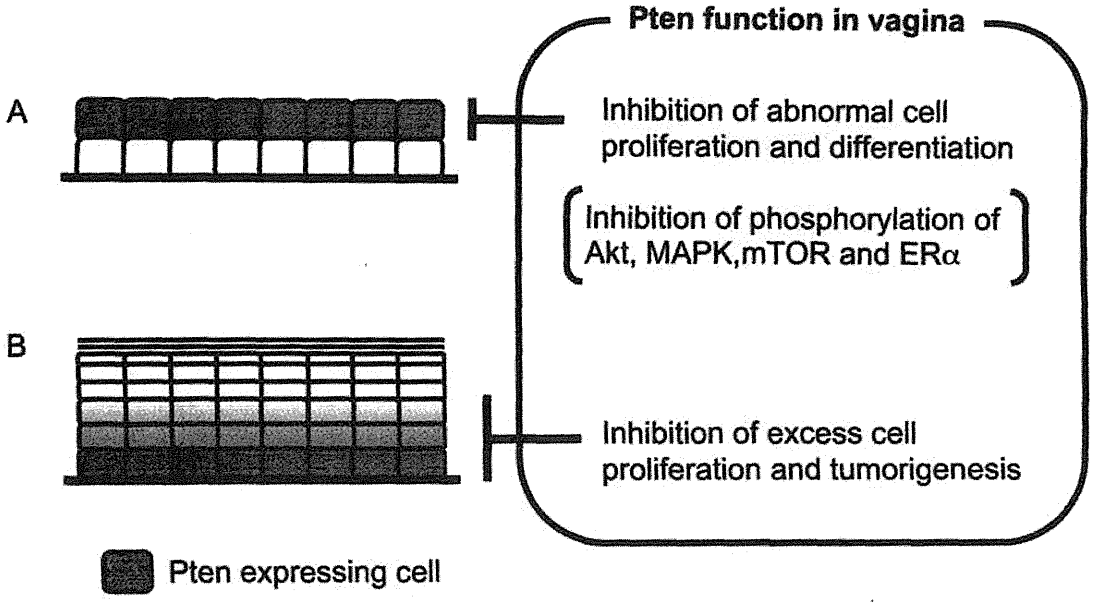
Fig. 8


Pten function in vagina
A
Inhibition of abnormal cell proliferation and differentiation
Inhibition of phosphorylation of Akt, MAPK,mTOR and ERα
B
Inhibition of excess cell proliferation and tumorigenesis
Pten expressing cell

in vivo 27: 333-338 (2013)

Epithelial–stromal Interactions in the Mouse Vagina Exposed Neonatally to Diethylstilbestrol

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Abstract. *Background: In neonate mice exposed to diethylstilbestrol (neoDES), vaginal epithelium shows persistent proliferation and stratification even after ovariectomy. Tissue recombination studies suggest that neonatally-estrogenized vaginal stroma can induce vaginal epithelial hyperplasia depending on the stromal age. This study examined the proliferative effect of the vaginal stroma from 8-day-old mice treated with DES on the vaginal epithelia of 8-day-old and adult mice. Materials and Methods: Vaginal epithelium and stroma from 8-day-old and adult mice was recombined, and grafted to ovariectomized host mice. Results: The 5-bromo-2'-deoxyuridine (BrdU)-labeled cells in the epithelium and the number of epithelial cell layers were not significantly different between epithelia from 8-day-old and adult mice when combined with stroma from 8-day-old control mice. BrdU-labeled cells in the vaginal epithelia from both age groups combined with the stroma from 8-day-old neoDES mice exhibited higher values. The epithelium from neoDES adult mice had a lower percentage of BrdU-labeled cells. Conclusion: The stroma from 8-day-old neoDES mice induces epithelial cell proliferation, but lower stromal cell proliferation.*

The mouse vagina consists of stroma and stratified squamous epithelium, with 2 to 12 cell layers. During estrous cycles, vaginal epithelial cells proliferate and stratify (up to 12 layers) and the superficial cells undergo keratinization in response to estrogen. Overiectomy induces a decrease in the number of epithelial cell layers, epithelial thickness and mitotic index, accompanied by an increase of apoptotic cells (1, 2).

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Key Words: Epithelial–stromal interactions, vagina, mouse, diethylstilbestrol.

