INTEGRATION OF DATA FROM THE *IN VITRO* LONG-TERM EXPOSURE STUDY ON HUMAN ENDOTHELIAL CELLS AND THE *IN SILICO* ANALYSIS: A CASE OF DIBUTYL PHTHALATE-INDUCED VASCULAR DYSFUNCTION

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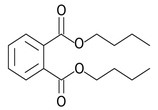
INTRODUCTION AND OBJECTIVES

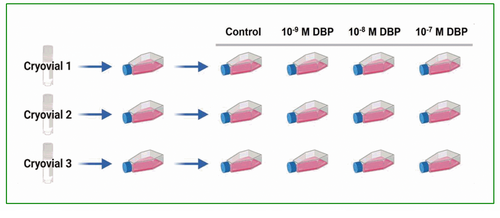
Dibutyl phthalate (DBP)



is a synthetic chemical used in many industrial and consumer products. DBP exhibits its effects mainly on the endocrine and reproductive system, but it can also affect the function of the vasculature, thereby contributing to the incidence of cardiovascular diseases (CVDs) in human population; however, the underlying mechanisms behind the DBP-induced vascular dysfunction are not fully understood. Aside from CVDs, endothelial dysfunction has also been associated with other human diseases such as diabetes, insulin resistance, chronic kidney failure, tumor growth, venous disease, and several viral infection diseases, thus widening the potential target organs affected by DBP and human diseases that DBP can contribute to.

Here, we sought to integrate the experimental data obtained from the long-term exposure of human vascular endothelial cells (ECs) to DBP and the *in silico* analysis to infer the molecular mechanism of DBP's action in vascular ECs and provide a possible association between DBP exposure and human diseases with vascular etiology.

METHODS AND DESIGN



EA.hy926 → human endothelial cell line

4-week-long exposure

Quantitative RT-PCR → mRNA expression analysis
alamarBlue™ → cell viability
Adhesion to gelatin → adhesion to extracellular matrix
Wound healing ("scratch") assay → cell migration
Griess assay → nitrite (NO_2^-) levels, as a proxy for nitric oxide (NO) production
List of enriched diseases → DBP-affected genes entered in the Comparative Toxicogenomic Database's (CTD) Set Analyzer tool
Cytoscape analysis → network visualization
Gene Ontology and KEGG pathway analysis → identification of biological processes, molecular functions, and specific signaling pathways for the DBP-affected genes

RESULTS

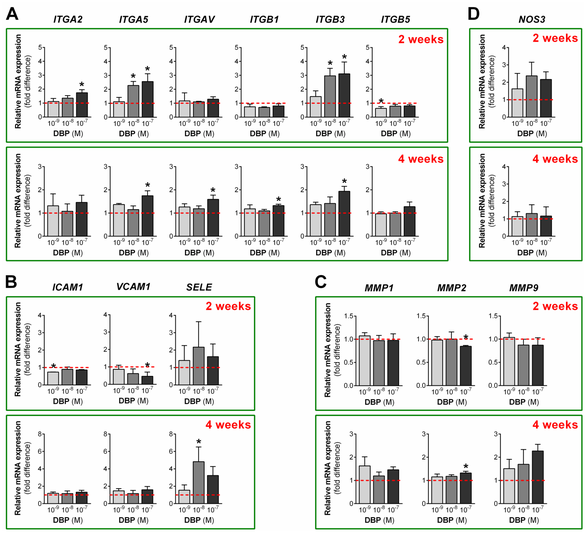


FIGURE 1. Gene expression during long-term exposure of EA.hy926 cells to DBP. The mRNA expression of the selected **(A)** integrins, **(B)** cellular adhesion molecules, **(C)** MMPs, and **(D)** NOS3. All results are expressed relative to the vehicle-treated control that was set as 1 (dashed line).

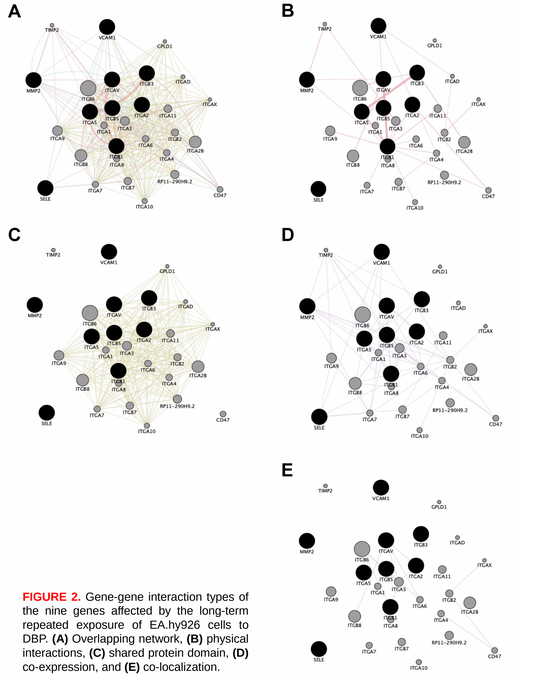


FIGURE 2. Gene-gene interaction types of the nine genes affected by the long-term repeated exposure of EA.hy926 cells to DBP. **(A)** Overlapping network, **(B)** physical interactions, **(C)** shared protein domain, **(D)** co-expression, and **(E)** co-localization.

