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Organochlorine pesticides induce epithelial to mesenchymal transition of human primary cultured hepatocytes

Nathalie Zucchini-Pascal [1,*], Ludovic Peyre [1], Georges de Sousa, Roger Rahmani [*]

Laboratoire de Toxicologie Cellulaire et Moléculaire des Xénobiotiques, INRA, UMR 1331 TOXALIM (Research Center in Food Toxicology), 06903 Sophia Antipolis, France

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ABSTRACT

Persistent organic pollutants (POPs) are a group of organic or chemicals that adversely affect human health and are persistent in the environment. These highly toxic compounds include industrial chemicals, pesticides such as organochlorines, and unwanted wastes such as dioxins. Although studies have described the general toxicity effects of organochlorine pesticides, the mechanisms underlying its potential carcinogenic effects in the liver are not well understood. In this study, we analyzed the effect of three organochlorine pesticides (dichlorodiphenyltrichloroethane, heptachlore and endosulfan) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the epithelial to mesenchymal transition (EMT) in primary cultured human hepatocytes. We found that these compounds modified the hepatocyte phenotype, inducing cell spread, formation of lamellipodia structures and reorganization of the actin cytoskeleton in stress fibers. These morphological alterations were accompanied by disruption of cell–cell junctions, E-cadherin repression and albumin down-regulation. Interestingly, these characteristic features of dedifferentiating hepatocytes were correlated with the gain of expression of various mesenchymal genes, including *vimentin*, *fibronectin* and its receptor *ITGA5*. These various results show that organochlorines and TCDD accelerate cultured human hepatocyte dedifferentiation and EMT processes. These events could account, at least in part, for the carcionogenic and/or fibrogenic activities of these POPs.

1. Introduction

A wide variety of chemicals are released every day into the environment from residential, commercial, agricultural and industrial sources. The exposure of organisms to these environmental contaminants is a major issue for public health policy. In particular, persistent organic pollutants (POPs) are toxic chemicals that adversely affect human health and the environment. The physicochemical characteristics of these compounds are such that they persist in the environment for long periods of time and can accumulate through the food chain. Indeed, they can be accumulated and stored in fats where they persist in the body. Thus, POPs are toxic chemicals that adversely affect human health and the environment around the world (Deichmann and MacDonald, 1971; El-Shahawi et al., 2010; Lim et al., 2010). Consequently, the Stockholm Convention in 2004 listed the first 12 POP chemicals (named the "Dirty Dozen"), including doxins, DDT and heptachlor. In 2011, nine compounds, including endosulfan, were added to the Stockholm Convention list. Various *in vitro*, *in vivo* and epidemiological studies indicate that all these POPs present a carcinogenic potential for human. The liver is the tissue most sensitive to intoxication by POPs, and more generally xenobiotics, due to its role in metabolism. However, and despite numerous studies suggesting the hepatotoxicity of these compounds, the cellular and molecular mechanisms underlying the toxicity and carcinogenic effects of POPs in human hepatocytes remain poorly understood.

The epithelial to mesenchymal transition (EMT) is a multistep process that is physiologically important in various processes, including embryonic development and tissue repair. It has also been implicated in a variety of diseases including fibrosis and the progression of carcinoma (Jou and Diehl, 2010; Kalluri and Weinberg, 2009). EMT is the process by which a polarized epithelial cell undergoes multiple biochemical and molecular changes such that it assumes a mesenchymal cell phenotype. Epithelial cells are defined as polarized cells that adhere to a basal membrane, form cohesive cell layers through intercellular junctions

Abbreviations: Ab, antibody; DAPI, 4′,6′-di-amidino-2-phenyl indole; DMSO, dimethyl sulfoxide; ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; FBS, fetal bovine serum; HCC, hepatocellular carcinoma; POP, persistent organic pollutants; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; DDT, dichlorodiphenyltrichloroethane.

* Corresponding authors. Address: Laboratoire de Toxicologie Cellulaire et Moléculaire des Xénobiotiques, INRA, UMR 1331, 06903 Sophia Antipolis, France. Tel.: +33 4 92 38 65 48; fax: +33 4 92 38 64 01.

E-mail addresses: zucchini@sophia.inra.fr (N. Zucchini-Pascal), rahmani@sophia.inra.fr (R. Rahmani).

[1] These authors equally contributed to this work.

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(i.e. adherens junctions, desmosomes and tight junctions) and communicate with one another through gap junctions. These various interactions slow the motility of cells within the epithelium. By contrast, mesenchymal cells do not display intercellular interactions, and are characterized by their ability to move on an extracellular matrix (ECM). The EMT process involves major phenotypic modifications that occur as a sequence of steps, including loss of apico-basal polarity, disruption of intercellular junctions, modifications of cell surface proteins (E-cadherin and integrins) and reorganization of the cytoskeleton (Firrincieli et al., 2010; Thiery, 2003; Ozdamar et al., 2005). All these events result in the fibroblastic-like phenotype and the acquisition of migratory and invasive properties. However, a classification has been proposed involving three different biological subtypes depending on the biological context in which EMT occurs (Kalluri and Weinberg, 2009). Type 1 EMT leads to the apparition of cells with a mesenchymal phenotype to create new tissue. This subtype is associated with implantation, embryo formation and organogenesis. All EMT processes implicated with wound healing, tissue regeneration and fibrosis constitute type 2 EMT. In contrast to type 1, this subtype is associated with inflammation and can lead to organ destruction. Type 3 EMT occurs in neoplastic cells and allows the final steps of cancer progression causing invasion and metastasis. Despite this classification based upon distinct biological processes, all three EMT type share a common program based on common cellular and molecular events.

