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Effect of atmospheric fine particles on epidermal growth factor receptor mRNA expression in mouse skin tissue

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ABSTRACT. We investigated the effect of atmospheric fine particles on epidermal growth factor receptor (*Egfr*) mRNA expression in mouse skin tissue and explored the effect of atmospheric fine particles on skin aging. Forty female BALB/c mice were randomly divided into four groups (each comprising 10 mice) as follows: a saline control group and low-, medium-, and high-dose atmospheric fine particle groups (1.6, 8.0, and 40.0 mg/kg, respectively) (fine particles were defined as those with a diameter of ≤2.5 μm, i.e., PM2.5). Each dose group was exposed to intratracheal instillation for 3 days. Twenty-four hours after the last exposure, real-time quantitative polymerase chain reaction was used to detect the expression of *Egfr* mRNA in the skin tissue of each mouse. The expression levels of *Egfr* mRNA in the medium- and high-dose PM2.5 groups were significantly higher ($P < 0.05$) than that in the control group, and were positively correlated with the dose.

Medium and high concentrations of PM2.5 can induce the expression of *Egfr* mRNA and promote skin aging.

Key words: Atmospheric fine particles; Epidermal growth factor receptor; Real-time fluorescent quantitative PCR

INTRODUCTION

Skin aging is a complex process involving physiological structure disorder and functional degradation of the skin, and it is influenced by both internal and external factors. It generally manifests as natural aging and photo-aging (Fisher et al., 2002). Natural aging is mainly affected by genetic and other endogenous factors; it gradually becomes apparent over time, and takes the form of fine wrinkles, and dry, loose, and rough skin (Guo et al., 2012). Skin covers the whole surface of the body and is therefore the most important bridge to the external environment. It is susceptible to aging, which can cause anxiety, depression, low self-esteem, and a series of psychological and social problems, thereby seriously impacting a person's life and work. Therefore, preventing and delaying skin aging have become the focus of intensive medical research (Knezevic et al., 2007).

The expression of epidermal growth factor receptor (*Egfr*) is an early event in skin aging, and plays a key role in the skin aging process (Hachiya et al., 2009). The link between atmospheric particulate pollution and skin aging has received the attention of many researchers of environmental epidemiology, but the exact mechanism behind the relationship is unknown. In this study, the effect of atmospheric fine particulates on *Egfr* mRNA expression in mouse skin tissue was investigated to determine that mechanism.

MATERIAL AND METHODS

Material

Animals

Forty BALB/c female mice weighing 18-20 g were provided by the Southern Medical University Experimental Animal Center.

Instruments and reagents

We used the following reagents: QIAE II Agarose Gel Extraction Protocol (Qiagen, Germany); RNA extraction reagent (Bioteck, Beijing, China), reverse-transcription-polymerase chain reaction (RT-PCR) reagents (Bioteck, Beijing, China), and reverse transcriptase (Invitrogen Corporation). The main instruments were: a Thermo Andersen G-2.5 high-volume sampler (Thermo Electron Corporation, USA), a UV spectrophotometer (US Bio-Rad Company), and a Strata gene Mx3000P real-time PCR instrument (Agilent, USA).

Primer sequences

TRIzol was used for genomic extraction and the following *Egfr* primers were designed

and synthesized: upstream primer: 5'-AGCTCACGCAGTTGGGCA-3'; downstream primer: 5'-TCTCATGGGCAGCTCCTT-3'.

Methods

PM2.5 sources and handling

The Thermo Anderson G-2.5 high-volume sampler was used to collect atmospheric PM2.5 from the roof of a building (no obvious sources of pollution, 12.2 m high) in the Haizhu District of Guangzhou City between August and September 2014 (fine particles were defined as those with a diameter of ≤2.5 μm, i.e., PM2.5). The PM2.5 filter membrane was cut into 1-cm^2 pieces, soaked in triple-distilled water, and treated with ultrasonic oscillation for 30 min. The PM2.5 fluid was then filtered using a six-layer gauze. After the soaking, oscillation, and filtering procedure had been carried out three times, the filtrate was vacuum freeze-dried into dry powder and stored at 4°C in a refrigerator. Prior to use, a specific weigh of the dry PM2.5 powder was added to Dulbecco's modified Eagle's medium culture solution (Gibco Co.) on a clean bench; the culture was ultrasonically vibrated for 15 min and sterilized to prepare different concentrations of PM2.5 solution.

Animal grouping and exposure

The 40 female BALB/c mice were randomly divided into four groups (each comprising 10 mice) as follows: a saline control group and low-, medium-, and high-dose atmospheric fine particle groups (1.6, 8.0, and 40.0 mg/kg, respectively). Each dose group was exposed to intra tracheal instillation for 3 days with an infusion volume of 1.5 mL/kg and an interval of 24 h.

