Environmental Research 140 (2015) 18–31

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Phenotypic malignant changes and untargeted lipidomic analysis of long-term exposed prostate cancer cells to endocrine disruptors

Carmen Bedia *, Núria Dalmau, Joaquim Jaumot, Romà Tauler

Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), c/ Jordi Girona 18-24, 08034 Barcelona, Spain

ARTICLE INFO

Article history:
Received 4 February 2015
Received in revised form
5 March 2015
Accepted 16 March 2015

Keywords:
Endocrine disruptors
Chronic exposure
Untargeted lipidomics
Prostate cancer
Chemometrics

ABSTRACT

Endocrine disruptors (EDs) are a class of environmental toxic molecules able to interfere with the normal hormone metabolism. Numerous studies involve EDs exposure to initiation and development of cancers, including prostate cancer. In this work, three different EDs (aldrin, aroclor 1254 and chlorpyrifos (CPF)) were investigated as potential inducers of a malignant phenotype in DU145 prostate cancer cells after a chronic exposure. Epithelial to mesenchymal transition (EMT) induction, proliferation, migration, colony formation and release of metalloproteinase 2 (MMP-2) were analyzed in 50-day exposed cells to the selected EDs. As a result, aldrin and CPF exposure led to an EMT induction (loss of 16% and 14% of E-cadherin levels, respectively, compared to the unexposed cells). Aroclor and CPF presented an increased migration (134% and 126%, respectively), colony formation (204% and 144%, respectively) and MMP-2 release (137% in both cases) compared to the unexposed cells. An untargeted lipidomic analysis was performed to decipher the lipids involved in the observed transformations. As general results, aldrin exposure showed a global decrease in phospholipids and sphingolipids, and aroclor and CPF showed an increase of certain phospholipids, glycosphingolipids as well as a remarkable increase of some cardiolipin species. Furthermore, the three exposures resulted in an increase of some triglyceride species. In conclusion, some significant changes in lipids were identified and thus we postulate that some lipid compounds and lipid metabolic pathways could be involved in the acquisition of the malignant phenotype in exposed prostate cancer cells to the selected EDs.

1. Introduction

Prostate cancer is the most commonly diagnosed visceral neoplasm and the second leading cause of cancer deaths in American men Jemal et al., (2008). Also, benign prostatic hyperplasia is a common benign neoplasm occurring in approximately 50% of men around 60 years. The initiation and progression of prostate cancer are still not well understood, but inappropriate levels of steroid hormones have been proposed to induce prostate carcinogenesis (Yeh et al., 2014; Prins et al., 2007).

In the context of a potential role of the environment in cancer development and progression, endocrine disruptors (EDs) have been an important subject of study. EDs are a class of environmental toxicants that interfere with the synthesis, secretion, transport, action or elimination of natural hormones. EDs are present in various commodities such as pesticide mixtures, plastic industry, cleaning and personal care products, industry sub products, and drugs. The major mechanism of exposure to EDs is food, as most of them bioaccumulate and are still present in the food chain. Many studies have evidenced that exposure to these chemicals in utero and during early life could result in birth defects, behavioral disorders, and cancer (Knower et al., 2014; Jeng, 2014; Kajta and Wojtowicz, 2013). In the case of prostate cancer, some epidemiological and animal-based studies suggest a direct association between EDs exposure and prostate cancer risk (Prins, 2008). Among all the EDs, three molecules have been chosen to carry out this work: aldrin, Aroclor 1254 and chlorpyrifos (CPF).

Aldrin is a chlorinated hydrocarbon molecule used as an insecticide on crops until 1970 and later used for killing termites until 1987 in the U.S. Other countries banned its use years later.

Abbreviations: BPA, bisphenol A; CE, cholesteryl esters; CL, cardiolipin; CPF, chlorpyrifos; DAG, diacylglycerol; EDs, Endocrine Disruptors; EMT, Endothelial-Mesenchymal Transition; GSLs, glycosphingolipids; MCC, Matthews Correlation Coefficient; MCR, Multivariate Curve Resolution; MMP-2, metalloproteinase-2; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PCBs, polychlorinated biphenyls; PLS-DA, Partial Least Squares Discriminant Analysis; SLs, sphingolipids; TAG, triacylglycerols; TIC, Total Ion Current Chromatogram; TNFα, Tumor Necrosis Factor Alpha; VIP, Variable Importance in Projection

* Corresponding author.
E-mail addresses: carmen.bedia@idaea.csic.es (C. Bedia), nuria.dalmau@idaea.csic.es (N. Dalmau), joaquim.jaumot@idaea.csic.es (J. Jaumot), roma.tauler@idaea.csic.es (R. Tauler).

http://dx.doi.org/10.1016/j.envres.2015.03.014
0013-9351/

In any case, aldrin is still present in the environment from these past uses. A recent epidemiological study has suggested that aldrin exposure is associated with increased risk of aggressive prostate cancer, (Koutros et al., 2013) although some other studies reported no causal relationship between aldrin exposure and human cancer risk (Hooker et al., 2014).

Polychlorinated biphenyls (PCBs) are stable, lipophilic compounds that accumulate in the environment and the food chain. Numerous studies have demonstrated a relationship between PCBs exposure and a variety of toxic effects, such as carcinogenicity, teratogenicity or reproductive toxicology in animals (Bell, 2014; El Majidi et al., 2014; Zani et al., 2013). Also, correlation between environmental and occupational exposures to PCBs and human prostate cancer has been reported (Kling et al., 1978; Hessel et al., 2004). Aroclor 1254, hereafter aroclor, is a mixture of 60 compounds representative of PCB environmental pollution. Several publications have demonstrated its ability to decrease sperm motility and count and to alter ventral prostate antioxidant system (Selvakumar et al., 2011; Murugesan et al., 2005). Also, a recent work reported a possible association between exposure to aroclor and the induction of a cell transformation process in rat prostate (Cillo et al., 2007).