The purpose of this study was to investigate the effects of organochlorine pesticides (DDT, heptachlor and endosulfan) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the EMT process in primary cultured human hepatocytes. Primary cultures of human hepatocytes are a very powerful *in vitro* model for investigating many aspects of liver physiopathology (Eisenbrand et al., 2002; Guguen-Guillouzo and Guillouzo, 2010). We found that all POPs tested disrupted the phenotypes of human hepatocytes, as revealed by the loss of epithelial features. The organochlorines and TCDD disrupted cell–cell contacts, characterized by the loss of E-cadherin from adherens junctions as a consequence of repression of the expression of its gene. These compounds accelerated the dedifferentiation process in cultured hepatocytes and led to reorganization of the actin cytoskeleton and induction of the mesenchymal marker vimentin. All these events were associated with excessive ECM production: the mRNAs for α1(I) collagen, fibronectin and its receptor α5β1 integrin were all overproduced. Most importantly, we report the first data evidencing the effects of organochlorines on EMT, a process implicated in liver fibrosis and carcinogenesis.

2. Material and methods

2.1. Cell culture and incubation experiments

All experiments on human tissue were in accordance with ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration. Hepatocytes from human liver were isolated and cultivated as previously described (Berry and Friend, 1969). Briefly, after isolation, cells were seeded onto type-1-collagen-coated dishes. Hepatocytes were cultured in medium I (William's medium E, 10% FBS, 50 UI/ml penicillin, 50 μg/ml streptomycin and 0.1 UI/ml insulin) for 18 h. Then, medium I was replaced by a similar one that did not contain serum and was supplemented with hydrocortisone hemisuccinate (1 μM) and bovine serum albumin (240 μg/ml). All data presented are the mean, or representive of results obtained from three independent experiments (i.e. results obtained on primary cultured hepatocytes isolated from three different donors).

2.2. Immunofluorescence staining

Human hepatocytes were grown on type-1-collagen-coated glass coverslips. After experimental procedures, the cells were washed with PBS, fixed with 4% paraformaldehyde and permeabilised in PBS containing 0.1% Triton. After washing, cells were blocked with 3% BSA in PBS and incubated for 1 h with primary antibodies: E-cadherin (1/500), β-catenin (1/450) and vimentin (1/400). Goat anti-rabbit IgG and goat anti-mouse IgG coupled to AlexaFluor 488 or 594 (Molecular Probes, Eugen, OR), respectively, were used as secondary Antibodies at a dilution of 1/500. After washing, slides were mounted and sealed in ProlongGold antifade reagent (Invitrogen). Images were acquired with an inverted fluorescence microscope (Nikon) equipped with a CCD camera (ORCA ER, Hamamatsu Photonics), at × 20 magnification.

2.3. Western blot analysis

The cells were treated for 72 h with increased concentrations of DDT, heptachlor and endosulfan (0.2, 2 and 20 μM). Hepatocytes were lysed in hypotonic buffer (25 mM HEPES, pH 7.5, 5 mM $MgCl_2$, 5 mM EDTA, 5 mM DTT, 2 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml pepstatin A) supplemented with 0.1% SDS. After concentration determination (BCA Protein Assay kit), proteins were loaded onto either 10% or 12% SDS–polyacrylamide gel and transferred onto PVDF membrane (Amersham Life Science, Buckinghamshire, UK). After blocking with 5% non-fat skimmed milk in TBS-T (10 mM Tris, pH 7.5, 140 mM NaCl, 0.1% Tween 20) for 1 h at 37 °C, membranes were washed and incubated with the corresponding primary antibody in TBS-T containing 3% BSA. After washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (anti-mouse immunoglobulin G or anti-rabbit immunoglobulin G, Promega, Madison, WI, USA). The signals were detected using ImmobilonWestern Detection Reagents (Millipore, Molsheim, France) and acquired using CCD camera (ChemiGenius2, SynGene). Then, semi-quantification was performed using the analysis software GeneTools.

2.4. RNA isolation and real-time RT-PCR

RNAs were isolated and cDNA synthesized as previously described (Zucchini et al., 2009). Quantitative PCR analysis was carried out with LightCycler®480 Probes Master (Roche), according to the manufacturer's instructions, together with FAM-labeled hydrolysis probes from the Universal Human Probe Library Set (Roche). Intron-spanning primers were designed with Universal Probe Library Assay Design Center software (www.roche-applied-science.com/sis/rtpcr/upl/index.jsp?id=uplct_030000). Calculations were carried out with *gapdh* as the endogenous control reference gene. Fold differences in gene expression were calculated with LightCycler software, taking into account the efficiency of amplification, determined from a standard curve obtained with the second-derivative maximum method.

