EXPRESSION OF ENDOPLASMIC RETICULUM OXIDOREDUCTASES (EROS) AND THEIR ROLE IN THE GI TRACT

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ABSTRACT

It has been shown that some ER redox enzymes are differentially expressed in stomach and oesophagus tissue. The tissues of the gastrointestinal system, which are subject to external changes of environment during the process of digestion, represent a novel area in which human ER oxidoreductases (Eros) can be studied.

Barrett's oesophagus is a common premalignant condition characterised by acid and bile reflux. We hypothesised that the development of metaplastic tissue in Barrett's may be associated with changes in the expression of Eros, and that the environment of gastric reflux could drive oxidative changes in the structure of Eros.

In this thesis, it is shown that Ero1α is expressed at a higher level in OE33 oesophageal adenocarcinoma cells than in OE21 oesophageal squamous carcinoma cells. Ero1β is not expressed in these cells. Altering pH or culture media or bile acid treatment does not cause any detectable changes in the expression or oxidation state of Ero1α, Ero1β or Protein Disulphide Isomerases (PDIs) in the OE21 and OE33 cell lines.

Human Ero1β was produced as a recombinant HIS-tagged protein, which was inactive when thioredoxin was used as a substrate, but could oxidise PDI *in vitro*. Attempts were made to produce redox-state specific antibodies against either Ero1α or Ero1β. Ero1α and Ero1β-HIS recombinant proteins were used to produce hybridomas, which were tested for Ero1α or Ero1β specificity in rodent tissue and cell lines.

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LIST OF ABBREVIATIONS

ADP	adenosine diphosphate
AGR2	anterior grade homolog 2
AMS	4-acetamido-4′-maleimidylstilbene-2,2′-disulfonic acid
APP	amyloid precursor protein
APS	ammonium persulfate
AS	active site
ATF6	activating transcription factor
ATP	adenosine triphosphate
BiP	binding protein, aka Grp78
bp	base pairs
BSA	bovine serum albumin
BSEP	bile salt export pump
bZIP	basic leucine zipper domain
CA	cholic acid
cAMP	cyclic adenosine monophosphate
CD	cell adhesion molecule: cluster of differentiation
CDCA	chenodeoxycholic acid
cDNA	complementary DNA
CDX2	caudal-type homeobox 2
CFTR	cystic fibrosis transmembrane conductance regulator
CLAP	chymostatin, leupeptin, antipain and pepstatin
CMC	critical micelle concentration
CoA	coenzyme A
CPY	carboxypeptidase Y
CRE	cAMP-response element
CYP	cytochrome P
DAPI	4',6-diamidino-2-phenylindole
DCA	deoxycholic acid
DMEM	Dulbecco’s modified Eagles’s medium
DNA	deoxyribonucleic acid
DTT	dithiothreitol
ECL	enhanced chemiluminescence
EDEM	ER degradation enhancing 1,2-mannosidase like protein
EDTA	ethylenediaminetetraacetic acid
EGF	endothelial Growth Factor
Endo H	endoglycosidase H
ER	endoplasmic reticulum

ERAD	ER-associated degradation
ERD2	ER lumen protein retaining receptor
Ero1α	endoplasmic reticulum oxidoreductase α
Ero1β	endoplasmic reticulum oxidoreductase β
ERp	endoplasmic Reticulum Protein
ERSE	ER stress response element
FAD	flavin adenine dinucleotide
FCS	fetal calf serum
GDEA	gastroduodenoesophageal anastomosis
GDP	guanosine 5′-diphosphoglucose
GI	gastrointestinal
Gls I	glucosidase I
Gls II	glucosidase II
GORD	gastro-oesophageal reflux disease
GPX	glutathione peroxidise
Grp78	glucose regulating protein, aka BiP
GSH	reduced glutathione
GSSG	oxidised glutathione
GST	glutathione S-transferase
GT	UDP-glucose:glycoprotein glycosyltransferase
GTP	Guanosine-5'-triphosphate
H_2O_2	hydrogen peroxide
HA	influenza virus hemagglutinin
HIF-1	hypoxia-inducible factor 1
HIS	Histidine tag
HSP	Heat Shock Protein
Ig	immunoglobulin
IP	immunoprecipitation
IP3R1	inositol triphosphate receptor type 1
IPTG	isopropyl β-D-1-thiogalactopyranoside
IRE1	inositol requiring kinase 1
LB	lysogeny broth
LCA	lithocholic Acid
MAM	mitochondrial membrane associated ER membrane
MAPK	mitogen-activated protein kinase
MEM	minimum essential medium
MES	2-(N-morpholino)ethanesulphonic acid
MHC	major Histocompatibility Complex
Mns I	mannosidase I

MNT	MES-NaCl-Tris lysis buffer
mRNA	messenger ribonucleic acid
NEF	nucleotide exchange factor
NEM	N-ethylmaleimide
NF-κB	nuclear factor kappa-B
NI-NTA	nickel-nitriloacetic acid
NMR	nuclear magnetic resonance
OD	optical density
ORF	open reading frame
OST	oligosaccharyl transferase
PAGE	polyacrylamide gel electrophoresis
PBD	protein binding domain
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDI	protein disulfide isomerase
PERK	protein kinase RNA-like endoplasmic reticulum kinase
Prx	peroxiredoxin
pK_a	acid dissociation constant
QSOX	quiescin-sulphydryl oxidase
RAMP	receptor activity-modifying protein
RC1	regulatory cysteines 1
RC2	regulatory cysteines 2
RER	rough endoplasmic reticulum
RNA	ribonucleic acid
ROS	reactive oxygen species
Rpl Proteins	ribosomal proteins
RPMI	Roswell Park Memorial Institute medium
rRNAse	reduced ribonuclease
RT-PCR	reverse transcriptase PCR
SC	shuttle cysteines
SDS	sodium dodecyl sulphate
SER	smooth endoplasmic reticulum
SOD2	superoxide dismutase 2
SRP	signal recognition particle
TAE	tris base, acetic acid and EDTA
TAP	transporter associated with antigen processing
TBS	tris-buffered saline
TCA	trichloroacetic acid
TEMED	N,N,N',N'-Tetramethylethylenediamine

TM	transmembrane domain
TRAM	translocating chain-associated membrane protein
TRX	thyoredoxin
UPR	unfolded protein response
VEGF	vascular endothelial growth factor
XBP1	x-box-binding protein-1

DECLARATION

I declare that the experiments described in this thesis were carried out by me in the School of Biological and Biomedical Sciences, University of Durham, under the supervision of Dr. Adam M. Benham and Mr YKS Viswanath. This thesis has been composed by myself and is a record of work that has not been submitted previously for a higher degree.

Graeme Ronald Watson

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DEDICATION

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