



Effects of Heated Tobacco Products' Extract from Cigarette Smoke on the Growth of Lung Cancer Stem Cells

Yasunari Kanda*

Division of Pharmacology, National Institute of Health Sciences, Kanagawa, Japan

Abstract

Several types of cancer, including lung cancer, have been linked to an increased risk of smoking by epidemiological studies. Cancer stem cells (CSCs), a minor cell population in tumors that contribute to drug resistance and recurrence, are the source of lung cancer. Aerosols made from heated tobacco products (HTPs) contain nicotine and harmful chemicals. However, the available evidence is insufficient to accurately determine whether HTPs are safer than smoking cigarettes. The effects of cigarette smoke extract (CSE) from HTPs on lung CSCs in lung cancer cell lines were the subject of this study. CSEs increased stem cell marker expression and lung CSC proliferation, according to our findings [1]. Cytokines and expression of the epithelial-mesenchymal transition (EMT) were also induced by CSE. These findings suggest that HTPs can in vitro induce CSCs in the lung.

Keywords: Heated tobacco products; Cigarette smoke extract; Cancer stem cells; Epithelial-mesenchymal transition

Introduction

Epidemiological investigations have proposed that cigarette smoking is a gamble factor for carcinogenesis and the movement of a few kinds of disease, including cellular breakdown in the lungs. Over 5000 chemicals can be found in cigarette smoke, including 79 that have been identified as oncogenic [2]. Socially and medically, it's important to know how active and passive smokers' health is affected by smoking cigarettes.

Cancer stem cells (CSCs), a minor cell population in tumors that contribute to cellular heterogeneity, have been linked to an increasing number of cases of lung cancer. CSCs are thought to be linked to cancer growth and recurrence due to their potential for high levels of drug resistance and tumorigenicity [3]. Recently, a detoxifying enzyme called aldehyde dehydrogenase (ALDH), which is responsible for oxidizing aldehydes inside cells, has been used as a functional marker to identify CSCs in clinical samples and cancer cell lines. Tobacco-specific nitrosamine 4-(methylnitrosamino) 1-(3-pyridyl)-1-butanone (NNK) induces the proliferation of lung CSCs in A549, and nicotine induces the proliferation of breast CSCs in MCF-7.

The vaporization of heated tobacco products (HTPs) results in aerosols containing nicotine and other chemicals, which are relatively new. There are a few HTPs that are monetarily accessible, including Glo by English American Tobacco Plc., IQOS by Phillip Morris Worldwide Inc., and Ploom S by the Japan Tobacco Inc. HTPs supposedly have decreased degrees of hurtful synthetics, like nicotine, tar, and carbonyl mixtures, when contrasted with ordinary cigarettes [4]. However, some chemicals, such as glycerol, propylene glycol, and glycidol, are known to be more abundant in HTPs than in cigarettes. The health effects of HTPs are drawing more and more attention as they become more widely available. As per the World Wellbeing Association (WHO), there is at present inadequate proof to presume that HTPs are less destructive than consumed cigarettes. As a result, the connection between HTPs and the development of cancer is still poorly understood [5].

We have explored the impacts of tobacco smoke remove (CSE) from HTPs on lung CSCs in vitro. Both stem cell and epithelial-mesenchymal transition (EMT) markers were induced by CSE from HTPs, as were CSC proliferation.

Materials and Methods

Cell culture

The American Type Culture Collection in Manassas, Virginia, USA housed the A549 cells, while the Japanese Collection of Research Bioresources in Tokyo, Japan housed the HCC827 and H1975 cells. These cells were refined in Dulbecco's adjusted Bird's medium (DMEM; Supplemented with 10% heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO, USA) HyClone, Logan, Utah, USA), 100 g/mL streptomycin (Gibco BRL, Carlsbad, CA, USA), and 100 U/mL penicillin [6].

ALDH assay

According to a previous report [12], the ALDEFLUOR kit from Stem Cell Technologies in Vancouver, British Columbia, Canada, was used to identify CSC populations with high ALDH enzyme activity. The cells were plated at a thickness of 1×10^5 cells in 60 mm culture dishes. The cells were treated for three days with each concentration of the CSE following overnight serum deprivation [7]. Cells were then suspended at a convergence of 1×10^6 cells/mL in an ALDH measure support containing the ALDH substrate BAAA (1 μ M) and hatched for 30 min at 37 °C. Diethylaminobenzaldehyde (DEAB, 15 M), a specific ALDH inhibitor, was used as a negative control. A FACS Aria II cell sorter (BD Biosciences, San Jose, CA, USA) was utilized to gauge and sort the ALDH-positive and - negative cells.