3. Results

3.1. Organochlorine pesticides perturb the phenotype of primary cultured human hepatocytes

Primary human hepatocytes were cultured in sub-confluent conditions with and without 20 μM of DDT, heptachlor or endosulfan and were analyzed for morphological changes by phase-contrast microscopy after 72 h of treatment. Concentrations of organochlorines have been selected upon cytotoxicity analysis. Cell viability evaluated by MTT dye reduction assay in primary cultured human hepatocytes, revealed IC_{50} of ≈126, 86 and 95 μM for DDT, heptachlor and endosulfan, respectively (data not shown). Based upon these data and the concentrations commonly used for *in vitro* studies in the literature, we selected 20 μM as the highest concentration tested in the present study. TCDD (25 nM) and TGF-β (2 ng/ml) were used as positive controls for cell plasticity and EMT induction (Diry et al., 2006; Caja et al., 2011). In control (vehicle-treated) cultures, cells had polygonal shapes and formed islets establishing cell-to-cell contacts with distinct intercellular borders, and thus displayed features typical of mature and differentiated cells. Cultures exposed to organochlorine exhibited notable morphological alterations. In particular, DDT and Heptachlore led to the acquisition of a spindle-shape cell phenotype and to the apparition of individual cells exhibiting starry-shape morphology, similar to the effects of TGF-β and TCDD (Fig. 1). Narrowing of the cytoplasm and lamellipodia structures (arrows) were observed after treatment with all compounds tested. Cells treated with endosulfan and TCDD presented more refringent intercellular borders (arrowhead), suggesting that these compounds led to cell detachment.

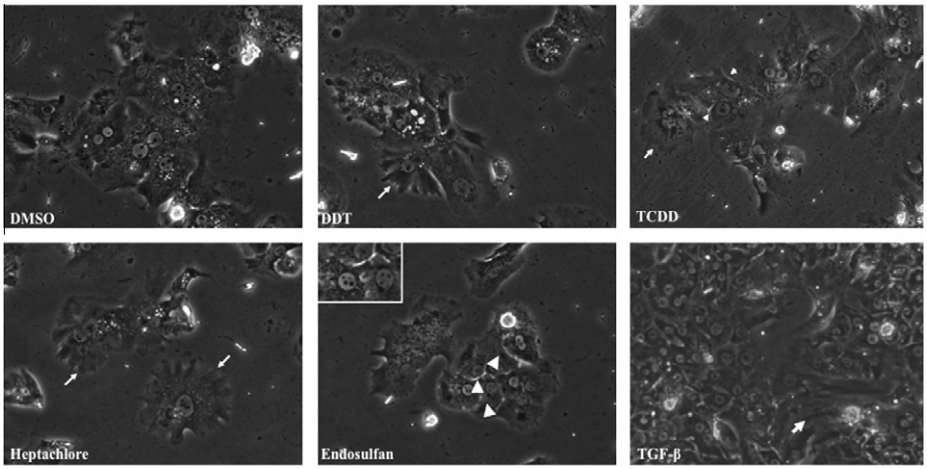

Fig. 1. Effects of organochlorines and TCDD on hepatocytes-cell morphology. Cells were exposed to 20 μM organochlorines (DDT, heptachlor and endosulfan), 25 nM TCDD and 2 ng/ml TGF-β for 72 h. Cells morphology was examined under a light microscope. Chemicals induced lamellipodia structures (arrows) and more refringent intercellular borders (arrowhead). The results shown are those from a single experiment representative of three independent experiments.

These observations show that primary human hepatocytes exposed to organochlorine pesticides acquired phenotypic characteristics suggesting a mesenchymal-like phenotype.

3.2. Organochlorine pesticides induce loss of epithelial characteristics

Adherens junctions are a major type of cell–cell junctions and they contribute to the maintenance of endothelial monolayer integrity. E-cadherin plays a key role in these structures; its cytoplasmic domain recruits several molecules including α-catenin and β-catenin (Aberle et al., 1996; Kemler, 1993). β-catenin binds directly to the intracellular domain of E-cadherin and to α-catenin, which connects the adherens junction complex to the actin cytoskeleton (Aberle et al., 1994; Hülsken et al., 1994; Jou et al., 1995). In view of the morphological features acquired by human hepatocytes following exposure to organochlorines, we visualized the E-cadherin/β-catenin complex by immunofluorescence analysis (Fig. 2). Control (vehicle-treated) cells contained substantial amounts of E-cadherin that co-localized with β-catenin at the plasma membrane, evidence of functional adherens complexes. In con-

