growth factors (EGF)

EGF is a family of proteins that stimulates proliferation response and differentiation in MG cells. Two primary receptor categories are related to EGF binding-signal: human epidermal growth factor receptor 2 (HER2), or ErbB2/neu in rodents [82], and a cognate receptor, epidermal growth factor receptor (EGFR), or ErbB1/HER1 [83]. HER-EGF signaling activates the complex phosphoinositide 3-kinases (PI3K)/AKT/mTOR cascade for proliferative and anti-apoptotic processes [84,85]. EGFR also initiates MAPK and STAT pathways, triggered by other hormones and growth factors (Figs. 2 and 3) [86,87].

The EGF-EGFR binding pathway is triggered from the transactivation

of G-protein coupled estrogen receptor (GPER) that increases MMP expression and stimulates the ERK phosphorylation for c-Myc mitogenic activity in the stroma, ducts, and TEBs [88,89]. This GPER-induction of MMPs is regulated by the c-Src pathway that amplifies mRNA expression [86]. Moreover, EGF determines an epithelial expansion toward stroma by a hyaluronan small-fragments type signal in the ECM [88]. Thus, EGF activity in stromal and epithelial compartments is protease-dependent, supporting the epithelial proliferation needs for stromal ECM relaxation [90]. MMPs are responsible not only for the proteolytic activity of extracellular components in the stroma [91] but also to promote the cleavage of the newly synthesized EGF molecule transmembrane precursors [92].

Amphiregulin (Areg) is a transmembrane glycoprotein, a precursor of the EGF family, synthesized in the epithelium and stroma [93]. Areg binds to EGFR type to promote fibroblast proliferative activity, stimulating secretion and autocrine action of fibroblast growth factor [94,95]. It also mediates estrogen-paracrine signals during ductal proliferation and progesterone-dependent morphogenesis in pregnancy [96–98]. Areg acts by increasing DNA synthesis through the ERK pathway that stimulates the upregulation of cyclin D1, modulating the progression of the cell cycle [99]. Furthermore, Areg induces R-spondin protein expression in luminal cells that synergize with Wnt to determine cell fate [100].

2.2.2. Transforming growth factor- β

TGF- β is a cytokine that presents dual activity: antiproliferative or mitogenic effects, depending on the tissue [61]. It modulates cell activity by MAPK and AKT pathways to provoke antiproliferative/apoptotic effects [101]. For involution regulation, TGF- β promotes ECM remodeling by its expression in myoepithelial cells and cross-regulation of the AKT pathway (Fig. 4) [102–104]. The last mechanism involves prolactin crosstalk, which stimulates PI3K/AKT activation for cell survival, inhibiting the TGF- β -apoptotic signaling [105]. After weaning, prolactin levels decrease and cease stimulation of PI3K/AKT, leading to apoptotic fate mediated by TGF- β [101,104]. TGF- β modulates cyclin-dependent kinase (CDK) activity and reduces c-Myc, the protein coded by the *Myc* gene that regulates cell cycle progression and expression in epithelial cells, acting as a ductal elongation inhibitor interrupting the epithelial cell cycle [106]. Indirectly, TGF- β regulates the expression of p21 and p53, which promote cell cycle arrest and pro-apoptotic induction during late lactation and involution [102,107].

TGF- β regulates ER+/PR+ cell fate is provided by activation of its cognate receptor, which promotes the nuclear accumulation of “small mothers against decapentaplegic” (Smad), a signal protein for cell development regulated by TGF- β , in its phosphorylated form [108]. In addition, estrogens and TGF- β interact during MG development. Estrogens present a TGF- β inhibition route, influencing Smad proteins through ER α signaling, promoting a ubiquitin-proteasome pathway for degradation of Smad [60]. This is a non-genomic mechanism of estrogens and TGF- β crosstalk, and an important co-regulation pathway which is not well established.

2.3. Adipokines

Adipokines are a class of hormonal-cytokines secreted mainly by adipocytes, which play an essential role in breast development [109]. Several adipokines, such as ghrelin, interleukin 6 (IL-6), adiponectin, and leptin are involved in mammary stroma remodeling and cell proliferation mechanisms [56].