Sphere-forming assay

As previously mentioned, the sphere-forming assay was carried out. In brief, cells were plated at a density of 5000 cells/mL in

*Corresponding author: Yasunari Kanda, Division of Pharmacology, National Institute of Health Sciences, Kanagawa, Japan, E-mail: kanda@nihs.go.au

Received: 28-Mar-2023, Manuscript No. ijm-23-96512; Editor assigned: 31-Mar-2023, PreQC No. ijm-23-96512(PQ); Reviewed: 14-Apr-2023, QC No. ijm-23-96512; Revised: 21-Apr-2023, Manuscript No. ijm-23-96512(R); Published: 28-Apr-2023, DOI: 10.4172/2381-8727.1000218

Citation: Kanda Y (2023) Effects of Heated Tobacco Products' Extract from Cigarette Smoke on the Growth of Lung Cancer Stem Cells. Int J Inflamm Cancer Integr Ther, 10: 218.

Copyright: © 2023 Kanda Y. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

serum-free DMEM supplemented with N2 (Gibco), 20 ng/mL basic fibroblast growth factor (R&D Systems, Minneapolis, MN, USA), and each concentration of the CSE on ultra-low attachment 24-well plates (Corning, Acton, MA, USA). The number of spheres was microscopically examined five days later. 100x magnification was used to capture sphere images [8].

qRT-PCR

After the cells were treated with CSE for 24 h, complete RNA was disengaged utilizing the TRIzol reagent (Thermo, Waltham, MA, USA), as per the producer's guidelines. A QuantiTect SYBR Green RT-PCR Kit (QIAGEN, Valencia, CA, USA) and an Applied Biosystems ViiA7 real-time PCR system were used for the qPCR assays. Normalization to GAPDH mRNA levels was used to determine the relative changes in transcript levels for each sample.

Results

CSE from HTPs induces the proliferation of ALDH-positive cells

To begin, we investigated the cytotoxicity of CSE made from three different HTPs—HTPa, HTPb, and HTPc—in three different lung cancer cell lines—A549, HCC827, and H1975. A549 cells' proliferation was slightly slowed by 0.3 percent and 1% HTPa, respectively. A549 cells' proliferation was slowed down by HTPb at concentrations greater than 0.3 percent. The A549 cells' proliferation was slightly slowed down by 0.3 percent HTPc. HTPs had no significant impact on proliferation in HCC827 or H1975. Using lung cancer cell lines (A549, HCC827, and H1975), we carried out an ALDH assay to investigate the effects of the CSE from HTPs on the CSCs. In A549, HCC827, and H1975 cells, HTPa dose-dependently induced the proliferation of ALDH-positive cells. HTPb was likewise found to prompt the expansion of ALDH-positive cells in a portion subordinate way in every one of the three cell types. ALDH-positive cells were also proliferated by HTPc in the A549 and HCC827 cell lines. Based on these findings, it appears that CSE promotes cell proliferation in ALDH-positive lung cells [9].

CSE from HTP induces sphere formation

We used a different CSC assay to test the CSCs' ability to form spheres in order to verify that the HTP's CSE had an effect on them. Like the ALDH measure, HTPa prompted circle development in the three cell lines in a portion subordinate way. The A549 cells developed spheres as a result of HTPb, whereas HTPc caused HCC827 and H1975 cells to form spheres. These outcomes affirm the impacts of the CSE from the HTPs on the multiplication of lung CSCs [10].

CSE from HTP induces the expression of stem cell markers

We examined the stemness markers Oct3/4, Nanog, and Sox2 in order to investigate the molecular mechanisms by which the CSE from the HTPs induced the proliferation of CSCs. ALDH-positive cells were significantly increased by HTPa (3% in three cell lines) and HTPb (0.01% in A549 and HCC827, and 3% in H1975). We chose 3% HTPc because it did not significantly increase the number of ALDH-positive cells. In the three cell lines, HTPa increased the expression of the three stem cell markers, just like the ALDH assay did. Except for Sox2, HTPb also increased the expression of stem cell markers in the A549 and HCC827 cells. Conversely, HTPc upregulated the outflow of undifferentiated organism markers in HCC827 cells. Based on these findings, it appears that the CSE from the HTPs promoted ALDH-positive cell proliferation in a variety of ways [11].

CSE from the HTPs induces the expression of EMT markers

There is mounting evidence that CSCs may have EMT potential; thusly, we dissected whether HTPs could actuate the declaration of EMT markers. HTPa, like the ALDH assay and stem cell markers, increased Twist and Snail expression and decreased E-cadherin expression in the three cell lines, indicating that HTPa induced EMT. HTPb promoted EMT in HCC827 and H1975 cells, but not in A549 cells. HTPc, on the other hand, did not cause EMT. These outcomes recommend that the impacts of the CSE from the HTPs on the EMT contrast among the HTPs.

Discussion

In this study, we discovered that CSEs from HTPs induced CSC marker expression and proliferation in the lung. In addition, the HTPs' CSEs induced the expression of EMT markers and inflammatory cytokines, indicating that HTP aerosols may contain harmful chemicals that could influence lung cancer development. These information recommend that HTPs are related with the improvement of cellular breakdown in the lungs [12].