Diethylstilbestrol (DES), a synthetic estrogen, was routinely prescribed to several millions of pregnant women from 1946 to 1971 for the prevention of abortion (3). It is now well-known that *in utero* exposure to DES induces vaginal clear-cell adenocarcinoma in young women (4). In mice, neonatal exposure to estrogens, including DES, induces ovary-independent persistent proliferation and cornification in vaginal epithelium (5). Vaginal epithelial cells from ovariectomized adult mice exposed neonatally to DES (neoDES) exhibit a decrease in the initial rate of cell proliferation and an altered sensitivity to epidermal growth factor and insulin *in vitro*, suggesting that epithelial cells isolated from DES-exposed vaginae in mice are different in sensitivity on hormones and growth factors, from those of control mice (6, 7). Thus, neonatal DES exposure alters the characteristics of epithelial cells and sensitivity to hormones and growth factors in the mouse vagina.

Cunha *et al.* (8) examined the epithelial-stromal interactions of ovary-independent vaginal hyperplasia using a tissue recombination technique. While recombinants of the epithelium and the stroma from adult control mice exhibited an atrophied epithelium in ovariectomized hosts, control epithelium associated with the stroma from adult mice treated with estrogen neonatally-exhibited hyperplasia, suggesting that the stroma from neonatally-estrogenized mice induced cell proliferation in the control epithelium. On the other hand, control epithelium from 8-day-old mice exhibited atrophied epithelium in ovariectomized hosts when it was recombined with 8-day-old mouse stroma treated with estrogen neonatally. These results indicate that vaginal stroma from 8-day-old mice has less inductive activity compared to adult vaginal stroma, or the epithelium from 8-day-old mice is not able to accept the proliferation signals from the estrogenized stroma.

The underlying stroma can induce and specify the regional characteristics of the epithelium (9). Uterine epithelium combined with vaginal stroma differentiates into a vaginal stratified squamous epithelium. Similarly, vaginal epithelium in association with uterine stroma exhibits uterine epithelial

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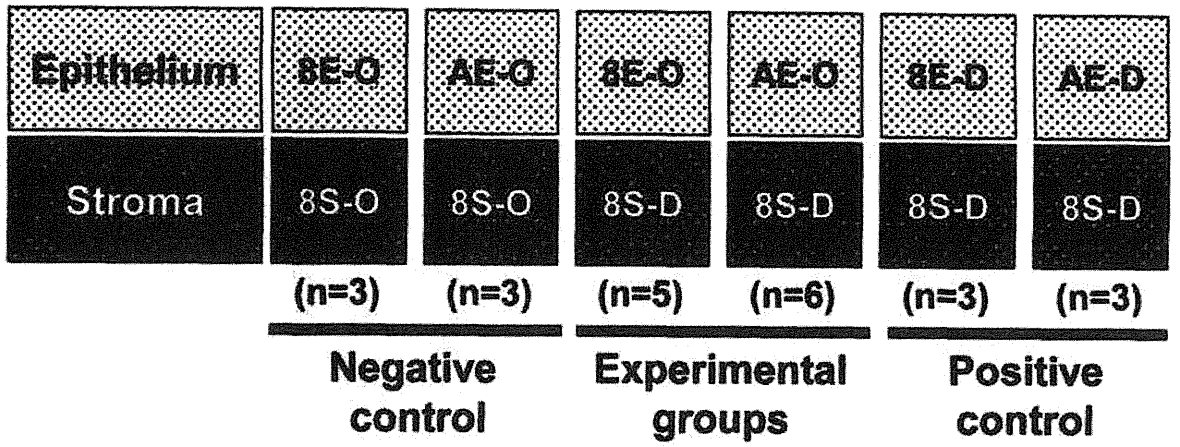

Figure 1. *Diagram indicating the tissue recombinant groups: 8S-O, stroma from vagina from 8-day-old mice; 8E-O, the epithelium from vagina from 8-day-old mice; AE-O, epithelium from adult mouse vagina; 8S-D, stroma from vagina from 8-day-old mice exposed neonatally to diethylstilbestrol (DES); 8E-D epithelium from 8-day-old mouse vagina exposed neonatally to DES; AE-D, epithelium from vagina from adult mice exposed neonatally to DES.*

differentiation. The developmental response of epithelia is age-dependent and neonatal epithelium has a higher sensitivity to induction signals from the stroma than the adult epithelium (9). Thus, the balance of epithelial sensitivity and stromal induction capacity may differ with age.

The aim of this study was to examine whether the vaginal stroma from 8-day-old neoDES mice induced continuous cell proliferation of untreated epithelium. Vaginal epithelium and stroma from 8-day-old neonatally DES- or oil-treated mice were separated, recombined with the epithelium from 8-day-old or adult mice, and transplanted into ovariectomized host mice, the epithelial morphology and proliferative activities of cells in the grafts were then examined.

