ASSESSMENT OF HYPOXIA ASSOCIATED MARKERS IN OESOPHAGOGASTRIC CANCER

A thesis submitted to The University of Manchester for the degree of Doctor of Medicine in the Faculty of Medical and Human Sciences

Ewen Alexander Griffiths

2006

School of Medicine

This project was undertaken in the Department of Gastrointestinal Surgery, South Manchester University Hospitals NHS Trust, Wythenshawe Hospital, South Moor Road, Manchester, M23 9LT and the Academic Department of Radiation Oncology, Christie Hospital NHS Trust, Christie Hospital, Wilmslow Road, Withington, Manchester, M20 4BX

DEDICATION

To Uncle Ron who died of gastric cancer.

To my dearest cousin Alex who will be sadly missed.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

15-Lox-1	15-lipoxygenase-1
5-FU	5-flurouracil
AB	antibody
ABC	avidin-biotin complex
AF	atrial fibrillation
APC	adenomatous polyposis coli
APeS	aminopropyltriethoxysilane
ARDS	adult respiratory distress syndrome
ARNT	aryl hydrocarbon receptor nuclear translocator
ASA	American Society of Anaesthesiologists
ASCOT	Assessment of Stomach and Oesophageal Cancer Outcomes and Treatment
AUGIS	Association of Upper Gastrointestinal Surgeons of Great Britain and Ireland
BCG	intra-vesical bacille Calmette-Guerin
BCL-2	B-cell CLL/lymphoma-2
BSG	British Society of Gastroenterology
CAG	cag pathogenicity island
CA-9	carbonic anhydrase-9
CD44	CD44 antigen
CDC25B	cell division cycle 25B
CDH1	E-cadherin gene
CEA	carcinoembryonic Antigen
c-erbB2	v-erb-b2 erythroblastic leukaemia viral oncogene homologue 2
CI	confidence interval
CLO	columnar-lined oesophagus
c-Met	met proto-oncogene (hepatocyte growth factor receptor)
COX-2	cycloxygenase-2
CR	complete response
CRM	circumferential resection margin
CT	computer tomography
CTA	clinical trial authorisation
CVA	cerebrovascular accident
DAB	3, 3'– diaminobenzidine
DCC	deleted in colon cancer
DNA	deoxyribonucleic acid
DRM	distal resection margin
ECF	epirubicin, cisplatin and fluorouracil
EDTA	ethylenediaminetetraacetic acid
EF-5	2-nitroimadazole compound
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ELISA	enzyme linked immuno-sorbent assay
EMR	endoscopic mucosal resection
Epo-R	erythropoietin receptor
EUS	endoscopic ultrasound
FGD	^{18}F-label glucose analogue 2-fluro-2-deoxy-D-glucose
FHIT	fragile histidine triad gene
GAMBO	goat anti-mouse biotinylated antibody
GI	gastrointestinal
GIST	gastrointestinal stromal tumour
Glut-1	glucose transporter-1
GOJ	gastro-oesophageal junction
GORD	gastro-oesophageal reflux disease
H&E	haematoxylin & eosin
H. pylori	helicobacter pylori
HGD	high grade dysplasia
HIF	hypoxia-inducible factor
HR	hazard ratio
HRE	hypoxia-responsive elements
hTERT	telomerase reverse transcriptase
IARC	International Agency for Research and Cancer
IGF	insulin like growth factor
IHC	immunohistochemistry
IL	interleukin
IM	intestinal metaplasia
IMP	investigational medicinal product
iNOS	inducible nitric oxide synthase
IRS	immunoreactive score
ISH	in situ hybridisation
Ki-67	MIB-1, proliferation antigen
K-Ras	v-Kiras2 Kirsten rat sarcoma viral oncogene homologue
K-sam	encodes fibroblast growth factor receptor 2
LDH	lactate dehydrogenase
LGD	low grade dysplasia
LREC	local research ethics committee
LRM	longitudinal resection margin
LTA	left thoracoabdominal approach
LVF	left ventricular failure
MALT	mucosa-associated lymphoid type B-cell lymphoma
MHRA	Medicines and Healthcare products Regulatory Agency
MMP	matrix metalloproteinase
Mono	monoclonal antibody
Morb	morbidity
Mort	mortality
MRC	Medical Research Council
MRI	magnetic resonance imaging
MSI-H	microsatellite instability-high
MUC	mucin gene
MVD	Micro-vessel density
NGT	nasogastric tube
NICE	National Institute of Clinical Excellence
nm23	non-metastatic protein cells 1
NO	nitric oxide

