CHIEF SCIENTIST OFFICE

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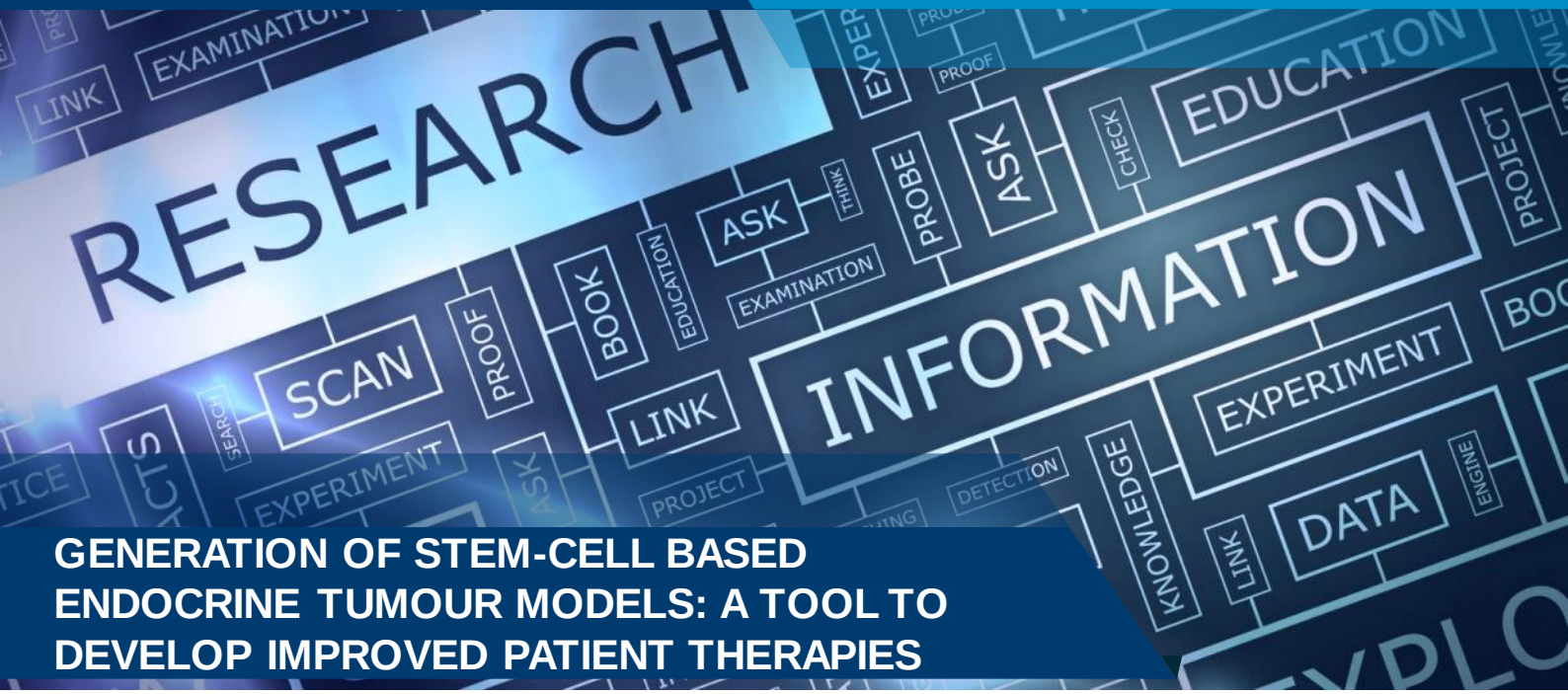
RESEARCH PROJECT BRIEFING