Leptin and adiponectin act in opposite ways during the development, lactation, and involution of the MG. Leptin induces cell-cell interaction between adipocytes and epithelial cells, promoting epithelium proliferation and differentiation (Figs. 2 and 3) [109,110]. Through this mitogenic ability, leptin also expands the stem and progenitor cell population in the epithelial compartment by stimulating the PI3K/AKT pathway [111]. Extramammary leptin is a significant effector in mice mammary tissue by stimulation of the hypothalamic-pituitary-ovarian

axis to produce estrogens [112]. Although leptin acts as an HGF inhibitor, in the presence of prolactin, it promotes mitogenic pathways [113]. In cows, prolactin increases leptin receptor (Ob-R) expression and leptin mRNA expression during lactation [114,115]. Ob-R binding triggers most intracellular proliferation pathways, such as MAPK, PI3K, JAK/STAT, and mTOR [116], and regulates lactogenesis and fatty acid synthesis in bovine MG [114].

Adiponectin, contrarily, activates antiproliferative and pro-apoptotic pathways, promoting 5' adenosine monophosphate-activated protein kinase (AMPK) cascade [117,118], which inhibits proliferative pathways, i.e., MAPK, AKT, Wnt, JAK, and PI3K (Fig. 4) [119–121]. Prolactin inhibits adiponectin release in the breast [122]. This lactation hormone and growth hormone regulate adiponectin transcription and its two receptors, ADIPOR1 and ADIPOR2 [118,122,123]. Both hormones modulate STAT5 binding to adiponectin gene promoter, aiming to decrease its expression [124]. Thus, adiponectin mRNA expression decreases during lactation when prolactin and GH are at high circulating levels [123].

2.4. Epigenetic Regulation and miRNA

The epigenetic regulation of MG development is crucial to specific aspects of cell differentiation, notably in the epithelial compartment [125]. Processes related to DNA methylation (DNAm), histone modifications, and miRNA expression play crucial roles in pathway crosstalk mechanisms, previously explained, and repressing/silencing of the expression of essential genes [126]. Briefly, DNAm and histone modifications alter the chromatin state, enabling the inhibition or promotion of genes during mammary development, whereas miRNA impacts mainly the post-transcriptional process [125,127,128].

2.4.1. DNA methylation

In general, DNAm is an epigenetic tool by which cells “lock” genes; this means that when methylation occurs, gene silencing takes place [125]. However, the impacts of DNAm vary according to the context since it may also modulate active gene transcription [129]. In MG, when DNAm takes place in tumor-suppressing genes it leads to carcinogenesis [130]. DNAm is mediated by DNA methyltransferase enzymes (DMTs) occurring in CpG (cytosine-phosphate-guanine) islands [126]. During MG development, the expression of DMTs increases (for hypermethylation) or decreases (for hypomethylation) in cells linked to epithelial-luminal differentiation, such as stem and progenitor cells [127]. DNAm in specific genomic regions contributes to the differentiation and self-renewal processes observed by reducing Ki-67 and SOX transcription factor expression in stem and luminal progenitor cells [131]. Thus, DNAm is associated with silencing genomic regions related to cell fate or expression of genes that determine the cell lineage. During lactation, genes linked to milk proteins are demethylated and start to be expressed in luminal epithelial cells [125]. Furthermore, methylation/demethylation events in DNA regulate the expression of oxytocin receptors in the MG, indirectly modulating this hormone action in lactation [132].

2.4.2. Histone modifications

Molecular alterations in histones, such as acetylation, ubiquitination, methylation, and demethylation, regulate DNA accessibility and transcription of specific chromatin sites [133]. These post-translational modifications present a variable role in progenitor, basal, and luminal epithelial cells during MG development [134,135]. Thus, histone-modifier proteins in mammary tissue regulate epithelial-mesenchymal transition and cellular hierarchy [127]; histone methyltransferases and demethylases control these modifications. The maintenance of this activity is performed by polycomb repressive complexes (PRCs), i.e., protein groups that mediate the transcriptional signal of essential proteins for cell fate. The most commonly studied PRCs in MG are the histone-lysine N-methyltransferase EZH2, which

regulates differentiation in TEBs and progesterone-induced proliferation, and the K demethylases group (KDM), which mediates luminal progenitor cell differentiation [136–139]. This regulation also indirectly impacts the pathway of paracrine differentiation induced by EGF and TGF- β [134].

