Cylindroma to Spiradenoma: Is Telomerase Involved?

Newcastle University

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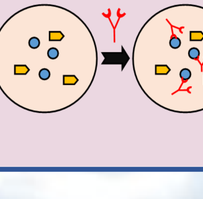
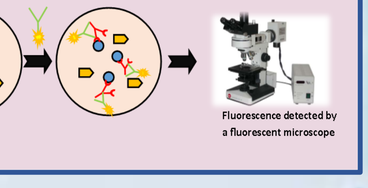
By: Lordi Anne Tickell **Email:** l.a.tickell@ncl.ac.uk **Supervisor:** Dr Gabriele Saretzki, Campus for Ageing and Vitality. **In collaboration with** Dr Neil Rajan, Institute of Genetic Medicine

Background

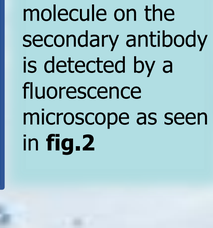
- A **mutation** in the **CYLD gene** results in a rare, benign and painless tumour called a **cylindroma**
- **Cylindroma** have been shown to progress into more painful tumours called **spiradenoma** (1)
- **Telomerase**, an enzyme which prevents chromosomal telomere shortening over time, has been implicated in the many properties of cancers (2)
    - Telomerase' 2 main components are **'TERC'** (an RNA template) and **'TERT'** (the catalytic component)



Scalp cylindroma (3)



Telomerase repairing a telomere



Cylindroma and spiradenoma (1)

Aim

- Determine whether **telomerase** is involved in the progression of **cylindroma** into **spiradenoma**

Methods

- **Immunofluorescence staining (IF)** of 8 tumour sections (**fig1, 2 and 3**), using antibodies that bind specifically to, and allow us to visualise the locations within a cell of:
    - **hTERT** (human TERT)
- **Telomeric Repeat Amplification Protocol (TRAP)** of tumour sections, to show levels of telomerase activity (**fig.2**)
- **Western Blot** to show if the antibody binds to its specific molecule (**fig.4**)

Figure 1. *IF Method*

The fluorescent molecule on the secondary antibody is detected by a fluorescence microscope as seen in **fig.2**

Results

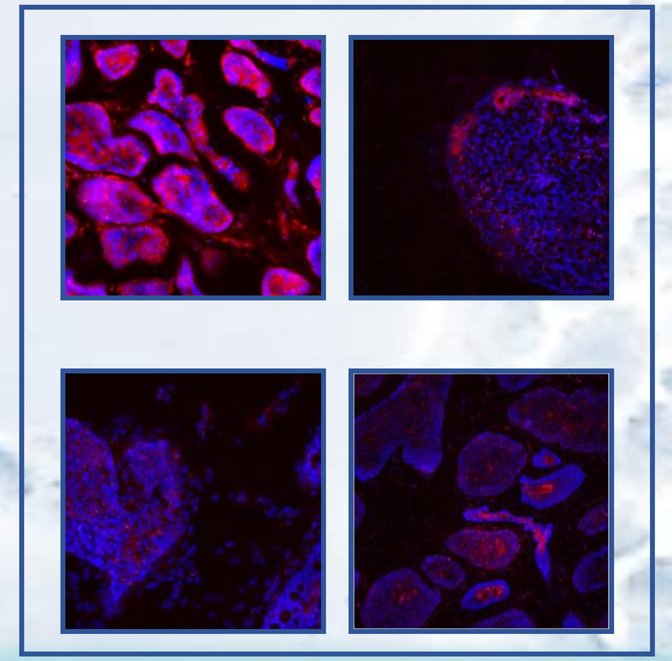


Figure 2. *Immunofluorescence Staining of tumour sections with hTERT antibody*

Initial staining showed **positive** results in most sections

hTERT is **red**. **Blue** is the **nuclear counterstain, dapi**

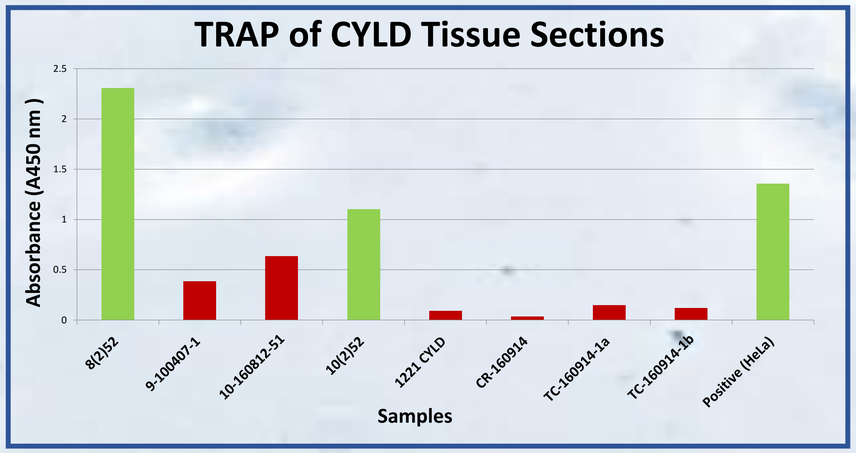


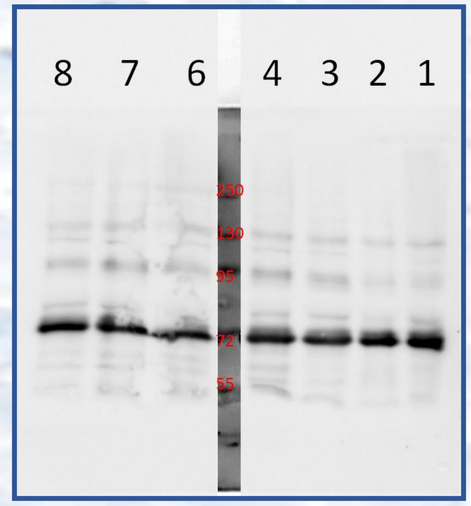
Figure 3. *TRAP assay of 8 tumour sections*

The majority of tumours had a **negative (red)** result except two, contrasting with the hTERT stains in **fig.2 (green)**. However, hTERT may be present in the cell but may not necessarily be active

Figure 4. *Western Blot to assess hTERT antibody specificity in tumour samples*

For hTERT, if specific, you would expect to see **one** dark band at **128 kDa** in each column.

Here the darkest bands are around **72 kDa**, with lots of other faint bands at different points. This shows that the hTERT antibody was **not specific**



Conclusions

Due to the short duration of the project, these experiments could not show us telomerase' role in the progression. However, the foundation for further study has been laid.

What Next?

- Identify an antibody that will bind **specifically** to hTERT
- A **double staining** of tumour sections using **anti-DKK2 antibody** – a way of quantifying which parts of a tumour are more cylindroma/spiradenoma (1) – and an anti-hTERT antbody.
- This will allow us to see if there is a difference in the level of hTERT in each tumour type.

Acknowledgements

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References

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3. Rajan, N., Ashworth, A. (2015). "Inherited cylindromas: lessons from a rare tumour." The Lancet Oncology 16(9): e460-e469