



Formaldehyde solutions in simulated sweat increase human melanoma but not normal human keratinocyte cells proliferation



M. Rizzi, PhD^a, B. Cravello^b, S. Tonello^a, F. Renò, PhD^{a,*}

^a Innovative Research Laboratory on Wound Healing, Health Sciences Department, Università del Piemonte Orientale, via Solaroli 17, 28100 Novara, NO, Italy

^b Associazione Tessile e Salute (Health and Textile Association), Corso Pella 2, 13900 Biella, BI, Italy

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ABSTRACT

Our skin is in close contact with clothes most of the time thus risking potentially noxious chemicals contact. One of the potentially harmful manufacturing by-products that can be released by textiles when sweating is formaldehyde, used as an anti-crease treatment. As it is known to be carcinogenic to humans and a potent skin sensitizer, the aim of this study was to investigate its effects on both normal human keratinocytes (HaCaT cells) and on a highly invasive malignant melanoma cell line (SK-MEL-28) in order to contribute to the definition of safety cut-off to be applied to the production processes.

Formaldehyde concentrations below the commonly accepted limits (10–50 μM) were obtained by diluting formaldehyde in simulated sweat (UNI EN ISO 105-E04). The effects on cell proliferation were evaluated by cell counting, while ERK pathway activation was evaluated by western blot.

Low concentrations of formaldehyde (10 μM) in both acidic and alkaline simulated sweat were able to increase malignant melanoma cell proliferation, while not affecting normal keratinocytes. Melanoma proliferation increase was greater in acidic (pH = 5.5) than in alkaline (pH = 8) conditions. Moreover, formaldehyde stimulation was able to induce ERK pathway activation.

The data obtained suggest the need for an even increasing attention to the potentially harmful effects of textile manufacturing by-products.

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1. Introduction

The skin represents the outer shell of the body and is crucial in permitting the interaction between the whole organism and the external environment. Anatomically, the skin is composed of two mutually interdependent layers: the dermis (the innermost) and the epidermis (the outermost). The epidermis is composed of several cell types among which keratinocytes predominate and contribute to innate immune response, thus playing a key role in protecting the organism from external threats (Grimstad et al., 2012; Köllisch et al., 2005). As any other tissue, the skin can develop benign tumors as well as malignant ones. Melanoma originates from melanocytes, neural crest derived, a pigment producing cells located in various anatomical sites and especially in skin basal layer (Palunic et al., 2016). Melanocyte transformation into tumor cells is usually a multistep process involving the interaction of environmental, genetic, and host (i.e. previous personal or strong family history of melanoma and atypical nevi phenotype) factors (Konstantinov et al., 2016; Palunic et al., 2016). Malignant melanoma

is one of the most aggressive skin cancers and its incidence has radically increased in the past decades, especially among the Caucasian population, with an annual incidence increasing at a greater rate than any other major cancer. Although it is less common than other skin tumors (i.e. basal cell and squamous cell carcinoma) and displays an overall mortality rate of around 20%, it accounts for the majority (~80%) of skin cancer related deaths (Palunic et al., 2016; Singh and Salama, 2016; Jeong et al., 2011; Mueller and Bosserhoff, 2009). Such increased incidence and morbidity is clinically testified by the 10-year survival rate for metastatic patients amounting to <10% despite advanced treatments, as metastatic disease is highly resistant to conventional therapies (Konstantinov et al., 2016; Palunic et al., 2016; Ma et al., 2013; Buommino et al., 2009; Köllisch et al., 2005).

The skin is mostly in contact with clothes and accessories that may represent an often ignored source of potentially noxious compounds. A prolonged contact with potentially harmful chemicals derived from clothing as manufacturing by-products can result in skin integrity damage, finally resulting in allergic or sensitization reactions in the short period whereas in the long run they can induce overproliferation of neoplastic cells (Rizzi et al., 2014).

Among the potentially noxious chemicals found in textiles as manufacturing by-products there is formaldehyde (FA), a highly reactive compound widely used in many industrial processes that can

* Corresponding author at: Innovative Research Laboratory for Wound Healing, Health Sciences Department, Università del Piemonte Orientale "A. Avogadro", Via Solaroli 17, 28100 Novara, Italy.

E-mail address: filippo.reno@med.unipmn.it (F. Renò).

enter the human body by inhalation, ingestion or through the skin (Tulpule and Dringen, 2013). For these reasons, formaldehyde exposure concerns both workers, elderly as well as children (Duong et al., 2011).

