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DNA polymerase beta is involved in the protection against the cytotoxicity and genotoxicity of cigarette smoke

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ABSTRACT

Reactive oxygen species (ROS) and oxidative DNA damage have been implicated in the cigarette smoke-induced cytotoxicity and genotoxicity. DNA polymerase β (polβ), a key base excision repair (BER) enzyme in repairing oxidative DNA damage, may play a crucial role in fighting against the cytotoxicity and genotoxicity of cigarette smoke. In this study, we applied a novel approach to collect cigarette smoke extract (CSE) and investigated the cytotoxic and genotoxic effects of CSE by using the mouse embryo fibroblasts that express wild-type of polβ (polβ$^{+/+}$), null of polβ (polβ$^{-/-}$) and overexpression of polβ (polβ^{oe}). Our results showed that polβ$^{-/-}$ cells treated with CSE exhibited a higher ROS level and more DNA single-strand breaks and chromosomal aberrations than that of polβ$^{+/+}$ and polβ^{oe} cells. These data suggested that polβ mediated-BER may involve in repairing the CSE-induced DNA damage and protection against the cytotoxicity and genotoxicity of CSE.

1. Introduction

Cigarette smoke is a major risk for many diseases including chronic obstructive pulmonary disease (COPD), cardiovascular diseases and various cancers (D'Agostini et al., 2008; Ambrose and Barua, 2004). According to a report from International Agency of Research on Cancer, almost 90% of lung cancers and 30% of other types of malignant tumor are closely associated with cigarette smoke exposure. Although cigarette smoke-related carcinogenesis has been intensively studied, its molecular mechanisms remain unclear. Cigarette smoke exposure could lead to the accumulation of excessive amount ROS through Fenton reaction mediated by metal ion and result in oxidative DNA damages (Yang et al., 1999; Samson, 1992). It has been proposed that cigarette smoke may increase cellular oxidative stress (Panda et al., 1999) and result in oxidative DNA damage including DNA single strand breaks (SSB), DNA double strand breaks (DSB) and DNA-protein cross-links (Faux et al., 2009; Kiyosawa et al., 1990; Phillips, 2002). In addition, cigarette smoke-induced reactive oxygen species (ROS) was suggested to be an important initiation factor and mediator in the carcinogenicity of cigarette smoke (Carter and Hamm, 2009). Based on these reports, it's conceivable that oxidative DNA damage via ROS should be involved in carcinogenicity of cigarette smoke exposure. Base excision repair (BER) is a major pathway responsible for recognition and removal of oxidized, alkylated or deaminated bases induced by environmental and endogenous stressors such as oxidation, alkylation and

Abbreviations: CSE, cigarette smoke extract; ROS, reactive oxygen species; BER, base excision repair; polβ, DNA polymerase beta; MTT, 3-[4,5-dimetho-thiazol-2-yo]-2,5-diphenyl tetrazolium bromide; SCGE, single cell gel electrophoresis; OTM, olive tail moment; DMSO, dimethyl sulfoxide.

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deamination (Samson, 1992; Demple and Harrison, 1994). Therefore, BER capacity should play a crucial role in combating oxidative DNA damages induced by cigarette smoke.

DNA polymerase beta (polβ), a key component in BER pathway, fills in a single-nucleotide gap with its DNA polymerase activity which is essential for repairing oxidative DNA damages (Belousova and Lavrik, 2010) and excises the 5′-terminal deoxyribose phosphate resulting from 5′-incision of an apurinic/apyrimidinic (AP) site by AP endonuclease 1 (APE1) (Liu and Wilson, 2012; Beard and Wilson, 2006). It has been well accepted that ROS-induced oxidative DNA damages such as oxidized bases, apurinic/apyrimidinic (AP) sites and single-strand DNA breaks are mainly repaired by DNA polymerase beta (polβ) dependent-BER pathway (Barzilai and Yamamoto, 2004; Sugo et al., 2000). Down-regulation of DNA polymerase beta increased the cellular sensitivity to DNA alkylating agent, methyl methanesulfonate (MMS) (Sobol et al., 2000), whereas enhanced polβ significantly reduced MMS-induced base lesions in mouse embryonic fibroblasts (Sobol et al., 2000). Recent works from our laboratory also showed that polβ deficient cells exhibited an elevated level of oxidative DNA damage, chromosomal breakage as well as the cellular hypersensitivity to anti-cancer drug bleomycin, which is known as a ROS inducer and consequentially leads to oxidative DNA damage (Liu et al., 2011). These observations suggest that polβ plays an important role in repairing oxidative DNA damage and maintaining chromosomal integrity and stability. Thus, we hypothesize that cigarette smoke-induced DNA damage might be repair through polβ-dependent BER pathway and polβ deficient could impair BER, leading to the accumulation of cigarette smoke-induced DNA damage and chromosomal breakage, thus exacerbating the cigarette smoke genotoxicity. In contrast, polβ proficiency should effectively repair cigarette smoke-induced DNA damage maintaining chromosomal integrity as well as combating the cytotoxicity and genotoxicity of cigarette smoke. To elucidate this hypothesis, we developed a novel method to collect and extract the mainstream of cigarette smoke, and observed the ROS generation, DNA single strand breaks and DNA damage repair, chromosomal breakage as well the cell viability in mouse embryo fibroblasts that express different levels of polβ to further evaluate the cytotoxicity and genotoxicity caused by cigarette smoke extract.

