ERBB3: A potential target for both early detection and treatment of Devil Facial Tumour 1 (DFT1)

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Abstract

Devil Facial Tumour 1 (DFT1) is one of two transmissible neoplasms of Tasmanian devils (*Sarcophilus harrisii*) predominantly affecting their facial regions. DFT1's cellular origin is that of Schwann cell lineage where lesions are evident macroscopically late in the disease. Conversely, the pre-clinical timeframe from cellular transmission to appearance of DFT1 remains uncertain demonstrating the importance of an effective pre-clinical biomarker. We show that ERBB3, a marker expressed normally by the developing neural crest and Schwann cells, is immunohistohemically expressed by DFT1, therefore the potential of ERBB3 as a biomarker was explored. Under the hypothesis that serum ERBB3 levels may increase as DFT1 invades local and distant tissues our pilot study determined serum ERBB3 levels in normal Tasmanian devils and Tasmanian devils with DFT1. Compared to the baseline serum ERBB3 levels in unaffected Tasmanian devils, Tasmanian devils with DFT1 showed significant elevation of serum ERBB3 levels. Interestingly Tasmanian devils with cutaneous lymphoma (CL) also showed elevation of serum ERBB3 levels when compared to the baseline serum levels of Tasmanian devils without DFT1. Thus, elevated serum ERBB3 levels in otherwise healthy looking devils could predict possible DFT1 or CL in captive or wild devil populations and would have implications on the management, welfare and survival of Tasmanian devils. ERBB3 is also a therapeutic target and therefore the potential exists to consider modes of administration that may eradicate DFT1 from the wild.

Introduction

Devil Facial Tumour 1 (DFT1) is one of two transmissible neoplasms of Tasmanian devils (*Sarcophilus harrisii*) predominantly affecting their facial regions. DFT1's cellular origin is that of Schwann cell lineage where lesions are evident macroscopically late in the disease. Conversely, the pre-clinical timeframe from cellular transmission to appearance of DFT1 remains uncertain demonstrating the importance of an effective pre-clinical biomarker.

ERBB3 is expressed in early embryonal development and plays an integral role in the development of the neural crest and Schwann cells. ERBB3 is one of four members of the Epidermal Growth Factor (EGF) family representing a complex group of type 1 transmembrane receptor tyrosine kinase (RTK) designated *EGFR/ERBB1/HER1, ERBB2/HER2, ERBB3/HER3* and *ERBB4/HER4*. ERBB3 is upregulated in a number of human cancers such as breast, colon, gastric, ovarian and prostate but seldom reported in animal cancers.

DFT1's expression of ERBB3 immunohistochemically expression led us to postulate that excess extracellular domain (ECD) may circulate in the host's plasma and present itself as a possible candidate biomarker for DFT1.

Methods

A pilot study of thirty-five Tasmanian devils including clinically healthy Tasmanian devils (CHD, n=15), clinically healthy devils with dermatopathy (CHDD, n=4), Tasmanian devils with clinical DFT1 (DFT1, n=8) and Tasmanian devils with cutaneous lymphoma (CL, n=12)

Briefly, paraffin embedded tissues were processed using standard Histological and Immunohistochemical methods using citrate buffer antigen retrieval, Biocare Medical universal HRP detection kit and monoclonal rabbit anti-human ERBB3 (Abcam, clone SP71, ab93739, 1:50). Serum ERBB3 levels were measured using the RayBio anti-human ERBB3 ELISA Kit according to the manufacturer's instructions. The ELISA standard curve was plotted using Prism v5 and results for each serum interpolated and corrected for dilution. The significance of differences in serum ERBB3 between groups was determined using a Kruskal-Wallis test with Dunn's Multiple Comparison utilizing Prism v5.

Results

DFT1 histology (Fig 1A) Haematoxylin and Eosin. ERBB3 IHC revealed moderate to strong expression in 75% of cells in all strains of DFT1 (Fig 1B). Higher magnification (Fig 1C). Devil skin and subcutis (Fig 1E), peripheral nerve was seldom positive for ERBB3 (red arrow) in keeping with downregulation of ERBB3 in the adult in contrast to DFT1 ERBB3 expression (black arrow). ERBB3 in Tasmanian devil lymphoid follicle (Fig 1F). Trigeminal nerve section (Fig 1I) showed ERBB3 expression in nerve bodies (black arrow) and occasional ERBB3 expression in the adaxonal area (red arrows). Positive control devil bowel (Fig 1G) and negative controls DFT1 (Fig 1D), bowel (Fig 1H) and Trigeminal nerve (Fig 1J).

