Investigation of MicroRNA's in the Gastroesophageal Reflux – Barrett's Oesophagus – Adenocarcinoma Sequence

Cameron Michael Smith

Submitted in total fulfilment of the requirments of the degree of
Doctor of Philosophy

June 2012

Flinders University
The Department of Surgery,
Flinders Medical Centre

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Summary

Gastrointestinal reflux disease can lead to the development of Barrett's oesophagus (conversion of oesophageal squamous epithelium to columnar epithelium with intestinal metaplasia) and oesophageal adenocarcinoma. However, the molecular mechanisms driving the reflux-Barrett's oesophagus-oesophageal adenocarcinoma sequence are not fully understood. MicroRNAs (miRNAs) are a class of small RNA molecules involved in almost every cellular process investigated.

In this study quantitative assessment identified seven differentially expressed miRNAs in Barrett's oesophagus compared with squamous epithelium including: increased expression of miR-21, miR-143, miR-145, miR-194, miR-215 and decreased expression of miR-203 and miR-205. miR-143, miR-145 and miR-205 were also increased in gastroesophageal reflux disease. MiR-143, miR-145 and miR-215 were decreased in oesophageal adenocarcinoma.

Gastroesophageal reflux disease: Unravelling roles for miRNAs in the oesophagus

Subsequent studies were performed to explore the biological consequences of these changes in miRNA expression. Investigation of increased miR-143, miR-145 and miR-205 levels in an oesophageal squamous cell line identified these miRNAs can regulate proliferation and apoptosis. We therefore hypothesized that these miRNAs might act as regulators of oesophageal epithelial restitution in response to reflux. Investigation of miRNA and mRNA expression in tissues identified correlations between miR-143 and both BMP4, a key promoter of columnar specific gene expression and *CK8*, a marker of a columnar phenotype. This data is consistent with a possible role for miRNA expression in development of Barrett's oesophagus.

Tumour suppressor miRNAs in oesophageal adenocarcinoma

Studies using an oesophageal adenocarcinoma cell line revealed that decreased miR-143, miR-145 and miR-215 expression likely contributes to a reduction in proliferative and apoptotic control in this cancer. Further, this reduction is likely

mediated by a number of miRNA directed changes in gene expression. In-situ hybridisation identified localisation of these miRNAs to the crypts within the Barrett's oesophagus epithelium. Dysplasia is thought to originate from the crypts of the Barrett's oesophagus epithelium, so we hypothesized that miR-143, miR-145 and miR-215 play a role in regulating proliferation and apoptosis in these crypts, with decreased expression promoting the development cancer development in these areas.

The miR-200 family: Involvement in Barrett's oesophagus and oesophageal adenocarcinoma

Decreased expression of miR-141 and miR-200c, members of the miR-200 family was found to distinguish Barrett's oesophagus from related gastric and intestinal epithelia. Bioinformatic analysis provided computational evidence that this decreased miRNA expression might contribute to the abnormal proliferative and apoptotic status of Barrett's oesophagus epithelium.

We observed decreased expression of the miR-200 family (miR-141, miR-200a, miR-200b, miR-200c and miR-429) and increased expression of *ZEB1* and *ZEB2* in oesophageal adenocarcinoma. The miR-200 family regulates the epithelial to mesenchymal transition, a key process in tumour metastasis, by targeting the transcription factors *ZEB1* and *ZEB2*. These results provided the first evidence implicating miRNAs in the epithelial to mesenchymal transition in oesophageal adenocarcinoma.

Moving forward

This study provides an exciting platform to build from, especially for further investigating a miRNA mechanism for the development of both Barrett's oesophagus and oesophageal adenocarcinoma. In addition, this study provides preliminary support for the development of miRNA based tools for (1) assessing the efficacy of reflux control, (2) classifying patients at risk of developing Barrett's oesophagus and oesophageal adenocarcinoma, and (3) therapies targeted towards modulating miRNA expression to reduce oesophageal adenocarcinoma tumour growth.

Declaration

I certify that this thesis 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.

Signed …….. Date …….

Acknowledgements

I would like to thank my supervisors Dr. Damian Hussey, Dr. Michael Michael and Prof. David Watson for supporting me throughout this project. While you have all helped me complete my project in different ways, your passion for research has been a great motivator for me.

Damian, I am very grateful for the opportunity to study in your laboratory. The friendly and dynamic lab that you have established has provided me with the ability to work in an efficient and relaxed environment. Your friendly, organised and enthusiastic approach has been a constant source of motivation and support.

Michael, your enthusiasm and encouragment has been constant throughout my project and this has helped me to establish new techniques and ask new and exciting questions. I have learnt a lot from your collaborative nature and this has helped me expand the scope of my project to many different areas.

David, the clinical focus that you have brought to this project has been extremely helpful. I have learnt a lot from discussion with you and have also been motivated by your ability to bring together many different sectors of the medical research community.