2.4.3. miRNA

miRNAs are a family of RNAs that do not code known proteins but can silence gene expression through translational suppression [140]. They are produced in the nucleus and go through a series of modifications in the cytoplasm [141]. The fact that a single miRNA may target several genes means that the downstream effects can considerably affect many cell functions [142]. The existence of oncosuppressor miRNA has been described and these are downregulated in cancer [143]. The primary mechanism of miRNAs action is the post-translational targeting of specific sequences of mRNA, which leads to the suppression of the encoded protein [144]. miRNAs can either act on cells in which they have been transcribed or affect other cells and tissues from their translocation by exosome vesicles [145]. After being transcribed, miRNA binds to the Argonaute protein (AGO)/RNA-induced silencing (RISC) complex, between the translated region (open reading frame - ORF) or after the stop codon (3' prime untranslated region - 3'UTR), which keeps it silenced until its activation [146].

In the MG, miRNAs have been described to have specific physiological impacts: they regulate post-transcriptional expression of genes directly involved in mammary duct branching and acini formation (Fig. 2) [147,148]. The mechanisms involved in duct elongation are matrix metalloproteinase expression levels which are also related to the expression of miRNA [149]. Specific miRNAs are expressed according to the developmental stage of the MG [128,150]. They attenuate the expression of methyltransferases and acetyltransferases that impact two epigenetic mechanisms: DNAm [151], and histones acetylation [152]. The potential of miRNA under normal and/or pathological conditions is due to the inhibition of mRNA translation or transcript degradation (see review - Klinge (2015)). During breast development and lactation, miRNA acts mainly by inhibiting the expression of mRNAs and proteins linked to cell death and/or methyltransferases that suppress proliferative signals (Fig. 3) [154]. The Smad complex, generated by the stimulus of exogenous TGF, recruits co-activators in the nucleus and induces acetylation and expression of the TGF gene in stromal cells [155].

During involution, cyclooxygenase-2 (Cox-2) controls cell proliferation and the progress of MG to the regression phase, modulated by miRNA families [148]. In the murine model, downregulation of miRNA affects transcription and translation of MAPK, CDKs, and proliferative factor receptors [156]. Several miRNAs reduce the stability or translation of ER α mRNA, decreasing the influence of estrogen in cells [153].

3. Bisphenol A

BPA is one of the most widely studied EDs. Humans and a wide range of species are commonly exposed to BPA in everyday life. BPA is a xenoestrogen directly related to alterations in reproductive organs [6, 157].

From a toxicokinetic point of view, the oral ingestion of BPA precedes transformation during the liver first pass metabolism, which converts it into BPA-glucuronide and sulfate conjugates, through a series of oxidation and hydroxylation reactions in hepatocytes due to the presence of cytochrome P450 [158]. Thus, the great majority of ingested BPA is metabolized within 5 h of its ingestion. Despite glucuronides have no estrogenic activity, they contribute to adipogenesis, as demonstrated in a murine preadipocyte cell line (3T3L1) [159] but other oxidative metabolites may have endocrine impacts, such as estrogenic activity [160]. Bisphenol S and Bisphenol F, two analogues that were proposed to replace BPA, have a similar metabolism [161]. BPA is considered a metabolic-endocrine disruptor with access to multiple cell levels (from cellular machinery to epigenetic fields) and multiple organs [162–165].

Although the hepatic metabolism of BPA is known to be quite fast in humans, unconjugated molecules have been detected in blood and urine samples, which means that non-oral routes, such as skin absorption, for example, may circumvent liver and intestinal metabolism [166]. According to Vandenberg et al., it is, however, difficult to identify all the sources of non-oral exposure to xenoestrogens [167]. In addition, bioaccumulation in fatty tissues is considerable due to its lipophilic feature [168]. Efforts by the Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA) have described a list of target-organs affected by BPA according to exposure doses [165].

In vivo experiments describing the impacts of BPA in the MG have applied a range of different doses. Frequently, doses are representative concentrations of ubiquitous human exposure, according to public health organizations (FDA, EPA, WHO; [169]). In this review we focused on discussing results from papers that have applied either doses considered safe or doses that are commonly found in human blood or urinary samples. However, it is important to clarify that non-monotonic dose response relations in MG physiology and morphology have been assigned to BPA exposure. In summary, there is a consensus that BPA may cause effects at low doses that are not present at high doses [170, 171]. Thus, it is crucial to take into consideration the possibility of a non-monotonic dose-response when comparing results obtained in different experimental designs, since the impacts of low doses may not be predicted by those of high doses. The main mechanisms by which BPA acts in the MG tissue and some consequences of exposure during windows of susceptibility will be discussed below.

