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Mesenchymal Stem Cell-Mediated Sodium Iodide Symporter Gene Therapy of Breast Cancer

A thesis submitted to the National University of Ireland as partial fulfilment of the requirements for the degree of Doctor of Philosophy (PhD)

by

James John Ryan BSc, MSc.

Under the supervision of Dr. Roisin Dwyer

And direction of Prof. Michael Kerin

Discipline of Surgery, School of Medicine, Clinical Sciences Institute, NUI Galway
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LIST OF ABBREVIATIONS

AD Aldehyde dehydrogenase
Ad5/CMV/GFP Adenovirus-5 carrying GFP gene with CMV promoter
Ad5/CMV/NIS Adenovirus-5 carrying NIS gene with CMV promoter
Ad5/MUC1/NIS Adenovirus-5 carrying NIS gene with MUC1 promoter
AdCMV-p53 Adenovirus carrying P53 gene with CMV promoter
BALP/c mouse strain used for cancer and immunology research
BCL2 B-cell lymphoma 2
BCSC Breast Cancer Stem Cell
CA-15 Cancer Antigen-15
CA27-29 Cancer Antigen 27-29
cAMP Cyclic adenosine monophosphate
CAR Coxsackie Adenovirus Receptor
CCR2 Chemokine Receptor Type 2
CD Cluster of Differentiation
CEA Carcinoembryonic antigen
cGy/mCi centi Gray/ milli Curie
CMV Cytomegalovirus
COOH carboxy
CREB cAMP response element-binding protein
DCIS Ductal Carcinoma in situ
DMSO Dimethyl Sulfoxide
DNA Deoxyribonucleic Acid
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<tr>
<td>E1A</td>
<td>Early 1A</td>
</tr>
<tr>
<td>E1B</td>
<td>Early 1B</td>
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<tr>
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<td>Early 2A</td>
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<td>Early 2B</td>
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<td>E3</td>
<td>Early 3</td>
</tr>
<tr>
<td>E4</td>
<td>Early 4</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal Growth Factor</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal Growth Factor Receptor</td>
</tr>
<tr>
<td>EMT</td>
<td>Epithelial to Mesenchymal Transition</td>
</tr>
<tr>
<td>ER</td>
<td>Estrogen Receptor</td>
</tr>
<tr>
<td>ERα</td>
<td>Estrogen Receptor alpha</td>
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<tr>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
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<tr>
<td>FOS</td>
<td>FOS oncogene</td>
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<tr>
<td>GFP</td>
<td>Green Fluorescent Protein</td>
</tr>
<tr>
<td>GVHD</td>
<td>Graft Versus Host Disease</td>
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<tr>
<td>HER2</td>
<td>Human Epidermal Growth Factor-2</td>
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<tr>
<td>HIF</td>
<td>Hypoxia Inducible Factor</td>
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<td>HMLER</td>
<td>human mammary epithelial cells expressing SV40 large-T antigen, the telomerase catalytic subunit, and H-Ras oncoprotein</td>
</tr>
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<td>hrs</td>
<td>hours</td>
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<tr>
<td>hTERT</td>
<td>human Telomerase reverse transcriptase</td>
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<td>I-</td>
<td>iodide</td>
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<td>K-BALP</td>
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<td>LOX</td>
<td>lysyl oxidase</td>
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<td>micro metres per second</td>
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<td>MAPK</td>
<td>Mitogen-activated Protein Kinase</td>
</tr>
<tr>
<td>mCi/m2</td>
<td>milli Curie per metre squared</td>
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<td>MCP-1</td>
<td>Monocyte chemoattractant protein-1</td>
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<td>Abbreviation</td>
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<tr>
<td>MFP</td>
<td>Mammary Fat Pad</td>
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<td>mRNA</td>
<td>messenger Ribonucleic Acid</td>
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<td>Mesenchymal Stem Cell</td>
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<tr>
<td>RARβ</td>
<td>Retinoic Acid Receptor beta</td>
</tr>
<tr>
<td>RAS</td>
<td>Protein controlling intracellular signaling networks</td>
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<tr>
<td>RGD</td>
<td>Arginine Glycine Aspartame</td>
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<td>RNA</td>
<td>Ribonucleic Acid</td>
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<td>RQ-PCR</td>
<td>Relative Quantitative-Polymerase Chain Reaction</td>
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<td>SCID</td>
<td>Severe Combined Immunodeficiency</td>
</tr>
<tr>
<td>SLC5A5</td>
<td>X-linked inhibitor of apoptosis protein</td>
</tr>
<tr>
<td>SP-1</td>
<td>specificity protein 1</td>
</tr>
<tr>
<td>SPECT/CT</td>
<td>Single-photon emission computed tomography</td>
</tr>
<tr>
<td>T₃</td>
<td>Triiodothyronine</td>
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<tr>
<td>T₄</td>
<td>Thyroxine</td>
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XXIII
<table>
<thead>
<tr>
<th>Acronym</th>
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<tr>
<td>TAF</td>
<td>Tumour Associated Fibroblasts</td>
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<tr>
<td>Tg</td>
<td>Thyroglobulin</td>
</tr>
<tr>
<td>TGFα</td>
<td>Tumour Growth Factor alpha</td>
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<tr>
<td>TGFβ1</td>
<td>Tumour Growth Factor beta 1</td>
</tr>
<tr>
<td>THRα</td>
<td>Thyroid Hormone receptor alpha</td>
</tr>
<tr>
<td>THRβ</td>
<td>Thyroid Hormone receptor beta</td>
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<tr>
<td>TNF</td>
<td>Tumour Necrosis Factor</td>
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<td>TNM</td>
<td>Tumour size (T)Nodal involvement (N) Metastasis (M)</td>
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<td>TRAIL</td>
<td>TNF-related apoptosis-inducing ligand</td>
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<td>TSH</td>
<td>Thyroid Stimulating Hormone</td>
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<td>Very Late Antigen-4</td>
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<td>XIAP</td>
<td>X-linked inhibitor of apoptosis protein</td>
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COMMUNICATIONS ORIGINATING FROM THIS WORK