Skin tissue RNA extraction

The TRIzol method was used; in accordance with the instructions, RNA was conventionally extracted from the back skin tissue of the mice, and cryopreserved at -80°C until required.

Real-time fluorescence quantitative PCR

The cDNA was synthesized by reverse transcription; 2 μg total RNA was used as the template, according to the manufacturer instructions provided with the PrimeScript® 1st Strand cDNA Synthesis Kit (TaKaRa). The reverse transcription reaction system was formulated to synthesize cDNA (the total system had a volume of 20 μL). The Mg^{2+} and primer concentrations were optimized for fluorescence quantitative RT-PCR.

The reaction system for real time PCR amplification comprised a volume of 25 μL (TaKaRa SYBR® Premix Ex Taq kit), and the PCR regimen was 95°C for 30s, 95°C for 5 s, and 60°C for 30s (for 40 cycles). An Agilent Stratagene fluorescence quantitative PCR instrument (Mx3000P) was used for fluorescence quantitative PCR.

Statistical methods

The Wilcoxon rank sum test was performed and the SPSS17.0 software was used for

analyses. Data are reported as “median (P25-P75)”; P < 0.05 was considered statistically significant.

RESULTS

Real-time PCR results

The expression levels of *Egfr* mRNA in the medium- and high-dose PM2.5 groups were significantly higher than in the control group (P < 0.05), and were positively correlated with the dose, as shown in Tables 1 and 2.

Table 1. Epidermal growth factor receptor (*Egfr*) mRNA expression levels in skin tissue.

Groups	Skin tissue
A (control)	1.417 (1.174-1.604)*†
B (low dose)	2.744 (2.118-3.107)†
C (medium dose)	4.503 (3.396-5.152)‡
D (high dose)	7.865 (6.597-8.485)‡§

‡,§,*,†Statistically significant differences compared with groups A, B, C, and D, respectively (P < 0.05).

Table 2. Correlation between airborne fine particulate matter concentration and epidermal growth factor receptor (*Egfr*) mRNA expression.

			GROUP	VOLU
Kendall’s tau_b	GROUP	Correlation coefficient	1.000	0.784 (**)
		P value (2-tailed)	-	0.000
		N	30	30
	VOLU	Correlation coefficient	0.784 (**)	1.000
		P value (2-tailed)	0.000	-
		N	30	30
Spearman’s rho	GROUP	Correlation coefficient	1.000	0.903 (**)
		P value (2-tailed)	-	0.000
		N	30	30
	VOLU	Correlation coefficient	0.903 (**)	1.000
		P value (2-tailed)	0.000	-
		N	30	30

Correlation significant at a P value of 0.01 level (2-tailed). GROUP represents different atmospheric concentrations of fine particulate matter; VOLU shows the expression level of *Egfr* mRNA in skin tissue; *Positive correlation between the two.

Correlation analysis of different concentrations of PM2.5 and *Egfr* mRNA expression

Different concentrations of PM2.5 were positively correlated with the expression of *Egfr* mRNA in the skin tissue (Kendall’s tau_b: r = 0.784, P = 0.000; Spearman’s rho: r = 0.903, P = 0.000, r > 0.40); there was a significant correlation between the two.

DISCUSSION

Research has shown that high expression of *Egfr* is closely related to human skin aging, and is not only an early event in the skin aging process, but can also be used as an early indicator of skin aging (Hachiya et al., 2009; Sakurada and Tsao, 2009). The latest research by Xu et al. (2006)

shows that UVB irradiation of immortalized keratinocytes can increase c-jun and c-fos expression, while this trend can be significantly reduced by *Egfr* antagonists. This strongly indicates that *Egfr* plays an important role in skin aging.

Atmospheric particles less than or equal to 2.5 μm (PM2.5 particles) are known as fine particulates or fine particles. Numerous studies have shown that PM2.5 particles contain large quantities of toxic and hazardous substances; they have a long residence time in the atmosphere and a long transmission distance, and not only affect human health and air quality but also human skin. These substances attach to the skin, resulting in skin metabolic cycle disorders; they cause blackheads, enlarged pores, horny accumulation, skin aging, etc. The results of this study suggested that there was no significant difference in the expression level of *Egfr* mRNA between the low-dose PM2.5 group and the control group, while the mouse skin exposed to medium and high concentrations of PM2.5 showed signs of aging. Moreover, medium and high concentrations of PM2.5 induced the expression of *Egfr* mRNA, which was positively correlated with the dose. This observation may be explained by the fact that the skin tissue has defense capabilities for a certain concentration of PM2.5, but beyond that concentration, the skin tissue's defense is impaired, leading to the high expression of *Egfr* mRNA. Therefore, by blocking *Egfr*, it is possible to eliminate or greatly weaken PM2.5-induced skin aging.

Conflicts of interest

The authors declare no conflict of interest.

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