# Hypoxic regulation of microRNA biogenesis

**KANCHANA VERONIKA BANDARA**

**Bachelor of Science (Honours)**

**Department of Gastroenterology**

**Department of Renal Medicine**

**School of Medicine**

**Faculty of Health Science**

**Flinders University**

**A thesis submitted for the degree of Doctor of Philosophy**

**December 2013**

# Table of contents

List of Figures ..... vi

List of tables ..... x

List of abbreviations ..... xi

Abstract ..... xvii

Declaration ..... xix

Publications and presentations ..... xviii

Acknowledgements ..... xxii

**CHAPTER 1 INTRODUCTION** ..... 1

1.1 Hypoxia ..... 2

1.2 Hypoxia and cancer ..... 3

1.3 Cell response to hypoxia ..... 5

1.3.1 Hypoxia inducible factor-1 (HIF-1) ..... 7

1.3.2 HIF independent pathways ..... 12

1.3.3 HIF expression and cancer ..... 13

1.4 MicroRNAs ..... 17

1.4.1 Discovery of microRNAs ..... 17

1.5 MicroRNA biogenesis ..... 18

1.5.1 DROSHA processing ..... 18

1.5.2 Nuclear export by Exportin-5 ..... 22

1.5.3 DICER processing ..... 23

1.6 Relationships between biogenesis proteins ..... 26

1.7 RNA induced silencing complex ..... 27

1.8 Gene regulation by microRNAs ..... 28

1.9 MicroRNAs and cancer ..... 30

1.9.1 Specific miRNAs function as oncogenes or tumour suppressors ..... 31

1.9.2 MicroRNAs and metastasis ..... 32
1.10 Mechanisms of miRNA dysregulation in human cancer ..... 33
1.11 Hypoxic regulation of miRNAs in cancer ..... 35
1.11.1 Expression and function of miR-210 in hypoxia ..... 36
1.11.2 Mechanisms involved in hypoxic regulation of miRNA expression ..... 37
1.12 Rationale for this study ..... 40
**CHAPTER 2 . MATERIALS AND METHODS** ..... 41
2.1 Cell culture procedures ..... 42
2.1.1 Cell lines and culture procedures ..... 42
2.1.2 Exposure of cultured cells to experimental conditions ..... 45
2.1.3 Transient transfection of cultured cells ..... 46
2.2 RNA protocols ..... 50
2.2.1 RNA extraction ..... 50
2.2.2 RNA quantitation ..... 51
2.2.3 Microarray analysis ..... 51
2.2.4. Relative quantification of real time RT-PCR for miRNA ..... 52
2.2.5 Relative quantification of real time RT-PCR for precursor and mature miRNA ..... 53
2.2.6 Relative quantification of real time RT-PCR for mRNA ..... 54
2.2.7 Statistical analysis ..... 55
2.3 Protein protocols ..... 57
2.3.1 Protein extraction ..... 57
2.3.2 Immunoblotting ..... 58
2.4 Measurement of luciferase acitivity in transiently transfected cells ..... 63
2.5 DICER activity assay ..... 64
2.5.1 Gel purification of plasmid DNA ..... 64