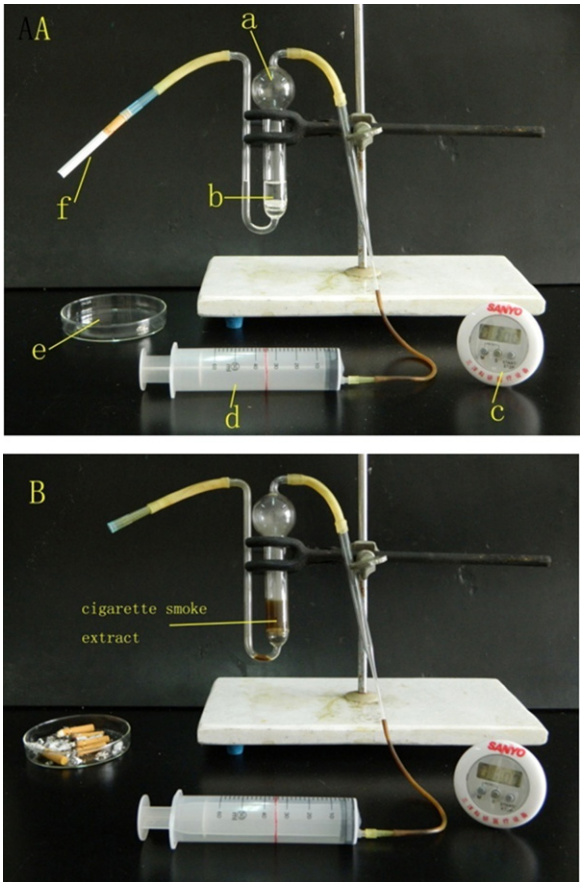

Fig. 1 – The collection of cigarette smoke extracts (CSE). (A) Cigarette smoke collecting device. (a) U-shape absorption glass tube with a perforated plate; (b) 5 mL of DMSO which serves as the absorption liquid; (c) timer; (d) a 50 mL plastic syringe; (e) a plate used for receiving the smoke ash; (f) the cigarette. A rubber tube holds all these parts together. (B) The final CSE collected by flow rate (35 mL puff volume, 2 s duration, 1 puff/min, smoked to a butt length within 2–3 mm of the edge of the tipping paper). The mainstream from five cigarettes was collected within 5 mL DMSO and the color of the absorption solution turned black in the end.

2. Materials and methods

2.1. *Preparation of cigarette smoke extract*

Cigarette smoke extract (CSE) was prepared according to the method developed by our laboratory. The cigarettes used in this study were Pride brand (China Tobacco Inc., Chengdu) containing 14 mg of tar and 1.4 mg of nicotine per cigarette. A U-shape absorption glass tube was used as absorption device and a 50 mL plastic syringe was served as a pump (Fig. 1A). The flow rate of the mainstream smoke of cigarette was strictly regulated by the US Federal Trade Commission (35 mL of air was pumped each time within 2 s and another pump was made 58 s later. Cigarette end was replaced by an ignited cigarette when the butt was about 2–3 mm). The mainstream smoke from five cigarettes was bubbled into 5 mL DMSO at 25 °C (Fig. 1B). Then, the CSE was transferred to a sterilized vial and stored at −20 °C. The CSE stock was diluted by culture medium for dosing cells, and the concentrations of CSE were illustrated as the volume ratio of CSE versus culture medium.

2.2. *Cell culture*

Polβ deficient cell line (polβ$^{-/-}$), wild-type cell line (polβ$^{+/+}$) and overexpression cell line (polβ^{oe}) were a kind gift from Dr. Samuel H. Wilson (NIEHS/NIH, USA). To verify polβ expression levels in the three cell lines, we examined polβ protein levels by using of western blot. The results exhibited that polβ protein was not detected in polβ null cells and wild-type cells expressed a moderate level of polβ protein, whereas polβ^{oe} cells expressed 2-fold higher protein than polβ wide-type cells. The three cell lines were cultured in Dulbecco's Modified Eagle