3.1. Changes in serum hormone availability

The serum concentration of hormones is a factor that actively impacts MG homeostasis and dynamics (Fig. 5A). BPA exposed adult rats (25 mg/kg/day) showed increases in the serum levels of E2 and progesterone; in these animals, there was a higher generation of reactive oxygen species (ROS) and lower presence of antioxidants in granulosa cells, leading to an increase in inflammatory markers in the ovary [172]. Thus, BPA impacts steroidogenesis through direct influence in ovarian cells, which alters serum steroid concentrations [173]. Perinatal and prepubertal exposures to BPA (25 and 250 ng/kg/day) play a role in disrupting the hypothalamic-pituitary-gonadal axis and/or the metabolic homeostasis of hormones in mouse models [174]. Thus, these alterations may lead to disruption effects, since BPA (50–5000 mg/kg/day) was also proven to increase E2 bioavailability by inhibiting enzymes that inactivate this hormone [175–178]. Contrarily, perinatally treated F1 female rats (0.05 mg/kg/day or 20 mg/kg/day) showed reduced serum levels of E2 and progesterone during gestation [179]. Perinatal exposure to BPA (0.5 or 10 mg/kg/day) also triggered a disrupting process in female CD-1 mouse offspring through the increase in length of estrous cycles, impacting the MG homeostasis, and inducing accelerated morphogenesis of the epithelial structures [180].

BPA (0–3000 μ g/kg/day) induced a dose-dependent decrease serum levels of leptin and adiponectin in mouse [181]. Obesity disorders are linked to modulation at the cellular level caused by BPA (Fig. 5A) – related to insulin and leptin resistance [182] and to the effect in human and murine preadipocyte differentiation [159] –, and changes in the pancreatic cell cycle, increasing the serum insulin level in dams exposed (10 or 100 μ g/kg/day) during pregnancy [183,184].

3.2. Changes in hormone receptor machinery and expression

Variations in serum estrogen concentrations impact the MG homeostasis mechanisms. However, similarly to what happens in response to variations in endogenous estrogen levels, disrupting pollutants influence the expression and activity of hormone receptors (Fig. 5B) [185, 186].

Xenoestrogens, similarly to endogenous estrogens, diffuse the cell membrane and bind to nuclear ERs (ER α and ER β) [187]. Therefore,

hormone receptor binding has been considered the central toxicity mechanism by which these chemicals impact homeostasis of the organism [188]. When available in high concentrations, BPA also binds to androgen receptors, antagonizing their functions [168]. Even though BPA binds to ER, with an approximately 10^{-4} fold lower affinity than E2 [189], it reduces the ability of E2 to activate ER α cells [190]. Since BPA presents more affinity to ER β [191–193], it may affect signaling of ER α by E2 [194]. Both receptor interactions have been proposed as responsible for triggering hormone-dependent tumors in the breast [195,196].

E2-ER binding is based on the affinity of the molecule to the ligand-binding domain of the receptor; for exogenous compounds, the presence of a para-hydroxy group assures this interaction [197]. Through binding, conformational changes in ER protein activate it, enabling genomic and non-genomic mechanisms [6]. However, studies comparing the binding features of compounds to ERs have shown that while E2 binds in similar ways to ER α and ER β , other chemicals do this in different

manners [198]. BPA was proven to exert an allosteric modulation, binding to a specific receptor site to increase agonist and antagonist functions [199]. A molecular modeling study revealed that BPA binding to the ER α “ligand-binding domain” disturbs the receptor-DNA recognition process [188], causing long-lasting epigenetic alterations that impact gene expression [200].

The ligand-receptor binding may trigger three different mechanisms. A genomic mechanism occurs based on binding of the activated receptor to EREs, specific nucleotide sequences in the DNA located near estrogen-regulated genes [201]. The second consists of an indirect genomic mechanism, which involves changes in gene expression as well, however, through interaction with other transcription factors, not EREs [202]. A non-genomic mechanism has also been described, based on the interaction of the ligand-receptor association with kinase receptors to influence other signaling pathways, impacting cell proliferation by stimulating ER α or reducing ER β signaling [203].

