Temperature-controlled Smoker: Based on Intelligent Health Management Technology

Haiyun Wang[1,†], Keyu Han[2,†], Ruihan Shi[3], Zhenxiang Guan[4] and Shiqi Huang[5,*]
[1]*College of Pharmacy, Zhengjiang University, Hangzhou, Zhejiang, 310058, China*
[2]*School of Changzhou bilingual school, Changzhou, Jiangsu, 213000, China*
[3]*Cardiff sixth form college, Cardiff, CF24 0AA, U.K.*
[4]*St.Johnsbury academy, St.Johnsbury, Vermont, 05819, U.S.A.*
[5]*Dalian No.24 High School, Dalian, Liaoning, 116001, China*

[†]*Contributed equally*

Keywords: Intelligent Health Management, Lung Cancer, Cigarette, Temperature, TTF-1, Napsin A, CK5/6, P40, P63.

Abstract: Intelligent Health Management (IHM) is a new industry formed by the integration of traditional health management, artificial intelligence, big data and other new information technologies. It comprehensively manages the individual and population health risk factors, and achieves the effective control of the occurrence and progress of diseases with limited resources. As a major health risk factor, smoking closely connects with the lung cancer, which is one of the leading causes of cancer death and the malignant tumors with the fastest increase in morbidity and mortality in the world. As the yield of smoke condensate (tar) from tobaccos differs in different burning conditions, the prediction is that the exposure to increasing temperature of smoking increases frequency and severity of lung cancer. This paper will measure markers of lung cancer using confocal microscopy with TTF1, Napsin A, p63, p40 and CK5/6 markers, mortality using survival curves, tumor number, tumor size, and employing mice as laboratory animals. Positive control could be treatment with radon or asbestos, negative control could be no smoke exposure. The results would be applied to temperature control in e-cigarette design, providing new lung cancer prevention strategy for the intelligent health management of smokers.

1 INTRODUCTION

Lung cancer is one of the malignant tumors with the fastest increase in morbidity and mortality and the biggest threat to the health and life of population (Lu, Yu, Yi 2019). Lung cancer is also the second most common cancer in 2020. It is major factor that causing of mortality in 93 countries, the incidence of lung cancer (11.4%) is only less than breast cancer, and lung cancer has the highest mortality rate (18.0%) (Sung, Ferlay, Siegel, Laversanne, Soerjomataram, Jemal, Bray 2021). A prediction in 2015 estimates that the total cancer deaths would reach 1 359 100 in the EU in 2015 (766 200 men and 592 900 women), while lung cancer rates rise 9% to 14.24/100 000 and becoming the cancer with the highest rate, reaching and possibly overtaking breast cancer rate (Malvezzi, Bertuccio, Rosso, Rota, Levi, Vecchia, Negri 2015).

Smoking is closely related to the occurrence of lung cancer. Tobacco smoke can lead to abnormal DNA methylation in cells, which in turn decreases the expression of tumor suppressor genes, which in turn increases the expression of KRAS gene, which promotes cell growth. In 2012, a whole-genome sequencing analysis of smokers and nonsmokers with non-small cell lung cancer by researchers at the University of Washington found that the overall mutation rate was more than 10 times higher in smokers than in nonsmokers, and that nearly 50 percent of smokers had Kirsten rat sarcoma viral oncogene homolog mutations compared with nonsmokers (Govindan, Ding, Griffith, Subramanian, et al.). In addition, scientists have studied smokers and non-smokers and found that both cancer patients and healthy people show different changes in DNA methylation compared with non-smokers.

Tobacco tar is the product of incomplete combustion of organic matter under anoxic condition. It is a mixture of hydrocarbons, hydrocarbon oxides, sulfides and nitrous compounds, including