GENERATION OF STEM-CELL BASED ENDOCRINE TUMOUR MODELS: A TOOL TO DEVELOP IMPROVED PATIENT THERAPIES

AIMS

Tumours affecting the endocrine system (i.e. hormone producing glands) constitute a broad group of benign and malignant conditions that are associated with substantial morbidity. Although most endocrine tumours occur in a 'sporadic' setting (i.e. without a family history) they may also occur as part of a number of hereditary conditions, which include Multiple Endocrine Neoplasia Types 1-4. Although several genes have been identified to underpin the development of both sporadic and hereditary endocrine tumours (e.g. *MEN1, CDKN1B*), the mechanisms resulting in tumour formation are poorly understood. This is part reflects the limitations of existing disease models, which include 'knockout' mouse models or poorly representative cell lines (e.g. derived from non-endocrine cancers). To address this deficiency, we aimed to develop a novel approach to endocrine tumour modelling, combining the use of human induced pluripotent stem cells (which can be used to generate any cell type including endocrine cells) and gene-editing, with the aim of gaining new insights into the mechanisms of tumour formation as well as providing a valuable resource for drug discovery.

KEY FINDINGS

- Gene-editing of stem cells successfully facilitated the generation of a number of different cellular models each harbouring gene-specific mutations relevant to endocrine tumour formation. These cell lines underwent a process of 'differentiation' to establish endocrine-like cells to investigate gene function in a relevant cellular environment.
- Gene 'knockout' stem cell models were generated for several genes associated with hereditary and sporadic endocrine tumours (e.g. *MEN1*, *CDKN1B*) and effects on cell function assessed (e.g. cell morphology, proliferation, cell cycle, mitosis)
- Inactivation of the *Multiple Endocrine Neoplasia Type 1* (*MEN1*) gene impaired endocrine cell formation limiting the utility of the model. Modification of the approach allowed the introduction of a 'genetic switch' that facilitated inactivation of the *MEN1* gene once endocrine cells had formed thereby providing a powerful model for future studies.

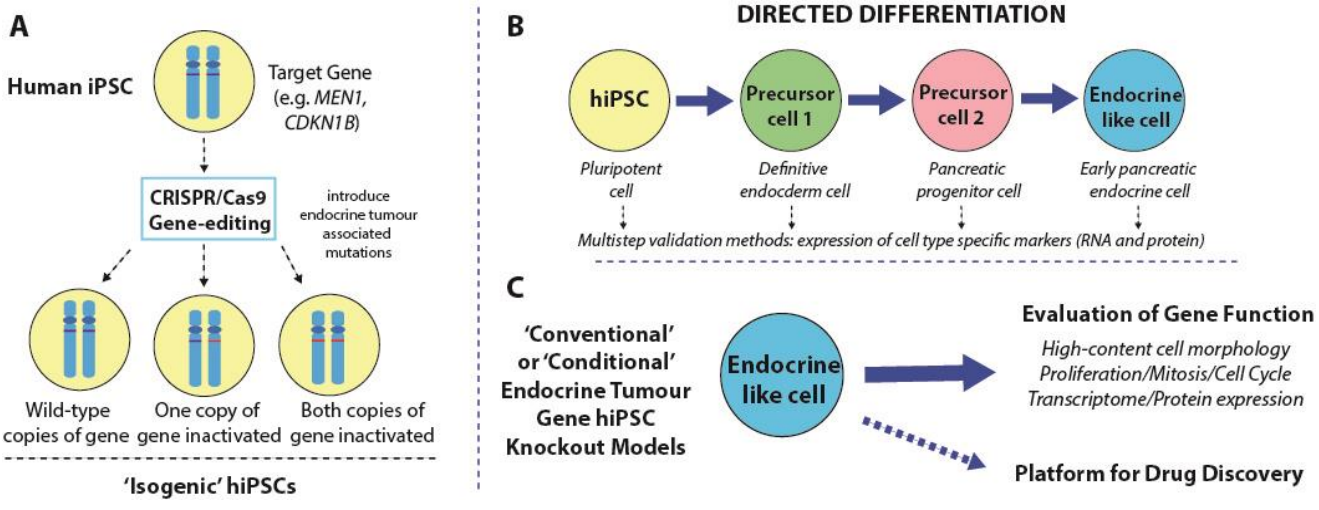
WHAT DID THE STUDY INVOLVE?