The agonistic effect of xenoestrogens mimics the transcriptional activation promoted by endogenous estrogens. It is worth considering that the agonist/antagonist activity created by EDs depends on the target gene and cell type in question [201]. Among the wide range of estrogen target tissues – comprising female and male reproductive tracts, bone, and the cardiovascular system – is the MG [204]. As previously stated, estrogen plays a significant role in the MG, among other hormones, for cell differentiation and proliferation [2]. The expression varied throughout the lifetime in a gerbil model, responding to the current hormonal profile [205].

3.3. Interference in molecular pathways

Perinatal BPA exposure (250 ng/kg/day) in CD-1 and C57Bl6 mice increases MG estrogen sensitivity [206]. This sensitiveness implicates changes in the expression of proteins related to proliferation, such as Ki-67 [207], which presents an increase in its cytoplasmic expression related to breast tumors [208] and upregulation of p16 and cyclin E, to induce proliferation [209]. In contrast, BPA (5 mg/kg/day) also increases the caspase-3 cascade related to the involution process in albino rat mammary tissue [207]. Caspase is a cell death protein that could switch the normal apoptotic process response to necrotic activation [210].

EGFR response in breast cells is BPA-induced (1 μ M, cell culture) through the GPER/EGFR transduction pathway [211]. ERK is rapidly activated by the GPER cascade after BPA exposure (10 μ M, cell culture) in breast cancer cell lines [212]. Furthermore, Perrot-Applanat et al. hypothesize that BPA and exogenous estrogens [206] could induce overexpression of Areg in TEBs of different species during the proliferative period [213]. Thus, BPA can increase MG sensitivity to EGFs, even in cells that lack ER, through access to the GPER cascade [211], which interestingly shows a BPA-regulation of EGF via non-estrogenic pathways in MG. Furthermore, in MG of rat offspring prenatally exposed to BPA (250 μ g/kg/day), a higher expression of AKT/ERK and a lower expression of TGF- β provokes modulation of proliferative signaling [214]. BPA also interacts with estrogen-related receptor γ (ERR- γ), an orphan receptor to which BPA binds strongly [168]. BPA-induced ERR- γ activity (10 nM, cell culture) can modulate MMPs by ERK/AKT cascade, increasing the expression of MMP-2 and MMP-9 [215] and, consequently, impacting the composition and organization of MG tissue.

It is known that BPA modulates multiple receptor targets by epigenetic reprogramming [164]. From a tumorigenic perspective, BPA (1–10 nM, cell culture) up-regulates VEGF expression in breast tumor cell lines, supporting invasiveness and malignancy by means of an ER-dependent mechanism that promotes angiogenesis in vivo [216]. By inducing high sensitivity to progesterone, perinatal exposure BPA (5 mg/kg/day) in C57/Bl6 mice triggers Wnt-4 and Nf-kB in MG, stimulating a proliferative process in MSC [217] and an increase in RANKL expression that participates in progesterone signaling of MG in exposed rats (0, 25, 250 μ g/kg/day, dose-dependent) [218]. Through NF-kB and