Chlorpyrifos (CPF) is an organophosphate insecticide that acts on the nervous system of insects as an acetylcholinesterase inhibitor. Although it has been described as moderately toxic to humans, it remains one of the most widely used organophosphate insecticides. Exposure to CPF has been linked to neurobehavioral and neurodevelopmental effects (Saunders et al., 2012; Grandjean and Landrigan, 2014). Concerning prostate cancer, a very large prospective cohort study reported a correlation between chlorpyrifos exposure and prostate cancer risk in farmers (Alavanja et al., 2003).

DU145 is an androgen-independent prostate cancer cell line model, known to express estrogen receptor beta (ERβ) and lacking both estrogen receptor alpha (ERα) and androgen receptor (AR) (Linja et al., 2003; Guerini et al., 2005). Nevertheless, aldrin, aroclor and CPF have shown to interact with ERβ among other intracellular targets (Luft et al., 2009). This receptor has been implicated in mediating effects of EDs; (Kuiper et al., 1998) in the case of PCBs, for example, they induce a significant reduction of ERβ in anteroventral periventricular nucleus of brain rats (Salama et al., 2003).

Lipids are molecules that modulate cellular processes such as cellular differentiation, proliferation, apoptosis and senescence, and thereby contribute to the homeostatic control of tissue growth and vascularization. These functions have shown to be altered in tumor cells, to allow them to grow locally and to metastasize to distant sites (Schulze and Harris, 2012; Hanahan and Weinberg, 2000). Therefore, a malignant phenotype or transformation towards a metastatic profile should be reflected in a characteristic lipidic signature.

Although the ED contaminants above mentioned have been the subject of epidemiological, animal and cell studies that involve them in cancer risk and progression, the phenotypic changes of a long-term exposed prostate cancer cells to EDs and the potential roles of lipids in the development of these changes has been never reported. We hypothesized that these contaminants would induce malignant alterations in DU145 prostate cancer cells when used at non-toxic concentrations and long exposure times (50 days), and that these changes would be accompanied by alterations in lipid levels and composition. The aim of this research was to investigate the potential malignant changes in cell phenotype after the ED exposure and to characterize the lipid profile of cells that accompany these changes. The novelty of this work not only resides in the exploration of the malignant effects induced by the EDs from the lipid point of view, but also in the lipidomic analysis approach used to characterize these changes. In this study, an untargeted lipidomic analysis has been performed using chemometric analysis tools which enabled the exploration of LC-MS data without any previous pre-conceived idea about the lipid candidates. In contrast to the targeted lipidomic studies, in which only a limited number of predefined lipid-specific signals are investigated, the untargeted approach used in this work had the advantage that potentially offered the discovery of novel interesting lipid molecules and metabolic pathways involved in the phenotypic changes observed.

2. Materials and methods

2.1. Reagents

Aldrin, Aroclor 1254, CPF, bisphenol A (BPA), tumor necrosis factor alpha (TNFα), tetrazolium bromide salt (MTT), cell culture media and reagents were obtained from Sigma. Analytical grade methanol and chloroform were purchased from Merck and Carlo Erba, respectively. HPLC Gradient Grade acetonitrile was from Fischer Chemicals. Lipid standards were obtained from Avanti Polar Lipids.

2.2. Cell line and culture

DU145 prostate cancer cells were obtained from the American Type Culture Collection. This cell line was cultured in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin, at 37 °C in a humidified atmosphere containing 5% of CO2.

2.3. Treatment of cells

The solutions of all the contaminants were prepared at 10 mM in DMSO. The concentration used for the chronic exposure (1 μM) was chosen on the basis of the non-toxicity at 72 h, observed in an MTT cytotoxicity assay (data not shown). From the 10 mM solutions, intermediate solutions of 100 μM were prepared diluting the concentrated solutions with non-supplemented RPMI. The vehicle solutions were also prepared with the same amounts of DMSO. DU145 cells were seeded in a 6-well plate at density of 2×10^5 per well, and 20 μl of each solution was added in the corresponding well to a final volume of 2 ml of supplemented RPMI (final concentration of DMSO 0.01%). Cells were cultured in standard conditions and diluted every 3 days. Cultures were treated after every passage to complete the 50-day treatment.

2.4. MTT proliferation assay

Cell viability was determined by using the 3–(4,5-dimethylthiazol-2-yl)–2,5–diphenyltetrazolium bromide assay on 96-well plates, according to the manufacturer's instructions.

2.5. Wound healing assay

Cells originated from the 50-day treatment were seeded in 24-well plates at a density of 1×10^5 cells per well and left grown to be confluent. The cell monolayers were scraped with a sterile 100 μl tip to create a denuded area perpendicular to the red line previously drawn in the external face of the well bottom. Cells were washed twice with PBS and non-supplemented RPMI was added (500 μl/well). Pictures of line intersections were taken from each well using a lens magnifier (1 ×, Nikon SMZ1500) fitted with a digital camera (Nikon DS-Ri1), and then plates were placed again in the incubator for 16 h. After incubation, pictures of the same