

https://dspace.org/

# Short hairpin RNA-directed knockdown of epidermal growth factor receptor in human oesophageal squamous carcinoma cell lines

**Killick, Mark Andrew**

**2008-05-27T12:52:55Z**
http://hdl.handle.net/10539/4896

Downloaded from a DSpace repository. https://dspace.org/

## TABLE OF CONTENTS

**Abstract** ..... iii

**List of Figures** ..... viii

**List of Tables** ..... x

**CHAPTER 1** ..... 11

1.1 Cancer overview ..... 11
1.2 Causative agents in cancer ..... 11
1.2.1 Insensitivity to antigrowth signals ..... 12
1.2.2 Evasion of apoptosis ..... 13
1.2.3 Limitless replicative potential ..... 14
1.2.4 Sustained angiogenesis ..... 14
1.2.5 Tissue invasion and metastasis ..... 15
1.2.6 Self sufficiency of growth signals ..... 15
1.3 Epidermal growth factor receptor (EGFR) ..... 17
1.3.1 Molecular description of EGFR ..... 17
1.3.2 EGFR signalling pathways ..... 18
1.3.3 The mitogen-activated protein kinase pathway (MAPK) ..... 18
1.3.4 Phosphatidylinositol 3- kinase cascade ..... 19
1.3.5 Alternative EGFR activation pathways ..... 20
1.4 General characteristics of oesophageal cancer in humans ..... 20
1.4.1 Adenocarcinoma ..... 21
1.4.2 Human oesophageal squamous cell carcinoma (HOSCC) ..... 21
1.4.3 Epidemiology of HOSCC ..... 22
1.4.4 Molecular characteristics of HOSCC ..... 22
1.5 Molecular therapeutics in cancer ..... 23
1.5.1 Antibodies ..... 24
1.5.2 Antisense ..... 24
1.5.3 Tyrosine kinase inhibitors ..... 25
1.6 Molecular therapies targeting EGFR ..... 25
1.7 RNA interference ..... 26
1.8 Exploiting the RNAi pathway. ..... 27
1.9 Objectives ..... 30
1.10 Aims ..... 30

**Chapter 2** ..... 31

2.1 Design and selection of RNA's to be used for knockdown ..... 31
2.2 Small interfering and short hairpin RNAs (siRNA and shRNA) ..... 31
2.3 siRNA/ shRNA design ..... 32
2.4 Materials and methods ..... 33
2.4.1 siRNA selection ..... 33
2.4.2 shRNA design ..... 34
2.4.3 shRNA synthesis: ..... 35
2.4.4 Verification of complete shRNA expression cassette ..... 36
2.5 Results ..... 37

2.5.1 Modification of siRNAs to shRNAs ..... 38
2.5.2 Verification of the shRNAs cassette sequences ..... 41
2.6 Discussion ..... 43
**CHAPTER 3 ..... 46**
3.1 Construction and validation of reporter systems ..... 46
3.2 siRNA/shRNA target efficacy reporter system ..... 46
3.3 Reporter system description ..... 47
3.4 Materials and methods ..... 47
3.4.1 Design of the shRNA efficacy reporter system ..... 47
3.4.2 Construction of the shRNA efficacy reporter system ..... 48
3.4.3 Plasmid preparation for shRNA efficacy reporter system ..... 48
3.4.4 Ligation of 3xEGFR target into the psiCHECK and pCIeGFP plasmid ..... 49
3.4.5 Transformation of Escherichia coli DH5α ..... 49
3.4.6 Confirmation of insert ..... 50
3.5 Results ..... 51
3.6 Discussion ..... 54
**CHAPTER 4 ..... 57**
4.1 EGFR shRNAs knockdown efficiency assay ..... 57
4.1.1 psiCHECK 2.2 dual luciferase reporter system ..... 57
4.1.2 pCIeGFP reporter system ..... 58
4.2 Materials and methods ..... 59
4.2.1 Mammalian transfection plasmid description ..... 59
4.2.2 Suitable DNA plasmid isolation for mammalian cell transfection ..... 59
4.2.3 DNA Quantification ..... 60
4.2.4 Mammalian cell culture and transfection ..... 60
4.2.5 Dual luciferase assay ..... 61
4.2.6 pCIeGFP EGFR shRNA efficiency reporter system ..... 62
4.3 Results ..... 62
4.3.1 psiCHECK dual luciferase reporter system ..... 62
4.3.2 pCIeGFP 3 x EGFR target reporter system ..... 64
4.4 Discussion ..... 70
**CHAPTER 5 ..... 72**
5.1 The potential use of RNAi in cancer therapy ..... 72
5.2 Knockdown of EGFR in HOSCC ..... 72
5.3 Materials and methods ..... 73
5.3.1 HOSCC cell culture and transfection ..... 73
5.3.2 Whole cell extraction 74
5.3.3 Protein estimation ..... 74
5.3.4 SDS-PAGE ..... 75
5.3.5 Western Blotting ..... 75
5.3.6 Densitometery ..... 76
5.4 Results ..... 76
5.4.1 Knockdown of endogeous expressed EGFR ..... 78
5.4.2 Estimation of transfection efficiency ..... 81
5.5 Discussion ..... 83

**CHAPTER 6 ..... 88**

6.1 General discussion ..... 88
6.2 Knockdown of endogeneously over expressed EGFR ..... 88
6.3 Vision for the future ..... 90
6.3.1 Avenues for Exploration ..... 90
6.3.2 Consideration for clinical application ..... 91
6.4 Conclusion ..... 91

**References: ..... 92**

**APPENDIX ..... 100**

**Tissue culture ..... 100**

**Protein estimation ..... 101**

**Sample preparation for SDS-PAGE ..... 102**

**SDS-PAGE ..... 102**

**Western Blot ..... 103**