Wang, H., Han, K., Shi, R., Guan, Z. and Huang, S.
Temperature-controlled Smoker: Based on Intelligent Health Management Technology.
DOI: 10.5220/0011368300003444
In *Proceedings of the 2nd Conference on Artificial Intelligence and Healthcare (CAIH 2021)*, pages 326-332
ISBN: 978-989-758-594-4

benzopyrene, radioactive isotopes and so on. During smoking, there are more than 3000 kinds of chemical substances in the tobacco tar produced by tobacco combustion, among which multi-chain aromatic hydrocarbons and nitrosamines have strong carcinogenic activity. What is worse, the content of polycyclic aromatic hydrocarbons in tobacco tar is much higher than that in tobacco itself in both types and quantities. During smoking, tobacco tar enters the respiratory tract of smokers with smoke flow. The polycyclic aromatic hydrocarbons (PAHs) in tar, a carcinogenic substance, are mostly produced during smoking. Then tar is deposited in human lungs and accumulates on the surface of lung. As a result, multi-chain aromatic hydrocarbons and nitrosamines involved in tar can lead to DNA damage of bronchial epithelial cells through a variety of mechanisms, making oncogenes activated and tumor suppressor genes inactivated, thus causing cell transformation and finally cancerization.

There are several markers are used in immunohistochemical staining of this experiment. First of all is TTF-1(Thyroid transcription factor 1). It is a nuclear protein with a relative molecular weight of 38× 103 and belong to a group of the NKx2 transcription factors. The regulation of thyroid tissue is one of the important functions of this marker. It also has high sensitivity and specificity in lung adenocarcinoma with serosal effusion.

Next is P63 protein. It belongs to P53 protein family. P63 protein's germline mutations are associated with severe mammary developmental defects in both rodents and humans. Different p63 isoforms have been identified, some of which (DeltaNp63) are preferentially expressed in the epithelial basal cells of different organs and have been considered as possible markers of stem cells (Barbareschi, Pecciarini, Cangi, Macrì, Rizzo, Viale, Doglioni 2001).

Then comes p40 protein. Actually, it is a subtype of the P63 protein mentioned before. It is commonly expressed in the basal cell layer or progenitor cell layer of layered epithelial tissues, basal cells of certain glandular epithelium and thymic epithelial cells. p40 protein staining yields high sensitivity as well as high specificity for distinguishing SQC from ADC, neuroendocrine carcinomas, and malignant mesothelioma (Tatsumori, Tsuta, Masai, Kinno, Taniyama, Yoshida, Suzuki, Tsuda 2014).

Finally, here comes CK5/6 and Naspin A. The former one is a basal cytokeratin with high molecular weight (58Kda and 56Kda) (Kriegsmann, Cremer, Zgorzelski, Harms, Muley, Winter, Kazdal, Warth, Kriegsmann 2019). In normal tissues, basal cells of squamous and ductal epithelium and some squamous germinal cells, myoepithelial cells, mesenchymal cells are positive, and glandular epithelial cells are negative (Gaydarov, Martinelli-Kläy, Lombardi 2021). Therefore, it can be used for differential diagnosis of squamous cell carcinoma and adenocarcinoma, mesothelioma and adenocarcinoma. It can also be used for differential diagnosis of benign and malignant ductal epithelial hyperplasia.

Normally, the tobacco carcinogen produced in the process of smoking is about 1~6‰ of the weight of the original tobacco, and the production of tobacco carcinogen has a certain relationship with the frequency of smoking. The more times smokers suck in a unit of time, the more carcinogen is produced. Smoking three puffs per minute produces almost twice as much carcinogen as smoking one puff per minute. The amount of carcinogen produced is also related to the length of the cigarette, because when the cigarette is lit, the tar smoke produced by the cigarette passes through the unburned part of the cigarette, some of it is absorbed by the tobacco. As the light gets closer and closer to the end, almost all of the carcinogen produced goes into the smoker's respiratory tract. The ratio of the front to the back of a cigarette is about 1:1.4, which is why cigars, pipe cigarettes, and hookah cigarettes all produce less tar than paper cigarettes (Hecht 2006).

Tar's generation, enrichment, increment has a close relationship with smoke local lighting temperature. The majority of tobacco carcinogen produced under 700—900℃, but during smoking, cigarette lighting local temperature up to 600-900 ℃, and at the same time the blazing red parts of the temperature up to 980-1050℃, in the interval between two smoking, the temperature dropped about 100-150 ℃. Thus, In the smoking process, most of tobacco tar can be produced and that will affect human health seriously. This phenomenon is also the major cause of increasing popularity of e-cigarette. E-cigarette is an up-to-date item that mimics a cigarette and has the same look, smoke, taste and feel as a cigarette. it represents alternative-to-smoking products which produce a visible aerosol that the user inhales. They simulate the psych behavioral aspects of smoking dependence and deliver the chemical component of the smoking dependence, nicotine (Konstantinos, Gene, Stephen, Riccardo, Jonathan 2016). It vaporizes nicotine and turns it into vapor for the user to smoke. Compared with traditional cigarette, most e-cigarette liquid evaporates at about 220 degrees Celsius, which is mainly a physical change. It is not easy to produce harmful substances,

