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Effects of B[a]P-UVA Co-Exposure on Epigenetic Marks of Isolated Skin Cells and Impact of an *Arundo donax* L. Extract of These Changes

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Abstract

Background: Extrinsic aging results from environmental stressors such as UVR or pollutants. While the effects of single pollutants are better understood, those of their combination remain poorly scrutinized. **Objective:** Building on a study showing downregulation of several processes upon co-exposure to B[a]P and UVA, we investigated changes induced by epigenetic marks. **Materials and Methods:** Human primary fibroblasts and HaCaT cells were exposed to B[a]P and UVA. After 24 hours, exposed and unexposed cells were compared to assess DNA methylation. Focusing on HaCaT, multiplex assays enabled quantifying histone H3 modifications and evaluating four splicing factors (SRSF1, SRSF3, SFPQ, and SF3B1) by immunohistochemical labeling. The expression of keratinocyte-/fibroblast-relevant genes was assessed by RT-qPCR. Finally, the impact of an *Arundo donax* L. extract added 24 hours before B[a]P-UVA co-exposure was analyzed. **Results:** Exposure to B[a]P-UVA raised DNA methylation (HaCaT: ×3.6, fibroblasts: ×1.9), an increase prevented by the extract. In HaCaT cells, B[a]P-UVA increases the frequency of S10P (+38%). When exposure was preceded by extract treatment, the frequency of several methylations was impacted. B[a]P-UVA only induced the expression of SRSF1 and SFPQ in HaCaT (+46% and +34%). Treatment with the extract abolished this effect. Co-exposure increases the expression of inflammation-related genes (IL-1α, IL-1β) in HaCaT cells and decreases those of AQP3, KRT15, and SOD2. The extract has little effect on these changes. In primary fibroblasts, exposure to B[a]P-UVA lowered the expression of LOXL2, LUM, and TGFBR2 (−38%, −59%, and −51%, respectively), and the extract did not affect these modifications. **Conclusion:** Within 24 hours, a single B[a]P-UVA co-exposure changes epigenetic marks of skin cells but has only mild effects on gene expression. An *Arundo donax* L. extract can prevent part of the epigenetic marks' changes and

could stimulate the expression of some genes in primary fibroblasts.

Keywords

Extrinsic Aging, DNA Methylation, Histone H3, Splicing Factors, *Arundo donax* L.

1. Introduction

Skin aging results from the cumulative effects of two superimposed mechanisms [1]. The first one is intrinsic aging. Genetically determined, it is due to the passage of time and affects all tissues, leading to relatively mild aging signs: fine lines, xerosis, and laxity [2]. Yet, the skin is constantly exposed to, and a major target of, environmental stressors that damage it in a process known as extrinsic aging. Extrinsic aging is characterized by more pronounced aging signs: coarse wrinkles, uneven pigmentation, and solar lentigos. Besides, pollutants can lead to several skin diseases, including skin cancer [3] [4].

Among the widespread skin stressors, the deleterious effects of UV Radiation (UVR) on all skin compartments have long been recognized [5]. While UVB (290 - 315 nm) and UVA (315 - 400 nm) are of particular importance, near-infrared radiation (770 - 1400 nm) also plays a role [2]. Still, the impact of UVR depends on skin pigmentation, DNA repair, antioxidants, and other endogenous protective mechanisms. Furthermore, responses to acute and chronic exposures differ in the skin reaction they induce, and photoaging essentially results from daily exposure to low doses of radiation [6].

Nevertheless, UVR is not the only skin stressor. Since first reported [7], it is clear that air pollutants (smog, ozone, particulate matter, organic compounds, *etc.*) also have multiple deleterious effects, leading at the level of the skin to aging signs, pigmentary disorders, and a wide range of pathologies [8]. Among the most widespread pollutants is Benzo[a]pyrene (B[a]P), a Polycyclic Aromatic Hydrocarbon (PAH) mainly resulting from incomplete combustion of fossil fuels and other organic compounds, including automobile exhaust and cigarette smoke [9]. Besides being a potent carcinogenic compound [10], skin exposure by inhalation or direct absorption activates detoxification mechanisms, results in oxidative stress, and exacerbates inflammation [11] [12]. It also impairs the barrier function, facilitating the entry of other pollutants [7] [13].

It should be pinpointed that the results mentioned above are from the analysis of single stressors [12]. While useful, such an approach is far from real-life conditions in which the skin is subjected to the simultaneous assault of multiple stressors. Despite the awareness of this complexity and efforts to unravel how stressors interact, their synergistic effects are poorly understood, although there is evidence that they do. This is the case for specific PAHs such as B[a]P, which present increased toxicity upon UV irradiation [14] [15]. Considering the health threat of pollution, there is an urge to better apprehend the effects of pollutants and their mixtures.