2.5.2 *In Vitro* Transcription for RNA Radio-labelling with UTP ..... 64

2.5.3 End labelling Decade markers ..... 66

2.5.4 Detection of human DICER Activity ..... 67

2.5.5 Preparing a denaturing PAGE gel ..... 68

**CHAPTER 3 . DICER REGULATION BY HYPOXIA** ..... 70

3.1 Introduction ..... 71

3.2 Aims ..... 73

3.3 Exposing cells to hypoxia ..... 73

3.4 Hypoxic regulation of *DICER* mRNA expression ..... 76

3.4.1 The effect of hypoxia on *DICER* mRNA expression in cancer cell lines ..... 76

3.5 Hypoxic regulation of DICER protein levels ..... 81

3.5.1 The effect of hypoxia on DICER protein levels in breast cancer cell lines ..... 81

3.5.2 The effect of hypoxia on DICER protein levels in human umbilical vein endothelial cells (HUVECs) ..... 85

3.6 Discussion ..... 87

3.7 Chapter summary ..... 89

**CHAPTER 4 MECHANISMS OF DICER REGULATION BY HYPOXIA** ..... 90

4.1 Introduction ..... 91

4.2 Aims ..... 93

4.3 Hypoxia inducible factor dependent regulation of DICER ..... 93

4.3.1 DICER mRNA and protein levels after HIF hydroxylase inhibition ..... 93

4.3.2 DICER mRNA and protein levels after HIF inhibition ..... 100

4.3.3 DICER mRNA and protein levels after HIF overexpression ..... 105

4.3.4 DICER mRNA and protein levels in RCC4 cells with +VHL/-VHL ..... 108

4.4 Prolyl hydroxylase dependent regulation of DICER ..... 111
4.5 MicroRNAs involved in DICER regulation ..... 117
4.5.1 DICER regulation by miR-210 ..... 117
4.5.2 DICER regulation by miR-103 and miR-107 ..... 121
4.5.3 DICER 3'UTR regulation in hypoxia ..... 125
4.6 Proteasomes in DICER regulation ..... 127
4.7 Discussion ..... 129
4.8 Chapter summary ..... 132
**CHAPTER 5 HYPOXIC REGULATION OF MIRNA BIOGENESIS PROTEINS.** ..... 133
5.1 Introduction ..... 134
5.2 Aims ..... 135
5.3 Hypoxic regulation of DROSHA mRNA and protein levels ..... 135
5.4 Hypoxic regulation of DGCR8 protein levels ..... 137
5.5 Hypoxic regulation of XPO5 protein levels ..... 138
5.6 Hypoxic regulation of TARBP2 mRNA and protein levels ..... 139
5.7 Hypoxic regulation of AGO2 mRNA and protein levels ..... 141
5.8 Co-ordinated expression of miRNA biogenesis proteins ..... 143
5.8.1 Relationship between DICER and TARBP2 ..... 143
5.8.2 Relationship between DICER and DROSHA ..... 146
5.9 Discussion ..... 148
5.10 Chapter summary ..... 151
**CHAPTER 6 EFFECTS OF HYPOXIA ON MIRNA EXPRESSION AND FUNCTION** ..... 152
6.1 Introduction ..... 153
6.2 Aims ..... 155
6.3 Mature and precursor miRNA expression in hypoxia compared to normoxia ..... 155

6.3.1 Mature and precursor miRNA expression analysis by microarrays .... 155

6.3.2 Mature and precursor miRNA expression analysis by real time RT-PCR .... 179

6.4 MicroRNA mediated gene suppression is affected by hypoxia .... 182

6.5 Role of DICER in miRNA processing .... 185

6.6 Discussion .... 188

6.7 Chapter summary .... 192

**CHAPTER 7 DISCUSSION** .... 194

7.1 DICER regulation by hypoxia .... 195

7.2 Mechanisms of DICER regulation in hypoxia .... 197

7.3 Hypoxia down regulates other miRNA biogenesis proteins .... 201

7.4 Effect of hypoxia on mature miRNA expression and function .... 204

7.5 Significance .... 209

7.6 Future directions .... 210

7.7 Conclusion .... 212

**References** .... 213

# List of Figures

Figure 1.1 Oxygen dependent gene regulation by hypoxia inducible factor-1α ..... 10

Figure 1.2 Genes activated by hypoxia-inducible factors (HIFs) that are involved in tumour progression. ..... 15

Figure 1.3 Schematic diagram of the canonical miRNA biogenesis pathway. ..... 21

Figure 3.1 *CAIX* mRNA expression in MCF7 cells in hypoxia vs. normoxia. ..... 75

Figure 3.2 miR-210 expression in MCF7 cells in hypoxia vs. normoxia. ..... 75

Figure 3.3 *DICER* mRNA expression in MCF7 cells after exposure to varying concentrations of hypoxia. ..... 77

Figure 3.4 Viability of SKBR3 cells following hypoxia. ..... 77

Figure 3.5 *DICER* mRNA expression in MCF7 cells in hypoxia vs. normoxia. ..... 79

Figure 3.6 *DICER* mRNA expression in HT29 cells in hypoxia vs. normoxia. ..... 79

Figure 3.7 *DICER* mRNA expression in SKBR3 cells in hypoxia vs. normoxia. ..... 80

Figure 3.8 DICER protein levels in MCF7 and SKBR3 cells in hypoxia vs. normoxia. ..... 82