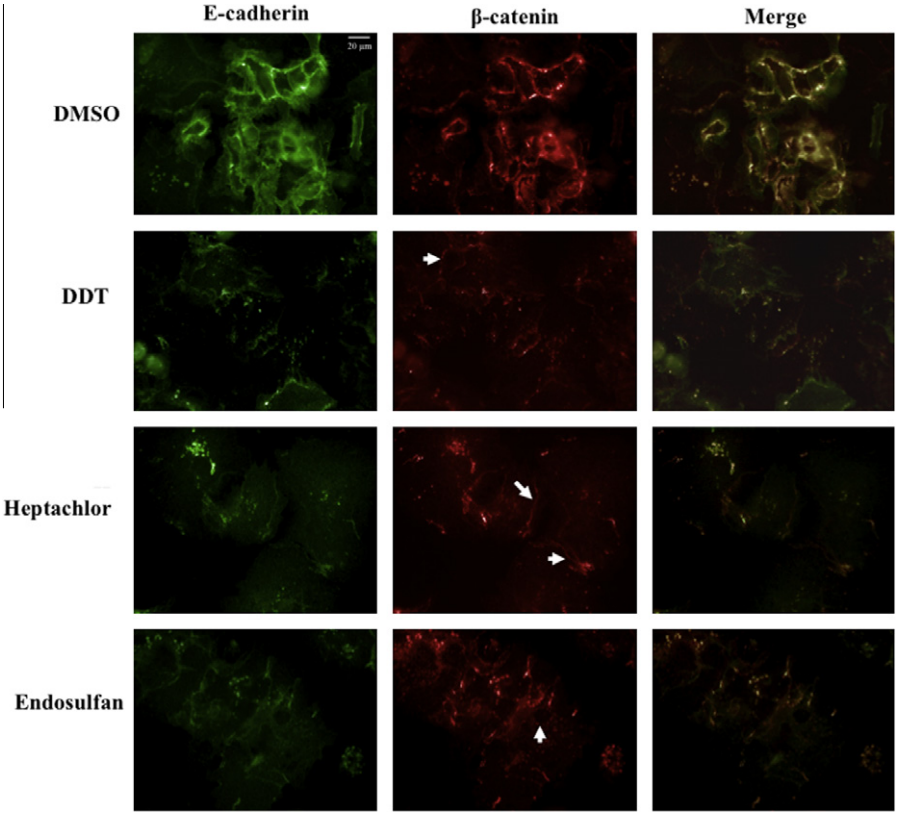

Fig. 2. Organochlorines disrupts adherens junctions. Human hepatocytes were grown on coverslips and treated either with DMSO (0.25%) or 20 μM organochlorines (DDT, Heptachlor, Endosulfan). 72 h later, cells were fixed and processed for indirect immunofluorescence for the detection of the E-cadherin (green)/β-catenin (red) complex. Co-localisation was done in the merge column in yellow color. Representative of three separate experiments (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