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ABSTRACT

**Introduction:** The Sodium Iodide Symporter (NIS) facilitates iodide accumulation in the thyroid and radioiodide imaging and treatment of thyroid disease. Studies have suggested that elevated levels of NIS expression in malignant breast tissue may facilitate diagnosis, imaging and treatment of breast cancer. Alternative approaches, such as Adenovirus-based NIS gene therapy is limited by vector immunogenicity and an inability to specifically target tumours. Mesenchymal Stem Cells (MSCs) may represent appropriate cellular vehicles as a result of their proven tumour tropism and immune privilege. The aim of this project was to determine the presence, relevance and regulation of native mammary NIS expression and to explore the potential of MSC-mediated NIS gene therapy of breast cancer.

**Methods:** Expression of NIS, and putative regulators (Retinoic acid receptors (RAR), Estrogen receptor (ER), Phosphoinositide-3-kinase (PI3K), and Thyroid hormone receptors (THR)) were determined by Relative Quantitative-Polymerase Chain Reaction (RQ-PCR) in 100 breast tissue specimens that included 15 controls. *In vitro* the effects of individual and combined estradiol, retinoic acid (RA) and thyroxine stimulation on NIS expression was determined in breast cancer cell lines. MSCs were engineered to express NIS and characterised in terms of phenotype and persistence of NIS expression and function. The distribution of systemically injected MSC-NIS in non-invasive disease and labelled MSCs in metastatic murine breast cancer models was determined over time.

**Results:** NIS gene expression levels were significantly higher in malignant tissue compared to normal but even higher in benign tissue. Significant positive correlations in gene expression suggested relationships between NIS and putative regulators: RARα, RARβ, ERα and THRβ which were confirmed by estradiol, RA and thyroxine stimulation of NIS expression *in vitro*. Combined stimulation with RA and thyroxine had a synergistic effect on NIS expression. MSCs were successfully engineered to express NIS with no significant impact on phenotype observed. A cytotoxic effect on adjacent breast cancer cells was also demonstrated using Iodide$^{131}$ *in vitro*. In animal models, initial ectopic engraftment was shown to deplete...
over time except in malignant tissue and tumour-targeted MSC tropism as well as successful delivery of transgene to tumour sites was observed.

**Conclusion:** This thesis presents novel data on the presence and relevance of mammary NIS expression in human tissues. It also supports a regulatory role for estradiol and retinoic acid, and introduces the potential for thyroid hormones to stimulate mammary NIS expression. The phenotype and migratory behaviour of labelled and Ad5/CMV/NIS infected MSCs demonstrated here strongly support the potential of MSC-mediated NIS gene therapy of breast cancer