

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

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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

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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
<i>ABPASD</i>	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

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

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