NSAID	non-steroidal anti-inflammatory drugs
NSCLC	non small cell lung cancer
O/N	overnight
OGD	oesophago-gastro-duodenoscopy
PCR	polymerase chain reaction
PDT	photodynamic therapy
PE	pulmonary embolism
PET	positron emission tomography
PGE_2	prostaglandin-E2
PNCA	proliferating cell nuclear antigen
Poly	polyclonal antibody
POSSUM	Physiological and operative severity score for enumeration of mortality and morbidity
PPI	proton pump inhibitors
PRM	proximal resection margin
Prosp	prospective
PTEN	phosphatase and tensin homolog deleted on chromosome ten
R&D	research & development
RAMBO	rabbit anti-mouse biotinylated antibody
RB	retinoblastoma
Retro	retrospective
RNA	ribonucleic acid
RNS	reactive nitrogen species
ROS	reactive oxygen species
RT	reverse transcriptase
RT	room temperature
SAGOC	Scottish Audit of Gastric and Oesophageal Cancer
SCC	squamous cell carcinoma
SIP1	SMAD-interacting protein 1
Smad4	Also called Dpc4; tumor suppressor in the TGF-β signalling pathway
SMUHT	South Manchester University Hospitals NHS Trust
SPECT	single photon emission computed tomography
TBS	Tris-buffered saline
TGF-α/β	Transforming growth factor-α/β
TMA	tissue microarray
TNF-α	tumour necrosis factor-α
TNM	tumour node metastases staging system
TNT	Tris-HCL/NaCl/Tween
TP	thymidine phosphorylase
tp16	tumour protein 16
tp53	tumour protein 53
TSA	tyramide signal amplification
TUR	transurethral resection of bladder tumour
twist-1	twist homologue 1
UICC	International union against cancer
UTI	urinary tract infection
VEGF	vascular endothelial growth factor
VHL	von-hippel-lindau protein
WHO	World Health Organisation
YC-1	3-(5'-hydroxymethyl-2'-furyl1)-1-benzylindazole

ABSTRACT

THE UNIVERSITY OF MANCHESTER

ABSTRACT OF THESIS submitted by Ewen A. Griffiths for the Degree of Doctor of Medicine and entitled 'Assessment of hypoxia associated markers in oesophagogastric cancer' July 2006.

Introduction: There is a need to increase understanding of oesophagogastric cancer biology and develop methods for determining prognosis. Hypoxia is implicated in the aetiology and prognosis of a number of cancers, but has not been studied in oesophagogastric cancer. It is important to understand the patient and tumour characteristics that might influence biological data. For example, tumour length and circumferential resection margin (CRM) status have not been adequately assessed as prognostic markers in oesophageal cancer.

Aims: 1) To investigate the relationship of tumour length and CRM with other histopathological variables and survival in patients with surgically treated oesophageal cancer. 2) To establish a retrospective database of patients with gastric and gastro-oesophageal junctional (GOJ) cancer; analyse relevant clinico-pathological prognostic factors prior to molecular marker analysis. 3) To investigate HIF-1α and HIF-2α expression as prognostic markers in gastric and GOJ cancer. 4) To investigate HIF-1α and other related markers (HIF-2α, Epo, Epo-R, Glut-1, Ki67, VEGF) in oesophageal and gastric adenocarcinoma carcinogenesis. 5) To establish a prospective study to measure tumour hypoxia (by pimonidazole staining).