A hallmark of intrinsic aging and stressor-induced deleterious effects is oxidative stress and inflammation, which ultimately result in cellular senescence, impaired tissue homeostasis, and phenotypic alterations (**Figure 1**) [16]. Besides these direct effects, intrinsic aging and pollutant exposure have indirect impacts mediated by epigenetic alterations [17]-[25]. These epigenetic changes encompass DNA methylation, histone modification (mostly methylation or acetylation), and RNA interference [17], which, without altering the DNA sequence, lead to heritable changes in gene expression. Several studies pinpoint the role of these changes in intrinsic aging and upon skin assault by pollutants, whether UVR or organic pollutants like B[a]P, and have started deciphering the underlying mechanisms [17]-[24]. Epigenetic modifications are not only induced by aging and pollutants; they are an integral part of the aging process [25], and the changes they cause to the accessibility of DNA are critical in determining the proteins expressed and their levels of expression, including DNA repair pathways that preserve genomic integrity and cell survival [26] [27].

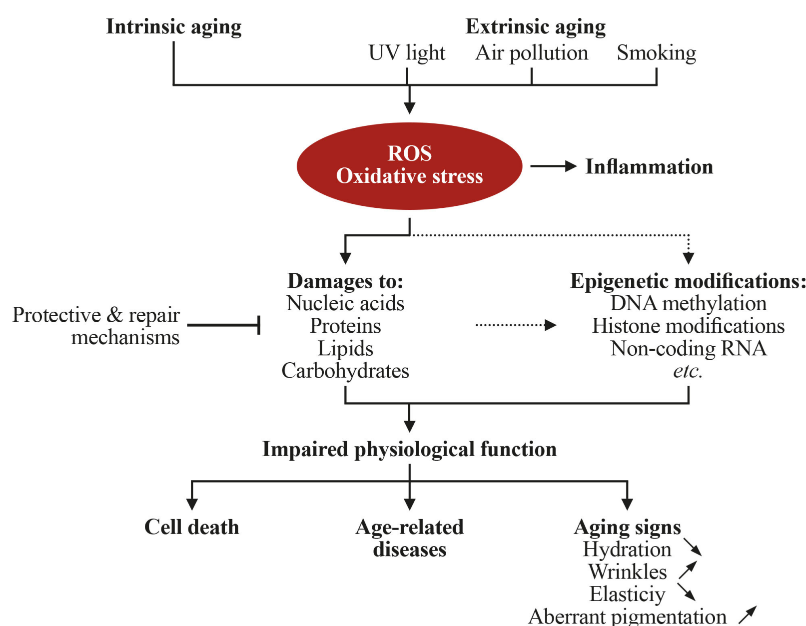


Figure 1. Potential mechanisms of environmental stressors on skin aging.

Apart from its role in regulating gene expression, several reports also indicate that epigenetics is essential to control alternate splicing and polyadenylation, leading to alternate mRNA isoforms [28]. If alternate splicing/polyadenylation explains the massive proteomic complexity compared to the relatively limited number of genes, it is now clear that such mechanisms significantly contribute to the regulation of many biological processes [28]. Whether environmental stressors also induce alternate mRNA processing has never been addressed.

A previous mass-spectrometry-based proteomic analysis revealed that contrary to UVA alone, B[a]P and UVA co-exposure of *ex vivo* skin explants leads to the overexpression of proteins involved in many processes but to the downregulation

of those implicated in epidermal development, keratinocyte differentiation, and redox processes [29]. To gain further insight into the skin reactions to environmental stressors, we evaluated two aspects of the modifications occurring upon B[a]P and UVA co-exposure. The first one is epigenetics. We analyzed the changes happening to global DNA methylation and histone H3 upon co-exposure, which are major epigenetic markers. Due to their crucial role in the proteome, we also assessed the expression of four splicing factors that were identified as showing differentially expressed upon co-exposure [29]. Finally, we evaluated the effect of an *Arundo donax* L. extract containing compounds known to influence epigenetic marks on some of the co-exposure-induced alterations evidenced.

2. Materials and Methods

2.1. Human Fibroblast Isolation and Culture

Fibroblasts were isolated by serial culture from abdominoplasty or mammoplasty surgical discards of three female donors (39, 51, and 64 years old) [30]. Each line was separately cultured in Eagle's Minimum Essential Medium (EMEM) deprived of L-glutamine (Thermo Fisher Scientific, MA, USA) that was supplemented with 10% inactivated fetal calf serum (Dominique Dutscher, France), 100 U/ml of penicillin, 100 µg/ml of streptomycin (Sigma-Aldrich), and 2 mmol/l of L-glutamine (Sigma-Aldrich, MI, USA). They were incubated at 37˚C under 5% CO_2 until reaching passage three or four.