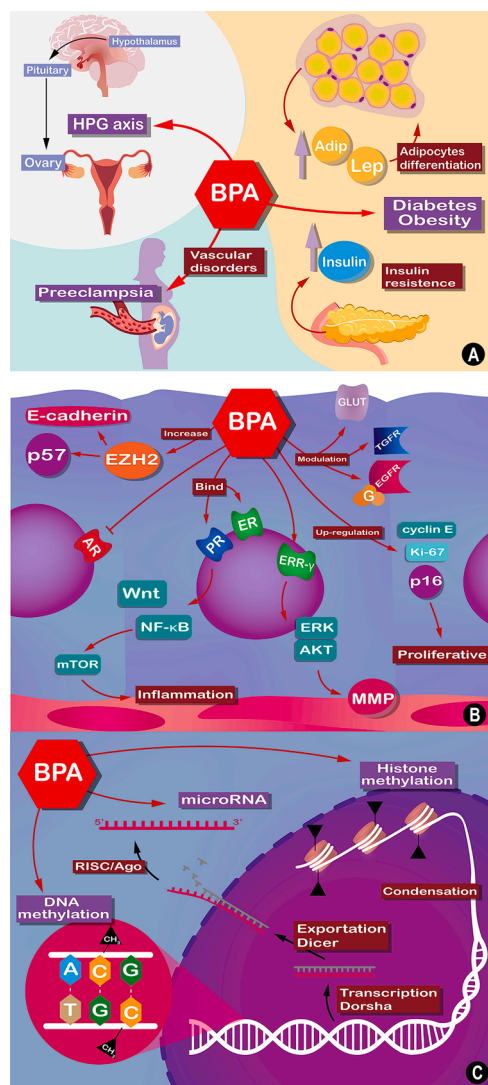


Fig. 5. Mechanisms by which bisphenol A induces mammary gland alterations. (A) Short- and long-term changes in morphological and, consequently, pathological aspects of the mammary gland. (B) Molecular pathways disrupted by BPA: binding to ER and PR to trigger inflammatory response through *mTOR* via *Wnt/NF-kB*; binding *ERR- γ* to trigger *ERK/AKT* pathways for stromal remodeling by *MMP* expression; modulating membrane receptors, such as *TFGR*, *EGFR*, and proteins, such as *GLUT*; and increasing *EZH2* protein synthesis, as an epigenetic disruption mechanism, and/or up-regulating proliferative proteins (*cyclin E*, *Ki-67*, *p16*). (C) Epigenetic mechanisms disrupted by BPA.

mTOR signaling, perinatal exposure to BPA diluted in drinking water (10 µg/mL/day) also promotes tissue inflammation and autophagic activity in MG from rats female offspring [219], creating a suitable microenvironment for cancer establishment [11].

In general, exposure to BPA is linked to the incidence of epigenetic alterations, related to the production of ROS and oxidative stress [220]. Changes in the modulation of oxidative mediator synthesis lead to disruption in the functions of imposing cellular organelles, such as mitochondria [221,222], and triggering of cell signaling pathways that induce the expression of apoptotic proteins [223]. In women in pre- and post-menopause phases, exposure to BPA increases the production of inflammatory and oxidative markers, with postmenopausal women being more susceptible due to their lower concentrations of serum E2 [224].

3.4. Epigenetic and miRNA alterations

There is evidence of epigenetic involvement in the majority of morphological alterations caused by increased exposure to EDs. Epigenetic alterations are caused by environmental or hereditary stable gene expression changes without modification in DNA sequences [225]. Perinatal exposure to factors that shift gene expression patterns may cause epigenomic reprogramming in mammals during embryogenesis [226]. This window of susceptibility is highly responsive to EDs, which control the epigenetic inheritance of adult diseases by modulating DNA epigenetic changes in reproductive cells [227]. These changes may shift the expression pattern by silencing or activating genes based mainly on methylation and/or modifications in histones [228].

Since DNAm, post-translational histone modification and noncoding RNAs are key regulators during MG development and differentiation, the impact of endocrine disruptors on these events will be discussed (Fig. 5C).

3.4.1. Changes in methylation patterns and histones

DNAm was confirmed to be sensitive to environmental exposure, and the methylation patterns vary according to cell types [168]. Previous experiments have confirmed this by the shift in mice offspring coat color after maternal exposure to a BPA formulated diet (50 mg/kg) [229]. Usually, a decrease in DNAm is present when perinatal exposure to this ED occurs, but no changes were observed after adult CD-1 mice exposure (5 mg/kg) [230], suggesting that methylation effects depend on the developmental phase of exposure.

In vitro experiments revealed that BPA (0.1 and 0.01 nM) changes the methylation pattern of MCF-10 F cells, by increasing the expression of genes involved in DNA repair and decreasing the expression of genes involved in apoptosis [231]. In MCF-7 cells, BPA exposure (0.1 and 10 nM) can alter the methylation pattern in several ER-dependent regions compared to BPS and BPF [232]. This disruptor is known to promote hypermethylation of oncogenes and hypomethylation of tumor suppressor genes [233]. The potential effect of neonatal exposure of rats to BPA (2.4 µg/day) on methyltransferase gene expression may be involved in this mechanism [234]. Alteration in methyltransferase expression is a known mechanism of action of BPA in metabolic disorders. In rats, for example, DNA hypermethylation after BPA perinatal exposure (50 µg/kg/day) was attributed to oxidative stress [235].