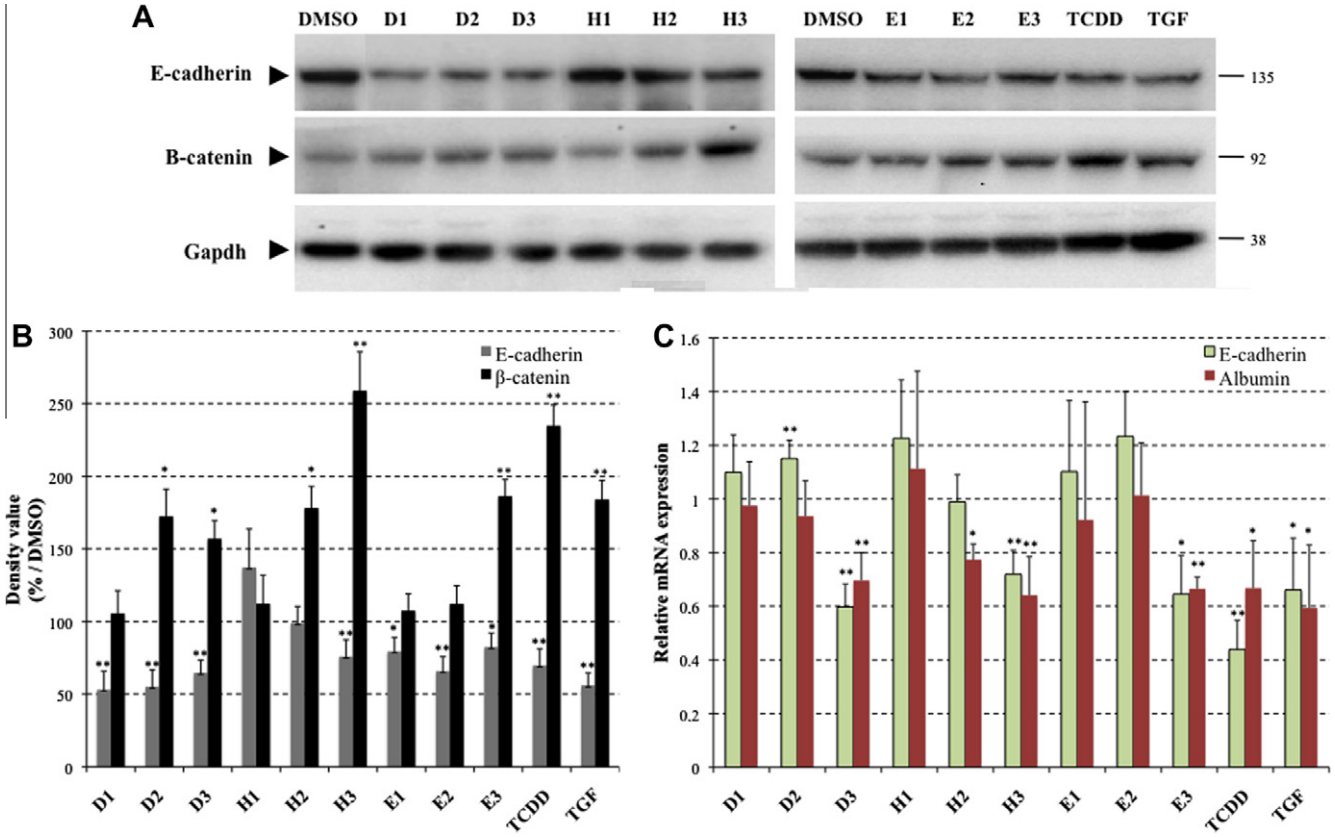

Fig. 3. Effect of organochlorines and TCDD on epithelial hallmarks of human hepatocytes. Hepatocytes were treated with organochlorines [DDT (D), heptachlor (H) and endosulfan (E)] at 0.2 (1), 2 (2) and 20 (3) μM, 25 nM TCDD and 2 ng/ml TGF-β for 72 h. (A) Cells were lysed and E-cadherin (E-Cadh) and β-catenin 1 (β-cat) protein levels were analyzed by Western blotting. (B) Band densitometry was performed after the acquisition with a CCD camera and the results are defined as the ratio between treated cells versus DMSO-treated cells normalized by Gapdh. (C) Real-time quantitative PCR was used to quantify the mRNA of E-cadherin and albumin in human hepatocytes exposed to organochlorines, TCDD and TGF-β for 48 h. Relative mRNA expression levels (normalized with respect to *gapdh*) were determined and mRNA levels in DMSO-treated cells were set to 1. Error bars indicate the means ± SEM of triplicate determinations from three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$.

trast, human hepatocytes exposed to organochlorines contained much less, and discontinuous, E-cadherin and β-catenin staining. These differences indicate that organochlorine treatment led to disassembly of the E-cadherin/beta-catenin complex.

The amounts of E-cadherin protein in the DDT-, heptachlor- and endosulfan-treated hepatocytes were reduced and were similar to that in hepatocytes exposed to TCDD and TGF-β (Fig. 3A and B). Similarly, E-cadherin transcript abundance was significantly reduced by organochlorine pesticides and positive control treatments (Fig. 3C). The total β-catenin level increased, in a dose-dependent manner, in cells exposed to DDT, heptachlore and positive controls (Fig. 3A and B), without mRNA induction (data not shown). These results are consistent with the treatments resulting in β-catenin stabilization.

We analyzed the expression of the *albumin* gene, encoding a protein that serves as a marker of mature and functional hepatocytes: the synthesis of albumin is an indicator of the differentiated state of cultured hepatocytes (de Juan et al., 1992). All chemical treatments resulted in a lower than control abundance of the albumin mRNA (Fig. 3C). Therefore, DDT, heptachlore, endosulfan and TCDD accelerate the dedifferentiation of primary cultured hepatocytes and appear to induce the EMT process.

3.3. Organochlorine pesticides induce gain of mesenchymal markers

We tested whether repression of E-cadherin and dedifferentiation by organochlorines were accompanied by the acquisition of the mesenchymal marker vimentin, an intermediate filament protein normally found in cells of mesenchymal origin or in migrating epithelial cells. Co-immunofluorescence staining experiments revealed a fibrillar rearrangement of vimentin in the cytoplasm of hepatocytes exposed for 72 h to organochlorines or TCDD (Fig. 4). These changes occurred in parallel to polarization of the F-actin stress fibers throughout the cells; in control conditions, actin was organized into a cortical pattern at the cell-to-cell junctions.

Control primary human hepatocytes cultured for 96 h (72 h mock-treatment tested 24 h post-plating) expressed vimentin mRNA and protein (Fig. 5A and B), consistent with the dedifferentiation of primary hepatocytes observed during cell culture (Godoy et al., 2010). Both organochlorines and positive controls increased the levels of vimentin mRNA and protein (Fig. 5A and B).

We tested whether the acquisition of the mesenchymal phenotype was accompanied by the production of ECM. We assayed the mRNA for *α1(I) collagen* and the α5 transcripts encoding fibronectin receptor (*ITGA5*) after 72 h of treatment (Fig. 5C and D). DDT, Heptachlore and TGF-β significantly increased the amounts of the mRNAs for α1(I) collagen and ITGA5. TCDD up-regulated ITGA5 mRNA, but not α1(I) collagen mRNA. Consistent with these findings, fibronectin protein was up-regulated by all organochlorines, TCDD and TGF-β (Fig. 5B), with endosulfan having the smallest effect (Fig. 5E).

Our various findings demonstrate that organochlorine pesticides induce an EMT program in primary cultured hepatocytes.