2.2. HaCaT Origin and Culture

HaCaT cells (#T0020001, batch: 0003798, AddexBio, CA, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) deprived of sodium and pyruvate (Invitrogen, MA, USA) that was supplemented with 10% inactivated fetal calf serum (Dominique Dutscher, France), 100 U/ml of penicillin, and 100 µg/ml of streptomycin (Sigma-Aldrich). They were maintained at 37˚C under 5% of CO_2.

2.3. Experimental Plan

The same experimental plan was applied to all experiments. On day 0, cells were seeded at a final density of 10^5 cells/ml. After 48 hours, B[a]P was added to the medium (25 nmol/l final concentration). After 30 minutes of incubation (37˚C, 5% of CO_2), cells were rinsed once with Phosphate Buffered Saline (PBS) and resuspended in PBS before being subjected to a 2.5 J/cm^2 UVA-irradiation (BIO-SUN irradiation system, Vilber Lourmat, Germany). After replacing the PBS buffer with culture medium, cells were further incubated for 24 hours (37˚C, 5% of CO_2) before being harvested and analyzed.

When applicable, this experimental plan also included testing an organic *Arundo donax* L. extract (batch ARDC2108, Greentech, France). This water extract was supplemented to the culture medium 24 hours after the initiation of cell culture at a final concentration of 0.01% (v/v); therefore, 24 hours before B[a]P and UVA co-exposure. Its partial characterization revealed its richness in sugars (19.8% of

dry weight) and polyphenols (4.5% of dry weight). Besides classical plant flavonoids, these polyphenols include unusual flavonoids, including catechins and flavones, such as luteolins.

2.4. Quantification of Total DNA Methylation

DNA from B[a]P-UVA treated or untreated cells was extracted using the DNeasy Blood and Tissue Kit (#68504, Qiagen, Germany) according to the manufacturer's recommendations. After diluting all DNA samples to a concentration of 20 µg/ml, 5-methyl cytosines were quantified using the Global DNA Methylation Assay Kit (#ab233486, Abcam, UK) following the recommended protocol. Experiments were performed on three different cultures of human primary fibroblasts and in triplicate on independent culture batches of HaCaT cells.

2.5. Quantification of Histone H3 Modifications

Total histones were purified from HaCaT B[a]P-UVA treated or untreated cells using the Histone Extraction Kit (#ab113476, Abcam, UK). Histone H3 modifications were quantified using the Histone H3 Modification Multiplex Assay Kit (#ab188910, Abcam, UK) following the manufacturer's protocol. Quantifications were performed on three independent cultures of HaCaT cells.

2.6. Immunohistochemical Quantification of Splicing Factors

Before immunohistochemical reactions, cells were fixed with 4% paraformaldehyde and saturated with 2% BSA for 25 minutes. SRSF1 and SRSF3 were labeled with mouse monoclonal antibodies (#103 and 1H4G7, respectively, Invitrogen, MA, USA). Both were revealed and quantified using a goat anti-mouse secondary polyclonal antibody cross-adsorbed to Alexa Fluor™ 633 (#A21050, Life Technologies, CA, USA). SFPQ and SF3B1 were labeled using rabbit polyclonal antibodies (#HPA054689 and HPA050275, respectively, Merck, Germany), which were then quantified with the same goat anti-rabbit secondary antibody cross-adsorbed to Alexa Fluor™ 546 (#A12379, Life Technologies, CA, USA). Phalloidin labeling (#A12379, Life Technologies, CA, USA) was used to counterstain actin filaments.

Each experimental condition was evaluated on three different batches of HaCaT cells. Images were observed using an inverted Axio Observer 2 microscope (Zeiss, Germany) and analyzed with the Visilog 7.3 software (Noesis, France). For each marker, 40 fields of three independent stained HaCaT cell line cultures were analyzed, and results were standardized to those of the intensity of the actin counterstaining.

2.7. Gene Expression Analysis by qRT-PCR

RNAs were prepared using the RNeasy Micro Kit (#74004, Qiagen, Germany) following instructions. After determining reaction yield and quality (NanoDrop™ 2000/2000c, Thermo Fisher Scientific, MA, USA), mRNAs were reverse transcribed thanks to the RT2 Easy First Strand Kit (#330421, Qiagen, Germany).

Quantification of gene expression was performed by qRT-PCR using pre-coated RT^2 Profiler PCR Arrays (#330231, Qiagen, Germany) and a LightCycler@ 480 (Roche Diagnostics, Switzerland). The analysis included 11 epidermal relevant genes, 12 dermal relevant genes and two housekeeping genes used as internal standards: RPLP0 (60 S acidic ribosomal protein P0) and GAPDH (glyceraldehyde 3-phosphate dehydrogenase). Results from triplicate experiments performed on three independent HaCaT cell cultures and three different fibroblast lines are expressed as $2^{-\Delta\Delta Ct}$ using data from unstressed-untreated HaCaT/fibroblasts as references.