The presence of FA as a manufacturing by-product in textiles is of great clinical interest as there is mounting evidence correlating such aldehyde to adverse health effects. Moreover, FA is carcinogenic to humans, as well as a potent skin sensitizer (Duong et al., 2011; Bosetti et al., 2008; Naya and Nakanishi, 2005).

Formaldehyde, as well as other textile manufacturing by-products, could activate ERK (extracellular-signaling regulated kinases) signaling pathway and induce very different dose-dependent cellular effects, ranging from apoptosis to enhanced cell proliferation, in both normal and tumor cell lines (Rizzi et al., 2014; Aizenshtadt et al., 2012; Szende and Tyihák, 2010; Freick et al., 2006; Tyihák et al., 2001).

Although up to now there are no mandatory regulations for formaldehyde presence in textiles, the mounting evidence strongly relate it to adverse health effects thus fostering many standards or voluntary labels acting in the textile industry (i.e. the European Ecolabel, the National Technical Report UNI/TR 11359 and many private marks (Oeko-Tex, Bluesigned, Aafa RLS, and so on)) to regulate its presence in the final product.

A previous study (Rizzi et al., 2014) demonstrated that neutral pH aqueous solutions with formaldehyde concentrations lower than normally indicated limits by international standards and labels were able to induce a significant increase in melanoma cells proliferation.

As FA present on textiles as a manufacturing by-product could be released from clothes upon sweat extraction, the aim of the present study was to evaluate the effects of FA diluted in simulated sweat (according to UNI EN ISO 105-E04 directive) on an in vitro model of normal keratinocyte (HaCaT) and melanoma (SK-MEL-28) human cell lines in order to contribute to the definition of safety cut-off to be applied to the production processes.

2. Materials and methods

2.1. Cell culture

Human melanoma cell line SK-MEL-28 was a kind gift of Prof. Daniela Taverna from Molecular Biotechnology Center, Department of Molecular Biotechnology and Health Sciences, University of Turin whereas HaCaT cells were purchased from Cell Lines Service GmbH (Eppenheim, Germany).

Spontaneously immortalized keratinocytes (HaCaT), isolated from human adult skin (Boukamp et al., 1988), and SK-MEL-28 cells, a highly metastatic melanoma cell line (Jeong et al., 2011), were grown as previously described (Rizzi et al., 2014; Renò et al., 2013).

2.2. Simulated sweat solutions

Simulated sweat solutions were prepared according to UNI EN ISO 105-E04 directive, describing the composition of two artificial fluids with acidic (5.5) and basic (8) pH. Briefly, simulated basic sweat is composed of 0.5 g/L L-histidine monohydrochloride monohydrate ($C_6H_9O_2N_3 \cdot HCl \cdot H_2O$), 5 g/L sodium chloride (NaCl), 5 g/L sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), whereas simulated acidic sweat is composed of 0.5 g/L L-histidine monohydrochloride monohydrate ($C_6H_9O_2N_3 \cdot HCl \cdot H_2O$), 5 g/L sodium chloride (NaCl), 2.2 g/L sodium phosphate monobasic dehydrate ($NaH_2PO_4 \cdot 2H_2O$). In both solutions pH was adjusted to the desired value by adding sodium hydroxide (NaOH).

2.3. Cell treatments

According to effects observed in a previous study, cells were treated with growing concentrations (10–50 μ M) of formaldehyde (FA) (Sigma Aldrich, St. Luis, MO, USA) (Rizzi et al., 2014) aqueous solutions made in

simulated sweat, according to UNI EN ISO 105-E04 directive. Starting acidic and basic solutions were diluted in DMEM (Dulbecco's Modified Eagle's Medium) without FBS (Fetal Bovine Serum) just before cell treatment to obtain intermediate concentrations, whereas cell treatment was done in complete medium specific for each cell line. Tested concentrations were chosen taking into account both FA IC_{50} (3.03×10^{-4} M) (Sakaguchi et al., 2007) and limits described in the private label Oeko-Tex and in the volunteer European label (Ecolabel) guidelines (20 ppm, corresponding to 1 mg/L or 3.33×10^{-5} M in the elution solution) for such compound in textiles (Rizzi et al., 2014).

To evaluate formaldehyde effects on cell proliferation, treatment timing (2 days for HaCaT cells and 3 days for SK-MEL-28) was chosen according to the observed cell duplication time (approximately 48 h for keratinocytes and 72 h for melanoma cells).