In pregnant exposed rats, BPA (6 or 52 mg/kg/day) changed the β-casein gene methylation pattern, promoting alterations in the secretion of this protein [236]. Epigenetic changes involving DNAm and remodeling of chromatin have been related to the process of carcinogenesis. Exposure during the perinatal window also impacts the methylation patterns of offspring MG: significant differences in the methylation pattern of MG are shown on the 50th postnatal day when comparing control and perinatally exposed (250 µg/kg/day) rats; changes in the initiation site of the alpha-lactalbumin gene are noteworthy [237], contributing to the development of neoplastic lesions in adulthood.

Environmental BPA-exposure that leads to carcinogenic changes may also be induced by histone methylation [238], which causes gene silencing. As an example of histone methyltransferase, PRC2 is known to

induce gene silencing and tumorigenesis. Its catalytic subunit, EZH2, is responsible for providing the methyltransferase activity [239]. In addition to increasing EZH2 mRNA and protein expression in MCF7 cells, BPA also increases the EZH2 mRNA and protein expression in the MG from in utero exposed adult mice [238]. EZH2-mediated methylation also impacts p57 and the E-cadherin genes, thus influencing cell cycle regulation and cell-cell adhesion, impacting cell proliferation [240] and cancer invasiveness [241]. In addition, the Homeobox A10 gene (Hoxa10), which encodes the homeobox transcription factor protein, was hypomethylated in mice after in utero exposure to BPA, causing alterations in gene expression programming and in the binding of ER to the ERE of Hoxa10, increasing the responsiveness of cells to estrogens [230].

3.4.2. Changes in microRNA expression

Among the factors that modulate miRNA expression, hormone signaling is of great importance, as estrogens and androgens are the main modulators of miRNAs in the reproductive system [242]. The cleavage of pri-miRNA into precursor miRNAs during its biogenesis in the nucleus may be repressed by ERα interactions [243]. Technically, ERα and androgen receptors affect this process through their gene transcription regulation [153]. In human cell lines, E2 induces alterations in miRNAs, as demonstrated by sequencing techniques [244]. Estrogen binds to ERα and ERβ to activate response elements in some miRNA promoters, i.e., miRNA-21, -155, and -124 [245].

Therefore, exposure to endocrine disruptors such as xenoestrogens may also affect the expression of certain types of miRNAs. This modulation even attributes them with a role as biomarkers since the quantities present in extracellular fluids may reflect environmental modifications [142,246]. The disruptors DDT and BPA (10 µM) are known to down-regulate miR-21 in MCF-7 cells [247]. The effects of miRNAs on the estrogen activation pathway may also influence ER coactivators [153, 247]. In goat mammary epithelial cells, for example, miR-135b down-regulated prolactin [248]. In ewes, embryonic exposure to BPA (0.5 mg/kg/day) upregulated miRNAs related to endothelial cell damage (miR-217) and cell cycle arrest (miR-608) in adult offspring [249]. Goats and ewes are models of constant mammary gland activity, due to their use for persistent milk production; this aspect obviously characterizes their MG as different within regards to human and rodent models, which are the most widely discussed herein. However, they are a very interesting model for obtaining mammary epithelial cell lines [250].

Therefore, the impacts of ED exposure during phases where MG shows considerable growth and differentiation may involve long-term epigenetic modifications through miRNAs. Specifically, in breast cancer, miRNA expression is related to the type of neoplasia developed by the patient, which, consequently, is a valuable tool to detect and evaluate stages in breast cancer, since serum analysis can be performed [251]. Thus, since EDs are regulators of miRNAs expression due to hormone receptor binding, there may be a relation between ED exposure and breast cancer susceptibility.

4. What are the consequences of these disturbances during the developmental windows?

BPA exposure and a normal environment are compared in Table 1, which summarizes the consequences of disruption during different windows of susceptibility. Morphological alterations in the epithelium and a higher susceptibility to breast cancer are the major impacts of ED exposure during perinatal development. Higher numbers of TEBs, dilated ducts, and alveolar structures [252] are MG alterations present after gestational exposure. In adulthood, the impacts of perinatal exposure to BPA include an increase in collagen fibers and in the incidence of hyperplasia [207,236,253], inflammation [254,255], and the presence of proliferative cells [256,257]. In the MG, EDs increase susceptibility to malignant alterations, especially during critical periods of development, when tissue is prone to growth and differentiation [258].

