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The Study of Radon α Particles Exposure in Mice and the Mechanism of the Lung Cancer Caused by Radon Which Increase the Protein of RAGE and S100A6 in the Tissue in Bronchial

Yujun Chen

The King's Academy, Sunnyvale 94085, United States.

Abstract

Previous studies have proved that radon has certain damage to DNA. In this study, through the next-generation sequencing, two-dimensional gel electrophoresis and analysis image, western blot, and chest radiograph to find out the relationship between radon exposure and lung cancer. According to the other research, S100A6 and RAGE both are cancer-related protein, so can explain to the influence of radon exposure to lung cancer. If exposure under radon with high concentration, it may cause lung cancer by the mechanism of when it decays to emit high-energy flourishing α particles to the lower respiratory tract, and Long-term retention there cause damage and even malignant transformation of locally exposed bronchial epithelial cells, ultimately cause the lung cancer.

Keywords

Radon α particles, Lung Cancer, RAGE and S100A6.

1. INTRODUCTION

Radon is a chemical element, the symbol Rn and atomic number 86, which belong to the noble gas group. Radon is a naturally radioactive soil gas that commonly came out from radium, thorium, and uranium in soils and rocks. On account of Radon is a colorless, odorless and tasteless gas, it is hard to detect in human life [1]. However, Radon is really common in human life, this component can be emitted from earth and stone, then influence human health.

Radon itself is harmless, but when Radon decays to emit high-energy flourishing α particles, it is one of the most harmful substance to human body. Furthermore, Radon's concentration is the key to determining if damage to humans. In the open environment, it has low concentration in air so Radon gas is not enough to harm human health. However, in a confined space, such as inside a house, high concentration of radon will be present, causing harm to human body.

In addition, according to United States Environmental Protection Agency (EPA) estimates, radon is the second leading cause of lung cancer (LC) in United States [2]. Furthermore, international research about examined the data on 68,000 underground miners who were exposed to a wide range of radon levels led by National Cancer Institute(NCI), they examine the relationship of radon exposure and human LC is real [3]. Moreover, the well-established association of the genomic region 15q25 to lung cancer might be influenced by exposure to radon among uranium miners [4].

One of the important protein in the lung cancer is RAGE. On account of receptors for advanced glycation end-products (RAGE) is a type of inflammatory gene and it is a multi-ligand cell surface receptors really common express in lung cancer. Therefore, according to the experiment about the influence of nicotine to the lung cancer, the result shows the expression of RAGE in the lung cancer mice have increased. [5].

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In the research of LC, the bronchial epithelial cell is lining most of the respiratory tract as respiratory mucosa, and at the beginning of the respiratory cycle. It also has a substantial barrier function for the toxin and allergens into the lung [6]. Therefore, choose the bronchial epithelial cell as the subject to discover the influence of Radon to LC.

Therefore, this study does research on the relationship between Radon and LC. In this study we used the in vivo study of mice, Two-dimensional gel electrophoresis and western blot to analysis the main protein in the cell. This paper report when Radon decays to emit high-energy flourishing α particles to the lower respiratory tract, and how does Radon causing the gene variants, and the mechanism about how does the gene variants damaged bronchial epithelial cells, thus cause the LC.

2. MATERIALS AND METHODS

2.1. Exposure Conditions, and Animals

2.1.1 Exposure Conditions

Radon chamber (made by Dong-hua University, China), at a concentration of 10,000 Bq/m3, 12 h/d for up to cumulative doses of 100, 200, or 400 work level months (WLM), respectively [7].

2.1.2 Animals

Forty healthy male Mus musculus (house mice) will be divided into three experimental groups randomly (inhale radon gas) and set up one control group (inhale oxygen gas without radon), each group has ten mice.

2.1.3 Positive Control Group animals

Prepared ten healthy male Mus muscles (house mice) put in the experiment box, which full fide the high concentration nicotine gas. Then to make sure some of the mice in these ten get lung cancer.

2.2. The in Vivo Study

2.2.1 Next-generation Sequencing

Take all the forty healthy mice DNA sample, use the new method of next-generation sequencing to test DNA sequencing. This is the high-throughput method for evaluation of tri-nucleotide mutation patterns generated by chemical exposures, and link with cancer. Furthermore, it can get at least more than one result about the mutation in the DNA, so is improve the efficient of the lab.

