The Ageing Lung:

Investigating telomere-associated damage in response to cigarette smoke exposure and in chronic lung disease.

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Introduction & Aims

- Telomeres are repeated structures of the DNA formed by the bases TTAGGG at the end of chromosomes.
- Telomeres are covered in a group of proteins called shelterin which act as a cap to protect the DNA from damage.
- When the shelterin covering the telomeres becomes detached, telomeres are exposed or “uncapped” which activates the DNA damage response (DDR).
- Exposed telomeres become co-localised with DDR factors such as a marked histone protein called γH2A.X (DNA normally wraps around histone proteins in order to stabilise their structure).
- When γH2A.X is located at a telomere it indicates damage and can be indicative of senescence otherwise known as biological ageing.
- We know that cigarette smoke causes damage to telomeres. The first aim of this research was to find out whether the damage was reparable in cells and lung tissue.
- The second aim was to find out whether telomere damage was increased in patients with adult-onset asthma.

Keywords

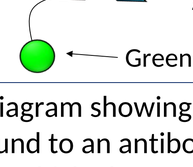
- **Telomere**: repeated structure at the end of chromosomes
- **Foci**: sites of DNA damage (ie. γH2A.X)
- **Telomere associated foci (TAF)**: when DNA damage is located at a telomere
- **Immuno-FISH**: way of staining cells with fluorescence in order to see DNA, telomeres and damage
- **Senescence**: deterioration of cells which leads to biological ageing

Methods

Experiment 1: Mice were exposed to cigarette smoke for 48 hours and then allowed to recover for either 24 hours or 7 days before lung tissue was extracted. This tissue was compared to lung tissue of control mice which had not been exposed to smoke. In order to measure DNA damage at telomeres, tissue was stained by the immuno-fluorescence *in situ* hybridisation (immuno-FISH) method, outlined below, and then imaged with a fluorescence microscope.

Experiment 2: Lung cells grown in culture were exposed to 15% cigarette smoke and then allowed to recover for either 24 hours, 4 or 7 days. These cells were compared to control cells which had not been exposed to smoke. The cells were then stained by the immuno-FISH method to measure DNA damage at telomeres.

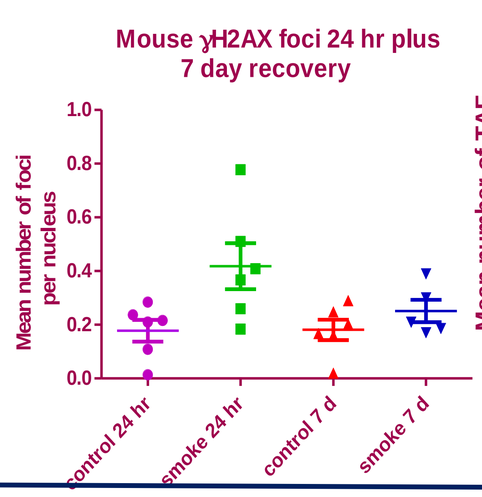
Experiment 3: Lung tissue from human patients with adult-onset asthma was extracted during lung surgery and then stained by the immuno-FISH method in order to find out whether telomere damage was increased in patients with adult-onset asthma. The asthma tissue was compared to lung tissue of patients without asthma.

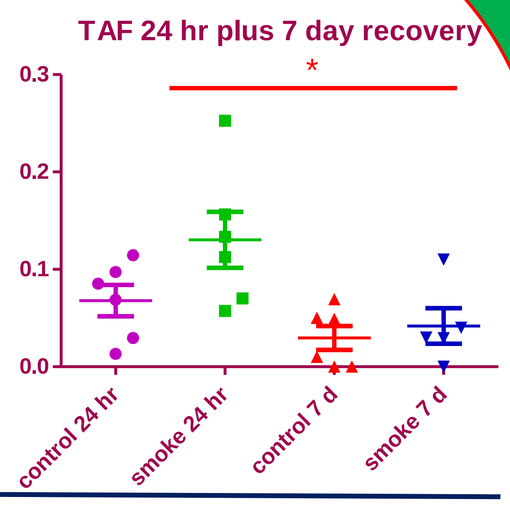


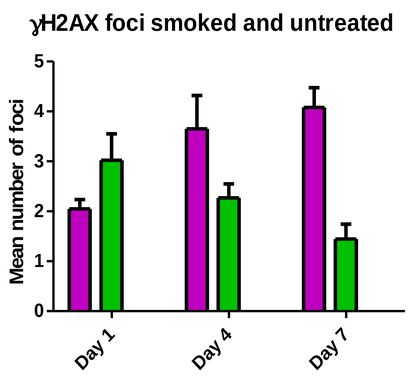
Results

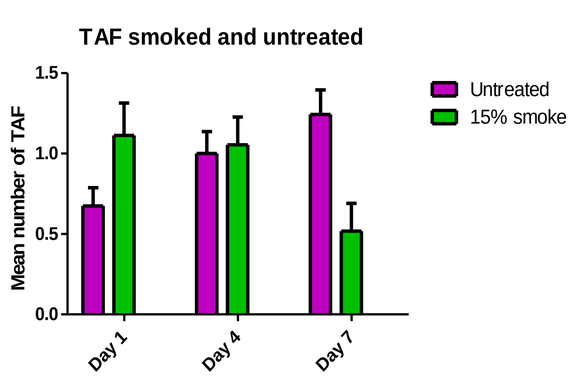
Experiment 1

The first graph shows the γH2A.X foci (DNA damage) in the lung tissue of mice exposed to cigarette smoke and their controls. They were allowed to recover for 24 hours or 7 days before tissue was extracted. It shows an increase in damage in smoke exposed mice compared to controls and a decrease in DNA damage when mice are allowed to recover for 7 days. However the decrease isn’t statistically significant. The second graph shows the frequency of DNA damage co-localised at telomeres in mouse lung tissue. It shows an increase in telomere associated foci (TAF) and then a statistically significant decrease (shown by the red asterisk and line) in TAF when mice are allowed 7 days to recover.