Materials and Methods

Animals. C57BL/6J mice purchased from CLEA Japan Inc. (Tokyo, Japan) were given commercial diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum*. All animals were housed under controlled lighting (12 h light/12 h dark) and temperature (23-25˚C) conditions and maintained in accordance with the NIH guide for the Care and Use of Laboratory Animals (10) and all experiments were approved by the Institutional Animal Care Committee of the Yokohama City University (#20402).

Treatments, tissue separation and recombination. Neonatal C57BL/6J mice were daily injected subcutaneously with 3 μg DES (Sigma Chemical Co., St. Louis, MO, USA) dissolved in 0.02 ml sesame oil or the oil vehicle-alone for five days from the day of birth (=day 0). Vaginae were dissected from oil- or DES-treated 8-day-old mice and 90- to 120-day-old mice ovariectomized 10 days before termination, then incubated with 1% trypsin (Gibco BRL, Gaithersburg, MD, USA) in Hank's balanced salt solution (HBSS) (Sigma Chemical Co.) for 90 min at 4˚C. After trypsinization, tissues were placed into 20% fetal bovine serum (Sigma Chemical Co.) in HBSS for 5 min, and vaginal epithelium and stroma were separated by gentle mechanical manipulation using fine forceps. The procedure of tissue recombination was performed according to previous reports (8, 9). Six types of recombinants were prepared (Figure 1): Stroma from vaginae from 8-day-old mice (8S-O) plus epithelium from vaginae from 8-day-old mice (8E-O); 8S-O plus epithelium from vaginae from adult mice (AE-O); stroma from vaginae from 8-day-old mice exposed neonatally to DES (8S-D) plus 8E-O, 8S-D plus AE-O; 8S-D plus epithelium from vaginae from 8-day-old mice exposed neonatally to DES (8E-D); and 8S-D plus epithelium vaginae from adult mice exposed neonatally to DES (AE-D). Tissue recombinants were incubated on agar plates overnight at 37˚C in humidified, 5% CO_2 atmosphere in air.

Grafting, harvesting and histological analysis. Tissue recombinants (n=2-3 of each group) were transplanted under the renal capsules of ovariectomized host mice (n=3-6) for two weeks. Hosts were injected intraperitoneally with 5-bromo-2'-deoxyuridine (BrdU) (Sigma Chemical Co.) (10 mg/100 g body weight) 2 h before sacrifice to identify proliferative cells. Host mouse uterus and vaginae were weighed to confirm the lack of estrogenic stimulation at dissection. Grafts were fixed in 10% formalin neutral buffer solution (Wako Pure Chemical Industries, Osaka, Japan), embedded in paraffin and sectioned at 6 μm of thickness. Sections were stained with hematoxylin and eosin for histological analysis.

BrdU immunostaining. To determine the number of proliferative cells on the grafts, BrdU immunostaining was performed. Sections were mounted on glass slides coated with 2% 3-aminopropyltriethoxysilane (Sigma Chemical Co.), de-paraffinized, hydrated with graded ethanol and rinsed in phosphate-buffered saline (PBS) (pH 7.4). Endogenous peroxidase was blocked by 0.3% H_2O_2 in methanol for 10 min and immersed in 2 N HCl for 20 min at room temperature to denature the genomic DNA, then neutralized in 0.1 M sodium tetraborate buffer (pH 8.5). After washing with PBS, sections were incubated with 1% trypsin (Sigma Chemical Co.) for 30 min at 37˚C for antigen retrieval.

Sections were incubated with 1% bovine serum albumin (BSA) in PBS (BSA/PBS) for 20 min at room temperature and incubated