2.2.2 Radon Exposure

Put three experimental groups randomly in radon chamber which a concentration of 10,000 Bq/m3, 12 h/d for up to cumulative doses of 100, 200, or 400 work level months (WLM) three different concentration of radon. The concentration controlled by computer. And then put the control group in the normal chamber which only have oxygen. Food and water were available ad libitum during exposure. All the mice stay in their chamber for 14 days.

2.2.3 Next-generation Sequencing 2

After accepted radon exposure, detect DNA of all the mice in this experiment, using the Next-generation Sequencing method (repeat step 2.2.1) to find out if mice's DNA changed or mutated. Record all the data.

2.3. Distinguish Main Protein in the Mutation

2.3.1 Preparing test samples [7]

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Mice anesthetize by chloral hydrate at 1ml per 100g weight and use tracheal lavage to "wash" mice's tracheal. Furthermore, extract protein from the bronchial of mice. The tissue need chill with liquid nitrogen and grind the small tissue. Then dilute supernatant with the lysate (2 M thiourea [Amersco, USA], 4% w/v CHAPS [Bio-Rad, USA], M urea [BBI, USA], 65 mM dithiothreitol [DTT; BBI, USA], 40 mM Tris [BBI, USA], 1 mM phenylmethylsulfonyl fluoride [PMSF; BBI, USA], and 0.5% IPG buffer [Amersham Biosciences, USA]) and incubate 20 mint in room temperature.

2.3.2 Two-dimensional gel electrophoresis and Analysis image

In the first dimension, mix the ten samples equally which get from one group of experiment group and use the IPG strip from 3-11, to separate each protein. Using a plastic container and laying the pH strip in it, then pour the mixing sample on the strip. Repeat this step for other two experiment groups samples and one control group sample. Then wait until each protein line show up on the strip. In the second dimension, put this strip vertically on the SDS-PAGE to test the weight of each protein. The gel was stained with Coomassie brilliant blue R250 (Roxo-Rosa et al., 2006) wait around 2.5 hours and scanned with an Charged camera systems (ChemiDoc™ MP) to determine the moleculer weight, pl, and presence/absence and up or down regulation of proteins. The digital image was analyzed with the ImageMaster 2D Platinum 5.0 software (Amersham Biosciences, USA). The software can storage and structuring large amount of data collected from 2D image. Then use the EXQuest™ spot cutter (Bio-Rad, USA) to accurately locates and excises protein bands or spots from 1-D and 2-D gels or blots. It then loads them into 96- and 384-well micro plates or 96-tube racks for downstream processing and analysis [7].

2.3.2 Database search

According to the Two-dimensional gel electrophoresis result, using the protein's pH scale and weight to distinguish what that protein is through the NCBInr database.

2.3.3 Western Blot

The cassette opened by opening key and laying in the tray with blotting buffer for 20 minutes on the rocking platform. Then pre-soaked fiber path with blotting buffer and lay fiber path on the gel holder (on the black plastic). Next, lay the blotting paper on the top of fiber path without bubble between those two material. Then lay the blotting gel on the top of blotting and take out the nitrocellulose membrane make it wet first and lay it on the gel. The last is putting another fiber path and hold the gel holder. Then insert the gel holder into SDS-PAGE machine, and waiting around two and half hours. Next, take out the gel holder and lay in the container full of blotting buffer. Then carefully take out the gel membrane and immerse membrane and put it in a container with 25 milliliters of blocking solution and incubated 15-20 minutes at room temperature. Next is pour out blocking solution and then add 10 milliliters primary antibody incubate 15-20 minutes pour it and and add the washing buffer. Then, add the 10 milliliters second antibody as same as the primary antibody steps. At the end, use the substrate to wash the membrane.

2.4. Chest Radiograph

Mice anesthetize by chloral hydrate at 1ml per 100g weight, and lay each mice on the Chest radiograph machine to take the image.

2.5. Positive Control Group Data

Choose all the mice who get the lung cancer from the nicotine inhale group, repeat the step Next-generation Sequencing and Western Blot to detect the amount of RAGE in the mice who have lung cancer.