but traditional cigarette always has chemical reaction during combustion. However, for some special groups, using e-cigarette also involves drawbacks, particularly for COPD patients. Experiment shows that in airway cells from patients with COPD, aerosols from an e-cigarette were associated with similar toxicity to cigarette smoke (Carioli, Malvezzi, Bertuccio, Boffetta, Levi, La Vecchia, Negri 2021.).

At present, e-cigarettes have already developed temperature-controlled models, smokers can adjust the voltage and resistance value of a specific temperature-controlled box to get their favorite e-cigarette taste, such temperature regulation technology can be applied to intelligent health management system. Health management is the process of monitoring, analyzing, evaluating and predicting the health status and risk factors of individuals and groups, and intervening in health risk factors through health consultation and guidance, In this research, If we figure out the relationship between cancer rates and smoking temperature, A rigorous intelligent temperature control system could be developed and used on e-cigarettes to monitor a smoker's mouth temperature in real time and automatically cool when it is above a certain level to minimize the rate of getting cancer.

2 MATERIALS AND METHODS

2.1 Materials

Silica gel (≥99.0%), muffle furnace, control console, quartz reactor (volume≈1.6 cm^3). All of the reaction processes should comply with International Organization for Standardization of cigarette smoking.

160 male mice (aged 6-8 weeks and 18-22g), surgical instruments for dissection, reagent for preparation of sectioning (4% paraformaldehyde, saline, paraffin, PBS, etc), immunohistochemical staining kit, marker (TTF, Napsin A, CK5/6, P40 and P63 antibody), goat anti-rabbit IgG.

2.2 Tobacco Combustion Products (Jebet, Kibet, Kinyanjui, Yamori 2018)

Build reactor assembly and pyrolysis product capture unit. The combustion temperature starts from 200℃, and products are collected every 100℃ the temperature increases. Oxygen is delivered to the reactor for tobacco combustion. The whole combustion process continues for 5 minutes. According to the temperature, six experimental groups of products were obtained.

Tobacco tar condenses as a volatile gas phase component in an ice bath and is collected through a delivery pipe. At the end of each process, weigh all products to ensure that the mass difference of products was not more than ±5%.

2.3 Modeling and Grouping

160 male mice aged 6-8 weeks and 18-22g are weighed and randomly divided into 8 groups with 20 rats in each group.

A. Positive control group: continuous radon exposure for one month;

B. Negative control group: no smoke exposure, normal culture for one month;

C. Experimental group 1-6: each group is exposed to different tobacco smoke, whose pyrolysis temperature are 200℃, 300℃, 400℃, 500℃, 600℃ and 700℃, treat each group for a month.

During the experiment, all mice are given a normal diet, and each group is treated specifically as required by radon gas or tobacco smoke on the day of preparation. The body weights of mice are observed daily.

2.4 Specimen Preparation

After anesthetizing the mice, the mice are first perfused with normal saline and then perfused with 4% paraformaldehyde. Dissect the mice to judge the number of tumors and take out the tumors. The length and width of each tumor are measured with vernier calipers, and the tumor volumes are calculated according to the formula.

Tumors are placed in 4% paraformaldehyde, fixed for 4h, then paraffin embedded and sectioned after gradient dehydration.

2.5 Immunohistochemical Staining

The paraffin sections that have been cut will be baked for 2h, dewaxed, hydrated, antigenic repaired, endogenous peroxidase blocking, sealed, and then labeled with anti-TTF, anti-Napsin A, anti-CK5/6, anti-P40 and anti-P63 respectively according to the protocol. Incubate at room temperature for 30 to 60 minutest, and soak in PBS or TBS for 15 minutes.

Goat anti-rabbit IgG is incubated with secondary antibody at 25℃ for 1h, and soak in PBS or TBS for 15 minutes. DAB and H&E staining procedures are performed according to the protocol.