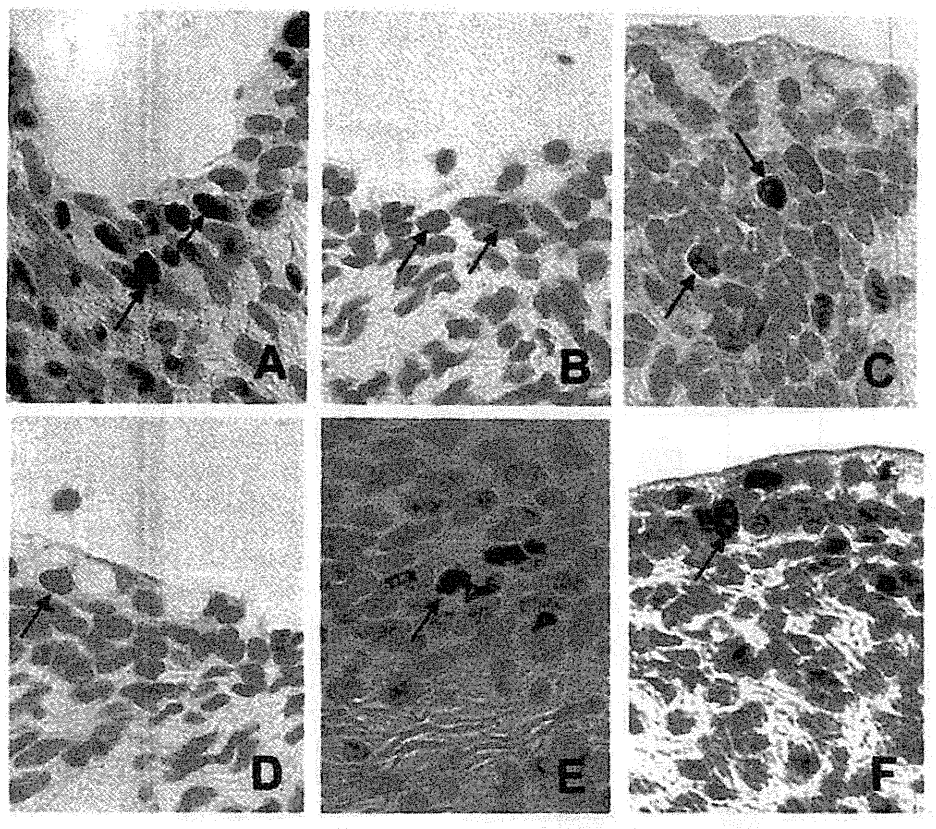

Figure 2. *Representative histological sections of 5-bromo-2'-deoxyuridine (BrdU) immunostaining of the tissue recombinants, 8S-O plus 8E-O (A), 8S-O plus AE-O (B), 8S-D plus 8E-O (C), 8S-D plus AE-O (D), 8S-D plus 8E-D (E) and 8S-D plus AE-D (F), respectively. (See lagend to Figure 1 for groups). BrdU-labeled cells are shown by arrows. Bar=20 μm. The cells indicated by arrows do not appear to stain as densely in all photos.*

with anti-BrdU-peroxidase, Fab fragments (1:15 dilution; Roche Diagnostics GmbH, Mannheim, Germany) in 1% BSA/PBS at 4°C overnight. Peroxidase visualization was performed using 2 mg/ml diaminobenzidine (DAB) (Sigma Chemical Co.) dissolved in 0.05 M Tris/HCl buffer (pH 7.4), containing 1 M imidazole (Sigma Chemical Co.) and 0.1% hydrogen peroxide for 1 min. Sections were counterstained with hematoxylin. The labeling index (%) was estimated by counting the number of BrdU-labeled cells per 200 cells in the grafted epithelium and stroma, separately, in three randomly-selected sections (n=3-6).

Statistical analysis. Data are expressed as the mean±standard error. Two-tailed Student's *t*-test after application of F-test was used for the comparison of the two mean values. A statistically significant difference was defined as $p<0.05$.

Results

Tissue recombinant groups performed in this study are shown in Figure 1. All-tissue recombinants exhibited a squamous epithelial morphology, with 2-3 cell layers typical of normal vaginal epithelium (Figure 2). In the 8S-D plus AE-O group, mucous cells were observed on the superficial cell layers. BrdU-labeled cells were found in both epithelium and stroma in the all-tissue recombinants (Figure 2, arrows).

The percentage of BrdU-labeled cells in the epithelium of 8S-D plus 8E-O was significantly higher than that of 8S-O plus 8E-O (Figure 3A). In the epithelium of 8S-D plus AE-O, the percentage of BrdU-labeled cells was significantly higher than that of 8S-D plus AE-D (Figure 3A). No significant difference was found in the percentage of BrdU-labeled cells in the epithelium between 8S-D plus 8E-O and 8S-D plus AE-O (Figure 3A), however, the percentage of BrdU-labeled cells in the epithelium of 8S-D plus AE-D was significantly lower compared with that of 8S-D plus 8E-D and 8S-D plus AE-O (Figure 3A).