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3. POSSIBLE RESULTS

3.1. Result 1: Radon Cause the Mutation in DNA and Lung Cancer Correlated with Proteins RAGE and S100A6 in Bronchial Tissue after Radon Exposure

3.1.1 DNA sequence (before and after exposure)

Through the method of NGS, know the sequence of mice DNA before and after expose under the radon chamber. An experiment group in the highest concentration radon chamber, it ten out of ten mice, has the mutation of their DNA. An experiment group which in the lowest concentration radon chamber have eight out of ten mice have the mutation on their DNA. And the control group which has eight out of ten mice have the mutation. Each mutation happens in mice there are more than thousands change in mice's DNA, especially the mice in the highest concentration radon room. Although the number of mice gets the mutation as same as the lowest concentration experiment group, the number of mutation happen in DNA the experiment group much more than the control group.

3.1.2 Distinguish the Specific Protein

3.1.2.1 2-DE images

There are four 2-DE images, in total with 500-600 protein spot on the images. In those 500-600 protein spot, there are around 9 up-regulated spots and 6 down regulate spots.

3.1.2.2 Identification of Protein

Among the 15 unregulated protein spots, 9 up-regulated were identified as zinc, α-enolase, tubulin β, heat-shock protein 27 (HSP27), β-actin, thioredoxin (TRX), receptor for advanced glaciation end products (RAGE), S100A6, and annexinA2, and 6 down regulated spots were cytochrome c oxidase, serine proteinase inhibitor, γ-actin, aldehyde dehydrogenase (ALDH), myosin light polypeptide 4, and LOC500450 protein [7].

3.1.3 Western Blotting

Through western blotting to analysis the all the 15 up regulate and down regulate spots, discover the amount of RAGE and S100A6 are much more then other proteins in the radon-inhaled group. And these two expression patter are consistent with the result show in the 2-DE image.

Fig 1. Through western blotting confirm the amount of RAGE and S100A6 exist in mice much more than the common protein GAPDH

3.1.4 Chest radiograph

At a quantity of 100 WLM radon exposure mice group, there is 1 out of 10 mice lung has shadow in the chest radiograph image. At a quantity of 200 WLM radon exposure mice group, there are 2 out of 10 mice lungs have shadow in the chest radiograph image. At a quantity of 400 WLM radon exposure radon exposure mice group, there are 5 out of ten mice lungs have shadow in the chest radiograph image. In the control group, non-radon inhale mice, there is 1 mice lung have shadow in the chest radiograph image.

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3.1.5 Positive Control Group

In the group of nicotine inhale mice, all the mice which get the lung cancer their DNA have the mutation and each mutation happens in mice there are more than thousands change in mice's DNA. And expression of RAGE are increased and much more than GAPDH in mice [5].

3.2. Result 2: Radon Cause the Mutation in DNA and Lung Cancer, Which Have Not Correlation with Specific Proteins

3.2.1 DNA sequence (before and after exposure)

Through the method of Sanger sequencing, know the sequence of mice DNA before and after expose under the radon chamber. An experiment group in the highest concentration radon chamber, it eight out of ten mice have the mutation of their DNA. An experiment group that in the lowest concentration radon chamber has eight out of ten mice have the mutation on their DNA. And the control group which have eight out of ten mice have the mutation. Furthermore, all the mutation mice have the similar number of mutations in their DNA.

3.2.2 Distinguish the Specific Protein

3.1.2.1 2-DE images

The experiment results are as same as 3.1.2.1.

3.2.2.2 Identification of Protein

The experiment results are as same as 3.1.2.2.

3.2.3 Western Blotting

Through western blotting to analysis the all the 15 up regulate and down regulate spots, discover all the protein have the similar amount both in the radon-inhaled group and oxygen-inhale group. Also, those similar amount mean all of those protein expression are increased.

3.2.4 Chest radiograph

At a quantity of 100 WLM radon exposure mice group, there is 1 out of 10 mice lung has shadow in the chest radiograph image. At a quantity of 200 WLM radon exposure mice group, there are 2 out of 10 mice lungs have shadow in the chest radiograph image. At a quantity of 400 WLM radon exposure radon exposure mice group, there are 1 out of ten mice lungs have shadow in the chest radiograph image. In the control group, non-radon inhale mice, there is 2 mice lung have shadow in the chest radiograph image.