4. Discussion

In this study, we demonstrate that organochlorine pesticides considerably disrupt the phenotype of primary cultured human

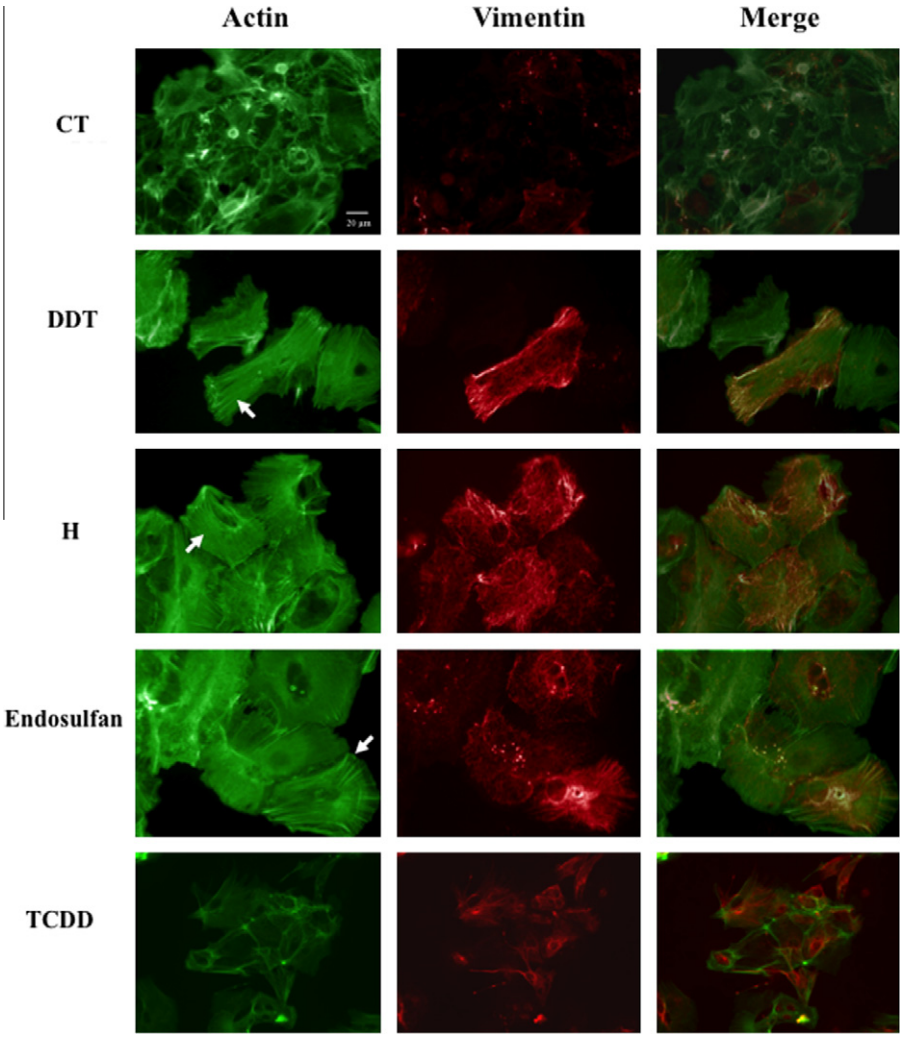

Fig. 4. Organochlorines and TCDD disorganize the actin cytoskeleton increases the expression of vimentin in human hepatocytes. (A) Cells were exposed to 20 μM organochlorines (DDT, heptachlor and endosulfan), 25 nM TCDD and 2 ng/ml TGF-β for 72 h. Then, cells were fixed and processed for indirect immunofluorescence for the detection of F-actin by AlexaFluor 488-conjugated phalloidin staining (green) and vimentin (red). Co-localisation was done in the merge column in yellow color. Representative of three separate experiments. (B and C) Hepatocytes were treated with organochlorines [DDT (D), heptachlor (H) and endosulfan (E)] at 0.2 (1), 2 (2) and 20 (3) μM, 25 nM TCDD and 2 ng/ml TGF-β for 72 h. Protein and mRNA levels were analyzed by Western blotting and real-time quantitative PCR, respectively. The relative amount of *vimentin* was expressed as a ratio to *gapdh*, and the *vimentin* level in DMSO-treated cells was set to 1. Data are expressed as the mean ± S.D. $^{*}P < 0.05$ and $^{**}P < 0.01$ obtained by analysis of variance ANOVA followed by post hoc multiple comparison testing (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

hepatocytes. We report that these pesticides cause the loss of epithelial features, characterized by the repression of E-cadherin expression, the dissociation of intercellular junctions and the decreased expression of albumin. This was accompanied by structural modifications with the apparition of lamelipodia and modifications of the actin cytoskeleton reorganized into stress fibers. All these events related to epithelial character loss occurred in parallel to the gain of mesenchymal markers, notably vimentin, and the synthesis of the ECM proteins α1(I) collagen, fibronectin and its receptor α5 integrin. This disrupted phenotype was closely related to EMT. Note however that hepatocytes cultured for 72 h acquired a modified morphology and expressed a mesenchymal marker: vimentin. This is suggestive of a dedifferentiation process. Indeed, it has been demonstrated that EMT is a process characteristic of the dedifferentiation of hepatocytes *in vitro* (Godoy et al., 2010); hepatocytes cultured in monolayers dedifferentiate, thereby losing their specialized liver functions, and die by apoptosis (Tuschl and Mueller, 2006; Bailly-Maitre et al., 2002). After a few days of culture in collagen-coated plates, rat hepatocytes spread and acquire a fibroblast-like shape (Tuschl and Mueller, 2006). Consequently, we conclude that DDT, heptachlore, TCDD and TGF-β accelerate the dedifferentiation process of primary cultured human hepatocytes, potentiating EMT.