In contrast, BrdU-labeled cells in the stroma of 8S-D plus AE-O was significantly reduced compared to those of 8S-D plus 8E-O, 8S-O plus AE-O and 8S-D plus AE-D (Figure 3B). The percentage of BrdU-labeled cells in the stroma of 8S-D plus 8E-O was significantly lower than that of 8S-O plus 8E-O (Figure 3B). The number of epithelial cell layers in 8S-D plus AE-O was significantly higher compared to that of 8S-O plus AE-O (Figures 2 and 3C).

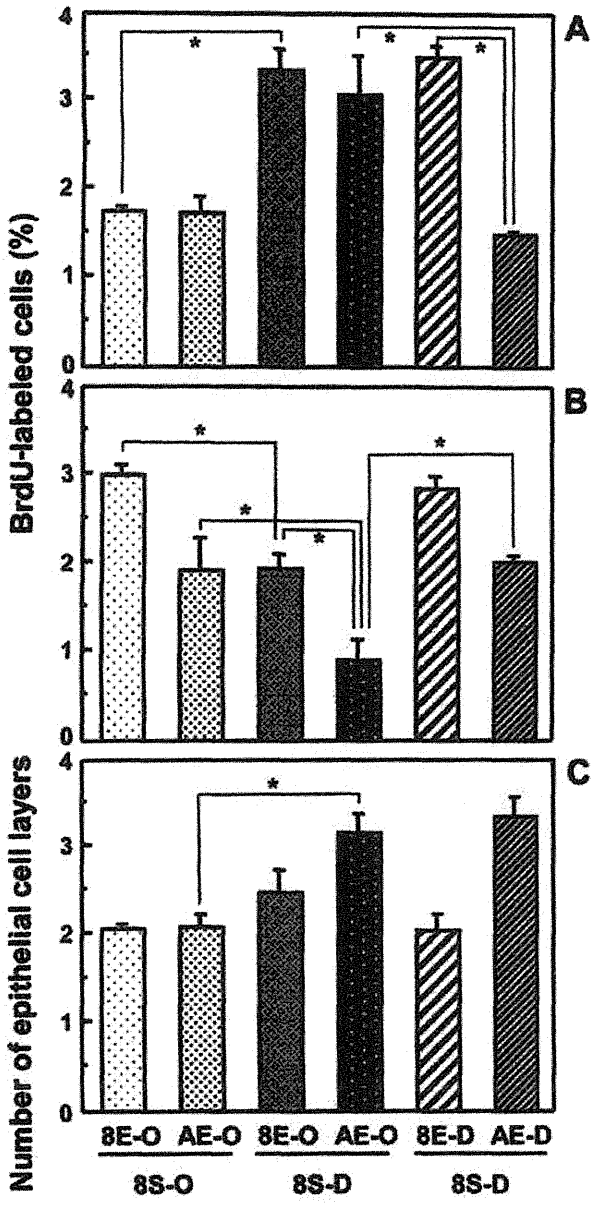

Figure 3. *Percentage of 5-bromo-2'-deoxyuridine (BrdU)-labeled cells in the vaginal epithelium (A) and stroma (B) of tissue recombinants and the number of cell layers in the epithelium of tissue recombinants (C). *Significant difference at $p<0.05$. (See legend to Figure 1 for groups).*

Discussion

Epithelial–stromal interactions are important in many organs. In male and female genital tracts, the underlying stroma induces and specifies the regional characteristics of the epithelium (11). In tissue recombination experiments, heterotypic recombinants composed of vaginal stroma and uterine epithelium usually exhibit vaginal morphogenesis. Therefore, stroma has an inductive effect on epithelial cells. The developmental responses of these heterotypic recombinants are age-dependent (9). Recently, we reported that fibroblast growth factor (FGF)7- and FGF10-FGFR2IIIb from the vaginal stroma stimulate differentiation in vaginal epithelial cells *via* the mitogen activated protein kinase (MAPK)1/3 pathway (12). Cunha *et al.* (8) also examined the epithelial-stromal interactions of ovary-independent vaginal hyperplasia using a tissue recombination technique and reported that the vaginal stroma from estrogenized mice induces stratification in the untreated vaginal epithelium.