2.4. Cell proliferation

In order to evaluate the effects of formaldehyde on cell proliferation, 5×10^3 SK-MEL-28 cells and 2×10^3 HaCaT cells were seeded in 48 well plates and treated with test chemicals. At the end of the experiment, cells were fixed, and stained with crystal violet dye photographed at $4\times$ magnification using an optical microscope (Leica ICC50HD). Each experiment was performed in triplicate. The counting procedure has been performed as previously described (Rizzi et al., 2014). Cell density was expressed as percentage on control values \pm standard error of the mean (S.E.M.).

2.5. Western blot

For time course experiments, 1×10^6 cells were treated for different times (0 min, 5 min, 30 min, 60 min, 120 min) with the formaldehyde concentration giving the maximum proliferation result. Cell lysis, protein quantification and western blot determination of ERK and phospho ERK expression was performed as previously described (Rizzi et al., 2014).

2.6. Statistical analysis

ANOVA test followed by Bonferroni's post-hoc test were done for statistical analysis. Statistical procedures were performed with the Prism 4.0 statistical software (GraphPad Software Inc., CA, USA). Probability values of $p < 0.05$ were considered statistically significant. For proliferation studies, statistical analysis was performed comparing cell density observed at each concentration tested to the control. For western blotting experiments, statistical analysis was performed comparing signal intensity observed at each time point to control (T0, unstimulated) conditions.

3. Results

3.1. Formaldehyde effects on normal human keratinocytes

Experiments aimed at evaluating the effects of formaldehyde on normal human keratinocytes proliferation showed that the lowest FA concentration tested (10 μ M), when dissolved in simulated sweat prepared according to UNI EN ISO 105-E04 directive, was not able to affect normal human keratinocytes proliferation (Fig. 1). In fact, as shown in Fig. 1, when FA was diluted in acidic solution, a slight, even if not statistically significant, increase in cell proliferation was observed, whereas in alkaline conditions cell proliferation did not differ from control. Moreover, when the compound was tested at higher concentrations (25 and 50 μ M), its effects on HaCaT cells proliferation varied depending on the artificial sweat pH. In particular, 25 μ M acidic FA treatment resulted in a nearly unvaried proliferation effect compared to control conditions, whereas 50 μ M acidic solution started to induce toxic effects