There are also so many people throughout the Flinders Medical Centre that I would like to acknowledge and thank. Thank you to Mary Leong, George Mayne, Tingting Wang, Stuti Srivastava, Olga Sukocheva, Jakob Kist, Rainer Haberberger, Tim Chataway, Gino Saccone, Ann Schloithe, Dani Dixon, Kim Griggs for your assistance, encouragment and support throughout my project. I would also like to acknowledge and thank Dr Richard Hummel for his friendly motivation and humour which has been of great assistance to me throught his time in the lab.

I feel a special mention and thank you should go out to the PhD office shared by many on level 3. Lauren Thurgood, you were the first to finish, and showed us all that it could be done. George Barretto and Alison Elder, thank you for all of the

laughs and constructive discussions we shared. You both made the office a fun and enjoyable place to work.

Thank you to the staff in Anatomical Pathology at SA Pathology, Flinders Medical Centre for assistance with tissue processing and staining of tissue slides. I would also like to acknowledge work by Grace Tan, Richard Hummel and Stuti Srivastava. Grace used PCR to assess the expression of *IL6, CK14, BMP4, CK8, CK20, CDX2, HNF1*α and *GATA4* in samples from patients with ulcerative oesophagitis. Richard performed additional miRNA PCR analysis to allow miRNA expression to be compared between Barrett's oesophagus, tissue control and ulcerative oesophagitis patient groups. Stuti performed transfections, RNA isolations, and PCRs for predicted miRNA targets. In addition, Stuti performed western blot experiments for KRAS, ZEB2, YES, RTKN, DTL, HDAC7 and GAPDH in protein isolate from cells transfected with miR-143, miR-145 or miR-215. I would also like to thank Dr Peter Rabinovitch for kindly providing the Barrett's oesophagus and dysplastic cell lines Qh, Ch and Gi.

This study was funded by a Competing Project Grant from the National Health and Medical Research Council of Australia. I was supported by a Flinders University Research Scholarship and a PROBE-NET PhD scholarship funded by a Strategic research Partnerships Grant from the Cancer Council of New South Wales. These funding sources have allowed this project to be completed in a timely fashion for which I am very grateful.

I would like to thank my parents John and Debbie Smith as well as my sister Allannah. As always, your love and support has been without limits. To all my other family and friends, I thank you for providing both encouragement and laughter, as these have been a great motivator for completing my thesis.

Finally, I would like to thank my wife Siobhan Smith for her love and support that she provides on a daily basis. Thanks for being there for the emotional rollercoaster that comes part and parcel with every PhD project.

Publications

Publications arising directly from this thesis:

Smith CM, Michael MZ, Watson DI and Hussey DJ. microRNA expression in oesophageal adenocarcinoma and proximal gastric adenocarcinoma. *World Journal of Surgery* 2009; **33** (Supp1).

Smith CM, Watson DI, Michael MZ, Hussey DJ. MicroRNAs, development of Barrett's esophagus, and progression to esophageal adenocarcinoma. *World Journal of Gastroenterology* 2010; **16**(5): 531-537.

Wijnhoven BP, Hussey DJ, Watson DI, Tsykin A, **Smith CM**, Michael MZ, and South Australian Oesophageal Research Group. MicroRNA profiling of Barrett's oesophagus and oesophageal adenocarcinoma. *British Journal of Surgery* 2010; **97**(6): 853-861.

Smith CM, Watson DI, Leong MP, Mayne GC, Michael MZ, Wijnhoven BP, Hussey DJ. miR-200 family expression is downregulated upon neoplastic progression of Barrett's esophagus. *World Journal of Gastroenterology* 2011; **17**(8): 1036-1044.

Smith CM, Michael MZ, Watson DI, Tan G, Astill D, Hummel R, Hussey DJ. Impact of gastroesophageal reflux disease on miRNA expression, location and function. Manuscript in preparation for submission to *Digestive Diseases and Sciences.*

Other Publications:

Barreto SG, Bazargan M, Zotti M, Hussey DJ, Sukocheva OA, Peiris H, Leong M, Keating DJ, Schloithe AC, Carati CJ, **Smith CM**, Toouli J, Saccone GT. Galanin receptor 3 - a potential target for acute pancreatitis therapy. *Neurogastroenterology and Motility* 2011; **23**(3): e141-151.

Hummel R, Watson DI, **Smith CM**, Kist J, Michael MZ, Haier J, Hussey DJ. Mir-148a improves response to chemotherapy in sensitive and resistant oesophageal adenocarcinoma and squamous cell carcinoma cells. *Journal of Gastrointestinal Surgery* 2011; **15**(3): 429-438.

Selected conference presentations arising from the thesis

Surgical Research Society of Australasia, Adelaide, SA, November 2008. **Smith CM**, Michael MZ, Watson DI, Bright T, Wijnhoven, BP, Dijckmeester WA, Mayne GC, Astill D and Hussey DJ. miR-143 expression in gastroesophageal reflux disease and Barrett's oesophagus.

