

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