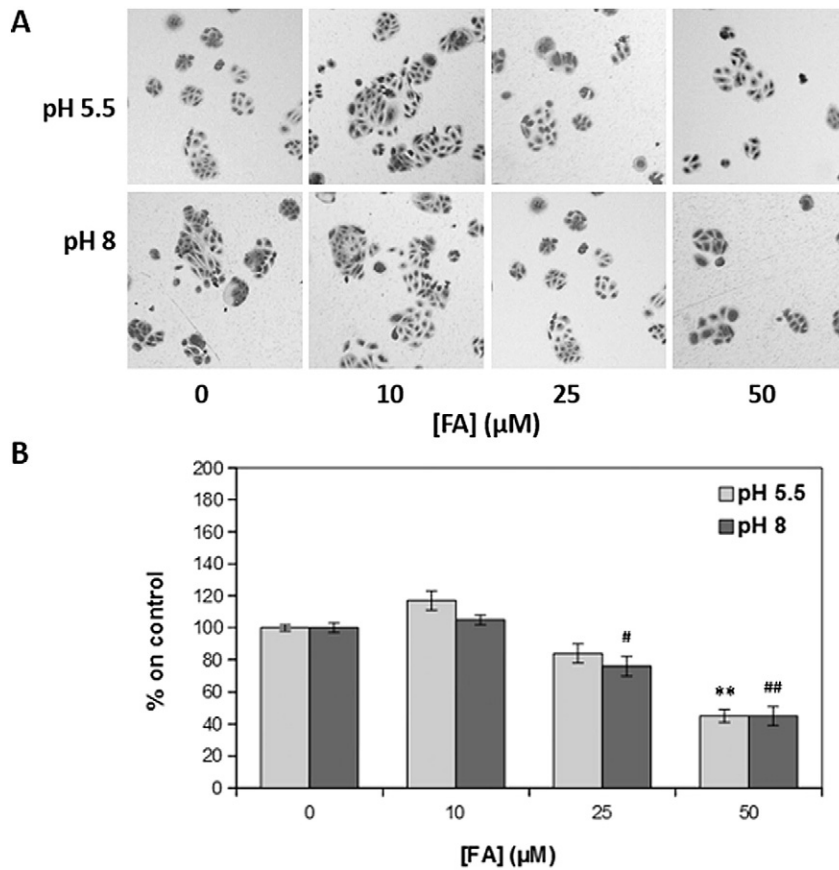


Fig. 1. Effects of formaldehyde (FA) on HaCaT cells proliferation. A) representative images (magnification 4 \times) of control and FA treated cells. B) quantification of FA diluted in acidic (pH = 5.5, light gray bars) or basic (pH = 8, dark gray bars) simulated sweat effects on normal human keratinocytes cell proliferation. Results represent the mean value of three independent experiments and are expressed as mean values \pm standard error of the mean (S.E.M.). ** $p < 0.01$; # $p < 0.05$; ## $p < 0.01$.

($p < 0.01$), resulting in a reduction of about 60% in cell proliferation (Fig. 1, light gray bars).

On the other hand, when test solutions were prepared in basic simulated sweat (pH = 8), initial toxic effects started to be evident at 25 μM ($p < 0.05$) and to worsen at 50 μM ($p < 0.01$), resulting in a reduction of about 60% in cell proliferation (Fig. 1, dark gray bars).

Even if a lack of any statistically significant effect of low FA concentrations on HaCaT cells proliferation was observed, ERK (Extracellular signaling Regulated Kinase) activation pathway was investigated.

As shown in Fig. 2, stimulation with 10 μM FA in simulated sweat resulted in slight variations in ERK signaling pathway activation, depending on artificial fluid pH. When the aldehyde was diluted in acidic simulated sweat, it only induced a slight and transient (approximately 20%, $p < 0.01$) ERK activation after 60 min stimulation, as highlighted by ERK/pERK ratio compared to basal ERK activation state (Fig. 2, light gray bars). On the other hand, FA stimulation at basic pH results in a slight decrease in ERK signaling (maximum reduction of approximately 20%), starting from 5 min stimulation (Fig. 2, dark gray bars). Such results closely retrace proliferation profile with the late (60 min) slight increase in ERK phosphorylation occurring at acidic pH, and a slight decrease in ERK pathway activation following alkaline stimulation.

3.2. Formaldehyde effects on human melanoma cells

The effects of formaldehyde on human melanoma cells were evaluated in a highly metastatic cell line (SK-MEL-28) (Jeong et al., 2011). While having no appreciable effects on normal keratinocytes, both acid and alkaline stimulations at the lowest FA concentration (10 μM) positively affected melanoma cell proliferation (Fig. 3). Interestingly

such effect was pH dependent, as acidic stimulation strongly increased cell proliferation (approximately 80% increase, $p < 0.001$) (Fig. 3, light gray bars), whereas the effect of alkaline stimulation was less marked (approximately 40% increase, $p < 0.05$) (Fig. 3, dark gray bars). Furthermore, when FA was tested at higher concentrations (25 and 50 μM), independently from the simulated sweat pH, it did not significantly affect SK-MEL-28 cells proliferation.

SK-MEL-28 proliferation was strongly affected by 10 μM FA stimulation: ERK pathway was evaluated under such experimental conditions, as this intracellular signaling cascade is known to play a key role in tumor proliferation (Rizzi et al., 2014; Aizenshtadt et al., 2012; Chuang et al., 2000).

As shown in Fig. 4, stimulation with 10 μM FA in simulated acidic sweat resulted in a significantly transient increase in ERK activity (approximately 50% increase, $p < 0.001$), with an activation peak after 5 min stimulation, as highlighted by pERK/ERK ratio (Fig. 4, light gray bars). On the other hand, when FA was diluted in basic artificial sweat, ERK activation peak (approximately 50% increase, $p < 0.001$) appeared later, starting after 60 min stimulation (Fig. 4, dark gray bars). Also for melanoma cells, ERK activation profile closely retraced the results observed in cell proliferation studies: the higher increase in cell proliferation occurred after acid stimulation related to an early and strong increase in ERK phosphorylation (reaching the maximum peak after 5 min stimulation) compared to basal condition. The lower proliferation increase observed after alkaline stimulation, instead, correlates with a late and sustained ERK activation (peaking after 60 min and remaining also after 120 min), thus giving a possible explanation for observed reduced effect on cell proliferation when compared with acid stimulation.