Figure 3.9 DICER protein levels in MCF7 and SKBR3 cells hypoxia vs. normoxia. 83

Figure 3.10 DICER protein levels in MCF7 and SKBR3 cells hypoxia vs. normoxia. ..... 84

Figure 3.11 DICER protein levels in HUVECs in hypoxia vs. normoxia. ..... 86

Figure 4.1 HIF-1α protein levels after exposure to HIF hydroxylase inhibitor dimethyloxalyl glycine (DMOG). ..... 95

Figure 4.2 *DICER* mRNA expression in MCF7 cells after exposure to HIF hydroxylase inhibitors. ..... 96

Figure 4.3 *DICER* mRNA expression in SKBR3 cells after exposure to HIF hydroxylase inhibitors. ..... 97

Figure 4.4 DICER protein levels in SKBR3 cells after exposure to HIF hydroxylase inhibitors. ..... 99
Figure 4.5 HIF-1 repression in SKBR3 cells after HIF-1α inhibition in hypoxia vs. normoxia. ..... 101
Figure 4.6 *DICER* mRNA expression in SKBR3 cells after HIF-1α suppression in hypoxia vs. normoxia. ..... 102
Figure 4.7 *DICER* mRNA expression in MCF7 cells after HIF-1α and HIF-2α suppression in hypoxia vs. normoxia. ..... 103
Figure 4.8 DICER protein levels in SKBR3 cells after HIF-1α inhibition in hypoxia vs. normoxia. ..... 104
Figure 4.9 HIF-1α protein levels in SKBR3 cells after HIF over expression. ..... 106
Figure 4.10 *DICER* mRNA expression in SKBR3 cells after HIF-1α and HIF-2α over expression. ..... 106
Figure 4.11 DICER protein levels in SKBR3 cells after HIF-1α and HIF-2α over expression. ..... 107
Figure 4.12 miR-210 expression in RCC4 cell lines in normoxia. ..... 109
Figure 4.13 DICER expression in RCC4 +VHL and RCC4 -VHL cell lines. ..... 110
Figure 4.14 Prolyl hydroxylase-2 dependent regulation of *DICER* mRNA. ..... 112
Figure 4.15 Prolyl hydroxylase-2 dependent regulation of DICER protein. ..... 113
Figure 4.16 Prolyl hydroxylase-1 dependent regulation of DICER. ..... 115
Figure 4.17 Factor inhibiting HIF-1 dependent regulation of DICER. ..... 116
Figure 4.18 Human *DICER* 3' UTR. ..... 116
Figure 4.19 miR-210 expression after miR-210 mimic and miR-210 antagomir. ..... 118
Figure 4.20 *DICER* mRNA and protein levels after over-expressing miR-210 in normoxia. ..... 119

Figure 4.21 *DICER* mRNA expression after over-expressing miR-210 and inhibiting miR-210 in hypoxia vs. normoxia. ..... 120
Figure 4.22 miR-103 expression in hypoxia vs. normoxia. ..... 122
Figure 4.23 miR-107 expression in hypoxia vs. normoxia. ..... 122
Figure 4.24 *DICER* mRNA expression after inhibiting miR-103 and miR-107 in hypoxia vs. normoxia. ..... 123
Figure 4.25 DICER protein levels after inhibiting miR-103 and miR-107 in hypoxia vs. normoxia. ..... 124
Figure 4.26 *DICER* 3'UTR not targeted by miRNAs in hypoxia. ..... 126
Figure 4.27 DICER regulation by proteasomes. ..... 128
Figure 5.1 DROSHA mRNA and protein levels in hypoxia vs. normoxia. ..... 136
Figure 5.2 DGCR8 protein levels in hypoxia vs. normoxia. ..... 137
Figure 5.3 XPO5 protein levels in hypoxia vs. normoxia. ..... 138
Figure 5.4 TARBP2 expression in hypoxia vs. normoxia. ..... 140
Figure 5.5 AGO2 expression in hypoxia vs. normoxia. ..... 142
Figure 5.6 Relationship between DICER and TARBP2 protein levels. ..... 144
Figure 5.7 Relationship between DICER and TARBP2 protein levels. ..... 145
Figure 5.8 Relationship between DICER and DROSHA protein levels. ..... 147
Figure 6.1 Mature miRNA expression in hypoxia (0.1% $O_2$ 16 h) vs. normoxia. ... 156
Figure 6.2 miR-210 expression in MCF7 cells in hypoxia vs. normoxia. ..... 158
Figure 6.3 Precursor miRNA expression in hypoxia (0.1% $O_2$ 16 h) vs. normoxia. 159
Figure 6.4 Ratios between precursor/mature miRNA expression in hypoxia (0.1% $O_2$ 16 h) vs. normoxia. ..... 160
Figure 6.5 Principle component analysis plot of microRNA expression values from MCF7 cells in hypoxia (0.1% $O_2$ 16 h) vs. normoxia. ..... 162
Figure 6.6 Mature miRNA expression in hypoxia (0.1% $O_2$ 48 h) vs. normoxia. ... 165