We discovered that all three HTP-derived CSEs promoted cell proliferation. The impacts were somewhat unique among the HTPs, as each was made at an alternate temperature [HTPc (200 °C) < HTPa (240 °C) < HTPb (300-350 °C)], proposing that their synthetic parts contrasted. It is also known that the smoking conditions have an impact on the components of the HTPs. After smoking HTPs, it's important to think about the concentrations of the chemicals in the air and blood [13].

We have previously demonstrated that tobacco-specific N-nitrosamine 4-(methylnitrosamino) 1-(3-pyridyl) 1-butanone (NNK) activates Wnt signaling to increase the number of ALDH-positive A549 cells [6]. Despite the fact that NNK is known to cause cancer, it has been reported that HTPa and HTPb contained lower levels of NNK than conventionally burned cigarettes. The expression of the Wnt target gene Dkk1 was not increased by HTPa or HTPb. We discovered that HTPa- and HTPb-induced EMT markers were consistent with the ALDH assay. It has also been reported that nicotine, benzo[a]pyrene, formaldehyde, isoprene, acrylonitrile, and N-nitrosornicotine (NNN) induce EMT markers in cigarette smoke [14]. These synthetic substances have additionally recently been distinguished in the standard spray of HTPa and HTPb. HTPa significantly induced EMT markers in comparison to HTPb. NNN may be a potential chemical that causes EMT in HTPs due to the fact that its concentration in HTPa mainstream aerosol is higher than in HTPb mainstream aerosol. Only nicotine has been examined in the concentration of chemicals in HTPs smokers' blood to this point. Analyzing and identifying additional chemicals involved in the CSE-induced proliferation of lung CSC via EMT is necessary for future research [15].

Acknowledgement

None

Conflict of Interest

None

References

1. Talhout R, Schulz T, Florek E, Benthem J, Wester P (2011) Hazardous compounds in tobacco smoke. *Int J Environ Public Health* 8: 613-628.
2. Saygin C, Matei D, Majeti R, Reizes O, Lathia JD (2019) Targeting cancer stemness in the clinic: from hype to hope. *Cell Stem Cell* 24: 25-40.

3. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J (2007) ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 1: 555-567.
4. Hirata N, Sekino Y, Kanda Y (2010) Nicotine increases cancer stem cell population in MCF-7 cells. *Biochem Biophys Res Commun* 403: 138-143.
5. Hirata N, Yamada S, Sekino Y, Kanda Y (2017) Tobacco nitrosamine NNK increases ALDH-positive cells via ROS-Wnt signaling pathway in A549 human lung cancer cells. *J Toxicol Sci* 42: 193-204.
6. Uchiyama S, Noguchi M, Takagi N, Hayashida H, Inaba Y (2018) Simple determination of gaseous and particulate compounds generated from heated tobacco products. *Chem Res Toxicol* 31: 585-593.
7. Simonavicius E, McNeill A, Shahab L, Brose LS (2019) Heat-not-burn tobacco products: a systematic literature review. *Tab Control* 28: 582-594.
8. Helen GS, Jacob P, Nardone N, Benowitz NL (2018) IQOS: examination of Philip Morris International's claim of reduced exposure. *Tab Control* 27: 30-36.
9. Forster M, Fiebelkorn S, Yurteri C, Mariner D, Liu C, et al. (2018) Assessment of novel tobacco heating product THP1.0. Part 3: comprehensive chemical characterisation of harmful and potentially harmful aerosol emissions. *Reg Toxicol Pharmacol* 93: 14-33.
10. Horinouchi T, Miwa S (2021) Comparison of cytotoxicity of cigarette smoke extract derived from heat-not-burn and combustion cigarettes in human vascular endothelial cells. *J Pharmacol Sci* 147: 223-233.
11. Hirata N, Yamada S, Yanagida S, Ono A, Nishida M, et al. (2022) Lysophosphatidic acid promotes the expansion of cancer stem cells via TRPC3 channels in triple-negative breast cancer. *Int J Mol Sci* 23: 1967.
12. Yanagida S, Satsuka A, Hayashi S, Ono A, Kanda Y (2021) Chronic cardiotoxicity assessment of BMS-986094, a guanosine nucleotide analogue, using human iPS cell-derived cardiomyocytes. *J Toxicol Sci* 46: 359-369.
13. Tsuji K, Yamada S, Hirai K, Asakura H, Kanda Y (2021) Development of alveolar and airway cells from human iPS cells: toward SARS-CoV-2 research and drug toxicity testing. *J Toxicol Sci* 46: 425-435.
14. Kim S, Hwang K, Choi D, Choi K (2018) The cigarette smoke components induced the cell proliferation and epithelial to mesenchymal transition via production of reactive oxygen species in endometrial adenocarcinoma cells. *Food Chem Toxicol* 121: 657-665.
15. Zhang N, Zhu T, Yu K, Shi M, Wang X, et al. (2019) Elevation of O-GlcNAc and GFAT expression by nicotine exposure promotes epithelial-mesenchymal transition and invasion in breast cancer cells. *Cell Death Dis* 10: 343.