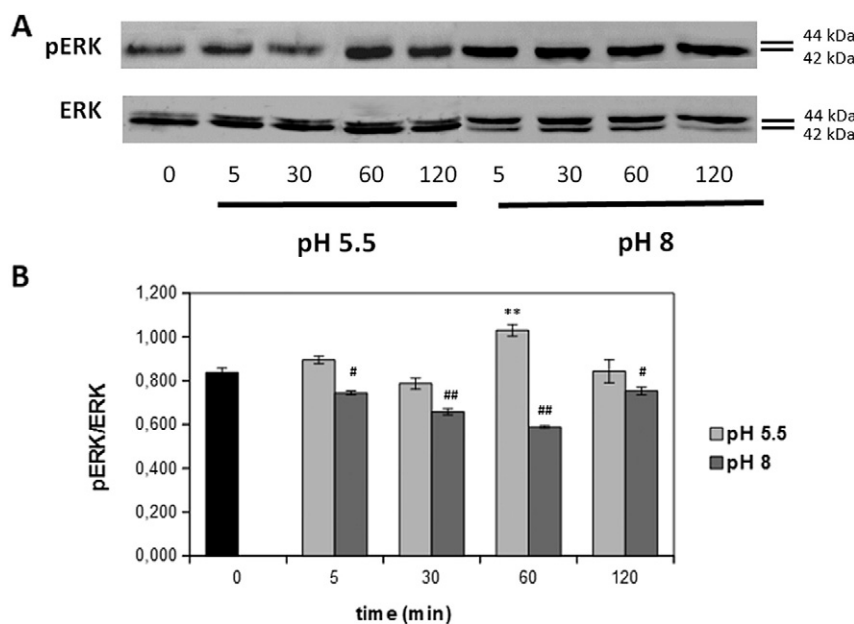


Fig. 2. Formaldehyde (FA) induced ERK phosphorylation in HaCaT cells. A) representative western blot images obtained by stimulating the cells with 10 μ M FA for different times. B) densitometric quantification of ERK phosphorylation. Results represent the mean value obtained from three independent experiments and are expressed as mean values \pm standard error of the mean (S.E.M). Light gray bars represent acidic stimulation while dark gray bars represent FA stimulation at basic pH. ** $p < 0.01$; # $p < 0.05$; ## $p < 0.01$.

4. Discussion

The skin is in touch with clothes for a long time: clothing thus represents an often ignored source of potentially noxious substances as many chemicals that are potentially harmful to human health may remain on textiles as manufacturing by-products and can be released upon sweat extraction. Thanks to its aqueous nature, sweat can induce chemical solubilization of such compounds, making them more easily accessible to cells. Among the potentially noxious manufacturing by-products that can be released by textiles there is formaldehyde, which is widely used in textile industry in anti-crease treatments (De Groot et al., 2010).

Many chemical manufacturing by-products found in textiles, such as formaldehyde, are classified as potentially carcinogenic to humans, but only some countries have mandatory legal guidelines regulating their levels in clothes and accessories. Considering formaldehyde, only some countries (i.e. Japan and Finland) have legally limited FA content for textile fabrics to reduce the risk of contact dermatitis and other adverse effects among their population (De Groot et al., 2010). To bridge such regulatory gap, some standards or voluntary labels (i.e. European Ecolabel, the National Technical Report UNI/TR 11359) and private marks (Oeko-Tex, Bluesigned, Aafa RLS, and others) have introduced limits to regulate their presence in the final textile products.

The present study aims at evaluating the effects of formaldehyde diluted in simulated sweat prepared according to UNI EN ISO 105-E04 directive on cell proliferation and ERK signaling pathway activation in normal human keratinocytes and in a human malignant melanoma cell line.

In a previous study (Rizzi et al., 2014), it has been demonstrated that neutral pH aqueous FA solutions were able to increase cell proliferation and ERK activation in two differently invasive melanoma cell lines.

In the present study attention was focused on normal human keratinocytes (HaCaT cells) and on a highly invasive melanoma cell line (SK-MEL-28) in order to evaluate whether there are any differences in FA induced cellular responses in normal and tumorigenic cells.

To reach a more physiological experimental environment, formaldehyde was diluted in artificial fluids mimicking sweat composition under different physiological conditions. In fact, exocrine sweat is known to be characterized by an acid pH whereas apocrine sweat is characterized by an alkaline pH (Fishberg and Bierman, 1932).