The Australian Society for Medical Research, Adelaide, SA, June 2009. **Smith CM**, Michael MZ, Watson DI and Hussey DJ. miRNA expression in gastroesophageal reflux disease and Barrett's oesophagus.

International Surgical Week, Adelaide, SA, November 2009. **Smith CM**, Michael MZ, Watson DI and Hussey DJ. MicroRNA expression in oesophageal adenocarcinoma and proximal gastric adenocarcinoma

Lorne Genome Conference, Lorne, VIC, February 2010. **Smith CM**, Michael MZ, Watson DI and Hussey DJ. Altered microRNA expression in gastroesophageal reflux disease.

Australian Health and Medical Research Congress, Melbourne, VIC, November 2010. **Smith CM**, Tan G, Michael MZ, Watson DI and Hussey DJ. Altered miRNA expression in gastroesophageal reflux disease and miR-143 expression in Barrett's oesophagus.

Keystone Symposia: microRNAs and non-coding RNAs and cancer, Banff, Canada, February 2011. **Smith CM**, Michael MZ, Watson DI and Hussey DJ. Down regulation of miR-143, miR-145 and miR-215 is directly linked with neoplastic hallmarks of oesophageal adenocarcinoma.

Abbreviations

In text abbreviations

GORD	gastroesophageal reflux disease
BE	Barrett's oesophagus
HGD	high grade dysplasia
EAC	oesophageal adenocarcinoma
miRNA	microRNA
IPL	interpapillary basal layer
PBL	papillary basal layer
H&E	hematoxylin and eosin
ABPASD	alcian blue periodic Schiff diastase
Pri-miRNA	primary miRNA
TRBP	trans-activation-responsive RNA-binding protein
miRNP	miRNA-containing ribonucleo-protein particles
RISC	RNA induced silencing complex
HITS-CLIP	high throughput sequencing of RNA's being isolated following immuno-precipitation of RISC complexes
MQ	milliQ
SDS	sodium dodecyl sulfate
EDTA	ethylenediaminetetraacetic acid
cDNA	complementary DNA
Het-1A	cell line derived from oesophageal squamous tissue
Qh	Barrett's oesophagus cell line
Ch, Gi	High grade dysplasia cell lines
OE-19	oesophageal adenocarcinoma cell line
Tris	tris(hydroxymethyl)aminomethane
TBE	tris-borate-EDTA
DMEM	Dulbecco's modified eagle medium
DEPC	diethyl pyrocarbonate
DIG	digoxigenin
LNA	locked nucleic acid

SSC	saline sodium citrate
HCl	hydrochloric acid
NBT	nitro blue tetrazoliumchloride
kDa	kilodalton
ISH	in-situ hybridisation
RT-PCR	real-time polymerase chain reaction
RT	reverse transcription
EMT	epithelial to mesenchymal transition

Gene Names used in text

BMP4	bone morphogenic protein 4
CDX2	caudal type homeobox 2
HNF1α	HNF1 homeobox A
NFKβ	nuclear factor kappa B
GATA4	GATA binding protein 4
RARX	retinoic acid receptor X
PDCD4	programmed cell death 4
CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2
EGR1	early growth response 1
DUSP10	dual specificity phosphatase 10
SLAMF7	SLAM family member 7
GEM	GTP binding protein overexpressed in skeletal muscle
ANKRD1	ankyrin repeat domain 1
CYR61	cysteine-rich, angiogenic inducer, 61
H2AFX	H2A histone family, member X
RTKN	rhotekin
PABPC4	poly(A) binding protein, cytoplasmic 4
NUAK2	NUAK family, SNF1-like kinase, 2
HDAC7	histone deacetylase 7
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
YES	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1

DTL	denticleless homolog (Drosophila)
CXCR2	chemokine (C-X-C motif) receptor 2
TGFβ	transforming growth factor, beta 1
IL6	interleukin 6
CK	cytokeratin
ZEB1	zinc finger E-box binding homeobox 1
ZEB2	zinc finger E-box binding homeobox 2
PRKCE	protein kinase C, epsilon
PI3K	phosphoinositide-3-kinase, catalytic, alpha polypeptide
AKT	v-akt murine thymoma viral oncogene homolog 1
AP1	jun proto-oncogene
EGR3	early growth response 3
HS3ST1	heparan sulfate (glucosamine) 3-O-sulfotransferase 1
RPS6KB1	ribosomal protein S6 kinase, 70kDa, polypeptide 1
mTOR	mechanistic target of rapamycin
MUC1	mucin 1, cell surface associated
FSCN1	fascin homolog 1, actin-bundling protein (Strongylocentrotus purpuratus)
JAMA	F11 receptor
TSPAN8	tetraspanin 8
MAPK	mitogen activated protein kinase 1
ERK	mitogen activated protein kinase 1
JNK	mitogen activated protein kinase 8
P38	mitogen activated protein kinase 14