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Chemico-Biological Interactions 155 (2005) 31–42
Chemico-Biological Interactions
www.elsevier.com/locate/chembioint

Induction of apoptosis in the lung tissue from rats exposed to cigarette smoke involves p38/JNK MAPK pathway

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Received 2 March 2005; received in revised form 13 April 2005; accepted 13 April 2005

Abstract

Smoking is a major cause of human lung cancer. Past studies suggest that apoptosis might influence the malignant phenotype, but little is known about the association between apoptosis and cigarette smoke (CS)-induced lung pathogenesis. Using an in situ cell death detection kit (TA300), the association of CS with apoptosis was determined in a concentration-dependent manner. Furthermore, the expression of related proteins were investigated in the terminal bronchiole areas of the lung tissue from rats exposed to CS. Results showed that the expression of phosphotyrosine proteins was increased significantly in lung tissue of rats exposed to CS from 5 to 15 cigarettes. Using Western blotting and immunoprecipitation assay, Fas, a death receptor, was proved just be one of these phosphotyrosine proteins. CS triggered activation of MAP kinase (p38/JNK or ERK2) pathway, which led to Jun or p53 phosphorylation and FasL induction links Fas phosphorylation. Further, smoke treatment produced an increase in the level of proapoptotic proteins (Bax, t-Bid, cytochrome c and caspase-3), but a decline in Bcl-2, procaspase-8 and procaspase-9 proteins. Thus, CS-induced apoptosis may result from two main mechanisms, one is the activation of p38/JNK-Jun-FasL signaling, and the other is stimulated by the stabilization of p53, increase in the ratio of Bax/Bcl-2, release of cytochrome c; thus, leading to activation of caspase cascade.

Keywords: Apoptosis; Cigarette smoke; Lung pathogenesis; MAP kinase; Caspase cascade

Abbreviations: CS, cigarette smoke; MAPK, mitogen-activated protein kinases family; JNK, c-Jun N-terminal kinase; ERK2, extracellular signal regulating kinase2; FasL, Fas ligand; PKC-α, protein kinase C-α; STAT1, signal transducer and activated of transcription; MEK1, MAPK/ERK kinase1; Bax, Bcl-2 associated X protein; t-Bid, truncated Bid; Bcl-2, B cell lymphoma-2; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis

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doi:10.1016/j.cbi.2005.04.008

1. Introduction

Epidemiological evidence has long suggested that smoking is a major cause of human cancer in various tissue sites [1–4]. However, the mechanisms by which cigarette smoke (CS) induces cancer remains unknown, and among the 3800 compounds identified in CS, a large number exert mutagenic and carcinogenic activity [5]. In addition, high concentrations of reactive oxygen species (ROS) and nitrogen oxides (NO_x) produced from CS [6–8] may also be in part responsible for its carcinogenicity [9]. Molecular toxicological investigations have demonstrated that CS can elevate expression of heme oxygenase [10] and alter regulation of the c-fos gene at the transcriptional level [11]. However, the mechanism underlying the CS-induced expression of c-fos and other signal proteins remains to be elucidated. Recently, it was found that exposure to CS results in oxidative stress stimulation of inducible NO synthase (iNOS) and c-fos via regulation of protein tyrosine phosphorylation and MAP kinase which in turn may promote lung pathogenesis [12]. Furthermore, CS has been reported to have the ability to induce DNA single strand breaks in cultured human and rodent cells [13–18]. It was previously reported that a positive correlation exists between the number of cigarettes smoked per day and both the 8-hydroxyguanosine (8-OH-G) levels and their repair activities in human peripheral leukocytes and lung tissue [19,20]. Recently, peroxynitrite was identified in aqueous CS fraction [21], and this has been shown to react with guanine and DNA in vitro to form 8-nitroguanine (8-NO_2-G) [22,23]. It was also recently found that gaseous NO_x can induce 8-NO_2-G formation in human lung fibroblast cells and in tobacco cigarette smokers [24] and smoke-exposed Wistar rats [25].

The inappropriate regulation of apoptosis is associated with a variety of diseases, ranging from neurodegenerative disorders to malignancy [26]. Past studies suggest that apoptosis might influence the malignant phenotype and promote tumor progression [27]. Apoptosis involves two main pathways: death receptor and mitochondria death pathway [28]. The two apoptotic pathways converge at activation of caspase, leading to apoptosis. In addition to caspases, the members of the mitogen-activated protein kinases family (MAPK) are also pathways which mediate apoptosis. The MAPK superfamily of serine/threonine kinases is activated by numerous extracellular stimuli and involved in signal transduction cascades that play an important regulatory role in cell growth, differentiation and apoptosis [29,30]. Three major mammalian MAPK have been described: the extracellular signal regulating kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK. However, the diverse MAPK members are activated in response to different extracellular stimuli and have distinct downstream targets, thus serving different roles in cellular responses. ERK is activated primarily by mitogenic stimuli such as growth factors leading to cell growth and survival [31]. On the other hand, JNK and p38 are activated by UV irradiation, DNA damage, hydrogen peroxide, heat and osmotic shock, resulting in apoptotic cell death [32].

In the past two decades, significant progress has been made in understanding the molecular and cellular pathogenesis of lung cancer. However, the mechanism behind the CS-induced apoptosis and related proteins remains unclear. To elucidate the association between apoptosis and CS-induced lung pathogenesis, an animal exposure system was designed to investigate apoptosis and the expression of related proteins in the terminal bronchiole areas of the lung tissue of rats exposed to CS.

2. Materials and methods

2.1. Chemicals

Tris–HCl, EDTA, SDS, phenylmethylsulfonyl fluoride, leupeptin, Nonidet P-40, deoxycholic acid, sodium orthovanadate, aprotinin and polyclonal antibody against β-actin were purchased from Sigma chemical Co. (St. Louis, MO). Protein assay kit was obtained from Bio-Rad Labs. (Hercules, CA). In Situ apoptosis detection kit, TACS Blue Labe & Kit (TA300) were purchased from R and D Systems Inc. (MN, USA). Polyclonal antibodies against PY54, PY-20, c-Raf-1, MEK1, PKC-α, phosphor-STAT1, phospho-p53 (Ser392) and p53 were obtained from Transduction Lab. (KY, USA). Antibodies against Fas, FasL, phospho-ERK, Bcl-2, Bax, Bid, cytochrome c, caspase-8, caspase-9 and caspase-3 were from Santa Cruz Biotechnology (CA, USA), and those against phospho-p38 MAP kinase (Thr180/Tyr182), phospho-