Formaldehyde is the simplest aldehyde, as it is composed of one carbon, one oxygen and two hydrogen atoms and, because of its small size, can quickly penetrate cell walls and membranes. From a chemical point of view, it is an electrophilic molecule that can be attacked by various nucleophilic species of biological interest.

Living organisms can interact with formaldehyde of both endogenous or exogenous origin, mediating different effects at cellular level, depending on its dose (Szende and Tyihák, 2010).

In vitro, FA reacts with a plenty of functional groups from both proteins and DNA, finally resulting in different chemical modifications, whose nature, yield and half-life strongly depend on reaction conditions (i.e. pH, temperature) (Hoffman et al., 2015).

In vitro experiments with analytically pure formaldehyde (0.1–10 mM) showed a clear dose dependence in cellular effects. Tyihák and coworkers reported that high FA concentrations (10 mM) cause a high degree of cellular damage, moderate concentrations (1 mM) increase apoptosis, whereas low concentrations (0.1 mM) are able to enhance cell proliferation and reduce apoptosis. Interestingly, these authors noticed that tumor cells seem to be more sensitive than normal cells to FA effects (Szende and Tyihák, 2010; Tyihák et al., 2001).

Formaldehyde rapidly crosses biological membranes. As it has been demonstrated that it can efficiently bind and block protein functionality of viruses, it is reasonable to assume that it can work equally well on cellular systems, protein complexes and associated biological processes (Suterland et al., 2008).

Chemically, formaldehyde acts as a bifunctional cross-linking agent, able to inactivate, stabilize or immobilize proteins, without perturbing their tertiary structure, finally resulting in quite stable in vivo modifications, with cross-link half-lives of ~10–20 h depending on the cell type and experimental conditions (Hoffman et al., 2015; Metz et al., 2004).

Depending on the peptide sequence, formaldehyde-induced chemical modifications could be represented by the reversible formation of methylol groups and Schiff bases or the more stable methylene groups. The formation of such modifications is influenced by various factors, among which local environment and pH play a key role (Tulpule and Dringon, 2013; Metz et al., 2004; Conaway et al., 1996; Steinhart et al., 1946).

Formaldehyde not only reacts with proteins but also with DNA, resulting in the formation of crosslinked DNA-protein adducts, a well