Findings: 1) Both tumour length and CRM status were independent prognostic factors in surgically treated patients with oesophageal cancer. 2) A retrospective database of 251 patients was established. Only additional surgical resection of the spleen or pancreas and ASA grade were independent predictors of prognosis. 3) In 177 patients for whom tissue was obtained, HIF-1α expression had no prognostic significance. However, HIF-1α expression pattern was a significant predictor of survival on univariate analysis; patients with HIF-1α expression at the invasive edge had a median survival of only 18 mths compared with 33 mths in HIF-1α negative tumours. HIF-2α expression was a prognostic factor on univariate analysis. Neither HIF-1α nor HIF-2α had independent prognostic significance. 4) The expression of the hypoxia associated markers increased significantly from normal tissue to invasive malignancy in both the oesophageal and gastric carcinogenesis models. 5) A prospective study was established after LREC and R&D approval was obtained. Data for the first 9 patients enrolled showed intra and inter-tumoral variation in hypoxia.

Conclusions: 1) The development of imaging approaches for assessing tumour length pre-operatively would be of value. CRM should continue to be reported on routine histopathology. 2) There are clinico-pathological and prognostic differences between GOJ and other gastric tumours. A standard classification of GOJ tumours should be adopted internationally. 3) The dependence of HIF-1α as a prognostic factor on staining pattern may be due to its differential regulation of down-stream molecules. As neither HIF-1α or HIF-2α had independent prognostic significance, they are unlikely to play a role as single markers of prognosis. The high expression of HIF-2α suggests its further study as a therapeutic target would be of value. 4) HIF-2α should be assessed as a predictive marker of disease progression in patients with Barrett's dysplasia. 5) Some oesophagogastric cancers are strongly hypoxic.

DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

COPYRIGHT STATEMENT

PREFACE

Ewen A Griffiths graduated MB ChB from Dundee University in 2000. He undertook basic surgical training in the North East of England and passed the Membership of Royal College of Surgeons (Glasgow) examination in 2003. Work carried out in this thesis was completed whilst he was employed as a Surgical Research Fellow in the Department of Gastrointestinal Surgery, South Manchester University Hospitals NHS Trust and the Academic Department of Radiation Oncology, Christie Hospital NHS Trust. In February 2006 he was appointed to the North West general surgical specialist registrar rotation and plans to sub-specialise in Oesophagogastric surgery.

ACKNOWLEDGEMENTS

First and foremost I would like to thank my supervisors Dr Catharine West and Mr Ian Welch for their help and all the support they have given me over the last two years. I also greatly appreciate the priceless assistance that Helen Valentine provided with the laboratory side of the project and her patience with me whilst I learnt new techniques. In addition I am indebted to Dr Susan Pritchard for all the hard work she has put in scoring slides and helping with the pathological aspects of the projects. I also thank Dr Stephen McGrath for being the second slide scorer.

There are many other people I would like to thank; without them this project would never have been as successful as it has proved to be:

- Staff at Wythenshawe Hospital; in particular staff of the Department of Histopathology, especially Dr Paul Bishop and Nigel Martin. Staff of the Clinical Audit Department, who where invaluable in obtaining patient records. Special credit is due to Carol Toner and Toni Yao obtaining hundreds of medical notes and to Stephen Bullough for searching the NHS tracking database. In addition, thanks are due to Fran Mellor, Upper GI Cancer Nurse Specialist and Mr Simon Galloway for helping to recruit patients into the prospective study. I am also most grateful to Jane Ellis for secretarial support and to Vivienne Benson and Susie Rushton, Pharmacy Department, for helping with the pimonidazole study.
- I would like to thank Prof Pat Price, Dr Jo Cresswell, Claire Brooks, Anne Mason and James Cullen from the Academic Department of Radiation Oncology for their help in supporting my research.
- I would like to thank Zoe Brummel and Nina Whitchelo who as medical students helped in initiating some of the projects which were developed in this thesis.
- I am grateful to Julie Morris, Head of Medical Statistics, Wythenshawe Hospital and David Ryder, Medical Statistics, Christie Hospital for helping me with statistical analysis. Also, Francesca Buff, Gray Institute, helped in the analysis of the Barrett's carcinogenesis data – for which I'm very grateful.
- Finally I would like to thank all of the patients who kindly agreed to participate in the prospective study.