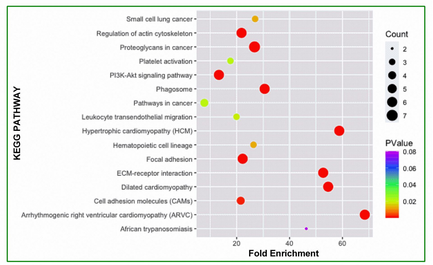


FIGURE 3. Functional annotation pathways of KEGG for the nine genes affected by the long-term repeated exposure of EA.hy926 cells to DBP.

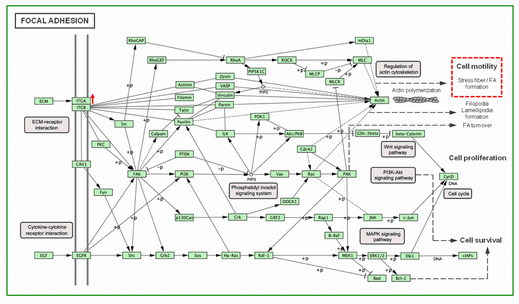


FIGURE 4. Schematic analysis of the integrin signaling pathway. The nine DBP-affected genes were entered in the DAVID tool and analyzed using the KEGG pathway analysis.

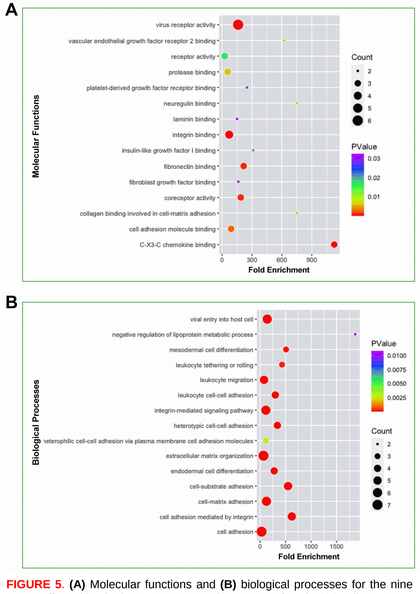


FIGURE 5. **(A)** Molecular functions and **(B)** biological processes for the nine genes affected by the long-term repeated exposure of EA.hy926 cells to DBP.

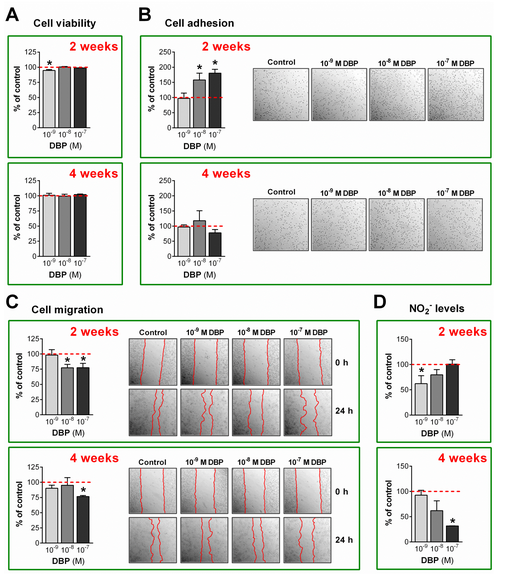


FIGURE 6. Viability, adhesion, migration, and NO production of EA.hy926 cells during long-term exposure to DBP. **(A)** Cell viability. **(B)** Cellular adhesion to gelatin was observed after 30 min under the inverted microscope. The adhesion percentage was quantified using the SRB assay. **(C)** Cell migration was assessed under the inverted microscope. The migration percentage was quantified using the ImageJ software. **(D)** NO_2^- levels in the cell culture media. All results are expressed relative to the vehicle-treated control that was set as 100% (dashed line).

TABLE 1. The top 10 diseases linked with the nine genes affected by the long-term repeated exposure of EA.hy926 cells to DBP.

	ID	Corrected p-value
Cardiovascular diseases		
– Cardiovascular disease	MeSH: D002318	1.87E-10
– Vascular disease	MeSH: D014652	6.18E-10
– Myocardial ischemia	MeSH: D017202	5.67E-06
– Heart disease	MeSH: D006331	1.44E-05
Cardiovascular diseases/Nervous system diseases		
– Cerebrovascular disorder	MeSH: D002561	2.66E-07
– Stroke	MeSH: D020521	1.56E-06
– Cerebral hemorrhage	MeSH: D002543	9.36E-06
Immune system disease		
– Urticaria	MeSH: D014581	5.79E-06
– Autoimmune diseases	MeSH: D001327	2.33E-05
– Multiple sclerosis	MeSH: D009103	1.30E-04

CONCLUSIONS

Our results provide novel information regarding the molecular mechanism of DBP's action in vascular ECs and contribute to the evidence that exposure to DBP may constitute a risk factor for the occurrence of different diseases with vascular etiology. Additional *in vivo* and *in vitro* studies are needed to improve the confidence in the chain of molecular events involved in DBP's action in vascular ECs and the association with human vascular diseases.