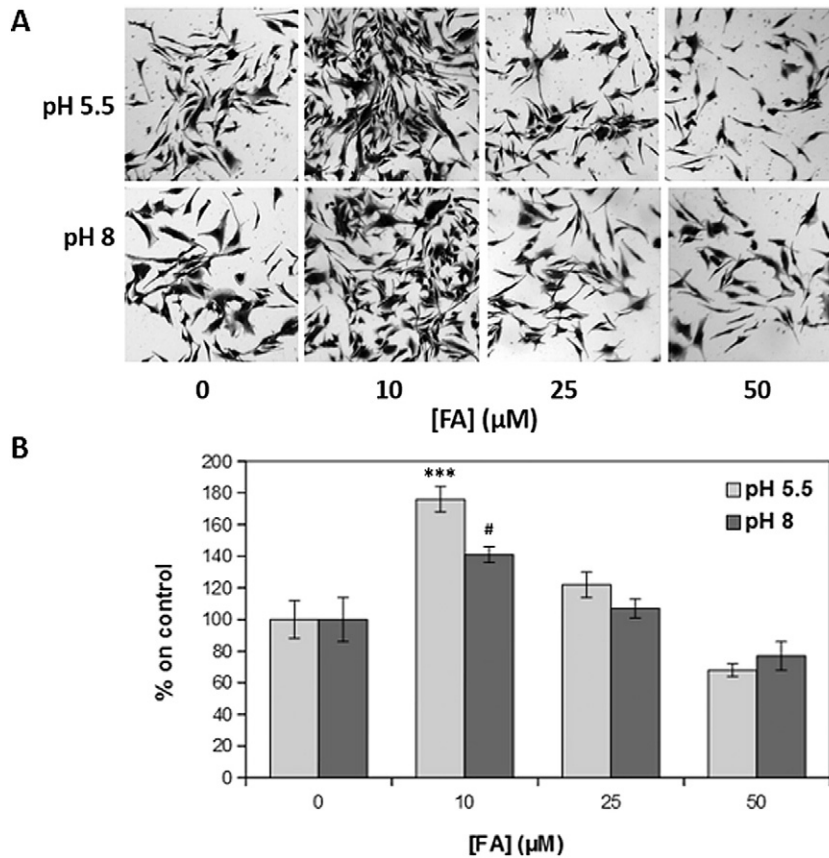


Fig. 3. Effects of formaldehyde (FA) on SK-MEL-28 cells proliferation. A) representative images (magnification 4 \times) of control and FA treated cells. B) quantification of FA diluted in acidic (pH = 5.5, light gray bars) or basic (pH = 8, dark gray bars) simulated sweat effects on human malignant melanoma cell proliferation. Results represent the mean value of three independent experiments and are expressed as mean values \pm standard error of the mean (S.E.M.). *** $p < 0.001$; # $p < 0.05$.

known abnormality in the early carcinogenic process. As low concentrations of FA were shown to be able to increase cell proliferation, such effect could induce the accumulation of cells bearing abnormal DNA-proteins crosslinks, thus enhance its genotoxicity and carcinogenicity (Szende and Tyihák, 2010).

As an increase in cell proliferation is known to be a critical factor in chemical-induced carcinogenesis (Starr and Gibson, 1985), in the present study attention has been focused on FA ability to differently affect normal and tumor cell responses. As shown in the results section, low concentrations (10 μM) of formaldehyde diluted in both acidic or basic simulated sweat were not able to increase normal human keratinocytes proliferation. In addition, it has been observed that the higher concentration tested (25 and 50 μM) were able to induce a toxic response.

On the other hand, when FA diluted in both acidic or basic simulated sweat was tested at the lower concentration (10 μM) on SK-MEL-28 cells, it was shown to increase cell proliferation, with a bigger increase in acidic conditions. The higher concentrations tested (25 and 50 μM), indeed, did not significantly reduce cell proliferation compared to control in both acidic and basic pH conditions.

Because of these results, ERK signaling pathway activation has been studied. ERK is an upstream activator of > 150 substrates involving such signaling cascade in the regulation of critical cellular responses, including transcription, translation, mitosis and apoptosis (Brys et al., 2016). In particular, ERK pathway is strongly involved in regulating cellular responses such as mitogenesis and differentiation, as it has been demonstrated that its transient activation mediates cell proliferation, whereas its persistent activation mediates cell growth arrest and differentiation (Chuang et al., 2000).

Data shown in the results section correlate acidic FA (10 μM) induced increase in SK-MEL-28 proliferation with an early transient

activation of ERK signaling pathway, thus confirming the previously described formaldehyde involvement in its activation in various normal and tumor cellular models (Rizzi et al., 2014; Aizenshtadt et al., 2012; Chuang et al., 2000).

Alkaline FA (10 μM) induced increase in SK-MEL-28 proliferation was lower than the effect observed after acidic stimulation and ERK activity peak appeared later than at pH 5.5: it could be hypothesized that under these conditions such tardive signaling pathway activation was not sufficient to induce a greater increase in proliferation.

On the other hand, HaCaT cells stimulation with formaldehyde (10 μM) at both acidic or alkaline pH did not result in a statistically significant increase in cell proliferation, even if ERK signaling pathway is activated. The lack of a clear effect on cell proliferation in this cell line might be due to an insufficient activation of the signaling pathway, as the model of normal human keratinocytes adopted (HaCaT cells) displays a basal level of ERK activation greater than the one observed in SK-MEL-28 cells.

Melanoma is classified according to the presence of 4 significantly mutated genes, namely BRAF, RAS, NF1, Triple Wild Type (Triple-WT) (Konstantinov et al., 2016). In particular, epidemiological studies highlighted that nearly half of the patients with an advanced melanoma diagnosis harbor a valine to glutamine substitution in codon 600 of the serine-threonine kinase BRAF (V600E phenotype) (Brys et al., 2016; Eroglu and Ribas, 2016).

Observed results correlating FA stimulation with an increase in cell proliferation are even more interesting as the malignant melanoma experimental model adopted (SK-MEL-28 cells) harbors BRAF (V600E) mutation. Such hot spot mutation, in fact, enhances cellular sensitivity to MEK inhibition, finally resulting in both cyclin D1 protein expression downregulation and G₁ cell cycle arrest (Konstantinov et al., 2016; Kim et al., 2012; Solit et al., 2006).