The morphological alterations in the gland during these windows are based on tissue remodeling occasioned by hormonal signaling [259]. According to Fenton and Birnbaum, EDs may cause irreversible impacts in tissue architecture related to hormone binding and receptor activation, but not limited to this mechanism [13].

Thus, it is reasonable to connect the possible impacts of chemicals that cause hormonal balance changes and alter the long-term homeostasis of the gland. It is important to bear in mind that perinatal exposure to chemicals (a window of susceptibility) implicates transgenerational effects by impacting the development of primordial germ cells [260], leading to impacts on embryo development. These effects are transmitted not due to DNA mutations but due to epigenetic changes in the germline or embryonic context-dependent transmission cells during development [10]. In addition, epigenomic changes caused by in utero exposure induce long-term alterations in mammary morphology since mammary stem cell differentiation, lineage determination, and development are functions regulated epigenetically, employing DNAm and miRNA [125].

The period of perinatal development is critical regarding these influences so that epigenetic changes in parents can be transmitted to children [164]. According to Skinner et al., the sequence of events that culminates in pathological effects starts in the epigenetic action, passing through alterations in the reading of DNA, protein expression, and organ physiology until it causes tissue disorder [261].

Yaoi et al. report that maternal exposure to BPA can change approximately 0.3 % of the CpG regions, causing hyper or hypomethylation in the developing brain's genetic material [262]. These authors also reinforce that methylation caused by BPA is loco-specific and dependent on the developmental stage of the organ. Regarding possibly affected genes, it is believed that they involve deregulation of, for example, genes encoding hormone receptors, altering their expression, or long-term activity [263]. Specifically, in MG cells, some genes known to be affected by BPA in vitro action are involved in DNA repair (BRCA1, BRCA2, BARD1, CtIP, RAD51, and BRCC3) and the regulation of apoptosis (PDCD5 and BCL2L11) [231].

During the post-weaning involution window of susceptibility, MG is characterized by a pro-inflammatory environment that may facilitate metastasis and tumor development [264]. The weaning period may also lead to a critical window of estrogenic signaling, consisting of a risk factor to the development of breast cancer, which can be worsened in the presence of EDs [265]. When sensitized by EDs such as BPA, this pro-neoplastic microenvironment develops characteristics for the growth of local tumors by hormonal imbalance or interferences in molecular pathways, to accelerate the apoptosis or overstimulate proliferative activity [29,52]. In postmenopausal women, serum BPA was positively associated with elevated mammographic density, confirming a relation between EDs and a higher breast cancer risk [177]. Both molecular and physiological changes converge or start from an epigenetic point of view, and epigenetic regulation (or disruption) affects MG structure [152].

5. Final remarks and conclusion

The MG comprises a complex organ where a dynamic development/involution system is present to allow the necessary plasticity for the early nourishment of mammals. It constitutes a warning system for risk assessment of EDs, which disturb MG homeostasis and cause long-term alterations mainly related to malignant pathologies. Although some aspects have been elucidated regarding the mechanisms involved in these processes, much remains to be unveiled, especially because cohort and epidemiological studies are the only options to evaluate human effects, and experimental designs for animals do not usually mimic all the possible exposure routes.

Reviewing the literature led us to raise a particular concern regarding the lack of studies about the impacts of gestational and lactational exposure in mothers, since most studies focus on perinatal and pubertal exposures. It is also important to emphasize that BPA acts

not only on ERs but also on multiple hormone receptors and epigenetic targets in cells, triggering a plethora of adverse effects. Thus, BPA is a metabolic-endocrine disruptor that is dose-dependent – different dosages of exposure can trigger different effects in organs – and have effects on multiple cell levels – interfering in processes from metabolism to morphology.

In conclusion, MG development windows are orchestrated by several molecules and pathways that respond to the effects of ubiquitous industrialized chemical compounds. BPA contributes to the establishment of the MG neoplastic predisposition by acting through several mechanisms. This review synthesizes some lessons learned in the scientific field, contributing to understanding of MG dynamics and responses to deleterious factors.

Declaration of Competing Interest

The authors declare no conflict of interest.

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