3.2.5 Positive Control Group

The experiment result as same as 3.1.5

3.3. Possible Results Summery

Possible Result #	In Vivo Study	2-D electrophoresis	Western Blot	Chest Radiograph
1	Experiment Group Mutation > Control Group Mutation	15 unregulated protein spots	Only the expression of RAGE and S100A6 increased much more than common amount in the radon-inhaled groups. The control group keep in regular range	Higher concentration of radon inhale have higher percentage have the shadow in the lungs
2	Experiment Group Mutation ≥ Control Group Mutation	15 unregulated protein spots	All of those protein still in the normal amount of expression in both control and experiment groups	The percentage of mice have shadow in their lung is the radon inhale group and the control group are similar

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4. DISCUSSIONS

Lung cancer has a complicate disease mechanism, according to the previous study has reported the mutation on former uranium miners because of the radon exposure. In this report, the possible results give the two results of the experiment. On account of the mutation will happen anywhere and anytime, the mutation happen on mice is not the surprise event. The mutation happens most in the radon inhale groups and also sometimes accompany with the RAGE and S100A6 appearance. Therefore, infer the unregulated protein have the connection with DNA mutation in mice.

The positive control group has the reliable results get from other research paper and experiment, which shows having lung cancer mice caused by nicotine inhale the most obvious change in their protein is RAGE. And the study also discover that lead to mice incremental dilation of alveolar spaces through the x-ray. Therefore, we have the compared sample to define if the lung cancer happen in our experiment groups.

For the possible result one, compare with the control group the amount of DNA mutation is much more than common ratio. And the nicotine inhale group as the positive control group also has the similar amount of DNA mutation with experiment groups. Furthermore, based on the 2-DE images result know there are 6 up regulated proteins and 9 down regulated proteins. The expression in those 15 unregulated proteins RAGE and S100A6 have uncommon increase both on experiment groups and positive control groups. Therefore, it shows the correlation between the lung cancer and RAGE and S100A6, because positive control group already make sure the lung cancer happen on those mice and compare that with the experiment group will know the relation between those are the increase of expression both on RAGE and S100A6.

For the possible result two, the mutation of mice's DNA also happened, but in experiment groups still much more than in the control group. Therefore, through the result get from the Next-generation Sequencing, radon to human health have some influence but not that much. Because DNA mutation happen at any time, and then we cannot to judge radon is the only reason to cause the mutation. Furthermore, because of the western blotting result show there are no specific unregulated protein have any tend to increase or decrease amount compare with normal level. Moreover, positive control group have obvious increase expression of RAGE and S100A6 then cause the lung cancer, and in the possible results two the mice have lung shadow in the radiograph no more than twenty percent. Then can infer radon-inhale will influence DNA mutation and cause some genetic defect, but have not strong evidence to prove that will cause lung cancer.

Through analysis more than thousands mutation in the DNA that happen in three experiment groups and one control group mice. The control group mice have the mutation obviously less than any mice received the radon gas exposure. In a large database, we selected the mice with the most similar DNA mutations from each experimental and control groups. Although the variation in their DNA was roughly the same, through western blotting test the mice's bronchial tissue and showed that the highest concentration of exposure mice had the greatest increases in RAGE and S100A6. Therefore, according to the possible result, we speculate that on account of radon cause DNA mutation and also cause RAGE and S100A6 increased beyond the norm standard are the important reason cause lung cancer. Furthermore, these two protein can as the signal to define the beginning of the LC, on account of the more mice lungs have shadow in their chest radiograph in the radon-inhale groups than the oxygen-inhale group have.

5. CONCLUSION

Our study investigates the Radon effect on the mice especially on their lung and the correlation protein with lung cancer. Through analysis the DNA sequence and the 2-DE images,

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we find out the AGE and S100A6 there two proteins are the potential effect on the lung cancer. And these two could as the signal to detect if lung cancer appear in patient's body. However, regardless of the experiment results, further investigations are required to study if other unregulated protein are correlate with cancer and if radon also influence other organ and cause the cancer. Furthermore, through the specific step to know in which stage of the lung cancer the expression RAGE and S100A6 will increase more than regular amount and until which amount of radon inhale will cause mice will get the a mild symptom of lung cancer. Through this experiment result, also can do the further study about if RAGE and S100A6 exist in other kind of cancer and illness.

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