The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	Generation of MYB-NF1B translocation in salivary gland cells using CRISPR/Cas9 genome editing as a model for adenoid cystic carcinoma
Short Project Title:	**Generation of MYB-NF1B translocation using CRISPR-Cas9 as a model for adenoid cystic carcinoma of the salivary gland**

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DIVISIONAL AFFILIATION	
Primary Division:	Cancer Biology
Primary Team:	Development and Cancer

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Adenoid cystic carcinoma (ACC) of the salivary glands is characterised by slow growth, extensive perineurial invasion, frequent metastasis and low survival rates. Standard of treatment is limited to surgery to the primary site, which is often followed by post-operative adjuvant radiotherapy. Currently, no systemic agent has been found to be effective and response rates to conventional chemotherapy drugs are <10% with appreciable toxicity. This disease, therefore, represents an area of high unmet medical need.

Recent next generation sequencing studies have shown a low and diverse mutation rate in ACC with the only common genetic aberration being MYB-NFIB translocations, which are present in more than 50% of cases [1, 2]. These translocations have been proposed to promote overexpression of the MYB transcription factor either by stabilizing the protein and/or providing novel enhancers for expression [3]. Therefore fusion genes involving MYB have been proposed to be major drivers of ACC development.

Molecular studies in ACC have been hampered by the lack of bona fide cell lines, particularly those containing MYB translocations. The only verified models are patient-derived xenograft tumours, which tend to be slow growing and difficult to manipulate for in depth analysis. The aim of this studentship is to generate the MYB-NF1B translocation in cells of the salivary gland to establish models of ACC disease.

Recent studies have described the generation of ex vivo 3D self-organising structures, termed organoids, derived from many organs including colon, prostate and pancreas. Maimets et al [4] have described the generation of organoids from cells with stem/progenitor properties derived from mouse salivary glands. These organoids had structure and cell type properties similar to that of normal glands and when implanted into mice were able to rescue salivary function in irradiation damaged salivary glands.

PROJECT AIMS

1. To generate a MYB-NF1B translocation model using CRISPR/Cas9 and homology directed repair technology on cell lines and 3D organoid structures derived from mouse salivary gland cells

2. To analyse the tumour phenotype and drug sensitivities in vitro cultures and in vivo

3. To assess the role of salivary cell lineages in adenoid cystic carcinoma tumour development

RESEARCH PROPOSAL

1. Generation of a genetic model for ACC.

The genetic modification methodology that will be used is based on the study by Vanoli et al [5] to generate the EWSR1-WT1 translocation in cells. To generate MYB-NF1B translocations within the endogenous loci, guide RNAs corresponding to both genes that will promote Cas9 directed specific double-strand breaks in the DNA will be used. A donor plasmid with homology arms to promote homology-directed repair-mediated translocation will be included. This donor plasmid will also contain a drug resistance marker to select for integrated events. A refinement of this process will be the integration of loxP sites such that the selectable marker is removed by Cre recombinase once a drug resistant clone has been generated. Initial studies will be performed in 293T cells to test our methodology and improve efficiency.

Once the methodology has been tested in cell lines, 3D organoid models derived from mouse and human salivary glands will be used as donor cells for genetic modification. Self-organising 3D organoids are amenable to these types of manipulations as they are clonal in that each structure is derived from single stem/progenitor cells, can be drug selected and passaged many times and banked when frozen.

2. In vitro and in vivo analysis of MYB-NF1B translocation model

Genetically modified 3D models will be analysed in vitro and will be implanted in the salivary gland of mice to study their in vivo growth. These models will be analysed in various ways. Paraffin embedded tissue will be generated and immunohistochemistry will be performed to analyse the structure and the expression of tissue and cell type markers. Comparison of their phenotype to that of normal salivary glands and adenoid cystic carcinomas derived from patients will be performed. Perineurial association and metastatic properties of the in vivo implanted cells will also be assessed. Similar experiments with cells derived from ACC patient-derived xenografts (PDX), which will serve as phenotypic models, are currently being performed in the lab. Transcriptomic and chromatin immunoprecipitation (ChIP) analysis will be performed to determine the transcriptional programme regulated by the translocated MYB protein. Their profile will also be compared to that found in human ACC samples. In addition, drug sensitivity studies will be performed and compared to those found in studies using PDX samples. If feasible, 2D cell line generation from either 3D or in vivo genetically modified models will be performed.

3. Assessment of salivary cell lineages in ACC development.

The cell of origin for ACC is not well established, due to the lack of models to study this question. Salivary glands contain various cell types, including acinar, myoepithelial and ductal cells. To investigate if the MYB translocation can drive ACC formation in specific cell types, we will perform FACS sorting on salivary gland

cells using antibodies specific to either acinar or myoepithelial cells and carry out the CRISPR/Cas9 genetic modification on these populations.

Outcome and Impact

The main outcome of this studentship is the generation of relevant genetic models of the MYB-NF1B translocation event in cells of the salivary gland to understand how it drives the formation of ACC, which is of crucial importance to developing novel ways to treat this disease.

LITERATURE REFERENCES

1. Ho AS, Kannan K, Roy DM, Morris LG, Ganly I, Katabi N, Ramaswami D, Walsh LA, Eng S, Huse JT *et al*: The mutational landscape of adenoid cystic carcinoma. *Nat Genet* 2013, 45(7):791-798.
2. Stephens PJ, Davies HR, Mitani Y, Van Loo P, Shlien A, Tarpey PS, Papaemmanuil E, Cheverton A, Bignell GR, Butler AP *et al*: Whole exome sequencing of adenoid cystic carcinoma. *J Clin Invest* 2013, 123(7):2965-2968.
3. Drier Y, Cotton MJ, Williamson KE, Gillespie SM, Ryan RJ, Kluk MJ, Carey CD, Rodig SJ, Sholl LM, Afrogheh AH *et al*: An oncogenic MYB feedback loop drives alternate cell fates in adenoid cystic carcinoma. *Nat Genet* 2016, 48(3):265-272.
4. Maimets M, Rocchi C, Bron R, Pringle S, Kuipers J, Giepmans BN, Vries RG, Clevers H, de Haan G, van Os R *et al*: Long-Term In Vitro Expansion of Salivary Gland Stem Cells Driven by Wnt Signals. *Stem Cell Reports* 2016, 6(1):150-162.
5. Vanoli F, Tomishima M, Feng W, Lamribet K, Babin L, Brunet E, Jasin M: CRISPR-Cas9-guided oncogenic chromosomal translocations with conditional fusion protein expression in human mesenchymal cells. *Proc Natl Acad Sci U S A* 2017, 114(14):3696-3701.

CANDIDATE PROFILE

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)

Pre-requisite qualifications of applicants: e.g. BSc or equivalent in specific subject area(s)	See ICR's minimum entry requirements
Intended learning outcomes: Please provide a bullet point list (maximum of seven) of the knowledge and skills you expect the student to have attained on completion of the project.	1. Knowledge of cancer biology and therapy 2. Familiarity with CRISPR/Cas9 genome editing technology 3. Working with 3D organoid models 4. Analysis of gene function in vitro and in vivo