2.8. Statistical Analysis

Except for qRT-PCR, for which results are expressed as average $2^{-\Delta\Delta Ct}$, all results are presented as mean ± standard error of the mean of their relative variation compared to the untreated-unstressed control, but statistical analyses were carried out on raw data. According to Shapiro-Wilk tests, only data from the immunohistochemical quantification have a normal distribution and were compared using an ANOVA followed by Tukey HSD *post-hoc* tests. All other data were compared using a Friedman test followed by pairwise Wilcoxon tests.

3. Results

3.1. Effect of B[a]P-UVA and *Arundo donax* on DNA Methylation

As a first approach to studying the effect of B[a]P and UVA co-exposure on epigenetic marks of skin cells, we analyzed the total DNA methylation of HaCaT keratinocytes and primary fibroblasts.

Relative quantification (Figure 2) showed that, compared to unexposed controls, B[a]P and UVA co-exposure led to a 3.6 ± 0.7-fold increase ($p < 0.001$) in the methylation of HaCaT cell total DNA. Even if less pronounced, a significant pollutant-induced 1.9 ± 0.3-fold increase ($p = 0.034$) in DNA methylation was also evidenced in primary fibroblasts.

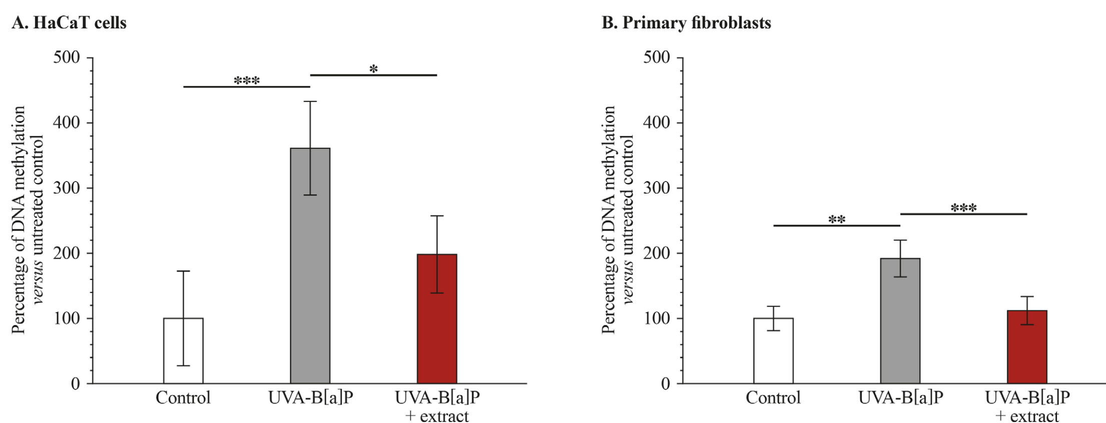


Figure 2. Relative quantification of total DNA methylation in (A) HaCaT cells and (B) primary fibroblasts. Results are expressed versus unstressed-untreated control HaCaT cells or primary fibroblasts. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Compared to co-exposure alone, pretreatment with the *Arundo donax* L. extract reduced DNA methylation by 45.2% in HaCaT cells (p = 0.025). In primary fibroblasts, the decrease of 41.6% was also significant (p < 0.001). Consequently, pretreatment with the extract resulted in methylation levels that were not significantly different from those of unexposed and untreated controls (p = 0.514 for HaCaT cells and p = 0.329 for primary fibroblasts).

3.2. Effect of B[a]P-UVA and *Arundo donax* on Histone H3 Modification

Another important aspect of epigenetic regulation is histone post-translational modifications. They determine their interaction with DNA and, therefore, gene accessibility. We focused on histone H3 and analyzed 21 of its possible modifications in HaCaT cells (**Figure 3**).

After B[a]P and UVA co-exposure, a single modification became more frequent, S10P, whose frequency increased by +38.4 ± 8.7% (p = 0.003). Compared to the untreated control, pretreatment with the *Arundo donax* L. extract before pollutant co-exposure induced several significant variations. K36me1, K36me3, and S10P became more frequent (+26.6 ± 4.3%, p = 0.003; +35.1 ± 3.9%, p = 0.026; and +50.5 ± 5.6%, p < 0.001), while K27me1, K36me2, and K79me3 became less frequent (−24.6 ± 6.0%, p = 0.041; −12.1 ± 5.0%, p = 0.045; and −6.9 ± 5.1%, p = 0.017). Three of these modifications were significant not only compared to the untreated control but also to B[a]P and UVA co-exposure. This is the case of K36me1 (p = 0.045), K36me3 (p = 0.017), and K79me3 (p = 0.049). The other three modifications were observed at frequencies that are not significantly different whether cells were subjected to pollutants alone or after treatment with the *Arundo donax* L. extract (K27me1: p = 0.0554, K36me2: p = 0.250, and Ser10P: p = 0.411).