2.6 Staining Evaluation and Interpretation (Cintrón, Martínez, Jusino, Conte-Miller, Mendoza 2021)

Screen entire slides under light microscope to find areas with brown signal (positive expression) in DAB and H&E sections. For TTF-1, P40 and P63, successful staining pattern is nuclear staining. For Napsin A and CK5/6, successful staining pattern is membrane labeling. Control slides are used to delete the effect of nonspecific signal and background from positive signal.

Using Image-pro-plus (IPP) to select area of interesting (AOI) on the picture, measure the integral optical density (IOD) of this area, select and measure effective statistical area, and calculate IOD/area (mean density).

2.7 Data Processing and Analysis

All final data are statistically analyzed using SPSS 20.0.

(1) The survival of mice is analyzed by Kaplan-Meier survival curve;

(2) Collect IOD/area values of TTF-1, Napsin A, CK5/6, P40 and P63 of mice in each group, all data are expressed as mean ± standard deviation ($\overline{x} \pm s$), and variance between different groups are analyzed by student t test (α=0.05).

3 POSSIBLE RESULTS

3.1 Possible Result 1

The number and average size of tumors in lung tissue and IOD values of TTF-1, Napsin A, CK5/6, P40 and P63 are all positively related to the smoking temperature, while the survival rate of mice is negatively related to the smoking temperature (The slope of the Kaplan-Meier estimator is negative, and decreases as the temperature increases).

Table 1 shows that the number and average size of tumors in lung tissue and IOD values of TTF-1, Napsin A, CK5/6, P40 and P63 are all positively related to the smoking temperature, while the survival rate of mice is negatively related to the smoking temperature (The slope of the Kaplan-Meier estimator is negative, and decreases as the temperature increases).

Table 1: Possible Result 1.

	Experimental Groups (smoke exposure)						Positive Control	Negative Control
	200℃	300℃	400℃	500℃	600℃	700℃	Radon exposure	no exposure
number of tumors	+	+	++	++	++	+++	+++	-
size of tumors	+	+	++	++	++	+++	+++	-
IOD	+	+	++	++	++	+++	+++	-
survival rate	-	-	--	--	--	---	---	+

Note. In all the following tables, "+" represents a larger numerical value of the variables, while "-" represents a smaller survival rate compared with the negative control group. IOD represents the IOD mean values (density) of TTF-1, Napsin A, CK5/6, P40 and P63.

3.2 Possible Result 2

Table 2 shows that the number and average size of tumors in lung tissue and IOD values of TTF-1, Napsin A, CK5/6, P40 and P63 are all negatively related to the smoking temperature, while the survival rate of mice is positively related to the smoking temperature (The slope of the Kaplan-Meier estimator is negative, and increases as the temperature increases).

Table 2: Possible Result 2.

	Experimental Groups (smoke exposure)						Positive Control	Negative Control
	200℃	300℃	400℃	500℃	600℃	700℃	Radon exposure	no exposure
number of tumors	+++	++	++	++	+	+	+++	-
size of tumors	+++	++	++	++	+	+	+++	-
IOD	+++	++	++	++	+	+	+++	-
survival rate	---	--	--	--	-	-	---	+

3.3 Possible Result 3

Table 3 shows that the number of tumors in lung tissue and the IOD values of TTF-1, Napsin A, CK5/6, P40 and P63 are positively related to related to the smoking temperature. However, the size of tumors is negatively related to the smoking temperature, while the survival rate of mice is positively related to the smoking temperature (The slope of the Kaplan-Meier estimator is negative, and increases as the temperature increases).

Table 3: Possible Result 3.

	Experimental Groups (smoke exposure)						Positive Control	Negative Control
	200℃	300℃	400℃	500℃	600℃	700℃	Radon exposure	no exposure
number of tumors	+	+	++	++	++	+++	+++	-
size of tumors	+++	++	++	++	+	+	+++	-
IOD	+	+	+	++	++	+++	+++	-
survival rate	---	--	--	--	-	-	---	+

3.4 Possible Result 4

Table 4 shows that the number and average size of tumors in lung tissue and IOD values of TTF-1, Napsin A, CK5/6, P40 and P63 always have the reverse relationship with the smoking temperature compared with that of the survival rate of mice. Their values reach peaks or valleys at the same interval within the temperature zone, and fall or rise when approaching the two extreme points of the temperature zone.