Serum ERBB3 levels are shown in figure 2. Serum ERBB3 in the Fifteen Tasmanian devils without neoplasia ranged from <30-663 pg/ml with a median of 32 pg/mL (30 – 220; interquartile range). Serum ERBB3 levels in the eight Tasmanian devils (devils 16-23) with clinical DFT1 ranged from 766-18,254 pg/ml with median of 3051 pg/mL (1060 – 10879; interquartile range. In the twelve Tasmanian devils with cutaneous lymphoma (devils 24-35) serum ERBB3 levels ranged from <30-20,021 pg/ml with a median of 1485 pg/mL (289 – 7901; interquartile range). In summary, serum ERBB3 levels are elevated in devils with DFT1 and CL when compared to clinically healthy devils.

Discussion

ERBB3 had previously avoided scrutiny due to its kinase inactivity; however, ERBB3 has now been the subject of intense investigation over the past decade and recognised now as a potent partner of the epidermal growth receptor family. ERBB3 upregulation during developmental, dedifferentiation and regenerative processes encapsulates the Schwann cell's inherent plasticity and imparts certain characteristics of malignant transformation advantageous to transmission of DFT1. Our pilot study has shown for the first time that ERBB3 is consistently expressed immunohistochemically and that ERBB3 is also elevated in the serum of Tasmanian devils with advanced DFT1 and cutaneous lymphoma. Therefore, our research indicates that serum ERBB3 has the potential to be utilised as a biomarker of DFT1 or CL in Tasmanian devils to assist conservationists in the management and welfare of Tasmanian devils and species survival. The simplicity of the ELISA Serum ERBB3 methodology is easily incorporated into routine laboratory batch testing, equally it can be applied to include rapid turnaround of results for urgent cases. Extension of this research is necessary to include greater numbers of healthy Tasmanian devils both with and without visible injuries, devils with large and small DFT1 lesions as well as pre-clinical DFT1. This will firmly establish the normal reference range for serum ERBB3 from which potential pre-clinical DFT1 devils may be identified. In addition, because ERBB3 is now recognised as a therapeutic target, potential exists to consider modes of administration in addition to existing whole cell vaccination such as ERBB3 monoclonal antibody, peptide or xenogeneic vaccines including checkpoint inhibitors. A combinatorial immunotherapeutic approach will enhance cytotoxic destruction, provide long-term immunity from DFT1 and therefore eradicate this transmissible tumour from the wild