Figure 6.7 Precursor miRNA expression in hypoxia (0.1% $O_2$ 48 h) vs. normoxia. 167

Figure 6.8 Ratios between precursor/mature miRNA expression in hypoxia (0.1% $O_2$ 48 h) vs. normoxia. 168

Figure 6.9 DICER protein levels in MCF7 cells after transfections with siRNAs targeting DICER. 170

Figure 6.10 Mature miRNA expression after DICER inhibition vs. normoxia. 171

Figure 6.11 Precursor miRNA expression after DICER inhibition vs. normoxia. 174

Figure 6.12 Ratio between precursor/mature miRNA expression after DICER inhibition vs. normoxia. 176

Figure 6.13 Principle component analysis plot of microRNA expression values from MCF7 cells in hypoxia (0.1% $O_2$) 48 h, normoxia and *DICER* inhibition. 178

Figure 6.14 Mature and precursor let-7a expression in hypoxia vs. normoxia. 180

Figure 6.15 Mature and precursor miR-21 expression in hypoxia vs. normoxia. 181

Figure 6.16 Mature and precursor miR-185 expression in hypoxia vs. normoxia. 181

Figure 6.17 Schematic representations of the reporter constructs. 183

Figure 6.18 miRNA processing is affected by hypoxia. 184

Figure 6.19 RNA gel showing pre-miRNA processing in hypoxia vs. normoxia. 186

Figure 6.20 Pre-miRNA processing in hypoxia vs. normoxia. 187

# List of tables

Table 2.1 Human derived cell lines ..... 44

Table 2.2 List of siRNAs ..... 47

Table 2.3 List of plasmid constructs ..... 49

Table 2.4 List of pre-miRNA and miRNA assays ..... 56

Table 2.5 List of mRNA assays ..... 56

Table 2.6 List of antibodies ..... 62

Table 6.1 miRNAs up regulated in MCF7 cells after exposure to hypoxia (0.1% $O_2$) vs. normoxia for 16 h. ..... 157

Table 6.2 miRNAs down regulated in MCF7 cells after exposure to hypoxia (0.1% $O_2$) vs. normoxia for 16 h. ..... 157

Table 6.3 miRNAs up regulated in MCF7 cells after exposure to hypoxia (0.1% $O_2$) vs. normoxia for 48 h. ..... 166

Table 6.4 miRNAs down regulated in MCF7 cells after exposure to hypoxia (0.1% $O_2$) vs. normoxia for 48 h. ..... 166

Table 6.5 miRNAs up regulated in MCF7 cells after DICER inhibition vs. normoxia. ..... 172

Table 6.6 Mature miRNAs down regulated in MCF7 cells after DICER inhibition vs. normoxia. ..... 173

Table 6.7 Pre-miRNAs up regulated in MCF7 cells after DICER inhibition. ..... 175

Table 6.8 Pre-miRNAs down regulated in MCF7 cells after DICER inhibition. ..... 175