Table 4: Possible Result 4.

	Experimental Groups (smoke exposure)						Positive Control	Negative Control
	200℃	300℃	400℃	500℃	600℃	700℃	Radon exposure	no exposure
number of tumors	+	+	++	+++	++	+	+++	-
size of tumors	+	+	++	+++	++	+	+++	-
IOD	+	+	++	+++	++	+	+++	-
survival rate	-	-	--	---	--	-	---	+

4 DISCUSSIONS

4.1 Possible Result 1

The number and average size of tumors in lung tissue and IOD values of TTF-1, Napsin A, CK5/6, p40 and P63 are all positively related to the smoking temperature whilst the survival rate is negatively correlated

The result indicates that the combustion products at the highest temperature have most harmful effect on lung. In this study, we showed the first time that increasing temperature of smoking will increase frequency and severity of lung cancer. A similar conclusion was reached by Audriy Jebet, 2018, they have demonstrated that various masses of tobaccos from different cigarettes may yield different amounts of smoke condensate (tar) depending on the nature of tobacco, tobacco additives and tobacco growing conditions, we have verified that using immunohistochemical staining produces similar results. The experimental research results will hopefully serve as useful feedback information for improvements for contemporary cigarette.

4.2 Possible Result 2

The number and average size of tumors in lung tissue and IOD values of TTF-1, Napsin A, CK5/6, p40 and P63 are all negatively related to the smoking temperature whilst the survival rate is positively correlated

The results contradict the hypothesis, so the hypothesis does not hold, with lower temperature, the mice are more likely to survive. One limitation should be noted here, when mice are directly exposed under smoke exposure, the raised temperature may create other risk factor for mice to catch the cancer, or kill the mice directly which will affect the survival rate. However, this problem could be solved if we consider lowing the temperature of smokes simultaneously not change the composition of the smoke.

4.3 Possible Result 3

The number and average size of tumors in lung tissue and IOD values of TTF-1, Napsin A, CK5/6, p40 and P63 has no relationship with the survival rate of the mice and burning temperature of smoking.

The results are only partially support the hypothesis, the number of the tumor and IOD values of TTF-1, Napsin A, CK5/6, p40 and P63 are positively correlated with the smoking temperature, whilst the size of tumor and the survival rate are negatively correlated. The first possible reason for this contradiction is the temperature has no relationship with all these factors. To prove this point of view, the same series of experiment need to be settled for three more times, if the results are same as before, the supposition is being denied. Second possible cause is there are operate misses during the experiment. Repeat the trial and strictly follow every step on instruction might change the results or deny the hypothesis.

4.4 Possible Result 4

The number and average size of tumors in lung tissue and IOD values of TTF-1, Napsin A, CK5/6, p40 and P63 reached maximum value at 500℃ then the value decreases.

This experiment cannot prove the validity of the hypothesis, but provided temperature intervals where the chance to catch the cancer is the highest. One major reason for this phenomenon might be there is an optimum temperature for tobacco to release the maximum volume of combustion products. Further study like repeat the trial using 425, 450, 500, 525,550,575℃ to find the temperature for the top risk for the maximum number and the largest size of tumors with the highest IOD value and lowest survival rate. Hence, our novel findings may provide new insights into control the temperature of smoking to lower the risk of having cancer.

5 CONCLUSIONS

The possible result 1 can confirm that the higher the burning temperature of the tobaccos, the greater the possibilities for smokers to get cancer, and the possible result 2 proved that these two factors are negatively correlated, and then for the possible result 3, the temperature only influence the size of tumor while the survival rate will be higher with the increasing of the temperature. Possible results 4 provided a range of temperature for the highest cancer mortality, which may give a feasible method to decrease the incidence of cancer among the smokers worldwide.

This experiment considered temperature as a variable factor, whereas under the limited laboratory facilities, only some reasonable possibilities can be provided. If the experiment can be further improved and implemented into the temperature control of e-cigarette, the e-cigarette would cease ignition to decrease the release of nicotine when it reaches the most harmful temperature, and the whole process is supposed to connect with terminal monitoring program. This invention can become a new achievement in the field of health management, and decrease the cancer rate among the smokers worldwide.

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