Organochlorines, like TCDD and TGF-β, repressed E-cadherin expression. E-cadherin is a universal epithelial marker that plays a key role in the maintenance of cellular integrity, and its repression marks early EMT (Zeisberg and Kalluri, 2004; Li et al., 2003). The link between the loss of E-cadherin function and the occurrence of EMT in liver disease is well documented. For example, decreased E-cadherin expression has been observed in approximatively 40% of hepatocellular carcinoma samples (Yang et al., 2009). E-cadherin loss of function is associated with invasion and mestastasis of cancer cells, and other pathological conditions, such as fibrosis. Liver fibrosis is accompanied by the loss of E-cadherin, which promotes the process of EMT (Cho et al., 2010; Kaimori et al., 2007). Interestingly, we found that DDT and hepatchlor induced the mRNAs for α1(I) collagen and ITGA5, and up-regulated fibronectin. These two components of the ECM are

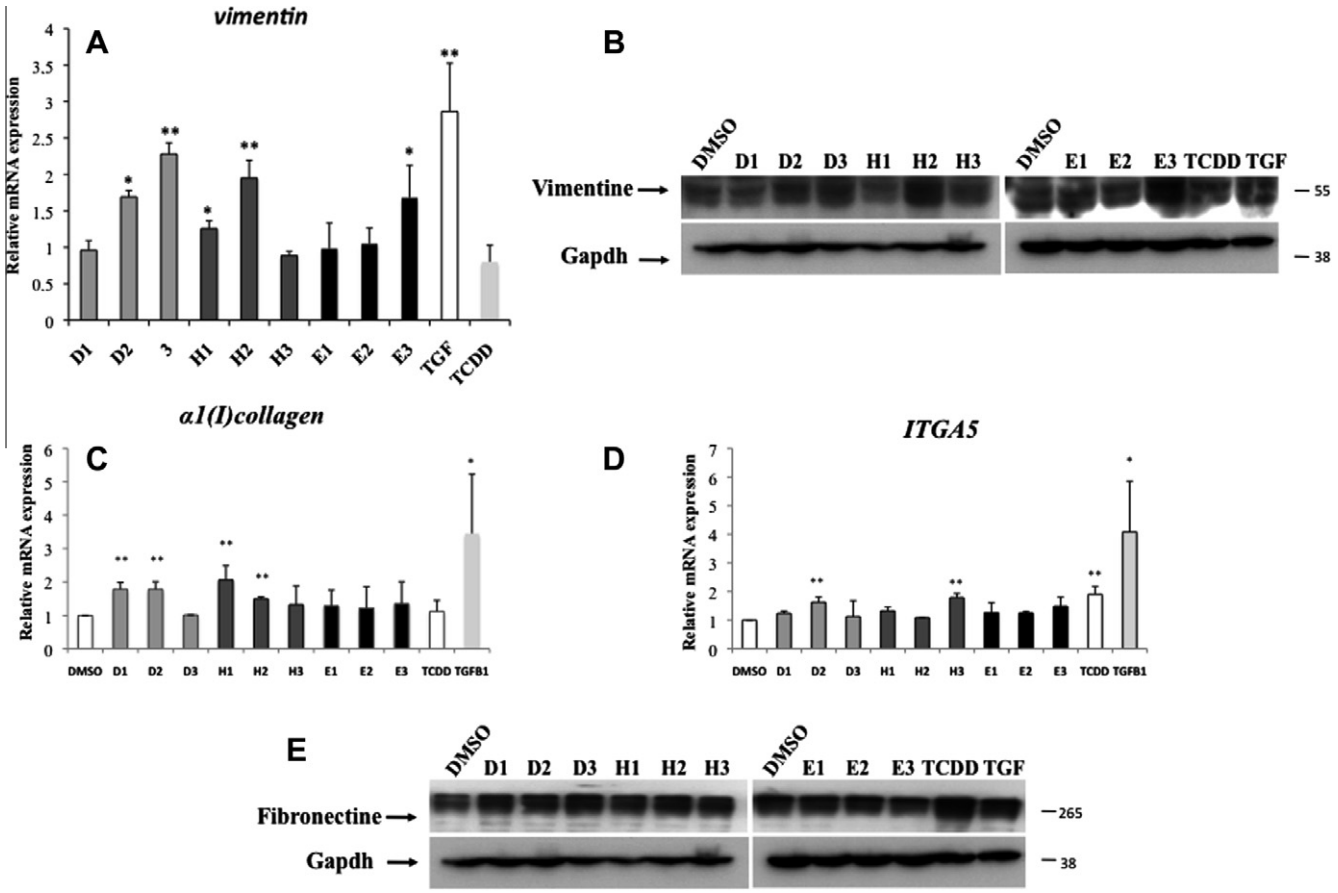

Fig. 5. Effects of organochlorines and TCDD on the ECM. (A) Hepatocytes were treated organochlorines [DDT (D), heptachlor (H) and endosulfan (E)] at 0.2 (1), 2 (2) and 20 (3) μM, 25 nM TCDD and 2 ng/ml TGF-β for 72 h. (A) The relative amounts *α1(I)collagen* and *ITGA5* genes were assessed by real-time RT-PCR and were expressed as a ratio to *gapdh* with the *α1(I)collagen* and *ITGA5* levels in DMSO-treated cells set to 1. Data are expressed as the mean ± S.D. $^{*}P < 0.05$ and $^{**}P < 0.01$ obtained by analysis of variance ANOVA followed by post hoc multiple comparison testing. (B) Cells were lysed and fibronectin protein level was analyzed by Western blotting.

