Early Detection of Cancer Using Circulating Tumour DNA: Feasibility and Field Trial Using the Unique Tasmanian Devil Transmissible Cancer

Investigators

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Project

Summary

Circulating tumour DNA (ctDNA) has the potential to be used as a new type of early detection method for cancer, but the unique genomic changes in each cancer and the low levels of ctDNA in early cancer make this challenging to develop and assess. The goal of this project is to develop an assay for the detection of ctDNA in Tasmanian devils affected by Tasmanian devil facial tumour disease (DFTD), a clonally transmissible cancer spread by biting. As DFTD is a single clonal lineage, a ctDNA assay developed against common somatic mutations could be used to develop a preclinical diagnostic test for the disease, as well as to measure its incubation period. This offers a unique opportunity to study the potential of ctDNA for early detection of cancer. This project involves development of a novel application for cancer early diagnosis and has implications for understanding the dynamics and growth of a cancer that has “metastasised” through an animal population.

Background

Tasmanian devil facial tumour disease (DFTD) is a clonally transmissible cancer that is spread between devils by allogeneic transfer of living cancer cells by biting (Figure A). First observed almost twenty years ago, the disease has caused widespread Tasmanian devil population decline, and is threatening this unique marsupial species with extinction (Hawkins et al., 2006). Current efforts to save the species involve the establishment of isolated captive breeding programmes; however, the lack of availability of a preclinical DFTD diagnostic test and uncertainty about the disease’s incubation period necessitate lengthy quarantine periods for captive individuals.
Recent studies of human cancer have revealed that tumour-derived DNA, carrying somatic mutations uniquely present in the cancer genome, can frequently be detected in patient blood (reviewed in Haber and Haber and Velculescu, 2014). This so-called circulating tumour DNA (ctDNA) can be used as a non-invasive and sensitive biomarker for cancer progression, as its concentration appears to correlate with tumour burden (Dawson et al., 2013; Diehl et al., 2008). However, as the set of somatic mutations carried by each patient’s cancer is unique, the cancer must first be diagnosed, biopsied and generally profiled before each patient’s personalised ctDNA assay can be developed. Thus ctDNA cannot currently be used for early diagnosis of cancer, and rigorous exploration of its potential is extremely challenging (Haber and Velculescu, 2014).

The unique clonal nature of DFTD presents an opportunity to develop ctDNA biomarkers for use in DFTD preclinical diagnosis. A subset of the somatic mutations acquired early in the DFTD lineage will be shared by all or most DFTD tumours (Figure B); ctDNA assays to detect these mutations would thus be universal to DFTD. Early diagnosis of DFTD would not only assist with Tasmanian devil quarantine and conservation management, but would also provide tools to estimate the DFTD incubation period, i.e. time from an individual receiving an infectious bite to showing clinical signs of DFTD. Knowledge of the length of this period is essential for mathematical modelling of the DFTD epidemic, and would provide insight into DFTD tumour growth rates as well as broadening our understanding of cancer cell colonisation of new environments, a process akin to metastasis.

This project would serve as a proof-of-principle study for a novel application of ctDNA technology as a tool for early detection of cancer, as well as providing insight into the dynamics of transmission of an unusual cancer in Tasmanian devils.

**Project structure and outline**

This project is divided into three one-year phases, outlined below. Consideration of the time involved in training a student in new laboratory and bioinformatics techniques have been included in the estimated timings. A discussion of the techniques involved and experience to be gained is included below.
**Phase I: Feasibility study and assay development**

Duration: One year

We will first perform a feasibility study by searching for evidence for DFTD DNA in plasma from two devils with overt, large and metastatic DFTD tumours. This will involve plasma DNA extraction and PCR. Next, we will sequence complete genomes of ten “index” DFTDs together with matching hosts, collected from geographically diverse locations in Tasmania. Bioinformatics analysis will be performed to identify structural variants common to these DFTDs but absent in hosts. We will then perform PCR screens for these candidate mutations on a panel of 500 DFTDs and 500 normal devils to confirm DFTD-specificity.

By the end of this phase we will have:

- Determined if it will be feasible to design a ctDNA assay for the early detection of DFTD
- Identified universal DFTD genetic markers for use in DFTD detection

**Phase II: Assay optimisation**

Duration: One year

We will optimise a multiplex-PCR assay for the detection of a panel of pan-DFTD DNA markers in plasma. Quantitative and digital PCR methods will be used to perform ctDNA quantification, and a series of standards of known concentration will be developed to calibrate the assay. We will perform experiments on plasma from devils with overt DFTD and DFTD systemic metastasis to determine if ctDNA concentration correlates with DFTD tumour size and tumour burden.

By the end of this phase we will have:

- Determined the optimal conditions for the assay
- Determined the quantitative limits of the assay
- Developed a panel of positive and negative controls for use in assessing assay performance

**Phase III: Field trial**

Duration: One year

DFTD ctDNA will be measured in longitudinal plasma samples collected from animals before and after the onset of DFTD clinical symptoms. The data will be used to estimate DFTD incubation period. The student will travel to Tasmania to perform a field study to estimate the proportion of individuals in affected populations with preclinical DFTD.
By the end of this phase we will have:

- Performed a field trial to assess the predictive value of our assay in determining DFTD disease outcome
- Estimated the DFTD incubation period
- Incorporated DFTD incubation period into mathematical models of DFTD spread
- Transferred skills to conservation scientists to implement the assay in routine field work and quarantine management

Relevance to cancer

This project has obvious implications for our understanding of an unusual transmissible cancer, and may have immediate impact on animal welfare and conservation management. However, this project also has broader relevance to cancer more generally. In addition to serving as a proof-of-principle for a novel application of ctDNA technology for early cancer detection, this project will improve our understanding of the dynamics of DFTD tumour establishment, a process with similarity to metastasis of human cancers. Furthermore, early cancer diagnosis and the development of sensitive non-invasive methods for cancer detection are priority areas in cancer research; this project will provide training for a student in this area, and is a compact and high impact project which will feasibly be completed within three years.

References


Further information

The project outlined in this proposal will involve a new collaboration within the Cambridge Cancer Centre between the Department of Veterinary Medicine and the CRUK Cambridge
Institute. Dr Elizabeth Murchison’s laboratory investigates transmissible cancers, and has the samples and bioinformatics expertise required for this project; Dr Nitzan Rosenfeld is a leader in the field of noninvasive cancer diagnostics, and is an expert in ctDNA detection and analysis.

Please also note that some consumables funding to support this project has already been secured from the Save the Tasmanian Devil Program and the National Science Foundation.