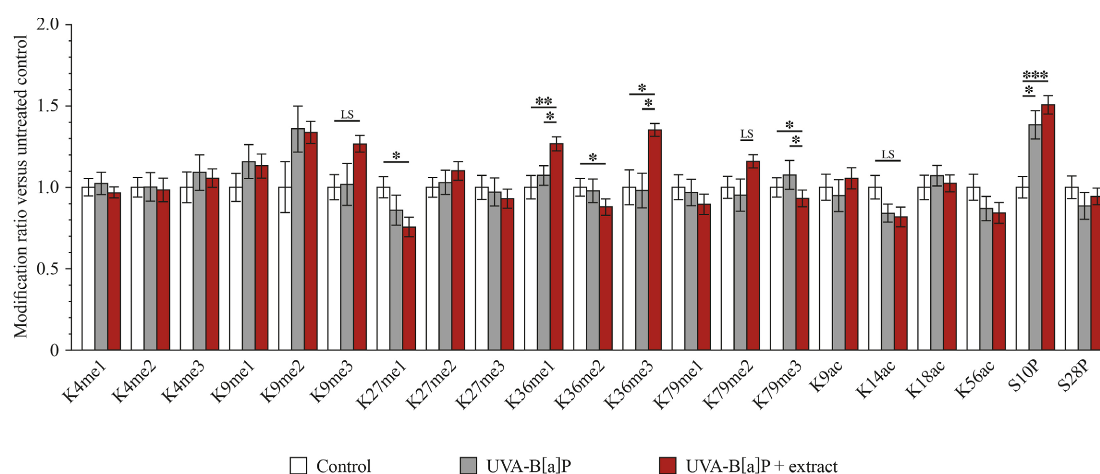


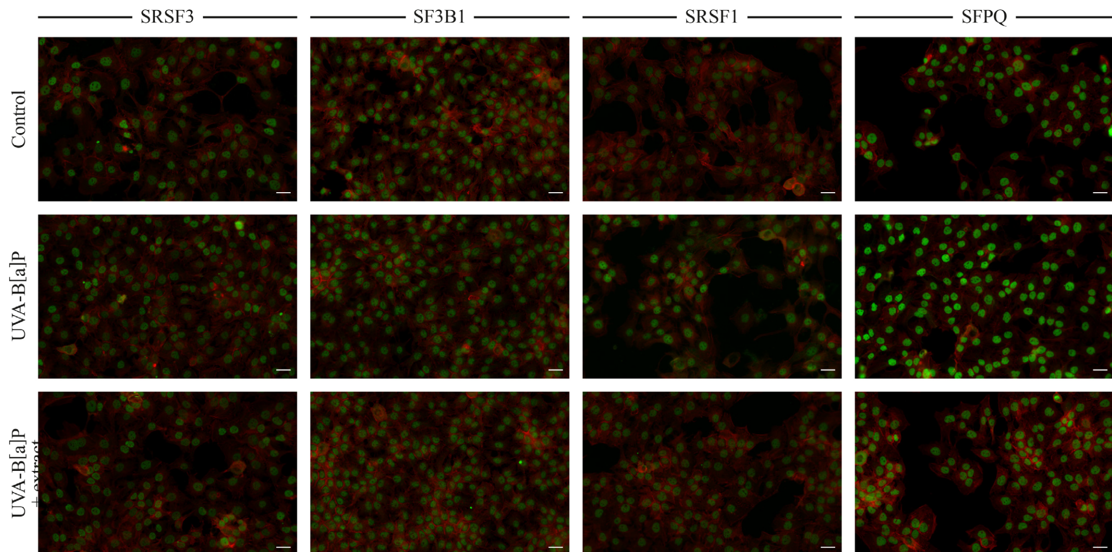
Figure 3. Relative quantification of 21 possible histone H3 modifications. Results are expressed versus unstressed-untreated control HaCaT cells. LS: p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001.

3.3. Effect of B[a]P-UVA and *Arundo donax* on the Expression of Splicing Factors

Having previously identified four splicing factors differentially expressed upon

co-exposure of skin explants to B[a]P and UVA, but not to UVA alone [29], we further investigated their fate. Thus, we performed immunohistochemical quantification of their labeling in HaCaT cells subjected or not to B[a]P-UVA and the *Arundo donax* L. extract (**Figure 4**).

Contrary to what was observed in skin explants in which SRSF3 (Serine and Arginine Rich Splicing Factor 3) was induced, and SF3B1 (Splicing Factor 3b Subunit 1) was repressed, these two splicing factors presented no significant variation in HaCaT cells whatever condition tested.