In the present study, all-tissue recombinants exhibited a squamous epithelium that consisted of two to three cell layers. The percentage of BrdU-labeled cells in the epithelium of 8S-D plus 8E-O recombinants was significantly higher than that of 8S-O plus 8E-O, suggesting that the stroma from 8-day-old mice exposed neonatally to DES induced epithelial cell proliferation. In the 8S-D plus AE-O recombinants, mucification of vaginal epithelium was observed in the superficial cell layers, indicating that AE-O recombined with 8S-D could be stimulated by the DES-exposed mouse stroma. In addition, epithelial age did not correlate with epithelial cell proliferation induced by the stroma because no significant difference was found in the epithelium between 8S-D plus 8E-O and 8S-D plus AE-O groups. Our previous report showed that activated Hedgehog signaling stimulates epithelial cell proliferation in neonatal uterus and vagina but inhibits stromal cell proliferation in neonatal uterus (13), therefore, neonatal exposure to DES could alter these signaling pathways from the stroma. The number of vaginal epithelial cell layers was not different among groups except 8S-D plus AE-O, suggesting that 8S-D also has the ability to induce stratification and cornification only in the epithelium from adult mice. Interestingly, AE-D exhibited low cell proliferation even if it was recombined with 8S-D, therefore, the epithelium from adult mice exposed neonatally to DES did not respond to the stimulative signals from the stroma.

The percentage of BrdU-labeled cells in the stroma of 8S-D plus 8E-O recombinants was significantly decreased compared with that in 8S-O plus 8E-O, suggesting that the stroma from 8-day-old mice exposed neonatally to DES exhibited less cell proliferation. In the case of adult epithelium recombinants, neonatally DES-exposed stroma also exhibited decreased cell proliferation. In addition, the percentage of BrdU-labeled cells in the stroma of 8S-D plus AE-O recombinants was significantly decreased compared with that in 8S-D plus 8E-O. This fact suggests that stromal cell proliferation is affected by the age of the recombined epithelium. Therefore, adult vaginal epithelium inhibited stromal cell proliferation, but vaginal epithelium from 8-day-old mice did not. The epithelium from both 8-day-old and adult mice exposed neonatally to DES did not inhibit the stromal cell proliferation. Thus, the epithelium from DES-exposed mice may lose its inhibitory effects on the stroma.

Immature uterine stroma but not adult stroma exhibits 17β-estradiol (E_2)-induced cell proliferation (14). Uterine and vaginal stromal cells from immature mice are mitogenically stimulated by E_2 *in vitro* (15) and by DES *in vivo* (16). Normal uterine growth is independent of the ovaries and adrenals prior to postnatal day 10 (17). These results indicate that immature stromal cells can proliferate with or without E_2 compared to adult stroma. This allows us to speculate that paracrine regulatory signals from adult epithelial cells prevent proliferation of the stroma. In rat uterine stromal cells, $p27^{Kip1}$ protein, a cell cycle-dependent kinase inhibitor (CKI) and exerting a negative control on cell-cycle progression, is detected by immunohistochemistry and E_2 induces a heterogeneous and a 'gradient-like' expression pattern of $p27^{Kip1}$ (18). The expression of $p27^{Kip1}$ protein could be affected by the epithelial tissue, resulting in regulation of uterine and vaginal stromal cell proliferation. $p21^{Cip1}$, another CKI, acts as a positive cell cycle regulator in mouse uterine epithelium (19). These results suggest that $p27^{Kip1}$ and $p21^{Cip1}$ might be positive and/or negative regulators of the cell cycle in the stroma of female reproductive organs affected by the epithelium. Further studies are needed to investigate the role of $p27^{Kip1}$ in both vaginal epithelium and stroma.

In conclusion, vaginal stroma from 8-day-old neoDES-mice induces vaginal epithelial cell proliferation, however, the epithelium of adult mice exposed neonatally to DES did not respond to the stroma. The vaginal epithelium of adult mice inhibits cell proliferation of stroma from 8-day-old mice neonatally-exposed to DES, but not of the epithelium. Therefore, the characteristics of the epithelium and the stroma are permanently changed by neonatal exposure to DES.

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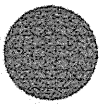

ENVIRONMENTAL HEALTH

COMMENTARY Open Access

Science and policy on endocrine disrupters must not be mixed: a reply to a "common sense" intervention by toxicology journal editors

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See related Editorial: http://www.ehjournal.net/content/12/1/70/abstract

Abstract

The "common sense" intervention by toxicology journal editors regarding proposed European Union endocrine disrupter regulations ignores scientific evidence and well-established principles of chemical risk assessment. In this commentary, endocrine disrupter experts express their concerns about a recently published, and is in our considered opinion inaccurate and factually incorrect, editorial that has appeared in several journals in toxicology. Some of the shortcomings of the editorial are discussed in detail. We call for a better founded scientific debate which may help to overcome a polarisation of views detrimental to reaching a consensus about scientific foundations for endocrine disrupter regulation in the EU.