# List of abbreviations

| | |
|---|---|
| 4EBP1 | eIF4E-binding proteins |
| AGO2 | Argonaute 2 |
| ALDOA | Aldolase A |
| AMPK | AMP-activated protein kinase |
| ANGPTL4 | Angiopoietin-like 4 |
| AP-1 | Activating protein 1 |
| APS | Ammonium persulphate |
| ARNT | Hydrocarbon receptor nuclear translocator |
| ARS2 | Arsenite-resistance protein 2 |
| Asn-803 | Asparagine residue 803 |
| ATP | Adenosine triphosphate |
| Bcl-2 | B-cell lymphoma 2 |
| BCL2L11 | Bcl-2 Like 11 |
| CAIX | Carbonic anhydrase IX |
| CAPS | N-cyclohexyl-3-aminopropanesulfonic acid |
| CBC | Cap-binding complex |
| CBP/p300 | Cyclic adenosine monophosphate (cAMP)-response-element-binding protein (CREB) binding protein |
| cDNA | Complementary DNA |
| ChIP | Chromatin immunoprecipitation |
| ChIP-Seq | ChIP coupled with next generation high throughput sequencing |
| CLL | Chronic lymphocytic leukaemia |
| COX-2 | Cyclooxygenase-2 |

| | |
|---|---|
| C-P4H1 | Collagen prolyl-4-hydroxylase |
| CREB | Cyclic AMP response element binding protein |
| CXCR4 | C-X-C chemokine receptor type 4 |
| DDX17 (p72) | DEAD box RNA helicase |
| DDX5 | DEAD/H box 5 |
| DDX5 (p68) | DEAD box RNA helicase |
| DFO | Desferrioxamine |
| DGCR8 | DiGeorge syndrome critical region 8 |
| DMSO | Dimethly sulfoxide |
| DNA | Deoxyribinucleic acid |
| dsRNA | Double stranded RNA |
| DTT | Dithiothreitol |
| DUF283 | Unknown Function 283 |
| dUTP | Deoxyuridine triphosphate |
| ECL | Enhanced chemiluminescence |
| EDN1 | Endothelin 1 |
| EDTA | Ethylenediaminetetraacetic acid |
| eEF2 | Eukaryotic elongation factor 2 |
| eEF2K | eEF2 kinase |
| EGR-1 | Early growth response-1 |
| EGRF | Epidermal growth factor receptors |
| eIF2α | Eukaryotic initiation factor 2α |
| EMT | Epithelial to mesenchymal transition |
| EPAS1 | Endothelial PAS domain protein |
| EPO | Erythropoietin |
| ERα | Estradiol |

| | |
|---|---|
| ETC | Electron transfer chain |
| $FADH_2$ | Flavin adenine dinucleotide |
| FBS | Fetal bovin serum |
| FIH-1 | Factor inhibiting HIF-1 |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase |
| GDP | Guanosine diphosphate |
| GFP | Green floursence protein |
| GLUT | Glucose transporters |
| GTP | Guanosine triphosphate |
| GW182 | Glycine tryptophan repeat containing protein of 182 kDa |
| HIF: | Hypoxia inducible factor |
| hnRNP A1 | Heterogenous nuclear ribonuclear protein |
| HNSCC | Head and neck squamous cell carcinomas |
| H-Ras | Transforming protein p21 |
| HREs | Hypoxia responsive elements |
| HRMs | Hypoxically regulated miRNAs |
| Hsp70 and Hsp 90 | Heat shock proteins |
| HuR protein | Human antigen R protein |
| HUVEC | Human umbilical vein endothelial cells |
| IGF2 | Insulin-like growth factor ii |
| IL-8 | Interleukin 8 |
| IPAS | Inhibitory PAS domain |
| ISCU1/2 | Iron-sulphur cluster assembly protein |
| K-Ras | V-Ki-ras2 Kirsten rat sarcoma viral oncogene |
| L1CAM | L1 cell adhesion molecule |
| LOX | Lysyl oxidase |

| | |
|---|---|
| MAPK | Mitogen-activated protein kinase |
| MEF | Mouse embryonic fibroblasts |
| miRNA | microRNA |
| miRtrons | Short intronic hairpins |
| MNT | Max binding protein |
| mRNA | Messenger RNA |
| mTOR | Mammalian target of rapamycin |
| MYC (c-MYC) | Myelocytomatosis oncogene |
| NADH | Nicotinamide adenine dinucleotide |
| NC | Negative control |
| NF-κB | Nuclear factor kappa light chain enhancer activated B cells |
| ODD | Oxygen dependent degradation domain |
| $p70^{s6k}$ | Protein Ser-Thr kinase that phosphorylates the ribosomal S6 subunit, |
| PACT | Protein activator of PKR |
| PAS domain | Per/Arnt/Sim domain |
| PBS | Phosphate buffered saline |
| PDGF | Platelet-derived growth factor B |
| PDK1 | Pyruvate dehydrogenase kinase 1 |
| PERK | Endoplasmic reticulum resident kinase |
| PHD1 | Prolyl hydroxylase domain 1 |
| PHD2 | Prolyl hydroxylase domain 2 |
| PHD3 | Prolyl hydroxylase domain 1 |
| PI3K | Phosphoinositide 3-kinase |
| PIWI | P-element induced wimpy testis |