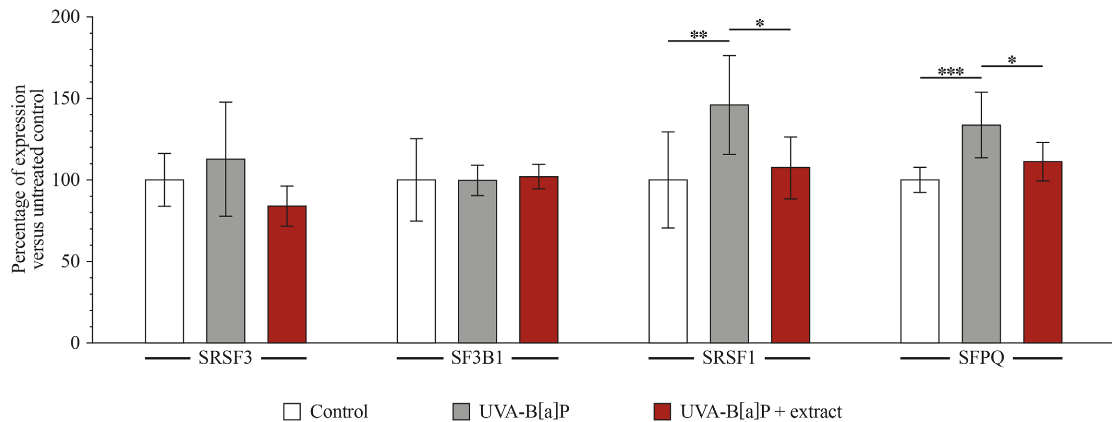


Figure 4. Immunohistochemical labelling of SRSF3, SF3B1, SRSF1, and SFPQ splicing factors. (A) Representative immunohistological images (scale bar: 20 μm); (B) Relative quantification of the labelling. Results are expressed versus unstressed-untreated control HaCaT cells. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

SRSF1 (Serine and Arginine Rich Splicing Factor 1) and SFPQ (Splicing Factor Proline and Glutamine Rich), which showed increased expression in skin explants subjected to B[a]P and UVA, revealed similar expression variations in HaCaT cells. The combination of the two pollutants led to a 46.0 ± 10.6% increased SRSF1

expression (p = 0.005) and a 33.6 ± 7.2% increase in SFPQ expression (p < 0.001) compared to the unstressed and untreated control.

Pretreatment with the *Arundo donax* L. extract before subjecting HaCaT cells to B[a]P-UVA led to a significant decrease in expression levels compared to cells that were only subjected to pollutants: −26.3 ± 4.4% for SRSF1 (p = 0.019) and −16.8 ± 3.3% for SFPQ (p = 0.014). Consequently, pretreatment with the *Arundo donax* L. extract restored expression to levels that were not significantly different from those of the untreated control (p = 0.833 for SRSF1 and p = 0.288 for SFPQ).

3.4. Effect of B[a]P-UVA and *Arundo donax* on Skin Gene Expression

To further gain insight into the effects of B[a]P and UVA co-exposure and to that of the *Arundo donax* L. extract, we assessed the expression levels of several genes important to epidermis and dermis biology, comparing them to those of untreated-unstressed control HaCaT cells or primary fibroblasts (Figure 5).