Keywords: Endocrine disrupting chemicals, Environment, Health, Precautionary principle, Regulatory toxicology

Commentary

"Common sense is the collection of prejudices acquired by age eighteen"

- Albert Einstein

As experts and practitioners of endocrine disrupter research, several of whom were invited to prepare some recent international status reports of the topic [1-4], we, the authors, would like to comment on the recent editorial "Scientifically unfounded precaution drives European Commission's recommendations on EDC regulation, while defying common sense, well-established science and risk assessment principles" by Dietrich et al. [5].

We are concerned that the Dietrich editorial appears to be intended as an intervention designed to impact imminent decisions by the European Commission concerning endocrine disrupting chemicals (EDCs), countering the views recently expressed by the 129 signatories of the Berlaymont Declaration on endocrine disrupters [6] and by the Collegium Ramazzini [7]. Given the prominent nature of the authors as members of several EU scientific committees and the importance of these decisions, we would have expected a more accurate analysis of the situation. In contrast, the editorial confuses and conflates several aspects of the current debate that are important to clarify. In general, their fears appear to be founded on a 'common sense' that largely ignores the continued efforts of many scientific expert groups at European and international level as well as the expertise and competence of European decision makers.

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First, in describing endocrine systems as "... play[ing] a fundamental role in the physiological response to changes in the environment with the aim of keeping an organism's response within the homeostatic space" Dietrich et al. seek to define the endocrine system in overly simplistic terms to reduce the task of identifying endocrine disruption to making distinctions "between those effects that are within this adaptive range and effects that go beyond the boundaries of this space and thus can be called adverse" [5]. It is perplexing that editors of international toxicology journals seem to be unaware of the fact that endocrine systems also have a programming role during development, and that disruption of these programming events leads to irreversible effects that go far beyond disturbances of homeostasis [1]. Such phenomena (for example disruption of androgen action in fetal life and the malformations that arise from this) have been described for decades in the scientific literature and provide some of the cause for concerns about endocrine disrupting chemicals. These and other clearly demonstrated cases necessitate the identification of specific windows of vulnerability and this poses considerable challenges to established toxicity testing paradigms, all of which Dietrich et al. [5] ignore.

Thresholds and no thresholds

Dietrich et al. [5] claim that the "currently drafted EU framework" is based on an *a priori* default assumption of no thresholds for regulating endocrine disrupters, but no document is referenced to substantiate this claim. The latest publicly available document from the European Commission is the Report of the Endocrine Disrupters Expert Advisory Group (ED EAG) published by Directorate General Joint Research Centre (JRC) [8] which is intended to provide the underpinnings of the future EU regulatory framework for endocrine disrupters. The Report was prepared by an expert group comprised of 43 members from competent authorities representing 19 member countries of the European Union as well as other stakeholders including environment and health, NGOs and the industry-funded scientific association, ECETOC. The circumstances that led up to this Report are at odds with the claim by Dietrich et al. [5] that the proposed regulatory framework "is based on virtually complete ignorance of all well-established and taught principles of toxicology and pharmacology, of opinions raised by the European Commission's own competent expert authority (...), and of critical statements made by EU member states...". In the JRC document [8], no reference is made to a presumed *a priori* assumption of no thresholds for endocrine disrupters.

From a scientific standpoint, the issue of the existence of a threshold for endocrine disrupters and other non-genotoxic toxicants remains under debate. As Dietrich et al. [5] rightly point out, absence of effect cannot be statistically demonstrated in an experimental setting. It derives from this that regardless of the mode-of-action and the existence or non-existence of a mechanistic threshold, such a threshold cannot be demonstrated experimentally. If science prides itself in the robustness of its experimental approach to evidence, it should be stressed that the current argument can be modelled or theorised upon, but cannot currently be definitively experimentally tested. Regarding the claim that "...the weight of evidence (...) clearly demonstrates the presence of threshold for non-genotoxic compounds including EDCs...", Dietrich et al. [5] ignore that this evidence is far from established. In international toxicology journals, not under the editorship of Dietrich et al. [5], widely accepted biometrical and mathematical principles about the impossibility of establishing thresholds at the level of populations, independent of the status of the chemicals in terms of genotoxicity or non-genotoxicity have been elaborated [9,10].