| | |
|---|---|
| PMSF | Phenylmethylsulfonyl fluoride |
| POLR3K | Polymerase (RNA) III (DNA directed) polypeptide K |
| Pre-miRNA | Precursor miRNA |
| Pri-miRNA | Primary miRNA |
| Pro-402 and Pro-564 | Proline residue-402 and 564 |
| PVDF | Polyvinylidene difluoride |
| pVHL | Von Hippel Lindau protein |
| PWM | Position weight matrix |
| RAD52 | RADiosensitive protein 52 |
| Ran-GTP | Ran guanosine triphosphate |
| RCC | Clear cell renal carcinoma |
| RISC | RNA induced silencing complex |
| RL | Renilla luciferase |
| RLC | RISC loading complex |
| RNA | Ribonucleic acid |
| RNAi | RNA interference |
| RNU6B | U6B small nuclear RNA |
| RPC5 | RNA polymerase III subunit C5 |
| rpS6, | Ribosomal protein S6 |
| RT PCR | Reverse transcription polymerase chain reaction |
| SDS PAGE | Sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| shRNAs | Short hairpin RNA |
| SIP1 | SMN interacting protein 1 |
| siRNA | Small interfering RNA |
| ssRNA | Single stranded RNA |

| | |
|---|---|
| TAF9B | Transcription initiation factor TFIID subunit 9B |
| TARBP2 | Tar binding protein 2 |
| TEMED | Tetramethylethylenediamine |
| TP53 | Tumour protein 53 |
| tRNAs | Transfer RNA |
| UTR | Untranslasted region |
| VA1 RNA | Adenoviral RNA |
| VEGF | Vascular endothelial growth factor |
| XPO5 | Exportin 5 |
| Y-RNAs | Non-coding cytoplasmic localized RNAs |
| ZEB1 | Zinc finger E box-binding homeobox 1 |

# Abstract

Hypoxia is a key feature of many cancers and the presence of hypoxia is associated with more aggressive and metastatic tumours. MicroRNAs are 17-22 nucleotides, non-coding, single stranded RNA that are important regulators of gene expression. Functional studies show that microRNAs are involved in regulating many cellular processes including developmental timing, cell differentiation, cell proliferation and cell death. The expression levels of many microRNAs are deregulated in human disease conditions including cancer. In addition to deregulation of specific microRNAs in cancer, it has emerged that most tumour cell lines and cancers are characterised by global reductions in microRNA expression when compared to adjacent normal tissue. Cancers are commonly characterised by hypoxia and also by global reductions in the levels of mature microRNAs.

This thesis examined the hypothesis that hypoxia mediates the global reduction of microRNAs through repressive effects on microRNA biogenesis proteins. Cancer cell lines were exposed to hypoxia and manipulations of hypoxia inducible factor (HIF) and HIF hydroxylase activity. The effects of hypoxia on the mRNA and protein levels of enzymes involved in microRNA biogenesis (DICER, DROSHA, TARPB2, DCGR8, XPO5) were determined by RT PCR and immunoblotting. The effect of hypoxia on microRNA biogenesis and function was determined with microarrays, RT PCR, activity assays and reporter assays.

In two breast cancer lines (MCF7 and SKBR3), a colorectal cancer cell line (HT29) and a non-cancer cell line (HUVEC) there were significant reductions of DICER mRNA and protein levels after exposure to hypoxia. This effect was independent of HIF but dependent on the HIF hydroxylase PHD2 and was partly mediated by feedback effects by microRNAs. Furthermore, several other proteins with critical

roles in microRNA biogenesis such as DROSHA, DGCR8, TARBP2 and XPO5 also showed significant and co-ordinated repression under hypoxic conditions. The significant and consistent reduction in the levels of proteins with central roles in microRNA biogenesis under hypoxia did not have a substantial effect on the expression levels of mature microRNAs over the time course of these experiments. Even though hypoxia exerted only modest effects on the production of mature microRNAs, a significant influence of hypoxia on the function of exogenously introduced precursor microRNA was observed. These observations provide further and important interfaces between oxygen availability and gene expression and a potential mechanistic explanation for the reduced levels of microRNAs observed in some cancers. They provide further support for the existence of feedback mechanisms in the regulation of the microRNA biogenesis pathway and the relative stability of microRNAs.