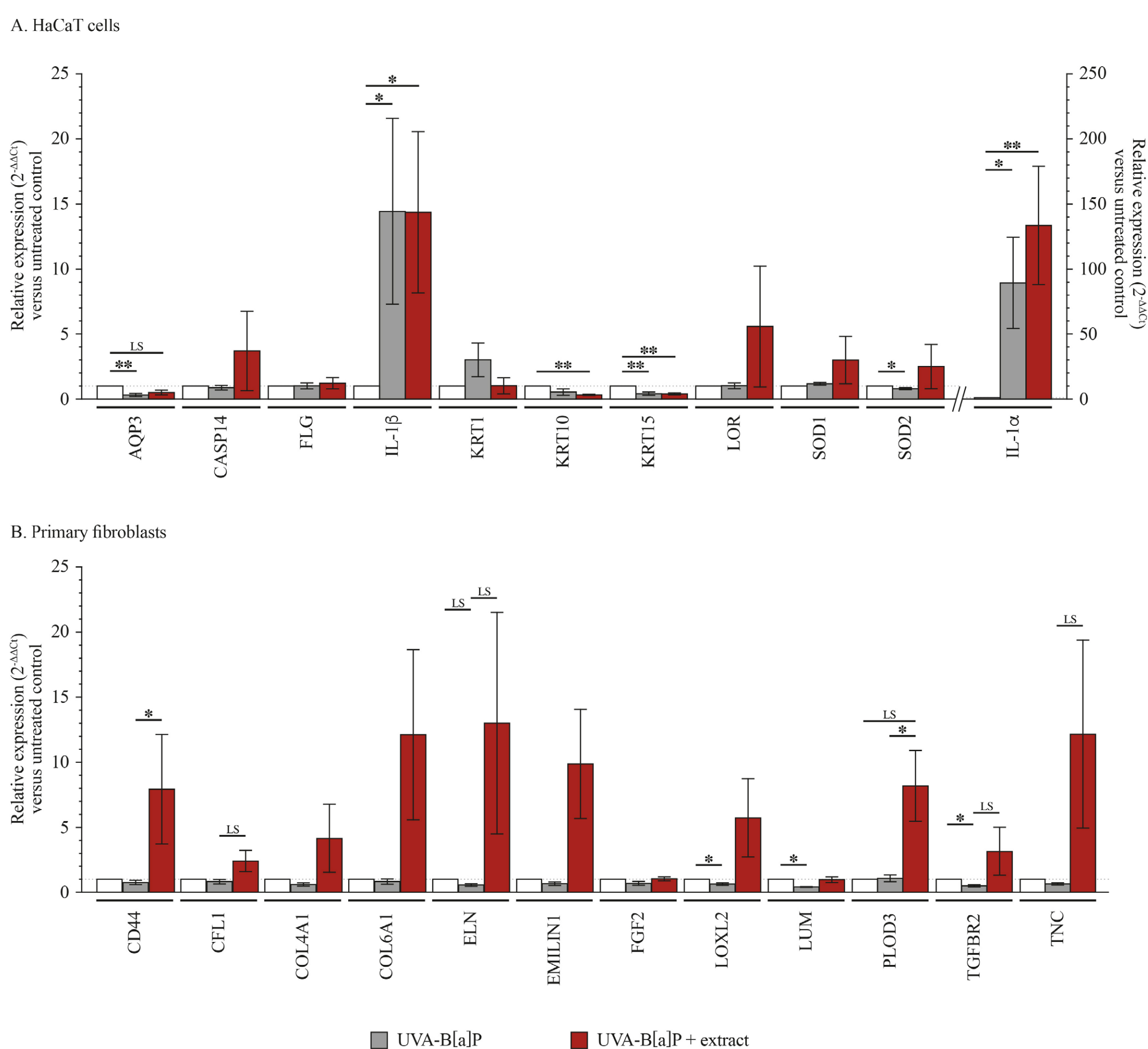


Figure 5. Relative expression ($2^{-\Delta\Delta Ct}$) of relevant (A) epidermal marker genes in HaCaT cells and (B) dermal marker genes in primary fibroblasts. Results are expressed versus unstressed-untreated control HaCaT cells or primary fibroblasts. LS: < 0.1, $^{*}p < 0.05$, $^{**}p < 0.01$.

In HaCaT cells, the inflammation-related genes IL-1α and IL-1β showed significantly higher expression upon B[a]P and UVA co-exposure (89.4-fold and 14.4-fold increase, respectively, p = 0.020 for both). The other genes presenting significant variations showed decreased expression: AQP3 (−49.6%, p = 0.008), KRT15 (−59%, p = 0.009), and SOD2 (−20%, p = 0.039). Pretreatment with the *Arundo donax* L. extract before co-exposure had little effect on these pollutant-induced variations. Still, it also affected SOD2 (p = 0.122), which variation became non-significant. Besides, the non-significant decrease in KRT10 expression observed upon co-exposure alone became significant (−68%, p = 0.009).

In primary fibroblasts, changes induced by B[a]P and UVA co-exposure were somewhat limited. Among the 12 genes scrutinized, only LOXL2, LUM, and TGFBR2 showed significantly decreased expression (−38%, p = 0.047; −59%, p = 0.023; −51%, p = 0.047, respectively), while that of ELN was only at the limit of significance (−45%, p = 0.059). Contrary to HaCaT cells, pretreatment with the *Arundo donax* L. extract before co-exposure increased the expression of many genes compared to exposure to pollutants, but results showed high variability. Thus, only the increase in the expression of CD44 (10.6 folds) and PLOD3 (7.7 folds) was significant (p = 0.023 and p = 0.047, respectively). Three other genes presented an expression increase that was at the limit of significance: ELN (23.5 folds, p = 0.059), TGFBR2 (6.5 folds, p = 0.078), and TNC (19.2 folds, p = 0.059).

4. Discussion

While the individual impacts of environmental stressors are well-documented, there is a scarcity of studies exploring their impact when combined. However, we are constantly exposed to such mixtures, and they are accumulating evidence that they result in exacerbated reactions, heightened disease risks, and increased mortality [31]-[34].

In a previous study focusing on the epidermal layer of skin explants, several processes were identified as being affected by B[a]P and UVA co-exposure compared to UVA alone [29]. Building upon these results, we used similar co-exposure conditions, primarily focusing on changes affecting epigenetic marks in a preliminary exploratory study. It is important to specify that our study exclusively evaluated the effects of B[a]P and UVA co-exposure. Without control exposures to UVA or B[a]P alone, we cannot exclude the influence of a single stressor or conclusively attribute results to co-exposure, nor can we ascertain a synergistic effect of both stressors.