the most ubiquitous and powerful mediators of fibrosis. Liver fibrosis is the liver's wound-healing response to various chronic injuries, including chronic hepatitis, non-alcoholic steatohepatitis (NASH), and exposure to xenobiotics (for example alcohol, drugs, and pesticides) (Henderson and Forbes, 2008; Bataller and Brenner, 2005). This state results from the excessive secretion of ECM components including α1(I) collagen and fibronectin (Benyon and Iredale, 2000). The end-stage irreversible consequence of progressive liver fibrosis is cirrhosis associated with complications such as portal hypertension, hepatic encephalopathy and HCC. Indeed, cirrhosis is a major preneoplastic condition in the majority of HCCs because fibrotic or cirrhotic states may be a predisposing risk factor for HCC (Jeng et al., 2007) and may be the consequence of environmental chemical carcinogens including hydrocarbons, organochlorine pesticides, and plant toxins (Leong and Leong, 2005). Type 2 EMT is a major process involved in fibrosis. It has been suggested that the occurrence of EMT in fibrotic adult organs protects epithelial cells against injury and repairs tissue, promoting ECM production (Firrincieli et al., 2010). The origin of fibrogenic cells or activated myofibroblasts responsible for the ECM in fibrotic liver is still unclear. Indeed, freshly isolated mouse hepatocytes exposed to transforming growth factor-β (TGF-β), a key mediator of the initiation and the progression of liver fibrosis, enter an EMT process, acquiring a mesenchymal phenotype and resulting in collagen deposition (Kaimori et al., 2007). This *in vitro* study was consistent with the finding *in vivo* that mouse hepatocytes can undergo EMT to contribute substantially to fibroblastic cells in CCl_4-induced liver fibrosis (Zeisberg et al., 2007). However, contradictory data support the idea that hepatocytes do not undergo EMT in liver fibrosis in mice (Taura et al., 2010). One explanation of these contradictory findings is that liver fibrosis may be a process regulated in time, in which liver cells engaged in EMT may subsequently pass through a mesenchymal to epithelial transition (MET). The modification of the morphology of human hepatocytes cultured *in vitro* and the deregulation of genes implicated in the fibrosis process upon organochlorine pesticides suggest that these xenobiotics induce an EMT process that could be involved in a fibrosis status. These observations are in accordance with the idea that hepatocytes could undergo EMT process to generate fibrogenic cells (Caja et al., 2011; Zeisberg et al., 2007).

Our results contribute to explaining the effects of some environmental contaminants on the liver. Indeed, chemicals including organochlorines are hepatotoxic and are potential carcinogens, as suggested by laboratory experiments (Leong and Leong, 2005). Few epidemiological studies have examined the possible relation between exposure to pesticides and the incidence of cancer. However, a link between water contaminated with DDT and increased death rates among Chinese farmers has been established (Lau, 2008). Moreover, significant excess incidence of liver cancer was reported after occupational exposure to DDT (Brown, 1993; Cocco et al., 1997). DDT also induces liver tumor in laboratory animals (Turusov et al., 1973; Tomatis and Turusov, 1974). DDT, heptachlor and endosulfan are classified as non-genotoxic hepatocarcinogens, like TCDD. This means that their carcinogenicity in the liver is attributed to dysregulation of various signaling pathways (those associated with apoptosis, proliferation, oxidative stress, or cytochrome P450 induction, for example). Mechanisms have been proposed in which TCDD might be indirectly genotoxic, either through

the induction of oxidative stress or by altering the DNA-damaging potential of some endogenous compounds (Park et al., 1996; Slezak et al., 1999; Graham et al., 1988). Other data from rat models suggest that the pro-carcinogenic potential of TCDD could be ascribed to suppression of apoptosis liver (Stinchcombe et al., 1995; Luebeck et al., 2000). Activation of the aryl hydrocarbon receptor (AhR) by TCDD triggers a signaling cascade responsible for cell plasticity and cell motility (Diry et al., 2006). Moreover, a common signature of non-genotoxic carcinogens seems to be the alteration of Gap junctional intercellular communications (GJIC). Indeed, such disruption is one of the principal disorders observed during carcinogenesis (Krutovskikh and Yamasaki, 1997; Cronier et al., 2009). Several lines of evidence indicate that various non-genotoxic carcinogens (including TCDD, endosulfan, DDT and heptachlore) inhibit connexin-mediated GJIC (Bager et al., 1997; Wärngård et al., 1996; Cowles et al., 2007; Ruch et al., 1990). This disorder is also observed during EMT, as the loss of cell-to-cell adhesion with the disintegration of tight, adherens, and gap junctions characterize this process. The EMT process is central to liver carcinogenesis because it is involved in both early-stage (i.e. participating in the establishment of fibrosis which constitutes a pre-neoplasic lesion) and late-stage (i.e. regulating invasiveness and metastasis) events.

In conclusion, our study demonstrates that organochlorines perturb the phenotype of primary cultured hepatocytes, potentiating the dedifferentiation of cells and inducing an EMT program. These findings provide further insight into the carcinogenic action of environmental contaminants in the human liver.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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