# Declaration

'I certify that this work does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text';

Kanchana V. Bandara

# Publications and presentations

**Peer reviewed publications-submitted**

Bandara, K.V., Michael, M.Z. and Gleadle, J.M. Hypoxia represses microRNA biogenesis proteins in breast cancer cells. Submitted to BMC Cancer.

**Poster presentations**

Bandara, K.V., Michael, M.Z. and Gleadle, J.M. Hypoxic regulation of miRNAs in cancer. ASMR SA Division: Annual Scientific Meeting. Adelaide, Australia. 2011

Bandara, K.V., Michael, M.Z. and Gleadle, J.M. Repression of Dicer expression by hypoxia. Advances in Hypoxic Signaling: From Bench to Bedside (Q4). Keystone Symposia Conference, Fairmont Banff Springs, Banff, Alberta, Canada. February 12 - 17, 2012

Bandara, K.V., Michael, M.Z. and Gleadle, J.M. Dicer regulation by hypoxia. 6th Australian Health and Medical Research Congress 2012. Adelaide, Australia, 2012

Bandara, K.V., Michael, M.Z. and Gleadle, J.M. Dicer regulation by hypoxia. Flinders Centre for Innovation in Cancer Annual Research Day. Bedford Park, Australia. 2013

**Oral presentations**

Bandara, K.V., Michael, M.Z. and Gleadle, J.M. Hypoxic regulation of miRNAs in cancer. Adelaide RNA special interest group meeting. Adelaide, Australia. 2013

Bandara, K.V., Michael, M.Z. and Gleadle, J.M. Hypoxic regulation of miRNAs in cancer. Flinders Clinical and Molecular Medicine cluster seminar series. Bedford Park, South Australia. 2013

Bandara, K.V., Michael, M.Z. and Gleadle, J.M. Hypoxic regulation of miRNAs in cancer. Flinders Clinical and Molecular Medicine cluster seminar series. Bedford Park, South Australia. 2012

# Acknowledgements

I would like to sincerely thank Prof. Jonathan Gleadle for his guidance, encouragement, brilliant advice and enthusiasm for my project over the years, and also for contributions of time and support during the preparation of the thesis. I would like to thank Dr. Michael Micahel for allowing me to conduct my research in his laboratory, for providing guidance, encouragement and expert advice during my PhD and the final thesis preparation. I'm grateful to Prof Keryn Williams for her support and advice during my PhD studies.

I would like to acknowledge the funding bodies; Flinders Clinical and Molecular Medicine and Lyn Wrigley Trust (FMC Foundation) for providing the funds for my project.

I'm very grateful to all present and past lab colleagues for their support throughout my PhD journey. I would like to thank Letitia Pimlott, Karen Humphreys and Kym McNicholas for their constant support, helpful discussions and optimism. I'm very grateful to Hayley McFetridge, for going out of her way to help me during the final stages of my PhD research.

I'm also grateful to Roshini Somashekar, Jean Winter and Joanne Wilkins of Gastroenterology for technical support and helping me on numerous occasions. I would like to acknowledge Dr. Tim Chataway, Nusha Chegeni and Dr. Alex Colella at Flinders Proteomics facility, for their technical advice. Also thank Anne Rogers for technical advice. I'm grateful to Mark Van der Hoek from the Adelaide microarray facility for assisting with the miRNA microarrays, Dr Dan Peet and Greg

Goodall lab, Adelaide for providing me with reagents. Claudine Bonder at IMVS, Adelaide, for generously providing me with Human umbilical vein endothelial cells.

Finally, a very big thank you to my wonderful husband Peveni Rajapakse who has been a rock throughout and endlessly encouraged me, put up with the unavoidable side effects of doing a PHD while attempting to have a life. To my family, particularly my mother, thank you for your love, support, and unwavering belief in me. Without you, I would not be the person I am today.