

ASSESSMENT OF HYPOXIA ASSOCIATED MARKERS IN OESOPHAGOGASTRIC CANCER

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DEDICATION

To Uncle Ron who died of gastric cancer.

To my dearest cousin Alex who will be sadly missed.

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LIST OF ABBREVIATIONS

15-Lox-1	15-lipoxygenase-1	FGD	¹⁸ F-label glucose analogue 2-fluro-2-deoxy-D-glucose
5-FU	5-flurouracil	FHIT	fragile histidine triad gene
AB	antibody	GAMBO	goat anti-mouse biotinylated antibody
ABC	avidin-biotin complex	GI	gastrointestinal
AF	atrial fibrillation	GIST	gastrointestinal stromal tumour
APC	adenomatous polyposis coli	Glut-1	glucose transporter-1
APeS	aminopropyltriethoxysilane	GOJ	gastro-oesophageal junction
ARDS	adult respiratory distress syndrome	GORD	gastro-oesophageal reflux disease
ARNT	aryl hydrocarbon receptor nuclear translocator	H&E	haematoxylin & eosin
ASA	American Society of Anaesthesiologists	H. pylori	helicobacter pylori
ASCOT	Assessment of Stomach and Oesophageal Cancer Outcomes and Treatment	HGD	high grade dysplasia
AUGIS	Association of Upper Gastrointestinal Surgeons of Great Britain and Ireland	HIF	hypoxia-inducible factor
BCG	intra-vesical bacille Calmette-Guerin	HR	hazard ratio
BCL-2	B-cell CLL/lymphoma-2	HRE	hypoxia-responsive elements
BSG	British Society of Gastroenterology	hTERT	telomerase reverse transcriptase
CAG	cag pathogenicity island	IARC	International Agency for Research and Cancer
CA-9	carbonic anhydrase-9	IGF	insulin like growth factor
CD44	CD44 antigen	IHC	immunohistochemistry
CDC25B	cell division cycle 25B	IL	interleukin
CDH1	E-cadherin gene	IM	intestinal metaplasia
CEA	carcinoembryonic Antigen	IMP	investigational medicinal product
c-erbB2	v-erb-b2 erythroblastic leukaemia viral oncogene homologue 2	iNOS	inducible nitric oxide synthase
CI	confidence interval	IRS	immunoreactive score
CLO	columnar-lined oesophagus	ISH	in situ hybridisation
c-Met	met proto-oncogene (hepatocyte growth factor receptor)	Ki-67	MIB-1, proliferation antigen
COX-2	cyclooxygenase-2	K-Ras	v-Kiras2 Kirsten rat sarcoma viral oncogene homologue
CR	complete response	K-sam	encodes fibroblast growth factor receptor 2
CRM	circumferential resection margin	LDH	lactate dehydrogenase
CT	computer tomography	LGD	low grade dysplasia
CTA	clinical trial authorisation	LREC	local research ethics committee
CVA	cerebrovascular accident	LRM	longitudinal resection margin
DAB	3, 3'-diaminobenzidine	LTA	left thoracoabdominal approach
DCC	deleted in colon cancer	LVF	left ventricular failure
DNA	deoxyribonucleic acid	MALT	mucosa-associated lymphoid type B-cell lymphoma
DRM	distal resection margin	MHRA	Medicines and Healthcare products Regulatory Agency
ECF	epirubicin, cisplatin and fluorouracil	MMP	matrix metalloproteinase
EDTA	ethylenediaminetetraacetic acid	Mono	monoclonal antibody
EF-5	2-nitroimidazole compound	Morb	morbidity
EGF	epidermal growth factor	Mort	mortality
EGFR	epidermal growth factor receptor	MRC	Medical Research Council
ELISA	enzyme linked immuno-sorbent assay	MRI	magnetic resonance imaging
EMR	endoscopic mucosal resection	MSI-H	microsatellite instability-high
Epo-R	erythropoietin receptor	MUC	mucin gene
EUS	endoscopic ultrasound	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	SCC	squamous cell carcinoma
NSCLC	non small cell lung cancer	SIP1	SMAD-interacting protein 1
O/N	overnight	Smad4	Also called Dpc4; tumor suppressor in the TGF- β signalling pathway
OGD	oesophago-gastro-duodenoscopy	SMUHT	South Manchester University Hospitals NHS Trust
PCR	polymerase chain reaction	SPECT	single photon emission computed tomography
PDT	photodynamic therapy	TBS	Tris-buffered saline
PE	pulmonary embolism	TGF-α/β	Transforming growth factor- α/β
PET	positron emission tomography	TMA	tissue microarray
PGE₂	prostaglandin-E2	TNF-α	tumour necrosis factor- α
PNCA	proliferating cell nuclear antigen	TNM	tumour node metastases staging system
Poly	polyclonal antibody	TNT	Tris-HCL/NaCl/Tween
POSSUM	Physiological and operative severity score for enumeration of mortality and morbidity	TP	thymidine phosphorylase
PPI	proton pump inhibitors	tp16	tumour protein 16
PRM	proximal resection margin	tp53	tumour protein 53
Prosp	prospective	TSA	tyramide signal amplification
PTEN	phosphatase and tensin homolog deleted on chromosome ten	TUR	transurethral resection of bladder tumour
R&D	research & development	twist-1	twist homologue 1
RAMBO	rabbit anti-mouse biotinylated antibody	UICC	International union against cancer
RB	retinoblastoma	UTI	urinary tract infection
Retro	retrospective	VEGF	vascular endothelial growth factor
RNA	ribonucleic acid	VHL	von-hippel-lindau protein
RNS	reactive nitrogen species	WHO	World Health Organisation
ROS	reactive oxygen species	YC-1	3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole
RT	reverse transcriptase		
RT	room temperature		
SAGOC	Scottish Audit of Gastric and Oesophageal Cancer		

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-1 α and HIF-2 α expression as prognostic markers in gastric and GOJ cancer. 4) To investigate HIF-1 α and other related markers (HIF-2 α , 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-1 α expression had no prognostic significance. However, HIF-1 α expression pattern was a significant predictor of survival on univariate analysis; patients with HIF-1 α expression at the invasive edge had a median survival of only 18 mths compared with 33 mths in HIF-1 α negative tumours. HIF-2 α expression was a prognostic factor on univariate analysis. Neither HIF-1 α nor HIF-2 α 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-1 α as a prognostic factor on staining pattern may be due to its differential regulation of down-stream molecules. As neither HIF-1 α or HIF-2 α had independent prognostic significance, they are unlikely to play a role as single markers of prognosis. The high expression of HIF-2 α suggests its further study as a therapeutic target would be of value. 4) HIF-2 α 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.

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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.

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