Adversity of effects

It is also unclear where the claim by Dietrich et al. [5] that "the currently drafted EU framework for EDCs foresees a priori regulation of agents that may show presumably endocrine-mediated effects in some experimental system (*in vitro, in silico, in vivo...*)" derives from. The JRC report clearly states that for a substance to be identified as an endocrine disrupter, evidence not only of an endocrine mode-of-action but also of an adverse effect is required, as well as some plausible link between mode-of-action and adversity. This is consistent with the widely accepted IPCS definition [11] of endocrine disrupters which the JRC report accepted.

Concerning assays or endpoints that would be considered adequate for assessments of evidence of adverse effects, the JRC report makes detailed reference to level 4 or level 5 of the assays included in the OECD Conceptual Framework for the assessment of endocrine disrupters. This framework is the result of expert efforts over many years [12]. Although many endpoints relevant to endocrine disruption are not included in the OECD study guidelines, the tests that form part of the current framework are validated, robust, reproducible methods that have been tested in many laboratories before approval to ensure consistent, valid results that are also recognised worldwide under the OECD Mutual Acceptance of Data. These can hardly be qualified as "irrelevant tests" as Dietrich et al. [5] have done.

A priori assumption of human relevance

Referring to a statement by the European Commission (again not referenced) that "relevance of the data to

humans should be assumed in the absence of appropriate data demonstrating non-relevance", Dietrich et al. [5] declare: "The mere statement demonstrates the lack of attention paid by the European Commission to the weight of scientific evidence that clearly demonstrates the presence of a threshold for non-genotoxic compounds including EDC". Here, the authors conflate the statistical impossibility of demonstrating the absence of effects (and thresholds) with the issue of demonstrating human relevance of toxicity data derived from testing on animals. In doing so they reveal ignorance of important risk assessment principles elaborated in an IPCS Framework document [11] for assessing the human relevance of non-cancer endpoints [13]. The default assumption under that framework is of human relevance, unless there is evidence of toxicodynamic or toxicokinetic differences between the animal test species and humans that shows that the effect seen in animals is not expected to occur in humans. The applicability of that default assumption was tested through a number of case studies [13]. The alternative *a priori* assumption (that effects seen in animals are not relevant for humans) would be unworkable and would undermine the sense of conducting toxicological testing in animals at all.

"Scientifically unfounded precaution", and the distinction between hazard assessment and risk management

The most worrying aspect of the editorial by Dietrich et al. [5] is the blurring of the border between what constitutes science and what belongs to the realm of political, societal and democratic choices.

The Precautionary Principle is enshrined in European Law in the EC Treaty as well as in International Law [14]. This principle was elaborated at the 1992 Rio Conference on the Environment and Development, during which the Rio Declaration was adopted. Principle 15 states that: "in order to protect the environment, the precautionary approach shall be widely applied by States according to their capability. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation" [14]. Defined in this way, the precautionary principle is a legal concept for addressing scientific uncertainty, and not a scientific concept. Its interpretation and application is a matter for politicians and lawyers. The state of the science on endocrine disruption has been reviewed and summarised in several recent reports published by the UNEP/WHO or commissioned by the European Commission [1,2,8,15]. Already over 10 years ago, it was concluded that the state of the science justified regulatory action [13]. Decisions as to what kind of action may be justified by the level of available evidence and proportionate to the potential risks is a matter for politicians and risk managers, and not the exclusive domain of scientists. Yet Dietrich et al. [5] express strong reservations regarding the application of EU law but do not engage with the scientific basis for concern, or with widely published scientific evidence.

In contrast, the JRC report [8] made a clear distinction between hazard identification and characterisation on the one hand, which they considered within the remit of their expertise, and risk management on the other.

Scientific truths about endocrine disruption as a phenomenon resulting from disturbances of the programming effects of the endocrine system during development seem to have been ignored by Dietrich et al. [5]. It is to be hoped that this editorship of international toxicological journals will be able to engage in a better founded scientific debate which may help to overcome a polarisation of views detrimental to reaching a consensus about scientific foundations for endocrine disrupter regulation in the EU.

Abbreviations

EC: European communities; ECETOC: European centre for ecotoxicology and toxicology of chemicals; ED EAG: Endocrine disrupter expert advisory group; EDC: Endocrine disrupting chemical; EU: European union; IPCS: International programme for chemical safety; NGO: Non-governmental organisation; OECD: Organisation for economic cooperation and development.

Competing interests

All authors declare that they have no competing interests. Several of the authors were invited by the European Commission and UNEP/WHO, as scientific experts, to prepare some recently published international reports on state of the science of endocrine disrupters.

Authors' contributions

A core group of the authors first drafted the manuscript and circulated it for comments. All authors contributed actively to the revision of the draft. All authors approved the final version.

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