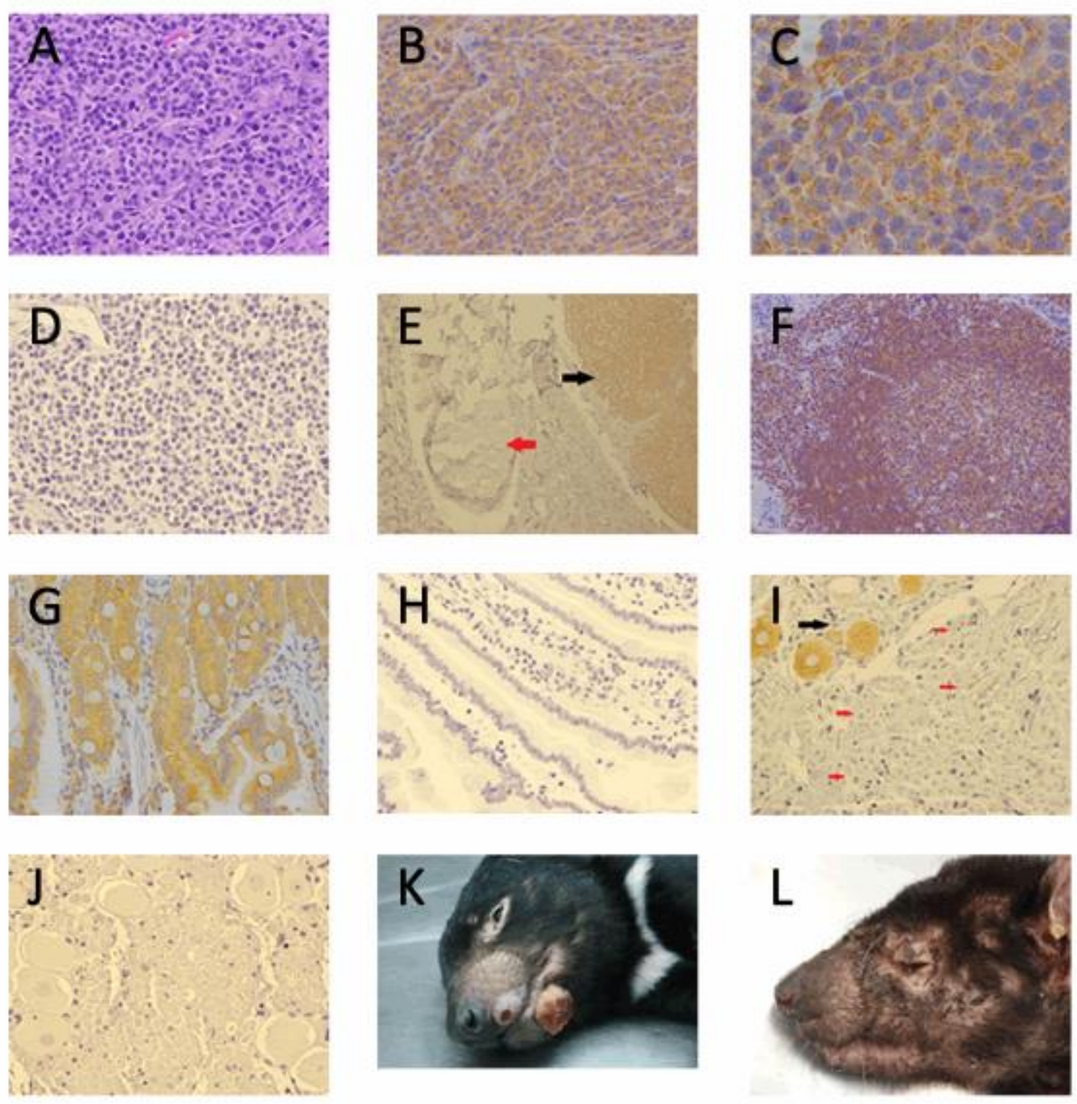

Figure 1. DFT1 staining and skin manifestation.
(A) Haematoxylin and Eosin stained DFT1 x40, (**B)** ERBB3 Immunohistochemical expression in DFT1 strain 3 x40, (**C)** ERBB3 immunohistochemical expression in DFT1 strain 3 x100, (**D)** DFT1 negative control, (**E)** Tasmanian devil skin and subcutis section with peripheral nerve (red arrow) and DFT1 (black arrow) x10, **(F)** Tasmanian devil lymph node ERBB3 expression lymphoid follicle x20, (**G)** Tasmanian devil bowel ERBB3 positive control x40, (**H)** ERBB3 IHC negative control bowel, (**I)** trigeminal nerve shows ERBB3 positive nerve body (black arrow) and occasional adaxonal ERBB3 positivity (red arrows) x40, (**J)** ERBB3 IHC negative control trigeminal nerve, (**K)** Tasmanian Devil gross appearance of DFT1. Photo credit: DPIPWE archive, (**L)** Tasmanian devil gross appearance cutaneous lymphoma. Photo credit DPIPWE archive.

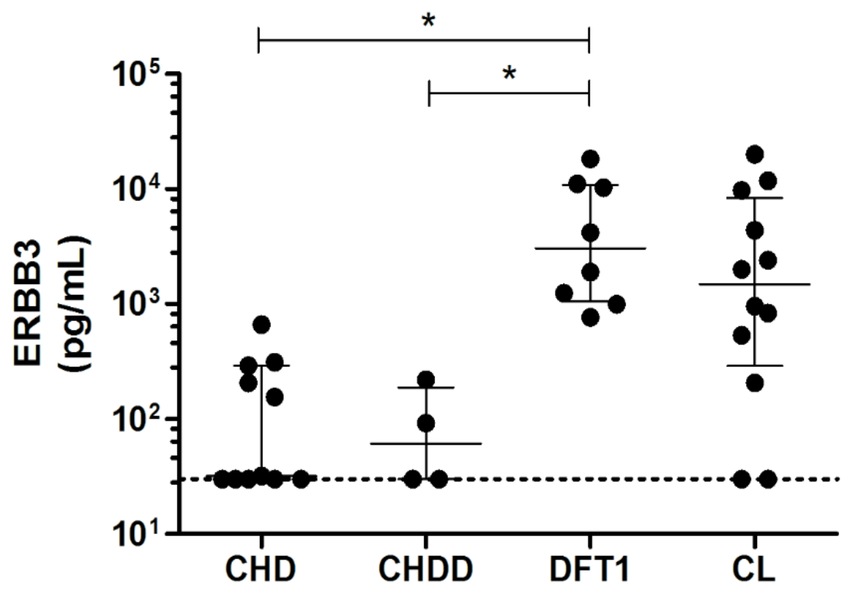

Figure 2. Serum ERBB3 levels in Tasmanian devils.
Serum ERBB3 levels were measured by ELISA in clinically healthy Tasmanian devils CHD (n=11), clinically healthy Tasmanian devils with dermatopathy CHDD (n=4), clinically diagnosed DFT1 (n=8) and those with cutaneous lymphoma CL (n=12). Horizontal dashed line indicates the limit of detection of the ELISA assay at 30 pg/mL. Results of individual devils are shown with the median and interquartile range identified by the whiskers. Significance testing using a Kruskal-Wallis test with Dunn's Multiple Comparison Testing shown with * representing $p < 0.05$.

Acknowledgements and References

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