While the effects of environmental stressors on DNA methylation and their consequences on embryonic development or human health are well documented [35]-[39], their impact on the skin remains largely unexplored. Nevertheless, a few studies have shown that UV radiation alters the methylation profile of epidermal cells, resulting in global and site-specific modifications [23]. Changes in DNA methylation profiles were also evidenced in isolated primary fibroblasts, but only after repeated exposure and after a few days [40]. Our study revealed that co-

exposure to B[a]P and UVA increases total DNA methylation in HaCaT cells and primary fibroblasts. A single exposure and 24 hours are sufficient to highlight these changes.

Histone modification is another factor exerting genome-wide control. The only modification we evidenced upon B[a]P and UVA co-exposure is the phosphorylation of Ser10 in the tail of histone H3, a prominent modification [41] [42]. This increased frequency is consistent with its higher frequency upon UV radiation, via the MAP kinase cascade [43]. Besides, in mice, lung exposure to cigarette smoke leads to the activation of IκB kinase α, which also results in increased S10P frequency [44]. Yet, such a modification has never been evidenced in epidermal cells subjected to stressors. Besides, it is generally associated with S28 phosphorylation and closely linked to the methylation of K9 and K14 [41] [42], modifications we did not highlight in our analyses. Thus, the biological effects of this increased S10P frequency are difficult to establish without further studies, especially since it promotes two opposite processes: chromosome condensation during mitosis and gene transcription during interphase [41] [42] [45].

Changes in mRNA splicing represent another process that significantly affects the proteome by modifying the mRNA isoforms produced [28]. A previous study on epidermal cells of skin explants co-exposed to B[a]P and UVA identified four differentially expressed splicing factors [29]. The analysis of HaCaT cells subjected to the same treatment revealed that only SRSF1 and SFPQ are overexpressed, a discrepancy probably due to the different experimental systems. Thanks to their ability to bind nucleic acid and numerous proteins, these two proteins are implicated in mRNA splicing and several processes related to RNA metabolism [45]-[49]. Best studied for their involvement in disease development, they are also stress-response elements. Both relocate to the cytoplasm under stress, being involved in alternate splicing and ultimately participating in stress response. The fact that SRSF1 is overexpressed under stress, as we have evidenced, has never been proven to the best of our knowledge. Yet, using lung epithelial cells, Zhao *et al.* recently showed that small particulate matter induces several proteins involved in mRNA splicing, including SRSF1 [50].

These major genome-wide changes in epigenetic marks induced by environmental stressors would be expected to lead to substantial modifications in gene expression. While such changes have been observed in skin explants after repeated exposure over seven days [29], our study identified limited changes in the expression of keratinocyte-/fibroblast-relevant genes following a single co-exposure period and 24 hours. Most notably, inflammation genes were induced, but other affected genes showed only mild downregulation.

In addition to assessing the effects of co-exposure, we evaluated the impact of an *Arundo donax* L. extract. This rhizomatous tall grass, native to Asia, thrives almost worldwide thanks to its highly efficient clonal reproduction. Thus, it exhibits low genetic variability but is believed to have successfully adapted to diverse environments thanks to its epigenetic variability [51] [52]. The partial characterization of

the extract we used revealed its richness in polyphenol. Besides common plant flavonoids, these polyphenols also encompass unusual ones. This is the case of catechins, compounds best studied in green tea. They are not only potent antioxidants but also modulate DNA methylation [53]-[55], as do luteolins, another flavonoid found in the extract [56]-[58].

In our model system, whether HaCaT cells or primary fibroblasts, the extract prevents the hypermethylation of DNA induced by co-exposure. It also hinders the overexpression of SRSF1 and SFPQ. While the extract did not affect Ser10 phosphorylation of histone H3 induced by co-exposure, it led to an increased methylation frequency of several residues. This is the case for K9me, with which S10P is generally associated, which could favor gene transcription [41] [42] [45]. In HaCaT cells, the extract has little influence on gene induction/repression induced by co-exposure. Yet, it leads to increased expression of some markers (ELN, PLOD3, and TNC), which seems related to the extract itself, possibly indicating a reinforcement of the extracellular matrix. While the full impact of the extract requires further work, it still seems of interest to partially reverse pollutant-induced changes in epigenetic marks.

5. Conclusion

The findings collectively demonstrate that co-exposure to B[a]P and UVA affects gene expression, as previously evidenced [29], and modifies epigenetic marks as well as mRNA processing. These changes are induced by a single co-exposure and manifest within 24 hours after cells are subjected to stressors. Some of these modifications can be reverted by the *Arundo donax* L. extract evaluated. However, a limitation of this study is that we cannot ascertain whether the changes observed result from a synergistic effect of co-exposure. This point will be of great interest for further analysis, possibly using chemometric-related statistical tools. Another point that will be of great interest to elucidate is the consequences of these alterations.

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Declaration

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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