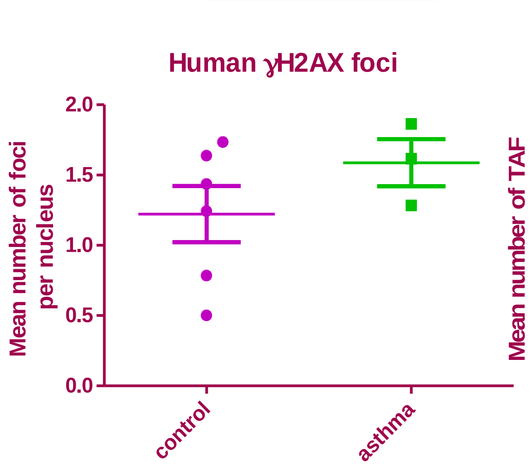


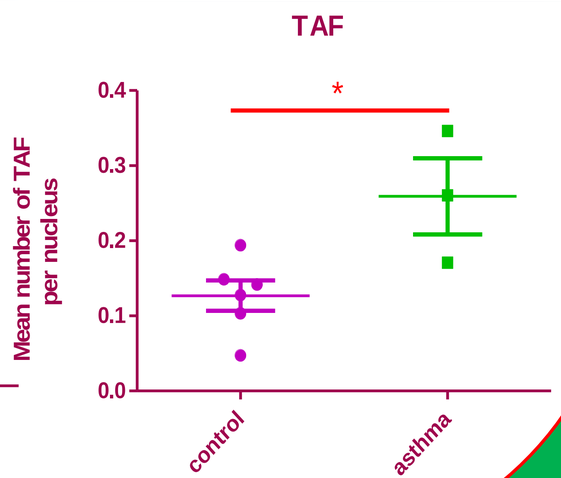
Experiment 2

The first graph shows DNA damage foci in control lung cells and lung cells exposed to 15% smoke. The cells were left to recover for either 1, 4 or 7 days before damage was counted. It shows a decrease in DNA damage when cells were allowed longer to recover. The second graph shows TAF in controls and lung cells exposed to 15% smoke after recovery times of either 1, 4 or 7 days. The graph shows a slight decrease of TAF between 1 an 4 days recovery an then a large decrease with 7 days recovery. However the results were inconclusive as the control cells showed a lot of variability and the TAF results of 1 and 4 days were not significant.

Experiment 3

The first graph shows DNA damage in lung tissue of human patients with adult-onset asthma as well as tissue from control patients without asthma. It shows a slight insignificant increase in DNA damage. The second graph shows DNA damage associated with telomeres in the lung tissue of patients with adult-onset asthma as well as controls. The results show a statistically significant increase in telomere associated damage in the tissue of patients with asthma compared to the control lung tissue (shown by the red asterisk and line).





Immuno-FISH Technique

Specific antibodies which are conjugated to a marker are applied to the tissue or cells and bind exclusively to either telomeres or DNA damage (Figure 1). When specific light is shone on to the tissue, the marker on the antibody emits a colour. DNA damage lights up green and telomeres appear red (Figure 2). When the telomere and damage co-localise it is called telomere associated foci (TAF) and indicates senescence.

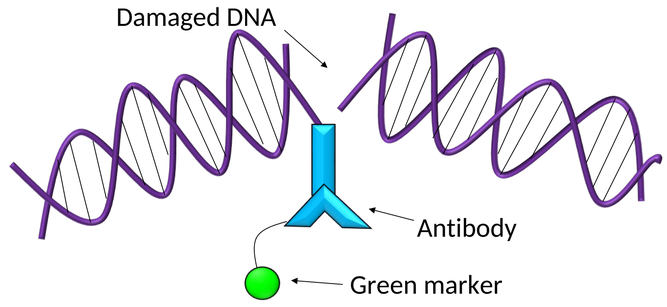


Figure 1: Diagram showing damaged DNA strands bound to an antibody conjugated to a marker which glows green under certain light.

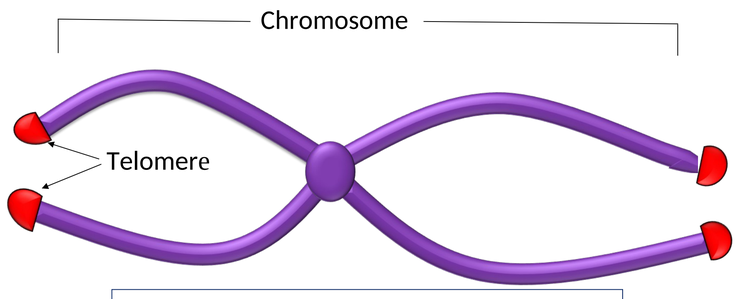


Figure 2: Diagram showing a chromosome (purple) with telomeres at the end. When antibody binds telomeres, the marker glows red under certain light.

Conclusions

- In experiment 1 telomere damage appeared to decrease when the mice were allowed time to recover indicating a cellular repair mechanism which fixes DNA damage before the cells enter senescence.
- Experiment 2 showed inconclusive results and needs further investigation in order to determine whether cultured cells show telomere repair when allowed recovery time.
- Experiment 3 showed that lungs of patients with adult-onset asthma had increased telomeric damage indicating that lung tissue may enter senescence when they lungs become asthmatic. However this pilot data only includes 3 samples of asthmatic tissue meaning that further investigation is needed to confirm this.

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