Cylindroma to Spiradenoma: Is Telomerase Involved?

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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)

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)

Results

![Image of immunofluorescence staining](Image)

**Figure 1.** IF Method

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

**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 antibody.
- This will allow us to see if there is a difference in the level of hTERT in each tumour type.

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References