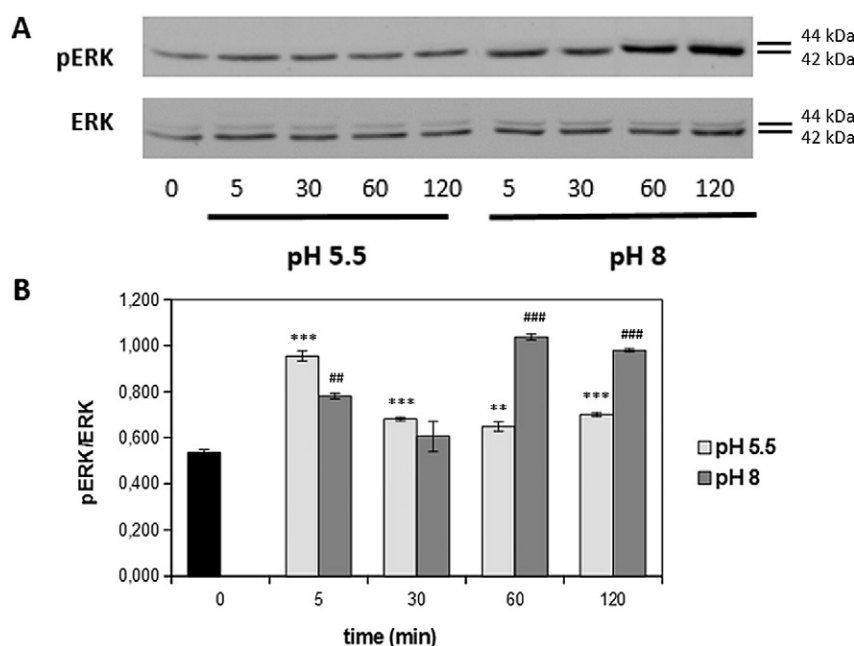


Fig. 4. Formaldehyde (FA) induced ERK phosphorylation in SK-MEL-28 cells. A) representative western blot images obtained by stimulating the cells with 10 μ M FA for different times. B) densitometric quantification of ERK phosphorylation. Results represent the mean value obtained from three independent experiments and are expressed as mean values \pm standard error of the mean (S.E.M). Light gray bars represent acidic stimulation while dark gray bars represent FA stimulation at basic pH. ** $p < 0.01$; *** $p < 0.001$; ## $p < 0.01$; ### $p < 0.001$.

Formaldehyde metabolism is known to generate reactive oxygen species (ROS) (Pongsavee, 2013; Duong et al., 2011), known to interfere with intracellular signaling pathways activation, cell proliferation, cytokines and transcription factors activation and even apoptosis (Asatiani et al., 2011). Moreover, studies on neural cells showed that FA is able to interfere with glutathione (GSH) cycle, increasing its export from the cells and thus increasing oxidative stress levels (Tulpule and Dringen, 2013).

Redox state plays a key role in maintaining cell and tissues homeostasis and an impairment in such balance could affect cell growth and differentiation, along with cell signaling and even apoptosis (Asatiani et al., 2011). Moreover, recent studies have highlighted that cellular responses to ROS vary according to their concentrations: high ROS levels mediate toxic cellular effects (i.e. protein oxidation and DNA damage) whereas low ROS levels are known to enhance cell proliferation (Valko et al., 2007; Boonstra and Post, 2004; Halliwell and Whiteman, 2004).

ROS could induce MAPK activation and in particular ERK signaling pathway activity (Son et al., 2011): such stimulation, along with chemical modifications that could be induced by formaldehyde treatment in cellular proteins acting as key regulators of cell survival responses may provide a plausible explanation for the observed increase in melanoma cell proliferation.

5. Conclusion

Data presented showed that formaldehyde, a widely used aldehyde, representing a very common textile industry manufacturing by-product, could increase tumor cell proliferation. Such results not only agree with the previously described ability of neutral pH FA solutions to increase melanoma cells proliferation (Rizzi et al., 2014), but also show that this chemical, when diluted in simulated sweat, thus more closely mimicking the physiological condition, is able to induce a strong proliferative response in the highly invasive melanoma cell line SK-MEL-28.

Considering that the skin is in close contact with clothes for a long time and that sweat can extract chemical manufacturing by-products from fabrics, thus making them more easily accessible to cells, our data suggest that an even increased attention should be paid to the

potential noxious effects of such compounds, in order to define safety cut-offs to be applied to production processes.

Transparency document

The Transparency document associated with this article can be found, in online version.

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