Evaluation of Nitric Oxide Synthase Expression in Healthy and Fibrotic Human Lung Cells

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Pictured above are healthy lung cells isolated from a 17 year old male
Have you ever wondered how a cut or scrape heals itself?

Shown below is a picture of an open wound. When a wound begins to heal, cells beneath the skin that contain muscle fibers start pulling together to contract the tissues beneath the scab.

http://www.bolandcell.co.za/MYOFIBROBLAST.html
What can go wrong?

Fibrosis: When specialized cells called myofibroblasts do not stop working once a wound is healed.

- The picture on the right shows how a normal fibroblast (#1), always present in the tissues, becomes a myofibroblast (#3), a specialized cell necessary for a wound to contract and heal.
- The problem begins when the healing is complete and the myofibroblasts do not die or return to the fibroblast state (#1).
- This can occur in many tissues including lung, liver, heart, and kidney creating scarring.

What is IPF?

The Sharma Lab is concerned with what causes Idiopathic Pulmonary Fibrosis (IPF).

- IPF is scarring of the lung tissues.
- This disease causes loss of ability to receive oxygen.
- IPF also can lead to other deadly symptoms such as: collapsed lung, lung infections, lung cancer, and blood clots in the lungs.

Why are we concerned?

- There is not a cure for this disease.
- People usually only live 3-5 years after diagnosis.
- Doctors do not know what causes IPF.

Our hypothesis is that the presence of Nitric Oxide (NO) impacts the progression of this incurable disease.

The Role of Nitric Oxide (NO)

• It has been shown that Nitric Oxide (NO) is implicated in the advancement of fibrosis at the molecular level.

• It has been speculated that NO leads to increased accumulation of myofibroblasts.

• Electrode-based analysis has been used previously to determine the amount of NO in cultured, rat lung myofibroblasts.

Nitric Oxide Synthase (NOS)

• A NO producing enzyme that can be monitored through measurement of emitted NO levels.
• The activity of the enzyme can be manipulated by using NOS inhibitors.
• There are 3 different forms of NOS. Only two of the three are normally expressed outside of the nervous system: iNOS and eNOS.
• We are looking for these two in lung cell extracts.

Experimental Approach

- We grow lung cells in culture.
- We collect the cells & lyse them to release the proteins.
- We perform immunoprecipitation, using antibodies to selectively concentrate the protein of interest.
- We run a gel using electrophoresis to separate the proteins by size.
- Next, we transfer the proteins from a gel onto a membrane.
- Then we use a technique called Western Blotting to verify the presence of a particular protein.
Western Blot

Western Blotting is used to detect proteins on the transferred membrane.

1. We block the membrane overnight to prevent non-specific interactions between the membrane and the antibody used to detect the protein.
2. A primary antibody is used first to bind to a protein of interest.
3. Then the secondary is applied. It should bind to one of the primary antibodies.
4. Last, a substrate is added onto the membrane after being washed.
5. A purple band indicates where the secondary antibody bound.

http://en.wikipedia.org/wiki/Western_blot
This is a Western Blot of samples from healthy lung cells using different antibodies for the immunoprecipitation.

The lanes contained the following:
1. Protein mass ladder
2. eNOS control
3. 0% whole cell lysate
4. eNOS IP 0%
5. iNOS IP 0%
6. uNOS IP 0%
7. 10% whole cell lysate
8. eNOS IP 10%
9. iNOS IP 10%
10. uNOS IP 10%

The eNOS antibody was used for western blotting.
What do these results tell us?

• Our goal was to see a band indicating that Nitric Oxide Synthase (NOS) is present. The molecular weight of NOS is between 130-150 kDa. On the previous slide, we see a band across the top that could possibly tell us that NOS is present!

• However, this was not always the case. We did not always see a band between 102-150 kDa. Instead, we only saw the smaller bands around 25-50 kDa, which told us that the secondary antibody was binding to something too small to be NOS and was probably antibody fragments left over from the immunoprecipitation.
Conclusion

• NOS does not appear to be present in the untreated healthy or diseased lung cells.
• We are currently treating the cells with growth factors to see if this increases the detectable levels of NOS.
• More experiments will be required to verify the conditions that allow us to detect NOS.

Picture shown is an example of myofibroblasts in culture.

Future Experiments

a) Run more samples of cells over a wider range of passage numbers.
b) Add growth factors to the culture media to see if changes in NOS expression levels are observed.
c) Perform immunocytochemistry to see if there are differences in the levels of characteristic muscle fibers such as actin and fibronectin.

Picture shown is a gel under the UV light.
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