The study combined several laboratory-based methods to establish the cellular models. This included: methods to characterise and validate the stem cell models including assessment of pluripotency (i.e. the ability to differentiate into all major cell types); a variety of gene-editing methods based on 'CRISPR' strategies to introduce the appropriate disease-associated mutations (i.e. those occurring in patient-derived sporadic and/or hereditary endocrine tumours (Figure: Part **A**); and differentiation methods to generate the formation of endocrine-type cells (Figure: Part **B**). Once the respective cell lines were established, multiple approaches were used to validate the cellular models (e.g. DNA sequencing, pluripotency assessment), before going on to assess the consequences of gene disruption on cellular processes relevant to tumour formation (e.g. microscopy; cell proliferation and division; cell cycle control; changes in gene and protein expression (Figure; Part **C**).

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

Combining human induced pluripotent stem cells (hiPSCs) with CRISPR/Cas9 gene-editing facilitated the generation of several genetically-defined cellular models harbouring mutations found in patient-derived endocrine tumours. This facilitated the comparison of cell lines that were essentially identical except for changes in the gene under study (i.e. 'isogenic' cell lines (Fig. **A**). These studies demonstrated that inactivation of either one or both copies of the *MEN1* or *CDKN1B* genes, respectively, did not affect initial pluripotency, although the subsequent ability to form other cell types was impaired in the *MEN1* 'knockout' cells. To address this, we used an alternative approach, developng a 'conditional' *MEN1* 'knockout' model, in which the *MEN1* gene could be inactivated following endocrine differentiation, thereby allowing the consequences of *MEN1* loss to be assessed in relevant cell types. Transcriptome analysis (i.e. global RNA sequencing) of endocrine lineage cells derived from this model identified pathways potentially regulated by *MEN1,* providing possible novel insights into endocrine tumour formation. These studies provide proof-of-concept for the combined use of stem cells and gene-editing to generate pre-clinical human endocrine tumour models which in turn may provide new insights into disease mechanisms.

Figure: Research Overview



WHAT IMPACT COULD THE FINDINGS HAVE?

This project has several potential impacts:

- For Academia/Industry: Cellular disease models based on hiPSCs and gene-editing offer unlimited quantities of genetically-defined cells for translational research as well as providing a powerful pre-clinical platform for drug discovery. Thus, the models generated in this project are suitable for high-throughput phenotypic screening with therapeutic compound libraries to look for potential relevant cellular effects (e.g. synthetic lethality) and offer future collaborative opportunities with both academia and industrial partners.
- For Patients: There is an unmet clinical need for new treatments for advanced endocrine tumours, with studies of traditional model systems (e.g. mouse models, existing cancer cell lines) failing to translate into major clinical benefit. The generation of pre-clinical human disease models harbouring patient-specific mutations offer potential opportunities to identify and develop personalised treatment approaches.
- For Policy: These studies support the 'Replacement' principle of 'The 3Rs' (i.e. 'Replacement, Reduction, Refinement') by promoting the use of non-animal methods in scientific experimentation and disease modelling.

HOW WILL THE OUTCOMES BE DISSEMINATED?

Early work related to the project has been presented at national scientific meetings, and the intention is to submit recent studies to upcoming national/international scientific-clinical meetings. Thereafter, the work will be submitted to peer-reviewed scientific journals in one or more research manuscripts. I am also keen to engage with additional academic/industrial partners to maximise the utility of the cell lines and will look to establish new collaborative partnerships. Existing partnerships with relevant patient groups will also be used to disseminate research findings to the wider public.

CONCLUSION

Stem cell and gene-editing methods provide a new approach for endocrine disease modelling, potentially offering a more relevant cellular environment to investigate gene function and the mechanisms of tumour formation than existing disease models. These approaches also offer an attractive platform to undertake high-throughput drug screening with the aim of identifying new treatment strategies.

RESEARCH TEAM & CONTACT

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Additional Information

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